CLINICO-PATHOLOGICAL EFFECTS OF EXPERIMENTAL *ASCARIS SUUM* INFECTION IN YANKASA LAMBS

BY

IBRAHIM ISAH

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA

MARCH, 2016

CLINICO-PATHOLOGICAL EFFECTS OF EXPERIMENTAL *ASCARIS SUUM* INFECTION IN YANKASA LAMBS

BY

Ibrahim ISAH, DVM (ABU) 2010
(M.Sc/Vet.Med./4055/2011-2012)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN VETERINARY PARASITOLOGY

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

MARCH, 2016

DECLARATION

I declare that the work in this dissertation entitled "**Clinico-Pathological Effects of Experimental *Ascaris suum* Infection in Yankasa Lambs**" has been carried out by me in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Ibrahim ISAH ____________________ ______________

Signature Date

CERTIFICATION

This dissertation entitled, "**CLINICO-PATHOLOGICAl EFFECTS OF EXPERIMENTAL *ASCARIS SUUM* INFECTION IN YANKASA LAMBS**" by Ibrahim ISAH, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. O.J. Ajanusi ____________________ ____________________
Chairman, Supervisory Committee Signature Date

Dr. N.P. Chiezey ____________________ ____________________
Member, Supervisory Committee Signature Date

Prof. L.B. Tekdek ____________________ ____________________
Member, Supervisory Committee Signature Date

Dr. O.O. Okubanjo ____________________ ____________________
Head, Department of Veterinary Signature Date
Parasitology and Entomology,

Prof. Kabir Bala ____________________ ____________________
Dean, School of Postgraduate Studies, Signature Date
A.B.U. Zaria

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Allah (to whom be the Glory) who gave me the life and strength that enabled me start and complete this research work. I am grateful to my parents for their unflinching support, sincere prayers and persistent patience. ‘My Lord! Bestow thy mercy upon my parents, as they raised me up when I was little’.

I would like to express my profound gratitude to my supervisors: Profs. O.J. Ajanusi, L.B. Tekdek, and Dr. N.P. Chiezey for their invaluable guidance, unwavering support, and enduring patience throughout the period of this study. May God Almighty reward you abundantly. Amen! I am also deeply grateful to the Head of Department of Parasitology and Entomlogy, Dr. O.O. Okubanjo for his guidance and support. I also would like to thank Profs. I.A. Lawal and A.J. Natala as well as Dr. I.D. Jatau for their advice, physical and moral support.

I acknowledge the contributions of my lecturers from all the Departments of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. My sincere gratitude goes to Ahmadu Bello University, Zaria for providing me with the opportunity to carry out the M. Sc. Programme and for the financial support rendered. The assistance offered to me by the technical staff of the Helminthology Laboratory, Clinical Pathology Laboratory and other Laboratories of the Faculty of Veterinary Medicine is well appreciated and duly acknowledged. Many thanks indeed.

This project could not have been completed without the assistance and support given to me, in different ways by my relatives, friends and colleagues. People like Drs. Alhassan B. Yunusa, Muhammed Babashani, H. B. Aliyu and my esteemed wife, Maryam Adam will forever be remembered for their support and understanding. I thank you all and may Allah bless you!

ABSTRACT

The effects of *Ascaris suum* infection in Yankasa lambs were investigated. Twenty four Yankasa lambs aged 6-8 months were purchased and randomly allocated into two groups (G1 and G2). The lambs in G1, consisting of 16 animals, were orally infected with 1500 infective *A. suum* eggs daily for seven days while G2, consisting of 8 animals served as the uninfected control. All the experimental animals were closely monitored for 10 weeks, during which faecal samples were collected and analysed; haematological and biochemical parameters were evaluated. A total of seven animals (six from the infected and one from the control group) were sacrificed on days 7, 14, 28 and 56 post-infection (p.i) for larval/worm recovery, pathological and histopathological examinations. Clinical signs observed in the infected group were cough and dyspnoea. Significant differences ($P<0.05$) between the mean respiratory and pulse rates of the infected (28.03 and 83.78 beats/min) and those of the control (23.84 and 81.08 beats/min) groups, respectively, were observed on day 14 p.i. Non-significant ($P>0.05$) higher eosinophil counts were observed in the infected group than in the control group on days 7, 28 and 35 of infection. There were significant differences in the counts of white blood cells, neutrophils, lymphocytes and monocytes at various weeks of the experiment between the infected group and the control. The values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in G1 increased significantly ($P<0.05$) on day 14 p.i. The infected group also had a significant increase in the mean values of creatinine and urea on day 28 p.i. The liver of the infected animals sacrificed on days 7, 14, 28 and 56 of infection showed varying degrees of diffuse whitish areas of necrosis. Similarly, histopathology revealed different levels of mononuclear cellular infiltration in the liver of the infected animals. In two of the infected animals, the kidneys were congested. By comparison, the corresponding organs in the control were normal. Eight *A. suum* larvae were recovered from the lung of the infected animal sacrificed on day 28 of infection. However,

no eggs were detected in the faeces and the infection did not have any influence on body weight changes, Packed Cell Volume (PCV), total proteins, albumins, globulins and haemoglobin concentration. It is concluded, based on the findings of this study that *Ascaris suum* is infective to Yankasa lambs but is only slightly pathogenic and may not reach patency.

TABLE OF CONTENTS

TITLE PAGE .... **ii**
DECLARATION .... **iii**
CERTIFICATION .... **iv**
ACKNOWLEDGEMENTS .... **v**
ABSTRACT .... **vi**
TABLE OF CONTENTS .... **viii**
List of Figures .... **x**
List of Plates .... **xi**
List of Tables .... **xii**
List of Appendices .... **xiii**
ABBREVIATIONS, GLOSSARY AND SYMBOLS .... **xiv**
CHAPTER ONE **1**
1.0 INTRODUCTION **1**
1.1 Background of the Study **1**
1.3 Justification of the Study **2**
1.4 Aim and Objectives of the Study **3**
1.5 Research Questions **4**
CHAPTER TWO **5**
2.0 LITERATURE REVIEW **5**
2.1 Introduction **5**
2.2 Life cycle of *Ascaris suum* **6**
2.3 Epidemiology of Ascariosis **7**
2.4 Clinical Manifestations of Ascariosis **11**
2.5 Pathology and Economic Importance of Ascariosis **11**
2.6 The Hepato-Tracheal Migration in Ascariosis **13**
2.7 *Ascaris suum* in Lambs and Kids **16**
2.8 *Ascaris suum* in Cattle **17**
2.9 Diagnosis **18**
2.9.2 Diagnosis in the laboratory 21
2.10 Control Strategies **24**
2.10.1 Chemotherapy 25
2.10.2 Alternative control measures 26
2.11 Management Practices **27**

2.12 Vaccination Studies **30**
CHAPTER THREE **32**
3.0 MATERIALS AND METHODS **32**
3.1 Experimental Animals and Management **32**
3.2 Experimental Design **32**
3.3 Isolation of Infective Eggs **32**
3.4 Inoculation **33**
3.4 Clinical Observations **33**
3.5 Examination for Lesions and Larval Recovery **34**
3.6 Larval Recovery **34**
3.7 Faecal Egg Examination **35**
3.8 Haematological Examination **35**
3.9 Data Analysis **36**
CHAPTER FOUR **37**
4.0 RESULTS **37**
4.1 Egg Recovery and Culture **37**
4.2 Clinical Signs **37**
4.2.1 Vital Parameters 39
4.2.2 Live Weight Changes 43
4.3 Haematological Findings **45**
4.4 Biochemical Parameters **54**
4.5 Pathological Findings **62**
4.5.1 Gross pathology 62
4.5.2 Histopathology 67
4.6 Larval Recovery **72**
CHAPTER FIVE **75**
5.0 DISCUSSION **75**
CHAPTER SIX **79**
6.0 CONCLUSIONS AND RECOMMENDATIONS **79**
6.1 Conclusions **79**
6.2 Recommendations **79**
REFERENCES **80**
APPENDICES **94**

List of Figures

Figure	Title	Page
4.1:	Mean (± SEM) respiratory rates in the *A. suum*-infected and control lambs	40
4.2:	Mean (± SEM) temperatures in the *A. suum*-infected and control lambs	41
4.3:	Mean (± SEM) pulse rates in the *A. suum*-infected and control lambs	42
4.4:	Mean (± SEM) white blood cell counts (10^9/L) in the *A. suum*-infected and control lambs	49
4.5:	Mean (± SEM) eosinophil count (10^9/L) in the *A.suum*- infected and control lambs	50
4.6:	Mean (± SEM) neutrophil count (10^9/L) in the *A. suum*-infected and control lambs	51
4.7:	Mean (± SEM) lymphocyte count (10^9/L) in the *A. suum*-infected and control lambs	52
4.8:	Mean (± SEM) monocyte count (10^9/L) in the *A. suum*-infected and control lambs	53
4.9:	Mean (± SEM) AST serum activity (IU/L) in the *A. suum*-infected and control lambs	55
4.10:	Mean (± SEM) ALT serum activity (IU/L) in the *A. suum*-infected and control lambs	56
4.11:	Mean (± SEM) ALP serum activity (IU/L) in the *A. suum*-infected and control lambs	57
4.12:	Mean (± SEM) urea serum concentration (mmol/L) in the *A.suum*-infected and control lambs	58
4.13:	Mean (± SEM) creatinine serum concentration (µmol/L) in the *A. suum*-infected and control lambs	59

List of Plates

Plate	Title	Page
I:	Infective eggs of *Ascaris suum* after 30 days of culture at 30˚C...........	38
II:	Tiny, white, pin-point necrotic foci on the liver of a lamb slaughtered on day 7 after oral infection with 10,500 infective *A. suum* eggs...	63
III:	Liver of the other infected lamb slaughtered on day 7 p.i. with relatively less diffuse, tiny, faint, pin-point necrotic foci...	64
IV:	Liver of an uninfected control animal slaughtered on day 7 p.i. showing no observable lesions..	65
V:	Liver of a lamb slaughtered 14 days after trickle-infection with 10,500 infective *A.suum* eggs; showing larger, whitish necrotic areas...	66
VI:	Photomicrograph of a section of the liver of *Ascaris suum*-infected lamb slaughtered on day 14 p.i..	68
VII:	Photomicrograph of a section of the kidney of a lamb slaughtered 7 days after infection with 10,500 infective *A. suum* eggs..	69
VIII:	Photomicrograph of a section of the kidney of a lamb slaughtered 56 days after infection with 10,500 infective *A. suum* eggs..	70
IX:	Lung slide of a lamb slaughtered 7 days after infection with 10,500 infective *A. suum* eggs, showing no obvious changes..	71
X:	One end of one of the larvae recovered from the lungs of a lamb slaughtered on day 28 after infection with 10,500 infective *A. suum* eggs...............	73
XI:	The other end of the larva...	74

List of Tables

Table	Title	Page
2.1:	Results of prevalence studies showing that infections with *A. suum* in pigs remain highly prevalent throughout the world	9
2.2:	Parameter(s) investigated in experimentally infected model organisms	15
4.1:	Mean (± SEM) body weights of the infected and control Yankasa lambs	44
4.2:	Mean (± SEM) Packed Cell Volume (%) in the *A. suum*-infected and control lambs	46
4.3:	Mean (± SEM) haemoglobin concentration (g/dL) in the *A. suum*-infected and control lambs	47
4.4:	Mean (± SEM) total protein concentrations (g/dL) in the *A. suum*-infected and control lambs	48
4.5:	Mean (± SEM) serum albumin concentration (g/L) in the *A. suum*-infected and control lambs	60
4.6:	Mean (± SEM) serum glucose (mmol/L) in the *A. suum*-infected and control lambs	61

List of Appendices

Appendix	Title	Page
I:	Mean (± SEM) respiratory rates in the *A. suum*-infected and control lambs	94
II:	Mean (± SEM) temperatures in the *A. suum*-infected and control lambs	95
III:	Mean (± SEM) pulse rates in the *A. suum*-infected and control lambs	96
IV:	Mean (± SEM) values of haematological parameters of the Yankasa lambs in the experimental groups during the pre-infection period	97
V:	Mean (± SEM) white blood cell counts (10^9/L) in the *A. suum*-infected and control lambs	98
VI:	Mean (± SEM) eosinophil count (10^9/L) in the *A.suum*- infected and control lambs	99
VII:	Mean (± SEM) neutrophil count (10^9/L) in the *A. suum*-infected and control lambs	100
VIII:	Mean (± SEM) lymphocyte count (10^9/L) in the *A. suum*-infected and control lambs	101
IX:	Mean (± SEM) monocyte count (10^9/L) in the *A. suum*-infected and control lambs	102
X:	Mean (± SEM) AST serum activity (IU/L) in the *A. suum*-infected and control lambs	103
XI:	Mean (± SEM) ALT serum activity (IU/L) in the *A. suum*-infected and control lambs	104
XII:	Mean (± SEM) ALP serum activity (IU/L) in the *A. suum*-infected and control lambs	105
XIII:	Mean (± SEM) urea serum concentration (mmol/L) in the *A.suum*-infected and control lambs	106
XIV:	Mean (± SEM) creatinine serum concentration (µmol/L) in the *A. suum*-infected and control lambs	107
XV:	Almost inconspicuous white pinpoint foci on day 28 p.i	108
XVI:	Even more nearly inconspicuous white pinpoint foci on day 56 p.i	109

ABBREVIATIONS, GLOSSARY AND SYMBOLS

Symbols	Meaning
G1	Group 1 (comprising of infected lambs)
G2	Group 2 (uninfected control)
AST	Aspartate transaminase
ALT	Alanine transaminase
p.i.	post initial infection
PCV	Packed Cell Volume
EPG	Egg per gram
cm	Centimetres
WHO	World Health Organization
˚C	Degree centigrade
L_2	second stage larva
L_3	third stage larva
L_4	fourth stage larva
A. suum	*Ascaris suum*
MAFF	Ministry of Agriculture, Fisheries and Food
ELISA	Enzyme-linked immunosorbent assay
spp	species
UV	Ultraviolet
A. lumbricoides	*Ascaris lumbricoides*
ABU	Ahmadu Bello University
KOH	potassium hydroxide
rpm	revolutions per minute
M	Molar
µ	microns
EDTA	Ethylenediaminetetraacetic acid

WBC	Total white blood cell count
mm	Milimetre
GIT	gastro-intestinal tract
H & E	Haematoxylin and Eosin
Hb	Haemoglobin
TP	total proteins
g/dl	grams per decilitre
ALP	Alkaline phosphatase
vs	versus
IU/L	International Units per Litre
mmol/L	Millimoles per litre
μmol/L	Micromoles per litre
A.D.C.C	Antibody-dependent cell-mediated cytotoxicity

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The large round worm of pigs, *Ascaris suum*, is reported to migrate in the tissues of a wide range of animals, including sheep (Fitzgerald, 1962; Johnson, 1963; McDonald and Chevis, 1965; Vassilev, 1960). Ascariosis is the most important internal macro-parasitism present in farmed pigs worldwide (Stewart and Hoyt, 2006). In abattoirs, the presence of milk spots in the livers of affected animals represents considerable losses due to offal condemnations (Barker *et al*., 1993). Milk spot liver is a well-established terminology used to denote the whitish healing foci occurring in the liver stroma when *Ascaris suum* larvae are immobilized by the host's inflammatory reaction (Kelly, 1993). Estimates of daily *Ascaris* female egg production generally are in the range of 200,000 eggs (Sinniah, 1982) but the number of eggs a female produces decreases with worm load (Sinniah and Subramaniam, 2009)

In Nigeria, pigs and sheep are mostly reared on extensive and semi-intensive systems of management (Ajala and Osuhor, 2004) and *Ascaris suum* is a very fecund parasite producing eggs that are resistant. So, the chances of sheep acquiring the infection through contaminated pasture are high. In view of this, it was considered worthwhile to evaluate the possible infectivity and pathogenicity of *Ascaris suum* in sheep.

1.2 Statement of Research Problem

Ascaris suum can cause significant clinical manifestations and reduce carcass quality in cattle and sheep. However, in areas of industrialized farming systems, the clinical impact of *Ascaris suum* may be limited since most farms are specialized for a single type of livestock, and pig slurry is seldom applied on ruminant grazing areas. In contrast, in more extensive livestock

production systems with mixed species or in areas where livestock are roaming freely, as is the case in many developing countries such as Nigeria, the impact of *Ascaris suum* in abnormal hosts might be higher, although this may not have been documented (Celia, 2012).

The pathological lesions of internal organs in sheep associated with migrations of the larvae of *Ascaris suum* have been described in natural infection (Clark *et al*., 1989 and Paciejewski, 1993). Mature *Ascaris suum* has been observed in the intestine or bile ducts of lambs at several abattoirs (Johnson, 1963; Harcourt and Costema, 1973; Sauvageau and Frechette, 1980).

Despite these reports, information on the impact of *Ascaris suum* infection on sheep is scarce. The recent "Neglected Infectious Diseases" published by the Bill and Melinda Gates Foundation in January 2012 referred to *Ascaris* as "one of six neglected diseases where there may be opportunities to reduce the disease burden but where there are knowledge gaps" (Celia, 2012). This research was to evaluate the possible clinico-pathological and haematological changes that might occur in experimental *Ascaris suum* infection in Yankasa lambs.

1.3 Justification of the Study

In lambs, *Ascaris suum* may also be a cause of clinical pneumonia as well as 'milk spot' lesions, resulting in condemnation of livers (Urquhart *et al*., 2000). Worms in the bile ducts have been shown to cause pathological changes (Harcourt and Costema, 1973). Condemnation of 70% of the lamb livers from one farm in the (UK) was associated with *Ascaris suum*, (Mitchell and Linklater, 1980) and the pathological changes were similar to those described in an earlier *Ascaris suum*-suspected case in lambs (Harcourt and Costema,

1973). Yellow-white plaques (15 mm in diameter) were observed on the liver from a lamb that also harboured *Ascaris spp.* in the bile ducts (Sauvageau and Frechette, 1980). However, other researchers have reported an apparent lack of macroscopic milk spots in lambs experimentally infected with *Ascaris suum* (Fitzgerald, 1962; Brown *et al*., 1984). Young adults of *Ascaris suum* are occasionally found in the small intestine of sheep (Urquhart *et al*., 2000). Clinical manifestations due to intestinal worms are unknown (Celia, 2012).

As is evident from the foregoing, there remains more to be done to get a clearer picture of the infectivity and pathogenecity of this infection in different breeds of sheep. Therefore, it is hoped that the findings of this study will shed some light on the clinical manifestations and pathological changes that may be caused by *Ascaris suum* infection in Yankasa lambs.

1.4 Aim and Objectives of the Study

The aim of the study is to evaluate the infectivity and identify the possible clinical manifestations of experimental *Ascaris suum* infection in Yankasa lambs.

The objectives of the study were to:

a) Evaluate the infectivity of *Ascaris suum* to Yankasa lambs using patency, EPG and larval/worm recovery as criteria.

b) Evaluate the clinical effects of *Ascaris suum* infections on Yankasa lambs.

c) Investigate the pathological effects of *Ascaris suum* infections on Yankasa lambs.

1.5 Research Questions

a) Is *Ascaris suum* infective and pathogenic to lambs upon experimental infection?

b) Does *Ascaris suum* infection in lambs result in any clinical signs?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

A. suum is a widespread parasitic nematode that causes infection in pigs with high prevalence rates in host populations (Roepstorff *et al*., 1998; Nansen and Roepstorff, 1999). The prevalence of *A. suum* infection varies with geographical region and farm management practices but few swine herds are totally free of infection (Roepstorff *et al*., 1999; Roepstorff, 2003). Porcine ascariosis interferes with the health and performance of pigs while resulting in reduced feed-intake to gain ratios and liver condemnation, culminating in economic losses (Stewart and Hale, 1988).

Larvae of certain nematode species of the suborder Ascaridata are capable of migrating in the organs of non-specific hosts (McDonald and Chevis, 1965; Borella *et al*., 1966). This ability is also possessed by larvae of *Ascaris suum,* which cause pathomorphological changes in the liver and lungs of infected lambs and calves (Aitken and Sanford, 1968). Such an infection, however, occurs only rarely in practice. Non-specific hosts usually come in contact with infective *Ascaris* eggs in joint enclosures or on pasture grounds manured with pig slurry (Borland *et al*., 1980; Gunn 1980; Mitchel and Linklaler 1980; Gibson and Lanning 1981), or when pigs and cattle are grazed on the same pasture grounds (Thansborg *et a1*., 1999). Male and female adult worms measure 15-25 cm and 20-35 cm respectively. Estimates of daily *Ascaris* female egg production generally are in the range of 200,000 eggs (Sinniah, 1982) but the number of eggs a female produces decreases with worm load (Sinniah and Subramaniam, 2009). Unembryonated ova enter the environment via the faeces and can remain viable in the soil for up to 15 years (WHO, 1967). During embryonation, larvae undergo two moults in the

egg (Geenen *et al*., 1999). Grazing animals are therefore repeatedly infected usually with small numbers of eggs.

In this chapter, a general overview will be provided on the life cycle, pathology and migratory pathway of *Ascaris suum*. Other areas, such as clinical manifestations and diagnosis of ascariosis will also be discussed.

2.2 Life cycle of *Ascaris suum*

Pigs transmit the infection by passing *Ascaris* eggs with the faeces. These eggs develop in the soil to fully embryonated eggs within 4–6 weeks at temperatures between 18 and 20 °C (Meyer *et al*., 1978; Mejer *et al*., 2000). The larva emerging from the egg is not a second-stage larva (L_2) as was previously presumed but rather a third-stage larva (L_3) covered by a loosened second-stage cuticle (Fagerholm *et al*., 2000). Hosts become infected *via* the faecal-oral route (Fagerholm *et al*., 2000). After oral intake, hatching is induced by the altering chemical and physical factors of the new environment. The larvae inside the egg get stimulated to secrete proteinases and chitinases, which presumably help them in degrading the different layers of the eggshell from the inside out (Geng *et al*., 2002; Hinck and Ivey, 1976). When the larvae emerge from the egg, they start their hepato-tracheal migration by penetrating the wall of the caecum and upper part of the colon (Murrell *et al*., 1997). Then, the L_3s are transported through the mesenterial blood veins to the liver where they get stuck in the capillaries and destroy liver tissue in order to get to the efferent blood vessels (Douvres *et al*., 1969; Murrell *et al*., 1997). The larvae move through the blood stream to the lungs via the capillary system in about 7 days post- infection where they penetrate the alveoli, move up the respiratory tree, and eventually get swallowed again. From 8 days p.i. onwards, the L_3s finally return to the small intestine where they start their first ecdysis inside the host to reach

the L_4 stage by day 14 p.i. The worms reach maturity at about 6 weeks post-infection and adult females begin to excrete fertilized eggs. Unembryonated ova enter the environment *via* the faeces and can remain viable in the soil for up to 15 years (WHO, 1967).

2.3 Epidemiology of Ascariosis

A. suum infections seem to be present in pig farms all over the world. However, few countries have up-to-date information on its prevalence. Many of the prevalence studies were performed decades ago, making the results unrepresentative of the current situation.

Table 2.1 provides an overview of the studies in the literature reporting the prevalence of *A. suum*. Different studies applied different methods to determine the presence of *Ascaris* in the pigs. Some used the percentage of rejected livers as a measure for parasite exposure; others checked the presence of worms by examining the intestinal tract at slaughter or by coprological examination of faecal samples to detect parasite eggs. This Table shows the results of studies that were performed in different countries, at different times and settings. Because of this, it is not possible to draw conclusions concerning epidemiological trends of ascariosis on pig farms. However, the Table does indicate that *A. suum* is still prevalent on a large number of pig farms throughout the world, and that the situation has not changed significantly over the last few decades. For example, two comparable studies from Denmark performed in 1989 and 2010; show that the situation has remained roughly the same with *A. suum* being present on 88% and 76% of the investigated farms respectively (Celia, 2012).

Strong evidence exists that under in-door conditions, massive infection of piglets with *A. suum* usually occurs shortly after arrival in the highly infectious fattening units and not in the farrowing pens (Beloeil *et al*., 2003; Nilsson, 1982; Roepstorff and Nansen, 1994). Several studies have shown that *A. suum* egg excretion was nearly absent in weaners despite the,

sometimes substantial, presence of eggs in the farrowing pens of intensive herds (Joachim *et al*., 2001; Lai *et al*., 2011; Roepstorff, 1997; Roepstorff and Nansen, 1994). Here, the increased hygienic standards in combination with the low humidity in the farrowing pens may significantly reduce the presence of favourable microenvironments for egg survival.
On the other hand, in piglets raised under outdoor conditions, significant transmission is thought to occur soon after birth and pigs are infected before fattening (Roepstorff and Murrell, 1997; Roepstorff and Nansen, 1994)

There are three major reasons why *Ascaris* is still so prevalent in current high- intensity pig farms. First, because it has a direct life cycle, and is therefore not reliant on other organisms for its transmission to new hosts. Second, the female parasitic worms are highly fecund and are capable of producing hundreds of thousands of eggs per day that contaminate the surroundings instantaneously. A study by Mejer and Roepstorff, (2006), showed that pigs that are naturally exposed to a paddock contaminated with *A. suum* show the highest egg excretion 17 weeks after being introduced onto the paddock. After this point, egg counts drop again. The lifespan of adult *A. suum* worms can be over 1 year, which is significantly longer than the average lifespan of a fattening pig these days. Therefore, once adult worms are present in fattening pigs, egg shedding increases with the age of the pigs unless the worms are cleared from the intestine by anthelmintic therapy. Third, the eggs of *A. suum* are generally considered to be highly resistant to external environmental factors, suggesting their possible survival for up to several years in the appropriate conditions (Nilsson, 1982).

