

EFFECT OF VACCINATION OF GOATS WITH H-GAL-GP AND H11 ANTIGENS
FROM
INTESTINAL MEMBRANE CELLS OF *HAEMONCHUS CONTORTUS*

A Thesis

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ABSTRACT

Extracts of adult *Haemonchus contortus* were purified and used as a vaccine against the blood feeding parasite in goats as previously studied in sheep. The proteins used were H11 and H-gal-GP, hidden gut antigens from the microvillar membrane of the gut of the worm and combined with Quil A as adjuvant. The antigens were then administered to a group of goats kept on concrete then artificially infected with *H. contortus*. The control group received Quil A injections and was also infected. This study was performed to analyze the effects of the H11/H-gal-GP vaccine when given to goats as compared to sheep. The trial showed that IgG levels peaked three weeks after the first vaccine and remained high throughout the remaining booster series but began to wane after artificial infection. However, the IgG levels remained significantly higher than in the controls throughout the entire study. Overall mean fecal egg counts (FEC) were significantly higher in the controls and packed cell volume levels were significantly higher in vaccinated goats compared to controls from Week 3 post infection to the end of the study. A booster vaccine given Week 7 pi caused a sharp increase in IgG levels, elimination of worm burdens and decrease in FEC in the vaccinated group. Ninety-six percent fewer *H. contortus* adults were recovered at necropsy in vaccinated group compared to controls and >96% reduction in FEC after booster vaccine given during established infection. This study shows that the H11/H-gal-GP vaccine was sufficient in protecting goats after challenge infections but is shorter lived than when given under the same conditions in sheep as shown in previous trials. Booster vaccine given when infection levels are rising are effective in eliminating infections, reducing FEC and

therefore may be used in place of an anthelmintic to control haemonchosis in goats as in sheep.

Chapter One

INTRODUCTION

Haemonchus contortus (Nematoda, Trichostrongyloidea) is a blood sucking intestinal helminth that lives in the abomasum of small ruminants worldwide. This parasite can be devastating to producers as it causes decreased production levels due to clinical signs such as anemia, edema, and death. Economic losses are especially increased in tropical and subtropical regions where *H. contortus* thrives and consumption of goat meat is higher than other food animals. Control programs in the past included pasture management strategies combined with intensive anthelmintic treatment and prophylaxis which was effective in reducing losses of meat and wool in sheep and goats. There are anthelmintics still available but multiple drug resistant *H. contortus* strains have quickly developed and producers and veterinarians are now faced with seeking alternative methods of treatment and prevention (Sangster, 1999; Miller et al., 1987 and 1994; Jackson et al., 2001, Terrill et al., 2001).

Researchers worldwide have been studying new strategies and novel approaches to the control of *H. contortus* in hopes to alleviate the current dependency on anthelmintics that are becoming less efficacious (Waller, 2004). Copper-oxide wire particle boluses have shown very positive results in reducing FEC in recent work but much research is left to ensure the safety of the copper in sheep and goats (Burke et al., 2004). Condensed tannin (CT)-containing forages are another new approach to controlling haemonchosis (Paolini et al., 2003; Athanasiadou et al., 2000). Research studies have proven that when given CT-containing hay, established infections are reduced and animals are generally healthier. Condensed tannins are found in forages

such as *Serecia lespedeza* and chicory and the amount of the forages that must be consumed by the animal to be efficacious is still being studied. Nematophagous fungi have been researched such as *Duddingtonia flagrans*, which affects all worm larvae in feces (Fontenot et al., 2003; Terrill et al., 2004). The primary objective of this method is to reduce parasite larval contamination of pastures which could take years. Breeding animals for genetic resistance to parasites has been studied at length and some sheep breeds such as the Gulf Coast Native, Barbados Blackbelly and St. Croix have shown resistance to nematode infections (Miller et al., 1998; Li et al, 2001). Since these breeds are not high producers such as the Suffolk breed, there is reluctance by the sheep industry to incorporate the resistant sheep into their flocks.

Although the above approaches are promising, there is still much work to be done to incorporate them into parasite control programs that do not continue to rely on anthelmintic use. Another approach being studied is the development of a vaccine against *H. contortus* that could give protection from the parasite and not lead to resistance in the future. Several methods of vaccine components such as irradiated larvae, cysteine proteases, somatic antigens, whole nematode antigen, and hidden gut antigens have been researched with variable results (Urquhart et. al., 1966; Knox et al., 2005; Alunda et al., 2003; Kabagambe, et al., 2000; Smith et al., 2001). The most consistently successful attempts have been vaccination using proteins extracted from the microvillar surface of the intestinal tract of *H. contortus* with some reductions in worm burdens higher than 90% (Knox and Smith, 2001). These antigens that are found in the gut of the parasite and are not present on the worm's surface are "hidden" from the host under normal infection conditions. The animal mounts an immune response to the antigens contained in the

vaccination and then the parasite is affected after it ingests blood containing the antibodies. H11 and H-gal-GP antigens are thought to be involved in the breakdown of peptides that are produced by digestion of dietary protein and therefore the mechanism of protection after vaccination could be by antibody-induced disruption of nutrient uptake (Newton and Meeusen, 2003).

Historically, the trials using hidden gut antigens, H11 and H-gal-GP specifically, have been carried out in sheep only. There is a tremendous lack of research in the responsiveness to goats to these vaccines. Sheep and goats show different susceptibilities to parasite infections and usually require different treatment protocols when using anthelmintics. Pomroy et al., 1985 showed that sheep grazing along with goats under the same conditions had lower fecal egg counts than the goats. In order to further study the immunological response in goats to hidden gut vaccines, the present trial was conducted by using a vaccine combination of H11 and H-gal-GP membrane antigens in a group of weaned goats against haemonchosis. The hypothesis is that there is a significant difference in FEC, PCV, worm burdens, and antibody levels in goats vaccinated with H11/H-gal-GP compared to controls after artificial infection with *H. contortus*.

Chapter Two

REVIEW OF LITERATURE

2.1. *Haemonchus contortus*

Haemonchus contortus or the “Barber Pole Worm” is the most deadly parasite in sheep and goats in tropical and subtropical regions worldwide. This parasite also affects cattle, deer and other exotic ruminants. It is a highly pathogenic, blood sucking nematode causing clinical signs of anemia, hypoproteinemia, edema, and death (Bennett, 1983; Smith and Sherman, 1994). *H. contortus* has a short life cycle of less than 3 weeks. Adult nematodes in the abomasum lay eggs that are passed in feces onto pasture. With favorable conditions such as moisture, heat and humidity, the eggs quickly hatch and larvae develop to infective 3rd stage larvae in soil and manure. The infective larvae then climb up blades of grass and are ingested by grazing animals. In the abomasum, these larvae will go through a 4th stage before reaching adulthood. These 4th stage larvae when present in large amounts can be quite dangerous as they also feed on blood but are not producing eggs leading to false negative results on fecal examination of suspect animals (Smith and Sherman, 1994). Adults and larval stages (4th and 5th) can be recovered from the abomasum of an infected animal at necropsy and identified by their distinguishing features as described by Whitlock, 1960 and Georgi, 1974. Eggs on fecal exams are typically identified then quantified by the modified McMaster technique (see Appendix B, Whitlock, 1948; Thienpont et al., 1979).

The transmission season for *H. contortus* can be year round in environments that are warm and wet. Larvae survivability on pasture may be for as long as one year when conditions are favorable. In cooler regions, larvae may live on pasture for several weeks

to months. Immunity to this parasite is slow to develop in sheep and goats and it tends to wane around parturition. This phenomenon is known as the “peri-parturient rise.” The rise is referring to the increase in susceptibility to the parasite and result of high number of eggs produced. The fecundity of *H. contortus* is enormous in that a single worm may lay up to 5,000 eggs per day. In larger herds, especially grazed on short grass, problems with haemonchosis seem to increase exponentially (Georgi, 1974; Smith and Sherman, 1994). As shown in a previous study by Pomroy et al., 1985, goats especially will have difficulty dealing with haemonchosis on overgrazing conditions when compared to sheep as they are less likely to elicit an effective immune reaction to the parasite leading to a higher FEC in goats than sheep even when raised together in equal conditions.

2.2 Anthelmintic resistance

With the age of modern anthelmintics, parasitologists have often recommended strategies that maximized the benefits of treatment, ignoring issues of resistance. This has led to an over-reliance and over-use of anthelmintics and a loss of common sense management practices. The drugs are often used prophylactically rather than therapeutically. The prevalence of multiple drug resistant *H. contortus* is alarmingly high. Veterinarians and producers are at risk of having no effective anthelmintics in the near future (Sangster, 1999; van Wyk, 2001; Waller, 2004).

Anthelmintic resistance is defined as the ability of worms to survive treatments that are generally effective at the recommended dosages. This has become a major threat to the current and future control of parasites in small ruminants and horses. Development of resistance occurs when treatment eliminates worms whose genotype renders them susceptible. Worms that are resistant survive treatment and pass on their resistant genes

to the next generation. Resistant worms accumulate and finally treatment failure occurs. The clinical definition of drug resistance is when there is <95% reduction in fecal egg count (FEC). The population of susceptible worms that are on pasture at the time of treatment and are “in refuge” from the drug are known as the “refugia.” Worms in refugia provide a pool of susceptible genes that dilute the resistant genes in that population of worms. Until recently, worms in refugia have been overlooked as the most important component of drug resistance selection (van Wyk, 2001).

