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Epidemiology of Strongylate Nematode and Coccidia (Eimeria) Infection in Sheep With Special Reference to Breed Resistance.

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EPIDEMIOLOGY OF STRONGYLATE NEMATODE AND COCCIDIA (*EIMERIA*) INFECTION
IN SHEEP WITH SPECIAL REFERENCE TO BREED RESISTANCE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Interdepartmental Program
in
Veterinary Medical Sciences

by
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December 1994

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To my cousin, Kalpana.

ACKNOWLEDGEMENTS

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ABSTRACT

In the United States, the annual production losses in small ruminants due to internal parasites has been estimated at $45 million. In 1992, 1993 and 1994, breed specific responses to naturally acquired strongylate nematode and coccidia (*Eimeria*) infections were compared in three classes (suckling lambs, weaned lambs, and mature ewes) of pure-bred Suffolk and sheep native to Louisiana (Native) which were grazed together on infective pasture. Parasitological (fecal egg counts and nematode counts) and hematological (packed cell volumes, total immunoglobulin levels, total and class-specific anti-*Haemonchus contortus* antibody levels and eosinophil counts) data were collected.

In suckling Native lambs, resistance to *H. contortus* infection developed by 7-10 weeks of age, manifested by reduced fecal egg counts, high packed cell volumes and reduction in adult nematode burdens and a slower decline in maternal antibodies. In contrast, suckling Suffolk lambs remained unresponsive to strongylate nematode infection and suffered mortality.

Anthelmintic treatments administered to weaned Native lambs were not detrimental to the protective responses to *H. contortus*. Weaned Suffolk lambs and mature ewes continued to remain susceptible to strongylate nematode infection and susceptibility was complicated by anthelmintic resistance.

Total *H. contortus* specific immunoglobulin levels indicated that both breeds became immune competent by three to four months of age. Total immunoglobulin levels and anti-*H. contortus* IgM, IgA, IgG1 and IgG2 levels were lower in weaned Suffolk lambs compared to Native lambs. No differences were seen in the blood eosinophil counts between the weaned lambs and mature ewes of the two breeds.

The variability demonstrated in the parasitological and packed cell volume data indicates that breed substitution or possibly cross-breeding could alleviate production losses and the problems associated with anthelmintic resistance.

In 1992 and 1993, no differences were detected between the overall coccidial oocyst output of both lambs and mature ewes of the two breeds. *Eimeria crandallis* was the predominant species. In suckling lambs, maximum numbers of *E. crandallis* oocysts excreted in feces occurred between six to nine weeks after turnout and no clinical coccidiosis occurred.

INTRODUCTION

In the United States, internal parasites such as strongylate nematodes and coccidia cause serious economic losses to the sheep industry (Drummond et al., 1981). These losses are mainly due to poor performance in ewes, unthriftiness and death of lambs and cost of anthelmintics. Traditionally, anthelmintics have been used either prophylactically or curatively to control pathogenic parasites of sheep such as *Haemonchus contortus* and *Trichostrongylus colubriformis*. Widespread development of anthelmintic resistance and increasing concern about the environmental impact of anthelmintic use, however, necessitate the need to investigate alternative control procedures such as rotational grazing, vaccination of susceptible sheep and breeding for increased nematode resistance.

The epidemiology of strongylate nematodes mainly depends on the nematode species and the prevalent weather and climatic conditions of a geographic area (Coles, 1986). A thorough knowledge of epidemiological factors and their interactions is essential for strategic application of anthelmintics and for implementation of sound pasture management programs. In the northern United States, hypobiosis during fall and winter and confinement of sheep during winter limit the number of generations of *H. contortus* per year to one or two (Herd et al., 1984a; Capitini et al., 1990). But in the southern United States, the hot rainy summer and relatively mild winter conditions could provide excellent microclimatic conditions for uninterrupted development of free-living stages of strongylate nematodes leading to heavy chronic parasitism and consequent financial losses, especially, in domestic breeds of sheep on pasture based production systems (Miller, J.E., personal communication).

Since, there is evidence that host defense mechanisms are needed for chemotherapeutics to eliminate pathogens (reviewed by Doenhof and Davies, 1992), selection for anthelmintic resistance would be more important in situations where susceptible breeds of sheep are maintained on pasture with suppressive anthelmintic treatments in climatic zones which could support development of many nematode generations per year. The premise of rotational grazing is to reduce the need for such anthelmintic treatment by removing animals from pasture which would allow the free-living stages

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of parasitic nematodes to die, thus, breaking the life cycle and reducing reinfection. To achieve this, pastures have to be rested for longer than the survival time of infective larvae [L3] (Levive et al., 1975). Whilst pasture rotation increases the grazing capacity of the land considerably, free-living stages can develop quickly and L3 can survive for long periods of time rendering this approach of control unreliable (Levine et al., 1975; Banks et al., 1990).

Vaccines containing various sources of nematode antigens (metabolic enzymes, structural proteins, and excretory/secretory products) have been developed to control economically important sheep nematodes such as *H. contortus*, *T. colubriformis*, *Ostertagia circumcinta* and *Oesophagostomum columbianum* (reviewed by Emery and Wagland, 1991). However, effectiveness of such vaccines have only been determined under controlled experimental conditions and large scale field trials have not yet been conducted to demonstrate control under normal management conditions. One problem with vaccines is that, so far, they fail to protect young lambs which are immune incompetent and thus are subjected to severe parasitism.

In sheep, variation in resistance to nematode infection is genetically determined and heritable (Gray and Woolaston, 1991). Therefore another means of control could be achieved by selection for resistance. If it could be proven by cost-benefit analysis that genetically resistant sheep would bring better financial returns, then one could replace a susceptible breed with a resistant breed or select resistant individuals within the susceptible breed to be used in appropriate breeding programs to build up flock resistance.

It is encouraging to see that though *H. contortus* has a very high biotic potential and a relatively short generation time, it is not capable of adapting to host immune mechanisms even after several passages in resistant hosts (Woolaston et al., 1992). However, there are reports to indicate that *T. colubriformis* can adapt to host resistance (Gray and Woolaston, 1991). The prerequisites to the practicalities of breeding for nematode resistance are identification of genetically resistant breeds and a knowledge of factors which can interact with the development and perpetuation of acquired

resistance. A knowledge of effector mechanisms is also essential to devise methods to induce protection in the susceptible sheep by vaccination or immunotherapy.

Research conducted in Ohio and elsewhere in the United States showed that breeds such as St. Croix, Florida Native, and Barbados Blackbelly, and their cross-bred progeny are more resistant to strongylate nematode infection than domestic breeds (Yazwinski et al., 1981; Courtney et al., 1985; Gamble and Zajac, 1992). Previous studies with mature ewes, ewe lambs and tracer lambs maintained on the same and separate pastures at the Central Research Station, Louisiana Agricultural Experimental Station indicated that Native sheep compared to Suffolk sheep had superior nematode resistant qualities (Lemarie, 1985; Miller et al., 1993).

The principle breed raised in Louisiana is the Suffolk. The native (Native) breed in Louisiana is an upgraded white-faced scrub sheep originating from early introductions into the area. The Native sheep is primarily a wool breed and constitutes only a fraction of the commercial sheep population now found in Louisiana. Phenotypically, although they resemble the Native sheep found in other Gulf Coast States (Alabama, Mississippi and Florida) whether genetic differences exist among these Native sheep is not known (Miller, J.E., personal communication). The Native sheep possess many hardiness qualities that make the sheep excellent for those that want to pursue a low-input and sustainable type sheep operation (Fernandez, J.M., personal communication). They thrive in the hot, humid southern United States without the benefit of anthelmintic treatments, remain productive with the consumption of poor quality forage with no supplementation, and do not have any lambing or lamb-rearing difficulties.

The predominant strongylate nematodes found in sheep in Louisiana are *H. contortus* and *T. colubriformis* (Lemarie, 1985). *Oesophagostomum columbianum*, *T. axei*, *Cooperia* spp., and *Nematodirus* spp. are rarely found.

The primary objectives of the present study were to determine the comparative susceptibilities of suckling Native and Suffolk lambs to strongylate nematode infection when subjected to natural challenge on the same pasture and how repeated anthelmintic treatments would

influence the expression of resistance in weaned lambs and ewes of the two breeds. Studies were also conducted to determine anti-*H. contortus* antibody levels between the two breeds.

When compared to parasitic gastroenteritis due to strongylate nematode infection, little or no attention is generally paid to clinical coccidiosis in mature sheep on pasture because clinical disease is relatively rare. Ovine coccidia research in the United States has covered a wide range of disciplines (Lotze, 1953; Shah, 1963; Mahrt and Sherrick, 1965; Levine and Ivens, 1970; Fitzgerald and Mansfield, 1978; Horton and Stockdale, 1979; Foreyt et al., 1981a,b). These studies include descriptions of new species and life histories, determinations of prevalence, efficacy of anticoccidials under experimental conditions, and outbreak investigations. However, there has been very little research related to coccidia (*Eimeria*) in sheep during the last 15 years. Previous studies at Louisiana State University suggested that there was a difference in coccidia infection between Suffolk and Native lambs (Miller, unpublished observations). Therefore, another objective of the present study was to investigate the interaction of *Eimeria* spp. and gastrointestinal nematode infection in the Suffolk and Native breeds.

REVIEW OF LITERATURE

The epidemiology of strongylate nematode infection in sheep is a complex interaction among the host, parasite, and the external environment. Therefore, the review of literature is primarily focused on discussing the environmental factors influencing this triad and variation in host responses to infection.

1. Development and survival of free-living stages of strongylate nematodes

The infection cycle can be divided into two phases. One is the development of eggs to L3 on pasture and other phase is the development from L3 to adult in the host. The life cycle of strongylate nematodes is direct and the host is infected by ingesting the L3 with herbage. Age, breed, availability of susceptible host, favorable microclimatic conditions, survivability of L3, hypobiosis, peri-parturient rise in fecal egg counts, and management practices all determine infection levels in the host.

The infection potential of pastures depends on the environmental conditions (rainfall, temperature, soil moisture, microhabitat, and microclimate) which influence hatching of eggs, and development and survival of the first-stage (L1), second-stage (L2), and L3 larvae (Levine, 1963). Temperatures between 20-30°C ensure the greatest survival of eggs. Maximum rate of egg development proceeds at a temperature range of 15-27°C when soil water discharge is not more than 2 cm (Levine, 1963). Temperatures below 7°C inhibit development (Crofton et al., 1965; Vlassoff, 1982). When pasture growth is supported with irrigation, temperatures above 15°C result in faster development of eggs of *H. contortus* yielding a maximum of four generations per year (Uriaorte and Valderrabana, 1989).

Cold temperatures are detrimental to both eggs and L3 of *H. contortus*, but only the eggs of *T. colubriformis* (Levine et al., 1974; Beveridge et al., 1989). In the winter, intermittent development can occur even when the minimum ground level temperature falls below 4°C if the temperature in fecal pellets is above 7°C (Besier and Dunsmore, 1993a).

Without moisture, thermal energy can not affect development of eggs. Moisture also protects the free-living stages from desiccation. A 25 mm rainfall spread across a 10 day period produces optimum moisture conditions for development (Coles, 1986). However, the distribution of rainfall in the few days following deposition of feces is more critical than the total amount received (Besier and Dunsmore, 1993b).

Laboratory studies indicated that increasing relative humidity from 70 to 100% positively influences the rate of development of eggs to L3 (Levine, 1963). A reduction in the relative humidity from 100 to 85% would completely abolish the development of eggs to L3 at 20-35°C. Under laboratory conditions, a combination of 100% relative humidity and 30°C will optimize development of *H. contortus* and *T. colubriformis* eggs to L3 within 3 to 4 days (Hsu and Levine, 1977).

The survival of L3 larvae of *H. contortus* and *T. colubriformis* is associated with low temperatures, high rainfall and the presence of a large quantity of pasture herbage (Beveridge et al., 1989; Besier and Dunsmore, 1993b). Infective larvae of *H. contortus* can survive up to 256 days at 4°C, 128 days at 20°C, and 64 days at 25°C and 35°C (Todd et al., 1976). When temperatures are conducive for larval survival, rainfall becomes the limiting factor (Onyali et al., 1990). In the northern United States, during the summer and fall grazing seasons using pasture rotation, L3 can survive in large enough numbers on pasture, that has been free of sheep for 48 days, to cause severe infections in lambs during the second rotation (Levine et al., 1975).

Thus, there is an inverse relationship between the optimum temperature for development of eggs to L3 and for survival of L3. The infection potential of pasture depends on the fraction of eggs reaching the L3 stage, the number of days L3 can survive, and the number of degree days (optimum temperatures for a specified number of days) required for the larvae to reach the L3 stage (Levine, 1963). The number of degree days required for the development from eggs to L3 is critical in determining infection potential (Levine, 1963).

Horizontal movement of L3 from fecal pellets to the external environment is followed by vertical migration which is also influenced by rainfall, temperature and relative humidity (Rose, 1964; Callinan and Westcott, 1986). Herbage height is another factor which can influence infection potential. In an average summer, wet pastures with lush herbage can provide a more dangerous source of infection than well drained pastures (Rose, 1964).

2. Source of strongylate nematode infection for ewes and lambs on pasture

Since the development and survival of free-living stages are dependent on weather and climatic conditions, it is important to identify the factors conducive to the initiation and propagation of infection. For ewes and lambs on pasture, strongylate nematode infection in the spring can originate from the peri-parturient rise in fecal egg counts which has three sources (Connan, 1971; Gibson and Everett, 1972; Herd et al., 1983; Taylor et al., 1990):

(1)   Relaxation of immunity resulting in increased fecundity of nematodes.

(2)   Maturation of hypobiotic larvae.

(3)   Overwintered larvae on previously grazed pasture.

The peri-parturient rise in ewe fecal egg counts is a universal phenomenon and, as the name indicates, occurs around the time of lambing (Schillhorn vanVeen and Ogunsuri, 1978; Lemarie, 1985; Taylor et al., 1990). Experiments with goats kidding the whole year demonstrated that the peri-parturient rise and hypobiosis are totally independent events (Fakae, 1990). The peri-parturient rise can occur in sheep lambing during the dry season (Schillhorn vanVeen and Ogunsuri, 1978). In year round or in accelerated lambing programs, the peri-parturient rise can be a continuous source of infection for lambs throughout the year (Agyei et al., 1991). Apart from being the source of infection for lambs, ewes may also suffer from acute haemonchosis during the peri-parturient rise period (Connan, 1971; Thomas and Ali, 1983; Taylor et al., 1990). Depression of immunity due to hormonal changes at parturition (Reinecke, 1989) is the underlying cause of the peri-parturient rise.

Physiologically, hypobiosis is equivalent to diapause in insects and it ensures the survival of the parasite through times of the year that are detrimental to survival of free-living stages (Connan, 1971; Schillhorn vanVeen and Ogunsuri, 1978; Ikeme et al., 1987; Captini et al., 1990). In a study of parasite population changes in a winter rainfall area in Australia, abomasal nematode counts performed on a flock of naturally infected spring-born lambs indicated that *H. contortus* could overwinter almost entirely in the host as hypobiotic larvae (Thomas and Waller, 1979). In England, hypobiosis occurs during the fall and both *H. contortus* and *Ostertagia* spp. undergo hypobiosis equally well (Connan, 1971).

While the strain of *H. contortus* infecting sheep in the northern United States undergoes 40-100% hypobiosis during winter, only a slight degree of hypobiosis occurs in the strain parasitizing sheep in the southern United States (Herd et al., 1984a; Lemarie, 1985; Capitini et al., 1990). Lemarie (1985) conducted monthly necropsies on Suffolk and Native tracer lambs that grazed with their respective ewe flock. In Suffolk tracers, when the mean *H. contortus* burden reached a peak of 51,090 in October, only 5% of the population was early fourth-stage larve (L4). During the period of this presumed hypobiosis, substantial numbers of nematodes were also present in late L4 and early adult stages. These findings convinced the authors that density dependent constraints, acting upon the establishment of nematodes, was probably the major factor influencing the observed hypobiotic trend of *H. contortus* and hypobiosis may not be prominent in the life cycle of *H. contortus* in the Gulf Coast region of the United States

Winter hypobiosis of *H. contortus* in the northern United States has now been established to be an obligatory survival mechanism independent of external stimuli such as decreasing temperature and photo period (Captini et al., 1990). In the southern United States, because hypobiosis is minimal (Miller, unpublished observations) increasing numbers of adult nematodes might cause severe haemonchosis in late summer and fall.

Haemonchus contortus L3 from the previous year's lamb crop can overwinter on pasture in enough numbers to cause infection in spring-born lambs (Kerboeuf, 1985). In Canadian spring conditions, transfer of dewormed ewes and lambs to contaminated pastures resulted in lambs acquiring two waves of infections; the first originating from overwintered larvae and the second from the peri-parturient rise (Thomas and Boag, 1972). Thus, hypobiotic and overwintered larvae contribute to the overall peri-parturient rise in the spring. Anthelmintic treatment of parturient ewes with an anthelmintic effective against hypobiotic larvae and adult nematodes is therefore necessary to prevent the occurrence of clinical haemonchosis and the resultant pasture contamination (Taylor et al., 1990).

In Louisiana, overwintered larvae may be an important contributor to peri-parturient rise. Serial fecal egg counts of ewes and lambs which grazed naturally infective pastures and nematode burdens of concurrently grazing tracer lambs indicated that strongylate nematode infections started increasing in early spring reaching pathogenic level from late spring through summer and declined to non pathogenic levels the remainder of the year (Lemarie, 1985; Miller, unpublished observations).

3. Anthelmintic treatment and provision of "safe" pasture for ewes and lambs

Lambs and ewes grazing infective, poor quality pastures can develop chronic haemonchosis manifested by chronic anemia, severe loss of body condition and death (Allonby, and Urquhart, 1975). Under these circumstances, suppressive anthelmintic treatments in 21 day cycles would be the preferred choice for control.

Various workers have proposed different treatment programs for parturient ewes to control the peri-parturient rise (Darvill et al., 1978; Zhou and Schillhorn vanVeen, 1986; Taylor et al., 1990). Pre-lambing anthelmintic treatment has been shown to postpone the peri-parturient rise and increase milk yield in ewes (Darvill et al., 1978). Zohu and Schillhorn vanVeen (1986) achieved effective control by treating housed ewes after lambing. The authors argued that since anthelmintics might not be 100% effective against hypobiotic larvae, treating ewes postpartum would allow time

for the completion of development of hypobiotic larvae to adult nematodes, which would then be susceptible to treatment.

Another study showed that if ewes are treated at lambing, the peri-parturient rise could be effectively suppressed; however, there was no major impact on the egg output of lambs during the summer (Thomas and Boag, 1972). In the northern United States, maturation of hypobiotic larvae in housed ewes lambing early in the spring and pasture contamination from late lambing ewes grazing contaminated spring pastures resulted in a peri-parturient rise two to four weeks after the start of lambing (Herd et al., 1983). Based on these findings, the authors formulated an anthelmintic treatment protocol for sheep producers in the northern United States targeting the hypobiotic larvae and adult nematodes in the early and late lambing ewes, respectively.

Lambs of susceptible sheep breeds are known to develop heavy nematode infections after weaning for which anthelmintic treatment is warranted (Southcott, 1971). Hence, provision of "safe" pastures for ewes and lambs following a dose of anthelmintic at weaning is important to prevent reinfection. A "safe" pasture is not necessarily free of infective larvae, but is one in which infectivity is sufficiently reduced so that infection increases slowly when susceptible stock graze. Though, increase of infections to pathogenic levels cannot be excluded, weaning lambs on to "safe" pasture following anthelmintic treatment has been shown to delay the occurrence of acute haemonchosis during summer in the Netherlands (Eysker, 1982).

When "treat and move" strategies are adopted, at least 72 hours should elapse before putting animals on the new pasture to allow fertile nematode ova to reach minimum levels in feces which will reduce contamination of the "safe" pasture. A single lamb heavily infected with *H. contortus* that escaped treatment caused failure of a 'treat and move' strategy (Herd et al., 1984b). The benefits of better weight gains, lower nematode burdens and extra wool production in ewes have been demonstrated in sheep grazing "safe" pastures. (Mitchell and Fitzsimons, 1983; Mitchell and Fitzsimons, 1984; Waller et al., 1987a,b; Fawcett and McDonald, 1988). When weaned lambs are subjected to substantial parasite challenge, the superimposed stress of weaning can predispose lambs

to heavy infections resulting in reductions in packed cell volumes and weight gain (Watson, 1991).

This would further justify the need for provision of "safe" pasture for weaned lambs.

For spring grazing of lambs and ewes, "safe" pastures can be established by grazing the

pasture with unrelated hosts such as cattle from late summer until the start of lambing in late or

early spring (Waller et al., 1987b). Co-grazing sheep and cattle with or without anthelmintic

treatment has also been shown to reduce the levels of infection to sheep (Donald and Waller, 1982).

Synchronization for lambing in the fall would help avoid the adverse effects of

haemonchosis on the growth and production of market and replacement ewe lambs.

4. Inducing protection against strongylate nematode infection in sheep

The outcome of vaccination studies with conventional antigens varied with the breed and the

age of sheep (Manton et al., 1962; Urquhart et al., 1966a; Bradley et al., 1973; Duncan et al., 1978).

Following the failure of oral vaccination with irradiated *H. contortus* L3 to induce protection in

three-month-old Blackface lambs attempts were made to stimulate protective immunity by employing

different immunization techniques (Urquart et al., 1966ab). These techniques included the use of

Freund's adjuvant alone or combined with *Fasciola* antigen, intraperitoneal injection of normal L3,

reduction in the number of irradiated L3, and substitution of a serial daily challenge exposure to a

single challenge dose. However, none of these modifications conferred protection.

Age dependent effects on the maturation of immune responses to *H. contortus* was clearly

evident when Blackface lambs aged seven months or more developed protective immunity by

vaccination with irradiated *H. contortus* L3 followed by challenge with normal L3 (Urquart et al.,

1966b).

In parasite-free Merino lambs, sonicates of adult nematodes but not the L3 and exsheathing

fluid, produced significant reductions in *H. contortus* of challenge infections (Adams, 1989). In

Florida Native lambs less than six months of age, a significant reduction (59%) in adult *H. contortus*

counts was obtained when lambs were vaccinated with a high molecular weight fraction (mw >

30,000 KD) of an inoculum consisting of 50% somatic extract and 50% excretory/secretory products of L3 (Neilson and Van DeWalle, 1987).

Substantial levels of protection have been achieved against *H. contortus* in Clon Frost and crossbred (Suffolk x Grayface and Finn x Dorset) lambs by using a "hidden" antigen, the integral membrane extract of intestines dissected from adult nematodes (Munn et al., 1987; Smith, 1993). The "hidden" antigen did not afford protection against *O. circumcinta* or *Nematodirus battus*. Further, serum from lambs that had naturally acquired *H. contortus* did not react with the gut membrane extracts (Smith, 1993). Subsequently, a functional protein, H 11, was isolated from the gut membranes of *H. contortus* which was then cloned and expressed in active form with baculovirus-sf9 insect cell system (Munn et al., 1993). Since, response to vaccination is genetically determined (Windon and Dineen, 1981) and sheep are never exposed to internal nematode gut proteins, cross-bred lambs that do not respond to vaccination procedures using conventional nematode antigens, were protected against haemonchosis when vaccinated with H 11 (Smith and Smith, 1993).

The lethal consequences of antibody or complement mediated adherence of inflammatory cells to L1 or L3 (Mackenzie et al., 1980) and the presence of cross reactive and highly antigenic glycoproteins in the cuticle of the L3 (McGillivery et al., 1989; Cox et al., 1989; Cox et al., 1990) suggest that the nematode surface can be a target for protective immunological reactions leading to the expulsion of L3. Sheep vaccinated systematically with surface extract from exsheathed *H. contortus* L3 were partially protected and developed high serum antibody reactivity against surface extract and whole viable exheathed L3 (Turnbull et al., 1992). Vaccination with collagens and other peptides found deep in the cuticle of *H. contortus* L3 failed to induce protection (Boisvenue et al., 1991).

The effectiveness of autogenous vaccine using a crude antigen produced from adult *H. contortus* was tested in naturally infected Suffolk lambs and ewes at Louisiana State University

(Miller, unpublished observations). Based on the serial fecal egg counts of the vaccinated and control animals, this vaccine conferred 75 % protection in ewes and 49% protection in lambs.

Cuticular surface antigens of exsheathed *H. contortus* L3 and L4 have been characterized by surface labelling and immuno-blotting (Cox et al., 1989; Cox et al., 1990). The SDS soluble proteins of exsheathed L3 and L4 consist of a few major antigens that were glycosilated, stage specific, and refractory to collagenases. Thus, if the surface of the L3 and L4 of strongylate nematodes contain protective antigens, breed differences can be defined in terms of antigen recognition patterns by screening the surface antigens against the post-infection sera from sheep of different breeds.

5. Within- and between-breed resistance in sheep to strongylate nematode infection

In the United States, genetic resistance was observed in the 1950's when a series of studies showed different intensities of strongylate nematode infection in sheep grazing naturally infective pastures, and progeny testing demonstrated that the resistance was heritable (Whitlock, 1955; Whitlock, 1957; Whitlock and Madsen, 1957). Among the five breeding rams, the ram dubbed "Violet" produced progeny that were remarkably resistant to infection. From these observations, it was suggested that control of strongylate nematodes could be achieved by elimination of sires and dams whose progeny were susceptible.

Two decades later, Australian workers described a Merino ram that sired progeny highly resistant to *H. contortus* and *T. colubriformis* and named that ram the "Golden Ram" (reviewed by Gray, 1987). By selective breeding, lines of Merino and Romney sheep (resistant, susceptible, and random-bred) that vary parasitologically and immunologically in their responses to *H. contortus* and *T. colubriformis* have been derived in Australia and New Zealand, respectively (Gray and Woolaston, 1991).

Resistance varies among individuals within a breed (Barger, 1989; Woolaston, 1992). The experiment conducted by Dinnen et al (1978) where three-month-old Merino lambs segregated into responder and non-responder groups following vaccination and challenge with irradiated L3 of *T. colubriformis* clearly demonstrated within-breed variation in resistance. Comparably, when

observations were made on the peri-parturient rise in lines of Merino ewes selected as lambs for

different levels of resistance to *H. contortus*, ewes bred for increased resistance had lower mean fecal

egg counts than those not selected or bred for decreased resistance (Woolaston, 1992).

Age is one other important factor contributing to the within-breed variation in resistance to

strongylate nematode infection. The age factor became evident when attempts to vaccinate two to

four-month-old Blackface lambs with irradiated *H. contortus* L3 failed to induce protection

(Urquhart et al., 1966a; Duncan et al., 1978; Ross et al., 1978) whereas vaccination of seven-month-

old lambs did induce protection (Urquhart et al., 1966b). When lambs from responder and non-

responder parents were vaccinated with irradiated *T. colubriformis* L3 at 8 to 12 weeks of age, the

progeny from responder parents had significantly lower fecal egg counts than those from non-

responder parents (Windon and Dineen, 1981). This indicated that genetic differences might be more

important than age per se in determining the levels of resistance to infections even in young lambs.

The degree of between-sheep variation of any parameter of resistance is given by the

correlation between repeated measurements of the parameter on the same animal. The correlation sets

an upper limit to a trait's heritability (Falconer, 1981). Selection of individuals for increased

nematode resistance would be meaningful only if the between-sheep variation is high and significant.

Response to selection is determined by the heritability estimates of resistance parameters.

Heritabilities for fecal egg counts and lymphocyte stimulation indices estimated by between-sire

analysis of variance and sire offspring correlations were of moderate magnitude and comparable to

those for production traits yielding responses to selection for *H. contortus* and *T. colubriformis*

resistance in sheep (Gray and Woolaston, 1991).

Breed comparison studies have established parasitological (fecal egg count, nematode

burden), hematological (packed cell volume, hemoglobin type, eosinophil count) and performance

(weight gain) differences between breeds following natural infections or artificial inoculations. As

judged by fecal egg counts monitored for two years in naturally infected ewes, susceptibility to *H.

contortus* was found to increase in the order of Red Maasai, Black Head Persian, Merino, Dorper,

Corridale and Hampshire (Preston and Allonby, 1979). Scottish Blackface and Finn Dorset ewes were experimentally infected with *H. contortus* and detailed parasitological and patho-physiological comparisons were made (Altief and Dargie, 1978a,b). After a primary infection, compared to Blackface, Finn Dorset sheep suffered serious abomasal pathology. The effects of reinfections were more pronounced in the Finn Dorset sheep while the Blackface expelled nematodes.

Some resistant breeds of sheep, possibly due to their long term association and co-evolution with nematodes in the absence of anthelmintic intervention, develop a strong degree of protective resistance early in life. In grazing experiments with two-month-old St. Croix and Dorset lambs, St. Croix lambs shed significantly fewer *H. contortus* eggs as early as six weeks after being placed on contaminated pasture and had more than 99% fewer nematodes than Dorset lambs (Gamble and Zajac, 1992). When experimental infections (10,200 *H. contortus*) were induced in three-month-old wether lambs, Dorset x Barbados Blackbelly cross-bred lambs had longer prepatent periods and a higher percentage of hypobiotic larvae 17 days post-infection than Dorset x Barbados Blackbelly x Suffolk cross-bred and Dorset lambs (Yazwinski et al., 1981). Similarly, Courtney et al (1985) found that 6 to 16 week old St. Croix and three-quarter St. Croix (quarter Dorset or Rambouillet) lambs, compared to domestic lambs of similar age that had Suffolk, Finn, Dorset and Rambouillet blood in different proportions, were able to acquire resistance to *H. contortus* about 8 weeks after either natural or artificial infection.

6. Methods of selection of sheep for increased resistance to strongylate nematode infection

The potential value of genetic resistance has to be demonstrated by the performance of resistant sheep under conditions of natural challenge. Selection methods that utilize natural infections should identify the time during which within-flock variation reaches the maximum. In a naturally selecting population, over dispersion of parasite burdens is typical and is attributed to genetic variation (Barger, 1985a). Over dispersion often results when a relatively small proportion of hosts carry a large proportion of the parasites. Thus, compared to a susceptible breed, a resistant breed of sheep would have a relatively large number of individuals available for selection.

Progeny with superior nematode resistant capabilities can be obtained if selection is made on both rams and ewes (Windon and Dineen, 1981). The polygenic nature of inheritance of resistance and long generation interval in sheep contribute to the lengthily time lapse before improvement of flock performance becomes evident.

Selection of sheep based on parasitological and/or hematological parameters would be time consuming and sheep would have to be subjected to infection either naturally or artificially. Modern molecular biological techniques may assist identification of resistant individuals soon after birth by use of genetic markers. Some of the Class I and Class II genes found within the major histocompatibility complex (MHC) and other genes which are linked to the MHC have been associated with resistance to parasitic infections in sheep (reviewed by Kennedy, 1990). Outtridge et al (1988) described a Class I antigen (SY-1) that could identify Merino lambs resistant to *T. colubriformis*. They found that 61 lambs with SY-1 had significantly lower fecal egg counts than 63 lambs which had other lymphocyte antigens. The lambs having this marker did not show any reductions in body weight or wool production.

Restriction fragment length polymorphism can be used to define the polymorphism present in the Class II MHC loci. Various restriction enzymes and DNA probes can be employed to define unique band patterns for different haplotypes (Bell et al., 1987). Associations can then be made between the band patterns and resistance or susceptibility.

Microsatellites are multialleic, abundant, and uniformly distributed markers exhibiting Mendelian inheritance. They, together with families in which a disease condition is segregating, are used to compare the common segregating linkage between the markers and the disease. The advantage of using microsatellites is they can be detected by polymerase chain reaction (Reapply and McDonald, 1992). The potential of using microsatellite markers for predicting resistance in the neonatal sheep cannot be realized fully until future correlational studies establish the patterns of segregation of gene markers with resistance in families.

7. Mechanism of resistance and susceptibility to strongylate nematode infection

Genetic differences influence resistance or susceptibility to strongylate nematode infection in sheep. Resistance to strongylate nematode infection can be categorized into three types (Emery and Wagland, 1991):

(1)   Innate resistance.

(2)   Resilience, in which the host has the ability to mitigate the deleterious effects of parasitism and maintain production, and reproduction.

