

Immunologic Responses in Florida Native Sheep
Experimentally Infected with *Haemonchus contortus*

By

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IMMUNOLOGIC RESPONSES IN FLORIDA NATIVE SHEEP EXPERIMENTALLY-INFECTED
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The host responses of worm-free Florida Native lambs to infection with *Haemonchus contortus* were investigated for the elucidation of "resistance factors" which may play a role in maintaining lower worm burdens. Physiological and immunological measurements were made on worm-free lambs divided by blood hemoglobin type prior to infection and post-infection during a sequential necropsy. Immunoelectrophoresis, electrophoresis and indirect hemagglutination for antibody titer, demonstrated which protein fractions and antibodies predominated in the serum and abomasal mucous, how they changed with the parasitic infection and the relationship to blood hemoglobin type. Physiological factors monitored included PCV, blood hemoglobin levels and fecal egg count.

Infected animals showed maximum blood loss, demonstrated by lowered PCV's and hemoglobin level, at 26 days after infection. Subsequently,

increased gamma-globulin and a decreased albumin-to-globulin ratio were observed. The increased gamma-globulin fraction may be related to the antibody activity (exhibited by indirect hemagglutination testing) in both serum and abomasal mucous which increased significantly after experimental infection with *H. contortus*. The identification of the immunoglobulins (IgA, IgG and IgM) responses against the parasite presented evidence that IgM and IgG were most prominent in serum, and IgA and IgG were the most prominent in mucous. Complement proteins were also shown to increase substantially after parasitic infection.

The percentages of the abomasal mucous and blood serum proteins were established. Characterization of these proteins, including the immunoglobulins, by immunoelectrophoretic techniques, revealed a maximum of 8 and 10 proteins in serum from worm-free and parasitized lambs, respectively. Mucous extracted from the abomasum exhibited 5 to 7 proteins regardless of infection status.

INTRODUCTION

Morbidity and mortality losses caused by trichostrongylid parasites are of major economic importance in worldwide sheep production. Figures by the United States Department of Agriculture (U.S.D.A., 1965) estimated annual losses of \$21 million in sheep due to the trichostrongylids which include: \$7 million due to deaths, \$11 million to morbidity and \$3 million to wool loss. Becklund (1961) found economic losses due to parasite mortality of sheep on 15 farms in southern Georgia averaged \$1,233 per farm. He also found on the Georgia Coastal Plain region an average loss of \$289 per farm on 23 sheep farms.

Haemonchus contortus, according to Whitlock (1955a), is the only gastrointestinal nematode which causes a primary disease. This recognizable disease, called haemonchosis, produces a hemorrhagic anemia (Richard et al., 1954, Campbell and Gardiner, 1960). Blood loss caused by *H. contortus* was determined by Baker et al. (1959) who tagged sheep erythrocytes with radioactive chromium 51. They determined that *H. contortus* removed an average of 0.08 ml of blood per worm per day. Clark et al. (1962), also using radioactive chromium 51 and iron 59, found blood loss in experimentally infected lambs to be 0.049 ml per worm per day.

Clinical symptoms of haemonchosis vary from the peracute form where the parasite causes rapid death, to a sub-clinical form which is essentially asymptomatic. Heavily parasitized lambs may exhibit growth reduction, permanent stunting (Spedding, 1956), weakness, muscular

trembling, pale mucous membranes, cold extremities, rapid weak pulse, increased respiratory rate and edema with accompanying "bottle jaw" swelling under the jaw (Levine, 1968; Tetzlaff, 1970). Animals less severely infected have lowered resistance and may be susceptible to secondary infection. These animals are unthrifty, listless and have dry, harsh wool (Levine, 1968; Tetzlaff, 1970).

The losses incurred in sheep carrying worm burdens at the sub-clinical level have been reported to be substantial. Gordon (1958) demonstrated a drop in milk production in lactating ewes experimentally infected with *H. contortus*. Spedding (1955) and Spedding et al. (1958) have shown a reduction in growth rate as much as 30% in ewes having normal pasture parasite burdens. Brunsdon (1963) found that lambs treated with thiabendazole weighed 30 pounds more than controls at slaughter and produced 49% more wool.

Due to the short period from uptake of infective larvae to ova production (14-21 days) and the fact that infective larvae are resistant to freezing and drying (Monnig, 1956), control of haemonchosis by management practices (pasture rotation or dry lot feeding) may not be effective. Anthelmintic drug control is, at present, the most effective and economical means to reduce worm burdens. Although, where these compounds are under continuous use, there have appeared resistant strains of *H. contortus* (Theodorides et al., 1970). The appearance of these strains can cause the need of increased frequency of treatments and, with the rising costs of anthelmintics, this is of economic concern in sheep production.

Immunity and genetic resistance in sheep against internal parasites have been reported frequently in the literature. Observations by Evans

et al. (1963), Evans and Whitlock (1964), Loggins et al. (1965a, 1965b), Jilek and Bradley (1969), Radhakrishnan et al. (1972) and Bradley et al. (1973) suggest a correlation between hemoglobin type, hematocrit value and severity of infection with *H. contortus* infections. Evans and Whitlock (1964), Jilek and Bradley (1969) and Radhakrishnan et al. (1972) found consistently higher hematocrit values in hemoglobin type A. This physiologic factor (greater erythrocyte volume) may allow these sheep a better chance of surviving parasite challenge. Loggins et al. (1965b) Jilek and Bradley (1969), Radhakrishnan et al. (1972) and Bradley et al. (1973) found Florida Native sheep were more resistant to infection with *H. contortus* than Rambouillet sheep. Florida Native sheep appear to have some type of "resistance factor" due to the fact that they are able to undergo self cure more readily than Rambouillets and had significantly more larval forms of *H. contortus* than adult worms (Radhakrishnan et al., (1972).

Arrested development of *H. contortus* has been well documented, although the factors governing this phenomenon are poorly understood. Dineen et al. (1965), Dineen and Wagland (1966), Soulsby (1966), Wagland and Dineen (1967), Michel (1968) and Donald et al. (1969) attribute the arrested development of *H. contortus* to resistance of the host while Blitz and Gibbs (1972a) ascribe it, at least in part, to an environmental diapause-inducing condition (decreasing photoperiod).

The objectives of the present investigation were to characterize the host responses of worm-free Florida Native lambs to infection with *H. contortus* and to elucidate the "resistance factors" that have a role in maintaining lower adult worm burdens. Hematological data, serum collection and abomasal mucous extraction were the main considerations

in the determination and interpretation of these "factors." Sequential necropsy of infected worm-free lambs from pre- through post-patency gave an unique insight into the immunologic mechanism occurring in lambs, in both the humoral and mucous secretory systems, while the parasitic infection proceeded. The following physiological and immunological measurements were performed: (a) hematocrits, (b) hemoglobin levels, (c) fecal ova counts, (d) electrophoresis of serum and mucous, (e) immunoelectrophoresis of serum and mucous using anti-sera and *H. contortus* antigen and (f) indirect hemagglutination. Through the analyses of lamb serum and abomasal mucous, additional information was gained concerning which protein fractions and immunoglobulin classes they contained, how they changed with age and whether there were statistical relationships to environment, hemoglobin types or other genetic factors.

LITERATURE REVIEW

Life Cycle, Morphology and Metabolism of *Haemonchus contortus*

Haemonchus contortus (Rudolphi, 1803), commonly known as the "barber pole worm," is found principally in the abomasum of ruminants worldwide. The males are 10-20 mm long and are reddish brown in color. Females range from 18-37 mm in length and have characteristic white ovaries spiralled around their reddish blood-filled intestine giving the "barber pole" appearance.

The male *H. contortus* is identified by a bursa having elongated lateral lobes and long slender rays. A small dorsal lobe of the bursa is asymmetrically located around the left lateral lobe supported by a Y-shaped dorsal ray. Its spicules are 0.46-0.51 mm long. In the female, the vulva is covered by an anterior flap. This vulvular flap usually will be large and conspicuous (linguiform), but can be diminished to a knob-like structure in some specimens (Levine, 1968).

The mature female produces from 4,000 to 10,000 ova daily. These ova measure 70-85 μ by 41-48 μ and are expelled in the feces of the host as a 16 to 32-cell embryo. Continual mitosis of the embryo is dependent on temperature, moisture and metabolic oxygen (Jilek, 1968; Levine, 1968; Tetzlaff, 1970). Cleavage is highly determinant, meaning germ cell lines are segregated and can be followed as to which structures they may become. Morphologically, the cells within the egg form a morula, blastula, gastrula and then elongate to a vermiform embryo (Levine, 1968). The elongated

embryo shows defined organs, three cell layers (endoderm, ectoderm and mesoderm), germ cells, gut and stomodeum. The first stage larvae of *H. contortus* hatch within 14-19 hours. This first stage is rhabditiform and actively feeds on bacteria and other microorganisms. The second stage larvae, which appear within 1-2 days, are also rhabditiform. The third stage (L_3) or infective stage is stronglyliform and sheathed, and is found 4-7 days after hatching.

The infective stage is characteristically 682-780 μ in length, has 16 intestinal cells and a globular buccal cavity. The tip of the tail to the end of the sheath is 65-78 μ with a kink found in the sheath tail. The intestinal cells contain granules which will be used to maintain the infective larvae (Lapage, 1968). These larvae are negatively geotropic and positively phototropic except for strong sunlight and can exist for months if temperature and moisture conditions are adequate (Levine, 1968; Soulsby, 1965). In the morning or evening hours, infective larvae may migrate up blades of grass where they are available to be ingested by the grazing animal. Exsheathment is triggered after ingestion by physical and chemical components such as temperature and CO_2 -carbonic acid concentrations (Rogers, 1962). This triggering mechanism causes secretion of exsheathing fluid containing leucine aminopeptidase from a region around the excretory cell. The enzyme acts on an area about 20 μ back of the anterior end for release of the L_3 larva (Lapage, 1968; Levine, 1968; Rogers, 1962; Silverman, 1965).

The released third stage larvae migrate to the paramucosal lumen at the surface of the mucosa or become lodged in the epithelial processes of the mucosa (Silverman, 1965; Tetzlaff, 1970). Ecdysis occurs after

3 days and a fourth stage attaches to the mucosa with its buccal capsule and ingests blood. In 9 to 11 more days, after growth and development, another ecdysis occurs and fifth stage or immature adults emerge. They attach to the abomasal mucosa where they also ingest blood. The mouth of the fifth stage now has a dorsal lancet which has two thorn-type points. Within 6 to 8 more days, morphological and physiological development is finished and the parasite is a functional blood sucking adult.

Metabolic pathways of nematode ova, larvae and adults have been studied extensively (Smith, 1965). Cheng (1973), Levine (1968) and Rogers (1962) present chemical formulas of these pathways. Glycogen is the main source of stored energy in the ova and sheathed infective larvae. Energy is released by the Embden-Meyerhof route of phosphorylating glycolysis. Phosphorylation occurs as in vertebrate tissue but neither arginine phosphate or creatine phosphate have been detected (Rogers, 1962). Lactic acid may not be the end product of anaerobic metabolism and pyruvic acid may also be metabolized which suggests that it may be involved in production of lower fatty acids which are secreted (Rogers, 1962). Data for *H. contortus* ova and larvae show respiratory quotients of 0.58-0.60 and 0.64, respectively. Adult *H. contortus* are found to contain a type of hemoglobin which transports oxygen (Lapage, 1968; Levine, 1968).

Pathogenesis

Haemonchus contortus causes primary damage by sucking blood. Both the fourth stage larvae and adults suck blood, consequently damaging the abomasal mucosa by their attachment and piercing activities. The anemia produced is proportionally related to the numbers of adult worms present, yet cannot be correlated to the numbers of eggs per gram of feces (Andrews, 1942; Kingsbury, 1965). Blood first appears in the feces 6-12

days after infection (Clark et al., 1962). Boughton and Hardy (1935), using an abomasal fistula, observed varying degrees of petechiation on the abomasal mucosal surface indicating sites of recent attachment. They observed the parasites attach themselves by a striking motion of the head and neck. Attachment lasted for approximately 12 minutes after which detachment occurred leaving wounds which continued to hemorrhage for an additional 7 minutes.

Hematological changes associated with anemia caused by *H. contortus* may include erythrocytes showing anisocytosis, polychromasia, Howell-Jolly bodies and punctate basophilia (Levine, 1968). Serum proteins and albumin in parasitized lambs may show a decrease, while the alpha-1-, alpha-2-, beta- and gamma-globulins may all be increased (Kuttler and Marble, 1960; Leland et al., 1960). Wilson and Turner (1965) noted that even moderate mixed nematode infections (mainly *H. contortus*) caused a decrease in the serum albumin to globulin ratio and an increase in serum gamma-globulin. Decreased total serum protein, albumin concentrations and albumin to globulin ratios were also seen in Florida Native and Rambouillet lambs infected with *H. contortus* (Bradley et al., 1973). Eosinophil and lymphocyte infiltration in abomasal tissue of lambs was observed several weeks after infection with *H. contortus* (Bradley et al., 1973; Malczewski, 1971). Increased lymphocytes and lymphoid hyperplasia were also observed in sheep resistant to *H. contortus* (Silverman, 1965).

Anemia as low as 3-4 grams of hemoglobin per 100 ml of blood can be present in *H. contortus* infected animals (Levine, 1968). Clinically, the gums, conjunctiva and mucous membranes are pale. Edematous swelling

under the jaw ("bottle jaw"), constipation, weakness, cold extremities, listlessness, dull, dry, harsh wool and unthriftiness may be seen (Levine, 1968; Tetzlaff, 1970). Parasitized animals may die suddenly, symptoms may persist for weeks before dying, or recovery may occur leaving stunting or reduced muscle growth (Spedding, 1956).

Epidemiology and Ecology of *H. contortus*

The mode of infection of sheep with *H. contortus* infective larvae is by grazing on infected pastures or ingestion with their feed or water. When pasture conditions are favorable, the infective stage (L₃) is reached within 2.5 days to 2 weeks (Levine, 1968; Levine et al., 1975). These conditions are complex in nature. Levine (1968) describes them to be a combination of climatic and micrometeorologic. Additional conditions such as the terrain and soil type, nature and type of vegetation, degree of stocking and number of nematode species competing for space are important factors.

Oxygen has also been found to be obligatory for the adult female to lay eggs (LeJambre and Whitlock, 1967) and for egg development to occur on the ground (Shorb, 1944). Optimal temperature for development (60 hours) is 33.3°C (Berberian and Mizelle, 1957). Levine (1968) states that Hsu in 1967 studied the effects and relationships of temperature and relative humidity to the development of *Trichostrongylus colubriformis* and *H. contortus*. He found that *H. contortus* needed relative humidities above 85%. Rose (1963) reported that desiccation severely reduced *Haemonchus* larvae developing from sheep feces.

The habitat of the larvae is in a thin layer at the surface of the ground. Conditions can be different in this microhabitat than

above ground where weather is usually measured. Gordon (1948) introduced bioclimatographs to help recognize the relationship of temperature and precipitation to the epidemiology of gastrointestinal parasites of ruminants. Levine (1963) introduced parasite profiles of geographic regions and discussed in detail the effects of weather and climate on the bionomics of ruminant nematode larvae. He considered the potential transmission period of *Haemonchus* to lie between mean monthly temperatures of 15 to 37°C when the soil water deficiency was not more than 2.0 cm.

Monnig (1956) reported that infective larvae are active climbers and can withstand desiccation and freezing. They are negatively geotropic and positively phototropic to soft light, a state seen after sunrise and before sunset (Rees, 1950; Soulsby, 1965).

A means of control of *H. contortus* and other trichostrongyles might appear to be the elimination of the larvae and eggs on the ground. Several authors have described methods for soil treatment but no satisfactory method is mentioned that is safe, efficient, and inexpensive. Crofton (1949) found that removal of sheep for at least 12 days reduced the number of larvae on a pasture. He found reductions of 57% when sheep were removed 3 weeks and 90% at 4 weeks. He believed plowing and reseeding would eliminate larvae from a pasture. Soulsby (1965) and Levine et al. (1974), in contrast, have reported that under favorable conditions *H. contortus* infective stage larvae could survive 1.5-3.5 months.

Immunology and Resistance

The idea that metazoan parasites stimulated an immune response was first reported by Stoll (1929), using *H. contortus*. He infected lambs with the parasite and observed that after placing them on pasture

their fecal egg counts rose to high levels. After several weeks he found a dramatic fall in egg counts, some to negative values. He correlated this phenomenon to a loss of adult worms. Even after subsequent reinfection with large numbers of infective larvae, the lambs remained refractory to reinfection. Stoll termed this phenomenon "self cure and protection." In 1930, Stoll and Nelson reported that this resistance was humoral, based on intradermal reactions to saline extracts of *H. contortus*. Stumberg (1933) substantiated this fact by using a cutaneous anaphylactic test in which he detected antibody against *H. contortus* in dilutions of 1:50,000.

Hawkins and Cox (1945) found that serum obtained from sheep that had undergone a natural infection with the trichostrongyles (mainly *H. contortus*) caused precipitates around the mouth, excretory pore, anus or cuticle of exsheathed larvae. There were no precipitates in suspensions of ensheathed larvae in immune sera or larvae in sera of lambs that had been raised parasite free except for coccidia or *Strongyloides*. Silverman (1965) also obtained similar results. Antibodies that cause these precipitates at the physiological orifices were believed to be "functional" by Oliver-Gonzales (1946), meaning that they contribute directly to resistance.

Stewart (1950a,b,c) has demonstrated that a complement fixing antibody response occurs after infestation with *H. contortus* or *Trichostrongylus* spp. He found a correlation between the fall of ova counts and the rise in antibody titer in experimentally infected sheep. In field studies he observed 7 periods of "self cure." On each occasion most sheep which had a drop in ova counts also showed a rise in serum

titer. This occurrence was similar to the result when infective larvae of *H. contortus* were superimposed upon an existing infection producing "self cure." This was contrary to the reports by Ross and Gordon (1933) and Gordon (1948) in which they concluded that acquisition of resistance to *H. contortus* by previous infestation was uncertain; that evidence did not indicate that "self cure" was a manifestation of resistance and that it occurred close to periods after rain. Stewart (1950c) found the reason "self cure" takes place in naturally grazing flocks after rain was because this caused large doses of infective larvae to mature and be ingested.

The immunological reaction and subsequent protection depends on the availability of the infective larvae (Stewart, 1950a). Even though infective larvae are continuously available, infection is maintained at a low level (Soulsby, 1958). If non-immune sheep were placed on this type of pasture they would probably acquire heavy burdens of gastrointestinal parasites. In Florida, weather conditions are such to allow the above situation to be maintained year round or for at least longer periods of time. In areas where conditions become unfavorable for larval development there is a depression of the immune status due to lack of stimulation by infective larvae. This is confirmed by a persistent fall in antibody titer in which "Spring rise" (characteristic increase of ova count) occurs (Soulsby, 1957).

The mechanisms of "self cure" in lambs cause a response by the host which results in the loss of part or the whole *H. contortus* burden. These mechanisms have been postulated and the causes have been shown to be varied in nature. Stewart (1953) found that at the time of "self cure" there was a significant rise in blood histamine as well as

antibody level. If antihistamine drugs were given at this time the phenomenon did not occur, yet there was still an increase in antibody titer. This reaction was characteristic of an allergic sensitization with an edematous condition of the mucous membrane of the abomasum. This was substantiated by Stewart (1955) who reported that abomasums of previously non-exposed lambs remain flaccid and normal when large doses of exsheathed larvae were injected into the abomasum. In hypersensitized and resistant lambs, the abomasum had increased peristalsis and segmentation in 10 minutes. Within 1 hour the abomasum was pale and edematous. Histological examination of the mucosa of animals that undergo self cure show edema and aggregation of eosinophiles (Soulsby, 1958).

Soulsby (1965) suggests resistance to parasites may be due to a change at the environmental site caused by the parasite itself. An alteration of oxygen tension associated with pH could be such a product of infection which is seen in inflammatory reactions. Christie (1970) found the activity of fourth stage *H. contortus* larvae damage the function of the cells of the gastric epithelium. Hydrogen ion concentrations are increased and the pH of these cells which is near neutral drops dramatically to pH 1.8 to 3.5. This is unfavorable to the development and persistence of the adults. Ejection of adult worm populations and "self cure" could be explained because of these changes after intake of large doses of larvae.

Arrested development of larvae is an important immunologic-related occurrence. It is of particular significance, for it depicts the primary means of overwintering for *H. contortus* in temperate regions (Blitz and Gibbs, 1972a). Large numbers of fourth stage larvae and low

numbers of adults are frequently observed during the winter months (Blitz and Gibbs, 1972b; Gibbs, 1967). It is maturation of these larvae in the spring that contributes to the characteristic increase in the number of ova at this time called "Spring rise" (Field et al., 1960; Gibbs, 1967; Parnell, 1962; Procter and Gibbs, 1968). O'Sullivan and Donald (1970) hypothesized on the importance of hormonal changes in lactating ewes which depressed their immunological capacity resulting in a stimulation of dormant larval stages to mature. This is only part of the answer as Spring rise is also observed in wethers and young virgin ewes (Brunsdon, 1964; Croften, 1958).

The factors initiating arrested larvae are generally felt to be from high levels of resistance, whether from previous exposure or inherent mechanism (Dineen et al., 1965; Dineen and Wagland, 1966; Wagland and Dineen, 1967; Donald et al., 1969). Blitz and Gibbs (1972a) have also added another dimension to the mechanisms of arrested development by presenting evidence that showed if larvae were cultured in the laboratory at constant temperature and in darkness, and then exposed for 4 to 6 weeks to environmental conditions similar to those prevailing during September they would become inhibited following ingestion by worm-free lambs. They believe two factors are operating: that preinfective and infective *H. contortus* are sensitive to diapause-inducing stimuli (decreasing photoperiod or temperature) causing the inhibition and that resistance from the host will prevent worms from developing as demonstrated by Dineen and co-workers (1965). Bradley et al. (1973) reported significantly higher levels of larvae in Florida Native lambs than Rambouillet lambs indicating that either one or both of the aforementioned mechanisms may be in operation.

It is believed the larval stages are the important immunizing agents inducing "self cure and protection" (Soulsby, 1965). Reports by Silverman (1965) and Silverman and Patterson (1960) point to the antigenicity of the fourth and early fifth larval stages and that the antigens are released during growth and development. Soulsby et al. (1959) and Soulsby and Stewart (1960) obtained serological evidence of a noticeable reaction to exsheathing fluid at the time of "self cure."

The relationship of age to the production of immunity against *H. contortus* in sheep has also been studied. Manton et al. (1962) found lambs infected with larvae of *H. contortus* at 2-4 months of age to be unable to develop immunity while lambs 10-12 months of age could. Urquhart et al. (1966a, 1966b) in vaccination studies against *H. contortus* found lambs 1-3 months of age unable to develop immunity and lambs 7 months old produced a high degree of protection. Tetley (1959) found no differences in susceptibility between worm-free Romney lambs 6-10 or 3-6 months of age. Similar results were reported by Dineen and Wagland (1966) between sheep 320 and 455 days of age, though Silverman (1965) and Silverman and Patterson (1960) reported laboratory infected sheep aged 8-12 months showed longer parasite life cycles than sheep aged 4-6 months, indicating some resistance.

Resistance to parasitic infections has also been correlated with genetic factors. As early as 1932 Clunies-Ross reported observations on genetic resistance of sheep to infections with stomach worms. Stewart et al. (1937) reported that the Romney Marsh sheep were more resistant to *Ostertagia* sp. than other breeds they used. Scrivner (1964a, 1964b, 1967) found genetic resistance to ovine ostertagiasis, which could be

transmitted by the ram in Targhee sheep. Emik (1949), Warwick et al. (1949) and Whitlock (1955a, 1955b, 1958) reported resistance to the trichostrongyles (mainly *H. contortus*) was genetically transmitted. Loggins et al. (1965a, 1965b) believed that genetic factors were responsible for parasitic resistance in Florida Native sheep in comparison to the Southdown, Hampshire or Rambouillet sheep.

Jilek and Bradley (1969) found high frequencies of hemoglobin type a (Hb A) in Florida Native sheep which were believed to be more resistant to *H. contortus*. This was in agreement with Evans et al. (1963) who reported that sheep with Hb A were infected with fewer adult *H. contortus* than other hemoglobin types. Evans and Whitlock (1964) found sheep with hemoglobin type A had higher total volume of circulating erythrocytes and hematocrit values than either types B or AB. Radhakrishnan et al. (1972) confirmed that Florida Native sheep with Hb A had consistently higher packed cell volume (PCV) values than other hemoglobin types but reported no data that would confirm that hemoglobin types were indicators of resistance against *H. contortus* or that Hb A sheep were less susceptible to such infections. If sheep with Hb A do have more circulating erythrocytes, they might be better able to withstand the effects of *H. contortus*.

Radhakrishnan et al. (1972) did find significantly lower numbers of adult worms in Florida Native lambs in comparison to Rambouillet lambs. This observation was substantiated by Bradley et al. (1973) who gave 1 or 2 oral doses of *H. contortus*. They reported Florida Native lambs had higher levels of larval stages, prolonged prepatent period (21 days) and a more rapid "self cure" than Rambouillet lambs. Silverman (1965) also reported a delayed prepatent period (20 days) in

resistant sheep as compared to susceptible sheep (15 days). Florida Native sheep may possess "resistance factors" which enable them to be resistant toward *H. contortus* infection without prior exposure or to initiate a more rapid "self cure" (Bradley et al., 1973).

Natural resistance to *Haemonchus* spp. in animals not previously exposed, at least post-natally, has been noted. Fourie (1931) reported a great deal of difficulty in producing a sufficient number of typical cases of pure haemonchosis. Urquhart et al. (1962) reported low peak egg counts and low numbers of adult worms at slaughter 30 to 40 days after challenge in 50 per cent of sheep given 10,000 larvae. Brambell et al. (1964) dosed 6 young sheep with 1000,000 larvae but found high numbers of worms in only 1 animal. Bitakaramire (1966) found low numbers of worms in 5 sheep challenged with 50,000 larvae. Christie (1970) demonstrated the ability of sheep to resist large doses of *Haemonchus* sp. by administering 3,000,000 infective larvae to 3 resistant and 2 worm-free lambs. Christie (1970) believed age was a very important factor in classifying natural resistance. This was substantiated by Dineen et al. (1965) and Wagland and Dineen (1967) where 27 total deaths among 58 lambs aged 2 to 4 months were attributed to haemonchosis. Dineen and Wagland (1966) had no deaths among 40 lambs aged 7 months given similar doses of infective larvae.

