Haemonchus contortus Infections in Alpacas and Sheep

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ABSTRACT

The blood feeding nematode *Haemonchus contortus* infects the abomasum of small ruminants and compartment three (C-3) of camelids. Heavy infections may cause severe anemia and death. Alpacas were first introduced into the U.S. in the 1980s. Although not true ruminants, alpacas may become infected with *H. contortus* and develop the same clinical signs as sheep and goats. Even though alpacas may become infected with the parasite, prior research by Hill et al. (1993) and Green et al. (1996) indicates alpacas may be more resistant to parasitic infection because they found lower numbers of eggs in the feces of alpacas compared to small ruminants. For our research, we hypothesized that given the same exposure to experimental infection, alpacas would be less susceptible than sheep to *H. contortus*. Experiment 1 was conducted with adult male alpacas (23) and sheep (12) housed in pens to prevent additional exposure to *H. contortus*. All animals were dewormed orally with a cocktail of fenbendazole, levamisole, and ivermectin. *Haemonchus contortus* infective larvae were administered orally to alpacas and rams in the following groups:1) 20,000 larvae as a single dose (bolus, n=6 both alpacas and sheep), 2) 20,000 larvae in daily doses of 4,000 larvae for 5 days (trickle, n=5 for alpacas, n=6 for sheep). Two additional groups of alpacas (n=6 each) received either 50,000 larvae as a bolus infection, or in daily doses of 10,000 larvae for 5 days (trickle). Fecal egg counts (FEC) were determined every 2 days from 14 to 42 days post infection (PI) and then at 5 day intervals until day 62 PI. Packed cell volume (PCV), FAMACHA scores, weight, and body condition scores were evaluated weekly. In general, mean FEC were lower in alpacas than sheep (p<0.01), and mean alpaca PCV was affected less by infection than sheep PCV. Experiment 1 results are consistent with our hypothesis that alpacas are less susceptible to *H. contortus* infection than sheep; however we were unable to determine whether alpaca FEC reflected fewer adult worms or only reduced *H. contortus* egg production compared to sheep. Experiment 2 was conducted with 16 alpacas and 12 rams and all animals were orally dewormed as in Experiment 1.

Haemonchus contortus infective larvae were administered orally to alpacas in the following groups: 1) 20,000 larvae as a single dose (bolus, n=8), and 2) 20,000 larvae in daily doses of 4,000 larvae for 5 days (trickle, n=8). Ram groups (n=6 each) were the same as the alpaca groups. Fecal egg counts were determined at 5 day intervals from days 14 to 49 PI for bolus infected animals and to day 54 PI for trickle infected animals. FAMACHA scores were evaluated weekly. Packed cell volume was evaluated at the beginning and end of the study. All animals were euthanized 49 days after the last infection day. At euthanasia, abomasa and C-3 were harvested for determination of total worm burden and the pH was determined for each sheep rumen and abomasum and each alpaca C-1 and C-3. Mean FEC and total worm burden were significantly lower in alpacas than sheep (p<0.0001 for both FEC and total worm burden). Rumen pH in sheep was higher than C-1 pH in alpacas, but abomasal pH in sheep was significantly lower than C-3 pH in alpacas. Bolus infected sheep had lower FAMACHA scores than the other groups, and PCV was lower on the last day of sampling than the first day of sampling in all groups. The results of Experiment 2 also support our hypothesis that alpacas are less susceptible to *H. contortus* infection than sheep. However, it is unclear whether the differences are the result of physiological stomach differences between host species or whether other factors, such as immunity or parasite strain are important.

Dedication

To my family, especially my parents, Sharon and Benny Casey, and Cameron Hughes for all their love, support, and encouragement.

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Haemonchus contortus and Life Cycle

Haemonchus contortus is an abomasal (Soulsby, 1965) and compartment 3 (C-3) blood feeding nematode of small ruminants and camelids (Gillespie et al., 2010). The common name of the parasite is the barber-pole worm because the white reproductive tract is wrapped around the blood-filled intestine making it resemble a barber's pole (Sutherland and Scott, 2010). *Haemonchus contortus* belongs to the order Strongylida and family Trichostrongylidae. The parasite has a small buccal cavity with a single tooth, called the lancet, which is made of cuticle and is used to slice host mucosa for feeding (Soulsby, 1965; Zajac, 2006).

Haemonchus contortus has separate sexes and adults can be seen with the naked eye. Adult females are 20-30 mm in length, tapered at both ends, and usually have a vulvar flap (Soulsby, 1965; Zajac, 2006). Males are 10-20 mm in length and are tapered at the anterior end. The posterior end of the male has a copulatory bursa and spicules with a barb at the end used to hold open the female's genital opening during mating (Schmidt and Roberts, 2005) (Figure 1).

The life cycle of *H. contortus* is direct, and the prepatent period is 17-21 days in small ruminants. Adult worms only live for a few months and reproduce sexually in the host (Soulsby, 1965; Zajac, 2006). Females may produce thousands of eggs per day (Zajac, 2006) that come out in the feces of the host (Soulsby, 1965; Zajac, 2006). Under ideal conditions (hot: 31°C-34°C, humid: relative humidity above 85%) (O'Connor et al., 2006), larvae hatch from eggs in the feces and feed on bacteria (Soulsby, 1965; Zajac, 2006). These are the first stage larvae (L1) that hatch in about 2 days, and in as little as 8-12 hours, the larvae molt, removing the cuticle. After molting, the second stage larvae (L2) grow rapidly and continue to feed on bacteria. After a second molt, larvae enter the infective third stage (L3) about 5 days after coming out in the feces. These larvae retain the cuticle of the second stage as a protective sheath or covering, which protects the L3 from unfavorable weather conditions. The sheath also covers the buccal cavity, which prevents larvae from feeding. Larvae survive on stored energy as they travel from the fecal mass to the vegetation to be consumed by a grazing host, such as a sheep (Sutherland and Scott, 2010). Once ingested, larvae exsheathe in the forestomach and travel to the abomasum where they begin to feed. Larvae then molt to the fourth stage (L4) in about 1.5-2 days after infection, and grow rapidly as well as feed on blood (Soulsby, 1965). The L4 can be found in the anterior region of the fundus and are almost completely absent in the pylorus (Dash, 1985). This

may be an unfavorable environment for fourth stage larvae. During the fourth stage, larvae develop a buccal capsule that looks like a truncated cone. About 4-6 days after an animal is infected, male larvae have a swollen and thick posterior end where the tail is short, conical and smooth. Females have a longer tail that tapers. Later in the fourth stage, the buccal cavity slightly changes shape and has a more rounded appearance and the walls appear thicker than previously. The male has a more distinct posterior end that is blunt and the spicules and bursa are visible. In female larvae, the formation of the vagina, uterus, and ovary can be seen. Between 9 and 12 days after infection, the larvae molt one final time to the pre-adult stage (L5). The buccal cavity looks the same as a mature adult, and contains the dorsal lancet that is curved and has two thorn-shaped points. In the male, the spicules are visible and the bursa is well developed. The rays are distinct but are bunched together. The female vulval opening is more distinct, and the vagina is funnel shaped and leads into the genital tube. The ovaries and uterus are also well developed in the pre-adult stage. Further growth and development occurs during this stage, and mature adults are marked when the female's ovarian tubes are coiled around the intestine and the uterus contains eggs. Males have very distinct spicules and well defined genital tube that frequently contains a mass of spermatozoa. Mature males and females are usually present by 18 days after infection, but could take up to 21 days to fully mature (Soulsby, 1965). Adult worms are found in the posterior fundic region of the abomasum closer to the pylorus, whereas L4 are found in the anterior region, which may be a selection by adult worms of a more favorable feeding site, or an avoidance of the host reaction in the area parasitized by earlier larval stages (Dash, 1985) (Figure 2).

Hypobiosis. Like many other trichostrongylid nematodes, *H. contortus* is able to undergo a state of arrested development known as hypobiosis (O'Connor et al., 2006). Hypobiosis occurs within the host. Larvae do not develop directly into adults but remain as L4 in the mucosal wall for weeks or months. During this time, larvae are metabolically inactive, and the proportion of hypobiotic worms is often greatest when conditions outside the host are least favorable for parasite development and eggs shed in the environment would be unlikely to survive. The mechanisms of hypobiosis are not fully understood. Environmental conditions (season and climate), host immunity, and genetics have been implicated (Captini et al., 1990).

Pathogenesis

Low worm burdens may have little effect on an animal, but higher worm burdens may have a large impact (Zajac, 2006). Haemonchosis is the disease associated with severe *H. contortus* infections (Baker et al., 1959; Schmidt and Roberts, 2005). Clinical signs typically seen with this disease are severe anemia, seen as pale mucous membranes, and submandibular edema, known as "bottlejaw," resulting from hypoproteinemia. Haemonchosis can result in decreased appetite, weight loss, and death (Baker et al., 1959). As a result, the normal packed cell volume (25-44.5% for alpacas and 27-45 for sheep) and total protein (4.7-7.3g/dL for alpacas and 6-7.5 for sheep) are severely decreased (Hoffman, 2006; Aiello et al., 2010). Susceptibility to disease varies from animal to animal due to genetics and the environment.

Distribution and Importance

Haemonchus contortus has a worldwide distribution with concentrations in the tropics and subtropics where there are high temperatures and a lot of rainfall. The parasite can also be found in more temperate areas, such as the United States. In the southeast U.S., *H. contortus* predominates over other gastrointestinal nematodes, such as *Teladorsagia* and *Trichostrongylus* species and can be found in large numbers because of the warm and humid environment (O'Connor et al., 2006). The optimum environmental temperatures for *H. contortus* are 31°C-34°C (O'Connor et al., 2006), but it can be found in temperatures as low as 10°C and as high as 36°C (Zajac, 2006). In the western U.S. where it is drier, *H. contortus* is more likely to be a problem in irrigated areas. High humidity (more than 85%) is important for the parasite because a lot of moisture in the air helps protect it from desiccation at high temperatures (O'Connor et al., 2006). *Haemonchus contortus* does not endure the cold very well (Zajac, 2006), and eggs do not hatch below 9°C (O'Connor et al., 2006).

In most areas where alpacas and sheep are found, parasites are also found. Based on pathogenicity, it is reasonable that *H. contortus* is one of the most important disease problems in the small ruminant industry because it is a major cause of production loss (Zajac, 2006). Also, the anthelmintic resistance of *H. contortus* is becoming a problem in camelids. For example, Gillespie et al. (2010), reported resistance was present to two of the three classes of anthelmintic drugs on three llama/alpaca farms in Georgia. These scientists have demonstrated that multiple anthelmintic resistance in llama and alpaca gastrointestinal nematodes is an emerging problem in

the United States. In another study conducted by Sarre et al., (2012), doramectin resistance was found on an alpaca farm in Belgium. They reported resistance was mainly to *H. contortus.* Sarre et al., (2012) also reported that was the first time doramectin resistance had been reported in European alpacas. However, resistance was previously reported in European ruminants mostly to the benzimidazoles (Sarre et al., 2012). These scientists have demonstrated that drug resistance of *H. contortus* is not only a U.S. problem but could be a problem in other parts of the world. *Haemonchus contortus* has also been found in cattle but clinical disease is rare.

Camelids and Sheep

History of Camelids. The ancestors of today's camelids evolved between 11 and 9 million years ago in North America, and the family Camelidae dates as far back as 35 million years (Hoffman, 2006). Three million years ago, camelids migrated from North America to South America via the Isthmus of Panama and into Asia by crossing the land bridge between Alaska and Siberia. Camelids became extinct in North America 12,000 years ago and are survived by today's South American camelids (SAC), as well as the one-humped camel (*Camelus dromedaries*) in Africa and the two humped Bactrian camel (*Camelus bactrianus*) in Asia (Hoffman, 2006).

Characteristics of Camelids. Camelids are herbivorous, even toed ungulates with padded feet. They belong to the order Artiodactyla with pigs, hippopotamus, bison, deer, and goats and are in the suborder Tylopoda. The camelid family includes camels (*Camerlus dromedaries or bactianus*), llamas (*Lama glama*), guanacos (*Lama guanicoe*), vicuñas (*Vicugna vicugna*), and alpacas (*Vicugna pacos or Lama pacos*) (Fowler, 1998; Hoffman, 2006). Camelids are large animals with long, slender necks and long legs. These animals are alert and have big, round eyes and wool covered bodies. All six species have the same diploid number of chromosomes (2n=74) and are able to interbreed and produce live, fertile offspring (Fowler, 1998; Hoffman, 2006). In addition, camelids are modified ruminants because they have a different stomach and digestive tract arrangement. This allows camelids to survive in the harsh environments of the Andes where they are naturally found (Fowler, 1998; Hoffman, 2006).

Importance of Alpacas. Alpacas are becoming more important in the United States and worldwide because the number of alpacas has increased since their introduction to the U.S. from South America to at least 200,000 as of 2010 (Long, 2010). Although they do not compete with cattle as meat producing animals, alpacas do have some value, such as in fiber production. Also, the natural adaptations of alpacas to harsh environments means alpacas do not have to be placed on the highest quality pastures unlike most livestock. Because the number of alpacas in the United States is growing, it is now even more important to gain control of parasites like *H. contortus* that can be detrimental to animal health.

History of Sheep. Sheep and goats were the first livestock species to be domesticated about 11,000 years ago in southwest Asia. They were mainly raised for meat, but wool was found to be a useful product by the fourth and fifth millennium B.C. in southwest Asia and Europe (Chessa et al., 2009). Sheep then spread across Europe, Asia, and into Africa, developing into various breeds.

Most hair sheep breeds originated in Africa. Some breeds originated in the desert of South Africa, while others are from the tropical areas of West Africa and further developed in the Caribbean with the introduction of the slave trade. The St. Croix and Barbados Blackbelly breeds are hair sheep from West Africa and the Caribbean, and these breeds thrive in conditions of heat and humidity (Notter, 2000; Bradford, 2005). Hair sheep, specifically the Barbados Blackbelly, were first introduced into the United States in the early 1900s. They were sent to Texas to be further bred and even crossed with Rambouillet and mouflon to create the Barbado breed. In 1960, the Virgin Island White sheep (also known as St. Croix sheep) were imported to the U.S. from the island of St. Croix and were bred with U.S. breeds to create the Katahdin. The St. Croix and Barbados Blackbelly breeds are still found in the U.S. and have thrived over the years (Bradford, 2005).

Characteristics of Sheep. Sheep are small ruminants and have become a worldwide commodity. They belong to the order Artiodactyla with camelids, suborder Ruminantia, and family Bovidae (Church, 1988). Sheep are found in an assortment of sizes, colors, and breeds around the world and are considered to be intermediate grazers, meaning they tend to eat lower quality grasses but will also eat flowers and twigs of woody species. Their incisors are located at

the front of the lower jaw and have wedge shaped cutting surfaces that allow clipping of plants against the opposing gum pad of the upper jaw (Martin and Bryant, 1989).

