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Ostertagia ostertagi: Population dynamics under pasture and confinement conditions with particular reference to the inhibition phenomenon

Eddi, Carlos Solomon, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1989

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OSTERTAGIA OSTERTAGI: POPULATION DYNAMICS UNDER PASTURE AND CONFINEMENT CONDITIONS WITH PARTICULAR REFERENCE TO THE INHIBITION PHENOMENON

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
Veterinary Medical Sciences

by
Carlos S. Eddi
D.V.M., University of Buenos Aires, 1975
M.S., Louisiana State University, 1987
May, 1989

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DEDICATION

To my teachers of veterinary parasitology in Argentina: Drs. Roman Niec, J.J. Boero, Nicolas Gelormini, Amar S. Thakur and J.J. Lombardero.

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ABSTRACT

This experiment was conducted to further define epidemiological events involved in the induction, duration and maturation phases of inhibited development of Ostertagia ostertagi in naturally infected calves and to characterize some aspects of the immune response associated with the inhibition phenomenon.

During March, April and May 1988, 3 groups of 18 calves were grazed on the same experimental pasture for 3 weeks. At the end of each grazing period, 2 calves of each group were slaughtered for analysis of worm population; the remaining calves were divided into 2 subgroups of 8 calves. One subgroup was returned to pasture while the other was placed in confinement. At 2-week intervals herbage samples were collected for larval recovery.

Large numbers of O. ostertagi early 4th-stage larvae (EL4) were observed in calves placed on pasture in March and April, while calves introduced in May had smaller numbers of parasites and lower percentages of EL4. Ostertagia ostertagi larval inhibition was attributed to seasonal factors. Similar level of EL4 in grazed and confined calves, suggested that inhibition was not

induced by a density-dependent phenomenon. Maturation occurred in September,i.e., as spontaneous and marked qualitative changes in the worm population. This supported the theory of a similarity of nematode larval inhibition and diapause in insects as a means of survival during periods of environmental adversity. No differences were observed in plasma pepsinogen values among the 3 groups at the time of maturation.

Numbers of O. ostertagi and the total number of worms in grazed calves and those in confinement were not different. Lengths of adult worms and numbers of eggs in utero were significantly smaller ($P<0.05$) in grazed calves through September. However, differences in vulval flap, lengths of EL4 and lengths of spicules were not significant. Grazed calves exhibited higher IgG titers. No response of lymphocytes stimulated with O. ostertagi L3 antigen was observed until the end of July, but a significant lymphocyte proliferation occurred in August. Abomasal lymph node lymphocytes displayed higher levels of proliferation than those from internal iliac lymph nodes and peripheral blood.

LITERATURE REVIEW

INTRODUCTION

Although gastrointestinal (GI) parasitism in cattle may be attributed to a number of species, Ostertagia ostertagi is well recognized as the most important gastrointestinal helminth of cattle in temperate areas of the world (Levine, 1968; Anderson et al. 1969; Armour, 1970; Bayly, 1977). Clinical disease is characterized by diarrhea, submandibular edema and rapid weight loss (Anderson et al. 1965; Armour et al. 1973). Under certain circumstances, parasitic development of a proportion of ingested larvae temporarily ceases for periods of up to several months, producing a syndrome in which deaths can occur (Armour and Ougborne, 1982; Armour, 1974).

Although several species of Ostertagia are found in cattle, it is O. ostertagi which is associated with most recorded clinical outbreaks and is considered by Lichtenfels et al. 1988, as the major species responsible for the disease. This species occurs in the abomasum, chiefly in cattle, but occasionally has been observed in other ruminants. While Bisset (1980) and Coop et al. (1985), successfully establishmed O. ostertagi in lambs,

Herlich (1958), Bessanov (1959) and Pandey (1971), failed to produce patent infections in sheep through experimental inoculations. On the other hand, Williams (1987), obtained patent infections in 73 out of 86 experimentally infected goats.

Occasionally, O. bisonis and O. leptospicularis have been associated with causing disease in cattle (Worley and Sharman, 1966; Bisset, 1980; Armour et al. 1980). Ostertagia lyrata is commonly reported in association with O. ostertagi, but in significantly lower numbers. Because of this, Lichtenfels et al. (1988) considered it a minor species.

Ostertagia ostertagi was first described by Ostertag in 1890, who named it Strongylus convolutus (McKellar, 1986). Two years later, Stiles renamed it as Strongylus ostertagi (Skrjabin, 1954), and Ransom assigned the present name in 1907 (Suderman, 1979). The first report on the pathogenic effect of O. ostertagi in cattle was in the United Kingdom by Gardener (1911). From that time there has been a myriad of reports published on its significance in Europe (Rose, 1961; Anderson et al. 1965; Armour et al. 1973; Taylor, et al. 1973; Mitchell, 1976; Raynaud and Bouchet, 1976a), North America (Vegors, 1958;

Ciordia and Bizzel, 1963; Goldberg, 1968; Williams and Bilkovich, 1971), South America (Niec, 1979; Fiel et al. 1985; Steffan et al. 1985; Suarez 1985; Entrocasso, (1987), and Oceania (Anderson, 1971; Brunsdon, 1971; Smeal et al. 1977).

TAXONOMIC POSITION AND MORPHOLOGY

Although the parasite has been classified within different genera and the taxonomic system of the Nematoda has undergone many changes (Skrjabin et al. 1954; Drodz, 1967), the genus Ostertagia has not been extensively revised. The present classification follows that of Yamaguti (1961), with modifications proposed by Anderson et al. (1974). The taxonomic position of O. ostertagi in the Phylum Nemathelminthes, (Schnieder, 1873) is shown below:

CLASS: Nematoda, Rudolphi, 1808

SUBCLASS: Secernentea, Dougherty, 1958

ORDER: Strongylida, Molin, 1861

SUPERFAMILY: Trichostrongyloidea, Cram, 1927

FAMILY: Trichostrongylidae, Leiper, 1912

Genus: Ostertagia, Ransom, 1907

species: ostertagi, Stiles, 1892.

The morphology of O. ostertagi has been reviewed by Levine, (1980) and Soulsby (1982). The life history has been described by Threlkeld (1946, 1958), which was the first report of measurements of larval stages at various times during the course of development. Douvres (1956) provided important contributions on the morphogenesis of the parasitic larvae and Rose (1969), studied in detail the day to day changes undergone by the parasite in the host after experimental infections.

The cuticle of O. ostertagi has approximately 25 to 30 longitudinal, cuticular ridges called synlophe (Lichtenfels et al. 1988). Freshly isolated worms are usually a transparent brown color and for this reason, O. ostertagi is commonly known as the "medium" or " brown stomach worm" (Levine, 1978).

The anterior end of the adult worm is less than 25 μm wide and the buccal capsule is inconspicuous (Soulsby, 1982). Adult females are approximately 8 to 9 mm in length with the vulvar opening located 1.2 to 1.5 mm from the posterior end. The vulva is adjacent to, and covered by a cuticular extension, generally known as a flap. Michel et al. (1972) reported that the rate of development of the flap was more variable than that of

the rest of the worm, partly because in some worms the flap remained in an undeveloped state. This arrested development of the flap affected more worms in older calves than in young, suggesting that the development of the flap might be associated with host resistance (Michel, 1972a; 1972b). Adult males are smaller than females and are approximately 6.5 to 7.5 mm long with a well-developed copulatory bursa at the posterior end. The bursa is composed of proconus, two lateral lobes and one dorsal lobe, with an accessory bursal membrane situated anterior on the dorsal side. The spicules of O. ostertagi are slender, measuring approximately 210 to 230 μm in length and each ends in three bluntly-hooked processes. The gubernaculum is 50-60 μm long and 14 μm wide, and along with the spicules, can also be observed in the bursa (Soulsby, 1982). It is elongated, oval-shaped, and rounded in front with a thin offshoot in the back (Skrjabin, 1954).

Eggs of O. ostertagi are typically strongylid in appearance and thin-shelled. When freshly deposited by the female, eggs contain around 6 to 8 blastomeres. Eggs measure around 75-85 μm long and 40-50 μm wide. This is usually sufficient for differentiation of O.

ostertagi eggs from those of Trichostrongylus spp., since the latter have an asymmetric pole; sides of O. ostertagi eggs are symmetric to each other (Cunliffe and Crofton, 1953). First stage larvae (L1) measure around 300 to 400 μm in length (Threlkeld, 1946), while second stage larvae (L2) which can be observed on the second day after hatching, are approximately 800 μm long (Skrajabin, 1954). First and second stage larvae, have a clearly defined rhabditiform esophagus.

With rate of development dependent on temperature, moisture and aeration conditions (Rose, 1961), the free-living larvae will undergo two molts within the fecal pat to become infective, third stage larvae (L3). The infective L3 of O. ostertagi is approximately 700 μm long and is relatively simple in structure.

The mouth is surrounded by three lips. The buccal cavity is small and tubular leading into a claviform oesophagus. The intestine is composed of 16 nucleated cells joined posteriorly to a slender rectum. The excretory system is situated in the anterior part of the body and consists of an excretory pore, a short excretory duct and two excretory gland cells (Threlkeld, 1946). The larvae are enclosed in a loose cuticular sheath

resulting from the second ecdysis; this sheath affords protection from drought and temperature extremes (Soulsby, 1982).

At approximately 24 hours after ingestion, parasitic L3 are very similar to free-living infective L3. The sixteen intestinal cells may still be clearly visible. The only obvious change is the loss of the outer loose cuticle resulting from the second ecdysis (Rose, 1961; 1969). However, two days after infection, the intestinal cells are no longer sharply delineated although the outer cuticle is clearly separated from the body around the head and the tail. The sexes at this time can be differentiated by means of the position of the multicellular genital primordium and while in female larvae it is approximately 150 μm from the tip of the tail, in males it is more than 250 μm from the tip of the tail (Rose, 1961). The head or anterior end of the parasitic L3 in the host is essentially the same as that of the free-living L3.

After the third ecdysis is completed, fourth-stage larvae (L4) undergo marked changes in the reproductive system. The genital primordium of the four-day old male larva is somewhat rectangular in shape and consists of

a double row of cells. Four ovoid cells can be seen at the end of the intestine. The genital primordium of the female larva consists of a rounded body from which two finger-like projections extend equidistantly, one in an anterior, and the other in a posterior direction. The head of the juvenile fourth-stage larva (EL4) is similar to that of the L3 (Manual of Veterinary Parasitology Laboratory Techniques, 1977), but in later fourth-stage larvae, a cap-like cuticular structure exists, which is also present in the adult worm. After the fourth day of infection, EL4 measure roughly 1100 μm in length. Larvae grow until the eleventh day, when they are approximately 4.5 mm. long After the twelfth day of infection the fourth ecdysis (second parasitic ecdysis) is completed and the larvae lose the outer loose cuticle. The fifth-stage or young adult (L5) was described for the first time by Douvres (1956). It measures approximately 5 mm in length (Rose, 1969). Further development of the immature L5 involves basically growth and maturation of the reproductive cells, so that day to day changes are not so prominent as during development of the L4 stage. The head of the L5 worm has a cuticularized peribuccal structure of indented, hexagonal shape. Cervical papillae

are present in both male and female worms and in the male, a pair of papillae are present just anterior to the bursa. During the fifth-stage, virtually all structures of the worm remain unchanged but an overall increase in size occurs (Rose, 1961).

LIFE CYCLE

Ostertagia ostertagi has a direct life cycle which involves free-living (eggs, L1, L2 and infective L3) and parasitic stages (parasitic L3, L4, L5 and adult worms). Under natural conditions animals are infected with O. ostertagi by ingestion of L3 on grass. Following ingestion, the L3 is known to exsheath in the rumen (Armour, 1970), although exsheathed O. ostertagi larvae have been observed in ingesta obtained from sheep fitted with oesophageal fistulas. It may be that mastication or salivation of the host also provides a stimulus for exsheathment (McKellar, 1984). Under artificial conditions, L3 of trichostrongylids have been induced to exsheath in solutions of dissolved gaseous carbon dioxide at 38 °C (Rogers, 1966) or sodium chloride solution (Leland, 1963). Exsheathment under natural conditions has been extensively studied in Trichostrongylus spp. by

Rogers, 1960. The phenomenon consists of two stages: in the first, host factors activate the parasite to yield "exsheathing fluid", thought to be a specific leucine aminopeptidase. In the second stage, the "exsheathing fluid" attacks the sheath, causing it to fracture and allowing the parasite to escape. In a series of micrography studies, Rogers and Sommerville (1960) reported that the fluid is produced around the base of the esophagus and emerges from the excretory pore between the larval cuticle and the sheath. A ring-shaped area of weakness results causing the anterior tip of the sheath to detach and the larva wriggles free. Parasitic L3 migrate to the abomasum where they penetrate deeply into the glandular pits. On the fourth day after infection, larvae undergo the first parasitic molt and remain in the 4th stage. Fourth stage larvae undergo morphological changes until day 12. Approximately 15 days post-infection, the immature fifth-stage worm or young adult emerges from the gastric glands, having increased in length by seven to 10 times to 8-10 mm (Armour, 1970). While spermatogenesis in males may be completed as soon as day 15 after infection, females may contain mature ova on day 16 (Rose, 1969). Eggs produced by female worms

may be found in the feces at approximately 21 days after ingestion of the L3 (Rose, 1969). However, as will be discussed later, development may take up to four or five months, following inhibition of larvae at the EL4 stage (Armour and Duncan, 1987; Armour and Bruce, 1974). Peak egg output usually occurs 25 days after infection and then declines logarithmically (Michel, 1969). Eggs deposited by adult females in the abomasum pass out in the feces of the host at the morula stage and hatch after 24 hours producing an active L1 in the fecal pads (Smith, 1976). L1 hatch and molt to become L2, and both stages feed on coliform bacteria in the feces (Smith, 1976). The L2 then molts to become an infective L3. During the second ecdysis the cuticle of the L2 is retained (Taylor, 1930) and the L3 is thus rendered resistant to conditions of drought and extremes of temperature. The L3 ingested by grazing animals repeat the infection cycle.

Many years ago it was observed that when cattle were infected with O. ostertagi during autumn and early winter in the U.K. (Martin et al. 1957; Anderson et al. 1965) or in cold temperate areas of North America (Smith and Perreault, 1972), and during the spring-summer in the southern states of the United States (Williams and Knox,

1976) large numbers of larvae temporarily ceased development at the EL4 stage for periods of up to several months (Armour et al. 1969; Armour and Bruce, 1974). This phenomenon is usually described as arrested larval development or inhibited larval development or hypobiosis (Armour and Duncan, 1987). The inhibited parasite remains in the abomasal mucosa for periods of weeks or months, while conditions on pasture are inimical to development and/or survival of free-living stages; this has been hypothesized as a means by which O. ostertagi can survive adverse conditions of winter or hot and dry conditions of summer (Armour and Ogbourne, 1982). However, inhibited larval development may also arise as a manifestation of host resistance and possibly as a result of both of these factors acting together (Anderson et al. 1965; Michel, 1969; Michel, 1974; Michel et al. 1979). Since inhibited larval development is a phenomenon of considerable epidemiological and clinical importance in veterinary medicine, it will be considered later in more detail.

EPIDEMIOLOGY

According to Armour (1980) the term epidemiology has

for many years been defined as the study of outbreaks of readily transmissible infectious diseases that spread within and between host populations. Host, management, and environmental factors are responsible for the propagation of parasites and of the development of clinical disease. In North America, seasonal patterns of ostertagiasis were described for the northern (Smith and Perreault, 1972; Malczewski et al. 1975; Shillhorn-Van Veen and Melancon, 1984) and southern regions (Williams and Bilkovich, 1973; Suderman, 1979; Ciordia, 1979; Craig, 1979; Brauer, 1983; Williams, 1983; Williams et al. 1986b).

Winters in Europe are severe, as in the northern temperate regions of North America. The description by Michel (1970) of a single peak in larval numbers of O. ostertagi occurring during August and September each year followed by high larval mortality during the winter months, was confirmed by several workers in Europe (Rose, 1970; Nilsson and Sorelius, 1973; Armour et al. 1979). When new calves were placed on pasture at the end of the summer or in early autumn, they were at risk to O. ostertagi Type I disease, because their time of placement on pasture coincided with high levels of pasture

contamination (Malczewski, 1975). Many investigations have been carried out on the survival of L3 on pasture. Goldberg and Rubin (1956) reported from Maryland that O. ostertagi overwintered on pasture, since more than 2900 worms were recovered from tracer calves that grazed in August-September, while more than 100 were recovered in December and April-May respectively. Drudge, et al. (1958) confirmed overwinter survival in Kentucky over a 4-year period. O. ostertagi was present in necropsies of calves that grazed pasture contaminated with parasites the previous year but which was left unoccupied during the winter. In Wyoming, Schwink (1963) reported that feces containing eggs of Cooperia oncophora and Ostertagia spp. were placed on experimental plots at several periods to study development and survival of larvae on pasture. Both parasites survived on all plots after more than a year of exposure. In a similar trial, Goldberg (1968) observed in Maryland that the average monthly temperatures required for development of O. ostertagia larvae were 55 to 70.3°F and monthly precipitation of 1.4 to 3.7 cm, with a maximal recovery from the herbage at 69.8°F. When cattle fecal pats containing eggs of O. ostertagia, C. punctata and

Oesophagostomum radiatum were deposited on pasture in Maryland at the beginning of summer, peak recovery of larvae from pats occurred at 1 to 2 weeks after initial exposure and final recovery at 8 to 9½ weeks. Over 80% of the larvae recovered were within 13 cm of the fecal pats, and times of peak and final recovery were 4 and 24 weeks respectively for O. ostertagia.

Under severe winter conditions of the maritime provinces of Canada, Smith (1972) observed that parasite-free calves grazing on naturally infected paddocks for 18-day periods, acquired very few O. ostertagi over a second winter. However, Slocombe (1974) in southwestern Ontario, reported that O. ostertagi was one of several nematode parasites of cattle that overwintered on pasture for 2 successive years.

In the southern temperate areas of the United States, Williams and Bilkovich, (1971) carried out a study to determine the effect of weather conditions on the development and survival of O. ostertagi. Feces were placed on plots as compact pads, scattered in small portions and as pads or watery feces. The percentage recovery of infective larvae was usually higher on plots with dispersed feces than from plots with fecal pads.

No differences were observed in the percentage yield of larvae from plots with large or small fecal pads, and the effect of form of feces on survival of infective larvae was considered negligible (Williams and Bilkovich, 1973). Larvae were recovered for as long as 8 months (October to May), but average survival time in this period was 6 months. Development of eggs and preinfective stages to L3 was delayed for 2 to 6 weeks by cold weather during December, January and February. Length of survival and percentage recovery of larvae were lowest during the summer months. While optimal conditions for development of larvae to the L3 stage occurred during October and November and in March and April (13 to 23°C mean air temperature and 7.5 to 17.0 cm total monthly precipitation), optimal conditions for survival of infective L3 were considered to be 8 and 23°C mean air temperature and 7.5 to 17.0 cm total monthly precipitation.

In a study of the effects of various constant temperatures on development of the free-living stages of some nematodes, Ciordia and Bizzell (1963) reported that the rate of development of the eggs and larvae of O. ostertagia in fecal cultures accelerated as the

temperature increased. The developmental rate varied substantially between cultures, and the optimum temperature, based on rate of development and proportion of eggs giving rise to infective L3, was 25°C. No development beyond the gastrula stage occurred below 6°C, and even with temperatures above 32°C, development was quick, but the mortality rate of L1 and L2 was high.

Under laboratory conditions Pandey (1972) observed hatching of O. ostertagi eggs in distilled water at temperatures of 4 to 35°C. Infective L3 developed in fecal cultures at 10 to 35°C, while the rate of development increased with temperature. Although L3 survived in distilled water for up to one hour at 50°C, 8 hours at 45°C, 2 days at 40°C and 42 weeks at 20°C, at 4 and 1°C, 90 and 75% respectively, of infective L3 were still alive after one year. However, less than 1% of infective L3 were alive when repeatedly frozen at -10°C and thawed at 25°C, 35 times in 137 days (Pandey, 1972).

Infective larvae display random movement which requires a water medium (Crofton, 1954), and the migration of these larvae onto herbage is favored after rainfall, which softens the fecal pats. A thin film of moisture is required for active movement of the larvae

on the herbage although the larvae cannot migrate against gravity in a fluid medium, and generally precipitation washes many larvae down to the soil surface (Soulsby, 1982). Controversial results were observed on lateral migration of the infective L3. Goldberg (1970) reported recovering 80 percent of the larvae within 13 cm of the dung pat. Bairden et al. (1979), Fincher and Stewart (1979), Armour et al. (1980a, 1981) and Al Saqur et al. (1982) reported migration of the L3 into the soil to re-emerge later. Vertical and lateral transmission passively transported by earthworms (Gronvold, 1979), dungbreeding flies of the genus Psycoda (Jacobs et al. 1968), and by spores of the fungus Pilobolus spp. (Bizzell and Ciordia, 1965) as reported in dispersion of Dictyocaulus viviparus, were also reported.

DISEASE CLASSIFICATION AND CLINICAL SIGNS

Martin et al. (1957) reported 10 outbreaks of severe chronic diarrhea and emaciation in young dairy cattle on farms in Scotland during the spring and early summer of 1956. The only consistent finding at necropsies from each outbreak was the presence of large numbers of O. ostertagi and the only constant pathological alteration

was a marked degree of granularity and thickening of the abomasal mucosa. In 8 of the outbreaks, the cattle had been housed for up to 5 months before the onset of clinical signs. As some of the Ostertagia found at necropsy were immature, and due to the asymptomatic prepatent period during the housing of the animals without larval ingestion, the authors concluded that the disease could be explained by an inhibition of development of the worms responsible for the chronic diarrhea and the "atypical parasitic gastritis". However, it was not until 1965, after a series of field investigations of parasitic gastritis due to Ostertagia, that Anderson et al. (1965) could classify the syndrome into three phases: Type I, pre-Type II and Type II disease.

Type I Ostertagiasis was described as a clinical entity resulting from rapid acquisition of large numbers of larvae that complete development to the adult stage about 3 weeks after infection (Anderson et al. 1965). Younger calves from weaning to 15 months of age were the most severely affected. Although mortality may be low, morbidity can be high because more than 70 per cent of animals in a herd may be affected (Armour et al. 1973).

The principal clinical signs were profuse, green diarrhea, marked weight loss, loss of appetite, and a dry, hardened skin (Armour et al. 1973). As the disease progresses, the eyes become sunken and the coat has a rough appearance (Anderson et al. 1965). At necropsy, the characteristic feature of Type I disease is the presence of a large number of worms, the majority of which are adult parasites (Williams, 1986a). Type I disease is usually present from July to October in cold temperate areas of the world (Armour et al. 1973); it occurs most commonly between January and April in warm temperate regions (Williams et al. 1985).

The pre-Type II phase is the period extending from the time when cattle acquire inhibition-prone larvae until the inhibited worms resume development to maturity (Williams et al. 1986a). During this phase, cattle may have thousands or even hundreds of thousands of EL4 present in the abomasal mucosa with no apparent clinical signs (Anderson et al. 1971). Animals generally appear healthy, although some may be unthrifty. Where large numbers of adult worms are present, a mild diarrhea and poor growth may be observed in some animals (Armour, 1970; Williams et al. 1986a). In cold temperate areas,

the pre-Type II phase occurs between late autumn and early spring, while in warm temperate climates it occurs from spring until late summer and fall. Michel et al. (1976), showed that under winter conditions of the United Kingdom, there is a steady turnover of the Ostertagia population in the host, including arrested larvae.

Type II disease occurs at a time when parasitic gastroenteritis is not generally expected (Anderson et al. 1971). The main clinical signs are profuse diarrhea that occasionally is intermittent and may last for 7-10 days (Armour et al. 1973; Selman et al. 1976), rapid and marked weight loss, pallor, and subcutaneous edema in approximately 40 per cent of clinical cases (Raynaud, 1978b; Burger et al. 1978). Animals become extremely weak and are unable to stand and have sunken eyes. In some cases a moderate anemia is present (Armour, 1970). At necropsy large numbers of O. ostertagi are present, and generally more than 25 per cent are in the EL4 stage (Wedderburn, 1970). While morbidity is usually low, mortality is often high in affected animals even after multiple treatments with anthelmintics (Anderson, 1971; Smith and Perreault, 1972). However, in recent years, the newer benzimidazoles and ivermectin with high

efficacy against EL4 have proven to be highly useful in prophylaxis of the disease (Williams, 1986a).

Type II ostertagiasis is mainly a problem in yearling steers and 2-year-old heifers in late gestation (Anderson, 1971), although cases among older animals, particularly bulls are common enough (Selman et al. 1976). Prognosis in cases of Type II disease is poor and affected animals often remain in poor condition for several months. Type II disease occurs during late winter and spring in cold temperate climates and during late summer through fall in warm temperate regions (Michel, 1969a).

In France, Raynaud and Bouchet (1976; 1977) described an "oedema disease", considering it to be a new type of ostertagiasis. Edematous abomasal mucosae, 2 or 3 times thicker and heavier than normal, were observed, and the gastric folds were filled with a watery edema giving the appearance of fetal membranes. As very few parasites were observed in association with this condition, it was suggested that it may be the result of an allergic reaction to the larval stage (Raynaud and Bouchet, 1976a, 1976b, 1977; Raynaud, 1978a, 1978b).

