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Factors Affecting Susceptibility Of Goats To Coccidia

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FACTORS AFFECTING SUSCEPTIBILITY OF GOATS TO COCCIDIA

by

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BIOGRAPHICAL SKETCH

Yaser Ahmed was born in Rabak, Sudan in 1971. He graduated from high school among the top one percent on the national level. He was admitted to School of Science in 1989. In 1993 he received his Bachelor of Veterinary Science (BVSc) from University of Khartoum, Sudan. He studied both veterinary medicine and animal production. Yaser was awarded two prizes of merit as an outstanding student in both general chemistry and biochemistry. He practiced veterinary medicine and surgery at University of Khartoum animal farm and clinic. He participated in many workshops, seminars, and field trips conducted by the University of Khartoum. After his graduation as a veterinarian, he worked in an animal clinic for large animals and poultry, and he helped local farmers and animal producers in livestock management.

Yaser moved to the U.S., where he worked as a research assistant at North Carolina Agricultural and Technical State University. He was selected as the recipient of the 2006-2007 Interdisciplinary Biotechnology and Biodiversity Fellowship Award. Yaser has participated in the Gamma Sigma Delta Show case of Excellence poster Competition.

His research has been presented at state, national and international meetings. His work was supported by the USDA Evans Allen program and the SAES Agricultural Research program. He plans to pursue a PhD in Public Health in the future.

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LIST OF ABBREVIATIONS

ACD	Acid Citrate Dextrose
BW	Body Weight
BCS	Body Condition Score
C	Celsius
CD	Cluster Differentiation
cDNA	Complementary DNA
DC	Dendritic Cells
DNA	Deoxy Ribonucleic Acid
epg	Eggs Per Gram of Feces
FEC	Fecal Egg Count
g	Gram
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GIN	Gastrointestinal
IFN-γ	Interferon gamma
IL-10	Interleukin-10
IL-8	Interleukin-8
Kg	Kilogram
L1	First Stage Larvae
L2	Second Stage Larvae
L3	Third Stage Larvae
L4	Fourth Stage Larvae
LPS	Lipopolysacchride

µg	Microgram
µg/ml	Microgram per Milliliter
µl	Micro Liter
ml	Milliliter
mm	Millimeter
mRNA	Messenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
NK cells	Natural Killer cells
NRAMP1	Natural Resistance Associate Macrophage Protein
PAMP	Pathogen-Associated Molecular Patterns
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PRR	Pattern Recognition Receptors
RNA	Ribonucleic Acid
Rnase	Ribonculease
rpm	Rotations per Minute
RT	Room Temperature
RT-PCR	Reverse Transcriptase PCR
spp	Species
TLR	Toll-like receptors
WBCDC	White Blood Cell Differential Count

ABSTRACT

Ahmed, Yaser. FACTORS AFFECTING SUSCEPTIBILITY OF GOATS TO COCCIDIA. **(Advisor: Dr. Mulumebet Worku),** North Carolina Agricultural and Technical State University.

Natural Resistance Associate Macrophages Protein one (*NRAMP1*) gene encodes a transmembrane protein that regulates divalent cation transport that hinders intracellular pathogen proliferation. The objective of this study was to determine how *NRAMP1* expression and other factors may be associated with coccidia infections in goats. Clinically healthy Boer, Spanish, and Spanish-Boer cross goats (N=140) from the NCA&T Small ruminant unit were used. The FAMACHA© score, body condition score, body weight, and age were recorded. Fecal samples were evaluated for the levels of *haemonchus* eggs and coccidia oocytes using McMaster slides. Blood samples were evaluated for packed cell volume and white blood cell differential counts.

Genomic DNA was extracted from blood on FTA cards according to the manufacturer's protocol (N=40). RNA was extracted using the ZR Whole-Blood Total RNA Kit (N=12). The RNA samples were reverse-transcribed and the cDNA was obtained using the Ambion-Retroscript kit. Specific primers for *NRAMP1,* IL-8, IL-10, Interferon gamma, and CD14 were used for RT PCR, to detect *NRAMP1* in genomic DNA and to determine CD14 and cytokine gene expression from isolated RNA. Samples amplified using primers for GAPDH were used as loading controls.

The effect of breed, age, and infection status on selected health parameters, gene identification and expression were analyzed using SAS ANOVA. Coccidia fecal egg count was significantly related to age ($p<0.05$). Expression of *NRAMP1*, IL-10 and IL-8 was significantly increased with increased fecal coccidia egg counts ($p<0.05$). *haemonchus* fecal egg count was significantly related to FAMACHA score, PCV, and to increased percentages of neutrophils, eosinophils, and monocytes in blood. Expression of CD14 and IL-8 was significantly increased in goats with high *haemonchus* fecal egg counts ($p<0.0114$). A 443bp region of the *NRAMP1* gene was identified in genomic DNA from 50% of goats tested. This variation may be associated with gene polymorphisms. Distinct gene expression patterns may be associated with the innate immune response to coccidia and nematodes in goats. Age appropriate management strategies will help control coccidiosis in goats.

CHAPTER 1

INTRODUCTION

The number of meat goats in the United States has increased from 415,000 (in 1987) to 3.1 million (in 2010) and in dairy goats from 129,000 to over 390,000 (NASS, 2010). In North Carolina interest in meat goat production has increased rapidly during the past eight years because of the increased demand for goat meat (Luginbuhl, 2007; Dubeufa, et al., 2004).

Internal parasites represent the greatest threat to the goat industry (Sahlu et al., 2009). Coccidiosis and haemonchosis are serious diseases in many animal species, including goats (Kahn and Line, 2010). Goats are more susceptible to nematode parasites than sheep (Lightbody et al., 2001; Lloyd, 1987). Animals selected for breeding based on their natural genetic resistance against several diseases especially gastrointestinal parasites has been used by farm animal producers. The immune response to pathogens in farm animals is influence by the host, the pathogen and the environments (Caron et al., 2004). Previous studies concluded that when animals were exposed to pathogens some of them were susceptible others were resistance. Studies of resistance to *Salmonella pullorum* in poultry and *Brucella suis* in swine found a significant role of the host genetic makeup in the manifestations of the disease (Cameron et al., 1942; Roberts and Card, 1926).

Research on meat goat genetic resistance to disease is limited (Sahlu et al., 2009). Studies in sheep showed that genetic resistance to gastrointestinal parasite depends on individual immune response which varies among and within breeds (Bishop and Morris, 2007). Several studies on meat goats concluded that genetic variability of resistance exist between and within breed (Mandonnet et al., 2001; Fakae et al., 1999; Vlassoff et al., 1999 ; Baker et al., 1998).

Previous studies concluded that *NRAMP1* provides natural resistance against intracellular pathogens such as *Leishmania* (Bradley et al., 1977), *Salmonella* (Lissner et al., 1983), and *Mycobacterium* (Gros et al., 1981). The influence of *NRAMP1* on resistance to different intracellular pathogens has been studied both in vitro (Lissner et al., 1983) and in vivo (Gros et al., 1981). Infection with the intracellular protozoa parasites of *Eimeria* species results in coccidiosis in goats. This potentially fatal disease can be economically devastating for goat producers (Foreyt, 1990). There is no published evidence on the role of *NRAMP1*expression in response to coccidiosis in goats.

The objective of this study was to determine how *NRAMP1* expression may be associated with coccidia infections in the goat. The effects of breed, age and co-infection with strongyles parasites were also investigated.

CHAPTER 2

LITERATURE REVIEW

2.1 Goat Meat Industry Past, Present and Future

Goats have been used throughout history by humans for their milk, meat, hair, and skins (Hirst, 2008; Mussman, 1982). Some farmers prefer breeding goat over other livestock due to their relatively low cost of breeding stock, easy handling, adaptability and tolerance to a variety of environmental climate, high reproductive rate, and effective conversion of limited resources into meat, milk and hides (Balicka, 1999; Harper and Penzhorn, 1999). Goat meat trade is profitable and attractive to many farmers and breeders in southern and southeastern United States (Glimp, 1995).

2.2 Goat Breeds

The Food and Agriculture Organization of the United Nations identified about 570 goat breeds worldwide (Scherf, 2000). Among them are Spanish, Boer, and their crosses. The Boer goat was introduced into the United States in 1993 (Sahlu et al., 2009). They are characterized by their outstanding growth rate, good body conformation, carcass quality, and genetic traits (Van Niekirk and Casey, 1988). As a result, Boer goats have been used by many breeders to improve growth rate and carcass qualities of indigenous breeds through crossbreeding (Newman and Paterson, 1997). Spanish goats were

introduced to US from Spain through Mexico. Spanish goats are characterized by their ability to survive adverse climate conditions and parasitic resistant when compared with Boer goats (Luginbuhl, 2007).

Through crossbreeding Boer goats improve weights at birth, weaning, breeding, sexual maturity, and an increase in kidding rate (Cameron et al., 2001). When compared with Spanish goats, Boer goats have shown advantage over Spanish goats due to their heavy weight (Cameron et al., 2001). The average daily gain for Spanish crosses (Boer x Spanish) is higher than Spanish purebred (Cameron et al., 2001). Boer crosses have higher body weight gain than Spanish goat (Garza and Garza, 1997; Lewis et al., 1997). A detailed study evaluated the growth rate, feed consumption, and carcass traits of Spanish and Boer x Spanish cross kids (Waldron et al., 1995). It was concluded that at birth, Boer x Spanish kids weights were higher by 0.26 kg at birth and 2.1 kg at 8 month as compared to purebred Spanish kids (Shrestha and Fahmy, 2007).

2.3 Prevalence of Parasites in Goats

Parasitic infection in livestock is one of the significant threats to a wide range of animals. It leads to economic loss from both clinical and sub-clinical infection, including, low growth performance, decrease in productivity, increase in treatment cost, and mortality (Mahmoud et al., 2003).

The USDA reported that 74% of sheep in the United States experienced gastrointestinal nematode parasitism (USDA-APHIS-VS, 2003). In goats 91% were

treated for internal parasitic diseases at the Auburn University Veterinary Medical Teaching Hospital during 1993 to 2000 (Pugh and Navarre, 2001).

2.4 Coccidiosis of Goats

Coccidiosis is a worldwide economically important serious disease in many species of livestock, including, cattle, sheep, goats, pigs, and poultry. The disease is caused by protozoa of the genera *Eimeria*. It can lead to acute invasion and destruction of intestinal mucosa. Infection is characterized by diarrhea, fever, loss of appetite, weight loss, emaciation, and sometimes death. There are numerous species of *Eimeria* that can affect goats, but not all of them are pathogenic.

Coccidia need only one host to complete their life cycles. Coccidia are host specific (coccidia of goats, for example, cannot infect cattle or the opposite), and there is no cross immunity between species of coccidia (Kahn and Line, 2010). Despite the fact that the *Eimeria* species are species specific, there are some species that infect both sheep and goats; *Eimeria caprovina* (Norton, 1986), *Eimeria pallida, and Eimeria punctata* (Chevalier 1966). *E arloingi, E christenseni, and E ovinoidalis* are highly pathogenic in kids. Goat coccidiosis causes high mortality in kids that may reach 58% (Jalila et al., 1998; Levine, 1986), but its greater economic importance lies in the lowered productivity that it causes (Radostits et al., 2000).

Goats are susceptible to coccidiosis under adverse conditions, for example, inadequate nutrition, improper sanitation, or overcrowding, stresses due to weaning, long transporting, or sudden changes of feed (Smith and Sherman, 1994; Craig, 1986).

Animals between 1 mo and 1 yr old are more susceptible to *Eimeria* infection than older animals (Mahmoud et al., 2003), the latter, most often are resistant to clinical coccidiosis but may have inapparent infections. However, adults can be sources of infection to young animals.

