NEMATODE INFECTION EFFECTS ON IMMUNOPATHOLOGICAL RESPONSE TO VACCINE AND *MYCOPLASMA CAPRICOLUM CAPRIPNEUMONIAE* ANTIGENS IN GOATS

BY

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DECLARATION

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DEDICATION

To Almighty God for His abundant grace, life and health bestowed upon me a mere earthling.

To my loving wife, Anne and children; Carson, Rono, Kemboi, Chiri and Cheptoo who stood by me, prayed for my success and put up with my busy schedule.

To my loving Mum for her ceaseless prayers

ABSTRACT

Contagious Caprine Pleuropneumonia (CCPP), caused by *Mycoplasma capricolum capripneumoniae (Mccp)*, is a highly contagious disease of goats with high morbidity and mortality in naïve goats and a major threat to food security. The objective of this study was to investigate the impact of mixed nematode infection on immune responses to vaccine and native *Mccp* antigens and the resultant pathology in goats. To investigate the humoural response to vaccine antigen, 40 goats aged 9 – 12 months were randomly allocated to four groups of ten each. Group A were orally inoculated with infective stages of nematodes followed by immunization with inactivated *Mccp* vaccine after 3 weeks. Group B were not inoculated with nematodes but immunized as Group A. Group C were inoculated with infective stages of nematodes but not vaccinated as in A. Group D was neither inoculated nor vaccinated. To investigate pathology in response to native *Mccp* antigens 24 goats divided into four groups of six were used. Group E_1 without and E_2 with detectable nematode were inoculated intratracheally with *Mccp* organisms. F_1 without and F_2 with detectable nematode were used for contact transmission investigation were brought in contact with inoculates on the 7^{th} day post inoculation. Clinical observations and records were done daily at 8.30 am, blood for sera analysis was collected weekly, while pathological data was collected at post-mortem. Analysis of Variance and Tukey Honest Significant Difference, a post hoc test, multiple comparisons of means were performed using R statistical packages (Rx64 3.2.4 revised). The results showed that immune response to *Mycoplasma* vaccine antigens in nematode infected group (A) was significantly lower than that in vaccinated non-nematode infected (B) group ($p<0.05$). Red and gray lung consolidations were significantly different statistically ($p< 0.05$) thus corresponding to the observed clinical signs, fibrin deposition along with pleural effusions in nematode infected groups. There was a high morbidity in group E2 (4/6) compared to E1 (1/6) that necessitated euthanasia for welfare reasons. Fibrous adhesion, a sign of chronic disease was more pronounced in none nematode infected groups though not statistically significant ($p> 0.05$). Evidence from this study indicates that worm infection impacts negatively on immune response to *Mccp* infection, vaccine antigens and the resultant pathological picture. Thus I recommend that deworming exercise should be carried out before planned vaccinations are carried out.

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LIST OF ACRONYMS AND ABBREVIATIONS

AAMΦ	Alternatively activated macrophages
ADCC	Antibody dependent cellular cytotoxicity
APC	Antigen-presenting cells
ASAL	Arid and semi-arid lands
CCPP	Contagious Caprine Pleuropneumonia
cELISA	Competitive Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic acid
IL	Interleukin
KEVEVAPI	Kenya Veterinary Vaccines Production Institute.
KNBS	Kenya National Bureau of Statistics
LPS	Lipopolysaccharides
Mab	Monoclonal antibody
MALP-2	Mycoplasma activating lipoprotein-2
MHC	Major Histocompatibility Complex
MoLFD	Ministry of Livestock and Fisheries development
NFκB	Nuclear Factor - *Kappa* B
OIE	Office International des Epizootics
PAMP	Pathogen associated molecular patterns
PRRs	Pattern recognition receptors
RNA	Ribonucleic acid
TLR	Toll like Receptor
TNF-α	Tumour necrotic factor-*alpha*
UN-DESA	United Nations- Department of Economic and Social Affairs
VSG	Variant Surface Glycoproteins

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Many rural households found in the arid and semi-arid lands (ASALs) practice livestock keeping as an important socio-economic activity under traditional pastoralist production system which constitutes 80% of the estimated 28 million goats in Kenya (MoLFD 2009). Carlos (2003:2) as described arid and semi-arid lands (ASALs) as hot dry lands with erratic rainfall patterns and with precipitation often less than 500 mm annually whose occupants are mostly pastoral communities. In these regions sheep and goats, among other livestock are kept mainly as one of the sources of animal proteins, skin, income, risk mitigation, property security, monetary savings, and other socio-economic and cultural functions (Shiferaw *et al.*, 2006). Assortment of factors that include a range of infections, shortage of pasture and browse occasioned by ever increasing drought incidences in the region limit realization of maximum benefits from the all important industry. Other complication includes occasional floods that more often than not result in depilated infrastructure, insufficient utilization of indigenous genetic resource, poor production systems and unreliable markets.

Contagious Caprine Pleuropneumonia (CCPP) is one of the most severe infectious diseases of goats, listed as notifiable by the Office International des Epizootics (OIE, 2017). The disease causes major economic losses in goat production in arid and semi arid lands (ASALs) KNBS (2015). It is highly contagious, usually with a morbidity of up to 100% and mortality ranging from 60 to up to 80% in goats that are immunologically naive to the infection or none vaccinated (Samiullah, 2013;Rurangirwa and McGuire 2012).

Most of the sheep and goat population in Kenya is found in the ASAL areas (MoLFD 2009) bordering the national game parks and reserves. The goats may sometimes graze together with the wild ruminants some of which are susceptible to the contagious caprine pleuropneumonia as reported by Arif *et al.,* (2007) on wild caprines in a game reserve in Qatar and in gazelles in the United Arab Emirates by Nicholas *et al.,* (2008). These suggest that they may act as reservoirs. This is coupled with ever increasing cross-border trade and the transboundary nature of CCPP makes the disease a regional threat that requires appropriate responses to protect the source of livelihoods of the livestock dependent citizens. Kipronoh *et al.,* (2016) confirmed in their study findings that regions along the international borders had a high sero-prevalance (Swai et al., 2013) and therefore there is a high risk of CCPP infection among these flocks. The current approved method of control is through vaccination /immunization though chemotherapy may curb further transmission if the intervention is instituted early in disease occurrence. The OIE prescribed vaccine antigen for the control of the disease is composed of inactivated whole *Mycoplasma capricolum capripneumoniae (Mccp)* cells that are concentrated and semi-purified with saponin as an adjuvant (OIE, 2014). The current form of vaccine used in Kenya to provide prophylaxis against CCPP is an inactivated *Mccp* suspended in saponin and is protective for up to 14 months. However revaccination is recommended annually (Rurangirwa *et al.*, 1987, OIE, 2014).

Mccp the causative agent for CCPP is a member of the order Mycoplasmatales and class Mollicutes (MacVey *et al.,* 2013 and OIE, 2014) a class which is ubiquitous in nature. Infections with Mollicutes are parasitic, host specific and characterized by a

range of chronic, subclinical but severely debilitating and sometimes extremely fatal disease in *per acute* CCPP cases as reported by Srivastava 2010 where a high mortality occurred in goats imported into the country.

The organism's cell morphology is extremely pleiomorphic and various shapes have been described including spherical, ring –shaped, pear-shaped, spiral or filamentous shaped (MacVey *et al.,* 2013). The Mollicutes lack the genetic capacity to produce a cell wall; instead they are bound by a single trilaminar membrane composed of proteins, glycoproteins, lipoproteins, phospholipids and sterols (MacVey *et al.,* 2013, Cordova *et al.*, 2016). Cholesterol in the membrane provides osmotic stability (MacVey *et al.,* 2013). They possess a small genome of approximately 540-1380 kb with a base composition poor in guanine-cytosine (G-C) content. The G+C mol % content of Mycoplasma DNA ranges approximately from 24to 40 % (MacVey *et al.,* 2013). Due to its limited genome the organism portrays a complex relationship with the host especially with regards to immunity, making it difficult to separate the pathogenicity of the organism from host own immune response against them and this regard, the host immune response has been reported to be intimately involved in resultant pathology (MacVey *et al.,* 2013).The chronicity of *Mycoplasma* infections suggests that the immune response is ineffective in completely eliminating infection once it is established and latent infections are a common feature.

There is little documented data on the effect of nematodes infection on immune responses to vaccine antigens in goats. Several investigators, using other animal models, reported that nematode could influence vaccine efficacy by modulating host immune responses in particular where Th1- like and cellular – dependent responses play a major role in immune responses Elias *et al.,* (2005) and Van Riet *et al.,* (2007)

also reported that *Schistosoma mansoni* a trematode helminth and *Onchorcerca volvulus* infection decreases efficacy of vaccine against tuberculosis and tetanus respectively. Worm infection generally do not lead to mortality, however, they are chronic in nature and may lead to considerable morbidity with consequent loss in productivity (McNeilly and Nisbet, 2014). Immunologically, chronic nematode infection is characterized by skewed Th2-type immune response as well as T regulatory response which modulates immune effecter functions against nematode and modify pathology that would ensue (Cooper and Eleftherianos 2016; McNeilly and Nisbet, 2014)

This regulatory network is associated with chronic nematode infections and is thought to prevent strong immune responses against parasitic worms allowing the long term survival and restricting pathology (Cooper and Eleftherianos 2016; McNeilly and Nisbet, 2014). The network is also postulated to be able to get in the way of immune responses to non-nematode antigens such as those from vaccines, co-infecting pathogens, allergens, and self-antigens perhaps leading in this fashion to modified immunopathology, disease outcome or altered course among individuals chronically infected with nematode (Urban *et al.,* 2007). This would also predict that nematode infected individuals might not respond optimally to vaccines and this is an important issue since most current vaccines and those being developed will be introduced in areas where these endoparasite infections are highly prevalent (Dabasa *et al.,* 2017).

1.2. Statement of the Problem

As the world population expands from the current 7.8 billion to more than 9.8 billion by 2050 (UN-DESA, 2017), demand for milk, meat and animal based products will rise steeply and therefore it becomes more imperative to assess ways and means of ensuring food security and maximizing animal production. It is anticipated that the

vast majority of this increase will come from emerging sub-Saharan economies (UN-DESA, 2017). Climatic change and increased need for animal proteins and the ever diminishing land size among households, a consequent of population increase, demand for evaluation of existing vaccines, development of new and effective vaccines to control endemic, emerging and re-emerging diseases that impact on livestock production. Small ruminants provide milk and meat for poor households for it is relatively affordable to slaughter a goat to provide meat than the larger stock (cattle and camel). They can also easily be converted into cash to buy food stuff for the family. Food security can also indirectly be realized from goat's products such as skins, fuel, manure for amending soils and source of employment for some members of the community as shepherds. A larger proportion of Kenyan small ruminant population is found in arid and semi-arid areas (MoLFD 2009) endemic for CCPP. These areas also border National game parks and reserves and more often than not small ruminants graze together with the wild ruminants. Contagious caprine pleuropneumonia was confirmed in captive wild ruminants kept in a wildlife reserve in Qatar where it involved wild goat (*Capra aegagrus*), Nubian Ibex (*Capra ibex nubiana*), Laristan monflon (*Ovis orientalis laristanica*), and Gerenuk (*Litocranius walleri*) (Arif *et al.,* 2007).This may suggest that our vast population of wild small ruminant are susceptible or may act as reservoirs. This coupled with increasing cross-border trade and the transboundary nature of CCPP (Kipronoh *et al.,* 2016) makes the disease endemic and a regional threat to food security that requires appropriate responses.

1.3 Justification of the study

The nature and more often the short course of the CCPP disease during outbreaks demands for rapid vaccine deployment for bio-security to ensure food security and

socio-economic stability of the people who depend on livestock for livelihoods. Food security concerns require systematic evaluation of confounding factors that may affect vaccine efficacy in vaccines used in disease control and improving livestock production. World Animal Health Organization has imposed strict guidelines governing international trade on live animals and their products and Kenya stands to benefit from goat meat (*Chevon*) export if caprine pneumonia among other OIE listed diseases can be effectively eradicated or controlled. This study sought to evaluate the effects of nematode infection, one of the possible confounders to the available vaccine, among others, in a population of livestock targeted for vaccination. The huge bill in terms of vaccine development demands that any confounders that would affect their efficacy need to be taken into consideration.

1.4 Main Objective

To determine immunopathological response in goats infected with gastrointestinal nematodes to *Mycoplasma capricolum* subsp. *capripneumoniae (Mccp)* infection and vaccine antigens.

1.4.1 Specific objectives

1. To determine the effect of mixed nematode infection on humoural response in goats to inactivated *Mccp* vaccine antigens.
2. To determine serum protein levels in goats infected with mixed nematodes in response to inactivated *Mccp* vaccine antigens.
3. To investigate the impact of mixed nematode infection on antibody titres in goats co-infected with *Mccp.*
4. To evaluate the influence of mixed nematode infection on pathological effects of *Mccp* in goats.

1.5 Hypothesis

Mixed gastro-intestinal nematode infection do not influences immunopathological response to inactivated *Mycoplasma capricolum capripneumoniae (Mccp)* vaccine and live *Mccp* antigens.

1.5.1 Specific Hypothesis

Antibody immune response to inactivated *Mccp* vaccine antigens is not suppressed by mixed nematode infection in goats.

1.5.2 Specific Hypothesis

Mixed nematode infections do not affect serum protein levels in goats vaccinated with inactivated *Mccp* vaccine antigens.

1.5.3 Specific Hypothesis

Antibody immune response in goats to *Mccp* bacterial antigens is not impaired by mixed nematode infection.

1.5.4 Specific Hypothesis

Mixed nematode infections do not influence immunopathologic response to *Mccp* infected goats.

CHAPTER 2

LITERATURE REVIEW

2.1 Contagious Caprine Pleuropneumonia presentation and transmission

Contagious caprine pleuropneumonia (CCPP) is a bacterial disease characterized by high morbidity of up to 100 % and mortality of up to 80 % in immunologically naive flocks, dyspnoea, mucopurulent nasal discharge, fibrinous pleuropneumonia afflicting mainly goats, caused by *Mycoplasma capricolum* subsp. *capripneumoniae (Mccp)* of the family *Mycoplasmaceae*. (Tigga *et al*., 2014). The aetiological agent is closely related to *M. capricolum* subsp. *capricolum* which causes a disease that involves prominent pathological lesions in various organs including arthritis, mastitis and conjunctivitis and more distantly related to other *M. mycodes* cluster such as *M. mycoides* subsp. *capri* and *M. leachii*.(Samiullah, 2013)

Transmission occurs predominantly by spread from animal to animal through direct contact and is mediated by aerosolization of the respiratory secretions more so when there is crowding (OIE, 2009). Introduction of an infected animal into uninfected flock is a common route of dissemination. Long distance spread of infective *Mycoplasma* can occur through wind under optimal conditions (Ostrowski *et al.*, 2011). MacVey *et al.*, (2013) reported that viable *Mycoplasma hypopneumoniae* has been recovered greater than nine Kilometres away from an infected source. Age, genetic predisposition, prevailing environmental conditions, overcrowding and concurrent infections all are involved in contributing to resistance to infection or to susceptibility and therefore, minimizing the predisposing stressors will lessen disease occurrence. Attachment to the host cells is the first step in infection establishment and this facilitated by their anionic surface layer on most Mycoplasma (Guo *et al*., 2017). Host

receptors for attachment are surface proteins especially glycol-conjugates that allow for colonization of the mucosal surface (Rottem *et al*., 2012).

2.2 Geographic Distribution of Contagious Caprine Pleuropneumonia

The clinical disease has been reported in nearly 40 countries of Africa, Asia and Eastern Europe although the etiological agent has been isolated in about 20 countries (Ozdemir *et al.,* 2005, Centikaya *et al.,* 2009 and Nicholas and Churchward, 2012). This could be attributed to few laboratories with expertise for isolating and culturing of *Mycoplasmas* (Nicholas and Churchward, 2012). The disease poses a significant threat to disease free zones and food security in terms of animal sourced proteins.

The disease was first described in Algeria in 1873, and shortly after that in South Africa in 1881(OIE, 2014). The aetiological agent, *Mccp,* was first isolated and identified in Kenya (OIE, 2014).

With improved diagnostic techniques, the disease has been described in several countries of the world, more so in Eastern African and Asia (OIE, 2009). *Mcpp*, the causal agent for contagious caprine pleuropneumonia (CCPP) was isolated in Thrace region of Turkey mainland Europe in 2004 (Ozdemir *et al.*, 2005).

A study based on clinical surveillance in the field, regional slaughterhouses and regular submission of suspected lesions to regional laboratories showed that CCPP aetiological agent, *Mccp,* could be detected by culture and specific polymerase chain reaction (PCR) in 37.5% of the samples (Centikaya *et al.,* 2009). Outbreaks were reported in Greece 2006, Iran 2006-2007, Ethiopia 2007, Oman in 2008-2009, Tajikistan in 2009, China in 2006 and Yemen (Nicholas and Churchward, 2012) and Pakistan 2008 (Awan *et al.*, 2010).

Mccp was also isolated in 15.4% of samples submitted from sheep in East Turkey (Centikaya *et al.,* 2009). A fatal outbreak of CCPP that killed over 300 goats was reported in Mauritius 2009 (Srivastara *et al.,* 2010) following introduction of goats from continental Africa. El-Deeb *et al.,* (2017) reported the first isolation of *Mycoplasma capricolum* subsp. *capripneumoniae* from two regions of the Kingdom of Saudi Arabia.

Mccp bacteria were isolated and hence CCPP confirmed in captive wild ruminants kept in a wildlife reserve in Qatar where it involved wild goat (*Capra aegagrus*), nubian Ibex (*Capra ibex nubiana*), Laristan monflon (*Ovis orientalis laristanica*), and Gerenuk (*Litocranius walleri*) (Arif *et al.,* 2007).

A fatal outbreak of pneumonia in endangered Markhors (*Capra falconeri*) in Tajikistan occurred in 2010 (Ostrowski *et al.,* 2011) though a different etiological agent was isolated and identified as *M. capricolum* subsp. *capricolum,* that causes a less lethal pneumonia in goats. The isolation of *Mccp* in sheep and wild ruminants' samples (Arif *et al.,* 2007, Centikaya *et al.,* 2009) brings the strict host-specificity of this pathogen into question. This is of particular importance to Kenya since most of the sheep and goat population is found in the arid and semi-arid lands that include or border the National game parks and wildlife reserves teeming with wild small ruminants. There is need therefore to devise control measures that ensure effective control in both domestic wild small ruminants.

2.3: Immune Response to Mycoplasma capricolum capripneumoniae.

Analyses of *Mycoplasmas* genome revealed their limited biosynthetic capabilities, seemingly having lost almost all the necessary genes important for biosynthesis of essential materials such as amino acids, fatty acids, cofactors, and vitamins (Rottem,

2003,Rivera-Tapia and Rodríguez-Preval, 2006) and therefore these organisms depend on host's microenvironment for supply of full spectrum of biochemical precursors required for the biosynthesis of macromolecules required by the pathogens (Rechnitzer *et al.,* 2013). Competition for these biosynthetic precursors by Mycoplasmas may upset host cell integrity and amend host cell function.

Non fermenting *Mycoplasma spp.* utilizes the Arginine Dihydrolase Pathway for generating ATP (Rechnitzer *et al.,* 2013) and rapidly depletes the host's Arginine reserves affecting protein synthesis, host cell division, and growth.

The attachment of Mycoplasmas to the surface of host cells is thought to interfere with membrane receptors or alter transport mechanisms of the host cell (Rottem *et al*., 2012). The disruption of the K^+ channels of ciliated bronchial epithelial cells by *Mycoplasma hyopneumoniae* that resulted in ciliostasis has been described in pigs (Rivera-Tapia and Rodríguez-Preval, 2006).

The host cell membrane is also vulnerable to toxic materials released by the adhering Mycoplasmas. Although toxins have not been associated with Mycoplasmas, the production of cytotoxic metabolites and the activity of cytolytic enzymes are well established (Shlomo, 2003). Oxidative damage to the host cell membrane by peroxide and superoxide radicals excreted by the adhering Mycoplasmas appears to be experimentally well-substantiated (Rottem *et al*., 2012).

Intimate contact of the Mycoplasma with the host cell membrane may also result in the hydrolysis of host cell phospholipids catalyzed by the potent membrane-bound phospholipases present in many Mycoplasma species (Shibata *et al.,* 1995). This could

trigger specific signal cascades as described by Rosenshine and Finlay (1993) or release cytolytic lysophospholipids capable of disrupting the integrity of the host cell membrane (Shlomo, 2003).

During the fusion process, Mycoplasma components are thought to be delivered into the host cell affecting the normal functions of the cell. Whole arrays of potent hydrolytic enzymes have been identified in Mycoplasmas (Shlomo, 2003). Most remarkable are the Mycoplasmal nucleases that may degrade host cell DNA.

Phosphorylation of cellular constituents by interacting cascades of serine/threonine and tyrosine protein kinases and phosphatases is a major means by which a eukaryotic cell responds to exogenous stimuli (Shlomo, 2003). The delivery of an active phosphoprotein phosphatase into the eukaryotic cell upon fusion may interfere with the normal signal transduction cascade of the host cell.

These events could eventually amend receptor recognition sites as well as affect the induction and expression of cytokines while modifying the cross-talk between the assorted cells in an infected tissue (Razin and Herrmann, 2002). Upon infection, cells of the innate immune system, in particular of the Monocyte/macrophage lineage, react to bacterial components such as modulins (Chambaud *et al.*, 1999) by release of proinflammatory cytokines and interferon gamma which in turn can lead to shock and death. The bacterium can possibly stimulate the macrophages to produce oxygen radicals, TNF-α, IL-6 and nitric oxide.

Nutrient absorption of *M capricolum* from the host cell membrane is probably the initiation of infection. The toxic oxidant (peroxynitrite) accumulation and the

hydrolytic enzymes produced by *Mycoplasma* also contribute tissue damage (Darzi *et al.,* 1998).

Being unable to synthesize own nucleotides, Mycoplasmas developed potent nucleases, either soluble ones secreted into the extracellular medium or membrane-bound nucleases (Bendjennat *et al.,* 1999) apparently as a means of producing nucleic acid precursors required for metabolism. It has been shown that, occasionally, secreted Mycoplasma nucleases are taken up by the host cells (Paddenberg *et al.,* 1998). Thus it was suggested that, for some Mycoplasmas, such as *M. penetrans*, cytotoxicity is mediated at least in part by a secreted Mycoplasma endonuclease that is cleaving DNA and/or RNA of the host cells (Bendjennat *et al.,* 1999), and the endonuclease activity of *M. bovis* was implicated in the increased sensitivity of lymphocytic cell lines to various inducers of apoptosis (Sokolova *et al.,* 1998).

Though *Mycoplasma* circumvent phagocytosis, they interact with mononuclear and polymorphonuclear phagocytes suppressing or stimulating them by a combination of direct and indirect cytokine-mediated effects for example the expression of proinflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, chemokines such as IL-8, Monocyte chemo attractant protein-1, macrophage inflammatory protein-1α, Granulocyte-Monocyte colony stimulating factor as well as prostaglandins and nitric oxide (Shlomo, 2003). It is thought that for many Mycoplasmas, induction of cytokine production is a major virulence mechanism. The induced array of cytokines has a wide range of effects on the eukaryotic host cell and is recognized as important mediators of tissue pathology in infectious diseases (Rottem *et al*., 2012).

Potent molecules and mediators released by cells in response to Mycoplasmas and Mycoplasma-derived expression factors enhance display of major histocompatibility complex (MHC) class I and class II antigens and of costimulatory and cell adhesion molecules in leukocytes and endothelial cells, induce recruitment and extravasations of leukocytes to the site of infection and cause local tissue damage (Ruuth and Praz 1989, Henderson *et al.,* 1996).

Mycoplasma infections are not necessarily associated with strong inflammatory responses, and some Mycoplasmas colonize the respiratory and urogenital tracts with no apparent clinical symptoms. It is therefore alluring to speculate that in addition to triggering the production of proinflammatory cytokines, certain organisms have the capacity to downregulate NFκB or to induce anti-inflammatory cytokines such as IL-4, IL-10, IL-13, or transforming growth factor-β, contributing to the complex network of synergistic and antagonistic influences induced by Mycoplasmas on cells of the immune system with subsequent overall immunomodulation (Rottem *et al*., 2012).

The downstream signalling events that follow the Toll Like Receptor (TLR)-mediated activation by *Mycoplasma* lipoproteins leading to cytokine synthesis seem to be similar to the intracellular events induced by lipopolysaccharides (LPS). The TLRs have a cytoplasmic domain that is homologous to the IL-1 receptor. Thus it is likely that TLR2 activates the NF-κB pathway and perhaps other proinflammatory pathways as well, via their interactions with IL-1 receptor signalling genes (Lien *et al.,* 1999*)*. According to Shlomo (2003), *M. fermentans* lipoproteins or the lipoprotein-derived MALP-2 lipopeptide activate NF-κB and activating protein-1(AP-1), the two transcription factors plays a central role in the induction of proinflammatory cytokines, as well as the mitogen-activated protein kinase family members including extracellular

signal-regulated kinases 1 and 2, c-Jun amino-terminal kinase, and p38 mitogen activated kinases (Rottem *et al*., 2012).

Different *Mycoplasma spp*. has been shown to stimulate B cells, T cells, or both non-specifically. Shlomo (2003) reported that antibodies of different specificities, with no affinity for Mycoplasma antigens, are generated both in vitro and in vivo following exposure of lymphoid cells to different Mycoplasmas possessing B cell mitogens. Various *Mycoplasma spp.* expressed molecules are potent stimulators of T-cell-derived cytokines such as IL-2, interferon-α, or IL-4. It has been observed that they exert multiple amplifying effects on phagocytes and lymphocytes and affect the balance between Th1 and Th2 populations of $CD4^{+}$T cells, thereby influencing the direction of the subsequent effector phases of the immune response, and augmenting natural killer cell activity (Shlomo, 2003).

In order for Mycoplasma or any other pathogen to survive within its host tissues, evasion of the host immune system is of paramount importance and consequently they have devised diverse stratagem to circumvent host immune mechanisms (Sanchez-Vargas *et al.,* 2008). When *Mycoplasma* is inhaled, it is bound by the host cell membrane and its surface-exposing lipoproteins induce strong antigenic reactions.

The two key mechanisms used by these microbes that have been studied at length are molecular mimicry and phenotypic plasticity, which make certain that the Mycoplasmas or other pathogens are not fully or efficiently recognized by the host's immune system (Razin and Herrmann, 2002 and Sanchez-Vargas *et al.,* 2008) and thus ensuring their survival.

Molecular mimicry refers to antigenic epitopes or expression molecules shared by different Mycoplasmas and host tissue cells and are proposed as putative factors involved in evasion of host immune responses and/or induction of auto reactive antibodies observed during infections with certain Mycoplasmas. However, the highly evolved antigenic variation in this organism makes it hard for the host immune system to produce proper and effective antibodies to contain or even eliminate the infection (Shlomo, 2003 and Citti *et al.,* 2010).

Mycoplasmas are endowed with phenotypic plasticity and are able to change its antigenic make-up that can be achieved through random changes of expression of single or multiple genes depending on the prevailing environmental conditions. By oscillating at high frequency Mycoplasma genes, as in Variant Surface Glycoproteins (VSG) on the surface membranes of Trypanosomes and consequent generation of diverse immunoglobulin with low specificity, allow for numerous combinatorial antigenic repertoire and enormous array of antigenic variants (Shlomo, 2003 and Citti *et al.,* 2010). Transformation of the surface antigenic repertoire and consequently the capability of a micro-organism to rapidly vary the immunogenicity of these components, allows the pathogen to avoid recognition or outdo a host's immune system and thus ensure its survival to be able to pass its genes to the next level like all other living things.

A Mycoplasma population may impulsively and randomly generate distinct lipoprotein populations with a variety of antigenic phenotypes that will survive the specific host response capable of eliminating the predominant types. This molecular switching events leading to the generation of antigenic variant repertoire are reversible, and the

escape variants produced through random genetic variation must inherit the ability to produce, at a high frequency, a wide range of antigenic phenotypes (Shlomo, 2003 and Citti *et al.,* 2010).

Random phenotypic switching, an important evolutionary bonus to the pathogen, can be realized even before the onset of a specific immune response to the infective agent (Oelschaeger and Hacker, 2013). Through modification of the specificities of variant receptors or adhesion factors in the entire cell population, there is an improved probability that a given variation will succeed in finding the preferred receptors molecules on the mosaic of different tissues displayed by the host (Oelschaeger and Hacker, 2013; Shlomo, 2003). It may also provide the Mycoplasma, during the course of parasitic life, the flexibility to reach and adapt to different niches within the host where distinctive receptors may be required for colonization.

Despite the apparent limited genetic information that Mycoplasmas contain, the number of Mycoplasma genes involved in diversifying the antigenic nature of their cell surface is unexpectedly high. Genetic mechanisms of antigenic variation emerging from the Mycoplasma studies can be broadly divided into three categories that include variation by homopolymeric repeats that provide for favourite targets for insertions or deletions of nucleotides thus enabling the microbe to switch genes on and off (Deitsch *et al*., 2009).