Anatomy and Physiology of Gastrointestinal Tract of Camelids and Sheep

Camelids. Ruminants are considered the most efficient herbivorous animals at digesting fiber. Acquisition of nutrients depends on the symbiotic relationship between the host and microbial population in an anaerobic fermentation system, not on enzymes (Hoffman, 2006). Camelids, are not considered "true ruminants" like sheep and goats because they have distinct anatomical and physiological differences in their digestive tracts. However, camelids do have an expanded forestomach for microbial fermentation of food, and they ruminate, which is also known as chewing the cud. They have a three compartment stomach (C-1, C-2, and C-3), unlike the four chambers (rumen, reticulum, omasum, and abomasum) in sheep and goats (Vallenas et al., 1971; Church, 1988, Hoffman, 2006).

Compartment one (C-1) is divided into cranial and caudal sacs by a transverse sulcus (Vallenas et al., 1971; Martin and Bryant, 1989; Hoffman, 2006). The walls of both sacs are covered by recessed thin-walled sacculations lined by glandular mucosa in the ventral part, while exposed surfaces are covered by stratified squamous epithelium in the dorsal part of the compartment (Vallenas et al., 1971; Martin and Bryant, 1989). Exposed mucosa is non-papillated and folds are present but not permanent. Because the submucosa is very loose, the mucosa is easily folded, and the prominence of folds varies with the contraction of the stomach, while existing folds are eliminated by stretching. During stomach contractions, the saccules evert and empty contents into the lumen of C-1 (Vallenas et al., 1971; Hoffman, 2006). Glandular saccules in C-1 secrete large amounts of bicarbonate in association with an uptake in C-1 digesta from the lumen. This may contribute to buffering of digesta in C-1 and C-2 (Martin and Bryant, 1989). The main function of this glandular region is rapid absorption of solutes and water, which is much higher than in sheep or goats (Martin and Bryant, 1989). In addition, this compartment has a higher pH and holds on longer to C-1 fluid than the rumen of sheep, which is thought to allow higher bacterial yields for fermentation (Martin and Bryant, 1989; Dulphy et al., 1997).

Compartment two (C-2) is connected to C-1 and is lined by glandular and stratified squamous epithelium like in C-1 (Vallenas et al., 1971; Martin and Bryant, 1989). This compartment also has saccules as in C-1, but they do not evert during the contraction cycle

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(Vallenas et al., 1971). Compartment 2 contains liquid ingesta, whereas the dorsal portion of C-1 has more dry and finely minced roughage, and more fluid contents are located ventrally in C-1 (Vallenas et al., 1971).

Compartment three (C-3) is connected to C-2. This compartment is lined entirely by glandular mucosa and varies in arrangement along the length of C-3. The mucosa of the lesser curvature of the initial ⅕ of C-3 is arranged in a netlike pattern. The mucosa along the greater curvature of the initial ⅕ is arranged in nonpermanent folds, while permanent pleats cover the next three-fifths of the compartment. The mucosa over the greater curvature in the terminal one-fifth is very thick, and corresponds to the sheep abomasum because it contains gastric glands. The mucosa along the lesser curvature is not as thick and contains the pyloric glands, which are located along the entire pyloric circumference in the terminal one-fifth of C-3 (Vallenas et al., 1971; Martin and Bryant, 1989). Of this one-fifth, half of it has abundant parietal and chief cells, and the rest, toward the pylorus, has mucous-secreting cells (Martin and Bryant, 1989).

The pylorus, the last part of C-3, empties into the duodenum. The process of digestion and absorption in the small intestine is identical in ruminants and nonruminants. The hindgut does some fermentation also but not as much as the forestomach. Absorption of water, some volatile fatty acids, vitamins, and minerals is the primary purpose of the hindgut (Hoffman, 2006).

Forestomach motility is important for fermentation because it ensures that food is continually exposed to microbes for degradation. Two phases (A and B) of motility have been observed in alpacas. In phase A, the ventricular groove contracts, followed by a rapid contraction of C-2 (Martin and Bryant, 1989; Hoffman, 2006). Then the ventricular groove contracts again accompanied by the caudal portion of C-1. Phase B begins when the cranial part of C-1 and C-2 contracts and the caudal part of C-1. Phase B repeats 3-6 times in a cycle before resting and starting a new cycle (Martin and Bryant, 1989; Hoffman, 2006). Forestomach motility is continuous and occurs longer than in sheep (Martin and Bryant, 1989), and as a result, they have more homogenized stomach contents and have been observed to be more resistant to bloat. Ruminant motility is slower, and phases (α and β) occur in a markedly different fashion (Hoffman, 2006).

Sheep. Sheep have a four-compartment stomach (rumen, reticulum, omasum, and abomasum). The first three compartments (rumen, reticulum, and omasum) are lined with nonglandular mucosa for fermentation, while the abomasum is lined with glandular mucosa for chemical digestion (Church, 1988).

The rumen is the largest of the four compartments and is divided into dorsal and ventral ruminal sacs. The rumen mucosal surface is covered by ruminal papillae, which are used for absorption and increase the surface area of the rumen (Church, 1988). This compartment contains many different microbes including bacteria, fungi, and protozoa, which are important because of their fermentation properties. Motility in the rumen is essential for continuous mixing and movement of contents for digestion. Because the rumen and reticulum are close in proximity and there is continuous mixing and movement between the two compartments, they are often discussed as a single compartment.

The reticulum and rumen are partially separated but have different functional purposes. The reticulum looks like a honeycomb (Harfoot, 1978; Church, 1988) and moves ingested food into the rumen or omasum and helps in regurgitation during rumination (Church, 1988). The esophagus opens into an upper part of the reticulum, called the cardia. A ventricular groove, functions in suckling ruminants to allow milk to flow directly to the abomasum but is usually open in adults for digesta to flow into the reticulum and rumen (Harfoot, 1978; Church, 1988). The reticulum has the ability to contract to a fraction of its size, which allows mixing and movement of feed into and out of the rumen, regurgitation of digesta from rumen to mouth, movement of contents to the omasum, and release of fermentation gases (Church, 1988).

The omasum is connected to the reticulum and entrance is through the reticulo-omasal opening (Harfoot, 1978; Church, 1988). The omasum is composed of many layers of tissue all with absorptive epithelial lining, and small papillae line the compartment (Church, 1988). This compartment controls particles of digesta leaving the reticulorumen to go to the abomasum. It is generally the smallest of all four compartments and only allows small particles to enter for more absorption of nutrients like volatile fatty acids, water, and minerals (Church, 1988).

The abomasum is connected to the omasum and is the fourth compartment of the stomach (Harfoot, 1978; Church, 1988). It has three different regions (cardiac, fundic, and pyloric). The cardiac region has gastric glands lined with secretory columnar epithelium (Church, 1988). The secretory cells secrete hydrochloric acid and pepsinogen during digestion (Harfoot, 1978;

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Church, 1988). The fundic region has many parietal cells and chief cells that secrete pepsin and hydrochloric acid, and many are located in the gastric pits of this region. Stratified squamous epithelium lines the pyloric region and secretes mucous (Church, 1988). The inner surface of the abomasum has many folds as in other compartments that increase the surface area to allow for more acid and pepsin secretion. Also, these folds help prevent stratification of feed because it allows digesta and enzymes to mix making feed more homogenized. Because of the presence of hydrochloric acid, the pH of the abomasum is often less than 3 (Church, 1988).

Camelid and Sheep Immunity to *Haemonchus contortus*

Camelid Immunity. The camelid immune system is complex and not well understood. There are some similarities to the sheep immune system, but there are some differences as well. As in sheep, camelids have neutrophils, eosinophils, basophils, lymphocytes, and monocytes (Hamers and Muyldermans, 1998). Also, antibodies of the humoral immune response of camelids are unique because they consist of two heavy chains and no light chains (Odbileg et al., 2004), and up to 75% of camelid antibodies belong to the IgG subclasses (Hamers and Muyldermans, 1998). The role of heavy chain antibodies in the humoral immune response are not fully understood (Odbileg et al., 2004). In 1996, Green et al. found that development of serum antibodies to gastrointestinal nematodes increases with age in alpacas as it does in sheep, and they reach "steady state" or adult levels by about 24 months of age. This is slower than seen in sheep, in which it takes about 8 months to develop to adult levels in some breeds. Green et al. (1996) also suggest that alpacas are able to suppress fecal egg counts because they observed occasional fecal egg counts in alpacas and antibody levels, but they also mention that the absence of eggs does not mean adult worms are not present or eliminated from the body because the relationship between fecal egg counts and worm burden can only be determined at slaughter, which was not performed in this study.

Even though camelids are susceptible to similar diseases and parasites that affect sheep, the result of diseases can be affected by cytokine responses. Cytokines are important in T-cell differentiation, cell-mediated, and humoral immunity. As in sheep, Th-2 responses are elicited in alpacas by parasitic helminths, like *H. contortus*. Cytokines interleukin 4 (IL-4), IL-10, and IL-13 are produced, and they likely have similar activities to those of other mammals, such as

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sheep. These cytokines have been connected to regulation of the immune response in camelids (Odbileg et al., 2004).

Sheep Immunity. The sheep immune system is a complex system as in other mammals but is necessary to fight off disease and induce resistance. Resistance to *H. contortus* and other gastrointestinal nematodes is an inheritable genetic characteristic. A higher level of resistance is associated with some sheep breeds, like the St. Croix and Barbados Blackbelly breeds. Even though some breeds may be more resistant to the parasite, they do not completely reject the disease, but have a lower worm burdens as compared to susceptible breeds, such as the Dorset or Suffolk breeds (Gill, 1991).

The mechanisms of how sheep acquire resistance to *H. contortus* are not completely clear (Saddiqi et al., 2011). Resistance is an individual characteristic associated with age, breed, and previous exposure. The innate and adaptive immune systems are both important in protecting the host from *H. contortus* infection (Meeusen et al., 2005). In the innate system, the complement pathway is one of the first responses (Meeusen et al., 2000). When larvae enter the gastrointestinal system, and activate the complement pathway, C3a and C5a peptides are generated, which recruit eosinophils to the infection site independently of specific mechanisms, like CD4+ cells and IL-5. At this time, the parasite secretes chemoattractants that recruit more eosinophils and neutrophils, which help strengthen the inflammatory response (Reinhardt et al., 2011). The increase in eosinophils is important in innate immunity where complement mediates cytotoxicity of eosinophils against larvae without specific antibodies (Meeusen et al., 2000).

When larvae are expelled from the body, the reaction can be immediate or delayed. Immediate expulsion of larvae occurs in when mast cells and globule leukocytes (intraepithelial mast cells) attack larvae before they are able to enter abomasal glands. Hypermotility, gastric hypersecretion, hyperplasia, and increased mucus production are also important mechanisms in immediate expulsion (Miller, 1996; Balic, et al., 2002). Histamine has also been associated with immediate expulsion, and high concentrations have also been found in the abomasal mucosa of resistant sheep. These high concentrations promote hypersecretion and hypermotility, which is detrimental to the fecundity and motility of the worms (Hohenhaus and Outteridge, 1995). In addition, histamine helps antibodies move to the lumen of the abomasum (Miller, 1996).

Delayed expulsion happens when a specific immune response occurs against larvae in the abomasal glands. Various systems, cells, and antibodies regulate this expulsion, including the complement system, CD4+ T lymphocytes, antibody dependent eosinophil cytotoxicity, immunoglobulin A (IgA) and IgE antibodies (Balic et al., 2002). When the complement pathway is activated and mast cells degranulate, eosinophils from the tissue and blood increase in nonspecific and specific responses. Complement also recruits eosinophils to the abomasum wall and helps in eosinophil cytotoxicity against *H. contortus* larvae. Various proteins are then released from cells, which is due to degranulation (Rainbird et al., 1998). Also, eosinophils produce cytokines, which indicates they may have a regulatory function in the immune response (Behm and Ovington, 2000).

In sheep, natural and artificial infections promote the production of specific antibodies. Antibodies of the abomasum have been found to be more important than serum antibodies against gastrointestinal nematodes (Martinez-Valladares et al., 2005). When IgE production is induced, antibody dependent cytotoxicity in eosinophils, mast cells, and macrophages is also induced. Increased IgE levels have been linked with resistance to gastrointestinal nematodes in small ruminants (Pernthaner et al., 2005; Pernthaner et al., 2006; de la Chevrotiere et al., 2011). Also, the production of IL-4, IL-5, and IL-10 generally means there is a Th-2 response, which is associated with the presence of helminths (Tizard, 2009). In susceptible sheep breeds, lymphocytes of the abomasum do not develop cytokines that are associated with a Th-2 response in initial infection, but in later infections, cytokine production increases. Even though resistance does not reach the same level as genetically resistant sheep, the increased production of these cytokines could contribute to the increased resistance in susceptible sheep (Balic et al., 2002).

Commercial Anthelmintics to Control *Haemonchus contortus*

Alpacas and sheep that need treatment for GI parasites are given anthelmintics, even though there is no drug approved for use in alpacas in the U.S. (Fowler, 1998; Hoffman, 2006). In the 1960s, broad spectrum anthelmintics were introduced for treatment of ruminants and other livestock (Zajac, 2006). This changed the livestock industries, and anthelmintics were found to be very low-cost and cost effective for owners (Stear et al., 2006). Three groups of modern anthelmintics are available for use in small ruminants: 1) benzimidazoles, 2) cholinergic agonists

(imidazothiazoles and tetrahydropyrimidines), and 3) macrocyclic lactones (avermectins and milbemycins) (Stear et al., 2006; Zajac, 2006).

Benzimidazoles. The benzimidazoles, such as albendazole and fenbendazole, are a class of broad spectrum anthelmintics (Zajac, 2006) that were first introduced in 1961 (Caffrey, 2012). These anthelmintics bind to and interfere with specific sites on beta-tubulin and inhibit the making and assembly of microtubules (Sutherland and Scott, 2010; Caffrey, 2012). Losing cytoplasmic microtubules decreases the transport of secretory granules and enzyme secretion with the cytoplasm (Sutherland and Scott, 2010) as well as glucose uptake by adults and larvae. This drains the nematodes of glycogen stores and energy, and immobilizes the parasite to die. Benzimidazoles are also not easily absorbed and are not very water soluble (Caffrey, 2012). By withholding food for 12-24 hours in small ruminants, absorption usually improves because after ingestion, the medication slowly travels through the GI tract of the animal (Zajac, 2006).