Blood, Serum, Mucous and Immunoglobulin Proteins

Harris and Warren in 1955 described 3 types of hemoglobin proteins in ewes: 1) a fast moving hemoglobin, 2) a slow moving hemoglobin and 3) a combination of the faster and slower hemoglobins. Subsequently, Evans et al. (1956) labeled these types as Hb A, Hb B and Hb AB, respectively. These hemoglobin types are genetically determined by simple

Mendelian relationships, Hb A and Hb B being allelic and co-dominant (Evans et al., 1956; Huisman et al., 1965). Another hemoglobin type, Hb C (first designated Hb N) has been reported, but was usually found in either young lambs (less than 1 month of age) or animals which are severely anemic (Efremov and Braend, 1966; Vliet and Huisman, 1964). Evans and Whitlock (1964) correlated a relationship between hemoglobin types and packed cell volume; Hb A being greater than Hb B and Hb AB being intermediate. This observation was also substantiated in this study (see Results Table 2).

The first report of the serum proteins found in the normal adult sheep was by Silverstein et al. (1963). Immunoelectrophoresis with rabbit anti-whole adult sheep serum yielded 21 arcs of precipitation. These consisted of prealbumin, albumin, 3 alpha-1 proteins, 6 alpha-2 proteins, 9 arcs in the beta-1 protein area and 4 arcs in the beta-2 protein and gamma-globulin region. The beta-2- and gamma-globulin arcs were similar to those seen in other mammalian sera and were designated beta-2M- (later changed to IgM; W.H.O., 1964), beta-2A- and gamma-globulin. Jonas (1969) examined the immunoglobulin response of sheep to *Salmonella typhimurium* or human erythrocytes by immunoelectrophoresis using antisera from guinea-pigs which had been injected with suspensions of the above antigens treated with sheep sera or various body fluids. He reported two gamma-globulins (fast and slow), IgM, two arcs parallel to the two gamma-globulins, 1 beta-globulin arc and 2 weak alpha-globulin arcs. Preliminary evidence indicates that the last 3 proteins may be components of complement. Subsequent work by Jonas in 1972 using third stage *H. contortus* larvae treated with serum from parasite free or parasitized sheep to produce antisera in rabbits found 3 additional beta-globulins

and 1 additional alpha-globulin. Evidence was presented to indicate that the beta- and alpha-globulins may be components of the sheep complement system.

Silverstein et al. (1963) observed that the typical gamma-globulin showed a "gull wing" appearance, indicative of a fast and slow protein which are different but cross reacting. Leland et al. (1960) after examining sera by electrophoresis from lambs infected with *Trichostrongylus axei* reported various changes in the gamma-1- and 2-globulins associated with the parasitic infection. Jonas (1969) and Jonas et al. (1972) found separate fast and slow gamma-globulins in sheep serum. Tomasi and Bienenstock (1968) reported fast gamma-1- and slow gamma-2- immunoglobulins in bovine colostrum. Jonas (1969) also reported fast and slow gamma-globulin in sheep synovial, pericardiac and Graafian follicle fluid, colostrum and 4-day milk.

Dobson (1966) using sheep infected with *Oesophagostomum columbianum* found intestinal mucous exudate to contain gamma-, alpha- and beta-globulins, albumin and mucoprotein. Antibody titer determined by passive hemagglutination was low in control and high in infected animals especially when mucous came from areas of infection. Electrophoretic patterns from non-infected sheep showed high levels of mucoprotein. After first infected with *O. columbianum* relative concentrations of the mucoprotein diminished because of increased alpha- and beta- proteins. When a second infection was administered, decreasing mucoprotein was caused mainly by an increase in gamma-globulin.

Serum protein changes in sheep with natural or experimental nematode infections (especially *H. contortus*) are frequently recorded. Endrejat

(1956) first compared serum proteins from parasitized and nonparasitized sheep. He reported marked increases in gamma-globulin and decreases in albumin. Shumard et al. (1957) reported an increased albumin to globulin ratio (designated A/G) in lambs with mixed parasite infections. Kuttler and Marble (1960) reported similar results in lambs infected with *T. axei*. Turner and Wilson (1962), Wilson and Turner (1965) and Bradley et al. (1973) also reported decreased A/G ratios in parasitized sheep.

MATERIALS AND METHODS

Experimental Design

This investigation used 47 Florida Native lambs reared worm-free. They were divided into 3 groups according to hemoglobin type (Hb A, Hb B, Hb AB) determined by electrophoresis¹ using cellulose-acetate membranes with tris-ethylenediamine-tetra acetic acid-borate buffer (.13M, pH 8.9-9.3) at 400V for 50 minutes. Each hemoglobin group was randomly divided into 2 sub-groups, an infected and control (non-infected). The Hb A group consisted of 20 animals, 9 infected and 11 controls. The Hb B had 17 animals, 9 infected and 8 controls. The Hb AB group had 10 lambs, 7 infected and 3 controls.

Prior to lambing, 114 ewes in the flock from which the lambs would be selected were examined for worm burdens and tested for hemoglobin type and hemoglobin level. Correlations between the amount of worms, hemoglobin type and hemoglobin levels are presented in the results and discussion sections.

Twelve weeks prior to experimental infection with infective larvae of *H. contortus*, weekly fecal, blood and serum samples were collected for ova examination, hematological observation and serum analysis, respectively. Sampling began at 2.5 months of age and continued until

¹Microzone Electrophoresis System, Beckman Instruments Inc., Fullerton, California.

5.5 months of age when experimental infection occurred. The infection dose was given each lamb based on the equation $(110 + \text{body weight of lamb}) \times \text{body weight of lamb} = \text{number of larvae to use}$. The equation was intended to produce an infective dose which caused a decrease of hematocrit values at 10 days post-infection (Tetzlaff, 1970).

On day 0 (day of infection) 1 lamb (Hb A) was euthanized and necropsied for base line study. Subsequent scheduled necropsy of infected lambs and controls began 1, 7, 12, 16, 21, 26, 30, 33 and 38 days post-infection (Table 1). Blood, serum and fecal samples were

Table 1. Scheduled Necropsy of *H. contortus* Infected Lambs and Controls.

Day 1		Day 7		Day 12	
<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>
14*	A	132*	A	10*	A
19	A	120	A	17	A
147*	B	109*	B	117*	B
128	B	122	B	11	B
15*	AB	27*	AB		
124	AB	139	AB		
Day 16		Day 21**		Day 26	
<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>
118*	A	114*	A	115*	A
110	A	24	A	23	A
21	A	113*	B	22*	B
127*	B	126	B	141	B
134	B			16*	AB
137*	AB	**patency occurs			
Day 30		Day 33		Day 38	
<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>	<u>Lamb No.</u>	<u>Hb Type</u>
111*	A	12*	A	123*	A
9	A	13	A	25	A
121*	B	28*	B	31*	B
30	B	29	B	136*	AB
130*	AB	119*	AB		
112	AB				

*Infected with *H. contortus*.

collected on necropsy days. At necropsy, animals were inspected grossly for any abnormalities. The abomasums were then ligated, separated and collected.

Abomasums were opened and washed (washings collected for parasite examination), with all adult parasites collected and counted. Abomasums were then placed in 50 to 100 ml of 0.85% saline at 4°C for 12 hours. This temperature causes expulsion of the mucous from the goblet cells (Dobson, 1966). The tissues were then placed in HCl-pepsin solution for digestion of tissue and recovery of larval parasite stages (Herlich, 1956). The washings and dissolved tissues were washed through an 100 mesh screen sieve (0.149 mm openings) to collect larvae and adult parasites. Immature parasitic stages of *H. contortus* were identified according to Douvres (1957).

Mucous extracts were concentrated with vacuum dialysis. This process uses 1/4 inch dialysis tubing attached to a funnel placed into a filtering flask and put under vacuum for 24 hours. Measured protein concentrations similar to that of serum were attained by refractometer¹ analysis. Mucous and serum were all stored at -20°C which gives no serum protein changes (Kuttler and Marble, 1959).

Experimental Animals

Florida Native lambs were raised under worm-free conditions in concrete-floored pens. The ewes and their lambs were placed in pens within 24 hours of lambing. Pens and feed and water troughs were washed

¹AO T/C Refractometer, American Optical Instrument Co., Buffalo, New York.

daily to avoid fecal contamination. Individual fecal samples were examined weekly by a modified McMaster technique (Whitlock, 1948) to verify nematode parasite control. After weaning at 60 days of age, blood samples were taken weekly by jugular vein puncture from each lamb. Five ml of blood were collected in a Vacutainer^R ¹ tube containing EDTA as an anti-coagulant and 5 ml collected into a Vacutainer tube without anti-coagulant for serum collection. Ewes and lambs were fed according to National Research Council standards.

Fecal samples of several lambs at 1 month of age revealed *Strongyloides* sp. ova. Therapeutic doses of thiabendazole were given to all lambs. No additional ova were detected until experimental infection. Infection with this parasite was believed not to be through contamination but by pre-natal infection (Pfeiffer, 1962) or through the colostrum or milk as reported in swine (Batte and Moncol, 1966).

Haemonchus contortus Inoculum

Infective larvae of *H. contortus* for use in experimental infections were initially isolated from the University of Florida sheep flock using ova identification techniques (Monnig, 1956) and infective larvae identification (Keith, 1953; Skerman and Hillard, 1966). Infective larvae were tested for viability by first giving them to 2 Finnish Landrace rams which had recently undergone anthelmintic treatment with thiabendazole. Subsequent collection of ova for culturing purposes to obtain larvae for antigen was derived from these rams.

Infective larvae were obtained by fecal-vermiculite cultures at

¹Vacutainer^R Becton, Dickinson and Co., Rutherford, New Jersey.

27°C for 7 days. These cultures were then put into cheesecloth and placed in a Baermann apparatus which consists of a clamped funnel and wire mesh sieve. Warm water is added to the funnel until contact with the cultured material. After several hours, the larvae attracted to the warm water moved through the cheesecloth and collect at the bottom of the funnel where they are drained off into shallow petri dishes for pooling and storage at 10°C. The larvae were washed and allowed to settle, and the dilution adjusted so that each ml of fluid contained 1000 larvae. The lambs were infected with a 40 ml syringe equipped with an 8 inch flexible metal tube that was rubber coated. The tubing was inserted into the esophagus of the lamb and the correct larval dose was expelled. This was followed by passing 1 washing of distilled water through the syringe.

Hematology and Immunology

Hematocrit values were obtained using a microcapillary technique. Microcapillary tubes were filled with blood and sealed at one end with plastic clay and centrifuged at 11,500 r.p.m. for 5 minutes in a Model MB centrifuge.¹ Values were obtained using a microcapillary tube reader² which gave the packed cell volume measured as per cent (%).

For hemoglobin concentration determination, the cyanmethemoglobin method was employed (Anonymous, 1965a). This technique employs the use of 5.0 ml of cyanmethemoglobin reagent mixed with 0.02 ml of blood by

¹International Equipment Co., Needham Heights, Massachusetts.

²Ibid.

inverting several times. The contents are transferred to a cuvette and read against a reagent blank using a spectrophotometer.¹ The wavelength used is 540 m μ and the reading converted into grams of hemoglobin per 100 ml (Hb grams%) of blood using a standard curve.

Mucoprotein and serum protein fractionation was carried out by electrophoresis² on cellulose acetate membranes using a barbital buffer (pH 8.6) at 300V for 30 minutes. After a staining and clearing process (Anonymous, 1965b), membranes were scanned on a densitometer³ which produced density curves. These curves were divided into areas representing discrete fractions (mucoprotein, albumin, alpha-, beta- and gamma-globulins) in which the area under the curve could be determined, giving relative percents (%) of these proteins. Total protein was determined by refractometer,⁴ thus giving relative amounts of the fractions (mg/ml).

Antibody titers in serum and mucous exudate were measured by indirect hemagglutination (IHA). This test involves the use of erythrocytes coated with the antigen for which the animal has made antibodies or with which the antibodies will cross-react. If the serum has activity through a series of dilutions, the erythrocytes will

¹G. K. Turner Associates, Palo Alto, California.

²Microzone^R Electrophoresis System, Beckman Instruments, Inc., Fullerton, California.

³Model R-110, Beckman Instruments Inc., Fullerton, California.

⁴AO T/C Refractometer, American Optical Instrument Co., Buffalo, New York.

settle to the bottom of the test wells indicating positive or negative reaction. This test is sensitive (0.003 μg antibody/ml) and can be used in conjunction with other tests (Kagan and Norman, 1974). IHA microtiter test (Kagan and Norman, 1974) was done using Microtiter^R equipment.¹

Immuno-electrophoretic analysis of lamb serum and mucous exudate was performed on electrophoretic apparatus.² Samples were tested for their activity for immunoglobulins (IgG, IgA and IgM), gamma-globulin, beta-globulins, alpha-globulins, albumin and *H. contortus* antigen. Rabbit anti-sheep IgG, gamma-globulin and serum;³ Rabbit anti-ovine globulins and serum;⁴ and Rabbit anti-bovine IgG, IgA and IgM⁵ were used in test analysis. Rabbit anti-bovine immunoglobulins were found to be cross reactive with sheep serum.

Antigen for use in IHA and the diffusion phase of immuno-electrophoresis was obtained from pooled larvae and fresh *H. contortus* adults from necropsied lambs by a modified method described by Dobson (1966). One ml of centrifuged (2000 r.p.m. for 15 minutes) packed larvae were disintegrated using a tissue grinder and then transferred into 5 dram containers with 3 mm glass beads and shaken for three 15-minute intervals

¹Cook Engineering Co., Medical Research Division, Alexandria, Virginia.

²Microzone^R Electrophoresis System, Beckman Instruments, Inc., Fullerton, California.

³ICN Pharmaceuticals, Inc., Cleveland, Ohio.

⁴Colorado Serum Co., Denver, Colorado.

⁵Hiles Laboratories, Inc., Kankakee, Illinois.

on a Vortex Genie Mixer^R.¹ Volume was brought to 5 ml in physiological saline. Similar procedures were used with 200 adult worms brought to 2 ml volume.

Statistical Analysis

Analysis of variance, regression coefficients program and statistics of fit for dependent variables was carried out with the aid of the IBM 360-65 computer at the University of Florida. The "Z" two-tailed test was also used (Mendenhall, 1971). Variables analyzed include PCV, blood hemoglobin levels, serum proteins (albumin, beta-globulin and gamma-globulin), abomasal mucous proteins (albumin and gamma-globulin), ova counts, total protein, serum antibody (larval and adult antigen test). Comparisons made were pre-infection by blood hemoglobin type and pre- and post-infection with regard to infected or non-infected status by (a) Hb type (b) infection (c) Hb type by time (d) time by infection and (e) Hb type by infection.

¹Scientific Products, Inc., Evanston, Illinois.

RESULTS

Hemoglobin Levels and *Haemonchus contortus* Ova Counts from Florida Native Ewes Prior to Lambing

Hemoglobin Levels (gms. %) and ova counts (eggs per gram) of *H. contortus* (based on morphology, Skerman and Hillard, 1966) were taken on 114 ewes divided by hemoglobin type (Hb type). The data is presented in Appendix I. Average values for hemoglobin levels and ova counts, respectively, were Hb A, 10.6 gms. % and 352.6 e.p.g., Hb B, 10.3 gms. % and 324.3 e.p.g. and Hb AB, 10.1 gms. % and 312.8 e.p.g. Statistical comparison between Hb type using the Z test (Mendenhall, 1971) showed no differences in hemoglobin levels or ova counts.

Comparison of Packed Cell Volume, Hemoglobin Level, Albumin, Beta-Globulin, Gamma-Globulin and Total Serum Protein Between Hemoglobin Types in Worm-Free Lambs Prior to Experimental Infection

Sampling data for mean packed cell volume (Appendix II), hemoglobin level (Appendix III) albumin, beta-globulin, gamma-globulin and total serum protein (Appendix IV) prior to experimental infection with *H. contortus* is summarized in Table 2.

Table 2. Statistical Comparison of Packed Cell Volume, Hemoglobin Level, Serum Albumin, Beta-Globulin, Gamma-Globulin and Total Serum Protein Between Blood Hemoglobin Types in Lambs.

Hb Type	No. of Samples	PCV (%)	Hb Level (gms. %)	Means			Total Protein
				Albumin	Beta-Globulin	Gamma-Globulin	
A	19	36.9	15.9	3.0	0.43	1.4	6.1
B	17	30.5	13.3	2.9	0.40	1.2	5.8
AB	10	34.1	15.1	2.9	0.42	1.3	6.0
Overall Means	46	33.9	14.8	2.9	0.42	1.3	6.0

Analysis of variance of the packed cell volumes based on the "F" test found that the blood hemoglobin types were significantly different ($p < 0.01$). Type A was significantly greater than type B ($p < 0.01$), type A was greater than type AB ($p < 0.01$) and type B is less than type AB ($p < 0.01$).

Analysis of blood hemoglobin levels in the lambs, based on the F test, found the blood hemoglobin types to be significantly different ($p < 0.01$). Type A was significantly greater than type B ($p < 0.01$), type AB was greater than B ($p < 0.01$), but types A and AB were not different.

Analysis of the serum proteins (albumin, beta and gamma) showed no differences between the blood hemoglobin types. Analysis of variance of total serum proteins showed types A larger than B ($p < 0.05$) but no differences between types A and AB or B and AB.

Nematode Recovery in Lambs Experimentally Infected with *Haemonchus contortus* from Scheduled Necropsy.

The inoculation doses of infective larvae, recovery of larvae, early 5th stage and adults from abomasal contents, abomasal digest and mucous exudate, the total recovery and percent recovery are shown in

Table 3. Ova were first detected on the 21st day post-infection (see Appendix V). Adult worms were first recovered in low numbers (8 total) on 6/18 which corresponds to 21 days post-infection. Early 5th stage larvae were seen until the end of the experiment period.

Changes in Packed Cell Volume and Blood Hemoglobin Levels in Florida Native Lambs During Experimental Infection with *Haemonchus contortus*.

Mean packed cell volumes and blood hemoglobin levels based on sampling data prior to experimental infection were compared to values taken at necropsy in infected and control animals (see Appendices II and III). The differences between infected and controls were contrasted with and without regard to hemoglobin types.

Figure 1 shows the sequential changes in the packed cell volume in infected and non-infected lambs with regard to hemoglobin type. Testing by use of regression coefficients, analysis of variance and statistics of fit for dependent variables found no differences between blood hemoglobin types but found statistical differences at day 26 and 33 post-infection. These days correspond to a period of marked variation of these points in Figure 2. The "T" test using the sum of squares from the above test found a significant change at day 26, ($p < 0.05$) and at day 33, ($p < 0.1$).

Statistical analysis of blood hemoglobin levels found no difference with respect to hemoglobin type but produced "F" values showing a significant difference ($p < 0.04$) between infected and non-infected animals. Figure 3 illustrates the changes of the hemoglobin levels in the lambs infected and non-infected with *H. contortus* with respect to hemoglobin type. The variability of the levels at each collection period is very marked. Figure 4 shows these shifts without regard to blood hemoglobin

Table 3. Nematode Recovery From Scheduled Necropsy of Lambs Infected with *Hemonchus contortus*

Date	Sheep No. (Type)	Abomasal Contents		Abomasal Digest		Mucous Recovery		Total Recovery		Total Dose	% Recovery
		Larvae ²	Early 5th Adult	Larvae	Early 5th Adult	Larvae	Early 5th Adult	Larvae	Early 5th Adult		
5/29	14 A	0	0	0	0	0	0	0	0	8,856	0.0
	147 B	0	0	0	0	0	0	0	0	7,584	0.0
	15 AB	0	0	0	0	0	0	0	0	8,424	0.0
6/4	132 A	0	0	15	0	114	0	616	15	10,296	6.1
	109 B	0	0	0	0	404	0	404	0	11,744	3.4
	27 AB	0	0	468	28	100	0	568	28	14,136	4.2
6/9	10 A	0	0	4	760	346	0	350	760	6,191	17.9
	117 B	0	0	8	528	212	0	220	528	9,072	8.2
6/13	118 A	0	0	0	966	0	16	0	982	10,200	9.6
	127 B	0	0	0	828	0	30	0	858	12,856	6.5
	137 AB	0	0	0	794	0	0	0	794	8,375	9.5
6/18	114 A	0	0	0	806	5	0	0	806	7,616	10.6
	113 B	0	0	0	170	3	0	0	170	9,471	1.8
6/23	115 A	0	0	0	429	0	0	0	428	8,616	7.7
	22 B	0	12	0	584	304	0	0	584	9,859	9.1
	16 AB	0	0	0	616	50	0	0	616	10,375	6.6
6/27	111 A	0	0	0	192	314	0	0	192	11,375	6.3
	121 B	0	0	0	178	312	0	0	178	11,859	5.7
	130 AB	0	0	0	262	474	0	0	262	10,431	2.1
7/2	12 A	0	0	0	230	316	0	0	230	9,744	6.8
	28 B	0	0	0	200	334	0	0	200	10,431	6.4
	119 AB	0	0	0	328	178	0	0	328	8,856	7.3
7/7	123 A	0	0	0	32	604	0	0	12	10,136	6.3
	31 B	0	116	0	114	762	0	0	114	8,424	10.6
	136 AB	0	0	0	64	552	0	0	64	8,609	7.2

¹Hemoglobin type
²4th stage

Figure 1. Sequential Changes in Packed Cell Volume in Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus* Divided By Hemoglobin Types.

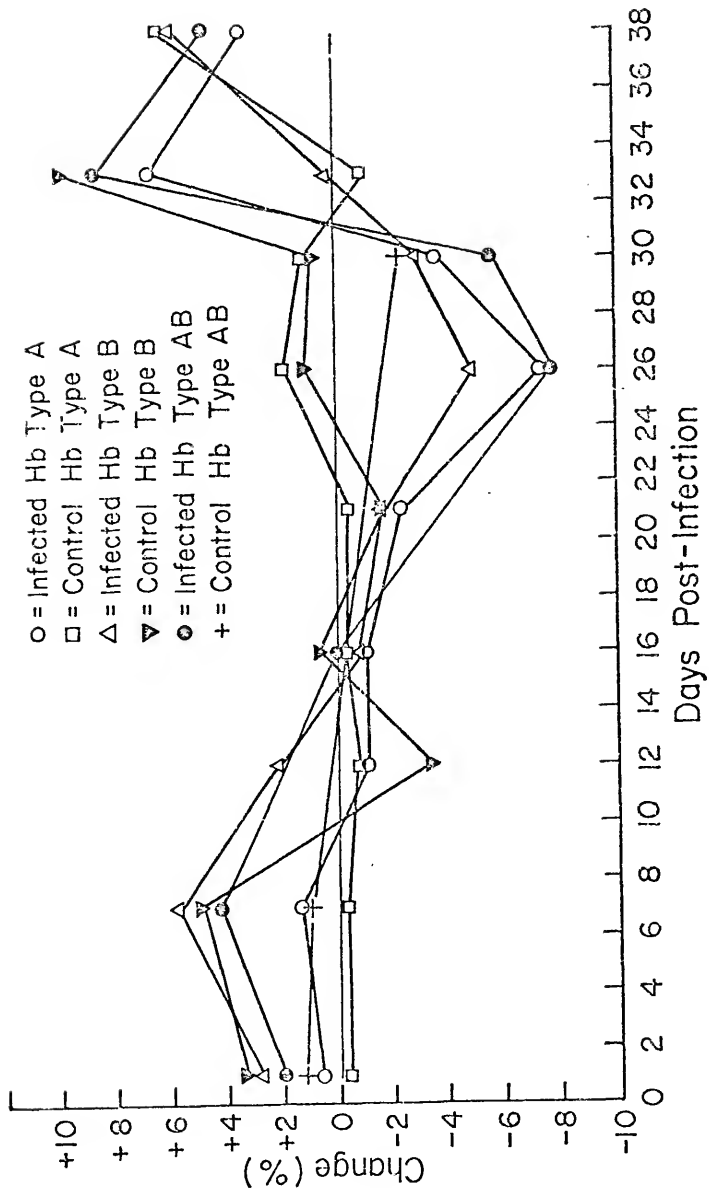


Figure 2. Sequential Changes of Packed Cell Volume in Lambs
Infected and Non-Infected with *Haemonchus contortus*
Without Regard to Hemoglobin Types.

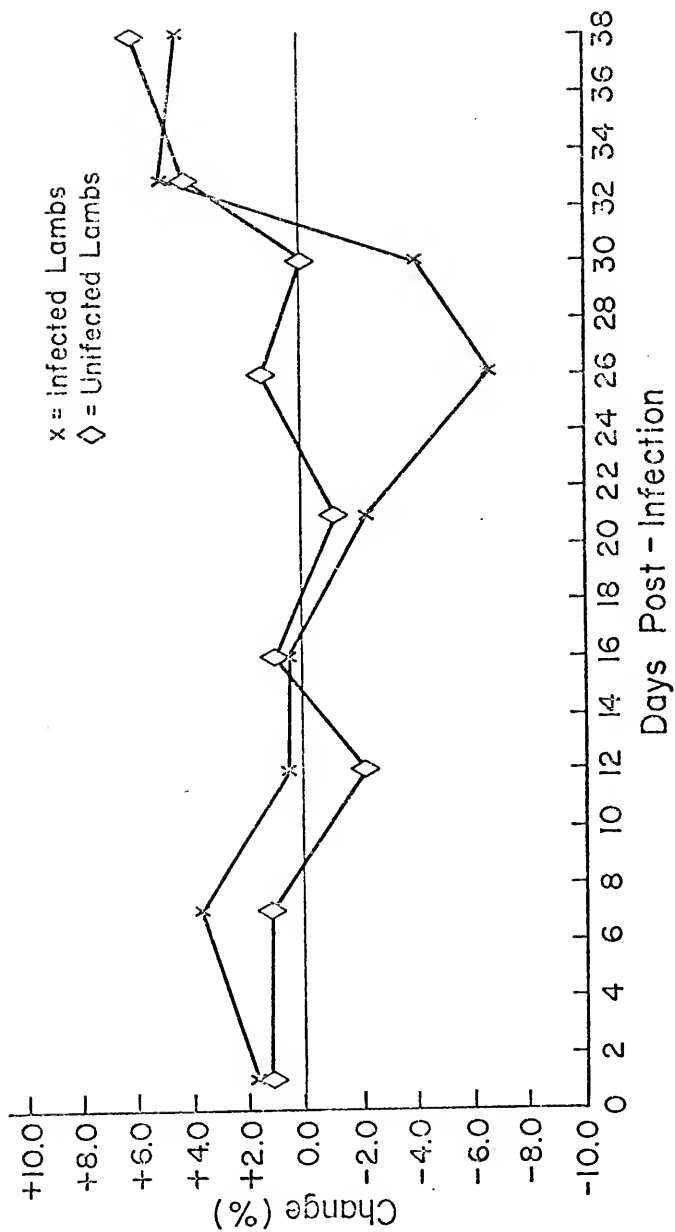


Figure 3. Sequential Changes in Hemoglobin Levels in Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus* Divided by Hemoglobin Types.