Variation of chromosomal rearrangements whereby there may be DNA inversion, gene conversion, duplication or deletion of tandem homologous blocks of DNA are used to regulate expression proteins and variation by reiterated coding sequence

domains (Oelschaeger and Hacker, 2013). Shlomo, 2003 undertook a comprehensive and comparative review on the genetic mechanisms generating antigenic variation of surface proteins in *Mycoplasmas* and other bacteria and on the role of antigenic variation in bacteria-host cell interactions. Many pathogenic bacteria and parasites utilize a collection of variable genes organized as gene families, allowing for the generation of an extensive repertoire of antigenic variants for maintaining surface variability (Oelschaeger and Hacker, 2013; Deitsch *et al*., 2009).

2.4 Nematode parasites of veterinary importance

Nematode members of the Strongyloidea the large bowel and Trichostrongyloidea, the abomasal and intestinal strongyles of ruminants are among the most commonly encountered nematode parasites in domestic animals (Whittaker et al., 2016) are among a group of multicellular organisms of veterinary, medical and economic importance as they infect food and game animals and humans provoking either fatal or often chronic disease (Moreau and Chauvin, 2010). In terms of food security, they are responsible for economic losses due to decreased milk yield, delays in attaining market weight for meat production and the cost incurred during development of anthelminthics and actual clinical treatment (Dabasa *et al.*, 2017, Badran *et al*., 2012).

Parasitic worms infesting domestic ruminants can be broadly classified into three taxonomic groups namely, nematodes, trematodes and cestodes some of them with complex lifecycles consisting of intermediate stages that require several hosts to complete their developmental stages (Moreau and Chauvin, 2010).

In each of these phases, in their life history, the parasites expresses various excretory-secretory substances with varying immunogenicity and can elicit or modify immune responses in the host.

The epidemiology of nematode infections of sheep and goats is influenced by several factors that include parasite factors, host characteristics, climatic factors and management systems used in livestock production. The incidence rate and severity of worm infection can be influenced by host factors such as genotype, age, level of nutrition, physiological status (lactation or gestation) and presence or absence of co-infections (Odoi *et al*., 2007).

Overstocking presents a huge problem and in addition to contributing to pasture degradation and soil erosion resulting in environmental destruction, the animals are forced to graze close to faecal material on the ground that results in increased uptake of large numbers of infective nematode stages (Ntonifor *et al*., 2013). The high stocking density also increases the level of contamination of the pasture with the free living stages of the nematodes (Stadalienė et al., 2015; Ntonifor *et al*., 2013). The crowding of watering points by different flocks particularly during dry spells may lead excessive contamination of surrounding pastures with eggs or larvae with consequent outbreaks of acute parasitic gastro-enteritis (Zvinorova et al., 2016). This may be exacerbated when the majority of the flock becomes susceptible to parasitic infection as a result of inadequate nutrition due to pasture and forage scarcity and confounding conditions such as age, pregnancy and lactation (Dagnachew *et al*., 2011).

Goats that are well nourished either through grazing, browsing or even stall raised are able to maintain their haemoglobulin levels as long their mineral supplement and more so iron and protein uptake is adequate (Risso *et al*.,2015).

Nematode transmission can occur through various ports of entry or modes such as *per os* with examples being *Strongyles*, through the skin (*Ankylostoma* sp. and

Strongyloides sp.), while others are passed through arthropod vector bites for instance *Onchorcerca* sp. (Moreau and Chauvin, 2010). The adult stages of nematodes inhabit various regions of the gut depending on the particular species. The female nematode lays eggs which are passed out by the host with its faecal material. These eggs then embryonate and hatch into first stage (L_1), the moult second stage (L_2), shedding their protective cuticle in the process and this then moult to third stage (L_3) larvae but retaining the cuticle from the previous moult, this double cuticled L_3 is the infective stage. After ingestion, L_3 develops through fourth stage (L_4) and fifth stage (L_5) larvae and mature in the abomasum for *Haemonchus* spp. and *Trichostrongyilus axei* while the maturation of *Trichostrongyilus columbroformis* in the small intestine and *Oesophagostomum* spp. occur in the large intestine (Badaso and Addis 2015).

The definitive and the intermediate host may be mammals, avian, reptiles, fish, molluscs and arthropods. They may be localized in organ lumen or tissues such as gut, liver traversing the parenchyma and bile ducts, lung, lymphatic vessels, conjunctivae (Moreau and Chauvin, 2010) eliciting various or sometimes no immunological responses.

Larvae of some gastro intestinal nematodes are also known to undergo a period of arrested development (hypobiosis) in case of adverse conditions to resume when there are favourable conditions for example at the onset of rains (Meradi *et al*., 2016; Roeber *et al*., 2013). This ensures that the parasite can pass its genes to the next generation or progeny.

The immune system is relatively inefficient in controlling nematode parasites and this may be due to these organism adaptations to a parasitic existence out of necessity or may have evolved to overcome or evade their host immune responses (Zaph *et al*.,

2014). Parasitic nematode are fully adapted obligates whose very survival depends on reaching some form of co-existence with the host until they attain the next stage in their life span (Parker *et al*., 2015). The long lifespan of nematode parasites in their hosts is evidence enough that they are adept at immune evasion, and it is clear that interference and modulation occur from the first events in infection otherwise they would be expelled immediately their antigens are detected by host immune responses (McNeilly and Nisbet, 2014).

Nematode do not simply ward off immune attack; rather, they influence and direct immune responses away from the modes most damaging to them, regulating the host immune response to create niches that optimize successful feeding and there long residency in the hosting organism to realize and effect reproductive activities(Cooper and Eleftherianos 2016). Strongyles and generally most helminthes are not known to replicate within their hosts and therefore their number in an individual is not more than the number that gained entry to the susceptible host at any given time or period of infection. For their guaranteed survival in their generations, they are usually known to cause mild or subclinical disease and as a rule, usually cause morbidity other than mortality because it would be not in their interest to be mortal. It is only when the nematode invades a host to which they are not fully adapted, immune compromised in one way or other, or in extremely large numbers that they tend to cause an acute or even mortal disease. One consistent attribute of gastro-intestinal geo-nematode infestation is the wide variation in parasite burden even within a flock (Moreau and Chauvin 2010). In that case, a minority in the livestock population may carry a heavier burden or large numbers of parasites whereas the majority of the same population harbours few worms. The scale of the worm burden in an individual host may be

determined by its genotype and its immune response to parasitic infestation. Within a flock or animal population, some individuals may be predisposed to a heavy infestation consequent to genetic, behavioural, nutritional, or other environmental factors. This scenario may also reflect divergence in exposure, susceptibility, or resistance to parasitic worms (Nabi *et al*., 2014).

Beside the above named factors, the innate immunity factors that influence parasite residence in the host include the presence of other parasites or pathogens within the same host (Alba-Hurtado *et al*., 2013; Sykes *et al*., 2010). The multi-stage presence in the gastro-intestinal tract may produce substances or factors that delay further development of larval stages of the same species within the same host (De Veer, *et al*., 2007). Multiple species infestation leads to competition for mutual habitation and nutrients in the gastro-intestinal tract thus influencing worm burden, location and the composition of the overall nematode population (McRae et al., 2015).

Host innate immune factors that have a bearing or influence worm burden among others include age, sex, and of paramount importance the genotype (McRae et al., 2015). Age and sex appear to impart their influence through hormones. To further the argument on hormonal influence on increased nematode egg, Zvinorova et al., (2016) observed that ewes showed a periparturent rise in faecal nematode ova, which coincides with lambing and the onset of lactation. This can be attributed to relaxation of immunity and the effect of increasing prolactin hormone that favours lactation.

Nematodes being multi-cellular and multistage organisms always present a huge challenge to the immune system in that each stage may be associated with different

structural, surface or excretory-secretory antigens. The parasites posses an extra coat, the cuticle, which provides extra protection to the nematode hypodermal plasma membrane (Page *et al.,* 2014). They also possess a loose coat that they can afford to cast it off when under attack minimizing damage from conventional immune barricades. Membrane attack complex of the complement system and perforin from cytotoxic T cells cannot penetrate nematode cuticles and hence an otherwise potent host immune response effector mechanism is evaded (Zhang *et al*., 2011). An effective immune response to infesting nematodes must either raise cells that destroy or weaken the defensive cuticle or strike them through soft spots on their surfaces such as the natural orifices like their digestive tract. Mature parasitic forms in the gastrointestinal tract are bathed in host enzymes, immunoglobulin A, and mucin(McRae et al., 2015), that forms an interactive mucosal defence system, while their feeding ends and alimentary tract encounter effector cells, cytokines, antibodies and complement(Moreau and Chauvin, 2010). Th_2 mediated immunoglobulin (Ig) E production is essential in controlling worm burdens through type-1 hypersensitivity reaction (Lee *et al*., 2011). A combination of nematode antigens with mast cell-bound IgE triggers mast cell degranulation and the release of vasoactive molecules and proteases (Allen and Maizels, 2011; Chen *et al.,* 2012 and Sagalme *et al.,* 2013). These stimulate gut smooth muscle to contract with increased vascular permeability of the intestinal capillary bed leading to an efflux of fluid into the intestinal lumen with increased gut motility resulting in dislodgement and expulsion of most worms.

Mast cell degranulation chemotactic products at the sites of nematode invasion attract eosinophils that in turn help in attempts to eliminate the worms (Mukai *et al*., 2016; Hepworth *et al*., 2012). Th_2 cell cytokines such as interleukin (IL)-5 change bone

marrow dynamics mobilizing the eosinophil pool with subsequent eosinophilia (Spencer *et al*., 2010). Parasites therefore may induce biphasic eosinophil migration. The first being provoked by mast cell and or parasite products, and the second by Th2 cytokines the chief being IL-5.

Shi (2004) suggested that eosinophils can be induced *in vivo* to begin expressing MHC II complexes and co-stimulatory molecules that are required for T lymphocyte to be functionally activated. Eosinophils are recruited to the focal points of infection where they are thought to modulate immune responses through an assortment of mechanisms following engagement of receptors they express for cytokines(Spencer *et al*., 2010), immunoglobulins and complement leading to secretion of a repertoire of interleukins, chemokines, growth factors and lipid mediators (Hogan *et al.,* 2008).

Eosinophils can destroy nematode parasites because they possess Fcε Receptors enabling them to bind to antibody- coated parasites, degranulate, and release their attack molecules directly onto the nematode cuticle (Makepeace *et al*., 2012). With the array of geo-nematode infestation this defence arm may not be effective against all worms. Antibodies may block enzymes required in adult worm metabolism or impede egg production thus leading to reduced pasture contamination with subsequent reduction of parasite transmission within the herd. Other immunoglobulin isotypes may also play a protective role towards nematode infection control and among these mechanisms may include antibody-mediated neutralization of larval produced proteases that aid in their migration and establishment, jamming of the anal and oral orifices by immune-complexes, prevention of shedding of the cuticle and blocking of stage development by antibodies directed against moulting products (Shi, 2004).

Th1 immune response component may be of little value to host immunity to nematode infection though they it is known to play a role in promotion of antibody class switching (McRae et al., 2015). Nevertheless, CD8 cytotoxic T lymphocytes may attack migratory larval stages or those that are deeply embedded in the gastro-intestinal mucosa (Sorobetea, *et al*., 2018). It is also possible that sensitized T cells may lead to development of type IV hypersensitivity reaction attracting mononuclear cells to the site of larval invasion leading to granuloma formation with subsequent arrest of migratory larvae (Sitchaungsi and Sirivichayakul, 2013).

In spite of an array of immune mechanism mounted by animals to defend themselves from worm establishment, morbidity and more often than not mortalities suggest that these responses may not be very effective in warding off successful infections.

A well adapted worm parasite is able to survive and reproduce in a parasitic relationship even in a host with competent immune system. The strategies employed to surmount or evade the host defences include parasite loss of antigenicity through molecular mimicry, masking by absorption of host antigens, antigenic variation though not marked as seen in protozoa, shedding of gylcocalyx, a potent immunogenic and activator of alternative complement pathway, blocking of antibodies and induction of tolerance (Cooper and Eleftherianos 2016; Moreau and Chauvin, 2010).

To camouflage themselves and for their own good, nematode may attempt to synthesize and finally express non-polymorphic MHC or even blood group antigens on their surface in order to identify themselves with their host. During their growth larval change their surface antigens and in this they are able to survive in the host tissues (Cooper and Eleftherianos 2016).

Immunosuppression may also contribute to the survival of parasitic nematode in their hosts (Doligalska and Donskow-Łysoniewska 2012). The quantity of infective stage larvae that effectively invade the susceptible host, the number of tissue migratory parasites and the count of the settled adult forms and their reproductive ability depend on the activity of the host immune system (Makepeace *et al*., 2012; Doligalska and Donskow-Łysoniewska 2012). Therefore it can be said that manipulation of immunological recognition, effectiveness of immune responses to foreign antigens and the resultant protective responses are the mechanism affect parasite population and survival in the host. In the evolutionary sense both parasite products and host immune system are adjusted to their intimate relationship (Doligalska and Donskow-Łysoniewska 2012). Parasitic worms tend to settle in privileged sites in the host which is reflected in the distinct location of larvae and adults in the host. Parasites need a suitable and non-hostile place of residence to propagate and therefore pass their genes to their progeny ensuring survival of species. The state of immune unresponsiveness protects growing and fragile juvenile stages during migration through the host tissues (Sun et al., 2013).

In concomitant infections with multiple co-infecting species, parasites network with one another through the host's immune system via mechanisms such as immune trade-offs and immunosuppression that was observed to able make the buffalos susceptible to bovine mycobacterium infection (Ezenwa and Jolles, 2011).

The maintenance of an immunosuppressed state in the host is in the best interest of pathogen and may also improve the fitness of the parasite (Hewitson *et al.*, 2009). This induced condition by nematode not only affects the incumbent parasite, but also

promotes new infection with further infectious larvae. Parasite acquisition is density-dependent and the number of parasites successfully establishing in the host may over time increase with the parasite burden in the host as long the conducive conditions are maintained. In long-lasting infections, immunosuppressive mechanisms prevent or limit parasite killing and expulsion; the ongoing infections do not elicit a strong host effector response; infection with one species predisposes for infection with other species and polyparasitism is common (Wang *et al.*, 2017; Ellis and McManus, 2009). Immunosuppression due to nematode infection is reflected in a dampened resistance to concurrent infections and poor response to vaccine antigen reflected by low seroconversion or none at all (Urban *et al.*, 2007).

Helminth infections induce regulatory T cells (Treg: Tr1, Th3) secreting IL-10 and transforming growth factor (TGF-β) (Doetze *et al.*, 2000) as well as $CD4^+CD25^+$ Treg expressing the Foxp3 transcription factor in the host (Cervi *et al.*, 2009 and Pacífico *et al.*, 2009). These regulatory T cells can alter the course of inflammatory disorders by increased production of IL-10 and TGF-β, together with induction of $CD25^+CD4^+$ $Foxp3^+$ T cells (Correale & Farez, 2007) whose gene expression favours immunosuppression or immunomodulatory pathways. This scenario may also represent a potential explanation regarding how exposure to a parasite could alter immune reactivity to unrelated stimuli.

Parasitic nematodes are known to release secretory-excretory products whose molecular structure and specificity may be changed during infection and most parasite immune evasion mechanisms depend on a form of molecular recognition between

parasite and host (Lightowlers & Rickard 1988 and White & Artavanis-Tsakonas, 2012).

Some studies have shown that infection with certain nematodes such as hookworm involves induction of nitric oxide production which is associated with impaired function of antigen-presenting cells and depletion of lymphocyte subpopulations (Dondji *et al.,* 2008); whereas Van Ginderachter *et al*., (2010) reported that myeloid cells derived from nematode infected animals exhibit antiproliferative properties.

Suppression of pro- inflammatory immune responses, through induction of immunosuppressive cytokines, mainly IL-10 and tumour growth factor (TGF) β (Johnston *et al.,* 2016), is dependent on the continued existence of parasites; *in vivo* T cell responses are restored following chemotherapy suggesting that modulation is an active process requiring the continued presence for release of nematode factors such as 'excretory-secretory' molecules into their environment which interact with host cells and molecules.

Parasite worm extracts have been reported to activate various macrophage subsets and among the most active in regulation of immune response are alternatively activated macrophages (AAMΦ) (Herbert *et al.,* 2004). Though most of the work and data collected is from work done on rodent models and human studies, there is growing body of evidence that ruminant nematode are similarly capable of modulating host immune response (Soulsby, 1987; Anthony *et al.,* 2007; Shin *et al.,* 2009; McNeilly & Nisbet 2014).

Extrapolation of findings from these models may to some extent give an explanation as to why there is sluggish development and low protective immunity to ruminant nematode and may present a number of consequences to livestock health and welfare in that the immunomodulatory molecules may either be viable vaccine candidates for vaccine development (McNeilly &Nisbet, 2014) or may have an influence on the susceptibility to concurrent infections or impact on responses to other livestock vaccine products.

So as to secure their survival, nematode parasites aim at producing considerable amounts of proteins and glycoproteins into the host environ, most of which posses the capacity of modulating antiparasite immune reaction(McSorley and Maizels, 2012). These molecules have the potential of interfering with critical phases in the immune response such as extravasations which may be blocked by parasite lectins and glycans (Rodrigues *et al.,* 2015) through binding to endothelial selectins, chemokine integrity. *Haemonchus contortus* and hookworms release proteases capable of degrading eotaxin (Robinson and Dalton 2011), release of host proteases is inhibited by nematode serpins with putative involvement in immune regulation and in parasite survival, attack by reactive nitrogen and oxygen intermediates by eosinophils and other effector cells is inhibited by nematode antioxidants such as glutathione S-transferase (Falcone *et al.,* 2004; Maizels *et al.,* 2004). Parasitic nematodes may also secrete cytokine homologues such as TGF-β (Johnston *et al.,* 2016) and produce protease inhibitors that are capable of blocking peptide antigen presentation and of eliciting an IL-10 response from macrophages (Ferragine *et al.,* 2013).

The efficiency of the innate response is crucial for invasion and survival of arriving larvae (Sorobetea, *et al.*, 2018). Immunosuppressive action of parasites can be primarily directed to antigen-presenting cells (APC) (Kapsenberg, 2003; Perrigoue *et al.*, 2008) and induction of suppressor/regulatory T cells and macrophages, with the common effect to selectively inhibition of local or systemic immune response.

Key attack points for selective immunoregulation conducted by parasites rely on (i) modulation of antigen recognition with changes in pathways of signal transduction; (ii) co-stimulation blockade; (iii) induction of regulatory cells; (iv) deviation to protective responses; (v) neutralization of proinflammatory cytokines; (vi) induction of anti-inflammatory cytokines and; (vii) modulation of leukocyte trafficking (Carvalho *et al.*, 2009; Dowling *et al.*, 2010).

Excretory – secretory products released by nematodes described as conserved molecular patterns associated with the pathogen (PAMP) may interact with the host pattern recognition receptor (PRRs) (Jackson *et al.*, 2009; Dowling *et al.*, 2010). Different carbohydrate moieties of nematode molecules are recognized by toll-like receptors (Medzhitov 2007; Dowling *et al.*, 2010) and the C-type lectins receptors on dendritic cells and macrophages (Cambi *et al.*, 2005). As a consequence of ligation, these dendritic cells will receive signals that are subsequently translated into different sets of Th1-, Th2-, or Treg-polarizing molecules. However, TLR ligation by nematode derived factors is recognized as a mechanism to limit of Th1 cytokine-mediated inflammation (Harnett and Harnett, 2010; Dowling *et al.*, 2010).

Mature DC generated in the immune system during nematode infection express relatively low levels of costimulatory molecules and proinflammatory cytokines

promoting proliferation of CD4- positive T cells with Th2 phenotypes (MacDonald and Maizels, 2008; Semnani *et al.,* 2008). Regulation of the host response starts from the recognition of the worms; nematode products are able to stimulate partially activated dendritic cells with suppressed expression of TLRs and activate factors which promote Th2 and T regulatory phenotypes (Jackson *et al.,* 2008). Some molecules which are released during tissue damage may interact with and induce anti-inflammatory effects (Ehlers and Ravetch, 2007).

This ability posed by nematode parasites to regulate or modify the host immune response through secretory-excretory products led some investigators such as Maizels *et al.,* (2004) to refer to nematode as master regulators of the host immune response, some effectively minimizing immune attacks meant to expel them and thereby ensuring their survival in the host for years and limiting host tissue damage. They strongly induce polarized T-helper type 2 (Th2) responses, elevated serum IgE titres, and eosinophil-rich inflammation infiltrates in the tissue (Barrett and Austen, 2009).

Nematode infections are therefore typically associated with marked eosinophilia, up regulated IgE production, mucous mastocytosis, and goblet cells hyperplasia (Anthony *et al.,* 2007, Shin *et al.,* 2009). These immune parameters are involved in different effector mechanisms highly depending on tissue infestation.

Many investigators have described several mechanisms against tissue-dwelling parasites (Patel *et al.,* 2009; Hewitson *et al.,* 2009; Danilowizc-Luebert *et al.,* 2011; White and Artavanis-Tsakonas, 2012). These migratory parasites are mainly larval stages of, for example, nematodes, *Dictyocaulus filaria* in goats or trematodes (*Schistosoma spp.*, *Fasciola spp.*), which migrate through tissue.

Antibody dependent cellular cytotoxicity (ADCC) is dependent on eosinophils, neutrophils, macrophages (Makepeace *et al.,* 2012), or platelets as effector cells and

Immunoglobulin (Ig) E, Ig G, or Ig A as antibodies (Moreau and Chauvin, 2010). The opsonized parasitic structures are destroyed by cells carrying receptors to the Fc fragment (RFc). The activation of these cells when the antibodies are fixed to the RFc lead to the release of products that are toxic to the worm such as major basic protein, Eosinophil cationic protein, eosinophil-derived neurotoxin, reactive nitrogen intermediates, among many others. ADCC is also able to immobilize nematode larval stages as they migrate through the gut mucosa (Moreau and Chauvin 2010; Harris and Gause 2011). Nitric oxide (NO), toxic to the worm, is released by classically macrophages activated by IFN-γ and TNF-α (Anthony *et al.,* 2007; Moreau and Chauvin 2010). Tissue-dwelling parasites have developed several evasive mechanisms to escape the effector immune response of the host. A well investigated example, *Fasciola sp.* escapes from the immune responses by different means as follows: (i) *Fasciola gigantica* produces superoxide dismutase which neutralizes superoxide radicals toxic for juveniles (young flukes). (ii) *F. hepatica* releases cathepsin L-protease which cleaves Ig E and Ig G involved in the ADCC. (iii) Juvenile flukes were found to be covered by IgM (Moreau and Chauvin, 2010).

While eosinophils do not express Fc receptor, IgM deposition on fluke tegument could inhibit eosinophil adhesion. IgG2 produced during fasciolosis in susceptible sheep has been also suspected to be a blocking immunoglobulin of the ADCC (Moreau and Chauvin 2010). Resistance in sheep to *Haemonchus contortus* is characterized by increased blood and tissues eosinophils, antigen specific Ig E levels, mast cells and cytokines IL-5, IL-13, TNF-α (Zhengyu *et al.,* 2016). This nematode is capable of modulating host immune response. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an enzyme produced by the parasite and is able to bind to the C3

complement protein thus inhibiting C3's function in the control of the parasite (Alba-Hurtado and Munoz-Guzman, 2013; Ortolani *et al.*, 2013)

Immune mediated resistance to nematodes can include both innate and adaptive components of the type 2 immune response. The cytokines IL-4, IL-5, IL-9, IL-13, and IL-21 produced by both Th2 cells and innate immunity cell population including Basophils, mast cells including type 2 innate lymphoid cells(ILC2s), promote specific effector mechanism that contribute to anti-parasite resistance(Anthony *et al.*, 2007).

Nematode can induce type 2 immune response (Maizels *et al.*, 2012) which involves innate and adaptive immunity that together can mediate a potent host protective response to nematode parasites or these cells will contribute to host resistance or may also promote host tolerance (co-existence with) nematode and this can include both anti-inflammatory and wound healing properties that are probably important when these metazoan parasites migrate in host tissues. (Allen and Maizels, 2011; Chen *et al.*, 2012 and Sagalme *et al.*, 2013).

$CD4^+$ Th2 cell is the key player in type 2 immunity (Zhu, 2015) through expressing type 2 cytokines as well as chemokines such as CC-chemokine receptor 3 (CCR-3) ligand, CC-chemokine 11(CCL11) also known as eotaxin-1. Barlow and McKenzie (2011) citing other workers reported that Nuocyte or 'natural helper' cells are among the first to produce type 2 cytokines following nematode infection. These cells create the condition that favours Th2 cell induction and after receiving signals from differential Th2 cells, they continue to release IL-13 and promote type 2 immunity (Allen and Maizels, 2011). In the absence of these innate helper cells Th2 cell immune responses during nematode infection is greatly impaired.

In the gastrointestinal tract the mucosal epithelial cells expresses IL-4Ra and acts as responders to promote goblet cell differentiation, the enhancement of mucus secretion and the production of resistin-like molecule beta(RELM-beta) which is an innate protein with direct anti-nematode activity (Gerbe, 2016). IL-4Ra ligation also stimulates intestinal muscle increased contractility and accelerated epithelial turn over and IL-18 and release of mast cell proteases that degrade tight junctions thereby increasing fluid flow that flush out nematode. These type 2 cytokine, IL-4 and IL-13, also are known to drive the alternative activation of microphages (Allen and Maizels, 2011). The antibodies produced from B cells in response to the resultant cytokine milieu also contribute substantially to these defensive mechanisms by diminishing worm fitness and fecundity. In tissues, parasites are open to attack by the full range of host innate effector arsenal, which includes microphages, neutrophils, eosinophils, Basophils and platelets. The ability of these effector cells to kill nematode is often dependent on one or more isotypes of specific antibody and the complement. Armed granulocytes or macrophages can release damaging reactive Oxygen and Nitrogen intermediates onto the invading nematode.

Immune components mediating anti-parasite resistance may include increased luminal fluid flow and intestinal contractility, antibody dependent mechanisms, mast cells production of mediators, secretion of mucous and resistin-like molecule-β (RELM-β) (De'Broski *et al*., 2009) and effector mechanism mediated or triggered by eosinophils and macrophages may also contribute to anti- resistance. Neutrophils essential in resistance to microbial pathogens, have also been associated with nematode infection (Allen *et al.,* 2015).

Nematode efficiently evade host immunity through a number of mechanisms including but not limited to promoting a strong regulatory T-cell response that diminishes type II

immunity, Immune deviation towards type I cytokine production and production of molecules with immune dampening properties. Shiegeo and Kazuyo quoting other workers suggest that the relatively new cytokines, thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 from epithelial cells induce Th2 immune response against nematode and allergens. TSLP acts to induce dendritic cells (DCs) capable of differentiating naïve CD4+ T cells to Th2 cells (Zieglar *et al.,* 2013). These TSLP-activated DCs produce CXCL8 and CCL24 attracting neutrophils and eosinophils respectively and also Th2 attracting cytokine CCL17 and CCL22 are produced (Zieglar *et al.,* 2013). However, these DCs do not produce TNF, IL-1β, IL-6, IL-10 or IL-12. Induction of Th2 cells by TSLP-stimulated DCs depends on OX40-ligand specifically induced by TSLP (Tomoki *et al.,* 2005)

2.5 Nematodes in Co-infections.

The majority of mammalian and non-mammalian species are co infected with pathogens (macro- and micro-parasites) that interact with each other to modify pathogen transmission, virulence and resulting pathology (Murphy *et al.,* 2013; Maizels and McSorley, 2016).

Investigators, in Senegal and Ethiopia, reported that a high prevalence of coinfection with nematodes (including *Ascaris lumbricoides* and *T. trichiura*) was associated with the severity of malaria attacks, higher densities of malaria parasites and lower haemoglobin concentrations(Le Hesran *et al.,* 2004; Degarege *et al.,* 2012). However, in separate studies in Malian and Thai subjects, nematode infections were associated with protection from acute malaria caused by infection with *Plasmodium falciparum* (Lyke *et al.,* 2005), decreased severity of renal pathology and jaundice (Nacher *et al.,* 2001), diminished severity of cerebral malaria (Nacher *et al.,* 2000). A

prospective study in Mali of the effects of filarial co-infection on the clinical course of malaria found that pre-existent filarial infection conferred protection against anaemia without necessarily affecting the severity of the malarial infection itself (Dolo *et al.*, 2012).

Overall, these epidemiological studies suggest that nematode infection in humans may alter the development of malaria not only by increasing the replication of *Plasmodium* parasites but also by modulating the severity of the pathological sequelae associated with malaria. Helminth coinfection has been shown to alter the cytokine response to malarial antigens and extracts: nematode-co infected children exhibited an overall higher IL-10 response to an extract of infected erythrocytes or to recombinant malarial antigens, without a concomitant decrease in IFN-γ production (Hartgers *et al.*, 2009; Diallo *et al.*, 2010).