Imidazothiazoles and tetrahydropyrimidines. Cholinergic agonists include drugs such as levamisole and pyrantel. The earliest drugs in this group appeared soon after 1958 (Caffrey, 2012). Levamisole is chemically different from pyantel, but both have the same mode of action (Zajac, 2006). These drugs act at specific acetylcholine receptors (Sutherland and Scott, 2010) to open acetylcholine-gated ion channels of the nematode body muscle. Binding to the nicotinic acetylcholine produces depolarization, contraction, and spastic paralysis, which allow the worms to be swept out of their normal location in the host (Caffrey, 2012).

Macrocyclic lactones. Macrocyclic lactones include ivermectin and moxidectin that interfere with chloride channel neurotransmission (Zajac, 2006). Specifically, these drugs act on the outer membrane layer of glutamate-gated chloride ion channels (GluCls). They act as allosteric modulators that increase the opening of GluCls that are found in the pharynx and neurons of worms (Caffrey, 2012). This inhibits body movement and pharyngeal pumping. It has been found that *Haemonchus* has multiple subtypes of GluCls and other ligand gated chloride channels that make it sensitive to this group of drugs (Caffrey, 2012). These drugs have been used extensively over the years against helminths and resistance has rapidly grown.

Anthelmintic Resistance. Drug resistance is a major threat to small ruminant and camelid industries, not only in the United States but also around the world (Waller, 1997). When modern anthelmintics were first introduced they were extremely effective, cheap, and safe for use on animals. Owners were encouraged to use drugs as a preventive measure, but relying on anthelmintics alone has led to resistance (Kaplan, 2004). Gastrointestinal nematodes become resistant to anthelmintics by two routes. Overly using drugs selects for individuals in a population that have a necessary mutation to survive. This includes resistant individuals that were already present before the introduction of anthelmintics and also where mutations occur subsequent to the introduction of drugs. Also, under-dosing animals selects for parasites that are resistant to sub-optimal drug exposure by removing susceptible worms from the population and leaves behind resistant worms. This allows the resistant parasites to persist and further spread resistance throughout the population (Sutherland and Scott, 2010). Besides human contributions to resistance, parasites also contribute by having great genetic diversity, high levels of gene flow into and out of the population by hosts, and by having naturally large populations in the environment as well as in various hosts (Kaplan, 2004). Anthelmintic resistance and parasitism by GI nematodes is a growing health concern in alpacas (Sarre et al., 2012). In order to help identify possible resistance to certain drugs a technique known as the fecal egg count reduction test (FECRT) has been developed. In this test fecal samples are collected from selected animals before being dewormed. Approximately 14 days later, another fecal sample is collected and the number of eggs enumerated. The percentage reduction in the number of eggs seen in the feces after deworming indicates whether or not there are worms that are resistant to the particular medication used (Anthelmintic Resistance Roundtable, 2005).

Alternative Control Methods. Alpacas and sheep with parasitic infections are often given anthelmintic treatments in order to improve animal health and well being, but using alternative control methods, not just anthelmintics to combat parasites, is a way to help keep a herd healthy. For example, only treating alpacas that are showing clinical signs is one way to slow the rate of resistance in an area. The refugia, or parasites that have not been exposed to a drug, is increased. Thus, fewer susceptible genes are removed from the population. The goal of selective deworming is to maintain as high a refugia as possible, which can be done by identifying those

animals that have the highest fecal egg counts as part of routine herd health checks (Kaplan et al., 2004).

The FAMACHA$^©$ system is a selective program that helps gain control of *H. contortus* This system was developed in South Africa and allows owners and producers to rank an animal's level of anemia (Kaplan et al., 2004; Burke et al., 2007). The FAMACHA$^©$ system works by scoring animals on a scale of 1 to 5 and is done by looking at the conjunctival membranes. A score of 1 or 2 is indicated as normal, while a score of 4 or 5 is anemic. The membranes of the eye are very pale at a 5. Using this method decreases the need to deworm all animals in a herd (Burke et al., 2007). Many producers use this system for sheep and has been deemed useful for alpacas (Cebra et al., 2014).

Pasture management is another alternative control measure that reduces the amount of larvae found on a pasture. By reducing the number of animals placed on a particular pasture, the number of eggs found on the pasture goes down (Stear et al., 2007). Another pasture management tool for parasite control is to perform rotational grazing. Allowing small ruminants and/or alpacas to graze a pasture and then removing them from that pasture for a period of time can reduce the amount of larvae. Areas where climates are more extreme than the southeastern U.S., such as the southwest U.S. are best suited for this because larvae are likely to die more quickly in these climatic extremes (Stear et al., 2007). Some internal parasitic nematodes, such as *Haemonchus* spp. or *Ostertagia* spp. are very good at thwarting control plans and are very damaging to alpacas and small ruminants.

Summary

Haemonchus contortus is a blood feeding nematode that infects the abomasum of small ruminants and compartment three of camelids. It is one of the biggest disease problems in the small ruminant industry (Zajac,2006), and anthelmintic resistance of *H. contortus* is a growing concern. Alpacas are South American camelids and are not considered to be true ruminants because they have a different anatomical and physiological arrangement of their stomach compared to small ruminants. Although alpacas are not true ruminants, alpacas may become infected with *H. contortus* and develop the same clinical signs as sheep and goats. Even though they may become infected with the parasite, they may be more resistant to *H. contortus*. Lower numbers of eggs have been observed in the feces of alpacas compared to sheep. The purpose of

the following experiments was to: 1) evaluate the response of alpacas and sheep to experimental
H. contortus infection at two dose levels of infective larvae using two dosing protocols in
Experiment 1, and 2) to determine the total worm burden of alpacas and sheep in an
experimental *Haemonchus contortus* infection using two dosing protocols in Experiment 2.

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Chapter 1

Haemonchus contortus infections in alpacas and sheep

Abstract

The blood feeding nematode *Haemonchus contortus* infects the abomasum of small ruminants and compartment three of camelids. Heavy infections may cause severe anemia and death. Although not true ruminants, alpacas may become infected with *H. contortus* and develop the same clinical signs as sheep and goats. We hypothesized given the same exposure to experimental infection; alpacas would be less susceptible than sheep to *H. contortus*. Experiment 1 was conducted with adult male alpacas (23) and sheep (12) housed in pens to prevent additional exposure to *H. contortus*. Animals were orally dewormed with fenbendazole, levamisole, and ivermecting 10 days before *H. contortus* infection. Infective *H. contortus* larvae were administered orally to alpacas and rams in the following groups:1) 20,000 larvae as a single dose (bolus, n=6), 2) 20,000 larvae in daily doses of 4,000 larvae for 5 days (trickle, n=6). Two additional groups of alpacas received either 50,000 larvae as a bolus infection, or in daily doses of 10,000 larvae (trickle). Fecal egg counts (FEC) were determined every 2 days from 14 to 42 days post infection (PI) and then at 5 day intervals until day 62 PI. Packed cell volume (PCV), FAMACHA$^©$ scores, weight, and body condition scores were evaluated weekly. Mean FEC was lower in alpacas than sheep ($p<0.01$) and mean alpaca PCV was affected less by infection than sheep PCV. Experiment 1 results suggest that alpacas are less susceptible to *H. contortus* infection than sheep, although we were unable to determine whether the lower alpaca FEC reflected fewer adult worms or only reduced *H. contortus* egg production compared to sheep. Experiment 2 was conducted with 16 alpacas and 12 rams and all animals were orally dewormed as in experiment 1. Infective *H. contortus* larvae were administered orally to alpacas in the following groups: 1) 20,000 larvae as a single dose (bolus, n=8), and 2) 20,000 larvae in daily doses of 4,000 larvae for 5 days (trickle, n=8). Ram groups (n=6 each) were the same as the alpaca groups. Fecal egg counts were determined at 5 day intervals from days 14 to 49 PI for bolus infected animals and to day 54 PI for trickle infected animals. FAMACHA$^©$ scores were evaluated weekly. Packed cell volume was evaluated at the beginning and end of the study. All animals were euthanized 49 days after the last infection day and abomasa and C-3 harvested for determination of total worm burden. In addition, at euthanasia, the pH of the rumen, C-1, abomasum, and C-3 were determined for each animal. Mean FEC and total worm burden were

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lower in alpacas than sheep. Rumen pH was higher in sheep than alpacas, but sheep abomasal pH was much lower than alpacas. Also, bolus infected sheep had lower FAMACHA$^©$ scores than the other groups, and PCV was lower on the last day of sampling than the first day of sampling in all groups. These results also support our hypothesis that alpacas are less susceptible to *H. contortus* infection than sheep, but it is unclear whether the differences are the result of physiological stomach differences between host species or whether other factors, such as immunity or parasite strain are important.

Introduction

Haemonchus contortus is an abomasal (Soulsby, 1965) and compartment 3 (C-3) blood-feeding nematode of small ruminants and camelids (Gillespie et al., 2010). It is responsible for the disease haemonchosis which may produce severe anemia and bottlejaw, decreased appetite, severe weight loss, and death. The parasite has a worldwide distribution with concentrations in the tropics and subtropics and is found in temperate regions like the United States. *Haemonchus contortus* is the biggest disease problem in the small ruminant industry in the U.S. and is becoming a problem in camelids because of increasing resistance to anthelmintics.

Alpacas (*Vicugna pacos*) are South American camelids (SAC) naturally found in the Andes around 13,500 feet. They belong to the order Artiodactyla with sheep, suborder Tylopoda, and family Camelidae with llamas. Alpacas were first introduced to the U.S. in the mid 1980s (Hoffman, 2006) and as of 2010, there are 200,000 alpacas in the United States (Long, 2010). Alpacas are not considered to be true ruminants because they have a three-compartment stomach (C1-C3) instead of four compartments (rumen, reticulum, omasum, and abomasum) found in sheep and goats. Compartment one is equivalent to the rumen and is probably where *H. contortus* larvae exsheathe when they enter the digestive tract. Compartment three is equivalent to the abomasum and is where *H. contortus* worms live and mate.

Even though alpacas are not considered to be true ruminants, they may become infected with *H. contortus* and develop the same clinical signs observed in sheep and goats. However, alpacas may be more resistant to the parasite than sheep. For instance, Hill et al. (1993) in New Zealand observed lower numbers of eggs in the feces of alpacas when they were co-grazed with sheep, and after following alpacas for over a year, we observed similar results (unpublished

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data). Based on these findings, we hypothesized that given the same exposure to experimental infection, alpacas would be less susceptible than sheep to *H. contortus*.

The purpose of our experiments was to evaluate the response of alpacas and sheep to experimental *H. contortus* infection at two dose levels of infective larvae using two dosing protocols in experiment 1 and to determine the total worm burden of alpacas and sheep in an experimental *Haemonchus contortus* infection using two dosing protocols in Experiment 2.

Methods

Experiment 1

Animals

The study was conducted at Virginia State University in Petersburg, VA and included 23 intact adult male alpacas (ages 3-16 years) and 12 intact adult rams (ages 3-4 years) consisting of six St. Croix and six Barbados blackbelly sheep that were selected at random from the breeding flock. Animals were moved to pens in an experimental feeding barn with accompanying small dirt lots unlikely to support development of infective *H. contortus* larvae. Alpacas and sheep were placed with 3 animals per pen within species, and, for a period of one week, animals were allowed to adjust to pens, nipple waterers, feeders, and diet. Hay was fed ad libitum, and a 12% crude protein supplement of corn and soybean was also provided daily at 0.5% of the combined weight of animals in each pen. The Virginia State University Institutional Animal Care and Use Committee approved all protocols.

Experimental protocol

After the adjustment period, animals were dewormed orally with fenbendazole (20 mg/kg for alpacas and 8 mg/kg for sheep) and levamisole (8 mg/kg both species). Levamisole was used in the form of a bolus given intact to rams but the bolus was crushed and suspended in water for use in alpacas because of the risk of choking. Ten days after anthelmintic treatment, fecal egg counts were greater than 5 eggs/gram in all animals. We found with fecal egg count reduction that the parasites were partially susceptible to fenbendazole and levamisole. However FEC after treatment were not as low as expected. Therefore, treatment with fenbendazole and levamisole was repeated in case there was inadequate dosing previously. Also, oral administration of ivermectin (0.2 mg/kg for both species) was used in case the remaining parasites were resistant

to fenbendazole and levamisole. Anthelmintic doses for alpacas were based on recommendations by Ballweber (2009). Fecal samples were collected again 10 days later to determine efficacy, and no parasite eggs were seen.

Sheep were randomly assigned to treatment groups. The two alpacas known to have the highest FEC in comparison to the rest of the herd were intentionally divided amongst the different treatment groups so as not to skew data and to reduce bias; the remaining alpacas were randomly assigned to treatment groups. All animals were orally administered infective larvae according to treatment allocation, where n=6 for all groups, except alpaca group 2 where n=5:

Alpacas: 1: 20,000 larvae given once (bolus infection),

2: 20,000 larvae as a trickle infection (4,000 larvae per day for 5 days),

3: 50,000 larvae given once as a bolus infection, and

4: 50,000 larvae as a trickle infection (10,000 larvae per day for 5 days).

Rams: 1: 20,000 larvae given once as a bolus infection, and

2: 20,000 larvae as a trickle infection (4,000 larvae per day for 5 days).

Animals in different treatment groups were commingled randomly within pens.

Previous research at Virginia Tech was conducted with lambs where 10,000 larvae were administered and found to be an effective dose to produce differences in FEC and packed cell volume and allow testing of resistance to *H. contortus* (Vanimisetti et al., 2004). This study used adult animals already immunized by natural infection, and we felt it was appropriate to use higher doses of larvae in order to obtain a response. Since our experience indicated that alpacas are less susceptible than sheep, doses of both 20,000 and 50,000 larvae were used to measure infection response.

Rectal fecal samples were collected every 2 days from 14-42 days post infection (PI), and then continued at 5-day intervals until 63 days PI. FAMACHA[©] scores and PCV were used to assess anemia at 7-day intervals starting 14 days PI. Weight and body condition scores were also recorded at 7-day intervals starting 14 days PI and continuing until 63 days PI. On the last day of sampling, animals were again treated with fenbendazole, levamisole, and ivermectin at the previously described dosages. The Virginia State University Institutional Animal Care and Use Committee approved all procedures.

Parasite

Haemonchus contortus infective larvae used to infect both alpacas and sheep were obtained from manure collected from experimentally infected sheep that were monoinfected with *H. contortus*. Feces from the sheep were cultured at room temperature for 10 days, and larvae were harvested using the Baermann technique (Zajac and Conboy, 2012). Larvae were then counted and stored in water at 4ºC until inoculation.