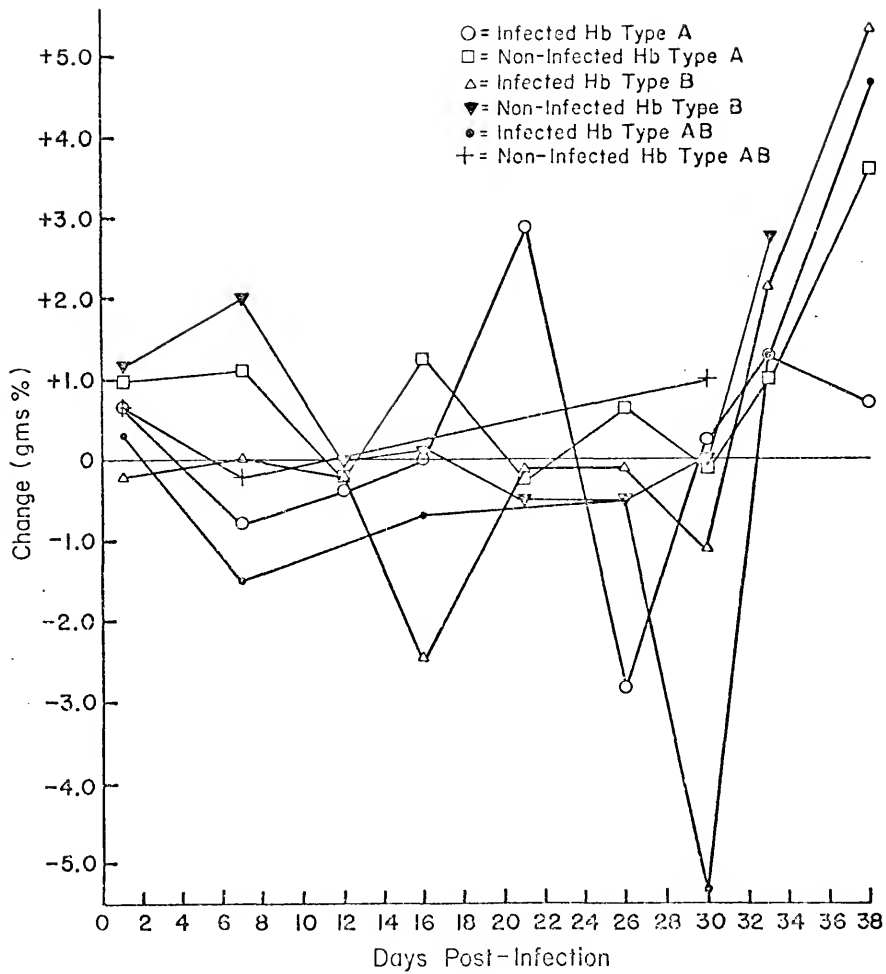
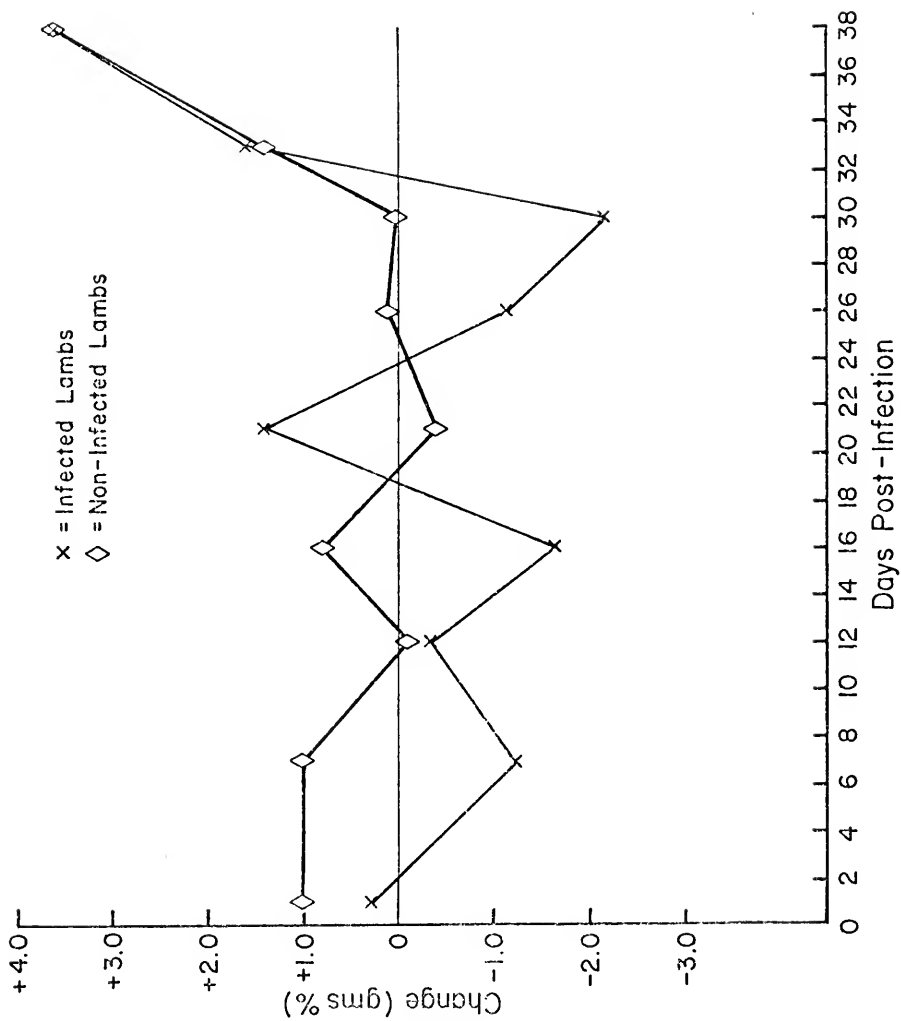


Figure 4. Sequential Changes in Hemoglobin Levels in Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus* Without Regard to Hemoglobin Types.



type. This figure also illustrates that the infected animals had more lower values throughout the infection period. The packed cell volumes and hemoglobin levels both increased after day 30 (see Figures 2 and 4).

Changes in the Serum Proteins in Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

Serum collected prior to infection was compared to serum at the day of necropsy. This data is presented in Appendix IV. The serum protein levels (gms. %) of albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin and gamma-globulin were then statistically analyzed for differences between the infected and control lambs after infection with *H. contortus*.

No significant differences between the infected and control lambs were apparent for albumin, alpha-globulins or beta-globulin. However, there was statistical significance ($p < 0.1$) in the differences of the amounts of gamma-globulins. The infected lambs had consistently higher values than the controls as shown in Figure 5. This can also be seen in Table 4 by looking at the differences between the changes of serum protein values. The analysis also indicated a decrease in the albumin-to-globulin ratio in infected lambs, with no relationship to hemoglobin type. Figure 6 shows this ratio decrease by hemoglobin type along with the average values (without regard to hemoglobin type).

The mean percentages of the serum proteins at each necropsy period are presented in Table 5. Significant trends are masked since comparisons of the animals prior to infection are not included as above and the differences in total proteins are not taken into account. The infected animals overall had lower albumin and higher gamma-globulin percentages.

Figure 5. Changes of Serum Gamma-Globulin in Florida Native
Lambs Infected and Non-Infected with *Haemonchus*
contortus Without Regard to Hemoglobin Types.

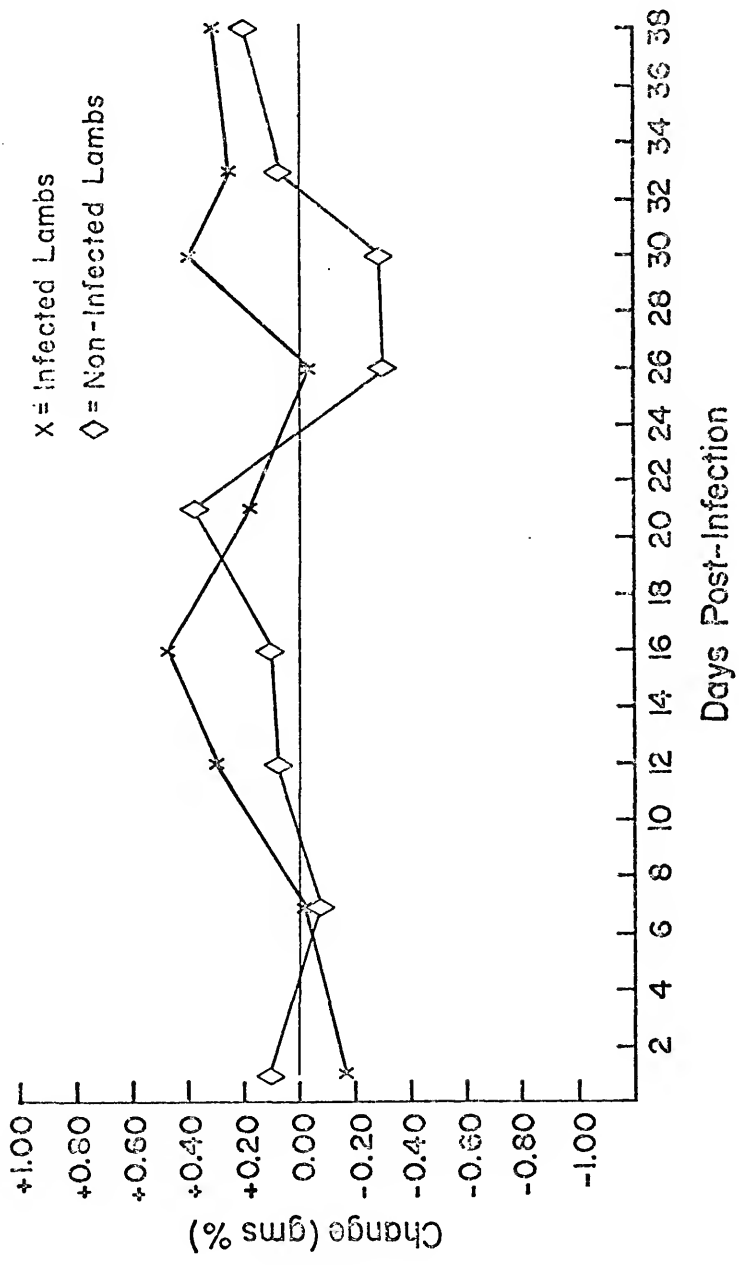


Table 4. Differences Between the Changes of Serum Proteins of Lambs Infected and Non-Infected with *Haemonchus contortus*

Sheep Type ¹	Post-Infection (Days)	Electrophoresis Values (gms %)					Gamma Average	A/G Ratio	A/G Average
		Albumin	Alpha-1	Alpha-2	Beta	Gamma			
A	1	+0.51	+0.96	-0.22	+0.07	+1.07		+1.68	
B	1	-0.43	-0.18	-0.26	+0.52	+0.03	-0.26	-0.37	+0.31
AB	1	-0.34	-0.28	-0.29	-0.05	+0.26		-0.38	
A	7	-0.23	-0.08	-0.10	-0.03	+0.22		-0.72	
B	7	+0.04	+0.09	+0.02	+0.26	+0.09	+0.03	-0.20	-0.12
AB	7	+0.40	-0.14	+0.16	-0.08	-0.21		+0.55	
A	12	+1.31	+0.09	+0.43	-0.71	+0.57	+0.22	+0.06	+0.72
B	12	+0.24	+0.16	-0.25	+0.03	-0.14		+1.49	
A	16	-0.68	-0.15	+0.60	-0.02	+0.54	+0.24	-0.90	-0.30
B	16	+0.37	-0.16	+0.25	+0.03	-0.07		+0.31	
A	21	-0.81	+0.15	+0.23	+0.59	-0.22	-0.18	+0.53	+0.25
B	21	-0.40	+0.10	+0.03	+0.17	-0.14		-0.04	
A	26	-0.11	+0.04	0.00	+0.03	+0.43		-0.90	
B	26	+0.72	-0.25	-0.15	+0.20	+0.17	+0.32	+0.20	-0.35
AB	26	-0.42	-0.10	+0.10	-0.19	+0.35		-0.46	
A	30	-0.06	+0.08	+0.22	-0.04	+0.60		-0.73	
B	30	-0.37	-0.15	+0.13	+0.21	+0.77	+0.29	-2.00	-0.63
AB	30	+0.34	-0.10	-0.54	+0.07	-0.50		+0.85	
A	33	-0.74	-0.03	-0.15	-0.27	+0.14		-0.59	
B	33	-0.81	-0.03	-0.19	-0.10	+0.09	+0.35	-0.66	-0.76
AB	33	-0.18	-0.15	+0.30	-0.18	+0.82		-1.04	
A	38	+0.21	+0.02	-0.46	-0.41	+0.70		-0.51	
B	38	-0.81	+0.10	+0.03	+0.05	+0.11	+0.32	-0.88	-0.63
AB	38	-0.69	+0.10	+0.06	+0.05	+0.16		-0.49	

¹ Hemoglobin type

Figure 6. Differences in the Changes of Albumin-to-Globulin
Ratio of Lambs Infected and Non-Infected with
Haemonchus contortus.

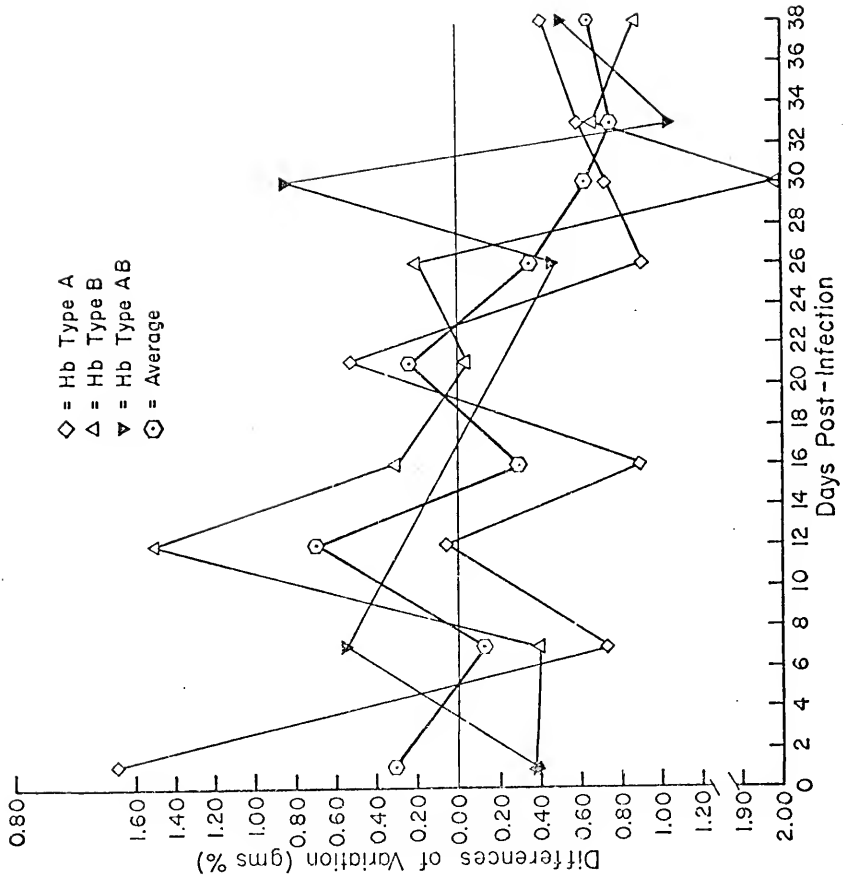


Table 5. Mean Percentages of Serum Proteins from Lambs Infected and Non-Infected with *Haemonchus contortus*.

	No. of Lambs	Day Post-Infection	Electrophoresis Values (%)			
			Albumin	Alpha	Beta	Gamma
Infected	3	1	42.0	23.9	11.8	22.3
Control	3	1	45.7	26.6	7.3	20.4
Infected	3	7	49.8	20.4	8.4	21.4
Control	3	7	53.9	21.2	7.2	17.8
Infected	2	12	50.2	19.2	9.2	21.3
Control	2	12	48.1	19.8	12.4	19.6
Infected	3	16	48.2	21.0	8.1	22.7
Control	3	16	46.3	20.3	10.2	23.3
Infected	2	21	45.8	22.5	8.4	23.1
Control	2	21	45.6	19.7	7.1	24.1
Infected	3	26	54.6	19.1	7.5	18.8
Control	2	26	50.7	20.5	7.7	21.0
Infected	3	30	41.7	22.8	9.2	26.3
Control	3	30	52.0	20.6	9.4	18.0
Infected	3	33	42.2	20.7	8.5	28.8
Control	2	33	49.9	20.5	8.8	20.8
Infected	3	38	44.4	17.8	7.6	27.0
Control	1	38	38.0	24.0	9.0	29.0
Total Infected	25	--	46.4	22.0	8.7	23.6
Total Control	21	--	48.6	21.5	9.0	20.9

Proteins in Abomasal Mucous Exudate from Lambs Infected and Non-Infected with *Haemonchus contortus*

Mean electrophoretic values of proteins found in abomasal mucous exudate during sequential necropsy are presented in Table 6. Total data is recorded in Appendix VI. Areas corresponding to albumin, alpha-globulin, beta-globulin and gamma-globulin were observed as was a large protein area designated as mucoprotein. This mucoprotein migrated within the area of the alpha and beta-globulins. Common characteristic electrophoretic patterns of the abomasal mucous are shown in Figure 7. The pattern in Figure 7a represents the albumin (1), alpha (2), beta (3), and gamma (4) protein areas. Note the migration of mucoprotein into the beta area. The pattern in Figure 7b exhibits migration of the mucoprotein past the beta area where it infringes on the alpha protein area, while Figure 7c shows a pattern with low amounts of mucoprotein.

The albumin, mucoprotein and alpha-globulin, mucoprotein and beta-globulin and gamma-globulin levels are presented graphically in Figure 8. Statistical analysis produced a significant "F" value ($p < 0.04$) for albumin comparison between the infected and control animals. There was a particular significance of albumin differences at day 30 ($p < 0.08$). No significant differences in gamma-globulin levels of infected versus control lambs were noted.

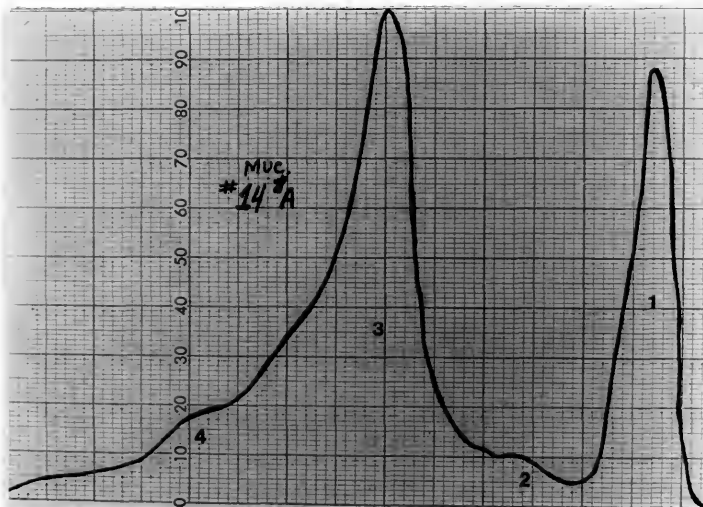
Antibody Evaluation in Serum from Lambs Infected and Non-Infected with *Haemonchus contortus*

Serum obtained at necropsy during the course of infection was tested by indirect hemagglutination (IHA). Sheep erythrocytes used in the test were either coated with antigen derived from adult or larval *H. contortus*. Significant changes in antibody titer between the controls and infected animals were noted using the test with adult antigen ($p < 0.08$) and

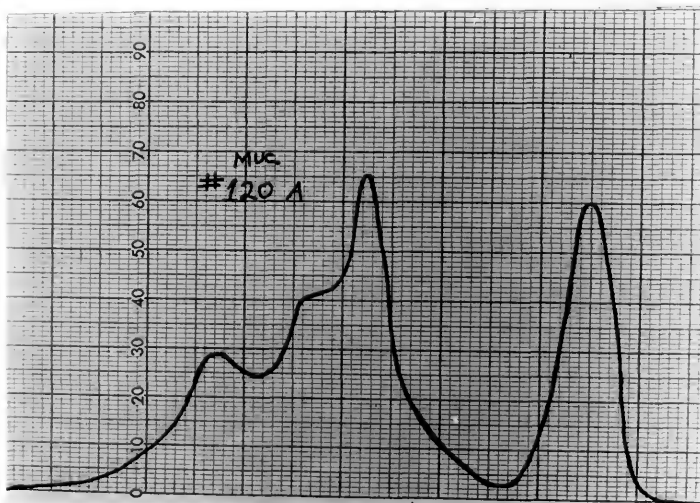
Table 6. Mean Percentages of Proteins in Abomasal Mucous Exudate from Lambs Infected and Non-Infected with *Haemonchus contortus*.

			Electrophoresis Values (%)			
			Albumin	Mucoprotein & Alpha-Globulin	Mucoprotein & Beta-Globulin	Gamma-Globulin
No. of Lambs	Day Post-Infection					
Infected	3	1	39.4	6.3	37.1	17.2
Control	3	1	38.9	9.1	38.6	12.2
Infected	3	7	38.3	10.1	36.6	15.0
Control	3	7	40.4	17.5	21.2	20.9
Infected	2	12	31.2	12.0	40.7	16.0
Control	2	12	33.9	6.9	43.5	16.1
Infected	3	16	36.9	10.4	30.8	21.9
Control	3	16	38.7	8.0	34.5	18.8
Infected	2	21	27.3	4.9	40.1	27.6
Control	2	21	31.7	6.3	38.6	23.3
Infected	3	26	21.8	20.9	34.4	22.9
Control	2	26	38.9	10.1	32.2	18.7
Infected	3	30	17.6	11.3	50.2	20.9
Control	3	30	38.0	9.3	28.2	24.5
Infected	3	33	34.1	10.7	29.2	26.0
Control	2	33	34.5	12.8	31.1	21.5
Infected	3	38	26.8	7.3	37.9	27.9
Control	1	38	34.4	6.1	34.1	25.4
Total Infected	25	--	30.5	10.6	37.2	21.7
Total Control	21	--	37.2	10.0	33.0	19.7

Figure 7. Characteristic Electrophoretic Patterns of Abomasal Mucous Exudate from Lambs Infected and Non-Infected with *Haemonchus contortus*.

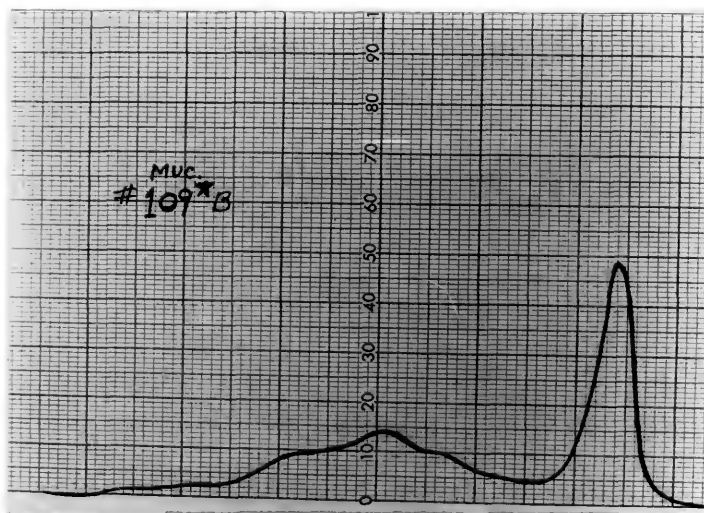


a.



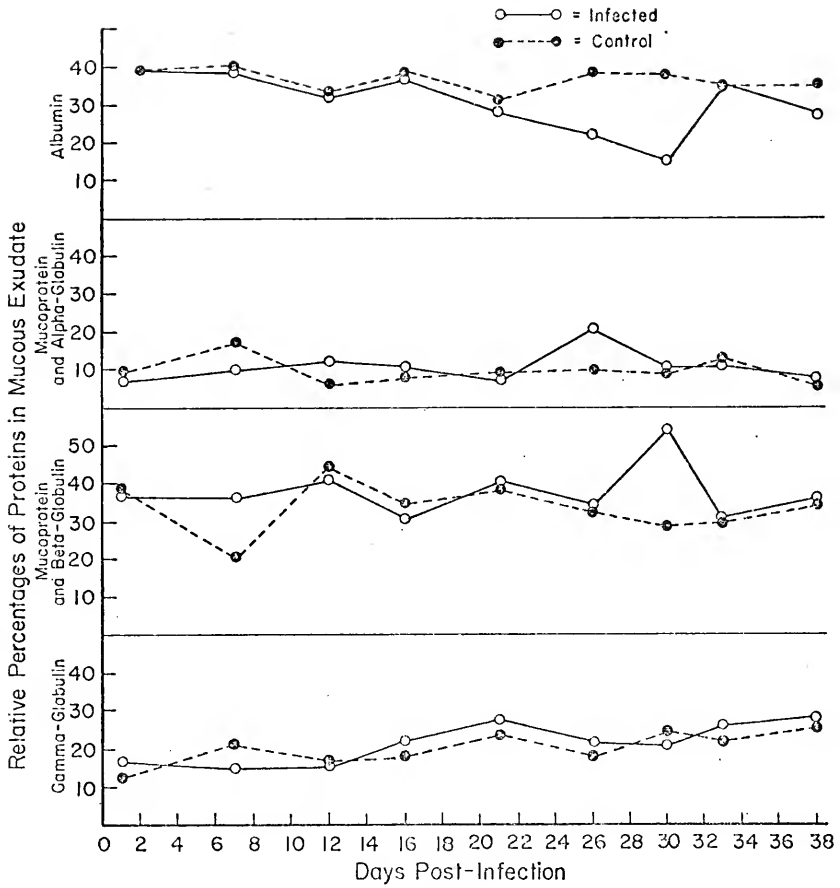
b.

Figure 7. "continued"



c.