Research has shown that there is anthelmintic resistance in *H. contortus* to benzimidazoles, imidothiazoles, and avermectins in sheep and goats throughout the world (Miller et al., 1987; Sangster, 1999; Jackson and Coop, 2000). Goats have a problem with multiple drug resistant strains of *H. contortus* particularly due to the difference in drug metabolism in goats and sheep. Goats tend to require a higher dose of certain anthelmintics than sheep for similar blood level profiles but this is often not recognized and underdosing occurs, leading to resistance at a rapid rate (Conder and Campbell, 1995; van Wyk, 2001). Studies conducted in the Gulf South region of the United States have demonstrated that ivermectin and moxidectin resistance can develop over a short period of time; even within a few years in particular herds of goats (Miller et al., 1994; Terrill et al., 2001).

General parasite control recommendations include decreasing stocking rates, keeping pastures and pen areas well drained, and periodically moving feeders or troughs to decrease transmission from heavily grazed areas. Short grasses in pens or around barns may need to be completely eliminated, as these are usually the most contaminated areas. Dilution strategies by mixing two or more livestock species on the same pasture

may also be helpful as sheep/goats and cattle or horses do not share most of the parasite species. Rotating the pastures between species may also be effective as one species will “vacuum” up the other species’ parasites; therefore, reducing contamination of pastures (Smith and Sherman, 1994; Barger, 1999).

2.3 Novel approaches to *H. contortus* control

Sheep and goat producers are currently facing a dismal future of total anthelmintic failure and no new drugs are on the horizon. With this in mind, researchers worldwide are diligently seeking alternatives or “novel approaches” for control of *H. contortus* in sheep and goats (Waller, 2004). One area of study has been identifying breeds of animals that have a genetic resistance to gastrointestinal nematodes. By breed substitution or crossbreeding, the dependency on anthelmintic treatment is considerably lessened (Miller et al, 1998; Li et al, 2001). Unfortunately, the resistant breeds such as Gulf Coast Native, are typically smaller in frame and are not readily accepted by producers that are accustomed to higher producers such as Suffolk.

The FAMACHA system of assessing anemia by an ocular conjunctiva color chart has been developed and shown to be an effective way of deciding which animals need treatment for haemonchosis (van Wyk and Bath, 2002; Vatta et al., 2002; Kaplan et al., 2004). Using this method should eliminate the practice of treating all animals in a herd or flock at frequent intervals; therefore, preserving the susceptible worms in refugia. This method may prove to be inconvenient to large scale producers since each animal must be evaluated as often as weekly during the peak *H. contortus* season.

Copper oxide wire particles have also been investigated as a treatment for haemonchosis in sheep and goats. When administered as an oral bolus, established

infections of *H. contortus* are alleviated and fecundity reduced (Burke et al., 2004 and 2005). This approach to treatment could reduce or eliminate the need for anthelmintics by producers but there is much to be done to determine the safety of multiple doses of copper while avoiding toxicity.

Feed supplements that contain substances such as condensed tannins that have a detrimental effect on gastrointestinal nematode populations have also been studied with varying degrees of success in sheep and goats (Athanasiadou et al., 2001; Paolini et al., 2003; Tzamaloukas et al., 2005). Condensed tannins decrease levels of protein degradation in the rumen and enhance amino acid production in the abomasum and small intestine which improves the overall health of the animal receiving the supplement. Some forages containing condensed tannins that have been studied for their anthelmintic properties are sericea lespedeza, quebracho, chicory and sulla. There appears to be reduction in fecundity of adult *H. contortus* as well as a reduction of established infections when given as a substitute for regular grass hay (Lange et al., 2005). The actual mechanism behind the reduced FEC and worm burdens when condensed tannin-containing forages is still debated. One theory is that since the animal is healthier, its immune response to the parasite is enhanced. There is certainly potential in this approach for reducing pasture contamination. Studies continue to determine the amounts of the supplements that are necessary to achieve the parasite reduction desired as well as a convenient delivery method for producer implementation.

Duddingotonia flagrans, a nematophagous fungus, is another novel approach that has researched for many years due to its potential in reducing pasture contamination (Fontenot et al., 2003; Terrill et al, 2004). The fungal spores must be fed daily to the

animals and there are no immediate anthelmintic effects; however, feeding the fungus while implementing another method of parasite control such as copper oxide wire particle treatment has been shown to be effective by Burke et al., 2005.

Vaccine development against *H. contortus* has been progressing for over three decades with varying results (Smith and Munn, 1990; Emery and Wagland, 1991; Newton and Meeusen, 2003). Strategies from the past range from oral vaccines to injection of irradiated larvae or cuticular collagen proteins with minimal to no success but as the immune system of the sheep and goats and the biology of the worm are studied further, advances continue in vaccine development (Urquhart et al., 1966; Mansfield et al., 1974; Smith and Christie, 1978; Boisvenue et al., 1991).

2.4 Recent developments in *H. contortus* vaccines

As advanced technologies have become available, much has been learned about the immune response of small ruminants to gastrointestinal parasites. Douch et al., 1996, studied nematode resistant sheep and noted that the larval migration inhibitory compounds in the gastrointestinal mucus appear to be associated with the increased numbers of mucosal mast cells and globule leukocytes present in gastrointestinal tissue when compared to non-immunized or susceptible sheep. Further research has been done to evaluate the cytokines and immunoglobulins in abomasal tissue and lymph nodes of sheep infected with *H. contortus*. Gill et al., 1992, found that seven days after infection with *H. contortus*, ovine abomasal tissue biopsies showed a six-fold increase in the amount of IgG₁, IgA, and IgM than uninfected controls. The immune responses elicited by the parasites in the small ruminant gastrointestinal tract are truly multifaceted which makes the development of a vaccine that targets the host response seemingly impossible.

With this in mind, vaccine technology has turned away from the natural host response and is now focused on directing a response against parasite proteins essential to parasite survival. Excretory/secretory proteins, somatic antigens, cysteine proteases, and hidden gut antigens are the topics of most recent literature regarding vaccine development (Knox and Smith, 2001; Newton and Meeusen, 2003).

Excretory/secretory products purified from somatic extracts of adult *H. contortus* used to immunize sheep have induced a protective immune response that led to a 32% reduction in FEC and 64% reduction in abomasal worm counts (Schallig et al., 1994; Schallig and Leeuwen, 1997). Somatic antigens from adult *H. contortus* (ASE) have been used in vaccination trials in sheep and caused a reduction of FEC post infection of up to 46%, reduced parasite burden at necropsy of 40%, and significantly higher anti-ASE IgG response in vaccinated lambs compared to controls (Alunda et al., 2003). An earlier study by Dominguez-Torano et al., 2000, using the specific somatic fraction p26/23 of adult *H. contortus* parasites showed a significant reduction (>60%) in mean FEC and mean worm burdens (61.6%) in the vaccinated group compared to the controls.

Recent work with cysteine proteases extracted from adult *H. contortus* has shown substantial protection against single challenge infections. These proteases were chosen due to their critical function in worm physiology such as nutrition and immune evasion. One trial using vaccinated lambs after challenge demonstrated significant reductions in FEC (56%) and final worm burdens (33%) when compared to nonvaccinated challenged lambs but no significance in IgG levels (Knox et al., 2005). Ruiz et al., 2004 conducted a similar trial using the cysteine protease vaccine in goats. His results showed a more

marked reduction in FEC (89%) and worm burdens (68%) along with a significant increase in IgG levels when compared to the controls.

Although the previously described vaccination strategies indicate that these proteins have immunoprotective effects against *H. contortus* challenges, the results are not particularly dramatic and cannot compare with efficacious anthelmintic treatment to eliminate parasites. However, research using antigens isolated from the microvillous membrane of the parasite gut have shown repeatedly substantial protection against *H. contortus* challenges (Jasmer and McGuire, 1991; Smith, 1993); specifically, the aminopeptidase complex H11 and a glycoprotein fraction containing galactose H-gal-GP. These proteins are described as “hidden” antigens because the infected animal does not become exposed to these antigens found in the gut of the parasite and therefore the animal has never acquired natural immunity to them. Smith et al., 1993 and 1999 describe the development of the H11 fraction vaccine method of extraction from adult *H. contortus* using lectins and gel filtration.

Andrews et al., 1997 conducted a trial in lambs using the H11 antigen vaccine to determine the length of duration of immunity in sheep when challenged with infection. He found that after receiving a series of H11 antigen, lambs infected with *H. contortus* 14, 84, 126 or 168 days later had a mean FEC reduction of 97%, 99%, 92% and 86% respectively and a reduction in mean worm counts of 87%, 94%, 92% and 62% respectively compared to controls. The peak in antibody levels was seen at 60 days after the first vaccination and was maintained throughout the study. Another trial by Andrews et al., 1995 analyzed the effect the H11 vaccine has on the periparturient rise in sheep. He found that immunized pregnant sheep challenged with *H. contortus* had a FEC

reduction of up to 99% in the last trimester. The lambs from these ewes also had antibodies to H11 through maternal transfer that offered moderate protection from infection with *H. contortus*. Other trials with H11 in sheep of varying breeds and ages have shown significant protection against anthelmintic-resistant and geographically different strains of *H. contortus* with consistently high reductions in worm burdens and FEC (Smith and Smith, 1993; Munn et al., 1993 and 1997; Newton et al., 1995).