Fecal Analysis

Efficacy of anthelmintic treatment was determined by the modified Wisconsin technique with a sensitivity of less than 1 egg per gram of feces (epg) (Zajac and Conboy, 2012).

All fecal samples during the experimental period were analyzed with the modified McMaster technique (Zajac and Conboy, 2012) using a sensitivity of 8 epg for alpacas and a sensitivity of 25 epg for sheep. Alpacas used in this study had been followed for over a year with routine fecal egg counts (FEC); low numbers of eggs were consistently observed in the feces of alpacas requiring a higher level of sensitivity in the test used for alpacas. Because FEC in sheep were consistently much higher than those of alpacas, use of the 8 epg sensitivity in sheep was not practical. To get a sensitivity of 25 epg for sheep using the modified McMaster technique, two chambered McMaster slides (Chalex Corporation, Ketchum, ID) were used to analyze 4 g fecal samples that were thoroughly mixed with 26 mL of a sugar salt solution (specific gravity 1.28) and strained through a double layer of cheesecloth. The strained material was then used to fill a two chambered McMaster slide. The grid in each chamber of the two chambered slide holds a volume of 0.15 mL for a total of 0.3 mL, which is 1/100th of the total volume of 30 mL. Therefore, the total number of eggs counted must be multiplied by 100 and divided by 4 to get the eggs per gram of feces, which is the same as multiplying the total number of eggs counted by 25 (Zajac and Conboy, 2012). A similar procedure was followed for alpaca FEC, except a three chambered McMaster slide was utilized and the eggs per gram calculated by multiplying the total number of eggs counted by 8.

Anemia Assessment

Anemia was assessed using packed cell volume (PCV) and FAMACHA[©] scores. Blood was collected for PCV by jugular venipuncture into heparinized tubes and an aliquot of each

sample placed in hematocrit tubes, which were centrifuged in a micro-hematocrit centrifuge for 5 minutes at 13,460 x G. After centrifugation, PCV was determined with a micro-capillary plate reader. Blood in heparinized tubes was centrifuged at 4°C for 10 minutes at 2,500 x G, and an aliquot of plasma was removed and placed on a refractometer to measure serum protein (Turner et al., 2012).

FAMACHA[©] scores were assessed by matching the color of the conjunctival mucous membranes of each animal to the FAMACHA[©] card and assigning a score of 1-5, where a score of 1 is considered not anemic and 5 is considered highly anemic (Kaplan et al., 2004).

Body Condition Scores

Body condition was measured by palpating the loin area behind the ribs and in front of the pelvis, and assigning a score of 1-10 (Thompson and Meyer, 1994; Hoffman, 2006), where a score of 1 is considered emaciated and 10 is considered grossly obese.

Statistical Analysis

Normal probability plots showed that FEC, FAMACHA[©], and body condition score were skewed. Subsequently, Fecal egg counts were compared between groups with the Wilcoxon Rank Sum test followed by Dunn's procedure of multiple comparisons that was used to make all pairwise comparisons between groups at every time point. FAMACHA[©] scores and body condition scores were also analyzed with the Wilcoxon Rank Sum test to compare the distribution of FAMACHA[©] and body condition scores of groups within species, followed by Friedman Chi Square where measurements were compared to the starting date with animal identification as a blocking factor. Because the packed cell volume (PCV) and serum protein were approximately normally distributed, the groups were compared at each time point, and time points compared to starting date within each group using mixed-model repeated-measures ANOVA. Host species comparisons were performed for groups receiving 20,000 larvae for FEC, PCV, serum protein, FAMACHA[©], and body condition score, and $p \leq 0.05$ was considered significant for all analyses.

Experiment 2

Animals

As in Experiment 1, the study was conducted at Virginia State University, Petersburg, Virginia with 16 intact adult male alpacas (ages 4-17 years) and 12 intact adult rams (ages 4-7 years) equally representing the Barbados Blackbelly and St. Croix breeds. All the alpacas from the first experiment and three sheep from Experiment 1 were used in Experiment 2, and all other sheep were selected at random from the breeding flock. All animals were housed and fed as in Experiment 1 with the exception that alpacas and sheep were placed with 4 animals per pen within species and were allowed to adjust to pens, nipple waterers, feeders and diet for 10 days. All protocols were approved by the Virginia State University or Virginia Tech Animal Care and Use Committees.

Experimental Protocol

After the adjustment period, animals were dewormed orally with fenbendazole, levamisole, and ivermectin as described for Experiment 1. Fecal samples were collected 10 days later to determine efficacy, and no parasites were observed.

Two alpacas and two sheep known to have the highest FEC in comparison to the rest of the herd were intentionally divided amongst the different treatment groups so as not to skew data and introduce potential bias by having them in the same group; the remaining alpacas and sheep were randomly assigned to fill treatment groups. All animals were orally administered infective larvae according to treatment allocation where n=8 for alpacas and n=6 for sheep:

Alpacas: 1: 20,000 larvae as a bolus (single) infection,

2: 20,000 larvae as a trickle infection (4,000 larvae per day for 5 days),

Rams: 1: 20,000 larvae as a bolus (single) infection, and

2: 20,000 larvae as a trickle infection (4,000 larvae per day for 5 days).

Animals in different treatment groups were commingled randomly within pens.

Rectal fecal samples were collected at 5 day intervals from 14-49 days PI for bolus infected animals and 14-53 days PI for trickle infected animals. FAMACHA[©] scores were used to assess anemia on day 1 and then at 5 day intervals from14-49 days PI. Packed cell volume (PCV) and weight were evaluated at the beginning and end of the study. At 49 days PI, bolus infected animals were transported to the Virginia Maryland Regional College of Veterinary

Medicine (VMRCVM) for euthanasia, and at 53 days PI, trickle infected animals were transported to the VMRCVM for euthanasia. The Virginia State University and Virginia Tech Institutional Animal Care and Use Committees approved all procedures.

Parasite

As in Experiment 1, *H. contortus* infective larvae used to infect alpacas and sheep were collected from manure of experimentally infected lambs only infected with *H. contortus* larvae. Feces from the sheep were cultured at room temperature for 10 days and harvested using the Baermann technique (Zajac and Conboy, 2012).

Fecal Sample Analysis

Efficacy of anthelmintic treatment was determined using the Modified McMaster technique with a sensitivity of 8 eggs per gram (epg) of feces for both species.

As in experiment 1, all fecal samples during the experimental period were analyzed with the modified McMaster technique (Zajac and Conboy, 2012) using a sensitivity of 8 epg for alpacas and a sensitivity of 25 epg for sheep.

Euthanasia and Total Worm Burden Determination

All bolus infected animals were euthanized 49 days PI and all trickle infected animals were euthanized 53 days PI using an overdose of barbiturates with secondary exsanguination. After euthanasia, the abomasum and C-3 were tied off in the animal to prevent movement of worms out of the abomasum or C-3 and were then removed from sheep and alpacas, opened along the greater curvature, washed with tap water to remove worms and contents and the washings retained in a graduated bucket. Ten percent of the total volume within the graduated bucket was collected and preserved with an equal volume of 10% neutral buffered formalin for later determination of worm burden. After the abomasum and C-3 were thoroughly washed, each stomach was placed in a metal pan mucosa side down and incubated in physiological saline overnight at 39°C to allow recovery of larvae. After overnight incubation, stomachs were rinsed with tap water and 10% of the total volume of washings was collected, and preserved in the same manner (Wood et al., 1995).

All worms were counted using a dissecting scope and worms were identified by sex and stage. Only complete worms or fragments with a bursa or vulval flap were counted. The eggs/g per female were determined by dividing the total number of adult females by the FEC on the day before euthanasia for bolus infected animals and the day of euthanasia for trickle infected animals.

pH Determination

The pH of the rumen and abomasum of sheep, and C1 and C3 of alpacas was determined using a portable pH meter (Accumet Portable AP5 pH meter, Fisher Scientific, Waltham, MA) that was standardized the morning of euthanasia with buffers of pH 4, 7, and 10. The probe of the meter was inserted in the contents of each chamber immediately following euthanasia. In addition, the pH was measured in rumen and C1 samples that were strained through 2 layers of cheesecloth as described in some protocols (Liu et al., 2009; Doyle et al., 2011). The pH of the abomasum was determined in the fundic region of the stomach where *H. contortus* is commonly found (Dash, 1985). Also, the pH of the fundic region of the lower 1/5 of C-3 was determined because that is where *H. contortus* is found.

Statistical Analysis

As in Experiment 1, FEC and FAMACHA[©] score were analyzed with the Wilcoxon Rank Sum test to compare the distribution of FEC and FAMACHA[©] scores, and Dunn's procedure of multiple comparisons was used to make all pairwise comparisons for all groups at each time point for FEC. Correlation analysis was used to assess the association between FEC and total worm burden. An analysis of variance followed by Tukey's procedure for multiple comparisons was used to analyze total worm burden, pH of the rumen, abomasum, C1 and C3, as well as the eggs/g per female, PCV, and weight because they were normally distributed. FAMACHA[©] and PCV were analyzed within species as in Experiment 1. The eggs/g per female was calculated by dividing the last day's FEC by the total number of female worms. Two bolus infected sheep died during the experimental period and were used only in calculating means for FEC and FAMACHA[©] graphs until their deaths, and p≤0.05 was considered significant for all analyses. All analyses were performed using JMP pro version 10.0.2 (Cary, NC, USA).

Results

Experiment 1

Fecal Egg Counts

In general, mean sheep FEC were higher than those of alpacas (p<0.01). Also, bolus infected sheep FEC were significantly higher than FEC of trickle infected sheep (p<0.02) (Figure 3A). Eggs were detected in the feces of bolus infected sheep on day 18, and the peak FEC of individual sheep varied. Also, one bolus infected sheep had a peak FEC of 50 epg and then had FEC of 0 thereafter. One trickle infected sheep had a peak FEC of 25 epg and then had FEC of 0 thereafter. These two sheep were exceptions. Also, bolus infected sheep had significantly higher FEC than 20,000 bolus infected alpacas (p<0.03), even though they were administered the same number of larvae using the same dosing protocol. However, there was no significant difference between trickle infected sheep and 20,000 trickle infected alpacas, even though they were also administered the same number of larvae using the same dosing protocol. With the exception of the 50,000 bolus infected alpaca group, eggs were detected in the feces of all other alpaca groups on day 16 PI. Eggs were detected in the feces of the 50,000 bolus infected alpaca group by day 26 PI. The number of larvae administered and the dosing protocol had no significant effect on FEC in alpacas. Even though both trickle infected alpaca groups had higher mean FEC than either group of bolus infected alpacas, the difference was not significant (Figure 3B). In addition, no alpaca group had a mean FEC higher than 1,000 epg at any time point.

Packed Cell Volume, Serum Protein, FAMACHA[©], Weight, and BCS

For all animals, the PCV fluctuated within the normal range of 25-44.5% (Hoffman, 2006). For only bolus infected sheep, the PCV declined significantly from the starting date over the course of infection (p<0.0004). Trickle infected sheep had a higher mean PCV than bolus infected sheep from day 21 to 62. Even though the 20,000 trickle infected alpaca group had a lower PCV than the other alpaca groups, no significant differences were detected in alpaca PCV (Figure 4).

Serum protein levels did not change significantly over time in any group and remained in the normal range (Hoffman, 2006) during the study. The bolus sheep group had higher serum protein than the trickle infected sheep group but the difference was not significant (p<0.59). The

20,000 trickle infected alpacas had higher serum protein than the 20,000 bolus infected alpacas (p<0.13) (Figure 5).

Mean FAMACHA[©] scores did not change over the course of the study for sheep (p<0.51) or alpacas (p<0.45). All groups fell between 1 and 3 out of 5 (Figure 6).

Body condition scores did not change over the course of the study for sheep (p<0.62) or alpacas (p<0.54). No group decreased below 5 or increased above 8 for body condition. With the exception of the 20,000 trickle infected alpaca group, body condition score was higher on day 63 than day 1 (Table 1).

Experiment 2

Fecal Sample analysis

When data for groups within species were combined, area under the curve analysis for FEC showed higher FEC in sheep than alpacas (p<0.0001) (Figure 7A). Eggs were detected in the feces of all groups on day 14 PI, with the exception of the bolus infected sheep group, when eggs were detected 19 days PI. The dosing protocol had no effect on fecal egg counts in alpacas or sheep. Bolus infected alpacas had a higher FEC than trickle infected alpacas (Figure 7B).

Total Worm Burden

There were no significant differences between bolus and trickle infected sheep in total worm burden (p<0.41), and there were no significant differences between bolus and trickle infected alpacas in total worm burden (p<0.99). Therefore, sheep and alpaca groups were combined for further analysis. Sheep had a higher mean total worm burden than alpacas (p<0.0001), and bolus infected sheep had the highest worm burden (Figure 8). Mean worm burdens were 708 (range of 120-2,120) for bolus infected alpacas, 541 (range of 20-1,970) for trickle infected alpacas, 4,895 (range 80-7,540) for bolus infected sheep, and 3,142 (range of 660-5,270) for trickle infected sheep.

Correlation of Total Worm Burden to Fecal Egg Counts

There was a strong positive correlation between the total worm burden and fecal egg counts for both alpacas (r = 0.87) and sheep (r = 0.80).

Eggs per Gram per Female

There were no significant differences between bolus and trickle infected sheep ($p<0.52$), and there were no differences between bolus or trickle infected alpacas ($p<0.94$) in female egg production. Therefore, sheep groups and alpaca groups were combined for further analysis. Egg production in sheep was at least two times higher than alpacas ($p<0.0001$). Trickle infected sheep had the highest number of eggs/g produced per female, and trickle infected alpacas had the lowest (Figure 9).

pH

The pH was about the same in the rumen of sheep as alpaca C-1 ($p<0.59$). Also, the pH increased in strained rumen and C-1 contents for all groups except the trickle infected sheep where it decreased, but there were no differences between unstrained and strained rumen and C-1 contents ($p<0.72$). The abomasal pH was much lower in sheep than alpacas ($p<0.0018$). The mean bolus infected sheep pH was 3.7, and the mean trickle infected sheep pH was 4.4. The mean trickle infected alpaca pH was 6.5, and the mean bolus infected alpaca pH was 6.2 (Figure 10).

FAMACHA[©], PCV, and Weight

There were no differences in alpaca FAMACHA[©] scores over the course of infection ($p<0.82$), and there were no differences in sheep FAMACHA[©] scores over the course of infection ($p<0.17$). FAMACHA[©] scores of all animals stayed between 1 and 3 out of 5 (Figure 11).

No differences were found between sheep groups or alpaca groups on day 0 or day 49 for PCV. The PCV was lower on day 49 than day 0 for all groups, but all groups were within normal ranges of 25-44.5% red blood cells on both days (Figure 12).