Table 2.1: Results of prevalence studies showing that infections with *A. suum* in pigs remain highly prevalent throughout the world

Year	**Country**	**Sample size and type**	**% Infected farms**	**% Infected pigs**			**Reference**
				Egg +	Worm +	Liver +	
1966	Sweden	200 sows			34%		(Roneus, 1966)
1974	Canada	90 pigs			39%		(Martin *et al*., 1974)
1976	The Netherlands	653,540 pigs				16%	(Tielen *et al*., 1976)
1980	Canada	2,500 pigs			37%	46%	(Polley and Mostert, 1980)
1980	Canada	2,500 pigs				44%	
1980	England	468 pigs			16%		(Pattison *et al*., 1980)
1989	Denmark	66 farms	88%	30%			(Roepstorff and Jorsal, 1989)
1990	Nigeria	1 ,000 pigs, 2 areas		53&10%			(Salifu *et al*., 1990)
1990	Canada	15 herds		32%	35%	82%	(Bernardo *et al*., 1 990c)
1993	Zimbabwe	7 128 pigs				17%	(Makinde *et al*., 1993)
1997	Belgium	20758 livers				36%	(Vercruysse *et al*., 1997)
1997	Canada	2,500 pigs				44%	(Wagner and Polley, 1997b)
1997	Canada	500 pigs			18%	50%	
1998	Denmark, Norway, & Sweden			25-30%			
1998	Iceland	516 herds		13 %			(Roepstorff *et al*., 1998)
1998	Finland			5%			
1999	'Denmark Denmark	413 farms	56% .				(Roepstorff *et al*., 1999)
1999		9 organic farms		33%			(Carstensen *et al*., 2002)

TABLE 2.1: Results of prevalence studies showing that infections with *A. suum* in pigs remain highly prevalent throughout the world

2000	China	100 outdoor pigs		37%			(Boes *et al*., 2000)
2001	Germany	13 farms		33%			(Joachim *et al*., 2001)
2004	Tanzania	70 pigs			44%		(Ngowi *et al*., 2004)
2005	The Netherlands	9 conventional farms	11%				
2005	The Netherlands	16 free range farms	50%				(Eijck and Borgsteede, 2005)
2005	The Netherlands	11 organic farms	73%				
2005	China	3,636 pigs		5%			(Weng *et al*., 2005)
2005	India	501 pigs			16%		(Gaurat and Gatne, 2005)
2008	Kenya	230 pigs			29%		(Nganga *et al*., 2008)
2010	USA	91 farms (finishing pigs)	39%				(Pittman *et al*., 2010b)
2010	USA	40 farms (sows)	25%				(Pittman *et al*., 2010a)
2010	Switzerland	90 conventional farms	13%				(Eichhorn *et al*., 2010)
2010	Switzerland	20 free range farms	35%				
2010	Sweden	2.4 million pigs				5%	(Lundenhcim and Holmgren, 2010)
2010	Denmark	79 farms, 1790 sows	76%	30%			(Haugegaard, 2010)
2011	China	916 pigs		15%			(Lai *et al*., 2011)
2011	Uganda	106 pigs from 56 households,		40%	73%		(Nissen *et al*., 2011)
2011	Tanzania	13,3 10 pigs				4%	(Mellau *et al*., 2011)
2011	Tanzania	731 pigs			8%		(Mkupasi *et al*., 2011)
2012	England	34,168 pigs				4%	(Sanchez- Vazquez *et al*., 2012)

Source: Celia, 2012

2.4 Clinical Manifestations of Ascariosis

A. suum infections rarely cause specific clinical symptoms in pigs (Hale *et al*., 1985), which explains why they are seldom associated with ascariosis by the farmers. The most obvious clinical symptom of ascariosis is the wheezing and coughing associated with the pulmonary migration of the L_3 around 7 days p.i (Bernardo *et al*., 1990a). Furthermore, the hepato-tracheal migration is associated with increased susceptibility to bacterial pathogens such as *Escherichia coli, Pasteurella multocida* and *Salmonella spp*. (Tjornehoj *et al*., 1992; Adedeji *et al*., 1989; Curtis *et al*., 1987). Therefore, if there is a concurrent infection with any of the above pathogens, the symptoms could be much vaguer.

2.5 Pathology and Economic Importance of Ascariosis

Although the presence of adult parasites in the small intestine might have an impact on the pig's productivity (Andersen, 1976; Bernardo *et al*., 1990b), it does not appear to have a major impact on the pig's health. The larval stages of this parasite on the other hand, do cause significant damage to the internal organs of its host in their attempt to successfully complete their hepato-pulmonary migration.

When the *A. suum* larvae migrate through the liver tissue of their host, significant tissue damage is caused, which appears as "white spots" on the superficial hepatic surface and within the liver tissue of infected pigs. These white spots are the most representative pathological lesions caused by migrating *A. suum* larvae in pigs, normally formed by the mechanical injury and inflammatory response induced by the migrating larvae (Roneus, 1966). In total, there are three types of white spots in *A. suum* infected pigs, including the compact and mesh-worked white spots, both of which are produced by eosinophilic

interstitial hepatitis, and the lympho-nodular type of spots formed by lympho-follicular hyperplasia (Nakagawa *et al*., 1983; Perez *et al*., 2001).

White Spots can be observed 3 days p.i. and start to resolve after about 2-3 weeks p.i. (Roepstorff *et al*., 1997; Eriksen *et al*., 1980). Depending on the numbers of white spots, the contaminated livers are trimmed or fully rejected at slaughter, resulting in obvious economic losses.

In order to complete their hepato-pulmonary migration, larvae need to pass through the lungs as well. As these larvae move into the alveolar and bronchiolar air spaces they cause direct physical damage to the lungs. This can subsequently promote the development of pneumonia or pleurisy (also known as pleuritis). The damage to the respiratory system can be recorded at the slaughterhouse and has been associated with the presence of *Ascaris* infection (Bernardo *et al*., 1990a; Flesja and Ulvesaeter, 1980; Nakagawa *et al*., 1983). It is obvious that the severity of the damage will depend on the amount of larvae that migrate through the lung (Miskimins *et al*., 1994) and that increased infection levels can easily cause respiratory distress and coughing. However, even after ingestion of high amounts of eggs, the *A. suum* infection itself very seldom causes lethal damage to the lung. It is rather the secondary, opportunistic, bacterial or viral infections that increase the chances of severe health problems. In addition, *A. suum* infections were observed to have a significant negative effect on the seroconversion and antibody levels to a *Mycoplasma hyopneumoniae* vaccine (Steenhard *et al*., 2009). Both the negative effects on the productivity caused by bacterial or viral infections and the costs of the treatment for these diseases contribute to the economic impact of ascariosis. Moreover, parasitism of adult *A. suum* in the intestine of pigs is also known to be responsible for villus atrophy, impaired absorption of vitamin A and temporary lactose

intolerance (Forsum *et al.*, 1981; Perez *et al.*, 2001; Copeman and Gaafar, 1972). Consequently, the infected pigs show decreased daily weight gain and feed conversion efficiency, which of course result in considerable economic loss.

Infections with *A. suum*, and especially the larval migration phase, have shown to have a significant negative impact on the economic profitability of a pig farm (Kipper *et al.*, 2011; Van Meensel *et al.*, 2010). The decreased health status of pigs, as a consequence of roundworm infection, is reflected in general production parameters like daily weight gain, feed conversion efficiency and meat quality (Bernardo *et al.*, 1990a; Hale *et al.*, 1985; Kanora, 2009; Kipper *et al.*, 2011; Knecht *et al.*, 2012; Stewart and Hale, 1988). However, in farms with low infection levels, the impact of infection is expected to be minimal and the economical profitability of active *Ascaris* control can be questioned (Roepstorff, 1997).

2.6 The Hepato-Tracheal Migration in Ascariosis

The life-cycle of *Ascaris suum* consists of three very important elements. The adult male, female and immature worms that live in the small intestine, the resistant egg stages that are produced by adult worms and passed out into the environment via the host faeces and the larval parasites that emerge from the hatched eggs into the host intestine and undergo a migratory pathway within the tissues of the porcine host. Each of these phases of infection contributes to the unique biology of the parasite, to its health impact and to a myriad of questions that arise in the mind of the researcher interested in *Ascaris*.

Ascaris is a parasite that exists not only as an adult worm in the intestine but also has a migratory pathway, known as a hepato-trachael migration (Sprent, 1954) that progresses from an infective egg (containing an L3 larva covered by an L2 cuticle) (Maung, 1978; Geenen *et al.*, 1999) hatching in the intestine, to a larva migrating via the portal blood vessels to the

liver. After migration within the liver, and some growth, the larvae advance to the lungs, penetrate the alveolar spaces and move to the pharynx where they are coughed up and swallowed, thereby returning to their former location within the small intestine where they mature into adults (Dold and Holland, 2011a). The explanation for the perpetuation of this perilous journey is far from understood. Smyth, (1994) speculated that such an extra-intestinal migration might represent "evolutionary baggage" left over from a previous lifecycle, perhaps originally based on skin penetration. However, other authors have argued that migration may confer significant fitness benefits given the associated risks of immune-mediated damage and mortality within the tissues (Read and Skorping, 1995; Mulcahy *et al*., 2005) and therefore by adopting this complex route, rather than just developing in the lumen of the small intestine where eggs originally hatch, the parasite gains a significant advantage. There is some evidence to suggest that migration confers enhanced parasitic growth (Read and Skorping, 1995). Jungersen *et al*. (1999) demonstrated that *A. suum* larvae infected intravenously in pigs, do not undergo larval migration, and develop more slowly. Data on larval ascariasis have mainly been accrued from animal model studies including the natural host of *A. suum*, the pig, and a range of abnormal hosts including lambs, cattle, rabbits, and small rodents such as guinea-pigs and mice (Table 2.2).

Table 2.2: Parameter(s) investigated in experimentally infected model organisms

Model organism	Parameter(s) investigated	Reference
Guinea-pig	Larval migration immunological response	Beraldo *et al*., 2012
Rabbit	Immunological Larval migration Hepatic pathology Pulmonary pathology	Berger, 1971
Gerbil	Larval migration	Cho *et al*., 2007
Rat	Larval migration	Yoshida, 1919
Mice	Larval migration	Douvres and Tromba, 1971
Pig	Larval migration	Douvres and Tromba, 1971
Cow	Larval migration	McCraw, 1975
Lamb	Larval migration	Ransom and Foster, 1919
Goat	Larval migration	Ransom and Foster, 1919

Source: Celia, 2012

2.7 *Ascaris suum* in Lambs and Kids

Adult *A. suum* has been observed in the intestine or bile ducts of lambs at a number of abattoirs (Johnson, 1963; Harcourt and Costema, 1973; Sauvageau and Frechette, 1980).

These infections were probably the result of exposure to pastures contaminated with pig faeces (Pedersen *et al*., 1992) as worms from lambs have been genetically characterized to be of pig origin (Nejsum, unpublished data). Worms in the bile ducts have been shown to cause pathological changes (Harcourt and Costema, 1973) but clinical manifestations due to intestinal worms are unknown (Celia, 2012).

As in cattle, transient lung symptoms 5-14 days p.i. have been reported for lambs and kids after experimental exposure to *A. suum* (Gaur and Deo, 1972; Brown *et al*., 1984). Symptoms included dry coughs, dyspnoea and pneumonia, and one animal died. Larvae were isolated from the lungs in both studies. About 70% of lamb livers from a farm were condemned on account of *A. suum* infection (Mitchell and Linklater, 1980) and the pathological changes were similar to those described in an earlier *A. suum* suspected case in lambs (Harcourt and Costema, 1973). Yellow-white plaques (15mm in diameter) were observed on the liver from a lamb that also harbored *Ascaris* spp. in the bile ducts (Sauvageau and Frechette, 1980). Histological examination of lamb livers after supposedly *Ascaris* spp. infection showed intensive eosinophil infiltration of the portal tracts, parasite tracks in the parenchyma and portal fibrosis (Harcourt and Costema, 1973).

However, other researchers have reported an apparent lack of macroscopic milk spots in lambs experimentally infected with *A. suum* (Brown *et al*., 1984; Fitzgerald, 1962). The lack of interlobular connective tissue in lambs is considered the main reason why “white spots” do not develop in this host animal (Brown *et al*., 1984). They are therefore critical to the reports

suggesting that condemnation of liver at abattoirs should be related to white spots caused by *A. suum* but do not refute that pathological changes take place in the liver. Numerous small disseminated greyish white nodules were observed in the liver following an experimenal infection; the space around the bile ducts and vessels was infiltrated with lymphocytic cells and developing foci were made up of lymphocytes and polymorphonuclear cells (Dubinsky *et al*., 2000).

2.8 *Ascaris suum* in Cattle

Several cases of clinical disease and even deaths due to *A. suum* have been reported in cattle, especially in young stock. Seven out of 17 heifers developed clinical signs of severe dyspnoea, coughing and forced expiration after turnout on a *A. suum*-contaminated pasture, and two animals eventually died (Morrow, 1968). In a total of three cases, acute respiratory distress, pneumonia or fatalities were registered 10 days after exposure either to pig manure in the feed or to pens previously used for pigs (Allen, 1961; McCraw and Lautenslager, 1971; McLennan *et al*., 1974). A sudden reduction in milk yield, respiratory symptoms, and eosinophilia were observed on two dairy farms after heifers had grazed pastures fertilized with pig slurry (Borgsteede *et al*., 1992). The animals tested positive for *A. suum* antibodies. A few worms were found in the bile duct of one calf on a farm whereas two other calves had 16 and 168 worms (Roneus and Christensson, 1977). Based on morphology, *Toxocara vitulorum* was excluded. Experimental infection studies support the above-mentioned clinical observations. *A. suum* larvae were thus recovered on day 10 p.i. (Henriksen and Krogh, 1979) and days 7-9 p.i. from the lungs of calves infected with embryonated eggs (McCraw, 1975). These findings coincide with the lung symptoms described in the above case reports. Greenway and McCraw, (1970) reported dyspnoea, coughing, and increased respiration rates, consistent with atypical interstitial pneumonia, as most pronounced 10-13 days after a

challenge infection in 7-week-old sensitized calves. Interestingly, the first sensitizing dose of 100,000 eggs was not enough to cause pronounced clinical signs. Similar lung pathology has also been described in yearling cattle that died after natural exposure to *A. suum* (Allen, 1961; Morrow, 1968). Pathology and clinical signs have been correlated with inoculation dose in surviving calves, but the link to mortality is less clear. Some animals have thus died after receiving "low doses" of 10,000-100,000 eggs whereas others have survived multiple doses adding up to 11 million eggs (Kennedy, 1954; McCraw and Greenway, 1970). *A. suum* has also been shown to cause varying levels of gross liver pathology in cattle. In one study, no milk spots or larvae were detected after inoculation of four 2-3-day-old calves with a single dose of 10,000 *A. suum* eggs (Kennedy, 1954), whereas milk spots were noted 3-5 days p.i. and larvae were isolated from the liver of 4-week-old calves infected with 2 million eggs (McCraw, 1975). Larval migration may have been underestimated due to suboptimal recovery methods. Alternatively, the difference in results may reflect the variation in age and/or dose level as it is likely that overall migration success is considerably lower in an inappropriate host. At slaughter, livers from cattle given 2000 or 5000 *A. suum* eggs were all condemned (Kennedy, 1954).

2.9 Diagnosis

Infections with *A. suum* are, most of the time, subclinical. Consequently, such infections typically remain unrecognized by farmers and their veterinarians. However, the use of correct diagnostic tools is essential for assessment of the infection status and the efficacy of the applied control strategies. The following methods are employed to diagnose the presence of *A. suum* infections on pig farms:

2.9.1 Diagnosis on the farm and at slaughter

2.9.1.1 Presence of the worm

Most farmers discover that their pigs harbour the infection by observing expelled adult worms after anthelmintic treatment (Theodoropoulos *et al.*, 2001). Although the expelled worms might not have conserved their exact shape and colour, a farmer should be able to detect these spaghetti-like organisms mixed with the faeces. When immature worms, which are notably smaller, are expelled, identification by the untrained eye might be harder.

The presence of adult worms in the small intestine of pigs can be registered at slaughter. However, except in experimental settings, the detection of worms in the intestine is hardly ever done in practice. If performed, one must keep in mind the time between last anthelmintic treatment and slaughter, as the larval stages in the intestine are small and easily overlooked. This could lead to wrong conclusions when no worms could be detected. The detection of adult parasites is, however, only a qualitative measure and not a quantitative one as the number of adult worms is not representative of the amount of migrating larvae the pig has been exposed to. Irrespective of the dose regimen, the number of worms that end up in the small intestine is generally inconsistent and independent of the intake of infective stages (Eriksen *et al*., 1992). Moreover, there appears to be an inverse relationship between the number of adults found in the intestine and the amount of eggs given during a single experimental infection dose (Roepstorff *et al*., 1997; Andersen *et al*., 1973) In addition, *Ascaris* populations are strongly aggregated within the pig population with few pigs carrying the majority of the worms while most of the pigs carry few or even no adult worms at all Roepstorff *et al*., 1997; Wagner and Polley, 1997; Boes *et al*., 1998).

2.9.1.2 Hepatic white spots

The most typical lesions caused by migrating *A. suum* larvae in pigs are the hepatic white spots. Upon the migration of larvae through the liver, the destroyed hepatic cells are replaced by interlobular depositions of fibrous tissue and cellular infiltrates, producing the characteristic white spots (Nakagawa *et al*., 1983; Perez *et al*., 2001). Milk spots have the property to heal, causing the liver to appear normal about 35 days after primary inoculation with *Ascaris* eggs (Copeman and Gaafar, 1972). In pigs that have primed immune responses due to vaccination or previous exposure to the parasite, white spots are larger, more distinct, and more persistent (Copeman and Gaafar, 1972; Eriksen, 1982; Vlaminck *et al*., 2011; Urban *et al*., 1985). This would suggest that white spot formation is the result of secondary immunological reactions to antigenic material released by migratory or dying larvae, which seems to be in accordance with previous studies showing a significant relationship between the number of white spots and antibodies against *Ascaris* antigens (Eriksen *et al*., 1980; Vlaminck *et al*., 2011; Lind *et al*., 1993; Frontera *et al*., 2003).

Although the number of liver lesions indicates the recent passing of migrating *A. suum* larvae, there exists no relationship whatsoever between the number of lesions and the number of adult parasites that eventually end up in the small intestine (Nilsson, 1982). In a study by Bernardo *et al*. (1990) the presence of white spots on the liver is more than twice as prevalent as worms in the small intestine at slaughter. The absence of worms in the intestine when white spots are visible on the liver can mean two things: (1) eggs have recently been ingested, but not enough time has elapsed to allow development of macroscopically detectable ascarids or (2) infection had been cleared by the host immune response but lesions had not yet resolved. When milk spots are present, it is highly unlikely that *A. suum* infection is absent.

The presence of milk spots has a high sensitivity, very low specificity, and high negative predictive value as a screening test for ascariosis in individual hogs (Bernardo *et al.*, 1990). Under continuous exposure, the number of white spots on the liver increases until weeks 6-9, after which there is a gradual decline towards lower levels (Eriksen *et al.*, 1992). This decline could be attributed to the build-up of immunity as continuous exposure to *A. suum* infections stimulates the development of strong protective immunity. Both Eriksen (1982) and Urban *et al*. (1985) suggested the existence of a pre-hepatic barrier in pigs repeatedly exposed to *A. suum* infections. This would impede freshly acquired larvae from reaching the liver and therefore prevent the formation of white spots. Hence, the number of liver white spots is a poor indicator of long-term *A. suum* exposure, as it only reflects recent larval migration. Livers might therefore look normal or only mildly affected at slaughter even though pigs have been exposed to high numbers of infective eggs during the course of their life.

The visual assessment of livers is also very subjective; especially when pigs with low numbers of white spots are slaughtered. The decision on whether or not an abnormality on the liver is considered a true white spot largely depends on the perception of the person doing the assessment. In addition, this usually has to be performed within an extremely short time span due to the high speed of slaughter.

2.9.2 Diagnosis in the laboratory

2.9.2.1 Faecal examination

Coprological examination is an important tool for monitoring worm infections in pig units and for maintaining effective worm control programmes. Examination of faeces for parasitic eggs may vary from a simple direct smear to more complex methods involving centrifugation and use of flotation fluids (MAFF, 1986). Flotation methods involve separating eggs from

faecal debris using a variety of flotation solutions with specific gravities which float worm eggs to the surface of the suspension. The most widely used and standard quantitative technique is the McMaster method (Gordon and Whitlock, 1939; Whitlock, 1948).

Ascaris eggs are round or oval shaped with a thick brown irregular wall and are easily recognized. The number of eggs excreted by a pig can give an indication of its intestinal worms burden because, in continuously exposed pigs, the quantity of eggs that are shed seems to be correlated with the number of adult worms in the intestine (Bernardo *et al*., 1990b; Nejsum *et al*., 2009). When using coprological examinations for the detection of *A. suum* infections in pigs, there is a significant possibility of false positive and false negative egg counts. False positives are the result of coprophagia and/or geophagia and their prevalence and magnitude depend on different management and housing factors (Boes *et al*., 1997). For diagnosis at farm level, however, these false positive samples are not as important as the false negatives. Boes *et al*. (1997) has shown that as much as 23% of the investigated pigs that harboured worms in their intestine did not excrete any eggs (Boes *et al*., 1997). These false negative results are possible when only immature worms are present or when only worms of a single sex are present. Evidently, these factors have an impact on the sensitivity of faecal examination for the diagnosis of *A. suum* infection.

2.9.2.2 Serology

Serological tests are widely used and generally accepted in the pig industry. Tests are available for a number of important bacterial and viral diseases in pigs (e.g. salmonellosis, mycoplasma infection, Aujeszky's disease, etc.). On the other hand, there is no commercial serological test currently available for ascariosis. The use of a serological method, however,

could in theory overcome the difficulties associated with the traditional methods (examination of faeces or livers) of diagnosing ascariosis in pigs.

It can be assumed that all pigs within a unit are exposed to a similar amount of migrating larvae as they reside in the same infective environment. In contrast, only few pigs in the population will eventually harbor adult worms in their intestine (Roepstorff *et al*., 1997; Wagner and Polley, 1997). Moreover, pig performance has been shown to be negatively associated with the amount of larvae migrating through the body of the host (Hale *et al*., 1985). Hence, serological diagnosis should be aimed at detecting larval exposure rather than the presence of adult worms.

Several studies have previously described the use of an Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the detection of anti-*Ascaris* antibodies in the sera of swine. In this regard, the use of different adult and larval extracts or excretory/secretory products has been evaluated (Urban *et al*., 1985; Lind *et al*., 1993; Frontera *et al*., 2003; Yoshihara *et al*., 1993; Roepstorff, 1998; Bogh *et al*., 1994; Vlaminck *et al*., 2012). Although most of these tests have been shown to be effective in diagnosing *A. suum* infections, the sensitivity and specificity of these tests are unclear. The reason for this is that traditional methods, like the detection of parasite eggs in the stool or white spots on the liver at slaughter, were used to categorize the pigs used for the validation of the serodiagnostic test. Due to the lower sensitivity of these traditional methods, there is a significant chance to include false negative samples in the evaluation of the ELISA.

Recently, for the validation of an experimental ELISA, Vlaminck *et al*. (2012) used sera from 190 pigs that were trickle-infected twice a week for 14 weeks, thereby ensuring that no false negative samples would be included for the validation of the ELISA test or the determination

of its cut-off value. In the same study it was also advised to test serum samples from older fattening pigs, as the number of sero-positive pigs increases with time (Vlaminck *et al*., 2012). This would reduce the chance of including false negatives when screening a farm for the presence of *Ascaris* infection using this sero-diagnostic technique. ELISA is currently being used to investigate whether or not the serological response to *A. suum* can be coupled to different production parameters of pigs. If so, test results could appeal more to the farmer. It would help improve his awareness on the topic and convince him of the fact that a more effective helminth control program could significantly improve farm profitability. In this way, serological testing could serve as a cost-effective, supportive tool for the veterinarian to assess the worm status of a farm and to evaluate whether changes in worm-control management are necessary or not.

2.10 Control Strategies

Transmission of *A. suum* among pig populations is dependent on factors such as housing system, hygiene, management practices and anthelmintic treatment (Nansen and Roepstorff, 1999). Hence, effective control of *A. suum* infections on pig farms relies not only on mass anthelmintic treatment, but also on effective general farm management and increased hygienic standards (Nilsson, 1982; Roepstorff and Jorsal, 1990). The moment *A. suum* is present on a farm; it is rather challenging to completely eliminate its presence. Although the aim of control programs against this parasite should not be to eradicate the parasite completely, a significant reduction in transmission intensity will readily result in a marked decrease of adverse effects on the health and productivity of the pig herd.

2.10.1 Chemotherapy

Prior to the arrival of broad-spectrum anthelmintics in the 1960s, treatment of worm infections in pigs was hardly ever applied. Since then, the control of parasitic infections on pig farms has become increasingly reliant on mass treatment with anthelmintic drugs. When selecting a drug for the control of *Ascaris* infections, several things need to be taken into account. Namely, (1) the margin of safety of the used compound, (2) the efficacy of the compound against the different stages of *A. suum*, (3) the spectrum of activity, (4) the mode of administration, and last but not least (5) the cost of the drug. All of the modern anthelmintics are found to be very safe. Treatments with doses exceeding the recommended dose did not show any clinical effect in pigs (Rochette, 1985). For the effective control of helminth infections on a pig farm it is advantageous to select an anthelmintic that kills the larval stages and has a broad spectrum of activity. Pyrantel, fenbendazole, flubendazole, and levamisole have shown activity against migrating stages of *A. suum* (Stewart *et al*., 1984; Thienpont *et al*., 1978; Kennedy *et al*., 1987; Oakley, 1974). Piperazine and pyrantel have restricted activity against the other common nematode infections (*Trichuris suis* and *Oesophagostomum spp*.) (Rochette, 1985) and are therefore not the drugs of choice when co-infections with these parasites are present on a farm. Anthelmintics of the macrocyclic lactone family (e.g. ivermectin, doramectin) have a significantly longer residual effect in comparison with the other anthelmintics and are also highly efficient against ectoparasites like mange mites (*Sarcoptes scabiei var suis*) and hog lice (*Haematopinus suis*) (Lichtensteiger *et al*., 1999; Lee *et al*., 1980; Yazwinski *et al*., 1997; Stewart *et al*., 1981; Logan *et al*., 1996).

To date, no studies have reported anthelmintic resistance in *A. suum* in pigs. Whether anthelmintic resistance would be easily induced in *A. suum* is unknown. However, the

development of anthelmintic resistance in *Oesophagostomum spp*. and in the closely related horse parasite *Parascaris equorum* (Boersema *et al*., 2002; Nareaho *et al*., 2011; Laugier *et al*., 2012; Nansen and Roepstorff, 1999) suggests that continued malpractice in this area might eventually lead to anthelmintic resistance in *A. suum*.

2.10.2 Alternative control measures

Over time, questions have been raised concerning the long-term impact of the massive application of these highly efficacious, broad-spectrum anthelmintic compounds on the environment. Due to the marginal absorbance rate of some of these drugs, high percentages of these substances are being excreted unchanged after oral or systemic administration (Farkas *et al*., 2003). Decomposing animal excrement/waste is spread all over the land, used for increased crop productivity, thereby also spreading the anthelmintic drugs and their residues. According to Spratt (1997), the world remains oblivious to what the possible long-term ecological effects of these drugs and their residues are on pasture fauna and flora. This concern, among others, is stimulating the need for alternative, more biological measures of parasite control.

In organic farming, the use of traditional synthetic drugs is not allowed, and therefore farmers prefer a phyto-pharmaceutical approach for the control of parasitic infections on their farms (Van Krimpen *et al*., 2010). Although many plants are suggested to have some nematocidal effects (Chitwood, 2002) later studies, e.g. by Van Krimpen *et al*. (2010) could not show any significant reduction on the worm load of infected pigs after treatment with a herb mixture (*Thymus vulgaris*, *Melissa officinalis*, and *Echinacea purpurea*), *Papaya* fruits, *Bolbo* leaf or complete *Artemesia* plants in comparison with untreated controls. Nonetheless, continued efforts could be made to standardize the plant extracts with good anthelmintic activity and formulate the best alternative herbal preparations to replace or complement synthetic drugs,

which are currently in use. In addition to phyto-pharmaceutical research, the possibility of using nematophageous fungi for the biological control of *A. suum* is currently being investigated. According to the study of Ferreira *et al*. (2011), mycelia of *Pochonia chlamydospori*a, which were added to the food of pigs, showed ovicidal activity against *A. suum* eggs after passage through the gastrointestinal tract.

In recent times, it has been shown that a number of infection parameters like worm burden, total egg output, and raised antibody levels as well as natural resistance to *A. suum* infections are heritable traits representing the close genetic control of immunity against this parasite (Skallerup *et al*., 2012; Nejsum *et al*., 2009).This, understandably, opens the door to selective breeding of pigs with increased resistance within the pig industry and could become relevant in free-range systems (Thamsborg *et al*., 2010).