Figure 8. Protein Content in Abomasal Mucous Exudate from Lambs Infected and Non-Infected with *Haemonchus contortus*.



larval antigen ($p < 0.008$). The test using larval antigen gave better responses than did the adult antigen. Differences in blood hemoglobin type approaching a significant level of 87%, and a level approaching 81% considering type by time from the tests using larval antigen were noted. Looking at these variations in Figure 9, the differences between Hb A, Hb B and Hb AB (Figures 9a, 9b and 9c, respectively) are actually a type by time relationship not due to one type having a better response. These responses occurred at different times; Type A showing early response, Type B varied response and Type AB having a later response.

Antibody Evaluation in Abomasal Mucous Exudate from Lambs Infected and Non-Infected with *Haemonchus contortus*

Abomasal mucous which had been concentrated to within ranges of serum protein levels was tested by IHA. Significant changes in antibody titer between the controls and infected animals were noted using adult antigen ($p < 0.00$) and larval antigen ($p < 0.004$ Table 7). Differences between blood hemoglobin types were not shown using adult antigen in the IHA test but had some differences at the 89% level using larval antigen. Mean responses of type B were slightly better than types A and AB which were very similar. As with serum antibody, Figures 10a, b and c (Hb A, Hb B and Hb AB, respectively) show similar responses between all types of lambs. Hemoglobin type A had an earlier response, type B had a varied response and type AB had a later response though these were not statistically significant.

Figure 11 plots mucous antibody titer without regard to blood hemoglobin types. Good responses were shown throughout the infection period particularly at day 12 and day 30. Indirect hemagglutination testing using adult antigen gave higher titer response.

Figure 9. Serum Antibody Against *Haemonchus contortus* Larvae and Adults from Sequential Necropsy of Infected and Non-Infected Lambs.

A. Hb Type A; B. Hb Type B; C. Hb Type AB.

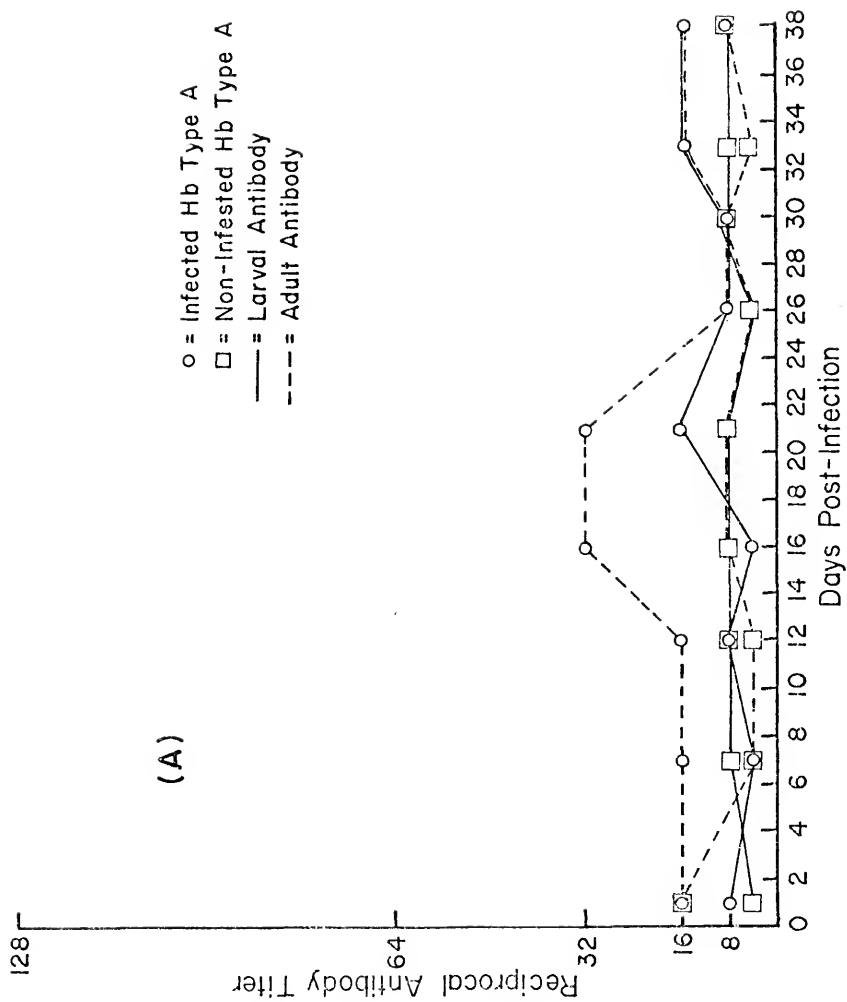


Figure 9. "continued"

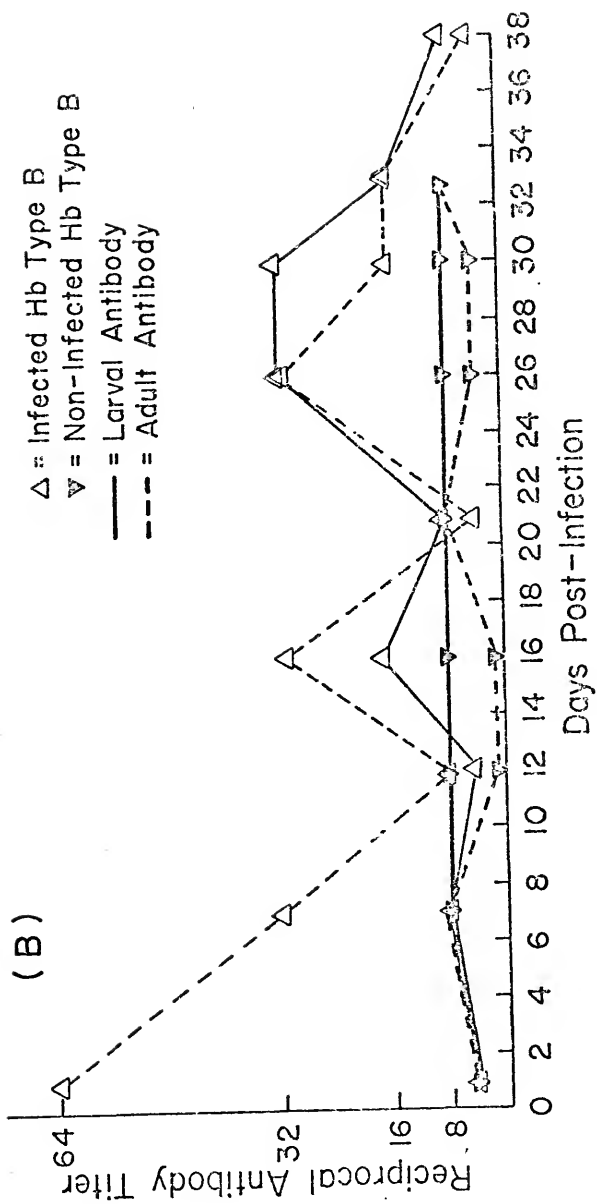


Figure 9. "continued"

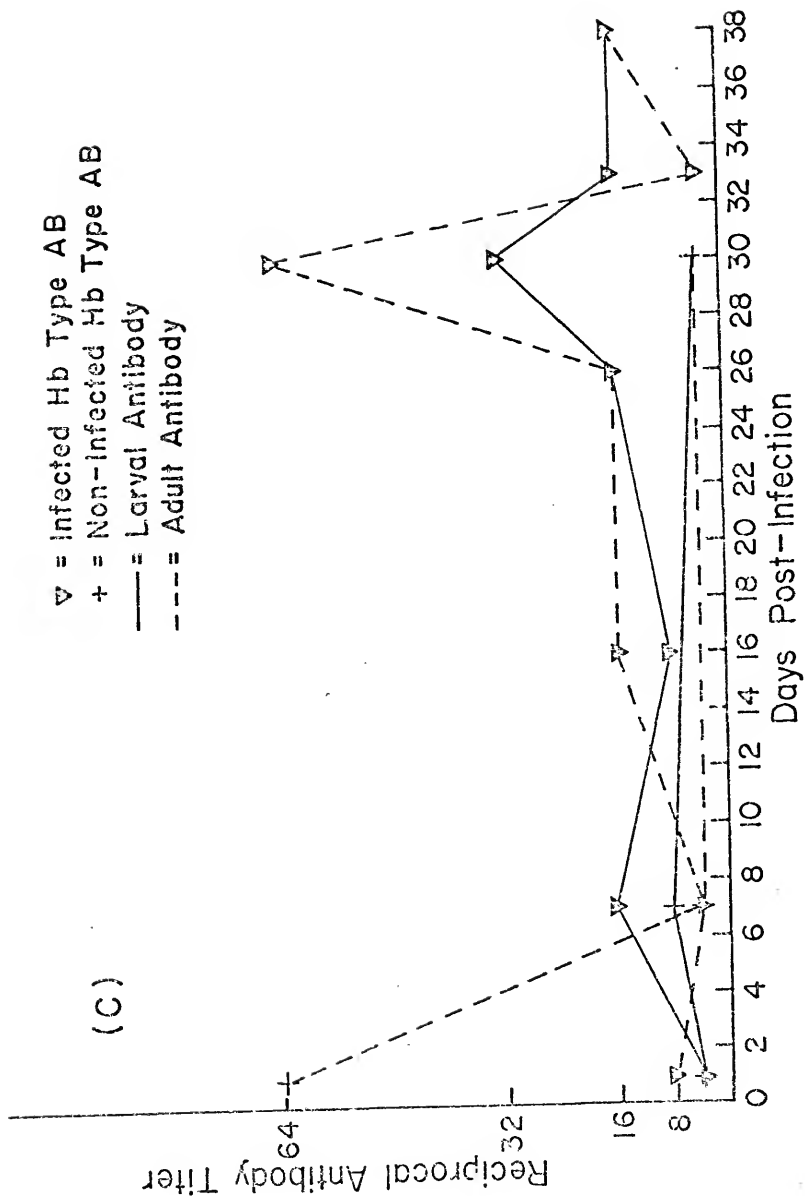


Table 7. Antibody Titer Against *Haemonchus contortus* in Serum and Abomasal Mucous Extraction from Sequential Necropsy of Infected and Non-Infected Lambs.

Date	Sheep No. (Type ¹)	Days Post- Infection	Ova Count	Mucous Titer ²		Serum Titer	
				Larval Antigen	Adult Antigen	Larval Antigen	Adult Antigen
5/29	14* A	1	0	32	64	8	16
	19 A	1	0	8	16	4	16
	147* B	1	0	16	256	4	64
	128 B	1	0	8	8	4	4
	15* AB	1	0	8	32	4	8
	124 AB	1	0	8	32	4	64
6/4	132* A	7	0	8	64	4	16
	120 A	7	0	8	8	8	4
	109* B	7	0	32	128	8	32
	122 B	7	0	16	16	8	8
	27* AB	7	0	128	64	16	4
	139 AB	7	0	8	16	8	4
6/9	10* A	12	0	32	256	8	16
	17 A	12	0	16	16	8	4
	117* B	12	0	16	32	4	8
	11 B	12	C	32	8	8	2
6/13	118* A	16	0	8	64	4	32
	110 A	16	0	16	32	8	8
	21 A	16	0	32	32	8	64
	127* B	16	0	64	128	16	32
	134 B	16	0	16	16	8	2
	137* AB	16	0	64	64	8	16
6/18	114* A	21	400	16	64	16	32
	24 A	21	0	16	16	8	8
	113* B	21	200	32	8	8	4
	126 B	21	0	8	8	8	8
6/23	115* A	26	2,200	32	16	8	8
	23 A	26	0	4	8	4	4
	22* B	26	1,400	64	128	32	32
	141 B	26	0	16	16	8	4
	16* AB	26	1,200	64	64	16	16
6/27	111* A	30	4,400	8	16	8	8
	9 A	30	0	16	16	8	8
	121* B	30	5,600	128	128	32	16
	50 B	30	0	16	16	8	4
	130* AB	30	6,000	128	256	32	64
	112 AB	30	0	8	8	4	4
7/2	12* A	33	7,600	32	32	16	16
	13 A	33	0	32	32	8	4
	28* B	33	5,800	64	32	16	16
	29 B	33	0	16	16	8	8
	119* AB	33	8,200	64	64	16	4
7/7	123* A	38	4,200	128	64	16	8
	25 A	38	0	8	16	8	8
	31* B	38	8,800	16	16	8	4
	136* AB	38	5,600	32	8	16	8

¹ Hemoglobin Type² Reciprocal titers using IHA

*Infected Lambs

Figure 10. Mucous Antibody Against *Haemonchus contortus* Larvae and Adults from Sequential Necropsy of Infected and Non-Infected Lambs.

A. Hb Type A; B. Hb Type B; C. Hb Type AB.

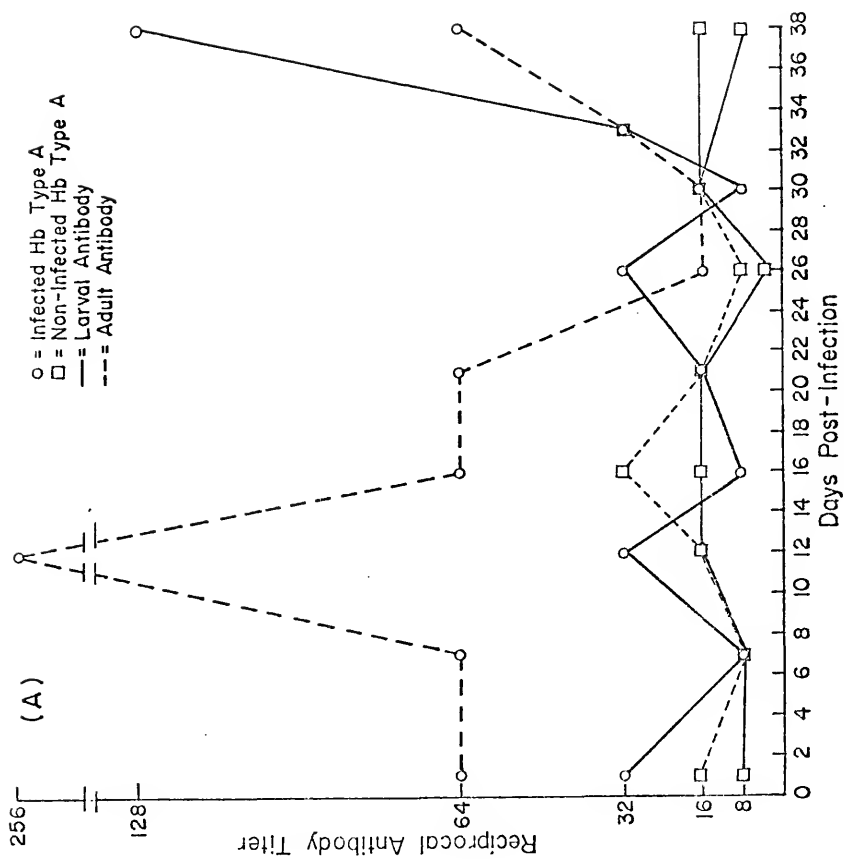


Figure 10. "continued"

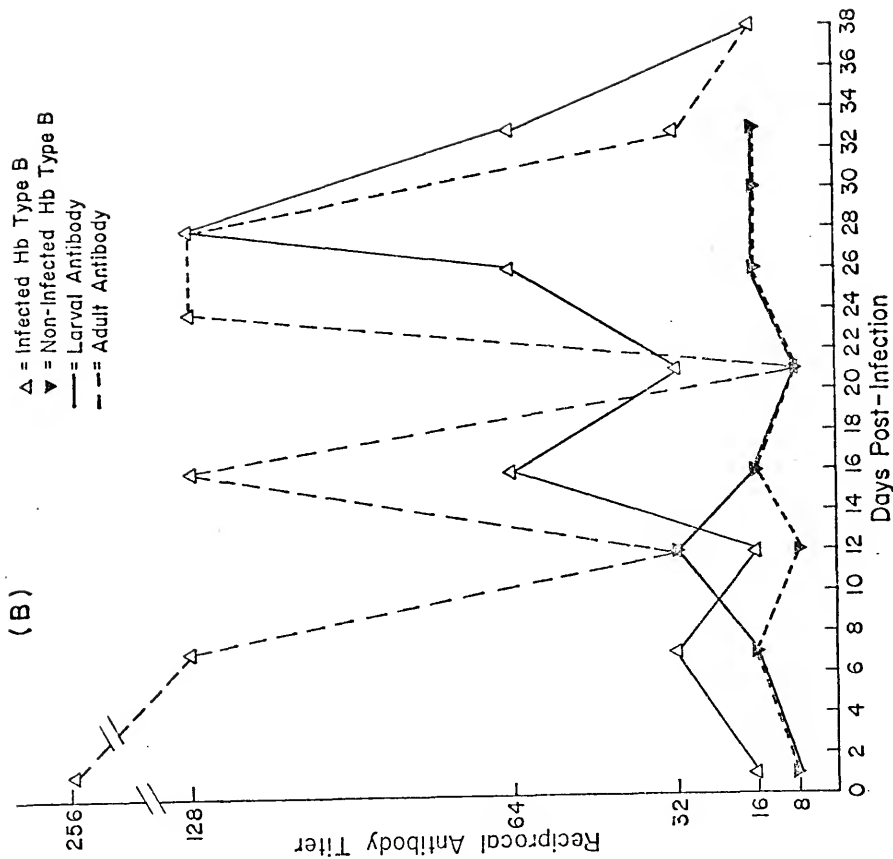


Figure 10. "continued"

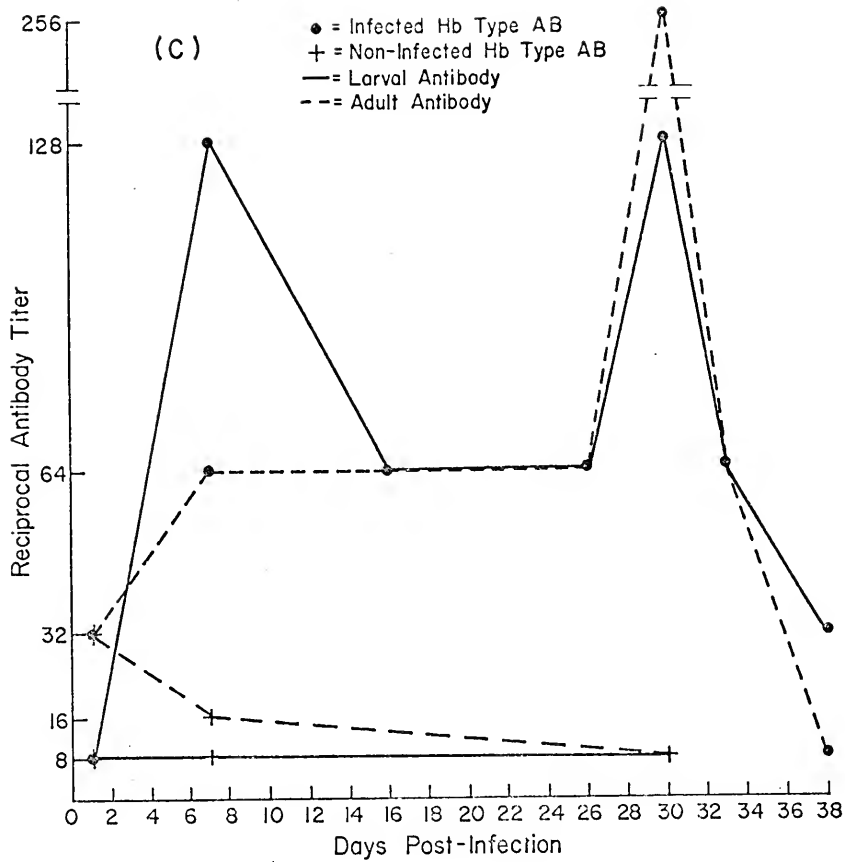
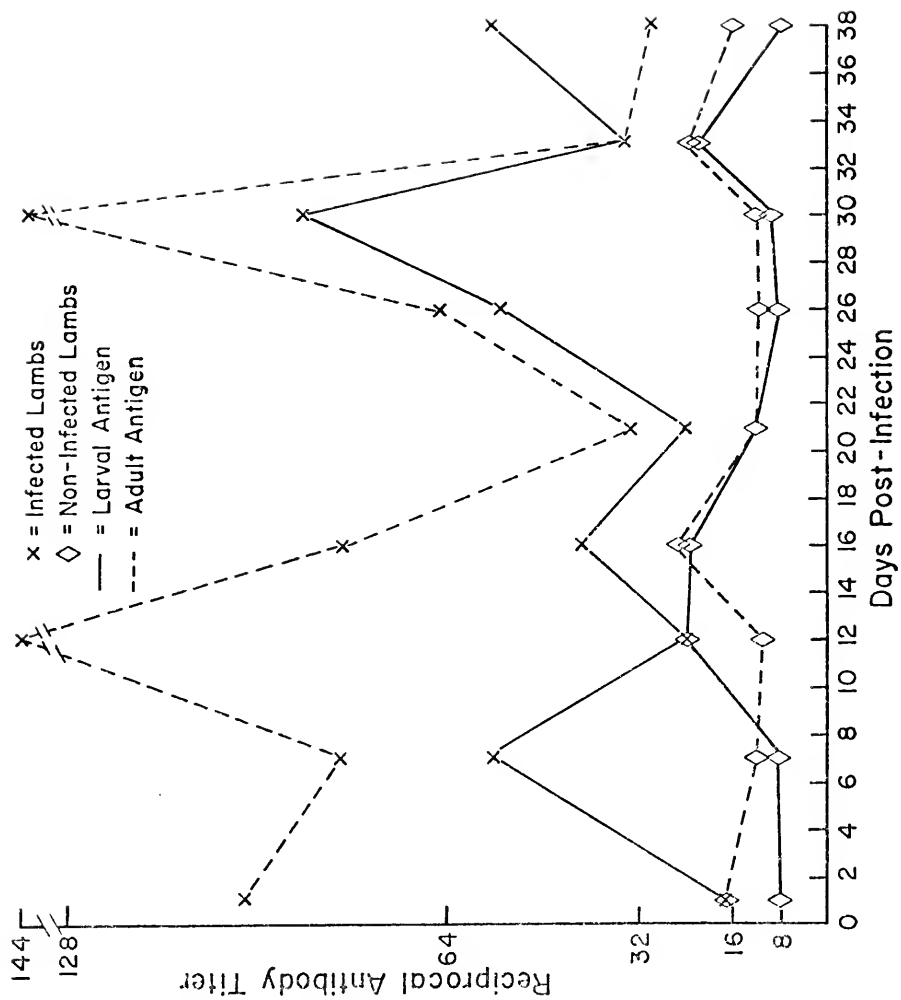


Figure 11. Abomasal Mucous Antibody Levels from Florida Native
Lambs Infected and Non-Infected with *Haemonchus*
contortus at necropsy.



Comparison of Mean Percentages of Proteins in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

Mean levels of the serum proteins as compared to abomasal mucous proteins are presented in Table 8. Substantial differences were seen in the albumin levels, but gamma-globulin was not substantially different. Alpha and beta proteins could not be compared due to the presence of mucoprotein.

Comparison of Antibody Titers in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

The results of IHA testing of serum and abomasal mucous exudate from lambs infected and non-infected with *H. contortus* are presented in Table 7. Higher titers were observed in the mucous than serum. Indirect humagglutination testing using adult antigen gave better responses than did larval antigen. There was no substantial upswing of titer levels at any point during infection, but there did appear to be a grouping of slightly higher responses at days 26 and 30. This time period corresponds to the time just after the parasite reaches patency.

Immunoelectrophoretic Characterization of Antibody and Proteins in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

The results of the immunoelectrophoretic analysis for immunological responses: gamma-globulin, IgG, IgA and IgM in serum and abomasal mucous are given in Tables 9 and 10, respectively. In both the sera and mucous, a strong reaction to anti-gamma-globulins and IgG was noted. The sera had no detectable reaction against IgA but good response for IgM, while the mucous had good IgA response and no detectable IgM response. In these instances responses appeared stronger in infected animals. There were no detectable responses in serum against antigen made from *H. contortus* adults or larvae. There were responses shown in mucous against

Table 8. Mean Percentages of Proteins in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*.

	No. of Lambs	Serum			Abomasal Mucous		
		Albumin	C.I.*	Alpha	Beta	Gamma	C.I.*
Serum (Infected)	25	46.4	±2.2	22.0	8.8	23.6	±2.2
Serum (Control)	21	48.6	±2.2	21.5	9.0	20.9	±1.6
		Mucoprotein		Mucoprotein & Beta		Mucoprotein	
		Albumin	C.I.	Alpha	Beta	Gamma	C.I.
Mucous (Infected)	25	30.5	±4.4	10.6	37.2	21.7	±2.5
Mucous (Control)	21	37.2	±3.0	10.0	33.0	19.7	±2.7

*95% Confidence Interval

Table 9. Immunoelectrophoretic Analysis of Serum from Sequential Necropsy of Lambs Infected and Non-Infected with *Haemonchus contortus*.

Date	Sheep No. (Type) ¹	Gamma- Globulin	IgG	IgA	IgM	<i>H. contortus</i> antigen	
						Adult	Larvae
5/29	14* A	+++	+++	--	+	--	--
	147* B	+++	+++	--	+	--	--
	15* AB	+++	+++	--	+	--	--
	19 A	+++	+++	--	+	--	--
	128 B	+++	+++	--	+	--	--
	124 AB	+++	+++	--	+	--	--
6/4	132* A	+++	+++	--	++	--	--
	109* B	+++	+++	--	++	--	--
	27* AB	+++	+++	--	+	--	--
	120 A	+++	+++	--	+	--	--
	122 B	+++	+++	--	++	--	--
	139 AB	+++	+++	--	+	--	--
6/9	10* A	+++	+++	--	+	--	--
	117* B	+++	+++	--	+	--	--
	17 A	+++	+++	--	+	--	--
	11 B	+++	+++	--	+	--	--
6/13	118* A	+++	+++	--	++	--	--
	127* B	+++	+++	--	+	--	--
	137* AB	+++	+++	--	+	--	--
	110 A	+++	+++	--	+	--	--
	21 A	+++	+++	--	++	--	--
	134 B	+++	+++	--	++	--	--
6/18	114* A	+++	+++	--	+	--	--
	113* B	+++	+++	--	+	--	--
	24 A	+++	+++	--	+	--	--
	126 B	+++	+++	--	+	--	--
6/23	115* A	+++	+++	--	+	--	--
	22* B	+++	+++	--	+	--	--
	16* AB	+++	+++	--	++	--	--
	23 A	+++	+++	--	+	--	--
	141 B	+++	+++	--	++	--	--
6/27	111* A	+++	+++	--	+	--	--
	121* B	+++	+++	--	+	--	--
	130* AB	+++	+++	--	+	--	--
	9 A	+++	+++	--	+	--	--
	30 B	+++	+++	--	+	--	--
	112 AB	+++	+++	--	+	--	--
7/2	12* A	+++	+++	--	++	--	--
	28* B	+++	+++	--	+	--	--
	119* AB	+++	+++	--	+	--	--
	13 A	+++	+++	--	+	--	--
	29 B	+++	+++	--	+	--	--
7/7	123* A	+++	+++	--	++	--	--
	31* B	+++	+++	--	+++	--	--
	136* AB	+++	+++	--	++	--	--
	25 A	+++	+++	--	+	--	--

¹Hemoglobin type

+++ = strong precipitation line
 ++ = good precipitation line
 + = detectable precipitation line
 -- = no detectable reaction

Table 10. Immunoelectrophoretic Analysis of Abomasal Mucous from Sequential Necropsy of Lambs Infected and Non-Infected with *Haemonchus contortus*.