The nematode-induced immune response may have several components that compromise the immune responses needed to keep the co-infecting agent in check, which include production of T_H2 cytokines most of which are known to be anti-inflammatory, activation of T regulatory cells and impaired function of antigen-presenting cells (Hewitson *et al.*, 2009). However, those same mechanisms, through dampening the activation of cells of the innate and adaptive immune systems, may potentially impair the early stages of certain co-infection (Webb, *et al.*, 2012).

Nematodes also stimulate potent regulatory cell populations of the innate and adaptive systems which differ from the effects mediated by cytokines of the Th2 subset of helper T cells (McSorley and Maizels, 2012). In contrast, pathogenic micro-organisms typically trigger a type 1 immune response which results in elevation in IL-12, IL-23

and IFN gamma and IL-17 (Anthony *et al.,* 2007). The potent response develops rapidly which is critical for the control of potentially lethal pathogens that can rapidly proliferate and disseminate throughout the host a rapid response that may come with a huge cost namely tissue damaging inflammation.

Attempts by co-infected hosts to place in control two or more classes of pathogenic microorganisms that generate different and potentially conflicting effector cell responses as in prevalent concurrent infections with nematode and other micro parasites may create a clinical and immunological dilemma in their control and resolution (Sagalme *et al.,* 2013, Gorsich *et al.,* 2014). Helminth excretory-secretory products can directly influence antigen presentation suppressing differentiation of Th1 and Th17 cells which are pro-inflammatory and supporting the development of the generally anti-inflammatory Th2 and T regulatory cells (Lightowlers and Rickard 1988; Hewitson *et al.,* 2009; White and Artavanis-Tsakonas, 2012). On the other hand, a population of macrophages and T regulatory cells that produce IL-10 and TGFβ may dampen the immune responses targeting microbial pathogens (Ma *et al.,* 2015).

Specific stimuli characteristic of nematode invasion trigger different aspects of the overall type 2 immune response that may include induction of tissue injury by nematode which induces release of cytokine alarmins (IL-25, IL-33 and TSLP), that promotes production of Th2 cytokines by ILC2 cells and Th2 cells and also by eosinophils and Basophils (Maizels *et al.,*2012). Though nematodes trigger characteristic infections associated with the biology of the specific parasite, they all evoke immune responses that share common features which include type 2 cytokines (IL-4, IL-5, IL-9, IL-13, IL-21, IL-25(17E) and alternatively activated

macrophages (M2a) (Kreider *et al.*,, 2007). M2 macrophages can be divided into three groupings distinguished by activation factors *in vitro*: IL-4 or IL-13(M2a or AAMϕ) (Suzuki *et al*., 2017), IL-10 or glucocorticoids (M2b) or immune complexes plus /or TLR ligands (M2c) (Ambarus *et al.*, 2012). Exposure of macrophages to IL-4 and IL-13 suppresses M1 (classically activated macrophages) activation and diverts their differentiation to M2 (alternatively activated) phenotype (Van Dyken and Locksley, 2013). Excretory-secretory nematode products can shut down dendritic cell synthesis of pro-inflammatory cytokines (Salgame *et al.*, 2013; Lumb *et al.*, 2017), chemokines and costimulatory molecules and also promote dendritic cell production of the immunoregulatory molecules IL-10 and TGF-β (Lumb *et al.*, 2017). Nematode infections have been shown in *vivo* to induce differentiation of a population of regulatory DCs that favour the generation of T reg cells (Adalid-Peralta, *et al.*, 2013). Among antigen-presenting cells, DCs are known to be the most critical for the initiation and also the maintenance of protective T cell responses to many infectious pathogens. In contrast to the exposure of DCs to microbial ligands of Toll-like receptors, exposure of DCs to nematode excretory-secretory products fails to upregulate the surface expression of costimulatory molecules such as CD40, CD80 and CD86 (Lumb *et al.*, 2017) and does not result in the synthesis of proinflammatory cytokines and chemokines such as IL-12, CCL2 (MCP-1) and TNFβ. Exposure to nematode not only inhibits the proinflammatory activation of DCs but also promotes an alternative regulatory agenda, which could further diminish or dampen down T_H1 and cytotoxic T lymphocyte responses (Adalid-Peralta, *et al.*, 2013). After being exposed to nematode, DCs promote the differentiation of T regulatory cells from naive T cell precursors or the further expansion of pre-existing T regulatory cell subpopulations (Salgame *et al.*, 2013). DC production of TGF-β and/or IL-10 in

response to exposure to nematode excretory-secretory products may be the critical mechanism underlying the ability of nematode infection to promote the population expansion of T regulatory cells. The above mechanism acts in synergy to produce a nematode-modulated immunoregulatory environment that compromises Th1 and Th17 response but favours T regulatory cell activity. Through immune modulation, infections can increase host susceptibility to other parasites (Abu-Raddad *et al.*, 2006), enhance the intensity of other pathogens infections and increase disease duration. Immune modulation is particularly strong in mixed infections involving micro-parasites and macro-parasites infection (Cizauskas *et al*., 2014). Intracellular pathogen (micro-parasites) generally induce adaptive immunity to mobilize Th1 cell responses (pro inflammatory cytokines, namely IFN, TNF-α, Interleukin (IL2)) while extracellular pathogens (macro-parasites) usually trigger Th2 responses (IL4, IL5, IL6) (Duque and Descoteaux, 2014). The pathways leading to and from Th1-type and Th2 type responses are mutually cross-regulated and thus mammalian host have difficulty in simultaneously mounting effective Th1 and Th2 responses (Abbas *et al.,* 1996). Strong polarizing infections may establish an immunological milieu that suppresses or amplifies that would be appropriate responses to infections to a second pathogen.

Fox *et al.,* (2000) working with mice demonstrated that *Heligmosoides polygyrus* a murine nematode infection prior to co-infection with *Helicobactor felis* had a substantial decrease in gastric expression of Type 1 cytokines such as IFN, TNF-α, IL1-β and an increased expression of IL-4, IL10, and TGF-β and these changes were associated with attenuation of gastric atrophy a scenario that changed with coinfection. This was contrasted by infection with *Toxoplasma gondii* prior to infection with *Helicobactor felis* which showed enhanced Type 1 immune response and potentiated gastric inflammation, atrophy and metaplastic changes (Stoicov *et al.,* 2004). These

two studies among others demonstrates that immunomodulation either suppression or enhancement of systemic or local immune responses by concurrent infection may have either beneficial or deleterious effect depending on the predominant pathogenic mechanism of the individual pathogen.

Nematodes are able to down regulate Th-1 like responses (pro-inflammatory) due to their high immunomodulatory activity or induce Th2/T regulatory-type immune responses. Working with mice, O'Neill *et al.,* (2001) showed that indeed F. Hepatica inhibits the Th1-like response induced by *Bordetella pertussis* and similarly, Flynn *et al.,* (2007) showed that *F. hepatica* is able to change predictive value of the bovine tuberculosis diagnosis by modifying the immune response against *Mycobacterium bovis.*

Nematodes are able to influence the course of co-infection depending on the effector mechanisms against the co-infecting microbial pathogen by either increasing resistance to pathogens whose protection is mediated by the Th2-like response while making the host more susceptible to pathogens that elicit Th1 like immune response (Elias *et al.,* 2005; Moreau and Chauvin 2010). Increases in the levels of the IL-4 including IL-5, IL-9, IL-13 and IL-21), activation and expansion of $CD4^+$ Th2 cells , plasma cells secreting Ig E, with rise in eosinophils , mast cells and Basophils counts (all of which can produce several types of Th2 type cytokines) typically characterizes Th2-type responses (Anthony *et al.,* 2007). By contrast, microbial infection which includes bacteria and viruses typically evokes pro-inflammatory IFN-gamma dominated Th-1 type response associated with increases in the number of TH-1 cells cytotoxic $CD8^+$ T cells, neutrophils and macrophages.

2.6 Mixed nematodes infection in vaccination outcome

Ideal veterinary vaccines must be pure, safe, potent and effective and they must be economical to the end user. Affordable and efficient animal vaccines encourage disease prevention avoiding reliance on antibiotics an important public health issue. Veterinary vaccines will continue to be an important tool to protect human health, animal health, food safety, and food security and must be accessible and economical (Roth 2011).

Biotechnology provide for platforms producing modern vaccines ranging from plasmid-based DNA to plant-based expressed systems to be used along with the more conventionally produced live or killed vaccines for control of human and veterinary acute viral, bacterial or protozoan diseases (Urban *et al.,* 2007; Josefsberg and Buckland, 2012). Equally sophisticated delivery systems include designer adjuvant formulations (Prysliak and Perez-Casal, 2016; McElrath, 2017) that utilize TLR ligands and other innate immune activators. The goal is to provide rational vaccine design and delivery models that safely induces quantitatively and qualitatively improved cell mediated and humoural immune responses appropriate for protection from challenging natural infection (Duque and Descoteaux, 2014). Confounders are numerous and differ when the target is prophylactic vaccination of an individual versus reduced risk of transmission of highly contagious agents (Knight-Jones *et al.,* 2014).

When population or herd vaccinations are required, specific factors such as age, sex, nutrition, health status, and genetic composition become unmanageable (Urban, 2007). Threats from infectious epidemics, dangers of emerging, re-emerging and zoonotic diseases presents a huge challenge to public health stakeholders who in turn must

consider, bio-security threat from select agent exposures and a global setting where co-infection of humans as primary targets and animals as reservoirs are likely to carry parasitic nematode. Specific vaccination requirements are consequences of immune modulating interaction inherent to co-evolutionary development of parasitism (Urban, 2007).

This study was undertaken to investigate the influence mixed nematodes has on immune responses to native Mycoplasma antigens for which there is little documentation. However, some investigators using mice and animal models have shown that nematode infection can influence vaccine efficacy by modulating host immune responses in particular when Th1- like and cellular – dependent responses are required (Elias *et al.,* 2005; Van Riet *et al.,* 2007; Urban *et al.,* 2007; Stelekati and Wherry, 2012; Stelekati *et al.,* 2014; Haben et al., 2014; Zaph *et al*., 2014). Elias *et al.,* 2005 and Van Riet *et al.,* (2007) reported that *Schistosoma* sp and *Onchorcerca volvulus* infection decreases efficacy of vaccine against tuberculosis and tetanus respectively. Urban *et al.,* (2007) and Steenhard *et al.,* (2009) were also able to show, in separate investigations, that *Ascaris suum* co-infection alters efficacy of vaccine against *Mycoplasma hypopneumoniae.* Working with mice, Su *et al.,* 2006 reported that *H. polygyrus* was able to down regulate the strong immunity against *Plasmodium chaubadi* induced by blood-stage antigens. However, there is no documentation on effects of nematode infection on infectious caprine disease vaccines.

Effects of nematode infection on vaccine efficacy among other confounders must be taken into account when using vaccines and also when developing new ones in particular when choosing adjuvants that are able to counter-balance the immune-modulator effects of concurrent nematode infection.

CHAPTER THREE

MATERIALS AND METHODS

The experimental study was carried out at microbiology and parasitology laboratories of Veterinary Sciences Research Institute, KARLO, and Muguga North. The laboratory component involved the isolation and culturing of *Mycoplasma capricolum* subsp. *capripneumoniae* and mixed stronglyloid infective stage (L_3) larvae, mainly *Haemonchus* spp. used in experimental infection. The experimental goats were housed in pens isolated from others.

3.1 Microbiological Methods

3.1.1 Growth Media for the isolation of Mycoplasma

Selection, handling of samples and preparation media (Modified Newing's tryptose broth/agar) for Mycoplasma growth was done as described in OIE Terrestrial Manual 2008. Rechnitzer *et al*., (2011) observed that *Mycoplasma* have small genomic size and their guanine cytosine ratio is also very low (23 to 40 mol %), so that only part of the total genome is presumably used for expression of genetic information leaving little room for housekeeping genes and consequently, *Mycoplasma* typically require intimate association with mammalian cell surfaces and display a complex nutritional requirements for in vitro growth. The characteristic, small size, close association to mammalian cells, and resistance to penicillin, are used to design special strategies for the recovery of *Mycoplasma* from clinical samples.

Mycoplasma lack cell wall, it was necessary to add adequate levels of protein to the culture media to maintain osmolarity. The media was prepared free of contaminants that could inactivate enzymes on the exposed membrane of these organisms. Bacterial

filters under negative pressure coupled with addition of penicillin and Thallium were used to prevent contamination

In culture, *Mycoplasma* can meet energy requirements in several ways. Glucose can be processed by a glycolytic pathway; Arginine and active Acetate can be degraded; or urea can be hydrolyzed to provide energy.

Mccp among other *Mycoplasmas* require an external source of sterols and fatty acids, so horse/pig/ goat serum was added to the media to serve as growth stimulant. Meat digests, peptones, beef extract and yeast extract were added provide the nitrogen, vitamins, amino acids and carbon in the media.

Sodium chloride was added to maintain the osmotic balance of the media. Yeast extract provides the preformed nucleic acid precursors that are required by *Mycoplasma* spp. and it also provided a heat-stable, low molecular weight mixture of vitamins, purine, pyrimidines, and other essential nutrients. Thallous acetate and penicillin were also added to the media to selectively inhibit the growth of other organisms.

3.1.2 Preparation of liquid media

Reagents used in preparation of Newing's Tryptose Broth for *Mycoplasma* were:

Bacto tryptose (Difco) 2.00% (w/v), Dextrose 0.20%, NaCl 0.50%, Na_2HPO_4 (anhydrous) 0.25%, Yeast extracts (Difco) 0.10%, Glycerol 0.50%, topped up with distilled water to make 100%

The media was prepared according to the procedure as described (OIE, 2008)

Ingredients were weighed and dissolved in distilled water in a flask by steaming for about 20 minutes.

When completely dissolved, the broth was allowed to cool to room temperature.

Inactivated pig serum was added at the ratio of 1volume pig serum to 10 volumes broth, and then heated to 56^0 C for 30 minutes.

Penicillin to control gram positive bacteria was added at a concentration of 200 i.u crystalline penicillin G (Glaxo) per ml of broth. Thallium acetate (BDH Ltd) was then added to control gram –negative bacteria at 0.05%. The pH of the broth was adjusted to 7.6 and 10 ml per Litre of 0.1% phenol red was added as the indicator. The broth was then filtered in 500ml Duran bottles using Seitz (EKS) pads under positive pressure at 15lb per sq. inch. The broth was thereafter incubated at 37^0C for 48 hours to detect any contamination. Uncontaminated agar was stored at 4^oC until the time of usage.

3.1.3 Preparation of solid media (Tryptose Agar)

The preparation solid media (Tryptose Agar) consisted of two components, namely, agar and serum that were prepared in three successive stages as described below:

1. Stage 1-preparation of the agar component

Ingredients

Bacto tryptose (Difco) 2.00% (w/v), NaCl 0.50%, Na_2HPO_4 (anhydrous) 0.25%

Glycerol 0.50%, Distilled water to top up to 100%

The above ingredients were dissolved in a flask by steaming in a water bath for 20 minutes. Then the broth was allowed to cool to room temperature and the pH was adjusted to 7.6. Then to every 100ml of broth, 1.5 gm of Difco (Bacto) agar was added. The resultant broth was sterilized by autoclaving for 15 minutes at 10lb per sq. inch. The media was stored at 4^oC until use.

2. Stage 2- serum component

Pig serum was inactivated at 56^{o}C for 30 minutes only. Then to every 30 ml of inactivated serum, was added: Yeast extracts (Difco) 3 gm per litre, crystalline Penicillin G (Glaxo) 500,000 i.u per L, 1 ml 1 % stock solution of Thallium acetate.

3. Stage 3- mixing of agar and serum component

The serum and the additives were sterilized by filtration through Seitz EKS pads. The filtered serum and additives were briefly warmed to 56^{0} C. The stock tryptose agar were melted in a steamer and allowed to cool to 56^{0} C in a water bath. 30ml of the serum component was added to each 100ml of tryptose agar.

3.2 Biological Specimens- Lung tissue

1. Cultivation of *Mycoplasma* organisms

A small piece (approximately 5 gm) of lung tissue that was harvested from a goat suspected to have died from contagious caprine pleuropneumonia in Narok was cut and minced using a surgical blade. The piece was placed inside the broth and incubated at 37 ^{0}C for 24 hours. Each broth culture was sub cultured by inoculating fresh broth medium with one tenth of their volume (about 10 micro litres) and titrates to the third bottle and cultures are put in the incubator for further incubation. This broth bottles were observed for growth indicated by colour change and then plated every 3 days until growth was evident.

2. Steps for Isolation of *Mycoplasma* bacteria in the Laboratory from infected lung tissue specimens

Day 0

Tryptose agar media that was ready for use and stored at 4^{o} C was placed in the incubator at $37^{o}C$ for 30 minutes to warm up before inoculating it with suspect specimen tissue.

Using a butcher's knife flamed to keep with sterile procedures, a big incision in a lung lesion was made and then a scalpel blade was used to chop out and minced about 5g of lung.

Using a sterile forceps the 5g piece of lung was placed aseptically (the bottle is passed over the Bunsen flame, then opened slightly and the 5g lung material dropped into the broth bottle, the mouth of the bottle is reflamed and the cap replaced tightly) into warmed up media.

The bottle was labelled indicating the date, identity of the source and placed into a metal rack and this procedure repeated for each specimen material that was processed then the inoculated specimens were recorded into the laboratory log book. Inoculated bottles were incubated at $37^{o}C$ and broth cultures were examined daily for evidence of growth.

Agar plates to be used in subculture were incubated in a humid atmosphere at 5% CO_2, 95% air.

Day 1

The cultures were removed from the incubator and placed onto a cardboard as an insulator on the bench to prevent temperature shock to the cultures that could have risen from a change in incubator temperature of $37^{o}C$ to bench temperature. Without shaking the bottles, the cultures were examined for colour change and filament

growth. Observations were recorded in the laboratory log book. Presence of gross turbidity was considered to indicate bacterial contamination. Each broth culture was sub cultured by inoculating fresh broth medium with one tenth of their volume and titrates to the third bottle (1/40) and cultures are put in the incubator for further incubation.

Day 2

The Day 1 procedure was repeated for day 2 in that cultures were examined and observation(s) recorded.

Day 3

Tryptose agar plates matching the number of specimens to be sub cultured were placed in the incubator for 30 minutes to dry excess condensation water.

As the agar dried, broth cultures were examined as for previous days and findings recorded. Dry agar plates were then labelled and the corresponding broth cultures sub cultured onto the agar by streaking the medium with a loop then Agar plates were sealed with parafilm to avoid loss of moisture. The plates were placed in a polythene bag tied and then incubated at 37 degrees.

Day 4 to21

Broth cultures were examined daily for evidence of growth and observations recorded. Colour change from red to yellow was an indication of growth, but confirmation that it was Mycoplasma growing was done through plate culture examination.

Plate cultures were examined every 1-3 days using a stereo microscope (x5-50 magnification). Colonies were examined for morphology and the typical ‘fried egg’ colony characteristic for Mycoplasma. Plate cultures that were negative were discarded after 14 days, while broth cultures were discarded after 21 days.

3.2.1 *Mycoplasma capricolum* subsp. *capripneumoniae* identification

Growth inhibition test was carried to identify Mycoplasma using a method first described by in Terrestrial Manual (OIE, 2014). It is the simplest and most specific of the tests available. The culture to be tested was warmed to 37 ^{0}C for at least 30 minutes. Four dilutions of the culture were prepared as neat, 10^{-1}, 10^{-2}, and 10^{-3}.

At the same time an appropriate number of plates with Mycoplasma media were dried by placing them into the incubator for 1 hour.

Each plate was flooded with the different dilutions of each of the test cultures and the plates left to dry for 30 minutes in the incubator. Wells were punched on the plate and filled with corresponding anti-serum using a pipette. Plates were allowed to dry and incubated at 37 ^{0}C for 2-6 days with daily observations. The plates were examined for growth and inhibition diameters measured. Positive inhibition is regarded as a zone of 2 mm or more.

3.3 Parasitological techniques

3.3.1 Faecal Egg Counts

Modified McMaster Technique (Zajac and Conboy, 2012) was used on faecal specimens collected directly from the rectum of the experimental goats for Faecal Egg Counts (FEC).

1. Sampling Requirements:

In order to perform this exercise, protective gear such as gloves, overalls and gumboots were required.

2. Sampling and processing procedure:

The sample were collected per rectal using a lubricated gloved hand then labelled with date and identification. The samples were to be placed in faecal bags then put into cool box to await transport to the laboratory.

3 gram faeces were weighed, placed in a suitable plastic jars and then homogenized. There after 42 ml of tap water was added and mixed well and then strained into a beaker.

The contents were thoroughly mixed and 15ml of the mixture was then put into a test tube. This was centrifuged at 1500 rpm for 5 minutes. After which the resultant supernatant was discarded. The pellet was resuspended with floatation mixture and then mixed well. The two chambers of McMaster egg count slide were filled with approximately 1 ml and allowed to settle. And then observed under ×10

The number of eggs were counted in each chamber and added together then the total multiplied by 50. This gave the number of eggs per gram of faeces in the goat.

3.3.2 Mixed Strongyloid Larvae culture technique

Procedure

Faeces were placed in suitable plastic jars then thoroughly mixed with vermiculite until a moist crumbly consistency was achieved; Fair compaction of the mixture before wiping out the debris surrounding the sides of the container above sample mixture line was done and then covered using a parafilm. The parafilm was then perforated using dissecting needle to facilitate air circulation and moistening. The urine cups were then labelled indicating identity, date of culture, and date for expected harvesting. Incubation was done at 26^{o}C for 14 days with periodic turning and wetting using a water sprayer as necessary to prevent desiccation.

At the end of the incubation period the sample was removed and placed on the bench. Parafilm was removed and lukewarm water added to top up the container and left on the bench for 10 minutes before turning it upside down on the petridish. The petridish was filled with lukewarm water and the sample left on the bench overnight.

By use of a pipette, the fluid was sucked from the petridish, a drop placed on another petridish, examined under a dissecting microscope to confirm presence of larvae and identification then all the larvae harvested as much as possible, placed in a falcon tube (about 20 mls) and then kept at 4 degrees centigrade in a fridge until use.

3.4. Experimental goats

Sixty-four CCPP free and unvaccinated small East African goats, aged between 9-12 months, were used in the study. The goats were bought from, Dol Dol, Laikipia County, and an area known to be free from the *Mycoplasma* disease and had not been vaccinated against CCPP. This status was confirmed serologically by latex agglutination and complement fixation tests. The goats were also screened for nematode infection by faecal egg counts.

The goats were then transported to Veterinary Sciences Research Institute- Muguga North. Upon arrival, they were weight and dewormed with Albendazole (Valbazen^{R} Norvatis) at 10mg/Kg body, ear tagged and vector control initiated. The goats were housed in well ventilated pens with provision for feed and drink, fed on good quality hay, mineral lick and on appropriate concentrates *ad libitum* and put under observation for four weeks.

3.4.1 Experimental Design

The goats were randomly allocated to four groups, (A, B, C, D) of 10 animals each. Nematode status was confirmed after the observatory period of 28 days.

Identification of the cultured mixed larvae by morphological differentiation before inoculation showed that the *Haemonchus contortus, Trichostrongylus* spp. and *Oesophagostomum* spp. were the most prevalent. To determine the effect of mixed nematode infection on immune responses to *M. capricolum* subsp. *capripneumoniae* vaccine antigens, after the initial 4 weeks for acclimatization, forty

(40) goats were randomly placed in four groups and nematode infection was done as follows:

Group A (Aw) were given 3000 infective stage (L3) larvae per os

Group C (Cw) were also given 3000 infective (L3) larvae per os

Groups B (Bnw) and (Dnw) were given normal saline per os

This is tabulated in table 3.1.

Table 3. 1: Mixed nematode larvae infection and vaccination with inactivated *Mccp* vaccine

Time (Days)	**Group A (n=10)**	**Group B(n=10)**	**Group C(n=10)**	**Group D(n=10)**
0(28th day of study)	Mixed larvae infection(300 L_3/ml of water then 10 ml pbs)	phosphate buffered saline	Mixed larvae infection (300 L_3/ml of water then 10 ml pbs)	phosphate buffered saline
35	EPG done and repeat nematode inoculation(100 L_3/ml of water then 10 ml pbs)	EPG done and repeat saline	EPG done and repeat nematode inoculation(100 L_3/ml of water then 10 ml pbs)	EPG done and repeat saline
42	EPG done	EPG done	EPG done	EPG done
49	Phlebotomy for serology. Vaccinated against CCPP	Phlebotomy for serology. Vaccinated against CCPP	Phlebotomy for serology and PBS	Phlebotomy for serology and PBS
56	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology
63	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology	Observation and Phlebotomy for serology
Daily Observation, weekly bleeding for sera and faecal collection every fourteen days for egg per gram analysis was done for 35 days then the experiment was terminated after 63 days.				

EPG- egg per gram. PBS- phosphate buffered saline CCPP-contagious caprine pleuropneumonia.

Goats in group A consisted of worm free animals artificially infected orally with 3000 mixed cultured larvae suspended in 10mls of water (300 L_3/ml) then flushed down with 10 ml of phosphate buffered saline (PBS). A repeat inoculum of 100 L_3/ml

contained in 10 ml of water was given a week after the first treatment. Three weeks after inoculation with infective larvae (49th day of study) the group was vaccinated with 1 ml of inactivated *Mycoplasma capricolum subsp. capripneumoniae* vaccine (caprivax^{R}) from Kenya Veterinary Vaccine Production Institute (KEVEVAPI).

Animals in group B consisting of ten goats (n=10) were screened for worms to confirm their nematode status as in group A and observation continued. Animals in this group were inoculated with given 10 ml of normal saline without nematode. Vaccination with *Mycoplasma* antigen was done on the same day (49th day of study) as described for group A.

Animals Group C consisting of ten goats (n=10) were inoculated with infective stages of nematodes (L_3) orally- 10mls of water containing 3000 larvae (300 L_3/ml) then flushed down with 10 ml of phosphate buffered saline. A repeat inoculums of 100 L_3/ml contained in 10 ml of water was given a week after the first treatment. This group of animals was not vaccinated.

Group D goats were 10 mls of phosphate buffered saline orally but were neither inoculated with worms nor vaccinated with Mycoplasma antigen.

All the goats in the four groups were bled for sera on weekly basis. Faecal samples for egg per gram counts were taken per rectal every fourteen days. The sera was frozen to be used later to monitor seroconversion in response to the vaccine using cELISA.

3.4.2 Experimental infection- inoculation with *Mycoplasma capricolum capripneumoniae*.

24 goats with natural worm infestation were used in this experimental study. They were randomly placed into four groups namely E_1, E_2, F_1 and F_2.

Groups E_1 and F_1 were dewormed with Albendazole (Valbazen^R Norvatis) at 10mg/Kg body. Groups E_2 and F_2 were not dewormed. All goats were under observation for 28 days after which they were treated as shown in the table 2.

Table 3. 2: Experimental infection- inoculation with *Mycoplasma capricolum capripneumoniae*

Time (days)	Group E_1 (not infected)	Group E_2(not infected)	Group F_1(contact only)	Group F_2(contact and worm infested)
Day 0	-Bleeding and faecal sample -Given 30ml *Mccp* culture, 15ml agar and 10 ml PBS	-Bleeding and faecal sample -Given 30ml *Mccp* culture, 15ml agar and 10 ml PBS	-Bleeding and faecal sample -Not inoculated	-Bleeding and faecal sample - Not inoculated
Day 7	-Bleeding for serum	-Bleeding for serum	-Bleeding for serum -Brought together with the infected group	-Bleeding for serum - Brought together with the infected group
Day 14-49	-Bleeding for serum - Faecal sample	-Bleeding for serum - Faecal sample	-Bleeding for serum - Faecal sample	-Bleeding for serum - Faecal sample

Group **E_1** consisting of 6 goats without detectable nematode were inoculated intratracheally with 30 ml of inoculum containing *M. capricolum capripneumoniae* organisms passaged to level 3, then 15 ml of agar followed by 10 ml of phosphate buffered saline as described by Wesonga *et al.*, (2004). Another set of animals, **Group E_2**, made up of 6 goats with mixed strongyloid nematodes was also inoculated intratracheally with 30 ml of inoculum containing *M. capricolum capripneumoniae* organisms passaged to level 3, then 15 ml of agar followed by 10 ml of phosphate buffered saline as described above.

A group of six goats (**Group F_1**) without detectable nematode was introduced on day 7 for contact transmission of the infectious agent animals and to monitor the course infection. Six other goats (**Group F_2**) with mixed strongyloid nematodes were also

introduced for contact transmission to see the effect of nematode on the clinical, antibody response and pathology to *M. capricolum capripneumoniae* infection.