2.11 Management Practices

For reasons of convenience, a scheduled application of anthelmintic drug seems to appeal to pig farmers (Nansen and Roepstorff, 1999). However, there are numerous aspects of farm hygiene and farm management that can have a major impact on the epidemiology of *A. suum*. Undoubtedly, the type of production system that is in use will be a key factor regarding the infection intensity on a farm (Nansen and Roepstorff, 1999). For obvious reasons, there is a higher diversity of parasite species and higher infection intensity on traditional farms and where pigs have access to outdoor facilities when compared to intensive indoor systems (Roepstorff and Nansen, 1994; Lai *et al*., 2011).

Farmers with fattening herds are advised to buy piglets from larger piglet producers with good management and hygiene (Joachim *et al*., 2001) and that were preferably treated prior

to parturition. Once *A. suum* is present on the farm, it is easily dispersed over the whole farm through moving animals and dirty materials and boots. Even flies have been shown to carry *A. suum* eggs (Forster *et al*., 2009).

Other management practices like the use of the all-in-all-out system and early weaning are linked to lower prevalence of *A. suum* (Joachim *et al*., 2001; Roepstorff and Jorsal, 1990; Roepstorff *et al*., 1999; Tielen *et al*., 1978). In the all-in-all-out production system, pigs are moved into and out of facilities in distinct groups with the hope that, by preventing the commingling of groups, the spread of disease is reduced. This practice also allows for a period of thorough cleaning and disinfecting between subsequent groups of animals. Early weaning of piglets (3-5 weeks) also seems to be associated with a reduced risk of *A. suum* infection (Roepstorff *et al*., 1999). However, it is likely that this can be attributed to the fact that both parameters are associated with other factors that are more important for parasite survival, like, for instance, the use of more traditional rearing methods or poorer general pen hygiene.

Reducing contact of pigs with their own faecal deposits or from pigs from previous rounds is important. Consequentially, housing of pigs on slatted floors seems to reduce the chance of parasite infestation compared to solid or partially slatted floors (Roepstorff and Jorsal, 1990; Tielen *et al*., 1978; Sanchez-Vazquez *et al*., 2010). Evidently, the stocking density in the pig house will also be important (Tielen *et al*., 1978), as chances of pens containing pigs with patent infections will increase. Positioning the water supply in the dung area instead of in the lying area or the feeding troughs seems to be associated with a decreased prevalence of *A. suum* (Roepstorff *et al*., 1999). Water spillage in areas where eggs are present will enhance the chances for survival of the eggs due to constant humidity. Pig urine seems to exhibit a

strong inhibitory effect on the development of *A. suum* eggs (Nilsson, 1982). Once in the environment, the eggs will embryonate until the infective larvae are present inside the egg. Only then is the egg infective for a new host. When environmental factors like temperature, oxygen availability, and relative humidity are suboptimal, the time for the eggs to become infective increases (Nilsson, 1982). This is reflected by the fact that white spot levels seem to vary within farms depending on the season. Presumably, the rising temperatures in spring and summer allow simultaneous development of infectious stage larvae in the eggs that have spent the winter in the pig pens. This would increase the amount of infective eggs being present in the environment and subsequently cause higher liver condemnation rates in summer and early autumn (Nilsson, 1982; Lai *et al*., 2011; Menzies *et al*., 1994; Sanchez-Vazquez *et al*., 2010).

Frequent cleaning of the pens, preferably after each round, is indispensable to destroy or reduce the amount of eggs in the environment. Clearly, the presence of rough and uneven surfaces would provide a good microenvironment for egg development and have an impact on the efficiency of cleaning protocols. Joachim *et al*. (2001) indicated that in older pens, the prevalence of *Ascaris* infections was significantly higher (63.0%) than in the new pens (27.9%). Moreover, Nilsson (1982) found high numbers of eggs (up to 3,000 eggs per gram) in the crevices of the floor between the slatted dung area and the resting area of the pen. Approximately half of these eggs appeared to be embryonated. In a study by Beloeil *et al*. (2003) 31% of the farms surveyed had residual dung containing *Ascaris suum* eggs in the fattening pens when restocking them with new fattening pigs, a practice favouring the transmission of *A. suum*. Even though careful cleaning of the pens with high pressure water will remove most of the residual dung, it seems ineffective to completely remove all the infective eggs (Nilsson, 1982). Although the use of disinfectants is effective against bacteria, most of them are rather ineffective against *A. suum* eggs (van den Burg and Borgsteede,

1987; Plachy *et al*., 1996; Massara *et al*., 2003). Instead, steam cleaning (Haas *et al*., 1998) and drying of the pens is a highly recommended management practice for killing roundworm eggs.

Moreover, sows should not be tethered and should be housed in groups in enriched environments (bedding, wallowing) for part of the gestation (Thamsborg *et al*., 2010; Roepstorff *et al*., 2011). As a result of poor management practices, the number of eggs surviving in the pens will increase and with it the prevalence of *Ascaris* and other pig parasites (Haugegaard, 2010). Good diagnostic assessments will be necessary to evaluate how these changes in farm management will affect parasite epidemiology.

2.12 Vaccination Studies

Pigs build up natural resistance against recurring infections rather quickly. Significant levels of protection were detected in pigs following one or multiple inoculations with infective eggs or L_3 of *A. suum* (Helwigh and Nansen, 1999; Eriksen, 1982). This provides some hope for future vaccination strategies. The development of vaccines against parasites is highly desirable. The search for a vaccine against *A. suum* has been on-going for about half a century.

Vaccination against *Ascaris* would offer better means of control than anthelmintic treatment since it would interfere with the infection, disease and transmission on a long-term basis. Because there is naturally acquired immunity against *A. suum*, vaccination should be feasible. Several researchers have developed experimental vaccines based on extracts, antigens or even UV-irradiated eggs of *A. suum*, with parasite reductions ranging from 58 to 99% (Chen *et al*., 2012; Vlaminck *et al*., 2011; Matsumoto *et al*., 2009; Islam *et al*., 2005; Tsuji *et al*., 2004;

Hill *et al*., 1994; Urban and Romanowski, 1985; Tromba, 1978). What we can conclude from these vaccination studies is that immunity against *A. suum* is inducible, which raises hope that one day vaccination against *A. lumbricoides* can also be achieved. However, most of these studies only evaluated the reduction of larvae in the lungs, while the ideal vaccine against *Ascaris* should mimic natural immunity and prevent larvae from penetrating the intestine and damaging the internal organs.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals and Management

Twenty four (24) Yankasa lambs, aged 6-8 months were purchased locally and were housed in fly and tick-proof pens of the Department of Veterinary Parasitology and Entomology, ABU, Zaria. While they were being acclimatized for two weeks, screening for internal and external parasites, treatment and prophylaxis were accomplished accordingly. The animals were fed twice a day with groundnut haulms, maize bran and *Digitaria spp* hay. Water and salt licks were provided *ad libitum.*

3.2 Experimental Design

The experimental animals were weighed, ear-tagged for identification and randomly allocated into two groups, G1 and G2. G1, the infected group, consisted of 16 animals while G2, the control group, consisted of 8 animals. The groups were kept in separate pens for a period of twelve (12) weeks.

3.3 Isolation of Infective Eggs

Eggs of *Ascaris suum* were obtained from female worms collected from the intestines of pigs slaughtered at slaughter slabs in Sabon Gari, Zaria.

The worms were collected in a beaker containing 50 ml of normal saline (0.9%), and transported to the Helminthology Laboratory Department of Veterinary Parasitology and Entomology, ABU, Zaria. The uteri of the worms were dissected out using forceps into a Petri dish and washed with 0.5 M KOH solution into a beaker (Fairbairn, 1961). The eggs were then agitated gently in the KOH solution for 30 minutes in order to dissolve the sticky

albuminous layer. The preparation was then placed in centrifuge tubes and centrifuged at 1500 rpm for 3 minutes. The supernatant was gently decanted, leaving about 0.5 ml which should contain the eggs. The eggs were then washed two times with distilled water and thereafter with embryonating fluid (0.1 M sulphuric acid) for the same period (Fairbairn, 1961. The eggs collected were suspended in the embryonating fluid, transferred to Petri dishes and incubated for 30 days at 27 ^{0}C (Dubinsky *et al*., 2000). They were then washed, and stored in distilled water at 4 ^{0}C until needed.

3.4 Inoculation

The eggs were gently rocked to achieve an even distribution. Eggs in 0.1 ml were counted under x10 objective of a light microscope. Each of the animals in group G1 was given, orally, 1500 infective eggs each day for a week. The dose was administered in a 1 ml-syringe and quickly followed with 20 ml of distilled water in order to pick up any hanging eggs in the mouth and ensure that the whole dose was consumed.

3.4 Clinical Observations

Daily physical examination was carried out. Temperature changes, respiratory and pulse rates were evaluated. Also, body weight changes were monitored weekly.

3.5 Examination for Lesions and Larval Recovery

On day 7 post- infection, two lambs from the infected group and one lamb from the control were sacrificed. On days 14, 28 and 56 post infection, one lamb each, from the infected group was sacrificed. The liver, heart, lung, kidneys and small intestine of each slaughtered lamb were observed for the presence of any gross lesions. Samples from these locations were fixed in 1% formalin solution, dehydrated in absolute alcohol, cleared in xylene and embedded in paraffin for preparation of fine blocks in paraffin wax. Sections of 5µ thickness were cut and stained with Haematoxylin and Eosin staining technique (Lillie, 1965). The sections were screened at low magnification (x100), after which areas having lesions were examined under high magnification (x200). To search for larvae, each organ was broken up into small (about 10 mm) pieces in normal saline in a petri dish. The suspension was examined by transferring a bit to a clean petri dish, adding saline (to make it translucent) and examining under a stereomicroscope at x10 objective.

3.6 Larval Recovery

Tissues from the liver, lung and heart of the sacrificed infected lambs were finely cut with scissors, crushed and the Baermann's technique (McCraw and Greenway, 1970) was employed to recover the larvae. The finely chopped tissues were suspended in a double layer of gauze, submerged in saline in Baermann apparatus, and left overnight (McCraw and Greenway, 1970). Afterwards, the fluid was allowed to gravitate from the stem of the funnel into centrifuge tubes and spun at low speed-500 rpm for 5 minutes so as to cause the larvae to gravitate. The sediment was then checked for the presence of larvae.

3.7 Faecal Egg Examination

Thrice weekly, beginning from day 7 post-infection, faecal samples were collected directly from the rectum of each animal to determine if the infections had reached patency. Faecal flotation technique (MAFF, 1986) was employed in order to find out if eggs were present.

3.8 Haematological Examination

1. Blood samples (5 ml each) from all animals were collected by jugular venipuncture into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA), as anticoagulant,. This was done on a weekly basis from day 0 to the end of the experiment that lasted for 12 weeks.

- Packed Cell Volume (PCV) was determined by the microhaematocrit method (Benjamin, 1978).
- Total White Blood Cells (WBC) were determined by using Neubauer haemocytometer.
- Differential leukocyte counts of blood smears were determined by the Battlement Method (Kelly, 1974).

2. Serum was harvested from clotted blood.

- The total serum proteins were determined by the Bieuret method. Serum albumin was determined by the use of Bromocresol green method (Weichselbaum, 1946) while the serum globulin fraction was determined as the difference between serum total protein and albumin fraction (Nnadi *et al.*, 2007).
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed according to the method described by Steven and Michael (2008).

3.9 Data Analysis

Data collected were expressed as means and their standard errors of mean (SEM) and presented as Tables and Charts. Data were analyzed using Graphpad Prism Software version 5.0. Significance of differences between group means was determined at $P \leq 0.05$. Student's t- test was used to test for differences between groups.

CHAPTER FOUR

4.0 RESULTS

4.1 Egg Recovery and Culture

A total of about 1.3 million eggs were recovered from the 5 female *A. suum* that were dissected, and after 30 days of culturing at 27˚C; most of the eggs (70%) became infective, with each egg containing a fully developed (Plate 1).

4.2 Clinical Signs

Cough and dyspnoea were noticed in the infected lambs; from day 7 after the initial infection until about 11 days after the last infection. No clinical signs were seen in lambs of the control group throughout the period of experiment.

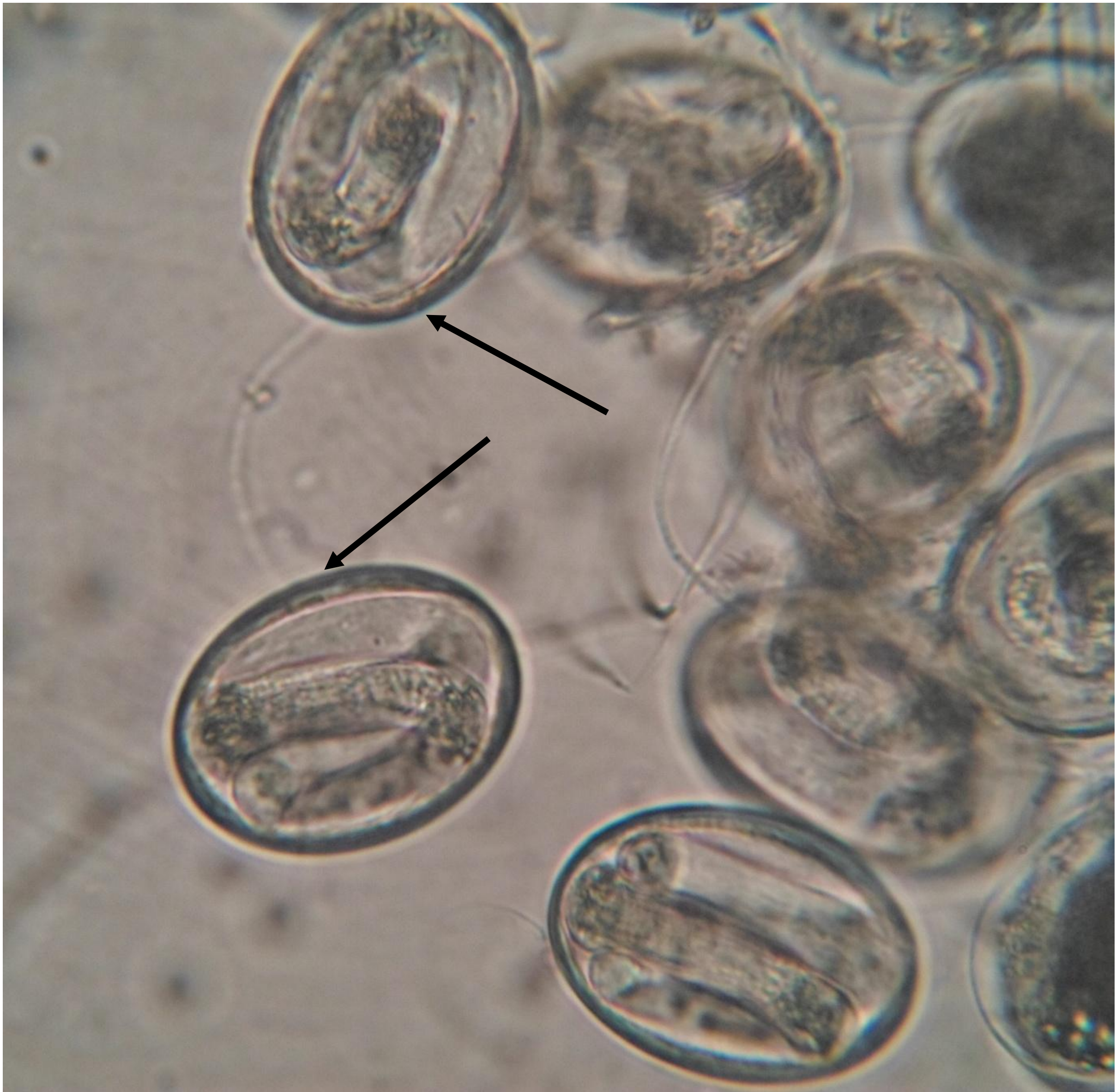


Plate I: Infective eggs of *Ascaris suum* (arrows) after 30 days of culture at 30˚C. (× **400)**

4.2.1 Vital Parameters

The mean (± SEM) temperatures as well as respiratory and pulse rates of the infected and the control groups for the 10-week experimental period are presented in Figures 1 to 3. The difference in the mean respiratory rates of the infected and the control groups was significant on day 14 of infection (Figure 1). The mean respiratory rate of lambs in the infected group was significantly higher ($P<0.05$) than that of lambs in the control group (28.03 ±0.31 vs 23.84 ± 0.28).

On the other hand, the mean temperatures of the infected group did not differ significantly ($P>0.05$) from those of the control group (Figure 2). However, the difference in the mean pulse rates in the infected and the control groups (83.78 ±0.21 vs 81.08 ± 0.98) (Figure 3) was significant ($P<0.05$) on day 14 of infection.

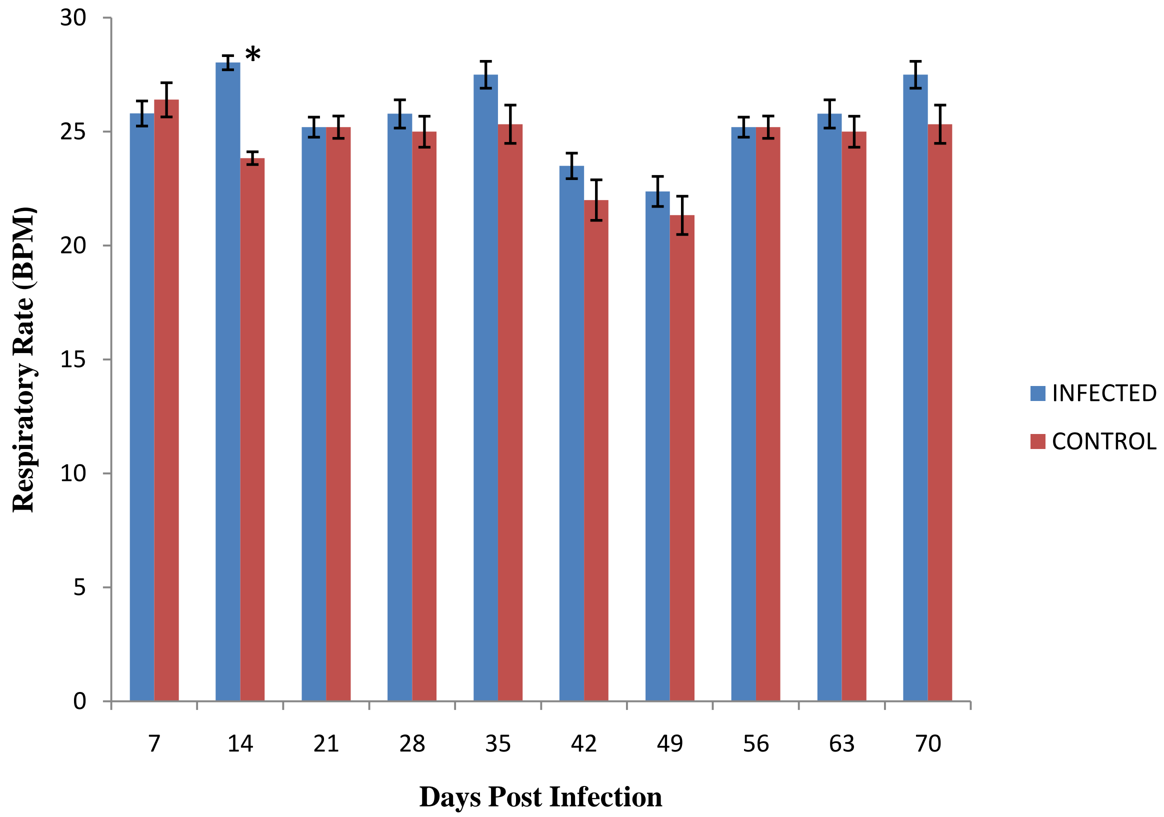


Figure 4.1: Mean (± SEM) respiratory rates (cycles/min) in the *A. suum*- infected and control lambs. *significantly different at $p < 0.05$

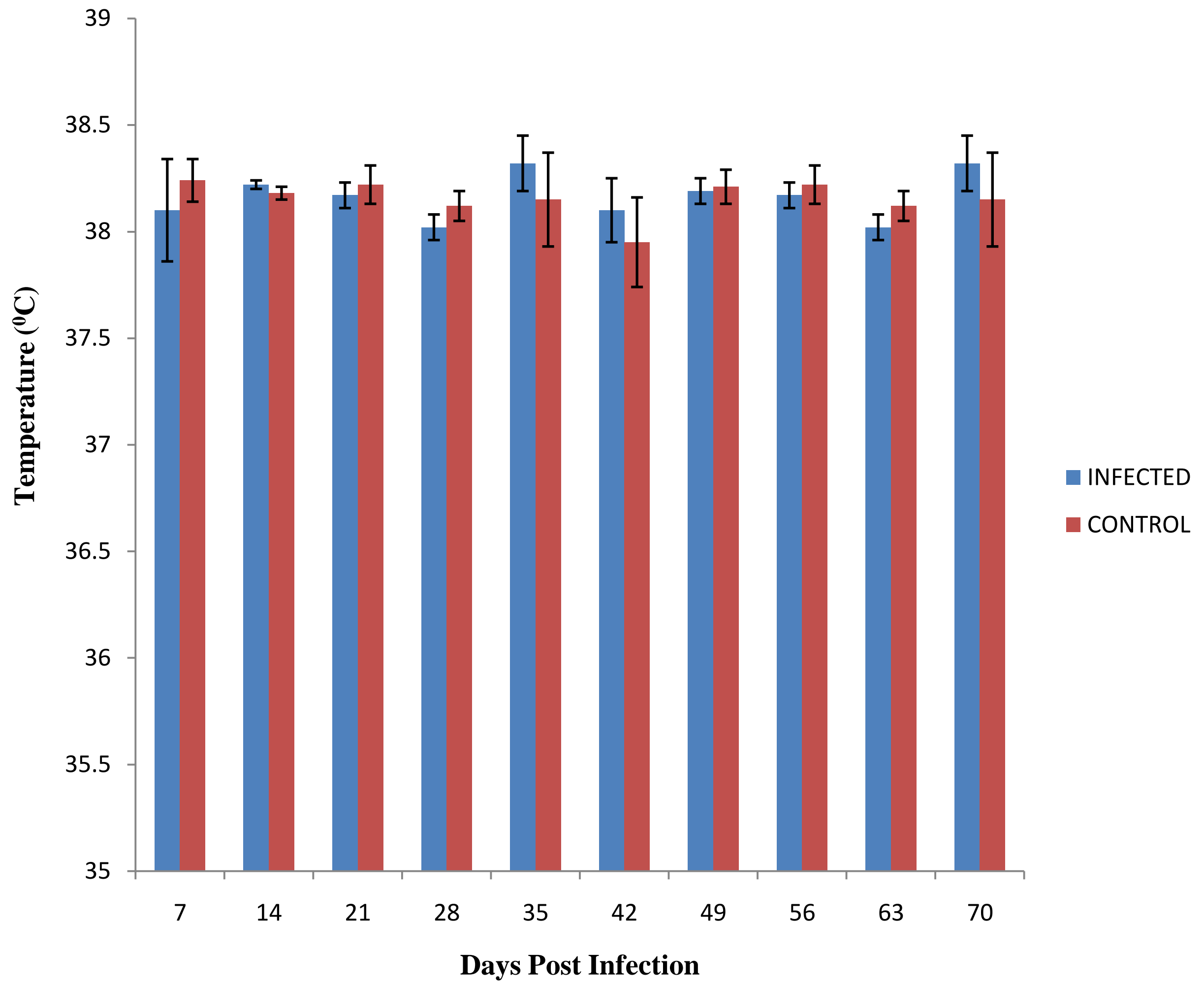


Figure 4.2: Mean (± SEM) temperatures in the *A. suum*-infected and control lambs.

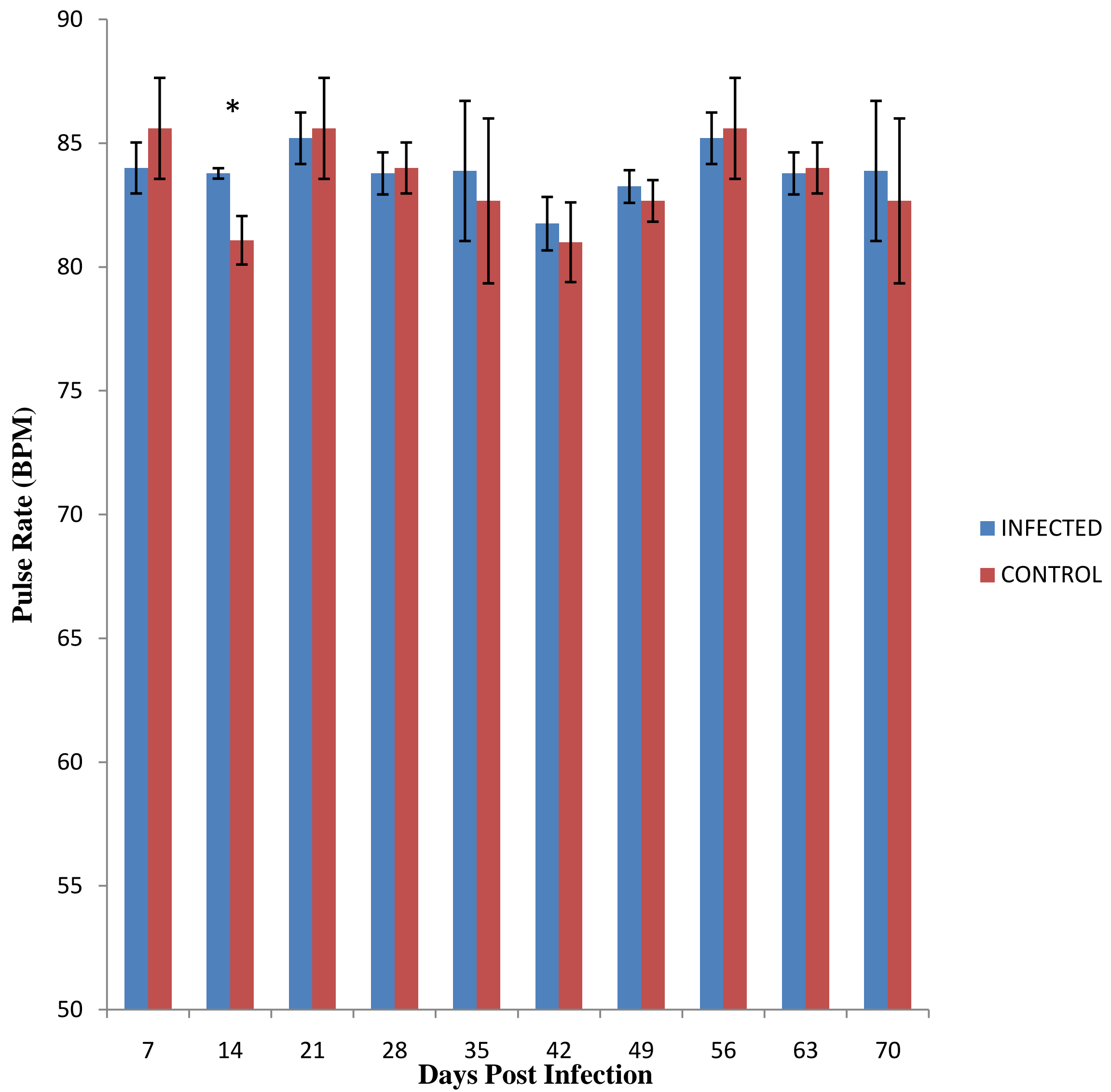


Figure 4.3: Mean (± SEM) pulse rates in the *A. suum*-infected and control lambs.
*significantly different at $p < 0.05$

4.2.2 Live Weight Changes

The mean (± SEM) body weights of the Yankasa lambs in the infected and control groups during the experimental period are presented on Table 4.1. The mean body weights of the lambs in the infected group were consistently lower than those of the control lambs but the differences were not significant (P>0.05).