The life cycle of coccidia consists of intracellular, extracellular, asexual, and sexual stages (Lillehoj and Okamura, 2003; Lillehoj and Lillehoj, 2000). Infection occurs when a susceptible goat ingests infective oocysts. Infected goats release oocysts to the environment through the feces. Sporulation of the occyst takes place under certain environmental conditions (temperature and humidity) and they become infective in several days. The sporulated oocyst has 4 sporocysts, each in 2 sporozoites, after ingestion by a susceptible goat, the sporozoites leave the oocyst, invade the intestinal mucosa or epithelial cells and develop into schizonts. The latter develop into merozoites which is the infective stage. Merozoites penetrate new cells and repeat the cycle. When a certain number of asexual generations are produced, the infective stage (merozoites) will become either mirco (male) or macro (female) gametocytes. In each generation a single macrogamete or a number of microgametes are produced in a host cell. Microgametes fertilize the macrogamete to develop an oocyst. Oocysts are discharged in the feces unsporulated containing resistant walls. Oocysts cannot survive in temperatures below 30°C or above 40°C; within this range, they may survive up to 1 year or more.

In goat coccidosis clinical signs occur as the result of invasion and destruction of intestinal epithelial cells and to some extent, the underlying connective tissue of the mucosa. This may lead to intestinal hemorrhage, diarrhea, bloody discharge, loss of

electrolytes, and dehydration. Blood hemoglobin or PCV is relatively low in severely affected animals. Necropsy findings may show lesions, congestion, hemorrhage or ulcer in the small intestine. The intestinal mucosa may appear yellow, white or pale.

The *Eimeria* life cycle is complex; it consists of intracellular, extracellular, asexual, and sexual stages. This results in innate and adaptive immunity at cellular and humoral level (Lillehoj and Okamura, 2003; Lillehoj and Lillehoj, 2000). The innate immune response to *Eimeria* includes physical barriers, phagocytes and leukocytes, and complement. The adaptive immunity is modulated by antibodies, lymphocytes, and cytokines. When an oocyst is ingested, its antigen is presented, intestinal antibody is produced and cell mediated immunity is activated (Trout and Lillehoj, 1996; Rose et al., 1984). Recent studies show that many cytokines are produced locally in response to coccidiosis in poultry (Min, et al., 2003), which were responsible for promoting protective immunity against *Eimeria* (Min et al., 2003; Yun et al., 2000; Lillehoj, 1998). In goats IFNγ and IL-4 play a role in the pathogenesis of goat coccidiosis (Ibarra and Alcala, 2007).

Intestinal coccidiosis can be diagnosed by the fecal flotation method using salt or sugar to examine fecal samples to identify oocysts. Saturated sodium chloride is usually used to concentrate and count parasite eggs. This technique is referred to as the McMaster techniques (Whitlock, 1948). Pathologic oocysts numbers could be found in hundreds or thousands per gram of feces. It is possible to find oocyst counts as high as 70,000 in kids without showing clinical signs, but weight gain may be affected (Hoste et al., 2001).

The number of oocysts identified in feces is affected by different factors, for example, type of *Eimeria* species, the number of infective oocysts ingested, animal age, breed, previous exposure, immunity status, and method of examination (Kahn and Line, 2010). Applying FEC only is not enough to diagnose coccidiosis, clinical signs and intestinal lesion should also be considered (Kahn and Line, 2010).

Coccidiosis can be controlled by reducing the number of oocysts ingested by young animals, which can be achieved by reducing animal exposure to the parasite. Methods include, applying proper sanitation methods to avoid feeding animals contaminated food and water, and giving colostrum to newborn animals. Young should be separated from the older animals, stresses should be avoided and introduction of new animals should be minimized. Amprolium and ionophorous, for example, are effective coccidiostats used in goats kids to prevent coccidiosis (Kahn and Line, 2010).

2.5 Haemonchus Contortus

Gastrointestinal nematode parasitic infection represents the main threat to livestock production worldwide. In small ruminants, *Haemonchus contortus* is the most common nematode parasite in south eastern USA and in areas with summer rainfall (Vatta and Lindberg, 2006; Chauhan et al., 2003). Young animals are more susceptible than adults**.**

The life cycle of *Haemonchus contortus* has two phases: a pre-parasitic phase followed by a parasitic phase. In the pre-parasitic phase, development happens outside the host. When eggs are passed into the feces, they undergo several developmental

stages until they hatch as first stage larvae (L1) then second stage (L2) and reach the infective stage in approximately two weeks if the temperatures is around 75°F, it may take longer during cold weather. First and second (L2) larval stages feed on fecal and soil bacteria. The infective stage L3, when swallowed from pasture by an animal during grazing, is passed to the abomasum, molts into L4 and penetrates the abomasal lining epithelial membrane and remains there until emerging as young adults L5. *Haemonchus contortus* females lay eggs after 2 to 3 weeks from the infection (Johnstone, 1998).

Clinical signs of Haemonchosis may include intermittent diarrhea followed by constipation, different levels of anemia, hypoproteinemia and edema, specifically under the lower jaw (bottle jaw), and in the abdomen. Acute infections may result in sudden death. Other signs include weight loss, weakness, rough coat, and anorexia.

Haemonchosis can be confirmed by conducting FEC using the McMaster technique, examining eye mucus membranes for anemia, or by applying FAMACHA score technique. On necropsy, *Haemonchus* worms are visible. Adult male *Haemonchus* are up to 18 mm long and females are up to 30 mm (Kahn and Line, 2010).

The FAMACHA score is a technique developed to be used by animal producers and farmers to improve their management of *Haemonchus contortus* infections (Burke et al., 2007). The name FAMACHA© is derived from the name of the inventor, Dr. Faffa Malan (FAffa MAlan CHArt). This system was developed in South Africa, and has been validated in the United States (Malan et al., 2001) and (Van Wyk and Bath, 2002). It categorizes animals, based on their ocular conjunctiva color into different anemia levels (1 to 5 scale) according to the FAMACHA© eye color chart (Vatta et al., 2001). In this

technique, only anemic animals need to be dewormed, which helps in reducing deworming. FAMACHA score system has been validated and identified as an effective tool in determining anemic sheep and goats in the southern US due to *H. contortus* infection (Kaplan et al., 2004).

The FAMACHA score also helps in solving the side effects of using chemical in the deworming which has led to increase in the number of anthelmintic resistant gastrointestinal nematodes (Mortensen et al., 2003 and Kaplan, et al., 2004). In the southern US, studies have shown that the FAMACHA score system is a useful tool for identifying anemic sheep and goats and that use of dewormers has been greatly reduced (Burke et al., 2007; Kaplan et al., 2004).

Haemonchosis can be controlled by reducing animal exposure to parasite by reducing pasture contamination, using FAMACHA score technique to indentify anemic animal and administering anthelmintics, and selecting animals that are resistant to *Haemonchus* for breeding, resistance to benzimidazoles, levamisole, and avermectins has been reported in sheep and goat (Kahn and Line, 2010).

2.6 The Immune System

The main role of the immune system is to distinguish organism's own cells and tissues (self) from non-self in order to protect against infection. Immune system has various mechanisms to eliminate non-self agents, this include inactivation, lysis, agglutination, precipitation, or phagocytosis of foreign cells (Kahn and Line, 2010). The Animals immune system is composed of two related functional elements, the innate

immune system and the adaptive immune systems (Hoffmann et al., 1999). Both function in coordination to protect against invading microorganisms (Medzhitov et al., 2002). Innate immunity represents the first line of defense against organisms, it acts rapidly in non specific way through anatomical barriers (skin, mucous membrane), secretions, and other cellular elements. Innate immunity lacks immunological memory of previous exposure. The adaptive immune system is the second line of defense, its response to pathogens is slower than the innate immunity response .However, it is specific, effective, and the protection it gives lasts longer (Bishop et al., 2007; Alberts et al., 2000; Adams and Templeton, 1998).

The immune system cells are originated in the bone marrow and produced by hematopoietic system. Macrophage, neutrophils, eosinophils, basophils, and dendritic cells are myeloid cells, while B lymphocytes, T lymphocytes and Natural Killer cells are lymphoid cells. Some of the cells are referred to as Leukocytes, or white blood cells (WBC), and they are classified into granulocytes and agranulocytes. Granulocytes consist of basophils, eosinophils, and neutrophils. Agranulocytes consist of monocytes, macrophages and lymphocytes (B-cells and T-cells). All play a role in defending the body against both infectious disease and foreign materials. Leukocytes are distinguished from each other by morphology and affinity towards histochemical stains. They differ in the site of their production, their life span in peripheral circulation, and the way of their stimulation release, and migration through blood circulation (Luster et al., 2005).

A differential WBC count is a useful method used in clinical diagnosis to determine the percentage of each WBC components in the peripheral blood by counting

the first 100 cells in blood smear. Differential WBC counts vary among the species. In goats 30 - 48 % of blood leukocytes are neutrophils, 1 - 8 % are eosinophils, 0 - 4 % are monocytes, 50 - 70 % are lymphocytes, 0 - 1% are basophils (Kahn and Line, 2010).

The lymphocyte is a type of immune cell that is capable of attacking foreign cells, cancer cells, and cells infected with a virus. The two main types of lymphocytes are B lymphocytes and T lymphocytes. T lymphocytes have a role in regulating the host immune responses. B lymphocytes regulate humoral immune response by releasing antibodies. T lymphocytes regulate activation of cell-mediated immunity such macrophages, natural killer cells (NK), cytotoxic T-lymphocytes, and cytokines (Gallya et al., 2011).

Monocytes function as phagocytic cells as part of the immune system response. After their formation, they enter the peripheral blood and then into the tissues to become permanent. When released, they mature into macrophages. Monocytes move fast to sites of infection and differentiate into macrophages and dendritic cells. In stained blood smear, moncytes are characterized by their large bipolar nucleous (Swirski et al., 2009).

Macrophages are large phagocytic leukocytes produced by monocyte differentiation. Macrophages play significant role in innate immunity by engulfing, destroying, and removing foreign organisms by phagocytosis. Macrophages present antigen to stimulate adaptive immunity (Khazen, et al., 2005; Janeway et al., 2001). Macrophages and dendritic cells provide the first line of cell-mediated defense by restricting the initial growth of infectious organisms and regulating the immunological reactions (Stafford et al., 2002). Macrophage has a role in cytokines production and

presentation of antigen to T cells through the MHC (Dekkers and Hospital 2002; Stafford et al., 2002).

Eosinophils are Pleiotropic multifunctional white blood cells which participate in various inflammatory responses, as well as regulator of innate and adaptive immunity and also in defending the host against parasitic infections (Young et al., 2006; Hogan et al., 2008). Peripheral blood eosinophils increase in response to parasites, gastrointestinal inflammation, and allergy. Their enzymes such as histaminases regulate histamine release by mast cells as a response to IgE stimulation. In a study of *Haemonchus contortus*, it was concluded that circulating eosinophils were greater in susceptible goat compared to resistant of goats (Bambou et al., 2009).

Basophils are white blood cells characterized by basophilic granules (blue stain) and multilobed nucleus they play a role in response to parasitic infection and their presence is rare in the peripheral blood. Its granules contain histamine (vasodilator) and a heparin (anticoagulant) (Voehringer, 2009).

Dendritic cells (DCs) are immune system cells that play a role in regulating innate and adaptive immunity responses by processing and presenting antigens to T and B lymphocytes (Banchereau, et al 1998). In response to intracellular pathogens, DC secrete interleukin-12, which stimulates T-helper cells to form Th1.The Th1 cells activate cell-mediated immunity (CMI) to produce interferon (IFNγ) and IL2. However, in response to extracellular pathogens, for example, helminth, dendritic cells (DC) secretes IL4 to stimulate the naïve TH cells into Th 2 differentiation. The Th2 cells activate antibody

production through the secretion of IL4, IL5, IL10, and IL13, which increase IgE production, eosinophils and mast cells (Kahn and Line, 2010).

2.7 Natural Resistance Associate Macrophages Protein One (NRAMP1)

The Nramp1 or Slc11a1 is a gene that encodes a transmembrane protein in the phagolysosome in macrophages, which regulates divalent cation transport (Gruenheid et al., 1997). This gene is expressed exclusively in myeloid cells. Nramp1 has a role in controlling intracellular iron homoeostasis, which influence the proliferation of intracellular pathogens (Forbes and Gros, 2001; Canonne-Hergaux et al., 1999).

Polymorphism is the occurrence of one of two or more variants of a particular DNA sequence, a gene, or chromosome in a population. Single nucleotide polymorphism (SNP) is the most common type of polymorphism which involves variation at a single base pair. Scientists studied the relationship between SNPs and diseases, drug responses, and other phenotypes. In *NRAMP1* polymorphism was studied in mice by replacing glycine with aspartic acid residue at position 169 of the protein. Mice with *NRAMP1* (glycine at position 169) were highly resistant to *Leishmania donovani* and *Salmonella typhimurium*, where as mice with the mutated *NRAMP1* (aspartic acid at position 169 instead of glycine) were highly susceptible (Vidal et al, 1995; Malo et al, 1994).