PATHOLOGY AND PATHOPHYSIOLOGY

The main lesions of ostertagiasis and pathophysiologic changes associated with them occur in the period immediately after large numbers of developing L4 complete development to the adult stage and emerge from the gastric glands (Armour, 1970). In a characterization of the disease under field conditions, Anderson et al. (1965), described that while abomasal pH was increased, and plasma pepsinogen levels were markedly raised, 5 types of lesions were associated with Type I ostertagiasis: presence of nodules; diffuse irregular hyperplasia giving a "Morocco leather" appearance to the mucosa; epithelial cytolysis; edema of the abomasal folds, and a variable amount of congestion. Although more than 80% of the worm population was arrested at the EL4 stage during the pre-Type II phase, no clinical evidence of infection was observed and there was practically no reaction around the inhibited larvae localized in the mucosa. Finally, the Type II phase occurs when large numbers of inhibited EL4 develop to maturity (Martin, 1957). Nodules and epithelial hyperplasia result and in many cases the "Morocco leather" or cobble-stone appearance of the mucosa is

observed. From the histopathological point of view, Snider et al. (1988) described three different phases in the Type I disease. The first phase was related to the development of the larvae through approximately day 17. A second phase was associated with the maturation and emergence of the adult parasite. The third phase was associated with changes that occurred up until the time that the mucosa returned to normal (Ritchie et al. 1966; Snider et al. 1988). Lesions in first phase were marked by extensive proliferation of undifferentiated epithelial cells for replacing the loss of normal cell populations, and an increase in mast cell populations in the mucosa. In the second phase there was a transformation of the glandular epithelium to a mucous epithelium and vast infiltration of eosinophils and neutrophils. In addition, plasma cells were present in the upper mucosa, while an extensive lymphoid nodule proliferation was observed close to the submucosa. The third phase was described as a healing process marked by an increase in globular leukocytes, which represented a portion of the mast cell population (Snider et al. 1988).

The Type II Ostertagia syndrome is closely related to the growth and maturation of DL4 and EL4 (Armour et

al. 1973). The mucosa shows a marked thickening and extensive nodular proliferation produced by the presence of large numbers of worms in varying stages of development, plus the presence of mucous cells and cellular infiltration of plasma cells, lymphocytes, macrophages, neutrophils and eosinophile. Finally, interstitial and submucosal edema is a major factor in thickening of the mucosa (Snider et al. 1988).

Because larvae cause mechanical damage to gastric glands during growth and development, particularly to the parietal and chief cells, it was concluded that this was the reason for the leakage of pepsinogen into the bloodstream and of albumin to the gut lumen (Ritchie et al. 1966; Jennings et al. 1966; Murray, 1969). However, later studies using horseradish peroxidase as a tracer, demonstrated that the mucosal permeability of infected and non-infected animals was similar (Stringfellow and Madden, 1979). Additionally, it was demonstrated that a rise in plasma pepsinogen occurred 48 hours after transplants of adult Ostertagia were made, not only into sheep (Al Saqur, et al. 1982), but also into calves (McKellar et al. 1986). According to McKellar et al. (1986), the mechanism by which plasma pepsinogen values

become elevated may be due to a variety of factors.

Increase of serum or plasma pepsinogen is understood to be a non-specific result of structural and biochemical changes in the abomasum (Schillhorn-Van Veen, 1988). However, measurement of plasma pepsinogen levels as a technique for reliable diagnosis of ostertagiasis, has been questioned. Anderson et al. (1965), found slightly elevated values (1,100 i.u./l) in calves with more than 100,000 L4. On the other hand, (Mylrea and Hotson, 1969; Brunsdon, 1971; Chiejina, 1978) it has been suggested that pepsinogen levels may increase with age. It was observed in the United Kingdom (Chiejina, 1977) that up to 5% of healthy adult cattle may have values in excess of 3,000 i.u./l. However, high levels in older cattle could very well be the result of previous abomasal damage caused by parasites or by other pathogenic factors unrelated to parasitism.

Brunsdon (1971) in New Zealand observed a highly significant correlation between pepsinogen levels and total adult abomasal worm burdens in calves under conditions of natural infection. Armour et al. (1979) in the United Kingdom showed not only a very good correlation between pepsinogen levels and adult worms

burdens in cattle, but also a high larval availability on pasture and abomasal damage, as might be expected during the first grazing season.

During the second grazing season, however, the same animals presented low worm burdens and only a moderate elevation of plasma pepsinogen. The same authors (Armour et al. 1979) suggested that the increase in pepsinogen levels was related to increased larval intake, but this was not accompanied by increased adult worm burdens. This reaction was considered to be a host immune response. This response might also be related to an allergic reaction in the mucosa after the ingestion of L3, generating the elevation of pepsinogen.

Using experimental infections, Snider et al. (1981), observed a direct correlation between plasma pepsinogen levels and the number of larvae in inoculation doses, the severity of clinical signs, as well as fecal egg count levels. While plasma pepsinogen in uninfected controls remained below 1000 i.u./l, all calves which were experimentally infected reached levels greater than 5000 i.u./l after 48 days of the extended inoculation period.

Working also under experimental conditions, Mckellar et al. (1986; 1987) observed that regardless of whether

calves were infected orally with L3 or with adult O. ostertagi by direct surgical transplantation, plasma pepsinogen levels were increased. Using fast-protein liquid chromatography, two forms of pepsinogen were identified, PG1 and PG2. However, because the presence of different proportions of the two forms were found in the plasma of cattle under different levels of experimental infections pepsinogen fractions were not useful in providing a key to the differential diagnosis (McKellar et al. 1988).

Since pepsinogen is mainly excreted by the kidneys (Uete et al. 1969), uropepsinogen levels in cattle were examined. Results obtained were not well correlated with pepsinogen levels in serum (Schillhorn-Van Veen, 1988). Mylrea and Hotson (1969), observed an increase of pepsinogen which they related to the age of cattle. According to Chalmers (1983), and as was already mentioned, such increases may well be the result of past abomasal damage due to parasites or other causes. Due to variations of results obtained using the same or different techniques, it is generally accepted that herd plasma pepsinogen levels are a much better indication of damage due to O. ostertagi infection than data obtained

from individual animals (Entrocasso, et al. 1986). Because abomasal function is to a great extent influenced by endocrine and paracrine hormones (gastrin, secretin, somatostatin, cholecystokinin), gastric inhibitory peptide levels should theoretically, have some modulation effect on pepsinogen secretion during ostertagiasis (Schillhorn-Van Veen, 1988).

Of the hormones mentioned above, only gastrin has been studied extensively (Reynolds et al. 1979; Entrocasso et al. 1986). Hypergastrinemia in sheep during experimental parasitic infections was reported by Anderson (1976; 1985), and Reynolds et al. (1979). Entrocasso (1986) and Schillhorn-Van Veen (1988) confirmed this event in cattle. Gastrin levels were increased three to tenfold during type I ostertagiasis, while the increment was between three to seven times in naturally occurring type II disease (Schillhorn-Van Veen, 1988). Hormone assays performed on samples from calves exposed to trickle infections, showed that the differences in gastrin levels between infected untreated calves and those protected by anthelminthics, were considerably greater than differences in plasma pepsinogen values. Peak gastrin levels in infected

calves fed hay alone, were found to be twice as high as values observed in animals maintained on a concentrate diet (Fox et al. 1988). While significantly increased serum gastrin concentration compared with preinfection values, was found in calves infected with O. ostertagi or T. axei, highly significant results were observed in calves infected with both parasites (Snider et al. 1988). It is generally considered that plasma pepsinogen and gastrin determinations may be highly useful for research purposes, but their reliability and practicality for individual animal situations and general clinical diagnoses are doubtful (Schillhorn-Van Veen, 1988).

PROTECTIVE IMMUNITY

Early investigations of the immune response of cattle to helminth parasites were mainly concerned with the development of immunodiagnosis procedures (Fife, 1971). Although immunization procedures against bacterial and viral diseases have been developed and resolved many important infectious diseases, the same has not been true for cattle helminths or helminth diseases generally with exception of successful vaccines (irradiated larvae) against Dictyocaulus viviparus in

cattle (Jarret et al. 1960), D. filaria in sheep (Sokolic et al. 1965), and Ancylostoma caninum in dogs (Miller, 1965). Attempts to protect cattle against O. ostertagi by varied immunization techniques have been unsuccessful (Armour, 1967). Although administration of single inoculations or multiple inoculations (oral) of infective L3 have elicited some degree of resistance, as manifested by reduced worm establishment, lower fecundity, and morphological changes, no significant protection against infection was observed (Michel et al. 1973; Herlich, 1976). Intraperitoneal inoculation of mixed parasitic larval stages grown in vitro and intravenous administration of exoantigens did not produce an immunity to subsequent oral challenge with O. ostertagi L3 (Herlich and Douvres, 1979; Herlich and Tromba, 1980). The failure of empirical immunization procedures to protect animals against helminth parasites, emphasizes the need for systematic studies of the mechanisms of host immune response to helminth infections (Terry, 1968).

In general, the effects of host immune response against helminth parasites may be summarized as follows:

(1) Alteration in development and morphology. According to Michel, et al. (1972a), development of the

vulval flap of O. ostertagi females is negatively affected by the host response. This observation was confirmed by Mckellar (1984), Frankena (1987), and Kloostermann (1984). It is widely accepted that worm length is a common parameter in determining normal growth and development characteristics of a parasite population (Michel et al. 1982; Frankena, 1987). Other parameters commonly used for measuring worm development, altered by immune response, are width of worms (Michel et al. 1971), dry weight or biomass (Christie et al. 1979) and length of spicules in males (Borgsteede and Hendriks, 1979).

(2) Impairment of fecundity is a parameter usually measured by counting the number of eggs in utero. Suppression of worm egg production resulting from a non-specific immunological reaction, has been reported by Kennedy (1980), O'Sullivan (1979), and Moqbel and Wakelin (1979).

(3) Inhibited or arrested development is a life cycle

characteristic of importance for O. ostertagi in cattle (Threlkeld and Johnson, 1948). It has been demonstrated in a number of investigations that hosts previously exposed to a variety of specific parasites, harbored

greater numbers of inhibited larvae than unexposed, naive animals (Donald et al. 1964; Dineen et al. 1965). However, Michel (1978), explained that the increase in inhibited larvae, was more likely an effect of adult worms being more rapidly lost from resistant animals, than from susceptible hosts. The same author pointed out that in worms like O. ostertagi of cattle with seasonal inhibition, host immune factors could well be involved in the induction of larval inhibition. After experimental infection of animals with a strain of O. ostertagi which had ceased to arrest in response to cold conditioning, Michel et al. (1979) concluded that "innate resistance" of older animals, when combined with seasonal conditioning effects on larvae, caused over 85% of the larvae in the natural challenge infection to become inhibited".

More recently, Snider et al. (1981), working with experimental infections in calves, showed that previously infected calves harbored greater numbers of EL4 of O. ostertagi than calves that had not been infected before challenge with multiple, increasing doses of infective L3. Because a lymphoid cell infiltration of the mucosa was observed, an immune response was suggested for the

greater levels of larval inhibition detected in the previously infected calves.

Immunity to O. ostertagi is acquired more rapidly in adults than in calves (Michel et al. 1979). However, the immunity to infection not only develops slowly, but also diminishes rather quickly. Armour (1967), demonstrated that immunity established after the first grazing season, was lost during winter housing. In a recent report, the same author confirmed that in spite of an initial immunity assessed by low fecal egg counts and the absence of clinical signs, second season cattle may accumulate large and potentially dangerous burdens of O. ostertagi EL4 (Armour et al. 1988). On the other hand, doubts have been expressed regarding a marked age-related immunity in ostertagiasis, because outbreaks of acute (Selman et al. 1976) and chronic (Cawley and Lewis, 1976) disease were reported in older cattle (Armour et al. 1973). Of all cattle age classes, yearlings appear to better withstand the effect of infection than younger animals and reduced establishment, stunting of worms and lower egg counts were reported in 15 month-old yearlings compared with three month-old calves (Smith, 1970).

Mechanisms of immunity to O. ostertagi remained

undefined. The relative importance of humoral and cellular elements in mediation of any degree of host immunity to O. ostertagi has not been determined (Klesius, 1988).

Length of worms and the percentage of adult females without vulval flaps has been shown to be affected by administration of immune-suppressive drugs like cortisone (Michel and Sinclair, 1969). Corticosteroid treatment of calves with an existing population of O. ostertagi inhibited EL4, did not trigger resumption of development of the EL4, but did appear to suppress the natural rejection of EL4. Immune-suppressed calves tended to have higher EPG counts, well-developed vulval flaps, parasites of greater length and significantly depressed lymphocyte counts in comparison to untreated controls (Michel and Sinclair, 1969; Prichard et al. 1974; McKellar, 1984; Frankena, 1987).

Few studies have been directed to the role of antibody response in O. ostertagi infections. According to Keus et al. (1981) and Klesius et al. (1986) antibody response to O. ostertagi from a quantitative point of view, is weak. An increase in anti-Ostertagia antibody titers was reported to occur simultaneously with

resumption of development of EL4 or an increase in numbers of adult worms of grazing animals (Kloosterman et al. 1984; Entrocasso et al. 1986). Serum IgG, IgM and IgA antibody responses to O. ostertagi have been detected with anamnestic IgG responses (Jensen and Nansen, 1978; Klesius et al. 1986; Klesius, 1988). However, the relative importance of the humoral response, is still basically unresolved. Snider et al. (1985), studied the cellular immune response in calves inoculated with O. ostertagi and C. punctata and in parasite-free calves to O. ostertagi larval antigen inoculation. A clear distinction in dermal reactions of calves to both O. ostertagi and C. punctata was observed. A delayed hypersensitivity response occurred in cattle infected with O. ostertagi, but not in Cooperia-infected cattle. While marked dermal reactions occurred in the O. ostertagi-inoculated calves at 24 and 48 hours, reactions were not seen in C. punctata-inoculated calves 24 hours post inoculation. In a subsequent study, Cross et al. (1987) showed an immediate hypersensitivity response by intradermal injections of a soluble L3 extract of O. ostertagi to infected cattle. The latter result confirmed previous suggestions of Snider et al. (1985),

regarding the possible involvement of IgE in the immune-mediated response to O. ostertagi. Although a decreased number of mast cells was noted in the skin injection sites, it is possible that the decrease was due to their degranulation (Klesius, 1988). Marked perivascular accumulations of eosinophiles and mononuclear cells were observed in the dermal reaction of O. ostertagi inoculated calves (Snider et al. 1985). In previous reports, it was indicated that inflammatory lesions in the abomasum of O. ostertagi-infected calves were mainly produced by eosinophile as a dominant cell type (Osborne et al. 1960; Ritchie et al. 1966; Ross and Dow, 1964).

As demonstrated by Washburn and Klesius (1984) and by Klesius (1985), soluble extracts of O. ostertagi L3 were capable of causing eosinophil chemokinesis and chemotaxis activity. The eosinophil chemotactic factor was considered to be an excretory-secretory material produced by O. ostertagi larval stages and adults (Klesius et al. 1986).

According to Wiggin and Gibbs (1987; 1988), the pathological changes present during ostertagiasis are not only due to a hypersensitivity response, but to the presence of eosinophils also. Even when this immune-

mediated response appeared to act in a protective fashion for the host, pathological change of varying intensity in host tissue also occurred (Klesius, 1988).

There is evidence that O. ostertagi induces suppression of cellular and antibody responses to infection. Through peripheral blood lymphoblastogenesis to soluble antigens and mitogens during primary and challenge infections, a transient immune suppression was reported (Klesius et al. 1984). Subsequently, it was demonstrated in several investigations that immunosuppression occurred in cattle during the prepatent period of O. ostertagi infection to both parasite antigens and non-specific mitogens (phytohemagglutinin-P, pokeweed mitogen and concanavalin-A) (Klesius et al. 1984; Snider et al. 1986).

Using Western blot and ELISA techniques, Cross et al. (1987) studied serological responses to a crude L3 antigen. A 32 kDa band was found when sera from cattle with pre-Type II ostertagiasis were tested and compared with sera from calves with Type I disease and uninfected animals. Although the larval antigen was considered to be cross-reactive or nonspecific, the 32 kDa fraction appeared to be a promising tool for future diagnostic

investigations of pre-Type II infection in cattle.

The capability of diagnosing the pre-type II phase of O. ostertagi infection in the live animal by an immunological procedure, rather than complex post-mortem and microscopic examination, could be extremely important, particularly in the case of cattle entering feedlots (Williams and Knox, 1988).

INHIBITED LARVAL DEVELOPMENT

The prepatent period of O. ostertagi is approximately three weeks. However, under specific conditions, parasitic larval development of a portion of ingested larvae temporarily ceases for periods of up to 3 to 6 months (Armour and Duncan, 1987). This phenomenon is described generically, as hypobiosis, or larval hypobiosis, and more specifically as inhibited larval development (ILD), retarded development or arrested larval development (ALD) (Williams, 1983). In the case of O. ostertagi, the inhibition occurs at the EL4 stage, i.e., at 4 or 5 days after infection and completion of the first parasitic molt.

Michel, (1974) suggested three criteria for determining the occurrence of true arrest or inhibition

of larval development:

1. the finding of large numbers of larvae at precisely the same stage of development in cattle when there has been no recent intake of infective larvae;

2. the finding that a significant proportion of the parasite population has remained at a precise point of development in animals which have been withheld from possible infection for a time longer than that required to reach the observed point of development;

3. the occurrence of a bimodal distribution in size or stage of development in a worm population in an animal not exposed to a corresponding pattern of infection.

Under the conditions in which inhibition of larval development occurs there is a large increase in a homogeneous population of EL4, which remain in a state of dormancy for prolonged periods of time (Martin et al. 1957; Anderson et al. 1965). The specific mechanism whereby O. ostertagi larvae become inhibited in development is not yet known. Martin et al. (1957), Ross (1963) and Michel (1963) attributed the phenomenon to host immunity acquired by cattle during grazing and natural infection. Anderson et al. (1965) and Armour, et al. (1969) postulated that inhibition of O. ostertagi

was associated with physiological changes in the parasite or possibly in the host in periods prior to the actual occurrence of inhibition, and that these changes were independent of the immunological response of the host. For these authors, inhibited larval development in cattle naturally infected with O. ostertagi, was primarily dependent on two factors:

(1) an innate susceptibility of a particular strain of larvae to inhibition;

(2) a conditioning effect of environmental factors (weather conditions, daylength), which acting on larvae on pasture, produced an optimal stimulus with subsequent inhibition of ingested larvae.

Anderson et al. (1965) and Armour et al. (1969) further postulated that inhibited development of O. ostertagi was a phenomenon similar to diapause which occurs in insect populations (Armour and Bruce, 1974). Michel (1967) found that in calves experimentally infected daily with O. ostertagi L3 at different inoculation levels, a constant percentage of the administered L3 accumulated in the host at the EL4 stage, suggesting that host immunity was not involved. However, some years later, Michel (1979) demonstrated that

acquired immunity by the host has a very important role, particularly in cases involving mature cattle, where the hypobiotic larvae have accumulated over a prolonged period. Michel's theory was supported by the fact that inhibited larvae will begin to mature under conditions known to depress host immunity, as during parturition (Michel et al. 1979) or during the stress of intercurrent disease (Michel et al. 1971).

A clear increase in the percentage of inhibited larvae was observed to occur at particular times each year (seasonally) both in helminth naive calves and those with prior experience of infection in different areas of the world (Anderson et al. 1969; Armour and Bruce, 1974; Williams et al. 1983; Williams et al. 1986). Williams et al. (1987), also found that certain seasonal variations in weather conditions may be an adequate stimulus to produce an earlier and gradual maturation of established inhibited larvae, as well as a reduction in trend of larvae on pasture to undergo inhibition.

Different levels of larval inhibition were artificially produced by conditioning O. ostertagi infective L3 and young helminth-naive calves in climatic chambers to simulate natural weather conditions (Armour

and Ogbourne, 1982). Chilling of infective L3 at 4°C for 5 to 10 weeks, readily induced inhibition in some strains of O. ostertagi, while other strains could not be conditioned by such treatment (Armour and Bruce, 1974). A significant percentage of "autumn conditioned" larvae became arrested at the EL4 stage after oral administration to calves, irrespective of whether the animals were kept in a spring, autumn or winter environment (Armour and Bruce, 1974). When infective L3 kept for various time periods at 18-20°C were administered orally into parasite-naive calves, the results positively demonstrated that chilling, as opposed to simple aging, was the stimulus to larval inhibition (Armour and Bruce, 1974).

In the United States, O. ostertagi has two different and defined patterns of inhibition. There is considerable evidence that in the southern states, inhibition of larval development occurs in spring (Williams and Knox, 1976; Williams et al. 1977; Williams et al. 1979; Craig, 1979). A similar pattern was observed on irrigated pastures in California (Baker et al. 1981), in Missouri (Brauer, 1983) and in Georgia (Becklund, 1962). However, there is substantial evidence

that inhibition of larval development in the northern states of the United States and Canada, follows a similar pattern to that described for northwestern Europe, i.e. in autumn (Smith and Perrault, 1972; Malczewski et al. 1975; Gibbs, 1979; Schillhorn-Van Veen et al. 1984; Herd, 1980).

Although it seems apparent that environmental or immunological factors are the main stimulus to larval inhibition in O. ostertagi, definitive mechanisms for induction, duration, and maturation in the phenomenon are still unknown. Several authors proposed that different strains of O. ostertagi possibly exist, some having a faculty for larval inhibition and others which lack this faculty (Armour et al. 1967a; Armour et al. 1967b; Sollod, 1967; Michel, 1967). This possibility was first detected when a strain of O. ostertagi originally from the Weybridge Laboratory (Armour et al. 1973) was assessed experimentally at Glasgow. The strain was maintained through several passages in calves at Glasgow University and failed to become inhibited in response to the environmental stimuli provided by autumn conditioning in a climatic chamber (Armour and Duncan, 1987).

Recent studies carried out in Australia (Smeal and

Donald, 1981) and in the U.S. (Frank et al. 1986) showed that inhibited larval development is a genetically based phenomenon and the propensity to arrest may be a heritable trait. When cattle infected with the local strain of O. ostertagi were exchanged between two different geographical regions with different climatic conditions (Louisiana and Ohio), in both environments, regardless of the season of pasture contamination, larval inhibition reached a maximum in the same season as in the environment of origin. Consequently, it was concluded that the propensity to larval inhibition in response to different environmental factors was genetically predetermined and the two isolates had not responded to the new environmental pressure. However, with extended time after several generations of selection, different isolates may respond to the new environmental conditions (Borgsteede and Eysker, 1987).

Michel et al. (1976), considered that as arrested larval development could be produced by several causes, the resumption of development by the EL4 stage, may be induced under at least 3 circumstances: (a) a small and constant number of larvae resumes development every day; (b) the degree of resumption of EL4 to the adult stage

is greatly increased when climatic conditions unfavorable to survival of free-living stages end; and (c) the increase in resumption of larval development may occur before favorable environmental conditions appear, as a result of a deterioration of host immunity.

While investigations at Glasgow University (Armour and Bruce, 1974) demonstrated that the EL4 matured approximately 4 months after infection and induction of inhibition, results of investigations at Weybridge (Michel et al. 1976a; Michel et al. 1976b) did not agree with this, and it was suggested that regular turnover of the O. ostertagi population occurred in the host. Because of this, it was added that maturation was not spontaneous as suggested by Armour and Bruce, (1974) although larger proportions of EL4 resumed development during spring at Weybridge.

Unfortunately, the mechanisms underlying resumption of development remain obscure. A switch in the hormonal state of the host could activate the resumption of the EL4, because greater anthelmintic efficiency was reported in cattle which received a luteinizing hormone and anthelmintic simultaneously (Cawley and Lewis, 1975). However, it is not clear if the observed improvement was

due necessarily to a reduction in the worm population. Treatment with pregnant mare serum, luteinizing hormone, stilbestrol (Armour et al. 1975), or luteinizing hormone-like activity of human chorionic gonadotrophin (Cummins and Callinan, 1979) did not improve the activity of anthelmintics against the EL4 stage.

An increase in circulating estrogens in cows close to parturition has been associated with immune-depression and resumption of EL4 development (Wedderburn, 1970). It is unlikely that immune-depression alone could be responsible for maturation of EL4 because calves that were experimentally immune-suppressed with corticosteroid did not yield evidence of resumed larval development (Armour, 1967; Prichard et al. 1974).

MATERIALS AND METHODS:

Approximately 65 Holstein bull calves acquired during fall at an age level of 2-6 weeks, in order to provide minimal age differences, were raised under conditions free of infection with helminth parasites to 4-5 months of age. A group of earliest acquired calves were used in the beginning phase of the experiment and later acquired calves in subsequent phases. The experiment was carried out on an 0.8 ha pasture, which was naturally contaminated with GI nematodes at the LSU Veterinary Medical Research Farm.

Three groups of calves were placed on the same contaminated pasture on 3 successive occasions in spring for both long term and restricted grazing and exposure to parasite infection.