Table 4.1: Mean (± SEM) body weights of the *A. suum*-infected and control Yankasa lambs

WEEK	INFECTED	CONTROL	P-values	Significance
1	18.20 ± 0.92	18.40 ± 0.72	0.420	NS
2	17.12±0.88	17.89±0.49	0.359	NS
3	16.40 ± 0.75	17.50 ± 0.70	0.417	NS
4	16.20±0.74	16.32±0.38	0.281	NS
5	15.67 ± 0.99	16.13 ± 0.39	0.510	NS
6	15.20 ± 0.58	16.24 ± 0.61	0.418	NS
7	15.60 ± 0.68	16.91 ± 0.58	0.424	NS
8	15.86 ± 0.68	16.98 ± 0.58	0.334	NS
9	15.92±0.39	17.00±0.16	0.442	NS
10	16.00 ± 0.71	17.18 ± 0.56	0.216	NS

NS- Not significant

4.3 Haematological Findings

The mean (± SEM) Packed Cell Volume (PCV), haemoglobin concentration (Hb) and total proteins (TP) are presented in Tables 4.2 to 4.4, respectively. The mean (± SEM) total white blood cell counts (WBC), eosinophils, neutrophils, lymphocytes and monocytes are presented in Figures 4 to 8, respectively.

In general, the mean PCV, haemoglobin and total protein concentrations in the infected group did not differ significantly (P>0.05) from those in the control group (Tables 4.2 to 4.4). Mean WBC was significantly higher (P<0.05) on days 7 and 35 of infection in the infected than in the control group (Figure 4). The mean WBC of lambs in the infected group were significantly higher on day 7 (P= 0.023) and day 35 (P= 0.047) than those of the control group [(6.8 ± 0.37 vs 6.00 ± 0.17) and (5.91 ± 0.12 vs 5.57 ± 0.26)] respectively. The mean eosinophil counts in the infected group were higher on days 7, 28 and 35 of infection than in the control group though, the differences were not statistically significant (P>0.05) (Figure 5).

Similarly, the mean neutrophil, lymphocyte and monocyte counts in the infected group were significantly (P<0.05) higher than those of the control group on days 56, 49 and 42 of infection respectively (see Figures 6-8).

Table 4.2: Mean (± SEM) Packed Cell Volume (%) in the *A.* suum-infected and control lambs.

Day	Infected	Control	P-value	Significance
7	33.00 ± 1.66	34.71 ± 2.71	0.578	NS
14	31.90 ± 1.52	33.83 ± 3.86	0.592	NS
21	36.90 ± 1.97	35.67 ± 1.50	0.676	NS
28	40.11 ± 6.51	38.50 ± 3.02	0.291	NS
35	38.91 ± 2.18	37.50 ± 2.79	0.701	NS
42	41.00 ± 3.11	41.4 ± 3.67	0.932	NS
49	30.30 ±1.30	33.67 ± 2.69	0.225	NS
56	30.75 ± 1.66	34.67 ± 3.23	0.268	NS

NS- Not significant

Table 4.3: Mean (± SEM) haemoglobin concentration (g/dL) in the *A. suum*-infected and control lambs

Day	Infected	Control	P-value	Significance
7	10.97 ± 0.55	11.53 ± 2.71	0.587	NS
14	10.55 ± 0.52	11.20 ± 1.28	0.592	NS
21	12.24 ± 0.66	11.70 ± 0.42	0.523	NS
28	13.36 ± 2.71	14.97 ± 0.98	0.514	NS
35	12.36 ± 8.19	13.42 ± 0.93	0.540	NS
42	13.71 ± 1.02	13.74 ± 1.22	0.983	NS
49	10.51 ± 0.73	11.18 ± 0.89	0.574	NS
56	10.22 ± 0.55	11.53 ± 1.07	0.465	NS

NS- Not significant

Table 4.4: Mean (± SEM) total protein concentrations (g/dL) in the *A. suum*-infected and control lambs

Day	Infected	Control	P-value	Significance
7	5.00 ±0. 32	5.31 ± 0.46	0.587	NS
14	5.07 ± 0.49	5.67 ± 0.20	0.421	NS
21	7.13 ± 1.34	5.57 ± 0.88	0.436	NS
28	4.18 ±0.62	5.68 ± 0.62	0.147	NS
35	5.45 ± 0.64	4.07 ± 0.49	0.166	NS
42	6.98 ± 0.88	6.78 ± 1.14	0.893	NS
49	5.94 ± 0.51	4.33 ± 0.77	0.574	NS
56	5.84 ± 0.21	5.75 ± 0.26	0.265	NS

NS- Not significant

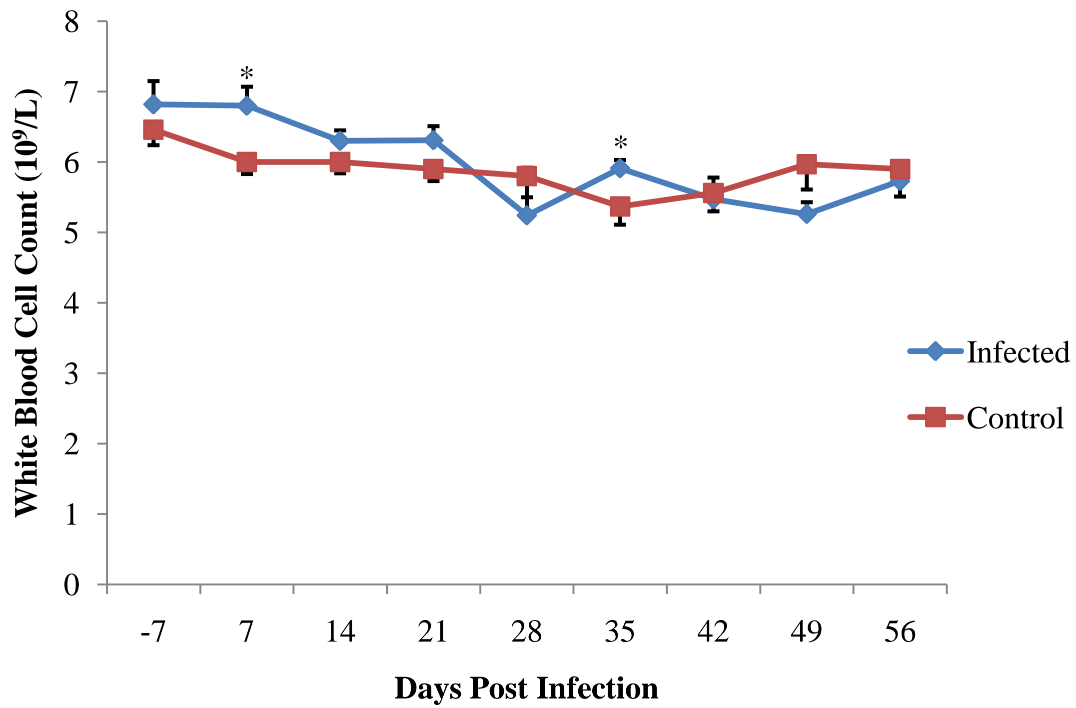


Figure 4.4: Mean (± SEM) total white blood cell count (10^9/L) in the *A. suum*-infected and control lambs * = significantly different at $p < 0.05$

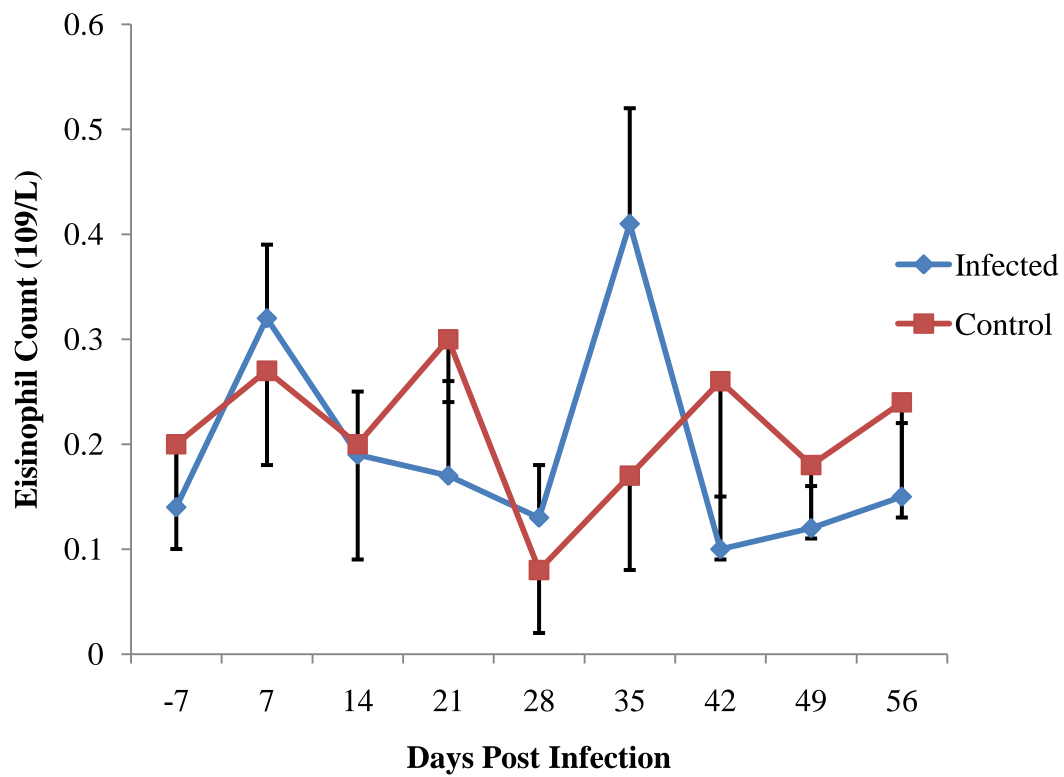


Figure 4.5: Mean (± SEM) Eosinophil count (10^9/L) in the *A. suum*-infected and control lambs

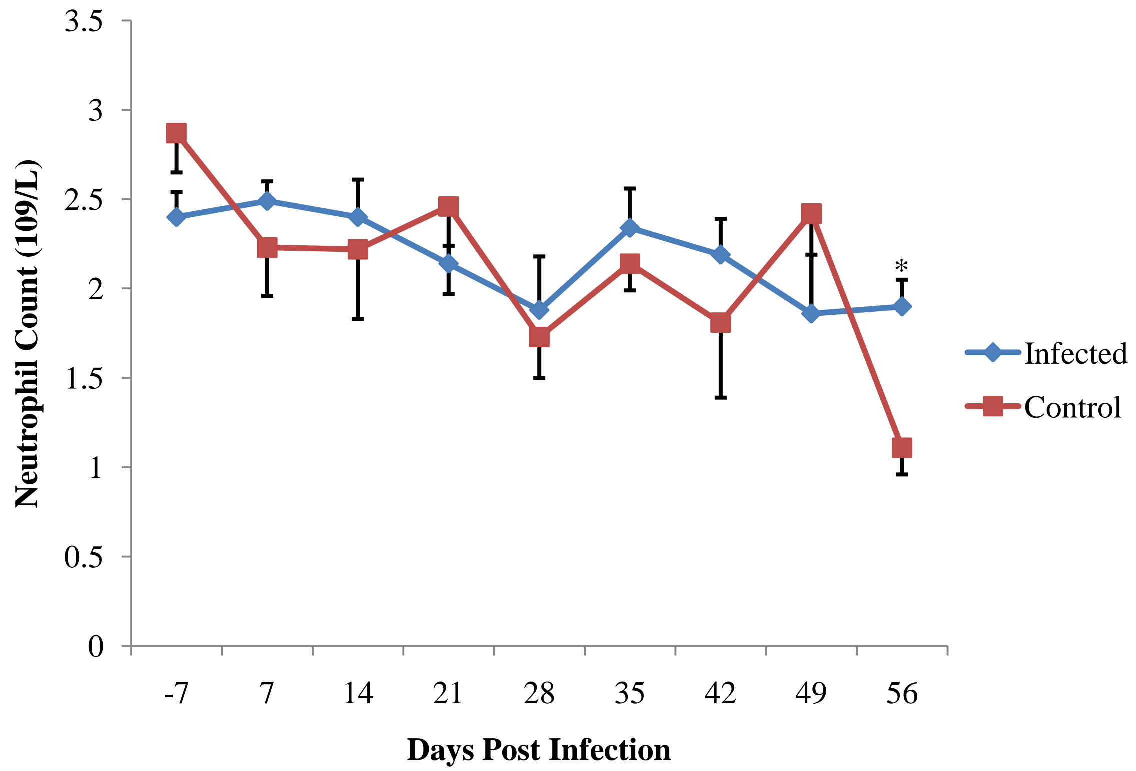


Figure 4.6: Mean (± SEM) neutrophil count (10^9/L) in the *A. suum*-infected and control lambs.

*** = significantly different at $p < 0.05$**

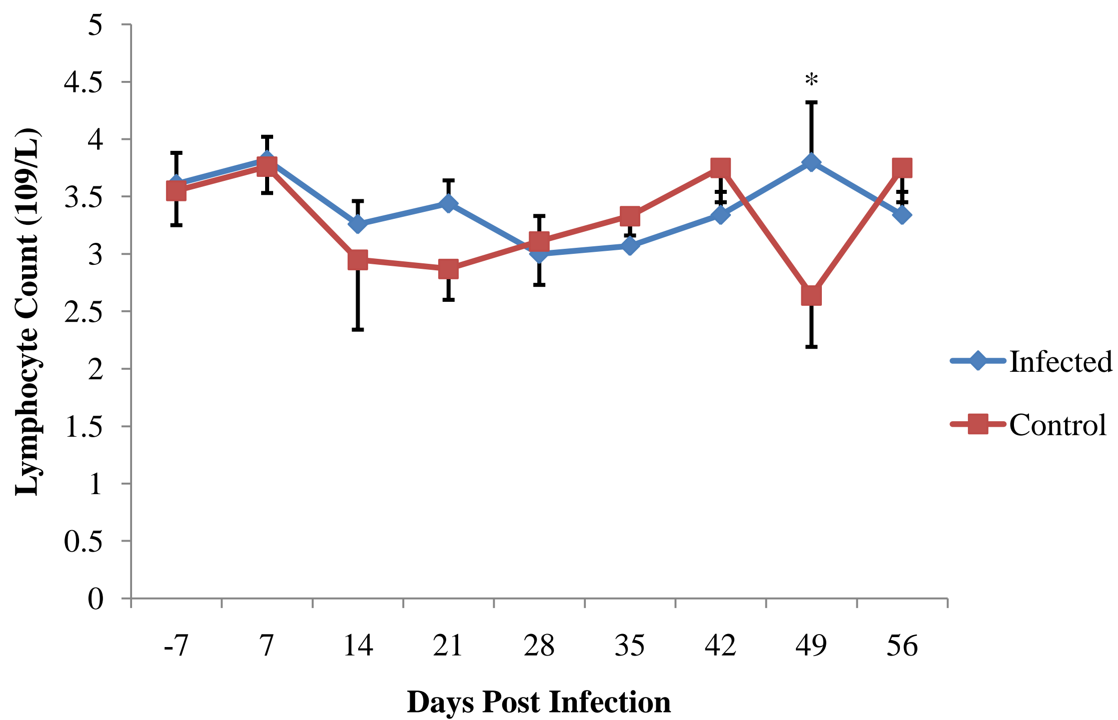


Figure 4.7: Mean (± SEM) lymphocyte count (10^9/L) in the *A. suum*-infected and control lambs.

*** = significantly different at $p < 0.05$**

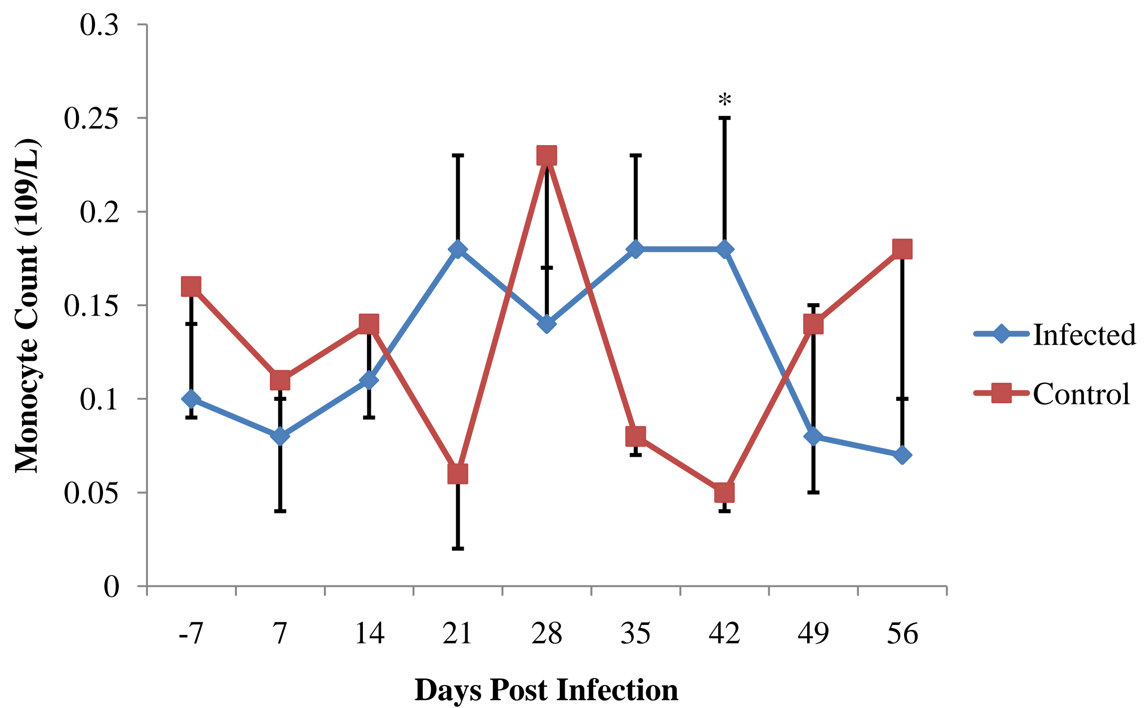


Figure 4.8: Mean (± SEM) monocyte count (10^9/L) in the *A. suum*-infected and control lambs.

*** = significantly different at $p < 0.05$**

4.4 Biochemical Parameters

The mean (± SEM) plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), urea and creatinine concentrations are presented in Figures 9 to 13. The mean (± SEM) plasma cocentrations of albumin and glucose are presented in Tables 4.5 and 4.6.

Mean plasma activities of AST (16.44 ± 4.47 vs 8.73 ± 1.24) and ALT (8.00 ± 2.18 vs 3.60 ± 0.54) were significantly higher ($P \leq 0.05$) in the infected than in the control lambs on day 14 of infection.

The mean concentrations of urea (7.85 ± 1.23 vs 4.35 ± 0.58) and creatinine (86.50 ± 11.99 vs 51.25 ± 6.84) of lambs in the infected group were significantly higher ($P < 0.05$) on day 28 of infection (Figures 12 and 13, respectively). However, there was no significant difference ($P > 0.05$) in the activities of ALP between the infected and the control groups. Similarly, there was no significant difference ($P > 0.05$) in the concentrations of albumin and glucose between the infected and control groups (Tables 4.5 and 4.6).

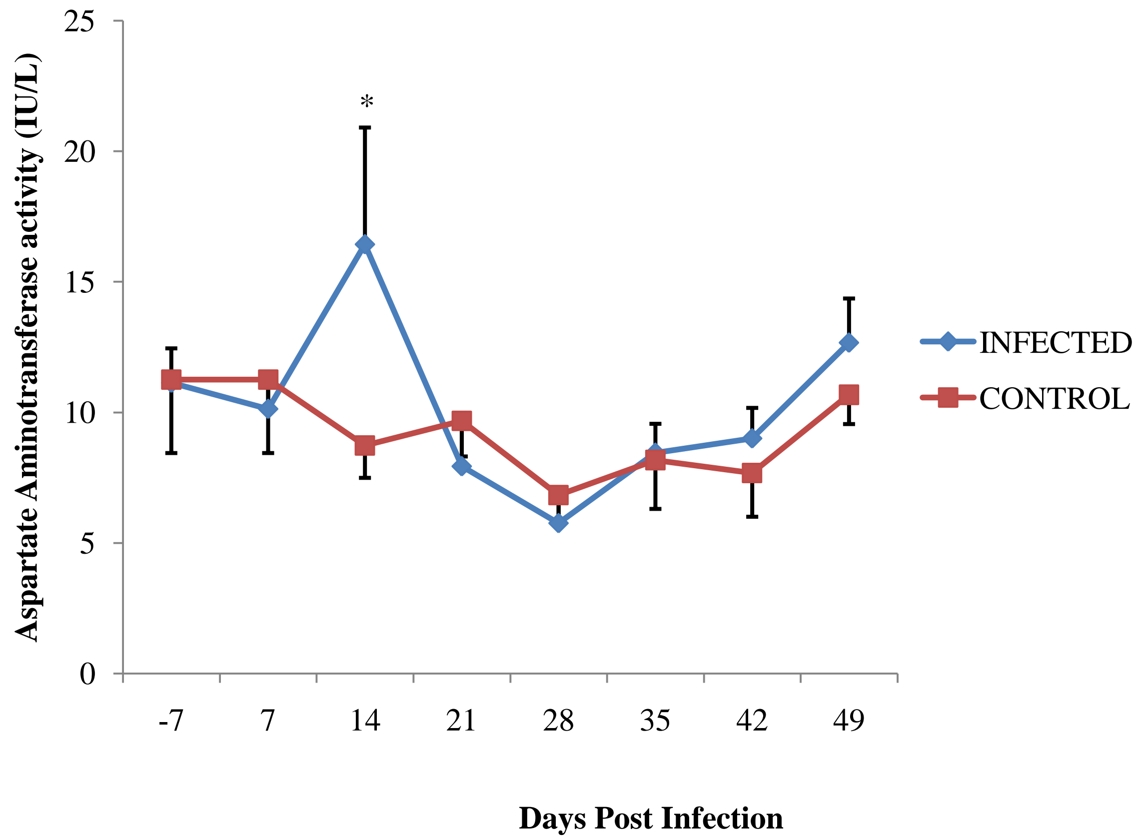


Figure 4.9: Mean (± SEM) AST serum activity (IU/L) in the *A. suum*-infected and control lambs.

*** = significantly different at p = 0.05**

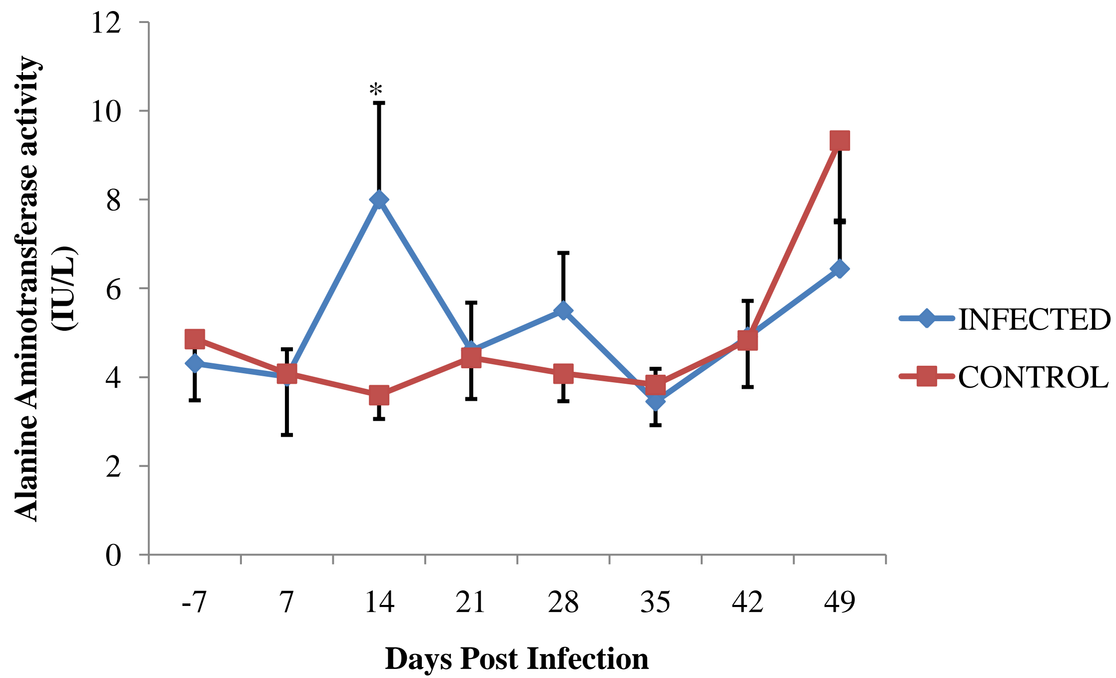


Figure 4.10: Mean (± SEM) ALT serum activity (IU/L) in the *A. suum*-infected and control lambs.