The course of infection was monitored through clinical examination for vital parameters such as rectal temperature, feeding, cough, nasal discharge, respiratory rates and sounds recorded at 8.30-10.00 AM for 35 days. Fever, severe clinical signs for 5 or off-feed 2 consecutive days respectively necessitated killing the experimental animals. Post mortem findings for the euthanized animals were recorded, blood for serology, pleural fluids and lungs specimens for culture were collected. Blood for serology was collected on weekly basis until termination of the trial for the surviving goats.

3.5 Post mortem examination

Goats killed on welfare reasons or at the end of trial were carefully opened. Pleural fluid was collected in sterile falcon tubes, thoracic cavity examined and lung pathology assessed noting any fibrin depositions, consolidation and adhesions. Lung samples were taken aseptically. Mediastinal lymph nodes were examined and airways opened up for examination.

The abdominal cavity was also examined for unusual lesions. The abomasums and the entire intestinal tract were taken out for worm recovery, count and identification.

Other organ systems were routinely examined to rule out any other cause of disease.

3.5.1 Lesion scoring

To assess the resulting pathology subsequent to intra-tracheal inoculation and in-contact infection with *M. capricolum capripneumoniae*, a comprehensive post mortem examination was performed at the termination of the study or euthanasia of experimental goats that were off feed for two consecutive days or running a fever of $\geq 41^0$C for 5 days incessantly.

The thoracic cavity was carefully opened and pleural fluid (when present) was harvested into sterile Falcon tubes. The lesions sought after included fibrin deposition on either parietal or visceral surface of the organs of the thoracic cavity, fibrous adhesions, hepatization (consolidation) whether red or grey, abscessation, involvement of the Mediastinal or bronchiole lymph nodes, haemorrhages, oedema or emphysema. Lesions were observed under natural light. Consolidation, adhesions and deposition were scored on a scale of 0-4 as follows:

Table 3. 3: Modified lesion scoring scale

LUNG LESION					
	No lesion	Mild lesion	Moderate lesion	Severe lesion	Extremely severe lesion and death
SCORE	0	1	2	3	4

This approach has been used in scoring pathological lesions caused by pathogens in other species other than goats. Even though it may not indicate virulence or pathogenicity of the infecting organism, it gives a reflection of the extent of the pathology resulting from all the factors brought into play following disease causation that includes those associated with host, agent and environmental factors.

The above scoring method was used in combination with the method used by Wesonga *et al.,* (2004) whereby lung lesions were classified as focal, multifocal or extensive affecting substantial part of each lobe of the lung and whether unilateral or bilateral.

3.6 Blood sample analysis and application of cELISA

Blood samples were investigated for serum late antibodies to *M. capricolum capripneumoniae* antigen on vaccination and latter on challenge infection by a modification of an ELISA test used as described by Wesonga *et al.,* (2004) using

competitive ELISA (c-ELISA). Blood specimens were taken fortnightly from all goats throughout the entire period of infection for testing at the end of experimental period.

c-ELISA contagious caprine pleuropneumonia (CCPP) kit is an enzyme immunoassay for the detection of antibodies directed against *Mycoplasma capricolum capripneumoniae* in individual caprine serum samples. The kit provides a specific detection using a monoclonal antibody "4.52" as described by Thiaucourt (1994). It detects antibodies that will appear following an infection or after immunization with a relevant CCPP vaccine. It can measure antibodies following a vaccination and allow for an evaluation of the vaccine or vaccination campaign quality.

The principle of the test involves micro plate coated with a Mccp purified lysate. The samples to be tested are premixed with a specific monoclonal antibody Mab4.52 in a preplate and the content transferred into the coated micro plate. Mccp specific antibodies present in the sample forms an immune-complex with Mccp antigen coated on the micro plate, competing with Mab4.52 for specific epitopes. The unbound material is washed away and anti-mouse antibody conjugate is added. In the presence of immune-complex between Mccp antigen and antibodies from the sample, Mab4.52 cannot bind to its specific epitopes and the conjugate is blocked from binding to Mab4.52. Conversely in the absence of Mccp-antibodies in the test sample, Mab4.52 can bind to its specific epitopes and the conjugate is free to bind to Mab4.52. Unbound conjugate is washed away and the enzyme substrate (Tetramethylbenzidine - TMB) added. In the presence of the enzyme, the substrate is oxidized and develops a blue compound becoming yellow after blocking. Subsequent colour development is inversely proportional to the amount of ant-Mccp antibodies in the test sample. The result for one test sample is expressed in percentage of inhibition by comparing the

optical density in the test well with the optical densities in the Monoclonal antibody control wells.

3.7 Serum Proteins Assay

Part of the collected sera was analyzed for globulins and albumins in goats from the various groups using an automated reader to determine if there was a significant difference in plasma proteins levels between the nematode infected vaccinates and non vaccinate groups.

3.8 Statistical Analysis

All data analyses were performed using R statistical package (Rx64 3.2.4 Revised) Analysis of variance was performed to ascertain the difference among and between the group means of antibody response to *Mycoplasma* vaccine and *Mycoplasma* bacterium antigen in goats with or without nematode. Serum protein levels were tested with a one way analysis of variance (ANOVA) to determine difference between the experimental groups P values less than 0.05 were statistically significant. To establish which of the groups differed from the others, a Post Hoc Test, Tukey honestly significant difference (HSD) test multiple comparisons of means giving 95% family-wise confidence level was performed on all sets of data.

3.9 Ethical consideration

Animal experimentation was conducted at the animal isolation facility run by KARLO- Veterinary Sciences Research Institute- Muguga North in agreement with stipulated laws on animal welfare and safety. Invasive procedures were performed by veterinarian (lead investigator) or veterinary paraprofessionals under supervision in strict adherence to aseptic techniques and laid procedures. Humane endpoint was when an experimental subject experienced 5 consecutive days of high fever (> 39.3^0C) or

off-feed for 2 days whereby a captive bolt stunning and exsanguinations was used to put the animal down.

This research was approved by the Institutional Animal Care and Use Committee of KARLO-Veterinary Science Research Institute, Muguga North upon compliance with all provisions vetted under and coded: KALRO-VSRI/ IACUC012/22092016.

CHAPTER FOUR

RESULTS

The results presented emanate from two sets of experiment involving a total of sixty four goats. In the first experiment, forty goats were used to investigate the effect of nematode infection on antibody response to killed *M. capricolum* subsp. *capripneumoniae* vaccine antigens. The second set of experiments involved 24 goats to generate data on the effect of worm infection on antibody response to live *M. capricolum* subsp. *capripneumoniae* antigens and the resulting pathology.

4.1 Faecal egg counts following experimental mixed nematode larvae infection

The 8 week long trial yielded the data that were analyzed and given in figures shown downstream.

The faecal egg count in groups A (Figure 4.1) increased with time from the initial count of non detectable egg per gram to the 8th week when the trial was terminated. There were detectable egg counts from the end of the second week post inoculation and subsequent exponential increase followed.

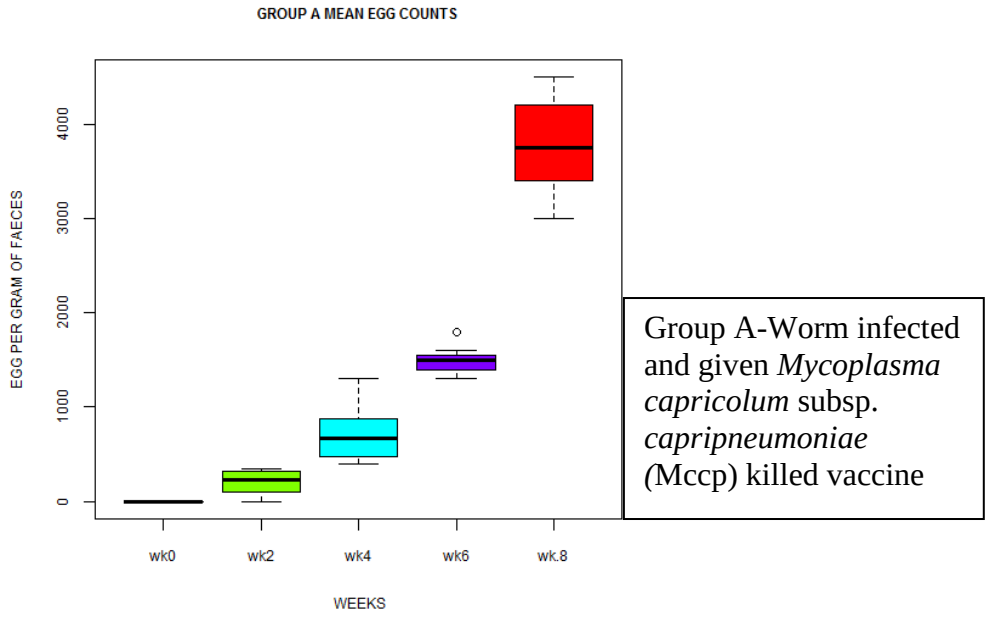


Figure 4. 1: Group A egg count in 8 week duration

Figure 4.1 shows a steady rise in faecal egg count from the end of the second week post larval inoculation reaching a highest recorded count of 4500 eggs at the end of the 8th week. The mean egg per gram for group A increased from non detectable in the first week to a mean of 3775 at the end of eight week when trial period ended.

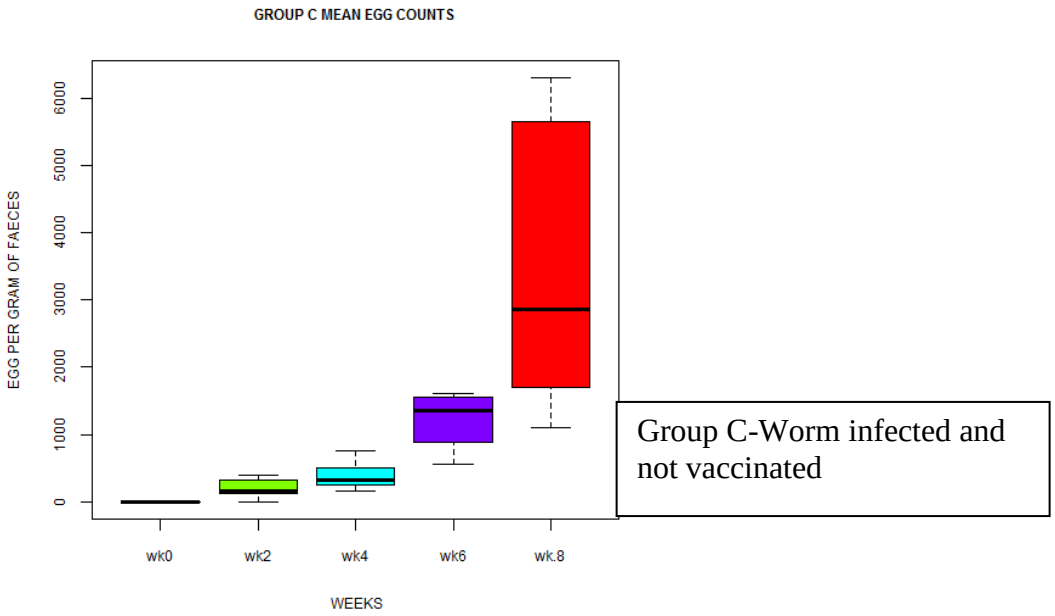


Figure 4. 2: Group C egg count in 8 week duration

The plot above shows a steady rise in faecal egg count at end of the second week post larval inoculation reaching a high count 6300 eggs in some goats accompanied with clinical signs that included pallor, submandibular oedema and loss of appetite. The same scenario as with Group B was also observed with group C having a mean of 3475eggs at the end of the 8th week. Interestingly, though the two groups were given the same dose of infective larvae, housed together with same feeding regime there were more outliers faecal egg counts from group C than with group A though the difference in means between the groups was not statistically significant. Both groups showed a similar growth pattern in faecal egg count as the worms matured.

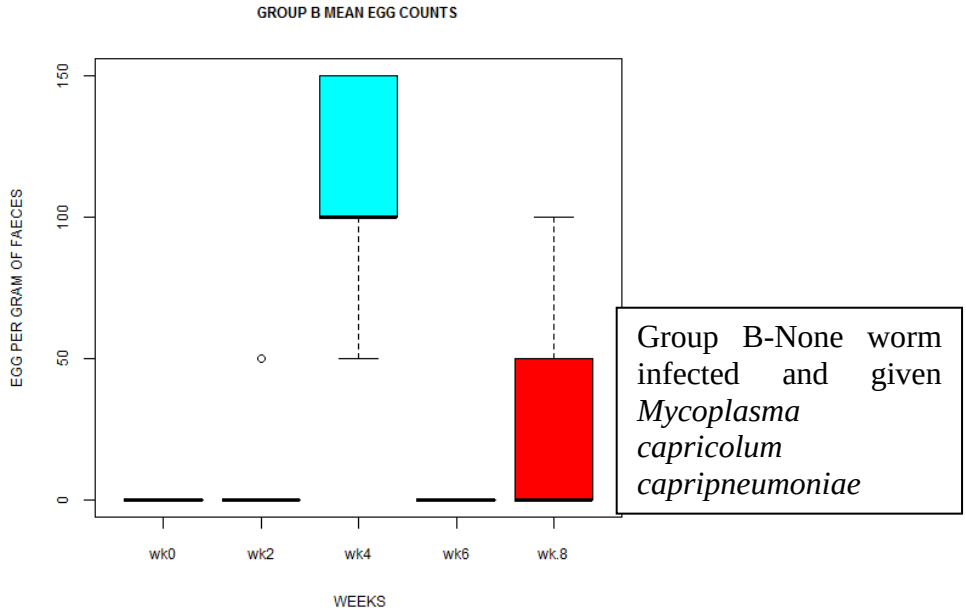


Figure 4. 3: Group B egg count in 8 week duration

Starting with non-detectable faecal egg count, some Group B goats' droppings had detectable nematode eggs at the end of the 4th and 8th week with the highest count being 150 and 100 eggs per gram of faeces respectively. Attempts were made to maintain them at zero egg per gram (epg) count were futile. On the 4th week the mean egg per gram for count for group B was 105.6.The goats were then dewormed with Albendazole (Valbazen^{R} Norvatis) at 10mg/Kg body to reduce worm infection to the lowest levels.

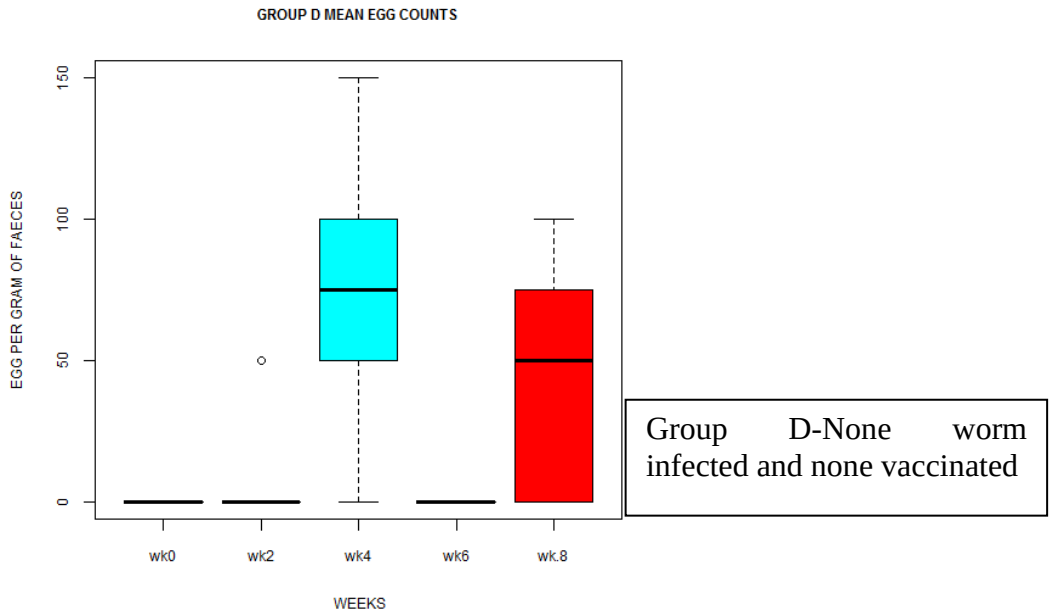


Figure 4. 4: Group D egg count in 8 week duration

Groups D had minimal but detectable egg counts with peaks on the 4th and 8th weeks of the trial. The peak count was 150 and 100 eggs per gram of faeces in each instance at the end of the 4th and 8th week respectively. Both group B and D were housed together and though they were supposed to be worm free there is a possibility that some goats had some hypobiotic parasites that started laying eggs by the fourth week or drug resistance was in play. In this study it was observed that dewormed goats maintained non-detectable faecal egg count status for only 4 weeks after which low levels were recorded.

The group means for the four treatments were plotted, as shown in Figure 4.5

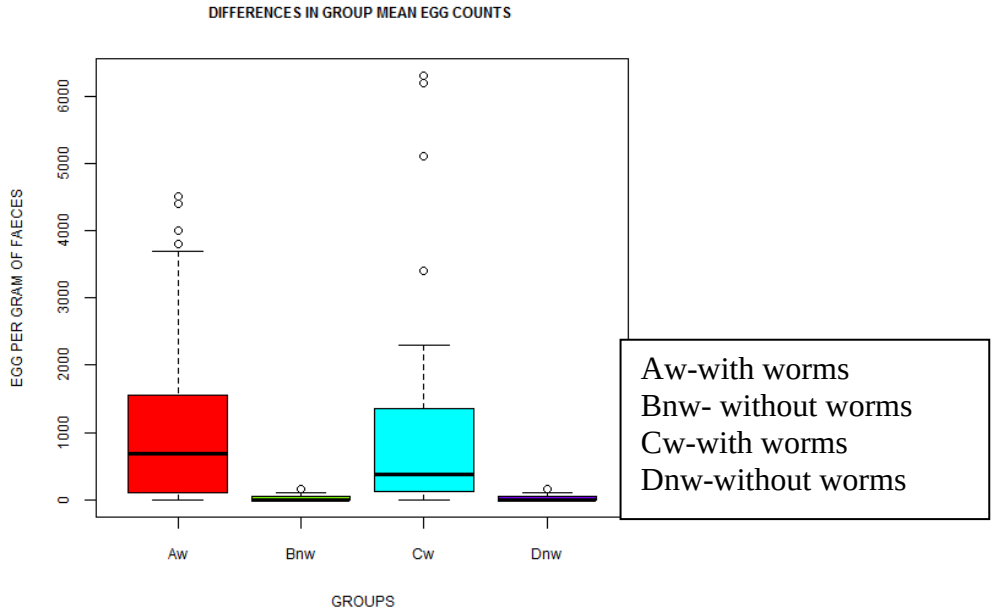


Figure 4. 5: Egg count variation between groups

One-way analysis of variance (not assuming equal variances) was run and yielded an output F value 15.146 and p value 7.098e-^{08} which is less than 0.05. There is a statistically significant difference between the group means.

To determine which groups are different from the others, a Tukey honestly significant difference multiple comparisons of means, a post hoc test or a post hoc pair wise comparison was performed with the output given below.

Table 4. 1: Tukey multiple comparisons of means for paired groups Aw, Bnw, Cw and Dnw

	Diff	**95% C.I**		**p-value**
		Lower	**Upper**	
Bnw-Aw	-1212.222222	-1803.8844	-620.5601	0.0000021
Cw-Aw	-186.250000	-795.0654	422.5654	0.8569985
Dnw-Aw	-1215.000000	-1823.8154	-606.1846	0.0000039
Cw-Bnw	1025.972222	434.3101	1617.6344	0.0000755
Dnw-Bnw	-2.777778	-594.4399	588.8844	0.9999993
Dnw-Cw	-1028.750000	-1637.5654	-419.9346	0.0001211

Aw-with worms
Bnw- without worms
Cw-with worms
Dnw-without worms

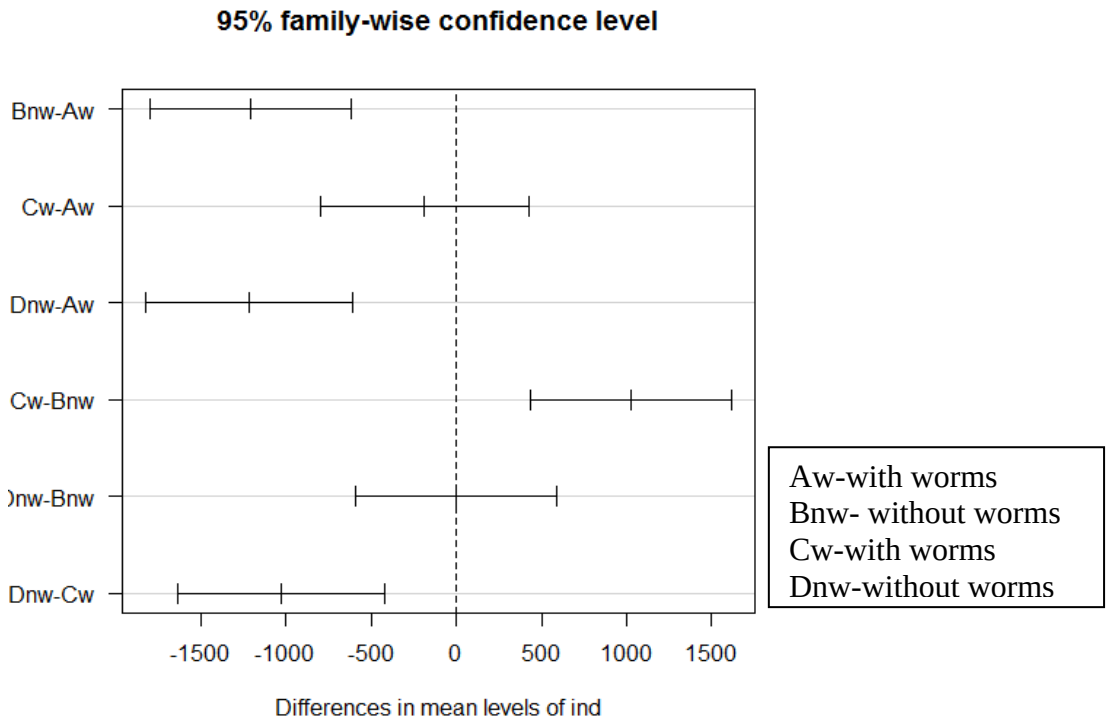


Figure 4. 6: Differences in mean faecal egg counts between paired groups.

From the table 4.1 and Figure 4.6, it is clear that the differences in means are significantly different between the groups Bnw-Aw, Dnw-Aw, Cw-Bnw and Dnw-Cw only. But there was no statistically significant difference in means between groups A and B though in the actual faecal egg counts group C had some of the highest egg per gram counts.

4.2 Leukocyte counts following experimental mixed nematode larvae infection and vaccination

4.2.1 Neutrophil count

A point leukocyte count was also performed to determine if there are variations in white blood cell count between vaccinated and none vaccinated goats with or without nematode infection.

Neutrophils are essential effector cells in response to tissue damage due to acute bacterial and a number of other pathogenic microorganisms. The polymorphonuclear cells kill or inhibit proliferation of many pathogens through generation of an array of reactive oxygen species along side or with microbiocidal peptides. Neutrophil count was done for the various groups and their means compared to determine any variation among the groups.

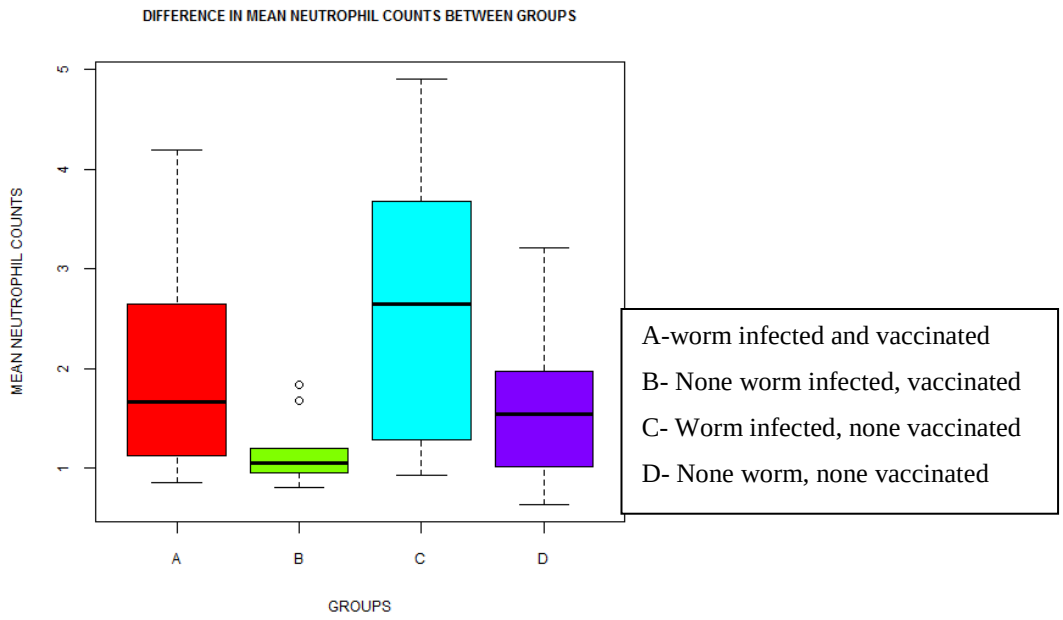


Figure 4. 7: Mean neutrophil counts in vaccinated and non-vaccinated nematode and non-nematode infected goats.

Figure 4.7 show that inter-group and intra-group means are different (some less, others more). Group C had the highest variation between themselves with group B individuals having the least intra-group mean variation. In this case it was necessary to perform analysis of variance so that differences can be brought out clearly.

From one-way analysis of variance in neutrophil counts group means the output was F-statistic = 3.0549 with a p-value equal to 0.04293 which is less than 0.05. There is a

statistically significant difference between the groups' neutrophil means with and without nematode infection. Nevertheless, this finding obligated further analysis to isolate where the difference between the groups lay.

To ascertain the groups that differed from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was conducted. The result of Tukey multiple comparisons of means analysis showed that the difference in means between group C and B (p-value = 0.0301684) is significant statistically, but there is no statistically significant difference between groups A and B, A and C, A and D, B and D, C and D (p-values =0.4104347, 0.5301547, 0.9052139, 0.8321904 and 0.2096518, respectively). The results of post hoc test were further plotted, Figure 8, showing 95% family- wise confidence level chart. The only line away from zero line was that comparing groups C and B confirming there statistically significant difference ($p<0.05$). The small variations within the other groups were statistically insignificant.

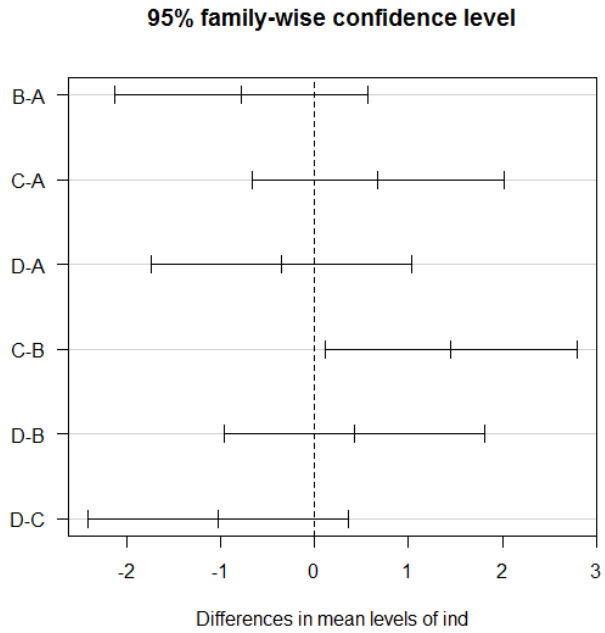


Figure 4. 8:Pair-wise comparison of means of neutrophil counts

4.2.2 Lymphocyte counts

The lymphocyte counts were compared within the groups and intergroup and the group means analyzed to find out if infection with nematodes has an influence on seroconversion.