Date	Sheep No. (Type) ¹	Gamma- Clobulin	IgG	IgA	IgM	<i>H. contortus</i> Antigen	
						Adult	Larvae
5/29	14* A	+++	+++	++	--	--	--
	147* B	+++	+++	+	--	--	--
	15* AB	+++	+++	++	--	+	--
	19 A	+++	+++	+	--	--	--
	128 B	+++	+++	--	--	+	--
	124 AB	+++	+++	+	--	--	--
6/4	132* A	+++	+++	++	--	+	+
	109* B	+++	+++	++	--	+	+
	27* AB	+++	+++	+	--	--	--
	120 A	+++	+++	++	--	--	--
	122 B	+++	+++	+	--	--	--
	139 AB	+++	+++	++	--	--	--
6/9	10* A	+++	+++	++	--	--	--
	117* B	+++	+++	++	--	+	--
	17 A	+++	+++	++	--	+	--
	11 B	+++	+++	+	--	+	--
6/13	118* A	+++	+++	+	--	--	--
	127* B	+++	+++	+	--	--	--
	137* AB	+++	+++	++	--	--	--
	110 A	+++	+++	+	--	--	--
	21 A	+++	+++	++	--	--	--
	134 B	+++	+++	++	--	+	--
6/13	114* A	+++	+++	+	--	+	--
	113* B	+++	+++	++	--	+	--
	24 A	+++	+++	++	--	+	+
	126 B	+++	+++	+	--	+	+
6/23	115* A	+++	+++	++	--	--	--
	22* B	+++	+++	+	--	--	--
	16* AB	+++	+++	++	--	--	--
	23 A	+++	+++	+	--	--	--
	141 B	+++	+++	+	--	--	--
6/27	111* A	+++	+++	+	--	--	--
	121* B	+++	+++	++	++	++	--
	130* AB	+++	+++	++	+	+	+
	9 A	+++	+++	+	--	--	--
	30 B	+++	+++	+	--	--	--
	112 AB	+++	+++	++	--	--	--
7/2	12* A	+++	+++	++	--	+	--
	28* B	+++	+++	+	--	+	--
	119* AB	+++	+++	+	--	--	--
	13 A	+++	+++	+	--	--	--
	29 B	+++	+++	++	--	--	--
7/7	123* A	+++	+++	+	--	+	+
	31* B	+++	+++	++	--	+	+
	136* AB	+++	+++	++	--	+	+
	25 A	+++	+++	+	--	--	--

¹Hemoglobin type

+++ = strong precipitation line

++ = good precipitation line

+ = detectable precipitation line

- = no detectable reaction

adult and larval antigen though more reactions were seen using adult antigen.

Typical immunoelectrophoretic patterns from control lambs are shown in Figure 12. Tests for responses against IgA, IgM, IgG, larval *H. contortus* (L.HC) and sheep serum fractions (A.S.) were developed using anti-sera to the above immunoglobulin classes or proteins. Figure 12a demonstrates that there are good IgM and IgG responses. Anti-sheep serum revealed: the immunoglobulins-G (1) and M (2), 1 additional beta protein, 2 alpha protein arcs (3) and albumin (4). Figure 12b from another non-infected lamb revealed no immunoglobulin-A response or reaction to larval *H. contortus*. The anti-sheep globulins (sheep serum minus albumin) responses revealed: good immunoglobulin-G (1), 3 arcs in the beta protein area (5), one arc being an identity with IgM, 3 alpha protein arcs (3) and albumin (4).

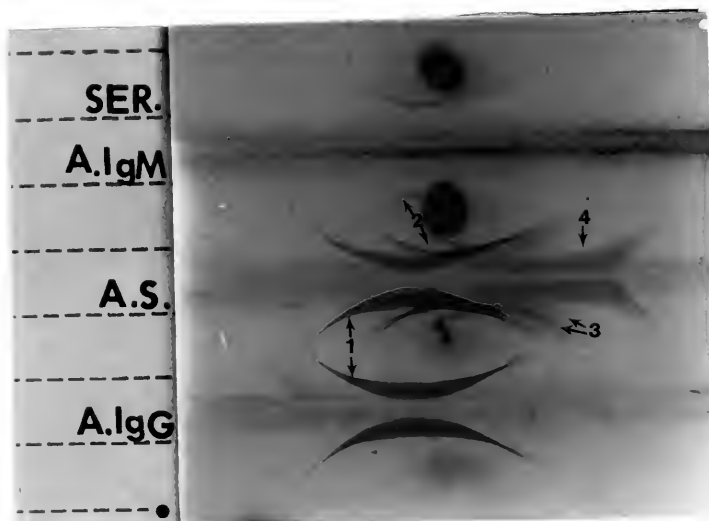
The patterns in Figure 13a from infected Florida Native lamb sera demonstrated good response to IgG. This IgG arc also revealed slow IgG (1) and fast IgG (2). There was an increase to 5 arcs in the beta protein area (3) and only 1 alpha arc (4). Although an increase in precipitation arcs were noted in most infected lambs, some lambs had similar patterns to non-infected lambs (Figure 13b). This figure reveals excellent IgM response (5) and two additional beta-proteins (3) two alpha proteins (4) and albumin (8). There were no detectable reactions to either larval or adult *H. contortus* antigen.

Immunoelectrophoretic patterns developed from abomasal mucous exudate are presented in Figures 14 and 15. Slow (1) and fast (2) IgG, 1 or 2 beta proteins arcs (3), 1 or 2 alpha protein arcs (4) and albumin

Figure 12. Characteristic Immunelectrophoretic Patterns of Serum from Worm-Free Florida Native Lambs.

Abbreviations used:

A.IgA=anti-immunoglobulin A; A.IgG=anti-immunoglobulin G; A.IgM=anti-immunoglobulin M; A.S.=anti-sheep serum; L.HC=larval *H. contortus*.



a.

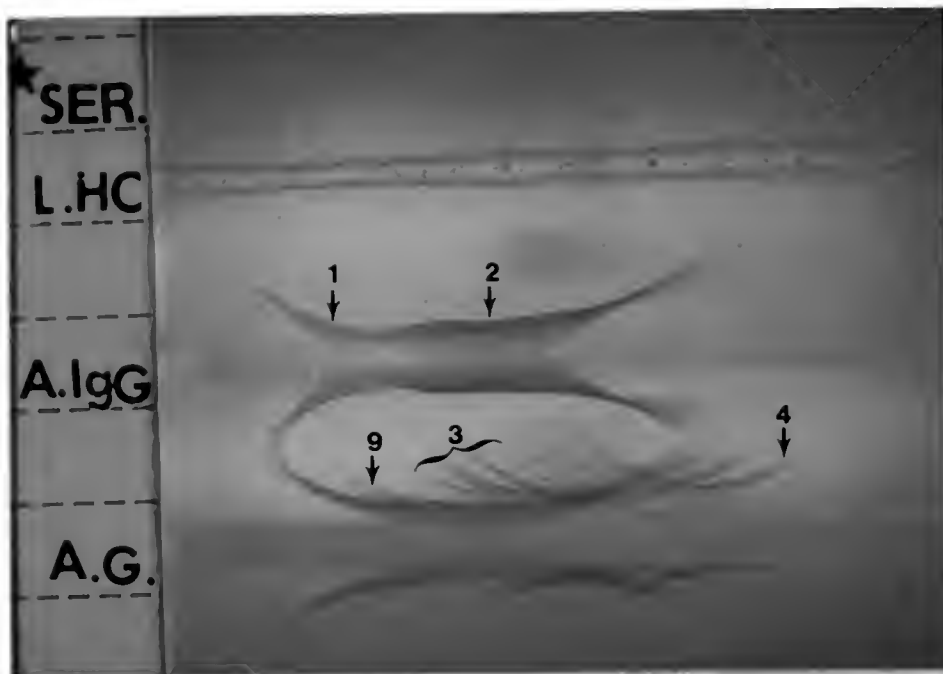


b.

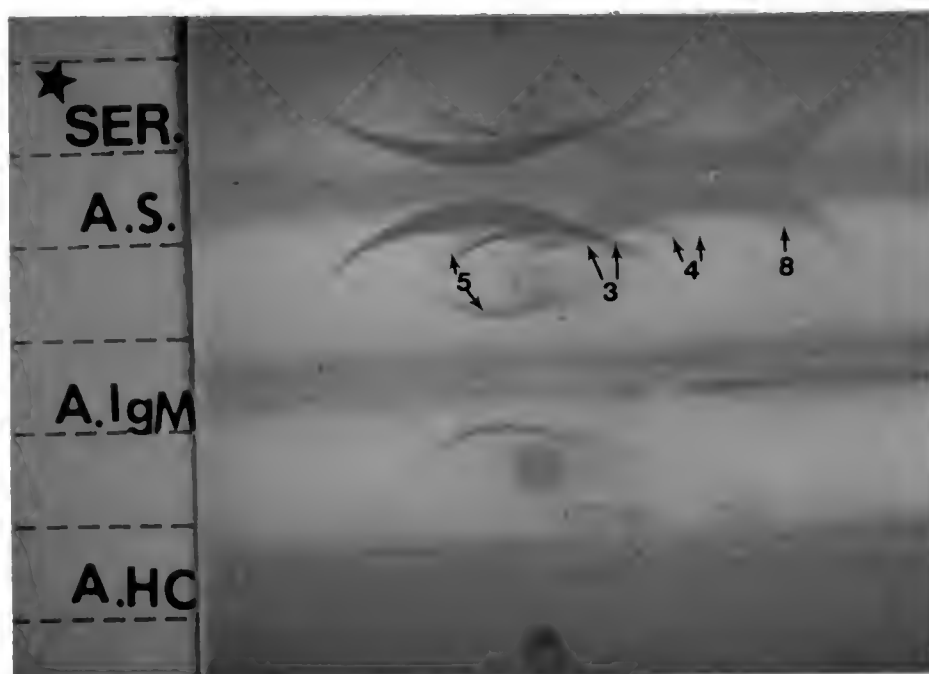
Figure 13. Characteristic Immuno-electrophoretic Patterns of Serum from Florida Native Lambs Infected with *Haemonchus contortus*.

Abbreviations used:

A.IgG=anti-immunoglobulin G; A.IgM=anti-immunoglobulin M; L.HC=larval *H. contortus* antigen; A.G.=anti-sheep globulins (serum minus albumin); A.S.=anti-sheep serum; A.HC=adult *H. contortus* antigen.



a.

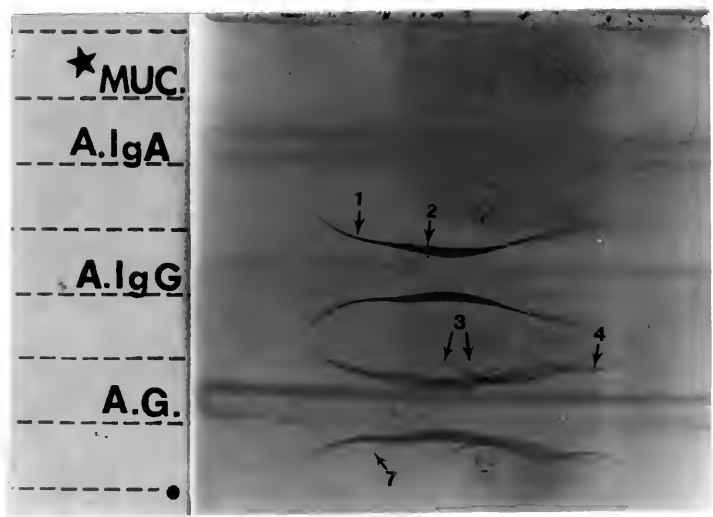


b.

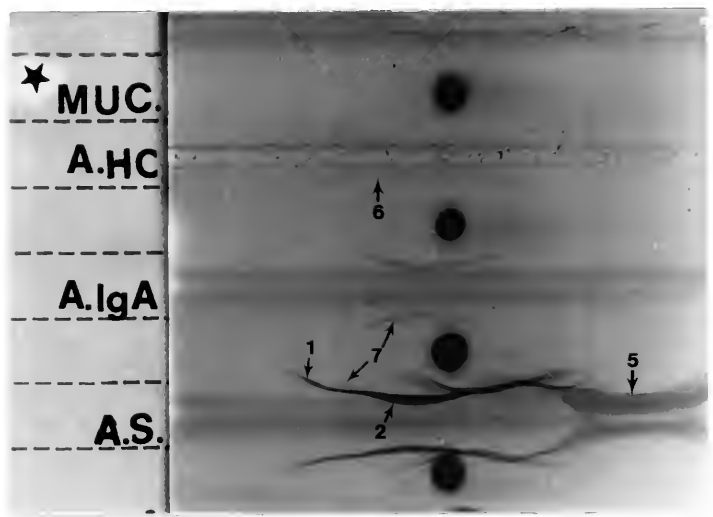
Figure 14. Immuno-electrophoretic Patterns from Abomasal Mucous Exudate in Parasitized Florida Native Lambs.

Abbreviations used:

A.IgA=anti-immunoglobulin A; A.IgG=anti-immunoglobulin G;
A.G.=anti-sheep globulins; A.S.=anti-sheep serum;
A.HC=adult *H. contortus* antigen; L.HC=larval *H. contortus* antigen.

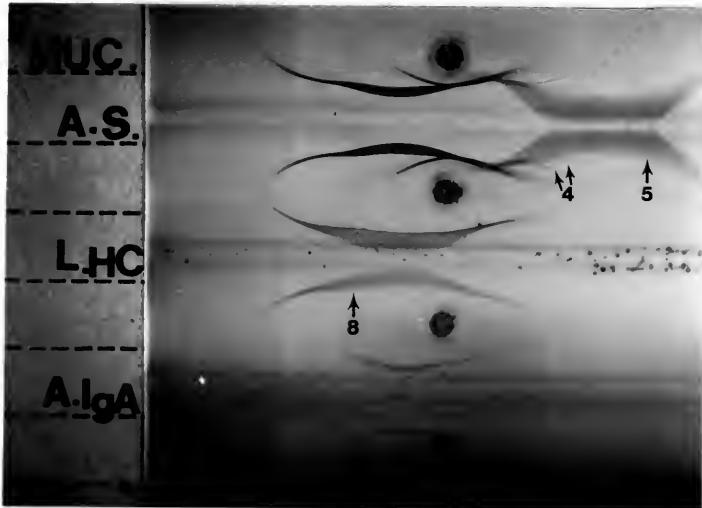


a.



b.

Figure 14. "continued"

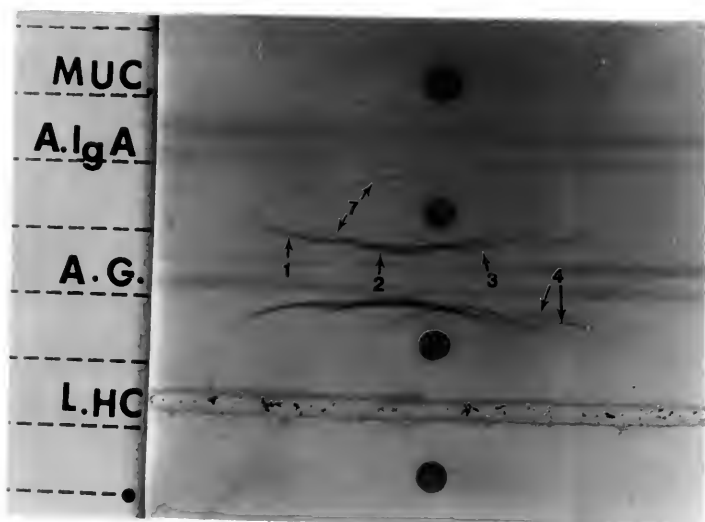


c.

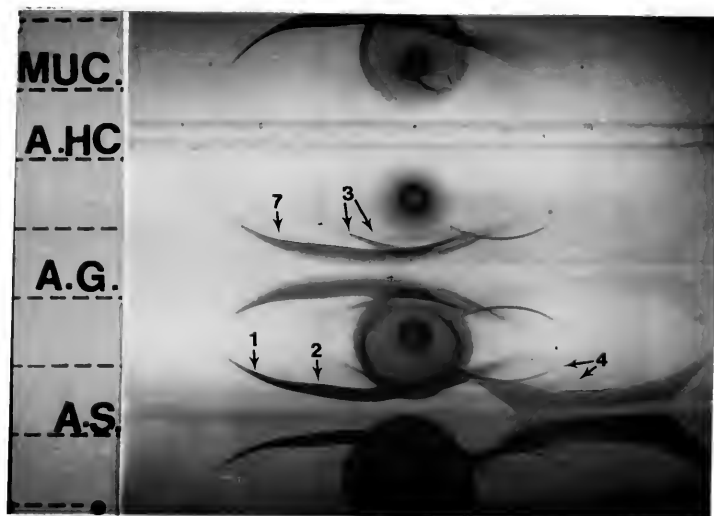
Figure 15. Immunelectrophoretic Patterns from Abomasal Mucous Exudate in Non-Parasitized Florida Native Lambs.

Abbreviations used:

A. IgA=anti-immunoglobulin A; A.G.=anti-sheep globulins;
A.S.=anti-sheep serum; A.HC=adult *H. contortus* antigen;
L.HC=larval *H. contortus* antigen.



a.



b.

(5) were characteristic of infected lambs. Similar patterns were seen in non-infected lambs (Figures 15a and b) except that only 1 beta protein was likely to be seen. Besides IgG, IgA response was excellent and produced an identity with a spur arising from the gamma protein area (Figures 14b, 7 and 15a, 7). An IgM response could not be detected with anti-IgM. Infected lambs also showed response against adult *H. contortus* antigen (Figure 14c, 8).

DISCUSSION

Relationship of Blood Hemoglobin Types to Blood Hemoglobin Levels and Natural Infection with *Haemonchus contortus* in Florida Native Ewes

Evidence of hemoglobin type differences in natural infection of Florida Native Ewes with *H. contortus* as determined by ova counts was not shown in sampling data taken on 114 ewes. This was in disagreement with Evans et al. (1963) and Jilek (1968) who reported fewer *H. contortus* in Hb A than other hemoglobin types. This was in agreement with Radhakrishnan et al. (1972) whose data did not suggest any differences in infection rates by hemoglobin type in Florida Native or Rambouillet sheep. In fact, Radhakrishnan et al. (1972) and Bradley et al. (1973) reported lower adult populations and egg counts in Hb AB than Hb A or Hb B lambs experimentally infected with *H. contortus*. The average blood hemoglobin levels in the three hemoglobin types revealed no differences, suggesting an even distribution of infection among the adult sheep. Perhaps, over time, immunologic factors initially different during first exposures become similar due to constant reexposure. This observation is substantiated by observations reported by Soulsby (1958), Levine et al. (1956), Bradley and Levine (1957) and Levine et al. (1975) in which sheep kept on the same pasture where infective larvae are continuously available have lower worm populations than sheep that are rotated to different pastures or have a non-immune status.

Relationships of Packed Cell Volume, Hemoglobin Level and Serum Proteins to Hemoglobin Types in Worm-Free Lambs

Packed cell volumes between blood hemoglobin types showed significant differences which is in agreement with reported literature. Hemoglobin type A had the highest erythrocyte volume, Hb B the least and Hb AB was intermediate between the other two types. The significance level of the PCV observation is considerably higher than the reports of Radhakrishnan et al. (1972) and Bradley et al. (1973). Reports by Evans and Whitlock (1964) and Jilek and Bradley (1969) indicating lower infection rates in sheep with Hb A are contradictory to the reports of Radhakrishnan et al. (1972) and Bradley et al. (1973) indicating lower helminth egg counts and fewer adult worms in Hb AB. These authors did report higher weight gains in Hb A though the statistical test was ambiguous. Both of these points will be discussed in more detail in a later section.

The blood hemoglobin values substantiate the report by Jilek and Bradley (1969) that Hb A was significantly greater than Hb B, Hb AB was greater than Hb B, but differences between Hb A and Hb AB were slight. Large fluctuations in hemoglobin levels were observed over the periods prior to and after infection (see Figure 3).

Differences in the blood serum proteins could not be correlated to Hb types. The total protein data did give an indication that Hb A was higher in total protein content than Hb B, though no differences were seen between Hb A and Hb AB or Hb B and Hb AB. Possible relationships may exist between higher PCV and hemoglobin levels and the higher total serum proteins in Hb A. These physiologic factors alone might give Hb A sheep the capacity to withstand the effects of *H. contortus*

infection as reported by Evans and Whitlock (1964), Jilek and Bradley (1969) and Bradley et al. (1973). The ability of certain breeds of sheep to resist parasitic infections is considered to be immunologic as well as physiologic in nature. These factors (immunologic and physiologic) are discussed in further detail using the Florida Native Sheep as a model.

Since differences in Hb type and individual animals were noted, experimental data collected after infection must take these facts into consideration. Therefore, a double control system was used. Collected data after infection with *H. contortus* was compared to sampling data prior to infection. These changes in themselves give significant data but to give further creditability, comparisons were also made to non-infected animals handled in a similar manner.

Nematode Recovery in Florida Native Lambs Experimentally Infected with *Haemonchus contortus*

The lower recovery rates in Florida Native lambs (Table 3) initially establishes that some factors are acting to keep infection at a lower than expected level. This is in agreement with similar results reported by Radhakrishnan et al. (1972) and Bradley et al. (1973) in Florida Native lambs.

Discussion of the Changes in Packed Cell Volume, Blood Hemoglobin Level and Serum Proteins in Florida Native Lambs Associated with *Haemonchus contortus* Infection

Maximum blood loss appeared at approximately 26 days after infection according to the results of the packed cell volumes and hemoglobin levels (Figures 2 and 4). Brambell et al. (1964) reported first blood losses in the feces of sheep 6 to 10 days after infection with *H. contortus*, with most blood loss occurring at 22 days. Bradley et al.

(1973) reported maximum blood loss at 24-26 days post-infection. Blood hemoglobin was a more sensitive test for the determination of blood loss since significant statistical differences ($p < 0.04$) were observed between infected and non-infected animals. The increase in PCV and hemoglobin after day 30 post-infection is believed to be a recovery due to increased hemopoiesis due to stimulation from the blood loss.

The increase in gamma-globulin and decreased albumin-to-globulin ratio (Figures 5 and 6) in the infected Florida Native lambs verify similar reports in the literature. Turner and Wilson (1962) and Wilson and Turner (1965) presented evidence that increased gamma-globulin content may be related directly to the degree of resistance. Identification of the serum proteins, immunoglobulins and antibody activity as determined by IHA will be discussed further.

Discussion of Abomasal Mucous Proteins from Lambs Infected and Non-Infected with *Haemonchus contortus*

The percentages of the abomasal mucous proteins are presented for the first time in sheep. These values were similar to those presented by Dobson (1966) for intestinal mucous exudate in Border Leicester x Merino sheep infected and non-infected with *O. columbianum*.

The largest differences between infected and control (non-infected) animals were noted with the albumin protein values. At the 30th day post-infection a significant decrease was noted in infected animals. This time sequence corresponds to shortly after the parasite matures and begins increased blood sucking activity (verified by PCV decrease). This response, coupled with increases in gamma-globulin, is a characteristic response seen in serum and can now be said to also occur in abomasal mucous in sheep.

Discussion of Antibody Activity in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

The activity of antibody in both serum and abomasal mucous increased significantly after experimental infection with *H. contortus*. This is similar to reports in the literature. Indirect hemmagglutination testing used antigen derived from larvae and adult worms. The larval antigen gave higher statistically significant results, although adult antigen gave higher titer responses. Differences in blood hemoglobin type were not observed in analysis of serum antibody activity but significant differences were approached using larval antigen in abomasal mucous titer analysis. The pattern being that Hb A had the earliest response, Hb B was varied and Hb AB was seen later in the infection. Responses verified the presence of antibody activity directed against the parasitic infection. Mucous had significantly higher titers than serum, especially after patency occurred (Figure 11). The area at the site of infection in the abomasum had the highest antibody activity, similar to the results of Dobson (1966) who found highest titers at the site of infection in the small intestine with *O. columbianum*.

Discussion of Immuno-electrophoretic Characterization of Antibody and Proteins in Serum and Abomasal Mucous from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*

In order to identify gamma-globulin responses against *H. contortus* immuno-electrophoretic patterns were developed using antigen (adult and larval) as well as anti-sheep serum, anti-sheep globulins and anti-immunoglobulins (IgA, IgG and IgM). The lack of precipitin reaction from the serum demonstrates that infected animals were not hyperimmunized by their contact with the parasites. This does not mean responses did not occur in the serum as antibody titer reveals reaction did occur.

Silverstein (1963) reported anti-antigen precipitin activity to occur principally in the 7S gamma-globulin (both fast and slow regions). Although antigen reaction was not revealed in serum, reaction was seen in mucous (Figures 14b, 6 and 14c, 8), as was a good gamma-globulin response (Tables 9 and 10). Identification of the gamma-globulins in serum revealed excellent IgG and IgM response. A spur was seen at the junction of the fast and slow IgG in the serum of some Florida Native lambs (Figure 13a, 9). Jonas (1969) also reported spurs at the junction of the fast and slow gamma-globulin in serum and various body fluids. A similar spur was seen in abomasal mucous (see Figures 14b, 7 and 15a, 7) which did form an identity with anti-IgA, indicating that IgA may also be responsible for some immunological response. The reason anti-IgA in serum did not develop a reaction cannot be explained since no immunological differences have been reported between serum and secretory IgA (Tomasi and Bienenstock, 1968). Consequently, these spurs from serum, mucous or body fluids may be similar in appearance, but not identical.