Cytokines are cell signaling proteins that function in stimulating and regulating the immune response (Mark and David, 2002). Helper T (Th) cells are classified into two subgroups, Th1 cells secrete cytokines IL-1, IL-2, IL-8, IL-12, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNFα) to regulate cell-mediated immunity and induce

inflammation and cytotoxicity. Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 to regulate humoral immunity and suppress the inflammatory actions of Th1 cells to regulate humoral immunity and suppress the inflammatory actions of Th1 cells (Liew, 2002; Else and Finkelman 1998; Mosmann and Coffman, 1989; Cher and Mosmann, 1987).

Interleukin 10 (IL-10) plays a significant role in suppressing inflammatory responses (anti inflammatory) by stimulating the production of type 2 cytokines and inhibiting the production of type 1 cytokines in Th1 cells, macrophages, and monocytes (Nockowski and Baran, 2005; Blumberg et al., 2001; Asadullah et al., 1998, and Vieira et al., 1991). Monocytes and T cells (Th2 cells) are main sources of IL-10 (Vieira et al., 1991).

Interlukin8 (IL-8) is an important inflammatory mediator that is responsible for the migration and activation of neutrophils. IL-8 is produced by monocytes, macrophages and other cells stimulated by IL1 and LPS (Matheson et al., 2006).

Interferon gamma (IFN γ) is a multifunction cytokine; it works as antiviral and macrophage activation and inhibition of the intracellular development of protozoan parasites (Liesenfeld et al., 1996; Dijkmans and Billiau, 1988; Murray, 1988; Houglum, 1983). Several cells may secrete IFN γ including CD4, T cells, CD8. T cells, NK cells, and macrophages. In mice, interferon IFN γ has been involved in early stage immune response to *Eimeria vermiformis* infection, increase in the production of IFN-γ was associated with genetically resistant mice to coccidia. Depletion of endogenous IFN-γ using a rat anti-IFN γ mAb resulted in increased oocyst output and mortality (Wakelin et

al., 1993). Interferon IFN γ has been shown to play an important role in host defense against *Eimeria* (Lillehoj and Choi, 1998, Rose et al., 1991, and Rose et al., 1989), *Leishmania* (Scott, 1991), *Plasmodium* (Schofield et al., 1987) and *Toxoplasma spp* (Suzuki et al., 1988). Several studies have also looked at associations between specific genes or markers and FEC. In particular, Coltman et al. (2001) found significant associations with a microsatellite within the interferon gamma gene in feral sheep.

Cluster of differentiation antigen 14 (CD14) is a gene that encodes a protein and functions as part of innate immune system, expressed mainly by macrophages (Setoguchi et al., 1989; Simmons et.al., 1989), monocytes, polymorphonuclear neutrophils (PMN) (Tobias and Ulevitch, 1993), B-cells (Schumann et al, 1990), and dendritic cells (Verhasselt et al, 1997). Cluster of differentiation 14 (CD14) functions as a co-receptor along with the Toll-like receptor TLR 4, to detect bacterial lipopolysaccharide (LPS), the main component of the cell wall of Gram-negative bacteria and stimulate LPS-dependent responses in a variety of cells (Darveau et al., 1995). The recognition of LPS by the immune cells involves CD14 (Haziot et al., 1998). Pathogens in general consist of pathogen-associated molecular patterns (PAMPs) which stimulate a group of receptors on the surface of the immune cells. The receptors which interact with PAMPs are called pattern recognition receptors (PRRs) and include CD14, the Toll-like receptors (TLRs), complement receptors, and other receptors (Janeway and Medzhitov, 2002). There is documented association between CD14 and IgE in man (Mitre and Nutman, 2006). The isotype IgE is known to be associated with the immune response to Haemonchus

contortus and other helminth infections. Further, CD14 is important in activating eosinophil cells essential to the anti-nematode response.

2.8 Genetic Resistance to Gastrointestinal Parasites

Selecting animals for breeding based on their genetic resistance to pathogens has been used in farm animals in order to provide natural resistance against several diseases. The genes that provide this resistance can also function as markers for prediction of genetic resistance against specific diseases (Adams and Templeton, 1998). Research on meat goat genetic resistance to disease is still limited, more studies were conducted in sheep than goat (Sahlu et al., 2009).

Studies investigated pathological, immunological and parasitological responses in goats after experimental infection with *H. contortus* (Fakae et al., 1999; Perez et al., 2001; Perez et al., 2003). In goats several studies concluded that variable genetic resistance to gastrointestinal parasites occurred between breeds (Pralomkarn et al., 1997; Baker et al., 1998, Baker et al., 2001, and Chauhan et al., 2003, Costa et al., 2000) and within breeds (Morris et al., 1997, Mandonnet et al., 2001; Chauhan et al., 2003). Specific goat breeds are more resistant to internal parasite than others. For example, east African goats are more resistant than Galla goats, the latter had higher FEC and lower PCV when compared to east African goats around the year especially during lactation period (Baker et al., 2001). In a different study, Kiko and Spanish breeds showed more resistant to internal parasites than Boer goats (Browning et al., 2007; Baker and Gray 2004). Gray (1991) reported that applying fecal egg counts (FEC) in natural parasitic

infection is useful method for detecting genetic resistance to GI nematodes. This is especially true during early parasitic exposure (Bishop et al., 1996; Morris et al., 2000) and its later stages (Khusro et al., 2004). Therefore, genetic variation between animals resistance to particular diseases exist (Albers et al., 1987).

2.9 Anthelmintic Resistance by Gastrointestinal Parasites

Parasitic resistance to anthelminitics posed a major threat to successful use of the drugs in farm animals (Rahman, 1994; Craig, 1993). Parasites resistance to some of these chemical agents, in some occasions, led to complete failure to eliminate the parasites (Burke and Miller, 2006). Anthelmintic resistance in *H. contortus* has been found in the areas where haemonchosis is endemic and farmers practice frequent anthelmintic treatment (Waller, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Animals Used for the Study

All one hundred and forty goats were selected from the herd at North Carolina Agricultural and Technical State University (NCA&TSU), Small Ruminant Research Unit (SRRU). All animals were ear tagged prior to the study. Goats used in the study were healthy, free of noticeable clinical signs for coccidiosis and haemonchosis, and were not under treatment during the study period.

Throughout the study all goats were kept in SRRU at NCA&TSU, and under balanced diet fed in adequate amounts, good sanitation, appropriate housing and equipment, and good management.

Breeds selected for the study were South African Boer, Spanish and Spanish Cross breed meat goats, which were all females. Age of goats used in the study ranged from 1 to 6 years old. Ages were determined based on SRRU records at NCA&TSU.

In the initial survey, all goats (N=140) were screened for coccidia, *haemonchus*, and packed cell volume. Sixty goats were then selected for further studies based on breed, age, and parasitic burden. Body weights were obtained by weighing each goat on a scale in pounds then, converted into kilograms. Body condition refers to the fleshiness of the goat which is scored from 1 to 5, where 1 is very lean, 2 is lean, 3 is moderately good

condition, 4 is fat, and 5 is obese. Body condition score was determined by physically handling the animal, assessing the rib areas using firm pressure with the fingers and running fingers down the goat's spine from the shoulders to the tail head, the degree of fat cushion over these bones determines the score.

3.2 Selection of Animals for Gene Expression

Out of sixty animals, twelve goats were selected for gene expression studies based on low and high fecal egg output for coccidia, age, and breed. Four goats from each of the three breed were selected, two of them were yearlings and two were adults.

3.3 FAMACHA© Eye Color Score

FAMACHA© chart is a parasite monitoring system used to correlate fecal egg count (parasitic load) with color of the ocular conjunctiva (Kaplan et al., 2004). In this method, animals were classified into 5 categories according to eye mucus color; category number 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly anemic; 4 = pink-white, anemic; 5 = white, severely anemic. Only goats classified under category number 3, 4, and 5 should be treated with anthelmintic.

3.4 Fecal Sample Collection

Fecal samples were collected by inserting the index and middle fingers directly in

to the rectum wearing sterile gloves for each animal. Samples collected were placed in clean zip- lock plastic bags.

3.5 Blood Collection

Blood samples for PCV, RNA isolation, and blood smears, were collected from the jugular vein of each animal into 7-ml BD Vacutainer® blood collection tubes containing 0.7 ml of the anti- coagulant acid citrate dextrose. Blood samples were collected in 2.5 ml PAXgene blood RNA tubes (QIAGEN Inc, Valencia, CA). The PAXgene tube contains a proprietary reagent that immediately stabilizes intracellular RNA for 3 days at room temperature (18-25°C) and 5 days at 2-8°C. FTA elute cards (Whatman Inc. Piscataway, NJ) were used to collect blood at room temperature for DNA isolation.

3.6 Laboratory Analysis

A modified McMaster technique was used for fecal egg count as described by Whitlock (1948). Two grams of feces were weighed and dissolved in 28 ml saturated sodium chloride to cause parasites eggs to float to the top of the liquid. Pellets were broken down using spatula. One milliliter syringe was used to transfer aliquots of the solution into both McMaster slide chambers and examined under an Olympus B X40 microscope using 10X magnification according to the Paracount-EPG™ Fecal Analysis Kit (Chalex Corporation, Wallowa, OR). Number of eggs for both coccidia and

Haemonchus contortus were counted in duplicate, the average was calculated, and then multiplied by 50 to get the eggs per gram (epg) of feces for each animal.

Heaprinzed hematocrit micro-Capillary Tubes (Drummond cat # 1-000-7500-HC/5) were used to collect blood in duplicates from BD Vacutainer® Blood Collection Tubes (BD®, Franklin Lakes, NJ). Micro-capillary tubes were sealed with clay sealant (Fisher Scientific Inc. Pittsburgh, PA) and placed in numbered duplicate sample slots, and then centrifuged for 10 minutes at 14,000 rpm in an IEC MB Micro Hematocrit centrifuge (Damon/IEC Division, Needham, MA). The capillary tubes were then placed in micro capillary reader to determine the PCV.

A thin Blood smear was prepared by placing a drop of blood (approximately 4 mm in diameter) on one end of a micro slide. A spreader slide was used at 45° angle to disperse the blood over the slide's length in order to space out cells far enough to be counted and differentiated. The slide was left to air dry at room temperature then stained with Sure Stain Wright CS-432 solution according to the manufactures Instructions (Fisher Scientific Inc. Pittsburgh, PA), slides were dipped in Wright stain for 10 seconds then in deionized water for 15 seconds, and finally rinsed by dipping in fresh deionized water for 10 seconds. The slides were left to dry at room temperature.

White blood cell differential counts were carried out by placing slides under 100X magnification of Olympus B X40 microscope using Sigma Mineral oil M5904. Counting was performed using Fisher Five Button Lab Counter (Fisher scientific Inc. Pittsburgh, PA). One hundred cells were counted to identify the percentage of lymphocytes, neutrophils, monocytes, eosinophils, and basophils.

3.7 DNA Isolation Method

The FTA® Elute Card (Whatman Inc. Piscataway, NJ) is a card that is impregnated with a patented chemical formula that lyses cells and denatures proteins upon contact causing the release of nucleic acids. Blood was collected on FTA card for DNA isolation. FTA Elute Cards were labeled with the appropriate goat ear tag identification one card was used for each sample. About 40µl of blood was pipetted onto the card in a concentric circular motion within the printed circle area. The FTA Elute Cards were stored at room temperature.

Using the 3mm Harris Uni-Core device (Whatman Cat. No. WB100039), 3mm (1/8") sample discs were removed from the center of FTA Elute card and transferred into a 1.5 ml microcentrifuge tube. Sterile H_2O (500 µl) was added to the tube and immediately pulse vortexed 3 times, for a total of 5 seconds. Using a pipette tip, the disc was removed from the wash, gently squeezed against the side of the tube and immediately transferred to a 0.5 ml microcentrifuge or PCR tube containing 30 µl of sterile. The H_2O discs were completely immersed in the H_2O by briefly centrifuging the tube for 10 seconds the tube was then transferred to a heating block at 95° C for 30 min. Tubes were vortexed 15 times during the incubation period. At the end of the incubation period tubes were removed from the block and pulse vortexed approximately 60 times then centrifuged for 30 seconds, to separate the matrix from the eluate which contained purified DNA which was stored at -20˚C until use. The FTA Elute matrix disc was removed and discarded.