Studies of the *Haemonchus* galactose-containing glycoprotein complex used in conjunction with H11 have also shown consistently highly immunoprotective against *H. contortus*. Smith and Smith, 1996 conducted a trial using the H-gal-GP complex polypeptides individually after dissociating the complex and showed that the individual peptides showed only partial protection against infection when compared to using the complex in its entirety. A different trial by Smith et al., 2000 showed that *Ostertagia ostertagi* glycoproteins extracted in the same method as *H. contortus* using lectins, demonstrated cross-protection in sheep challenged with *H. contortus* infections with FEC reductions between 81% and 91% and worm burden reduction of 57% to 84% when compared with controls.

The previously discussed trials using H11 and H-gal-GP were under experimental conditions therefore recently, trials using the antigens in sheep on naturally infected pastures have been conducted. In a trial lasting 11 months in South Africa, Smith et al., 2001 found that H11/H-gal-GP vaccinated lambs were protected from haemonchosis with a FEC reduction of >82% over a 4 month period of time but protective levels of antibodies were not maintained long enough to provide immunoprotection from subsequent reinfection. However, a booster of the vaccine given during the surge of

infection levels cleared the lambs of infection and restored the protective antibody levels showing that the vaccine could potentially replace an anthelmintic treatment. Another field trial conducted in Louisiana, USA, Kabagambe et al., 2000 looked at the vaccine combination in ewes grazing naturally infected pastures and found a 62% FEC reduction in susceptible vaccinated ewes compared to controls but no booster during infection was administered.

All of the previous successful trials using H11 and H-gal-GP have been with using the actual antigens extracted from the adult *H. contortus* worms. This is obviously not the most economically feasible method of mass producing a vaccine to make available to producers however, attempts at a recombinant version of the vaccine have been made but without success thus far. Trials in other species of worms have shown to be effective when using recombinant derivatives such as with *Schistosoma bovis* homologous vaccine in goats therefore; further studies with recombinant H11/H-gal-GP vaccines are not without hope (Boulanger et al., 1994). Another successful trial in a hookworm species in dogs similar to *H. contortus* was conducted using a recombinant aspartic hemoglobinase (Loukas et al., 2005). The quest for the efficacious *H. contortus* vaccine that will be both safe and economically feasible for use in sheep and goats continues.

Chapter Three

MATERIALS AND METHODS

3.1 Location and animals

The experiment was conducted at the School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana. Seven month old Boer cross, male goats were studied from October 2005 through January 2006. The goats were all raised under pastured conditions on the same farm located near LSU since birth.

3.2 Experimental design

In this trial, the *H. contortus* hidden gut antigens H11 and H-gal-GP were used to vaccinate goats under experimental conditions. After purchase, the goats were moved to covered concrete pens at LSU SVM and dewormed with levamisole (AgriLabs, St. Joseph, MO; 12 mg/kg PO) and albendazole (Valbazen[®], Pfizer, New York, NY; 20 mg/kg PO) for two consecutive days on a weekly basis for three weeks. At this time, fecal egg counts (FEC) were zero to one hundred and the goats were randomly allocated to the control and vaccinated groups containing eleven animals each. The vaccine containing H11 and H-gal-GP antigens and Quil A adjuvant prepared according to a protocol provided by Dr. W. D. Smith (Moredun Research Institute, Edinburgh, U.K.) was administered to the vaccine group three times at two week intervals (Week-5, Week-3, Week-1). The vaccine group also received a fourth dose of the vaccine during Week 7 post infection. The control group was given four injections of only Quil A at the same time points that the vaccine was given to the other group. Both groups were inoculated orally with a bolus of 5,000 *H. contortus* L₃ one week after the third vaccination date (Week 0). FEC and packed cell volume (PCV) were determined for both groups each

week and serum for antibody detection was also collected on a weekly basis. Necropsies were performed on three goats from each group Week 8 post infection of the trial and the remainders were sacrificed Week 10 post infection. The first necropsy was performed due to the deteriorating conditions of three controls from haemonchosis. The three vaccinated goats necropsied at the first date were chosen by highest FEC and lowest PCV levels at that time to match the chosen controls. The second necropsy group had uneven numbers (7 controls, 8 vaccinated) due to the death of one control goat during Week 4 of causes unrelated to haemonchosis. Gastrointestinal worms were collected and identified from all goats during necropsy. Enzyme linked immunosorbent assays (ELISA) were used to determine the presence of IgG in serum samples on all collection dates for both groups.

3.3 Vaccination preparation

3.3.1 Worm collection

Abomasal contents from sheep sacrificed 5 to 6 weeks after infection with *H. contortus* were collected and then filtered and rinsed with water. The worms were then hand picked and rinsed thoroughly with phosphate buffered saline (PBS, Sigma-Aldrich Co., Atlanta, GA) then frozen at -20°C in tubes containing PBS.

3.3.2 Preparation of extract of worm membranes

Frozen *H. contortus* adults (40 g) were thawed and dried using a vacuum filter funnel flask assembly (Corning Inc., Corning, NY; 500mL Filter System, .45uL Cellulose Acetate Low Protein Binding Membrane). The worms were ground up in ice cold homogenizing buffer made up of 500 ml PBS, 0.186g EDTA (tri-basic, Sigma-Aldrich Co.); pH 7.4 adjusted with concentrated HCl, 1 ml PMSF solution (0.174 g PMSF

(Sigma-Aldrich Co.) and 10 ml Isopropanol) using a motorized homogenizer (Tekmar Tissumizer, Cincinnati, OH), centrifuged at 16,000 rpm for 20 minutes at 4°C (Beckman Model J2-21, Fullerton, CA) and then the pellet was hand homogenized with buffer containing 0.1% Tween 20 (Sigma-Aldrich Co.) and centrifuged as before. The last step was repeated and the pellet was hand homogenized with buffer containing 2% reduced Triton-X 100 (Sigma-Aldrich Co.) and centrifuged at 37,800 rpm for 1 hour at 4°C (Beckman Coulter Optima XL-100K Ultracentrifuge) . This supernatant was retained and diluted three-fold with lectin wash buffer (LWB) consisting of 20 ml 1M Tris (Sigma-Aldrich Co.), pH 7.4, 250 ml 4M NaCl (Sigma-Aldrich Co.), 1g NaN₃ (Sigma-Aldrich Co.), 2ml 10mM MnCl₂ (Sigma-Aldrich Co.), 2ml 100mM CaCl₂ (Sigma-Aldrich Co.) made up to 2 L with dH₂O and the pellet discarded.

3.3.3 Preparation of lectin binding columns for antigen purification process

The chromatography columns (C10/10, GE Healthcare, United Kingdom) were set up in a cool room (5°C) prior to the purification steps with one 10 ml column containing agarose bound peanut agglutinin lectin (Vector Laboratories, Burlingame, CA) and the second 10 ml column containing agarose bound Concanavalin A (Con A) lectin (Vector Laboratories). The columns were packed halfway and the remainder filled with LWB containing 0.5% reduced Triton-X 100 then flow adaptors (AC 10, GE Healthcare) inserted to the top of the settled agarose bound lectin beads. The columns were connected to a peristaltic pump (TRIS Pump, Teledyne Isco, Lincoln, NE) and flushed overnight with LWB containing 0.25% CHAPS (Sigma-Aldrich Co.) at a rate of 5 ml/hr.

3.3.4 Elution of the lectin binding columns

Continuing to work in a cool room, the columns were set up so that the pump was connected to the peanut lectin column with the flow in the upward direction. The peanut column was then hooked up in series with the Con A column with the flow in the upward direction and the waste coming from the top of the Con A column being collected in a beaker. The diluted supernatant containing the *H. contortus* protein extract was pumped onto the lectin column set-up. Once the entire sample was loaded onto the columns, the columns were washed with LWB overnight at a rate of 7 ml/hr.

At this point, the lectin bound proteins were eluted from each column by adding buffer solutions containing sugars that compete with the glycoproteins for the lectins in the columns. The Con A column was removed from the assembly and the peanut column was connected to the pump so that the flow through the column was in the downward direction. Five ml of peanut elution buffer consisting of 3 g galactose (Sigma-Aldrich Co.) in 33ml LWB/0.25% CHAPS was pumped onto the column and the first 5 ml of eluate discarded. The ends of the tubing from the peanut column were connected to each other and the column incubated for two hours. The Con A column was hooked up to the pump as described for the peanut column and 5 ml of Con A elution buffer consisting of 1.3g 0.2M methyl glucopyranoside (Sigma-Aldrich Co.) and 1.3g 0.2M methyl mannopyranoside (Sigma-Aldrich Co.) into 33ml LWB/0.25%CHAPS was pumped onto the column. The first 5 ml of eluate was discarded and the column was disconnected from the pump and allowed to incubate for one hour with the tube ends connected to each other. After incubation, each column was connected to the pump and eluted separately as their specific elution buffers were pumped through the columns at a rate of 10 ml/hr.

Eluates were collected for two hours. The columns containing elution buffer were left overnight then eluates collected again for two hours.

3.3.5 Preparation of desalting column

Prior to the antigen purification process, 100 g of dry Sephadex G-25 (GE Healthcare) was soaked in desalting column wash buffer consisting of 10mM Tris/0.1% CHAPS; pH 7.4, made up to 5 liters overnight at room temperature. The swollen media was added to the 20 ml column (C 16/20, GE Healthcare) as with the lectin columns and the adaptor (AC 16, GE Healthcare) was inserted. The column was connected to the pump and flushed overnight with desalting column wash buffer at a rate of 250 ml/hr.