The anti-sheep serum or anti-sheep globulins developed with serum from worm-free lambs revealed a maximum of 8 proteins (Figure 12): slow and fast gamma-globulin, IgM, 2 additional beta proteins, 2 alpha proteins and albumin. Jonas (1969) reported 3 beta proteins (besides IgM) from parasite-free sheep which he designated beta-1, beta-4 and beta-5, and 3 alpha proteins designated alpha-1, alpha-2 and alpha-3. These immunoelectrophoretic patterns that he reported deviated from the patterns shown here in Florida Native lambs possibly because of age or breed differences, making labeling of beta protein arcs from Florida Native

Sheep difficult. These beta proteins were designated beta-1 and beta-2 (after Silverstein, 1968 and Jonas et al., 1972) in descending cathode to anode order as were the alpha proteins, alpha-1 and alpha-2 (Figure 16a). Jonas' et al. (1972) beta-5 protein arc which almost falls in the alpha protein region was not seen in either infected or non-infected lambs.

The 10 protein arcs developed from the serum of parasitized Florida Native lambs are similar to those reported by Jonas et al. (1972). The alpha-3 and beta-5 proteins as Jonas et al. (1972) describes them could not be detected in the Florida Native lamb. The proteins detected in these lambs were again labeled in a similar fashion as Jonas et al. (1972) but in descending (cathode to anode) order (see Figure 16b). This increase in the beta proteins in the parasitized lambs is attributed to an increase of the sheep complement system (Jonas, et al., 1972).

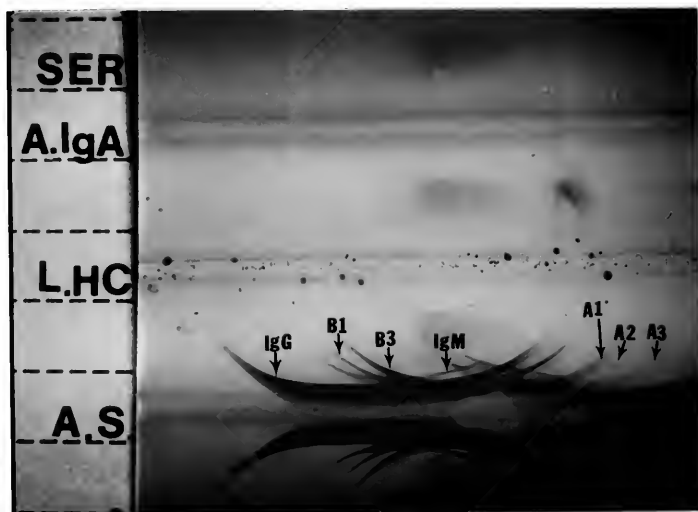
The immunoelectrophoretic patterns from abomasal mucous developed with anti-sheep serum or anti-sheep globulins revealed 5 to 7 proteins. Mucoprotein, as demonstrated in electrophoretic patterns, was not detected by this method. The proteins are identified and labeled in Figure 17 from cathode to anode. The IgA and IgG immunoglobulin reaction was the most prominent in mucous. The lack of increase in the beta proteins (complement) plus the increase in antibody activity (IHA) leads to the conclusion that much of the antibody activity is IgA, a non-complement fixing antibody.

In the higher vertebrates, IgM response accounts for most of the initial reaction (humoral) to foreign invasion (Weinstein, 1967). Jones et al. (1970) reported protective antibodies against *Nippostrongylus brasiliensis* in rat serum that contained IgM and other immunoglobulins

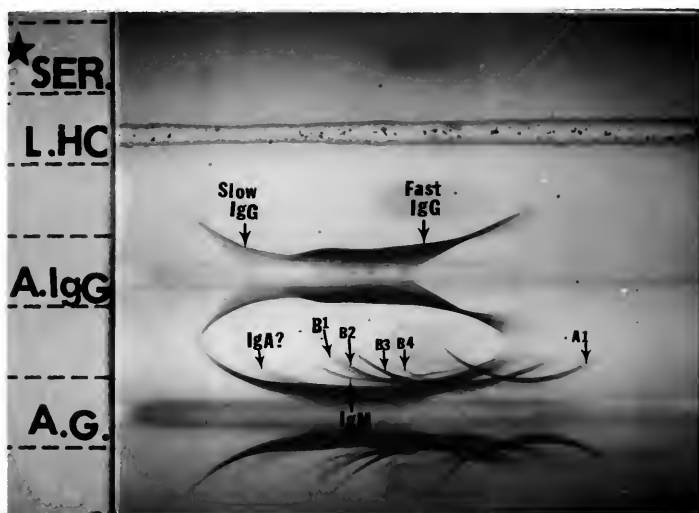
Figure 16. Identified Proteins in Serum from Florida Native Lambs Infected and Non-Infected with *Haemonchus contortus*.
a. infected lamb b. non-infected lamb

Abbreviations used:

A=alpha proteins; B=beta protein; A.IgA=anti-immunoglobulin A; A.IgG=anti-immunoglobulin G; A.G.=anti-sheep globulins; A.S.=anti-sheep serum; L.HC=larval *H. contortus* antigen.



a.

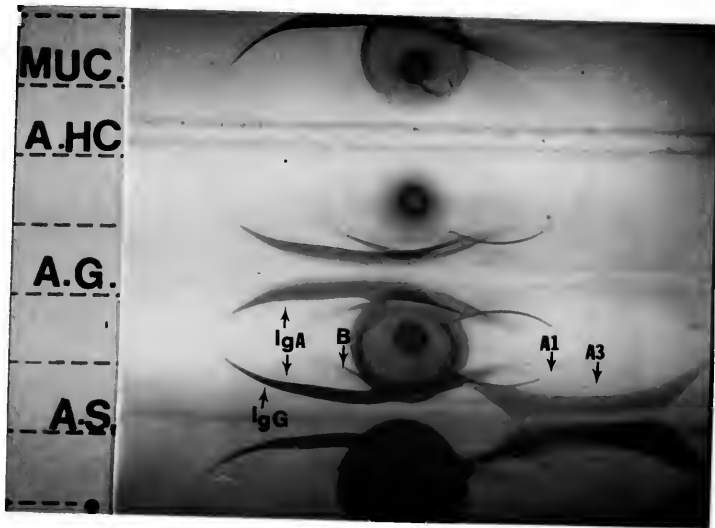


b.

Figure 17. Identified Proteins in Abomasal Mucous from Florida Native Lambs Infected with *Haemonchus contortus*.

Abbreviations used:

A=alpha protein; B=beta protein; A.G.=anti-sheep globulins;
A.S.=anti-sheep serum; A.HC=adult *H. contortus* antigen.



(7S gamma-1 and 7S gamma-2). This observation appears to be in part one of the reactions from Florida Native sheep serum directed towards the parasitic invasion. The role of complement in producing protective immunity against helminths has not been shown in the literature, but IgM has been demonstrated to be highly effective in cell lysis (Humphrey and Dourmashkin, 1965; Hoesler, 1972) and most efficient in binding complement (Glynn and Medhurst, 1967; Hoesler, 1972). Other actions of complement which may play a role in helminth immunity are the adherence of polymorphonuclear leucocytes to larval helminths (Morseth and Soulsby, 1969) and in phagocytosis (Ogilvie, 1970).

Tomasi and Bienenstock (1968) have suggested that IgA may be an important factor in the defence of the host in the intestine. Douvres (1962) and Dobson (1965, 1966) have demonstrated antibody against helminths in the intestinal mucous of sheep and cattle. In these reports the immunoglobulin classes were not determined. The major immunoglobulins demonstrated in abomasal mucous from Florida Native sheep were IgA and IgG.

The IgG antibody was found in both sera and mucous of Florida Native lambs. Evidence that this immunoglobulin has protection capacity has been reported against *Dictyocaulus viviparus* (Wilson, 1966) and *Nippostrongylus brasiliensis* (Jones et al., 1972) who found this immunity to be associated with the 7S immunoglobulin fraction, predominantly the 7S-gamma-1 fraction. This protection can be in the form of direct action, probably by neutralization of enzymes needed by the parasite (Weinstein, 1967), complement fixation (Humphrey and Dourmashkin, 1965) or enhanced phagocytosis.

Suggestions for Future Work

Immunoelectrophoretic precipitating arcs were not observed in serum against parasite antigen (larval or adult *H. contortus*), but increasing beta proteins (complement system) were seen. This phenomenon lends credence to the premise that the active serum immunoglobulin was principally IgG. This can be verified by treating samples with 2-mercaptoethanol followed by IHA testing. Reduction in antibody titer activity would indicate inactivation of IgM immunoglobulin.

Reaginic antibodies (IgE) have been identified following helminth infections in several animals including the sheep (Ogilvie, 1970). One of the most potent stimulus known for production of IgE is parasite infection (Jarrett, 1973). "Self cure" or sudden elimination of parasites (well documented in sheep with *H. contortus*) has been suggested to be related to reagin antibody (Jarrett, 1973). Characterization of IgE in the serum and abomasal mucous of sheep would add to the knowledge in this area and the role it plays in *H. contortus* infection. Since IgE is present in the humoral circulation in very small quantities but plasma cells that produce IgE are found in large numbers in tissues close to mucous surfaces (Ishizaka et al., 1969), it follows that its detection in mucous may demonstrate additional evidence as to why Florida Native sheep show resistance toward parasitic infection. Detection would be accomplished by either passive cutaneous anaphylaxis reaction or the radio-allergo-sorbent technique.

APPENDICES

APPENDIX I

Hemoglobin Levels and *H. contortus* Egg Counts from
Florida Native Ewes Prior to Lambing

Sheep No.	Type ¹	Hb level (gms. %)	Egg counts (per gm. feces)
011	A	9.5	0
013	A	10.9	0
014	A	9.5	800
017	A	12.6	0
018	A	6.7	400
026	A	10.6	200
027	A	8.5	0
031	A	10.6	0
039	A	9.5	0
047	A	9.5	0
053	A	10.6	400
070	A	6.7	200
101*	A	4.0	3,600
132	A	12.1	800
135	A	6.7	0
154	A	13.3	400
157	A	9.5	0
205	A	9.2	400
227	A	9.2	400
235	A	11.8	600
245	A	12.6	0
250	A	5.7	0
302	A	12.1	0
305	A	15.8	0
307	A	13.0	1,200
308	A	13.0	0
310	A	9.2	0
319	A	11.8	0
323	A	12.1	0
324	A	12.3	0
325	A	12.3	0
326	A	11.3	0
331	A	10.6	1,600
332	A	5.7	2,400
334	A	14.1	0
338	A	13.0	0
353	A	13.0	0
357	A	14.7	0
		$\bar{X}_A = 10.6$	$\bar{X}_A = 352.6$

Hemoglobin and Egg Counts (Continued)

Sheep No.	Type ¹	Hb level (gms. %)	Egg Counts (per gm. feces)
010	B	6.7	0
016	B	7.8	0
022	B	10.1	0
025	B	10.6	0
032	B	6.0	0
051	B	10.2	0
061	B	10.1	0
067	B	10.2	400
068	B	9.5	0
252	B	10.6	0
312	B	10.9	600
313	B	12.1	2,600
320	B	10.9	0
321	B	15.8	0
322	B	12.1	400
329	B	10.2	0
333	B	12.1	0
337	B	11.8	0
341	B	10.2	1,400
343	B	10.6	0
351	B	11.3	1,200
352	B	13.7	0
354	B	11.3	0
355	B	12.3	200
358	B	10.6	800
359	B	10.9	600
360	B	11.3	0
363	B	12.3	600
367	B	12.1	0
369	B	12.6	0
601	B	8.5	200
715	B	7.8	1,600
755	B	5.3	0
778	B	7.4	1,400
822	B	8.5	0
844	B	9.5	0
951	B	8.8	0
		$\bar{X}_B = 10.3$	$\bar{X}_B = 324.3$

Hemoglobin and Ova Counts (Continued)

Sheep No.	Type ¹	Hb level (gms. %)	Egg Counts (per gm. feces)
029	AB	12.1	0
042	AB	8.8	0
048	AB	9.2	0
049	AB	9.5	200
052	AB	10.2	0
059	AB	12.1	200
065	AB	14.0	0
112	AB	7.4	0
141	AB	13.3	0
156	AB	10.1	0
160	AB	8.1	0
207	AB	9.5	400
217	AB	7.8	1,200
224	AB	10.2	400
234	AB	10.2	3,600
239	AB	6.0	0
301	AB	10.9	200
303	AB	12.3	0
318	AB	12.3	0
327	AB	11.8	0
328	AB	11.3	0
330*	AB	13.3	0
336	AB	11.3	200
344	AB	12.3	200
349	AB	13.0	200
368	AB	11.3	600
764	AB	6.0	400
788	AB	10.1	200
813	AB	12.1	0
819	AB	7.8	400
820	AB	7.1	200
825	AB	7.1	0
832	AB	10.6	0
849	AB	5.0	2,200
853	AB	8.1	200
856	AB	9.5	200
909	AB	10.9	0
7125	AB	12.3	200
7155	AB	8.1	800
		$\bar{X}_{AB} = 10.1$	$\bar{X}_{AB} = 312.8$

¹Hemoglobin type

*Died one week later

APPENDIX II

Packed Cell Volumes (PCV) of Lambs Pre- and Post-Infection with *Haemonchus contortus*.

Sneep No.	Type ¹	PCV (%) on Weeks Pre-Infection										Mean	Necropsy (Days ²)	Change		
		10	9	8	7	6	6	5	4	4	3				2	1
14*	A	38	31	33	36	40	38	36	35	34	35	35	34	35.4	36 (1)	+0.6
19	A	48	41	41	40	42	41	41	39	38	36	38	39	40.3	40 (1)	-0.3
147*	B	32	27	27	26	26	25	25	28	25	24	24	25	26.2	29 (1)	+2.8
128	B	28	26	31	29	27	28	29	29	28	26	27	27	27.9	31 (1)	+3.1
15*	AB	38	34	32	34	31	34	34	32	32	31	32	32	33.0	35 (1)	+2.0
124	AB	38	35	32	34	32	36	34	33	34	34	31	34	33.9	35 (1)	+1.1
132*	A	41	40	37	40	39	42	39	39	37	37	38	35	38.7	40 (7)	+1.3
120	A	47	41	37	40	43	40	39	40	40	39	38	40	40.3	40 (7)	-0.3
109*	B	35	21	35	29	30	32	33	32	32	30	32	32	31.1	37 (7)	+5.9
122	B	33	31	29	30	29	31	30	27	28	25	26	28	28.9	34 (7)	+5.1
27*	AB	40	33	35	37	33	38	38	38	35	33	34	34	35.7	40 (7)	+4.3
139	AB	40	39	38	40	37	39	35	35	39	34	33	35	37.0	36 (7)	-1.0
10*	A	42	34	37	33	37	36	41	39	38	40	39	40	38.0	37 (12)	-1.0
17	A	45	38	39	39	41	39	40	40	36	36	36	38	38.9	38 (12)	-0.9
117*	B	28	24	24	25	26	30	25	27	26	25	25	24	25.8	28 (12)	+2.2
11	B	40	32	35	32	34	37	36	39	32	31	32	31	34.3	31 (12)	-3.3
118	A	39	34	38	37	38	37	36	34	33	34	35	35	35.8	35 (16)	-0.8
110	A	40	36	41	38	36	39	35	32	36	32	31	34	35.8	36 (16)	+0.2
21	A	40	31	35	38	40	37	39	38	36	36	33	34	36.4	39 (16)	+2.6
127*	B	36	34	32	33	33	35	37	34	33	32	32	30	33.5	33 (16)	-0.5
134	B	37	33	30	30	30	34	32	32	31	28	30	31	31.5	32 (16)	-0.5
137*	AB	40	37	37	34	32	35	36	34	31	32	30	30	34.0	34 (16)	0.0
114	A	50	41	39	36	35	34	33	33	33	35	32	34	36.3	34 (21)	-2.3
24	A	38	35	36	35	38	36	35	35	34	35	34	34	35.4	35 (21)	-0.4
113	B	36	33	32	36	38	36	35	36	35	33	34	33	34.8	33 (21)	-1.8
126	B	41	39	35	34	35	38	35	37	36	31	34	34	35.8	34 (21)	-1.8
115	A	36	43	38	39	41	40	38	38	35	35	38	38	38.3	31 (26)	-7.3

Packed Cell Volumes (Continued)

Sheep No.	Type ¹	PCV (%) on Weeks Pre-Infection										Mean	Necropsy (Days ²)	Change		
		10	9	8	7	6	6	5	4	4	3				2	1
23	A	42	39	40	39	41	41	35	36	35	37	34	36	37.9	38 (26)	+1.9
22*	B	32	31	29	27	33	33	33	29	29	27	28	27	29.8	25 (26)	-4.8
141	B	32	27	30	29	33	30	28	27	27	29	27	27	28.8	30 (26)	+1.2
16*	AB	40	35	36	36	40	39	37	37	36	30	31	30	35.7	28 (26)	-7.7
111*	A	34	29	34	35	39	32	35	33	31	32	33	35	33.5	30 (30)	-3.5
9	A	38	36	39	39	37	38	37	38	35	37	39	38	37.6	39 (30)	+1.4
121*	B	32	23	35	25	27	26	26	27	26	24	26	25	26.8	24 (30)	-2.8
30	B	34	30	30	33	31	31	31	31	29	30	32	31	31.1	32 (30)	+0.9
130*	AB	34	34	33	37	35	34	33	32	35	33	32	31	33.6	28 (30)	-5.6
112	AB	39	36	35	37	35	33	32	32	32	28	31	30	33.3	31 (30)	-2.3
12*	A	35	28	32	36	35	36	35	34	35	33	39	35	34.4	41 (33)	+6.6
13	A	41	36	38	34	37	35	34	32	32	33	35	32	34.9	34 (33)	-0.9
28*	B	36	26	26	32	38	34	32	30	29	29	27	29	30.7	31 (33)	+0.3
29	B	29	35	32	28	31	32	31	29	29	30	27	29	30.2	40 (33)	+9.8
119*	AB	31	29	26	30	27	30	30	32	30	28	31	29	29.4	38 (33)	+8.6
123*	A	43	38	36	39	37	38	37	36	37	36	36	38	37.6	41 (38)	+3.4
25	A	35	28	35	33	38	39	36	36	38	36	39	37	35.8	42 (38)	+6.2
31*	B	42	33	34	31	31	34	30	30	30	28	31	31	32.1	33 (38)	+5.9
136*	AB	39	34	36	33	38	37	35	35	34	32	36	35	35.3	40 (38)	+4.7

¹Hemoglobin type²Days Post-Infection

*Infected Lambs

APPENDIX III

Hemoglobin Levels¹ of Lambs Pre- and Post-Infection with *Haemonchus contortus*.

Sheep No. (Type ²)	Hb Level (gms. %) on Weeks					Mean	Necropsy (Days ³)	Change	Group Mean
	5	4	4	3	2				
14* A	13.9	16.9	17.0	16.1	16.5	16.1	16.8 (1)	+0.7	
147* B	11.3	12.8	11.6	11.4	11.8	11.8	11.6 (1)	-0.2	(+0.3)
15* AB	13.0	14.8	14.6	14.6	14.6	14.3	14.6 (1)	+0.3	
19 A	18.4	16.8	16.1	17.0	16.8	17.0	18.0 (1)	+1.0	
128 B	11.6	11.6	11.4	11.4	11.8	11.6	12.8 (1)	+1.2	(+1.0)
124 AB	15.7	15.3	14.6	14.6	14.8	15.0	15.7 (1)	+0.7	
132* A	17.0	16.5	16.5	16.1	16.5	16.5	15.7 (7)	-0.8	
109* B	15.7	13.9	13.9	15.0	15.0	14.7	14.7 (7)	0.0	(-0.8)
27* AB	16.8	16.1	13.0	13.5	13.0	14.5	13.0 (7)	-1.5	
120 A	18.7	17.6	15.0	15.7	15.3	16.5	17.6 (7)	-1.1	
122 B	13.0	11.6	12.8	12.8	12.8	12.6	14.6 (7)	+2.0	(+7.0)
139 AB	15.0	15.0	15.3	17.0	15.0	15.5	15.3 (7)	-0.2	
10* A	18.7	16.8	18.3	18.4	17.6	18.0	17.6 (12)	-0.4	(-0.3)
117* B	9.7	9.7	9.4	9.6	9.6	9.6	9.4 (12)	-0.2	
17 A	15.3	17.0	15.3	13.0	14.6	15.0	14.8 (12)	-0.2	(-0.1)
11 B	17.0	17.0	16.8	13.5	16.1	16.1	16.1 (12)	0.0	
118* A	15.7	15.0	15.3	15.3	15.0	15.3	15.3 (16)	0.0	
127* B	17.6	15.3	16.1	15.7	16.0	16.0	14.5 (16)	-2.5	(-1.6)
137* AB	16.8	15.7	15.0	15.7	15.3	15.7	15.0 (16)	-0.7	

Hemoglobin Levels (Continued)

Sheep No. (Type ²)	Hb Level (gms. %) on Weeks				Pre-Infection		Mean	Necropsy (Days ³)	Change	Group Mean
	5	4	3	4	3	2				
110 A	15.3	15.7	16.1	13.5	15.7	15.3	16.8 (16)	+1.5		
21 A	19.0	16.8	17.6	17.0	17.0	17.5	18.4 (16)	+0.9	(+0.8)	
134 B	13.9	13.5	12.8	13.0	13.9	13.4	13.5 (16)	+0.1		
114* A	13.0	13.9	12.8	13.0	13.0	13.2	16.1 (21)	+2.9	(+1.4)	
113* B	15.3	15.0	15.7	14.6	14.8	15.1	15.0 (21)	-0.1		
24 A	14.6	18.3	15.7	15.0	15.7	15.7	15.5 (21)	-0.2	(0.4)	
126 B	13.0	16.1	16.8	15.3	16.1	15.5	15.0 (21)	-0.5		
115* A	16.8	15.7	15.7	17.0	16.1	16.3	13.5 (26)	+2.8		
22* B	14.8	13.0	12.8	11.0	13.0	12.9	12.8 (26)	-0.1	(-1.1)	
16* AB	15.3	14.8	16.1	15.0	16.1	15.5	15.0 (26)	-0.5		
23 A	15.7	17.6	15.3	16.8	15.7	16.2	16.8 (26)	+0.6	(0.1)	
141 B	11.8	11.8	12.8	11.4	11.8	11.9	11.4 (26)	-0.5		
111* A	17.6	15.3	13.9	14.8	14.8	15.3	15.5 (30)	+0.2		
121* B	9.6	11.0	11.0	9.4	11.6	10.5	9.4 (30)	-1.1	(2.1)	
130 AB	15.3	15.0	14.8	13.9	14.6	14.7	9.4 (30)	-5.3		
9 A	19.0	15.7	17.0	18.3	18.7	17.7	17.6 (30)	-0.1		
30 B	13.9	14.6	15.7	14.8	14.8	14.8	14.8 (30)	0.0	(0.0)	
112 AB	17.0	16.1	15.0	11.4	15.7	15.0	16.0 (30)	+1.0		

Hemoglobin Levels (Continued)

Sheep No. (Type ²)	Hb Level (gms. %) on Weeks Pre-Infection					Mean	Necropsy (Days ³)	Change	Group Mean
	5	4	3	2	1				
12* A	16.8	14.8	13.0	14.6		14.8	16.1 (33)	+1.3	
28* B	14.8	13.0	11.6	11.4		12.5	14.7 (33)	+2.2	(+1.6)
119* AB	18.4	16.8	17.0	16.8		17.0	18.3 (33)	+1.3	
13 A	16.1	14.6	13.0	14.8		15.1	16.1 (33)	+1.0	(+1.9)
29 B	14.6	12.8	15.3	15.7		14.8	17.6 (33)	+2.8	
123* A	17.6	15.0	17.0	16.8		16.3	17.0 (38)	+0.7	
31* B	11.1	11.4	12.8	11.6		11.6	17.8 (38)	+5.4	(+3.6)
136* AB	13.9	13.9	13.0	13.9		13.7	18.4 (38)	+4.7	
25 A	14.8	13.9	14.8	14.6		14.8	18.4 (38)	+3.6	(+3.6)

¹ gms./100ml² Hemoglobin type³ Days post-infection

*Infected

APPENDIX IV

Changes in Serum Proteins in Florida Native Lambs
 Infected and Non-Infected with *Haemonchus contortus*.