From March 7 to March 14 Group 1 (n=18) calves were grazed on a small holding pasture with minimal parasite contamination in order to accustom the animals to grazing. After this first week of conditioning, group 1 calves were then grazed on the more heavily contaminated experimental pasture until April 4. On April 4, 2 calves from group 1 (tracers) were selected at random and

slaughtered at 12 days after removal from pasture to examine the level of pasture infectivity and worm population characteristics resulting from the initial grazing period of group 1. The remaining 16 calves were randomly divided into 2 subgroups. One subgroup of eight was placed in a concrete-floored pen (confinement) and maintained there until sequential slaughter of two animals on each of the following dates: June 21, July 27, August 30 and September 30. The remaining eight calves were returned to the contaminated pasture and grazed continuously until sequential slaughter of 2 animals on the same dates as indicated for calves in confinement.

In a similar manner, group 2 calves (n=18) were grazed first on the small conditioning pasture (April 4-April 18) and then on the primary experimental pasture from April 18 to May 5. Two (tracer) calves were again randomly selected and slaughtered after a 12-day holding period and the remaining 16 were divided into two subgroups of 8 for confinement and continuous grazing. Sequential slaughter dates for two calves from each of the group 2 subgroups were as designated for group 1 calves.

Group 3 calves (n=18) were conditioned on the small

pasture from May 2 to May 9, and then grazed on the primary experimental pasture from May 9 to May 30. Two calves (tracers) were again randomly selected and killed at 12 days after removal from pasture. The remaining 16 calves were divided into two subgroups of 8 for confinement and for continuous grazing on experimental pasture. Two animals of each of the group 3 subgroups were sequentially slaughtered on the same dates established for group 1 and 2.

From approximately the 1st to the 20th of June, July, August and September, 2 calves each month (also designated as tracer animals) were grazed alongside animals of group 1, 2 and 3 on the experimental pasture in order to continue assessment of monthly levels of pasture infectivity during the entire period of observation, i.e., from March through September.

Herbage larval recovery (L3/kg d.m.) As an additional technique for investigating levels of larval contamination on the experimental pasture, pasture herbage samples were taken between 7:00 and 8:00 a.m. at 2 week intervals using hand clippers. Samples were collected while walking along a w-shaped route at

approximately 10 m intervals. Total herbage collections were pooled in a single plastic bag and averaged approximately 1000 g depending on prevailing moisture levels.

A small portion (50g) of the initial herbage sample was removed for drying in an oven and calculation of the sample dry weight. Herbage samples were washed in tubs with 40 liters of tap water and a few drops of commercial detergent for 24 hours. All herbage was then thoroughly rinsed and discarded. Through settling and decantation in a series of smaller volume buckets and beakers, the fluid volume of samples was reduced to approximately 400 ml. Use of centrifugation in sucrose solution (1200 sp. gr.) was then necessary to separate larvae from remaining debris and silt. Larvae in the sugar solution were then resuspended in 4 liters of tap water to effect settling and recovery of the larvae in a small volume (10 ml) for counting and identification. Larvae were counted and identified in 4 separate drops of fluid from the thoroughly mixed 10 ml. The average number counted in the drops was used to calculate total numbers of larvae present in the sample. Total numbers of larvae in the pasture sample, determined by

extrapolation count from the 10 ml concentration, were expressed in terms of L3/kg of dry matter herbage.

Numbers of L3/kg. d.m. were examined for normality (Skewness, Kurtosis and W:Normal test statistic) (SAS System for Elementary Statistical Analysis, 1987). Raw values obtained were transformed into the Log_{10} (n +1). Correlation analyses (Pearson correlation coefficients) (SAS User's Guide: Statistics, 1985) were conducted for comparisons between herbage larval recovery and worm numbers recovered from tracer calves.

Fecal egg counts (EPG) At two week intervals also, individual rectal fecal samples were collected from all calves to determine egg per gram counts for an assessment of levels of infection and extent of egg output or pasture contamination on experimental pasture. The fecal egg count method used was a centrifugation-flotation procedure using sucrose as the flotation medium. Nematode eggs were floated under a 22 x 22 mm plastic coverslip suspended over a positive meniscus in tubes during centrifugation.

Egg counts were tested for normality (Skewness, Kurtosis and W:Normal test statistic) (SAS System for

Elementary Statistical Analysis, 1987). Counts were transformed to the Log_{10} (n+1) for statistical analyses. Egg count values were also correlated by Pearson coefficients with the total numbers of worms, total worms in the abomasum, and total numbers of O. ostertagi adults. Comparison of means after log_{10} (n+1) transformation, was tested by the General Linear Models procedure. Differences between means were tested by Least Means Squares (LSMeans) (SAS User's Guide: Statistics, 1985).

Fecal Cultures for determining generic composition of nematodes Pooled fecal samples of each calf group on pasture (after EPG counts) were cultured for harvest and identification of infective larvae on a monthly basis. Cultures consisted of feces mixed with vermiculite and necessary tap water; incubation was for 10 days at 24 °C. Larvae were recovered in Baermann funnels. The generic composition of L3 recovered from each calf group was expressed as a percentage.

Plasma pepsinogen (PP) analysis In order to detect

abomasal damage in calves during the experiment, PP values were determined. From the time that each calf group went to pasture initially, blood plasma samples were then collected continuously at 2-week intervals in EDTA-treated vacutainer tubes. PP analyses were determined by the method of Hirschowitz (1955), as modified by Koroto'ko and Islyamova, (1963). Test samples were incubated at 37ºC for 3 hours. After incubation, protein was precipitated with 10% trichloroacetic acid (TCA) and the liberated, phenolic amino acid tyrosine (non-precipitable with TCA) was estimated with Folin-Ciocalteau reagent. Absorbance was measured using a Coleman Junior II-A Spectrophotometer at 560 nm. Pepsinogen determinations were expressed as I.U. of tyrosine per liter.

Pepsinogen values were tested for normality (Skewness, Kurtosis and W:Normal test statistic (SAS System of Elementary Statistical Analysis, 1987). Pearson correlation coefficients were calculated between pepsinogen values and total number of worms in the GI tract, total number of worms in the abomasum, and total number of O. ostertagi EL4, developing 4th stage larvae (DL4) and

adults. Analysis of means after logarithmic transformation Log_{10} (n+1), were tested by General Linear Models procedure. Differences between means were tested by LSMeans (SAS User's Guide: Statistics, 1985).

Counting and identification of parasites recovered from principal and tracer calves at necropsy After slaughter and necropsy of all tracer calves and principal calves on scheduled days, the GI tract and lungs were removed intact and returned to the laboratory for processing.

The abomasum, small intestine, and cecum and colon were each ligated at anterior and posterior ends to be processed individually. The heart-lung complex, including the entire trachea, was processed for recovery of lungworms. The trachea, bronchi and bronchiles of smallest bore were carefully opened with blunt-end scissors and individual worms were recovered and counted. The GI tract was processed for worm recovery according to standard procedures used in the Ruminant Parasitology Laboratory of the Department of Veterinary Science (Williams et al. 1979; Williams et al. 1981).

Each section of the GI tract was placed in a 20

liter tub and opened lengthwise. Contents of each segment were washed into tubs and the mucosal surface was vigorously rubbed and further washed to remove all worms, mucus, and adhering digesta. The volume of washings in the tub was adjusted up to 20 liters with additional tap water. During thorough stirring to achieve a uniform suspension, 2 liter aliquots (10%) were collected by several small withdrawals of fluid and placed in screw-cap plastic bottles. Formaldehyde was added to a concentration of 5 to 10 %. Immediately after initial washing to remove adhering digesta, abomasa were examined for extent of gross pathology and assigned a score from 0 (normal) to 5 (severe). The examination took into account the number and nature of worm nodules or lesions, degree of tissue edema and congestion. Scores were tested for normality (Skewness, Kurtosis and W:Normal test statistics) (SAS System for Elementary Statistical Analysis, 1987). Differences among means scores were tested by General Linear Models and LSMeans (SAS User's Guide: Statistics, 1985).

Immediately after removal of contents and washings, each abomasum was then placed in a bucket of 7 liters volume. Approximately 1 liter of tap water was added to

the bucket and the abomasum was allowed to soak for 24 hours at room temperature (24-26 ºC); care was taken to insure that all mucosal surfaces remained submerged. The purpose of this procedure was primarily to allow immature stages, primarily EL4 to actively migrate out of the tissue. After soaking, the abomasal mucosa was rubbed briskly and kneaded in several washings with small volumes of tap water to remove all mucus and worms. All such washings were placed in the 7 liter bucket until the full volume of 7 liters was achieved. The abomasum was then discarded. While the 7 liter volume of abomasal mucosa washings (soak material) was being thoroughly stirred, a 1 liter aliquot (14.3%) was removed in 4 to 5 withdrawals and placed in a 1 liter, screw-cap plastic bottle. This collection was also preserved with formaldehyde at a concentration of 5 to 10 %. In preparation for worm counts and subsequent identification, worm collection bottles were carefully shaken to achieve a uniform suspension. This was poured into two beakers of 1 or 2 liter size. While fluid was being poured back and forth from one beaker to the other (to insure constant suspension), two 100 ml sub-aliquots were collected. All abomasal and small intestinal

subaliquots (100 ml) were further subdivided after careful mixing with a mag-mixer, into three 10 ml secondary subaliquots. This was quite often necessary because of large numbers of worms in abomasal and small intestinal collections. If fewer than 15 worms were recovered from secondary subaliquots, all worms in the entire 100 ml subaliquot were picked and counted. All final worm counts in 10 ml or 100 ml portions were determined by factoring volumes back to the original bulk volume of 20 liters or 7 liters (abomasal soak-wash). All worms counted were stored in 2 dram vials in 70 % ethanol plus 10 % glycerin. Worms were picked and counted from small amounts of subaliquot fluid in petri dishes of 100 mm (diameter) x 15 mm (depth) with a dissecting microscope. Identification of worm genera or species and stages of development was carried out with a compound microscope (100X) with worms being first cleared in lactophenol. Identification of immature nematodes was according to a description by Douvres (1956).

Worms counts were tested for normality (Skewness, Kurtosis and W:Normal test statistics) (SAS System for Elementary Statistical Analysis, 1987). Before

statistical analysis, the total worm counts were transformed by the formula Log_{10} (n). Percentages of EL4 were subjected to arcsin transformations (Steel and Torrie, 1985) and tested by General Linear Models procedures. Differences among means were tested using LSMeans (SAS User's Guide: Statistics, 1985).

Effect of immune response on worm populations

a. Worm counts

Worm counts and identification were carried out as described above. Total numbers of worms in the abomasum, total numbers of O. ostertagi and total number of worms in each animal were log_{10} transformed for normality and data were tested by General Linear Models procedures. Differences among means were tested by LSMeans (SAS User's Guide: Statistics, 1985).

b. Worm length

Random subsamples of at least 20 adult male and 20 adult female O. ostertagi were selected after counting, sex-differentiation, and identification of worms obtained from continuously grazed calves of group 1. The lengths of all worms in each subsample were measured. Similar-sized subsamples of 20 EL4 male and 20 EL4 female O.

ostertagi were also randomly collected and measured. Length of spicules was measured for 20 adult males.

Measurements and calculations were performed using a morphometry software package (Bioquant, R. & M. Biometrics, Nashville, TN), with an IBM personal computer (IBM, Corp., Boca Raton, FL) attached to a digitizing pad (Hipad Digitizer, Houston Instruments, Austin, TX). This was connected to a microscope equipped with a TV camera and a color monitor.

All data obtained from measurements were tested for normality (Skewness, Kurtosis and W:Normal test statistics) (SAS System for Elementary Statistical Analysis, 1987). Morphometry results are presented as means ± STD. Statistical analyses of measurements were tested by analysis of variance. Differences among means were tested by the Tukey test (SAS User's Guide: Statistics, 1985).

c. Number of eggs in utero

The number of eggs per female worm was determined from 20 specimens recovered from all continuously grazed calves of group 1. Eggs were clearly visible within worms (compound microscope, x100) while placed in lactophenol on microscope slides. Means were tested by analysis of

variance and the Tukey test (SAS User's Guide: Statistics, 1985).

d. Development of the vulval flap

Vulval flap development was scored from 1 (for flap absent) to 6 (for a completely developed flap) in at least 20 O. ostertagi female worms from continuously grazed calves in group 1. Means were tested by analysis of variance and the Tukey test (SAS User's Guide: Statistics, 1985).

Measurement of host immune response

Host immune activity was measured by determining antibody (IgG) response through the Enzyme-Linked ImmunoSorbent Assay (ELISA) (Klei et al. 1981) and the cell-mediated response by in vitro lymphocyte blastogenesis (Klesius et al. 1984).

a. Enzyme-Linked ImmunoSorbent Assay (ELISA):

Blood was collected monthly from all calves in groups 1, 2 and 3. Blood was allowed to clot for 1 hour at room temperature and 3 hours at 4 °C; serum was then harvested following centrifugation at 1,000 rpm for 30 minutes. Samples were stored at -20 °C in aliquots of approximately 2 ml until used. Standard positive and

negative samples were obtained from animals experimentally infected with O. ostertagia and parasite-free animals respectively. Standard dilutions of the positive reference serum used were 1:12.5, 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200.

Serum (IgG) was diluted in a 1:50 concentration, while conjugate dilution was 1:400. All dilutions of standard positive reference serum, and a 1:50 dilution of negative and test serum were performed in duplicate in each plate.

Antigen (Ag) was diluted in 0.1 M CO3 buffer (pH 9.6) and coated to the solid phase polystyrene plates by incubation for 3 hours in a water bath at 39°C. The plates were further incubated for 24 hours at 4°C. Unbound proteins were washed out using 0.1 M Phosphate Buffered Saline (pH 7.4) containing 0.5cc Tween 20 (PBS-Tween 20) and the sera diluted in PBS-Tween was added. Antibodies (IgG), when present in the sample and when specific for the Ag, were bound to the Ag. Serum samples were incubated in Ag-coated plates for 1 hour at room temperature, with constant shaking on a shaker table. Plates were washed with PBS-Tween 20, 3 times for 3 minutes. A peroxidase-labelled anti-IgG (antibovine-IgG

peroxidase conjugate, Sigma Chemical CO) prepared immediately before use, and diluted 1:400, was added to each well. Plates were incubated again for 1 hour at room temperature while shaking. Unbound, labelled anti-IgG was washed with PBS-Tween 20, 3 times for 3 minutes each and a substrate (80 mg of 5 amino-salicylic acid diluted 100 cc of distilled water and mixed 9:1 with 0.05% of hydrogen peroxide solution) sensitive to the peroxidase system was added. The substrate was allowed to react for 30 minutes at room temperature under dark conditions. The reaction was stopped with 0.1 N Na(OH). The amount of Ab in each sample was related to the amount of substrate that was converted. Substrate conversion was measured spectrophotometrically with a Microelisa Reader (Dynatech MR 700) at 450 nm. A mean standard curve was calculated with the Microelisa Reader, from all standard positive serial dilutions. Extinctions that differed more than 2 standard deviations from the comparable mean were not used. From these mean curves, regression lines were calculated. Valid regression lines were based on extinctions of at least 5 dilutions and had a correlation of at least 0.95.

All raw data obtained through the ELISA analyses

were tested for normality (Skewness, Kurtosis and W:Normal test statistics) (SAS System for Elementary Statistical Analysis, 1987). Logarithmic transformation of data was tested using General Linear Models procedures. Differences among means were analyzed by LSMeans (SAS User's Guide: Statistics, 1985).

Antigen preparation:

Several hundred gravid O. ostertagi female worms were recovered at necropsy from a calf experimentally infected with 100,000 L3 35 days earlier. Immediately after collection of the abomasal contents and washing and rubbing of worms from the mucosal surface, the material was clarified by washing over an 80 mesh sieve. The sieved material was then resuspended in tap water. Small portions of the material were then successively examined over several hours until a sufficient quantity of female worms was obtained. The worms were kept in tap water in a 100 ml beaker. The worms and some few eggs were then collected on filter paper in a small funnel. The mass of worms collected was then chopped into small pieces using a scalpel blade in order to free as many fertilized eggs as possible from the worm uteri. Chopped worms,

liberated eggs, and the filter paper on which they were isolated, was then hand blended into a vermiculite culture to which 100 grams of feces from a parasite-free calf and an appropriate amount of tap water were added. A 1000 ml beaker served as the culture vessel. This was covered with aluminum foil, in which a few small holes were made to provide for aeration. The culture was incubated at 24 to 26 °C for 10 days. Infective larvae were harvested in Baermann funnels. Approximately 900 L3 were recovered. These larvae were then used to establish a monospecific infection of O. ostertagi in a donor calf. The donor calf reached patency on day 18 and fecal cultures were prepared during the following 3 weeks in order to accumulate a total of approximately 25,000 L3. Once obtained, these larvae were used to reinoculate the same donor calf in order to build up the level of infection and produce a high rate of egg output for culturing large numbers of larvae. When this was accomplished, fecal cultures were prepared and harvested over the following 6 to 8 weeks to accumulate large numbers of L3 for antigen preparation. Larvae were stored at 4 °C until time for antigen preparation. L3 were exsheathed in a fresh solution of 1.3% sodium

hypochlorite (Clorox Co., Oakland, CA) for 15 minutes and then centrifuged for 5 minutes at 1000 rpm. The supernatant was discarded, while the sediment was rediluted with PBS. This step was repeated 5 times to assure that the L3 were washed free of sodium hypochlorite. The larvae were then concentrated in a vial and subjected to a three-time freeze-thaw technique for disruption, using a dry ice-acetone mixture (-70 ºC) for freezing and passage under a stream of luke warm water for thawing.

In order to stop any protease activity, 2.5 cc of Aprotinin (Sigma Chemical Co., St. Louis, MO) per 97.5 cc of larval suspension, was added to the system. The larval suspension was further disrupted by ultrasound using a Sonifier^{R} cell disrupter, model W350 (Bronson Sonic Power Co, Plainview, NY), with an energy setting at 50 for 8 minutes. The resulting suspension was extracted by stirring overnight at 4 ºC. Particles were removed from the suspension by ultracentrifugation at 100000 g for 10 minutes. The supernatant was collected, sterilized using Acrodisk (4.0 μm) and aliquoted into several vials of 250 μl each. Ag was stored at -70 ºC until used.

Protein concentration was determined spectrophotometrically by the method of Lowry et al. (1951) with a Micro ELisa Reader (Dynatech MR 700) set at 450 nu. Antigen concentration was obtained through the formula:

Ag con.= (Protein concentration x dilution factor)/Ag vol.

The protein-antigen concentration was 218 ug/cc.

Lymphocyte blastogenesis:

A lymphocyte blastogenesis assay was carried out on a monthly basis using peripheral blood mononuclear cells collected from blood samples of all the continuously grazed calves of Group 1. The assay was also performed every month, with blood and abomasal and internal iliac lymph nodes collected from tracer calves which grazed monthly with principal calves. Two parasite-free calves (comparable age to principal calves) were maintained in confinement throughout the experiment and served as uninfected controls.

Mononuclear cells from peripheral blood were separated from other blood cells by centrifugation with

Sepracell (density 1099 gm/cm^3, Sepratech Co., Oklahoma City, OK), at 1500 g for 20 minutes. Lymph node cells were obtained under aseptic conditions by gently teasing them from the excised and opened lymph nodes.

After 2 consecutive 10-minute washes by centrifugation at 300 g in RPMi 1640 (Gibco Laboratories, Grand Island, NY), 25mM Hepes buffer (Gibco) and 2mM L-glutamine (Difco, Detroit, MI), the predominantly lymphocyte layer was tested for viability using trypan blue. The cell suspension was standardized to a concentration of 3 X 10^6 viable cells using a hemocytometer and then treated with the following mitogens: phytohaemagglutinin 60 ug/cc , pokeweed mitogen 20 ug/cc (Sigma Chemical Co. St. Louis, MO).

O. ostertagi L3 antigen was prepared as described above in the ELISA section. Ag was used in concentrations of 0.5 ug/cc and 1.0 ug/cc. All dilutions were carried out in complete RPMI 1640 medium . All cultures were carried out in triplicate. Lymphocyte cultures containing mitogens were made in tissue culture multi-well plates with flat bottom wells (Costar^R, Data Packaging Co., Cambridge, MA). Lymphocyte cultures containing antigen were incubated in tissue culture

multi-well plates with round bottom wells (Linbro^{R}, Flow Laboratories Inc., Mc Lean, VA).

Plates covered with lids were incubated at 37º C in a humidified atmosphere containing 5% CO_2. Incubation time was 48 hours for mitogen-containing cultures, and 72 hours for Ag-containing cultures. After incubation, 0.5 μ Cu/cc ^{3}H Thymidine (New England Nuclear Corporation, Boston, MA) diluted in RPM1 1640 medium with 10% fetal bovine serum (HyClone^{R} Laboratories, Inc., Logan, UT) was added to the lymphocyte cultures.

Eighteen hours after radiolabeling, the cells from each well were harvested on to Titertek filter papers (Skatron AS, Norway) using a Titertek Cell Harvester (Skatron AS, Norway) and dried at room temperature while covered with aluminum foil. Filters were placed in scintillation vials filled with 3 cc of a scintillation fluid solution of 1 liter of Toluene (Mallinckrodt Inc., St. Louis, MO) and 40 cc of ChemFluor-I^{TM}, (ICN Radiochemicals, Irvine, CA).

Radioactivity of each vial was determined using a liquid beta-scintillation counter (Packard Tri-Carb^{R}, model 4530, Packard Instrument Co, Downers Grove, IL) with a gain set for ^{3}H count determination. Background

was determined by placing a blank filter disc in a vial containing only scintillation fluid. Radioactivity counts were carried out for 2 minutes.

Data were expressed as the net count per minute (NCPM). NCPM was calculated by subtracting the mean value of the unstimulated cultures from the mean triplicate, stimulated cultures. Mean values for the uninfected control calves were adjusted to 100% and all other values were analyzed for the percentage difference from the control.

Lymphocyte blastogenesis data were tested using a linear models procedure (SAS User's Guide: Statistics, 1985), and analyzed with the Duncan multiple range test to determine significant differences between means (Steel and Torrie, 1985).

METEOROLOGICAL DATA:

Monthly mean maximum and minimum temperature and total monthly precipitation for all months of the experiment and average monthly conditions of the last 25 years were obtained at a weather station approximately 4.8 km from the research site.

STATISTICAL ANALYSES:

Statistical analyses of data were carried out by the use of the statistical software SAS, Statistical Analysis System (SAS Institute Inc., Cary, NC.

Tests of hypotheses were carried out at 95% confidence levels (α=0.05), except for EPG values where the confidence level was established at 90% (α=0.10).

While large data bases were analyzed using the mainframe at Louisiana State University, the majority of the analyses were carried out using an IBM XT, with 640k of random access memory and a 30 Mb hard disk (International Business Machines Co. Racine, WI).

In order to allow for consistency in the reported results, numbers for all mathematical computations were rounded off using the system described by Blankenship and Campbell, 1976.

RESULTS

Meteorological conditions

There was little difference between maximum and minimum mean temperature during the experiment in comparison to 25-year averages (Figure 1). During the winter of 1988, maximum mean temperature ranged from 11.9 ºC to 20.9 ºC, while minimum monthly mean temperature ranged from 0.8 ºC to 8.8 ºC; during the spring, maximum mean temperatures ranged from 20.9 ºC to 29.6 ºC, while minimum mean temperatures ranged from 8.8 ºC to 14.6 ºC. During the summer period, maximum mean temperature ranged from 31.9 ºC to 32.7 ºC, while minimum mean temperature ranged from 19.7 ºC to 22.3 ºC.

Amounts and distribution of rainfall during the course of the experiment were in general, similar to the 25-year averages. However, current monthly rainfall amounts during the winter period were substantially higher than 25-year averages. In February, 373.1 mm were measured, and in March, 176.1 mm were observed. During May 1988, total rainfall was significantly lower than the 25-year average; only 29.0 mm were registered. The 25-year average for May was 126.8 mm.

From June through September (end of the experiment), only small differences were recorded; monthly rainfall amounts were always slightly less than 25-year averages.

Herbage larval counts on the experimental pasture

Total recovery of infective larvae from herbage decreased from 4,681 L3/kg d.m. in March to 227 L3/kg d.m. at the end of May (Figure 2). Counts increased from June to the end of July, reaching a peak of 2,916 L3/kg d.m. During the summer/fall period, herbage larval counts decreased significantly to levels of 261 L3/kg d.m.

O. ostertagi infective larvae followed the same pattern of total L3 counts. Although O. ostertagi was by far the predominant genus recovered during the entire experiment, total L3 counts were composed of a mixed population of Cooperia, Haemonchus and Oesophagostomum.

While 4,434 L3/kg d. m. of O. ostertagi were recovered in March, the few total L3 recovered at the end of May (227 L3/kg d. m.) consisted entirely of this genus. The peak observed at the end of July was predominantly O. ostertagi (1,823 L3/kg d. m.), while Cooperia spp. were second in prevalence (729 L3/kg d. m.). The few larvae recovered during September through

October, were also a mixed population of similar numbers of Ostertagi, Cooperia, and Haemonchus. Oesophagostomum was recovered in April and during September and October, and always in smaller numbers than the other genera.