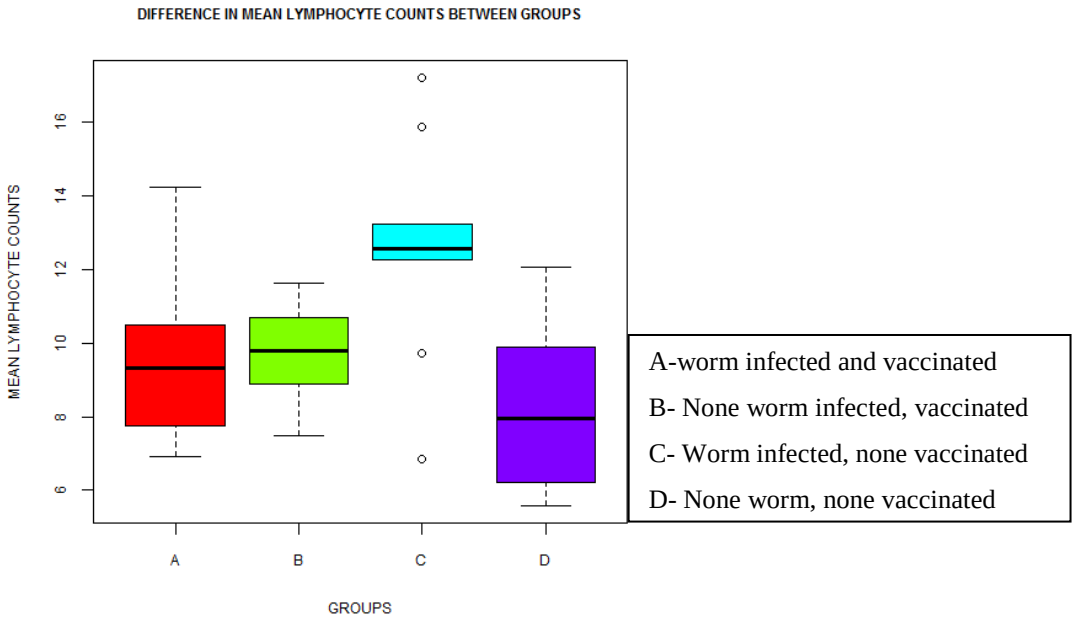


Figure 4. 9: Difference in mean lymphocyte counts between groups.

Figure 4.9 shows that group variance of means are different from each other and even within the groups. There was overlap of values between some groups. The differences in means between some groups could only be by chance and may not be significant statistically. In this case to performance analysis of variance was done so that the differences could be highlighted.

One-way analysis of variance in lymphocyte count means output gave F-statistic as 5.2502 with a p-value equal to 0.004787 which is less than 0.05. There is a significant difference between the groups with and without nematode infection. Nonetheless, this

finding compelled us to perform further analysis to isolate where the inequality between the groups rested.

To establish which of the group differed from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was performed (Figure 4.10) and showed that only groups C-A, C-B and D-C were the only paired group means that showed a significant difference.

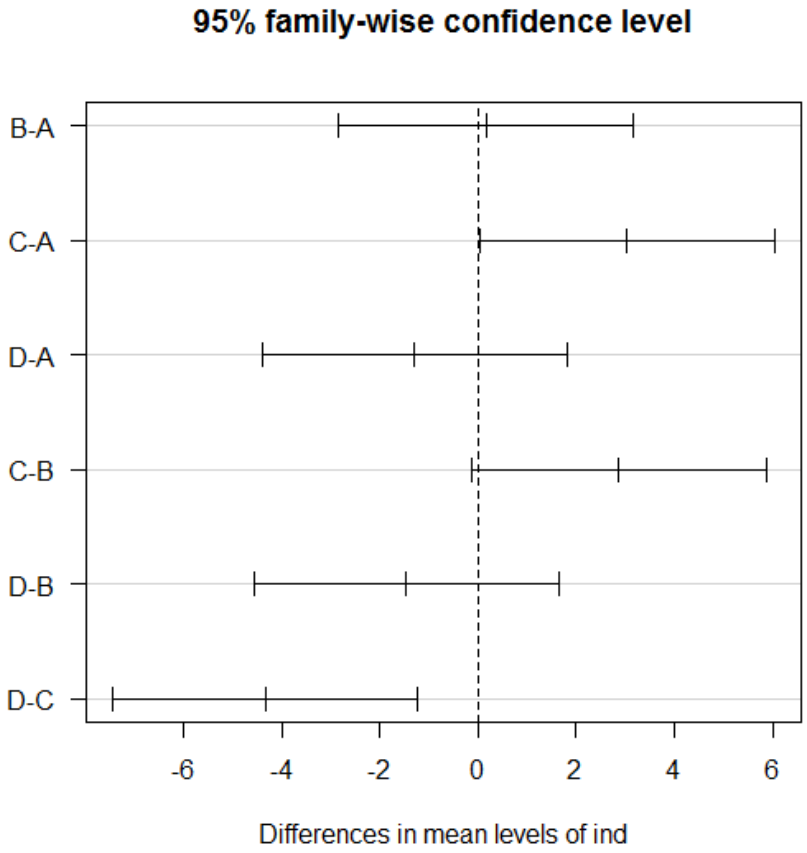


Figure 4. 10: Comparison of lymphocyte counts group means

4.2.3 Eosinophil counts

The eosinophil counts were compared within and between the groups. The group means were analyzed to find out if infection with mixed nematodes had an influence on eosinophil counts in the vaccinated groups.

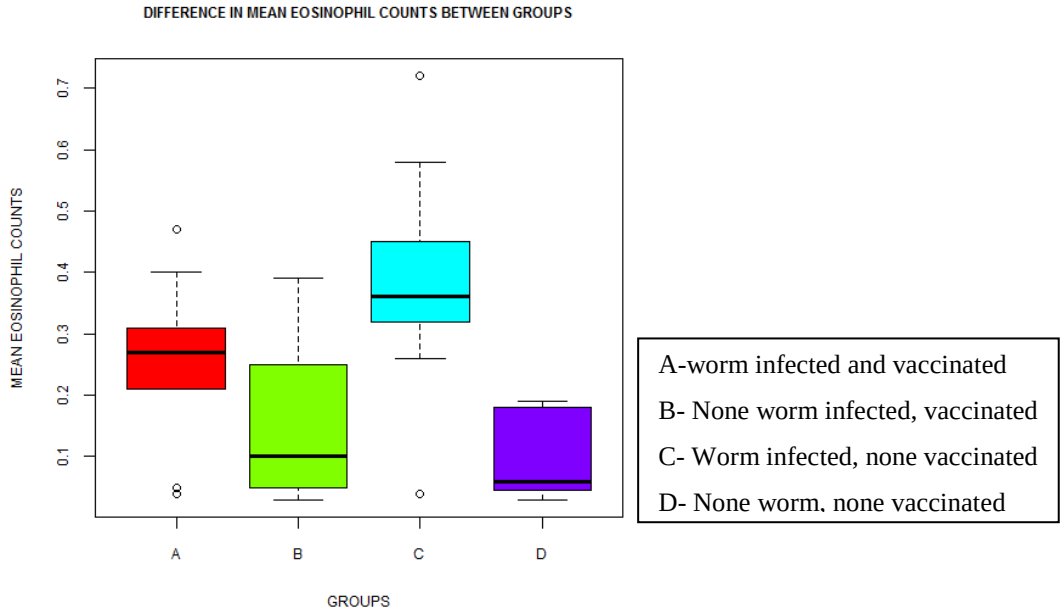


Figure 4. 11: Mean eosinophil counts between groups.

Groups A and C showed greater intra-group and intergroup variation in Eosinophil counts suggesting an impact from nematode infection on this leukocyte component of innate immunity towards metazoan parasites. Outliers were a prominent feature within each group.

From one-way analysis of variance in group eosinophil means output, the F-statistic is 6.7368 with a p-value equal to 0.001248 which is less than 0.05. There is a significant difference between the groups with and without nematode infection

To establish which of the group differed from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was performed that showed statistically significant differences only in paired groups C-B and D-C with p values = 0.0075966 and 0.0015341respectively. The others though

they differed from each other the variation was insignificant may be attributed to chance

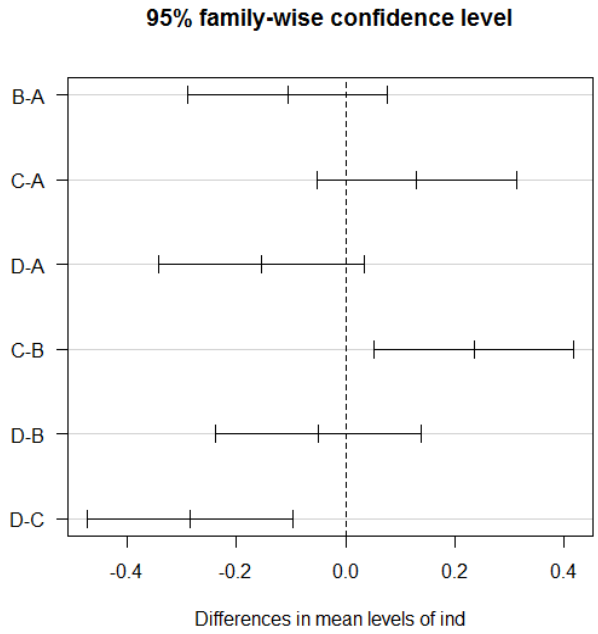


Figure 4. 12: Differences in eosinophil count group means

4.2.4 Monocyte count

Figure 4.13 shows that group C had a higher range in mean count values followed by Group A with outliers in both group A and C, suggesting that the nematode infected groups showed a wider range compared to non- worm individuals.

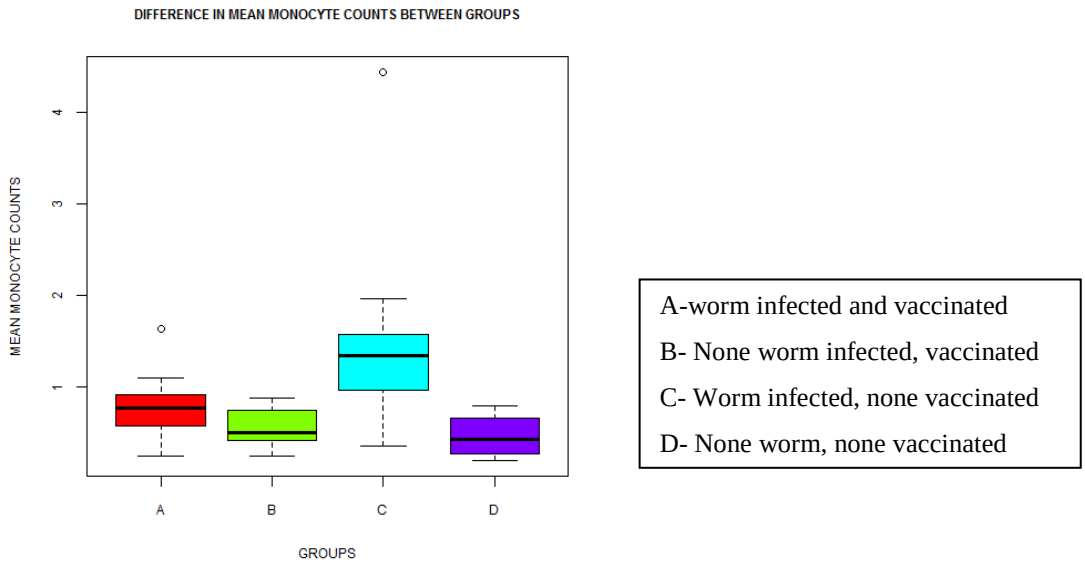


Figure 4. 13: Difference in mean Monocyte counts between groups.

A one way analysis of variance in group Monocyte count means was performed for the groups to find out if there was agreement in the said difference.

From the output the F-statistic is 5.0016 with a p-value equal to 0.006058 which is less than 0.05 hence there is statistically significant difference. There is a significant difference between the groups with and without nematode infection. Even so, this finding calls for further analysis to keep apart inequality between the groups. To establish which of the group differed from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was performed as with previous analytes. Groups C-B and D-C are the only paired group means that showed a significant difference.

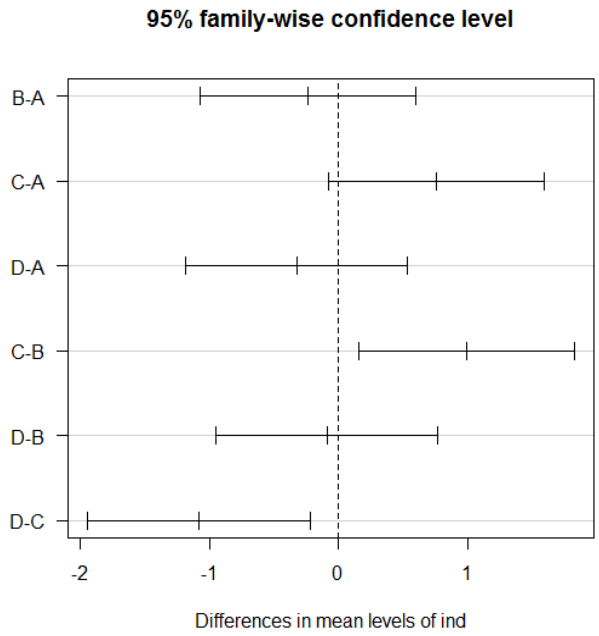


Figure 4. 14: Comparison of Monocyte counts group means

4.2.5 Basophil counts

Whole blood was taken from the goats for full haemogram with the basophil component for the entire groups being also subjected to analysis as with other haemogram components. A box and whisker plot (Figure 4.15) shows the output.

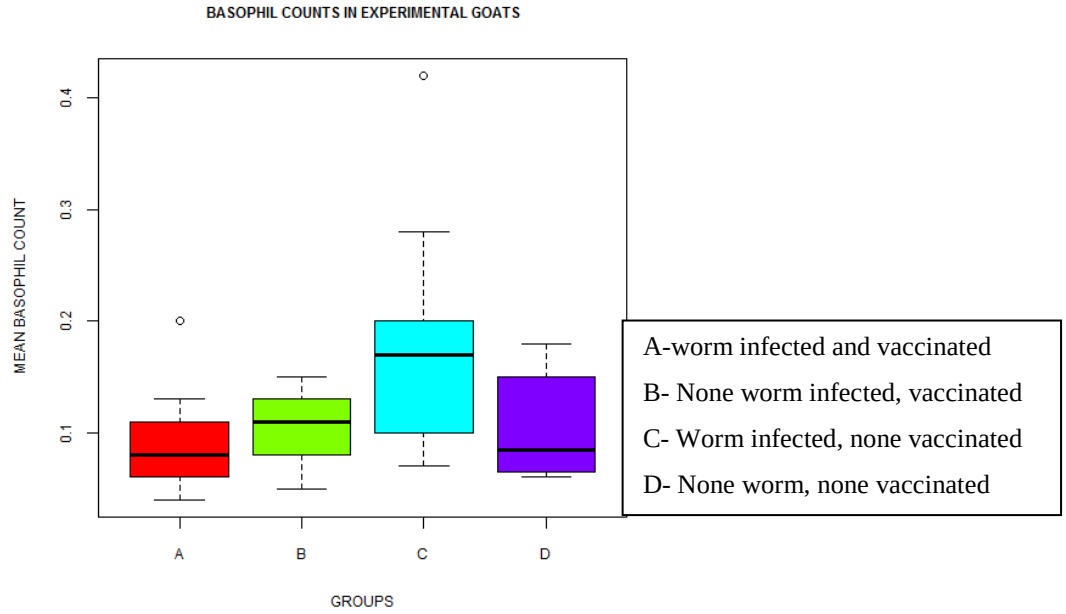


Figure 4. 15: Mean Basophil counts in experimental goats.

A one way analysis of variance in basophil group means was then performed for the groups to find out if there was agreement in the said difference.

From the output the F-statistic was 3.3947 with a p-value equal to 0.03006 which is less than 0.05 hence there is statistically significant difference. There is a significant difference between the groups with and without nematode infection. Despite that, this finding called for further analysis to isolate point of inequality between the groups.

To determine which one of the group differed from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was performed. Figure 4.16 shows the analysis output of the post hoc test.

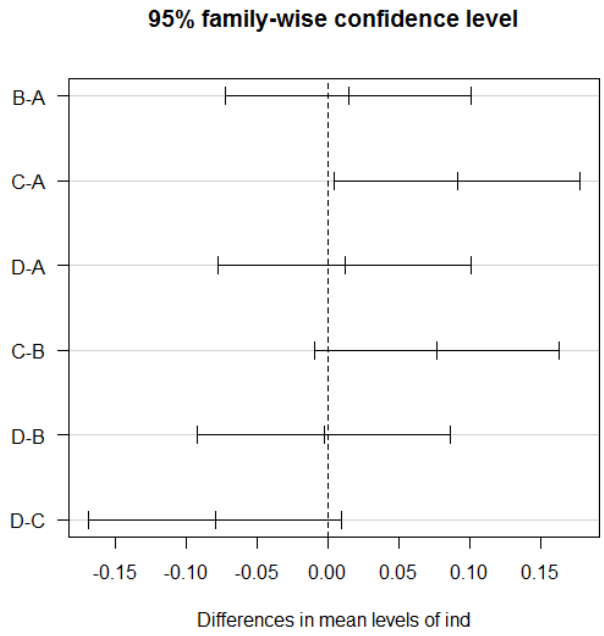


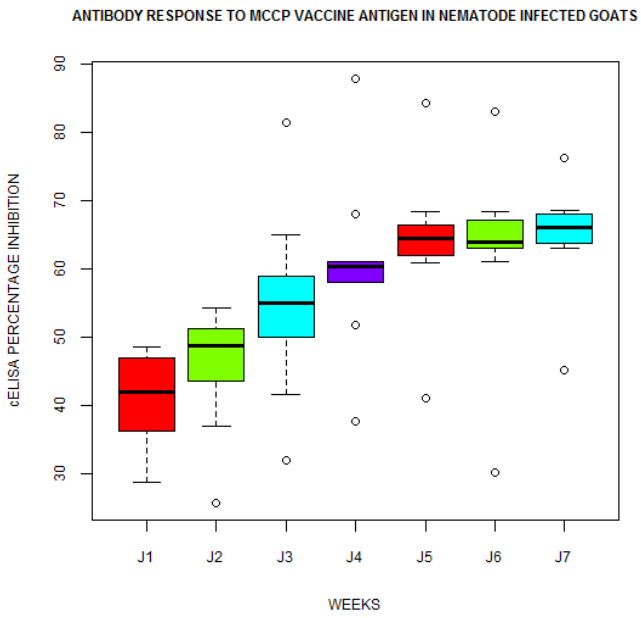
Figure 4. 16: Comparison of basophil counts group means

Figure 4.16 showing pair-wise comparison of basophil counts group means and 95%family-wise confidence level confirms that the only pair that showed significant variation were C-A (p-value=0.0360248) and that the other pairs showing little variation had no statistically significant difference.

4.3 Immune response to inactivated *M. capricolum capripneumoniae* vaccine antigen

Goats in groups A and B were vaccinated with an inactivated *M. capricolum capripneumoniae* vaccine. Groups C and D were given phosphate buffered saline as a placebo. Blood for sera was collected weekly and the end of trial. A cELISA was run on the samples to determine seroconversion and hence detect antibodies that appeared after vaccination in response to the killed vaccine antigens if any in the groups. A calculated inhibition percentage of >50%, as set by the producer of the kit, was deemed significant suggesting that there was an immune response to vaccine antigens.

At the end of the experiment all the sera were prepared and ran in one 96 well micro plate and the absorbance or optical density converted to percentage of inhibitions for each of the goat serum reading. For this test, positivity was set at 50% of percentage of inhibition. Goat serum samples with percentage of inhibition greater than or equal to 50% were considered positive for presence of *M. capricolum* subsp. *capripneumoniae* antibodies. The weekly percentages of inhibition values for the entire group were plotted to visualize the variation. The plot (Figure 4.17) shows increasing antibody titres given as percentage inhibitions. Antibody response reflected by percentage of inhibition means for group A (vaccinated when nematode infected) increased steadily to peak on the 7th week at 64.79 PI.



Group A-Worm infected and given killed *M. capricolum capripneumoniae (*Mccp) vaccine
KEY
J1=week 1, J2=week 2, .

Figure 4. 17: Antibody response to *M. capricolum capripneumoniae* vaccine antigens given in nematode infected (group A) goats.

Antibody response reflected by percentage of inhibition means for group B (vaccinated without detectable nematode eggs) increased steadily to peak at the 4th week PI=85.97 without appreciable increase until termination of study.

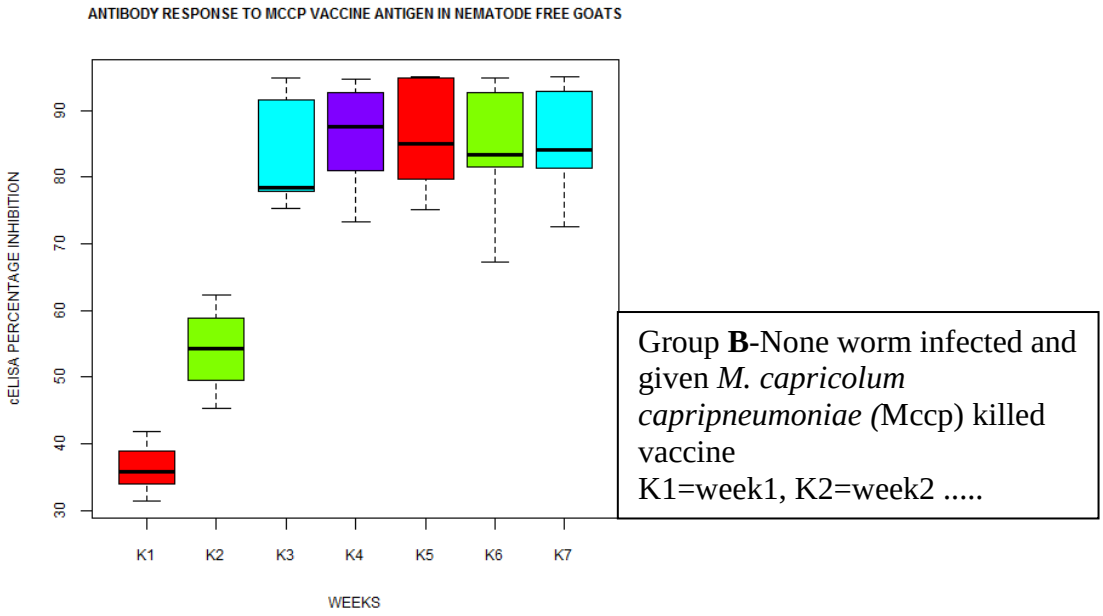


Figure 4. 18: Antibody responses to *M. capricolum capripneumoniae* vaccines antigens in (group B) goats.

Group C goats (not vaccinated) with one exceptional goat reading of 50.5 percent at the 2nd week had PIs that did not at any given time surpass the 50% PI mark which is a non significant antibody immune response in this kind of test.

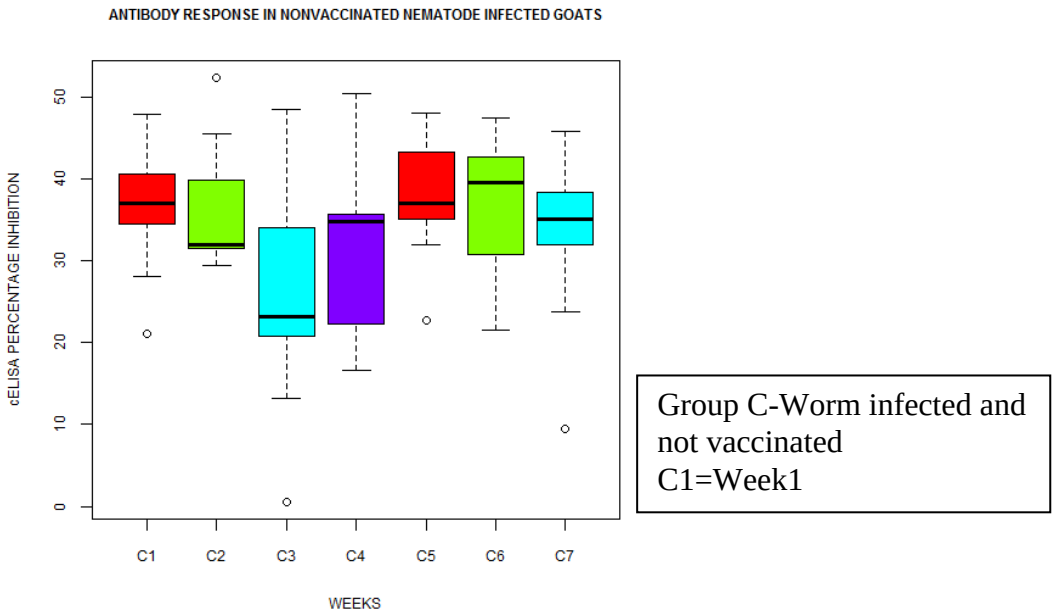


Figure 4. 19: Antibody response in none vaccinated (group C) goats with detectable nematodes infection.

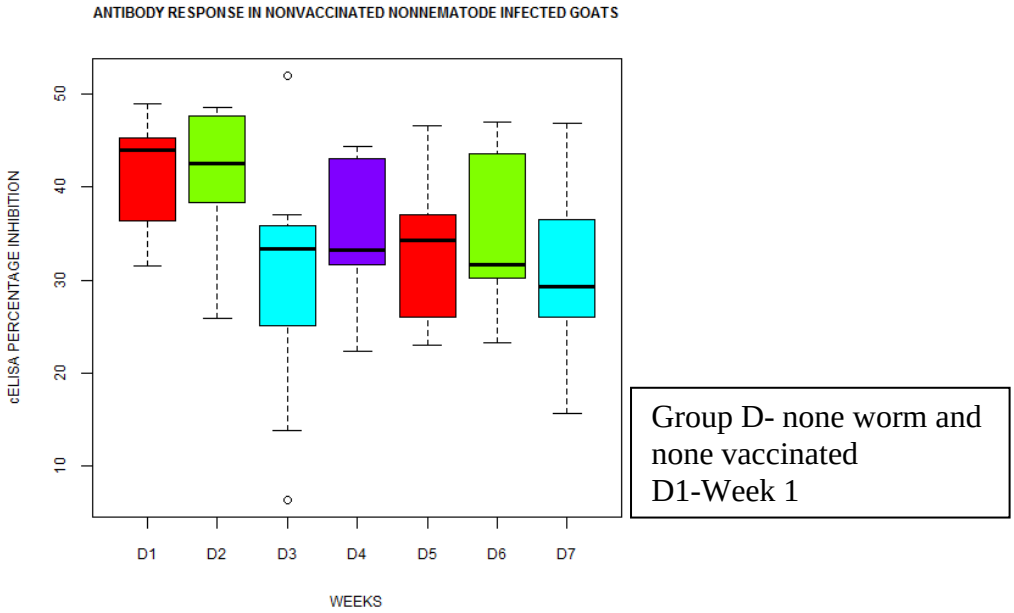


Figure 4. 20: Antibody response in non-vaccinated (group D) goats with no detectable nematodes infection.

In group D goats (not vaccinated), with only one exceptional goat reading of 51 percent at the 3th week, PIs did not at any given time surpass the 50% PI mark.

Figure 4.21 shows the difference in calculated mean cELISA percentage inhibition between the vaccinated nematode and none nematode infected goats.

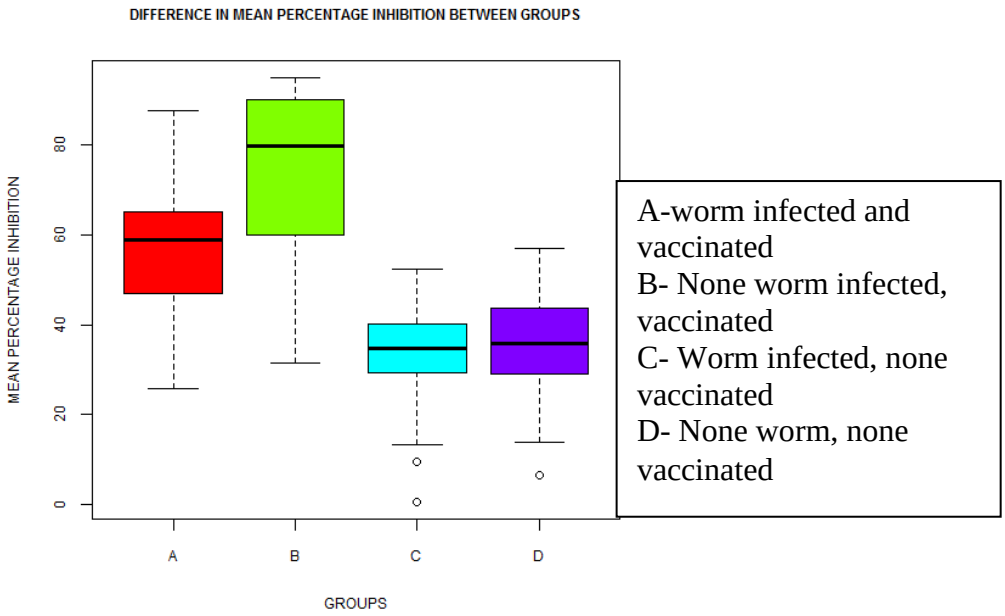


Figure 4. 21: Variation in group means antibody response.

Visually the above box plot shows that means are different (some less, others more). There was much overlap of values between some groups (for instance C and D). Hence, differences in means could have come about by chance (and we shouldn't reject the null hypothesis case). In this case it is imperative to perform analysis of variance so that differences can be highlighted.