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)			A/G ratio		
			Albumin	Alpha-1	Alpha-2		Beta	Gamma
2/28	14* A	2.50	2.25	0.22	0.87	1.30	2.56	0.88
3/7		2.75	2.10	0.21	1.20	1.18	2.61	0.80
3/21		3.25	2.44	0.47	0.67	0.52	2.70	0.90
4/4		3.75	3.20	0.24	0.84	0.36	2.05	1.56
4/21		4.25	2.87	0.19	0.94	0.34	1.67	1.72
5/2		4.75	3.29	0.09	1.09	0.45	1.48	2.22
5/9		5.00	2.80	0.13	1.16	0.34	1.67	1.68
Mean			2.56	0.22	0.96	0.64	2.10	1.21
Post-Infection ² (Day 1) Change		5.50	2.85 +0.29	0.40 +0.18	0.95 0.01	0.85 +0.21	1.25 -0.85	2.28 +1.07
2/28	19 A	2.50	3.19	0.84	0.95	0.28	1.04	3.07
3/7		2.75	2.97	0.75	0.60	0.50	0.97	2.85
3/21		3.25	2.88	0.58	0.90	0.19	0.96	2.97
4/4		3.75	2.92	0.72	0.67	0.31	1.18	2.47
4/21		4.25	2.84	0.20	1.42	0.38	1.26	2.25
5/2		4.75	3.03	0.69	0.62	0.67	0.99	3.06
5/9		5.00	3.08	0.29	1.63	0.29	1.41	2.18
Mean			2.99	0.58	0.97	0.37	1.11	2.69
Post-Infection (Day 1) Change		5.50	2.77 -0.22	0.31 -0.27	1.28 +0.31	0.51 +0.14	1.33 +0.22	2.08 -0.61

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	147* B	2.50	2.54	0.22	0.98	0.21	1.05	2.42
3/7		2.75	2.99	0.25	1.28	0.24	1.31	2.28
3/21		3.25	2.61	0.25	0.94	0.31	1.08	2.42
4/4		3.75	2.47	0.26	1.08	0.29	0.60	4.12
4/21		4.25	2.53	0.32	1.01	0.26	1.18	2.14
5/2		4.75	2.73	0.30	1.44	0.37	0.96	2.84
5/9		5.00	2.16	0.29	1.05	0.71	0.98	2.20
Mean			2.57	0.27	0.96		1.02	2.52
Post-Infection (Day 1) Change			5.50	2.01 -0.56	0.27 0.00	1.12 +0.16	0.68 +0.34	0.92 -0.10
2/28	128 B	2.50	3.67	0.24	0.88	0.78	0.92	3.99
3/7		2.75	3.78	0.27	0.62	0.96	1.17	3.23
3/21		3.25	2.27	0.21	0.87	0.62	1.73	3.66
4/4		3.75	3.09	0.12	0.85	0.61	1.53	2.02
4/21		4.25	2.91	0.11	0.82	0.47	1.40	2.08
5/2		4.75	2.86	0.22	0.72	0.66	1.24	2.31
5/9		5.00	3.12	0.34	1.21	0.45	1.17	2.67
Mean			3.10	0.18	0.85		1.30	2.38
Post-Infection (Day 1) Change			5.50	2.97 -0.13	0.36 +0.18	1.27 +0.42	0.47 -0.18	1.23 -0.07

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type 1)	Age (Months)	Electrophoresis Values (gms.%)					A/G ratio
			Albumin	Alpha-1	Alpha-2	Beta	Gamma	
2/28	15* AB	2.50	3.47	0.32	1.03	0.70	1.28	2.71
3/7		2.75	3.31	0.33	0.82	0.66	1.57	2.11
3/21		3.25	3.35	0.42	0.95	0.37	1.12	2.99
4/4		3.75	3.19	0.29	0.91	0.39	1.42	2.24
4/21		4.25	2.48	0.35	1.14	0.29	1.14	2.17
5/2		4.75	2.83	0.38	0.97	0.48	1.14	2.48
5/9		5.00	2.76	0.30	1.05	0.32	1.07	2.58
Mean			3.05	0.34	0.98	0.46	1.25	2.44
Post-Infection (Day 1) Change			5.50	2.35 -0.70	0.25 -0.09	1.05 +0.07	0.48 +0.02	1.66 +0.41
2/28	124 AB	2.50	2.92	0.22	0.94	0.36	1.15	2.55
3/7		2.75	2.65	0.17	0.75	0.35	0.67	3.95
3/21		3.25	2.96	0.18	0.98	0.24	1.03	2.87
4/4		3.75	3.67	0.41	0.93	0.70	1.09	3.36
4/21		4.25	3.31	0.19	1.01	0.39	1.10	3.00
5/2		4.75	2.96	0.19	0.68	0.39	1.38	2.14
5/9		5.00	3.06	0.23	1.19	0.35	1.07	2.86
Mean			3.07	0.22	0.93	0.40	1.07	2.87
Post-Infection (Day 1) Change			5.50	2.71 -0.36	0.41 +0.19	1.29 +0.36	0.37 -0.03	1.22 +0.15

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)					A/G ratio
			Albumin	Alpha-1	Alpha-2	Beta	Gamma	
2/28	132* A	2.50	3.31	0.23	0.95	0.32	1.18	2.80
3/7		2.75	2.66	0.26	1.10	0.35	1.93	1.38
3/21		3.25	2.82	0.27	0.98	0.27	1.76	1.60
4/4		3.75	3.22	0.41	0.99	0.41	1.47	2.19
4/21		4.25	3.10	0.43	1.01	0.54	1.01	3.07
5/2		4.75	3.03	0.33	0.81	0.44	1.18	2.50
5/9	5.00	3.29	0.09	1.19	0.43	1.20	2.74	
Mean			3.06	0.29	1.00	0.39	1.37	2.23
Post-Infection (Day 7)		5.50	3.05	0.18	1.02	0.37	1.47	2.07
Change			-0.01	-0.11	+0.02	-0.02	+0.10	-0.16
2/28	120 A	2.50	3.14	0.18	0.97	0.55	1.16	2.71
3/7		2.75	3.02	0.17	0.88	0.41	1.52	1.99
3/21		3.25	3.33	0.20	0.95	0.47	0.95	3.50
4/4		3.75	3.57	0.29	1.02	0.32	0.90	3.97
4/21		4.25	2.78	0.29	1.10	0.42	1.20	2.32
5/2		4.75	3.52	0.25	0.92	0.33	0.97	3.63
5/9	5.00	3.08	0.37	1.08	0.51	1.25	2.46	
Mean			3.20	0.25	0.99	0.43	1.13	2.83
Post-Infection (Day 7)		5.50	3.42	0.22	1.11	0.44	1.01	3.39
Change			+0.22	-0.03	+0.12	+0.01	-0.12	+0.56

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	109* B	2.50	2.93	0.39	1.13	0.29	1.16	2.52
3/7		2.75	2.41	0.39	1.21	0.47	0.91	2.64
3.21		3.25	2.83	0.35	1.04	0.46	0.92	3.07
4/4		3.75	3.16	0.01	0.98	0.52	0.92	3.43
4/21		4.25	2.90	0.20	1.16	0.20	1.03	2.82
5/2		4.75	2.81	0.25	1.03	0.28	0.92	3.05
5/9		5.00	3.13	0.25	1.04	0.36	0.92	3.40
Mean			2.88	0.26	1.08	0.37	0.97	2.97
Post-Infection (Day 7) Change			3.03 -+0.15	0.31 +0.05	1.06 -0.02	0.81 +0.44	0.99 +0.02	3.06 +0.09
2/28	122 B	2.50	3.23	0.40	0.96	0.13	1.18	2.74
3/7		2.75	2.88	0.34	1.00	0.48	1.00	2.88
3/21		3.25	3.16	0.09	0.79	0.09	1.47	2.14
4/4		3.75	3.05	0.82	0.97	0.34	0.82	3.72
4/21		4.25	2.78	0.28	1.12	0.41	1.40	1.98
5/2		4.75	3.06	0.31	0.78	0.31	0.83	3.68
5/9		5.00	3.26	0.27	1.10	0.37	1.09	2.99
Mean			3.06	0.35	0.96	0.30	1.11	2.76
Post-Infection (Day 7) Change			3.17 +0.11	0.31 -0.04	0.92 -0.04	0.46 +0.16	1.04 -0.07	3.05 +0.29

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	27* AB	2.50	2.64	0.18	0.97	0.44	2.27	1.16
3/7		2.75	2.18	0.22	0.62	0.62	2.07	1.05
3/21		3.25	3.23	0.45	0.97	0.23	1.42	2.27
4/4		3.75	3.36	0.57	0.86	0.58	1.33	2.53
4/21		4.25	2.85	0.35	1.11	0.44	1.96	1.45
5/2		4.75	2.85	0.35	0.86	0.26	1.38	2.06
5/9		5.00	3.26	0.32	1.00	0.49	1.63	2.00
Mean			2.91	0.35	0.91	0.44	1.72	1.69
Post-Infection (Day 7) Change			3.24 +0.33	0.23 -0.12	1.00 +0.09	0.38 -0.06	1.54 -0.18	2.10 +0.41
2/28	139 AB	2.50	3.66	0.27	0.94	0.33	1.10	3.33
3/7		2.75	3.41	0.30	0.91	0.45	0.94	3.63
3/21		3.25	3.13	0.38	0.95	0.32	1.23	2.54
4/4		3.75	2.95	0.25	1.16	0.25	1.40	2.11
4/21		4.25	3.35	0.29	0.92	0.42	1.01	3.32
5/2		4.75	2.86	0.32	1.31	0.32	1.20	2.38
5/9		5.00	3.29	0.19	1.12	0.49	1.01	3.26
Mean			3.24	0.29	1.04	0.37	1.13	2.87
Post-Infection (Day 7) Change			3.17 -0.07	0.31 +0.02	0.97 -0.07	0.39 +0.02	1.16 +0.03	2.73 -0.14

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type I)	Age (Months)	Electrophoresis Values (gms.%)				A/S ratio		
			Albumin	Alpha-1	Alpha-2	Beta		Gamma	
2/28	10* A	2.50	2.70	0.12	0.75	0.54	1.79	1.51	
3/7		2.25	2.13	0.16	0.73	0.40	1.26	1.69	
3/21		3.25	2.01	0.06	0.92	0.34	1.38	1.45	
4/4		3.75	2.54	0.31	1.14	0.57	0.93	2.73	
4/21		4.25	3.37	0.17	1.08	0.69	0.77	4.38	
5/2		4.75	2.96	0.23	0.64	0.50	0.77	3.84	
5/9		5.00	2.73	0.05	1.09	0.33	1.10	2.49	
Mean			2.63	0.16	0.91	0.48	1.14	2.31	
Post-Infection (Day 12) Change			5.75	3.56 +0.93	0.25 +0.09	1.31 +0.40	0.60 +0.12	1.97 +0.83	1.63 -0.68
2/28	17 A	2.50	3.50	0.31	1.04	0.14	1.01	3.46	
3/7		2.25	3.22	0.26	0.93	0.29	1.10	2.93	
3/21		3.25	2.99	0.22	0.93	0.22	1.33	2.25	
4/4		3.75	3.36	0.24	1.08	0.24	1.08	3.11	
4/21		4.25	3.22	0.21	0.84	0.51	1.01	3.18	
5/2		4.75	2.91	0.37	0.92	0.52	1.29	2.25	
5/9		5.00	2.97	0.16	1.06	0.39	1.51	1.97	
Mean			3.17	0.25	0.97	0.97	0.33	1.19	2.66
Post-Infection (Day 12) Change			5.75	2.79 -0.38	0.25 0.00	0.94 -0.03	1.16 +0.83	1.45 +0.26	1.92 -0.74

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	117* B	2.50	3.25	0.19	1.14	0.62	2.00	1.62
3/7		2.25	2.40	0.24	0.86	0.81	1.69	1.42
3/21		3.25	2.87	0.23	0.88	0.53	1.29	2.22
4/4		3.75	3.60	0.27	0.98	0.55	1.20	3.00
4/21		4.25	2.80	0.26	0.78	0.58	0.98	2.86
5/2		4.75	2.77	0.30	0.73	0.60	1.20	2.31
5/9		5.00	3.00	0.32	0.89	0.71	1.18	2.54
Mean			2.96	0.24	0.888	0.63	1.36	2.18
Post-Infection (Day 12) Change			5.75	+0.66	+0.05	+0.04	+0.09	-0.22
2/28	11 B	2.50	3.59	0.46	1.08	0.43	1.13	3.18
3/7		2.25	2.53	0.47	0.44	0.47	1.59	1.59
3/21		3.25	2.69	0.32	0.68	0.47	1.22	2.20
4/4		3.75	3.09	0.50	0.60	0.30	1.00	3.09
4/21		4.25	3.00	0.25	1.30	0.36	0.98	3.06
5/2		4.75	3.26	0.21	0.72	0.51	1.10	2.96
5/9		5.00	3.02	0.18	1.23	0.28	1.19	2.53
Mean			3.03	0.34	0.86	0.40	1.19	3.61
Post-Infection (Day 12) Change			5.75	+0.42	-0.11	+0.29	+0.06	-0.08

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)					A/G ratio
			Albumin	Alpha-1	Alpha-2	Beta	Gamma	
2/28	118* A	2.50	2.82	0.23	0.76	0.32	1.66	1.70
3/7		2.75	2.80	0.21	0.84	0.53	1.32	2.12
3/21		3.25	3.55	0.33	0.88	0.22	1.11	3.20
4/4		3.75	2.87	0.32	1.27	0.50	1.14	2.52
4/21		4.25	2.84	0.37	1.00	0.29	1.40	2.03
5/2		4.75	3.13	0.36	0.75	0.47	1.38	2.25
5/9		5.00	3.26	0.26	0.97	0.32	1.39	2.34
Mean			2.92	0.30	0.92	0.38	1.34	2.18
Post-Infection (Day 16) Change			6.00	+0.16	-0.05	+0.53	+0.33	+0.86
2/28	110 A	2.50	2.92	0.24	0.82	0.25	1.57	1.86
3/7		2.75	3.02	0.29	0.85	0.35	1.18	2.56
3/21		3.25	2.85	0.25	1.28	0.44	1.18	2.41
4/4		3.75	2.98	0.13	0.98	0.20	1.20	2.49
4/21		4.25	2.92	0.30	1.19	0.30	0.97	3.01
5/2		4.75	2.94	0.35	0.64	0.35	1.31	2.24
5/9		5.00	3.15	0.22	0.98	0.54	1.21	2.60
Mean			2.55	0.25	0.97	0.35	1.23	2.07
Post-Infection (Day 16) Change			6.00	+0.84	+0.10	-0.07	+0.35	+0.32

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio		
			Albumin	Alpha-1	Alpha-2	Beta		Gamma	
2/28	21 A	2.50	3.90	0.37	1.14	0.62	1.76	2.21	
3/7		2.75	2.71	0.31	0.83	0.43	1.52	1.78	
3/21		3.25	3.02	0.29	0.94	0.34	1.70	1.78	
4/4		3.75	3.15	0.40	1.10	0.35	1.39	2.27	
4/21		4.25	2.52	0.28	1.08	0.31	1.60	1.57	
5/2		4.75	2.94	0.28	1.31	0.28	1.38	2.13	
5/9		5.00	3.17	0.29	0.96	0.37	1.21	2.62	
Mean				3.06	0.32	1.05	0.39	1.51	2.03
Post-Infection (Day 16) Change			6.00	2.89 +0.11	0.32 -0.03	1.03 -0.09	0.80 -0.02	1.55 -0.30	1.86 +0.59
2/28	127* B	2.50	2.87	0.41	0.69	0.36	1.27	2.26	
3/7		2.75	2.68	0.52	0.52	0.24	1.44	1.86	
3/21		3.25	2.95	0.70	0.54	0.16	1.14	2.58	
4/4		3.75	3.41	0.32	0.98	0.23	0.75	4.55	
4/21		4.25	2.80	0.37	1.20	0.37	1.06	2.64	
5/2		4.75	2.94	0.27	0.41	0.86	1.11	2.65	
5/9		5.00	2.83	0.39	0.35	0.85	1.47	1.92	
Mean				2.93	0.43	0.67	0.44	1.18	2.48
Post-Infection (Day 16) Change			6.00	3.31 +0.38	0.28 -0.15	1.21 +0.54	0.64 +0.20	1.46 +0.28	2.27 -0.21

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)			Gamma	A/G ratio
			Albumin	Alpha-1	Alpha-2		
2/28	134 B	2.50	3.24	0.27	0.98	0.31	3.24
3/7		2.75	3.39	0.27	0.97	0.43	2.53
3/21		3.25	2.80	0.47	0.77	0.24	1.97
4/4		3.75	3.49	0.24	0.96	0.36	3.03
4/21		4.25	2.75	0.20	1.04	0.54	2.16
5/2		4.75	3.04	0.28	0.82	0.46	2.53
5/9		5.00	2.98	0.19	1.33	0.42	2.00
Mean			3.10	0.20	0.97	0.38	2.44
Post-Infection (Day 16) Change		6.00	3.11 +0.01	0.21 +0.01	1.31 +0.34	0.55 +0.17	1.92 -0.52
2/28	137* AB	2.50	3.21	0.23	1.17	0.37	2.87
3/7		2.75	3.21	0.33	0.92	0.37	2.72
3/21		3.25	3.11	0.23	0.96	0.33	2.91
4/4		3.75	3.23	0.25	0.82	0.21	3.26
4/21		4.25	2.88	0.27	1.14	0.32	2.69
5/2		4.75	3.07	0.34	0.98	0.31	3.04
5/9		5.00	2.86	0.39	1.16	0.20	2.40
Mean			3.08	0.28	1.02	0.27	3.35
Post-Infection (Day 16) Change		6.00	3.91 -0.22	0.13 +0.11	1.19 +0.14	0.40 -0.07	3.10 -0.25

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio		
			Albumin	Alpha-1	Alpha-2	Beta		Gamma	
2/28	114* A	2.50	3.84	0.25	1.12	0.62	1.67	2.30	
3/7		2.75	2.39	0.28	0.85	0.69	2.69	0.89	
3/21		3.25	2.87	0.27	0.96	0.37	1.33	2.16	
4/4		3.75	3.39	0.27	0.87	0.27	0.97	3.49	
4/21		4.25	2.71	0.31	1.23	0.34	1.21	2.24	
5/2		4.75	3.13	0.26	0.95	0.26	1.29	2.42	
5/9		5.00	3.03	0.30	1.18	0.41	1.18	2.57	
Mean				3.05	0.28	1.02	0.42	1.29	2.36
Post-Infection (Day 21) Change			6.00	2.92 -0.13	0.31 +0.03	1.21 +0.19	0.51 +0.09	1.63 +0.34	1.79 -0.57
2/28	24 A	2.50	2.79	0.50	0.90	0.18	1.73	1.61	
3/7		2.75	2.56	0.31	1.03	0.51	1.68	1.52	
3/21		3.25	3.16	0.48	0.57	0.33	0.96	3.29	
4/4		3.75	2.95	0.39	1.10	0.25	1.21	2.44	
4/21		4.25	2.70	0.28	1.33	0.40	1.58	1.71	
5/2		4.75	3.07	0.13	1.69	0.42	1.07	2.87	
5/9		5.00	2.78	0.27	1.33	0.43	1.29	2.15	
Mean				2.86	0.34	1.14	0.36	1.36	2.10
Post-Infection (Day 21) Change			6.00	1.92 -0.94	0.22 -0.12	1.10 -0.04	1.04 +0.68	1.92 +0.56	1.00 -1.10

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	113* B	2.50	3.15	0.38	0.93	0.28	1.35	2.33
3/7		2.75	3.14	0.34	0.63	0.34	1.16	2.71
3/21		3.25	3.16	0.33	1.08	0.29	1.23	2.57
4/4		3.75	3.32	0.39	0.88	0.44	1.17	2.83
4/21		4.25	3.10	0.26	1.07	0.38	1.58	1.96
5/2		4.75	3.15	0.41	0.99	0.51	1.45	2.17
5/9		5.00	3.35	0.27	0.91	0.36	1.40	2.39
Mean			3.19	0.34	0.94	0.37	1.33	2.40
Post-Infection (Day 21) Change		6.00	3.03	0.40	1.00	0.57	1.38	2.19
			-0.16	+0.06	+0.06	+0.20	+0.05	-0.21
2/28	126 B	2.50	3.39	0.38	1.12	0.49	0.91	3.72
3/7		2.75	3.03	0.29	0.84	0.38	1.05	2.88
3/21		3.25	2.95	0.29	0.74	0.30	1.02	2.89
4/4		3.75	3.36	0.45	1.02	0.38	1.08	3.11
4/21		4.25	2.45	0.37	1.03	0.50	1.45	1.69
5/2		4.75	2.77	0.30	1.05	0.30	1.18	2.35
5/9		5.00	2.82	0.26	1.28	0.23	1.51	1.97
Mean			2.97	0.33	1.01	0.37	1.17	2.54
Post-Infection (Day 21) Change		6.00	3.21	0.29	1.04	0.40	1.36	2.36
			+0.24	-0.04	+0.03	+0.03	+0.19	-0.18

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	115* A	2.50	3.61	0.21	1.31	0.37	1.60	2.14
3/7		2.75	3.34	0.18	1.01	0.45	1.32	2.53
3/21		3.25	3.20	0.19	1.20	0.40	1.40	2.28
4/4		3.75	3.58	0.23	1.12	0.35	1.71	2.09
4/21		4.25	3.37	0.18	1.20	0.39	1.35	2.49
5/2		4.75	3.34	0.39	0.78	0.44	1.03	3.24
5/9		5.00	3.25	0.25	1.05	0.54	1.40	2.32
Mean			3.38	0.23	1.10	0.42	1.41	2.90
Post-Infection (Day 26) Change			6.25	+0.12	+0.05	0.00	+0.51	1.25
2/28	23 A	2.50	3.84	0.27	0.95	0.56	1.78	2.16
3/7		2.75	2.89	0.26	0.74	0.58	2.32	1.24
3/21		3.25	3.51	0.23	0.66	0.59	1.19	2.95
4/4		3.75	3.49	0.26	1.04	0.39	1.42	2.46
4/21		4.25	2.75	0.23	1.19	0.46	1.46	1.88
5/2		4.75	2.51	0.23	1.29	0.36	1.46	1.72
5/9		5.00	2.60	0.18	1.47	0.43	1.71	1.52
Mean			3.08	0.24	1.05	0.48	1.62	1.90
Post-Infection (Day 26) Change			6.25	+0.23	+0.01	0.00	+0.06	1.03

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio		
			Albumin	Alpha-1	Alpha-2	Beta		Gamma	
2/28	22* B	2.50	3.31	0.22	0.90	0.39	1.77	1.97	
3/7		2.75	2.23	0.33	0.99	0.44	0.90	2.48	
3/21		3.25	2.94	0.19	0.93	0.29	0.63	4.67	
4/4		3.75	2.76	0.63	0.63	0.23	1.05	2.63	
4/21		4.25	2.49	0.18	0.85	0.42	0.96	2.59	
5/2		4.75	2.38	0.25	0.66	0.25	0.95	2.50	
5/9		5.00	2.73	0.23	0.86	0.39	0.89	3.07	
Mean				2.69	0.29	0.83	0.34	1.02	2.64
Post-Infection** (Day 26) Change			6.25	3.16 +0.47	0.13 -0.16	0.69 -0.14	0.43 +0.09	1.18 +0.16	2.68 +0.04
2/28	141 B	2.50	3.43	0.23	0.79	0.42	1.63	2.10	
3/7		2.75	1.97	0.15	0.57	0.91	2.20	0.89	
3/21		3.25	3.43	0.25	0.60	0.46	0.96	3.57	
4/4		3.75	3.39	0.26	0.71	0.52	1.11	3.05	
4/21		4.25	2.90	0.22	1.06	0.29	1.32	2.25	
5/2		4.75	2.97	0.27	0.66	0.51	1.29	2.30	
5/9		5.00	2.85	0.19	1.33	0.41	1.61	1.77	
Mean				2.99	0.22	0.82	0.50	1.44	2.08
Post-Infection** (Day 26) Change			6.25	2.74 -0.25	0.31 +0.09	0.83 +0.01	0.39 -0.11	1.43 -0.01	1.92 -0.16

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type ¹)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	16* AB	2.50	2.37	0.41	0.93	0.31	1.08	2.19
3/7		2.75	2.61	0.37	0.83	0.41	1.19	2.19
3/21		3.25	2.94	0.26	0.85	0.26	0.78	3.77
4/4		3.75	3.17	0.28	0.73	0.60	1.01	3.14
4/21		4.25	2.50	0.25	1.00	0.45	1.20	2.08
5/2		4.75	2.71	0.09	0.99	0.33	0.87	3.11
5/9		5.00	3.10	0.26	1.06	0.32	0.76	4.08
Mean			2.77	0.27	0.91	0.38	0.98	2.83
Post-Infection** (Day 26) Change			6.25	2.99 +0.22	0.13 -0.14	1.11 +0.20	0.38 0.00	0.89 -0.09
2/28	111* A	2.50	2.76	0.44	0.75	0.37	1.68	1.64
3/7		2.75	2.50	0.27	0.39	1.05	1.57	1.59
3/2		3.25	3.42	0.20	1.07	0.20	1.21	2.83
4/4		3.75	3.22	0.27	1.19	0.41	1.20	2.68
4/21		4.25	2.87	0.29	0.93	0.44	1.56	1.84
5/2		4.75	2.83	0.22	1.05	0.36	1.13	2.50
5/9		5.00	3.09	0.22	1.39	0.25	1.26	2.45
Mean			2.95	0.27	0.97	0.44	1.37	2.15
Post-Infection (Day 30) Change			6.25	2.72 -0.20	0.27 0.00	1.28 +0.31	0.60 +0.16	1.92 +0.55

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)			A/G ratio	
			Albumin	Alpha-1	Alpha-2		
	9 A						
2/28		2.50	3.72	0.35	0.98	0.99	3.74
3/7		2.75	3.52	0.24	0.93	0.55	3.35
3/21		3.25	3.30	0.37	0.93	0.23	2.62
4/4		3.75	3.43	0.27	0.76	0.76	3.17
4/21		4.25	2.78	0.20	1.12	0.54	1.92
5/2		4.75	3.15	0.45	0.80	0.45	3.00
5/9		5.00	2.93	0.33	1.22	0.35	1.99
Mean			3.26	0.31	0.96	0.45	2.74
Post-Infection (Day 30) Change		6.25	3.12 -0.14	0.23 -0.08	1.05 +0.09	0.65 +0.20	2.74 0.00
	121* B						
2/28		2.50	3.30	0.36	1.01	0.30	2.31
3/7		2.75	2.60	0.44	0.71	0.50	2.10
3/21		3.25	3.07	0.49	0.67	0.31	2.65
4/4		3.75	2.84	0.27	0.91	0.23	2.49
4/21		4.25	2.72	0.21	0.96	0.42	2.12
5/2		4.75	3.08	0.29	0.72	0.24	2.09
5/9		5.00	3.04	0.81	0.58	0.36	2.03
Mean			2.95	0.41	0.79	0.34	2.24
Post-Infection (Day 30) Change		6.25	2.76 -0.19	0.20 -0.19	1.12 +0.33	0.69 +0.34	1.42 -0.82

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	30 B	2.50	2.42	0.30	0.89	0.13	1.56	1.55
3/7		2.75	2.44	0.48	0.56	0.24	1.48	1.65
3/21		3.25	3.04	0.32	0.62	0.52	0.99	3.07
4/4		3.75	3.08	0.35	0.89	0.22	1.55	1.99
4/21		4.25	2.89	0.28	1.00	0.24	0.88	3.28
5/2		4.75	2.98	0.31	0.90	0.27	1.02	2.92
5/9	5.00	3.02	0.50	0.70	0.37	0.90	3.35	
Mean			2.84	0.36	0.79	0.28	1.20	2.37
Post-Infection (Day 30) Change		6.25	3.02 +0.18	0.32 -0.04	0.98 +0.20	0.41 +0.13	0.85 -0.35	3.55 +1.18
2/28	130* AB	2.50	2.85	0.26	0.74	0.36	1.68	1.70
3/7		2.75	2.62	0.25	0.43	0.60	1.89	1.38
3/21		3.25	2.93	0.42	0.55	0.21	1.39	2.11
4/4		3.75	2.88	0.63	0.87	0.23	1.57	1.83
4/21		4.25	2.54	0.28	1.42	0.38	1.48	1.72
5/2		4.75	3.08	0.30	1.18	0.46	1.18	2.61
5/9	5.00	3.02	0.27	1.67	0.60	1.43	2.11	
Mean			2.85	0.38	0.98	0.49	1.52	1.88
Post-Infection (Day 30) Change		6.25	3.15 +0.30	0.24 -0.14	1.62 +0.64	0.61 +0.12	1.58 +0.06	1.99 +0.11