3.3.6 Desalting the eluates

The Con A eluate was pumped onto the desalting column at a rate of 250 ml/hr and then followed with 40 ml desalting column wash buffer. The first 35 ml were discarded and the next 40 ml of eluate were collected. The column was flushed with 40 ml of desalting buffer to remove salt. The peanut eluate was pumped onto the desalting column after the buffer as described for the Con A eluate, followed with buffer and eluate collected. The peanut eluate was designated as H-gal-GP and the Con A eluate as H11.

3.3.7 Protein concentration

Each protein eluate was concentrated down by placing each into centrifugal filter units (Centriprep YM-10, Millipore, Billerica, MA) and centrifuging at 3000 rpm for 45 minutes at 4°C (IEC Centra-7R Refrigerated Centrifuge, International Equipment Co., Corston-Bath, United Kingdom). This brought the amount of protein down to approximately 3 ml per tube. The remaining amount was centrifuged again at 3000 rpm for 10 minutes at 4°C. This brought the amount per tube to approximately 1 ml. The H-

gal-GP and H11 proteins were then pooled separately and subsamples taken for protein analysis while the remainder was stored at -80°C.

3.3.8 Analysis of purified antigen

Quantitation of protein in each fraction was determined by using a microtiter plate-adapted bicinchoninic acid protein assay kit according to manufacturer's instructions (BCA Protein Assay Kit, Pierce Biotechnology, Rockford, IL).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine that the correct proteins were present in the two fractions. A slab gel modification of the procedure by Laemmli, 1970, was used for protein separation. Samples were reduced by heating at 100°C for 5 minutes in stock sample loading buffer consisting of 63 mM Tris-HCl at pH 6.8, 5% SDS, and 5% 2-beta-mercaptoethanol (Sigma-Aldrich Co.). The samples were then loaded onto the precast stacking gel (4-15% TRIS-HCL, Bio-Rad Laboratories). Two standard markers were run in adjacent lanes (PageRuler Protein Ladder, Fermentas, Hanover, MD; and SeeBlue Pre-Stained Standard, Invitrogen, Carlsbad, CA). The gel was run at 200V for 30 minutes (Bio-Rad Mini-PROTEAN II, Hercules, CA) then stained with Coomassie blue (Brilliant Blue R, Sigma-Aldrich Co.). Molecular weights were determined by comparing the samples with the known standard markers.

3.3.9 Vaccine formulation and administration

Each vaccine administered consisted of 100 µg of H-gal-GP and 100 µg of H11 combined with 5 mg of Quil A adjuvant (Brenntag Biosector, Fredericksund, Denmark) then diluted with PBS to a volume of 2 ml for the total vaccine. The control goats received 5 mg Quil A mixed with 2 ml PBS. The vaccines and adjuvant-only treatments

were prepared on each day of vaccination. The dose of each injection was divided equally and administered intramuscularly in the semimembranosus muscle of each hind leg.

3.4 Techniques

3.4.1 Fecal egg counts

Fecal samples obtained rectally were collected from all goats weekly from Week -5 through Week 10 post infection. The FEC, reported as the number of trichostrongyle type eggs per gram (EPG) of feces was determined by a modified McMaster technique using a salt solution consisting of 737g plain salt dissolved in 3000 ml of water. Two grams of feces were weighed out and then crushed in a cup. Thirty ml of salt solution was added to feces and the mixture stirred by hand then further mixed with a blender. While the solution was still agitated, a small amount was pipetted out and placed into McMaster slide chambers (Chalex Corporation, Issaquah, WA). Trichostrongyle-type eggs were counted inside the grids of each chamber then the total multiplied by 50 to get total eggs per gram (EPG).

3.4.2 Packed cell volume

Whole blood was collected from all goats via jugular venipuncture into 7 ml tubes containing K₃EDTA (BD Vacutainer Whole Blood Tubes, Becton, Dickinson, & Co., Franklin Lakes, NJ) as an anticoagulant on the same weekly schedule as the FEC. The blood was then transferred to microhematocrit tubes, sealed, and centrifuged (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickinson, & Co.) for 5 minutes. The PCV value was determined from the hematocrit reader inside the centrifuge.

3.4.3 Infective larval preparation

Feces from sheep with known infections of *H. contortus* was collected rectally and pooled. The feces was crushed, mixed with water in a culture pan and then combined with vermiculite until the mixture is moist but not wet. The pan was covered with aluminum foil with several holes to allow air circulation. The mixture sat at room temperature for 10 days and then was filtered through cheesecloth and tissue paper to recover larvae. The larvae were identified microscopically to be 98% *H. contortus* with the remainder being *Cooperia curticei* and *Trichostrongylus spp.* The larvae were refrigerated in water with penicillin G added until the infection day. All goats were given 5,000 infective *H. contortus* larvae diluted with 10 ml water in an oral bolus on Week 0 of this trial.

3.4.4 Necropsy techniques

Three goats from each group were necropsied at Week 8 post infection and the remainder was necropsied at Week 10 post infection. The goats were all euthanized with Pentobarbital Sodium 390 mg/ml/Phenytoin Sodium 50 mg/ml (Beuthanasia-D Special, Schering, Wayne, NJ) at a dose of 10 ml/100 lb body weight intravenously.

The abomasum, small and large intestine were removed from all goats. These parts were opened and washed and contents emptied into a bucket with 10 liters of water. Aliquots of 500 ml were placed into plastic bottles with formalin as a preservative.

3.4.5 Worm identification

The 500 ml aliquots of contents from the abomasum, small and large intestine from each goat were analyzed 100 ml at a time and parasites were recovered and placed

on microscope slides with lactophenol and coverslips. Once the parasites were counted and transferred to slides, they were identified by species, sex, and stage of development.

3.4.6 Enzyme-linked immunosorbent assay for antibody detection

Sera from whole blood collected weekly from all goats via venipuncture (BD Vacutainer Whole Blood Tubes, No Additive, Becton, Dickinson, & Co.) was stored at -20°C until ELISA's were performed. Serum samples were mixed with sodium azide (Sigma-Aldrich Co.) and refrigerated after thawing. Negative control sera from a 5 week-old kid with no prior exposure to H11 or H-gal-GP and no known infection with gastrointestinal nematode parasites was also mixed with sodium azide and refrigerated. The dam of the negative control kid had also not had any exposure to H11 or H-gal-GP antigens. The OD values for the negative control samples ranged from 0.04 to 0.2 which were consistently significantly lower than the baseline levels of all animals before given any injections in this study.

Polystyrene plates (Costar EIA/RIA, Corning, Inc.) were coated with 100 µl of H-gal-GP and 100 µl of H11 each diluted in 0.05M carbonate bicarbonate buffer, pH 9.6 (Sigma-Aldrich Co.) to give 1 µg/ml solution. The plates were covered with a microtiter plate sealer (SealPlate Microplate Adhesive Film, E & K Scientific) and refrigerated overnight. The plates were then washed 4 times with PBS and dried by tapping the plates onto absorbent papers to remove residual PBS. Serum samples from week -5 through week 7 (prior to final vaccine booster) were diluted with blocking solution (Starting Block T20 (PBS) Blocking Buffer, Pierce Biotechnology) at a 1:25 ratio while sera from weeks 8-10 (post final booster) are diluted at a 1:50 ratio. Two samples of negative control sera were also diluted at 1:25 and 1:50 and included on each plate. The plates

were blocked for 5 minutes with 200 µl/well of blocking buffer (Starting Block (PBS) Blocking Buffer, Pierce Biotechnology) then emptied. Fifty microliters per well of each diluted serum samples and controls were then added in duplicate to the plates and incubated sealed at room temperature for one hour. The plates were washed again 4 times with PBS and dried as previously described. Rabbit anti-goat IgG peroxidase conjugate (Sigma-Aldrich Co.) was diluted with T20 blocking buffer at a 1:50,000 ratio and the plates were coated with 100 µl/well of the diluted conjugate.

After the coating with conjugate, the plates were sealed and incubated for one hour at room temperature. The plates were then washed 4 times with PBS and dried as previously described. The next step was to coat the plates with 100 µl/well of the chromagen substrate (1-Step Ultra TMB-ELISA, Pierce Biotechnology) and incubate without a seal for 15 minutes in the dark at room temperature. The plates were then coated with 100 µl/well of 2M H₂SO₄ as a stop solution. The absorbencies were read at 450 nm by an ELISA plate reader (EL_x800 Universal Microplate Reader, Bio-Tek Instruments, Inc., Winooski, VT). Final OD values were determined by subtracting the negative control value from the results of the serum samples from the plate reader.

3.5 Statistical Analysis

The EPG, PCV, worm counts and ELISA OD data were analyzed by the SAS statistical package version 9.1.3. Repeated measures analysis of variance was used for comparisons of data over each time point with split-plot arrangement of treatments with treatment group and animals within treatment groups as main plot effects and time by group interaction as subplot effects (Devore and Peck, 2001). Significant differences were determined using a P value of <0.05. When a significant difference overall was

found, a Tukey's HSD test was used for main effect with pairwise comparison of least square means for interaction effects (Devore and Peck, 2001). FEC were log base e transformed to stabilize variance terms. Pearson correlation coefficient tests were used to analyze data sets for a linear association over time (Devore and Peck, 2001).