There were no differences in weight found among sheep groups or alpaca groups on day 0 or day 49. Weights were lower on day 0 than day 49 for all groups (Figure 13).

Discussion

The results of our experiments are consistent with our hypothesis that alpacas are less susceptible to *H. contortus* infection than sheep. Alpacas had lower FEC in both studies, as well

as a lower worm burden, and fewer eggs/g per female produced than sheep in Experiment 2. We believe this means *H. contortus* establishment and fecundity are reduced in alpacas.

Because we were unfamiliar with studies of experimental *H. contortus* infection in alpacas, we used both trickle and bolus infections in Experiment 1 with two different infection levels in alpacas. Trickle infections are more similar to repeated pasture infections, but they may not accurately represent natural infections. In natural infections on pasture, there is a continuous exposure to parasites through grazing, but a continuous exposure does not occur in trickle infections. Also, bolus infections are not natural, but usually result in a higher establishment rate in small ruminants (Gruner et al., 1994). Because there were no differences in alpacas based on numbers of larvae administered, in Experiment 2we used only the 20,000 larval dose that was being used in sheep.

In Experiment 1, the trickle infected alpaca groups had a higher FEC than the bolus infected alpaca groups, but this difference was not significant and this trend did not occur in Experiment 2.

In both experiments, FEC of trickle infected sheep were lower than FEC of bolus infected sheep although the difference was significant in Experiment 1 but not in Experiment 2. This may be explained by the small sheep group sizes in both experiments. Increasing group size would decrease the impact that sheep with a very low or very high FEC have on their groups. In addition, two bolus infected sheep died during the experimental period in Experiment 2, allowing us to determine the worm burden of only 4 bolus infected animals. The two sheep that died had low BCS at the beginning of the experiment and very high FEC, above 29,000 epg around their deaths. It is likely that *Haemonchus contortus* infection probably contributed to their deaths. This likely affected our results because we saw no significant difference between bolus and trickle infected sheep in total worm burden. If these two sheep had not died during the experimental period, we may have seen significantly higher worm burdens in the bolus infected sheep than in trickle infected sheep. In experiments one and two, there were no differences in FEC or worm burden among alpaca groups.

A limited number of animals were available for use in both experiments. Having a small sample size in general and having a different number of animals in each experiment may have decreased the power of our work. In addition, Virginia State University was decreasing their herd

size, but not all animals were to be removed from the herd, so a limited number of animals were available for a euthanasia trial.

There was a trend for alpacas that were the middle-high to the highest egg producers in Experiment 1 to also be the highest egg producers in Experiment 2, regardless of the number of worms administered or dosing protocol used, indicating that these animals may not reduce worm fecundity as well as other alpacas in the herd. Also, all of the same alpacas were used in both experiments, but only three sheep were used in both, which may have affected our results in that the mean FEC was much higher in sheep in Experiment 2 than Experiment 1. We could not determine whether or not the same trend observed in alpacas appeared in sheep. Using the same sheep in the same body condition and general fitness as in Experiment 1 may not have produced such high FEC and trends may have been observed in sheep from experiment to experiment. The three sheep that were the same in both experiments produced fewer eggs than the other sheep in Experiment 2, and two of the three sheep had the lowest FEC in Experiment 1. The third sheep had a higher FEC in Experiment 1, but was administered larvae as a trickle infection in Experiment 2 instead of a bolus as in Experiment 1, which may account for a lower FEC in Experiment 2.

Both experiments used a 25 epg sensitivity for sheep and an 8 epg sensitivity for alpacas, which may have affected our results because we may have overestimated sheep FEC. If all FEC were done with the same sensitivity, differences may not have been as great between alpacas and sheep. Nevertheless, even when alpaca and sheep FEC are completed with the same sensitivity, alpaca FEC are still much lower than sheep FEC (Hill et al., 1993; Green et al., 1996). Also, anthelmintic efficacy was determined by the modified Wisconsin technique, but the Modified McMaster technique was used in Experiment 2. These techniques have different sensitivities, but because of the anthelmintic efficacy in Experiment 1, we felt comfortable determining efficacy with a lower sensitivity in Experiment 2.

Animals used in these experiments were adults. Young animals would likely change our results because young animals are known to be more susceptible to parasitism than adults. This is likely due to a lack of developed immunity to the parasites. Green et al. (1996) reported that alpacas do not develop an adult level of antibodies to gastrointestinal parasites until about 23-26 months of age, which contrasts with some breeds of sheep that have adult levels by 8 months of age. They also reported that even with a delay in antibody development, alpacas still had a lower

FEC than sheep, which they contribute to a better mechanism of suppression of FEC in alpacas. Thus, if we used younger animals in our experiments, we may find a high FEC and higher establishment than in adults, but alpacas would likely still be lower than sheep.

Sheep used in experiments one and two were of the Barbados Blackbelly and St. Croix breeds. These breeds of sheep are known to be trichostrongylid parasite resistant compared to wool breeds (Gill, 1991). Even though these breeds are known to be more resistant, the sheep in our experiments still had higher FEC and worm burdens than alpacas. If we had used wool breeds in these experiments, we may have seen even greater differences in FEC and worm burdens in the two host species.

It is possible that parasite strain may have affected our results. These experiments used infective larvae cultured in the manure from experimentally infected sheep. The larvae were not from alpacas or a combination of alpacas and sheep. Therefore, the *H. contortus* larvae may have been better adapted to the sheep stomach than the alpaca stomach and would not have established as well in alpacas. In addition, the larvae used to infect the animals in Experiment 2 were not from the same culture as larvae used to infect the animals in Experiment 1. We also saw that fecal egg counts were consistently higher in both sheep and alpacas in Experiment 2 compared to Experiment 1. While larvae used in the both experiments were the same strain, they were from two separate culture periods and the larvae used in Experiment 2 had not been stored as long before use. Larvae used in Experiment 2 may have had better success in establishment compared to those used in Experiment 1.

The structural and physiological differences of the stomachs of alpacas and sheep could play a significant role in determining susceptibility to *H. contortus* infection. Normal C-1 pH ranges from 6-7, and normal rumen pH ranges from 5.8-7. We found the mean pH to be within normal limits and similar in alpacas and sheep. The pH of untrained rumen and C-1 contents was lower than strained rumen or C-1 contents for all groups except trickle infected sheep. The longer exposure to air that occurred in collected samples when they were strained through cheesecloth before measurement of pH may explain the increased pH. In both alpaca groups, unstrained C-1 pH was slightly higher than bolus infected sheep, but all three groups were lower than trickle infected sheep. It is possible the pH was not taken in the same part of the rumen or C-1 and may contribute to why trickle infected sheep had a higher mean pH than the other groups. Also, the day of harvest may have had an effect on pH and contributed to differences

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between bolus and trickle infected animals, which were collected on different days. Even though there were no differences in rumen and C-1 pH, our results were similar to results reported by Vallenas et al. (1973) and Dulphy et al. (1997), who reported a higher pH in alpaca C-1 than the rumen of sheep and Dulphy et al. (1997) reported similar results in llamas. The slightly higher pH in alpaca unstrained C-1 contents may contribute to a less successful establishment of *H. contortus*. It is possible that a higher pH does not allow *H. contortus* larvae to receive proper cues to stimulate the parasitic life cycle and exheathe or molt of the outer membrane surrounding infective larvae used for protection against desiccation (Sutherland and Scott, 2010), which means alpacas would have a lower worm burden than sheep.

The abomasum was more acidic than C-3. Normal abomasal pH is 3 or less, and normal pH in the distal one-fifth of C-3, which is the equivalent of the abomasum, is 2-3. Our mean abomasal and C-3 pH was higher in the present study. Human error may explain this. It is possible that while measuring the pH of C-3, the probe was not completely in the distal one-fifth and may explain why the pH is so much greater than the abomasum. In addition, the parasites themselves may explain the pH because some gastrointestinal nematodes such as *Haemonchus* spp. and *Ostertagia* spp. affect the mucosa and alter the pH of the stomach. Hertzberg et al. (2000) report that when the abomasal pH increases, parasites leave the mucus layer and move to the lumen. When this occurs, the worms are likely trapped in the moving digesta and are rapidly removed from the body. In addition, worms newly entering an already parasitized sheep with increased abomasal pH may not receive necessary cues to let them know they are in the abomasum, so they flow through to the small intestine instead of stopping in the abomasum. This study was conducted with *Ostertagia leptospicularis* but supports similar results observed with infections of *H. contortus* (Coop, 1971). The normal alpaca pH, which is higher than sheep may have a similar impact on *H. contortus* establishment.

Another physiologic factor affecting susceptibility in the two host species could be that the area where *H. contortus* is found in sheep is much larger than in alpacas. *Haemonchus contortus* is found in the distal one-fifth of the alpaca C-3, where there are very few ridges. In the abomasum, worms are found in a large portion of the fundus where there are many long folds (Figure 14). The distal one-fifth is a very small portion of C-3, and only the distal one-fifth is analogous to the abomasum and the monogastric stomach of non-ruminants. The larger area of the fundus where *H. contortus* is located is able to support a larger population of worms, unlike

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C-3, which may only support a small population because the area where *H. contortus* is naturally found is very small.

A further physiologic difference between the host species that could affect parasite susceptibility is the time it takes for larvae to travel to C-3. It takes food longer to travel to C-3 than the abomasum even when consuming similar diets (Pfister et al., 1989). In the Andes where alpacas are naturally found, they are used to consuming low quality forage and living in a harsher environment (Pfister et al., 1989). Because the forage is low quality, animals must extract as many nutrients as possible. This occurs by allowing food to pass through the digestive system at a much slower rate. Thus, if it takes larvae longer to reach C-3, it could be detrimental to larvae because newly exsheathed larvae need to reach C-3 to feed as soon as possible.

In addition to physiologic factors, the differences in innate and acquired immune response between alpacas and sheep may affect susceptibility. The immune response to *H. contortus* is a Th-2 response, and we know that eosinophils, mast cells, immunoglobulins A and E, as well as antibodies contribute to the response in sheep (Balic et al., 2002; Martinez-Valladares et al., 2005; Pernthaner et al., 2005; Pernthaner et al., 2006; Tizard, 2009; de la Chevrotiere et al., 2011). *Haemonchus contortus* is usually not eliminated from the host, but its fecundity is suppressed and resistance to new infection is induced. We also know antibodies contribute to the response in alpacas, but not much else is known about the alpaca immunological response to gastrointestinal nematodes. Alpaca immunology is an under studied area of alpaca health that needs to be analyzed further. Unfortunately, we were unable to measure any immunological responses in both studies, but it is logical to assume immunity to parasitism does play a role.

Conclusions

This research was conducted to evaluate the response of alpacas and sheep to experimental *H. contortus* infections. We believe these studies are a good start in the comparison of alpaca and sheep susceptibility to *H. contortus* infection and can be used to provide alpaca owners with information about alpaca resistance to the parasite. Our research is consistent with our hypothesis, as well as other studies that alpacas are less susceptible to *H. contortus* infection than sheep, but this does not mean alpacas cannot die from haemonchosis because they can and they do. We are reporting that when there is an equal opportunity of exposure to *H. contortus,*

alpacas have a lower worm burden and reduce fecundity better than sheep, but more research is needed.

Future research conducted with alpacas and sheep in regards to *H. contorus* infections should explore many avenues. For one thing, immunological testing should be conducted in general but also to examine the antibodies present during *H. contortus* infection, as well as examining the tissues where *H. contortus* is found for various cells, such as eosinophils and mast cells. It is also unclear whether or not most worms are immediately expelled from the body of alpacas or if there is delayed expulsion. In addition, future research should examine more physiological stomach differences between alpacas and sheep with regard to gastrointestinal nematode infections and how the gastrointestinal tract changes when animals go from unparasitized to parasitized. Future research should also use more animals in general, and use both young and adult animals to have a more uniform study population that is more applicable to alpaca farms throughout the U.S. Finally, alpacas and sheep involved in experimental infections may need to be infected with larvae grown from alpacas or a combination of alpacas and sheep to determine if larvae from alpacas are as pathogenic as larvae from sheep or are about the same in pathogenicity.

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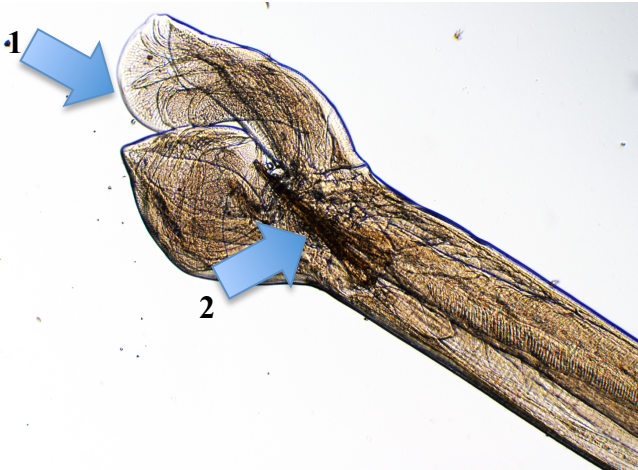
A.
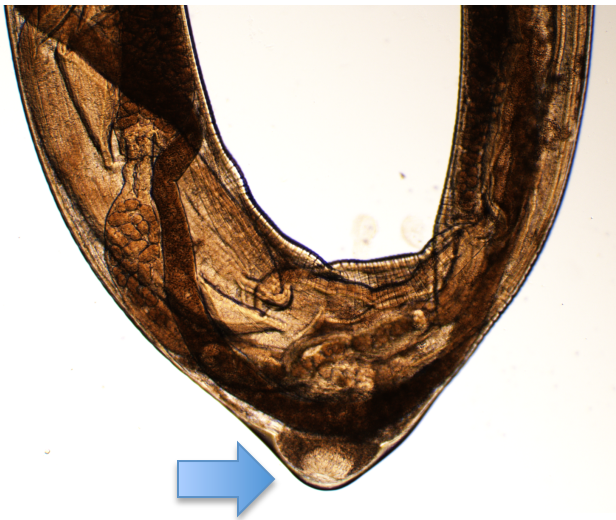
B.