One-way analyses of variance gave an output of F-statistic equals to 109.9 with a p-value equal to $<2e^{-16}$ which is less than 0.05. There is a significant difference between groups with and without nematode infection. In spite of this, this finding required further analysis to pinpoint where the difference between the groups lay.

To determine which groups were different from the others, a Post Hoc Test, Tukey multiple comparisons of means giving 95% family-wise confidence level was conducted. The analysis yielded the results tabulated in Table 4.2

Table 4. 2: Table showing 95% family-wise confidence level showing p-values for paired groups A, B, C and D

	diff	**95% C.I**		**p-value**
		lower	**Upper**	
B-A	17.496825	10.932584	24.061067	$2.7e^{-10}$
C-A	-22.407937	-28.972178	-15.843695	$1.2e^{-15}$
D-A	-20.298413	-26.862654	-13.734171	$2.9e^{-13}$
C-B	-39.904762	-46.469003	-33.340520	$< 2e^{-16}$
D-B	-37.795238	-44.359480	-31.230997	$< 2e^{-16}$
D-C	2.109524	-4.454718	8.673765	0.8395936

The result of Tukey multiple comparisons of means analysis (Table 4.2) shows that the difference in means between group C and D (p-value = 0.8396) is not significant statistically, but there is statistically significant difference between groups A and B (these are the two groups of a major interest), A and C, A and D, B and C, B and D (p-values = $2.7e^{-10}$, $1.2e^{-15}$, $2.9e^{-13}$, $< 2e^{-16}$, and $< 2e^{-16}$, respectively). Further attempts to illustrate the results were done and given in Figure 4.1 showing 95% family- wise confidence level differences in means and the ones far away from the zero line are statistically significant while those that straddle the line have insignificant difference.

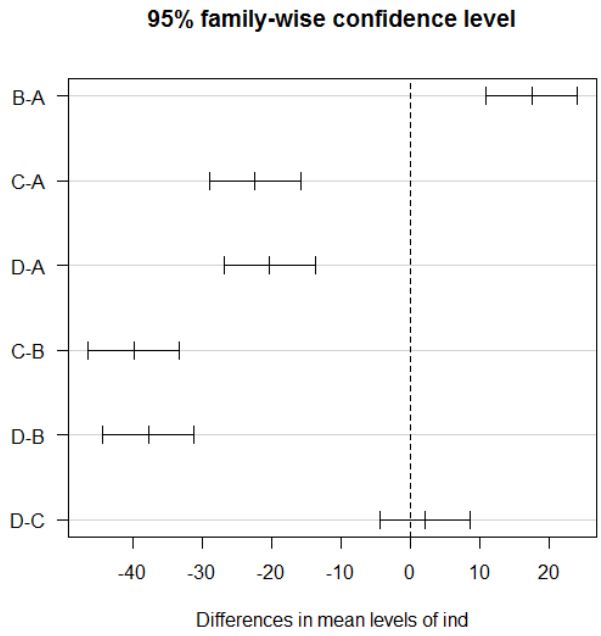


Figure 4. 22: Difference in means between paired groups A, B, C and D.

4.4 Serum protein levels in goats infected with nematodes in response to inactivated *Mycoplasma* vaccine antigens.

4.4.1 Globulins

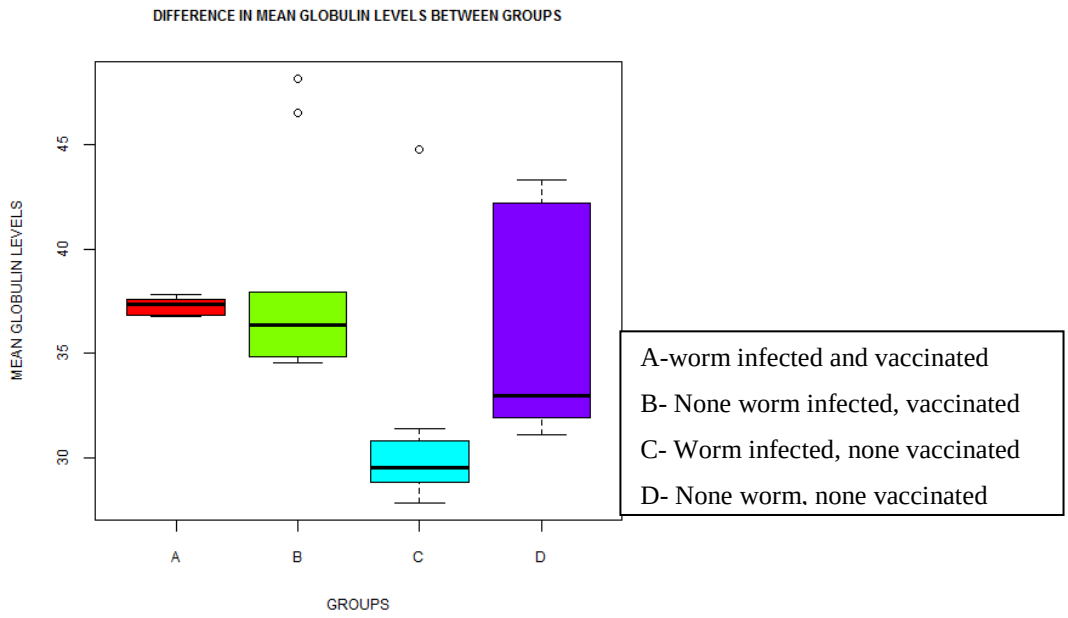


Figure 4. 23: Mean globulin levels in experimental goats.

From figure 4.23 it appears the globulin levels in group C are lower than those of the other groups. Group D registered a wide range of values overlapping those of groups A and B. Group B and C had outliers.

A one way analysis of variance was then performed for the groups to find out if there was agreement in the said difference. From the output the F-statistic is 4.5692 with a p-value equal to 0.009197 which is less than 0.05. There is a significant difference between the groups with and without nematode infection. In spite of this, this finding required further analysis to pinpoint where the difference between the groups lies.

To determine which groups were different from the others, a Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level was conducted. This confirmed that only pairs that showed significant variation C-A and C-B (p-value=0.0344204 and 0.0086006 respectively) the other pairs showed little variation that was not statistically significant.

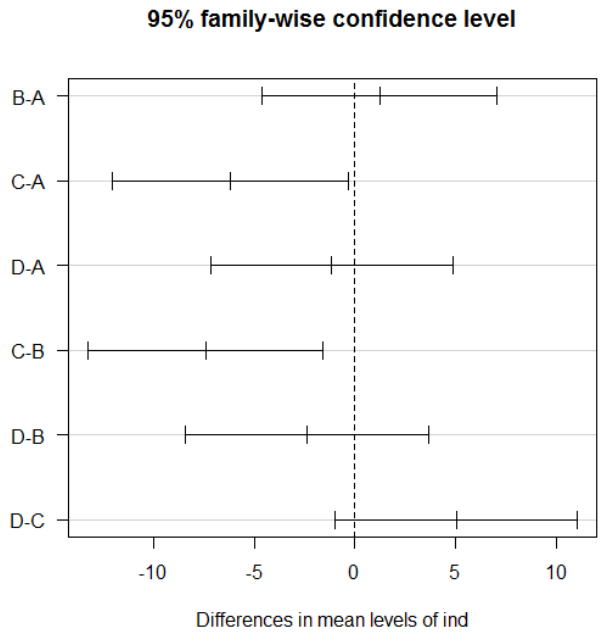


Figure 4. 24: Differences in group serum globulin means

4.4.2 Albumin Levels

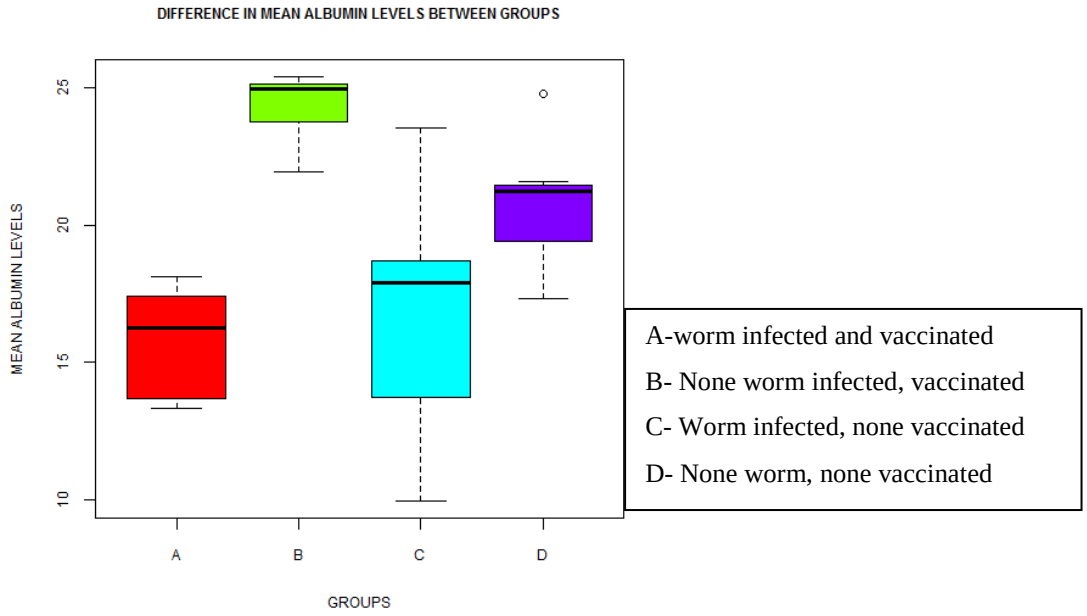


Figure 4. 25: Difference in albumin levels group means

From Figure 4.25, the albumin levels are higher and values vary less widely in group B than those of the other groups. Group C had among the lowest values recorded.

A one way analysis of variance was performed for the groups to find out if there was agreement in the said difference.

From the output, the F-statistic is 18.743 with a p-value equal to $4.032e^{-07}$ which is far less than 0.05. There is a significant difference between the groups with and without nematode infection. A post hoc test was performed for further analysis to pinpoint where the difference between the groups lies.

To confirm the groups that were different from the others, a Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level was conducted. The analysis yielded the results table 4.3

Table 4. 3: 95% family-wise confidence level showing p-values for paired groups

	diff	95% C.I		p-value
		lower	Upper	
B-A	8.5311111	4.9438945	12.1183277	0.0000020
C-A	0.4388889	-3.1483277	4.0261055	0.9871270
D-A	5.0681944	1.3705762	8.7658127	0.0041759
C-B	-8.0922222	-11.6794388	-4.5050056	0.0000050
D-B	-3.4629167	-7.1605349	0.2347016	0.0729068
D-C	4.6293056	0.9316873	8.3269238	0.0096496

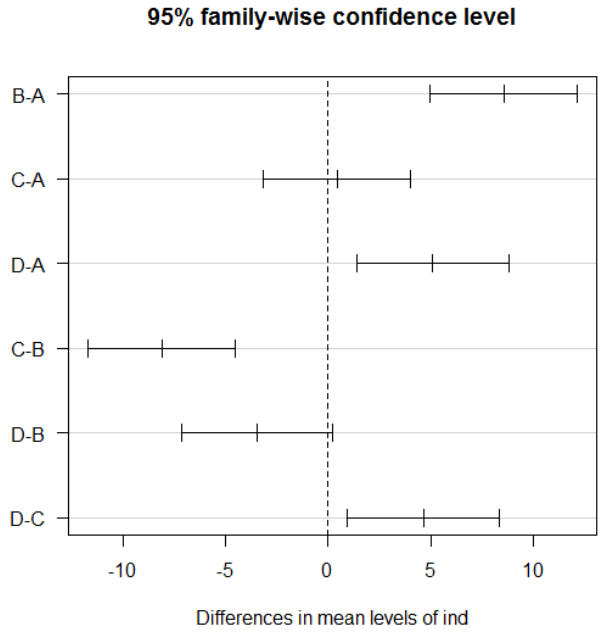


Figure 4. 26: Pair-wise comparison of albumin levels of group means

A pair-wise comparison of albumin level group means and 95%family-wise confidence level confirmed that only two pairs did not show significant variation C-A and D-B (p-value=0.9871270 and 0.0729068 respectively) while B-A, D-A, C-B and

D-C p-values(0.0000020, 0.0041759, 0.0000050 and 0.0096496 respectively) showed a variation that was statistically significant.

4.4.3 Albumin/Globulin Ratio

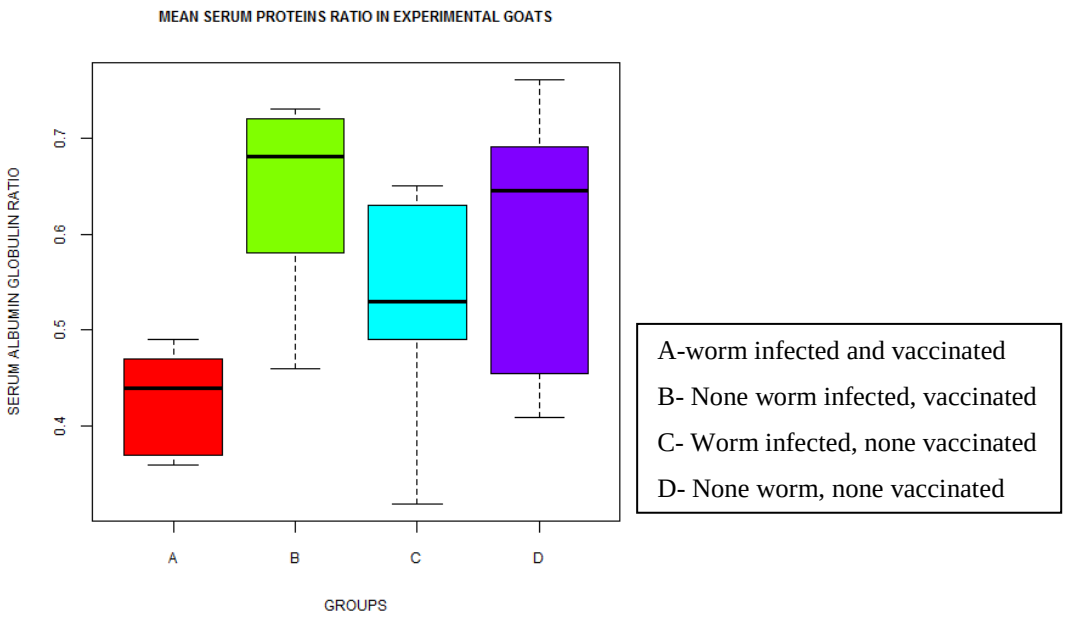


Figure 4. 27: Difference in group albumin-globulin ratio means

From Figure 4.27 the albumin-globulin ratio is higher in groups B and D than those of A and C. Group C had among the lowest values recorded.

A one way analysis of variance in group albumin globulin ratio means was performed for the groups to find out if there was agreement in the said difference.

From the output the F-statistic is 6.9574 with a p-value equal to 0.001031 which is far less than 0.05. There is a significant difference between the groups with and without nematode infection. A post hoc test was performed for further analysis to pinpoint where the difference between the groups lies.

To confirm the groups that were different from the others, a Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level was conducted.

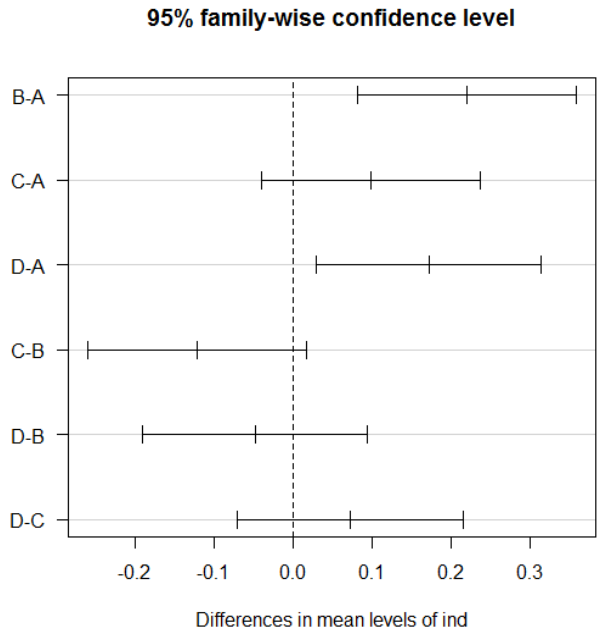


Figure 4. 28: Pair-wise comparison of group albumin-globulin ratio means

The post hoc test, Tukey HSD multiple comparisons of means (Fig. 4.28) revealed that B-A and D-A (p-values 0.0008435 and 0.0136828 respectively) were statistically significant. Whereas the group pairs C-A, C-B, D-B and D-C (p values 0.2338634, 0.1037849, 0.7937992 and 0.5207220 respectively) were not statistically significant.

4.4.4 Total protein levels

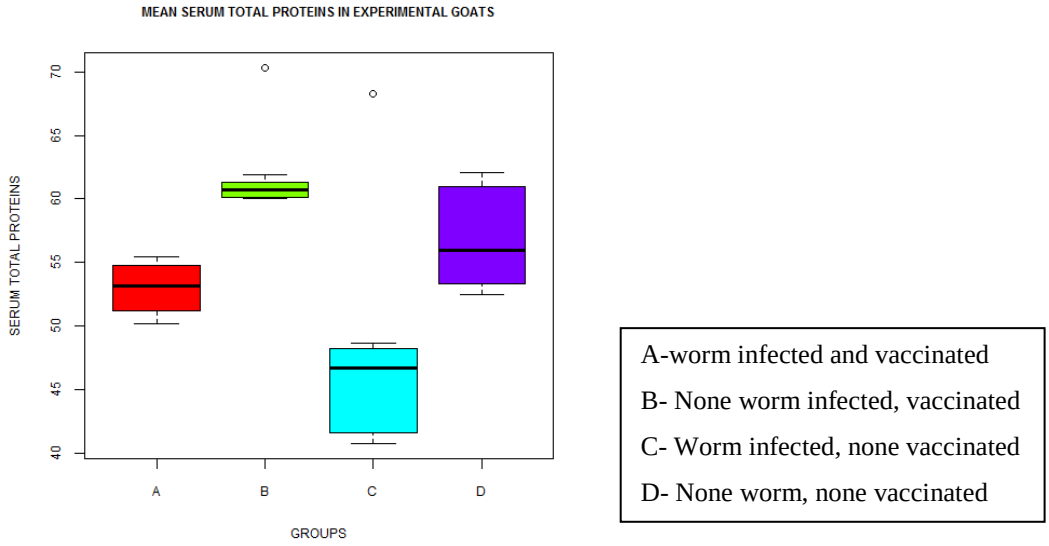


Figure 4. 29: Total proteins group means in the experimental goats

Figure 4.29 shows that group C had the lowest levels while group had higher values.

One –way analysis of variance in serum total protein group means was performed and from the output the F-statistic is 12.984 with a p-value equal to $1.155e^{-05}$ which is less than 0.05. There is a significant difference between the groups with and without nematode infection. Even so, this finding calls for further analysis to segregate inequality between the groups.

To establish which of the group differed from the others, a Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level was performed. The analysis output is given in Table 4.4.

Table 4. 4: Tukey multiple comparisons of serum total proteins group means

	Diff	**95% C.I**		**p-value**
		lower	**Upper**	
B-A	8.797778	2.265160	15.3303953	0.0049561
C-A	-5.727778	-12.260395	0.8048397	0.1022366
D-A	3.923889	-2.809779	10.6575569	0.4035225
C-B	-14.525556	-21.058173	-7.9929381	0.0000064
D-B	-4.873889	-11.607557	1.8597791	0.2231620
D-C	9.651667	2.917999	16.3853346	0.0026516

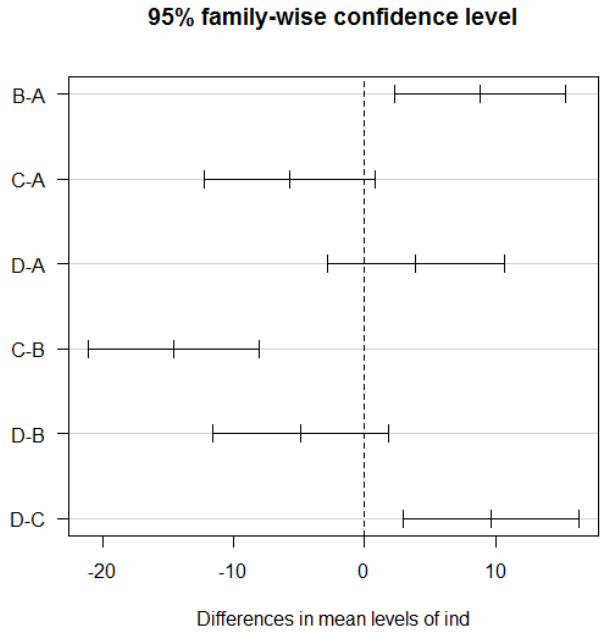


Figure 4. 30: Pair-wise comparison of group serum total protein means.

The post hoc test, Tukey HSD multiple comparisons of means (Fig. 4.30) revealed that B-A, C-B and D-C (p-values 0.0049561, 0.0000064 and 0.0026516 respectively) were statistically significant. Whereas the group pairs C-A, C-B, D-B and D-C were not statistically significant.

4.5 Impact of nematode infection on humoural response to *Mycoplasma* co-infection in goats

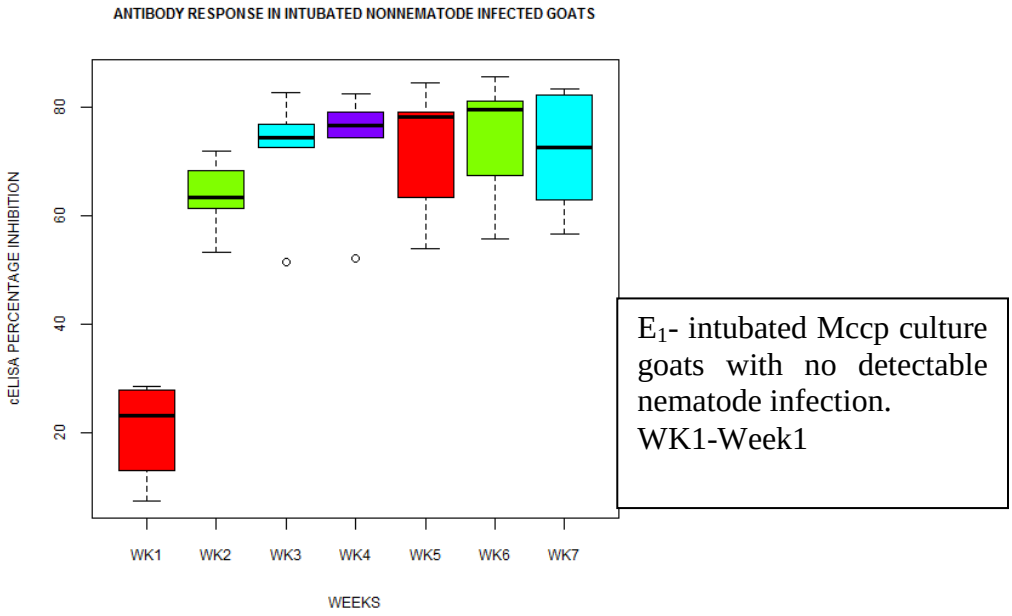


Figure 4. 31: Antibody response in group E_1- intubated goats with no detectable nematode infection.

Following inoculation, serum antibodies were detected at the end of the 1st week and increased to peak at 73.94 Percentage inhibitions after the 5th week as shown in Fig. 4.31 for group E_1 while it peaked at 71.6 in the 4th week for group E_2 (Figure 4.32).

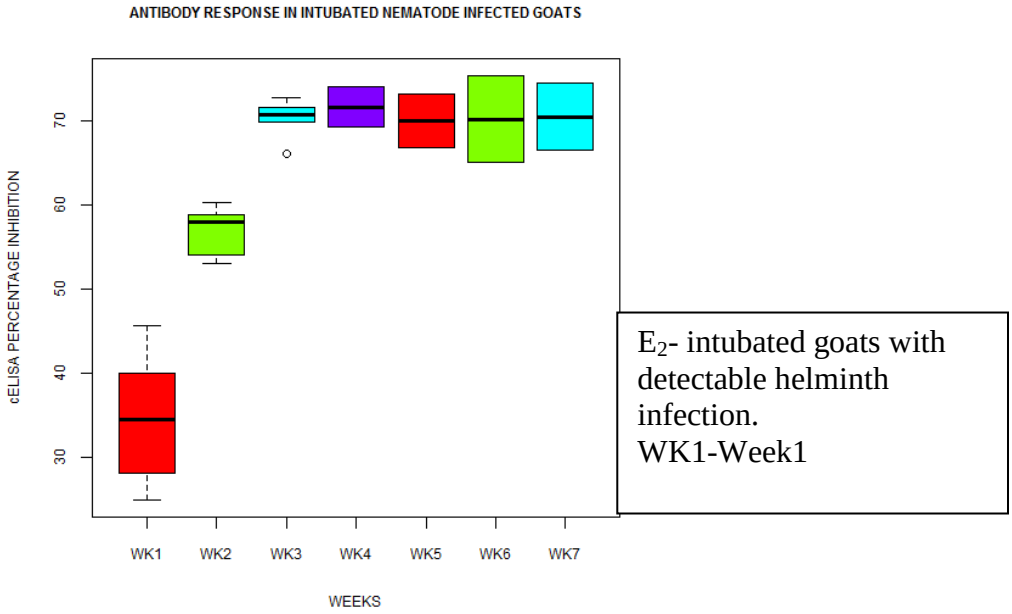


Figure 4. 32: Antibody response in group E2- intubated nematode infected goats

Group F1 goats serum antibodies peaked at 63.27 at the end of the 5th week after being brought in contact with inoculated goats shown in figure 4.33.

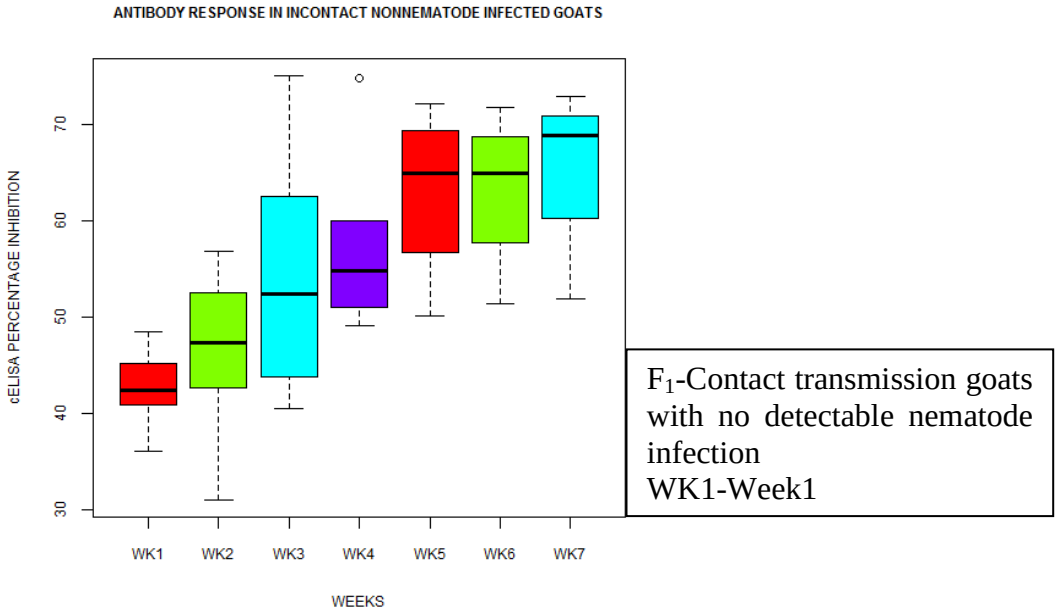


Figure 4. 33: Antibody response in group F_1- non nematode infected goats in contact transmission with intubates

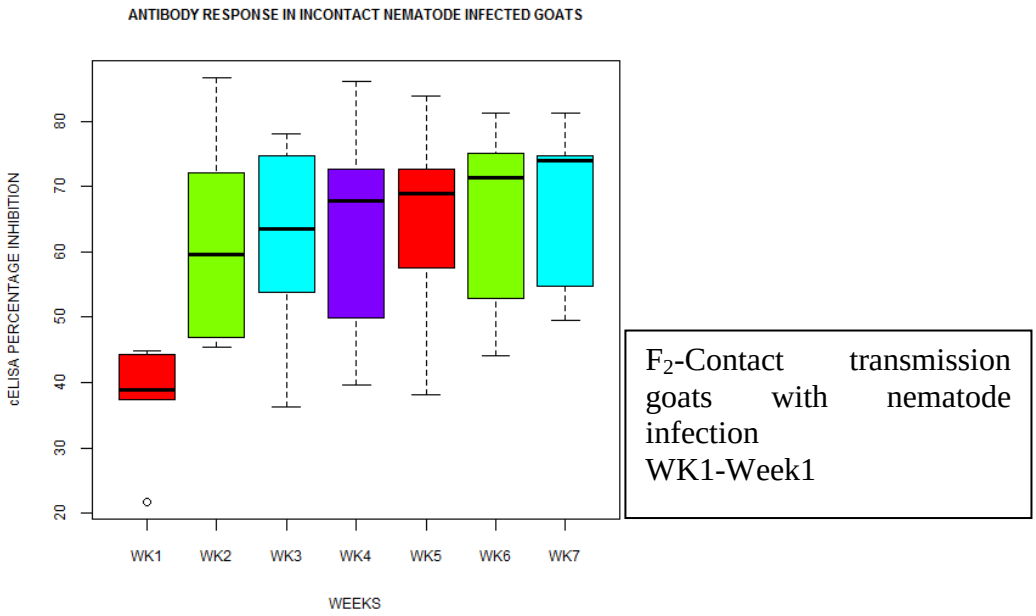


Figure 4. 34: Antibody response in group F_2- nematode infected goats in contact with intubates.