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/C ratio	
			Albumin	Alpha-1	Alpha-2	Beta		Gamma
2/28	112 AB	2.50	3.07	0.37	0.88	0.24	2.43	1.26
3/7		2.75	--	--	--	--	--	--
3/21		3.25	2.78	0.40	0.69	0.81	1.91	1.45
4/4		3.75	3.38	0.34	0.84	0.56	1.97	1.71
4/21		4.25	2.67	0.34	1.10	0.27	1.82	1.47
5/2		4.75	2.97	0.39	0.62	0.68	1.43	2.08
5/9		5.00	2.98	0.37	0.77	0.63	1.54	1.93
Mean			2.98	0.37	0.82	0.53	1.85	1.61
Post-Infection** (Day 30) Change			6.25	+0.64	-0.04	+0.10	+0.19	-0.44
2/28	12* A	2.50	3.25	0.48	1.00	0.21	2.25	1.44
3/7		2.75	2.65	0.50	0.63	0.44	1.77	1.50
3/21		3.25	2.83	0.24	1.22	0.29	1.80	1.57
4/4		3.75	3.56	0.23	1.27	0.34	1.60	2.22
4/21		4.25	3.07	0.30	1.12	0.35	1.65	1.86
5/2		4.75	2.98	0.26	0.91	0.56	1.38	2.16
5/9		5.00	3.21	0.24	1.18	0.36	1.70	1.89
Mean			3.08	0.32	1.05	0.36	1.74	1.77
Post-Infection** (Day 33) Change			6.50	-0.38	-0.09	0.00	+0.23	+0.18

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type ¹)	Age (Months)	Electrophoresis Values (gms.%)			A/G ratio			
			Albumin	Alpha-1	Alpha-2		Beta	Gamma	
2/28	13 A	2.50	2.82	0.40	1.41	1.07	0.90	3.13	
3/7		2.75	2.47	0.40	1.45	1.05	1.82	1.36	
3/21		3.25	2.70	0.32	0.71	0.13	1.43	1.89	
4/4		3.75	2.76	0.36	0.91	0.19	1.28	2.16	
4/21		4.25	2.66	0.33	0.88	0.39	1.33	2.00	
5/2		4.75	3.40	0.36	0.76	0.23	1.35	2.52	
5/9		5.00	2.94	0.35	1.01	0.38	1.42	2.07	
Mean				2.82	0.36	1.02	0.49	1.36	2.07
Post-Infection** (Day 33) Change			6.50	3.18 +0.36	0.30 -0.06	1.17 +0.15	0.45 -0.04	1.38 +0.02	2.30 +0.23
2/28	28* B	2.50	2.81	0.23	1.17	0.32	1.97	1.50	
3/7		2.75	2.45	0.16	0.91	0.44	2.14	1.14	
3/21		3.25	3.02	0.22	1.15	0.54	1.26	2.39	
4/4		3.75	2.51	0.37	1.05	0.36	1.81	1.39	
4/21		4.25	2.42	0.23	1.04	0.43	1.47	1.65	
5/2		4.75	2.92	0.28	1.11	0.31	1.27	2.30	
5/9		5.00	3.02	0.20	1.14	0.47	1.18	2.56	
Mean				2.74	0.24	1.08	0.41	1.57	1.72
Post-Infection** (Day 33) Change			6.50	2.61 -0.13	0.17 -0.07	1.02 -0.06	0.51 +0.10	1.78 +0.21	1.46 -0.28

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)				A/G ratio		
			Albumin	Alpha-1	Alpha-2	Beta		Gamma	
2/28	29 B	2.50	3.17	0.36	0.65	0.38	1.04	3.04	
3/7		2.75	3.46	0.16	0.81	0.56	0.97	3.57	
3/21		3.25	2.75	0.18	0.60	0.36	0.72	3.82	
4/4		3.75	2.95	0.36	0.81	0.41	0.98	3.01	
4/21		4.25	2.62	0.18	0.59	0.18	1.13	2.32	
5/2		4.75	2.81	0.24	0.75	0.38	1.11	2.53	
5/9		5.00	2.76	0.22	0.88	0.36	1.07	2.58	
Mean				2.11	0.24	0.73	0.38	1.00	2.11
Post-Infection** (Day 33) Change			6.50	+0.68	-0.04	+0.13	+0.20	+0.12	2.49 +0.38
2/28	119* AB	2.50	1.76	0.35	0.56	0.39	1.52	1.16	
3/7		2.75	1.71	0.74	0.46	1.10	1.58	1.08	
3/21		3.25	2.62	0.51	1.60	0.73	1.24	2.11	
4/4		3.75	2.96	0.54	0.76	0.38	2.24	1.32	
4/21		4.25	2.14	0.37	1.22	0.51	2.25	0.95	
5/2		4.75	2.49	0.27	0.77	0.36	1.30	1.91	
5/9		5.00	3.11	0.35	1.06	0.38	1.50	2.07	
Mean				2.40	0.44	0.92	0.55	1.52	1.58
Post-Infection** (Day 33) Change			6.50	+0.46	-0.19	+0.40	+0.01	+0.38	1.50 -0.08

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)					A/G ratio
			Albumin	Alpha-1	Alpha-2	Beta	Gamma	
2/28	123* A	2.50	1.78	0.23	0.84	0.42	1.32	1.35
3/7		2.75	1.71	0.74	0.46	1.10	1.58	1.08
3/21		3.25	3.32	0.13	0.83	0.51	1.21	2.74
4/4		3.75	2.85	0.22	0.99	0.20	1.44	1.98
4/21		4.25	3.11	0.19	0.84	0.66	1.41	2.20
5/2		4.75	3.27	0.17	1.20	0.33	1.43	2.29
5/9		5.00	3.37	0.11	0.72	0.68	1.32	2.55
Mean			2.77	0.26	0.84	0.56	1.38	2.01
Post-Infection** (Day 38)		6.75	2.62	0.22	0.74	0.32	2.29	1.14
Change			-0.15	-0.04	-0.10	-0.24	+0.91	-0.87
2/28	25 A	2.50	2.98	0.33	1.12	0.12	1.74	1.71
3/7		2.75	2.95	0.78	0.93	0.00	1.94	1.52
3/21		3.25	2.49	0.24	0.98	0.53	2.16	1.15
4/4		3.75	3.73	0.25	0.97	0.51	1.53	2.80
4/21		4.25	2.77	0.21	0.94	0.47	1.69	1.64
5/2		4.75	2.74	0.31	1.26	0.41	1.67	1.62
5/9		5.00	2.95	0.27	0.83	0.45	1.59	1.85
Mean			2.94	0.34	1.00	0.44	1.76	1.67
Post-Infection** (Day 38)		6.75	2.58	0.28	1.35	0.61	1.97	1.31
Change			-0.36	-0.06	+0.35	+0.17	+0.21	-0.36

Changes in Serum Proteins (Continued)

Date Pre-Infection	Sheep No. (Type)	Age (Months)	Electrophoresis Values (gms.%)					
			Albumin	Alpha-1	Alpha-2	Beta	Gamma	A/G ratio
2/28	31* B	2.50	3.49	0.37	1.10	0.21	1.62	2.15
3/7		2.75	2.53	0.27	0.63	0.27	1.78	1.42
3/21		3.25	3.00	0.14	0.78	0.37	1.21	2.48
4/4		3.75	3.12	0.08	1.16	0.12	1.12	2.78
4/21		4.25	2.65	0.26	0.93	0.33	1.12	2.37
5/2		4.75	2.77	0.24	0.88	0.41	1.20	2.31
5/9		5.00	2.88	0.14	0.91	0.24	1.42	2.03
Mean			2.92	0.25	0.91	0.28	1.35	2.16
Post-Infection** (Day 38) Change			6.75	2.79	0.23	1.27	0.52	1.68
			-0.13	-0.02	+0.36	+0.24	+0.33	-0.50
2/28	136* AB	2.50	3.96	0.58	1.08	0.27	1.42	2.79
3/7		2.75	2.92	0.26	1.18	0.53	1.40	2.08
3/21		3.25	3.37	0.25	0.89	0.25	1.24	2.72
4/4		3.75	2.50	0.58	0.75	0.64	1.92	1.30
4/21		4.25	2.63	0.53	0.90	0.47	1.46	1.80
5/2		4.75	3.43	0.09	1.06	0.26	1.45	2.36
5/9		5.00	3.44	0.20	0.99	0.31	1.35	2.55
Mean			3.18	0.36	0.98	0.39	1.46	2.18
Post-Infection (Day 38) Change			6.75	3.13	0.42	1.14	0.64	1.18
			-0.05	+0.06	+0.16	+0.25	-0.28	+0.47

1 Hemoglobin Type

2 at necropsy

*infected lamb

APPENDIX V

Serum Antibody Titer and Egg Counts from Lambs Infected and Non-Infected with *Haemonchus contortus* (Pre- and Post-Infection).

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²		
				Larvae	Adult	
2/28	14* A	2.5	0	Negative	2	
3/7			0	Negative	2	
3/14			0	Negative	2	
3/21		3.0	0	2	2	
3/28			0	2	2	
4/4			0	2	2	
4/14		4.0	0	2	2	
4/21			0	2	2	
4/25			0	2	2	
5/2		4.5	0	2	2	
5/9			0	2	2	
5/16			0	2	2	
5/29**		5.5	0	8	16	
2/28		19 A	2.5	0	Negative	4
3/7				0	Negative	2
3/14	0			Negative	2	
3/21	3.0		0	2	2	
3/28			0	2	2	
4/4			0	2	2	
4/14	4.0		0	2	2	
4/21			0	2	2	
4/25			0	2	2	
5/2	4.5		0	2	2	
5/9			0	4	2	
5/16			0	4	2	
5/29**	5.5		0	4	16	
2/28	147* B		2.5	0	4	4
3/7				0	4	4
3/14		0		2	2	
3/21		3.0	0	2	2	
3/28			0	2	2	
4/4			0	2	2	
4/14		4.0	0	2	2	
4/21			0	2	4	
4/25			0	2	4	
5/2		4.5	0	2	4	
5/9			0	2	4	
5/16			0	2	4	
5/29**		5.5	0	4	64	

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	128 B	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		5.0	0	2	4
5/16			0	2	4
5/29**			0	4	4
2/28	15* AB	2.5	0	2	4
3/7			0	4	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	2
5/29**			0	4	8
2/28	124 AB	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	Negative	2
3/21			0	Negative	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	4
5/9		5.0	0	2	4
5/16			0	2	4
5/29**			0	4	64

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	132* A	2.5	0	2	8
3/7			0	2	8
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	4	4
4/14		4.0	0	4	4
4/21			0	4	4
4/25		4.5	0	4	4
5/2			0	4	4
5/9		5.0	0	4	4
5/16			0	4	4
6/4**		5.5	0	4	16
2/28	120 A	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	4	4
6/4**		5.5	0	8	4
2/28	109* B	2.5	0	Negative	8
3/7			0	Negative	4
3/14		3.0	0	Negative	4
3/21			0	Negative	2
3/28		3.5	0	Negative	2
4/4			0	2	2
4/14		4.0	0	Negative	2
4/21			0	Negative	2
4/25		4.5	0	2	2
5-2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	2
6/4**		5.5	0	8	32

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	122 B	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	4
3/21			0	2	2
3/28		3.5	0	Negative	2
4/4			0		2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9			0	2	2
5/16		5.0	0	2	2
6/4**		5.5	0	8	8
2/28	27* AB	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9			0	2	2
5/16		5.0	0	2	2
6/4**		5.5	0	16	4
2/28	139 AB	2.5	0	2	2
3/7			0	2	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	4	2
5/2			0	4	2
5/9			0	2	2
5/16		5.0	0	4	4
6/4**		5.5	0	8	4

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	10* A	2.5	0	Negative	4
3/7			0	Negative	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		0	2	2	
5/16		5.0	0	2	2
6/9**		5.75	0	8	16
2/28	17 A	2.5	0	Negative	4
3/7			0	Negative	4
3/14		3.0	0	Negative	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		0	4	4	
5/16		5.0	0	4	4
6/9**		5.75	0	8	4
2/28	117* B	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	Negative	2
3/21			0	Negative	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	4
5/2			0	2	4
5/9		0	2	8	
5/16		5.0	0	2	8
6/9**		5.75	0	4	8

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	11 B	2.5	0	Negative	4
3/7			0	Negative	4
3/14		3.0	0	Negative	4
3/21			0	Negative	4
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		0	2	4	
5/16		5.0	0	2	4
6/9**			5.75	0	8
2/28		118* A	2.5	0	Negative
3/7	0			Negative	4
3/14	3.0		0	Negative	4
3/21			0	2	4
3/28	3.5		0	2	4
4/4			0	2	4
4/14	4.0		0	2	4
4/21			0	2	4
4/25	4.5		0	4	4
5/2			0	4	4
5/9	0		4	4	
5/16	5.0		0	8	8
6/13**			6.0	0	4
2/28	110 Δ		2.5	0	2
3/7		0		2	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		0	4	4	
5/16		5.0	0	4	8
6/13**			6.0	0	8

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	21 A	2.5	0	2	16
3/7			0	2	8
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	4	4
4/21			0	4	4
4/25		4.5	0	8	4
5/2			0	8	4
5/9		5.0	0	8	4
5/16			0	8	16
6/13**		6.0	0	8	64
2/28	127* B	2.5	0	2	4
3/7			0	4	4
3/14		3.0	0	4	4
3/21			0	4	4
3/28		3.5	0	4	4
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	4	2
5/9		5.0	0	4	2
5/16			0	2	4
6/13**		6.0	0	16	32
2/28	134 B	2.5	0	Negative	8
3/7			0	Negative	4
3/14		3.0	0	Negative	4
3/21			0	2	4
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	4	2
5/16			0	8	2
6/13**		6.0	0	8	2

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	137* AB	2.5	0	4	8
3/7			0	2	8
3/14			0	Negative	2
3/21		3.5	0	2	2
3/28			0	2	2
4/4			0	2	2
4/14			0	2	2
4/21			0	2	2
4/25			0	2	2
5/2		4.5	0	2	2
5/9			0	2	2
5/16			0	2	4
6/13**			0	8	16
2/28	114* A	2.5	0	Negative	2
3/7			0	Negative	2
3/14			0	Negative	Negative
3/21		3.0	0	Negative	Negative
3/28			0	Negative	2
4/4			0	Negative	Negative
4/14			0	Negative	Negative
4/21			0	Negative	Negative
4/25			0	Negative	Negative
5/2		4.5	0	Negative	Negative
5/9			0	Negative	Negative
5/16			0	2	2
6/18**			400	16	32
2/28	24 A	2.5	0	2	2
3/7			0	2	2
3/14			0	2	2
3/21		3.0	0	2	2
3/28			0	2	2
4/4			0	2	2
4/14			0	2	2
4/21			0	2	2
4/25			0	2	2
5/2		4.5	0	2	2
5/9			0	2	2
5/16			0	2	2
6/18**			0	8	8

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	113* B	2.5	0	2	2
3/7			0	2	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	2
6/18**		6.0	200	8	4
2/28		126 B	2.5	0	2
3/7	0			2	4
3/14	3.0		0	2	2
3/21			0	2	2
3/28	3.5		0	2	2
4/4			0	2	2
4/14	4.0		0	2	2
4/21			0	2	2
4/25	4.5		0	2	2
5/2			0	2	2
5/9	5.0		0	2	2
5/16			0	2	4
6/18**	6.0		0	8	8
2/28	115* A		2.5	0	2
3/7		0		2	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	2
6/23**		6.25	3,500	8	8

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	23 A	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		5.0	0	2	4
5/16			0	2	4
6/23**		6.25	0	4	4
2/28		22* B	2.5	0	Negative
3/7	0			Negative	2
3/14	3.0		0	Negative	2
3/21			0	2	2
3/28	3.5		0	2	2
4/4			0	2	2
4/14	4.0		0	2	2
4/21			0	2	2
4/25	4.5		0	2	2
5/2			0	2	2
5/9	5.0		0	2	2
5/16			0	2	2
6/23**	6.25		1,400	32	32
2/28	141 B		2.5	0	Negative
3/7		0		Negative	2
3/14		3.0	0	Negative	2
3/21			0	Negative	2
3/28		3.5	0	Negative	2
4/4			0	Negative	2
4/14		4.0	0	Negative	2
4/21			0	Negative	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	4
6/23**		6.25	0	8	4

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²		
				Larvae	Adult	
2/28	16* AB	2.5	0	Negative	2	
3/7			0	Negative	2	
3/14		3.0	0	Negative	2	
3/21			0	Negative	2	
3/28		3.5	0	Negative	2	
4/4			0	Negative	2	
4/14		4.0	0	Negative	2	
4/21			0	Negative	2	
4/25		4.5	0	Negative	2	
5/2			0	Negative	2	
5/9			0	2	2	
5/16		5.0	0	2	2	
6/23**		6.25	1,200	16	16	
2/28		111* A	2.5	0	2	4
3/7				0	2	4
3/14			3.0	0	2	4
3/21	0			2	4	
3/28	3.5		0	2	4	
4/4			0	2	4	
4/14	4.0		0	2	4	
4/21			0	2	4	
4/25	4.5		0	2	4	
5/2			0	2	4	
5/9			0	2	4	
5/16	5.0		0	2	4	
6/27**	6.25		4,400	8	8	
2/28	9 A		2.5	0	2	4
3/7				0	2	4
3/14			3.0	0	2	4
3/21		0		2	4	
3/28		3.5	0	2	4	
4/4			0	2	4	
4/14		4.0	0	2	4	
4/21			0	2	4	
4/25		4.5	0	2	4	
5/2			0	4	4	
5/9			0	4	4	
5/16		5.0	0	4	4	
6/27**		6.25	0	8	8	

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	121* B	2.5	0	Negative	Negative
3/7			0	Negative	Negative
3/14		3.0	0	Negative	Negative
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		0	2	2	
5/16		5.0	0	2	2
6/27**		6.25	5,600	32	16
2/28	30 B	2.5	0	2	4
3/7			0	2	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		0	2	4	
5/16		5.0	0	2	4
6/27**		6.25	0	8	4
2/28	130* AB	2.5	0	2	2
3/7			0	2	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	4
5/9		0	2	4	
5/16		5.0	0	2	4
6/27**		6.25	6,000	32	64

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	112 AB	2.5	0	Negative	2
3/7			0	Negative	2
3/14			0	Negative	2
3/21		3.0	0	2	2
3/28			0	2	2
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25			0	2	4
5/2		4.5	0	2	4
5/9			0	2	4
5/16			0	4	4
6/27**		6.25	0	4	4
2/28	12* A	2.5	0	Negative	2
3/7			0	Negative	2
3/14			0	Negative	2
3/21		3.0	0	2	2
3/28			0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25			0	2	4
5/2		4.5	0	2	4
5/9			0	2	4
5/16			0	2	4
7/2**		6.25	7,600	16	16
2/28	13 A	2.5	0	4	4
3/7			0	4	4
3/14			0	4	4
3/21		3.0	0	4	4
3/28			0	4	4
4/4			0	4	4
4/14		4.0	0	4	2
4/21			0	4	2
4/25			0	4	2
5/2		4.5	0	4	2
5/9			0	4	4
5/16			0	4	4
7/2**		6.25	0	8	4

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	28* B	2.5	0	4	4
3/7			0	4	4
3/14		3.0	0	2	4
3/21			0	2	4
3/28		3.5	0	2	4
4/4			0	2	4
4/14		4.0	0	2	4
4/21			0	2	4
4/25		4.5	0	2	4
5/2			0	2	4
5/9		5.0	0	2	4
5/16			0	2	4
7/2**		6.5	5,800	16	8
2/28	29 B	2.5	0	4	4
3/7			0	4	4
3/14		3.0	0	4	4
3/21			0	4	4
3/28		3.5	0	4	4
4/4			0	4	2
4/14		4.0	0	4	2
4/21			0	4	2
4/25		4.5	0	4	2
5/2			0	4	2
5/9		5.0	0	4	2
5/16			0	4	4
7/2**		6.5	0	8	16
2/28	119* AB	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	Negative	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9		5.0	0	2	2
5/16			0	2	2
7/2**		6.5	8,200	16	4

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	123* A	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9			0	2	2
5/16		5.0	0	2	2
7/7**		6.75	4,200	16	8
2/28	25 A	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	Negative	2
3/21			0	Negative	2
3/28		3.5	0	Negative	2
4/4			0	Negative	2
4/14		4.0	0	Negative	2
4/21			0	Negative	2
4/25		4.5	0	Negative	2
5/2			0	2	4
5/9			0	2	4
5/16		5.0	0	2	8
7/7**		6.75	0	8	8
2/28	31* B	2.5	0	2	2
3/7			0	2	2
3/14		3.0	0	2	2
3/21			0	2	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9			0	2	2
5/16		5.0	0	2	2
7/7**		6.75	8,800	8	4

Serum Antibody Titer (Continued)

Date	Sheep No. (Type ¹)	Age (Months)	Egg Count	Reciprocal Antibody Titer ²	
				Larvae	Adult
2/28	136* AB	2.5	0	Negative	2
3/7			0	Negative	2
3/14		3.0	0	Negative	2
3/21			0	Negative	2
3/28		3.5	0	2	2
4/4			0	2	2
4/14		4.0	0	2	2
4/21			0	2	2
4/25		4.5	0	2	2
5/2			0	2	2
5/9			0	2	2
5/16		5.0	0	2	2
7/7**		6.75	5,600	16	8

¹Hemoglobin Type

²Determined by IHA

*Infected

**Day of Necropsy

APPENDIX VI

Protein Values¹ in Abomasal Mucous Exudate from
Lambs Infected and Non-Infected with *Haemonchus contortus*
with Regard to Hemoglobin type.

Sheep No.	Type ²	Day Post-Infection	Protein Values (%)			
			Albumin	Mucoprotein & Alpha- Globulin	Mucoprotein & Beta-Globulin	Gamma- Globulin
14*	A	1	23.8	4.5	59.5	12.2
19	A	1	48.5	6.1	32.2	9.2
147*	B	1	42.3	8.3	30.3	19.1
128	B	1	34.1	12.6	40.4	12.9
15*	AB	1	52.1	6.1	21.4	20.4
124	AB	1	34.0	8.5	43.1	14.4
132*	A	7	35.5	11.3	35.5	17.7
120	A	7	24.2	37.4	19.2	19.2
109*	B	7	48.8	16.5	20.7	14.0
122	B	7	47.8	4.4	19.6	28.2
27*	AB	7	30.7	2.5	53.5	13.3
139	AB	7	49.1	10.7	24.8	15.4
10*	A	12	32.6	10.2	40.1	17.1
17	A	12	37.9	10.1	38.9	13.1
117*	B	12	29.9	13.8	41.3	15.0
11	B	12	30.0	3.7	48.1	19.2
118*	A	16	35.8	11.1	28.5	24.6
110	A	16	33.4	11.0	43.0	12.6
21	A	16	40.0	4.7	37.5	17.8
127*	B	16	30.5	14.7	39.0	15.8
134	B	16	42.7	8.3	23.1	25.9
137*	AB	16	44.5	5.5	24.8	25.2
114*	A	21	29.6	5.0	31.8	33.6
24	A	21	30.6	3.3	36.4	29.7
113*	B	21	25.0	4.9	48.4	21.7
126	B	21	32.9	9.3	40.9	16.9
115*	A	26	27.6	23.1	29.8	19.6
23	A	26	36.1	11.6	34.7	17.6
22*	B	26	18.2	27.5	34.1	20.2
141	B	26	41.8	8.6	29.8	19.8
16*	AB	26	19.7	12.0	39.3	29.0

Abomasal Mucous (Continued)

Sheep No.	Type ²	Day Post-Infection	Protein Values (%)			
			Albumin	Mucoprotein & Alpha- Globulin	Mucoprotein & Beta-Globulin	Gamma- Globulin
111*	A	30	14.5	1.9	63.1	20.5
9	A	30	36.7	5.9	30.1	27.3
121*	B	30	27.1	21.3	29.3	22.3
30	B	30	43.0	4.6	26.2	26.2
130*	AB	30	11.2	10.6	58.3	19.9
112	AB	30	34.3	17.3	28.3	20.1
12*	A	33	33.2	9.6	35.1	22.1
13	A	33	28.5	13.1	32.5	25.9
28*	B	33	38.7	14.5	25.3	21.5
29	B	33	40.6	12.5	29.8	17.1
119*	AB	33	30.5	7.9	27.1	34.5
123*	A	38	41.0	7.3	24.1	27.6
25	A	38	34.4	6.1	34.1	25.4
31*	B	38	27.2	9.9	40.2	22.7
136*	AB	38	12.3	4.8	49.5	33.4

¹Determined by Electrophoresis²Hemoglobin Type

*Infected Lamb

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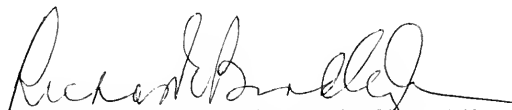
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BIOGRAPHICAL SKETCH

Jay Barry Klein was born at Miami Beach, Florida, on November 4, 1946. He received an Associate of Arts degree from Miami-Dade Junior College in May, 1966. His Bachelor of Science degree in Chemistry and Biology was received from the University of Miami in August, 1968. Beginning in September, 1968 until June, 1970, Mr. Klein taught basic science and mathematics at the Lear School in Miami, Florida. In January, 1971, he enrolled at the University of Florida and subsequently entered the graduate program in the Department of Animal Science. He received a Master of Science in Agriculture majoring in Parasitology in August, 1973.

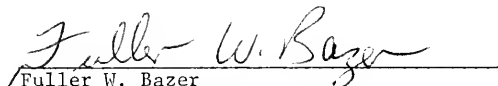
Jay Barry Klein was awarded a National Institute of Health Pre-Doctoral Traineeship in September, 1972. He is a member of Alpha Zeta Fraternity and the American Society of Parasitologists. He is married to Roberta Joyce and has a son, David and daughter, Jennifer.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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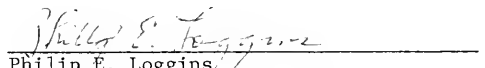
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