Figure 1. *Haemonchus contortus* **adult male bursa and female vulval flap. (A) male Haemonchus contortus adult. The arrows are pointing to the copulatory bursa (1) and spicules used to hold open the female worms genital opening (2). (B) female** *Haemonchus contortus* **adult. The arrow is pointing to the vulval flap.**
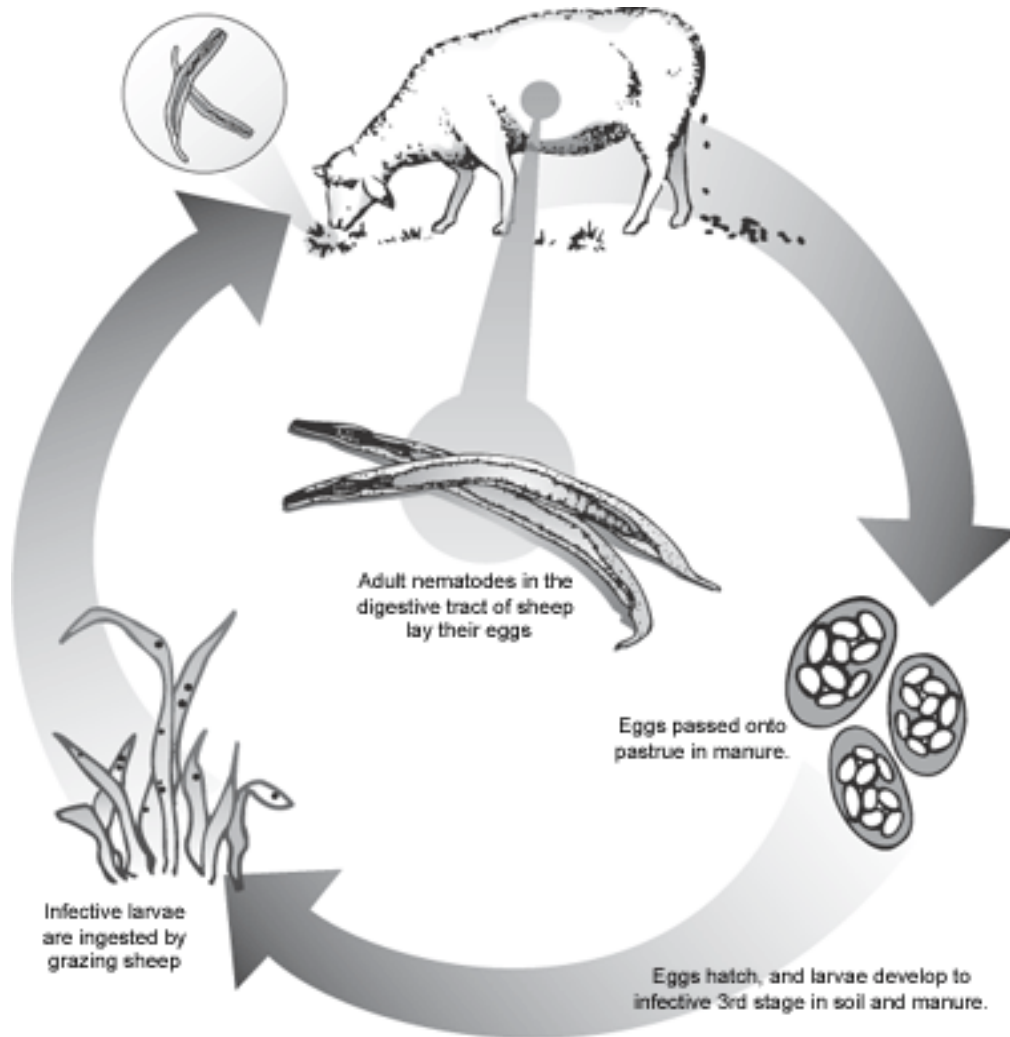
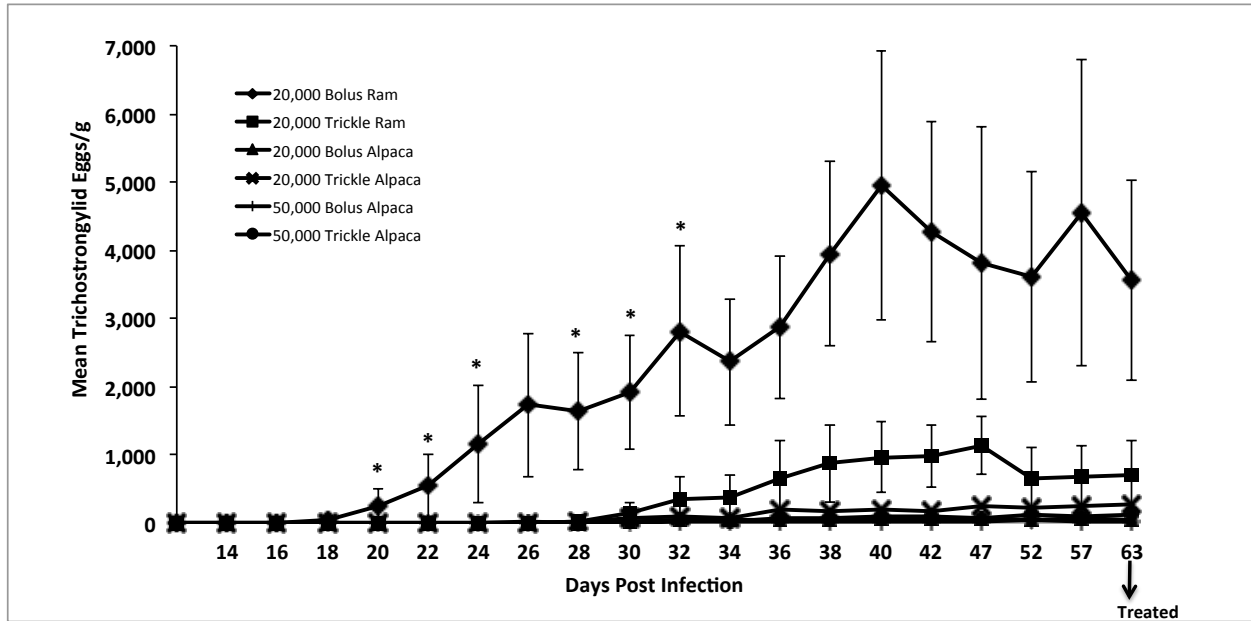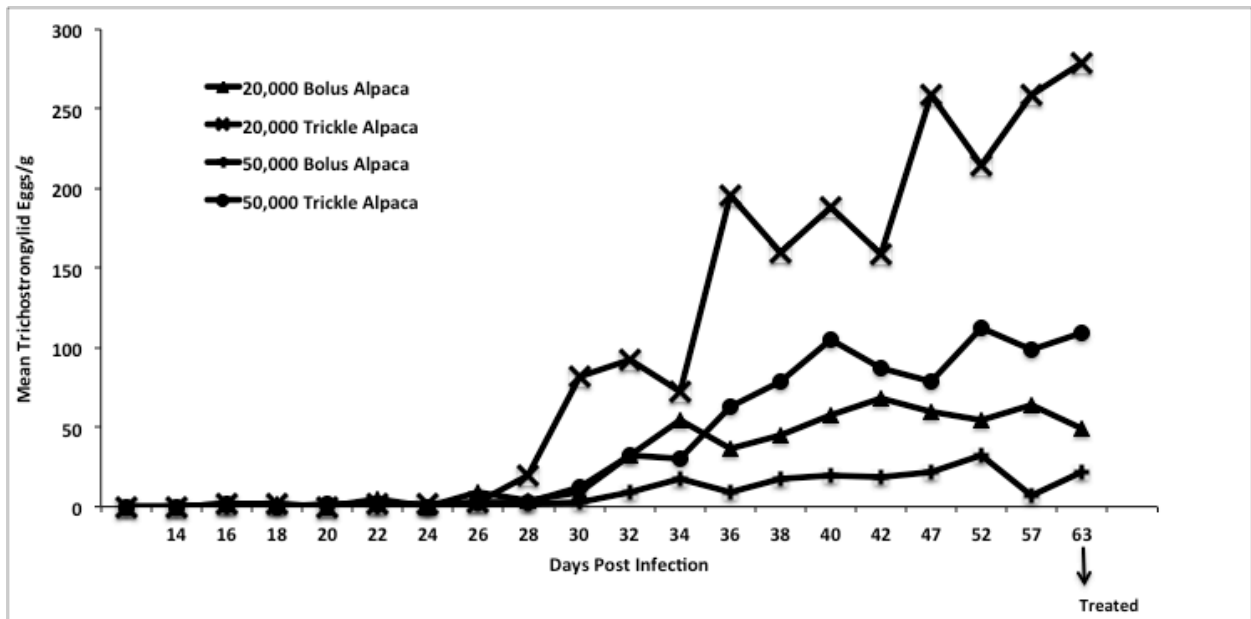**Figure 2. Lifecycle of trichostrongyle parasites, like *Haemonchus contortus* (Whittier et al., 2009).**

Figure 3A.