*** = significantly different at p < 0.05**

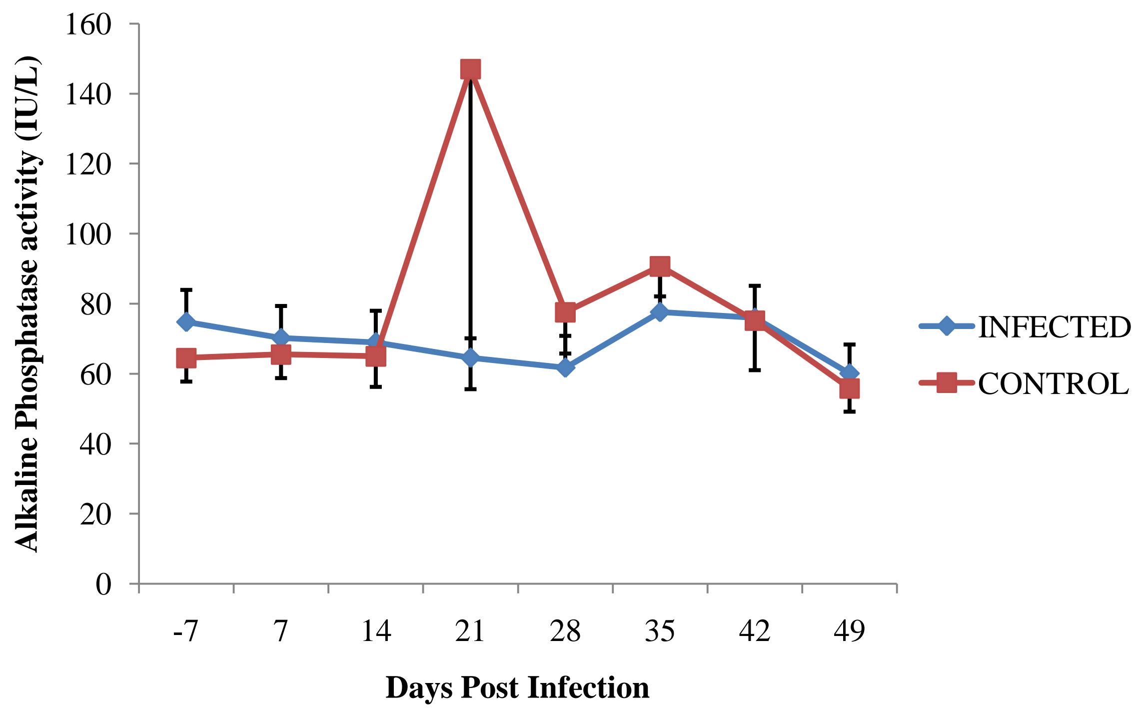


Figure 4.11: Mean (± SEM) ALP serum activity (IU/L) in the *A. suum*-infected and control lambs

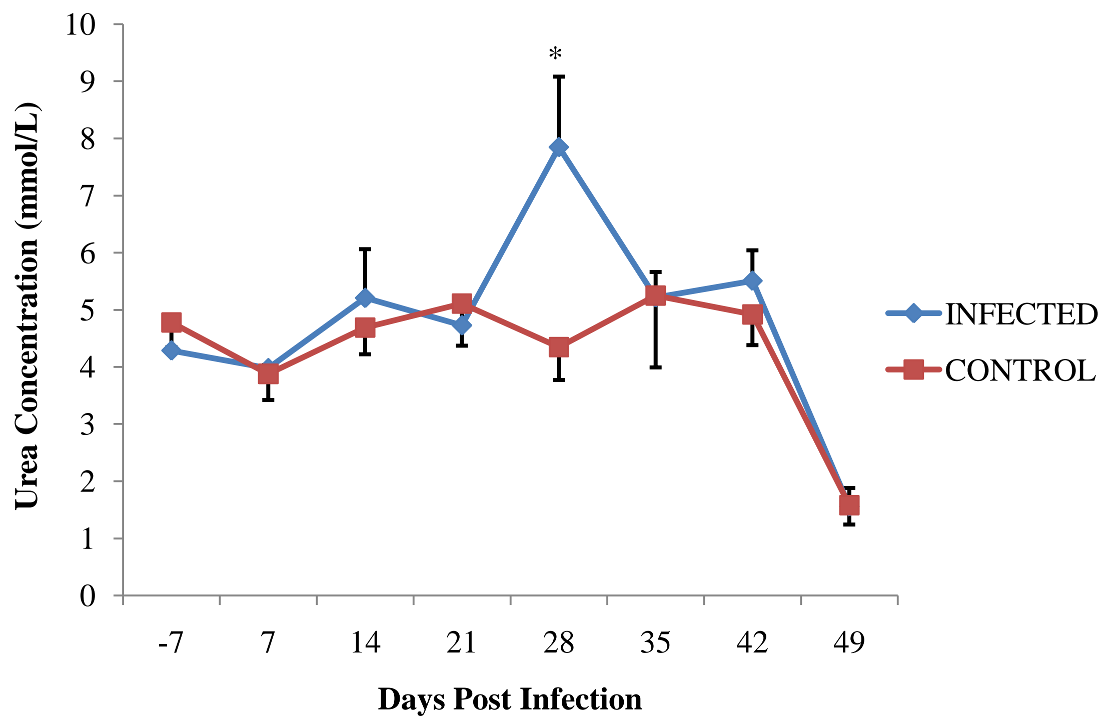


Figure 4.12: Mean (± SEM) urea serum concentration (mmol/L) in the *A. suum*-infected and control lambs.
*** = significantly different at $p < 0.05$**

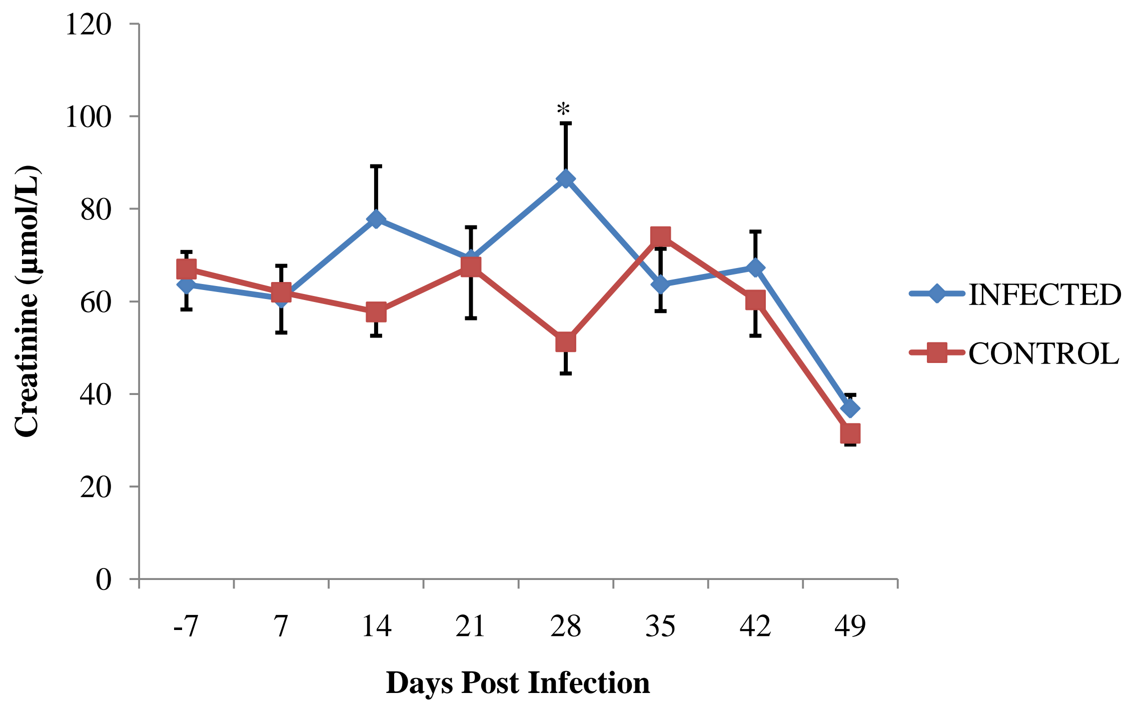


Figure 4.13: Mean (± SEM) creatinine serum concentration (µmol/L) in the *A. suum*-infected and control lambs.

*** = significantly different at $p < 0.05$**

Table 4.5: Mean (± SEM) serum albumin concentration (g/L) in the *A. suum*-infected and control lambs

DAY	INFECTED	CONTROL	P-value	Significance
7	44.56±2.38	37.88±4.98	0.152	NS
14	38.67±3.91	37.80±1.92	0.826	NS
21	36.13 ± 2.38	36.22 ±1.80	0.979	NS
28	31.63±5.05	38.92±1.28	0.112	NS
35	38.18±2.21	35.67±4.75	0.59	NS
42	34.55±2.20	30.83±6.19	0.500	NS
49	36.89±1.39	37.17±4.48	0.945	NS

NS- Not significant

Table 4.6: Mean (± SEM) serum glucose (mmol/L) in the *A. suum*-infected and control lambs

DAY	INFECTED	CONTROL	P-value	Significance
7	3.51±0.19	3.58±0.25	0.313	NS
14	3.36±0.30	3.45±0.18	0.783	NS
21	3.55 ± 0.22	3.39 ± 0.28	0.652	NS
28	3.61±0.29	3.23±0.13	0.192	NS
35	3.55±0.30	3.83±0.28	0.541	NS
42	3.91±0.31	3.40±0.27	0.298	NS
49	1.51±0.11	1.85±0.30	0.239	NS

NS- Not significant

4.5 Pathological Findings

4.5.1 Gross pathology

Each of the two infected lambs that were sacrificed 7 days after infection had tiny, white, pin-point lesions on the liver surface, although the lesions were more pronounced in one (Plate II) than the other (Plate III). In contrast, no lesions were noticed on the liver surface of the control animal sacrificed on the same day (Plate IV). Furthermore, no observable gross pathological findings were seen on the lungs, heart, kidneys and spleen of all the sacrificed animals.

By day 14 after infection, the foci had become larger (1×2 mm) and more noticeable (Plate V). However, by days 28 and 56 post infection the foci were almost inconspicuous (Appendices XV & XVI).

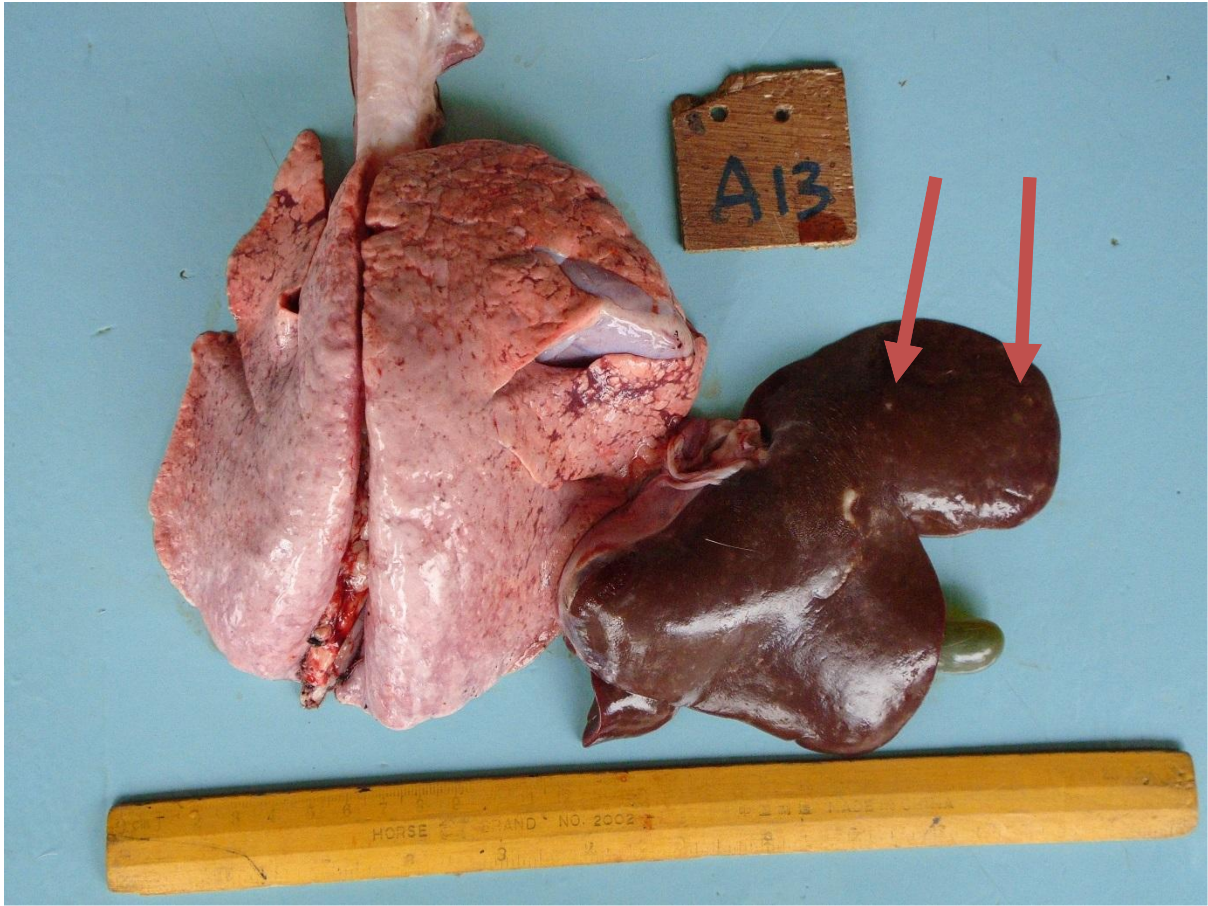


Plate II: Tiny, white, pin-point necrotic foci (Arrows) on the liver of a lamb slaughtered on day 7 after oral infection with 10,500 infective *A. suum* eggs.

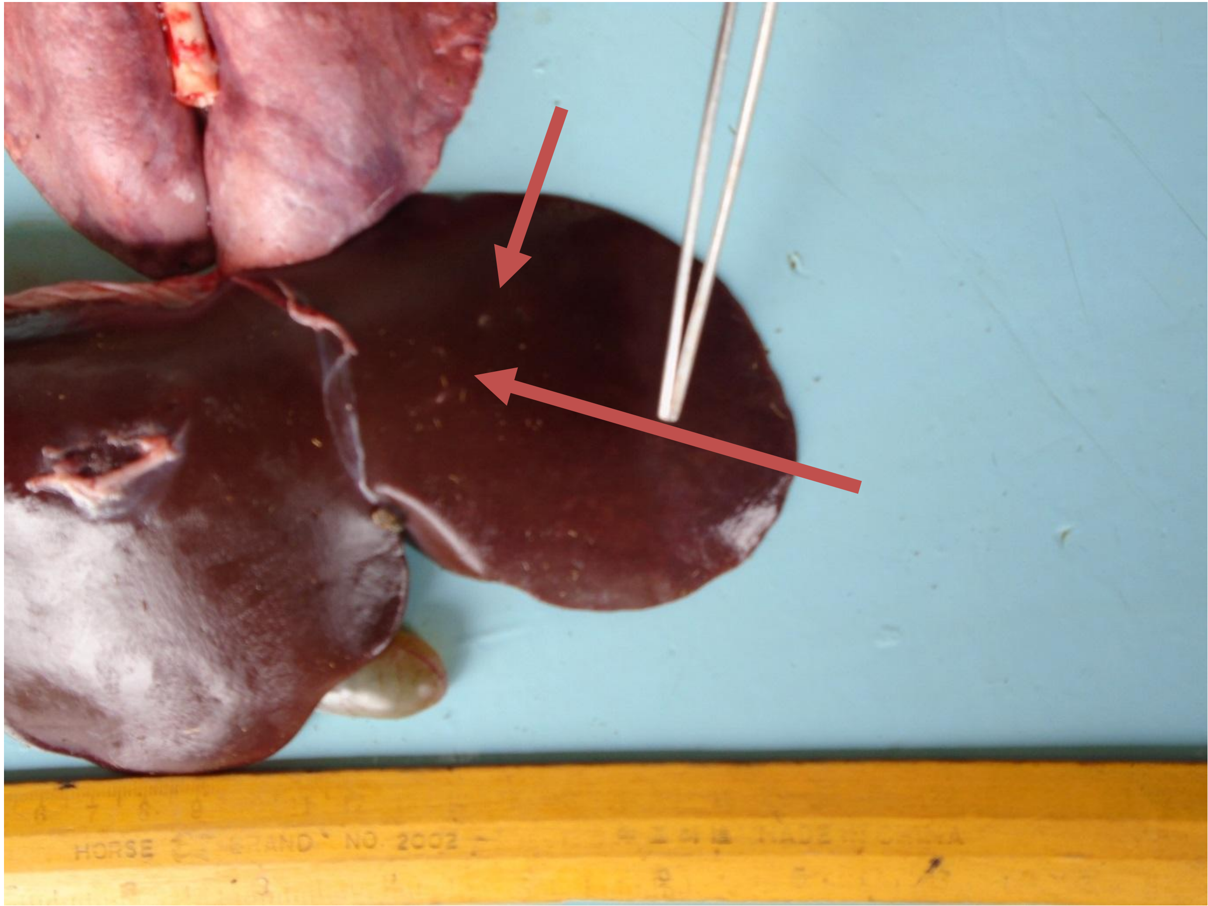


Plate III: Liver of the other infected lamb slaughtered on day 7 p.i with relatively less diffuse, tiny, faint, pin-point necrotic foci (arrows).

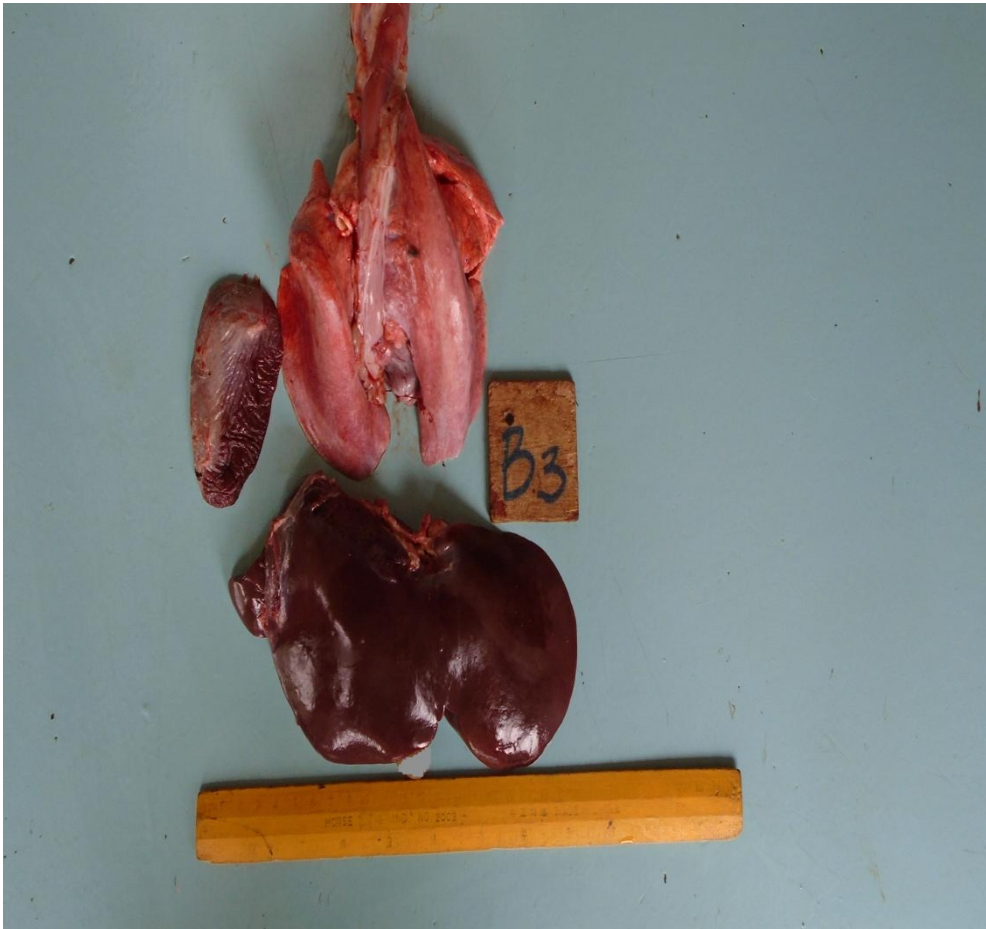


Plate IV: liver of an uninfected control lamb slaughtered on day 7 p.i, showing no observable lesions.

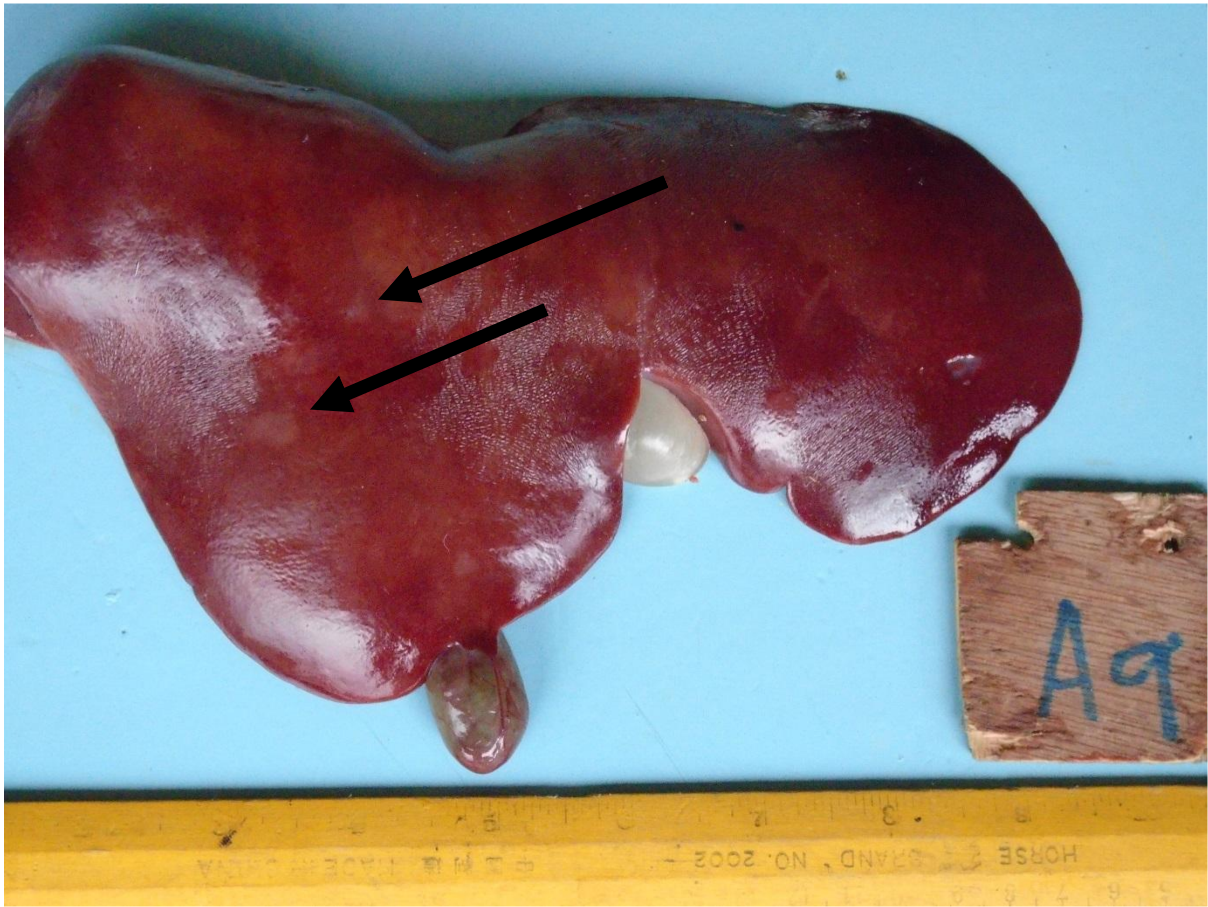


Plate V: Liver of a lamb slaughtered 14 days after trickle-infection with 10,500 infective *A. suum* eggs; showing larger, whitish necrotic areas (Arrows).

4.5.2 Histopathology

On days 7 and 14 of infection, areas of mononuclear cellular infiltration were observed in the liver parenchyma (Plate VI), while the kidney interlobular spaces were congested (Plate VII).

By days 28 and 56 p.i, the mononuclear cellular infiltration persisted in the liver, but to a lesser degree. It was also observed that the kidney of the animal sacrificed on day 56 p.i was congested (Plate VIII). Microscopically, no changes were noticed on the lungs (Plate IX). No lesions were observed in the corresponding organs of the uninfected control.

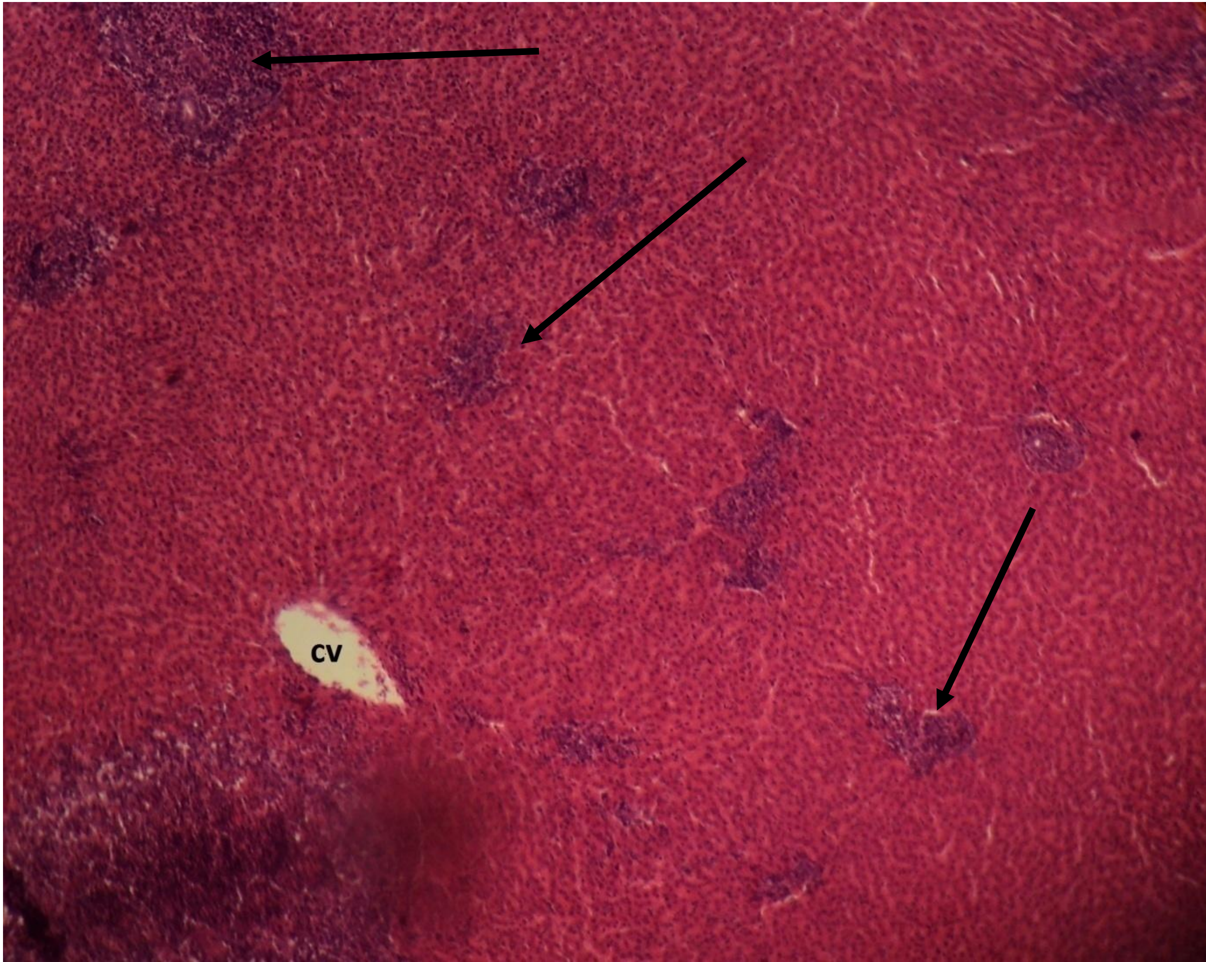


Plate VI: Photomicrograph of a section of the liver of *Ascaris suum*-infected lamb slaughtered on day 14 p.i. Note the areas of mononuclear cellular infiltration (arrows); **CV**-central vein (**H & E** × **200**).