(3)   Immunity (acquired resistance) which is complex, specific, diverse, aggressive, and anamnestic.

Innate resistance and resilience are present from birth and both have a physiological basis. In contrast, protective immunity to strongylate nematode infection develops in response to infection and can be transferred to non-immune sheep by inoculating whole lymph or washed lymphoid cells from gastric or thoracic duct of a donor sheep undergoing a challenge infection (Behnke and Parish, 1979; Smith et al., 1984). The following description is limited to aspects of host immunity. Protective immunity to strongylate nematode infection in sheep can be manifested in the following forms (Gordon, 1948; Radhakrishnan et al., 1972):

(1)   Shorter adult nematode length.

(2)   Suppression of fecundity.

(3)   Reduced establishment of incoming L3 (rapid expulsion).

(4)   Expulsion of adult nematodes.

(5)   Retardation of development of L3 and L4.

(6)   "Selfcure" after super infection.

In contrast to non-responder lambs, responder lambs vaccinated and challenged with *T. colubriformis* had reduced nematode length and male female ratio and fewer eggs in utero (Dineen and Windon, 1980). Suppression of fecundity has implications on the interpretation of fecal egg counts (Allonby and Urquhart, 1975). Inaccuracies may arise if fecal egg counts are used to predict

individual animal nematode burdens. Nevertheless, mean flock nematode burdens of *H. contortus* and changes in infection levels over time can be estimated from mean fecal egg counts with a high degree of precision (Roberts and Swan, 1981; Coyne et al., 1991).

Different sheep nematode species regulate their intra host populations differently (Barger, 1987). Newborn Merino lambs continuously on infective pasture developed resistance to *H. contortus* by four months of age (Barger, 1988). Serial nematode counts performed on Merino lambs undergoing trickle *H. contortus* infections and inoculated with radio-labelled *H. contortus* L3 two weeks prior to necropsy revealed that population regulation is mainly by manifestation of a state of resistance within three months after continuous exposure (Barger et al., 1985b). The state of resistance is characterized by the reduction in the establishment rate of L3 and concomitant mortality of established adult nematodes. Hyper-immune sheep rejected more than 90% of the challenged *H. contortus* L3 within 48 hours (Miller et al., 1983). Rejection of *H. contortus* L3 depends on the intake of L3 and is sensitive to corticosteroid treatment (Jackson et al., 1988). In three to four-month-old Merino lambs, resistance is sensitive to anthelmintic treatment and it has been found that treating young lambs during haemonchosis without moving them to "safe" pasture can adversely affect protective responses and lead to reinfections (Barger, 1988).

Seaton et al (1989) studied the dynamics of rapid expulsion of L3 following experimental trickle infection of sheep with radio-labelled *T. vitrinus* L3. As the infection progressed, establishment rate of L3 fell from 50% to almost 0% by two months. In Merino lambs, initiation of immune responses to *T. colubriformis* infections requires a threshold level of antigenic stimulation (Waller and Thomas, 1981). This threshold level is generally acquired within four months after exposure but in conditions of low larval availability, nematodes could continue to accumulate for seven months before being expelled. In studies that examined the effects of anthelmintic treatments in naturally infected Merino lambs, it was found that after the threshold levels were reached anthelmintic treatment hastened the development of immunity to *T. colubriformis* (Coop et al., 1984; Kerboueuf, 1986).

Developmental arrest of L4 (not true hypobiosis) can be initiated by host immune responses (Smith and Christie, 1979). Concerning the "selfcure" phenomenon, first grazing lambs and ewes can manifest "selfcure" late in summer or early fall but the full expression of "selfcure" is generally seen in mature ewes (Ayalew and Gibbes, 1973).

Hemoglobin (Hb) type has also been found to be associated with resistance to *H. contortus* (Jilek and Bradley, 1969). Florida Native ewes that had HbA or HbAB maintained higher Hb and packed cell volume levels than those possessing HbB (Jilek and Bradley, 1969; Allonby and Urquhart, 1976). Correlations were also found between Hb type and the degree of "selfcure" in sheep (Luffau et al., 1981). The Hb factor was not given consideration in breed resistant studies carried out in the last decade since a causal relationship between Hb type and resistance could not be established (reviewed by Gray, 1987).

Experimental *H. contortus* infections in immune competent, parasite-free (from birth) lambs indicated there was no difference in nematode establishment to primary infections between resistant and susceptible lines of Merino lambs (Gill, 1991) or between resistant St. Croix, Florida Native and susceptible (domestic breeds) lambs (Courtney et al., 1985; Zajac et al., 1990). Primary infections appeared to act in priming the initial immune response, as subsequent challenge infections clearly demonstrated reduced nematode burdens in resistant lambs.

Stimulation of immune responses requires that antigenic material be processed and presented by antigen presenting cells on their surface membrane in association with the appropriate host MHC molecule to T lymphocytes. T cells have two major functions. The T cell having the CD4 surface antigen act as a helper. They can for example help B cells to produce antibodies and induce macrophages to kill intracellular protozoa. Other subset of T cells bearing the surface antigen CD8 has a cytotoxic/immune regulatory function and are more important in removing virus infected host cells. There are two types of CD4$^+$ T cells. The subdivision is based on the cytokines they produce.

Acquired resistance to nematode infection is characterized by the development of humoral and cellular responses that are initiated by antigenic stimulation of CD4$^+$ T helper lymphocytes of

the Th2 type (Gill et al., 1993a; Else et al., 1993). The Th2 response is characterized by the in vitro production of interlukin (IL) IL-4, IL-5, IL-9 and IL-10 by restimulated mesenteric lymph node cells, tissue eosinophilia, mastocytosis and parasite specific IgG1, IgA and IgE anti-parasitic antibody responses. In contrast, in susceptible animals, the immunological reactions to nematode infections are mediated through the Th1 type of $CD4^+$ T helper cells and are not host protective. The non-protective Th1 type of cellular responses are characterized by the in vitro production of interferon-gamma (IFN-γ) and anti-parasitic IgG2a antibody response (Else et al., 1993). Depletion of $CD8^+$ T lymphocytes in the resistant line of Merino sheep did not alter the susceptibility to *H. contortus* indicating that CD8+ T cells may not have substantial involvement in the development of protective immunity against *H. contortus* (Gill et al., 1993a).

While anti-*H. contortus* antibody levels continued to decline until two and a half months of age in neonatal Merino lambs exposed to natural infections, similarly infected older sheep developed antibody responses (Watson and Gill, 1991). When adults and two-month-old Blackface lambs were vaccinated with irradiated *H. contortus* L3 and challenged, only the adults were protected with concomitant serum and abomasal antibody responses (Duncan et al., 1978). Thus, the inability to produce anti-*H. contortus* antibodies in adequate amounts may account for age dependent susceptibilities to strongylate nematode infection in sheep.

Acquired resistance to *H. contortus* infection in resistant lines of Merino lambs is characterized by the production of anti-parasitic antibodies and inflammatory cellular response in the peripheral blood and abomasal mucosa (Gill, 1991; Gill et al., 1993a). Following challenge infections with *H. contortus*, resistant Merino lambs developed significantly higher levels of IgG1 and IgA antibodies in serum, feces, and abomasal mucosa compared to random-bred lambs (Gill et al., 1993b). In vaccination studies, the failure to observe sustained and significant differences in anti-*H. contortus* antibody levels between resistant and susceptible breeds of sheep indicated that breed related susceptibilities to *H. contortus* might be due to qualitative differences in humeral immune responses (Neilson and Van DeWalle, 1987; Zajac et al., 1990; Gamble and Zajac, 1992).

In resistant sheep, cellular immune responses to *H. contortus* infection have been characterized by the production of two important effector cells, eosinophils and mast cells (Gill, 1991). The induction of these two inflammatory cells are under the control of the Th2 cytokines, IL-3, IL-4 and IL-5 (Urban et al., 1992). Studies with helminth infected laboratory animals demonstrated that antigen sensitized $CD4^+$ T cells secrete IL5 that regulates blood and tissue eosinophilia (Coffman et al., 1989). The importance of eosinophils in acquired resistance was realized when guinea pigs depleted of circulating eosinophils showed increased susceptibility to infections with *T. colubriformis* (Gleich et al., 1979). When Merino lambs and guinea pigs were vaccinated with *T. colubriformis*, individual variation in blood and tissue eosinophilia corresponded to responsiveness (Handlinger and Rothwell, 1981) suggesting that eosinophilia is not merely associated with infection but can be considered as a measure of host responsiveness.

The tropical Barbados Blackbelly breed, and it's crosses, developed higher levels of peripheral and abomasal eosinophilia in response to vaccination with *H. contortus* compared to domestic sheep (Yazwinski et al., 1981). Eosinophils bound to secretory IgA or complement in the gastrointestinal mucosa can degranulate and release neurotoxin and other harmful products such as major basic protein on the surface of L3 (Abu-Ghazaleh et al.,1989; Lombordi et al., 1990). Adherence of eosinophils through the Fc receptor of antibodies can eventually result in the death of larval nematodes (Mackenzie et al., 1980).

Upon stimulation with antigen, mast cells can release mono-amines, serine esterases and peroxidases and transform into globular leukocytes (Huntley et al., 1984). Naturally infected lambs born to resistant sires had significantly higher globular leukocytes in the intestinal lumen (Stankiewicz et al., 1993). A 15-40 fold increase in globular leukocytes was seen in the abomasal mucosa of naturally infected St. Croix lambs compared to Dorset lambs (Gamble and Zajac, 1992). Significant negative correlations between the density of globular leukocytes in the abomasal mucosa and nematode numbers and age related globular leukocyte responses seen in vaccinated sheep suggest

that globular leukocytes may be a major line of defense in protection against *H. contortus* and *T. colubriformis* (Smith and Christe, 1979; Douch, 1988).

Challenge infections with *T. colubriformis* stimulate the release of a range of inflammatory mediators including vasoactive amines, prostoglandins and slow reacting substances of anaphylaxis into the duodenum (Douch et al., 1983; Jones and Emery, 1991). Among the array of inflammatory mediators, slow reacting substances of anaphylaxis, which is made up of various leucotrines has been shown to possess the highest degree of inhibitory effect on larval migration (Douch et al., 1983). Gastrointestinal mucus finally incorporates all the inflammatory mediators, locally produced and serum derived immunoglobulins, enzymes and plasma proteins, and plays a significant role in entrapping and excluding the L3 from the niche (Miller, 1987).

In very young lambs, protective immunological responses to *H. contortus* infection can be down regulated by the induction of suppressor T cells (Shubber et al., 1984). Soya lambs born to infected ewes and fed colostrum were more susceptible to artificial *H. contortus* infection than lambs born to uninfected ewes and fed colostrum and lambs born to infected ewes and deprived of colostrum. Compared to lambs born to infected ewes and deprived of colostrum lambs born to infected ewes and fed colostrum exhibited higher levels of *H. contortus* antigen induced lymphoproliferative responses from birth to 20 weeks of age. These observations suggest that lymphoproliferative responses observed in Soya lambs were of a suppressor type and factors derived from infected ewe are responsible for the induction of these responses. In Soya lambs, suppressor T cell responses to *H. contortus* antigens can persist for a period of 2-3 months from birth (Shubber et al., 1984; Torgerson and Lloyd, 1992) and therefore may play a role in neonatal unresponsiveness.

8. Epidemiology of coccidia (*Eimeria*) infections in sheep on pasture

Coccidia are protozoan parasites infecting invertebrates and many classes of vertebrates including annelids, arthropods, amphibians, reptiles, birds and mammals (Pellerdy, 1974). The majority of the intestinal coccidia of sheep belong to the genus *Eimeria* of the phylum Apicomplexa. In sheep, *E. ahsata*, *E. ovina*, and *E. crandallis* which infect the ileum are relatively less pathogenic

than *E. ovinoidalis*, the sexual stages of which, cause severe damage to the cecum (Mahrt and Sherrick, 1965; Gregory and Catchpole, 1989; Norton, 1986). Size, shape and morphology of unsporulated and sporulated oocysts are criteria frequently used in speciation of coccidia. Other characteristics used for identification of ovine coccidia include prepatent period, kind of disease produced, site of infection and sporulation time (Lotze, 1953; Shah, 1963; Norton, 1986; Gregory and Catchpole 1989).

Oocysts are the only exogenous stage in the coccidian life cycle. Fayer (1980) in his extensive review of ruminant coccidia described the following characteristics of oocysts for the successful completion of the epidemiological cycle:

(1)   High biotic potential which is governed by inherent reproductive potential,

        immunity, nutrition of the host, strain of the parasite and the host, stress, and use

        of coccidiostats.

(2)   Capability to complete sporulation which is regulated by temperature, moisture,

        RH, oxygen tension, and sun light.

(3)   Survivability and infectivity.

When ingested by sheep, sporulated oocysts release sporozoites which then invade the host cells and undergo first asexual and then sexual reproduction producing thousands of oocysts. A dose of 50 oocysts of a single species has resulted in the shedding of as many as 6×10^9 oocysts in sheep during the subsequent 10 days (Pout, 1976). In ruminants, *Eimeria* could be a primary cause of enteritis in the young when raised in confinement and subjected to some form of stress (Fitzgerald, 1962; Parker et al., 1986; Gregory and Catchpole, 1989). Young lambs have been found to develop heavy infections and subsequently clinical disease a few weeks after turnout to pasture (Pout, 1973; Gregory et al., 1980; Gregory and Catchpole, 1989) or immediately after arrival into the feedlot (Christensen, 1940; Mahrt and Sherrick, 1965).

Eimeria spp. infections in pastured sheep has been established to be an age related phenomena with newborn lambs being highly susceptible and frequently manifesting heavy infections

with mixed species (Chapman, 1974; Gregory and Catchpole, 1989; Amarante, and Barbosa, 1992). Clinical eimeriosis in lambs can be correlated to the finding of very large numbers of oocysts of pathogenic *Eimeria* spp. in the feces. It was found that oocyst counts of lambs suffering from clinical eimeriosis were about ten times greater than their healthy peers (Gregory et al., 1980). However, this may not be necessarily true for all cases since lambs may die of acute coccidiosis before oocysts are shed or oocyst shedding following an acute infection may fall sharply after a peak leaving a critically ill animal with bloody diarrhea and reduced oocyst counts (Gregory et al., 1980). In addition to oocyst counts, the species responsible for an outbreak of clinical eimeriosis can be identified by examining the site and severity of lesions at necropsy (Norton, 1986).

Determination of prevalence of various species of *Eimeria* in breeder ewes can provide information on the potential transmission of pathogenic species from ewes to lambs. There is no evidence for a peri-parturient rise in oocyst count as occurs with nematode infections (Gregory et al., 1983). Nevertheless, for pasture born lambs, oocysts deposited by pregnant ewes during the winter months may be an important source of initiation of *Eimeria* spp. infections when lambs first start grazing and consuming some soil (Helle, 1970; Helle and Hilali, 1973).

9. Immunity to *Eimeria*, anticoccidial treatment and interaction of *Eimeria* spp. and strongylate nematodes in sheep

Immunity to coccidia infections is species specific and is initiated by the interaction of coccidial antigens and Th1 type of $CD4^+$ T lymphocytes in the intestinal mucosa (Wakelin and Rose, 1990). Activated $CD4^+$ T cells release IFN-γ which renders host cells refractory to invasion by sporozoites (Rose et al., 1991). Interestingly, it has been found that new born lambs are totally resistant to *Eimeria* spp. infection (Gregory and Catchpole, 1989) and protective immunity can be induced by artificially inoculating lambs less than one week of age with sporulated oocysts or keeping lambs of the same age in lambing pens having residual *Eimeria* contamination (Gregory and Catchpole, 1989; Gregory et al, 1989). Field studies conducted in the U.K. revealed that newborn lambs placed on pasture can develop solid immunity against the less pathogenic *E. crandallis* within

a few weeks after birth but their susceptibility to the most pathogenic *E. ovinoidalis* increased from

7-42 days after birth resulting in eimeriosis associated with reduced weight gain, diarrhea and death

of lambs in five to six weeks after turnout (Gregory and Catchpole, 1989).

Studies conducted with semi-confined lambs proved that subclinical eimeriosis could also

result in reduced weight gains (Horton and Stockdale, 1979; Foreyt et al., 1981a,b). Newborn lambs

too, gained more weight when infection was suppressed by treatment with effective coccidiostats

(Gregory et al., 1983; Gjrde and Helle, 1991). Treatment with the anticoccidial toltrazuril seven days

after turnout to pasture did not affect immunity to reinfections with *Eimeria* later in the grazing

season (Gjerde and Helle, 1991).

The interaction of *Eimeria* spp. and strongylate nematodes resulting from concurrent

infections can lead to depressions in host immunity against the nematode (Bristol et al., 1983; Upton

et al., 1987). Experimental infections with *E. crandallis* and *E. ovinoidalis* and *N. battus* in three to

five-week-old lambs resulted in a more severe enteritis and death than when the nematode infection

was delayed by two weeks (Catchpole and Harris, 1989). Simultaneous administration of

Nippostrongylus brasiliensis and *E. nieschulzi* to rats have been shown to lengthen the period of

patency and increase the total egg production of the nematode (Bristol et al., 1983; Upton et al.,

1987). A delay in the expulsion of challenge larvae of *Trichinella spiralis* was noted when mice

were infected with *E. nieschulzi* prior to challenge with *T. spiralis* (Dusynski et al., 1978). Though,

IFN-γ is a potent down regulator of the cytokines released from the Th2 type of CD4+ T

lymphocytes, systemic administration of anti IFN-γ antibodies to mice concurrently infected with *T.*

spiralis and *E. vermiformis* did not prevent the delay in expulsion of *T. spiralis* suggesting that

suppression of Th2 responses by *Eimeria* induced IFN-γ was limited to intestinal mucosa (Rose et

al., 1994).

MATERIALS AND METHODS

1. Sheep

This study was conducted at the Central Research Station Sheep Farm, Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge, Louisiana. In 1992, 1993, and 1994 Native and Suffolk ewes aged two to seven years were identified by ear tag. In each of the three study years, an equal number of ewes of each breed were bred to two rams in the fall and lambs were born in late spring. Parturient ewes were isolated and kept in lambing pens in the main barn. Within a few days after birth, lambs were identified by ear tag and tail docked. At six weeks of age, they were vaccinated against clostridial diseases.

2. Pasture

In 1992, a 4.8 hectare bermudagrass (permanent) pasture which had not been grazed by sheep for the previous eight months, was divided into four equal 1.2 hectare pasture plots. The plots were formed by cross-fencing. Two of the 1.2 hectare plots were overseeded with ryegrass in late fall to provide forage for winter and spring. In each year, ewes and lambs were turned out to one of the four pasture plots about a week after lambing. Ewes and lambs remained together on pasture until lambs were weaned collectively at 12 to 13 weeks of age. Lambs were kept in a weaning paddock for two weeks before being released to the original pasture. After weaning, the ewes and the lambs grazed separately in two of the four pasture plots in a crisscross fashion so that lambs could not cross fences to join dams. To maximize the use of forage and to ensure equal infection exposure, lambs and ewes were rotated between pasture plots. In both 1992 and 1993, all sheep remained on pasture until December. In 1994, lambs grazed until necropsy examinations were conducted at eight weeks of age. Sheep were temporarily removed from pasture for sampling, anthelmintic treatment, routine health and management purposes, and breeding. Lambs and ewes were supplemented with concentrate feed at the time of transition from permanent pasture to ryegrass (early fall) and during late pregnancy.

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3. Experimental designs

Epidemiological data on strongylate nematode and coccidia infection in Native and Suffolk

sheep was collected over a three year (1992-94) period. Strongylate nematode infection is described

for three categories of sheep: suckling lambs, weaned lambs, and ewes. For the description of

coccidial infection, suckling and weaned lambs were considered together.

1. 1992

Lambs (n=14 per breed) were born within a period of 27 days (February 2 to 28). Fecal

samples were collected directly from the rectum and blood samples were collected in serum (10 ml)

and EDTA (10 ml) tubes, by means of jugular venepuncture. Vacutainer tubes (Becton Dickinson

vacutainer system, Rutherford, NJ) and 21 gauge needles were used in bleeding. Both feces and

blood were collected at biweekly intervals alternating weekly until December 16 and 23 for lambs

and ewes, respectively. Lamb and ewe (n=9 Native and 8 Suffolk) sampling began with fecal

collection at two weeks of age and on March 4, respectively. Five Suffolk lambs died between 11

and 14 weeks of age.

Native lambs were randomly divided into two groups based on fecal egg counts. One group

of Native lambs and the group of nine remaining Suffolk lambs were designated to receive tactical

anthelmintic treatments as necessary when the packed cell volume of any of the lambs fell below 15

and/or mean fecal egg count increased to over 2500 eggs per gram of feces and clinical signs of

haemonchosis were observed. One other group of Native lambs remained untreated. The treated

Native and treated Suffolk groups received an initial ivermectin (Ivomec®, Merck AgVet, Rahway,

NJ) treatment (400µg/kg) at 13 weeks of age. After the initial treatment, another ivermectin

(400µg/kg) and four albendazole (Valbazen®, Smith Kline Beecham) treatments (10 mg/kg) were

administered on July 1, August 12, September 21, October 16, and November 27, respectively.

During this period, an additional six Suffolk lambs and one treated Native and one untreated Native

lamb died. The two Native lambs and two of the six Suffolk lambs died due to a coyote attack.

The same fecal egg count and packed cell volume criteria were used to determine treatment strategy for ewes. Ivermectin treatments (200 μg/kg) were administered on May 10, July 29, September 23, and October 16. Two Suffolk and no Native ewes died during this period.

Ivermectin and albendazole were administered by subcutaneous injection and as an oral drench, respectively.

2. 1993

In 1993, lambing for the two breeds was not synchronous. Native ewes (n=15) lambed from February 9 to March 1 and Suffolk ewes (n=15) lambed from March 6 to 14. Therefore, allocation of lambs to treatment groups was slightly different. Grouping of lambs was done as the lambs were born. The first born Native lambs (n=10) were assigned to the treated Native group and the lambs born later (n=6) were assigned to the untreated Native group. Suffolk lambs (n=13) were assigned to the treated Suffolk group. In lambs and ewes, the protocol for collection of feces and blood samples were similar to 1992. In lambs and ewes, sampling began with fecal collection at three weeks of age and on February 4, respectively. In order to avoid death in Suffolk lambs, as experienced in 1992, an albendazole (20 mg/kg) treatment was given at eight weeks of age. The treated Native lambs were given albendazole (20 mg/kg) treatment at the same time. Because of differences in the time of lambing, treated Native lambs were 12 weeks of age at the time of treatment.

Though the untreated Native group was maintained from birth, monitoring of these lambs commenced after weaning along with treated Native and treated Suffolk groups. The anthelmintic treatment plan for weaned lambs and ewes was similar to that described for 1992. Three anthelmintics were given to lambs as follows: levamisole (Tramisol®, American Cynamid, Wayne, NJ, 8 mg/kg) on June 11; albendazole (20 mg/kg) plus levamisole (16 mg/kg) on July 15, July 30, August 27 and September 30; and albedazole (20 mg/kg) plus ivermectin (400 μg/kg) on November 11. Levamisole was administered as a subcutaneous injection. Within five weeks after weaning four Suffolk lambs died. One more Suffolk lamb died on October 2.

Ewes were treated as follows: ivermectin (200 µg/kg) on March 27 and June 24; ivermectin (400 µg/kg) on August 6; and ivermectin (400 µg/kg) plus albendazole (20 µg/kg) on August 27 and October 14. Two Suffolk ewes died during this period. One Suffolk and two Native ewes were withdrawn from the study in April because they lost their lambs.

Necropsy examinations were conducted on four separate occasions to determine relative nematode burdens in lambs. During the suckling period, necropsy examinations were conducted on two occasions. Native and Suffolk lambs, four of each breed, which were paired at birth and grazed together with the monitor lambs, were removed from the pasture at 7 and 10 weeks of age and euthanized for necropsy examinations.

Necropsy examinations on weaned lambs were conducted on two occasions. Two lambs each from the treated Native and treated Suffolk groups which had post-treatment fecal egg counts of 0-50 eggs per gram of feces were removed from the pasture and euthanized for necropsy examinations on July 19 (38 days after a tactical anthelmintic treatment). For the second necropsy, three lambs from each of the three groups (treated Native, treated Suffolk and untreated Native) were removed from the pasture and necropsy examinations were performed on October 1 (30 days after a tactical anthelmintic treatment). While the lambs in the treated Native and treated Suffolk groups were selected for necropsy based on the post-treatment fecal egg counts (0-50 eggs per gram of feces), lambs in the untreated Native group were chosen randomly.

3. 1994

The study was limited to necropsy examination of suckling lambs. A cohort of 12 (six Native and six Suffolk) lambs paired at birth were euthanized and necropsy examinations were conducted at eight weeks of age.

4. Laboratory techniques

Nematode fecal egg counts in all lambs and ewes was performed by a standard double centrifugational flotation technique or a modified McMaster technique (Whitlock, 1948) using Sheather's sugar (Appendix) solution as the flotation medium and the results expressed as eggs per

gram. Pre- and post-treatment fecal egg counts were used to determine fecal egg count reduction percentages. The formula, 100 (1-x/y), where x and y represent pre- and post-treatment fecal egg counts, respectively was used in the determination of fecal egg count reduction. Fecal oocyst counts were also determined by using the same techniques and reported as oocysts per gram of feces. The fecal oocyst count determinations in Native lambs in 1993 were limited to the treated Native group only. In 1992, fecal samples were subjected to sugar flotation separately to determine the percentage infection with different species of *Eimeria* by differentiating oocysts according to descriptions given by Levine and Ivens (1970).

In 1992 and 1993, fecal cultures were performed every two weeks from May 27 to September 30. Fecal cultures were prepared by mixing feces, vermiculite and tap water. In 1992, individual fecal samples were cultured at 27-30°C and 80-100% relative humidity for 10 days. In 1993, fecal samples were pooled (two grams from each sample) before culture under the same conditions as in 1992. Larvae were harvested using the Baermann procedure and concentrated by centrifugation prior to identification. Whenever present in sufficient numbers, at least 100 larvae were identified by microscopic examination after heat killing for 15 seconds.

Peripheral blood leukocyte counts, specifically eosinophil counts were determined in weaned lambs and ewes indirectly by using differential leukocyte cell counts obtained from examining thin blood smears stained with Giemsa solution and total white blood cell counts determined using a Coulter counter. Packed cell volumes were determined by the microhematocrit technique (Schalam et al., 1975). Serum was separated by centrifugation at 3,000 rpm for 12 minutes and stored (-20°C) in sterile tubes until used.

At necropsy, the gastrointestinal tracts were processed for recovery of nematodes using established procedures (Miller et al, 1987). The abomasum, small intestine, cecum and colon were each ligated at the anterior and posterior ends to prevent the escape of nematodes into adjacent compartments of the gastrointestinal tract. The abomasum was opened along the greater curvature and was washed carefully with small quantities (two to three liters) of water. The procedure was

repeated two to three times. After each rinse, the contents were transferred into a bucket of approximately 15 liter capacity. Water was added to bring the contents of the bucket to 10 liters. Four separate 250 ml aliquots were combined into a one liter aliquot during constant agitation to ensure equal distribution of nematodes in the contents. After settling for one hour, 50 ml of the sample was decanted and replaced with an equal volume of formalin to preserve the nematodes in 5% formalin.

The abomasum was submerged in warm water and soaked overnight to allow immature nematodes to migrate out of the mucosa. After soaking, the abomasal mucosa was rubbed briskly and kneaded in several washings of small volumes (two to three liters) of water to remove all nematodes adhering to the mucosa. The washings were then brought to a final volume of 10 liters with tap water and a one liter sample was taken and preserved as described above. The small and large intestines (two to three meters of length including cecum) were slit open, separately, in different containers and the contents were emptied and the mucosal surface was washed with small quantities (two to three liters) of water. The opened small intestine was passed through fingers twice to remove any adhering nematodes. The volume of small and large intestinal contents were made up to 10 liters before a one liter sample was taken and preserved as described above.

Determination of the number of adult and immature nematodes was performed from a 10% aliquot of the one liter samples of abomasum contents and soak, and small intestine and large intestine contents. Identification of nematode species and stage of development was determined by microscopic examination. Nematodes were cleared in lactophenol. If the total number of nematodes recovered from the 100 ml aliquot was less than 10, then the rest of the nematodes from the remaining volume (900 ml) of the sample were recovered and identified.

5. Meteorological data

Monthly mean daily minimum and maximum temperatures and total monthly precipitation for all months of 1992 and 1993 were obtained from a weather station at the research site. Historical rainfall data was also obtained from the research site for a period of 23 years from 1969 to 1991.

6. Assay for total immunoglobulins

In 1992, serum samples taken on July 01 and July 29 from treated Native and treated Suffolk lambs were analyzed for total immunoglobulin levels using a radial immunodiffusion technique. The agarose gel plates containing anti-sheep immunoglobulins and reference standard sheep immunoglobulins were acquired (Bethyl laboratories, Inc). Serum immunoglobulin concentrations were determined from plots of precipitin ring diameter against immunoglobulin concentration of reference standards.

7. Enzyme linked immunosorbant assay (ELISA)

A crude adult antigen was produced from adult male and female *H. contortus* recovered at necropsy from a naturally infected ewe. The nematodes were homogenized over ice in cold PBS using a hand homogenizer. The nematode homogenate was centrifuged at 30,000 rpm for 30 minutes. The supernatant was removed. The protein concentration of the supernatant was determined by Lowry's method (Lowry et al, 1953), adjusted to 1.5 mg/ml and stored (-20°C) until used.

Alkaline phosphatase conjugated affinity purified rabbit anti-sheep IgG (H+L chains) antibody and alkaline phosphatase conjugated affinity purified donkey anti-mouse IgG (H+L chains) used in immuno assays were acquired as dried powders (Kirkegard and Perry Laboratories Inc. and Jackson Immunological Laboratories Inc., respectively). They were reconstituted with reagent quality water and stored (-20°C). The substrate for alkaline phosphatase p-NPP (p-nitrophenyl phosphate) was acquired as 20 mg substrate tablets (Kirkegard and Perry laboratories Inc.). A checkerboard analysis was conducted before the experimental ELISA in order to determine optimal antigen concentration and serum dilutions using known positive and negative sheep sera.

In 1992, anti-*H. contortus* total antibody levels in suckling and weaned lambs and ewes were determined using an ELISA procedure described by Neilson and Van DeWalle (1987) with some modifications. Antigen diluted to 2 μg/ml with carbonate coating buffer (Appendix) was dispensed in increments of 100 μl into each well of a 96 well microtiter ELISA plate and incubated overnight at room temperature. Plates were washed five times with a washing buffer (Appendix) and

drained. Then 200 µl blocking buffer (Appendix) was added to each well and plates were incubated

for one hour after which they were washed five times and drained. Test sera diluted 1:100 with

serum diluent (Appendix) were dispensed into wells in 100 µl quantities and plates were incubated

for two hours at 37°C. The plates were washed five times and 100 µl rabbit anti-sheep IgG enzyme

conjugate (0.5 µg/ml) was added and plates were incubated at room temperature for one hour. Plates

were washed five times and freshly prepared substrate, p-NPP, was added in 100 µl amounts to each

well. The reaction was allowed to proceed for 35 minutes for lamb sera and 20 minutes for ewe sera

in the dark at room temperature. Reaction was stopped by adding 50 µl/well of 5% EDTA and

absorbance was read at 410 nm using an automated ELISA plate reader. Controls with known

positive and negative sera and for rabbit anti-sheep immunoglobulin were included at all times. All

samples and controls were run in triplicate. Test results were standardized against plate to plate

variation by dividing the blank subtracted optical density values of the test sera by that of the

positive reference serum.

Class-specific anti-*H. contortus* antibody assays were performed only for weaned lambs in

1992. Mouse-anti sheep IgM, IgA, IgG1 and IgG2 were kindly supplied by Dr. Ken Beh, McMaster

Laboratory, Sydney, CSIRO, Australia. For isotype specific assay, serum was diluted 1:50 and used

in 50 µl quantities. Mouse anti-sheep IgM, IgG2, and IgA were diluted 1:100 and mouse anti-sheep

IgG1 was diluted 1:500. All antibody isotypes were used in 100 µl quantities. Donkey anti-mouse

antibody alkaline phosphatase conjugate was used in 100 µl quantities at 1 µg/ml concentration.