3.8 Isolation of RNA from Goat Peripheral Blood

Total RNA was extracted from blood collected in the PaxGene RNA collection tubes (QIAGEN Inc, Valencia, CA) using the ZR Whole-Blood Total RNA Kit (Zymo Research, Orange, CA), as per the manufacturers recommendation, briefly 600 µl ZR RNA Buffer was added to 200 µl whole-blood sample and mixed, the mixture was transferred to a Zymo-spin IIIC Column in a collection tube and centrifuged at 10,000 rpm (10,000-12,000 x g) for 30-60 seconds. Collection tube containing the flow-through was then discarded. RNA Prewash Buffer (400 µl) was added to the column, Centrifuged at 10,000 rpm for 30-60 seconds. RNA Wash Buffer (400 µl) was added to the column and centrifuged at 10,000 rpm for 30-60 seconds. DNase/RNase-Free Water (50 µl) was added directly to the column matrix which was then placed into an RNase-free 1.5 ml tube and centrifuged briefly to elute RNA which was stored at -70˚C until use.

3.9 Oligonucleotide Primers

Primers and PCR conditions for NRAMP1 and INF γ were selected according to previous studies performed by Judy, et al., (1998). Primers for IL-8 were used previously by Ables, et al., (2002). Primers for CD14 and GAPDH were studied by Shan et al., (2005). All primers used were purchased from MWG Inc, High Point, NC (Table 3.1).

Table 3.1. Primers used for the study

Gene	Primer Sequence	Length of PCR Product / bp
IL-10 Forward	5`-CGGCGCTGTCATCTGTTT-3`	571
IL-10 Reverse	5`-TCTTGGAGCATATTGAAGA-3`	571
INF- γ Forward	5`-CATCAAAGAAGACATGTTTC-3`	395
INF- γ Reverse	5`-TATGCCTTTGCGCTGGATCT-3`	395
GAPDH Forward	5`-GGCAAGTTCCATGCCACAGT`-3`	120
GAPDH Reverse	5`-GTCCCTCCACGATGCCAAAG-3`	120
CD-14 Forward	5`- GACGACGATTTCCGTTGTGT-3`	600
CD-14 Reverse	5`-TGCGTAGCGCTAGATATTGGA-3`	600
NRAMP1 Forward	5`-CATGAAGCCAACTGCCAAGG-3`	433
NRAMP1 Reverse	5`-GAAGCCTGCAAGATTGACCA-3`	433
IL-8 Forward	5`-GGAAAAGTGGGTGCAGAAG-3`	443
IL-8 Reverse	5`-GGTGGTTTTTTCTTTTTCATGG-3`	443

A multiplex PCR technique was used to amplify the PCR products for NRAMP1 and INFγ primers which were added simultaneously into each reaction tube. This method (Mutiplex PCR) was first used by Chamberlain et al, (1988). Each 25 µl reaction included 200 ng of goat genomic DNA, 5 µl 10X Long PCR Buffer, 2.5 µl PCR dNTP Mix, 1.25 µl for each of forward and reverse primer for NRAMP1 and INFγ, and 1 µl SuperTaq™ Plus DNA Polymerase (Ambion Inc, Austin, TX), which was added after initial denaturization at 94° C for 10 min, this was followed by 35 cycles at 94° C for 40 s, 61° C for 40 s 72° C for 40 s, and a final extension at 72° C for 10 min. The PCR

products were then loaded into 1.5% agarose gels in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA [pH 8.0]) buffer and visualized by ethidium bromide staining.

3.10 Genomic DNA and Primers

To detect the *NRAMP1* gene in genomic DNA, each 50-µl reaction included 200 ng of goat genomic DNA (N=40), 1.25 µl each of both NRAMP1 forward and reverse primer, 10X PCR buffer, and a 2.5 µl (2.5Mm) dNTPs, 1 µl superTaq plus DNA polymerase (Ambion Inc, Austin, TX) were added after initial denaturization at 94° C for 10 min, followed by 35 cycles at 94° C for 40 s, 61° C for 40 s, 72° C for 40 s, and a final extension at 72° C for 10 min. The PCR products were fractionated on 1.5% agarose gels in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA (pH 8.0) buffer and visualized by ethidium bromide staining.

3.11 Synthesis of cDNA

To prepare cDNA, isolated RNA from each goat was prepared and used at a concentration 1–2 µg of each sample. Two microliter of oligoDT primers was added (Ambion Inc, Austin, TX). The final volume was brought up to 12 µl by adding nuclease free water. The reaction was mixed, and then spun briefly. The sample was then heated for 3 minutes at 85°C, removed, spun briefly, and placed on ice. To this reaction 2 µl 10X RT Buffer,4 µl dNTP mix,1 µl RNase Inhibitor,and1 µl MMLV-RT Reverse Transcriptase (Ambion Inc, Austin, TX) were added so that PCR the final reaction was

20 µl. Samples were then mixed gently, spun briefly, and incubated at 44°C for 1 hr then at 92°C for 10 min to inactivate the Reverse Transcriptase. The cDNA produced was stored at - 20°C until use.

3.12 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was conducted in a thermocycler (MWG, USA). Each reaction consisted of 5 µl cDNA, 5 µl 10X Long PCR Buffer, 2.5 µl PCR dNTP Mix, 1.25 µl for each of forward and reverse primer for, and 1 µl SuperTaq™ Plus DNA Polymerase (Ambion Inc, Austin, TX), 1.25 µl forward and reverse for each of *NRAMP1*, INFγ, CD14, IL-10, and IL-8 primers. Experiments were conducted separately for, each primer. Total reaction volume was 50 µl. Samples were then amplified according to specific PCR conditions for each reaction as described in Table 3.2 (Judy, et al., 1998).

Table 3.2. Specific polymerase chain reaction conditions for NRAMP1 and INF- γ

PCR level	Temperature	Time involve
Pre-heating	94° C	10 min
Denaturation	94° C	40 s
Annealing	59° C	40 s
Post-heating	72° C	40 s
Final Extension	72° C	10 min

Polymerase chain reaction conditions for CD14, IL-8, IL-10, and GAPDH are shown in Table 3.3 according to previous studies conducted by Ables, et al., (2004) and Shan et al., (2005).

Table 3.3. Specific polymerase chain reaction conditions for CD14, IL-8, IL- 10, and GAPDH

PCR level	Temperature	Time involve
Pre-heating	94° C	5 minute
Denaturation	94° C	30 seconds
Annealing	56° C	30 seconds
Post-heating	72° C	30 seconds
Final Extension	72° C	5 minute

3.13 Analysis of PCR Product

The PCR products were analyzed by mixing 10 µl PCR reaction and 2 µl of 6x loading dye. The products were loaded into 1.5% agarose gel prepared in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA (pH 8.) buffer for separation by electrophoresis at 112 volts. PCR marker (G3161A, Promega, USA) was used. The gel was stained by ethidium bromide for 5 minutes followed by washing with deoinozed water and visualize under UV light. The Bio-rad gel docuamentation system was used to analyze the PCR samples.

3.14 Statistical Analysis

The statistical Analysis Software System SAS (SAS Institute, Cary, NC) was used for data analysis. Analysis of variance (ANOVA) was used to test if the model is significant. For example, age vs. body weight. Independent sample t-tests were used to analyze the relationship between expression and non expression of *NRAMP1* and body weight. Chi-square test was used to compare the effect of the three breeds on expression /non-expression of *NRAMP1*, the Chi-square gives only an estimate of the true probability value. When sample size is a small value (less than five) in one of the cell, Fisher's exact test was used instead of Chi-square test, for example, CD14 vs. breed.

CHAPTER 4

RESULTS

4.1 Selection of Animals for the Study

One hundred and forty goats were screened for age (years), body condition score, body weight (kg), FAMACHA score, PCV%, *haemonchus*, and coccidia eggs per gram of shedders. The results are presented in Appendix, Table 1. The average value for each of the above was 2.4, 3.75, 55.22, 2.74, 25.45, 588.54, and 1531.71 respectively.

Out of one hundred and forty goats, sixty goats (25 Boer, 25 Spanish, and 10 Spanish Cross) were selected for further study, based on low and high fecal egg shedders for coccidia (Appendix, Table 2). Animals with < 700 eggs per gram were classified as low. The average of coccidia eggs per gram of shedders was 295. Animals with average of 3610 coccidia eggs per gram were considered as high. Each group had thirty goats. Of the 60 goats chosen, there were 23 yearling and 37 adults. Health indicators in goats with high and low coccidia eggs per gram of feces were compared in Table 4.1.

4.2 Selection of Animals for Gene Expression Studies

Out of sixty animals, twelve goats were selected for gene expression based on low and high fecal egg output for coccidia, age, and breed. Four goats from each of the three breed were selected, two of them were yearling and two were adult.

Table 4.1. Comparison of health indicators in goats with high and low coccidia eggs per gram of feces

Health Parameters	Low Coccidia	High Coccidia
Age (years)	3.4	1.8
body condition score	3.6	3.2
body weight /Kg	57.2	44.1
FAMACHA score	2.4	3
PCV %	26.1	22.4
FEC for Haemonchus (epg)	548.6	1040
FEC for Coccidia (epg)	295.3	3610

4.3 Effect of Breed on Measures of Parasite Burden and Body Weight

Three different breeds of goat were studied (Boer, Spanish, and Spanish Cross). Comparisons between breed and each of body score condition (BSC), FAMACHA score system, and PCV were not significant ($p>0.05$) (Table 4.2).

Table 4.2. Effect of breed on health parameters

Breed	Age/ year	B. Wt/ (Kg)	BSC	FAMACHA	PCV %	FEC/H epg	FEC/C epg
Boer	2.8	51.5	3.4	2.9	22.9	1016	3294
Spanish	2.4	45.4	3.1	2.5	26.2	500	1098
Spanish Cross	2.5	60.0	3.9	2.7	23.0	932	768

Body weight comparisons for breed (N= 60) were not significantly different ($p>0.05$). However, Boer and Boer x Spanish goats tended to have higher average body weight (Figure 4.1).

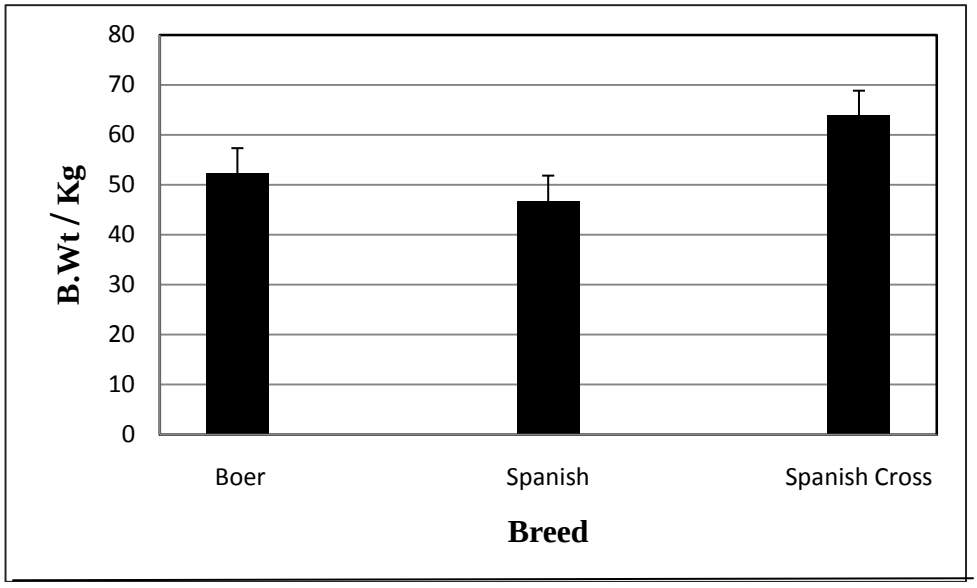

Figure 4.1. Effect of breed on mean body weight

The effect of breed (Boer, Spanish, and Spanish Cross) on each of *haemonchus* and coccidia eggs per gram (epg) of feces is not significant ($p>0.05$) (Figure 4.2). However, Boer goats are more susceptible to coccidiosis than Spanish goats. Breed had no significant effect on packed cell volume ($p>0.05$) (Figure 4.3).