Abomasal, intestinal and lung nematodes in tracer calves:

Assessment of worm burdens in tracer calves grazed monthly alongside the principal calves yielded information supportive of the levels of acquisition of the different species by principal calves (Tables 1 and 2). Greatest numbers of O. ostertagi were recovered during the grazing period in March (150,869), while a spring/summer decrease was evident from April to September (6,261, 4,602, 4,809, 1,987, 99 and 378 respectively) (Table 1).

Highest percentages of O. ostertagi EL4 were observed during the spring (57% and 69% in March and April, respectively) and decreased later except in September where a second peak of EL4 was observed (31%). However, the total number of O. ostertagi recovered in the latter case was significantly low (882 worms).

Levels of infection with Haemonchus sp. remained low throughout the experiment, with the smallest number

recovered in April (155) and the greatest number in September (6,199). Trichostrongylus axei infection remained negative or minimum from March to September.

Cooperia spp. were predominant in the small intestine (Table 2), and numbers of these parasites present in tracer calves ranged from 2,450 in April to 48,701 in September. Only minimal acquisition of Oesophagostomum radiatum and Trichuris sp. was observed from March trough September. Levels of infection remained negative or minimal (300-350) during the entire experiment.

The lung parasite, Dictyocaulus viviparus, was not detected from March to May, but a peak of 127 worms was observed in June. Smaller numbers were observed in July and August and no parasites were recovered in September.

The total numbers of infective larvae recovered from pasture grass samples, followed a similar pattern in relation to the total worm counts recovered from tracer calves (Table 3). The Pearson correlation coefficients of total numbers of infective larvae recovered from pasture and the mean total worm counts recovered from

tracer calves grazed monthly with principal calves (Table 4), was $r=0.69$, ($\alpha<0.05$).

Fecal egg counts in principal calves

Initial fecal egg counts in the three groups of principal calves were 0 at the time each group was placed on pasture. Group 1 calves (Figure 3) which were grazed continuously on contaminated pasture, reached a peak (619.3 e.p.g.) at the beginning of May. This was significantly greater ($P<0.1$) than the peak (242.4 e.p.g.) observed in group 1 confined calves. The continuously grazed calves had consistently higher fecal egg counts than confined calves. Fecal egg counts in confined calves decreased significantly ($P<0.1$) to low levels from July through September, when compared with those on pasture.

Egg counts in the continuously grazed calves of group 2 followed the same pattern of those kept in confinement until the end of June (Figure 4). A peak of 969.8 e.p.g. was observed in pastured calves in July; this was significantly greater ($P<0.1$) than mean fecal counts recorded in confined calves (80.0 e.p.g.). The curve of fecal egg counts in both subgroups followed a

similar pattern from late July through the end of August with means remaining statistically different ($P<0.1$). Calves on pasture had higher fecal eggs counts (292, 439.8, 323.3), when compared with those in confinement (134, 74.3, 13.5) respectively. Differences in counts of the two subgroups were also significant in September ($P<0.1$) with the pastured calves having values of 1153.5 and 2042.5 e.p.g., while those in confinement had 18.5 and 66 e.p.g., respectively.

The pattern of fecal egg counts in Group 3 principal calves was similar in both pastured calves and those in confinement (Figure 5). Calves on pasture had a peak of 775.4 e.p.g at the end of June, while those in confinement reached 849.3 e.p.g. in the middle of July. Counts in both groups decreased through the end of August (213 e.p.g for animals on pasture, and 125.3 e.p.g. for those in confinement). Egg counts from pastured calves were significantly greater ($P<0.1$) than those from calves in confinement during September only (532.5 and 451.5 e.p.g, vs. 92 and 68 e.p.g. respectively).

Generic composition of L3 in fecal samples of principal

calves

Identification of infective larvae from cultures of pooled fecal samples of the three groups of principal calves (Figures 5, 6 and 7), revealed that O. ostertagi was the most prevalent parasite through May. Cooperia spp. were present throughout the experiment. With the decrease in O. ostertagi after May, Cooperia spp., were of greater prevalence from June through August. However, in September fecal cultures, O. ostertagi was again the most prevalent parasite.

Numbers of Haemonchus sp. and T. axei observed, remained low or negative throughout the experiment. Oesophagostomum radiatum L3 were observed from June through September, but were most prevalent (30%) during August in Group 1 calves (Figure 5).

Population composition of O. ostertagi in principal calves

The population dynamics of O. ostertagi from calves that were grazed on pasture continuously and those that were maintained in confinement were analyzed first, by the use of actual numbers of worms, and then by use of $\log_{10}$ transformed numbers.

Worm recovery from principal calves placed on pasture during March, April and May and slaughtered at the end of June:

Group 1 calves which were placed on pasture in March (grazed 113 days) harbored a mean of 6,277 adults; 9,938 DL4; 42,064 EL4 (Table 5). The percentage of EL4 was 70%. Group 2 calves, which were placed on pasture in April, and grazed for 85 days, had a mean of 5,588 adults; 4,286 DL4 and 16,646 EL4 (62%). Group 3, which began grazing in March, (57 days), had a mean of 1,664 adults; 232 DL4, and 545 EL4 (22%).

Worm recovery in principal calves placed on pasture during March, April and May and slaughtered at the end of July:

Group 1 calves grazed the experimental pasture for 142 days (Table 6). The mean numbers of worms observed were 3,450 adults; 22,468 DL4, and 84,816 EL4 (76%). In group 2 calves, which grazed for 114 days, the mean numbers of adults, DL4 and EL4 recovered were 3,413; 2,523, and 4,408 (46%), respectively. In group 3 calves which were placed on pasture in May and grazed for 86

days, mean numbers of adults, DL4, and EL4 recovered were 8,989, 1,530, and 9,304 (46%), respectively.

Worm recovery from principal calves placed on pasture during March, April and May and slaughtered at the end of August:

Calves of group 1 (Table 7) which had been placed on pasture in March and grazed for 176 days, had mean numbers of 17,171 adults; 28,212 DL4, and 137,551 EL4 (75%). In group 2, which grazed for 148 days, the mean numbers of adults, DL4, and EL4 were: 1,480; 963, and 3,654 (59%) respectively. In group 3, which grazed for 120 days, the mean numbers of adults, DL4, and EL4 were 625, 718 and 791 (33%), respectively.

Worm recovery from principal calves placed on pasture during March, April and May and slaughtered at the end of September:

After grazing continuously for 206 days, the mean numbers of

adults, DL4, and EL4 in group 1 calves (Table 8) were: 64,656; 35,833, and 84,080 (44%) respectively. Group 2 calves, which placed on pasture in April and grazed for

179 days had mean numbers of 14,049 adults; 1,827 DL4, and 6,891 EL4 (22%). Group 3 calves, which grazed for 151 days, had 2,059 adults; 157 DL4, and 175 EL4 (7%).

Worm recovery from principal calves grazed on pasture for 1 month and then maintained in confinement until slaughter at the end of June:

In group 1 calves, which grazed in March and were then kept in confinement for 85 days (Table 9), the mean number of adults, DL4, and EL4 were 5,809; 14,951 and 78,342 (79%), respectively. In calves of group 2 which grazed in April and were confined for 54 days, the mean numbers of adults, DL4, and EL4 were 7,690; 14,456 and 68,179 (71%), respectively. Calves of group 3, which grazed during May, and were then confined for 29 days, had means of 1,344 adults, 12 DL4, and 174 EL4 (12%)

Worm recovery from principal calves grazed on pasture for 1 month and then maintained in confinement until slaughter at the end of July:

Calves from group 1 which grazed in March and were confined for 114 days (Table 10), had mean numbers of 4,091 adults, 6,465 DL4, and 89,854 EL4 (90%). Group 2,

which grazed in April and was confined for 83 days, had mean numbers of 553 adults, 296 DL4, and 1,738 EL4 (68%). Calves from group 3, which grazed in May and were confined for 58 days had 1,287 adults, 35 DL4, and 190 EL4 (17%).

Worm recovery from principal calves grazed on pasture for 1 month and then maintained in confinement until slaughter at the end of August:

Animals in group 1, (Table 11) which grazed during March, and were confined for 148 days, had mean numbers of 12,906 adults, 16,241 DL4, and 61,433 (68%) EL4. Group 2 calves (grazed in April and confined for 117 days) had mean numbers of 769 adults, 303 DL4, and 1,211 (52%) EL4. Group 3 which grazed in May, and was confined for 92 days had mean numbers of 1,203 adults, 450 DL4, and 2,276 (36%) EL4.

Worm recovery from principal calves grazed on pasture for 1 month and then maintained in confinement until slaughter at the end of September:

Group 1 calves, which grazed in March, and were confined for 179 days, (Table 12) had mean numbers of

48,722 adults; 15,378 DL4, and 35,697 (35%) EL4. Group 2 (grazed in April and confined for 147 days had mean numbers of 8,218 adults, 249 DL4, and 154 (2.6%) EL4. Group 3 (grazed during May and confined for 122 days) had mean numbers of 1,100 adults, and 100 DL4. No EL4 was recovered from this group in September necropsies.

A statistical comparison of mean numbers of O. ostertagi recovered at necropsy from principal calves that were grazed continuously or grazed for 1 month and then confined in pens, are presented in Table 13. In June necropsies, no significant differences were observed in group 1 between continuously grazed calves (58,279) and those kept in confinement (99,102). In September, however, mean numbers of O. ostertagi were significantly different between continuously grazed calves (184,569) and those maintained in pens (99,767). While there were no differences in worm burdens between animals kept in pens from June through September, significantly increased numbers of O. ostertagi were observed in calves slaughtered in September (184,569), when compared with those killed in June (58,279).

With the exception of calves in group 2 (confinement) killed in June (90,325), mean numbers of

O. ostertagi in group 2 were significantly lower than numbers in group 1. While mean numbers of O. ostertagi from calves kept in pens and killed in June (90,325) were significantly greater than those from calves killed in September (8,621), no significant differences were observed among the subgroups kept on pasture from June (26,520) through September (22,767). Mean numbers of O. ostertagi in pastured or confined animals killed in September, were not significantly different.

O. ostertagi worm burdens from animals in group 3 were significantly lower than those observed in group 1 and 2 calves from June through September. Mean numbers of O. ostertagi were not only not significantly different between animals kept on pasture (2,441) and killed in June, when compared with those maintained in pens (1,630), but also there was no increment in the mean number of O. ostertagi in pastured (2,391) or confined calves (1,200) from June through September.

The mean percentages of O. ostertagi inhibited EL4 recovered at necropsy of principal calves from June through September are presented in Table 14. No significant differences were observed in mean percentages of inhibited EL4 from group 1 continuously grazed or

confined calves from June through September. However, while mean percentages during June, July and August ranged from 68% to 90.5% in pastured and confined calves, September values (44% and 34.9%, respectively) indicated a decrease in populations of EL4. There were no significant differences among mean percentages from calves in group 2 whether pastured or confined, with exception of the significantly lower values for confined calves in September. However, while mean percentages of inhibition ranged from 44.5% to 71.5% in pastured and confined calves from June through August, levels of inhibition were substantially lower in both groups in September, particularly in confined calves (2.6%).

Mean percentage of inhibition was generally lowest in animals of group 3 which were introduced to contaminated pasture later (May) than those of groups 1 and 2 (Tables 9-12)). The percentage of inhibition in August-killed, group 3 calves was generally similar to that of other groups. However, significant differences were found between calves from groups 1 and 3. While there was no differences in the mean percentage of inhibition between animals on pasture or in confinement, calves killed in September had significantly lower mean

percentages (6.9% and 0%) than those killed from June through August (17.5% to 46.0%) (Table 14).

No correlation was observed between fecal egg counts and the numbers of adults, DL4, EL4 and total O. ostertagi recovered at necropsy from principal calves of any group (Table 17).

Pathology scores of abomasal mucosa from principal calves

Mean gross pathology scores for the abomasal mucosa of principal calves (pastured and confined) killed monthly are presented in Table 15. No consistent significant differences were observed for pathology scores of calves in the three groups. However, in group 1 (5.0, 5.0) and group 3 (1.7, 2.5), higher scores were recorded in September, for both pastured and confined calves than those observed in June for the three groups (group 1, 2.5, 3.2; group 3, 0.75, 1.6). In group 2, no differences were recorded, either between pastured animals (3.5, 2.0, 3.2, 3.7) or those in confinement (3.2, 2.5, 3.2, 3.0), or from beginning to end of the experiment. Classical lesions of ostertagiasis observed were umbilicated nodules, some of which were coalesced

into masses, with hyperemia and edema of the mucosa.

Total numbers of all worm genera (TNW) recovered at necropsy from principal calves

O. ostertagi was the most prevalent abomasal parasite observed throughout the experiment. Haemonchus sp. and T. axei were recovered in lower numbers (Tables 5, 6, 7, 8, 9, 10, 11 and 12). Worm burdens of the intestinal tract were predominantly Cooperia spp. Of the several species present, C. punctata and C. surnabada were most numerous with C. oncophora and C. pectinata being least numerous. Oesophagostomum radiatum and Trichuris sp. were observed in the cecum and large intestine. However, mean numbers of these parasites were never in excess of 2000 (Table 8). Calves in Group 1 always had greater numbers of worms than the other groups, with exception of the mean total numbers of worms (164,708) recovered in September from group 2 confined calves (Table 16). No significant differences were found between group 1 pastured or confined calves (Table 16). However, pastured calves that were killed in June, had significantly smaller numbers of worms than those killed in September (78,426 and 244,064 respectively). Total

numbers of worms in confined calves that were killed in June, did not differ significantly from those killed in September (102,243 and 101,347 respectively).

In group 2 pastured calves, mean total numbers of worms recovered in June, although smaller than numbers recovered in September, did not differ significantly (58,713 and 164,708 worms respectively). However, significantly smaller numbers (9,935 and 103,769 worms, respectively) were recovered in September than in June from group 2 confined calves (Table 16). On the other hand, significantly smaller numbers of worms were recovered from confined calves in July, August and September than from pastured calves (Table 16). Mean total numbers of worms recovered in June from pastured and confined calves of group 3 did not differ significantly from numbers recovered in September (Table 16). Although there were no significant differences between mean numbers of worms recovered from pastured or confined calves in June (14,043 and 9,553 respectively), significantly greater numbers of worms were recovered in July, August and September from pastured calves (148,769; 61,013 and 42,246) than numbers from calves in confinement (4,412; 8,163 and 2,235) respectively.

Plasma pepsinogen analysis

Mean values for calves in group 1 are shown in Figure 9. Both pastured and confined calves had an initial increase in mean pepsinogen values, (from 419 and 294 i.u./l to 1651 and 1877 i.u./l, respectively) during March and April. Values remained stable from the middle of May until the end of June. Clinically important levels (<1,800 i.u.) were not observed until July; at this time, values in pastured calves (1,954 i.u.) were significantly greater than those (924 i.u.) in confined calves. Although pepsinogen values for confined calves were also increased substantially from July onward, values for the pastured calves remained consistently greater ($P<0.05$) until the end of August. Mean values in pastured calves reached a peak of 2,930 i.u. in late August; at this same time, values in confined calves remained below the clinically important level (1,539 i.u.). Mean pepsinogen levels at the end of the experiment in September were 2,857 i.u. and 2,307 i.u. for pastured and confined calves respectively.

Initial pepsinogen values in April in the group 2 pastured and confined calves were 558 i.u. and 628 i.u.

respectively (Figure 10). Values in the pastured subgroups remained significantly greater than those of the confined calves from late May until early September. Mean pepsinogen values of pastured
calves reached a peak of 1,717 i.u. on August 15, while values for confined calves were 1,267. Final mean values for pastured calves in late September were 1,436 i.u., those in confined calves were 1,314.

Low initial mean plasma pepsinogen values were also observed for group 3 calves which were placed on pasture in May (Figure 11). These low values were 435 and 561 i.u. for the 16 calves which would be divided into confined and pastured subgroups after the first month of grazing. Both subgroups followed a similar pattern, with values below 1000 i.u. through June. Mean pepsinogen values were significantly greater in pastured calves from July 11 to August 15, reaching a peak of 1,914 i.u.; confined calves had a mean of 1,018 i.u. Mean values were similar for both subgroups at the beginning of September, but final values measured at the end of the experiment were higher in confined calves (1,681 i.u.) than in the pastured calves (1,475 i.u.).

Correlation analysis among PP and total numbers of

O. ostertagi adults, DL4, EL4, total O. ostertagi population and total numbers of worms in the abomasum, were r=0.60; r=0.78; 0.64; r=0.72 and r=0.71 respectively (Table 17).

Enzyme linked immunosorbent assay (ELISA) test

Anti-O. ostertagi antibody titers of group 1, measured by the ELISA test, are shown in Figure 12. At the time of the initial analysis on March 7, mean titers were 21.2 and 55.9 for pastured and confined calves, respectively. There were baseline values as the calves had not yet been subjected to infection exposure or divided into subgroups. A progressive increase was observed in both groups until May 30 when titers were 1,213.4 and 1,296.3 for pastured and confined calves, respectively. After this time, values for the two groups diverged. Titers for pastured calves reached a peak of 3,318.7 on July 25 and this was significantly greater than the corresponding titer (1,666.5) of the confined calves. At the end of the experiment on September 27, the mean antibody titer of pastured calves (3,008.5) was significantly greater than the mean in confined calves (869.2).

Mean anti-O. ostertagi titers of group 2 (Figure 13) at the initial examination on April 7, were 93.8 and 84.3 (baseline values) for pastured and confined calves, respectively. Values in both subgroups remained below 1,100 until May 30, but subsequently, significantly greater values were observed in continuously grazed calves on June 24 (2,659.3) and July 26 (2,408.9). Values observed for the confined calves on the corresponding dates were 882.8 and 967.1, respectively. Although mean antibody titers in pastured (2,111) and confined calves (1,628.4) were not significantly different on August 29, values for the pastured calves (5.763.0) were significantly greater than those of confined calves (1,513.3) at the end of the experiment (September 27).

Mean anti-O. ostertagi antibody titers in group 3 at the beginning of the experiment, (Figure 14), were 66.1 for pastured calves and 46.4 (baseline values) for those in confinement. Titers increased substantially from May 30 through June 24 (238.3 to 2,708.5 for pastured calves and 475.5 to 2,253.9 for confined calves). Mean titers were slightly increased in pastured calves on July 26 (to 2,997.6), but decreased slightly

(2,092.2) in confined calves. Considerable reduction in values was observed for both groups between July 26 and August 30, particularly for the confined calves, but pastured calves reached a peak mean titer of 3,152.3 on the final sampling date (September 27). This was significantly greater than the low mean titer of 712.0 in confined calves. No correlation was observed between ELISA titers and O. ostertagi adults, DL4, EL4 and total numbers of O. ostertagi (Table 17).

Lymphocyte proliferation assay in blood of continuously grazed principal calves

Calculation of the percentage difference between mean NCPM of uninfected control calves and continuously grazed principal calves of group 1 indicated no differences when lymphocytes were stimulated with PHA during week 0 and week 4 (Figure 15). However, a significant increase in the mean percentage NCPM of pastured principal calves (1098) was observed in week 8. The mean percentage NCPM in pastured calves was significantly greater than in uninfected control calves until week 24 to 28; differences were not significant thereafter.

The mean percentage NCPM of lymphocytes from pastured calves of group 1, stimulated with PWM (Figure 16), was similar to that of uninfected controls from week 0 to week 4. A significant increase in the mean percentage NCPM of pastured calves over that of uninfected controls was observed at week 8 (1,276) and week 12 (886). From week 16 to week 28, significant differences between mean percentages NCPM of pastured principal calves and uninfected controls were not observed.

After stimulation of lymphocyte cultures with O. ostertagi antigen at a concentration of 0.5 μg/cc the mean percentage NCPM from pastured calves was less than the mean percentage NCPM of uninfected controls (Figure 17), although differences were not significant from week 0 to week 20. A significant increase, was observed in the mean NCPM of pastured calves in week 24 only. When lymphocyte cultures were stimulated with O. ostertagi antigen at a concentration of 1 μg/cc (Figure 18) the mean percentage NCPM from pastured calves was similar to that of the uninfected controls from week 0 to week 16 (Figure 18). However, the mean percentage NCPM of pastured calves increased significantly in week 20 and

week 24, and then decreased to levels not significantly different from uninfected controls in week 28 (200).

Lymphocyte blastogenesis in lymph nodes and in blood of tracer calves

The mean percentages NCPM for lymphocyte cultures from blood, and abomasal and internal iliac lymph nodes from tracer calves stimulated with PHA are shown in Figure 19. Mean percentages NCPM from abomasal lymph nodes (43,408.2) were significantly greater than those from internal iliac lymph nodes (17,183.2), tracer calf blood lymphocytes (9,563.2) and, blood lymphocytes from uninfected controls calves (3,682.2).

Mean percentages NCPM from lymphocytes of abomasal lymph
nodes (48,307.4) (Figure 20), stimulated with PWM were significantly greater than those from lymphocytes of internal iliac lymph nodes (21,069.4), blood lymphocytes of tracer calves (20,443.3), and blood lymphocytes from uninfected controls (15,901.5).

The mean percentages NCPM from lymphocytes of abomasal lymph nodes (5,466.4) stimulated with O. ostertagi antigen (0.5 μg/cc) was significantly greater

than values from lymphocytes of internal iliac lymph nodes (365.5), blood lymphocytes of tracer calves (757.5), and blood from lymphocytes from uninfected control calves (573.7) (Figure 21). The same pattern was observed (Figure 22) when lymphocyte cultures were stimulated with O. ostertagi antigen at a concentration of 1.0 μg/cc, since significantly greater percentages NCPM were observed in lymphocytes of abomasal lymph nodes (9,521.2) in comparison to lymphocytes from internal iliac lymph nodes (700.2), blood lymphocytes from tracer calves (799.3) and from uninfected control calves (896.7).

Mean lengths of O. ostertagi adult worms

Mean lengths of O. ostertagi males and females are shown in Table 18. Mean lengths of O. ostertagi males recovered in March (7.5 mm), were significantly greater than those recovered in September (6.3 mm). Lengths of female worms varied from 9.7 mm, in those worms recovered in March to 7.0 mm for those recovered in September. The difference was statistically significant.

Mean lengths of O. ostertagi EL4

Mean lengths of O. ostertagi EL4 males recovered from March through September, ranged from 1.1 to 1.2 mm (Table 19). Values obtained in March were not significantly different from those obtained in September. A similar pattern was observed in the mean lengths of O. ostertagi EL4 females, which ranged from 1.2 to 1.5 mm; differences were also not significant in this case.

Vulval flap development

The mean vulval flap scores for parasites recovered from the first set of tracer calves (March) and group 1 pastured calves killed monthly from June through September, are shown in Table 20. Mean scores ranged from 4.1 in September to 5.0 in March and July. Mean scores of 5.0 for calves killed in March, were not significantly different from the 4.1 scores of calves killed in September (Table 21).

Numbers of eggs in utero of O. ostertagi females

Mean numbers of eggs in utero of O. ostertagi females recovered at necropsy from group 1 principal calves grazed on pasture continuously and from the first set of tracer calves (March), are shown in Table 21.

Mean numbers of eggs ranged from a low of 16.2 in August to a high of 25.5 in March. Mean numbers of eggs in March, were significantly greater than numbers observed in July (18.1), August (16.2), or September (16.5).

Spicule length of O. ostertagi males

Mean spicule lengths of O. ostertagi males recovered from group 1 principal calves maintained on pasture continuously and from the first set of tracer calves (March), are shown in Table 22.

Mean spicule lengths ranged from 224.4 mm in September to 232.5 mm in March. Mean spicule lengths were 231.9 mm in June, 230.9 mm in July and 231.1 in August. Although spicules measured in September were shorter than in March, no significant differences were detected at any time.

DISCUSSION

The weather pattern which prevailed during the present experiment was basically similar to the 25-year average for the Baton Rouge area with a few exceptions. The abundant rainfall during February and March of 1988 had a marked influence on the levels of pasture contamination and infectivity at the beginning of the experiment. Both the peak of pasture contamination evidenced by recovery of larvae from herbage samples and the peak of pasture infectivity indicated by worm counts in tracer calves, were particularly important in March. Maximal temperatures and rainfall, well below normal in April and May, appeared to be responsible for the small number of L3 recovered from herbage samples during May and June, and the significantly low worm burdens observed in tracer calves from April through the remainder of the experiment.

The epidemiological data obtained from pasture larval samples and worm counts in tracer calves in the present experiment confirmed previous observations in the southern United States, which established that inhibition-prone larvae were acquired by cattle primarily from late winter through spring (Williams et al. 1983;

1987). High percentages of EL4 were observed during March and April, but the percentage of EL4 and total numbers of O. ostertagi diminished from July through September as observed in data from tracer calves. Total O. ostertagi worm burdens and total numbers of all worms in tracer calves decreased from May to September, also confirming previous findings in Louisiana (Williams et al. 1983). However, while O. ostertagi populations decreased during summer, there was a slight increase in numbers of Haemonchus sp., Cooperia spp. and Oesophagostomum radiatum in tracer calves; this suggested that the infective L3 of these parasites may better survive the adverse conditions of hot and wet or hot and alternatively wet and dry weather conditions of summer in Louisiana (Williams and Mayhew, 1967; Williams and Bilkovich, 1971; 1973).