Coating, blocking and washing procedures were similar to that for total immunoglobulin assay.

Incubations with serum, class specific mouse anti-sheep antibodies and donkey anti-mouse alkaline

phosphatase conjugate were carried out at 37°C for one hour. For each antibody isotype, enzyme

substrate reaction was stopped after 20 minutes and absorbance was read at 410 nm by an ELISA

reader. Control with known positive and negative sera and for mouse anti-sheep antibody and donkey

anti-mouse antibody were always included in the test. Test results were standardized against plate to

plate variation by dividing the blank corrected optical density values of the test sera by the value for the positive reference serum.

8. Statistical analysis

While the fecal egg count and hematological data from suckling lambs were analyzed age specifically, data from weaned lambs and ewes were analyzed in relation to sampling date. For the statistical analysis of coccidia data, suckling and weaned lambs were considered together. Lamb and ewe coccidia data were analyzed with respect to age and sampling date, respectively. For the purpose of analyzing species specific oocyst levels, fecal oocyst counts from the two breeds of sheep were pooled prior to analysis. Since the fecal oocyst counts, fecal egg counts and nematode counts did not meet the requirements of normality and equal variances, they were transformed into Log_{10} (n+1) before being submitted to statistical analysis. Blood eosinophil counts were transformed into (n+10). All differences between breeds were considered significant at $P < 0.05$, unless otherwise indicated.

The overall differences between breeds for fecal oocyst counts, fecal egg counts, packed cell volumes, eosinophil counts and ELISA absorbance values in suckling and weaned lambs and ewes were determined by a univaritae measure analysis of variance. Differences between means were determined by Bonferroni-adjusted t-tests. In ewes, seasonal (spring, summer and fall) differences in fecal egg counts, packed cell volumes and eosinophil counts between breeds were determined by Student's t-tests.

Nematode counts (October, 1993) from the three groups of weaned lambs (treated Native, treated Suffolk and untreated Native) were compared by a one way analysis of variance. The differences between means were compared by Tukey's multiple mean comparison procedure. In 1994, the nematode counts of necropsied, suckling lambs were compared by Student's t-tests.

RESULTS

The monthly mean daily minimum and maximum temperatures and the total monthly rainfall for 1992 and 1993, and average monthly rainfall for 23 years recorded at the research site are illustrated (Figure 1). There were no noteworthy variations in the monthly mean daily minimum and maximum temperatures recorded. In 1992 and 1993, the highest mean monthly maximum temperatures were recorded during July and August, respectively. Except during the months of January, 1992 and December, 1993 when the mean minimum temperatures were below 7°C, the temperature range was otherwise not detrimental for larval development.

Considerable year to year variation was noted in the distribution of total monthly rainfall. Compared to the 23 year average, rainfall was noticeably greater during January, February, June and November, 1992 and January, April and October, 1993 and lower during April and May 1992 and July, August, September and December, 1993. Thus, the summer of 1993 was relatively drier than the summer of 1992. Other than in September, 1993, in all other months during the two year period, the total precipitation was always at or above the 75 mm level which when distributed evenly is considered to provide sufficient soil moisture conditions for larval development.

1. Strongylate nematode infection in suckling lambs

1. Nematode egg counts and packed cell volumes

In 1992, patent strongylate nematode infections were established in Native and Suffolk lambs at six weeks of age (Figure 2). Subsequent to four weeks of age, in both breeds, mean fecal egg count began to increase. In Native lambs, mean fecal egg count reached a peak (mean 2,136 eggs per gram of feces, range 0 to 5,000 eggs per gram of feces) at eight weeks of age and declined slightly by 10 weeks of age. In Suffolk lambs, mean fecal egg count continued to increase and reached a high of 17,808 eggs per gram of feces (range 4,100 to 41,000 eggs per gram of feces) at 12 weeks of age. The overall difference in mean fecal egg counts between the two breeds was highly significant (p < 0.0001) being accounted for at 10 and 12 weeks of age.

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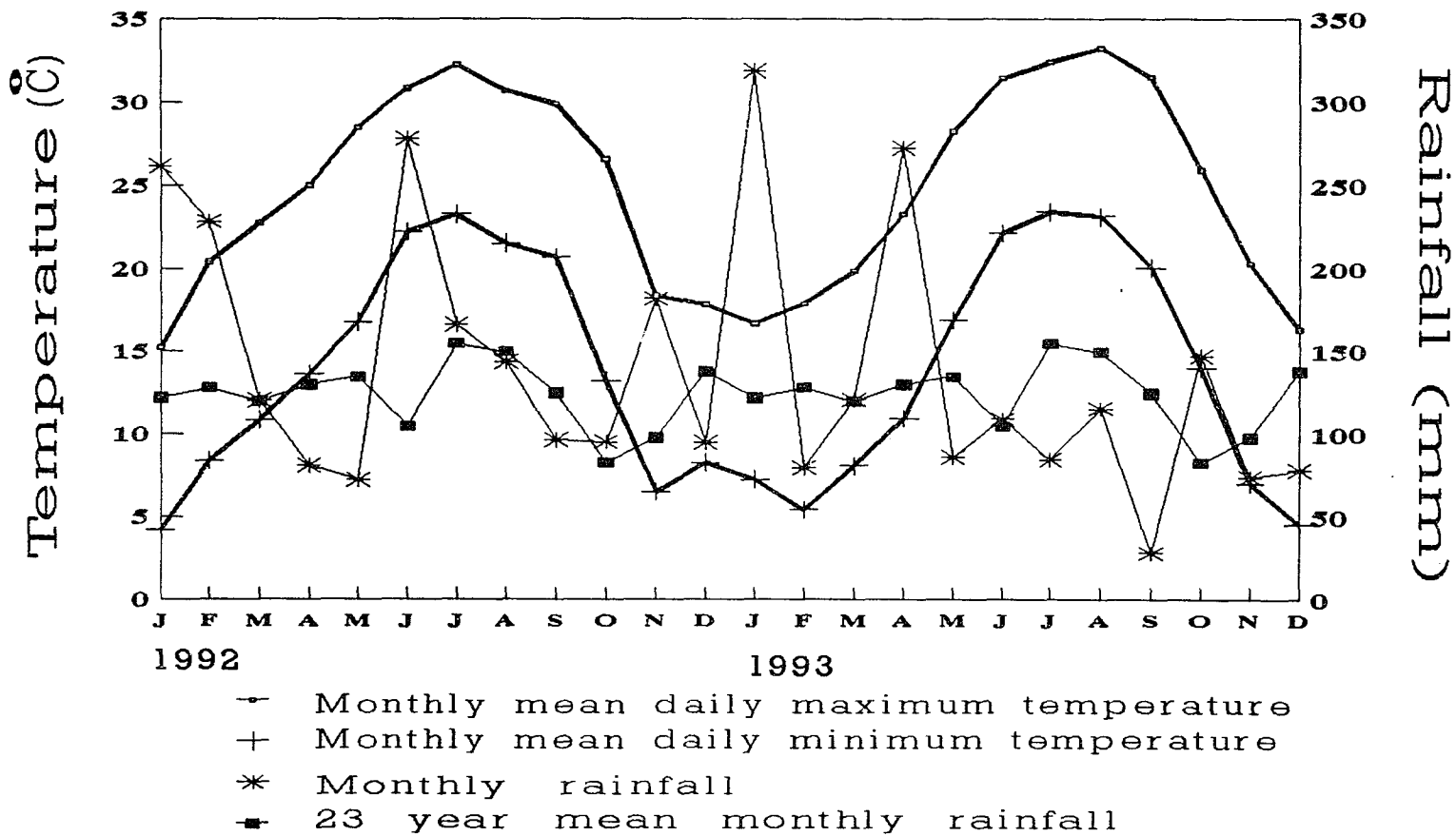
Figure 1. Temperature and rainfall patterns at the Central Research Station, Louisiana Agricultural Experimental Station.
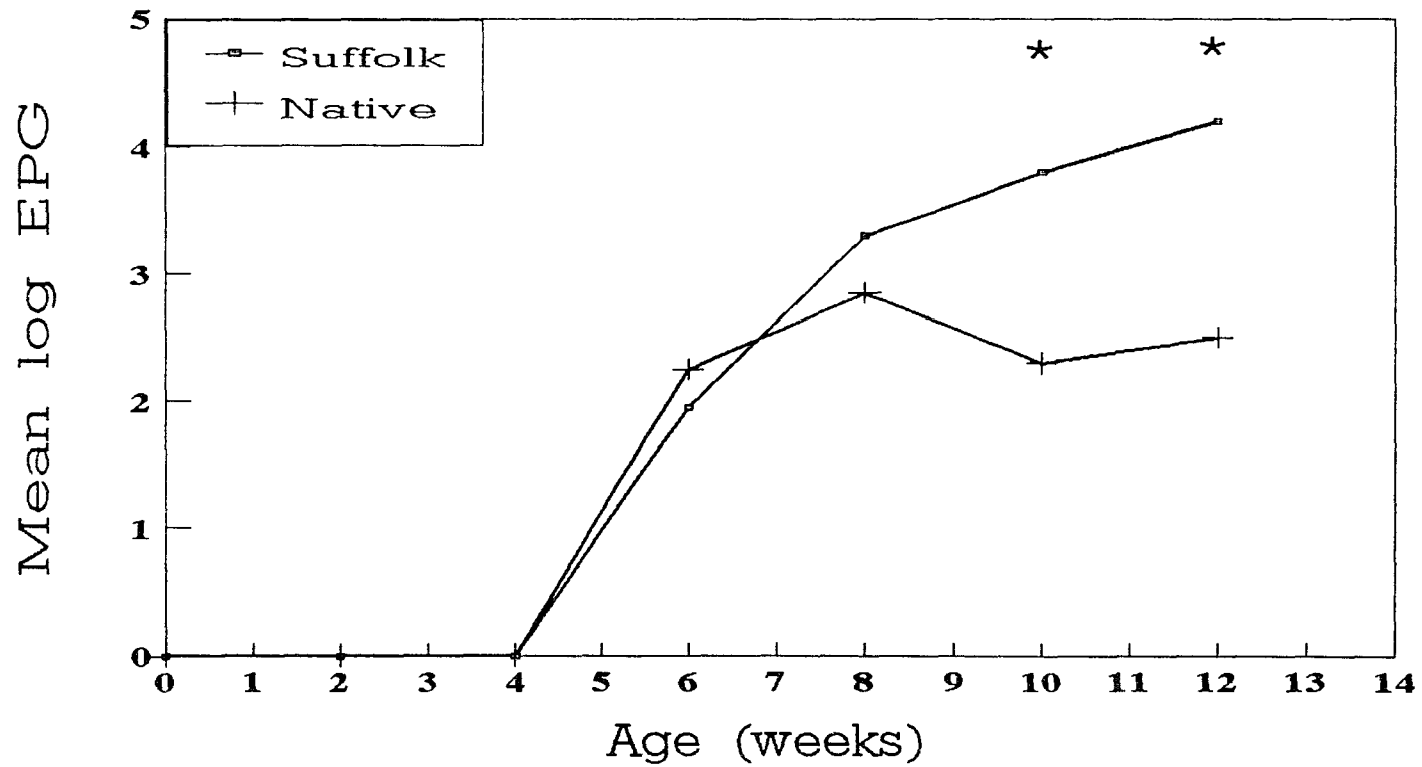
Figure 2. Mean fecal egg counts of naturally infected, suckling Native (n=14) and Suffolk (n=14) lambs in 1992. * indicates significant (p < 0.05) difference. One Suffolk lamb died at 11 weeks of age. EPG=Eggs per gram of feces.

There were essentially no changes in the packed cell volumes in Native lambs through 13

weeks of age (Figure 3). In contrast, the mean packed cell volume in Suffolk lambs was significantly

lower at 9, 11, and 13 weeks of age resulting in a highly significant (P < 0.0001) difference between

the overall means of the packed cell volumes of the two breeds. Five of 14 (35.7%) Suffolk lambs

died between 11 to 14 weeks of age and none of the Native lambs died.

In 1993, patent infections were established in both breeds (Figure 4). The pattern of mean

fecal egg count in Native lambs was similar to 1992 and reached a peak at nine weeks of age with a

mean of 580 eggs per gram of feces (range 50-1,150 eggs per gram of feces) and declined by 11

weeks of age. This peak was lower than that observed in 1992. To prevent death loss as occurred in

1992, Suffolk lambs required an anthelmintic treatment at eight weeks of age. This resulted in a

fecal egg count decline at nine weeks of age, with a subsequent increase at 11 weeks of age. Inspite

of this, there was a highly significant (p < 0.0001) difference between the overall means of the fecal

egg counts of the two breeds which was accounted for by significant differences at 5, 7, and 11

weeks of age. Changes in the packed cell volumes of the two breeds corresponded directly to the

changes in fecal egg counts (Figure 5). Native lambs maintained relatively stable packed cell

volumes while Suffolk lambs had a significantly lower mean packed cell volumes at 6, 8, and 12

weeks of age. The overall difference in packed cell volumes between the two breeds was highly

significant (p < 0.0001). Anthelmintic treatment of Suffolk lambs reduced the nematode burden

which accounted for the increase in packed cell volumes at 10 weeks of age.

2. Nematode counts—1993

The species of strongylate nematode genera found were *H. contortus*, *T. colubriformis* and

O. columbianum. In comparison with Suffolk lambs, Native lambs had 63.3% and 82.3% fewer

mean total number of nematodes at 7 and 10 weeks of age, respectively (Table 1). For *H. contortus*,

Native lambs had 64.1% and 93.2% , respectively, fewer than Suffolk lambs. In contrast, there were

37.5% fewer *T. colubriformis* in Native lambs at seven weeks of age and 29.6% more at 10 weeks of

age. Although mean *H. contortus* declined from 1,270 to 570 in Native lambs from 7 to 10 weeks of
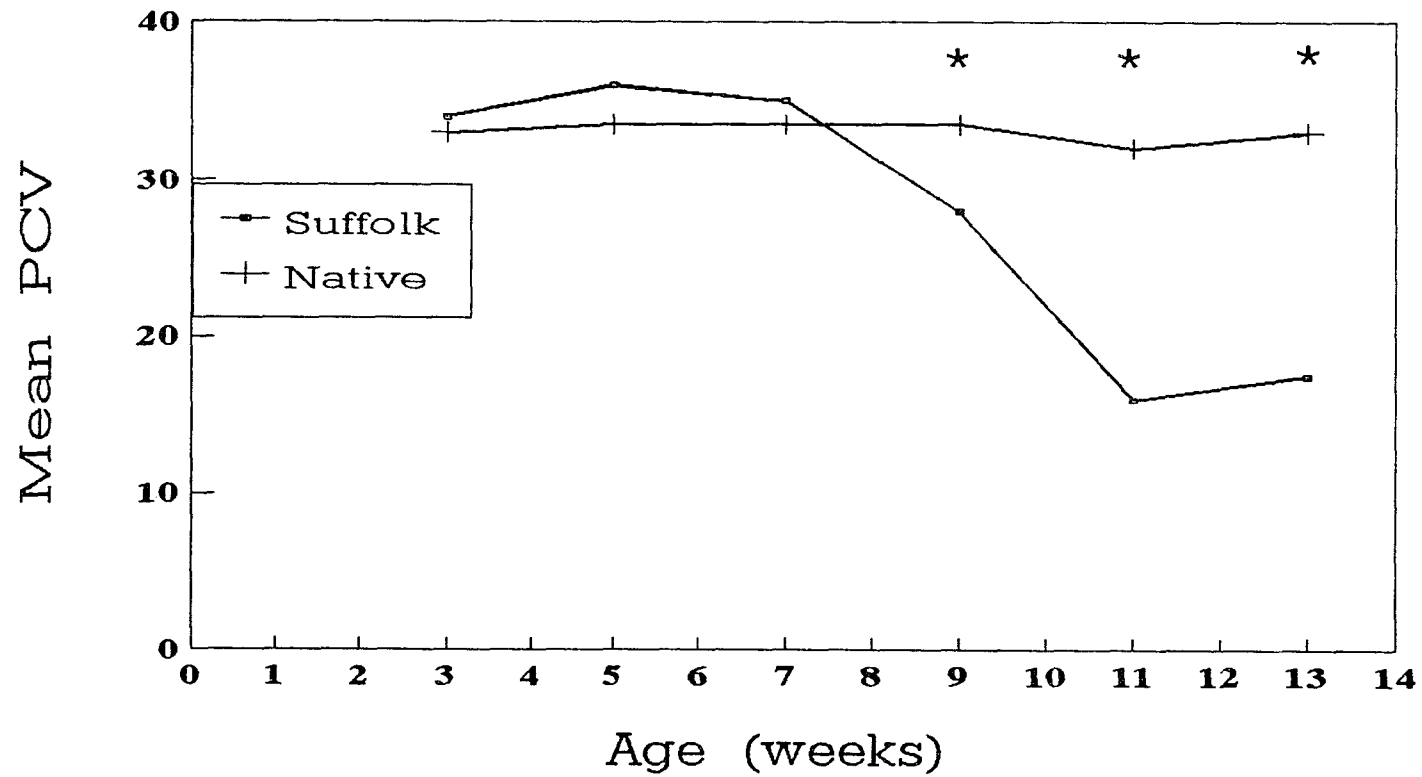
Figure 3. Mean packed cell volumes of naturally infected, suckling Native (n=14) and Suffolk (n=14) lambs in 1992. * indicates significant (p < 0.05) difference. Three Suffolk lambs died between 11-13 weeks of age. PCV=Packed cell volume.
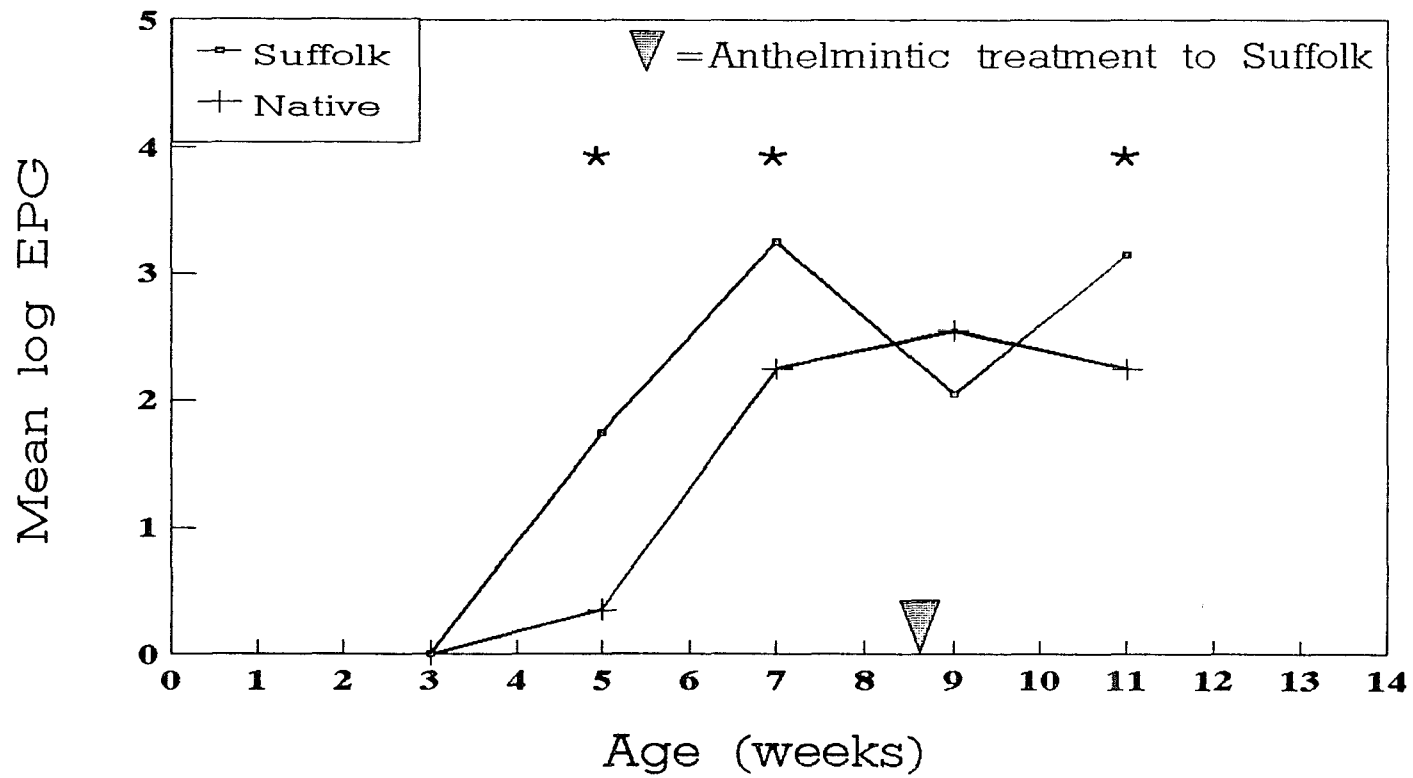
Figure 4. Mean fecal egg counts of naturally infected, suckling Native (n=10) and Suffolk (n=13) lambs in 1993. Suffolk lambs were treated with albendazole (20 mg/kg) at 8 weeks of age. * indicates significant (P < 0.05) difference. EPG=Eggs per gram of feces.
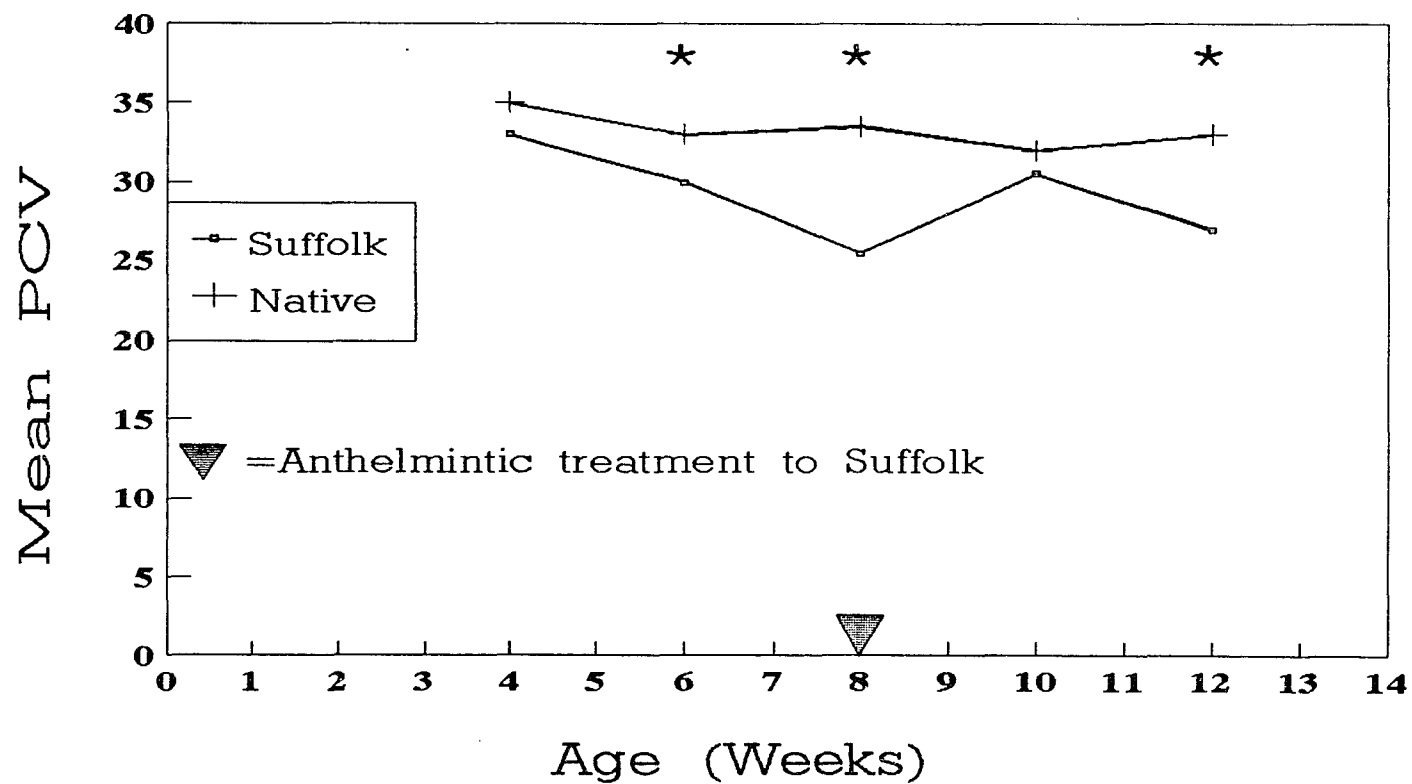
Figure 5. Mean packed cell volumes of naturally infected, suckling Native (n=10) and Suffolk (n=13) lambs in 1993. Suffolk lambs were treated with albendazole (20 mg/kg) at 8 weeks of age. * indicates significant (P < 0.05) difference. PCV=Packed cell volume.

age, an increase in *T. colubriformis* made the mean total nematode burden slightly greater. In Suffolk

lambs, mean total nematode counts increased from 3,665 to 9,190 from 7 to 10 weeks of age and *H.*

contortus represented the major proportion of this increase. Very few *O. columbianum* were found.

Table 1. Mean nematode counts of naturally infected, age-matched, suckling Native and Suffolk lambs in 1993.

Species	Age			
	7 weeks		10 weeks	
	Native (n=2)	Suffolk (n=2)	Native (n=2)	Suffolk (n=2)
Haemonchus contortus	1,270 (-64.1)[a]	3,535	570 (-93.2)	8,380
Trichostrongylus colubriformis	75 (-37.5)	120	1,050 (+29.6)	810
Oesophagostomum columbianum	0 (-100)	10	5 (0)	5
Total	1,345 (-63.3)	3,665	1,625 (-82.3)	9,190

[a]= % change (+/-) compared to Suffolk

3. Nematode counts—1994

In both breeds, nematode burdens were almost entirely represented by *H. contortus* (Table

2). Unlike 1992, the acquisition of *T. colubriformis* infections was low in Native lambs. At eight

weeks of age, compared to Suffolk, Native lambs had 88.4% and 89.1% fewer mean total number of

nematodes and mean *H. contortus*, respectively. A few *T. axei* were recovered from Native lambs.

Differences in total number of nematodes, *H. contortus*, and *T. colubriformis* were significant

between breeds.

Table 2. Mean±s.d. nematode counts of naturally infected, age-matched, suckling Native (n=6) and Suffolk (n=6) lambs at 8 weeks of age in 1994

Species	Native (n=6)	Suffolk (n=6)	Percentage[*] Difference
Haemonchus contortus	625[a]±467	5,758[b]±4,455	89.1
Trichostrongylus axei	20[a]±21	0.00[b]	—
Trichostrongylus colubriformis	46[a]±58	196[b]±165	76.5
Total	691[a]±494	5,955[b]±4,505	88.4

Within rows, means sharing dissimilar superscripts are significantly different.
Nematode counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.
* Native compared to Suffolk

4. Efficacy of anthelmintics and mortality

Ivermectin (1992) and albendazole (1993), both had fecal egg count reduction of greater than 95% in Native lambs and less than 85% in Suffolk lambs (Tables 3-6).

Table 3. The distribution and mean pre- and post-treatment (ivermectin, 400 μg/kg) nematode egg counts in feces of naturally infected, suckling Native lambs treated at 13 weeks of age in 1992

Lamb Number	Pre-Treatment[*]	Post-Treatment[*]	Percent Reduction
1	100	0	100
2	250	0	100
3	300	0	100
4	200	3	98.5
5	12,600	1,750	86.1
6	200	0	100
7	7,100	33	99.5
Mean	2,964	255	97.1

* Eggs per gram of feces

Table 4. The distribution and mean pre- and post-treatment (ivermectin, 400 μg/kg) nematode egg counts in feces of naturally infected, suckling Suffolk lambs treated at 13 weeks of age in 1992

Lamb Number	Pre-Treatment*	Post-Treatment*	Percent Reduction
1	4,100	1,650	59.8
2	8,950	200	97.8
3	8,200	650	92.1
4	13,159	5,250	60.1
5	32,250	1,250	96.1
6	14,850	900	77.8
7	18,650	900	95.2
8	21,850	4,850	77.8
9	9,900	3,450	82.7
Mean	14,656	2,122	82.2

* Eggs per gram of feces

Table 5. The distribution and mean pre- and post-treatment (albendazole, 20 mg/kg) nematode egg counts in feces of naturally infected, suckling Native lambs treated at 12 weeks of age in 1993

Lamb Number	Pre-Treatment*	Post-Treatment*	Percent Reduction
1	250	0	100
2	300	0	100
3	2,350	50	97.9
4	50	0	100
5	100	0	100
6	100	0	100
7	100	0	100
8	1,500	0	100
9	30	0	100
10	50	0	100
Mean	483	5	99.8

* Eggs per gram of feces

Table 6. The distribution and mean pre- and post-treatment (albendazole, 20 mg/kg) nematode egg counts in feces of naturally infected, suckling Suffolk lambs treated at 8 weeks of age in 1993

Lamb Number	Pre-Treatment[*]	Post-Treatment[*]	Percent Reduction
1	11,700	150	98.7
2	5,200	100	98.1
3	5,500	150	97.3
4	4,500	4	99.9
5	900	19	97.9
6	5,300	750	85.8
7	3,750	200	94.7
8	750	550	26.7
9	550	50	90.9
10	200	100	50
11	650	150	76.9
12	2,000	150	92.5
13	2,000	400	80
Mean	3,307.7	213.3	83.8

[*] Eggs per gram of feces

5. Total anti-*H. contortus* immunoglobulin levels—1992

Levels of anti-*H. contortus* antibodies were initially similar in both breeds at seven weeks of age (Figure 6). The breed x age interaction on antibody levels was not significant indicating that Native and Suffolk lambs had declining antibody concentrations that were diverging with the rate of decline more rapid in Suffolk lambs. A comparison of mean absorbance values revealed no significant differences between breeds on 7, 9, 11 or 13 weeks of age.