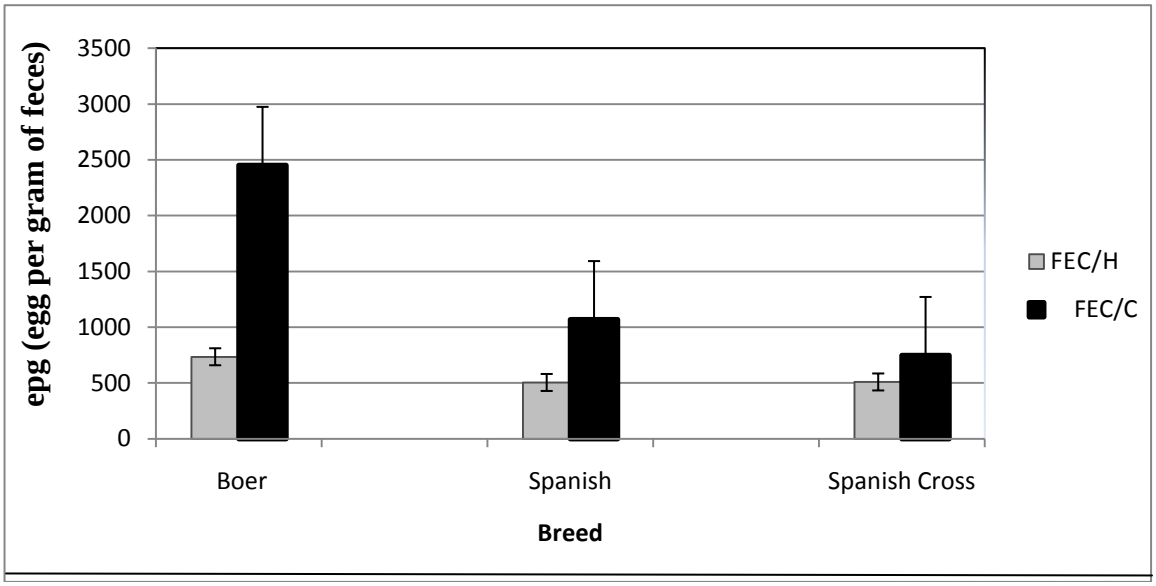

Figure 4.2. Effect of breed on coccidia and haemonchus levels

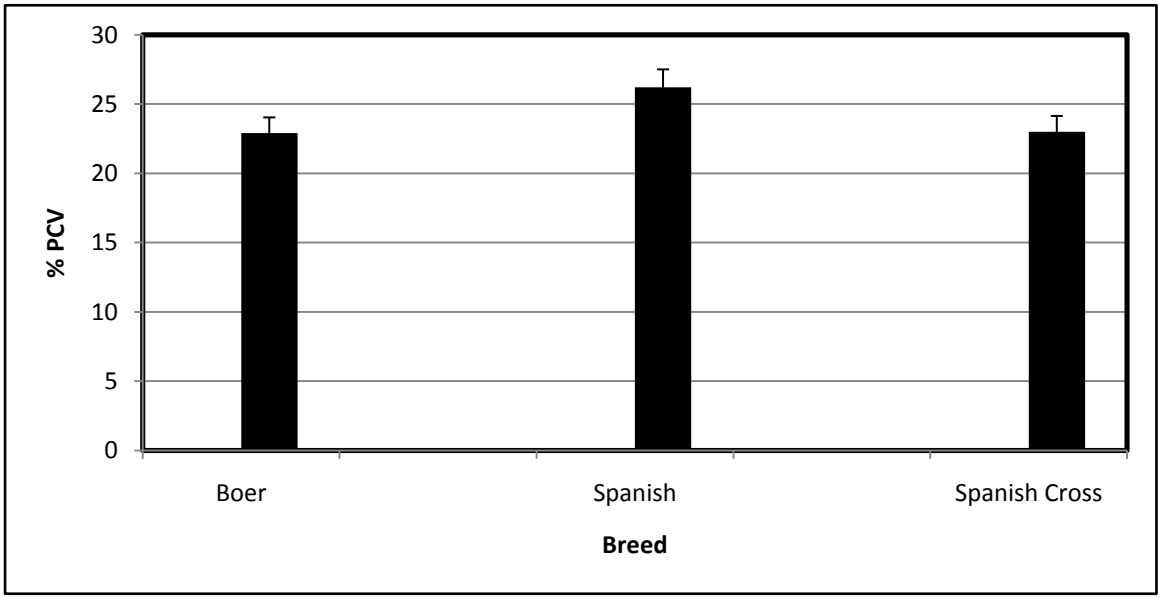

Figure 4.3. Mean packed cell volume among breeds

Spanish goats tended to have higher percentages of lymphocytes when compared with Boer goats and Boer x Spanish goats. However, Boer goats have higher percentage of Neutrophils, Eosinophils, and Monocytes when compared to Spanish goats and Boer x Spanish goats. The effect of breed on white blood cell differential count is shown in Figure 4.4 and Appendix, Table 3.

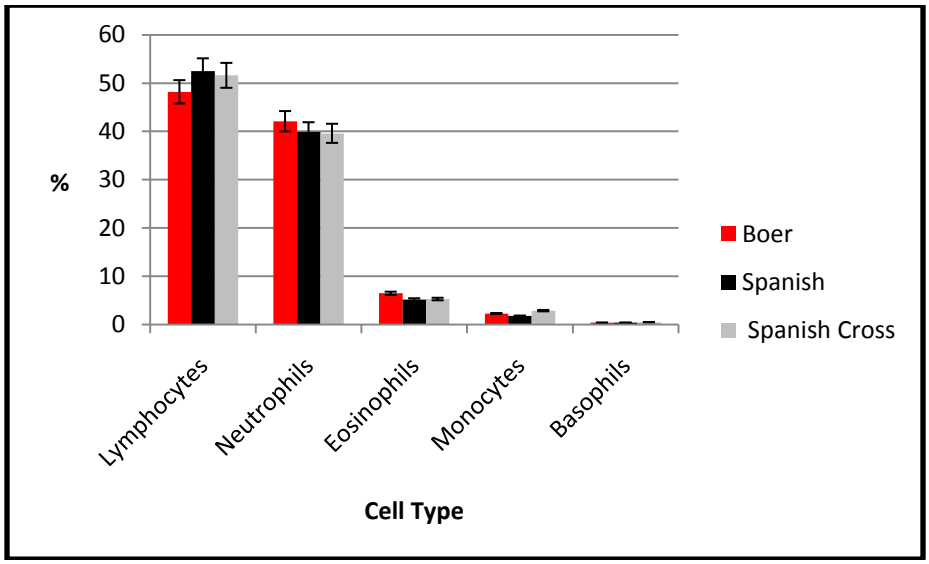

Figure 4.4. Mean white blood cell differential count among breeds

4.4 Effect of Age

Breed variation in mean health parameters for yearling and adult goats is presented in Table 4.3. Adult goats had better body condition ($p<0.0006$), heavier body weight ($p<0.0001$), and more resistance against internal parasites (FAMACHA $p<0.047$), than yearling goats. The correlation analysis results showed there was a weak negative

correlation between the body condition score and FAMACHA (r=-0.4023). However, age had no effect on PCV. Fecal egg count for *haemonchus* was not significantly related to age ($p>0.05$). Fecal egg count for coccidia was significantly related to age ($p<0.05$) (Figure 4.5).

Table 4.3. Breed variation in mean health parameters for yearling and adult goats

Age	B. Wt	BCS	FAMACHA	PCV	FEC/H	FEC/C
Yearling/Boer	43.0	3.1	3.2	20.7	1345.4	6659.0
Adult/ Boer	58.2	3.6	2.7	24.6	757.8	650.0
Yearling/Spanish	40.2	3.0	2.2	27.0	392.8	1757.1
Adult/ Spanish	47.4	3.1	2.5	25.8	544.1	827.0
Yearling/Spanish Cross	44.6	3.2	3.0	22.6	1120	1440
Adult/Spanish cross	73.3	4.5	2.5	23.6	775.0	208.3

Yearling ≤ 1 year. Adult > 1 year

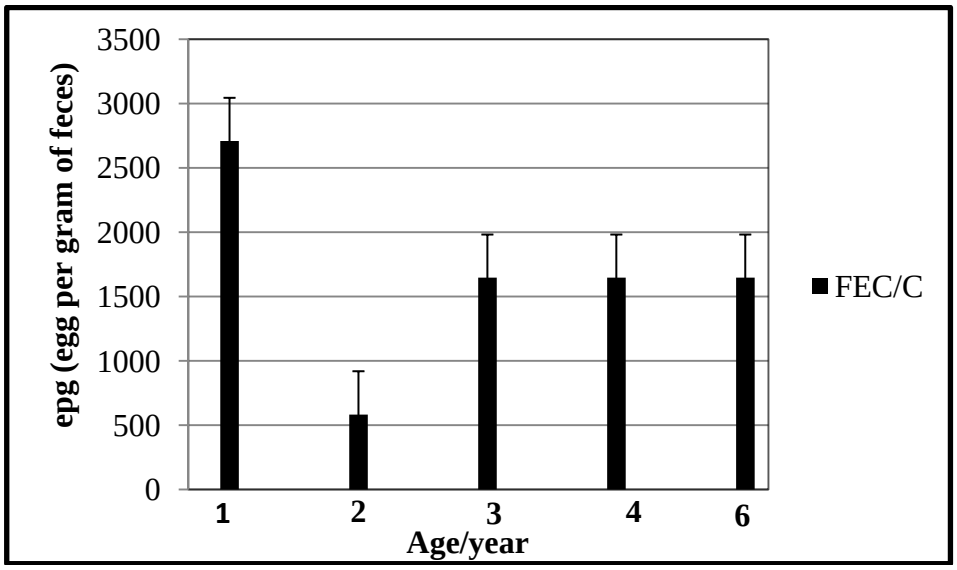

Figure 4.5. Effect of age on coccidia levels in goats

4.5 Effect of Infection

The fecal egg count for coccidia was not significantly correlated to white blood cells (Appendix, Table 4), FAMACHA score, and PCV ($p>0.05$). However, fecal egg count for *haemonchus* was significantly correlated to Lymphocytes ($p<0001$), Neutrophils ($p<0.0009$), Eosinophils ($p<0.0001$), and Monocytes ($p<0.0002$) (Table 4.4). FAMACHA score ($p<0.0007$) and PCV ($p<0.0003$) were also significantly correlated to Fecal egg count for *haemonchus*. Both coccidia and *haemonchus* have no effect on body condition score and body weight ($p>0.05$).

Correlation analysis of fecal egg counts of *haemonchus* and fecal egg counts of coccidia, body condition score (BCS), body weight (BW), FAMACHA score, and packed cell volume (PCV) is described in Table 4.5.

Table 4.4. Correlation analysis of haemonchus fecal egg counts vs WBCs

Description	r	p-value
Fecal egg count/ Haemonchus vs Lymphocytes	-0.91	<0001
Fecal egg count/ Haemonchus vs Neutrophils	0.83	0.0009
Fecal egg count/ Haemonchus vs Eosinophils	0.93	0.0001
Fecal egg count/ Haemonchus vs Monocytes	0.89	0.0002
Fecal egg count/ Haemonchus vs Basophils	-0.03	0.93

Table 4.5. Correlation analysis of haemonchus fecal egg counts vs select factors

Description	r	p-value
Fecal egg count/ Haemonchus vs Fecal egg count/ Coccidia	0.09961	0.7581
Fecal egg count/ Haemonchus vs BCS	0.09799	0.7619
Fecal egg count/ Haemonchus vs BW	0.31091	0.3253
Fecal egg count/ Haemonchus vs FAMACHA	0.83408	0.0007
Fecal egg count/ Haemonchus vs PCV	-0.86683	0.0003

Correlation analysis of fecal egg counts of coccidia and fecal egg counts of *haemonchus*, body score condition (BCS), body weight (BW), FAMACHA score and packed cell volume (PCV) is described in Table 4.6.