Figure 3B.
Figure 3. Fecal egg counts of sheep and alpacas in Experiment 1.
(3A) mean fecal egg counts of alpacas and sheep in Experiment 1. Vertical bars represent the standard error of the mean
(±SEM) and asterisks represent days of significance of bolus infected sheep and trickle infected sheep.
(3B) mean fecal egg counts (FEC) for alpacas only in Experiment 1.
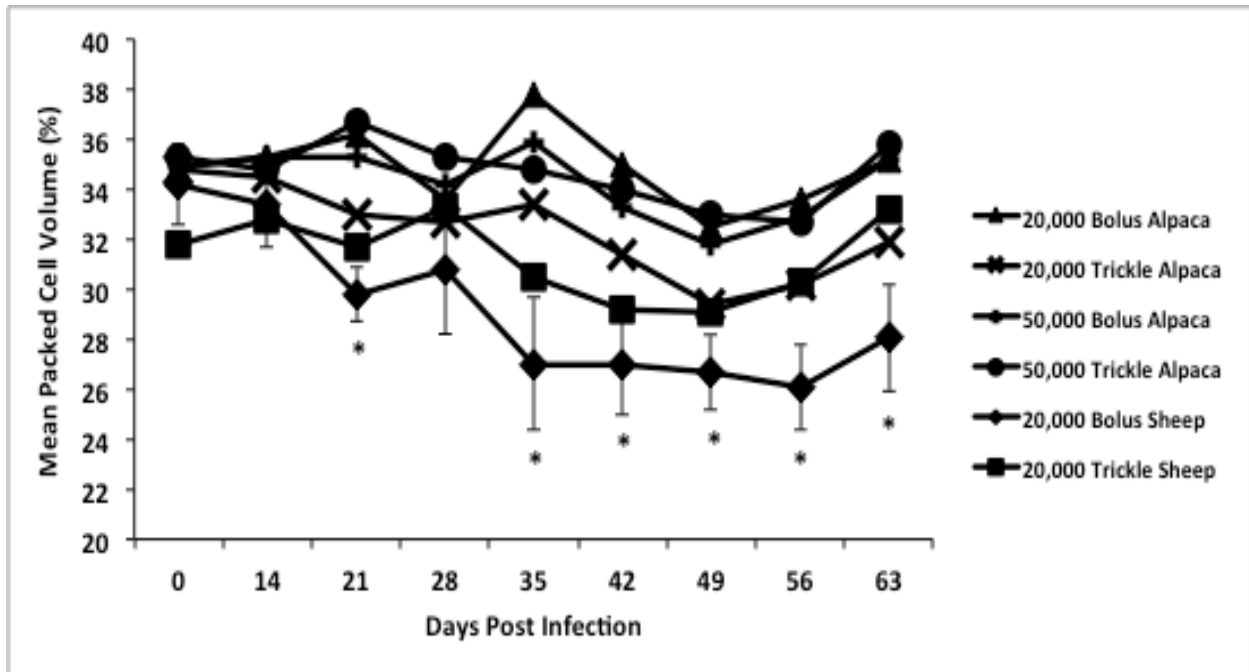
Figure 4. Mean packed cell volume for both alpacas and sheep in Experiment 1. Vertical bars represent the standard error of the mean (±SEM), and asterisks represent days of significance for the 20,000 bolus infected sheep from the starting date over the course of infection.
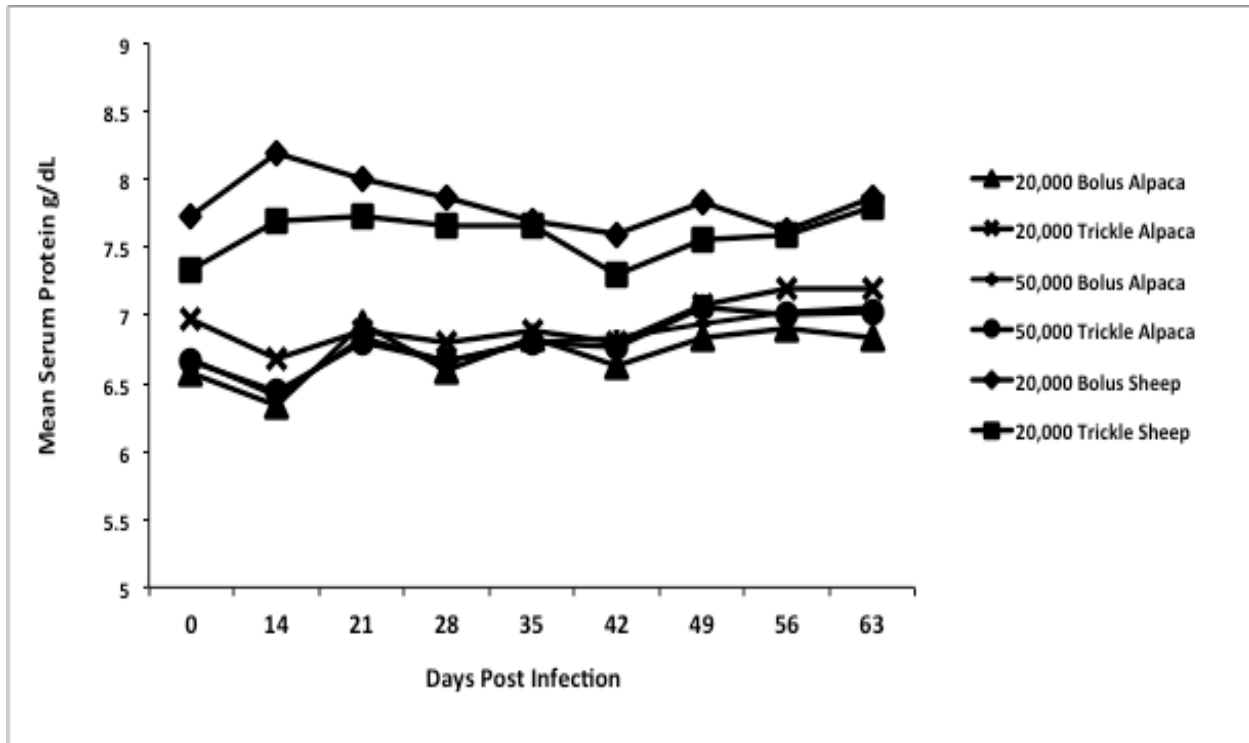
Figure 5. Mean serum protein (g/dl) for both alpacas and sheep in Experiment 1.
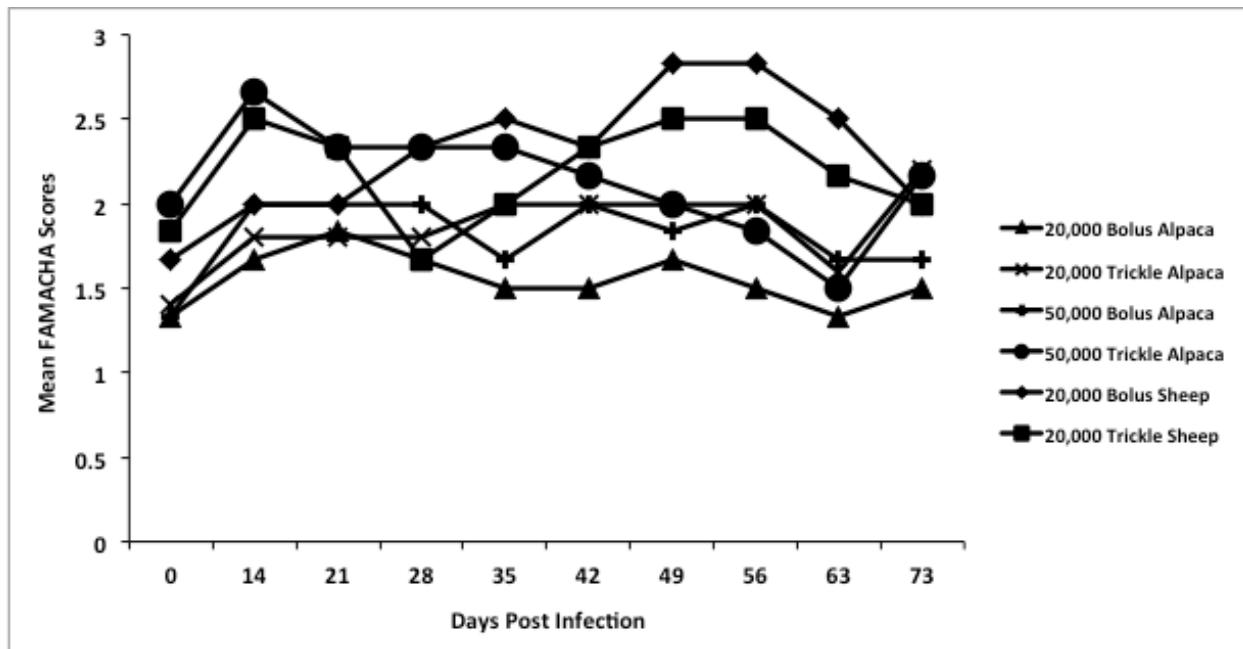
Figure 6. Mean FAMACHA© scores for alpacas and sheep in Experiment 1.
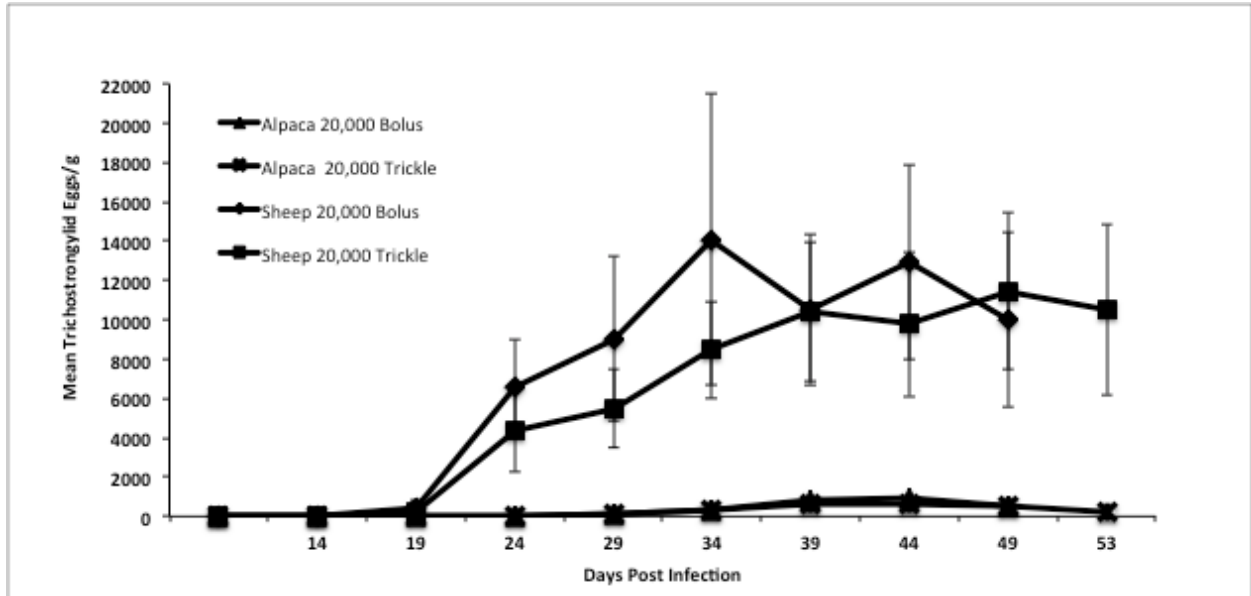
Figure 7A.
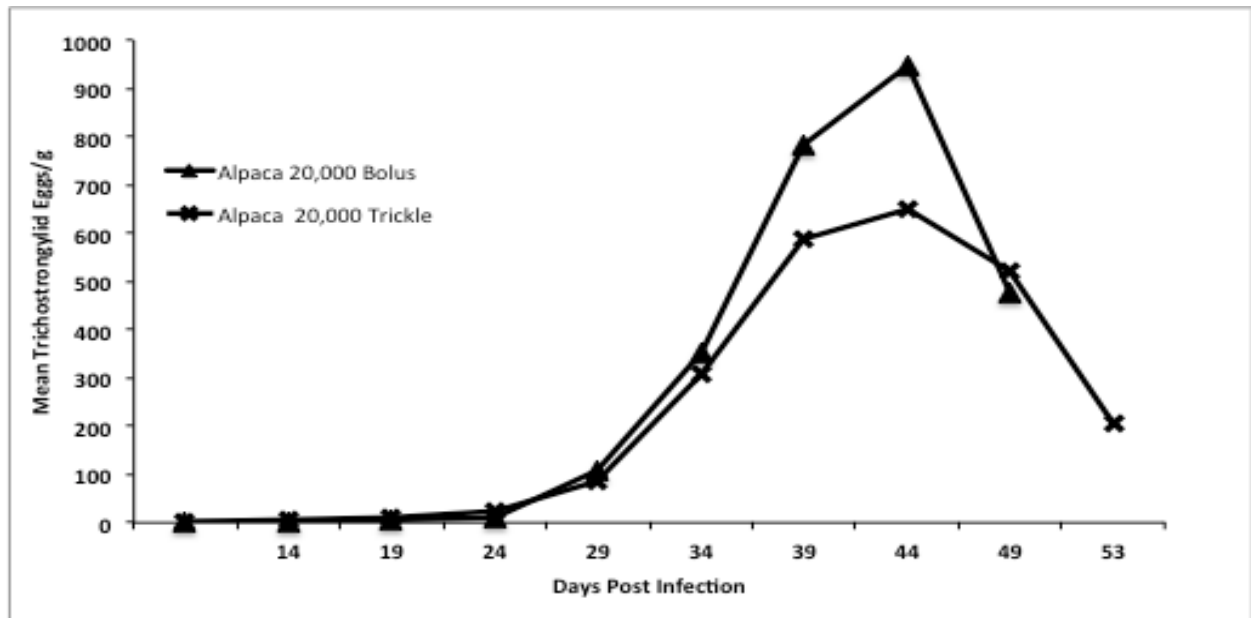
Figure 7B.