2. Strongylate nematode infection in weaned lambs

1. Nematode egg counts and packed cell volumes

In 1992, the mean fecal egg counts of treated Native lambs were lower than those of treated Suffolk lambs, which in turn were lower than untreated Native lambs except during the summer months when pre-treatment mean fecal egg counts exceeded the corresponding mean fecal egg counts of the untreated Native lambs (Figure 7). The mean fecal egg count of treated Native lambs was significantly lower than that of treated Suffolk lambs on all sampling dates prior to the first anthelmintic treatment (May 27 to June 24), on July 8, and on September 16 (immediately before the
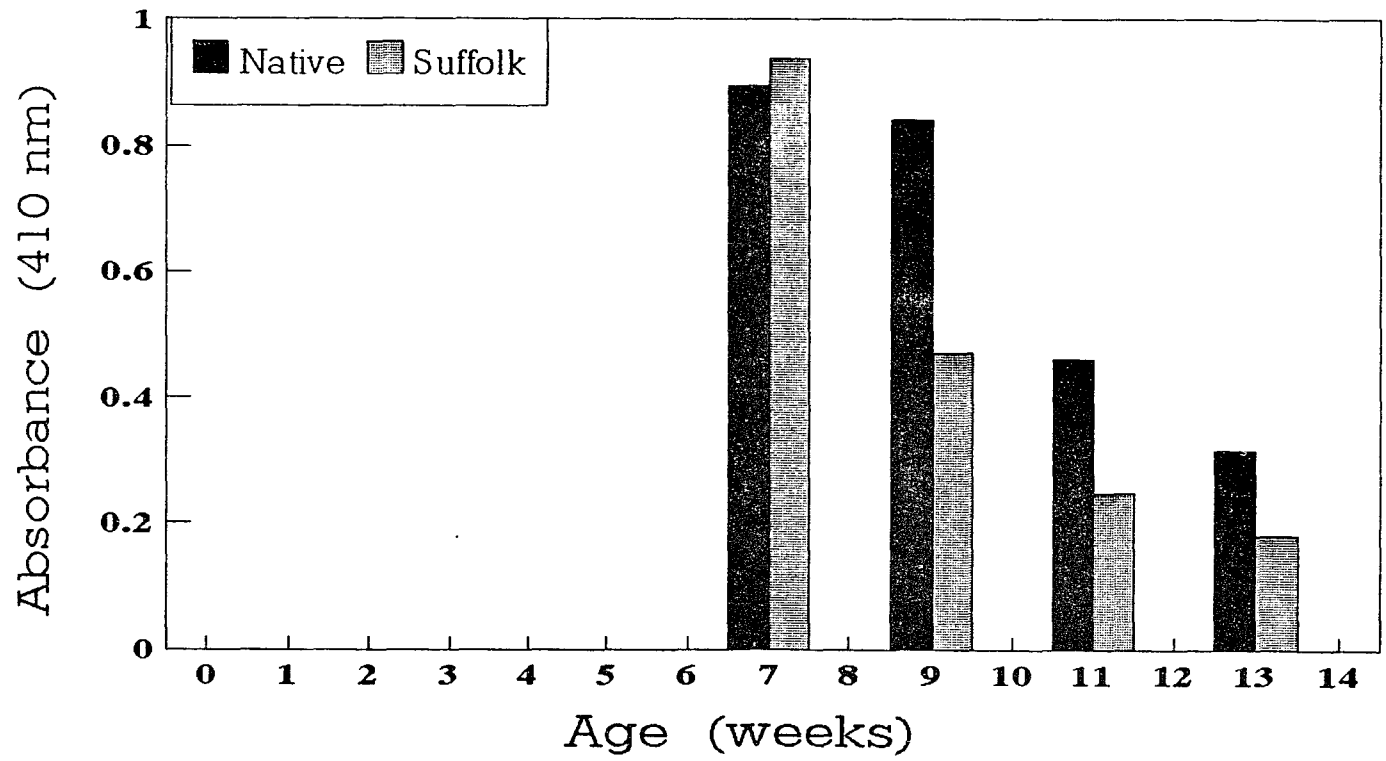
Figure 6. Mean total anti-*Haemonchus contortus* antibody levels in naturally infected, suckling Native (n=14) and Suffolk (n=14) lambs in 1992. Three Suffolk lambs died between 11-13 weeks of age.

Figure 7. Mean fecal egg counts of naturally infected, weaned, treated Native (n=7), treated Suffolk (n=9), and untreated Native (n=7) lambs in 1992. Treatment 1=ivermectin (400 µg/kg), treatments 2-5=albendazole (10 mg/kg). * indicates significant (p < 0.05) difference between the means of treated Native and treated Suffolk lambs. One treated Native lamb died on 8/20; 1, 1, 2, 1, and 1 treated Suffolk lambs died on 7/03, 8/13, 8/20, 9/22, and 11/20, respectively; one untreated Native lamb died on 8/20. EPG=Eggs per gram of feces.

third anthelmintic treatment). Mean overall fecal egg count of the treated Suffolk lambs was significantly higher than that of the treated Native lambs but not the untreated Native lambs (Table 7).

The treated Native lambs maintained the highest mean packed cell volume throughout the observation period (Figure 8). The mean packed cell volume of treated Suffolk lambs was the lowest at the beginning of the observation period, remained lower than treated Native lambs and was similar to untreated Native lambs from August 26 on. A comparison of the mean packed cell volumes of the treated Suffolk and treated Native lambs revealed significant differences on all sampling dates except the last two (December 2 and 16). The untreated Native lambs and treated Native lambs initially had similar packed cell volumes until July 1. Between July 1 and August 26, the untreated Native lamb mean packed cell volume steadily declined and after August 26 it remained relatively stable. The mean packed cell volumes of the untreated Native lambs were significantly lower than those of treated Native lambs on August 26, October 21, November 18, December 2 and 16. The difference in the overall mean packed cell volumes between all three groups was significant with treated Native > untreated Native > treated Suffolk (Table 7).

Four of the nine (44.4%) Suffolk lambs died due to haemonchosis during the observation period and each death occurred immediately prior to an anthelmintic treatment. Two more Suffolk lambs died on August 20 due to a coyote attack.

Table 7. Overall mean±s.d. fecal egg count (FEC) and packed cell volume (PCV) of Native and Suffolk weaned lambs—1992

Lamb Group	FEC (Eggs Per Gram)	PCV
Treated Native	$459^{a}\pm851$ (n=95)	$30.3^{a}\pm3.7$ (n=96)
Treated Suffolk	$2,194^{b}\pm3,011$ (n=89)	$22.4^{b}\pm5.7$ (n=87)
Untreated Native	$1,808^{b}\pm2,101$ (n=94)	$26.0^{c}\pm4.9$ (n=96)

Within columns, means sharing dissimilar superscripts are significantly different.
Fecal egg counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.

Figure 8. Mean packed cell volumes of naturally infected, weaned, treated Native (n=7), treated Suffolk (n=9), and untreated Native (n=7) lambs in 1992. Treatment 1=ivermectin (400 μg/kg), treatments 2-5=albendazole (10 mg/kg). * indicates significant difference between the means of treated Native and treated Suffolk lambs. + indicate significant (p < 0.05) difference between the means of treated Native and untreated Native lambs. One treated Native lamb died on 8/20; 1, 1, 2, 1, and 1 treated Suffolk lambs died on 7/03, 8/13, 8/20, 9/22, and 11/20, respectively; 1 untreated Native lamb died on 8/20. PCV=Packed cell volume.

In 1993, the nematode infection patterns in the three groups of lambs were similar to those observed during 1992 (Figure 9). However, two variations are noteworthy. One is that in 1993, the relative difference between the overall mean fecal egg counts of the treated Native and treated Suffolk lambs was greater. The mean fecal egg counts of treated Native lambs were significantly lower than those of treated Suffolk lambs on all sampling dates except on July 22, August 5 and November 25 immediately after the second, third and sixth anthelmintic treatments, respectively and on October 28. The second is that the untreated Native lambs had substantially higher fecal egg counts during the fall of 1993 and a precipitous drop occurred close to the end of the observation period. The difference in overall mean fecal egg counts between the three groups was significant with untreated Native > treated Suffolk > treated Native (Table 8).

The pattern of mean packed cell volumes of the three groups of lambs were also similar to those observed in 1992 (Figure 10). A comparison of mean packed cell volume values of treated Native and treated Suffolk lambs revealed significantly higher packed cell volume in treated Native lambs on June 3 to July 15, August 26, October 21 to November 18 and December 16. Compared to the mean packed cell volume values of treated Native lambs, the mean packed cell volumes in untreated Native lambs were significantly lower on August 26, October 21 to November 18, and December 16. The overall mean packed cell volume of the treated Suffolk lambs was significantly lower than that of the treated Native lambs but not the untreated Native lambs (Table 8).

Four of 13 (30.8%) Suffolk lambs died due to haemonchosis immediately after weaning and another died in late September.
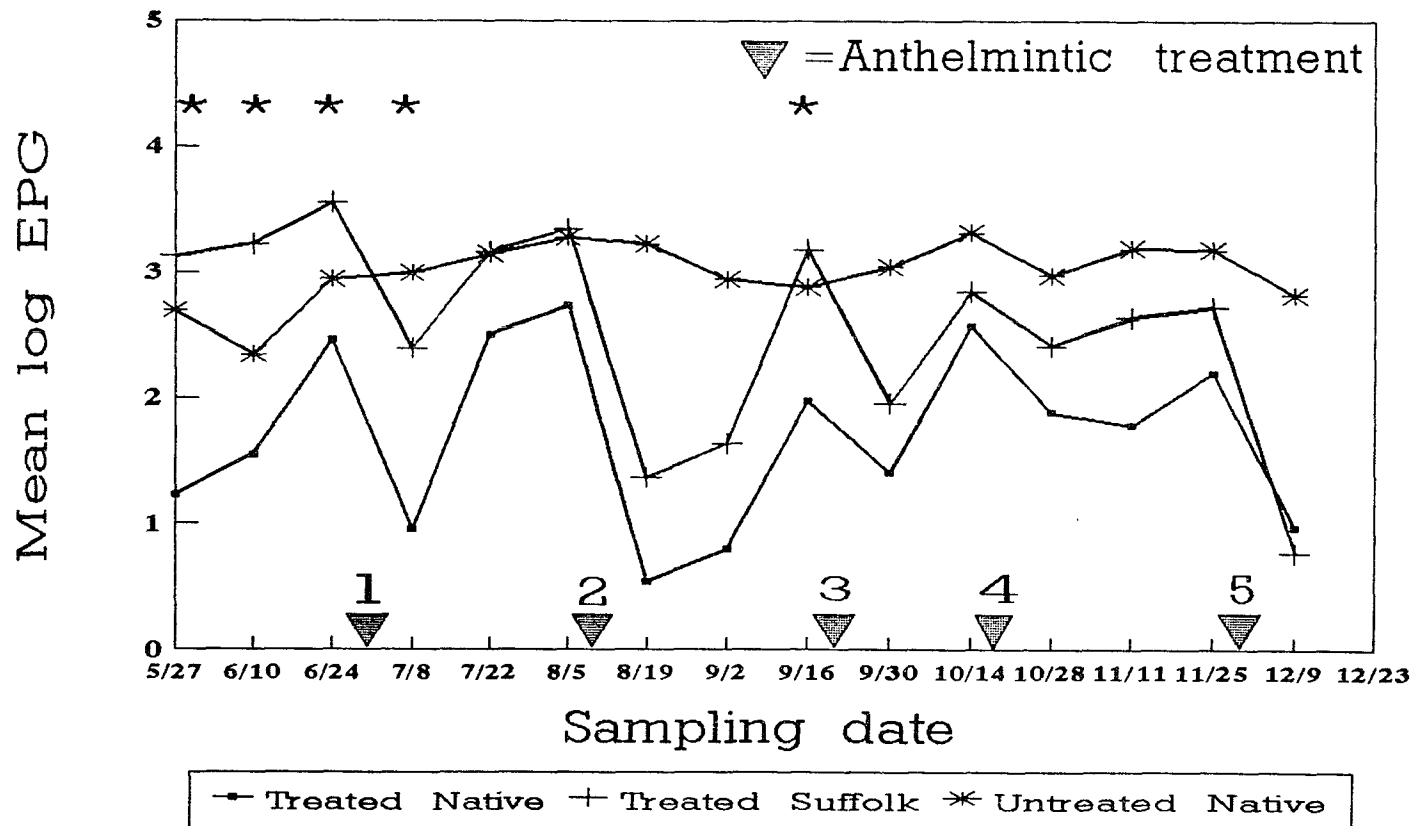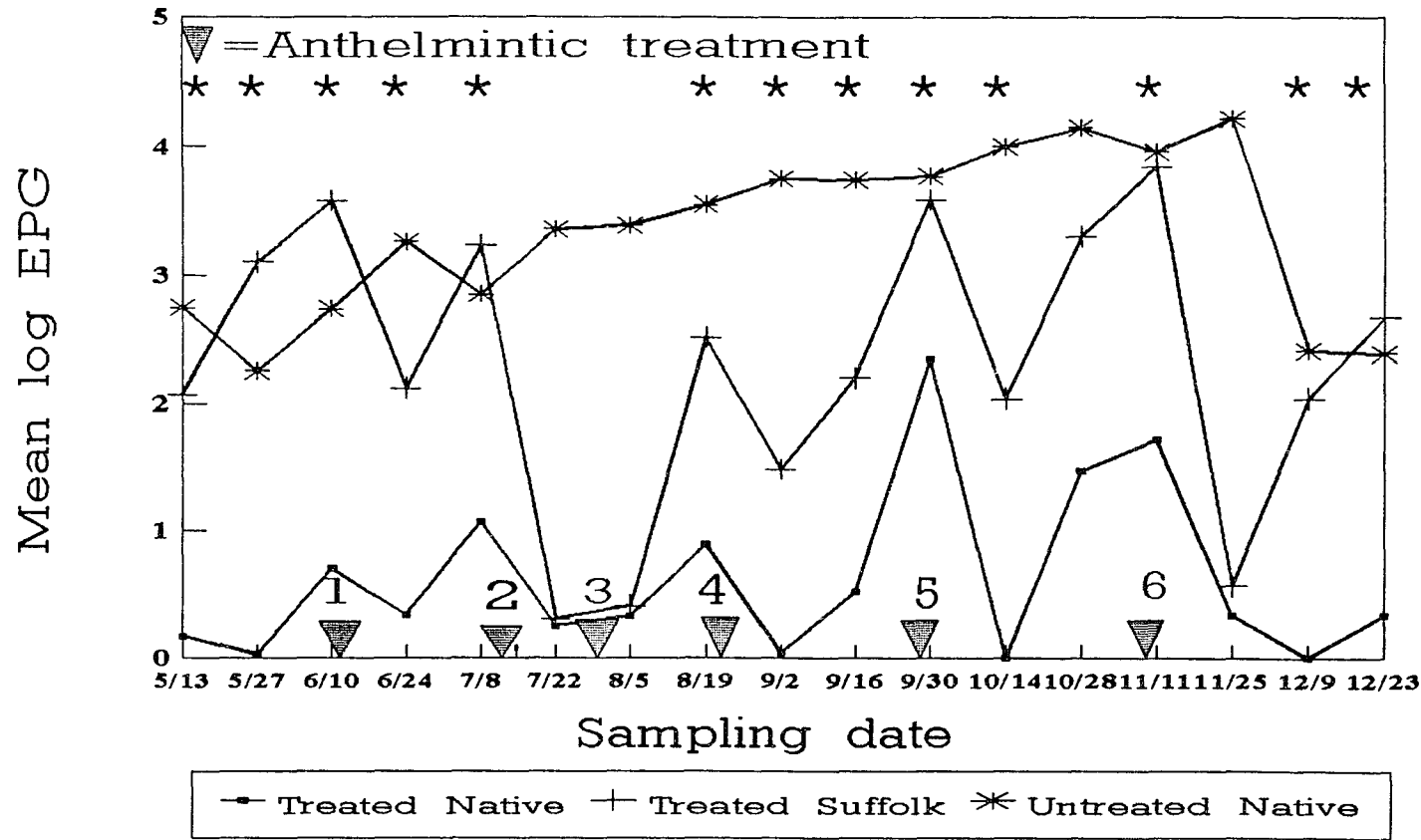
Figure 9. Mean fecal egg counts of naturally infected, weaned, treated Native (n=10), treated Suffolk (n=13), and untreated Native (n=6) lambs in 1993. Treatment 1=levamisole (8 mg/kg), treatments 2-5=albendazole (20 mg/kg) plus levamisole (16 mg/kg), treatment 6=albendazole (20 mg/kg) plus ivermectin (400 µg/kg). * indicates significant (p < 0.05) difference between the means of treated Native and treated Suffolk lambs. Two treated Native lambs were removed for necropsy on July, 19, 3 were removed for necropsy on October, 1; 4 treated Suffolk lambs died between 7/8 to 7/22, 2 were removed for necropsy on July, 19, 1 died on 10/02, 3 were removed for necropsy on October, 1; 3 untreated Native lambs were removed for necropsy on October, 1. EPG=Eggs per gram of feces.
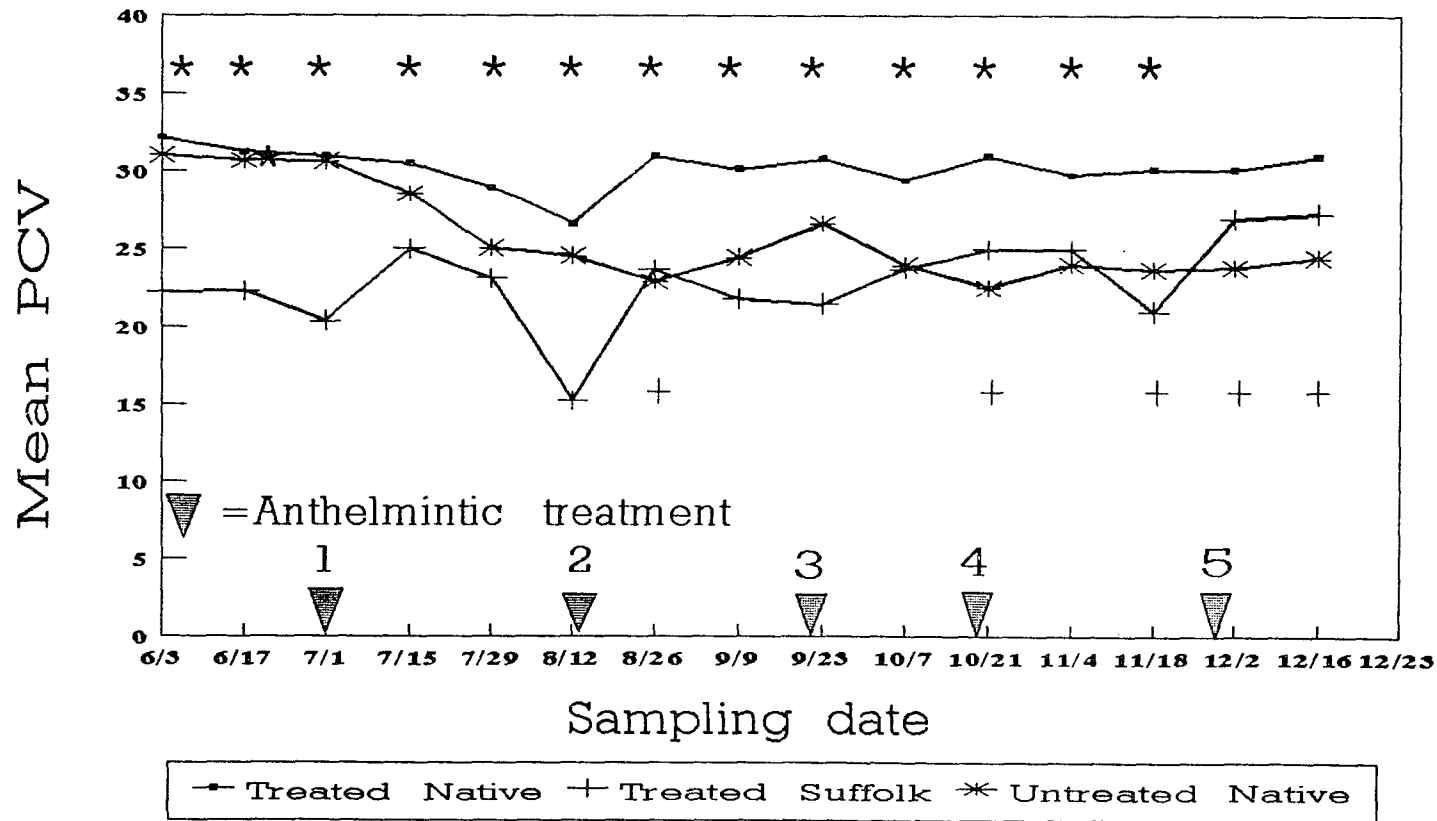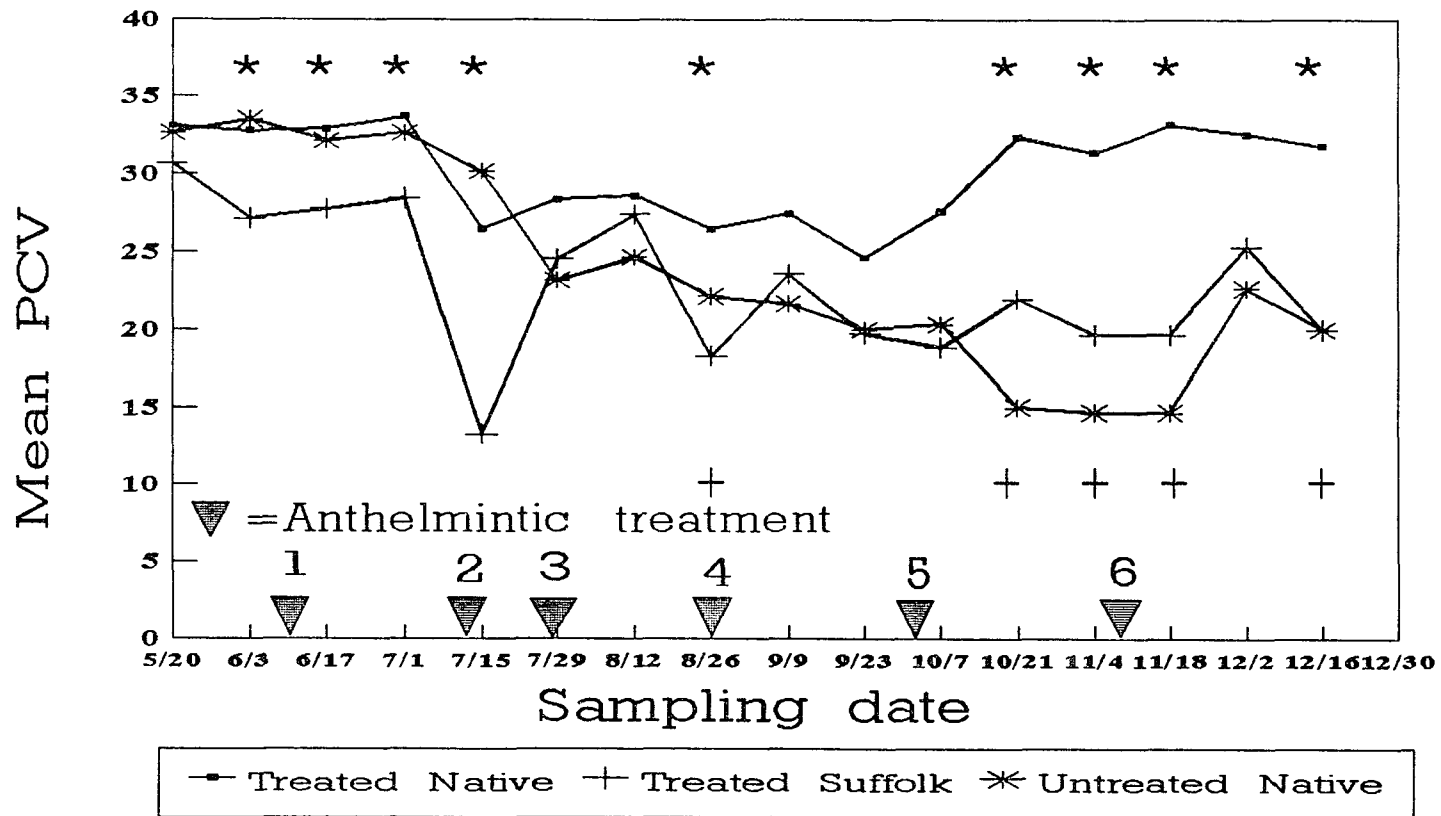
Figure 10. Mean packed cell volumes of naturally infected, weaned, treated Native (n=10), treated Suffolk (n=13), and untreated Native (n=6) lambs in 1993. Treatment 1=levamisole (8 mg/kg), treatments 2-5=albendazole (20 mg/kg) plus levamisole (16 mg/kg), treatment 6=albendazole (20 mg/kg) plus ivermectin (400 µg/kg). * indicates significant (p < 0.05) difference between the means of treated Native and treated Suffolk lambs. + indicate significant difference between the means of the treated Native and untreated Native lambs. Two treated Native lambs were removed for necropsy on July, 19, 3 were removed for necropsy on October, 1; 4 treated Suffolk lambs were died between 7/8 to 7/22, 2 were removed for necropsy on July, 19, 1 died on 10/02, 3 were removed for necropsy on October, 1; 3 untreated Native lambs were removed for necropsy on October, 1. PCV=Packed cell volume.

Table 8. Overall mean±s.d. fecal egg count (FEC) and packed cell volume (PCV) of Native and Suffolk weaned lambs—1993

Lamb Group	FEC (Eggs Per Gram)	PCV
Treated Native	93[a]±496 (n=127)	30.2[a]±4.4 (n=120)
Treated Suffolk	2,020[b]±3,497 (n=123)	24.1[b]±6.3 (n=116)
Untreated Native	6,568[c]±8,959 (n=84)	25.2[b]±7.8 (n=78)

Within columns, means sharing dissimilar superscripts are significantly different.
Fecal egg counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.

2. Larval differentiation

The population distribution of nematodes was represented by *H. contortus*, *T. colubriformis*, and *O. columbianum* larvae based on fecal larval cultures in 1992 and *O. columbianum* was not present in 1993 (Figures 11 and 12). In both years, the predominance of *H. contortus* larvae from treated Suffolk lambs corresponded well with their higher fecal egg counts. In contrast, when larvae could be recovered from treated Native lamb fecal cultures, both *H. contortus* and *T. colubriformis* were present.

In 1992, *T. colubriformis* larvae were predominant in the fecal cultures of untreated Native lambs at all times. In 1993, *T. colubriformis* larvae were also predominant until September 16 and 30 when *H. contortus* larvae were predominant.

3. Nematode counts—July, 1993

The mean nematode count in Native lambs was higher than Suffolk lambs for *H. contortus*, *T. axei*, and *T. colubriformis* (Table 9).

4. Nematode counts—October, 1993

Mean total, *H. contortus*, and *T. colubriformis* nematode burdens were highest in untreated Native lambs and lowest in treated Native lambs and the treated Suffolk lambs were intermediate (Table 10). A few *T. axei* were recovered from Native lambs. The mean *T. colubriformis* burden in
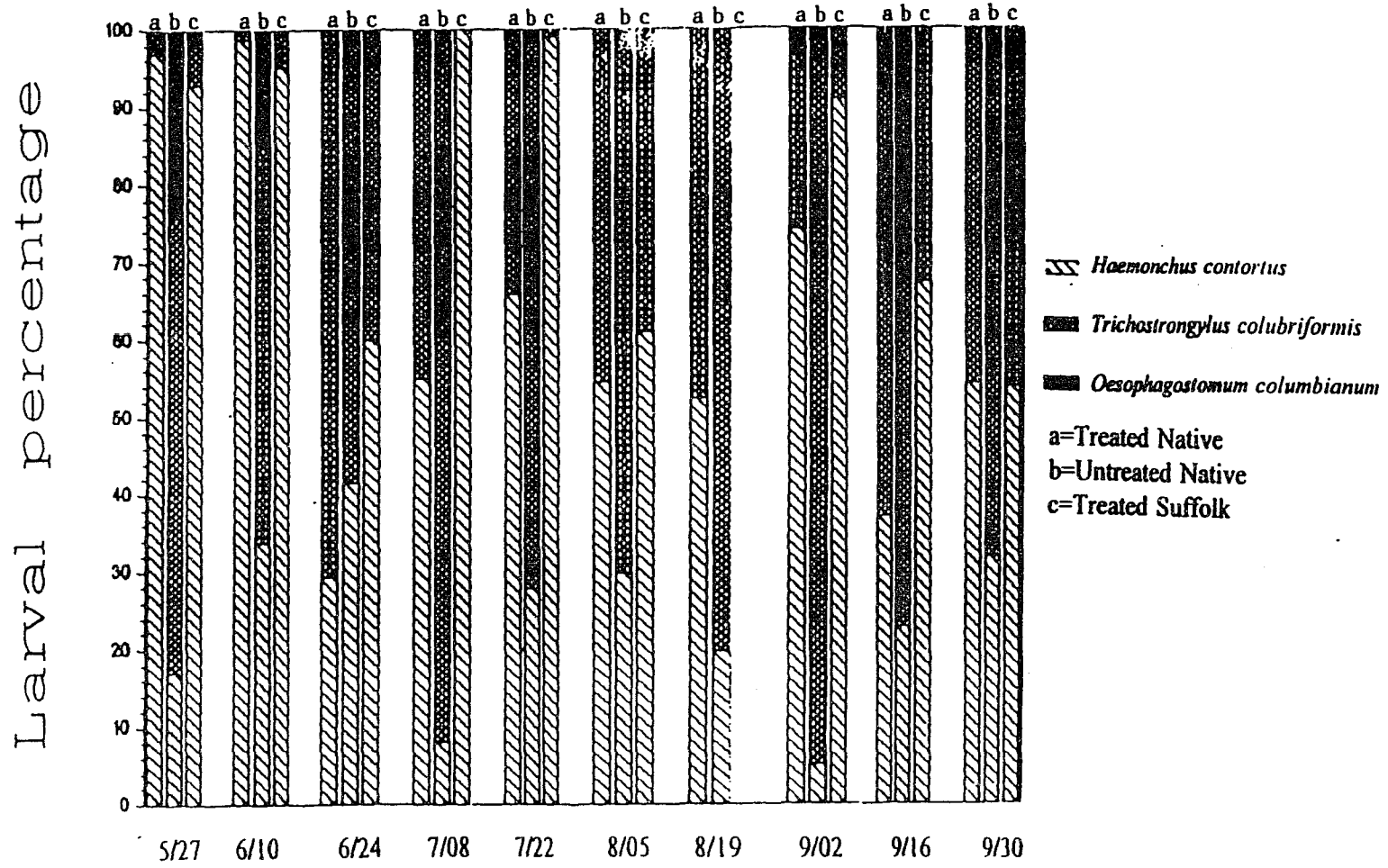
Figure 11. Population distribution of infective larvae cultured from individual fecal samples from treated Native, untreated Native and treated Suffolk lambs from May to September, 1992.
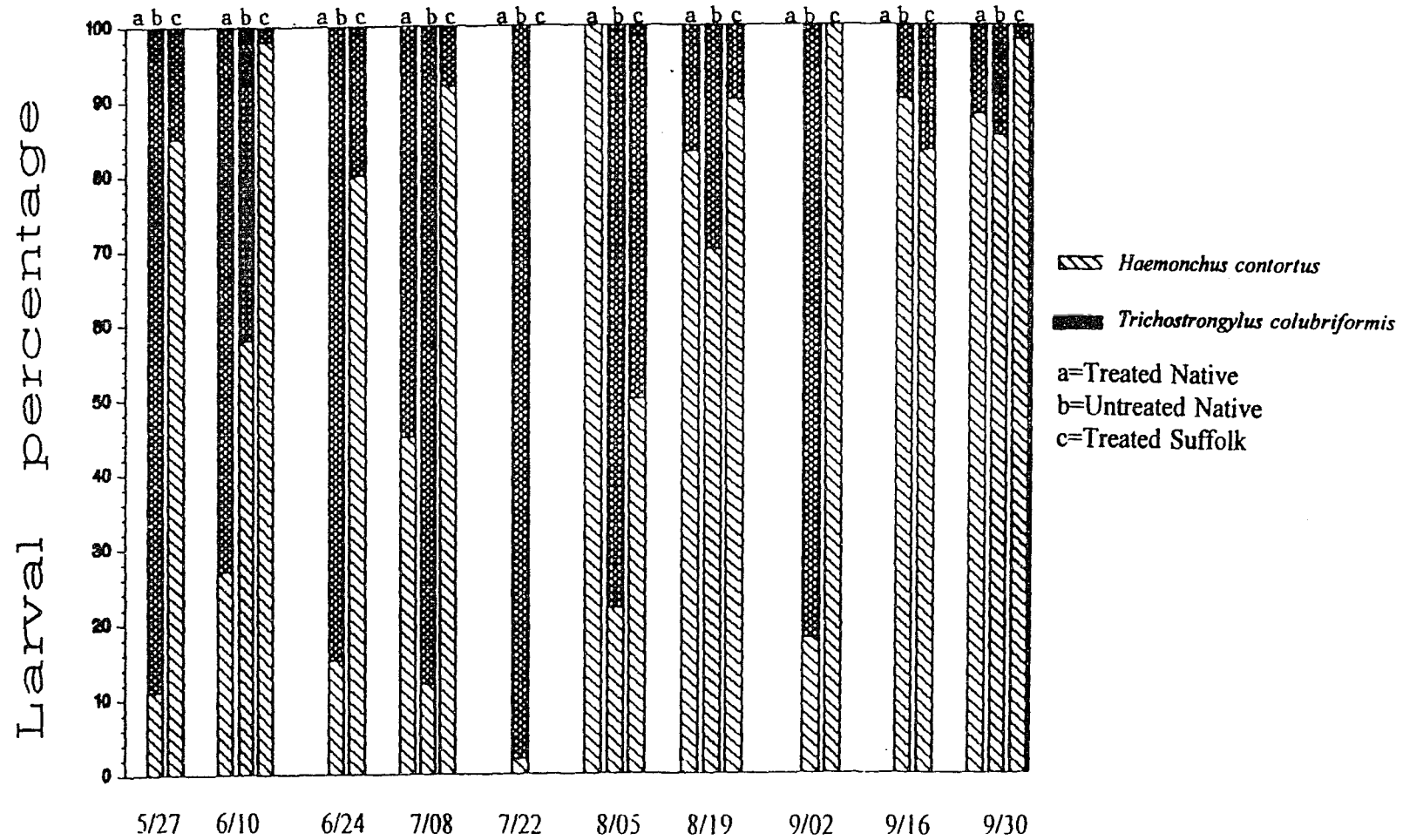
Figure 12. Population distribution of infective larvae cultured from pooled fecal samples from treated Native, untreated Native and treated Suffolk lambs from May to September, 1993.

treated Native lambs was significantly lower than that of untreated Native lambs. Mean total

nematode and *H. contortus* numbers did not differ significantly between the three groups.