The relatively good correlation (r=0.6955) between the total numbers of infective L3 recovered from herbage samples and the mean total worm counts recovered from tracer calves (grazed monthly with principal calves) during March through September, was in accord with results of previous investigations (Cabaret et al. 1982). Thus, these data provide support for pasture herbage

sampling as a reliable epidemiological technique, when the procedure is carried out properly.

Mean numbers of O. ostertagi recovered from group 1 principal calves which were placed on pasture in March, were significantly greater than in group 2 and group 3 calves, which were placed on pasture in April and May, respectively. Group 1 calves were exposed to a greater availability of L3 in March than the other groups in April and May. During the final month of the experiment in September, greater numbers of O. ostertagi were recovered from continuously grazed calves than in the confined calves. Although differences were not always statistically significant, the small number of samples (n=2 in each case), was probably responsible for the α error in which a false hypothesis could not be rejected (Steel and Torrie, 1985). A similar feature was observed when the mean percentage of O. ostertagi inhibited EL4 recovered from June through September was examined. Higher percentages of EL4 were observed from June through August than in September; a change in the O. ostertagi population was clearly visible in September when low percentages of EL4 were recovered (differences were not always statistically significant). However, increased

numbers of DL4 and adult worms recovered from principal calves in September indicated a large scale triggering of resumed development of spring-acquired EL4. Even though significant differences could not in all cases be demonstrated, the results provided good evidence that the maturation of EL4 was spontaneous in a particular time period (Armour et al. 1969; Armour and Bruce, 1974).

The regular turnover of O. ostertagi populations in the host during the period of larval inhibition as proposed by Michel et al. (1976), cannot be discounted in the present experiment because large numbers of O. ostertagi L3 always were observed in routine fecal cultures (for generic composition of potential pasture contamination) of feces from principal calves, and because there was continued possibility for pastured calves to acquire infection from pasture (low availability in summer, based on tracer calf data). However, if turnover of population did occur, it was not a necessary condition for larval inhibition, because calves in confinement acquired similar levels of EL4 as those that remained on pasture. Eggs counts in all continuously grazed calves were generally greater than those in confined calves; counts in pastured calves were

significantly higher in the last 3 months of the experiment. Although the second peak of total L3 and particularly, the peak of O. ostertagi L3 recovered from herbage samples at the end of July, were not well-correlated with the numbers of O. ostertagi recovered in tracer calves. Eggs counts of the 3 continuously grazed subgroups were significantly greater than those in confined calves after July. It was therefore possible that the peak of infective L3 on pasture in July was responsible for reinfection of principal calves with O. ostertagi, and consequent increase in numbers of adult worms and egg counts.

Acquisition of inhibition-prone larvae in the present work, followed the same pattern observed by Armour et al. (1973) in laboratory experiments and by Williams et al. (1983) under field conditions. Higher levels of EL4 were observed in those calves which were introduced to pasture in March and April in comparison to those introduced in May. It was clear that maximal transmission of infection occurred during early spring and decreased sharply in May, following extended dry weather which began in April. These results confirmed previous observations in Louisiana, which have indicated

that few to no O. ostertagi larvae remain on pasture after late May and early June (Williams et al. 1983; 1987).

As earlier reported by Williams et al. (1987), acquisition of inhibition-prone EL4 in the present work occurred during a relatively short period of time (March-April), when there was an increase in temperature associated with inadequate moisture. On the other hand, resumption of development took place during September, when temperatures decreased and moisture levels had increased earlier (July and August).

Larval inhibition of O. ostertagi may be associated with various factors such as acquired immunity by the host (Michel et al. 1979), a density-dependent mechanism of worm populations (Anderson et al. 1969; Smeal et al. 1977) and seasonal changes (Armour and Duncan, 1987). However, because principal calves placed on pasture in March, April and May had no previous exposure to O. ostertagi infection, host immunity would not likely have been a contributing factor to the high mean percentages of inhibited EL4 observed in the present experiment. Also, because the percentages of inhibited EL4 in continuously grazed calves were similar to those in

confined calves, density-dependent mechanisms (Schad, 1977) did not appear to be important.

Results of the present work have confirmed previous evidence in Louisiana that larval inhibition in the region is mainly related to seasonal factors, i.e., large numbers of inhibited larvae were found to occur simultaneously at a particular time of year in naive calves, without previous experience of infection (Williams et al. 1983;1987).

Although laboratory experiments at Glasgow (Armour et al. 1973) have shown that the duration of the period of inhibition was between 4 and 5 months, results in the present experiment indicated that inhibition lasted up to 6-7 months (March to September in group 1). Indication of significant maturation of EL4 in group 1, 2 and 3 did not occur until September.

Higher mean gross pathology scores of abomasal mucosa were observed primarily in continuously grazed group 1 calves that were exposed to infection initially in March, and sequentially killed from June through September. No differences were observed between groups kept continuously on pasture and those maintained in confinement. It is possible that levels of infection on

pasture were low during June through August and this could have been responsible for the similarity of pathology observed in both continuously grazed and confined calves. A significant gradation in scores of the 3 groups at the end of the experiment in September, indicated that the mucosal damage was related to the time period in which the animals acquired the initial infection. This was true, regardless of whether the calves were continuously grazed or confined and free of reinfection and/or to duration of pre-type II interval (Snider et al. 1988) and level of pasture infectivity during the period of infection. These results confirmed previous observations in Europe (Nansen et al. 1987), in which a delayed spring turnout of calves to pasture resulted in lighter infections with less pathological effects, than those in calves turned-out earlier. Although clinical signs of ostertagiasis were not observed at any time during the present experiment, higher mean gross pathology scores in group 1 calves were the result of edematous and congested abomasa, with practically the entire mucosal surface covered by umbilicated nodules (Snider et al. 1986; Williams et al. 1983; 1987).

Total mean numbers of O. ostertagi recovered at necropsy from principal calves in September, were higher, but not significantly different from those recovered in June. This was particularly important in calves of the three groups grazed on pasture continuously, because no worm count reductions related to immunity were observed after 5 months of continuous reinfection. Immunity to Ostertagia in cattle is controversial and apparently wanes from one season to the next (Armour et al. 1973). Entrocasso et al. (1986) have shown that yearling cattle which acquired infections of Ostertagia and Cooperia in their first grazing season were immune to natural challenge in their second grazing season. However, Armour et al. (1988) reported that unexpectedly large and clinically significant burdens of O. ostertagi inhibited EL4 were observed in second year grazing cattle, while fecal egg counts had remained low. On the other hand, under experimental conditions in which calves were infected with infective L3 or received surgical transplantation of adult worms, no significant differences in the percentage establishment of O. ostertagi adult worms were observed between calves previously infected and those which had been raised free

of parasite infection before the experiment (McKellar et al. 1987).

Results of antibody titers to O. ostertagi infections in the present experiment using an ELISA test, presented a similar pattern as observed in the results of plasma pepsinogen analysis. This confirmed observations of Entrocasso et al. (1986), who worked with naturally infected cattle in Scotland. However, while ELISA titers in continuously grazed calves were significantly increased at the end of the experiment when the O. ostertagi population was composed largely of adult worms and developing L4, titer in confined calves remained significantly lower than those on pasture, even during September as resumption in development of inhibited EL4 was also observed in this subgroup. As pointed out by Entrocasso et al. (1986) the higher titers of calves on pasture, may be due to constant reinfection, and maturation of inhibited EL4 (during type II phase); however this has not been adequately described. On the other hand, the increase in antibodies observed in continuously grazed calves subjected to constant reinfection confirmed the observations of Klesius et al. (1986). These investigators worked with experimental

infections and observed significant increases in IgG-antibody levels only after challenge inoculations, late in the course of an immunizing infection.

Even with significant increases in antibody response in the present work, no effect on worm burdens was observed. It is possible that this was a result of low antigenic stimulation from April through August due to the low level of pasture infectivity and concomitant low levels of reinfection (Williams et al. 1983). Another possible explanation was presented by Klesius et al. (1986) and Klesius (1988), in which it was pointed out that the role of antibody in limiting parasitic infections, appears to be the inhibition of metabolic processes and growth and reproductive capacities. This may be due to mechanical blockage of worm orifices, to inhibition of enzymes or to inhibition of feeding mechanisms (Klesius et al. 1986). Immunity to O. circumcincta, measured either in terms of loss or stunting of worms, was attributed to local IgA production and mucosal mastocytosis, although the actual mechanism which caused stunting, or worm expulsion remains a matter for speculation (Smith et al. 1986). Results in the present experiment confirmed previous observations, in

that mean lengths of O. ostertagi adult male and females worms, recovered from principal calves grazed on pasture continuously, were significantly smaller than worms recovered from March tracer calves, which grazed for only 25 days. Additionally, mean numbers of eggs in utero for O. ostertagi females from principal calves grazed continuously until September, were significantly lower than those recovered from March tracer calves. However, alteration of the vulval flap in female worms by host immune response, as described by Michel (1969c), was not confirmed in the present work. Although flap scores of O. ostertagi females recovered from continuously grazed principal calves (killed in September) were lower than those recovered from March tracer calves, the differences observed were not statistically significant. It is possible that significant differences would have been present, if the infections had persisted beyond September. This also introduce a question which has not been reported and concerns whether or not alteration of the vulval flaps and other morphological and reproductive capacities are affected in adults resulting from maturation of previously inhibited larvae as they are in normally developing worms (Williams, 1989).

The greater numbers of O. ostertagi adult worms observed in calves killed in September, was most likely due to the general maturation of spring-accumulated EL4. According to Williams et al. (1983), an increase in numbers of adult O. ostertagi and resulting cases of clinical parasitism was due to maturation of large numbers of EL4 rather than acquisition of new infection. An additional explanation for the presence of larger numbers of worms could be that immunity to O. ostertagi not only develops very slowly in calves over a lengthy period of exposure to the parasites (Michel, 1963), but is also incomplete (Klesius, 1988) and low (Keus et al. 1981).

The mean total numbers of all worm genera recovered at necropsy from principal calves corresponded in magnitud to that of O. ostertagi. Although no consistent significant differences were observed at the end of the experiment in September between calves grazed continuously and those maintained in confinement, greater numbers of worms were always recovered from continuously grazed calves throughout the experiment. In this case also, no evidence of immunity was observed. Even when total worm numbers consisted not only of O. ostertagi,

but also of Haemonchus sp., Cooperia spp., Oesophagostomum radiatum, and a few Trichostrongylus axei, more worms were recovered from those calves continuously grazed and killed in September, than in continuously grazed calves killed in June.

Fecal egg counts of continuously grazed calves increased, and remained at levels that were significantly different from those of calves kept in confinement in all three groups. On the other hand, while EPG in confined calves decreased after their brief monthly exposure to pasture in March, April, or May, counts of continuously grazed calves remained higher throughout the experiment, suggesting at least, a minimal level of reinfection during extended dry weather. This reinfection consisted not only of O. ostertagi, but also of Haemonchus sp., Cooperia spp. and Oesophagostomum radiatum. Pasture larval sampling indicated that even though numbers were minimal, some O. ostertagi L3 were also present in reinfection. Damage to the abomasal mucosa occurs as EL4 grow, develop in size to adult stage, and emerge from the gastric glands (Murray et al. 1970; Wiggin et al. 1987). This damage affects epithelial cells particularly. Depending on the level of infection and damage caused,

pepsinogen is not activated due to the lack of hydrochloric acid, but leaks into the circulation via the open "cell-tight junctions" (Armour and Ogbourne, 1982). Present results of plasma pepsinogen analyses in the 3 groups, showed 2 independent peaks in both pastured and confined subgroups. The first was observed approximately 1 month after calves were placed on pasture, while the second peak appeared during August-September. Obviously, the first peak was the result of infective L3 acquired during the spring, while the second was the result of the maturation of inhibited EL4 to adult worms. Higher PP values in group 1 were associated with greater levels of O. ostertagi infection than in the other two groups. Correlation (Pearson) of numbers of adults, DL4, EL4, total O. ostertagi and total numbers of worms in the abomasum (r^2=0.60, 0.79, 0.65, 0.72, and 0.71, respectively) with PP values, confirmed results of Entrocasso et al. (1986) and Schillhorn-Van Veen (1988). The highest level of correlation was observed between DL4 and PP. These results are not in accord with those of McKellar et al. (1987), who observed elevated PP values in previously worm-free calves which had O. ostertagi surgically transplanted into their abomasa. Although PP

values above 2,000 i.u./l were observed only in group 1 calves and values near 1,700 in group 2 and 1,900 in group 3, no evidence of clinical disease was observed at any time during the experiment.

Plasma pepsinogen values in continuously grazed calves were significantly higher than those in confined calves, particularly during July and August. However, both subgroups reached similar levels at the end of the experiment in September. Higher mean PP values in continuously grazed calves may be explained by constant reinfection, not with O. ostertagi alone, but also with Haemonchus sp. In this context it must be considered that a long period of time is required for the return of PP levels to normal values, and in animals continually exposed to reinfection, the recovery period is prolonged (Brunsdon, 1972). In contrast to the pastured calves, elevation of PP values in confined calves in September was with little doubt due to the simultaneous development of large numbers of EL4.

A significant increase in percentage NCPM was observed in PHA stimulated lymphocyte cultures from week 4 to week 20 when more than 70% of the O. ostertagi population was in the inhibited state. Mean percentages

NCPM decreased after week 20 to levels similar to those obtained in uninfected control calves until the end of the experiment (week 28) at the time the inhibited larvae began to mature to adult parasites.

Mean percentage NCPM for lymphocyte cultures stimulated with PWM were significantly higher than those of control calves from week 4 to week 12 only; values decreased and were similar to those of uninfected control calves from week 16 to week 28.

These data were the first to be obtained from lymphocyte blastogenesis responses in calves during pre-type II ostertagiasis. Previous studies under conditions of experimental type I infection with O. ostertagi and T. axei, showed a significant depression of responses to PHA 8 weeks after infection, while lymphocyte responses to PWM in infected and uninfected control calves did not differ (Snider et al. 1986). However, Cross et al. (1986) working with O. ostertagi experimental infections, could only confirm immunosuppression when cultures were stimulated with concanavalin A; no significant differences with uninfected control calves were obtained in those lymphocytes cultures stimulated with PHA. It is

important to mention that the conclusions of Cross et al. (1986) were not clear, because, while there was a suggestion that cattle continually exposed to O. ostertagi may be repeatedly immunosuppressed, in the same paper a reference is cited which indicated that prolonged or repeated exposure to O. ostertagi was necessary for the induction of a partial resistance (Holtenius et al. 1983).

On the other hand, Snider et al. (1986) did not observe any depression up to week 8 after experimental infection, when lymphocyte cultures were stimulated with concanavalin A. However, there was a significant elevation in mean percentage of NCPM at week 10. Snider et al. (1986) explained that differences obtained in the different experiments may have been due to differences in genetic background of the animals, environmental factors, mitogen characteristics or technique variations between laboratories. Kristensen et al. (1982) attributed such altered lymphocyte blastogenesis responses to several factors such as age, sex and nutritional status of the animals. Alternatively, different levels of conditions of experimental and natural infections and variations of immune response to

the several genera that infect cattle, may also be responsible for variation of results obtained by different investigators, including those of the present experiment.

The increase in mean percentage of NCPM in lymphocyte cultures stimulated with PHA and PWM may be the result of the presence of large numbers of O. ostertagi (in pre-type II and type II phases) which could elicit an immune response different in quality and quantity from the response produced under the more prompt type I phase. Additionally, the decrease in mean percentage NCPM, both in PHA and PWM stimulated lymphocytes cultures during the last period of the experiment (after week 16 and week 24), may have been due to a decrease in lymphocyte responsiveness during the nematode maturation phase. This has been shown to result in a rise in plasma histamine or histamine-like substances, which depressed responses to both mitogens (Snider et al. 1986) or due to generation of T-suppressor cells which are produced in response to the interaction of the immune system with the parasite antigen (Klesius et al. 1981).

Lymphocyte cultures from continuously grazed calves

of group 1, stimulated with O. ostertagi L3 antigen in two different concentrations (0.5 and 1 μg/cc), did not show any difference compared to uninfected controls from week 0 to week 16. However, a significant increase was observed in those cultures stimulated with 1μg/cc L3 O. ostertagi antigen from week 20 to week 24. Values decreased to levels equivalent with those of uninfected controls in week 28. Lymphocyte cultures stimulated with 0.5 μg/cc of O. ostertagi antigen reacted similarly, but the increase in mean percentage of NCPM was significant only during week 24. Taking into account that the main change in the O. ostertagi population from the inhibited EL4 to adult stage occurred during August/September (week 24 to 28), the present results confirmed previous data reported by Klesius et al. (1984), which indicated highly significant reactivity of lymphocyte cultures stimulated with O. ostertagi antigen during patent ostertagiasis. Results in the present experiment appeared also to support the existence of cell-mediated immunity during emergence of O. ostertagi from the glandular epithelium, but in the present case, during the type II phase of the infection. It is well known in many infectious diseases and parasitic infections, that after peak lymphocyte

stimulation has been reached, lymphocyte responses decrease and sensitive lymphocytes may no longer be detectable in peripheral blood after some weeks or months (Kristensen et al. 1982). It is also important to consider that the lack of lymphocyte response during the first weeks of infection, may be due to the fact that immunologically naive calves have relatively high frequencies of Ostertagia-specific cells in peripheral blood. After infection these frequencies decrease due either to an active suppression of the potential anti-Ostertagia response or an extra-vascularization of these cells to the site of infection (Gasbarre, 1986).

When PHA, PWM, and O. ostertagi L3 antigen (0.5 and 1 μg/cc) were used to stimulate leukocyte cultures from abomasal and internal iliac lymph nodes of infected tracer calves and compared with leukocytes obtained from peripheral blood of tracer calves, and those obtained from uninfected controls, mean percentage NCPM of leukocytes from abomasal lymph nodes always showed a significantly greater response. Although there were no relevant references for this observations, Hay et al. (1974) observed increased numbers of sensitized lymphocytes in the efferent lymph of single abomasal

lymph nodes of sheep after immunization. After presentation of specific antigen into a lymph node of sensitized calves, lymphocyte depletion could be the result. A selective removal of sensitized lymphocytes from the circulating pool might explain the negative or low lymphocyte reactivity to mitogen or antigens when peripheral blood lymphocytes are used (Kristensen et al. 1982).

Results of this dissertation research have provided confirmation of previous investigations which documented the population dynamics of Ostertagia ostertagi during the period of larval inhibition in Louisiana (Williams, 1986). The present work, however, has demonstrated additional perspectives of the inhibition phenomenon from both the parasite and host aspects. Measurement of morphological as well as reproductive characteristics were associated with quantitative changes in worm populations over time. These, and the analyses of cell-mediated and antibody-related immune responses, may be of possible support and relevance in understanding of ostertagiasis in yearling cattle. It was considered that the use of a relatively homogeneous lot of young dairy breed cattle raised free of infection with nematodes,

would provide appropriate baseline data for the various parameters examined. It may be considered also, that the qualitative and quantitative nature of the O. ostertagi inhibition phenomenon may vary to some degree from year to year depending primarily upon minor or major variation in weather conditions and consequent variation in host nutrition and immune response. It is the hope of this author that the present work offers additional understanding of a complex biological phenomenon which can be very critical in cattle production. It would be most gratifying if these data might stimulate additional research or be used to formulate even more precise and rational control measures for O. ostertagi than presently exist.

Table 1. Mean worm counts (abomasum) from tracer calves (n=2) grazed each month on experimental pasture with principal calves

Grazing period	(days)	Ostertagia ostertagi					Haemonchus sp.		Trichostrongylus axei	Total in abomasum
		Adult	DL_4	EL_4	$\%EL_4$	Total	Adult	Imm.		
3/7-4/4	(23)	29,042	36,284	85,543	57	150,869	1,467	0	0	152,336
4/4-5/5	(31)	287	1,668	4,306	69	6,261	155	50	0	6,466
5/2-5/30	(28)	2,748	783	1,071	23	4,602	737	0	18	5,357
6/9-6/30	(21)	898	1,903	2,008	42	4,809	2,192	18	0	7,019
7/6-7/28	(22)	404	1,290	293	15	1,987	1,703	0	0	3,690
8/4-8/25	(21)	99	0	0	0	99	1,448	0	0	1,547
9/1-9/22	(21)	610	0	272	31	882	6,199	374	50	7,505

DL_4 - developing 4th stage larvae; EL_4 - early 4th stage larvae.

Table 2. Mean worm counts from the intestinal tract and lungs of tracer calves (n=2) grazed each month on experimental pasture with principal calves

Grazing period	(days)	Lung	Small intestine	Large intestine	
		Dictyocaulus viviparus	*Cooperia* spp.	*Oesophagostomum radiatum*	*Trichuris* sp.
3/7-4/4	(28)	0	7,675	300	0
4/4-5/5	(31)	0	2,450	0	350
5/2-5/30	(28)	0	25,388	0	0
6/9-6/30	(21)	127	17,016	50	0
7/6-7/28	(22)	28	3,750	100	0
8/4-8/25	(21)	2	12,093	100	100
9/1-9/22	(21)	0	48,701	0	50

Table 3. Total numbers of infective larvae recovered from pasture grass samples and mean total worm counts recovered from tracer calves (n=2) grazed monthly with principal calves, from March to September 1988. Actual number and Log_{10} transformed values

	Total L_3/kg (dry matter)		Mean total worm counts	
March	4,681	(3.670)	158,875	(5.201)
April	2,717	(3.434)	9,127	(3.960)
May	1,450	(3.161)	31,213	(4.494)
June	641	(2.807)	24,284	(4.385)
July	1,371	(3.137)	7,540	(3.877)
August	1,446	(3.160)	13,940	(4.144)
September	261	(2.417)	56,185	(4.750)

Table 4. Pearson correlation coefficients of total numbers of infective larvae recovered from pasture grass samples and the mean total worm counts recovered from tracer calves (n=2) grazed monthly with principal calves

(r) = 0.6955
$P > R^1 = 0.0057^*$

[1] = Ho: R = 0

* = Significant at $\alpha = 0.05$

Table 5. Worm recovery from principal calves placed on pasture during March, April and May and maintained on pasture continuously until slaughter on June 28

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to June 28 (113 days)										
503	756	2,168	6,496	69	0	0	0	0	3,300	400	13,120
500	11,798	17,708	77,632	72	1,027	0	2,174	424	32,770	200	143,733
Mean	6,277	9,938	42,064	70	513	0	1,087	212	18,035	300	78,426
Group 2	Grazed April 4 to June 28 (85 days)										
524	7,508	6,801	24,477	63	689	0	1,034	1,862	8,202	1,502	52,075
527	3,668	1,772	8,816	62	0	0	0	0	50,999	100	65,355
Mean	5,588	4,286	16,646	62	344	0	517	931	29,600	801	58,713
Group 3	Grazed May 2 to June 28 (57 days)										
546	1,767	140	480	20	0	0	398	0	15,585	0	18,370
547	1,562	324	611	24	0	0	108	0	7,114	0	9,719
Mean	1,664	232	545	22	0	0	253	0	11,349	0	14,043

DL_4 - Developing 4th stage larvae; EL_4 - early 4th stage larvae.

Table 6. Worm recovery from principal calves placed on pasture during March, April and May and maintained on pasture continuously until slaughter on July 27

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to July 27 (142 days)										
502	4,227	18,548	96,015	81	307	0	2,696	307	37,632	400	160,132
505	2,674	26,389	73,617	72	762	0	763	4,654	19,489	0	128,348
Mean	3,450	22,468	84,816	76	534	0	1,729	2,480	28,560	200	144,237
Group 2	Grazed April 4 to July 27 (114 days)										
525	6,723	3,193	1,816	15	0	0	1,770	223	33,567	300	47,592
528	107	1,853	7,000	78	39	0	107	537	7,391	200	17,234
Mean	3,413	2,523	4,408	46	19	0	938	380	20,479	250	32,410
Group 3	Grazed May 2 to July 27 (86 days)										
542	9,905	1,700	11,668	50	0	0	9,792	0	195,189	700	228,954
544	8,074	1,361	6,941	42	0	0	20,080	2,057	29,875	200	68,588
Mean	8,989	1,530	9,304	46	0	0	14,936	1,028	112,532	450	148,769

DL_4 - Developing 4th stage larvae; EL_4 - early 4th stage larvae.

Table 7. Worm recovery from principal calves placed on pasture during March, April and May and maintained on pasture continuously until slaughter on August 30

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				*T. axei*		*Haemonchus* sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to August 30 (176 days)										
507	10,769	24,940	119,091	77	944	0	3,658	944	180,100	1,000	341,446
508	23,574	31,484	156,012	74	3,073	0	8,406	333	33,966	1,633	258,481
Mean	17,171	28,212	137,551	75	2,008	0	6,032	638	107,033	1,316	299,961
Group 2	Grazed April 4 to August 30 (148 days)										
523	2,561	1,082	5,525	60	0	0	1,624	69	64,745	100	75,706
529	400	842	1,784	59	100	0	200	0	17,118	500	20,944
Mean	1,480	963	3,654	59	50	0	912	34	40,931	300	48,325
Group 3	Grazed May 2 to August 30 (120 days)										
543	620	1,366	1,337	40	0	0	2,353	515	3,402	1,200	10,793
549	631	70	245	26	0	0	7,160	3,931	98,900	396	111,333
Mean	625	718	791	33	0	0	4,756	2,223	51,151	798	61,063

DL_4 - developing 4th stage larvae; EL_4 - early 4th stage larvae.