Chapter Four

RESULTS

4.1 H11/H-gal-GP vaccine

After elution of the protein fractions, a sample of each was taken for quantitation of protein concentration levels using a BCA protein assay kit. The peanut lectin eluate concentration was 2,290 $\mu\text{g/ml}$. The protein concentration present in the Con A lectin eluate was 314 $\mu\text{g/ml}$.

The fractions were then analyzed using SDS-PAGE to determine the proteins present in the eluates. Figure 1 demonstrates the bands present in the peanut lectin fraction lane were at the 230kDa and 170kDa regions when compared to the known protein ladder standards indicating the presence of H-gal-GP antigen. The Con A fraction lane contained a band at the 110kDa and 45 kDa regions indicating the presence of H11 antigen.

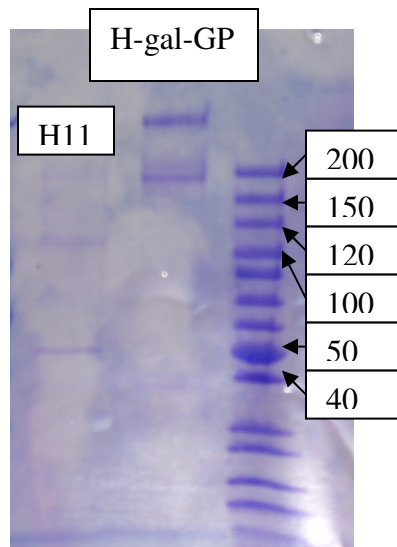


Fig. 1. SDS-PAGE gel indicating bands at 230kDa and 170kDa in H-gal-GP (middle) lane and 110kDa and 45kDa in the H11 (far left) lane compared to the known standards in the far right lane.

4.2 IgG antibody response to vaccine and adjuvant administration

Anti-H11 and anti-H-gal-GP IgG response was measured in serum samples taken weekly throughout the trial from both groups using ELISA optical density (OD) readings. Figure 2 demonstrates the IgG levels throughout the study of all animals. Control animals that received only Quil A adjuvant injections had consistently lower OD readings than those in the vaccinated group that received the H11/H-gal-GP with Quil A vaccine. The IgG levels in the vaccinated group increased during the initial vaccine and booster series but started to wane after infection Week 0. Although the levels slowly drop from Week 0 to Week 7 in the vaccinated group, the levels are still significantly higher than the control group. After the booster of the vaccine is given to the vaccine group during Week 7, the IgG levels increased sharply but again start to slowly decrease following the spike.

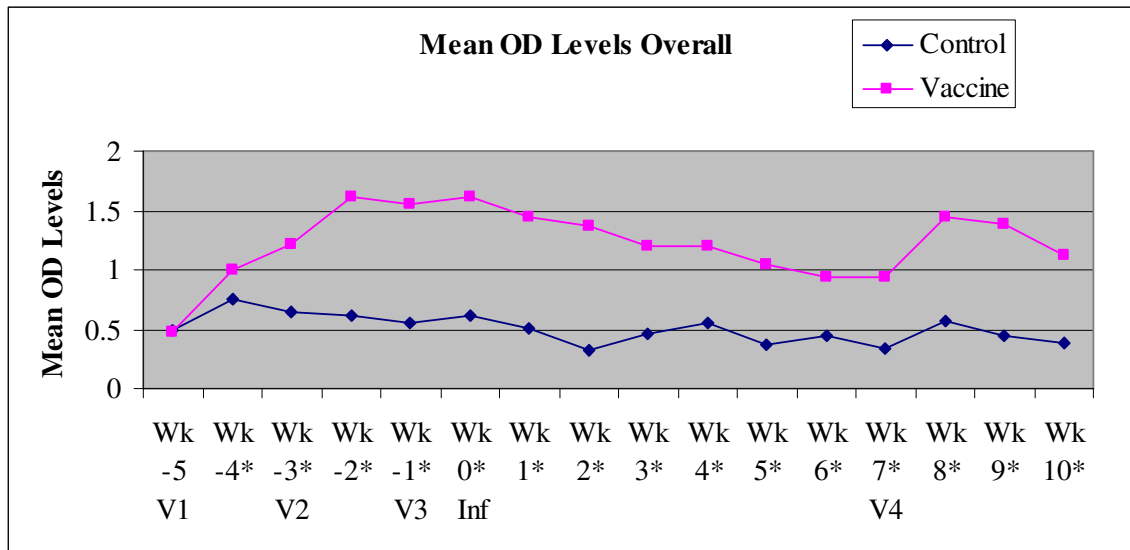


Fig. 2. IgG antibody response of goats given H11/H-gal-GP vaccine (vaccine group) or Quil A adjuvant only (control group) demonstrated by mean ELISA optical density (OD) readings. Differences are significant between groups at all time points after Week -5 and are indicated by an asterisk ($P < 0.05$). Injections were given Week -5, Week -3, Week -1 and Week 7. Infection with *H. contortus* larvae in both groups was at Week 0.

Since necropsies were performed on 6 animals during Week 8, there is no data for these animals after that time point. Figure 3 demonstrates the differences for each group from the beginning of the study until the necropsy dates. For the first necropsied group, there are significantly higher OD levels in the vaccinated group from Week -3 to Week 8 compared to the controls, and in the second necropsied group the levels are significantly higher in the vaccinated group from Week -4 to Week 10 compared to the control group.

4.3 Worm counts

Necropsies were performed Week 8 post infection in 3 goats in each group with the highest FEC and lowest PCV due to their deteriorating conditions from the haemonchosis. The remaining animals were necropsied at Week 10. The abomasal, small intestinal and the first meter of the large intestinal contents were sampled for worm recovery. All worms recovered from the abomasal contents from both groups at both necropsy dates were identified as adult *H. contortus*. No worms were found in the small intestine of all animals and only one goat from the control group necropsied at Week 8 had 10 adult *Oesophagostomum spp.* recovered from the large intestinal contents. Figure 4 illustrates the number of *H. contortus* adults that were present at Week 8 and Week 10 in the control and vaccinated groups. Worm counts were high in both groups at Week 8 with the greatest amount recovered from the control group. At week 10, there were significantly fewer worms found in the vaccinated group compared to the control group. This correlates with the decrease in FEC in the vaccinated group and the increase in FEC in the control group at Week 9.

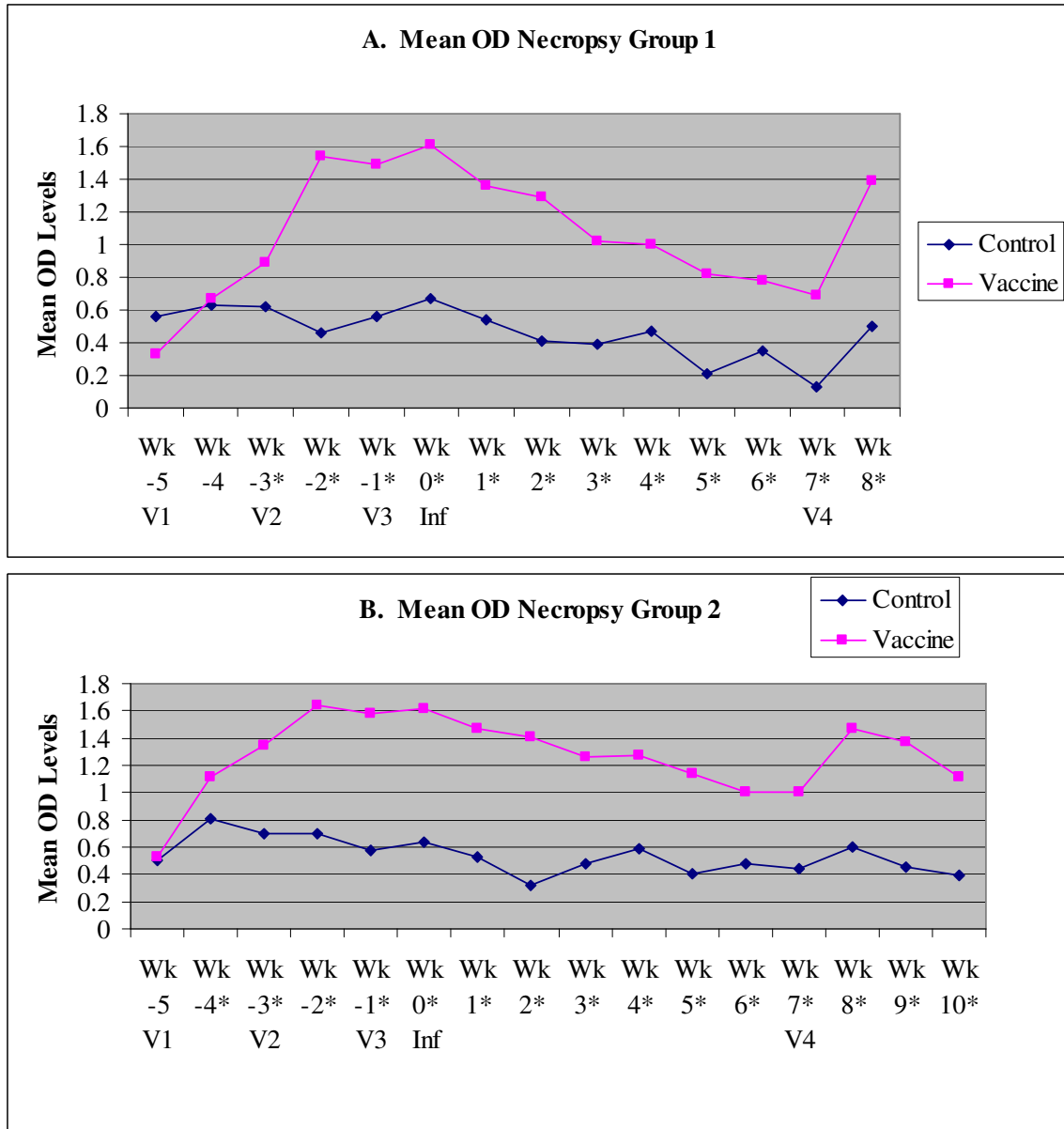


Fig. 3. Mean IgG levels of different necropsy date groups demonstrated by mean ELISA optical density (OD) readings A.) Necropsy group 1, necropsied Week 8, and B.) Necropsy group 2, necropsied Week 10. Significant differences indicated by an asterisk ($P < 0.05$).