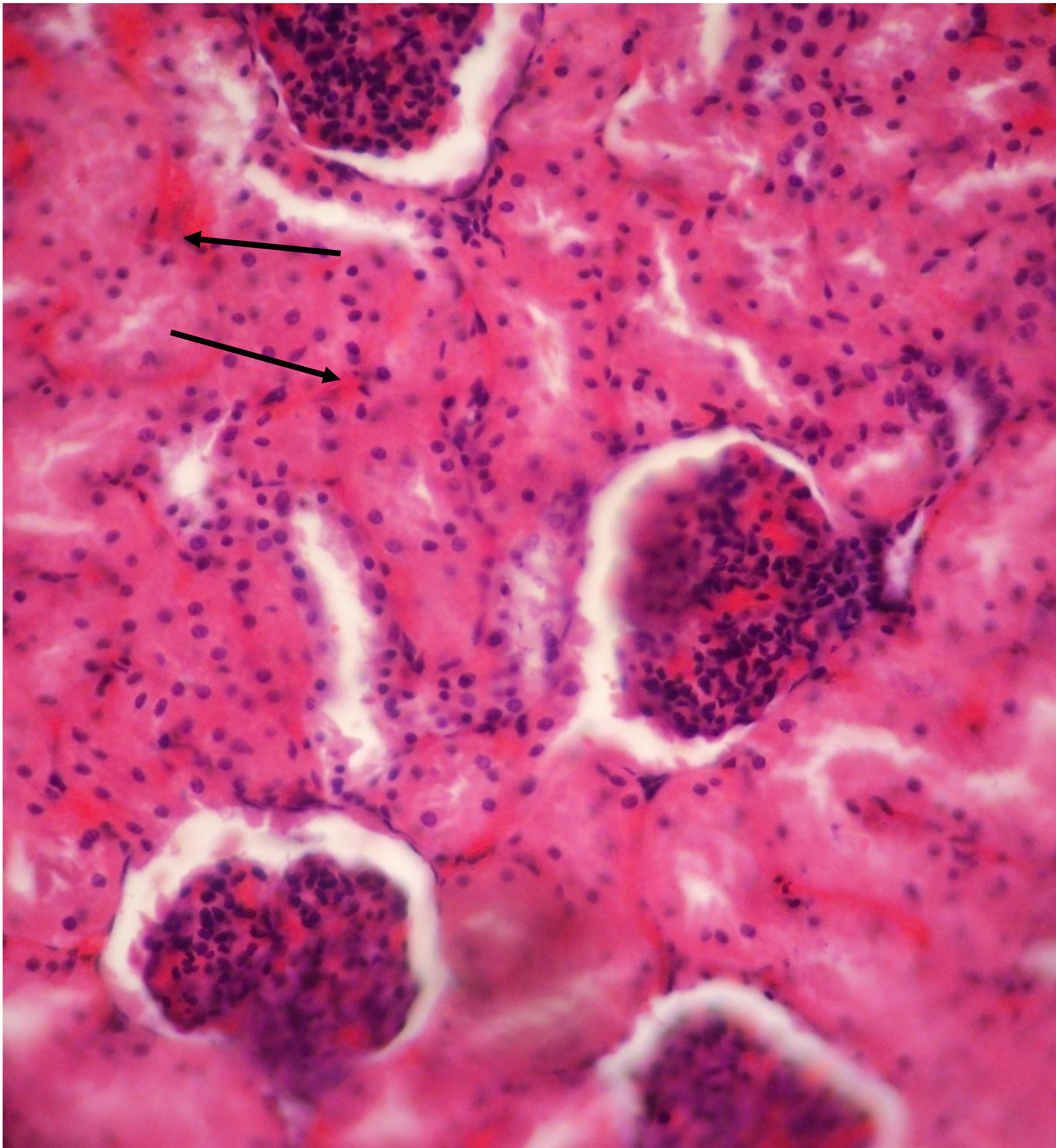


Plate VII: Photomicrograph of a section of the kidney of a lamb slaughtered 7 days after infection with 10,500 infective *A. suum* eggs. Note the congested intertubular spaces (arrows) (**H & E** × **400**).

.

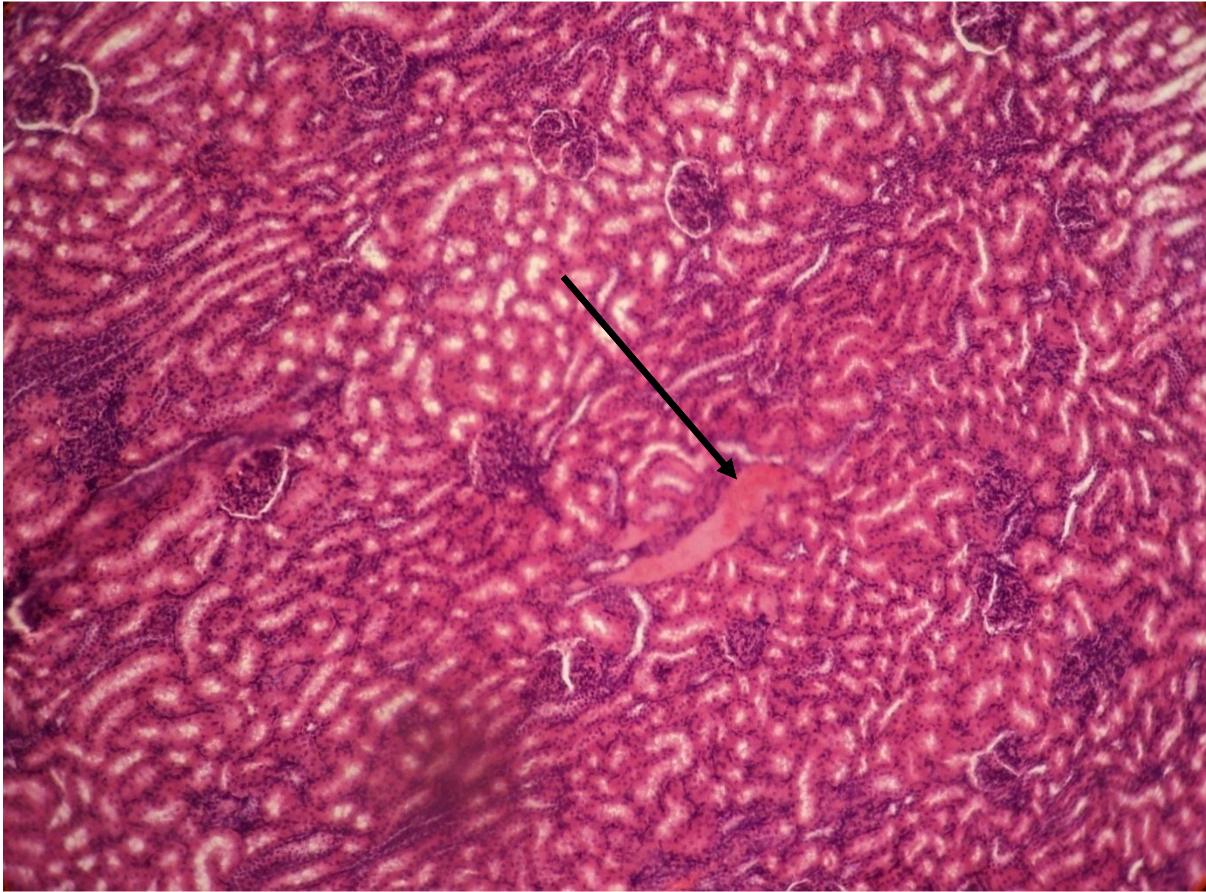


Plate VIII: Photomicrograph of a section of the kidney of a lamb slaughtered 56 days after infection with 10,500 infective *A. suum* eggs. Note the congested blood vessel (arrow) (**H & E × 200**).

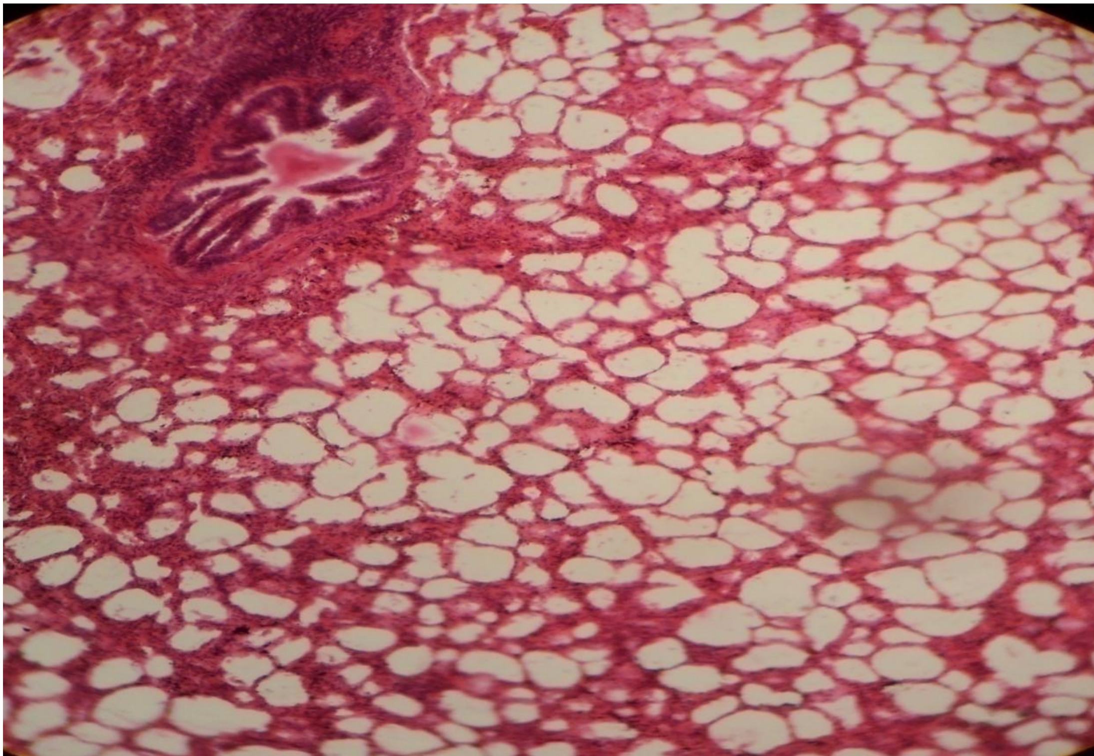


Plate IX: Lung slide of a lamb slaughtered 7 days after infection with 10,500 infective *A. suum* eggs, showing no obvious changes (**H & E × 200**)..

4.6 Larval Recovery

Eight (8) larvae were recovered from the lung of the infected lamb which was sacrificed on day 28 of infection. However, no larvae or adults were detected upon examination of the contents of the gastro-intestinal tract. Larvae were not recovered from any of the infected lambs sacrificed after day 28. Also, no larvae were detected in the lung or other organs of the uninfected control throughout the infection. The two ends of one of the larvae so recovered are shown in Plates X and XI.

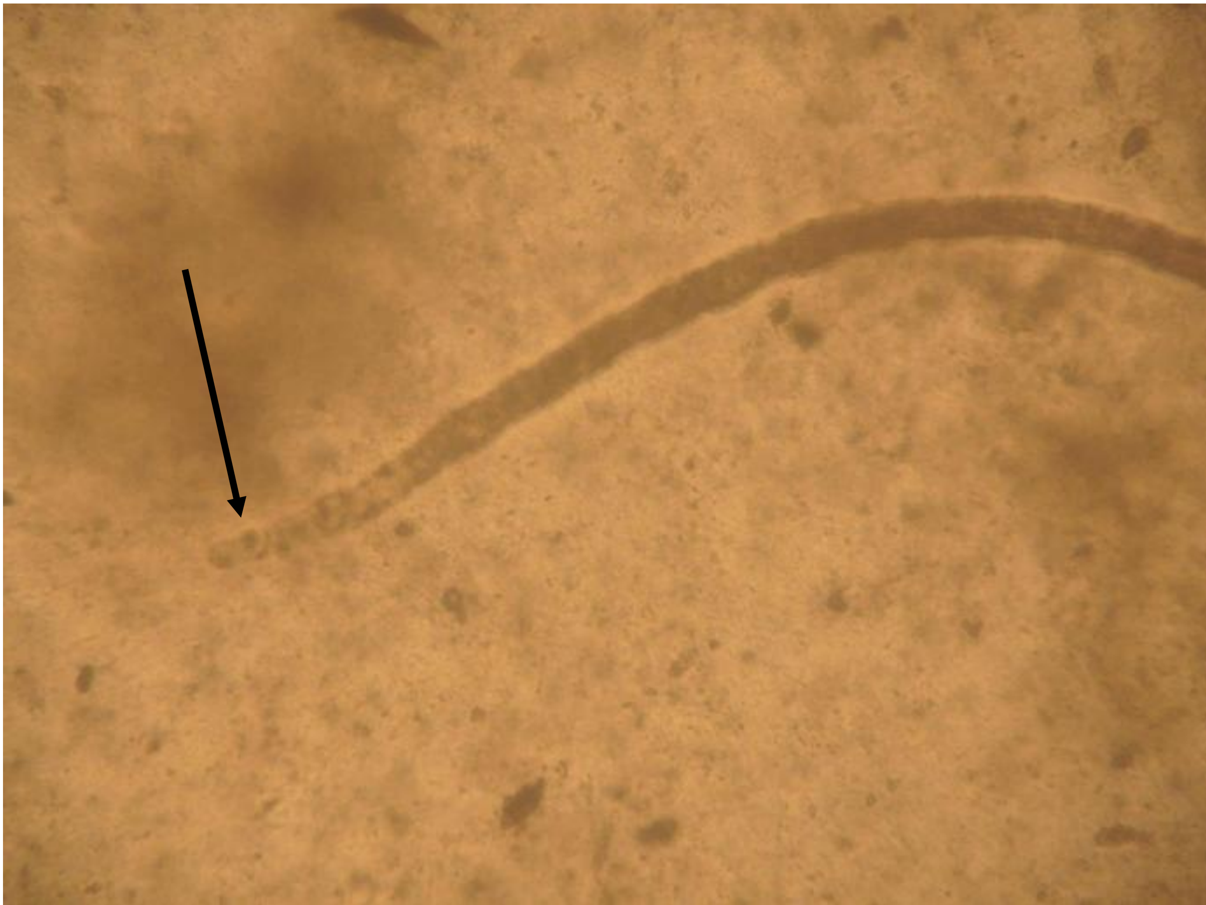


Plate X: One end of one of the larvae recovered from the lungs of a lamb slaughtered on day 28 after oral infection with 10,500 infective *A. suum* eggs (arrow). **(× 400)**

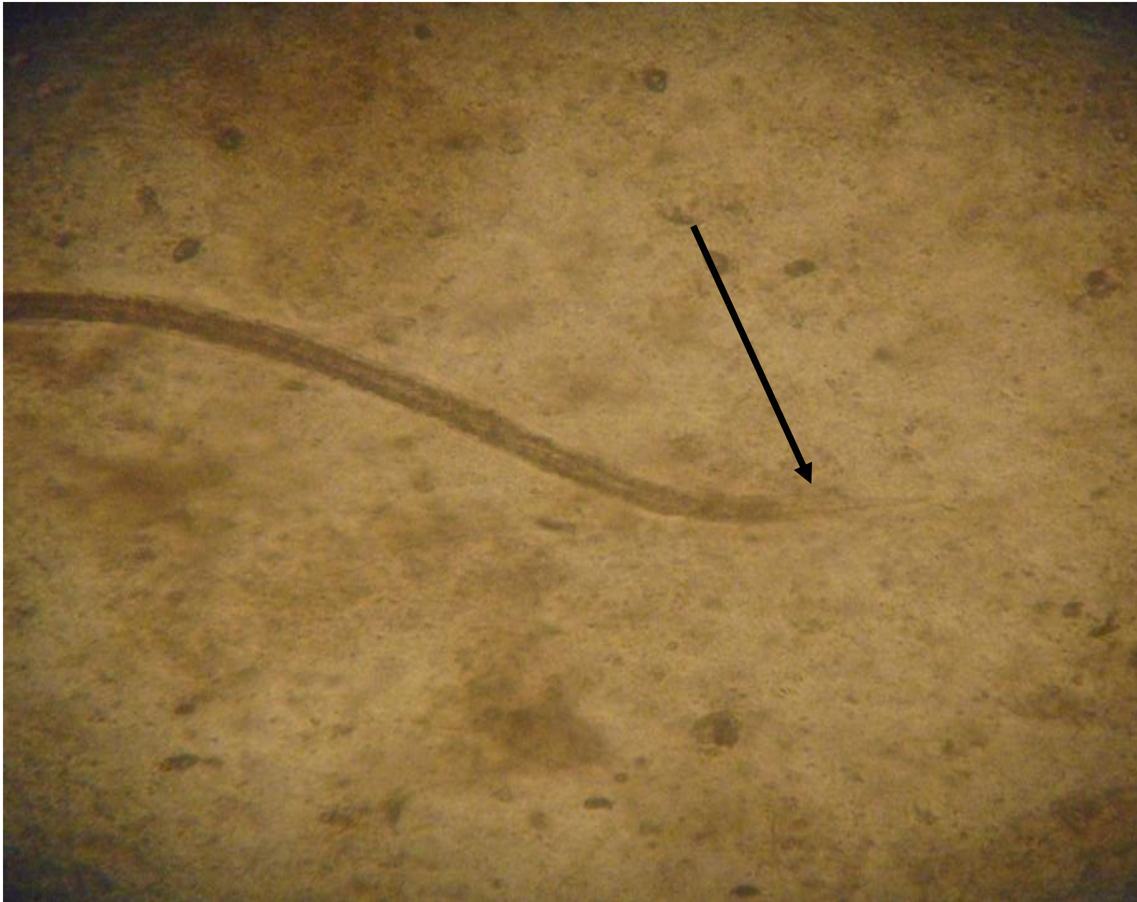


Plate XI: The other end of the larva (arrow). (× **400)**

CHAPTER FIVE

5.0 DISCUSSION

The effects of experimental *Ascaris suum (A. suum)* infection in Yankasa lambs were investigated. The findings of clinico-pathological lesions and *A. suum larvae* recovered from the lungs, following a trickle infection of lambs with 10,500 infective eggs are a strong proof that the infection had established in the infected lambs. However, the infection did not reach patency, likely because the Yankasa lambs may have been endowed with an ability to contain the parasite and also they were well-fed under the experimental set-up, which would have contributed to a boost in their immunity. In addition, the lambs were immunized before the infection against common parasitic and bacterial infections that could have, otherwise, occurred and caused depletion in their immunity.

The cough and dyspnoea observed in the infected animals were in agreement with the findings in the report of Krupicer *et al*. (1999) in which a prolonged infection of lambs with low doses of *A. suum* eggs resulted in a mild increase in the breath rate, which was accompanied with cough from day 6 of infection. Similarly, the infection in the present study did not appear to have significant effect on body weights. The significant difference in the respiratory and pulse rates on day 14 of infection could be attributed to the migration of the *A. suum* larvae in the lungs, which could have caused some damage to alveolar tissues thereby interfering with normal gaseous exchange.

The higher eosinophil count recorded in the infected than in the control group on days 7, 28, and 35 of infection may be an indication of increased mobilization of these cells from the bone marrow into the circulating blood. Since eosinophillia is a hallmark of parasitic infection, it is likely that the increase in circulating eosinophils was an attempt by the host to

kill the larvae. This is because degranulation of eosinophils has been reported to kill parasite larvae through the A.D.C.C. (Antibody-dependent cell-mediated cytotoxicity) (Butterworth, 1984). Previous report (Krupicer *et al*., 1999) had indicated similar finding of eosinophillia following an experimental infection of lambs with low doses of *A. suum* eggs. The decrease observed at some points during the course of the experiment in the infected group could be merely relative. This is because high neutrophil count could cause relative decrease in eosinophil count (Latimer, 2011).

The significant increase in the total white blood cell count on days 7 and 35 of infection in the infected group might be reflective of inflammatory process that might have been triggered by the migrating *A.* suum larvae in the liver and possibly other organs. Thus, the significantly higher neutrophil count recorded in the infected, compared to the control group on day 56 of infection, could be an indication of possible injury caused by the migrating larvae to the liver and perhaps, other organs.

Similarly, the significant increase in the lymphocyte count in the infected lambs might be an indication of attempt to develop specific immunity against the parasite. Likewise, it may be inferred that the increase in monocyte count, particularly on day 42 of infection, was a normal body response to clear itself of cell debris that may have accumulated as a result of damage to tissues by migrating *A. suum* larvae.

The non-significant changes observed in the values of PCV, haemoglobin and total proteins, as well as the levels of albumins and globulins might be an indication that the parasite was not very pathogenic to this host species. The elevation in the values of AST and ALT on day

14 of infection in the infected group may be an indication of liver damage, caused by the migrating *A. suum* larvae, and consequent escape of the enzymes into the extracellular compartment and, subsequently, into the blood. On the other hand, findings in this study of increased ALT and AST activities were contrary to findings in the reports of Dubinsky *et al.* (2000) who observed that a long-term infection of lambs with the dose of 100 and 1000 eggs for 23 days did not influence the activities of AST and ALT. However, the higher infective dose (1500 x 7 eggs) used in this study may have been responsible for this difference.

The significant increase in the values of urea and creatinine concentrations in the infected group on day 28 of infection may be suggestive of impairment of renal function, perhaps due to the congestion observed during histopathological examination. However, since larvae were not recovered and no gross lesions suggestive of larval migration were observed in the kidneys, it might be difficult to attribute the congestion to the parasite migration.

The occurrence of whitish pin-point gross lesions, which were eventually identified by histopathology examination to be infiltrating mononuclear cells on the liver on day 7 post-infection in this study, strongly suggests that larval migration had occurred in this organ. Previous study (Brown *et al.*, 1984) had reported small hepatic necrotic foci infiltrated with eosinophils and lymphocytes in lambs infected with higher dose of infective eggs of the *A. suum*. This study revealed that only sporadic lesions persisted from day 28 to day 56 post-infection, as was earlier reported by Roespstorff *et al.* (1997) and Dubinsky *et al.* (2000). This may be attributed to the progressive effacement of the lesions as the healing process proceeded.

In this present study, larvae were detected in the lungs of the lamb that was sacrificed on day 28 post-infection, but not in the other animals that were sacrificed at the later stage of the infection. The low rate of larval recovery may be due to the recovery method that was employed. Following very high single and repeated doses, *A. suum* larvae were recovered in higher numbers from the lungs of infected ruminants (Fitzgerald, 1962; Morrow, 1968; McCraw, 1975). Dubinsky *et al*. (2000) detected more larvae in the liver than in the lungs on day 7, but the larvae recovered from the lungs on day 21 were three times higher than those recovered from the liver. However, no larvae were recovered from the liver in this study. Likewise, no larvae were detected in the liver of infected lambs by Fitzgerald, (1962) and Clark *et al*. (1989). This may be ascribed to a different reaction of the liver tissue of pigs and sheep to migrating *A. suum* larvae (Brown *et al*., 1984). In addition, different breeds of sheep may have different reactions to the migrating *A. suum* larvae. Therefore, this study has shown that *Ascaris suum* is infective to sheep, causing few clinical signs and pathological effects.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1) *Ascaris suum* was infective to lambs but the infection did not reach patency.

2) Clinical signs were noticed but were less expressive.

3) The infection in lambs produced similar pathological effects as in pigs though not as remarkable.

6.2 Recommendations

1) Farmers should ensure that lambs are protected from grazing in such places where they may ingest pig slurry. Public enlightenment campaign on the dangers of such should be carried out.

2) Efforts aimed at controlling the infection in pigs should be intensified, which in turn will help in preventing its occurrence in lambs and other accidental hosts.

REFERENCES

Adedeji, S.O., Ogunba, E.O. and Dipeolu, O.O. (1989). Synergistic effect of migrating *Ascaris* larvae and *Escherichia coli* in piglets. *Journal of Helminthology*, 63: 19-24.

Aitken, M. M. and Sanford, J. (1968). Experimentally induced anaphylaxis in cattle. *Veterinary Record*, 82: 418-419.

Ajala, M.K. and Osuhor, C.U. (2004). Economic analysis of returns and cost structure in swine production in Kaduna state, Nigeria. *Tropical Journal of Animal Science*, 7: 11-18.

Allen, G.W. (1961). Acute atypical bovine pneumonia caused by *Ascaris lumbricoides*. *Canadian Journal of Comparative Medicine*, 26: 241-3.

Andersen, S. (1976). The influence of *Ascaris suum* infection upon growth rates in pigs. *Nordisk veterinaermedicin,* 28: 322-330.

Andersen, S., Jorgensen, R.J, Nansen, P. and Nielsen, K. (1973). Experimental *Ascaris suum* infection in piglets. Inverse relationship between the numbers of inoculated eggs and the numbers of worms established in the intestine. Acta Pathol Micr Sc, 81 (6): 650-6.

Barker, I.K., Dreumel, A.A.V. and Palmer, N. (1993). The alimentary system. *In*: Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (Eds.), *Pathology of Domestic Animals*, vol. 2, 4th Edn. Academic Press. Pp. 283–285.

Beloeil, P.A., Chauvin, C., Fablet, C., Jolly, J.P., Eveno, E. And Madec, F. (2003). Helminth control practices and infections in growing pigs in France. *Livestock Production Science,* 81 (1): 99-104.

Benjamin, M.M. (1978). *Outline of Veterinary Clinical Pathology*. The Iowa State University Press. P. 230.

Beraldo, W.T., Dias Da Silva, W. and Pudles, J. (2012). Antigenic properties of purified fractions from *Ascaris lumbricoides var. suum* on naturally sensitized guinea-pig. *British Journal of Pharmacology*, 17 (2): 236-44.

Berger, H. (1971). Experimentally induced patent infections of *Ascaris suum* in rabbits. *The Journal of Parasitology*, 57: 344-7.

Bernardo, T.M., Dohoo, I.R. and Donald, A. (1990a). Effect of ascariasis and respiratory diseases on growth rates in swine. *Canadian Journal of Veterinary Research*, 54: 278-284.

Bernardo, T.M., Dohoo, I.R. and Ogilvie, T. (1990c). A critical assessment of abattoir surveillance as a screening test for swine ascariasis. *Canadian Journal Veterinary Research*, 54 (2): 274-7.

Bernardo, T.M., Dohoo, I.R., Donald, A., Ogilvie, T. and Cawthorn, R. (1990b). Ascariasis, respiratory diseases and production indices in selected Prince Edward Island swine herds. *Canadian Journal of Veterinary Research*, 54: 267-273.

Boersema, J.H., Eysker, M. and Nas, J.W.M. (2002). Apparent resistance of *Parascaris equorum* to macrocylic lactones. *Veterinary Record*, 150 (9): 279-81.

Boes, J., Medley, G.F., Eriksen, L., Roepstorff, A. and Nansen, P. (1998). Distribution of *Ascaris suum* in experimentally and naturally infected pigs and comparison with *Ascaris lumbricoides* infections in humans. *Parasitology*, 117 (6): 589-96.

Boes, J., Nansen, P. and Stephenson, L.S. (1997). False-positive *Ascaris suum* egg counts in pigs. *International Journal of Parasitology*, 27(7): 833-8.

Boes, J., Willingham, A.L., 3rd, Fuhui, S., Xuguang, H., Eriksen, L., Nansen, P. and Stewart, T.B. (2000). Prevalence and distribution of pig helminths in the Dongting Lake Region (Hunan Province) of the People's Republic of China. *Journal of Helminthology*, 74: 45-52.

Bogh, H.O., Eriksen, L., Lawson, L.G. and Lind, P. (1994). Evaluation of an enzyme-linked immunosorbent- assay and a histamine-release test system for the detection of pigs naturally infected with *Ascaris suum*. *Preventive Veterinary Medicine*, 21 (3):201-14.

Borella, L.E. Adams, 1. G. and Malone, M.H. (1966). The role of histamine in acute experimental ascariasis. *Journal of Parasitology*, 52: 295·302.

Borgsteede, F.H.M., Deleeuw, W.A., Dijkstra, T., Alsma, G. and deVries, W. (1992). *Ascaris suum* infections causing clinical problems in cattle. *Tijdschr Diergeneeskd*, 117 (10): 296-8.

Borland, E. D., Keymer, I. F. and Counter, D. E. (1980). Condemnation of sheep livers probably due to ascariasis. *Veterinary Record*, 107: 265-266.