Table 9. Mean±s.d. nematode counts in weaned Native and Suffolk lambs 38 days (July, 1993) after an anthelmintic treatment

Species	Native (n=2)	Suffolk (n=2)
Haemonchus contortus	15,740	8,835
Trichostrongylus axei	111	0
Trichostrongylus colubriformis	82,000	11,350
Total	98,851	20,185

Table 10. Mean±s.d. nematode counts of weaned Native and Suffolk lambs 30 days (October, 1993) after an anthelmintic treatment and untreated Native lambs

Species	Native Treated (n=3)	Suffolk Treated (n=3)	Native Untreated (n=3)
Haemonchus contortus	3,300[a]±2,707	7,740[a]±7,155	8,395[a]±9,777
Trichostrongylus axei	110[a]±174	0[a]	72[a]±45
Trichostrongylus colubriformis	143[a]±163	1,933[b]±1,069	14,267[b]±9,585
Total	3,553[a]±2,930	9,673[a]±8,187	22,273[a]±19,033

Within rows, means sharing dissimilar superscripts are significantly different.
Nematode counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.

5. Total immunoglobulin levels

On both July 01 and 29, treated Native lambs had significantly higher immunoglobulin

concentrations than treated Suffolk lambs (Figure 13). The overall difference in total

immunoglobulin levels between the two breeds was highly significant ($p < 0.0001$).
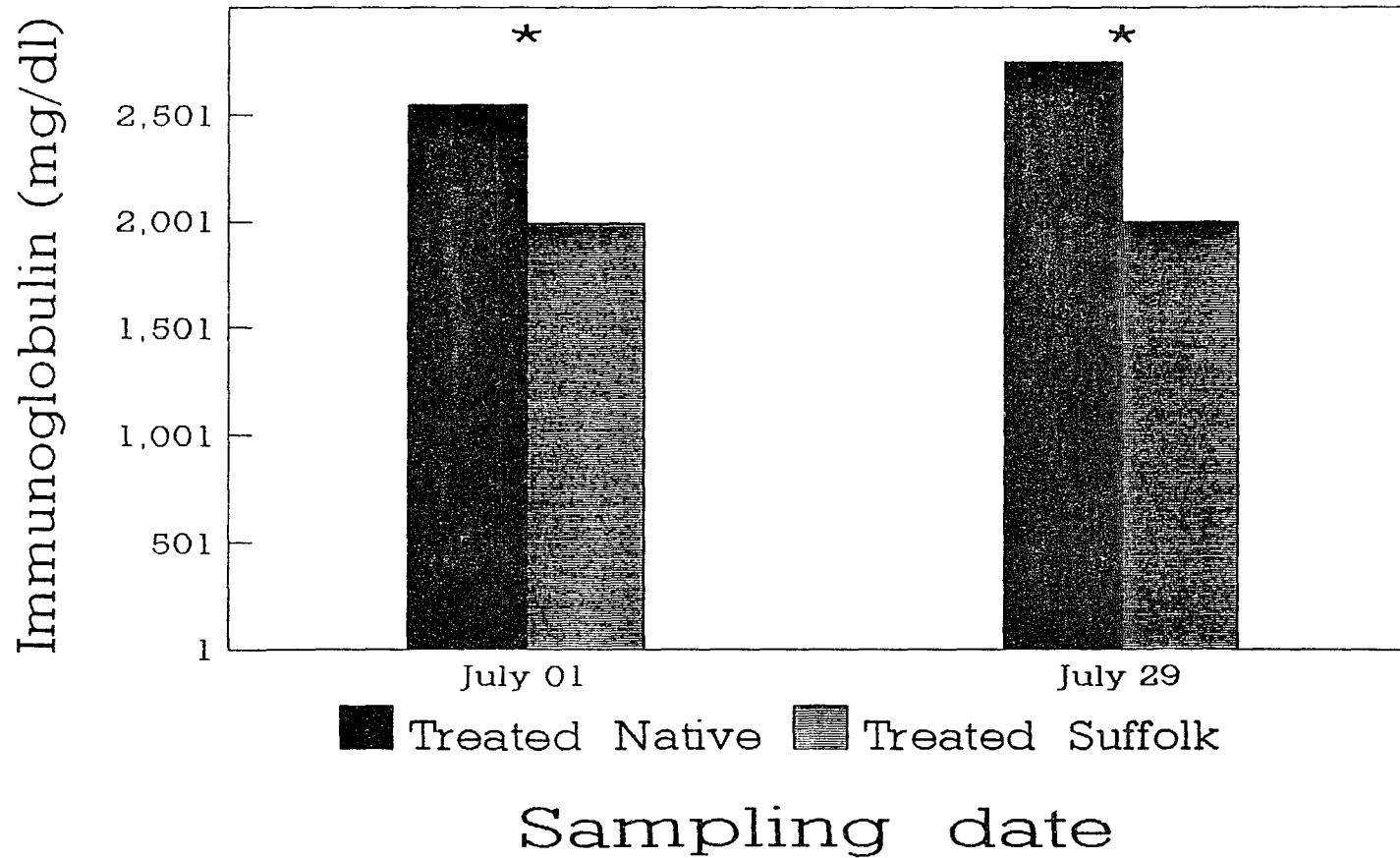
Figure 13. Mean total immunoglobulin levels in naturally infected, weaned, treated Native (n=6) and treated Suffolk (n=7) lambs in July, 1992. * indicate significant (p < 0.05) difference in mean total immunoglobulin levels between breeds on July 01 and July 29.

6. Total anti-*H. contortus* antibody levels-1992

The mean total anti-*H. contortus* antibody levels of treated Native lamb sera were always

higher than those for treated Suffolk lamb sera and changes in the antibody levels were similar in

both breeds (Figure 14). In both breeds, the antibody levels steadily increased from the first test date,

June 3 to reach peak levels on August 26. At the peak, a three to five fold rise in antibody levels

was seen in both breeds compared to the antibody levels on the first test date. The mean total

antibody levels of the two breeds were not significantly different at any time point.

7. Class-specific anti-*H. contortus* antibody levels-1992

The serum levels of class-specific anti-*H. contortus* antibodies of treated Native and treated

Suffolk lambs were determined at three time points after weaning (Figure 15). The patterns of the

antibody response of the four isotypes (IgM, IgA, IgG1 and IgG2) were broadly similar in both

breeds. Both Native and Suffolk lambs exhibited low levels of IgM, IgG1, IgG2 and IgA antibodies

initially (June 3). Subsequently, there was an increase in the levels of antibodies of all four isotypes

in both breeds (July 29). While further increases were seen in the levels of IgG1 and IgG2 antibodies

in both breeds (October 22), IgM and IgA levels declined. Reductions in the levels of Native IgM

were more profound than those for Suffolk IgM. The IgA decline in the two breeds was rather

slight. No significant differences were found in the levels of IgM, IgG1, IgG2 or IgA antibodies

between breeds on each of the three test dates.

8. Peripheral blood eosinophil counts

In both 1992 and 1993, eosinophilia was indicated by concurrent peaks in all three groups

of lambs (Figures 16 and 17). In 1992, the first peak of moderate magnitude was observed on July

29. A comparison of mean eosinophil counts of treated Native and treated Suffolk lambs revealed a

significantly higher mean eosinophil count in treated Native lambs on July 29. A second peak of

similar magnitude was seen between August 26 and September 9, and a third peak (more prominent

in treated Suffolk lambs) was observed on December 16. At this peak, treated Suffolk lambs had

significantly higher eosinophil counts than treated Native lambs. In 1993, the first peak of circulating
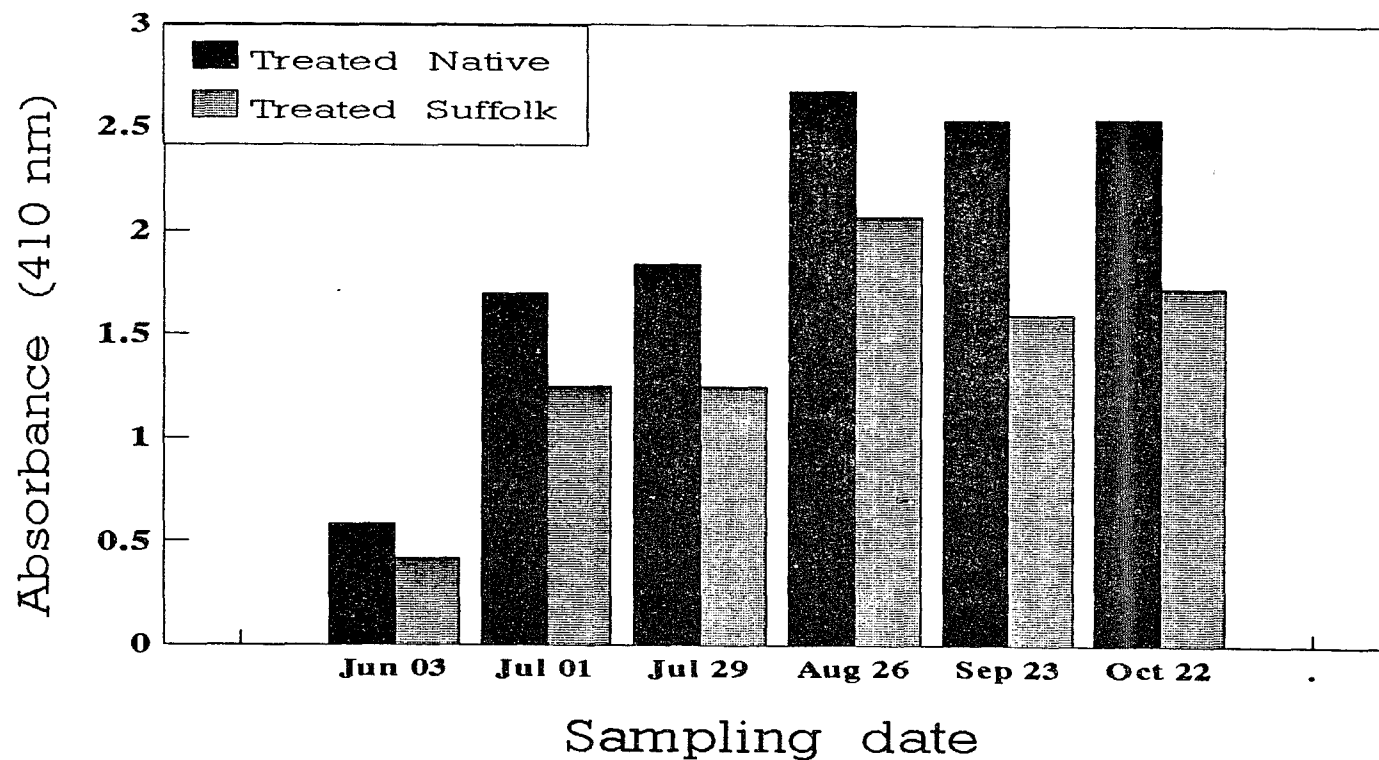
Figure 14. Mean total anti-*Haemonchus contortus* antibody levels in naturally infected, weaned, treated Native (n=7) and treated Suffolk (n=9) lambs in 1992. One treated Native lamb died on 8/20; 1, 1, 2. and 1 treated Suffolk lambs died on 7/03, 8/13, 8/20, and 9/22, respectively.

Absorbance (410 nm)

IgM
Treated Native
Treated Suffolk

4
3
2
1
0

Jun 03   Jul 29   Oct 22

IgA
Treated Native   Treated Suffolk

4
3
2
1
0

Jun 03   Jul 29   Oct 22

IgG1
Treated Native
Treated Suffolk

4
3
2
1
0

Jun 03   Jul 29   Oct 22

IgG2
Treated Native   Treated Suffolk

3
2
1
0

Jun 03   Jul 29   Oct 22

Sampling date
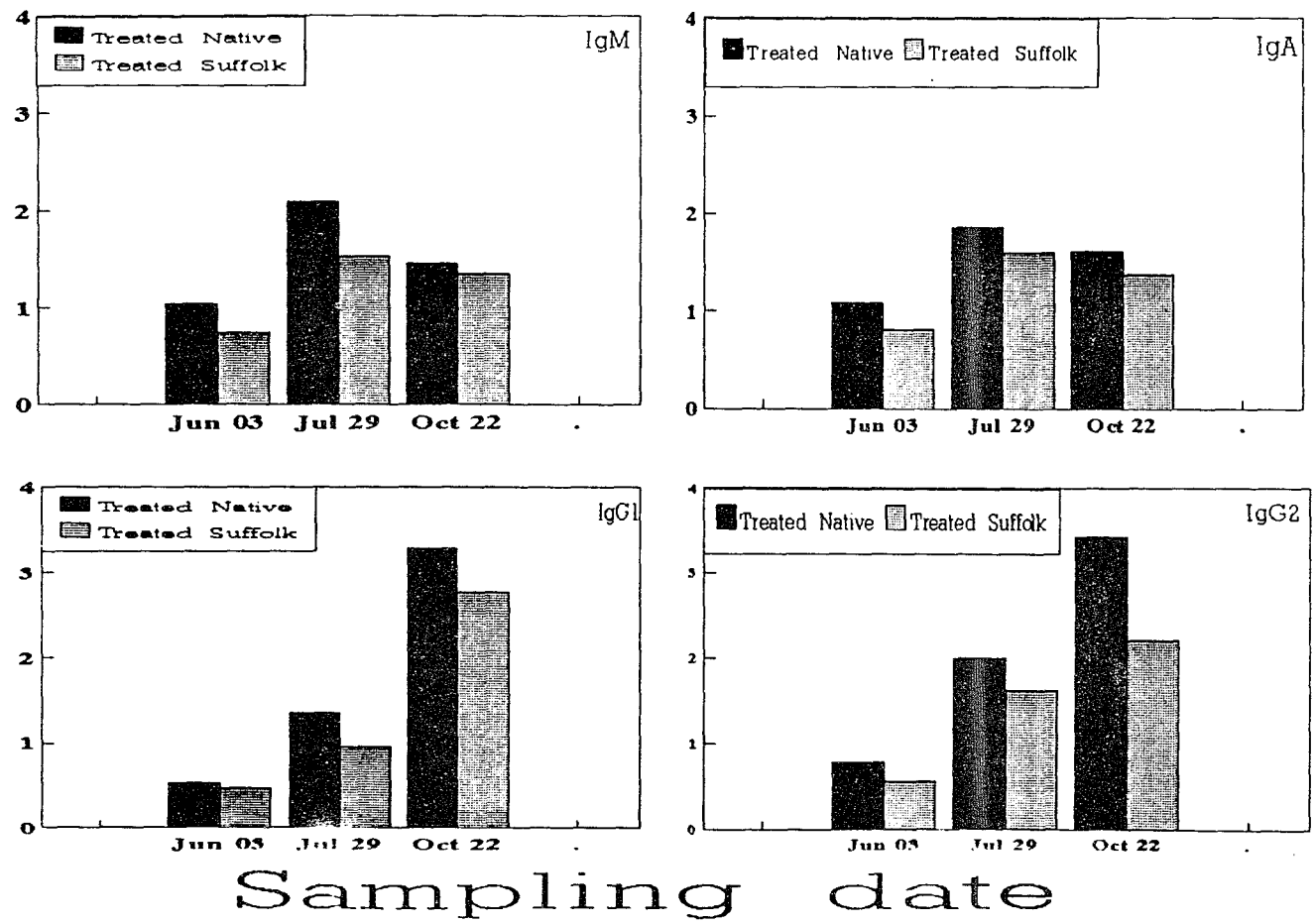
Figure 15. Mean anti-*Haemonchus contortus* IgM, IgA, IgG1 and IgG2 antibody levels in naturally infected, weaned, treated Native (n=7) and treated Suffolk (n=9) lambs in 1992. One treated Native lamb died on 8/20; 1, 1, 2, and 1 treated Suffolk lambs died on 7/03, 8/13, 8/20, and 9/22, respectively.

2,500

▽ =Anthelmintic treatment

Mean eosinophil / μl

1   2   3   4   5
▽   ▽   ▽   ▽   ▽

2,000

1,500   *   *

1,000

500

0

6/3 6/17 7/1 7/15 7/29 8/12 8/26 9/9 9/23 10/7 10/21 11/4 11/18 12/2 12/16 12/30

Sampling   date

━ Treated Native ＋ Treated Suffolk ✳ Untreated Native
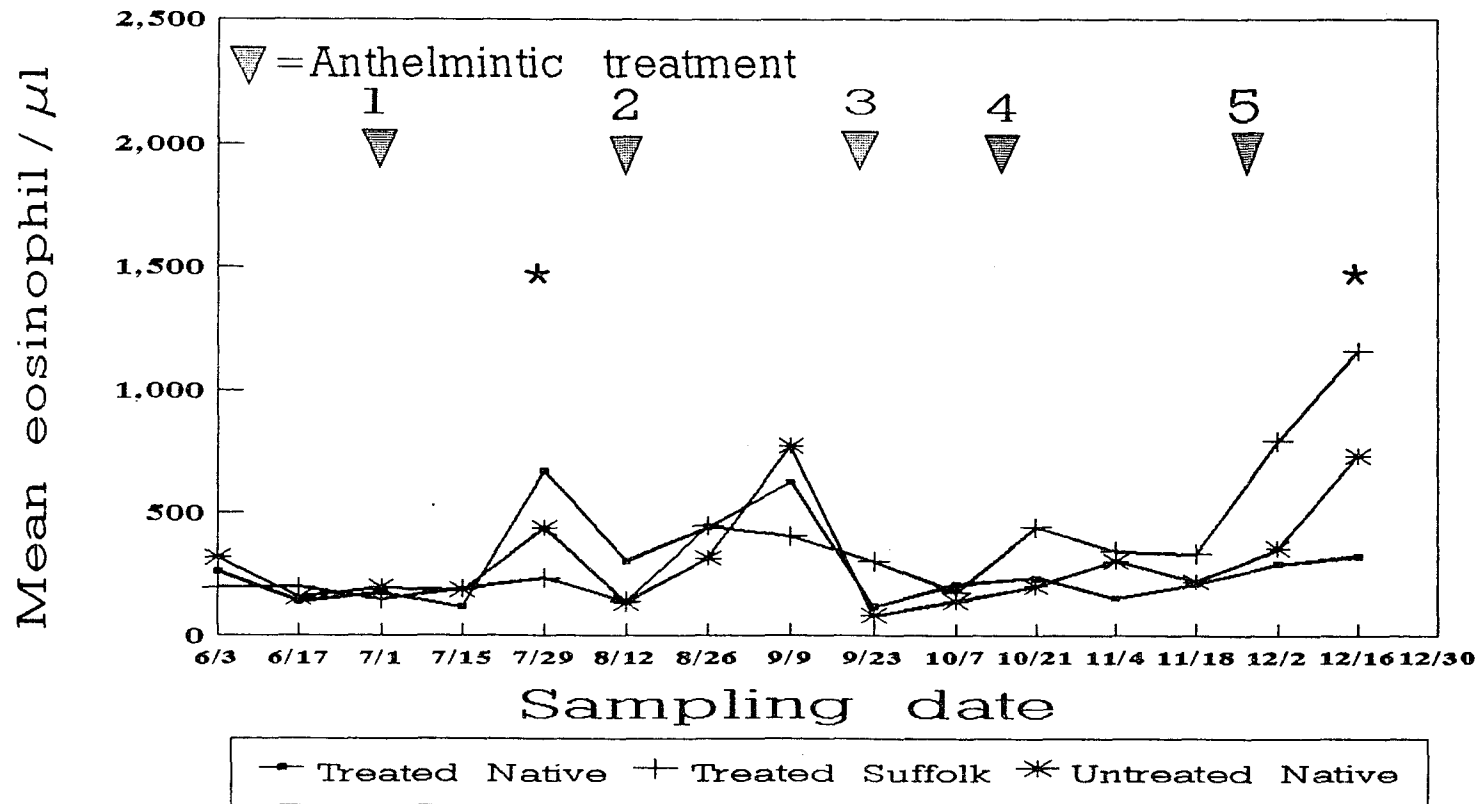
Figure 16. Mean circulating eosinophil counts of naturally infected, weaned, treated Native (n=7), treated Suffolk (n=9), and untreated Native (n=7) lambs in 1992. Treatment 1=ivermectin (400 μg/kg), treatments 2-5=albendazole (10 mg/kg). * indicates significant (p < 0.05) difference between the means of treated Native and treated Suffolk lambs. One treated Native lamb died on 8/20; 1, 1, 2, 1, and 1 treated Suffolk lambs died on 7/03, 8/13, 8/20, 9/22, and 11/20, respectively; 1 untreated Native lamb died on 8/20.

2,500

▽ =Anthelmintic treatment

*

2,000

Mean eosinophil / μl

1,500

1   2 3   4   5   6

▽   ▽ ▽   ▽   ▽   ▽

+

1,000

*

500

0

5/20 6/3 6/17 7/1 7/15 7/29 8/12 8/26 9/9 9/23 10/7 10/21 11/4 11/18 12/2 12/16 12/30

Sampling date

—•— Treated Native   —+— Treated Suffolk   —*— Untreated Native
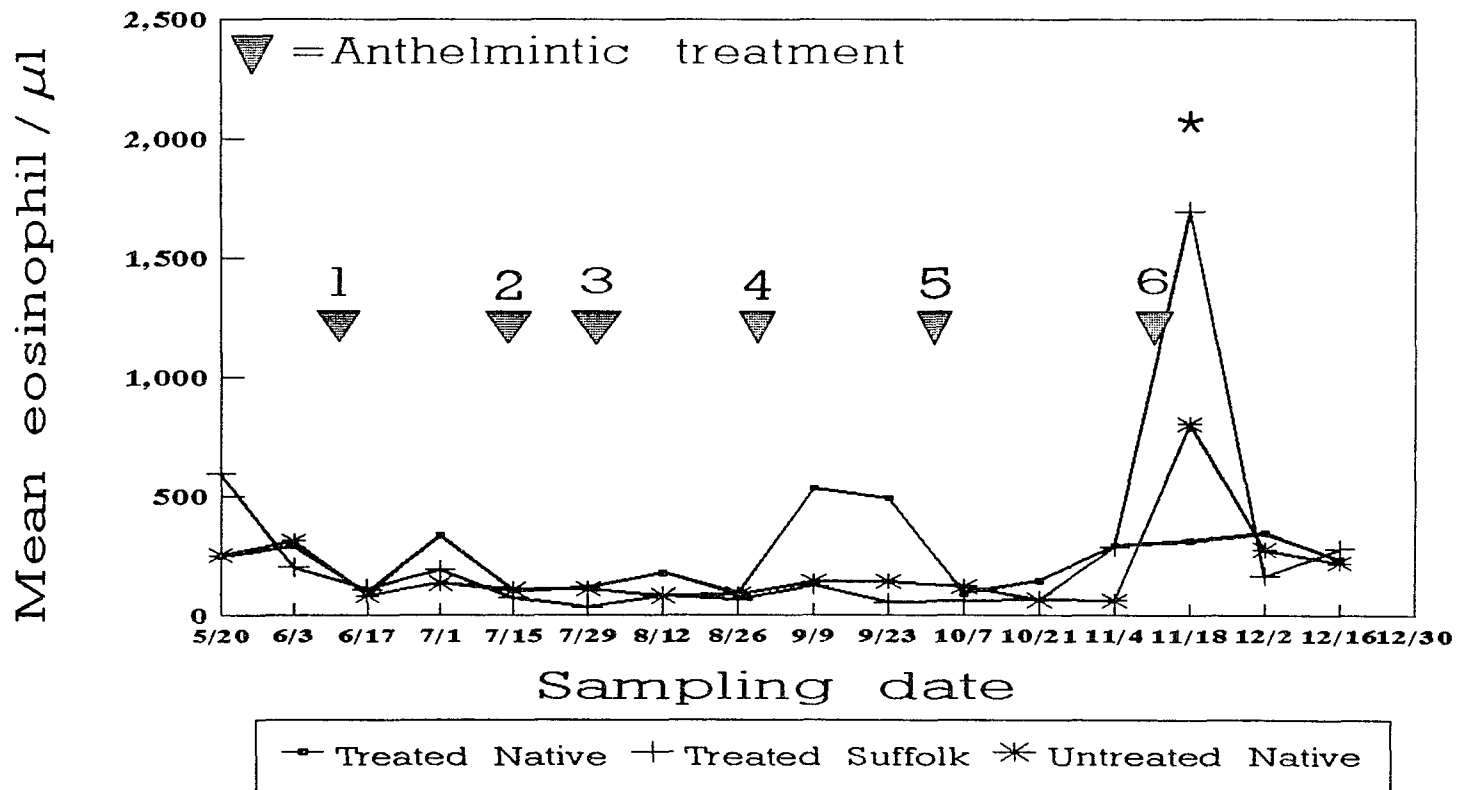
Figure 17. Mean circulating eosinophil counts of naturally infected, weaned, treated Native (n=10), treated Suffolk (n=13), and untreated Native (n=6) lambs in 1993. Treatment 1=levamisole (8 mg/kg), treatments 2-5=albendazole (20 mg/kg) plus levamisole (16 mg/kg), treatment 6=albendazole (20 mg/kg) plus ivermectin (400 μg/kg). * indicates significant (p < 0.05) difference between the means of treated Native and treated Suffolk lambs. Two treated Native lambs were removed for necropsy on July, 19, 3 were removed for necropsy on October, 1; 4 treated Suffolk lambs died between 7/8 to 7/22, 2 were removed for necropsy on July, 19, 1 died on 10/02, 3 were removed for necropsy on October, 1; 3 untreated Native lambs were removed for necropsy on October, 1.

eosinophil numbers was small (July 1) and the third peak (November 18) in treated Suffolk lambs

was notably larger. A comparison of mean eosinophil count values of treated Native and treated

Suffolk lambs revealed a significantly higher mean eosinophil count in treated Suffolk lambs on

November 18. In both 1992 and 1993, differences between the overall means of the eosinophil

counts of the three groups of lambs were not significant (Table 11).

Table 11. Overall mean±s.d. peripheral blood eosinophil counts in Native and Suffolk weaned lambs—1992 and 1993

Lamb Group	1992	1993
Treated Native	286±301 (n=96)	243±367 (n=119)
Treated Suffolk	299±418 (n=87)	220±363 (n=115)
Untreated Native	302±300 (n=94)	173±257 (n=78)

Eosinophil counts were (n+10) transformed prior to statistical analysis.

3. Strongylate nematode infection in ewes

1. Nematode egg counts and packed cell volumes

In 1992 and 1993, Native ewes maintained consistently lower mean fecal egg count than

Suffolk ewes (Figures 18 and 19). In both years, in Suffolk ewes, anthelmintic treatments were

followed by increases in fecal egg counts. Simultaneous but smaller increases were also observable

in the fecal egg count profiles of the Native ewes in 1992 and 1993. There were 13.3 and 15.3 fold

differences between the overall means of the fecal egg counts of the two breeds in 1992 and 1993,

respectively. In both 1992 and 1993, these differences between the overall mean fecal egg counts

were highly significant (P < 0.0001) and reflected the differences observed during the three grazing

seasons (Table 12 and 13).

In 1992 and 1993, Native ewes consistently maintained higher mean packed cell volume

from the beginning to the end of observations (Figures 20 and 21). In 1992, the differences between

means were significant on all sampling dates except on November 4 and 11. In 1993, initially both

groups of ewes had no differences between the mean packed cell volumes during the spring months.
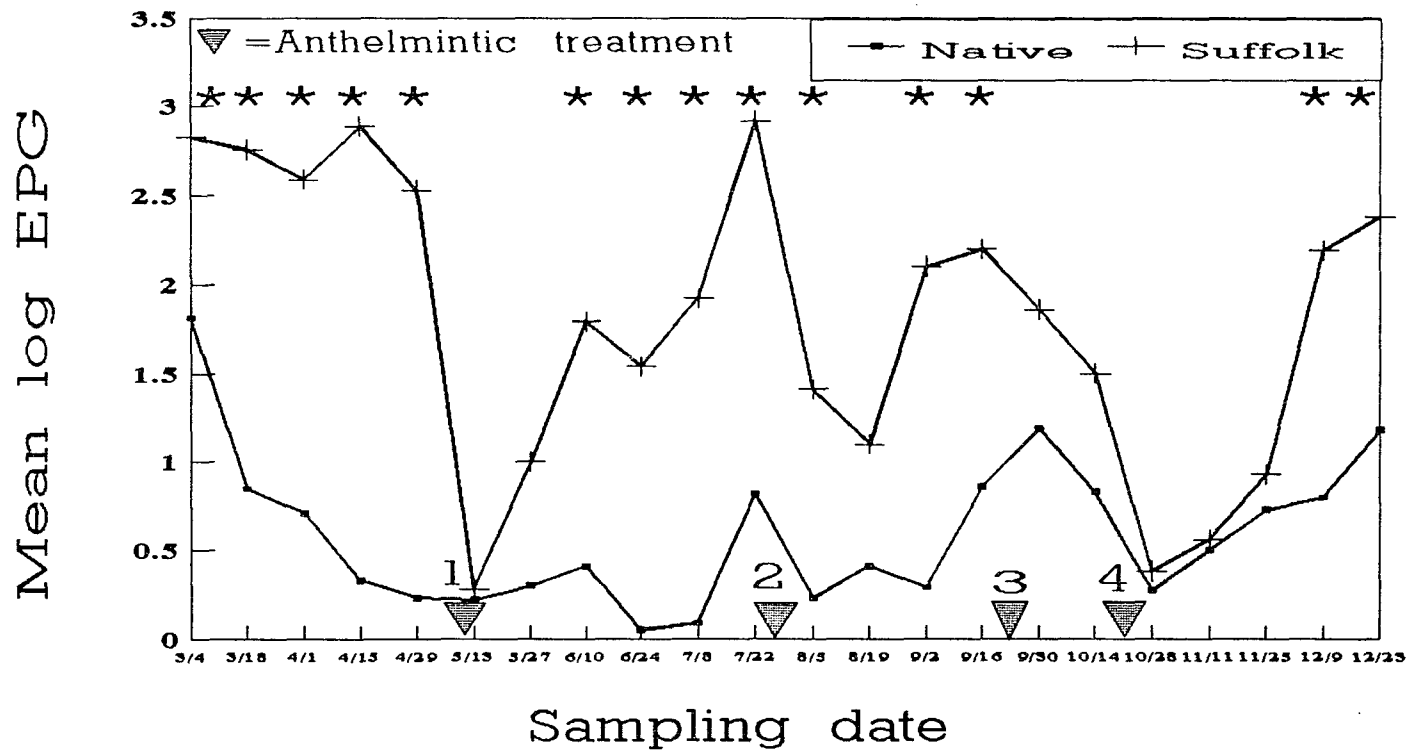
Figure 18. Mean fecal egg counts of naturally infected Native (n=9) and Suffolk (n=8) ewes in 1992. Treatments 1-4=ivermectin (200 µg/kg). * indicates significant ($p < 0.05$) difference. One Suffolk ewe died on 4/25, 1 died on 7/25. EPG=Eggs per gram of feces.