Group F2 goats serum antibodies peaked at 64.9 at the end of the 6th week after being brought together with inoculated goats shown in figure 4.34.

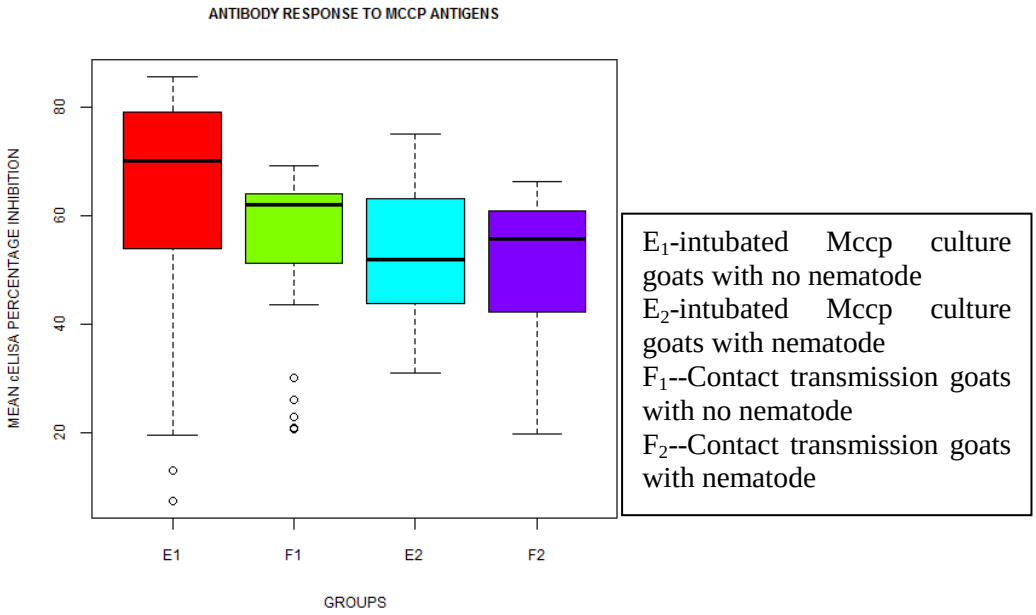


Figure 4. 35: Mean antibody response to Mycoplasma antigens between groups.

Figure 4.35 shows that means are different (some less, others more). But it also shows that each group present different effects of the nematode co-infection on immune responses to infection with *Mycoplasma capricolum capripneumoniae* organism, so that there is much overlap of values between some groups. Hence, differences in means could have come about by chance. Analysis of variance was performed to find out if there was difference in group means and also ascertain where the variation lay.

From the output the F-statistic is 3.825with a p-value = 0.01135 which is less than 0.05. There is a statistically significant impact of nematode co infection on antibody immune responses.

To determine which groups are different from the others, Tukey multiple comparisons of means, a Post Hoc Test or a post hoc pair comparison was performed.

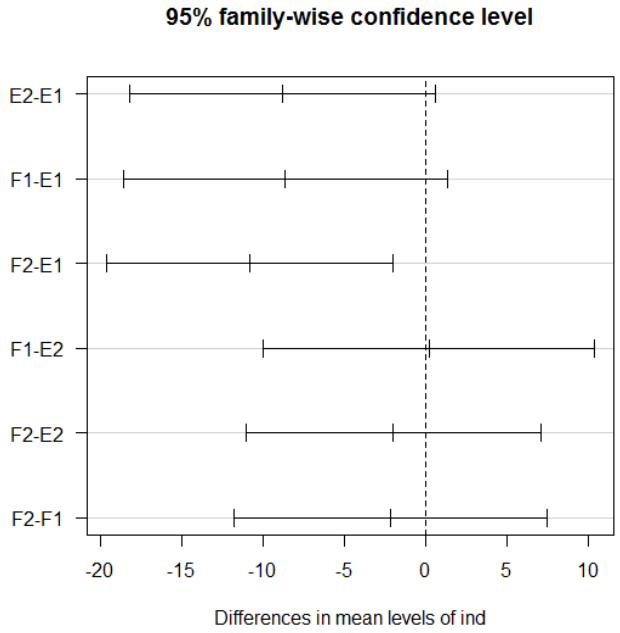


Figure 4. 36: Tukey multiple comparisons of percentage inhibition group means

Figure 4.36 showed that the pairs F2-E1 group means were significantly different statistically whereas the other pairs, though different, were not significantly different.

4.6 Influence of worm infection on pathological effects of *Mycoplasma capricolum capripneumoniae*

After the initiation of the study total of 9/12(75%) group 1 (tracheal inoculated) goats showed clinical signs of fever (Table 4.6), with 3/6 (50%) intubates (group E2- non-dewormed), 1/6 (16.7%) intubates (group E1-dewormed) euthanized due to severe clinical disease while 2/6 (33.33%) group F1 contact transmission goats were euthanized. 3/6 (50%) group F1 showed clinical signs of fever transiently. 1/6 (16.7%) group F2 contact transmission goat died.

Upon termination of study the overall analysis of post mortal findings across the groups showed 14/24(58.3%) had severe lesions, 9/24(37.5%) had mild lesions and 1/24 (4.16%) with normal lung texture.

The pathological findings included pleural effusions (Plate 4.5) that was straw coloured and became gel-like when left standing in open air. Acute fibrinous pneumonia was also observed in some cases, some with extensive red and grey hepatization (Table 4.5 and Plate 4.4), including overlying fibrinous pleuritis. Chronic cases showed severe pleural adhesions (Table 4.5, Plate 4.1and 4.2) with unilateral or bilateral lung involvement.

Table 4. 5: Characterization of gross lesions in goats, inoculated or in-contact goats, infected with *M. capricolum* subsp. *capripneumoniae*

Id	Group	Consolidation	Surface area in cm^2	Adhesion	Surface area in cm^2	Pleural fluids
651	E1	-	0	-	0	-
12		++	60	++	90	-
18		++	7.5	+	45	-
13		-	0	+++	119	-
21		+	36	++	95	-
27		-	0	-	0	-
44	E2	+++	152	++	90	230 mls
49		-	0	+	20	-
60		+++	338	+	Extensive. Fibrin deposit	-
65		++	120	+	60	-
42		+	79	+++	Fibrin deposit	200 mls
69		+++	162	+++	196	-
647	F1	-	0	++	24	-
19		-	0	+++	240	-
17		-	0	+	12	-
24		-	0	+	32	-
28		++	65	-	0	60 mls
16		+++	95	-	0	60 mls
63	F2	-	0	+++	156	-
48		+	30	+	Fibrin	-
66		-	0	-	0	-
52		+	40	+++	225	-
58		-	0	+	4	-
62		-	0	++	25	-

Table 4. 6: Clinical signs and symptoms in inoculated and contact transmission goats experimentally infected with *M. capricolum* subsp. *capripneumoniae*

Id	Group	Fever Days	Cough Days	Nasal discharge	Dull days	Anorexia	Extended neck	Dyspnoea
651	E1		+++	++	-	-	-	-
12		5	+	+	+	+	-	-
18		3	+++	+++	-	-	-	-
13		6	+	++	+	-	+	+
21		5	++	++	+	-	-	-
27		2	+	+	-	-	-	-
44	E2	2	+	+	-	-	+	+
49		3	+	++	+	-	-	-
60		6	+	+	+	-	+	+
65		5	+++	++	+	+	+	+
42		4	-	+	-	-	+	+
69		5	+	+	+	-	+	+
647	F1	2	-	++	+	-	-	-
19		4	+	++	-	-	-	-
17		4	+	++	-	-	-	-
24		3	++	+	-	-	-	-
28		4	+	+	+	+	+	+
16		2	+	+	+	+	+	+
63	F2	0	+	+	-	-	-	-
48		0	-	+	+	+	-	-
66		3	+	+	+	-	-	-
52		2	+	++	+	-	-	-
58		0	+	+	+	-	-	-
62		3	-	+	+	+	+	-

4.6.1 Fibrous adhesions

Following termination of study, necropsy revealed varying fibrous adhesions of the lungs to the thoracic cavity that ranged from focal to extensive lesions involving the entire lung surface leading to compromised function. 3/6(50%) of the intratracheally inoculated goats (group E1 without detectable worm infection) had fibrous adhesions one of them severe and given a score of 3 in a scale of 4. All the six goats belonging to group E2 (intratracheally inoculated goats with detectable worm infection) had fibrous adhesions of varying severity 3/6(50%) most extensive with one of them the awarded a score of 4. The contact transmission groups, F1 (without worms) and F2 (with

detectable worm infection), also had fibrous adhesion lesions of varying intensity ranging from 0-4. One particular case, goat 63 from group F2, had lung fibrous adhesion to thoracic cavity a sign of chronic disease and red hepatization a sign of acute disease process. This suggests that there was a relapse to acute phase of the disease. The lesion scores for the various groups were plotted and the output is depicted figure 4.36.

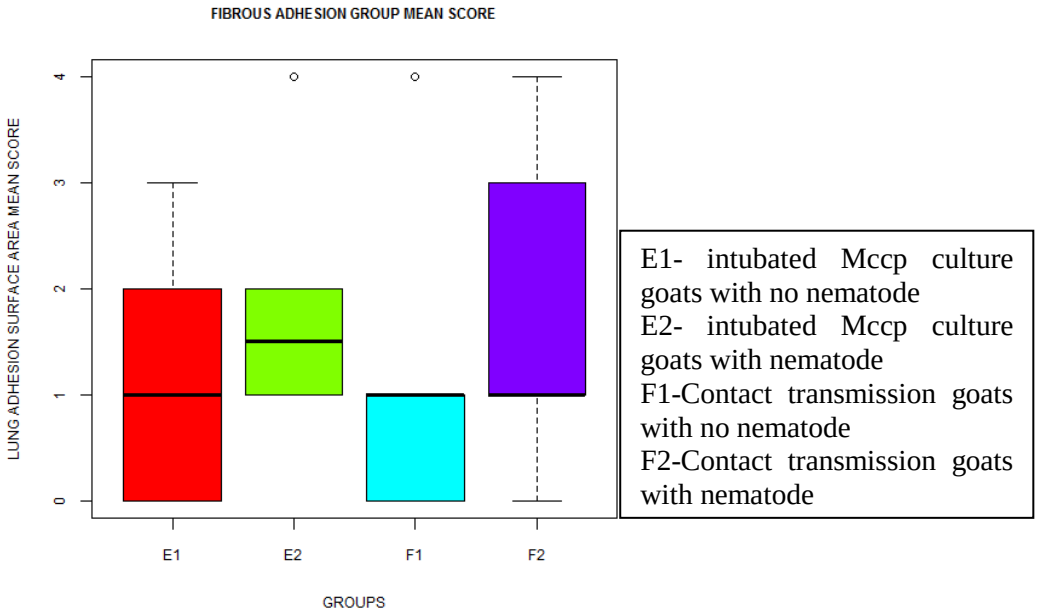


Figure 4. 37: Fibrous adhesion group mean score

Plate 4. 1: Lung fibrous adhesion to thoracic cavity Goat 18 E1compared with G66. Source, Author (2016)

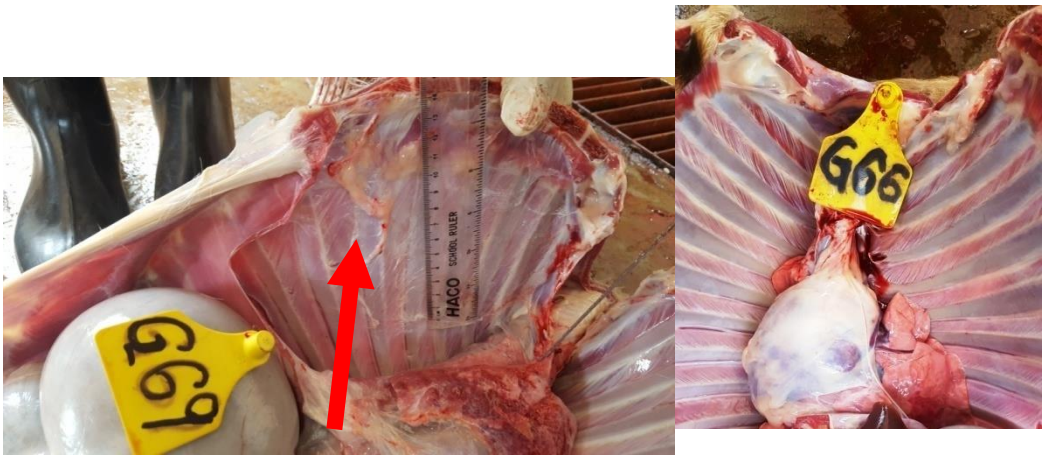


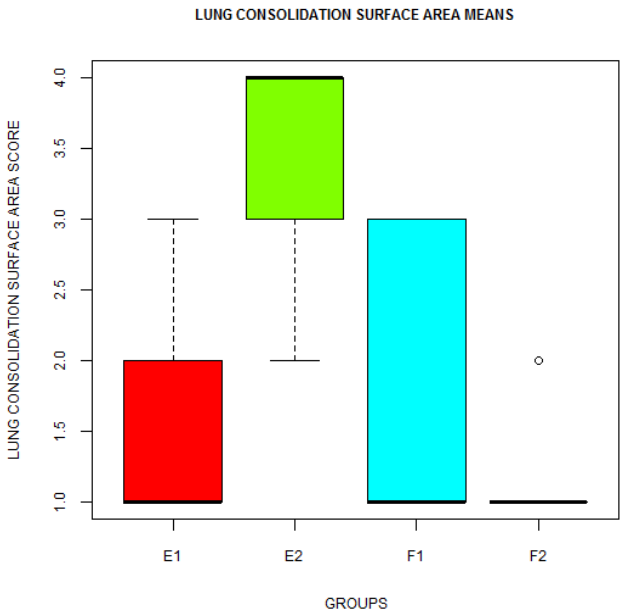
Plate 4. 2: Lung fibrous adhesion to thoracic cavity Goat 69 E2 compared with G66. Source, Author (2016)

Plate 4. 3: Lung fibrous adhesion to thoracic cavity Goat 63 F2. Source, Author (2016)

One-way analysis of variance in fibrous adhesion group means gave an output F value= 0.39535 with a p-value = 0.7577. There was no statistically significant difference between the groups.

4.6.2 Lung consolidation

More than 50% of the experimental goats' had lung consolidations varying from red to grey hepatization (Table 4.5). The lesion sizes also varied with the most extensive lesions observed in both inoculated and in contact goats with worms (Table 4.5). The overall analysis of post mortal finding across showed 14/24 (58.33%) goats had severe lesions, 7/24 (29.17%) goats had mild lesions and 1/24(4.17%) with normal lung texture. Consolidations were unilateral or bilateral with the diaphragmatic lobes more often affected.



E1- intubated Mccp culture goats with no nematode
E2- intubated Mccp culture goats with nematode
F1-Contact transmission goats with no nematode
F2-Contact transmission goats with nematode

Figure 4. 38: Lung consolidation group mean score. Source, Author (2016)

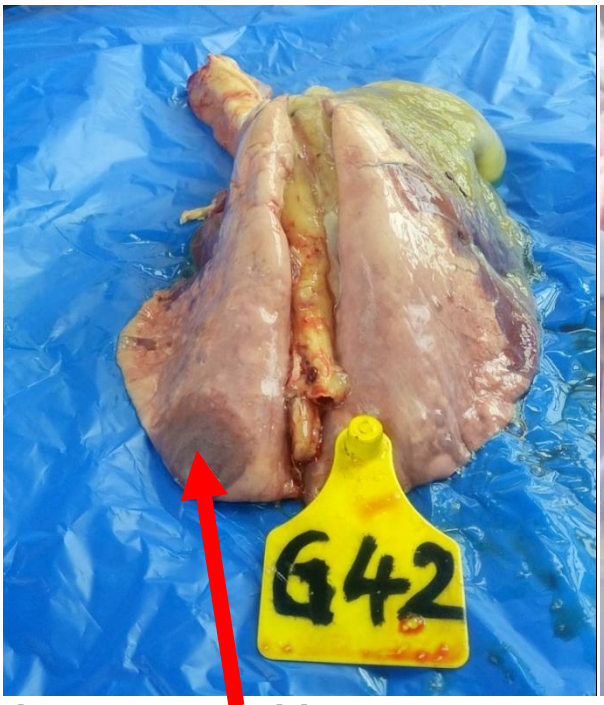


Plate 4. 4: Lung consolidation Goat 42 E2

Source, Author (2016)

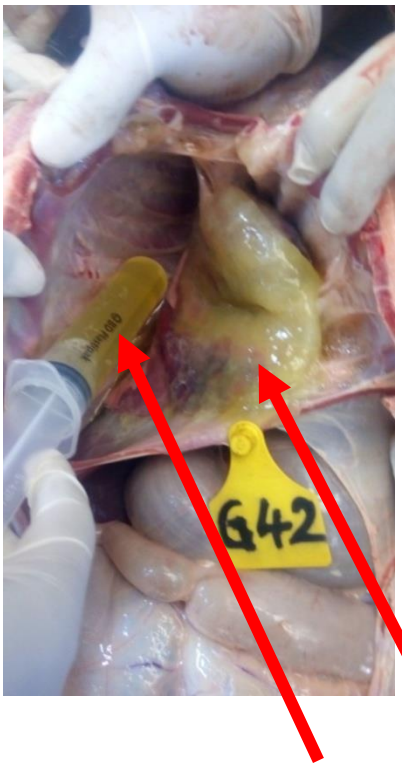


Plate 4. 5: pleural fluids& fibrin deposits goat 42

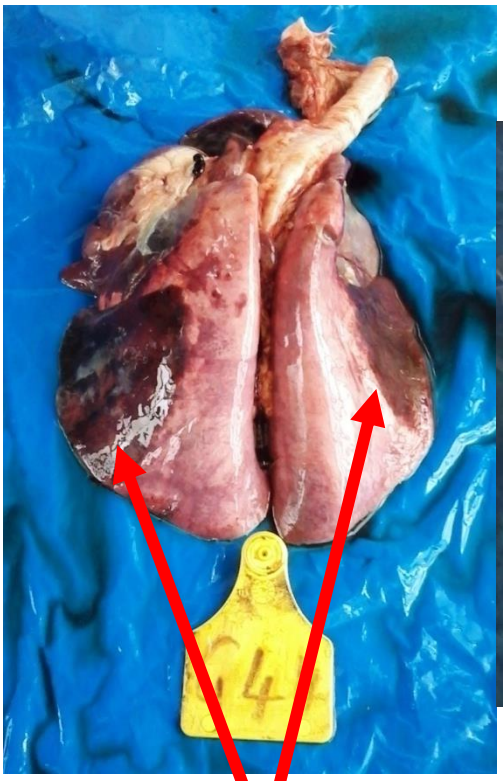


Plate 4. 6: Lung consolidation Goat 44 E2.

Source, Author (2016)

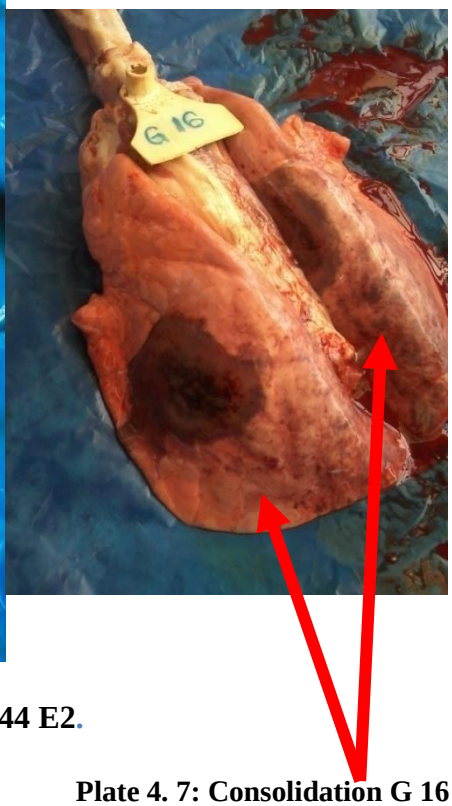


Plate 4. 7: Consolidation G 16 F1.

Source, Author (2016)

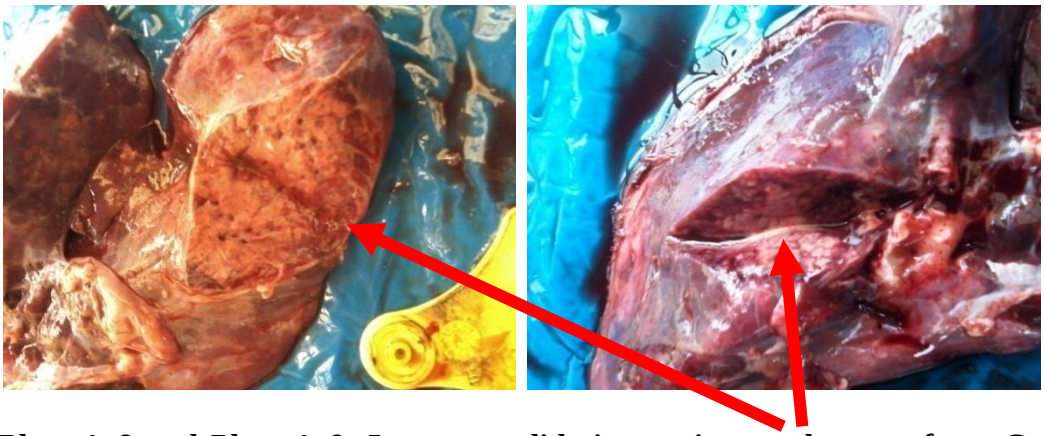


Plate 4. 8 and Plate 4. 9: Lung consolidation grainy cut lung surfaces Goat 44 group E2. Source, Author (2016)

From one-way analysis of variance in lung consolidation group lesion means the F-statistic is 10.021 with a p-value equal to 0.0003056 which is less than 0.05. There is a significant difference between the groups with and without nematode infection. Even so, this finding calls for further analysis to segregate inequality between the groups.

To establish which of the group differed from the others, a Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level was performed. The analysis output is shown in table 4.7.

Table 4. 7: Tukey multiple comparisons of lung hepatization (consolidation) group means

	diff	**95% C.I**		**p-value**
		Lower	**Upper**	
F1-E1	0.1666667	-1.1444921	1.4778254	0.9841099
E2-E1	2.0000000	0.6888412	3.3111588	0.0019574
F2-E1	-0.3333333	-1.6444921	0.9778254	0.8913307
F1-E2	1.8333333	0.5221746	3.1444921	0.0043982
F2-F1	-0.5000000	-1.8111588	0.8111588	0.7126972
F2-E2	-2.3333333	-3.6444921	-1.0221746	0.0003869

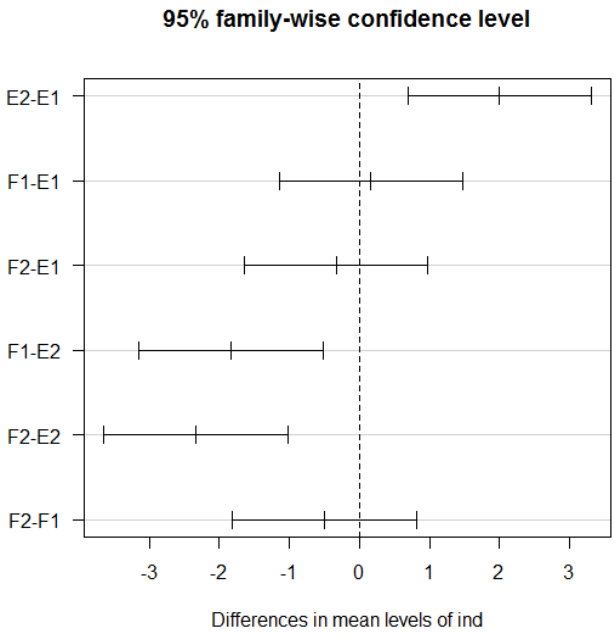


Figure 4. 39: Differences in group lung consolidation means levels

The post hoc test, Tukey HSD multiple comparisons of means revealed that E2-E1, E2-F1 and F2-E2 (p-values 0.0019574, 0.0043982 and 0.0003869 respectively) were statistically significant.

CHAPTER FIVE

DISCUSSIONS

5.1 Faecal egg counts following experimental mixed nematode larvae infection

The mean egg per gram for group A increased from non detectable in the first week to a mean of 3775 at the end of eight week when trial period ended. The exponential increase egg counts relates to worm growth, maturity and fecundity. Following inoculation, the larvae had to undergo developmental process from L_3 through L_4, a tissue stage in *Haemonchus* ssp. and some other strongyloids to adult stage. The same scenario was also observed with group C having a mean of 3475 at the end of the 8th week. Attempts were made to maintain groups B and D goats at zero egg per gram (epg) count were futile. On the 4th week the mean egg per gram for count for group B was 105.6 and that of group D was 100. This could be attributed to hypobiotic larval forms emerging to active forms, resistant larvae to anthiheminthic or re infection. It was seen that goats could maintain free or none detectable worm levels for five (5) weeks after treatment there after faecal egg count began to rise.

Among the individuals in the control group goat G647 had no detectable faecal egg counts for the 8 observed weeks. This also may be due to some level of resistance to worm infection because an initial count for the said goat during baseline counts for the pilot group was zero. Goats in groups B and D were dewormed again though the faecal egg counts were still low. At the end of 8th week of experimental study, group B had a worm mean count of 22.22 and D had a mean of 43.75. It is thought that hypobiosis phenomenon could explain the egg counts in B and D in spite of antihelminthic treatment or drug resistance. The worm count may not correlate with worm population but could also reflect worm prolificacy which may be also influenced by host immune

status. Group D registered a wide range of values overlapping those of groups A and B. There were many faecal egg count outlier values in Group B and C members.

5.2 Leukocyte counts following experimental mixed nematode larvae infection and vaccination

5.2.1 Neutrophil

In severe infections tissue injury leads to an inflammatory response seen as effusion of neutrophils, eosinophils and tissue fluid. On analysis there was a significant difference between the groups' neutrophil means with and without nematode infection (p= 0.04293). Tukey comparisons of means analysis, Figure 4.8 showed that the difference in means between group C and B (p-value = 0.0301684) was significant statistically, but there was no significant difference between the other groups. The findings agree with observations made by Ahmed *et al.,* (2015) for the neutrophil increase count was observed in nematode infected goats without killed vaccine antigen. The increase in neutrophils may have been due to phagocytic activity of polymorphonuclear and other inflammatory cells in response to particulate matter and debris of parasites. Neutrophils have been associated with nematode infection (Allen *et al.,* 2015). The ranges in neutrophil counts widely overlapped between groups which may not give sufficient information on effect of nematode on neutrophil responses to vaccine antigens. Innate immunity cells namely, the neutrophils, macrophages, dendritic cells among other cells that participate in immune responses possess pattern recognition receptors capable of recognizing various microbial antigens including those from killed vaccines. Chiao-Wen *et al.,* (2010) working on mice found out that neutrophils influxes lymph nodes or immunization centre following vaccination.

Comparing the mean differences among the pairs, A-D and B-D had p-values close to 1 meaning that their means did not vary widely. Group C had the highest group mean whereas B had the lowest. Group A and B were vaccinated while C and D were not. On the other hand, groups A and C had detectable nematode whilst B and D did not. These suggest that the statistically significant difference observed in the pair C-B can be attributed to worm infestation and not the vaccine.

5.2.2 Lymphocyte

Increase in lymphocyte count is usually associated with an increase in antibody and cell mediated immune response which may depend on the type of immunogenic stimulant. One-way analysis of variance in lymphocyte count means revealed a statistically significant difference between the groups that had and those that did not have nematode infection (p-value = 0.004787).

A comparison of group means at 95% family-wise confidence level analysis (figure 4.10) showed that groups C-A and D-C (p values 0.0465 and 0.00344 respectively) were the only paired group means that had a statistically significant difference. Groups A and C were housed together, both groups were nematode infected but group C were not vaccinated yet they showed a higher lymphocyte count than vaccinated group A. Increase in lymphocyte counts is usually associated with an increase in cell-mediated and antibody-mediated immunity. Group D (none nematode infected, none vaccinated) was housed differently from group C that had a higher count. This difference does not clearly link the response to the vaccine antigens but rather to nematode infection.