Brown, D., Hinton, M, and Wright, A.I. (1984). Parasitic liver damage in lambs with particular reference to the migrating larvae of *Ascaris suum*. *Veterinary Record*, 115: 300-303.

Butterworth, A.E. (1984). Cell-mediated damage to helminths. *Advanced Parasitology*, 23: 143-235.

Carstensen, L., Vaarst, M. and Roepstorff, A. (2002), Helminth infections in Danish organic swine herds. *Veterinary Parasitology*, 106: 253-264.

Celia, H. (2012). *Ascaris: The Neglected Parasite*. Academic Press. Pp. 107-420.

Chen, N., Yuan, Z.G, Xu, M.J, Zhou, D.H. and Zhang, X.X. (2012). *Ascaris suum* enolase is a potential vaccine candidate against ascariasis. *Vaccine*, 30: 3478-3482.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. *Annual Review of Phytopathology*, 40: 221-49.

Cho, S., Egami, M., Ohnuki, H., Saito, Y., Chinone, S. and Shichinohe, K. (2007). Migration behaviour and pathogenesis of five ascarid nematode species in the Mongolian gerbil *Meriones unguiculatus*. *Journal of Helminthology*, 81 (1): 43-7.

Clark, E.G., Von-Dewitz, A. and Acompanado, G. (1989). Spurious *Ascaris suum* infection in lambs. *Canadian Veterinary Journal*, 30: 903.

Copeman, D.B. and Gaafar, S.M. (1972). Sequential development of hepatic lesions of ascaridosis in colostrums deprived pigs. *Australian Veterinary Journal*, 48 (5): 263-268.

Curtis, S.E., Tisch, D.A., Todd, K.S. and Simon, J. (1987). Pulmonary bacterial deposition and clearance during ascarid larval migration in weanling pigs. *Canadian Journal of Veterinary Research*, 51: 525-527.

Dold, C. and Holland, C.V. (2011a). Ascaris and ascariasis. *Microbes and Infection*, 13 (7): 632-7.

Douvres, F.W. and Tromba, F.G. (1971). Comparative development of *Ascaris suum* in rabbits, guinea pigs, mice and swine in 11 days. Proceedings of the Helminthological Society of Washington, 38: 246-52.

Douvres, F.W., Tromba, F.G. and Malakatis, G.M. (1969). Morphogenesis and migration of *Ascaris suum* larvae developing to fourth stage in swine. *The Journal of Parasitology*, 55: 689 712.

Dubinsky, P., Svicky, E., Kovac, G.M Lenhardt, L., Krupicer, I., Vasilkova, Z., Dvorozonakova, E., Levkut, M., Papajova, I.And Moncol, D.J. (2000). Pathogenesis of *Ascaris suum* in reapeated infection of lambs. *Acta Veterinaria Brunensis*, 69: 201-207.

Eichhorn, L., Zimmermann, W., Gottstein, B., Frey, C.F., Doherr, M.G. and Zeeh, F. (2010). Gastrointestinal nematodes in Swiss pig farms - an explorative overview. In: 21st IVPS Congress, Vancouver, Canada, July 18-21, 2010. P. 808.

Eijck, I.A. and Borgsteede, F.H. (2005). A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in The Netherlands. *Veterinary Research Communications*, 29: 407- 414.

Eriksen, L. (1982). Experimentally induced resistance to *Ascaris suum* in pigs. *Nordisk veterinaermedicin*, 34 (6): 177-87.

Eriksen, L., Andersen, S., Nielsen, K., Pedersen, A. and Nielsen, J. (1980). Experimental *Ascaris suum* infection in pigs. Serological response, eosinophilia in peripheral blood, occurrence of white spots in the liver and worm recovery from the intestine. *Nordisk veterinaermedicin*, 32 (6): 233-242.

Eriksen, L., Nansen, P., Roepstorff, A., Lind, P. and Nilsson, O. (1992). Response to repeated inoculations with *Ascaris suum* eggs in pigs during the fattening period. I. Studies on worm population kinetics. *Parasitology Research*, 78 (3): 241-6.

Fagerholm, H.P., Nansen, P., Roepstorff, A., Frandsen, F., Eriksen, L., (2000). Differentiation of cuticular structures during the growth of the third-stage larva of *Ascaris suum* (Nematoda, Ascaridoidea) after emerging from the egg. *The Journal of Parasitology*, 86 (3): 421-427.

Fairbairn, D. (1961). The invitro hatching of *Ascaris Lumbricoides* eggs. *Canadian Journal of Zoology,* 39: 153-162.

Farkas, R., Gyurcso, A. and Borzsonyi, L. (2003). Fly larvicidal activity in the faeces of cattle and pigs treated with endectocide products. *Medical and Veterinary Entomology*, 17 (3): 301-6.

Ferreira, S.R., de Araujo, J.V., Braga, F.R., Araujo, J.M., Frassy, L.N. and Ferreira, A.S. (2011). Biological control of Ascaris suum eggs by Pochonia chlamydosporia fungus. *Veterinary Research Communications*, 35 (8): 553-8.

Fitzgerald, P.R. (1962). The pathogenesis of *Ascaris lumbricoides var. suum* in lambs. *American Journal of Veterinary Research*, 23: 731-736.

Flesja, K.I. and Ulvesaeter, H.O. (1980). Pathological lesions in swine at slaughter. III. Inter-relationship between pathological lesions, and between pathological lesions and 1) carcass quality and 2) carcass weight. *Acta veterinaria Scandinavica*. *Supplementum*, 1-22.

Forster, M., Klimpel, S. and Sievert, K. (2009). The house fly (Musca domestica) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Veterinary Parasitology*, 160 (1-2): 163-7.

Forsum, E., Nesheim, M.C. and Crompton, D.W.T. (1981). Nutritional aspects of Ascaris infection in young protein deficient pigs. *Parasitology*, 83: 497-512.

Frontera,. E, Carron, A., Serrano, F.J., Roepstorff,. A, Reina, D. and Navarrete, I. (2003). Specific systemic IgG1, IgG2 and IgM responses in pigs immunized with infective eggs or selected antigens of *Ascaris suum*. *Parasitology*, 127: 291-8.

Gaur, S.N.S. and Deo, P.G. (1972). Observations on the transmission of Ascaris lumbricoides (pig and human strains) in certain heterologous hosts. *Indian Journal of Animal Science*, 42 (4): 281-4.

Gaurat, R.P. and Gatne, M.L. (2005). Prevalence of helminth parasites in domestic pigs (*Sus scrofa domestica*) in Mumbai: an abbatoir survey. *Journal of Bombay Veterinary College*, 13: 100-102.

Geenen, P.L., Bresciani, J., Boes, J., Pedersen, A., Eriksen, L. and Fagerholm, H.P. (1999). The morphogenesis of *Ascaris suum* to the infective third stage larvae within the egg. *Journal of Parasitology,* 85: 616-22.

Geng, J., Plenefisch, J., Komuniecki, P.R. and Komuniecki, R. (2002). Secretion of a novel developmentally regulated chitinase (family 19 glycosyl hydrolase) into the perivitelline fluid of the parasitic nematode, *Ascaris suum*. *Molecular and Biochemical Parasitology*, 124: 11-21.

Gibson, G. McM. and Lanning, D.G. (1981). Liver damage in lambs. *Veterinary Record*, 109: 165.

Gordon, H.Mc. and Whitlock, H.V. (1939). A new technique for counting nematode eggs in sheep faeces. *Journal of Scientific and Industrial Research*, 12: 50–52.

Greenway, J.A. and McCraw, B.M. (1970). *Ascaris suum* infection in calves I. Clinical signs. *Canadian Journal of Comparative Medicine*, 34: 227-37.

Gunn, A. (1980). A case of *Ascaris suum* infection in lambs. *Veterinry Record*, 107: 581.

Haas, A., Platz, S., Eichhorn, W., Kaaden, O.R. and Unshelm, J. (1998). Effect of steam application based on microbiological and parasitologic test procedures. *Zentralblatt fur Hygiene und Umweltmedizin*, 201 (4-5): 337-47.

Hale, O.M., Stewart, T.B. and Marti, O.G. (1985). Influence of an experimental infection of *Ascaris suum* on performance of pigs. *Journal of Animal Science*, 60 (1): 220-225.

Harcourt, R. and Costema, P. (1973). Hepatic ascariasis in lambs. *Veterinary Record*, 92: 482-483.

Haugegaard, J. (2010). Prevalence of nematodes in Danish industrialized sow farms with loose housed sows in dynamic groups. *Veterinary Parasitology*, 168 (1-2): 156-9.

Helwigh, A.B. and Nansen, P. (1999). Establishment of *Ascaris suum* in the pig: development of immunity following a single primary infection. *Acta Veterinaria Scandinavica*, 40(2): 121-32.

Henriksen, S.A. and Krogh, H.V. (1979). Bovine infections of *Ascaris suum*. Preliminary observations. *Dansk Veterinær Tidsskrift*, 73: 126-32.

Hill, D.E., Fetterer, R.H., Romanowski, R.D. and Urban, J.F. Jr. (1994). The effect of immunization of pigs with *Ascaris suum* cuticle components on the development of resistance to parenteral migration during a challenge infection. *Veterinary Immunology and Immunopathology*, 42: 161-169.

Hinck, L.W. and Ivey, M.H. (1976). Proteinase activity in *Ascaris suum* eggs, hatching fluid, and excretions-secretions. *The Journal of Parasitology,* 62: 771-774.

Islam, M.K., Miyoshi, T. and Tsuji, N. (2005). Vaccination with recombinant *Ascaris suum* 24 kilodalton antigen induces a Th1/Th2-mixed type immune response and confers high levels of protection against challenged *Ascaris suum* lung-stage infection in BALB/c mice. *International Journal of Parasitology*, 35: 1023-1030.

Joachim, A., Dulmer, N., Daugschies, A. and Roepstorff, A. (2001). Occurrence of helminths in pig fattening units with different management systems in Northern Germany. *Veterinary Parasitology*, 96 (2): 135-46.

Johnson, A.A. (1963). Ascarids in sheep. *New Zealand Veterinary Journal*, 11: 69-70.

Jungersen, G., Fagerholm, H.P., Nansen, P. and Eriksen, L. (1999). Development of patent *Ascaris suum* infections in pigs following intravenous administration of larvae hatched in vitro. *Parasitology*, 119: 503-8.

Kanora, A. (2009). Effect on productivity of treating fattening pigs every 5 weeks with flubendazole in feed. *Vlaams Diergeneeskundig Tijdschrift,* 78: 170-175.

Kelly, W.R. (1974). *Veterinary Clinical Diagnosis*. 2nd Ed. Bailliere Tindall. Pp. 1-374.

Kelly, W.R. (1993). The liver and biliary system. In: Jubb, K.V.F., Kennedy, P.C. and Palmer, N. (Eds.), *Pathology of Domestic Animals*, vol. 2, 4th Edn. Academic Press. Pp. 375.

Kennedy, P.C. (1954). The migrations of the larvae of *Ascaris lumbricoides* in cattle and their relation to eosinophilic granulomas. *Cornell Vet*, 44 (4): 531-65.

Kennedy, T., Lucas, M.J. and Froe, D.L. (1987). Comparative efficacy of pyrantel tartrate, ivermectin and fenbendazole against experimentally induced immature *Ascaris suum* in pigs. *Agri- Practice*, 8 (2): 19-21.

Kipper, M., Andretta, I., Monteiro, S.G., Lovatto, P.A. and Lehnen, C.R. (2011). Meta-analysis of the effects of endoparasites on pig performance. *Veterinary Parasitology*, 181: 316-320.

Knecht, D., Jankowska, A. and Zalesny, G. (2012). The impact of gastrointestinal parasites infection on slaughter efficiency in pigs. *Veterinary Parasitology*, 184: 291-297.

Krupicer, I., Ondrejka, R., Svicky, E., Vasilkova, Z., Dvorolnakova, E., Dubinsky, P. and Moncol, D. J. (1999). Clinical and pathomorphological changes in the organism of lambs after long-term *Ascaris suum* infection. *Slovensky Veterinarsky casopis*, 24 (2): 93-97.

Lai, M., Zhou, R.Q., Huang, H.C. and Hu, S.J. (2011). Prevalence and risk factors associated with intestinal parasites in pigs in Chongqing, China. *Research in Veterinary Science*, 91: 121-124.

Latimer, K.S. (2011). Leukocytes. In: Duncan and Prasse's Veterinary Laboratory Medicine. *Clinical Pathology*, 5th Edn. Wileys-Blackwell. Pp. 45-61.

Laugier, C., Sevin, C., Menard, S. and Maillard, K. (2012). Prevalence of *Parascaris equorum* infection in foals on French stud farms and first report of ivermectin-resistant *P. equorum* populations in France. *Veterinary Parasitology*, 188 (1-2): 185-9.

Lee, R.P., Dooge, D.J. and Preston, J.M. (1980). Efficacy of ivermectin against *Sarcoptes scabiei* in pigs. *Veterinary Record*, 107 (22): 503-5.

Lichtensteiger, C.A., DiPietro, J.A, Paul, A.J, Neumann, E.J. and Thompson, L. (1999). Persistent activity of doramectin and ivermectin against Ascaris suum in experimentally infected pigs. *Veterinary Parasitology*, 82 (3):235-41.

Lillie, R.D. (1965). *Histopathologic Technique and Histochemistry*, 3rd Edn. Mc. Graw-Hill, New York. Pp. 525-558.

Lind, P., Eriksen, L., Nansen, P,. Nilsson, O. and Roepstorff, A. (1993). Response to repeated inoculations with *Ascaris suum* eggs in pigs during the fattening period. II. Specific IgA, IgG, and IgM antibodies determined by enzyme-linked immunosorbent assay. *Parasitology Research*, 79 (3): 240-4.

Logan, N.B., Weatherley, A.J. and Jones, R.M. (1996). Activity of doramectin against nematode and arthropod parasites of swine. *Veterinary Parasitology*, 66 (1-2): 87-94.

Lundenheim, N. And Holmgren, N. (2010). Prevalence of lesions found at slaughter among Swedish fattening pigs. In: 21st IVPS Congress, Vancouver, Canada, July 18-21. P. 925.

Makinde, M.O., Majok, A.A. and Hill, F.W.G. (1993). The Prevalence of Subclinical Diseases in Abattoir Pigs in Zimbabwe. *Preventive Veterinary Medicine*, 15: 19-24.

Martin, L.J., Gibbs, H.C. and Pullin, J.W. (1974). Gastrointestinal parasites of swine in Quebec. I. An incidence survey. *The Canadian Veterinary Journal*, 15: 72-76.

Massara, C.L., Ferreira, R.S., de Andrade, L.D., Guerra, H.L. and Carvalho Odos, S. (2003). Effects of detergents and disinfectants on the development of *Ascaris lumbricoides* eggs. *Cadernos de Saude Publica*, 19 (1): 335-40.

Matsumoto, Y., Suzuki, S., Nozoye, T., Yamakawa, T. and Takashima, Y. (2009) Oral immunogenicity and protective efficacy in mice of transgenic rice plants producing a vaccine candidate antigen (As16) of *Ascaris suum* fused with cholera toxin B subunit. *Transgenic Research*, 18: 185-192.

Maung, M. (1978). The occurrence of the second moult of *Ascaris lumbricoides* and *Ascaris suum*. *International Journal of Parasitology,* 8: 371-8.

McCraw, B.M. (1975). The development of *Ascaris suum* in calves. *Canadian Journal of Comparative Medicine*, 39: 354-7.

McCraw, B.M. and Greenway, J.A. (1970). *Ascaris suum* infection in calves. *Canadian Journal of Comparative* Medicine, 34: 227-237.

McCraw, B.M. and Lautenslager, J.P. (1971). Pneumonia in calves associated with migrating *Ascaris suum* larvae. *Canadian Veterinary Journal*, 12(4): 87-90.

McDonald, F. E. And Chevis, R.A.F. (1965). *Ascaris lumbricoides* in lambs. *New Zealand Veterinary Journal*, 13: 41.

McLennan, M.W, Humphries, R.B. and Rac, R. (1974). *Ascaris suum* pneumonia in cattle. *Australian Veterinary Journal*, 50 (6): 266-8.

Mejer, H. and Roepstorff, A. (2006). *Ascaris suum* infections in pigs born and raised on contaminated paddocks. *Parasitology*, 133: 305-312.

Mejer, H., Wendt, S., Thomsen, L.E., Roepstorff, A. and Hindsbo, O. (2000). Nose-rings and transmission of helminth parasites in outdoor pigs. *Acta Veterinaria Scandinavica*, 41: 153-165.

Mellau, B.L., Nonga, H.E. and Karimuribo, E.D. (2011). Slaughter stock abattoir survey of carcasses and organ/offal condemnations in Arusha region, northern Tanzania. *Tropical Animal Health and Production*, 43: 857-864.

Menzies, F.D., Goodall, E.A, and Taylor, S.M. (1994). The epidemiology of *Ascaris suum* infections in pigs in Northern Ireland, 1969-1991. *British Veterinary Journal*, 150 (2): 165-72.

Meyer, K.B., Miller, K.D. and Kaneshiro, E.S. (1978). Recovery of *Ascaris* eggs from sludge. *The Journal of Parasitology,* 64: 380-383.

Ministry of Agriculture, Fisheries and Food (MAFF), (1986). *Manual of Veterinary Parasitological Laboratory Techniques*. 3rd Ed.HMSO, London. Pp. 24-160.

Miskimins, D.W., Greve, J.H. and Baker, J.R. (1994). The Serious Effects of Ascarid Larval Migration on a Group of Market-Weight Swine. *Veterinary Medicine,* 89: 247-253.

Mitchel,l G.B. and Linklater K.A (1980). Condemnation of sheep due to ascariasis. *Veterinary Record*, 107: 70.

Mkupasi, E.M., Ngowi, H.A. and Nonga, H.E. (2011). Prevalence of extra-intestinal porcine helminth infections and assessment of sanitary conditions of pig slaughter slabs in Dar es Salaam city, Tanzania. *Tropical Animal Health and Production*, 43: 417-423.

Morrow, D.A. (1968). Pneumonia in cattle due to migrating *Ascaris lumbricoides* larvae. *Journal of American Veterinary Medical Association,* 153 (2): 184-9.

Mulcahy, G., O'Neil,l S,. Fanning, J., McCarthy, E. and Sekiya, M. (2005). Tissue migration by parasitic helminthes - an immunoevasive strategy? *Trends in Parasitology*, 21:2 73-7.

Murrell, K.D., Eriksen, L., Nansen, P., Slotved, H.C. and Rasmussen, T. (1997). *Ascaris suum*: a revision of its early migratory path and implications for human ascariasis. *The Journal of Parasitology,* 83: 255-260.

Nakagawa, M., Yoshihara, S., Suda, H. and Ikeda, K. (1983). Pathological studies on white spots of the liver in fattening pigs. *National Institute of Animal Health Quarterly*, (Tokyo) 23: 138-149.

Nansen, P. and Roepstorff, A. (1999). Parasitic helminths of the pig: factors influencing transmission and infection levels. *International Journal of Parasitology*, 29 (6): 877-91.

Nareaho, A., Vainio, K. and Oksanen, A. (2011). Impaired efficacy of ivermectin against *Parascaris equorum*, and both ivermectin and pyrantel against strongyle infections in trotter foals in Finland. *Veterinary Parasitology*, 182 (2-4):372-7.

Nejsum, P., Roepstorff, A., Jorgensen, C.B., Fredholm, M., Goring, H.H. and Anderson, T.J. (2009). High heritability for Ascaris and Trichuris infection levels in pigs. *Heredity*, 102 (4):357-64.

Nganga, C.J., Karanja, D.N. and Mutune, M.N. (2008). The prevalence of gastrointestinal helminth infections in pigs in Kenya. *Tropical Animal Health and Production*, 40: 331-334.

Ngowi, H.A., Kassuku, A.A., Maeda, G.E., Boa, M.E. and Willingham, A.L. (2004). A slaughter slab survey for extra-intestinal porcine helminth infections in northern Tanzania. *Tropical Animal Health and Production*, 36: 335-340.

Nilsson, O. (1982). Ascariasis in the pig. An epizootiological and clinical study. Acta *Veterinaria Scandinavica*, 79:1-108.

Nissen, S., Poulsen, I.H., Nejsum, P., Olsen, A., Roepstorff, A., Rubaire-Akiiki, C. and Thamsborg, S.M. (2011). Prevalence of gastrointestinal nematodes in growing pigs in Kabale District in Uganda. *Tropical Animal Health and Production*, 43: 567-572.

Nnadi, P.A., Kamalu, T. N. and Onah, D.N. (2007). The effect of dietary protein supplementation on the pathophysiology of *Haemonchus contortus* infection in West African Dwarf goats. *Veterinary Parasitology*, 148 (3-4): 256-261).

Oakley, G.A. (1974). Activity of levamisole hydrochloride administered subcutaneously against *A. suum* infections in pigs. *Veterinary Record,* 95 (9): 190-3.

Paciejewski, S. (1993). Infestation of lambs with *Ascaris suum* (Goetze, 1782). *Medycyna Weterrynaryjna*, 49: 31–33.

Pattison, H.D., Thomas, R.J. and Smith, W.C. (1980). A survey of gastrointestinal parasitism in pigs. *The Veterinary record*, 107: 415-418.

Pedersen, K., Monrad, J., Henriksen, S.A, Bindseil, B., Nielsen, J.S. and Jensen, E. (1992). Bovine infections of *Ascaris suum*. Preliminary observations. *Dansk Veterinærtidsskrift*, 75: 170-4.

Perez, J., Garcia, P.M., Mozos, E., Bautista, M.J. and Carrasco, L. (2001). Immunohistochemical characterization of hepatic lesions associated with migrating larvae of Ascaris suum in pigs. *Journal of Comparative Pathology*, 124 (2-3): 200-206.

Pittman, J.S., Shepherd, G., Thacker, B.J., Myers, G.H. and Fransisco, C.J. (2010a). Prevalence of internal parasites in a production system: part I - Sows. In: 21st IPVS Congress, Vancouver, Canada, July 18-21, 2010. P. 806.

Pittman, J.S., Shepherd, G., Thacker, B.J., Myers, G.H. and Fransisco, C.J. (2010b). Prevalence of internal parasites in a production system: Part II - Finishing pigs. In: 21st IPVS Congress, Vancouver, Canada, July 18-21, 2010. P. 807.

Plachy, P., Juris, P., Placha, I. and Venglovsky, J. (1996). Use of hydrated lime for disinfection of sewage sludge containing *Salmonella typhimurium* and *Ascaris suum* as model pathogens. *Veterinary Medicine*, 41 (8): 255-9.

Polley, L.R. and Mostert, P.E. (1980). *Ascaris suum* in Saskatchewan pigs: an abattoir survey of prevalence and intensity of infection. *The Canadian veterinary journal*, 21: 307-309.

Ransom, B.H. and Foster, W.D. (1919). Recent discoveries concerning the life history of *Ascaris lumbricoides*. *The Journal of Parasitology*, 5: 93-9.

Read, A.F. and Skorping, A. (1995). The evolution of tissue migration by parasitic nematode larvae. *Parasitology*, 111: 359-71.

Rochette, F. (1985). Chemotherapy of gastrointestinal nematodiasis in pigs. In: Vanden Bossche H, Thienpont D, Janssens PG, editors. Chemotherapy of Gastrointestinal Helminths. *Springer Verlag*, 463-86.

Roepstorff, A. (1997). Helminth surveillance as a prerequisite for anthelmintic treatment in intensive sow herds. *Veterinary Parasitology*, 73: 139-151.

Roepstorff, A. (1998). Natural Ascaris suum infections in swine diagnosed by coprological and serological (ELISA) methods. *Parasitology Research*, 84 (7): 537-43.

Roepstorff, A. (2003). *Ascaris suum* in pigs: population biology and epidemiology. PhD thesis, The Royal Veterinarian and Agricultural University, Copenhagen, Denmark.

Roepstorff, A. and Jorsal, S.E. (1989). Prevalence of helminth infections in swine in Denmark. *Veterinary Parasitology*, 33: 231-239.

Roepstorff, A. and Jorsal, S.E. (1990). Relationship of the prevalence of swine helminths to management practices and anthelmintic treatment in Danish sow herds. *Veterinary Parasitology*, 36 (3–4): 245–257.

Roepstorff, A. and Jorsal, S.E. (1990). Relationship of the prevalence of swine helminths to management practices and anthelmintic treatment in Danish sow herds. *Veterinary Parasitology*, 36 (3-4): 245-57.

Roepstorff, A. and Murrell, K.D. (1997). Transmission dynamics of helminth parasites of pigs on continuous pasture: *Ascaris suum* and *Trichuris suis*. *International Journal of Parasitology*, 27 (5): 563-72.

Roepstorff, A. and Nansen, P. (1994). Epidemiology and control of helminth infections in pigs under intensive and non-intensive production systems. *Veterinary Parasitology*, 54 (1–3): 69–85.

Roepstorff, A., Eriksen, L., Slotved, H.C and Nansen, P. (1997). Experimental Ascaris suum infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology*, 115 (4): 443-52.

Roepstorff, A., Mejer, H., Nejsum, P. and Thamsborg, S.M. (2011). Helminth parasites in pigs: new challenges in pig production and current research highlights. *Veterinary Parasitology*, 180 (1-2): 72 81.

Roepstorff, A., Nilsson, O., O'Callaghan, C.J., Oksanen, A., Gjerde, B. Richter, S.H. (1999). Intestinal parasites in swine in the Nordic countries: multilevel modelling of *Ascaris suum* infections in relation to production factors. *Parasitology*, 119 (5): 521-34.

Roepstorff, A., Nilsson, O., Oksanen, A., Gjerde, B., Richter, S.H., Ortenberg, E., Christensson, D., Martinsson, K.B., Bartlett, P.C., Nansen, P., Eriksen, L., Helle, O., Nikander, S. and Larsen, K. (1998). Intestinal parasites in swine in the Nordic countries: prevalence and geographical distribution. *Veterinary Parasitology*, 76: 305-319.

Roneus, O. (1966). Studies on the aetiology and pathogenesis of white spots in the liver of pigs. *Acta Veterinaria Scandinavica*, 7 (16): 100-112.

Roneus, O. and Christensson, D. (1977). Mature *Ascaris suum* in naturally infected calves. *Veterinary Parasitology*, 3 (4): 371-5.

Salifu, D.A., Manga, T.B. and Onyali, I.O. (1990). A survey of gastrointestinal parasites in pigs of the Plateau and Rivers States, Nigeria. *Revue d'elevage et de medecine veterinaire des pays tropicaux*, 43: 193-196.

Sanchez-Vazquez, M.J, Smith, R.P, Kang, S., Lewis, F., Nielen, M., Gunn, G.J. (2010). Identification of factors influencing the occurrence of milk spot livers in slaughtered pigs: a novel approach to understanding *Ascaris suum* epidemiology in British farmed pigs. *Veterinary Parasitology*, 173 (3-4): 271-9.

Sanchez-Vazquez, M.J., Nielen, M., Gunn, G.J. and Lewis, F.I. (2012). National monitoring of *Ascaris suum* related liver pathologies in English abattoirs: a time-series analysis, 2005-2010. *Veterinary Parasitology*, 184: 83-87.

Sauvageau, R. and Fre´chette, J.L. (1980). Hepatic ascariasis in a lamb. *Canadian Veterinary Journal*, 21 (2): 66.