Figure 19. Mean fecal egg counts of naturally infected Native (n=15) and Suffolk (n=15) ewes in 1993. Treatments 1-2=ivermectin (200 µg/kg), treatment 3=ivermectin (400 µg/kg), treatments 4-5=ivermectin (400 µg/kg) plus albendazole (20 mg/kg). * indicates significant (p < 0.05) difference. Two Native ewes were removed from the study on 4/5; 1 Suffolk ewe was removed from the study on 4/6, 1 died on 9/3, 1 died on 9/7. EPG=Eggs per gram of feces.
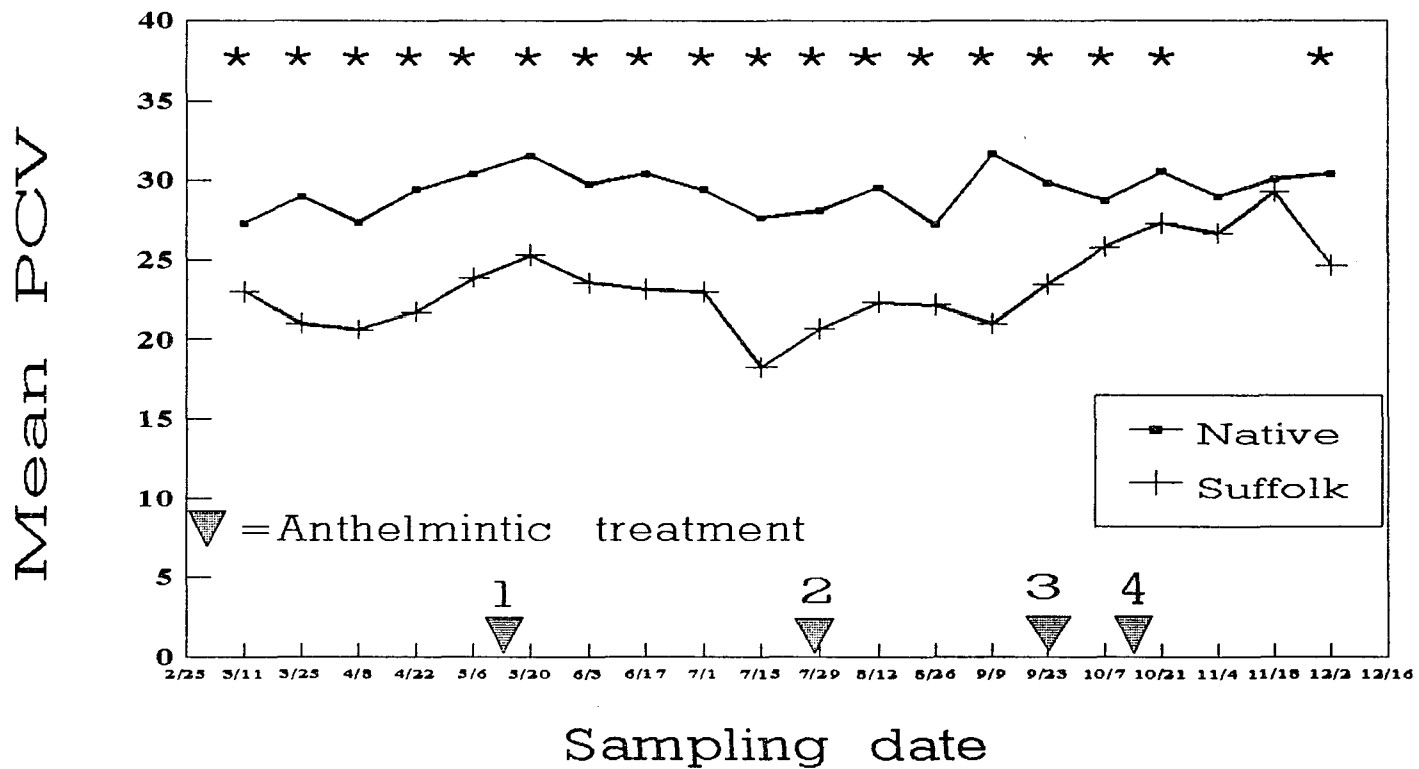
Figure 20. Mean packed cell volumes of naturally infected Native (n=9) and Suffolk (n=8) ewes in 1992. Treatments 1-4=ivermectin (200 µg/kg). * indicates significant (p < 0.05) difference. One Suffolk ewe died on 4/25, 1 died on 7/25. PCV=Packed cell volume.
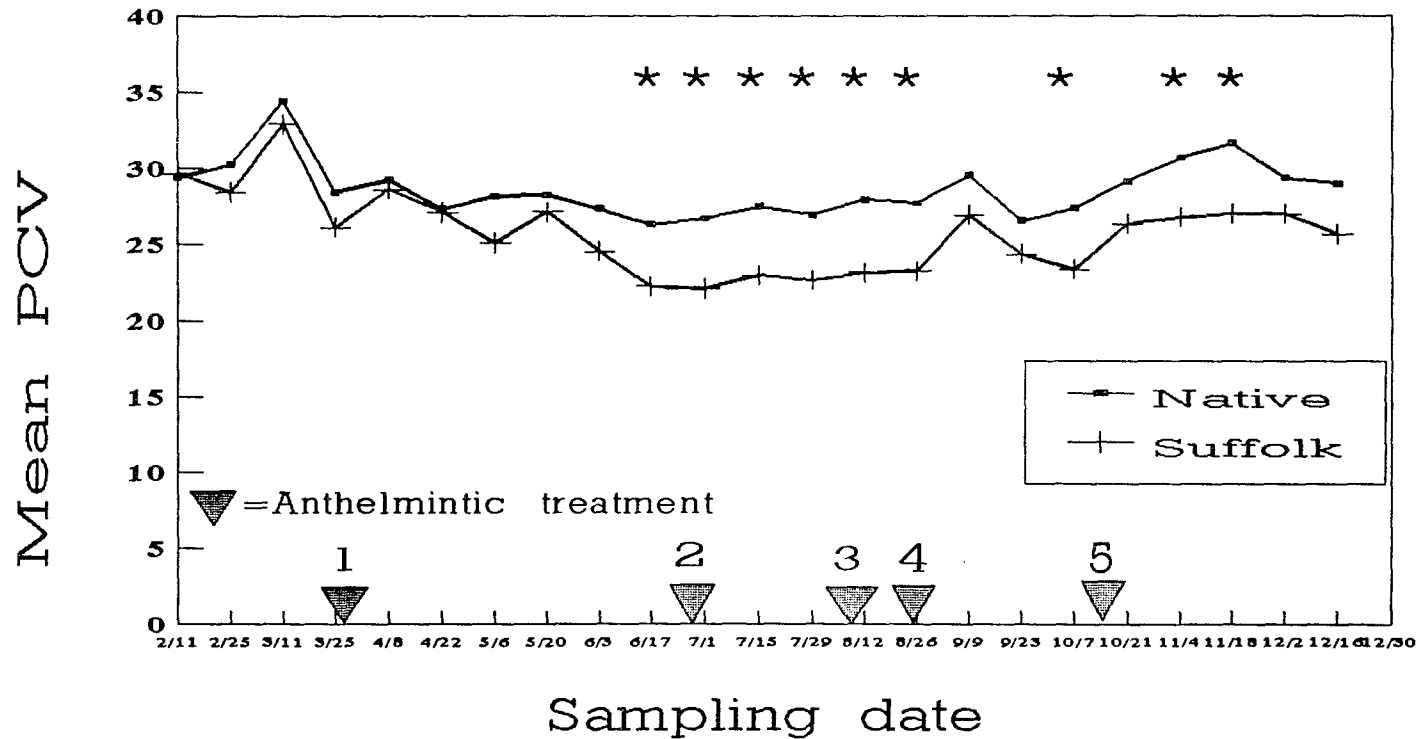
Figure 21. Mean packed cell volumes of naturally infected Native (n=15) and Suffolk (n=15) ewes in 1993. Treatments 1-2=ivermectin (200 µg/kg), treatment 3=ivermectin (400 µg/kg), treatments 4-5=ivermectin (400 µg/kg) plus albendazole (20 mg/kg). * indicates significant (p < 0.05) difference. Two Native ewes were removed from the study on 4/5; 1 Suffolk ewe was removed from the study on 4/6, 1 died on 9/3, 1 died on 9/7. PCV=Packed cell volume.

Thereafter, the mean packed cell volume of the Suffolk ewes declined and significant differences were continually observed from June 17 through August 26. In the fall, the mean packed cell volume of the Suffolk ewes remained low with significant differences being seen on October 7, November 4 and 18. In 1992 and 1993, difference between the overall means of the packed cell volumes of the two breeds was highly significant (p < 0.0001) and reflected the differences observed during the three seasons (Tables 14 and 15).

Table 12. Mean±s.d. fecal egg count (FEC) of treated Native and treated Suffolk ewes—1992

Season	FEC (Eggs Per Gram)		Level of Significance
	Native	Suffolk	
Spring	91±404 (n=63)	1,157±2,200 (n=52)	p < 0.0001
Summer	17±67 (n=63)	821±2,279 (n=45)	p < 0.0001
Fall	83±239 (n=63)	507±1,187 (n=42)	p < 0.01
Overall	64±274 (n=189)	852±1,986 (n=140)	p < 0.0001

Fecal egg counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.

Table 13. Mean±s.d. fecal egg counts (FEC) of treated Native and treated Suffolk ewes—1993

Season	FEC (Eggs Per Gram)		Level of Significance
	Native	Suffolk	
Spring	121±716 (n=91)	819±2,082 (n=96)	p < 0.0001
Summer	144±493 (n=91)	2,668±6,942 (n=95)	p < 0.0001
Fall	82±294 (n=103)	1,769±5,318 (n=71)	p < 0.0001
Overall	114±521 (n=285)	1,747±5,210 (n=262)	p < 0.0001

Fecal egg counts were $\log_{10}$ (n+1) transformed prior to statistical analysis.

Table 14. Mean±s.d. packed cell volumes (PCV) of treated Native and treated Suffolk ewes—1992

Season	PCV		Level of Significance
	Native	Suffolk	
Spring	29.3±3.7 (n=62)	22.6±5.3 (n=52)	p < 0.0001
Summer	29.2±3.7 (n=63)	21.5±4.5 (n=45)	p < 0.0001
Fall	29.8±3.6 (n=54)	26.2±4.5 (n=36)	p < 0.0001
Overall	29.4±3.7 (n=179)	23.2±5.2 (n=133)	p < 0.0001

Table 15. Mean±s.d. packed cell volumes (PCV) of treated Native and treated Suffolk ewes—1993

Season	PCV		Level of Significance
	Native	Suffolk	
Spring	27.9±3.0 (n=93)	25.8±5.9 (n=100)	p < 0.01
Summer	27.5±3.5 (n=91)	23.5±4.8 (n=93)	p < 0.0001
Fall	29.0±3.9 (n=90)	26.1±4.9 (n=60)	p < 0.0001
Overall	28.1±3.6 (n=274)	25.1±5.4 (n=253)	p < 0.0001

2. Total anti-*H. contortus* antibody levels—1992

Total serum anti-*H. contortus* antibody levels were determined in Native and Suffolk ewes at four time points (Figure 22). Native sera had consistently higher levels of antibodies than Suffolk sera. In both breeds, antibody levels increased from the initial low levels on April 8 to reach the maximum levels on June 19. In both breeds, antibody levels declined to lower levels on September 9 and increased again on December 3. There was no difference in antibody levels between breeds at any of the four test dates.

3. Peripheral blood eosinophil counts—1993

The mean eosinophil counts in both breeds were low in the summer and higher during the spring and the fall (Figure 23). There was no significant difference between the overall means of the
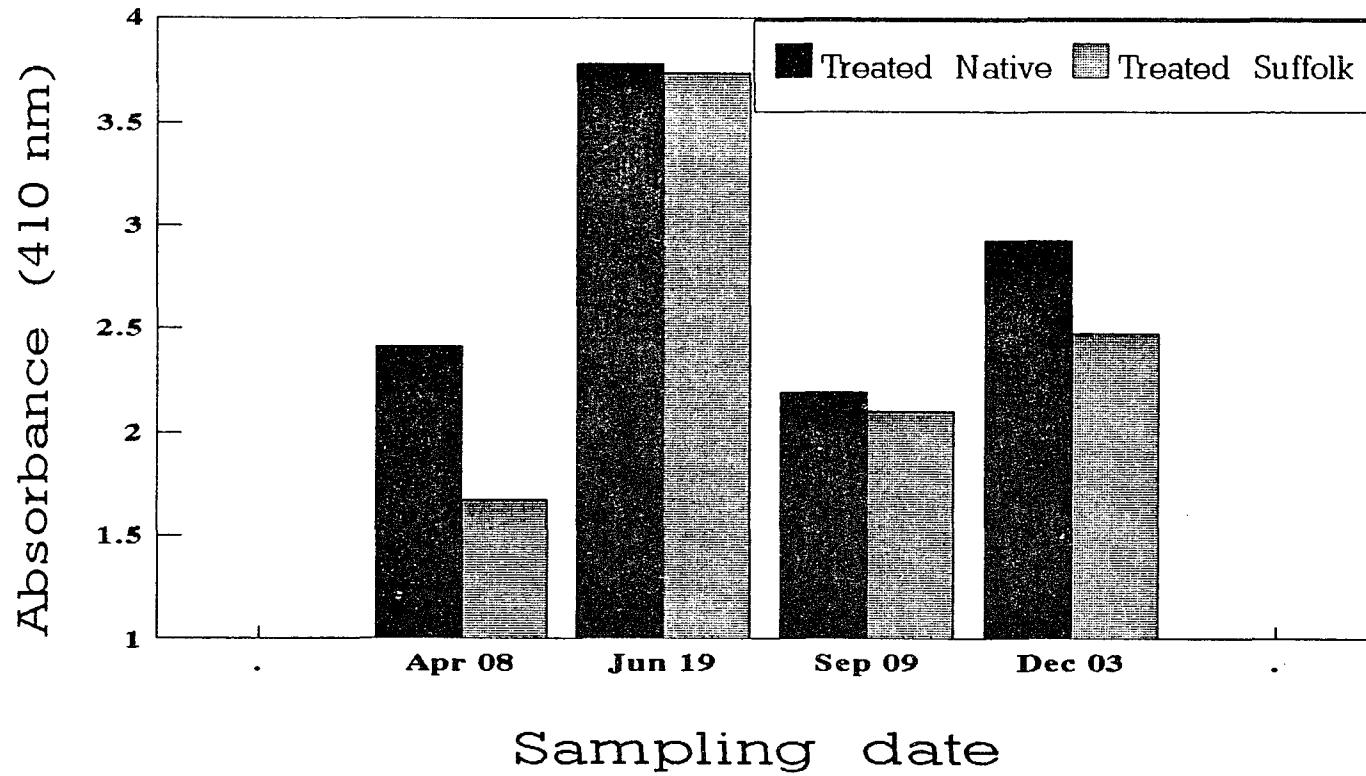
Figure 22. Mean total anti-*Haemonchus contortus* antibody levels in naturally infected, treated Native (n=9) and treated Suffolk (n=6) ewes in 1992.

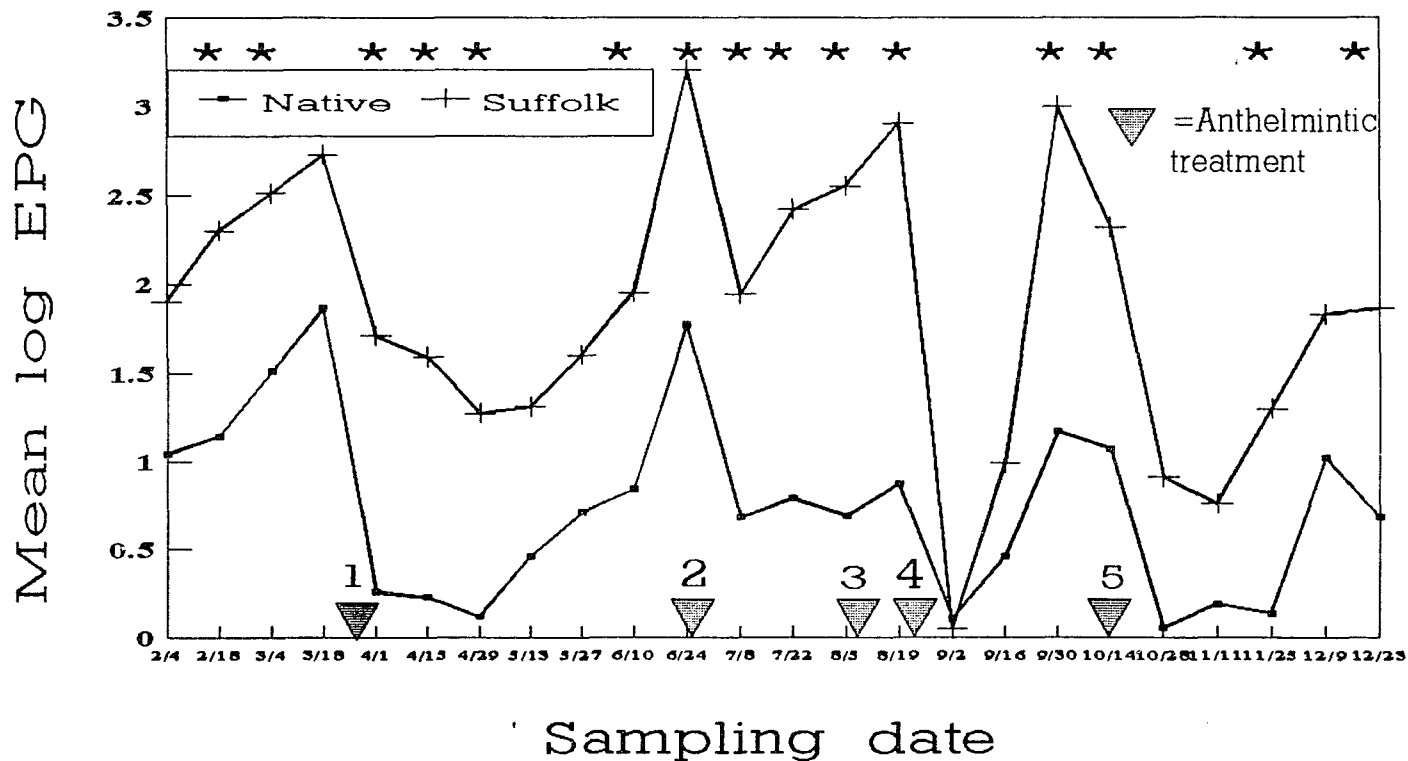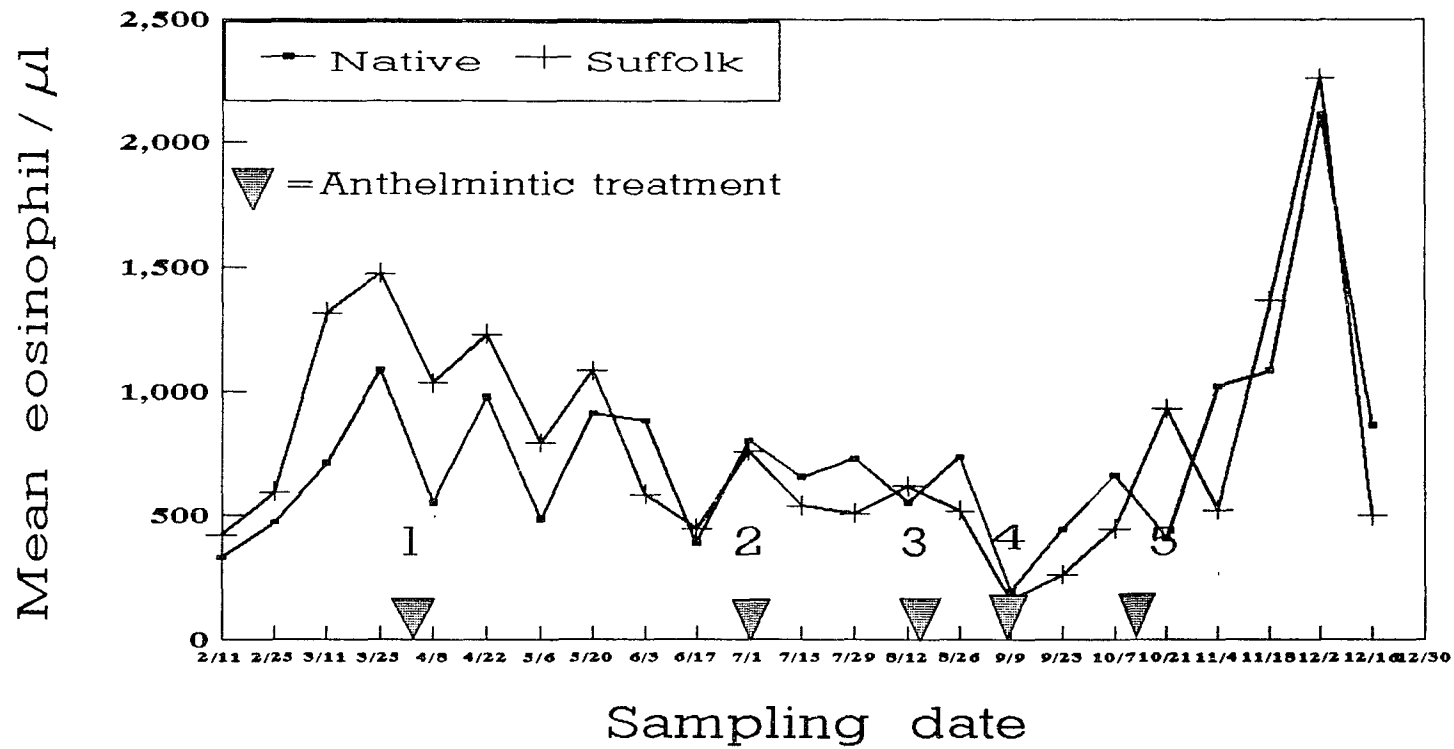Figure 23. Mean circulating eosinophil counts of naturally infected Native (n=15) and Suffolk (n=15) ewes in 1993. Treatments 1-2=ivermectin (200 μg/kg), treatment 3=ivermectin (400 μg/kg), treatments 4-5=ivermectin (400 μg/kg) plus albendazole (20 mg/kg). Two Native ewes were removed from the study on 4/5; 1 Suffolk ewe was removed from the study on 4/6, 1 died on 9/3, 1 died on 9/7.

eosinophil counts of the two breeds nor were differences detected when analyzed by

season (Table 16).

Table 16. Mean ±s.d. peripheral blood eosinophil counts of treated Native and treated Suffolk ewes—1993

Season	Native	Suffolk
Spring	763±593 (n=93)	952±801 (n=100)
Summer	585±457 (n=91)	492±492 (n=93)
Fall	920±900 (n=90)	1,086±1302 (n=59)
Overall	756±686 (n=274)	814±892 (n=252)

4. Coccidia (*Eimeria*) infections in lambs

1. Total fecal oocyst output

Oocysts were detected in the feces of lambs two and three weeks after birth in 1992 and

1993, respectively (Figures 24 and 25). In 1992, the mean total fecal oocyst counts for both breeds

increased steeply to reach a peak at six weeks of age. At this peak, the mean fecal oocyst counts for

Native and Suffolk lambs were 1.36×10^5 oocysts per gram of feces (range 6.5×10^2 to 1.11×10^6

oocysts per gram of feces) and 1.9×10^5 oocysts per gram of feces (range 1.45×10^3 to 9.85×10^5

oocysts per gram of feces), respectively. Thereafter, the mean total fecal oocyst counts for both

breeds declined to less than 10^3 oocysts per gram of feces at 24 weeks of age. The mean fecal oocyst

counts of both breeds then remained steady between 2×10^2 to 10^3 oocysts per gram of feces up to

the 44 weeks of age. Differences between the mean fecal oocyst counts between breeds were not

significant at any time between 2 to 44 weeks of age.

In 1993, the peak oocyst output was a little later than in 1992 and occurred at different

times for the two breeds. The Native lambs fecal oocyst count peak occurred at seven weeks of age

with a mean of 1.32×10^5 oocysts per gram of feces (range 2×10^2 to 3.95×10^5 oocysts per gram

of feces) and the Suffolk lambs peak occurred at nine weeks of age with a mean of 7.3×10^4 oocysts

per gram of feces (range 2.30×10^3 to 3.95×10^5 oocysts per gram of feces). Thereafter, Suffolk
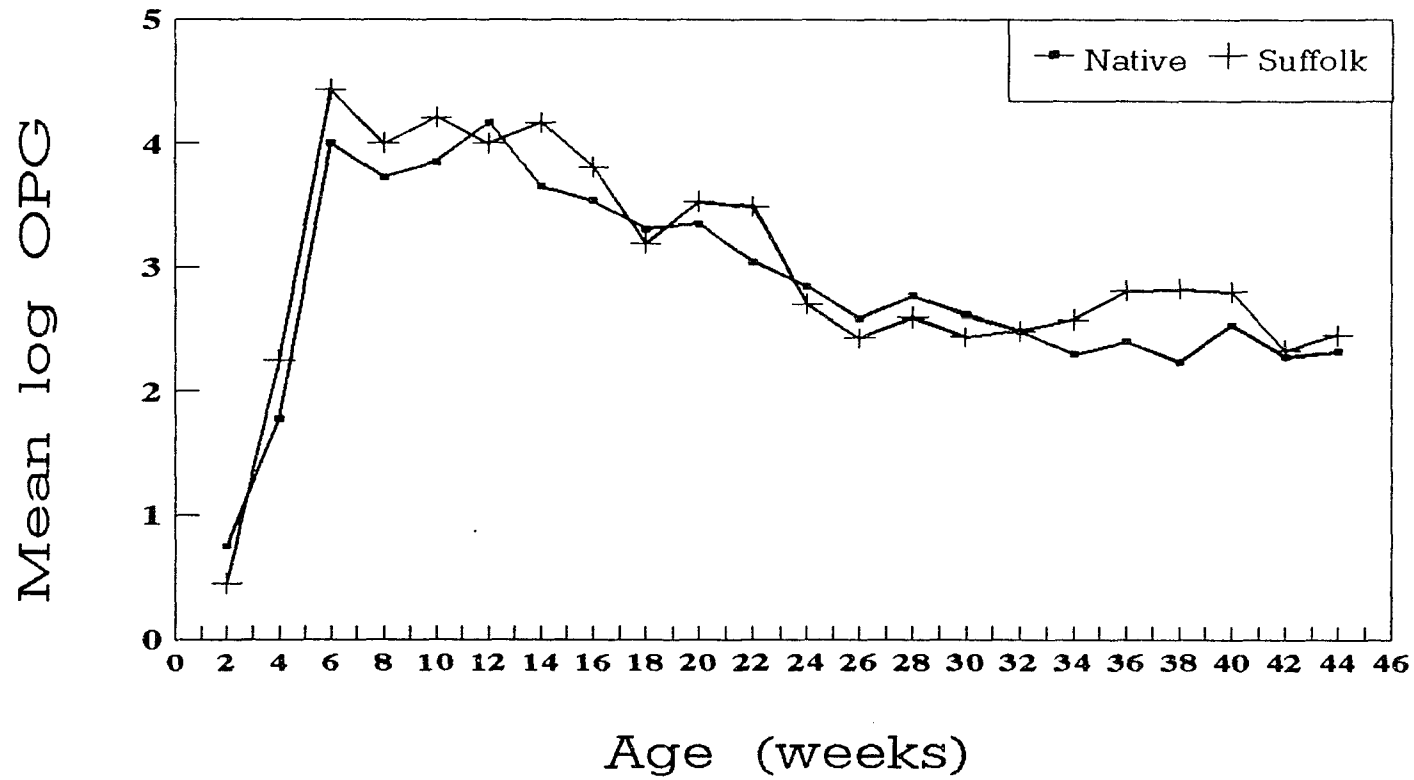
Figure 24. Mean total *Eimeria* oocyst counts of naturally infected Native (n=14) and Suffolk (n=14) lambs in 1992. Two Native lambs died at 26 weeks of age; 1, 2, 2, 1, 2, 1, 1, and 1 Suffolk lambs died at 11, 13, 14, 19, 25. 28, 32, and 40 weeks of age, respectively. OPG=Oocysts per gram of feces.
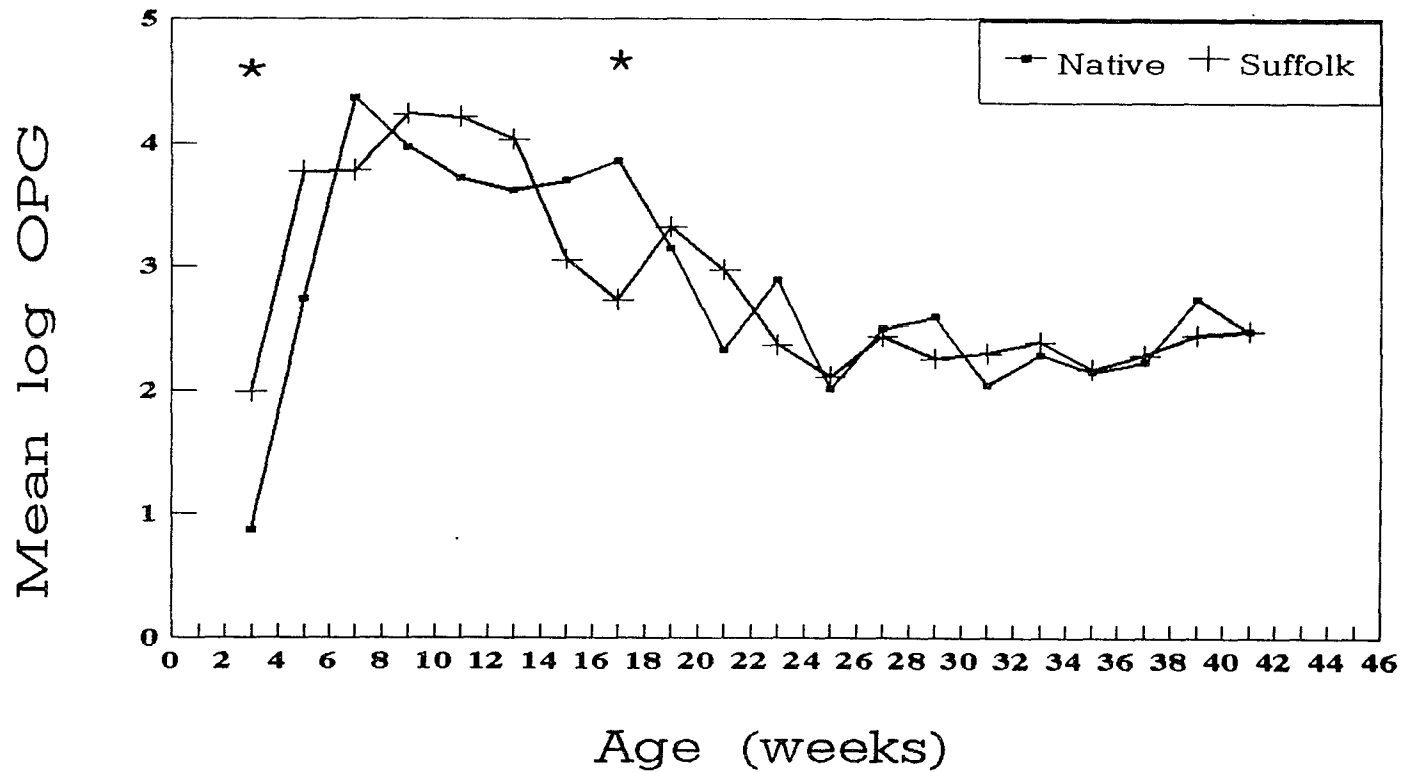
Figure 25. Mean total *Eimeria* oocyst counts of naturally infected Native (n=10) and Suffolk (n=13) lambs in 1993. * indicate significant (p < 0.05) difference. Two Native lambs were removed for necropsy at 22 weeks of age, 3 were removed for necropsy at 30 weeks of age; 4 Suffolk lambs died between 17-18 weeks of age, 2 were removed for necropsy at 22 weeks of age, 1 died on 29 weeks of age, 3 were removed for necropsy at 30 weeks of age. OPG=Oocysts per gram of feces.

lambs maintained a higher mean fecal oocyst count for a period of four weeks before dropping to

low levels at 15 weeks of age. Native lambs had a significantly higher mean fecal oocyst count than

Suffolk lambs at 3 and 17 weeks of age. From 19 to 33 weeks of age, both breeds had comparable

mean total fecal oocyst counts ranging from 10^2 to 10^4 oocysts per gram of feces. In both breeds,

mean total fecal oocyst counts then varied between 10^1 x 10^3 oocysts per gram of feces up to 41

weeks of age.