Figure 7. Fecal egg count of sheep and alpacas in Experiment 2.
(7A) mean FEC of alpacas and sheep in Experiment 2. Vertical bars represent the standard error of the mean (±SEM).
Because there were no differences within species, data was combined within species for further analysis.
(7B) mean FEC of alpacas only in Experiment 2.
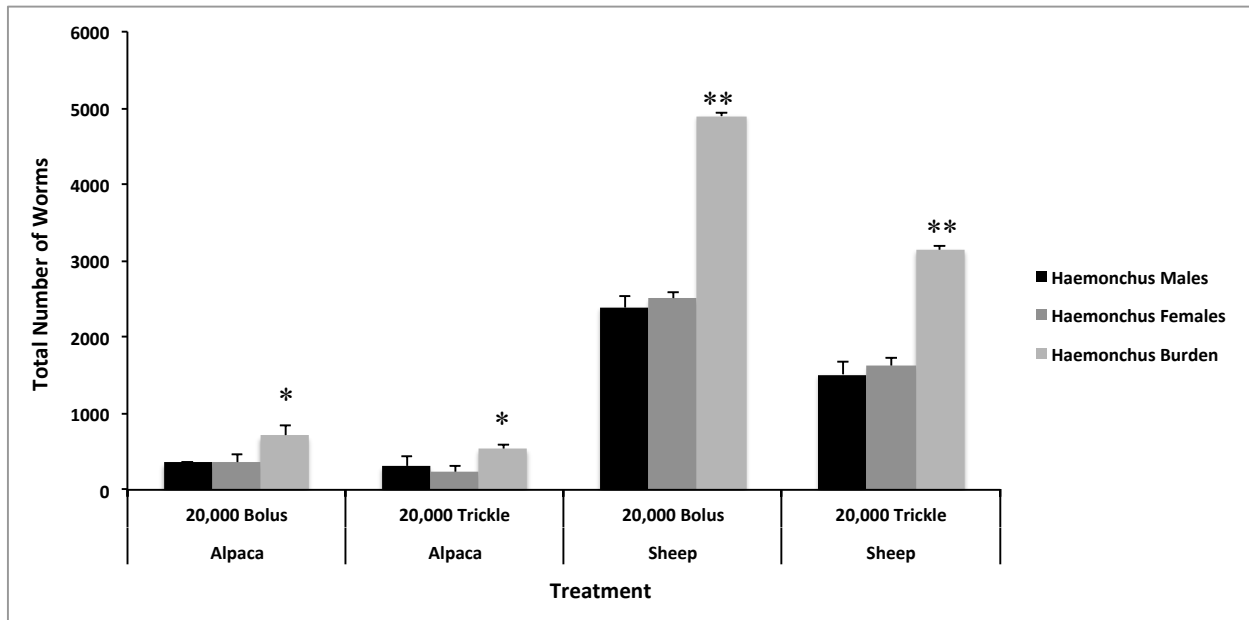
Figure 8. Total worm burden of alpacas and sheep in Experiment 2. Alpaca groups and sheep groups were combined for comparison. Columns with different superscripts are significantly different p<0.0001.
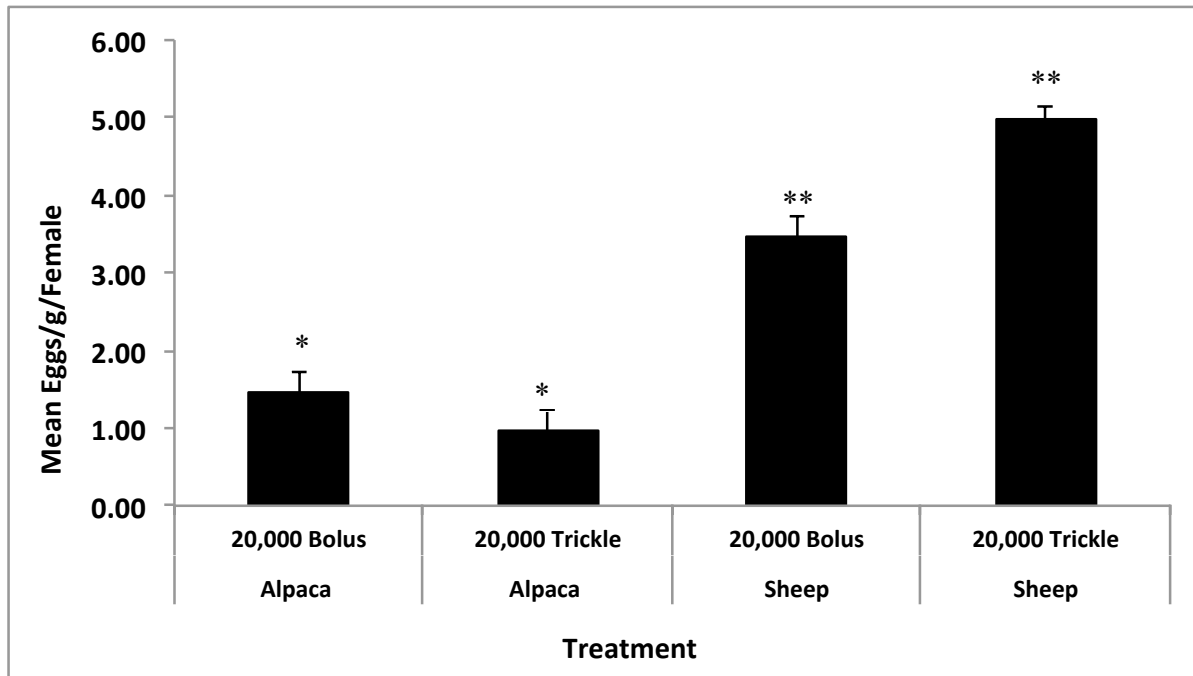
Figure 9. Mean eggs/g per female for alpacas and sheep in Experiment 2. Alpaca and sheep groups were combined for comparison. Columns with different superscripts are significantly different p<0.0001.
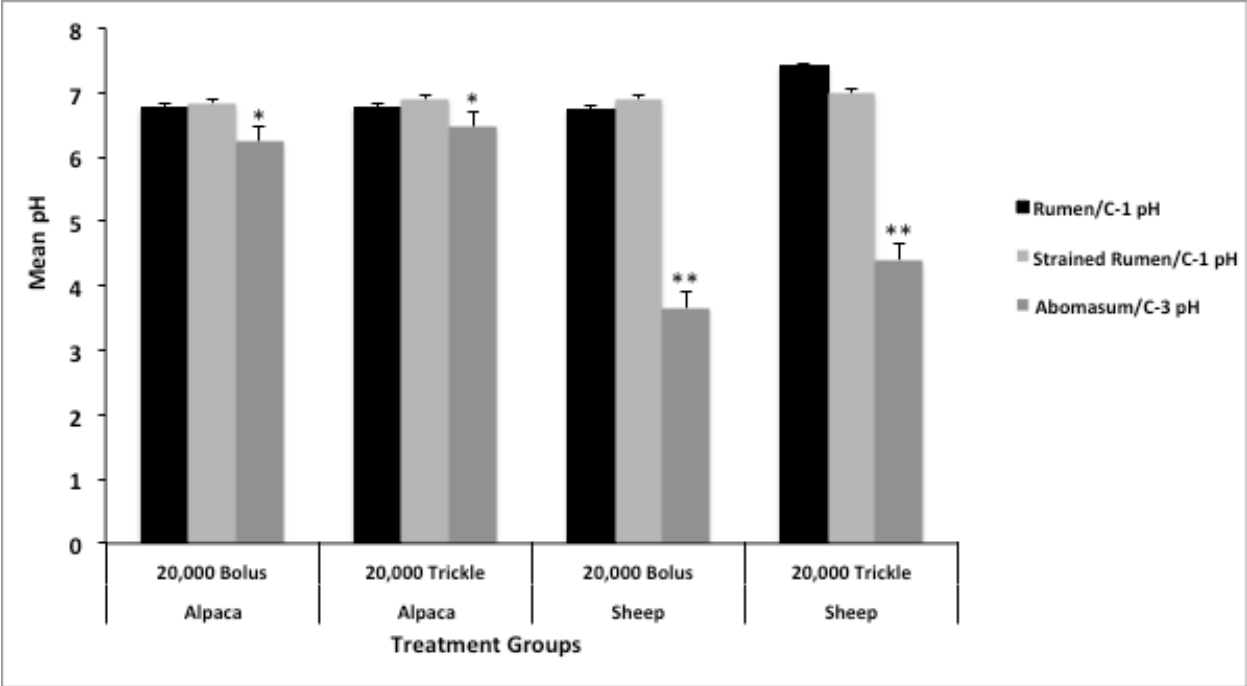
Figure 10. Mean pH of strained and unstrained rumen and C1 contents, as well as the pH of the abomasum, and C3 for alpacas and sheep in Experiment 2. Columns with different superscripts are significantly different p<0.0018. There were no significant differences in pH of the rumen and C-1.
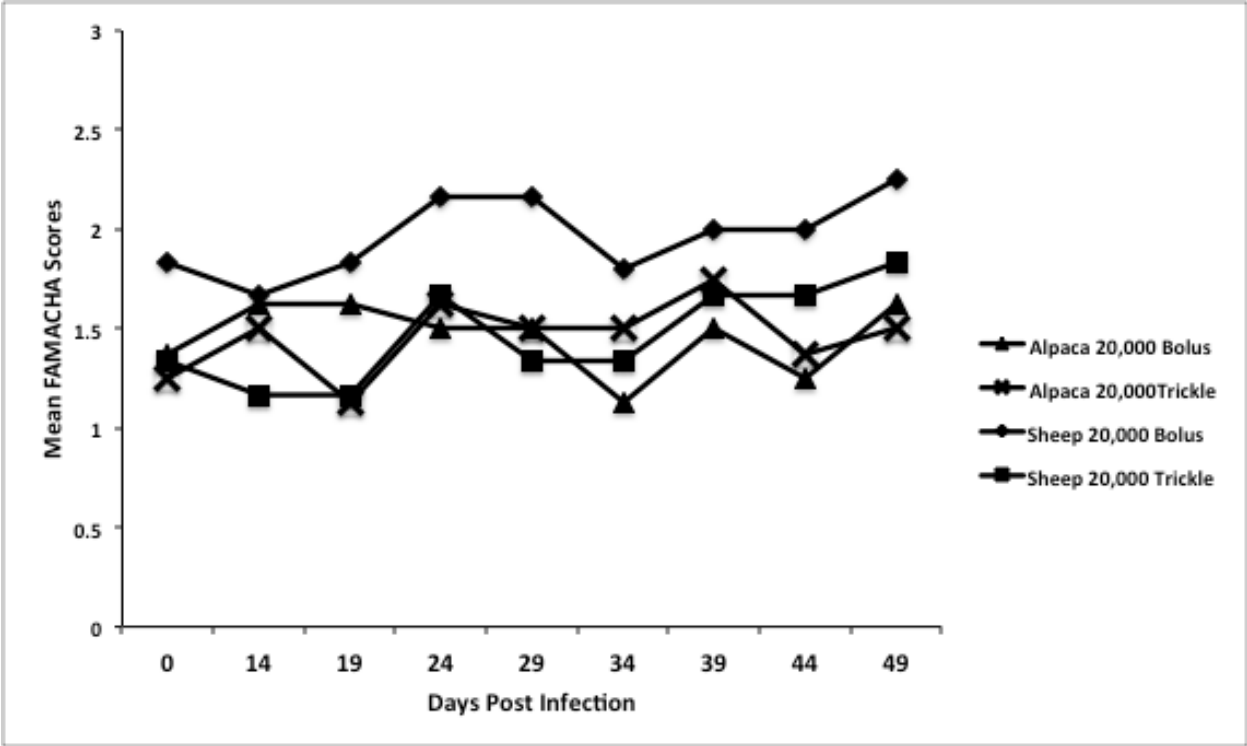
Figure 11. Mean FAMACHA© scores of alpacas and rams in Experiment 2. There was no significant difference in FAMACHA© score between groups over the course of infection.
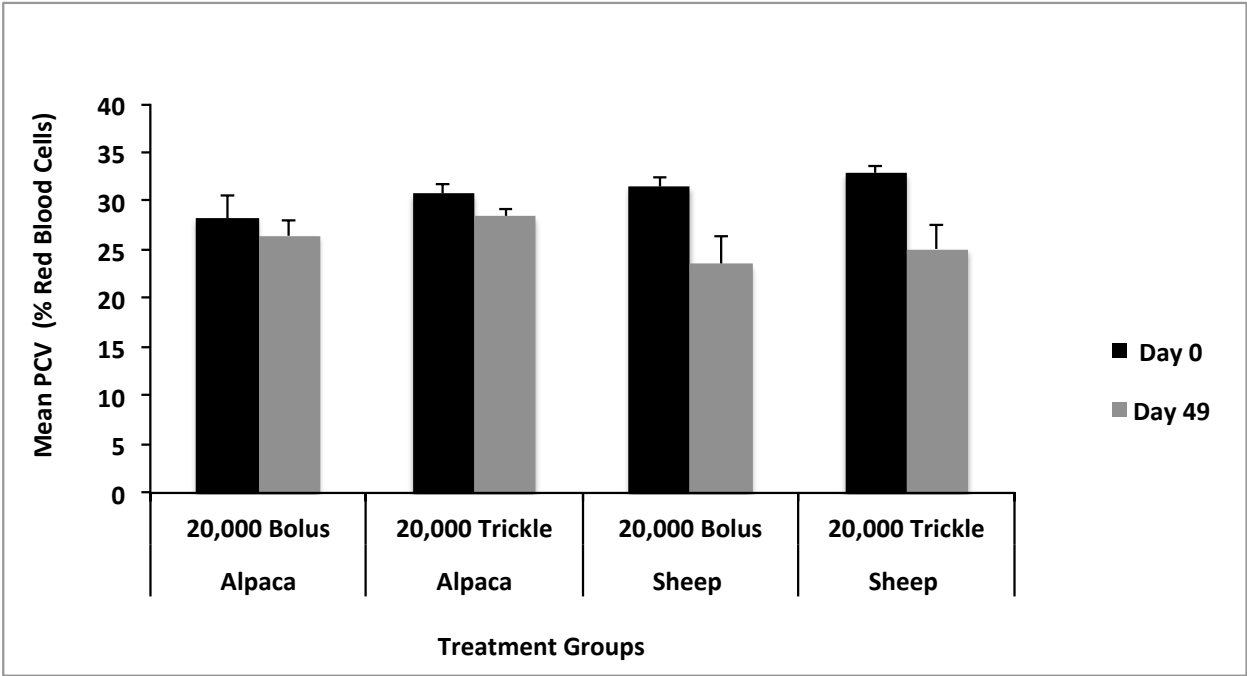
Figure 12. Mean packed cell volume (PCV) of alpacas and rams in Experiment 2. No significant differences were found between sheep groups or alpaca groups on day 0 or day 49 for PCV.
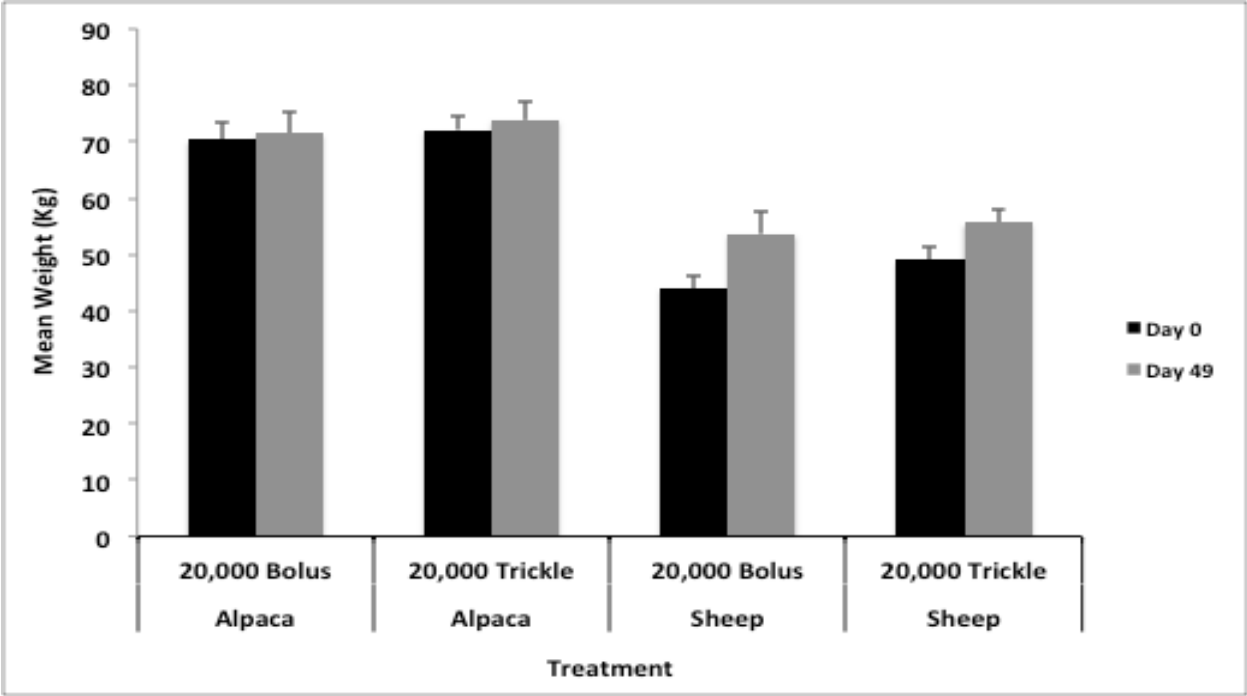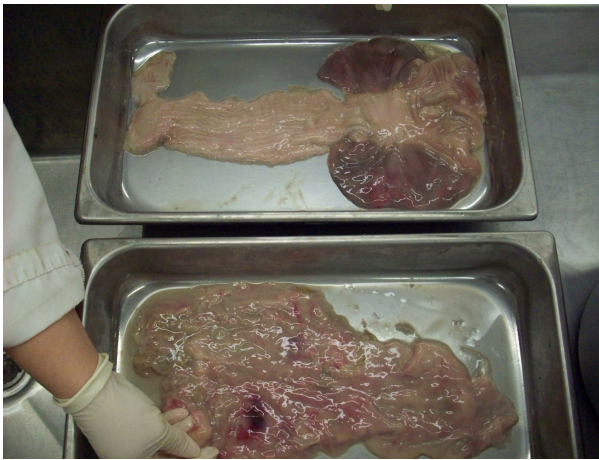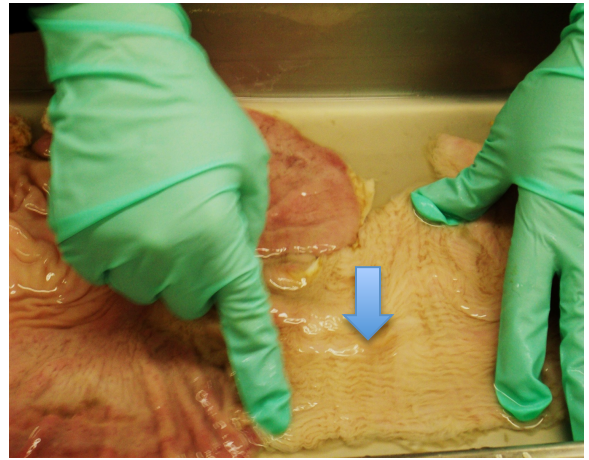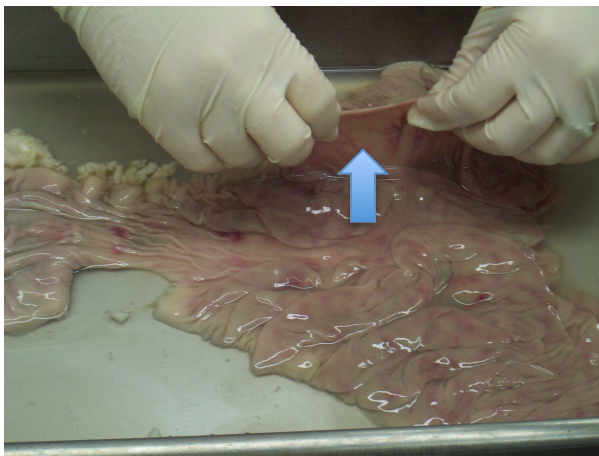
Figure 13. Mean weight (Kg) of alpacas and rams in Experiment 2. There were no significant differences in weight found between sheep groups or alpaca groups on day 0 or day 49.

Figure 14. Alpaca C-3 and sheep abomasum. (A) alpaca C-3 (top)and sheep abomasum (bottom). (B) short ridges or pleats (arrow) of the alpaca C-3. (C) long folds of the sheep abomasum (arrow).

Mean Body Condition Score for Alpacas and Rams		
Treatment	Day 0	Day 63*
20,000 Bolus Alpaca	7.2	7.3
20,000 Trickle Alpaca	6.6	6.0
50,000 Bolus Alpaca	7.3	7.8
50,000 Trickle Alpaca	7.0	7.3
20,000 Bolus Ram	6.5	7.0
20,000 Trickle Ram	6.2	7.3
*All animals were treated on day 63 and put on pasture.		

Table 1. Mean body condition score for alpacas and rams on days 1 and 63 in Experiment 1.