Table 4.6. Correlation analysis of coccidia fecal egg counts vs select factors

Description	r	p-value
Fecal egg count/ Coccidia vs Fecal egg count/ Haemonchus	0.09961	0.7581
Fecal egg count/ Coccidia vs BCS	0.24572	0.4414
Fecal egg count/ Coccidia vs BW	-0.04381	0.8925
Fecal egg count/ Coccidia vs FAMACHA	0.24984	0.4335
Fecal egg count/ Coccidia vs PCV	-0.23970	0.4530

4.6 Detection of NRAMP1

The gene encoding *NRAMP1* was differentially detected in genomic DNA (N=38) (Appendix, Table 5). Identification of *NRAMP1* in genomic DNA was not affected by breed. Figure 4.6 is a representative gel for *NRAMP1* in genomic DNA.

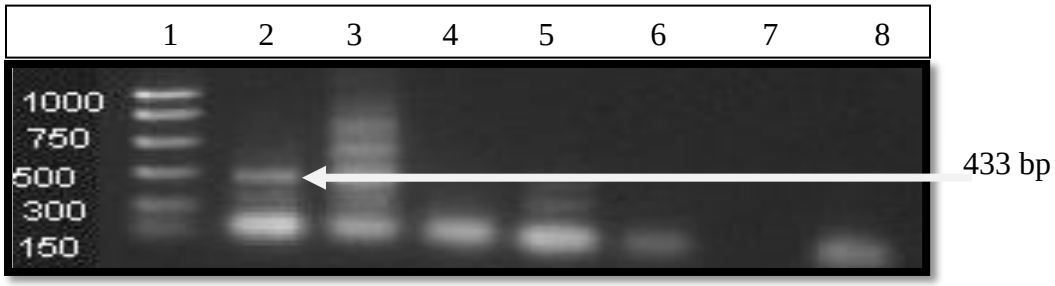

Figure 4 .6. Detection of NRAMP1 in genomic DNA in goat blood
Lane 1 is the PCR Marker, lane 2, 3, and 5 indicate detection of NAMP1. Lane 4 and lane 6 indicate no detection for NRAMP1. Lane 7 is a negative control. Lane 8 is a positive control (GAPDH) The arrow indicates the molecular weight of NRAMP1.

4.7 Expression of NRAMP1

The gene encoding *NRAMP1* was expressed with variability among animals (Figure 4.7). *NRAMP1* was significantly expressed with increased fecal coccidia egg counts ($p<0.05$) (Appendix, Table 6). Nramp1 was not significantly affected by fecal *haemonchus* egg counts (p=0.0677), PCV or BW (p-value=0.372).

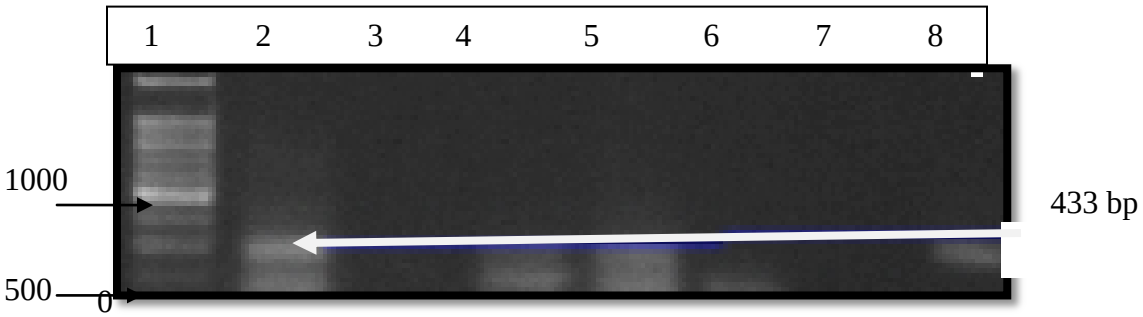

Figure 4.7. Expression of NRAMP1 in goat blood. Lane 1 is the PCR Marker, lane 2, 4, and 5 indicate expression of NAMP1. Lane 3 and Lane 6 indicate no expression for NRAMP1. Lane 7 is a negative control lane 8 is a positive control (GAPDH). The arrow indicates the molecular weight of NRAMP1.

The Gene encoding Interleukin 8 (IL- 8) was expressed in goat peripheral blood (N=12). IL-8 gene was differentially expressed in whole blood (Figure 4.8). Expression of IL-8 was associated with increased fecal egg counts for both coccidia and *haemonchus.*

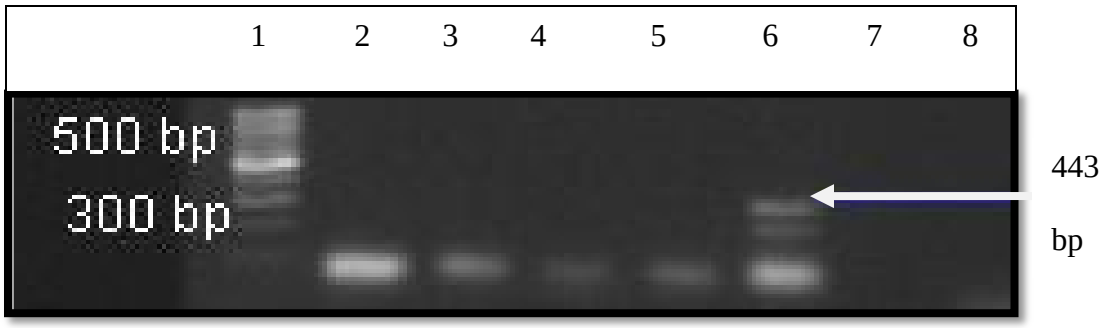

Figure 4.8. Expression of IL-8 in goat blood. Lane 1 is the PCR Marker. Lane 6 indicates expression of IL-8. Lane 2, 3, 4, and 5 indicate no expression for IL-8. Lane 7 is a negative control Lane 8 is a positive control (GAPDH). The arrow indicates the molecular weight of IL-8.

The Gene encoding Interleukin IL-10 was expressed in goat peripheral blood (N=12). IL-10 gene was differentially expressed in whole blood (Figure 4.9). Expression of IL-10, increased by coccidia infection ($p<0.05$).

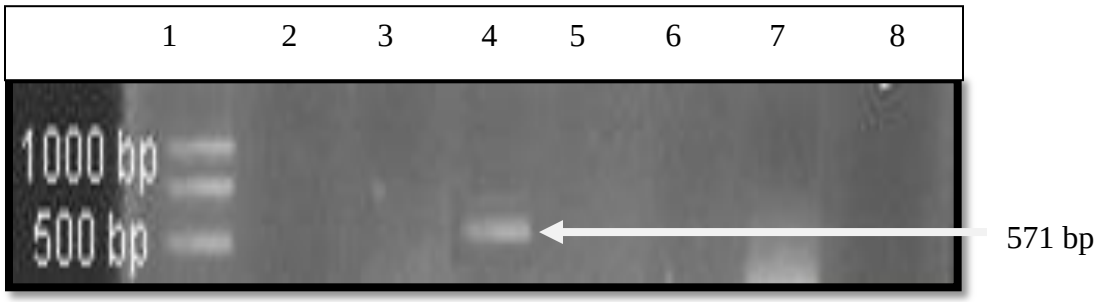

Figure 4.9 Expression of IL-10 in goat blood. Lane 1 is the PCR Marker. Lane 4 indicates expression of IL-10. Lane 2, 3, 5, and 6 indicate no expression for IL-10. Lane 7 is a positive control Lane 8 is a negative control (GAPDH). The arrow indicates the molecular weight of IL-10.

Cytokines expression at RNA level is described in Appendix, Table 6. The Gene encoding Interferon gamma (INF γ) (Figure 4.10) was expressed in goat peripheral blood (N=12). Interferon gamma gene expression was not affected by breed or associated with fecal egg counts for both coccidia and *haemonchus* (p>0.05).

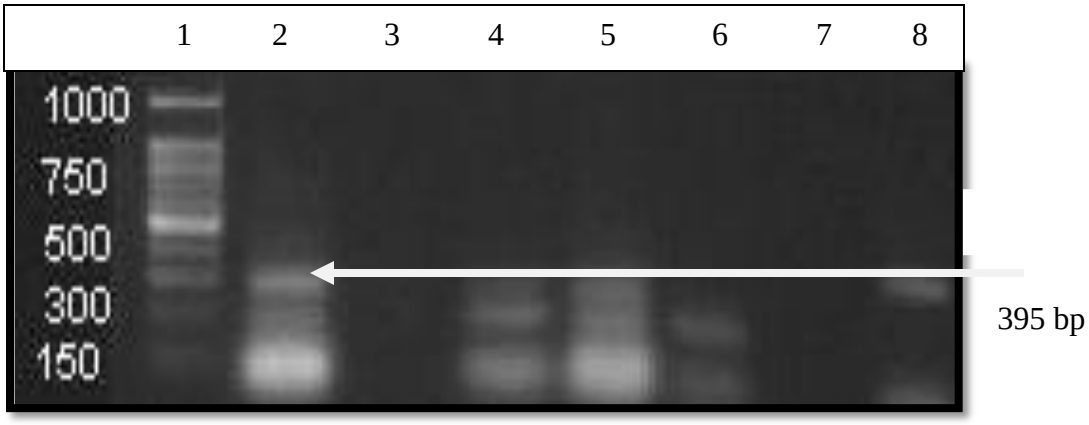

Figure 4.10 Expression of INF γ in goat blood. Lane 1 is the PCR marker. Lane 2, 4, 5 and 6 indicate expression of INF γ mRNA, lane 3 is empty, lane 7 is a negative control, and lane 8 is a positive control (GAPDH). The arrow indicates the molecular weight of INF γ.

Cluster of differentiation 14 (CD14) was expressed in goat peripheral blood (Figure 4.11). The expression of CD14 was significantly affected by increased fe counts for *haemonchus* (p<0.0114). Cluster of differentiation 14 (CD14) expressi not related to *NRAMP1* expression or coccidia fecal egg counts (p>0.05).

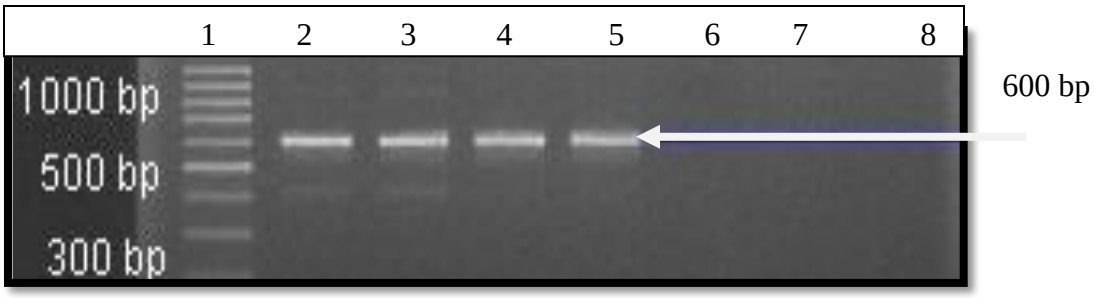

Figure 4.11. Expression of CD14 in goat blood. Lane 1 is the PCR marker. Lane 2, 3, 4, and 5 indicate expression of CD14, lane 6 and 7 are empty, and lane 8 is a negative control. The arrow indicates the molecular weight of CD14.

Figure 4.12. Expression of GAPDH in goat blood. Lane 1 is the PCR marker. Lane 2, 3, 4, 5, 6, and 7 indicate expression of GAPDH. Lane 8 is a negative control. The arrow indicates the molecular weight of GAPDH.