In both 1992 and 1993, differences between the overall means of the fecal oocyst counts of

Native and Suffolk lambs were not significant.

2. Species specific fecal oocyst output—1992

Nine species of *Eimeria* were identified from lambs. *Eimeria parva*, *E. crandallis*, *E.*

ovinoidalis, *E. faurei*, *E. ovina*, *E. granulosa* and *E. ashata* were detected first in lambs between two

to four weeks of age (Figure 26 and 27). The other two species *Eimeria pallida* and *E. intricata*

were detected first at six weeks of age.

Eimeria crandallis was the predominant species throughout the observation period. At the

peak of oocyst output, oocyst counts of *E. crandallis* ranged from 3.44 x 10^2 to 1.11 x 10^6 oocysts

per gram of feces with a mean of 1.61 x 10^5 oocysts per gram of feces which accounted for 98.8%

of the mean. None of the Native or Suffolk lambs manifested diarrhea or clinical signs suggestive of

coccidiosis during peak infection.

Eimeria ovina was the next predominant species. Output of *E. ovina* oocysts occurred in

moderate levels between 4 to 18 weeks of age. Output of *E. granulosa* oocysts showed an early peak

at eight weeks of age which declined by 16 weeks of age then recurred from 26 to 44 weeks of age.

Output of *E. faurei* and *E. ashata* oocysts were low to moderate and *E. ovinoidalis*, *E. pallida*, *E.*

parva and *E. intricata* were consistently very low throughout the entire observation period.
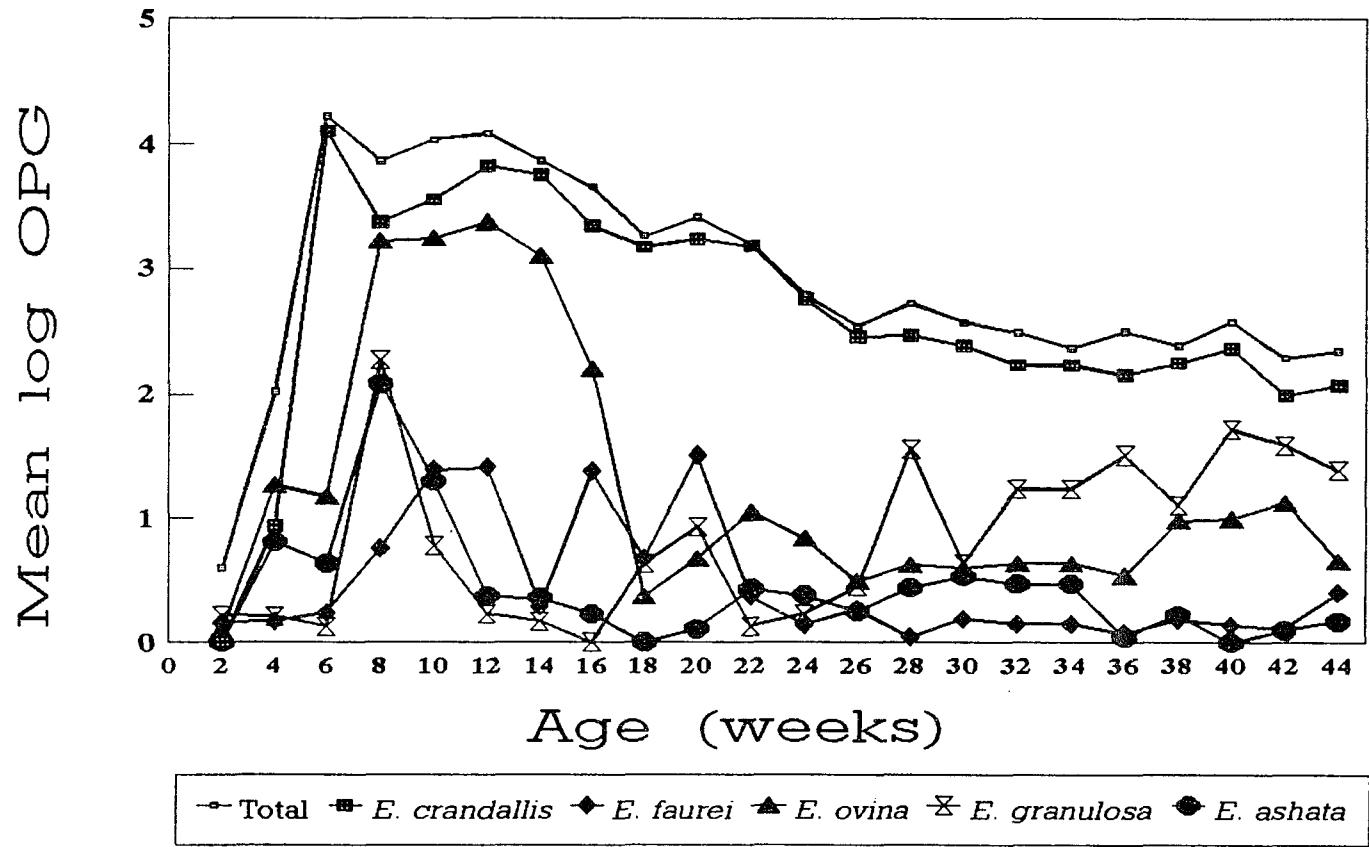
Figure 26. Age relationship of oocyst levels of different species of *Eimeria* in lambs (Native=14 and Suffolk=14) in 1992. Two Native lambs died at 26 weeks of age; 1, 2, 2, 1, 2, 1, 1, and 1 Suffolk lambs died at 11, 13, 14, 19, 25, 28, 32, and 40 weeks of age, respectively. OPG=Oocysts per gram of feces.
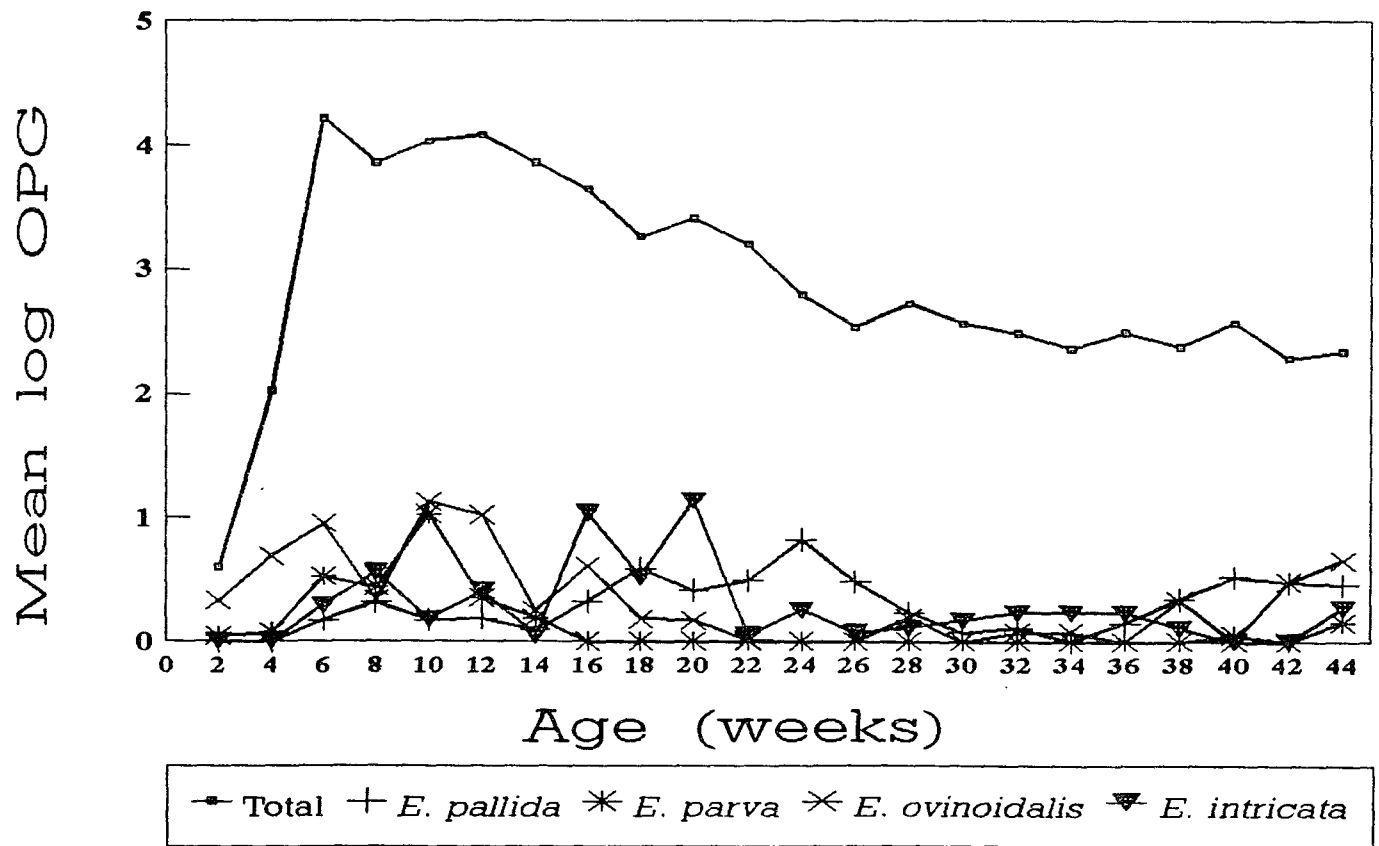
Figure 27. Age relationship of oocyst levels of different species of *Eimeria* in lambs (Native=14 and Suffolk=14) in 1992. Two Native lambs died at 26 weeks of age; 1, 2, 2, 1, 2, 1, 1, and 1 Suffolk lambs died at 11, 13, 14, 19, 25, 28, 32, and 40 weeks of age, respectively. OPG=Oocysts per gram of feces.

5. Coccidia (*Eimeria*) infections in ewes

<u>1. Total fecal oocyst output</u>

In both 1992 and 1993, mean fecal oocyst counts were high during the fall, low during the spring and the lowest during the summer in both Native and Suffolk ewes (Figures 28 and 29). In 1992, the mean fecal oocyst counts in Native and Suffolk ewes varied between 1 to 54 oocysts per gram of feces and 1 to 107 oocysts per gram of feces, respectively. In 1993, the mean fecal oocyst counts in Native and Suffolk ewes varied between 5 to 89 oocysts per gram of feces and 6 to 293 oocysts per gram of feces, respectively. Year to year variability in the magnitude of the mean fecal oocyst counts in the two breeds of ewes was not remarkable. In both years, from the beginning to the end of observations at any of the sampling date the mean fecal oocyst counts did not differ significantly nor the difference between the overall means of the fecal oocyst counts of the Native and Suffolk ewes was significant.

<u>2. Species specific fecal oocyst output—1992</u>

All nine *Eimeria* species found in lambs were also present in ewes. *Eimeria crandallis* was the predominant species during the three seasons and was the most common at all sampling dates except on May 13 and September 16 (Figures 30 and 31). There was a noticeable increase in oocyst output in *E. granulosa* in early fall with a peak on September 16. Increased *Eimeria ovina* oocyst output was evident during the spring and fall. Other species (*E. pallida, E. parva, E. ovinoidalis, E. faurei, E. ashata* and *E. intricata*) were recovered sporadically.
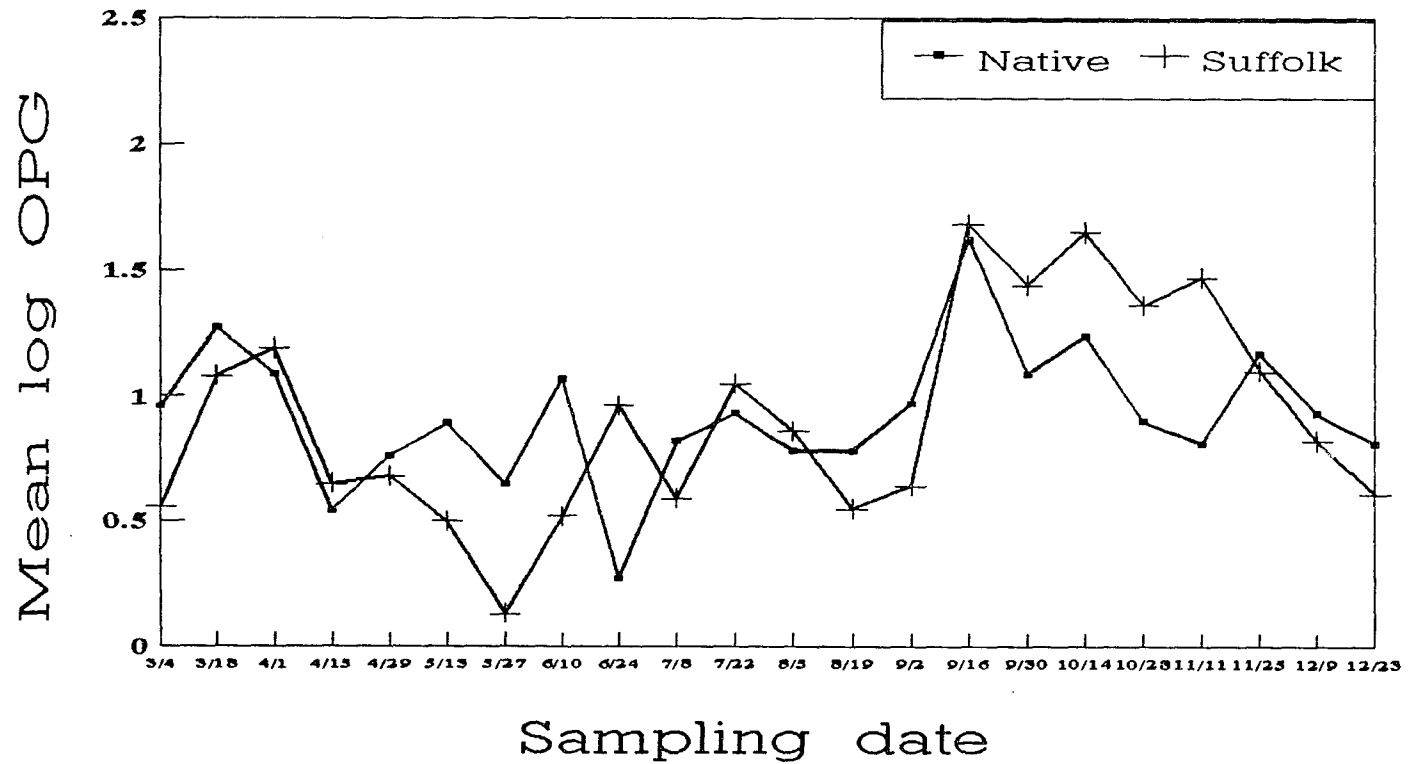
Figure 28. Mean total *Eimeria* oocyst counts of naturally infected Native (n=9) and Suffolk (n=8) ewes in 1992. One suffolk ewe died on 4/25, 1 died on 7/25. OPG=Oocysts per gram of feces.
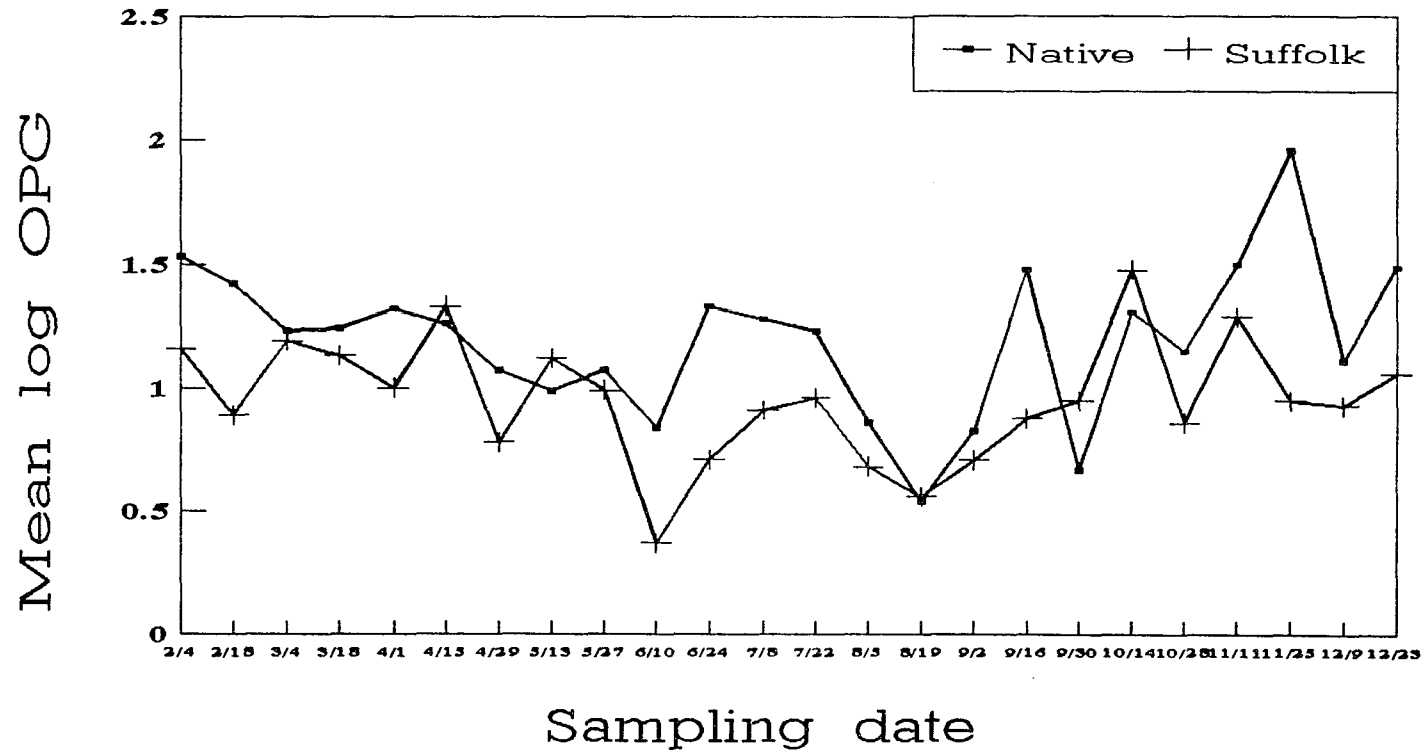
Figure 29. Mean total *Eimeria* oocyst counts of naturally infected Native (n=15) and Suffolk (n=15) ewes in 1993. Two Native ewes were removed from the study on 4/5; 1 Suffolk ewe was removed from the study on 4/6, 1 died on 9/3, 1 died on 9/7. OPG=Oocysts per gram of feces.
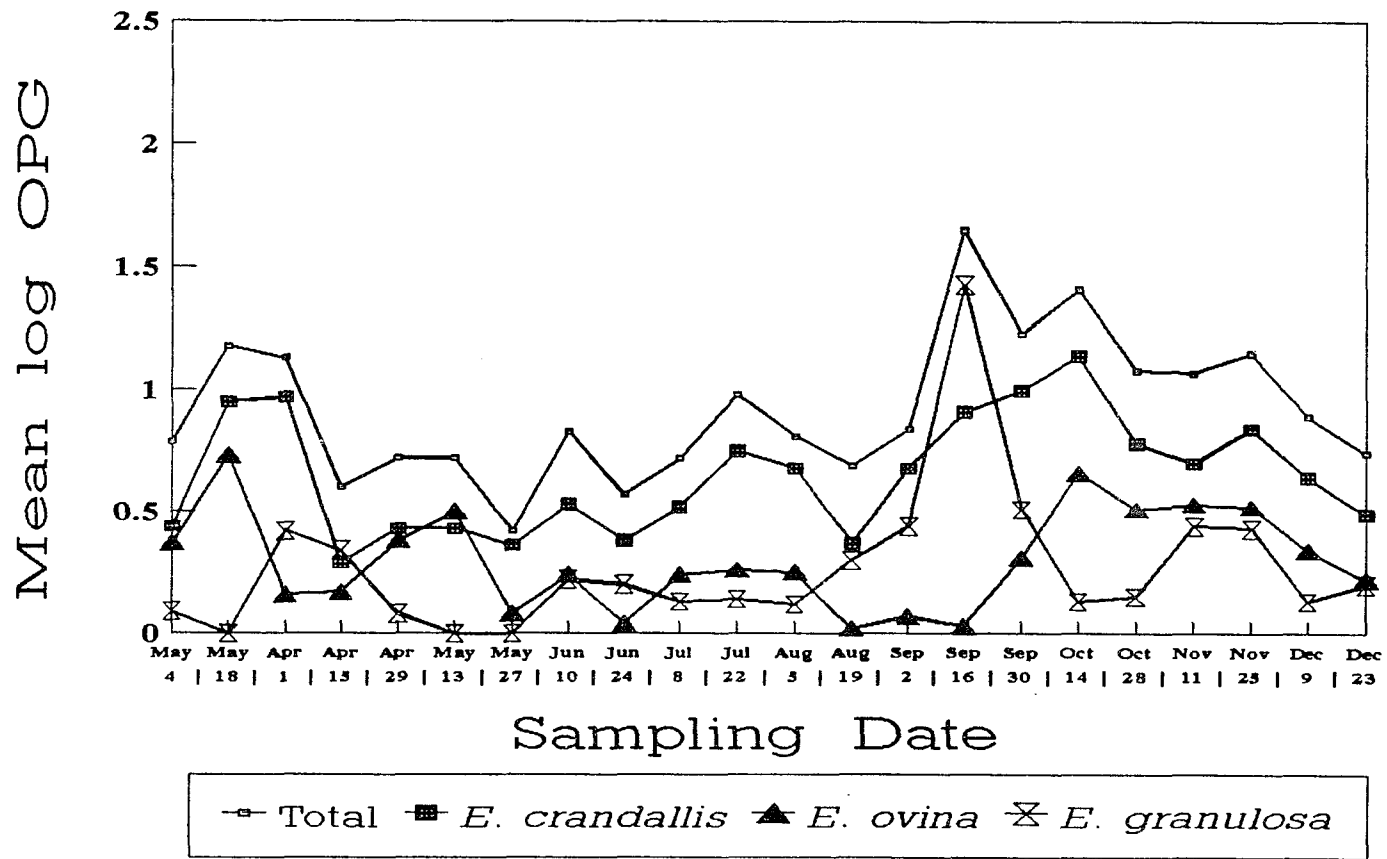
Figure 30. Relationship of oocyst levels of different species of *Eimeria* with sampling date in ewes (Native=9 and Suffolk=8) in 1992. One Suffolk ewe died on 4/25, 1 died on 7/25. OPG=Oocysts per gram of feces.
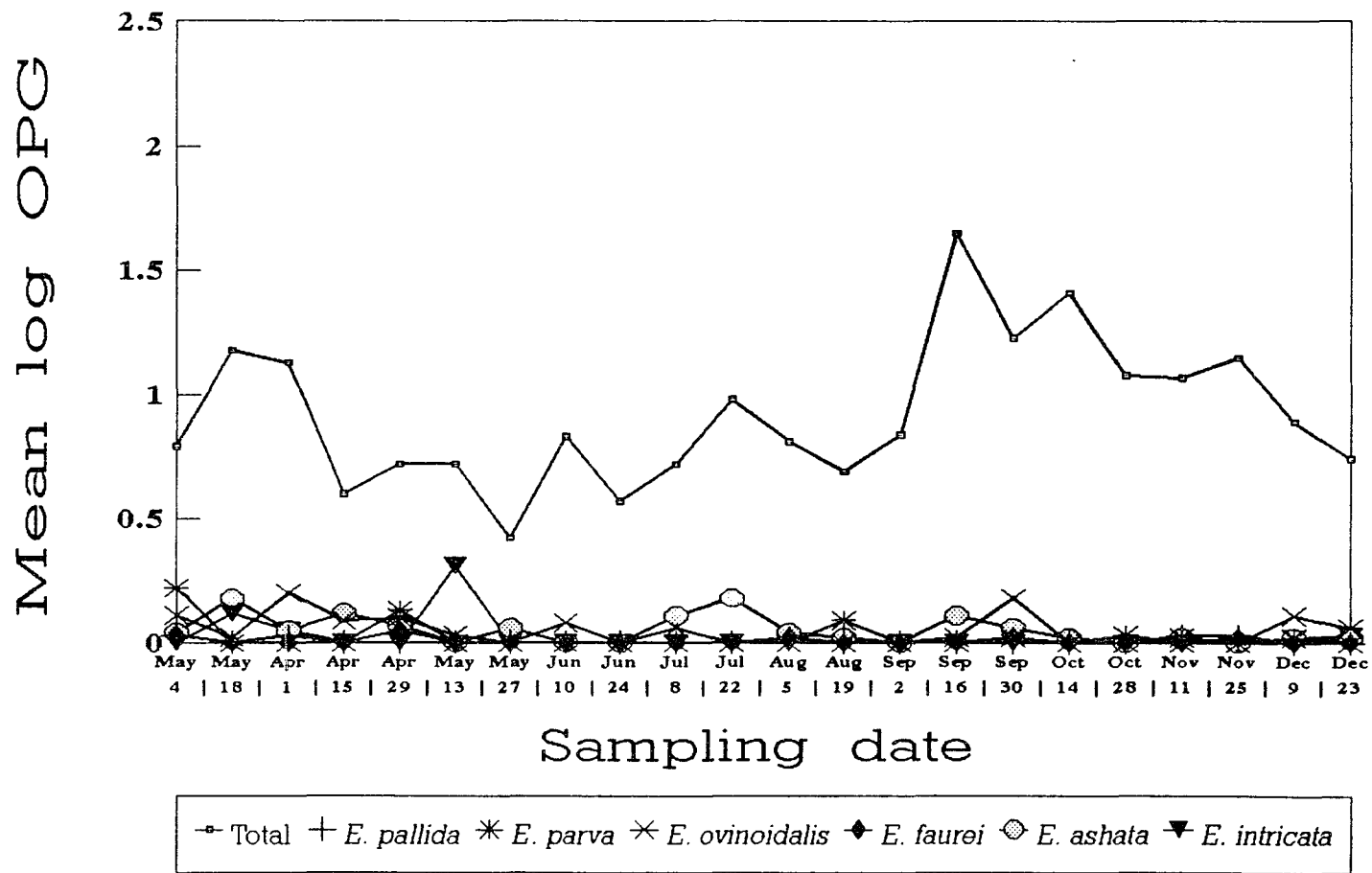
Figure 31. Relationship of oocyst levels of different species of *Eimeria* with sampling date in ewes (Native=9 and Suffolk=8) in 1992. One Suffolk ewe died on 4/25, 1 died on 7/25. OPG=Oocysts per gram of feces

DISCUSSION

1. Influence of breed on the susceptibility to strongylate nematode infection and efficacy of anthelmintics in suckling lambs

The dramatic and significant differences observed in the magnitude of fecal egg counts and packed cell volumes in the two consecutive years (1992 and 1993) indicated that suckling Native lambs are more resistant to strongylate nematode infection than Suffolk lambs. Increasing fecal egg counts with concomitant reductions in packed cell volumes, heavy nematode burdens, and high mortality indicated a state of unresponsiveness in Suffolk lambs. The recovery of fewer *H. contortus* from Native lambs in two years (1993 and 1994) indicated that resistance was directed specifically against *H. contortus* rather than *T. colubriformis*.

The recovery of fewer numbers of *T. colubriformis* in suckling Native lambs in 1994 compared to 1993 might be attributed to year-to-year variation in the availability of L3 on pasture. Prevalence of dry conditions in the summer months and December in 1993 may have adversely influenced the pasture availability of *T. colubriformis* L3 to suckling lambs in the spring of 1994. Likewise annual variation in the initial number of overwintered larvae on pasture and the degree of the peri-parturient rise (Figures 17 and 18) may have accounted for the differences observed in the fecal egg counts of Native lambs in 1992 and 1993. Nematode counts also confirmed that the differences in fecal egg counts between breeds was a true reflection of nematode burdens and not due to changes in nematode fecundity or an alteration of sex ratio which might also account for differences in fecal egg counts.

According to this study, naturally infected Native lambs acquired resistance to *H. contortus* by 7-10 weeks of age. Gamble and Zajac (1992) reported a similar 7-10 week post-exposure pattern of resistance in St. Croix lambs, compared to Dorset lambs, with natural *H. contortus* infection. However, in their grazing trial, the lambs were eight weeks old at first exposure and the resistance observed 7-10 weeks later coincided with what has been reported to be the earliest (about four months of age) that immune competency to *H. contortus* develops in pastured Merino lambs (Barger,

83

1988). Whether resistance in St. Croix or other resistant breeds of sheep can occur earlier than four months, as observed in the present study, has not been reported.

In the present study, lambs were released to pasture at one week of age and patent infections established by four to five weeks of age (Figures 2 and 4). Since the pre-patent periods of *H. contortus* and *T. colubriformis* infections are approximately three to four weeks (Soulsby, 1982), it appears that lambs started acquiring infection as soon as they were released to pasture. In this study, acquired resistance to *H. contortus* appeared to have developed over a six week period of grazing infective pasture. Thus, grazing for a minimum of six weeks appears necessary for the expression of resistance to *H. contortus* in Native sheep and perhaps other resistant breeds. The time period required for the manifestation of resistance can be influenced by time of lambing since lambs born in spring or summer may be subjected to heavy *H. contortus* infection in a shorter period than lambs born in late fall or winter. Other factors such as the degree of the peri-parturient rise, residual larval infection on pasture and severity of the preceding winter conditions can also affect the time required for the manifestation of resistance because they directly or indirectly influence the levels of pasture infectivity.

In experiments with naturally infective pasture, factors such as the distribution of L3 on the pasture and grazing behavior can influence the levels of nematode burdens in lambs. Consistent differences in the fecal egg counts, packed cell volumes and the nematode burdens between the two breeds of lambs for two consecutive years indicated that the first factor did not play a major role. No attempt was made to determine the grazing behavior of Native and Suffolk lambs in the present study.

In 1993, at 10 weeks of age, the total nematode burden in Native lambs compared to Suffolk was not only smaller but there was also a difference in species composition. While *H. contortus* constituted 91.2% of the total nematode burden in Suffolk lambs, *T. colubriformis* constituted 64.6% of the burden in Native lambs. Despite the fact that total nematode counts increased slightly from 7 to 10 weeks of age in Native lambs the mean fecal egg count declined

from 9 to 11 weeks of age as also observed in 1992. The relationship between fecal egg count and total nematode burden at 10 weeks of age in Native lambs can be explained by examining the composition of the nematode population which was largely *T. colubriformis*, a species not as prolific as *H. contortus* (Coyne et al., 1991).

The increased efficacy of ivermectin and albendazole in Native lambs as determined by the fecal egg count reduction test appeared to be due to more *T. colubriformis* and fewer *H. contortus*. A higher degree of susceptibility of *T. colubriformis* to anthelmintics possibly contributed to the enhanced efficacy of anthelmintics in Native lambs. By contrast, reduced efficacy of anthelmintics in Suffolk lambs could be due to the heavy nematode burdens composed primarily of resistant populations of *H. contortus*.

Both breeds of lambs had similar maternal antibody levels at seven weeks of age at which time they also had comparable mean packed cell volumes (Figures 3 and 6). From 9 to 13 weeks of age, antibody levels in Suffolk lambs declined at a faster rate than in Native lambs. This faster rate of decline coincided with depletion of packed cell volumes. In Native lambs, while antibody levels declined packed cell volumes remained stable.

Therefore, it appears that colostrally transferred antibodies may be protective in Native lambs acting to eliminate *H. contortus*. The role of anti-*H. contortus* antibodies passed in the milk of the Native ewe needs investigation. Endogenously synthesized anti-parasitic antibodies were detected in small quantities in the serum of five to six weeks old colostrum deprived Native lambs artificially inoculated with *H. contortus* (Bahirathan and Miller, unpublished observations). Thus, the possibility that immune mediated events leading to the synthesis of anti-*H. contortus* antibodies may have also contributed to the slower rate of decline of antibody levels in Native lambs cannot be discounted. In Suffolk lambs, colostrally transferred antibodies may not afford protection against *H. contortus* and it is possible that the faster decline in maternal antibodies was the result of the loss of antibodies during blood feeding activities induced by L4s and adults. The role of maternal antibodies in

conferring protection against *H. contortus* can be verified by experimentally infecting colostrum deprived and colostrum (Native colostrum) fed Native and Suffolk lambs.