Table 8. Worm recovery from principal calves placed on pasture during March, April and May and maintained on pasture continuously until slaughter September 30

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to September 30 (206 days)										
504	50,090	34,881	54,916	39	1,284	0	3,718	350	51,310	200	196,749
506	79,223	36,786	113,244	49	12,668	0	14,950	0	33,020	1,489	291,380
Mean	64,656	35,833	84,080	44	6,976	0	9,334	175	42,165	844	244,064
Group 2	Grazed April 4 to September 30 (179 days)										
522	13,020	2,188	5,793	28	268	0	23,977	3,292	162,661	1,399	212,598
526	15,078	1,466	7,989	30	326	0	13,800	0	78,059	100	116,818
Mean	14,049	1,827	6,891	29	297	0	18,888	1,646	120,360	749	164,708
Group 3	Grazed May 2 to September 30 (151 days)										
545	1,903	140	70	3	35	0	8,092	1,199	17,401	1,503	30,343
548	2,215	175	280	11	0	0	10,336	5,011	34,031	2,101	54,149
Mean	2,059	157	175	7	17	0	9,214	3,105	25,716	1,802	42,246

DL_4 - developing 4th stage larvae; EL_4 - early 4th stage larvae.

Table 9. Worm recovery from principal calves that were grazed on pasture for 1 month in March, April and May and then maintained in concrete-floored pens until slaughter on June 28

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to April 4 (28 days); confined April 4 to June 28 (85 days)										
512	5,531	20,491	90,162	78	0	0	0	0	3,600	400	120,183
513	6,087	9,412	66,522	81	313	0	670	0	1,100	200	84,304
Mean	5,809	14,951	78,342	79	156	0	335	0	2,350	300	102,243
Group 2	Grazed April 4 to May 5 (31 days); confined May 5 to June 28 (54 days)										
530	2,101	3,262	10,997	67	105	0	105	0	4,600	900	22,070
533	13,279	25,650	125,362	76	0	0	1,439	0	19,338	400	185,468
Mean	7,690	14,456	68,179	71	52	0	772	0	11,969	650	103,769
Group 3	Grazed May 2 to May 30 (28 days); confined May 30 to June 28 (29 days)										
550	200	0	445	17	0	0	200	0	7,800	0	8,645
551	2,125	35	174	7	101	0	237	0	6,500	0	9,172
555	1,707	0	209	11	0	0	5,227	0	3,600	100	10,843
Mean	1,344	12	274	12	340	0	1,888	0	5,967	33	9,553

DL_4 - Developing 4th stage larvae; EL_4 - Early 4th stage larvae.

Table 10. Worm recovery from principal calves that were grazed on pasture for 1 month in March, April and May and then maintained in concrete-floored pens until slaughter on July 27

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to April 4 (28 days); confined April 4 to July 27 (114 days)										
501	688	1,759	84,673	97	0	0	0	0	1,398	0	88,518
514	7,495	11,172	95,036	84	0	0	0	0	1,400	0	115,103
Mean	4,091	6,465	89,854	90	0	0	0	0	1,399	0	101,809
Group 2	Grazed April 4 to May 5 (31 days); confined May 5 to July 27 (83 days)										
535	535	100	1,450	70	0	0	0	0	1,300	0	3,385
537	572	492	2,027	66	0	0	0	0	1,200	0	4,291
Mean	553	296	1,738	68	0	0	0	0	1,250	0	3,837
Group 3	Grazed May 2 to May 30 (28 days); confined May 30 to July 27 (58 days)										
552	535	70	245	29	0	0	0	0	1,801	0	2,651
554	2,040	0	135	6	0	0	0	0	4,000	0	6,175
Mean	1,287	35	190	17	0	0	0	0	2,900	0	4,412

DL_4 - Developing 4th stage larvae; EL_4 - Early 4th stage larvae.

Table 11. Worm recovery from principal calves that were grazed on pasture for 1 month in March, April and May and then maintained in concrete-floored pens until slaughter on August 30

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to April 4 (28 days); confined April 4 to August 30 (148 days)										
515	15,748	22,223	77,007	67	0	0	0	0	1,000	0	115,978
517	10,064	10,260	45,859	69	0	0	304	0	1,297	0	67,784
Mean	12,906	16,241	61,433	68	0	0	152	0	1,148	0	91,881
Group 2	Grazed April 4 to May 5 (31 days); confined May 5 to August 30 (117 days)										
532	705	340	1,445	58	0	0	0	0	1,900	0	4,390
534	833	267	977	47	0	0	0	299	5,800	0	8,176
Mean	769	303	1,211	52	0	0	0	149	3,850	0	6,283
Group 3	Grazed May 2 to May 30 (28 days); confined May 30 to August 30 (92 days)										
540	1,404	105	0	0	0	0	99	35	1,300	0	2,943
556	1,003	795	4,552	72	33	0	100	0	6,900	0	13,383
Mean	1,203	450	2,276	36	16	0	99	17	4,100	0	8,163

DL_4 - Developing 4th stage larvae; EL_4 - Early 4th stage larvae.

Table 12. Worm recovery from principal calves that were grazed on pasture for 1 month in March, April and May and then maintained in concrete-floored pens until slaughter on September 30

Group and animal number	Abomasum								Total small intestine	Total large intestine	Total worm count
	Ostertagia ostertagi				T. axei		Haemonchus sp.				
	Adult	DL_4	EL_4	$\%EL_4$	Adult	Immature	Adult	Immature			
Group 1	Grazed March 7 to April 4 (28 days); confined April 4 to September 30 (179 days)										
510	45,376	9,672	24,577	31	0	0	0	0	2,100	100	81,825
511	52,068	21,084	46,817	39	0	0	0	0	800	100	120,869
Mean	48,722	15,378	35,697	35	0	0	0	0	1,450	100	101,347
Group 2	Grazed April 4 to May 5 (31 days); confined May 5 to September 30 (147 days)										
531	12,022	365	105	1	0	0	95	235	1,000	300	14,122
536	4,414	134	204	4.3	0	0	0	0	1,000	0	5,752
Mean	8,218	249	154	2.6	0	0	47	117	1,000	150	9,935
Group 3	Grazed May 2 to May 30 (28 days); confined May 30 to September 30 (122 days)										
557	1,100	100	0	0	0	0	35	0	1,000	0	2,235

DL_4 - Developing 4th stage larvae; EL_4 - Early 4th stage larvae.

Table 13. Mean numbers of Ostertagia ostertagi recovered at necropsy from principal calves that were grazed on pasture continuously or grazed for 1 month and then kept in pens

	June		July		August		September	
Group 1								
	(a)[2]		(a)		(a)		(a)	
Grazing	58,279	a[1]	110,734	ab	182,934	b	184,569	b
	(a)		(a)		(a)		(a)	
Confinement	99,102	a	100,410	a	90,580	a	99,797	a

a[1] = Values within rows having the same letters are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June		July		August		September	
Group 2								
	(a)[2]		(a)		(a)		(a)	
Grazing	26,520	a[1]	10,344	a	6,097	a	22,767	a
	(a)		(a)		(a)		(a)	
Confinement	90,325	a	2,587	b	2,283	b	8,621	b

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June		July		August		September	
Group 3								
	(a)[2]		(a)		(a)		(a)	
Grazing	2,441	a[1]	19,823	b	2,134	a	2,391	a
	(a)		(b)		(a)		(a)	
Confinement	1,630	a	1,512	a	3,929	a	1,200	a

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

Table 14. Mean percentage of Ostertagia ostertagi inhibited early 4th stage larvae recovered at necropsy from principal calves that were grazed on pasture continuously or grazed for 1 month and then kept in pens

	June	July	August	September
Group 1				
Grazing	(a)[2] 70.5 a[1]	(a) 76.5 a	(a) 75.5 a	(a) 44.0 a
Confinement	(a) 79.5 a	(a) 90.5 a	(a) 68.0 a	(a) 34.9 a

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within column having the same letter are not significantly different (P<0.05)

	June	July	August	September
Group 2				
Grazing	(a)[2] 62.5 a[1]	(a) 46.5 a	(a) 59.5 a	(a) 30.1 a
Confinement	(a) 71.5 a	(a) 68.0 a	(a) 44.5 a	(a) 2.6 b

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June	July	August	September
Group 3				
Grazing	(a)[2] 22.0 a[1]b	(a) 46.0 a	(a) 33.0 ab	(a) 6.9 b
Confinement	(a) 29.0 a	(a) 17.5 ab	(a) 36.0 a	(a) 0.0 b

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

Table 15. Mean gross pathology score* of abomasal mucosa from principal calves killed monthly

	June	July	August	September
Group 1				
	(a)[2]	(a)	(a)	(a)
Grazing	2.5 a[1]	4.2 b	2.2 a	5.0 b
	(a)	(a)	(b)	(a)
Confinement	3.2 ab	3.0 a	4.7 b	5.0 b

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letters are not significantly different (P<0.05)

	June	July	August	September
Group 2				
	(a)[2]	(a)	(a)	(a)
Grazing	3.5 ab	2.0 a	3.2 ab	3.7 b
	(a)	(a)	(a)	(a)
Confinement	3.2 a	2.5 a	3.2 a	3.0 a

a[1] = Values within rows having the same letters are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June	July	August	September
Group 3				
	(a)[2]	(a)	(a)	(a)
Grazing	0.75 a[1]	2.0 a	3.0 b	1.7 ab
	(a)	(a)	(a)	(a)
Confinement	1.6 a	2.5 a	1.5 a	2.5 a

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)
*Observed gross pathology was scored from 0 to 5 with 0 being normal and 5 being severe pathology

Table 16. Mean total numbers of all worm genera (TNW) recovered at necropsy from principal calves which grazed on pasture continuously or grazed for 1 month and then kept in pens

	June		July		August		September	
Group 1								
	(a)[2]		(a)		(a)		(a)	
Grazing	78,426	a[1]	144,237	ab	299,961	ab	244,064	b
	(a)		(a)		(a)		(a)	
Confinement	102,243	a	101,809	a	91,881	a	101,347	a

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June		July		August		September	
Group 2								
	(a)[2]		(a)		(a)		(a)	
Grazing	58,713	a[1]	32,410	a	48,325	a	164,708	a
	(a)		(b)		(b)		(b)	
Confinement	103,769	a	3,837	b	6,283	b	9,935	b

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letter are not significantly different (P<0.05)

	June		July		August		September	
Group 3								
	(a)[2]		(a)		(a)		(a)	
Grazing	14,043	a[1]	148,769	b	61,013	ab	42,246	ab
	(a)		(b)		(b)		(b)	
Confinement	9,553	a	4,412	a	8,163	a	2,235	a

a[1] = Values within rows having the same letter are not significantly different (P<0.05)
(a)[2] = Values within columns having the same letters are not significantly different (P<0.05)

Table 17. Correlation analysis of fecal egg counts (EPG), plasma pepsinogen (PP), and ELISA values with the total numbers of Ostertagia ostertagi, total numbers of worms in the abomasum and the total numbers of worms in the gastrointestinal tract

	Ostertagia ostertagi				Total No. of worms in abomasum	Total No. of worms
	Adults	DL_4	EL_4	Total		
EPG (r)	0.12036	-0.08621	-0.09503	-0.04768	-0.01357	0.43592
P>R[1]	0.5753	0.6888	0.6587	0.8249	0.9498	0.0332
PP (r)	0.60428	0.78555	0.64589	0.72399	0.71132	n/r
P>R	0.0017*	0.0001*	0.0006*	0.0001*	0.0001*	
ELISA (r)	0.03684	0.02407	-0.01571	0.00229	-0.00295	n/r
P>R	0.8643	0.9111	0.9419	0.9878	0.9891	

[1] = Ho: R=0.

* = Significant at $\alpha = 0.05$.

n/r = not reported.

EL_4 = early 4th stage larvae.

DL_4 = developing 4th stage larvae.

Table 18. Mean lengths (mm) ± standard deviation of (group 1) Ostertagia ostertagi adult worms recovered from principal calves (n=2) maintained on pasture and from the first set of tracer calves (n=2) used in March

Item	March	June	July	August	September
Females	9.7±1.1 a	9.0±1.2 a	7.1±1.2 b	7.0±1.0 b	7.0±0.8 b
Males	7.5±1.1 a	6.4±0.8 b	6.9±1.0 ab	6.9±1.2 ab	6.3±0.8 b

a = Values within rows having the same letters are not significantly different (P<0.05)

Table 19. Mean lenghts (mm) ± standard deviation of Ostertagia ostertagi early 4th stage larvae recovered from principal calves (group 1, n=2 per month in June through September) grazed on pasture continuously and from the first set of tracer calves (n=2) grazed in March

Item	March	June	July	August	September
Females	1.2±0.3 b	1.5±0.2 a	1.3±0.2 ab	1.3±0.2 ab	1.2±0.2 b
Males	1.0±0.2 a	1.2±0.2 a	1.1±0.3 a	1.1±0.2 a	1.1±0.2 a

a = Values within rows having the same letters are not significantly different (P<0.05)

Table 20. Mean vulval flap scores (mean ± standard deviation) of Ostertagia ostertagi females recovered from principal calves (group 1, n=2 per month in June through September) grazed on pasture continuously and from the first set of tracer calves (n=2) grazed in March

Item	March	June	July	August	September
Score	5.0±1.3 a	4.8±1.2 a	5.0±1.0 a	4.3±1.2 a	4.1±1.1 a

a = Values within rows having the same letters are not significantly different (P<0.05)

Table 21. Mean numbers of eggs *in utero* (mean ± standard deviation) of *Ostertagia ostertagi* females recovered at necropsy from principal calves (group 1, n=2 per month in June through September) grazed on pasture continuously and from the first set of tracer calves (n=2) grazed in March

Item	March	June	July	August	September
Mean	25.5 a	25.1 ab	18.1 bc	16.2 c	16.5 c
STD[1]	6.9	9.0	5.4	4.9	6.3

1 = Standard deviation
a = Values within rows having the same letters are not significantly different (P<0.05)

Table 22. Mean spicule length ± standard deviation of *Ostertagia ostertagi* males recovered from principal calves (group 1, n=2 per month in June through September) grazed on pasture continuously and from the first set of tracer calves (n=2) grazed in March

Item	March	June	July	August	September
Length (μm) a	232.5 a	231.9 a	230.9 a	231.1 a	224.4
STD[1]	23.8	23.5	25.3	16.7	28.0

1 = Standard deviation
a = Values within rows having the same letters are not significantly different (P<0.05)

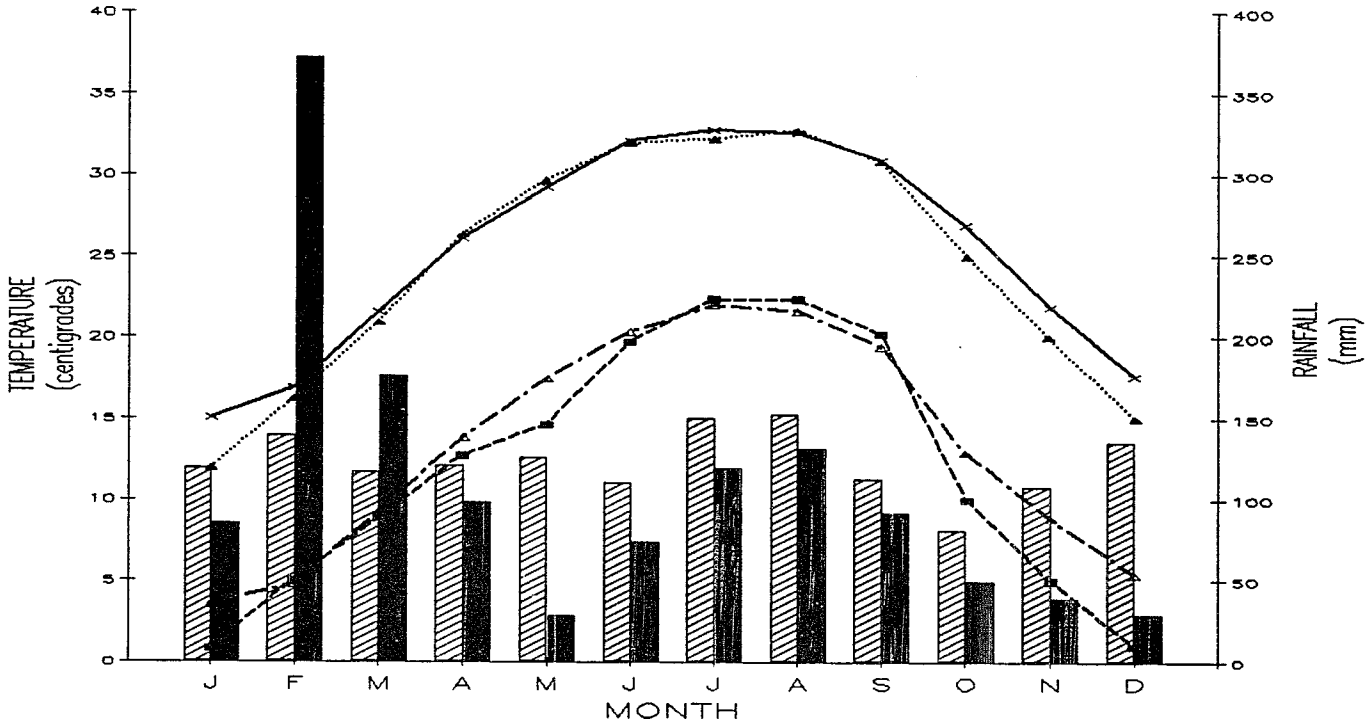

Figure 1: Maximum (—×—) and minimum (- - -) mean monthly temperatures and total monthly precipitation (▬) in 1988, in relation to 25-year average maximum (···▲···) and minimum (-■-) mean monthly temperatures and total monthly precipitation (▒)

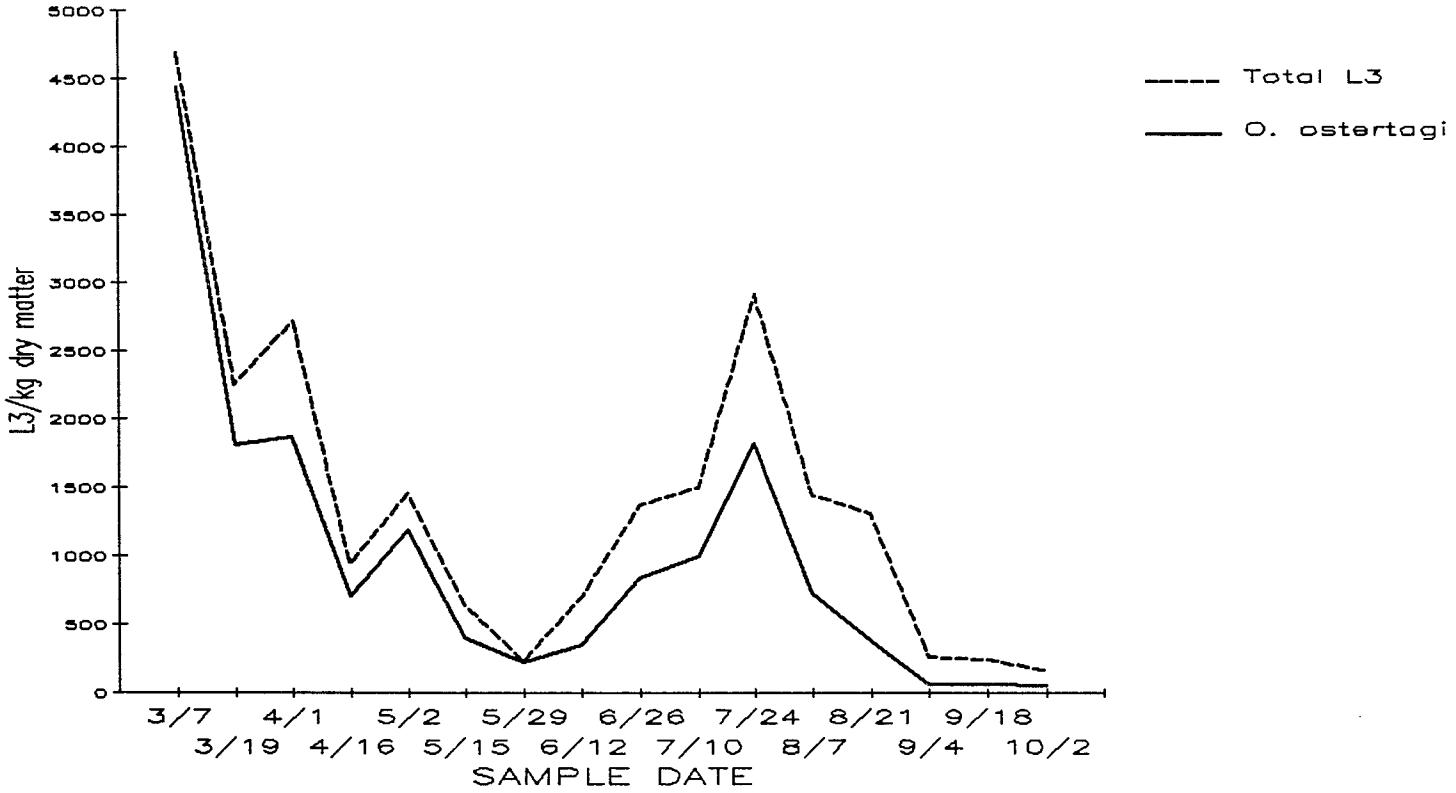

Figure 2: Recovery of infective larvae from herbage on the experimental pasture

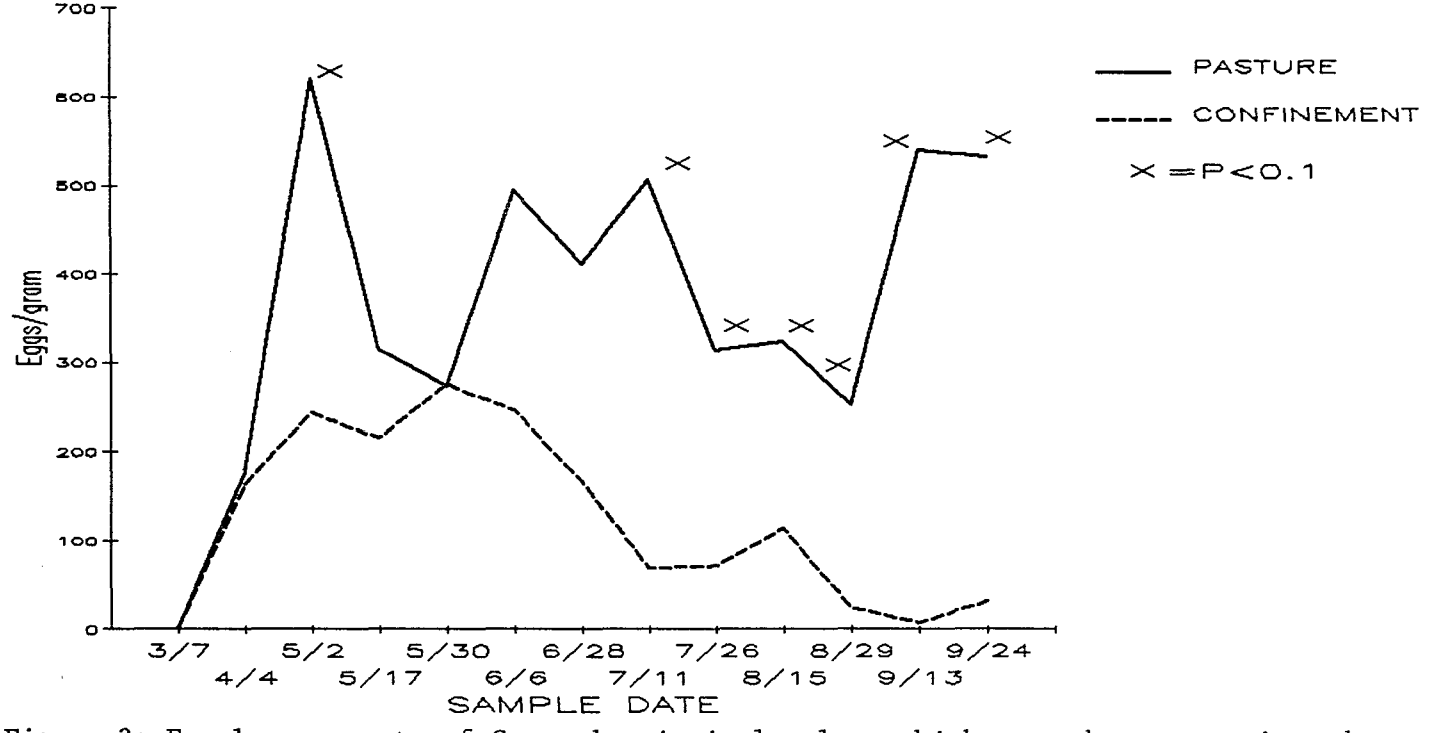

Figure 3: Fecal egg counts of Group 1 principal calves which grazed on contaminated pasture for 1 month beginnig March 7 and then were separated into equal subgroups of 8 for continuous grazing on pasture or for confinement in concrete-floored pens