4.8 Multiplex PCR to detect NRAMP1

The QIAGEN Multiplex PCR Kit (QIAGEN Inc Valencia, CA) was used to detect *NRAMP1*. The master mix contained pre-optimized concentrations of Hot Star Taq DNA Polymerase and MgCl2, plus dNTPs and a PCR buffer developed for multiplex reactions. The association of gene expression with measures of parasite infection is

summarized in Table 4.7. The pattern of gene in relation to coccdia and *haemonchus* fecal egg count is summarized in Table 4.8

Table 4.7. Summary of association of gene expression with measures of parasite infection

Comparison	Test	P-value
NRAMP1 +/- vs breeds	Fisher's Exact Test	1.0
NRAMP1+/- vs FAMACHA	Fisher's Exact Test	0.5475
NRAMP+/- vs FEC/H	Independent sample T Test	0.0677
NRAMP+/- vs FEC/C	Independent sample T Test	0.0278*
NRAMP +/- vs PCV	Independent sample T Test	0.1127
NRAMP+/- vs BW	Independent sample T Test	0.7501
CD14+/- vs breeds	Fisher's Exact Test	1.0
CD14 vs FAMACHA	Fisher's Exact Test	0.0909
CD14+/- vs FEC/H	Independent sample T Test	0.0114*
CD14+/- vs FEC/C	Independent sample T Test	0.3675
CD14+/- vs PCV	Independent sample T Test	0.0772
CD14 +/- vs.BW	Independent sample T Test	0.0619
c IL10 +/- vs breeds	Fisher's Exact Test	0.0545
IL10+/- vs FAMACHA	Fisher's Exact Test	0.4909
c IL10+/- vs FEC/H	Independent sample T Test	0.7873
cIL10 +/- vs FEC/C	Independent sample T Test	0.0026*
IL10+/- vs PCV	Independent sample T Test	0.3763
IL10+/- vs BW	Independent sample T Test	0.9380
IL8+/- vs FAMACHA	Fisher's Exact Test	0.0606
IL8+/- vs FEC/H	Independent sample T Test	0.0103*

Table 4.7. (Continued)

Comparison	Test	P-value
IL8+/- vs FEC/C	Independent sample T Test	0.0349*
IL8+/- vs PCV	Independent sample T Test	0.0038*
IL8+/- vs BW	Independent sample T Test	0.6189

* indicates that the analysis shows significant difference (p-value < 0.05)
+ Indicates Gene expression / - Indicate non gene expression

Table 4.8 Summary of the observed pattern of gene expression

Gene	Coccidia	Haemonchus
NRAMP1	+	-
CD14	-	+
1L-8	+	+
1L-10	+	-
INF γ	+	+

+ indicates significant expression ($p<0.05$) - indicates no significant expression ($P>0.05$).

CHAPTER 5

DISCUSSION

Internal parasites pose the greatest challenge to goat production (Sahlu et al., 2009). Coccidiosis is a worldwide economically important serious animal disease. Helminth infections are considered to be a major disease problem in domestic livestock worldwide (Levine, 1986).

Previous studies concluded that when animals are exposed to pathogens some of them were susceptible while others were resistant. Studies of resistance to intracellular parasites such as *Salmonella pullorum* in poultry and *Brucella suis* in swine found a significant role of the host genetic makeup in the expression of the disease (Cameron et al., 1942; Roberts and Card, 1926).

Coccidiosis is one of the most important diseases of sheep and goats in the United States because of its effect on productivity, especially in the growing of lambs and kids (Levine, 1986). Prevalence of *Eimeria* and helminth parasitic infections is common and contributes to production losses (Donkin and Boyazoglu 2004; Valentine et al., 2007). Current approaches to control coccidiosis focus on management and use of coccidiostats. Breed differences in the immune response to coccidia have been reported (Mandonnet et al., 2001; Fakae et al., 1999; Vlassoff et al., 1999; Baker et al., 1998). Sporozoites of *Eimeria bovis* are able to invade and to replicate in endothelial cells and cause gene transcription and up regulation (Taubert et al., 2006). Studies on the gene expression and

the immune response to *Eimeria* infecting goats are limited. More studies were conducted in sheep than in goats (Sahlu et al., 2009)

Natural resistance-associated macrophage protein one (*NRAMP1*) has been associated with innate resistance to unrelated intracellular pathogen infections, up-regulation of proinflammatory phagocyte functions, and susceptibility to diseases. *INRAMP1* is expressed in cells of the myelomonocytic linkage and also in the duodenum. *NRAMP1* has been in diverse organisms from, bacteria to man. Recent studies have reported expression in cows (Worku and Morris, 2009). To our knowledge this is the first report of the detection of *NRAMP1* in goat genomic DNA and its expression in coccidia infected goats.

Natural resistance-associated macrophage protein one regulates intracellular pathogen proliferation and inflammatory responses in mice, man, and cows (Vidal, 1993). Natural resistance-associated macrophage protein one (*NRAMP1*) exhibits a natural polymorphism with alleles termed resistant and susceptible. Alleles restrict or allow the proliferation of intracellular pathogens (Barton, 1999).

In the current study breed effect was not detected. However, polymorphisms were observed in the bands detected following amplification from genomic DNA. Analysis of sequenced amplicons may yield evidence of the presence of single nucleotide polymorphism since using the selected primer *NRAMP1* was not detected in the genomic DNA of animals.

Age also has been associated with expression of *NRAMP1* in human subjects following infection with intracellular parasites. Leung et al., (2007) study confirmed the

association between SLC11A1 (*NRAMP1*) and TB susceptibility and demonstrated for the first time that the association was restricted to females and the young age group. In the current study age association was not observed. However, only 30 yearling goats were sampled and a larger sample may be necessary to see this association. The association between *NRAMP1* and sex cannot be investigated because all animals selected for the study were females. The *NRAMP1* protein is a phosphoprotein and the extent of phosphorylation changes in response to inflammatory cytokines (Barton, 1999). Studies in goats indicate that cytokines may be a factor in the pathogenesis of goat coccidiosis (Ibarra et al., 2007).

Infections with *Eimeria* species have been observed to result in differential cytokine gene expression including production of Interferon gamma (Laurent et al., 2001), IL-10 (Rothwell et al., 2004), and IL-8 in cattle (Coussens, 2004). In the present study expression of *NRAMP1*, IL-10, and IL-8 were associated with coccidia infection. No significant differences were observed in the white blood cell differential count. The expression of CD14, a co-receptor for LPS binding, was evaluated in light of reports in porcine leukocytes, that LPS-induces expression of *NRAMP1*, a highly conserved in a time and dose dependent (Zhang et al., 2000). In the present study CD14 expression was not related to *NRAMP1* expression or coccidia infection.

Strongylid gastrointestinal nematodes are an important cause of disease and economic loss in small ruminants. The bloodsucking parasite *Haemonchus contortus* is the predominant species. In the current study goats were naturally infected with both coccidia (a protozoan) and gastrointestinal nematodes (*haemonchus*). Concomitant

infection with gastrointestinal nematodes did not impact the levels of coccidia oocysts detected. Studies in adult sheep have reported that, strongyles and coccidia were independently and negatively associated with host weight. The mechanism of action of gastrointestinal nematodes and coccidia was suggested to be independent (Craig et al., 2008). No effect was observed on body weight or body condition score in response to gastrointestinal nematodes and coccidia in the present study. However, gene expression studies and the immune response can be influenced by co-infection (Rodrigues et al., 2010). The expression of *NRAMP1* was not associated with infection with GI parasites. Infection with gastrointestinal nematodes impacted the % PCV and FAMACHA score supporting the identification of the majority of gastrointestinal eggs as being from the barber pole worm *haemonchus contortus*. The inflammatory cells neutrophils, eosinophils, and moncytes were significantly increased in response to strongyle infections. The increase in monocytes and *Haemonchus* egg counts was associated with an increase in CD14 expression. Previous studies indicate that expression of CD14 is associated with increases in eosinophils (Kedda, 2005) and IgE allergic reactions. Thus further studies are warranted to define the significance of this association in light of possible incidence of environmental bacteria and exposure to LPS.

CHAPTER 6

CONCLUSION

These results demonstrate that *NRAMP1* can serve as a molecular marker and therapeutic target against coccidia in goats. More studies on the association between the *NRAMP1* gene and innate immune response to pathogens may aid in understanding and enhancement of goat genetic resistance to pathogens.

This study also concluded that CD14 expression was significantly higher in goats with high *Haemonchus contortus* egg counts and was correlated with monocyte levels in blood CD14 was expressed with variability among animals. Infection with Coccidia was not related to CD14 expression or monocyte levels.

Genetic susceptibility to intracellular pathogens is linked to the *NRAMP1* gene, the significant part of natural resistance to disease can be inherited through crossbreeding including *NRAMP1*. However, it should be understood that natural resistance to parasitic infection is relative and not absolute, also selecting animals for breeding based on their natural resistance is not expected to give complete immunity against infectious diseases rather than to reduce the pathogenity of the disease and economic burden cause of the disease. Overall results provide information to support selection and management of animals based on, breed, age, parasitic infection, and genetic resistance which should be shared with animal producers.

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APPENDIX

Table 1. Initial survey of 140 goats from ruminant research unit NC A & T farm

Serial No.	Tag Number	Goat Breed	Sex	Age/ Year	PCV %	FEC/Haemonchus	FEC/Coccidia
1	Y05	Spanish	F	3	30	50	1100
2	381	Spanish	F	4	27	250	250
3	603	Spanish	F	2	25	300	1250
4	521	Spanish	F	3	31	50	100
5	435	Spanish	F	4	31	50	100
6	706	Boer	F	1	28	150	7500
7	701	Boer	F	1	18	1000	4750
8	700	Boer	F	1	19	1000	2200
9	750	Boer	F	1	21	700	1050
10	725	Boer	F	1	19	1000	2000
11	703	Spanish	F	1	25	300	700
12	709	Boer	F	1	24	400	1050
13	737	Spanish	F	1	32	0	1050
14	716	Spanish	F	1	19	1050	1150
15	711	Spanish	F	1	34	0	200
16	755	Spanish	F	1	29	100	500
17	2017	Boer	F	6	20	850	1500
18	2193	Boer	F	6	29	100	500
19	2034	Boer	F	6	26	300	400
20	2172	Boer	F	6	18	1550	300
21	615	Boer	F	2	23	500	50
22	4	SpanishX	F	4	22	550	200
23	12	SpanishX	F	4	23	500	150
24	8	SpanishX	F	4	22	550	150
25	16	SpanishX	F	4	25	350	100
26	621	SpanishX	F	2	33	0	650
27	705	SpanishX	F	1	17	2300	1850
28	713	Spanish	F	1	21	600	1250
29	739	SpanishX	F	1	29	50	1450
30	735	SpanishX	F	1	30	150	150
31	721	SpanishX	F	1	16	2500	2500
32	Y07	Spanish	F	3	27	700	300
33	605	Spanish	F	2	22	900	600

Table 1. (Continued)

Serial No.	Tag Number	Goat Breed	Sex	Age/ Year	PCV %	FEC/Haemonchus	FEC/Coccidia
34	386	Spanish	F	4	23	1600	0
35	6	SpanishX	F	4	17	2700	0
36	2195	Boer	F	6	24	600	100
37	2019	Boer	F	6	22	1200	1000
38	637	Boer	F	4	20	900	1200
39	9	Boer	F	4	27	500	500
40	824	Spanish	F	4	20	440	160
41	816	Spanish	F	3	20	1150	5100
42	842	Boer	F	1	16	5150	39100
43	808	Spanish	F	1	19	1300	7300
44	804	Spanish	F	3	26	500	1000
45	806	Boer	F	4	32	300	150
46	817	Boer	F	1	15	3150	3850
47	832	Boer	F	4	29	760	150
48	853	Boer	F	2	25	500	1100
49	834	Boer	F	1	28	100	5150
50	826	Boer	F	1	26	0	1850
51	813	Boer	F	3	30	2150	1800
52	835	Boer	F	2	20	400	350
53	812	Boer	F	1	14	2150	4750
54	805	Spanish	F	3	31	450	550
55	807	Spanish	F	1	31	0	1400
56	809	Spanish	F	3	19	700	1350
57	828	Spanish	F	2	30	160	600
58	830	Spanish	F	2	29	100	600
59	840	Spanish	F	3	24	1200	600
60	821	Spanish	F	3	25	650	400
61	434	Boer	F	1	19	900	2000
62	2022	Boer	F	6	21	700	1500
63	2035	Boer	F	6	26	300	500
64	2095	Boer	F	1	19	900	4000
65	2101	Boer	F	1	29	50	2000
66	2108	Boer	F	1	28	150	1500
67	2149	Boer	F	4	23	500	100
68	2162	Boer	F	6	26	300	300
69	2036	Boer	F	6	22	840	0