The numbers of adult male and female *H. contortus* were documented during the worm recovery and identification process for both groups (Table 1). The gender distribution appears to be even within the control and vaccinated groups but there are significantly fewer males than females recovered during Week 10 than Week 8.

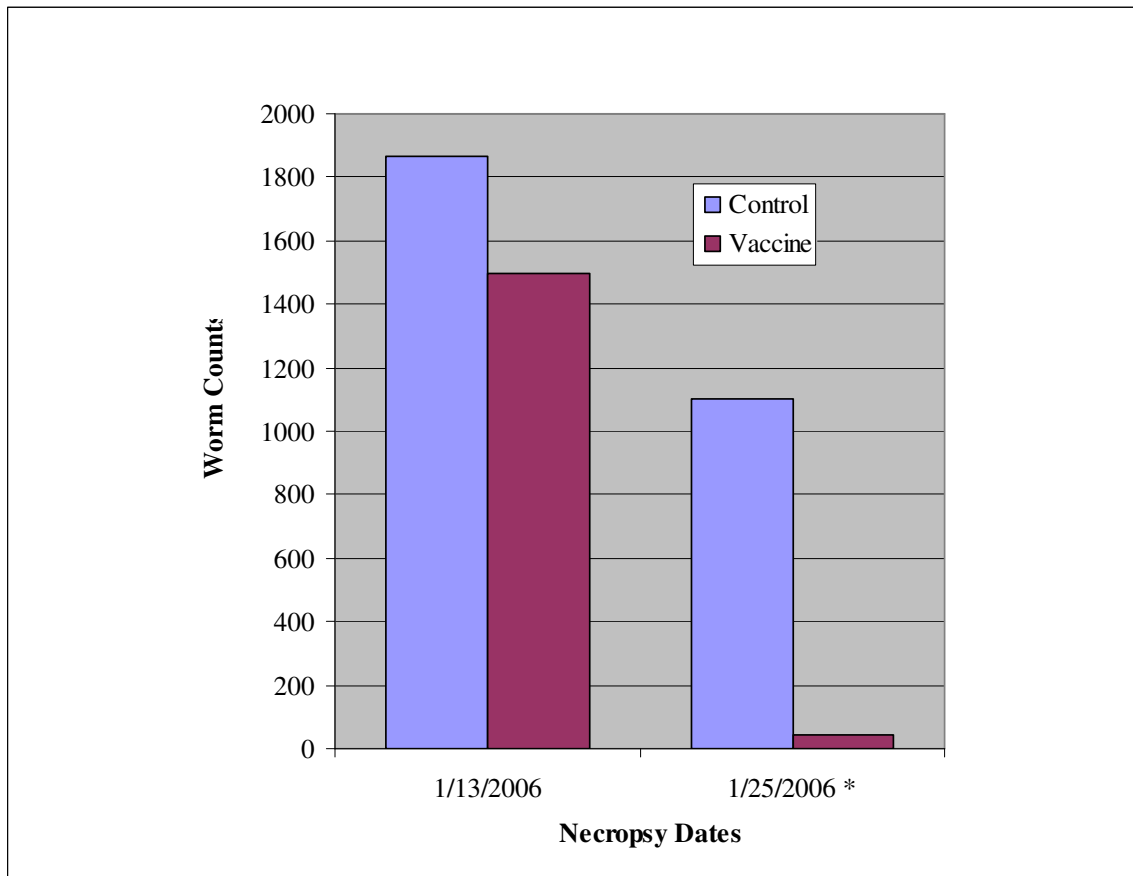


Fig. 4. Mean adult *H. contortus* counts recovered from the control and vaccinated groups at Week 8 (1/13/06) and Week 10 (1/25/06). The final H11/H-gal-GP booster was administered to the vaccinated group Week 7. A significant difference between control and vaccine group was seen during the second necropsy as indicated by an asterisk ($P < 0.05$).

Table 1. Mean male and female *H. contortus* adults recovered at necropsy from both groups.

Treatment	Week 8 Post Infection		Week 10 Post Infection	
	Male	Female	Male	Female
Control	833.33	1033.33	557.14	546.43
Vaccine	700	800	15.63	28.13

4.4 Fecal egg counts

The mean FEC for both groups from Week 3 post infection throughout the duration of the trial are presented in Table 2. Since the goats were dewormed before the study and kept on concrete to prevent incidental infections, the FEC of all animals are zero or close to zero until Week 3 post artificial infection of 5,000 infective *H. contortus* larvae. The mean FEC of the vaccinated group remain significantly lower than the control group throughout the entire trial. Three weeks after infection the control group FEC began to rise. This trend continued until Week 9 at which time the FEC dropped by 16.7% and continued to decrease at Week 10. The vaccinated group FEC began to rise at Week 4 but never reached the levels of the control group. The trend also continued until Week 8 at which time the FEC dropped but at Week 9 the FEC decreased significantly by 96.2%. When the FEC are log transformed, the vaccinated group still has a significantly lower FEC than the control group overall. When compared at each week of the study, the FEC are significantly lower at Weeks 4, 5, 6, 8, 9, and 10. The insignificance at Week 7 is likely due to the increasing FEC in the vaccinated group as the antibody levels were decreasing below protective levels at which time the vaccine was administered as a booster.

Table 2. Mean FEC in eggs per gram (EPG) of control and vaccinated group post infection with infective larvae. Vaccination was given to vaccine group at Week -5, Week -3, Week -1 and Week 7. _a Indicates significant differences at the time points between the groups for log transformed FEC (P < 0.05).

	Weeks Post Infection							
Treatment	3	4 _a	5 _a	6 _a	7	8 _a	9 _a	10 _a
Control	31.82	365.00	1700.00	2620.00	3255.00	5145.00	4285.71	3621.43
Vaccine	0.00	200.00	604.55	1090.91	1436.36	1331.82	50.00	193.75

Figure 5 illustrates the trend of the FEC for the control and vaccinated groups for the groups necropsied during Week 8 plotted with their corresponding OD levels.

Differences in mean FEC are significant from Week 5 through Week 8 of the trial. A trend in decreasing IgG levels in the vaccinated group from Weeks 4 through 7 is visible as Mean FEC in the vaccinated group rise during that time.

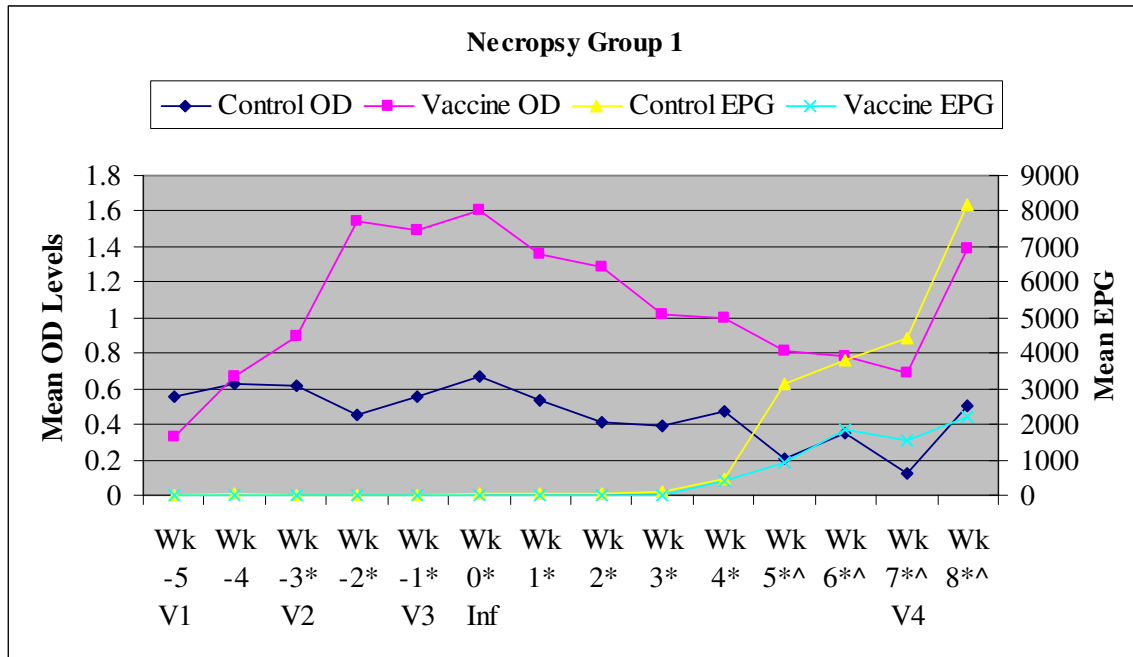


Fig. 5. Mean FEC in eggs per gram (EPG) and mean OD levels of control and vaccinated groups necropsied at Week 8. Injections were given Weeks -5, -3, -1, and 7 post infection at Week 0. Significant differences in FEC are indicated with * and for OD levels by ^ (P<0.05).