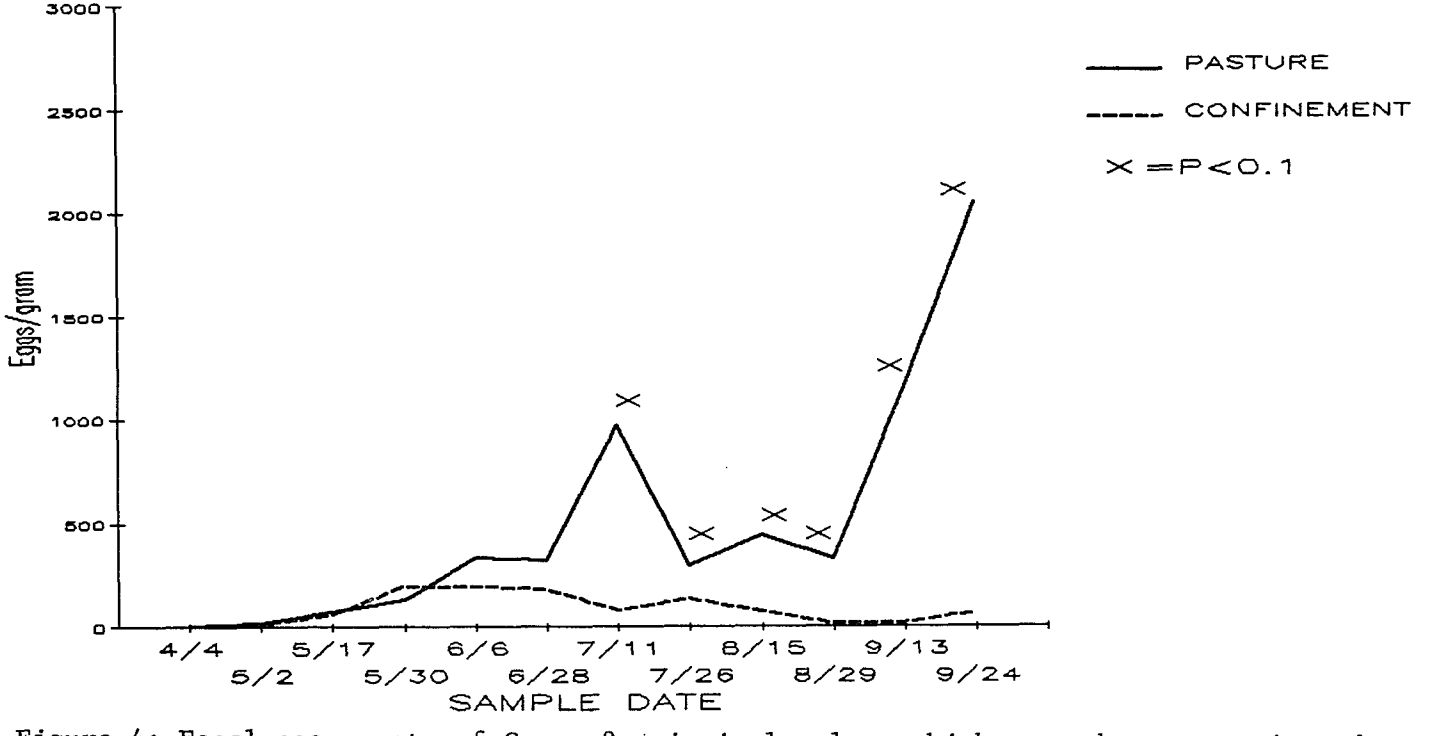

Figure 4: Fecal egg counts of Group 2 principal calves which grazed on contaminated pasture for 1 month beginning April 4 and then were separated into equal subgroups of 8 for continuous grazing on pasture or for confinement in concrete-floored pens

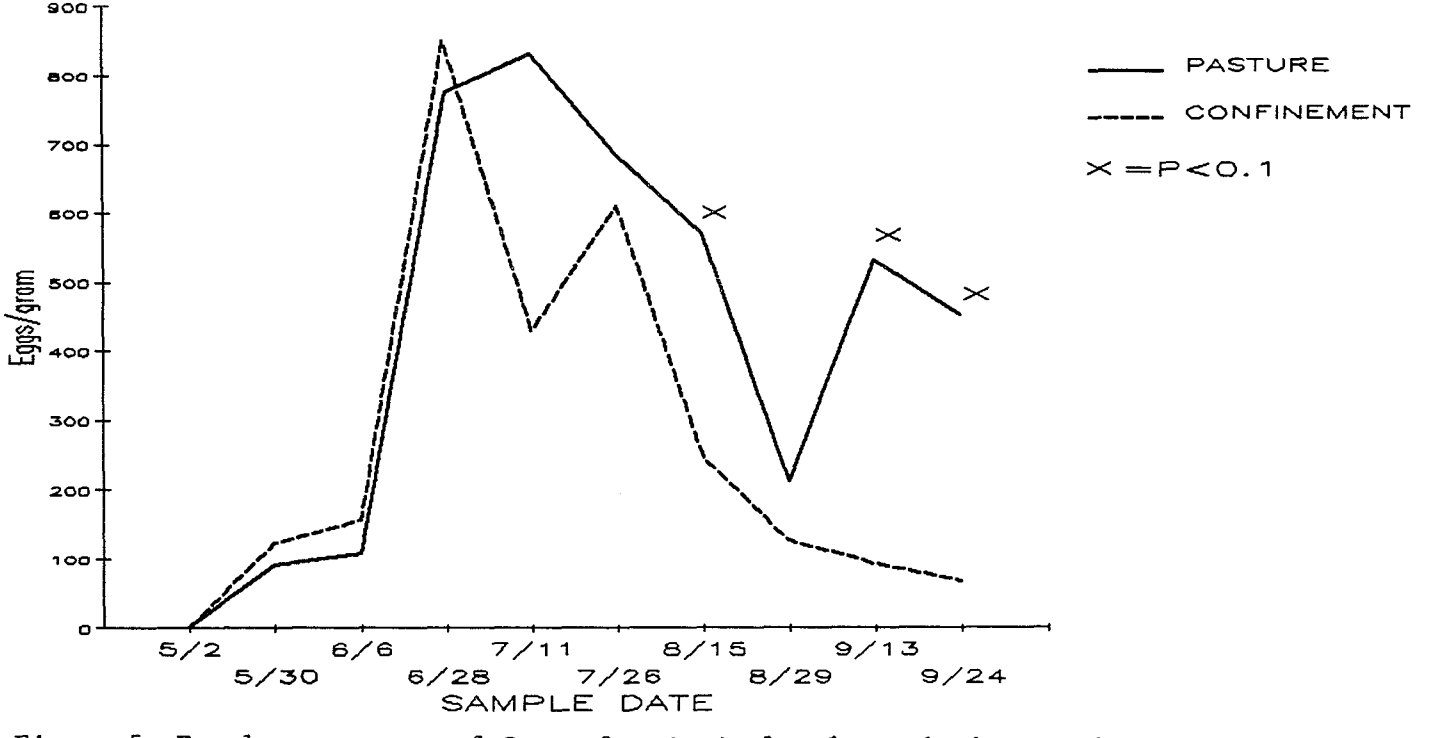

Figure 5: Fecal egg counts of Group 3 principal calves which grazed on contaminated pasture for 1 month beginning May 2 and then were separated into equal subgroups of 8 for continuous grazing on pasture or for confinement in concrete-floored pens

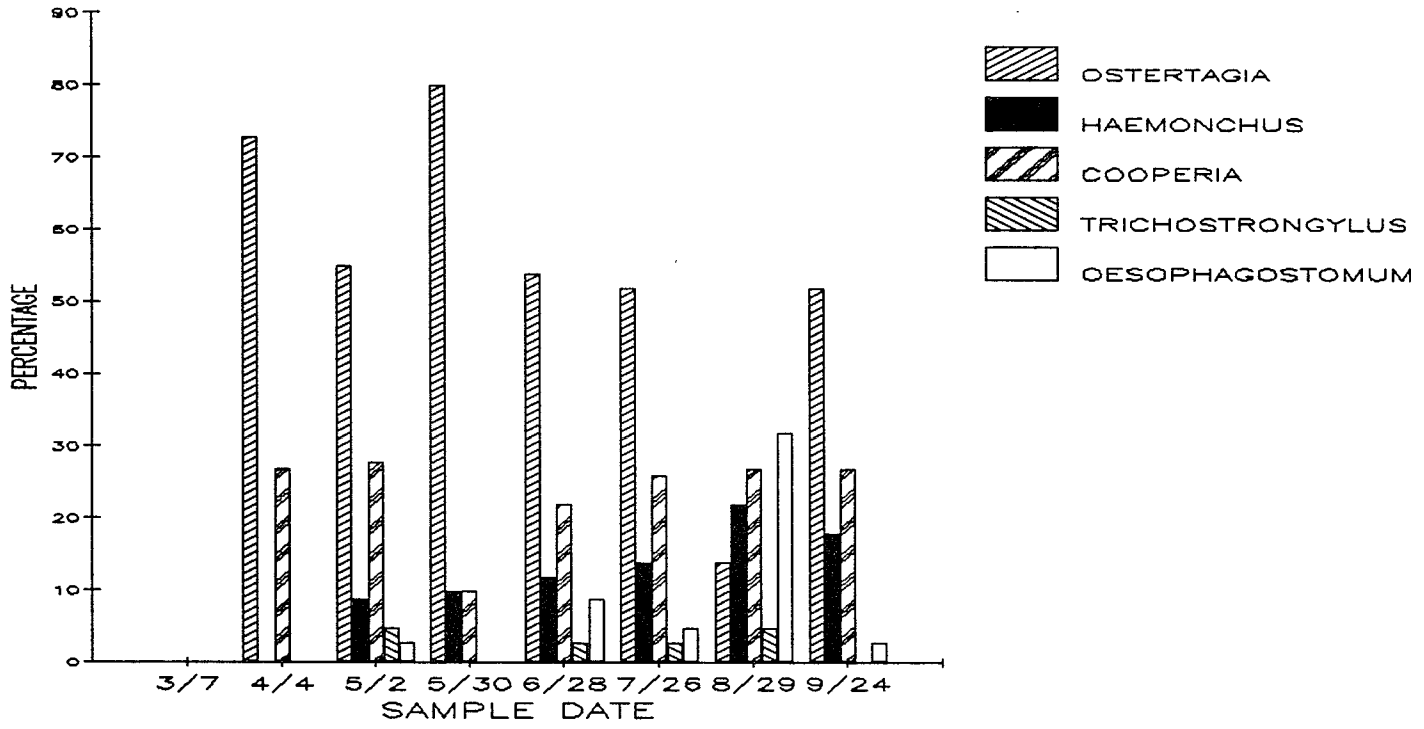

Figure 6: Generic composition of nematode eggs in fecal samples (Group 1); determined by identification of infective larvae from fecal cultures

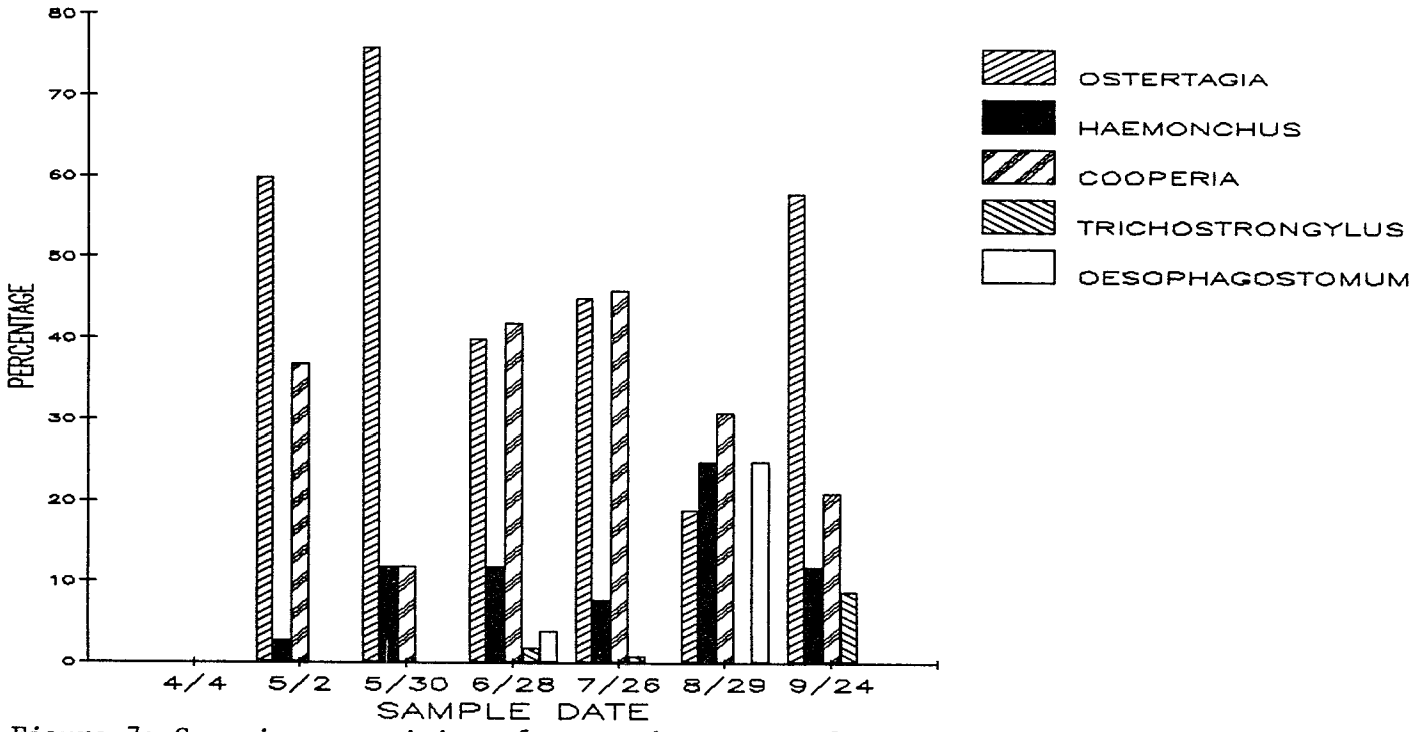

Figure 7: Generic composition of nematode eggs in fecal samples (Group 2); determined by identification of infective larvae from fecal cultures

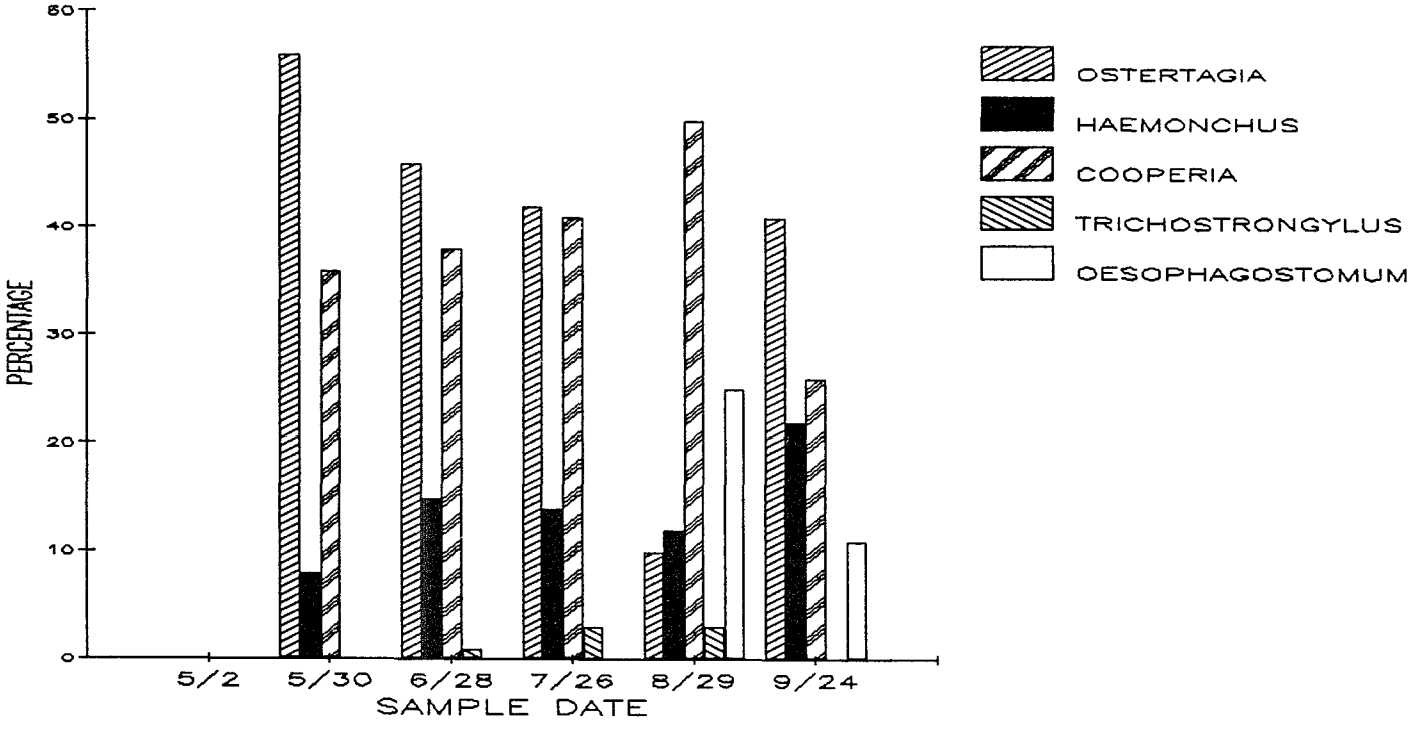

Figure 8: Generic composition of nematode eggs in fecal samples (Group 3); determined by identification of infective larvae from fecal cultures