Table 1. (Continued)

Serial No.	Tag Number	Goat Breed	Sex	Age/ Year	PCV %	FEC/Haemonchus	FEC/Coccidia
70	2173	Boer	F	6	30	100	600
71	2191	Boer	F	6	28	200	200
72	3005	Boer	F	1	21	700	1050
73	504	Spanish	F	1	31	0	940
74	509	Spanish	F	1	19	1300	1000
75	511	Spanish	F	1	26	150	1050
76	517	Boer	F	1	29	50	2000
77	519	Spanish	F	1	32	0	1000
78	524	Spanish	F	4	31	50	100
79	526	Boer	F	6	29	100	50
80	534	Boer	F	6	20	450	800
81	544	Spanish	F	3	31	150	50
82	547	Boer	F	1	24	350	1600
83	548	Boer	F	1	19	950	1850
84	550	Spanish	F	3	30	100	300
85	583	Boer	F	2	23	600	200
86	7	SpanishX	F	1	32	0	800
87	14	SpanishX	F	2	32	50	600
88	15	SpanishX	F	2	31	150	150
89	10	SpanishX	F	2	33	50	300
90	11	SpanishX	F	2	29	550	900
91	17	SpanishX	F	2	30	100	400
92	13	SpanishX	F	2	30	50	700
93	380	SpanishX	F	1	32	50	1200
94	388	SpanishX	F	1	30	100	1050
95	383	SpanishX	F	1	31	50	950
96	384	SpanishX	F	1	30	150	1800
97	385	SpanishX	F	1	29	100	1450
98	2016	SpanishX	F	1	29	300	1800
99	1	SpanishX	F	4	29	300	150
100	2	SpanishX	F	4	27	600	50
101	5	SpanishX	F	4	29	450	200
102	3	SpanishX	F	4	28	950	0
103	23	Spanish	F	1	30	100	200
104	387	Spanish	F	1	29	100	2000
105	433	Spanish	F	1	28	150	2400

Table 1. (Continued)

Serial No.	Tag Number	Goat Breed	Sex	Age/ Year	PCV %	FEC/Haemonchus	FEC/Coccidia
106	382	Spanish	F	1	26	1000	1200
107	315	Spanish	F	1		Dry	Dry
108	316	Spanish	F	1		Dry	Dry
109	305	Spanish	F	1	19	1200	1000
110	309	Spanish	F	1	19	1000	2000
111	527	Boer	F	1	23	1130	2040
112	506	Boer	F	3	22	500	520
113	530	Boer	F	1	24	Dry	Dry
114	546	Boer	F	1	25	1080	3900
115	536	Boer	F	1	30	90	3700
116	581	Boer	F	1	21	140	4880
117	562	Boer	F	1	22	1090	550
118	537	Boer	F	4	27	200	940
119	502	Boer	F	4	27	50	200
120	532	Boer	F	1	22	50	4350
121	531	Spanish	F	2	26	1030	1030
122	549	Spanish	F	3	26	330	360
123	584	Sapnish	F	1	18	470	1970
124	556	Spanish	F	1	29	100	2240
125	523	Spanish	F	3	26	600	550
126	514	Spanish	F	1	24	600	1520
127	503	Spanish	F	1	26	1600	2082
128	541	Spanish	F	2	27	400	440
129	522	Spanish	F	3	27	400	150
130	520	Spanish	F	1	22	1200	2000
131	535	SpanishX	F		23	Dry	Dry
132	533	SpanishX	F		32	Dry	Dry
133	540	SpanishX	F		26	Dry	Dry
134	518	Spanish	F		27	150	300
135	551	SpanishX	F		27	100	250
136	513	SpanishX	F		22	Dry	Dry
137	516	SpanishX	F		33	Dry	Dry
138	501	SpanishX	F		24	Dry	Dry
139	503	SpanishX	F		20	Dry	Dry
140	505	SpanishX	F		29	100	2000
139	503	SpanishX	F		20	Dry	Dry

Table 1. (Continued)

Serial No.	Tag Number	Goat Breed	Sex	Age/ Year	PCV %	FEC/Haemonchus	FEC/Coccidia
140	505	SpanishX	F		29	100	2000
139	503	SpanishX	F		20	Dry	Dry
140	505	SpanishX	F		29	100	2000
137	516	SpanishX	F		33	Dry	Dry
138	501	SpanishX	F		24	Dry	Dry
139	503	SpanishX	F		20	Dry	Dry
140	505	SpanishX	F		29	100	2000

SpanishX breed means BoerXSpanish goat breed

Table 2. Goats selected for the study based on parasitic burden

Doe Tag Number	Goat Breed	Age/ Year	BCS	B.Wt/ (Kg)	FAMACH Score	PCV %	FEC/ H	FEC/ C
Y05	Spanish	3	4	59	2	30	50	1100
381	Spanish	4	3	62	2	27	250	250
603	Spanish	2	3	49	3	25	300	1250
521	Spanish	3	4	77	2	31	50	100
435	Spanish	4	3	68	2	31	50	100
706	Boer	1	3	35	2	28	150	7500
701	Boer	1	4	59	4	18	1000	4750
700	Boer	1	4	52	4	19	1000	2200
750	Boer	1	3	44	3	21	700	1050
725	Boer	1	2	40	4	19	1000	2000
703	Spanish	1	3	38	3	25	300	700
709	Boer	1	3	45	3	24	400	1050
737	Spanish	1	3	43	1	32	0	1050
716	Spanish	1	3	44	3	19	1050	1150
711	Spanish	1	3	41	1	34	0	200
755	Spanish	1	3	46	2	29	100	500
2017	Boer	6	4	70	3	20	850	1500
2193	Boer	6	4	72	2	29	100	500
2034	Boer	6	5	102	2	26	300	400
2172	Boer	6	5	77	4	18	1550	300
615	Boer	2	5	84	3	23	500	50

Table 2. (Continued)

Doe Tag Number	Goat Breed	Age/ Year	BCS	B.Wt/ (Kg)	FAMACH Score	PCV %	FEC/ H	FEC/ C
4	SpanishX	4	3	49	3	22	550	200
12	SpanishX	4	5	75	3	23	500	150
8	SpanishX	4	5	90	3	22	550	150
16	SpanishX	4	5	98	2	25	350	100
621	SpanishX	2	5	79	1	33	0	650
705	SpanishX	1	3	45	4	17	2300	1850
713	SpanishX	1	3	45	3	21	600	1250
739	SpanishX	1	3	44	2	29	50	1450
735	SpanishX	1	3	45	2	30	150	150
721	SpanishX	1	4	44	4	16	2500	2500
Y07	Spanish	3	4	66	2	27	700	300
605	Spanish	2	4	60	3	22	900	600
386	Spanish	4	4	66	3	23	1600	0
6	SpanishX	4	4	49	3	17	2700	0
2195	Boer	6	4	77	2	24	600	100
2019	Boer	6	4	69	3	22	1200	1000
637	Boer	4	3	40	3	20	900	1200
9	Boer	4	3	45	3	27	500	500
824	Spanish	4	3	27	3	20	440	160
816	Spanish	3	4	41	2	20	1150	5100
842	Boer	1	3	38	4	16	5150	39100
808	Spanish	1	3	34	4	19	1300	7300
804	Spanish	3	4	45	3	26	500	1000
806	Boer	4	4	45	2	32	300	150
817	Boer	1	3	45	4	15	3150	3850
832	Boer	4	3	35	3	29	760	150
853	Boer	2	3	35	3	25	500	1100
834	Boer	1	3	35	2	28	100	5150
826	Boer	1	3	36	2	26	0	1850
813	Boer	3	2	40	2	30	2150	1800
812	Boer	1	4	44	4	14	2150	4750
805	Spanish	3	3	35	3	31	450	550
807	Spanish	1	3	36	2	31	0	1400
809	Spanish	3	2	30	4	19	700	1350
828	Spanish	2	3	35	2	30	160	600
830	Spanish	2	2	27	2	29	100	600

Table 2. (Continued)

Doe Tag Number	Goat Breed	Age/ Year	BCS	B.Wt/ (Kg)	FAMACH Score	PCV %	FEC/ H	FEC/ C
840	Spanish	3	2	30	4	24	1200	600
821	Spanish	3	2	30	2	25	650	400
835	Boer	2	2	24	3	20	400	350

Table 3. White blood cell differential count averages for each breed (N=60)

Breed	Lympho	Neutro	Eosino	Mono	Baso
Boer	48.2	42.1	6.5	2.3	0.4
Spanish	52.5	39.9	5.2	1.8	0.4
Spanish Cross	51.6	39.6	5.3	2.9	0.5

Table 4. Correlation analysis of fecal egg counts of coccidia and WBCs

Description	Test	R	p-value	Comment
FEC_C vs lympho	Correlation	-0.089	0.78	No correlation
FEC_C vs Neutro	Correlation	0.06	0.85	No correlation
FEC_C vs Eosino	Correlation	0.137	0.67	No correlation
FEC_C vs Mono	Correlation	-0.075	0.83	No correlation
FEC_C vs Baso	Correlation	-0.183	0.57	No correlation

Table 5. NRAMP1 Detection at Genomic DNA Level (N=38)

Serial Number	Doe Tag Number	Goat Breed	FEC/Hemonchus	FEC/Coccida	NRAMP1
1	Y05	Spanish	50	1100	+
2	381	Spanish	250	250	−
3	603	Spanish	300	1250	+
4	521	Spanish	50	100	
5	435	Spanish	50	100	−
6	706	Boer	150	7500	+
7	701	Boer	1000	4750	+
8	700	Boer	1000	2200	−
9	750	Boer	700	1050	+
10	725	Boer	1000	2000	−
11	703	Spanish	300	700	+
12	709	Boer	400	1050	+
13	737	Spanish	0	1050	+
14	716	Spanish	1050	1150	+
15	711	Spanish	0	200	−
16	755	Spanish	100	500	−
17	2017	Boer	850	1500	+
18	2193	Boer	100	500	−
19	2034	Boer	300	400	+
20	2172	Boer	1550	300	+
21	615	Boer	500	50	−
22	4	SpanishX	550	200	−
23	12	SpanishX	500	150	−
24	8	SpanishX	550	150	−
25	16	SpanishX	350	100	−
26	621	SpanishX	0	650	+
27	705	SpanishX	2300	1850	+
28	713	SpanishX	600	1250	−
29	739	SpanishX	50	1450	+
30	735	SpanishX	150	150	-
31	721	SpanishX	2500	2500	+
32	Y07	Spanish	700	300	−
33	605	Spanish	900	600	+
34	386	Spanish	1600	0	−
35	6	SpanishX	2700	0	−
36	2195	Boer	600	100	+
37	2019	Boer	1200	1000	
38	637	Boer	900	1200	+

Table 5. Continued

Serial Number	Doe Tag Number	Goat Breed	FEC/Hemonchus	FEC/Coccida	NRAMP1
39	9	Boer	500	500	−
40	824	Spanish	440	160	−
41	816	Spanish	1150	5100	
42	842	Boer	5150	39100	+
43	808	Spanish	1300	7300	

Detection of NRAMP1 at the genomic level is denoted by +
Undetected NRAMP1 at the genomic level is denoted by -

Table 6. NRAMP1 and cytokines expression at RNA level

NO.	ID#	Breed	FEC/H	FEC/C	NRAMP1	DC14	IL-10	IL-8	INF
1	701	Boer	1000	4750	+	+	+	+	+
2	2017	Boer	850	1500	+	-	−	+	+
3	2193	Boer	100	500	−	−	−	−	+
4	706	Boer	150	7500	+	−	-	+	+
5	716	Spanish	1050	1150	+	+	+	+	+
6	755	Spanish	100	500	−	−	-	−	+
7	Y05	Spanish	50	1100	+	−	−	+	+
8	381	Spanish	250	250	−	−	−	−	-
9	705	BXS	2300	1850	+	+	+	+	+
10	735	SpanishX	150	150	-	−	−	−	+
11	4	SpanishX	550	200	−	−	−	−	+
12	8	SpanishX	550	150	−	−	+	−	+

Gene expression at RNA level is denoted by +
Unexpressed gene at the RNA level is denoted by -