Sinniah, B. (1982). Daily egg production of *Ascaris lumbricoides*: the distribution of eggs in the faeces and the variability of egg counts, *Parasitology*, 84: 167-175.

Sinniah, B. and Subramaniam, K. (2009). Factors influencing the egg production of *Ascaris lumbricoides*: relationship to weight, length and diameter of worms. *Journal of Helminthology*, 65: 141-147.

Skallerup, P., Nejsum, P., Jorgensen, C.B., Goring, H.H., Karlskov-Mortensen, P. Archibald, A.L. (2012). Detection of a quantitative trait locus associated with resistance to *Ascaris suum* infection in pigs. *International Journal of Parasitology*, 42 (4): 383-91.

Smyth, J.D. (1994*). Introduction to Animal Parasitology*, 3rd ed. Cambridge University Press. Pp. 549.

Spratt, D.M. (1997). Endoparasite control strategies: implications for biodiversity of native fauna. *International Journal of Parasitology*, 27 (2): 173-80.

Sprent, J.F. (1954). The life cycles of nematodes in the family Ascarididae Blanchard 1896. *Journal of Parasitology,* 40: 608-17.

Steenhard, N.R., Jungersen, G., Kokotovic, B., Beshah, E. and Dawson, H.D. (2009). *Ascaris suum* infection negatively affects the response to a *Mycoplasma hyopneumoniae* vaccination and subsequent challenge infection in pigs. *Vaccine*, 27: 5161-5169.

Steven L.S and Michael A.S. (2008). Liver functions test. In: *Fundamentals of Veterinary Clinical Pathology*, second edition. Blackwell Publishing Company. Pp. 639-706.

Stewart, T.B. and Hale, O.M. (1988). Losses to internal parasites in swine production. *Journal Animal Science*, 66: 1548- 1554.

Stewart, T.B. and Hoyt, P.G. (2006). Internal parasites. In: Straw, E.B., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. (Eds.), *Diseases of Swine*, ninth edition. Blackwell Publishing. Pp. 904–905.

Stewart, T.B., Bidner, T.D., Southern, L.L. and Simmons, L.A. (1984). Efficacy of fenbendazole against migrating *Ascaris suum* larvae in pigs. *American Journal of Veterinary Research*, 45 (5): 984-6.

Stewart, T.B., Marti, O.G. and Hale, O.M. (1981). Efficacy of ivermectin against 5 genera of swine nematodes and the hog louse, *Haematopinus suis*. *American Journal of Veterinary Research*, 42 (8): 1425-6.

Thamgsborg, S. M., Roepstorff, A. and Larsen, M. (1999). Integrated and biological control of parasites in organic and conventional production systems. *Veterinary Parasitology*, 84: 169-186.

Thamsborg, S.M., Roepstorff, A., Nejsum, P. and Mejer, H. (2010). Alternative approaches to control of parasites in livestock: Nordic and Baltic perspectives. *Acta Veterinaria Scandinavica*, 52 (1): 27.

Theodoropoulos, G., Theodoropoulou, E. and Melissaropoulou, G. (2001). Worm control practices of pig farmers in Greece. *Veterinary Parasitology*, 97 (4): 285-93.

Thienpont, D., Vanparijs, O., Niemegeers, C. and Marsboom, R. (1978). Biological and pharmacological properties of flubendazole. *Arznei-Forschung*, 28 (4): 605-12.

Tielen, M.J., Truijen, W.T. and Remmen, J.W. (1976). The incidence of diseases of the lung and liver in slaughtered pigs as a criterion in the detection of herds in which the disease is a recurrent problem. *Tijdschrift voor diergeneeskunde*, 101: 962-971.

Tielen, M.J., Truijen, W.T., v d Groes, C.A., Verstegen, M.A., de Bruin, J.J. and Conbey, R.A. (1978). Conditions of management and the construction of piggeries on pig-fattening farms as factors in the incidence of diseases of the lung and liver in slaughtered pigs. *Tijdschr Diergeneesk*, 103 (21): 1155-65.

Tjornehoj, K., Eriksen, L, Aalbaek, B. and Nansen, P. (1992). Interaction between *Ascaris suum* and *Pasteurella multocida* in the lungs of mice. *Parasitology Research*, 78: 525-528.

Tromba, F.G. (1978) Immunization of pigs against experimental *Ascaris suum* infection by feeding ultraviolet-attenuated eggs. *The Journal of parasitology,* 64: 651-656.

Tsuji, N., Miyoshi, T., Islam, M.K., Isobe, T. and Yoshihara, S. (2004). Recombinant *Ascaris* 16-Kilodalton protein-induced protection against *Ascaris suum* larval migration after intranasal vaccination in pigs. *The Journal of Infectious Diseases*, 190: 1812-1820.

Urban, Jr. J.F. and Romanowski, R.D. (1985). *Ascaris suum*: protective immunity in pigs immunized with products from eggs and larvae. *Experimental Parasitology*, 60 (2): 245-54.

Urquhart, G. M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F. W. (2000). *Veterinary Parasitology*, 2nd Edition. Blackwell Science Ltd. London. Pp. 9-67.

Van den Burg, W.P. and Borgsteede, F.H. (1987). Effects of various disinfectants on the development and survival possibilities of the pre-parasitic stages of *Ostertagia ostertagi*, *Cooperia oncophora* and *Ascaris suum*. *Tijdschr Diergeneesk*, 112 (13): 769-78.

Van Krimpen, M.M., Binnendijk, G.P., Borgsteede, F.H. Gaasenbeek, C.P. (2010). Anthelmintic effects of phytogenic feed additives in *Ascaris suum* inoculated pigs. *Veterinary Parasitology*, 168 (3-4): 269-77.

Van Meensel, J., Kanora, A., Lauwers, L., Jourquin, J., Goossens, L. and Van Huylenbroeck, G. (2010). From research to farm: ex ante evaluation of strategic deworming in pig finishing. *Veterinary Medicine- Czech,* 55: 483-493.

Vassilev, I. (1960). The goat (*Capra hircus)* as host of *Ascaris suum* (Goeze, 1782). *Comptes Rendus De l'AcadémieBulgare Des Sciences,* 13: 75-78.

Vercruysse, J., VanHoof, D. and DeBie, S. (1997). Study on the prevalence of white spots of the liver in pigs in Belgium and its relationship to management practices and anthelmintic treatment. *Vlaams Diergeneeskundig Tijdschrift*, 66: 28-30.

Vlaminck, J,. Nejsum, P, Vangroenweghe, F., Thamsborg, S.M., Vercruysse, J. and Geldhof, P. (2012). Evaluation of a serodiagnostic test using *Ascaris suum* haemoglobin for the detection of roundworm infections in pig populations. *Veterinary Parasitology*, 189 (2-4): 267-73.

Vlaminck, J., Martinez-Valladares, M., Dewilde, S., Moens, L., Tilleman, K. and Deforce, D. (2011). Immunizing pigs with *Ascaris suum* haemoglobin increases the inflammatory response in the liver but fails to induce a protective immunity. *Parasite Immunology*, 33 (4): 250-4.

Wagner, B. and Polley, L. (1997). *Ascaris suum* prevalence and intensity: an abattoir survey of market hogs in Saskatchewan. *Veterinary Parasitology*, 73 (3-4): 309-13.

Wagner, B. and Polley, L. (1997a). Anthelmintic use on Saskatchewan pig farms: results from a postal survey. *Veterinary Parasitology*, 73: 299-307.

Weichselbaum, T. E. (1946). Calometric methods of determination of total serum protein. *American Journal of Clinical Pathology*, 16: 40-46.

Weng, Y.B., Hu, Y.J., Li, Y., Li, B.S., Lin, R.Q., Xie, D.H., Gasser, R.B. and Zhu, X.Q. (2005). Survey of intestinal parasites in pigs from intensive farms in Guangdong Province, People's Republic of China. *Veterinary Parasitology*, 127: 333-336.

Whitlock, H.V. (1948). Some modifications of the McMaster helminth egg-counting technique and apparatus. *Journal of Scientific and Industrial Research*, 21: 177–180.

World Health Organization (WHO), *Control of ascariasis (report of WHO committee*) (1967). *Technical Report Series*, 379: Pp. 1-39.

Yazwinski, T.A., Tucker, C., Featherston, H., Johnson, Z.and Wood-Huels, N. (1997). Endectocidal efficacies of doramectin in naturally parasitized pigs. *Veterinary Parasitology*, 70 (1-3): 123-8.

Yoshida, S. (1919). On the development of *Ascaris lumbricoides* Larva. *The Journal of Parasitology*, 5 (3): 105-15.

Yoshihara, S., Oya, T., Furuya, T. and Goto, N. (1993). Use of body fluid of adult female as an antigen in the enzyme-linked immunosorbent assay (ELISA) for diagnosis of swine ascariosis. *Journal of Helminthology*, 67 (4): 279-86.

APPENDICES

Appendix I: Mean (± SEM) respiratory rates (cycles/min) of lambs in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL
-7 (Pre-infection)	25.38 ± 0.47	25.00 ± 0.68
7	25.80 ± 0.55	26.40 ± 0.75
14	28.03 ± 0.31[a]	23.84 ± 0.28[b]
21	25.20 ± 0.44	25.20 ± 0.49
28	25.78 ± 0.62	25.00 ± 0.68
35	27.50 ± 0.59	25.33 ± 0.84
42	23.50 ± 0.56	22.00 ± 0.89
49	22.38 ± 0.66	21.33 ± 0.84
56	25.20 ± 0.44	25.20 ± 0.49
63	25.78 ± 0.62	25.00 ± 0.68
70	27.50 ± 0.59	25.33 ± 0.84

a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix II: Mean (± SEM) temperatures in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL
-7 (Pre-infection)	38.26 ± 0.07	38.11 ± 0.07
7	38.10 ± 0.24	38.24 ± 0.10
14	38.22 ± 0.02	38.18 ± 0.03
21	38.17 ± 0.06	38.22 ± 0.09
28	38.02 ± 0.06	38.12 ± 0.07
35	38.32 ± 0.13	38.15 ± 0.22
42	38.10 ± 0.15	37.95 ± 0.21
49	38.19 ± 0.06	38.21 ± 0.08
56	38.17 ± 0.06	38.22 ± 0.09
63	38.02 ± 0.06	38.12 ± 0.07
70	38.32 ± 0.13	38.15 ± 0.22

Appendix III: Mean (± SEM) pulse rates in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL
-7 (Pre-infection)	85.23 ± 0.83	85.60 ± 1.07
7	84.00 ± 1.03	85.60 ± 2.04
14	83.78 ± 0.21[a]	81.08 ± 0.98[b]
21	85.20 ± 1.04	85.60 ± 2.04
28	83.78 ± 0.85	84.00 ± 1.03
35	83.88 ± 2.83	82.67 ± 3.33
42	81.75 ± 1.08	81.00 ± 1.61
49	83.25 ± 0.66	82.67 ± 0.84
56	85.20 ± 1.04	85.60 ± 2.04
63	83.78 ± 0.85	84.00 ±1.03
70	83.88 ± 2.83	82.67 ± 3.33

a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix IV: Mean (± SEM) values of haematological parameters of the Yankasa lambs in the experimental groups during the pre-infection period.

Parameters	INFECTED	CONTROL
PCV (%)	38.28 ± 1.44	36.88 ± 1.88
HB (g/dL)	12.78 ± 0.47	12.28 ± 0.62
TP (g/dL)	5.20 ± 0.29	5.64 ± 0.43
WBC (10^9/L)	6.82 ± 0.33	6.46 ± 0.22
Neutrophils (10^9/L)	2.40 ± 0.14	2.87 ± 0.22
Lymphocytes (10^9/L)	3.61 ± 0.27	3.55 ± 0.30
Monocytes (10^9/L)	0.10 ± 0.04	0.16 ± 0.07
Eosinophils (10^9/L)	0.14 ± 0.06	0.20 ± 010
Basophils (10^9/L)	0.11 ± 0.05	0.00 ± 0.00
Band cells (10^9/L)	0.02 ± 0.01	0.06 ± 0.05

$p>0.05$

Appendix V: **Mean (± SEM) white blood cell counts (10^9/L) in *A. suum*- infected and control lambs**

Day	Infected	Control	P-value	Significance
-7	6.82 ± 0.33	6.46 ± 0.22		
7	6.80 ±0. 27^{a}	6.00 ± 0.17^{b}	0.023	S
14	6.30 ± 0.15	6.00 ± 0.16	0.216	NS
21	6.31 ± 0.20	5.90 ± 0.17	0.137	NS
28	5.24 ±0.68	5.80 ± 0.30	0.469	NS
35	5.91 ± 0.12^{a}	5.37 ± 0.26^{b}	0.047	S
42	5.47 ± 0.31	5.56 ± 0.26	0.823	NS
49	5.26 ± 0.17	5.97 ± 0.36	0.066	NS
56	5.73 ± 0.22	5.90 ± 0.39	0.217	NS

NS- Not significant; S- Significant; a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix VI: **Mean (± SEM) eosinophil count (10^9/L) in the *A. suum*- infected and control lambs**

Day	Infected	Control	P-value	Significance
-7	0.14 ± 0.06	0.20 ± 010		
7	0.32 ± 0.07	0.27 ± 0.09	0.679	NS
14	0.19 ± 0.06	0.20 ± 0.11	0.930	NS
21	0.17 ± 0.09	0.30 ± 0.06	0.310	NS
28	0.13 ± 0.05	0.08 ± 0.06	0.482	NS
35	0.41 ± 0.11	0.17 ± 0.09	0.109	NS
42	0.10 ± 0.05	0.26 ± 0.17	0.299	NS
49	0.12 ± 0.04	0.18 ± 0.07	0.420	NS
56	0.15 ± 0.07	0.24 ± 0.11	0.472	NS

NS- Not significant

Appendix VII: **Mean (± SEM) neutrophil count (10^9/L) in the *A. suum*-infected and control lambs**

Day	Infected	Control	P-value	Significance
-7	2.40 ± 0.14	2.87 ± 0.22		
7	2.49 ± 0.11	2.23 ± 0.27	0.075	NS
14	2.40 ± 0.21	2.22 ± 0.39	0.397	NS
21	2.14 ± 0.10	2.46 ± 0.49	0.691	NS
28	1.88 ± 0.30	1.73 ± 0.23	0.545	NS
35	2.34 ± 0.22	2.14 ± 0.15	0.688	NS
42	2.19 ± 0.20	1.81 ± 0.42	0.453	NS
49	1.86 ± 0.33	2.42 ± 0.56	0.148	NS
56	1.90 ± 0.31^{a}	1.11 ± 0.15^{b}	0.041	S

NS- Not significant; S- Significant; a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix VIII: **Mean (± SEM) lymphocyte count (10^9/L) in the *A. suum*- infected and control lambs**

Day	Infected	Control	P-value	Significance
-7	3.61 ± 0.27	3.55 ± 0.30		
7	3.82 ± 0.20	3.76 ± 0.23	0.855	NS
14	3.26 ± 0.20	2.95 ± 0.61	0.660	NS
21	3.44 ± 0.20	2.87 ± 0.27	0.106	NS
28	3.00 ± 0.33	3.11 ± 0.38	0.836	NS
35	3.07 ± 0.26	3.33 ± 0.17	0.262	NS
42	3.34 ± 0.20	3.75 ± 0.30	0.263	NS
49	3.80 ± 0.52[a]	2.64 ± 0.45[b]	0.013	S
56	3.34 ± 0.20	3.75 ± 0.30	0.232	NS

NS- Not significant; S- Significant; a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix IX: **Mean (± SEM) monocyte count(10^9/L) in the *A. suum*-infected and control lambs**

Day	Infected	Control	P-value	Significance
-7	0.10 ± 0.04	0.16 ± 0.07		
7	0.08 ± 0.02	0.11 ± 0.07	0.748	NS
14	0.11 ± 0.03	0.14 ± 0.05	0.741	NS
21	0.18 ± 0.05	0.06 ± 0.04	0.152	NS
28	0.14 ± 0.03	0.23 ± 0.06	0.177	NS
35	0.18 ± 0.05	0.08 ± 0.01	0.262	NS
42	0.18 ±0.07[a]	0.05 ± 0.01[b]	0.035	S
49	0.08 ± 0.07	0.14 ± 0.09	0.489	NS
56	0.07 ± 0.03	0.18 ± 0.08	0.186	NS

NS- Not significant; S- Significant; a,b Means with different superscripts in the same row differ significantly ($p < 0.05$)

Appendix X: Mean (± SEM) AST serum activity (IU/L) in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL	P-value	Significance
-7 (Pre-infection)	11.13±1.32	11.25±2.81	0.964	NS
7	10.13±1.32	11.25 ±2.81	0.964	NS
14	16.44±4.47[a]	8.73±1.24[b]	0.05	S
21	7.93 ±1.59	9.67 ± 1.36	0.463	NS
28	5.75±1.11	6.83±1.13	0.523	NS
35	8.45±1.11	8.17±1.87	0.889	NS
42	9.00±1.17	7.67±1.67	0.515	NS
56	12.67±1.69	10.67±1.12	0.396	NS

AST- Aspartate transaminase; [a,b]Means in the same row with different superscripts differ significantly; NS- Not significant; S- Significant

Appendix XI: Mean (± SEM) ALT serum activity (IU/L) in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL	P-value	Significance
-7 (Pre-infection)	4.31±0.62	4.86±1.38	0.669	NS
7	4.01±0.62	4.08 ±1.38	0.669	NS
14	8.00±2.18[a]	3.60±0.54[b]	0.023	S
21	4.60 ±1.08	4.44 ± 0.93	0.922	NS
28	5.50±1.30	4.08±0.62	0.288	NS
35	3.45±0.74	3.83±0.91	0.759	NS
42	4.91±0.81	4.83±1.05	0.956	NS
56	6.44±1.09	9.33±1.84	0.173	NS

ALT- Alanine transaminase; [a,b] Means in the same row with different superscripts differ significantly; NS- Not significant; S- Significant

Appendix XII: Mean (± SEM) ALP serum activity (IU/L) in the *A. suum*-infected and control lambs

Day	INFECTED	CONTROL	P-value	Significance
-7 (Pre-infection)	74.81±9.15	64.50±6.74	0.466	NS
7	70.21±9.15	65.50±6.74	0.466	NS
14	69.00±8.99	65.00±8.75	0.766	NS
21	64.60 ± 5.53	147.00 ± 91.41	0.254	NS
28	61.75±9.08	77.58±11.81	0.345	NS
35	77.64±11.55	90.67±8.56	0.456	NS
42	76.00±9.12	75.17±14.15	0.959	NS
56	60.11±8.24	55.83±6.66	0.269	NS

ALP- Alkaline phosphatise; NS- Not significant

Appendix XIII: Mean (± SEM) urea serum concentration (mmol/L) in the *A.suum*-infected and control lambs

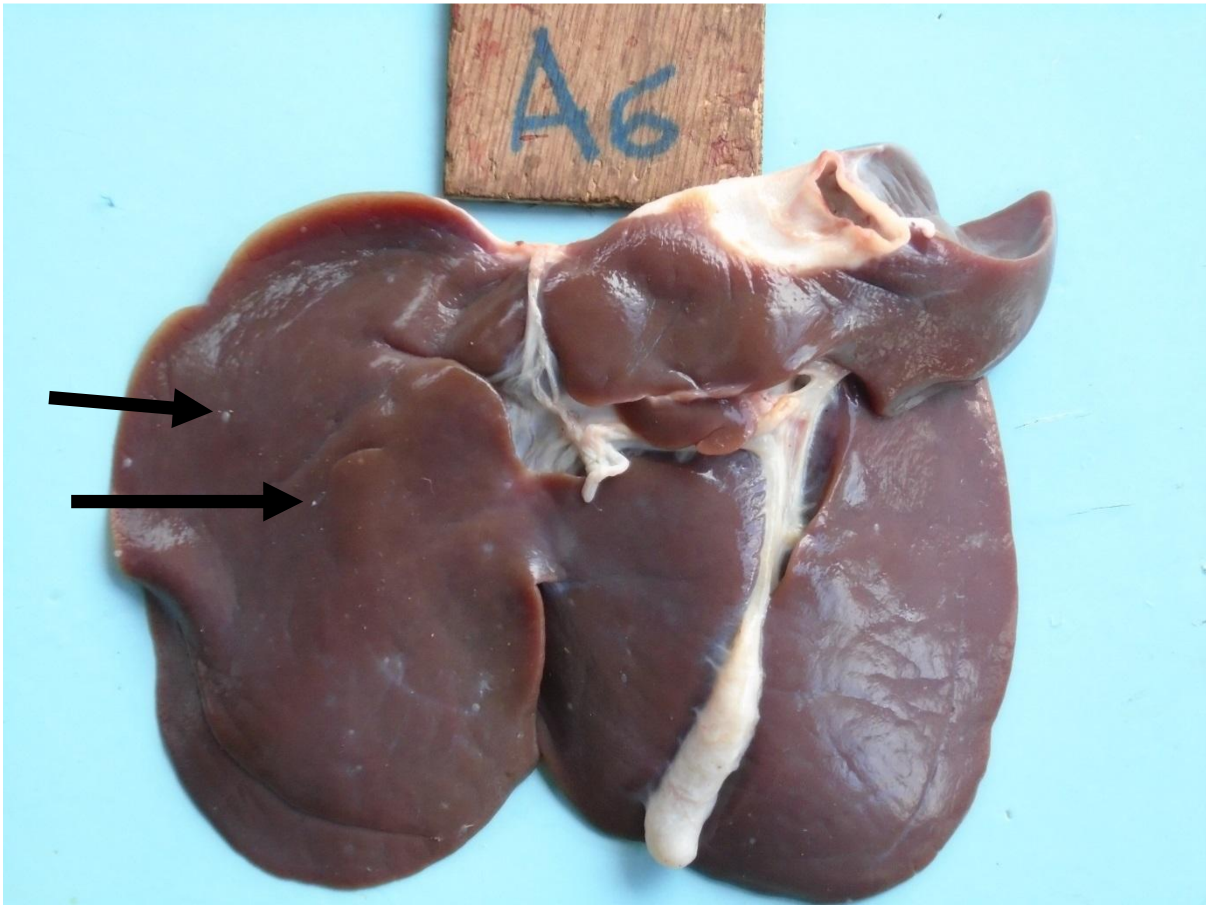
Day	INFECTED	CONTROL	P-value	Significance
-7 (Pre-infection)	4.29±0.053	4.78±0.46	0.561	NS
7	3.98 ±0.053	3.88±0.46	0.561	NS
14	5.21±0.85	4.69±0.47	0.561	NS
21	4.73 ± 0.49	5.11 ± 0.74	0.661	NS
28	7.85±1.23[a]	4.35±0.58[b]	0.01	S
35	5.22±0.44	5.25±1.26	0.977	NS
42	5.51±0.53	4.92±0.54	0.484	NS
56	1.56±0.32	1.58±0.34	0.963	NS

NS- Not significant; S- Significant; [a,b]Means in the same row with different superscripts differ significantly

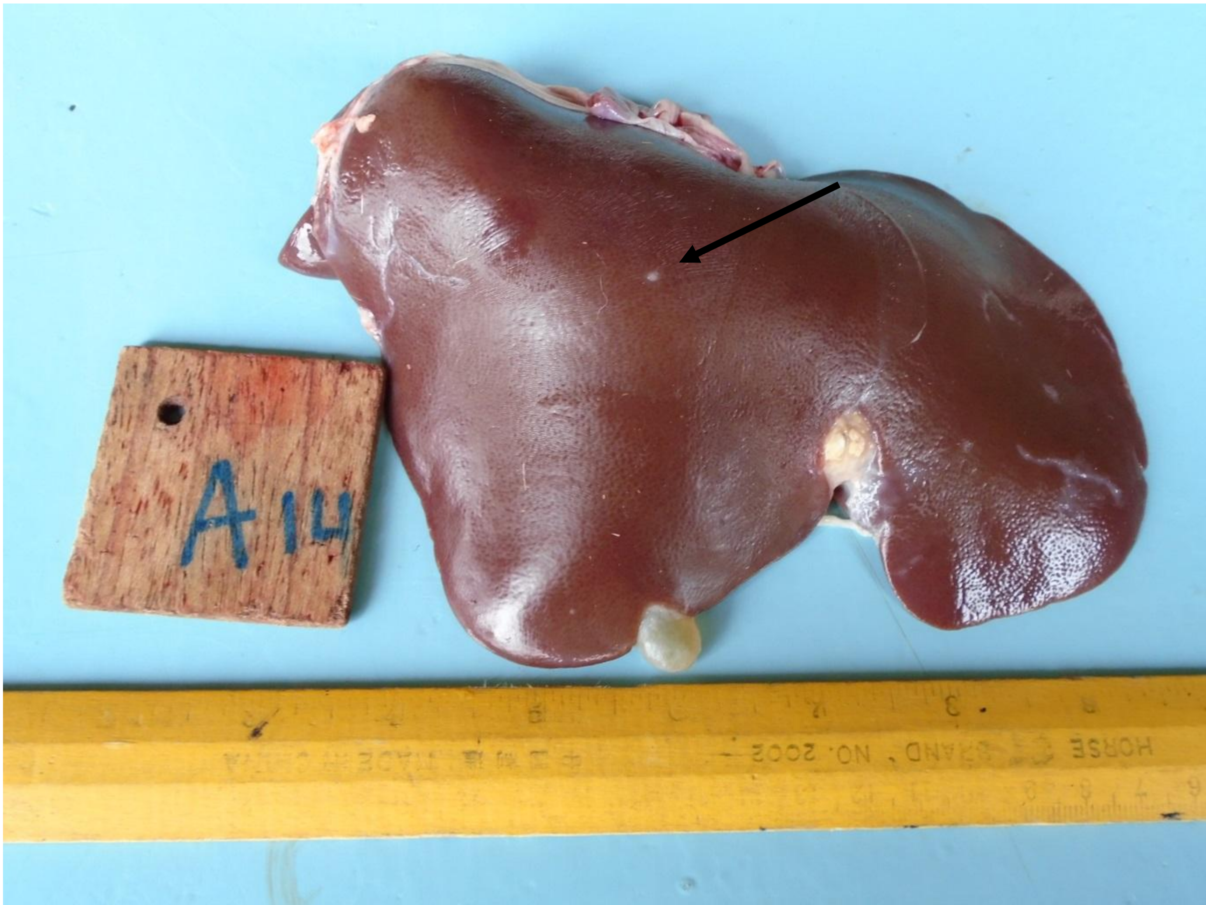
Appendix XIV: Mean (± SEM) creatinine serum concentration (µmol/L) in the *A.suum*-infected and control lambs

Day	INFECTED	CONTROL	P-value	Significance
-7 (Pre-infection)	63.63±7.05	67.00±8.76	0.777	NS
7	60.63±7.05	62.00±8.76	0.777	NS
14	77.78±11.42	57.73±5.17	0.082	NS
21	69.13 ± 6.89	67.44 ± 11.09	0.892	NS
28	86.50±11.99^{a}	51.25±6.84^{b}	0.013	S
35	63.64±7.72	74.00±16.11	0.519	NS
42	67.28±7.79	60.33±7.77	0.574	NS
56	36.89±2.89	31.50±2.45	0.210	NS

NS- Not significant; S- Significant; ^{a,b}Means in the same row with different superscripts differ significantly



Appendix XV: Almost inconspicuous white pinpoint foci on day 28 p.i. (arrows)



Appendix XVI: Even more nearly inconspicuous white pinpoint foci on day 56 p.i.(arrow)