Timing of anthelmintic treatment was the major determinant of mortality in Suffolk lambs. In 1992, when treatment with ivermectin was delayed until 13 weeks of age, fatal infections had been established in that five lambs died before treatment. In 1993, timely intervention with albendazole prevented morbidity and mortality.

2. Epidemiology, breed resistance and immunity to strongylate nematode infection in weaned lambs

Compared to treated Suffolk lambs, treated Native lamb mean fecal egg count and mean packed cell volume remained constantly lower and higher, respectively. In addition, as evidenced from larval culture the low mean fecal egg counts in treated Native lambs during haemonchosis seasons were comprised of *H. contortus* (overall mean of 62.3% and 68.4% in 1992 and 1993, respectively; Figures 11 and 12) and *T. colubriformis* (overall mean of 37.3% and 31.6% in 1992 and 1993, respectively). The high mean fecal egg counts in treated Suffolk lambs were comprised of *H. contortus* (overall mean 79.6% and 86% in 1992 and 1993, respectively) and *T. colubriformis* (overall mean 20% and 14% in 1992 and 1993, respectively). At the end of haemonchosis season, sacrificed treated Native lambs compared to treated Suffolk lambs had 57.2% fewer *H. contortus*. These findings indicate that repeated anthelmintic treatments were not detrimental to the protective responses developed by Native lambs to *H. contortus* in early neonatal life. They are contradictory to what has been reported in weaned Merino lambs by Barger et al (1988). In their study, every month during the haemonchosis season a group of Merino lambs grazing naturally infective pasture were randomly selected from the flock and given anthelmintic treatment. After three weeks of grazing, these lambs together with another group of randomly selected lambs were isolated and held in a pen for one week and necropsied to determine nematode burdens. The treated lambs had greater *H. contortus* burdens than untreated lambs. Thus, one anthelmintic treatment abrogated protective responses to *H. contortus* in weaned Merino lambs.

In contrast, weaned treated Suffolk lambs continued to remain susceptible to nematode infection and susceptibility was complicated by anthelmintic resistance which precipitated mortality. Recovery of greater numbers of *H. contortus* and *T. colubriformis* from sacrificed treated Suffolk lambs compared to treated Native lambs (October 1993) indicated that Suffolk lambs may be more susceptible to multiple species of strongylate nematodes.

In another study, to confirm the findings of natural infection in this study, six month old Native and Suffolk lambs exposed to natural infection from birth had their nematode burdens eliminated by anthelmintic treatment (Miller and Bahirathan, unpublished observations). Subsequently, they were inoculated with 20,000 *H. contortus* L3. Reduced fecal egg counts and stable packed cell volumes in Native lambs compared to higher fecal egg counts and reduced packed cell volumes in Suffolk lambs were consistent with the pattern of resistance observed in this part of the present study.

Though the mean *H. contortus* burden in sacrificed untreated Native lambs in October, 1993 was nearly equal to that in the treated Suffolk lambs, it should be noted that the mean *H. contortus* burden in the later was acquired within a month after anthelmintic removal of nematodes whereas that in the former was acquired during a period of eight months of continuous grazing. Similarly, when naturally infected 4.5 to 5.5 month old St. Croix x Dorset lambs were subjected to different anthelmintic treatment regimes and necropsied, it was found that the mean adult *H. contortus* burden of untreated control lambs was not greater than the lambs receiving treatment every three weeks (Courtney et al., 1983). Thus, it appears that in naturally infected weaned Native lambs, the recruitment and the loss of *H. contortus* are in a state of dynamic equilibrium that delineates the state of resistance.

The recovery of *T. colubriformis* in large numbers from sacrificed untreated Native lambs in October, 1993 indicated that infections with this species in Native lambs were cumulative. Under conditions of low larval challenge, Merino lambs exposed to natural infections continued to accumulate *T. colubriformis* for a period of seven months without evidence of immunity (Waller and

Thomas, 1981). Significant reductions in *T. colubriformis* burdens in treated Native lambs compared to untreated Native lambs indicated that repeated anthelmintic treatments eliminated *T. colubriformis* in Native lambs.

In July 1993, one of the two treated Suffolk lambs had only 200 *H. contortus* and no *T. colubriformis* which accounted for the lowered mean total nematode burden in treated Suffolk lambs (Table 9). One Native lamb had very high numbers of both, *T. colubriformis* and *H. contortus*. Therefore, the mean number of *T. colubriformis* and *H. contortus* in treated Native lambs were higher than in treated Suffolk lambs. The mean total nematode burden of treated Native lambs was unusually high and did not support the fecal egg count and packed cell volume data obtained from the rest of the treated Native lambs that manifested resistance to strongylate nematode infection under the same management conditions. Four factors alone or in combination could have contributed to this unusually high mean *H. contortus* and *T. colubriformis* burdens in treated Native lambs four weeks after weaning. They include chance of selecting susceptible individuals, heavier than normal exposure to strongylate nematodes on the weaning pasture, weaning stress, and/or anthelmintic abbreviation of challenge infections needed for priming the immune system. Within breed variation in susceptibility to *H. contortus* and *T. colubriformis* is an established phenomena (Woolaston, 1992; Dinnen et al., 1978). Because a few Natives could be relatively susceptible, the chance of selecting such a lamb was possible. Therefore, with the small number of treated Native lambs the chances of selecting one of those lambs is increased. Lambs can acquire heavy infection when they are confined in a small holding area which contains patches of pasture such as that used to wean lambs in the present study. Weaning stress has been found to delay the development of antibody responses to *H. contortus* in Merino lambs (Watson, 1991). Weaned lambs subjected to anthelmintic treatments may lose resistance to *H. contortus* and *T. colubriformis* and become reinfected when left on infective pasture (Barger, 1988; Gray et al 1992).

In 1992, there was no difference in the packed cell volumes of the treated Native and untreated Native lambs until late summer (August 26). Thus, Native lambs have the capability to

maintain a steady state of resistance to *H. contortus* during spring and summer months. Significant

reductions in the mean packed cell volumes of untreated Native lambs during fall were not

associated with an increase in mean fecal egg count. This suggests that chronic *T. colubriformis*

infection rather than newly acquired *H. contortus* infection may precipitate anemia in untreated

Native lambs during fall.

In 1993, fecal egg counts of untreated Native lambs gradually increased during summer

months indicating the establishment of *H. contortus* infections. Sharing the same pasture with

Suffolk lambs, which heavily contaminate pasture, successively for a second year may be a

contributory factor in the acquisition of such infections. In the fall, further increases in mean fecal

egg counts and substantial reductions in mean packed cell volumes indicated continuous

establishment of *H. contortus* infections in late summer through fall. This was verified by the shift in

larval population distribution from *T. colubriformis* to *H. contortus* at the end of summer (Figure

12). A dry spell in September followed by rainfall in October may also have contributed to the

increase in *H. contortus* burdens. Further, dry conditions may have forced the lambs to graze closer

to the ground and acquire heavy infection. Thus, resistance may indeed be relative and even resistant

breeds can become heavily infected when exposed to overwhelming *H. contortus* infection. Soluble

antigens derived from adult nematodes can abolish homologous immunity resulting in increased

fecundity, nematode size and survival time (Pritchard and Behnke, 1985). Such parasite derived

factors may have induced a similar phenomenon in untreated Native lambs. Though they became

heavily infected, none of the untreated Native lambs died during the summer and fall observation

period. The precipitous drop of mean fecal egg count in untreated Native lambs close to the end of

grazing season reflected a vigorous "selfcure" response which was followed by an increase in mean

packed cell volume. The capacity to hold packed cell volume during constant *H. contortus*

reinfections in Native lambs could only be possible by maintaining effective hemopoiesis. This

implies that Native lambs can not only respond immunologically but may also respond

physiologically to combat chronic strongylate nematode infections.

The development of *H. contortus* L3 can be adversely affected by increased abomasal pH (Blanchard and Westcott, 1994). The possibility that Native lambs may have a relatively higher abomasal pH than Suffolk lambs which could result in reduced establishment of *H. contortus* L3 was investigated following artificial infection of Native and Suffolk lambs with 20, 000 *H. contortus* L3 (Bahirathan and Miller, 1994 unpublished observations). There was no difference in abomasal pH between breeds.

Considering the fact that Native lambs were exposed to the massive pasture infectivity originating from the Suffolk lambs, it should be noted that the dynamics of the strongylate nematode population in Native lambs would have been different had the two breeds been maintained on separate pastures. In a previous study on the same research site, when Native and Suffolk ewes were maintained on separate pastures and pasture levels of L3 were estimated, the patterns of recovery of L3 were similar in both breeds but there were remarkable differences in the number (Lemarie, 1985). Compared to Suffolk pasture, Native pasture yielded very low and at times negligible numbers of *H. contortus* L3. Strikingly, the summer peak of activity of *H. contortus* L3 seen in the Suffolk pasture was virtually absent in the Native pasture. Therefore, it appears that Native lambs would not be exposed to heavy nematode infections unless they are co-grazed with susceptible lambs such as Suffolk.

Serial fecal egg counts of untreated Native lambs for two years suggested that weaned lambs carried maximum nematode burdens during the late summer-early fall periods of the year in Louisiana. Previous studies with naturally infected Native and Suffolk ewes also indicated a similar time period for the acquisition of maximum nematode burdens (Lemarie, 1985). The practical implications of these findings are that within-breed variation in nematode burdens will be greater during the late summer-early fall period and, therefore, any selection for resistance experiments utilizing natural strongylate nematode infections should be conducted during this period. The results of the present study confirmed that under Louisiana conditions pasture-raised Native lambs would remain clinically normal until late summer. This implies that with some exceptions, Native lambs

can be raised on pasture without anthelmintic treatment until this time. However, the effect of subclinical strongylate nematode parasitism on weight gain of lambs has to be considered when making treatment decisions.

Continuous and active presence of L3 on pasture is confirmed by the observation of increases in mean fecal egg counts in Suffolk lambs after each anthelmintic treatment (Figures 7 and 9). When environmental conditions are not limiting, the rate of development to L3 may surpass the death rate. Therefore, the completion of the life cycle of *H. contortus* is preserved, resulting in the development of multiple generations of nematodes (Uriaorte and Valderrabana, 1989). In the present study, at the research site, although maximum daily temperatures in summer reached as high as 32-35°C, adequate rainfall (at or above 100 mm/month, Figure 1) ensured soil moisture which could maintain pasture availability of L3. Therefore, year-round conditions were favorable for development and survival of L3 on pasture.

Experimentally induced infections in Merino lambs of different age groups demonstrated that initiation of antibody responses to *H. contortus* antigens is determined by the age of the lamb (Watson and Gill, 1991). In Native and Suffolk lambs, immune responses are clearly evident from increasing total anti-*H. contortus* antibody levels from about three months of age. The persistence of resistance to *H. contortus* in Native lambs was accompanied by increased levels of anti-*H. contortus* antibodies. However, similar increases in anti-*H. contortus* antibody levels were also observed in Suffolk lambs. Therefore, in contrast to the fecal egg count and packed cell volume, significant differences in anti-*H. contortus* antibody levels were not seen between breeds.

The results obtained from the current study are similar to observations of Gamble and Zajac (1992) on anti-*H. contortus* antibody responses in the serum of weaned St. Croix and Dorset lambs. In their study, resistance that developed in two month-old parasite-free St. Croix lambs following the exposure to natural *H. contortus* infections was not associated with sustained differences in post-exposure antibody levels between breeds. These observations together imply that although susceptible breeds of lambs can eventually become immune competent by repeated infections, anti-parasitic

antibodies may not necessarily be protective. Blood loss resulting from the parasite's feeding habits (causing hemorrhage) appears to be the cause of sub-normal levels of endogenous anti-*H. contortus* antibodies in weaned Suffolk lambs as observed with maternal antibodies in suckling Suffolk lambs.

The observation of significantly higher total immunoglobulin concentrations in treated Native lambs compared to treated Suffolk lambs during haemonchosis provide additional evidence that loss of immunoglobulins during blood loss could be the cause for reduced levels of anti-*Haemonchus* antibodies in Suffolk lambs. Reductions in total immunoglobulin levels have also been noted in Scottish Blackface and Finn Dorset sheep four to six weeks after artificial *H. contortus* infection (Abbott et al, 1985).

Stankilwicz et al (1994) studying the effect of fenbendazole on peripheral blood lymphocytes and human red blood cells and ovalbumin induced antibody responses found reduced lymphocyte blastogenesis and antibody responses in treated lambs compared to control lambs. In our study, since Native and Suffolk lambs were subjected to the same anthelmintic treatments, the consistently low levels of antibodies in Suffolk lambs can not be explained by the possible inhibitory effects of anthelmintics on the immune system.

In the present study, since the assay for IgG1 was conducted with a higher secondary antibody dilution, IgG1 appears to be the predominant anti-*H. contortus* antibody produced in weaned lambs of the two breeds and the levels of IgG1 can not be directly compared with the levels of any one of the other three antibody isotypes. Periodic measurements of class-specific antibodies showed clear-cut patterns for different antibodies. Initial IgM responses seem to be followed by increases in IgG1 and IgG2 antibodies in both breeds. The association of increased levels of IgG1 and IgG2 antibodies with reduced fecal egg counts in treated Native lambs compared to treated Suffolk lambs suggests that these two antibodies may be protective in the Native. The slightest decline in the levels of IgA antibodies seen in lambs during the period of peak transmission may have been due to a greater depletion of gastric IgA which maintains the serum levels (Gill et al., 1992).

Anti-*H contortus* (anti-L3) IgM and IgG2 responses, which developed in response to artificial challenge infections, in the sera of resistant Merino lambs were low and were not associated with protection (Gill et al., 1993b). In contrast, IgA and IgG1 antibodies were seen in significantly larger amounts in resistant lambs than random-bred lambs. IgG1 and IgA exert anti-parasitic activities either by acting directly upon parasite metabolism or indirectly by acting in conjunction with granulocytes (Abu-Ghazaleh et al., 1989; Mackenzie et al., 1980; Bottijer et al., 1985; Smith et al., 1985; Lombardi et al., 1990).

Schallig et al (1994) also studied the anti-*H. contortus* (anti-L3 and anti-adult) IgM, IgG1 and IgG2 responses in 10 month-old Texel sheep. He demonstrated moderate to large increases in all three antibodies to L3 and adult nematodes following experimental challenge infection. What role IgG2 may have in protection against *H. contortus* in Native and other breeds of sheep is yet to be determined.

The reason that might account for the susceptibility of weaned Suffolk lambs to *H. contortus* notwithstanding the capability to generate the Th2 antibodies, IgA and IgG1, which are supposedly host protective (Urban et al, 1992) is that since *H. contortus* L3 penetrates the abomasal mucosa and undergoes development (Rahman and Collins, 1990), there is a possibility that Suffolk lambs might be quantitatively or qualitatively deficient in a local inflammatory cellular component (such as mast cells or eosinophils) that cooperatively interacts with cytophilic anti-*Haemonchus* antibodies to effect expulsion. Protective immunological responses elicited in ponies that were immunized with irradiated *Strongylus vulgaris* L3 consisted of anmnestic eosinophilia, distinct eosinophil staining and degranulation characteristics and a strong surface antibody response against L3 (Monahan, 1993). Thus protective immunity against *S. vulgaris* larval migration appears to be mediated primarily by nematode surface directed antibody dependent cell mediated defense. Merino lambs vaccinated with surface extracts from *H. contortus* L3 were protected against artificial challenge infection with a concurrent antibody response (Turnbull et al., 1992) indicating that antibody dependent cell mediated defense prevented the establishment of invading L3. Therefore,

differences may be present in the inflammatory cellular responses in the abomasal mucosa of *H. contortus* infected Native and Suffolk lambs.

In treated Native and treated Suffolk, the two summer peaks in mean eosinophil counts were seen to coincide with increases in mean fecal egg counts following anthelmintic treatments indicating that both breeds can develop eosinophilic responses during haemonchosis (Figures 16 and 17). In 1992 and 1993, the third peak in mean eosinophil counts in the two groups was not seen with a concurrent increase in mean fecal egg counts and therefore, probably represented a response to *T. colubriformis* infection during late fall. *Trichostrongylus colubriformis* becomes the predominant nematode at this time of year as observed in tracer lambs (Miller, unpublished observations). The present study failed to establish a relationship between peripheral eosinophil counts and resistance to naturally acquired strongylate nematode infection.

Eosinophilia is generally a characteristic feature of infections with tissue invading parasitic nematodes. Significant differences in abomasal eosinophil counts were seen between resistant and random-bred lines of Merino lambs following challenge infections with *H. contortus* (Gill, 1991). Between-breed variation in the degree of abomasal eosinophilia was also observed following challenge infections with *H. contortus* (Bradley et al., 1973). Compared to Rambouillet lambs, Florida Native lambs manifesting resistance to *H. contortus* had severe eosinophilic infiltration in the abomasum.

The number of globular leukocytes at the site of infection is the only parameter that showed consistent and significant association with resistance in studies that examined within- and between-breed variation in resistance to *H. contortus* in sheep (Douch, 1988; Gill, 1991; Gamble and Zajac, 1992). The finding of a significant association between lumenal globular leukocytes, which are derived from tissue globular leukocytes, and larval migration inhibitory activity in the *H. contortus* infected abomasum of resistant sheep accentuates the effector role of globular leukocytes in anti-*H. contortus* immune responses in sheep (Stankiewicz et al., 1993). As an addendum to the present study, abomasal and small intestinal tissue samples were taken at the necropsy of suckling and

weaned lambs in 1994. The tissue sections are currently being examined to determine the quantitative and qualitative differences in eosinophils and globular leukocytes between Native and Suffolk lambs.

The role of CD4$^+$ T cells in protection against *H. contortus* in weaned Native lambs was investigated in a separate experiment in our laboratory (Miller and Bahirathan, unpublished observations). A cohort of six Native and three Suffolk lambs were grazed on naturally infective pasture from birth to three months of age. The lambs were removed from pasture and given an anthelmintic to remove the nematode burdens. They were then kept nematode-free and fed a concentrate feed. At six months of age, the Native lambs were divided into two groups. Peripheral blood CD4$^+$ T cells of one group of three Native lambs were depleted by a series of intravenous inoculations with a monoclonal antibody directed against the CD4 antigen. One day after the start of depletion, these lambs and the control lambs (three Native and three Suffolk) were inoculated with 20,000 *H. contortus* L3/lamb. Depletion of CD4$^+$ T cells was verified by flowcytometry.

Fecal egg counts and packed cell volumes indicated that ablation of peripheral CD4$^+$ T cells did not abrogate resistance to *H. contortus* in Native lambs. In the six nondepleted lambs, there was a positive correlation (r=+0.68) between the fecal egg count and peripheral blood CD4$^+$ T cell percentage 35 days post-infection. These observations are contradictory to what has been reported on lines of Merino lambs selected for resistance to *H. contortus* in Australia (Gill et al, 1993a). In that study, host responses to *H. contortus* (mucosal mast cell hyperplasia, eosinophilia and anti-parasitic antibody responses) were inhibited by anti-CD4 monoclonal antibody treatment and depleted lambs had significantly higher fecal egg output and nematode burdens compared to control lambs. Thus, it seems that within- and between-breed variation in resistance to *H. contortus* in sheep may have different effector mechanisms.

We employed two strategies to overcome the problem of anthelmintic resistance in Suffolk lambs. When fecal egg count reduction testing demonstrated that an anthelmintic regimen was

ineffective, we switched anthelmintic in an attempt to increase efficacy. When this strategy failed, combinations of chemically unrelated anthelmintics were used.

In 1992, ivermectin at double the dosage was ineffective when used in suckling lambs at 13 weeks of age and in weaned lambs on July 1 (Figure 7). Therefore, we switched to albendazole on August 12. At normal dosage albendazole was effective in controlling infection in both Native and Suffolk lambs until the end of the study.

In 1993, following the failure of albendazole at double the normal dosage in suckling Suffolk lambs, levamizole was used at normal dosage on June 11 (Figure 9). However since this was not effective in Suffolk lambs, on July 15 we used a combination of albendazole and levamizole both at double the normal dosage. Subsequently, this anthelmintic combination was used three more times. For the first two times this combination brought the mean fecal egg counts in both breeds to near zero. On August 27 and September 30 the combination was ineffective in Suffolk lambs. Therefore, on November 11 we used a different combination (albendazole and ivermectin both at double the normal dosage) which was very effective in both breeds.

Therefore, it appears that when nematode populations resistant to multiple anthelmintics are present, modified treatment strategies such as those adopted in the present study may not be relied upon for long term control.

3. Epidemiology, breed resistance and immunity to strongylate nematode infection in ewes

Compared to Suffolk ewes, Native ewes exhibited a high degree of resistance to strongylate nematode infection irrespective of the grazing seasons and repeated anthelmintic treatments did not influence the resistance. How this resistance in Native ewes is related to anthelmintic treatments is difficult to determine because an untreated control group of Native ewes was not maintained. The effect of the presumably higher level of pasture larval challenge expected from mixed grazing with Suffolk ewes on the susceptibility to infection in Native ewes can only be determined by having such a control group. Post-treatment peaks in mean fecal egg counts were high for Suffolk ewes and

practically negligible for Native ewes indicating the inability of reinfections to induce protection in Suffolk ewes.

If hypobiosis of *H. contortus* is not important in mature ewes in Louisiana, then control of nematode infection in Suffolk ewes and their newborn lambs can be augmented by adopting a "treat and move" to "safe" pasture strategy at lambing. Fecal egg count profiles indicated that in 1992 and 1993 when the peri-parturient rise was curtailed by anthelmintic treatment, it took more than two months for the nematode populations to reestablish. This delay in the acquisition of nematode infection was probably due to transfer of ewes (after weaning) to a "safe' pasture. The initial low mean packed cell volumes observed in Suffolk ewes in 1992 is a carryover from previous infection. Larger *Haemonchus* burdens as indicated by higher fecal egg counts and lower packed cell volumes was a consistent finding in Suffolk ewes during summer and early fall. These may lead to weight loss and possibly adverse effects on reproduction such as reduced fertility and conception rates since sheep are bred in fall.

During 1992, normal dosage of ivermectin resulted in satisfactory fecal egg count reductions for the four treatments administered. The initial fecal egg count reduction was more pronounced than the subsequent fecal egg count reductions. In 1993, on March 27 ivermectin was effective at normal dosage in both breeds. It was less effective in Suffolk ewes on June 24. Therefore, dosage was doubled on August 6. However both breeds failed to respond. This failure of ivermectin necessitated a switch to an albendazole-levamisole combination which reduced the mean fecal egg counts to near zero in both breeds. Prolonged and/or more profound efficacy of ivermectin in mature Suffolk ewes compared to suckling and weaned Suffolk lambs suggest the presence of age related differences in pharmacokinetics of ivermectin and/or an interaction between protective responses and ivermectin.

While increases in the total anti-*H. contortus* antibody levels were accompanied by low fecal egg counts in Native ewes, comparable levels of antibodies in Suffolk ewes were also observed. This, together with the finding of slower disappearing serum antibodies in suckling Native lambs

compared to suckling Suffolk lambs showing resistance to *H. contortus*, strengthens the concept that protective antibodies may exist in Native sheep.

In both breeds, compared to the mean eosinophil count peaks in summer, the peaks in late fall were higher (Figure 23). As observed with the fall mean eosinophil count peaks in weaned lambs, the high late fall peaks in ewes did not occur concurrently with substantial increases in mean fecal egg counts and therefore, probably represented responses to *T. colubriformis* infection rather than *H. contortus* infection. No differences were found between the mean eosinophil counts of the two breeds in any of the three grazing seasons. It is possible that under natural exposure conditions, peripheral eosinophil responses due to strongylate nematode infection may have been obscured by eosinophilic responses induced by other factors such as concurrent bacterial diseases such as footrot and allergens.

In summary, substantial variation in resistance to strongylate nematode infection was revealed between the three classes (suckling lambs, weaned lambs and mature ewes) of Native and Suffolk sheep by comparing the parasitological and hematological parameters under conditions of natural infections assuming identical challenge. Breed substitution followed by selective breeding or cross-breeding is therefore indicated as another approach to alleviate the problems associated with the use of chemotherapeutics for nematode control in sheep in Louisiana and other areas with similar environmental conditions.

Breed substitution is a simple, inexpensive and quick method to increase the sustainability of sheep production in parts of the United States and world where *H. contortus* is a major constraint on sheep production. To evaluate resistance and production performance, Native x Suffolk lambs were compared to pure-bred Native and Suffolk lambs while grazing naturally infective pasture (Barras and Miller, unpublished observations). Cross-bred lambs gained more weight than Native lambs, exhibited resistance like Native lambs and required fewer anthelmintic treatments during the haemonchosis season compared to Suffolk lambs. Genetic correlation with resistance and the influence of selection for resistance to strongylate nematode infection on the susceptibility to other

pathogens are other factors that need to be investigated before incorporating resistance into breeding programs.

4. Epidemiology and breed resistance to coccidia (*Eimeria*) infections in lambs and ewes

Coccidial infections comprised of multiple species of *Eimeria* are common in pasture raised lambs and ewes in Louisiana. Peak oocyst output was seen in lambs by six to nine weeks after turnout to pasture and was probably in response to a higher level of oocyst challenge on pasture than in lambing pens because lambs start eating forage and some soil beginning at two to three weeks of age. Gregory and Catchpole (1989), studying coccidial infections in sentinel lambs, observed peaks of coccidial activity and diarrhea within five to six weeks after the lambs were placed on pasture. In the present study, though lambs had coccidial oocysts in high numbers during the period of risk, clinical signs were not observed. This could be attributed to the differences in the prevalence of species present. *Eimeria crandallis* was the most prevalent species constituting more than 98% of the mean total oocysts per gram of feces and is not very pathogenic when compared to *E. ovinoidalis* that causes severe diarrhea in newborn lambs (Gregory and Catchpole, 1989). *Eimeria ovina* and *E. granulosa* were the second and the third most prevalent species and constituted less than 1.2% of the mean total oocysts per gram of feces. Differences in the levels of oocyst output of *E. crandallis*, *E. ovina* and *E. granulosa* between breeds were not significant. Therefore, the coccidia data of the lambs and ewes of the two breeds were pooled for the analysis of species specific oocyst output.

Eimeria ovinoidalis was not very prevalent in the present study. This is contradictory to the observations of daSilva and Miller (1991) who reported that *E. ovinoidalis* was the most prevalent species in Suffolk ewes in 1988 from the same location as this study. Since *E. ovinoidalis* is present in the coccidian fauna, variation in infection levels are probable following changes in climatic, management and/or ecological conditions.

Though lambs were able to support heavy multiplication of *E. crandallis* without manifesting clinical signs, the effect of these subclinical infections on the growth of lambs needs to be investigated since it has been shown that anticoccidial treatment of suckling lambs can effectively

suppress the initial peak of coccidial activity without interfering with the development of protective responses and bring weight gain benefits (Gregory et al., 1983; Gjerde and Helle, 1991). One other way by which lambs on pasture may benefit by timely administration of anticoccidal treatments is by removal of any harmful effects that may be precipitated from the interactions of *Eimeria* and strongylate nematodes in concurrent infections.

The fecal oocyst count profiles of ewes indicated the presence of suitable environmental conditions for oocyst development in the spring and the fall. The low levels of oocyst output seen in ewes during the summer months suggest that summer conditions might not be favorable for oocyst survival. In 1992 and 1993, increases in fecal oocyst counts of *E. crandallis* in ewes during fall/winter was followed by massive output of the same species in the successive spring in newborn lambs indicating that overwintered oocysts may serve as the source of initial infection to lambs on pasture. Reinfection was the cause of subsequent cycles of eimerian infections seen in lambs.

In 1993, the observation that Suffolk lambs had significantly higher mean total oocysts per gram of feces compared to Native lambs at three weeks of age may be due to exposure to higher levels of residual infection in Suffolk lambs in lambing pens that were previously occupied by Native lambs. The observation of significantly higher mean total oocysts per gram of feces in Native lambs compared to Suffolk lambs at 17 weeks of age may be due to differences in exposure levels since the two breeds of lambs were born at different times.

The dramatic drop in oocyst output in both Native and Suffolk lambs at six months of age indicated the development of a solid immunity to eimerian infections. The absence of a significant breed effect on fecal oocyst count in ewes and first grazing lambs, in which initial levels of oocyst output can be considered to reflect the degree of susceptibility, suggested that there is basically no difference in susceptibility to eimerian infections between Suffolk and Native breeds. This implies that Native and Suffolk sheep may have similar effector mechanisms against *Eimeria* spp. infection.

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APPENDIX

I. Sheather's sugar

Combine 454 g of granulated sugar (sucrose) and 355 ml of Tap water. Dissolve

sugar in hot tap water directly or add sugar to hot water over a low heat and stir.

II. Buffers

(a) Coating buffer, 0.1M, pH 9.6. Solution A: 8.401g of $NaHCO_3$+0.2g NaN_3.

Dilute to 1 liter with distilled water. Solution B: 10.599g of Na_2CO_3+0.2g NaN_3.

Dilute to 1 liter with distilled water. While stirring and monitoring, slowly add

solution A to Solution B until pH 9.6 is obtained. Store at +4°C.

(b) Washing buffer, 1 ml Tween-20+18g NaCl. Bring to 2 liters with distilled

water.

(c) Phosphate buffered saline,pH 7.3. Combine 2.14g $Na_2HPO_4.12$ H_2O or 0.8485g

Na_2HPO_4 and 0.54g KH_2PO_4 and 9.8g NaCl. Bring to 1 liter with distilled water.

(d) Blocking buffer (conjugate diluent). PBS+1% BSA+0.05% Tween-20

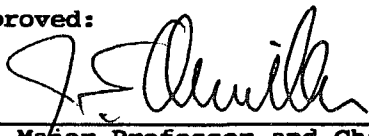(e) Serum diluent. PBS+1M NaCl+0.1% TritonX-100+1% BSA
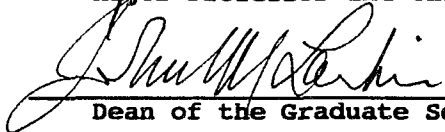
VITA

Mahesan Bahirathan was born on October 29, 1959 in Jaffna, Sri Lanka, the son of Eliathamby Mahesan and Vathilingam Puspawathy. He earned the Bachelor of Veterinary Surgery degree (BVSc) from the Faculty of Veterinary Medicine and Animal Science of the University of Peradeniya in Sri Lanka in 1985. He was employed by the Department of Parasitology, Faculty of Medicine at the same University where he completed the Master of Philosophy (MPhil) degree in Parasitology on June, 1988. From 1988 to 1989, he served in the Department of Biochemistry, Faculty of Medicine as an Assistant Lecturer. In 1990, he joined the Veterinary Research Institute as a Research Officer. In 1991, he was awarded a full four year Assistantship by the Louisiana State University to work in the Department of Epidemiology and Community Health as a Graduate Research Assistant. He enrolled as a doctoral student in the same Department. He was awarded the Doctor of Philosophy degree in Veterinary Medical Sciences in December, 1994. He has a number of research communications and publications and presented scientific papers in several meetings in the United States and Sri Lanka. Mahesan Bahirathan is married to Kalpana and they have a son, Branavan.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Mahesan Bahirathan

Major Field: Veterinary Medical Sciences

Title of Dissertation: Epidemiology of Strongylate Nematode and Coccidia
                             (Eimeria) Infection in Sheep with Special Reference
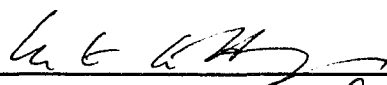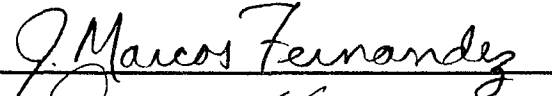   to Breed Resistance

Approved:

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Major Professor and Chairman

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Dean of the Graduate School
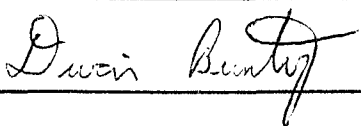
EXAMINING COMMITTEE:

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Date of Examination: _____

October 21, 1994   _____