Figure 6 shows the Mean FEC and OD levels for the group necropsied during Week 10. A trend is seen here in the vaccinated group as IgG levels decrease at Week 4, FEC in the vaccinated group begin to rise but after the booster at Week 7, the IgG levels spike and the FEC drop in the vaccinated group but continue to increase in the control group. Significant differences are seen in FEC for this group from Week 6 to Week 10 of the trial.

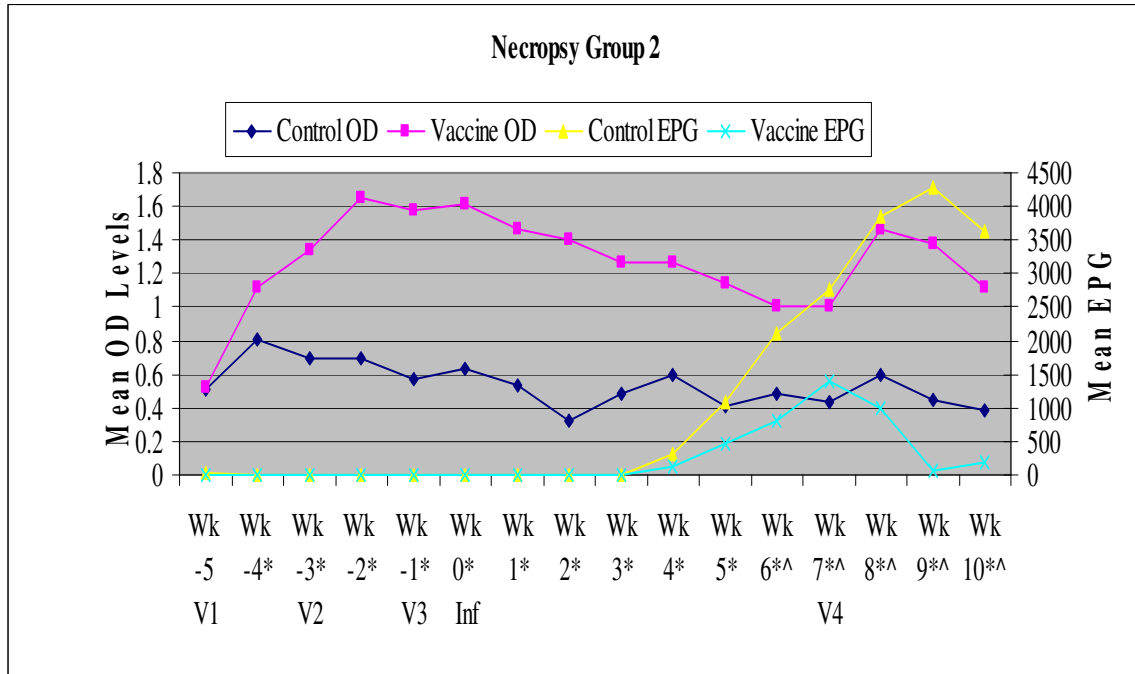


Fig. 6. Mean FEC and in eggs per gram (EPG) and mean OD levels of control and vaccinated groups necropsied at Week 10. Injections were given Weeks -5, -3, -1, and 7 post infection at Week 0. Significant differences in FEC are indicated with * and for OD levels by ^ (P<0.05).

4.5 Packed Cell Volume

Packed cell volume (PCV) was monitored weekly for both groups throughout the trial. From Week -5 to Week 1 post infection the PCV levels were equal for the vaccinated and control groups. Beginning with Week 2, the control group PCV began to decrease and continued to drop until Week 9, when the levels increased concurrently with the drop in FEC shown previously (Table 3). The vaccinated group PCV levels did not decrease until Week 4 post infection but remained higher than the control group PCV at each time point after infection. The PCV levels were significantly higher in the vaccinated group than the controls from Week 3 to Week 10. The PCV levels in the vaccinated group remained steady throughout the remainder of the trial after the drop at Week 4.

Table 3. Mean packed cell volume (PCV) levels for the control and vaccinated groups shown in weekly intervals from Week 1 post *H. contortus* infection through Week 10. The final H11/H-gal-GP vaccine booster was given to the vaccine group Week 7.
^a Significant differences between groups at these time points (P <0.05).

	Weeks Post Infection									
Treatment	1	2	3 _a	4 _a	5 _a	6 _a	7 _a	8 _a	9 _a	10 _a
Control	27.45	26.45	25.09	23.20	20.50	19.00	18.70	17.30	19.57	18.71
Vaccine	27.91	27.36	28.36	25.73	25.00	24.18	24.00	24.09	25.50	25.88

Figure 7 illustrates the PCV levels as the FEC change in the group necropsied during Week 8. PCV levels are significantly higher in this vaccinated group as compared to this control group at Week 8. As previously mentioned, the control animals were chosen for necropsy at this time due to their deteriorating conditions from haemonchosis and the corresponding vaccinated group was chosen based on the lowest PCV levels and highest FEC but were not showing clinical signs of haemonchosis.

Figure 8 depicts the PCV levels plotted against the FEC for the group necropsied at Week 10 of the trial. PCV levels are significantly higher in this vaccinated group from Week 4 through Week 10 as compared to this control group. It is noted that as FEC rise for each group, PCV levels decrease; however, the changes are less in the PCV levels of the vaccinated group.

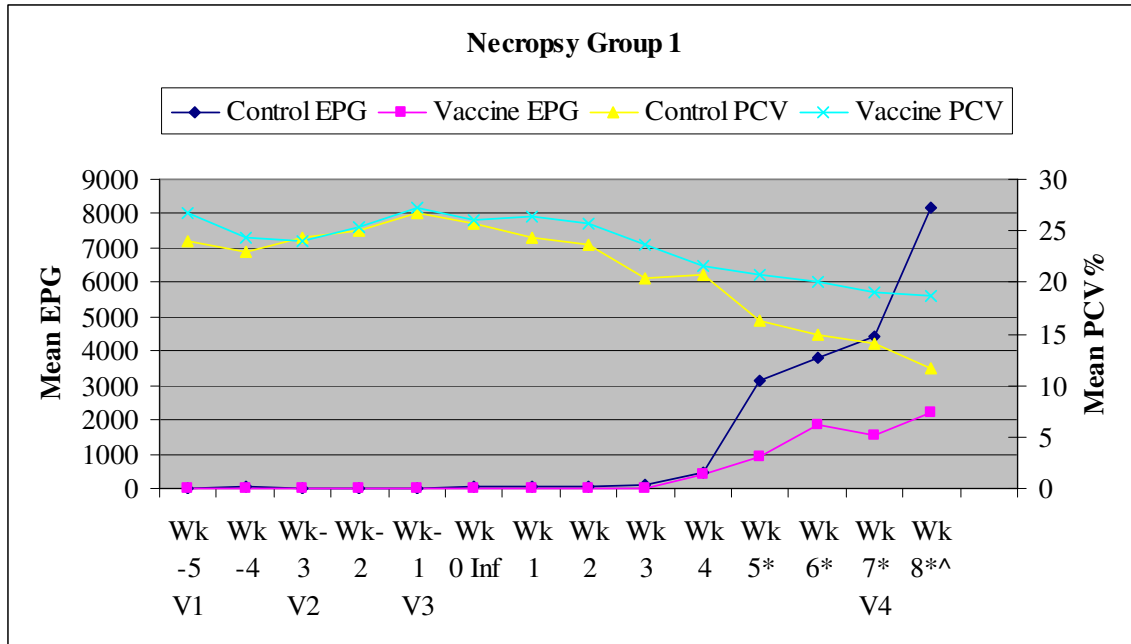


Fig. 7. Mean packed cell volume (PCV) % for control and vaccinated groups necropsied at Week 8 shown weekly throughout duration of trial. Significant differences in FEC are indicated with * and for PCV levels by ^ (P < 0.05). Injections were administered at Week -5, Week -3, Week -1 and Week 7 post infection with *H. contortus* larvae at Week 0.