5.2.3 Eosinophil

There was relative increase in eosinophil counts in the mixed nematode infected groups could have been due to endoparasite induced secretion of Th2 cytokines

consistent with the findings of Spencer *et al*., (2010). One-way analysis of variance in eosinophil group means showed that there was a significant difference between the groups with and without nematode infection (p-value equal to 0.001248) similar to the findings of Ahmed *et al.,* (2015). Tukey HSD multiple comparisons of means showed that there was a statistically significant difference between the means of groups C-B and D-C (p values, 0.0075966 and 0.0015341 respectively) the common factor here was nematode presence or absence. Both groups A and C had worms but group A eosinophil count mean was lower than that of group C, which had the highest mean, though not statistically significant (figure 4.12). Group A had a higher mean eosinophil count than group B though not statistically significant. Group C was overwhelmed by worm burden in spite of having been housed and fed the same way with those of group A the only underlying difference being nematode infection.

5.2.4 Monocyte

Damage-associated molecular patterns and molecular- associated molecular patterns are recognised by pattern recognition receptors following tissue damage and presence of migratory stages of infecting nematodes leading to an inflammatory response. Analysis of variance in Monocyte count group means showed a statistically significant difference between the groups with and without nematode infection (p= 0.0060). The significant difference was observed in groups C-B and D-C (p- values 0.0147493 and 0.0092267 respectively) shown in figure 4.14. The Monocyte count group mean reflected what was observed for the Eosinophil counts. The increase in Monocyte, Neutrophil and Eosinophil counts reflects increased phagocytic activity of these innate immunity cells on particulate matter and debris of parasites.

5.2.5 Basophil

Nematodes induce a skewed strong type 2 immune response characterized by high levels of IgE and increased numbers of eosinophils, mast cells and basophils. A one-way analysis of variance in Basophil count group means showed a significant difference between the groups with and without nematode infection (p-value = 0.030). Tukey multiple comparisons of means (**Figure 4.16)** confirmed that the only pair that showed statistically significant variation was C-A (p-value=0.0360248) the other pairs showed little variation that was not statistically significant.

5.3 Impact of nematode infection on humoural immune responses of goats to *Mycoplasma capricolum capripneumoniae* vaccine antigens

The immune response to the vaccine antigens varied between and within the vaccinated groups with a clear demarcation from the non-vaccinated groups. Antibody response reflected by percentage of inhibition (PI) means for group A (vaccinated when nematode infected) increased steadily though lower than was observed in group B (vaccinated without detectable nematode eggs) (Figs. 4.17 and 4.18 respectively). In groups C and D (not vaccinated) PIs did not at any given time surpass the 50% PI mark which is a non significant antibody immune response in this kind of test. Figure 4.21 showed that means were different and there was much overlap of values between some groups (e.g. C and D). There was a significant difference between vaccinated groups with or without nematode infection and those that were not vaccinated (p-value <0.000).

Tukey multiple comparisons of means analysis (Table 4.2) showed statistically significant difference between groups A and B, A and C, A and D, B and C, B and D (p-values = $2.7e^{-10}$, $1.2e^{-15}$, $2.9e^{-13}$, $< 2e^{-16}$, and $< 2e^{-16}$, respectively). The comparisons

were confirmed at 95% family- wise confidence level (Figure 4.22). The bar lines that crosses the zero line corresponded to none statistically significant differences. When group A (vaccinated with worms) was compared with group B (vaccinated without worms) there was a statistically significant difference (p-values = $2.7e^{-10}$) suggesting that there was a strong nematode influence on antibody response. This compares well with reports from some studies using other models that showed that nematode infection can influence antibody response to vaccine antigens by modulating host immune responses in particular when Th1- like and cellular – dependent responses are required (Stelekati and Wherry, 2012; Stelekati *et al.,* 2014). The difference in antibody response in worm infected and none infected groups also concurs to some degree with the findings of Elias, *et al.,* 2005 and Van Riet *et al.,* (2007) that *Schistosoma* sp and *Onchorcerca volvulus* infection decreases effective immune response and efficacy of vaccine against tuberculosis and tetanus respectively. Urban Jr., *et al.,* (2007) and Steenhard *et al.,* (2009) were also able to show, in separate investigations, that *Ascaris suum* co-infection alters efficacy of vaccine against *Mycoplasma hypopneumoniae.*

Working with mice, Su, *et al.,* 2006 reported that *H. polygyrus* was able to down regulate the strong immunity against *Plasmodium chaubadi* induced by blood-stage antigens). Figure showed that groups C-A, D-A, C-B and D-B were compared a statistically significant difference was observed for the paired groups whereas group D (none vaccinated, none nematode infected) and C (nematode infected none vaccinated) were not. This clearly suggested that the difference in antibody response was due to vaccine antigens as indicated by percentage inhibition less than the cut off point for groups C and D. The lower antibody response in group A compared with that of group

B also suggested that the internal parasites had an impact on immune responses to bystander antigens such as those from infective nematodes and the inactivated vaccine. This was in agreement with reports by Salgame *et al.,* (2013) and Chatterjee *et al.,* (2015) that antigen presenting dendritic cells after being exposed to nematode will influence immune response to disease and vaccine antigens through promotion of the differentiation of T regulatory cells from naive T cell precursors or the further expansion of pre-existing T regulatory cell subpopulations.

5.4 Serum protein levels in goats infected with mixed nematodes in response to Mycoplasma vaccine antigens.

5.4.1Globulins

Mean globulin levels for groups A, B, C and D were 37.26, 38.48, 31.07 and 36.09 respectively. Groups A and B, the vaccinated goats, had higher means than groups C and D, none vaccinated, with group D having a higher mean than group C. From the box plot (Figure 23) it appeared that the globulin levels in group C were lower than those of the other groups. Since this group was not vaccinated, any changes in globulin levels would be attributed to either increased production due to inflammatory reaction or decreased levels due to poor nutrition, disease process or loss through parasitism and this collaborates with what was reported by Ahmed *et al.,*. (2015). Given that there was no indication of any other clinical disease apart from clear parasitism as indicated by faecal egg counts in group C, the low globulin levels in this group reflected serum protein depletion by the worms (Chaichisemsari *et al.,* 2011, Ahmed *et al.,* 2015). The highest faecal egg counts were recorded in this group and had the most adverse clinical presentation of pallor, submandibular oedema and loss in body condition.

A one way analysis of variance in group means performed for the groups confirmed the observed difference (p-value = 0.0092) which is less than 0.05. There was a statistically significant difference between the groups with and without nematode infection.

A Post Hoc Test, Tukey HSD multiple comparisons of means giving 95% family-wise confidence level (Fig. 4.24) had showed that the difference lay on two pairs C-A and C-B (p values 0.0344 and 0.0086 respectively). Both groups A and C had detectable worm infection but their globulin levels varied. Group A members had been vaccinated while goats that belonged to group C were not. Group B members were vaccinated but had no detectable worm infection. Globulins are a positive acute phase proteins whose levels increase with the level of inflammation and parasitic infection in ruminants (Chaichisemsari *et al.*, 2011). In this study, there was a slight increase in globulin levels in groups A and B. It is here suggested that difference could have been brought about by vaccination for both group A and B were vaccinated and their globulin means varied though not statistically significant. This is in agreement with studies made by Aikhuomobhogbe and Orheruata (2006). There was a difference between B and D (not vaccinated) though statistically not significant it reflected some globulin increase in response to vaccine antigens.

5.4.2 Albumin levels

The albumin levels showed a greater variation (Figure-25) among the groups reflecting their worm burdens. Group B had a higher albumin levels with less variation compared with other groups. Group C had among the lowest levels with the greatest variation recorded reflecting the adverse effect of the gastrointestinal nematodes and

varied individual responses to infection. This observation agrees with Chaichisemsari *et al.*, (2011) studies on naturally worm infected sheep and also with was that observed by Ahmed *et al.* (2015).

A one way analysis of variance proved existence of a significant difference in albumin levels between the groups with and without nematode infection p-value equal to $4.032e^{-07}$. A pair-wise comparison of albumin level group means (Fig.4.26 and Table 4.3) confirmed that only two pairs did not show significant variation C-A and D-B (p-value=0.9871270 and 0.0729068 respectively) while B-A, D-A, C-B and D-C p-values(0.0000020, 0.0041759, 0.0000050 and 0.0096496 respectively) showed a variation that was statistically significant. The greatest difference lay in B-A and C-B pairs that is the worm laden and apparently worm free groups. The albumin concentration decreased inversely with increased parasite load of gastro intestinal worm infection. Albumin is a negative acute phase protein, whose concentration is decreased in inflammatory or parasitic infections (Kaneko *et al.*, 2008). The significant decrease in albumin levels recorded in worm laden goat groups (Groups A and C) in comparison to apparently worm free group individuals could be attributed to increased plasma leakage through blood sucking and perforation caused by parasites such as *Haemonchus* spp. on the gastric mucosa, selective loss due to albumin smaller size and osmotic sensitivity to fluid movement or increased breakdown and protein malabsorption through the damaged gastro- intestinal mucosa blood sucking abomasal worms . The presence of nematode in the gut may also interfere with protein metabolism and absorption with a net protein deficiency resulting in hypoproteinemia.

5.4.3 Albumin-Globulin Ratio

Figure 4.27 showed a that albumin-globulin ratios were higher in groups B and D (non-nematode infected groups) than those in A and C (nematode infected groups) (p values less than 0.05) this agreed with reports from Jas *et al.,* (2008) and Ahmed *et al.,* (2015) that nematode infection influence the serum protein components ratio either through globulin synthesis stimulation decreased albumin through leakage. A one way analysis of variance in albumin-globulin ratio group means confirmed the observed difference between the groups with and without nematode infection a p-value equal to 0.001031. This was in agreement with the reports by Ahmed *et al.,* (2015). Tukey HSD multiple comparisons of means (Fig.4.28) showed that B-A and D-A (p-values 0.0008435 and 0.0136828 respectively) were statistically significant suggesting that the difference noted between groups A and B could have included increased synthesis of globulin fraction in the vaccinated group B goats in response to vaccine antigens. The other groups did not vary significantly meaning that even though there were differences it could not directly linked to infection in group C as was in groups A and B.

5.4.4 Total protein levels

Protein loss into the intestinal tract either through blood sucking by parasites or through leakage from damaged mucosa causes a decrease in serum total protein. During infestation with haematophagous strongyles such as Haemonchus spp. considerable quantities of host proteins are lost into the blood suckers gut per day while some is lost with faeces. The result showed that group C had the lowest levels while group B goats had higher values (Fig. 4.29). There was a significant difference between the groups with and without nematode infection (p-value equalled to $1.155e^{-}$

^{05}) markedly less than 0.05.This is consistent with findings reported by Aikhuomobhogbe and Orheruata (2006). Even so, this finding called for further analysis to segregate inequality between the groups.

A post hoc test, Tukey HSD multiple comparisons of means (Table 4.4) revealed that B-A, C-B and D-C (p-values 0.0049561, 0.0000064 and 0.0026516 respectively) were statistically significant. Whereas the group pairs C-A, C-B, D-B and D-C were not statistically significant. The nematode infection activates the host's immune responses which results in heightened immunoglobulin synthesis increasing globulin fraction with subsequent decrease in albumin: globulin ratio in infected goats. But comparing groups A and B (Fig. 4.30) the latter group has a higher mean ($p< 0.005$) meaning that nematode infection influenced immune response to killed vaccine antigens.

5.5 Impact of mixed nematode infection on antibody response to *Mycoplasma* co-infection in goats

Antibody response varied between and within the groups following inoculation and natural transmission *Mccp*. The antibody titres rose from a group mean of 20.53 percentage inhibition in none worm infected E_1 post inoculation to a peak at 73.94 in the 6^{th} week post inoculation and when compared with the inoculated worm infected group E_2 group titres rose from 34.53 to peak at 71.6 in the 4^{th} week. There was some difference in group means between groups E_1 and E_2 though not a statistically significant. This concurs with observation reported earlier whereby it was shown that nematode infection had an influence on antibody response and resulting pathology in coinfections (McSorley and Maizels, 2012, Maizels and McSorley, 2016).

The box plot (Figure 4.35) showed that the group means were different (some less, others more). But it also showed that each individual group member presented different effects of the nematode co-infection on immune responses to infection with *M. capricolum* subsp. *capripneumoniae* organism, so that there was much overlap of values between some groups. This reflected individual immune response given the different underlying genetic constitution of each experimental subject as each one has different capability to respond to the bacterial antigens. The highest mean percentage inhibition in group E1 (intratracheally inoculated group without detectable nematode eggs) 73.94 was attained in the 6^{th} week post inoculation (PI). Group E_2 (intratracheally inoculated group with detectable nematode infection) had a mean of 70.15, group F_1 (contact transmission group without detectable nematode infection) at week 6 had a mean of 63.27 but the highest value of 65.67 on the 7^{th} week post inoculation. Group F_2 (contact transmission group with detectable nematode infection) had mean percentage inhibition value of 64.9 on the 6^{th} week and the highest group mean whose value was 66.8 on the 7^{th} week post inoculation. At a glance when one compares groups E_1 and E_2 mean percentage inhibition at the 6^{th} week PI whose difference is nematode infection, it suggests that worm infection dampened immune response to Mycoplasma antigens and this is in agreement with Ma *et al.*, (2015). The varying antibody immune response and clinical signs between and within the groups may have been influenced by 'secretory-excretory' from nematode leading to lower antibody titres observed in nematode infected groups p-value <0.01135 in agreement with previous studies (Hewitson *et al.,* 2009.,Webb *et al*., 2012).

When groups F_1 and F_2 were compared at 6 weeks we found out that their means were not statistically different. Hence, differences in means could have come about by

chance or may be due to study group size. Most of the group E (intubated) goats 9/12 (75%) showed clinical signs of fever, cough and nasal discharge. Due to severe clinical disease 67% (4/6) intubates (group E_2- nematode infected), 16.7 % (1/6) intubates (group E_1- with no detectable nematode infection) euthanized. This showed that Mycoplasma caused a severe disease in the nematode infected group compared with none worm infected group. 50 % (3/6) group F_1 showed clinical signs of fever transiently and 33.3% (2/6) group F_1 contact transmission goats were euthanized. 16.7% (1/6) group F_2 contact transmission goat died. From the clinical picture of the study groups and individual animals it can be deduced that nematode infection exacerbates Mycoplasma infection in goats. Analysis of variance in group means showed that there was an impact of nematode co-infection on antibody immune responses among the experimental groups (p-value <0.01135).

Upon intratracheal inoculation of goats with *M. capricolum subsp. capripneumoniae* bacteria the first experimental goat to show clinical signs was observed on day 7. Incubation period for contagious caprine pleuropneumonia is commonly 6 to 10 days. The disease course in those goats that showed overt signs that included a high fever of 40.8^{o} C, dyspnoea, cough, nasal discharge and off feed compared well with other reports (Arif *et al.,* 2007, Srivastara *et al.,* 2010, Rurangirwa and McGuire 2012). Three goats were euthanized for animal welfare reasons after coming down with an acute disease. These three goats were from group E_1 (worm infected) signifying this group was highly susceptible and this agreed with the earlier observation in other disease conditions (Abu-Raddad *et al.,* 2006).

In human studies, nematode coinfection has been shown to alter the cytokine response to malarial antigens and extracts (Hartgers, *et al.,* 2009; Diallo, *et al.,* 2010) leading to

aggravated disease with severe clinical signs and pathology. Stelekati *et al.,* 2014 working on mice revealed that chronic infections affected transition of immune effector to memory cells thus modifying disease progress and protection. This is brought about by altered cytokine milieu occasioned by chronic or bystander infections such nematode. The altered cytokine elaboration due to worm infection provides a microenvironment that impacts on both cellular and antibody response to bystander or co infection antigens. The nematode-induced immune response may have several components that compromise the immune responses needed to keep the co-infection in check, including the production of T_H2 cytokines, activation of T regulatory cells and impaired function of antigen-presenting cells. However, those same mechanisms, through dampening the activation of cells of the innate and adaptive immune systems, may potentially impair the early stages of certain co-infection (Webb *et al*., 2012).

5.6 Influence of worm infection on pathological effects of *Mycoplasma capricolum capripneumoniae*

Gross pathological findings were consistent with contagious caprine pleuropneumonia. Pathology due to Mccp is known to be restricted to the thoracic cavity where it can be unilateral or bilateral damage of the lungs and the pleura. The lesions observed in this study included fibrous adhesions, fibrin deposition, pleural effusions and lung consolidation which may be red or gray hepatization.

5.6.1. Fibrous adhesion

Fibrous adhesion was observed on post mortem across the groups with some having focal lesions while others had extensive lung fibrous adhesion to the thoracic cavity

and unilateral lung collapse this agreed with the works of Wesonga *et al.,* (2004). Fibrous adhesions were evident and some are shown in plates 4.1, 4.2 and 4.3. There was variability in lesion scores reflecting individual immune response reaction to infection. Strong polarizing infections may establish an immunological milieu that suppresses or amplifies that would be appropriate responses to infections to a second pathogen (Fox *et al.,* 2000). Generally, group E_1 experimental goats had lower scores of fibrous adhesion with respect to surface area. This group was intratracheally inoculated and had no detectable worm infection at the time of experimental infection. Plate 4.1 shows extensive left lung adherence to the thoracic rib cage. All the six goats belonging to group E_2 (intratracheally inoculated goats with detectable worm infection) had fibrous adhesions of varying severity with 50% of the group members having the most extensive lesions and one of them was awarded a score of 4. The contact transmission groups, F_1 (without worms) and F_2 (with detectable worm infection), had fibrous adhesion lesions of varying intensity ranging from 0-4. One particular case, goat 63 from group F_2, had extensive lung pathology that included lung fibrous adhesions to the thoracic cavity a sign of chronic disease and red hepatization a sign of acute disease process. The same scenario was also observed with goat 69 belonging to group E_2 in which the whole right lung had fibrous adhesion to the thoracic cavity with restricted function. In spite of this, group F_2 goats had less pathology with respect to fibrous adhesions compared with group F_1. It appears that innate and adaptive immune response to Mycoplasma observed as adhesions is compromised in worm infected goats as evidenced by less adhesions.

Despite the above post mortal observation , a one-way analysis of variance in fibrous adhesions group mean scores showed that there was no statistically significant

difference between the experimental groups (p-value = 0.7577) though clinical and pathology suggested individual and group differences.

5.6.2 Lung Consolidation

More than 95% of the experimental goats showed lesions with varying severity. 50% of the experimental goats showed bilateral or unilateral lung consolidation that ranged from red to grey hepatization on post mortem. The lesions were confined to the thoracic cavity and these findings were in agreement with observations of previous studies on classical contagious caprine pleuropneumonia (Wesonga *et al.*, 2004, Arif *et al.*, 2007, Rurangirwa and McGuire 2012, MacVey, *et al.*, 2013, OIE. 2014). Figure 4.37 shows lung consolidation (hepatization) group means scores with a wide over lap of values signifying varied intra- and intergroup immune response to Mycoplasma organism in the presence or absences of nematode infection. Host susceptibility to *Mccp* infection is determined by the vigour of the immune response. Too strong an inflammatory response induces production of free radicals leading to acute tissue damage and consolidation whereas an inadequate immune response allows for chronic disease development and widespread fibrous deposition.

On post mortem several goats (4 out of 6 from E_2 inoculates with nematode infection and 5 out of 12 contact transmission goats) on post mortem showed grey and red areas of hepatization (consolidation) in their lungs, in some cases pathological lesions were found affecting more than one lobe and animals showed marked pleuritis and pleural effusion depicting the adverse reaction to the infecting organism. It was found out that majority of the goats with severe pathology (extensive gray and red hepatization (Plate 5) with an accompanying fibrin deposition (Plate 4) and increased pleural fluids) on

post mortem belonged to group E_2. This showed the disease was more severe in the mixed nematode infected group than in the non-nematode infected. 25% of the experimental goats had pleural effusion a finding consistent with acute form of the disease. The pleural fluid was thick and straw coloured with two of the goats giving a yield of more than 200 mls. The cut surface of some affected lungs (Refer to plate 7 and 8) revealed a fine granular texture with grey hepatization.

From one-way analysis of lung consolidation group means output, the F-statistic is 10.021 with a p-value equal to 0.0003056 which is less than 0.05. This showed a statistically significant difference in hepatization between the groups with and without nematode infection. Even so, this finding called for further analysis to segregate inequality of scores between the groups. The post hoc test, Tukey HSD multiple comparisons of means revealed that E_2-E_1, E_2-F_1 and F_2-E_2 (p-values 0.0019574, 0.0043982 and 0.0003869 respectively) were statistically significant. The clinical signs with pathological findings and statistical results for groups E_2-E_1 showed there was a negative impact of nematode infection on host immune response. The finding also proved Koch's postulate in that a similar pattern of clinical and pathological findings were reproduced after isolation of putative causal organism followed by experimental infection of the research goats.

CHAPTER 6

CONCLUSION

From the above discussion, there is evidence indicating that worm infection has a negative influence on immune response to bacterial infection and bystander antigen such as those from vaccines. Immune response to inactivated Mycoplasma vaccine antigens in nematode infected groups was lower than that in none nematode infected group (p value=$2.7e^{-10}$). None nematode infected group had higher means suggesting that nematode infection affects immune response to vaccine antigens.

The clinical signs indicated that the disease was more severe in worm infected individuals in both tracheal inoculated and contact transmission goats. Antibody titres in response to live Mycoplasma antigens were lower in worm infected goats but none statistically significant though there a significant pathology. Fibrous adhesions, a sign of chronic disease was more pronounced in none nematode infected group. These groups had more fibrous adhesions according to scoring adopted and visual assessment of the lesion. This suggested that there is a tendency towards chronic disease that may lead to remission and thus increasing the chances of survival in none nematode infected groups. Vaccine induced protection may also be positively affected by nematode control programmes in that in our study the dewormed vaccinated group had a higher antibody response (p value=$2.7e^{-10}$). Classical clinical and pathological findings in contagious caprine pleuropneumonia such as fibrin deposition, pleural effusions, red and gray hepatization, were frequent in the nematode infected groups with some worst episodes observed (p-value equal to 0.0003056). Mycoplasma infection in the presence of nematode co-infection would lead to high mortality (p-values 0.0019574) and acute disease (4 out of 6 from group E_2 goats). Conversely, helminthiasis control may compromise the capability of the host to tolerate microbial

infections as evidenced more fibrous adhesions in chronic cases that were more prevalent in the none worm infected groups. Though resistance to those microbes may be enhanced, exacerbation of the associated harmful inflammation, read, hepatization, pleural effusions fibrin deposits and fibrous adhesion in the absence of parasitic worm-induced immunoregulatory networks, could potentially contribute to increased disease severity. Given that the nature of antigen determines the category of immune response, the interplay between the effects of parasitic coinfection on host microbial resistance and forbearance will differ with the particular antigen involved and with the gravity of the metazoan or microbial infection. Multicellular parasitic metazoans release of factors that can potently influence host immunological function. This complex response includes both type II immune response as well as innate and adaptive immunoregulatory compartments. It is therefore recommended that livestock be dewormed before vaccination is carried out.

To further characterize the effect of nematode infections on the host response to microbial pathogens and vaccines more extensive randomized studies with the use of relevant measurable biological indicators such as specific cytokines to assess actual immune responses are required. However animal welfare concerns and the cost involved restrict animal use in such experimental studies. Further studies are required to elucidate how nematode intrinsically reprogram adaptive immune response cells, namely, antigen specific T and B cells reactive to microbial pathogens. Another point to be looked into is how *Mycoplasma* induces the severe pathology, read, consolidation observed in the lungs of some of the infected goats and which of the cells of innate response are involved.

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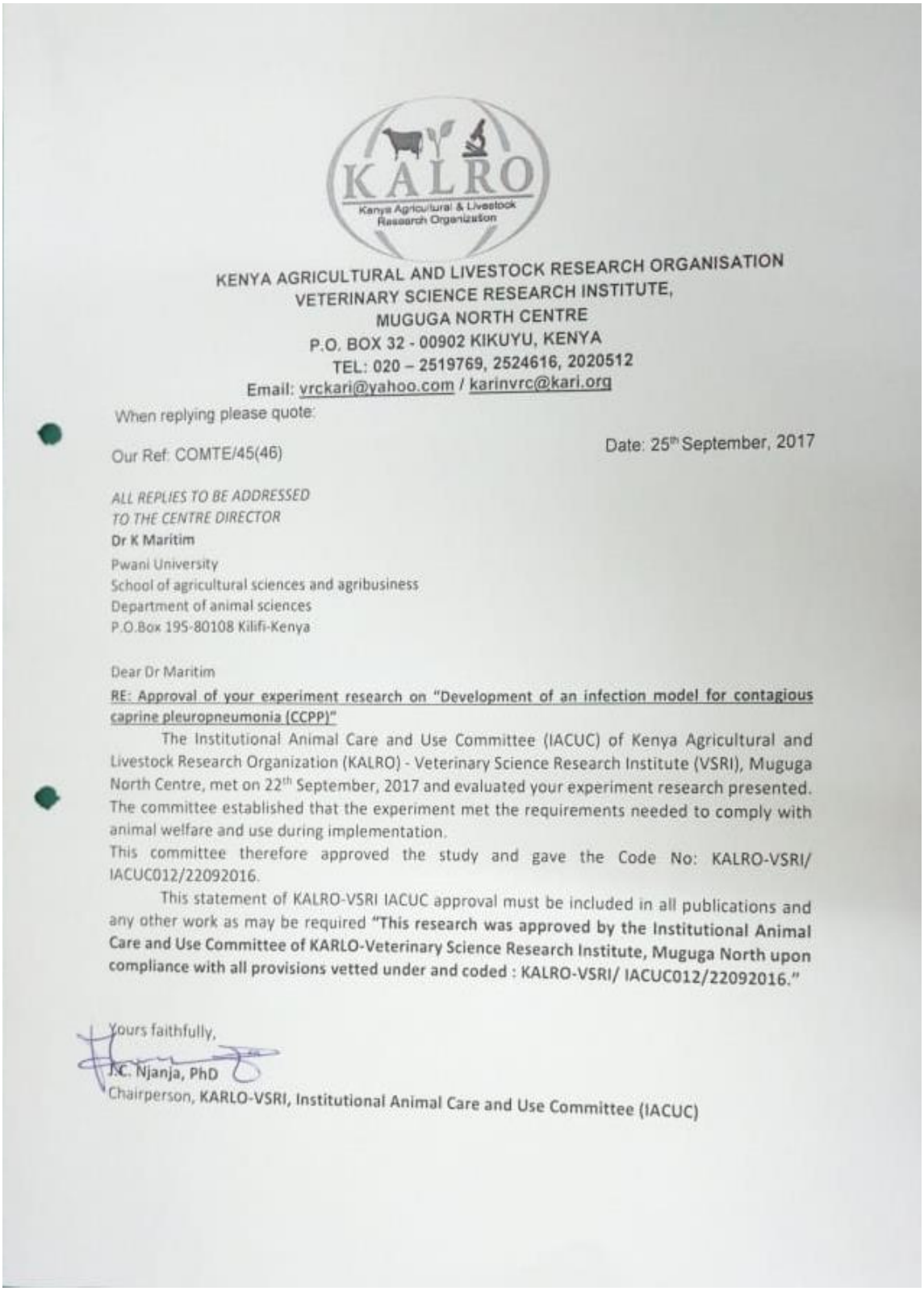
APPENDICES

APPENDIX I. ETHICAL APPROVAL



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When replying please quote:

Our Ref: COMTE/45(46)

Date: 25th September, 2017

ALL REPLIES TO BE ADDRESSED
TO THE CENTRE DIRECTOR
Dr K Maritim
Pwani University
School of agricultural sciences and agribusiness
Department of animal sciences
P.O.Box 195-80108 Kilifi-Kenya

Dear Dr Maritim

RE: Approval of your experiment research on "Development of an infection model for contagious caprine pleuropneumonia (CCPP)"

The Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO) - Veterinary Science Research Institute (VSRI), Muguga North Centre, met on 22th September, 2017 and evaluated your experiment research presented. The committee established that the experiment met the requirements needed to comply with animal welfare and use during implementation.

This committee therefore approved the study and gave the Code No: KALRO-VSRI/ IACUC012/22092016.

This statement of KALRO-VSRI IACUC approval must be included in all publications and any other work as may be required **"This research was approved by the Institutional Animal Care and Use Committee of KARLO-Veterinary Science Research Institute, Muguga North upon compliance with all provisions vetted under and coded : KALRO-VSRI/ IACUC012/22092016."**

Yours faithfully,

J.C. Njanja, PhD
Chairperson, KARLO-VSRI, Institutional Animal Care and Use Committee (IACUC)