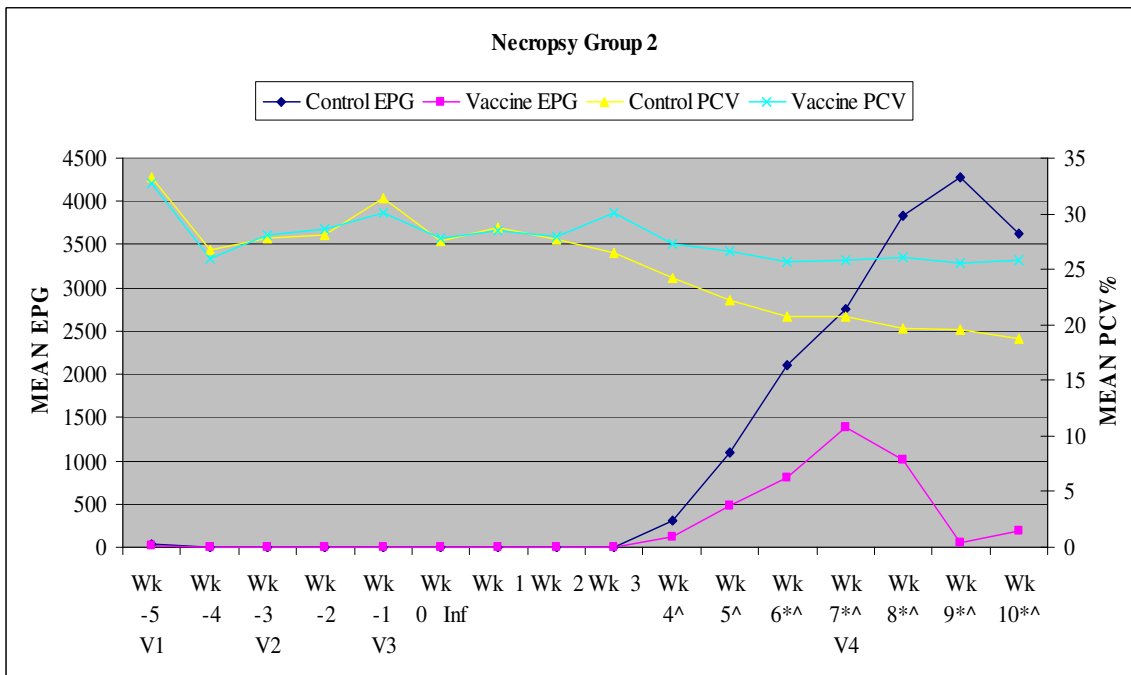


Fig. 8. Mean packed cell volume (PCV) % for control and vaccinated groups necropsied at Week 10 shown weekly throughout duration of trial. Significant differences in FEC are indicated with * and for PCV levels by ^ (P < 0.05). Injections were administered at Week -5, Week -3, Week -1 and Week 7 post infection with *H. contortus* larvae at Week 0.

Chapter Five

DISCUSSION AND CONCLUSIONS

This study showed that the aminopeptidase complex H11 and the *Haemonchus* galactose-containing glycoprotein (H-gal-GP) antigens extracted from the intestinal membrane of *H. contortus* administered with Quil A adjuvant had an immunoprotective effect in goats. After the initial series of vaccinations, the antibody levels began to decrease slowly but remained high enough to offer protection against clinical haemonchosis as demonstrated by the consistently lower FEC and higher PCV throughout the study in the vaccinated group when compared to the control animals. After challenge with *H. contortus* infective larvae, the vaccine failed to prevent infection from the parasites but the establishment of adult worms was significantly less than the control group and clinical signs of haemonchosis were not evident. This was shown at the first necropsy at Week 8 post infection, when the vaccinated group had only slightly fewer *H. contortus* recovered compared to controls, yet the animals necropsied from the control group were severely anemic and clinically ill from haemonchosis.

The antibody levels after the initial series of vaccinations maintained constantly at or above OD 0.8 and then increased dramatically to 1.4 after the final booster. Previous trials with sheep have shown that the antibody levels need to be at least OD 1.2 to give immunoprotection from *H. contortus* infections (Munn et al., 1997). This observation has been the guideline for determining at which time to administer booster dosages of the vaccine throughout research studies in the past (Ruiz et al., 2004).

The most striking immunoprotective effect shown by the goats receiving the vaccine was the elimination of the established *H. contortus* infection after the booster was given at Week 7 post infection. This is evident by the spike in IgG levels, the 96% reduction in FEC and the 96% fewer worms recovered at necropsy than the control group. The results of this trial along with previous studies using sheep further indicate that the H11/H-gal-GP vaccine may be used as a treatment due to the curative effect when given during a challenge infection with *H. contortus* (Smith et al., 2001). The increase in antibody levels preceded the decrease in FEC by one week when a vaccine booster was given during challenge infection as indicated in prior research with sheep (Smith et al., 2001; Ruiz et al., 2004).

Upon examination of the data from the animals that were sampled for the duration of the entire trial, it is noted that after the booster was given at Week 7, the FEC begin to drop in the vaccinated group and continue to rise in the control group. This decrease in FEC coincides with the spike in IgG one week after booster; however, the second week after booster had a significantly greater decrease in FEC in the vaccinated group than that seen in the week of the IgG increase.

A correlation between antibody levels and log FEC was found only at Week 8 in the vaccinated group with a correlation coefficient of -0.66. This was one week post final booster when there was a significant increase in antibody levels. These results are similar to those in field trials and certain experimental studies in sheep (Newton et al., 1995; Smith and Smith, 1996; Kabagambe et al., 2000). However, this contradicts certain previous studies that did find correlations between antibody levels and FEC and worm counts in vaccinated groups (Munn et al., 1993).

The overall worm counts were significantly lower in the vaccinated group as compared to the controls for both necropsy dates. There was no significant difference between controls and vaccinated goats during the first necropsy which was just one week post booster. As mentioned before, the FEC decrease after the final vaccine was one week after the spike in antibody levels and this is likely the case for the worm counts as well. A similar trial with necropsies performed on dates between the antibody increase and the drop in FEC needs to be done to confirm when the expulsion of the worms correlates with antibody spikes and FEC decreases. During the second necropsy, the worm counts were significantly lower in the vaccinated group compared to controls.

Also, there were significantly fewer male *H. contortus* adults recovered than females in the vaccinated group which is contrary to many studies that found more males than females during necropsy (Smith et al., 1993; Smith and Smith, 1993; Newton et al., 1995; Smith et al., 1999). This could be due to the male to female ratio of the larvae used to infect these animals which is an unknown variable.

Although this study was performed under experimental conditions with the goats kept on concrete and given *H. contortus* infections by oral bolus, the results mimic that of the field trials in sheep kept on pastures and naturally acquiring infections. The previous studies in sheep under artificial infections and controlled environment tend to have a longer lived immunity when given the H11/H-gal-GP vaccine. In this study, the immunogenicity began to wane and by Week 7 post infection, a vaccine booster was needed to clear the increasing infection levels in the vaccinated group. One explanation for the decrease in IgG after infection could be the actual removal of antibodies from the bloodstream as the L₄ stage of the parasite begins to feed in the abomasum. There are

definite differences in susceptibility in goats to *H. contortus* infections under field conditions; therefore it is to be expected under experimental conditions as well. Also, many of the prior vaccine studies used Freund's complete or incomplete adjuvant as opposed to the Quil A adjuvant used here. This could lead to differences in immunoprotection since Quil A is not as strong as Freund's adjuvant.

After extensive literature review, it appears that this is the first trial to be performed on goats using the H11/H-gal-GP hidden gut antigen vaccine. It is important to establish that the vaccine will also work in goats as they also suffer from multiple drug resistant *H. contortus* worldwide. Goats are depended on as a livelihood by millions of poor farmers in underdeveloped countries; therefore, it is critical that their health must not be overlooked. As shown in this study, there is a difference in the length of immunoprotection when given these antigens as a vaccine under experimental conditions as compared to sheep and the same may be true under field trials as well. Future work must include trials with H11/H-gal-GP vaccine administered to goats acquiring natural infection on pasture.

As has been previously discussed, the natural vaccine containing hidden gut antigens H11 and H-gal-GP is immunogenic in both sheep and goats; however, mass production of such a vaccine would not be economically feasible. The harvesting of enough *H. contortus* adults to sufficiently vaccinate a herd of goats would require the infection and necropsy of many sheep or goats. Quality control issues such as hosts that are free of disease prior to harvesting the parasites as well as the cost of such animals and the time consuming processing of the antigens deter pharmaceutical companies from attempting to get such a product approved by the FDA. At this time, however, the

recombinant antigen preparations have not been successful in prompting an immune response in vaccinated animals that is effective against challenge infections with *H. contortus*. Baculovirus-expressed H11S molecules and inactive *E. coli*-expressed H11-1 active site domain have been attempted as recombinant products for native H11 but at this time have not been successful (Newton and Meeusen, 2003). Recombinant versions of H-gal-GP have been difficult to develop as the natural effective form is a complex of proteins ranging in size from 31 to 230kDa. When the complex is broken down the protection from challenge infection is only partial and at least three of the metalloprotease (MEP) homologues present in the H-gal-GP complex do not contribute to protection (Smith et al., 1999). Recombinant expression and efficacy testing for a thrombospondin homologue, a galectin and a cystatin component of H-gal-GP have also not been protective (W.D. Smith, pers. comm.). Despite these setbacks, research in development of an efficacious recombinant vaccine against *H. contortus* has not been abandoned.

In conclusion, this study has found that H11 and H-gal-GP are immunogenic when used together as a vaccine against *H. contortus* infection in goats. The vaccine induced an increase in IgG levels that were maintained at higher levels than the controls throughout the entire study and although did not prevent establishment of infection, the infection levels were slower to develop and vaccinated animals did not show signs of haemonchosis as did the controls. This study also shows that the vaccine can be given during an existing infection to eliminate worm burdens in place of an anthelmintic. This seems to be necessary as there has been no long-term memory of immunity shown to these hidden gut antigens at this time. Continued work must be done in order to develop

an efficacious recombinant product that will be economical and convenient for producers so that the dependence on anthelmintics is reduced. This work must include trials with not only sheep but also goats so that the finished product may be marketed to all small ruminant producers and proper administration of the product is understood in all species.

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VITA

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