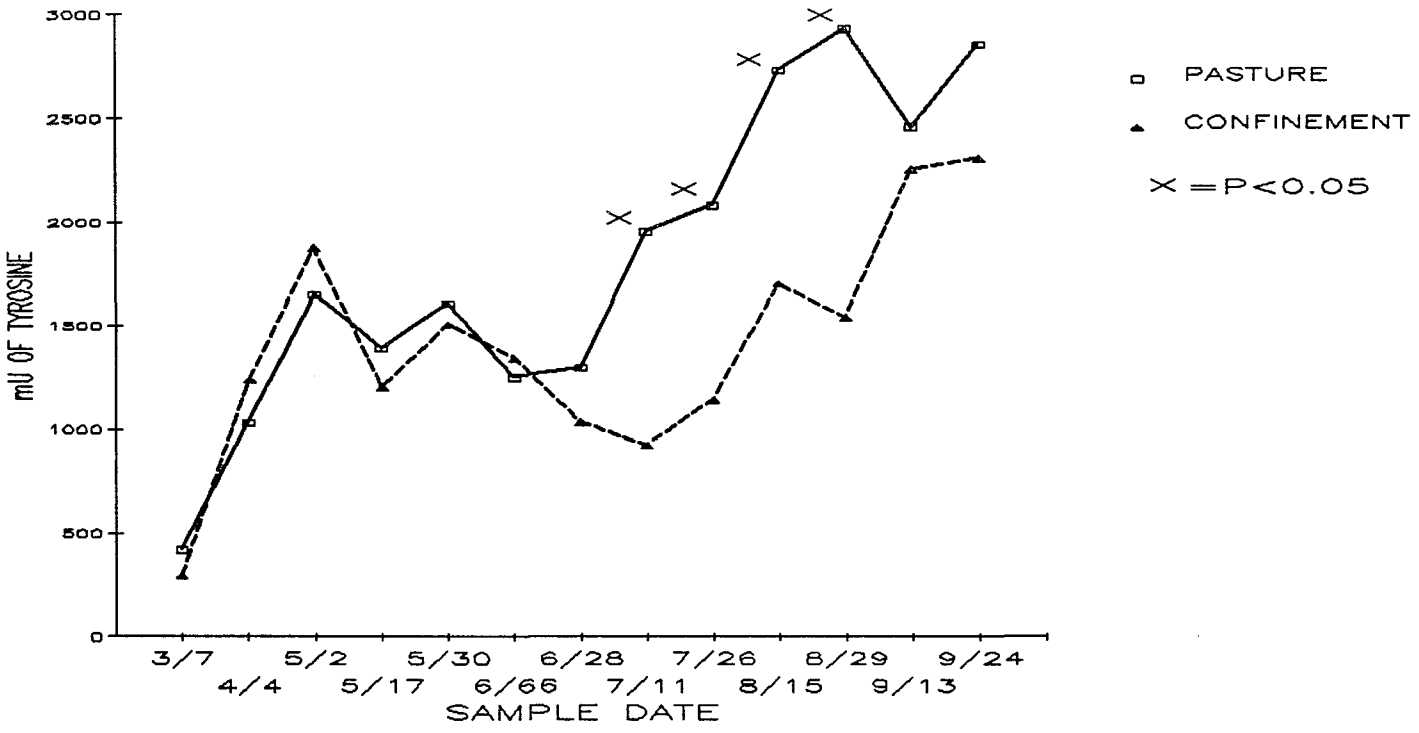

Figure 9: Plasma pepsinogen values from Group 1 calves

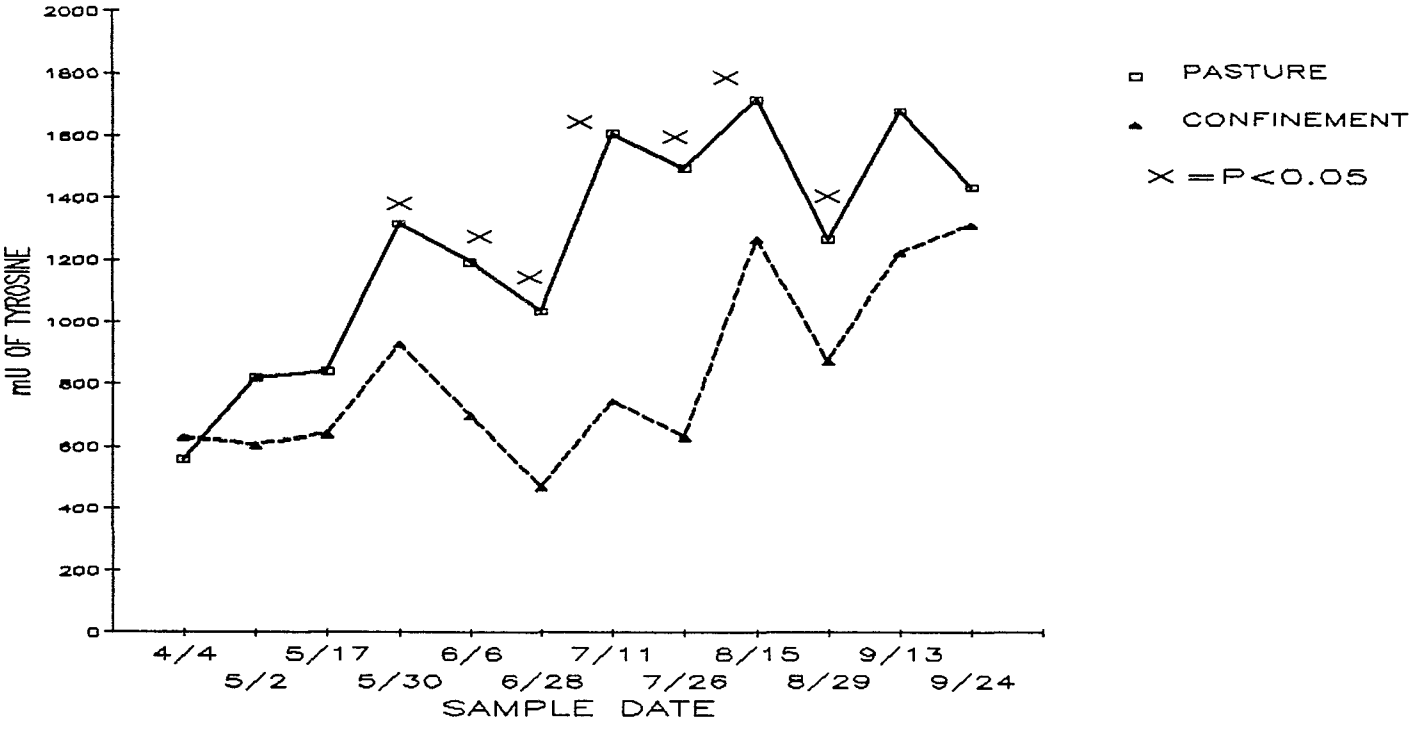

Figure 10: Plasma pepsinogen values from Group 2 calves

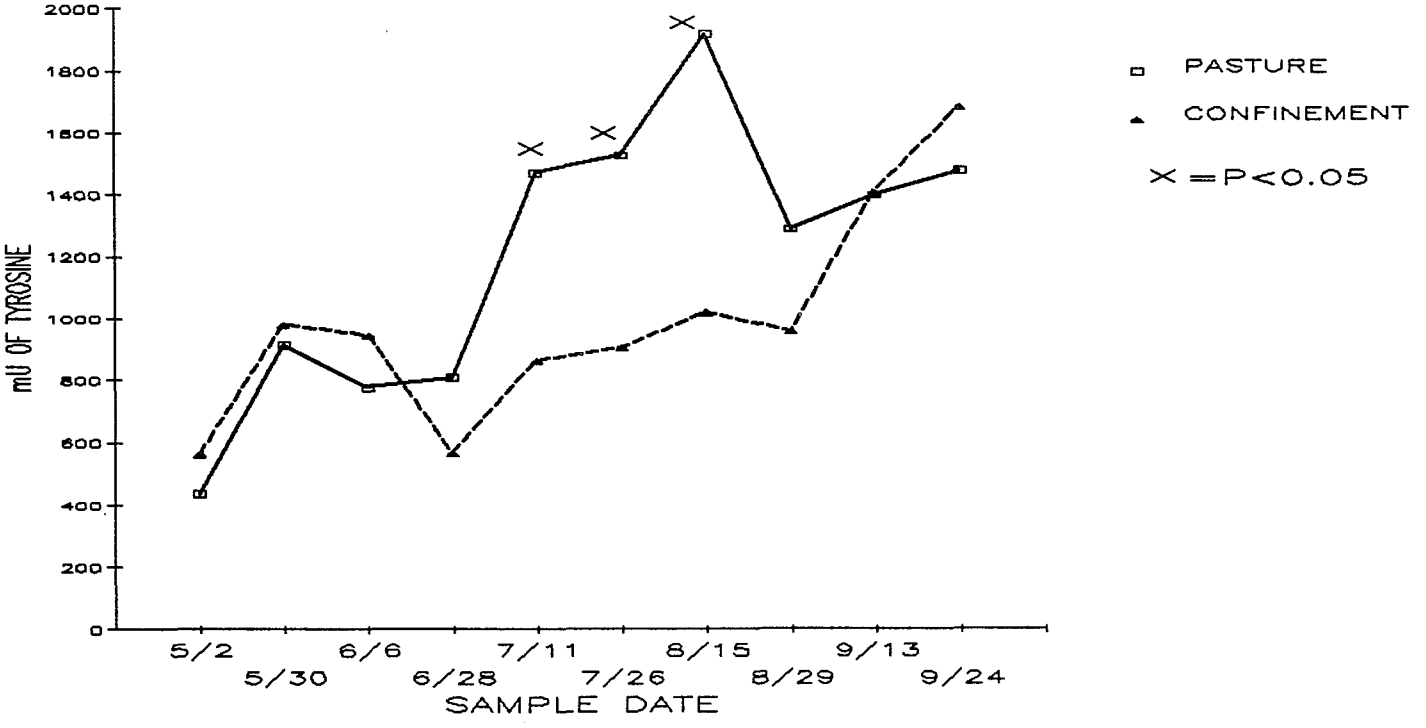

Figure 11: Plasma pepsinogen values from Group 3 calves

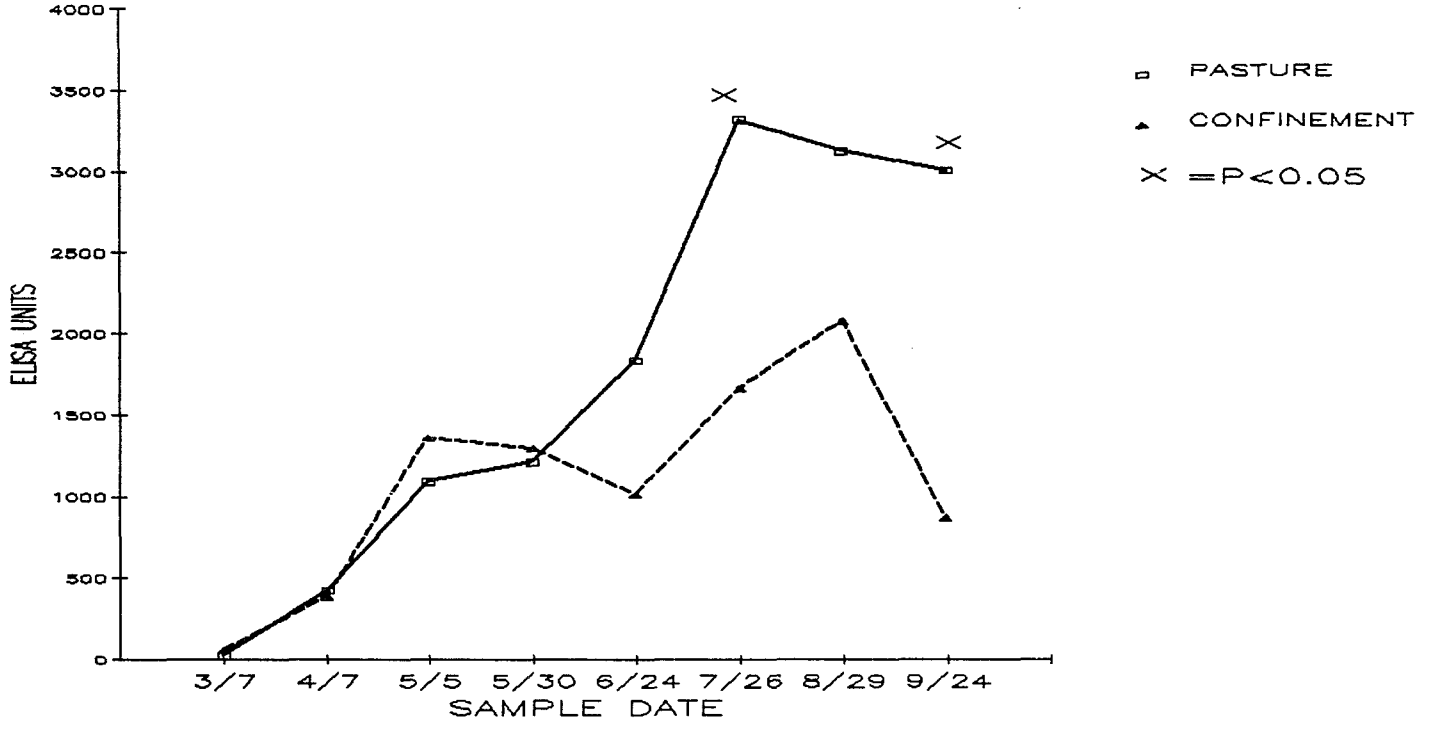

Figure 12: Measurement of antibody titers (ELISA) in pastured calves and calves held in confinement (Group 1)

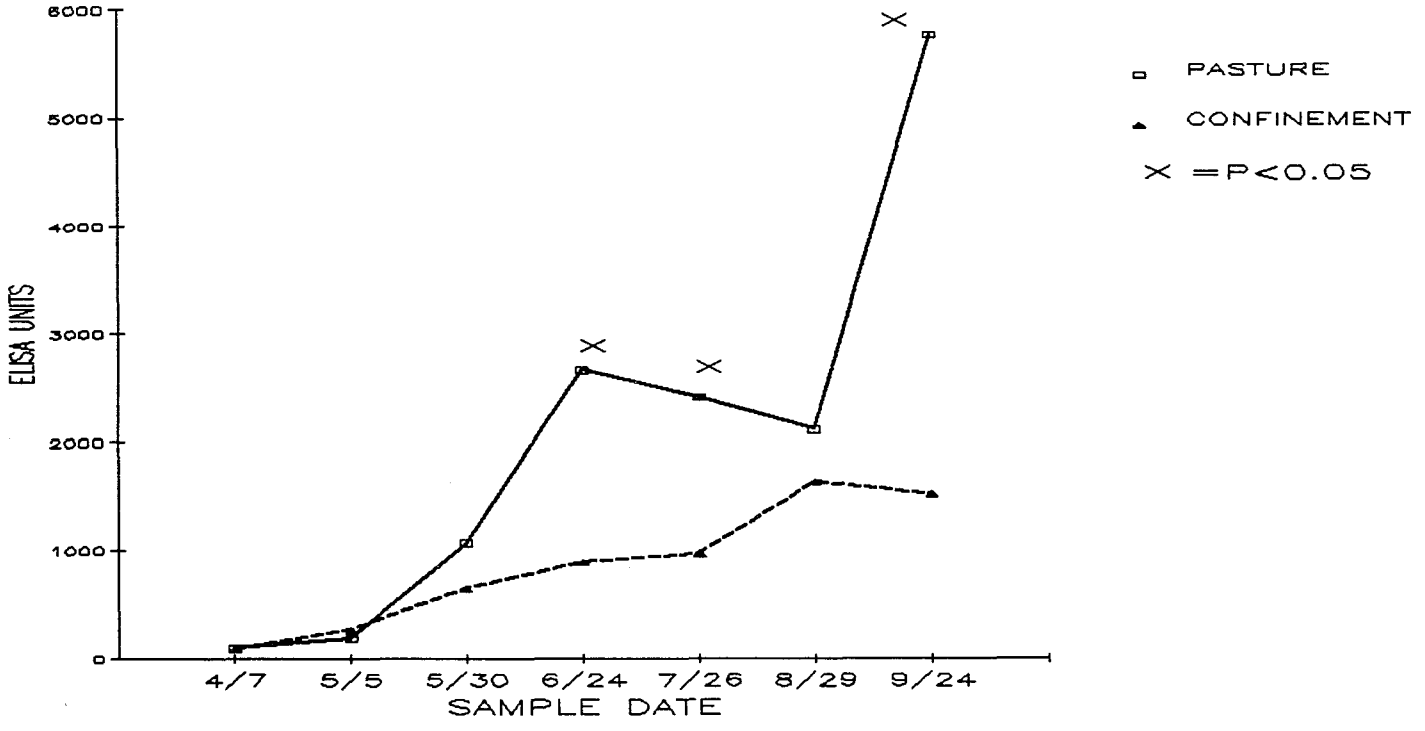

Figure 13: Measurement of antibody titers (ELISA) in pastured calves and calves held in confinement (Group 2)

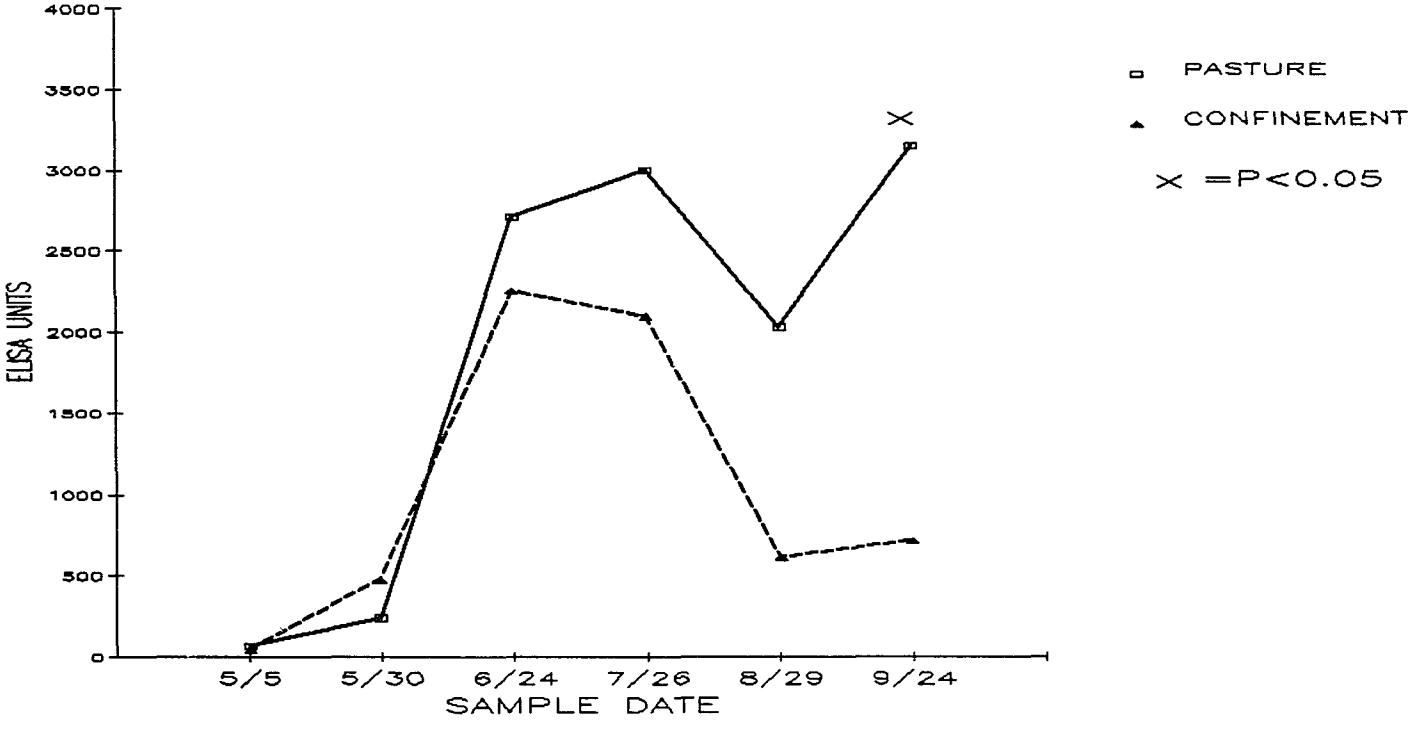

Figure 14: Measurement of antibody titers (ELISA) in pastured calves and calves held in confinement (Group 3)

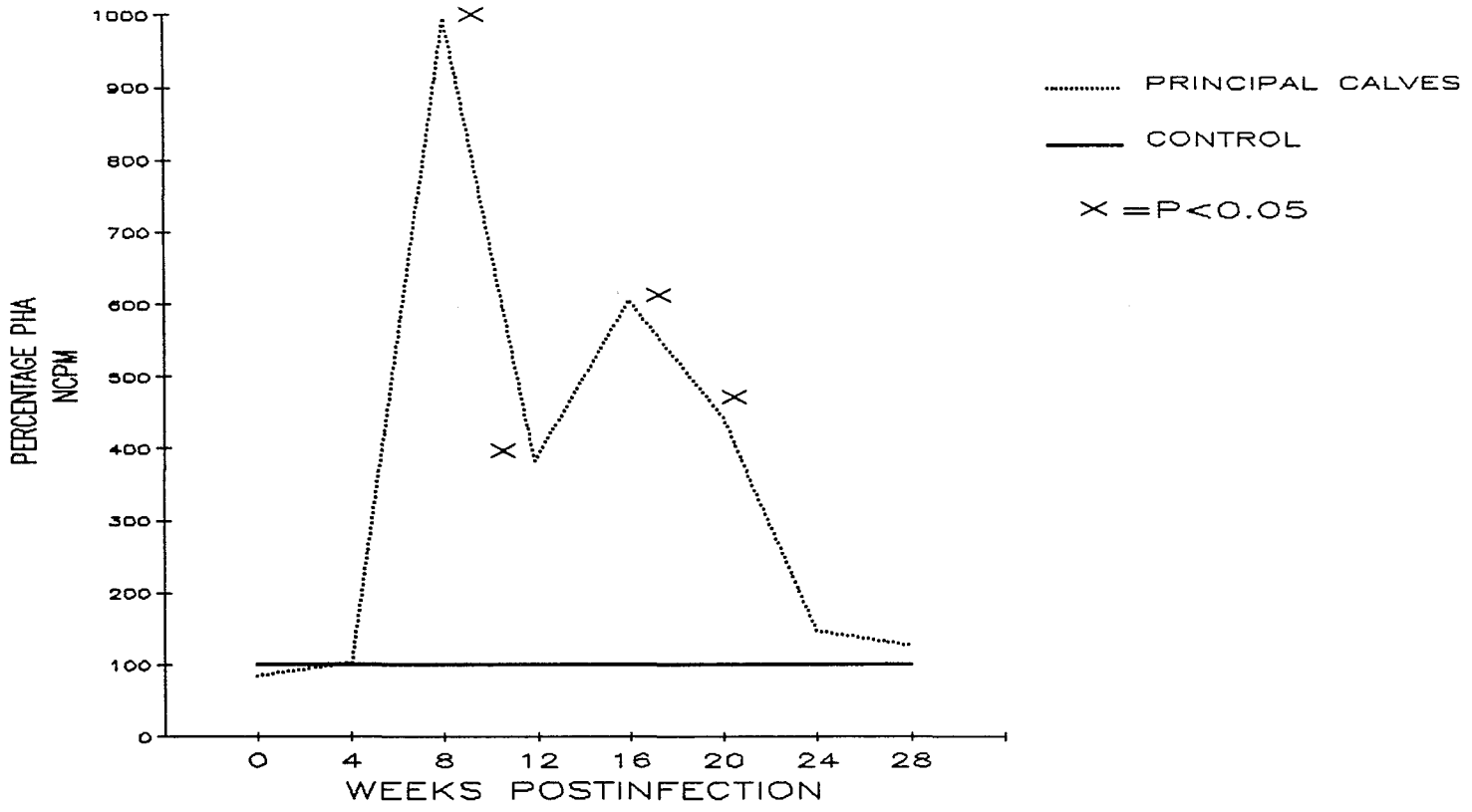

Figure 15: Phytohemagglutinin stimulation of peripheral blood lymphocytes from principal calves grazed on pasture, expressed as mean percentage of stimulation in control calves (n=2)

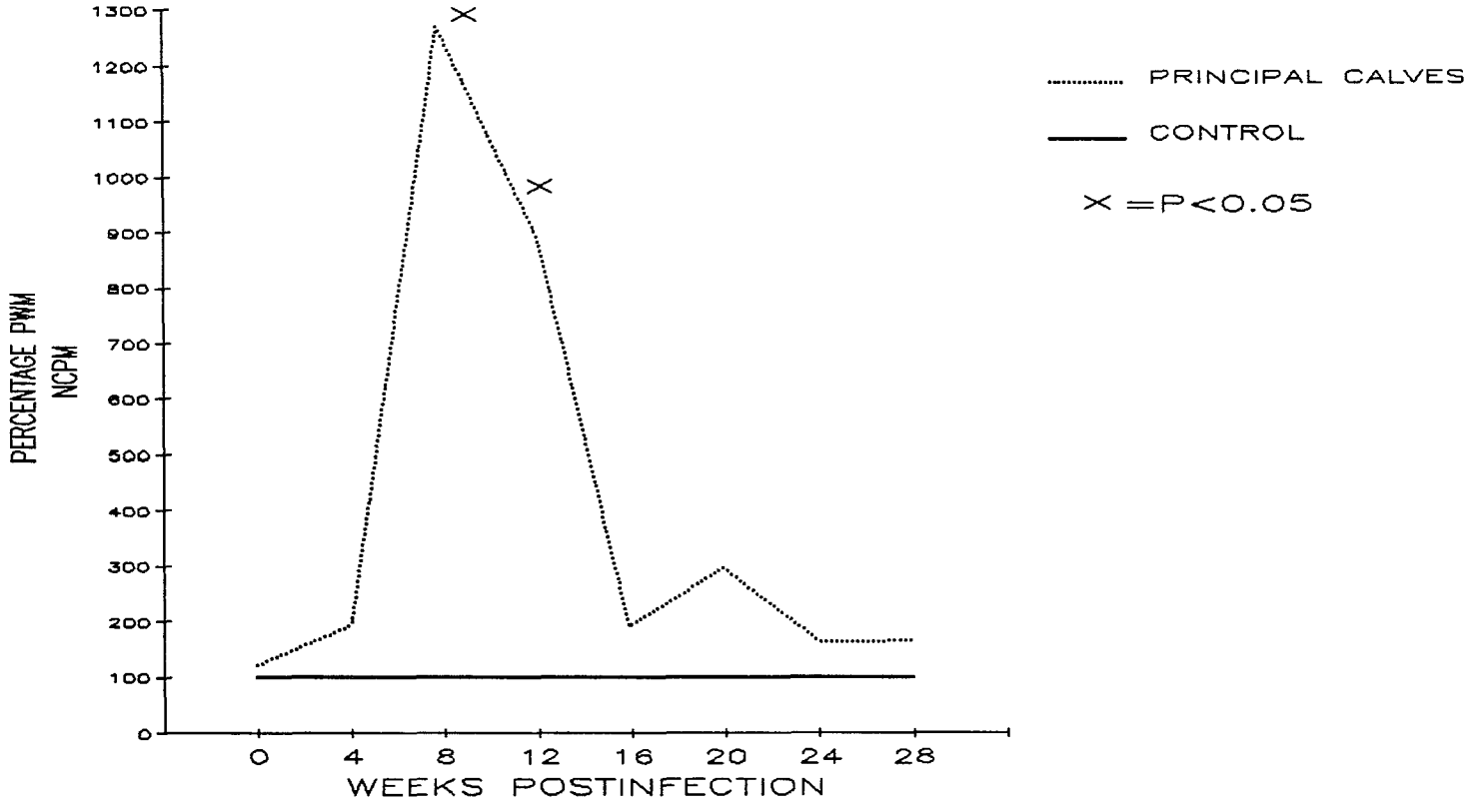

Figure 16: Pokeweed mitogen stimulation of peripheral blood lymphocytes from principal calves grazed on pasture, expressed as mean percentage of stimulation in control calves (n=2)

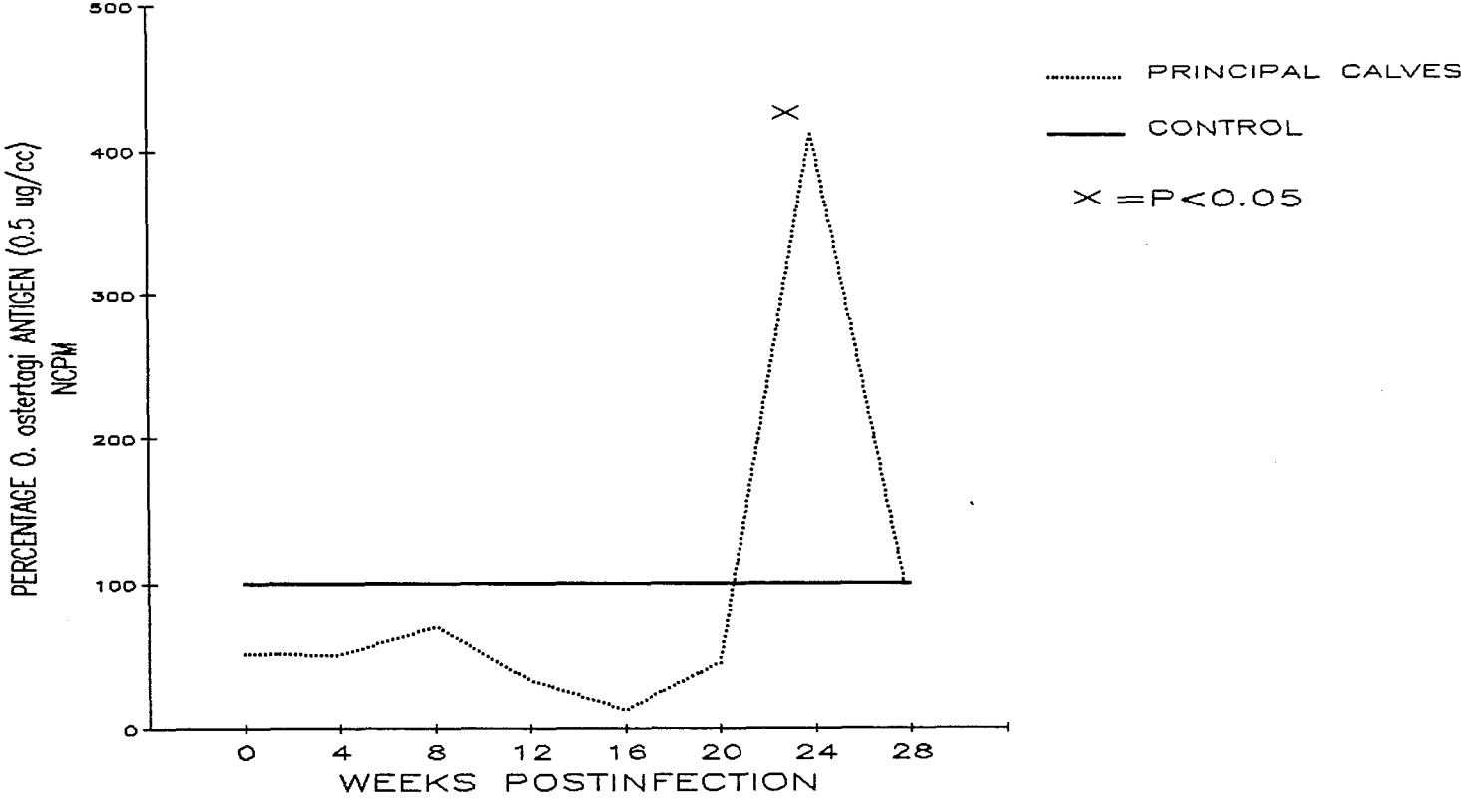

Figure 17: O. ostertagi antigen (0.5 ug/cc) stimulation of peripheral blood lymphocytes from principal calves grazed on pasture, expressed as mean percentage of stimulation in control calves (n=2)

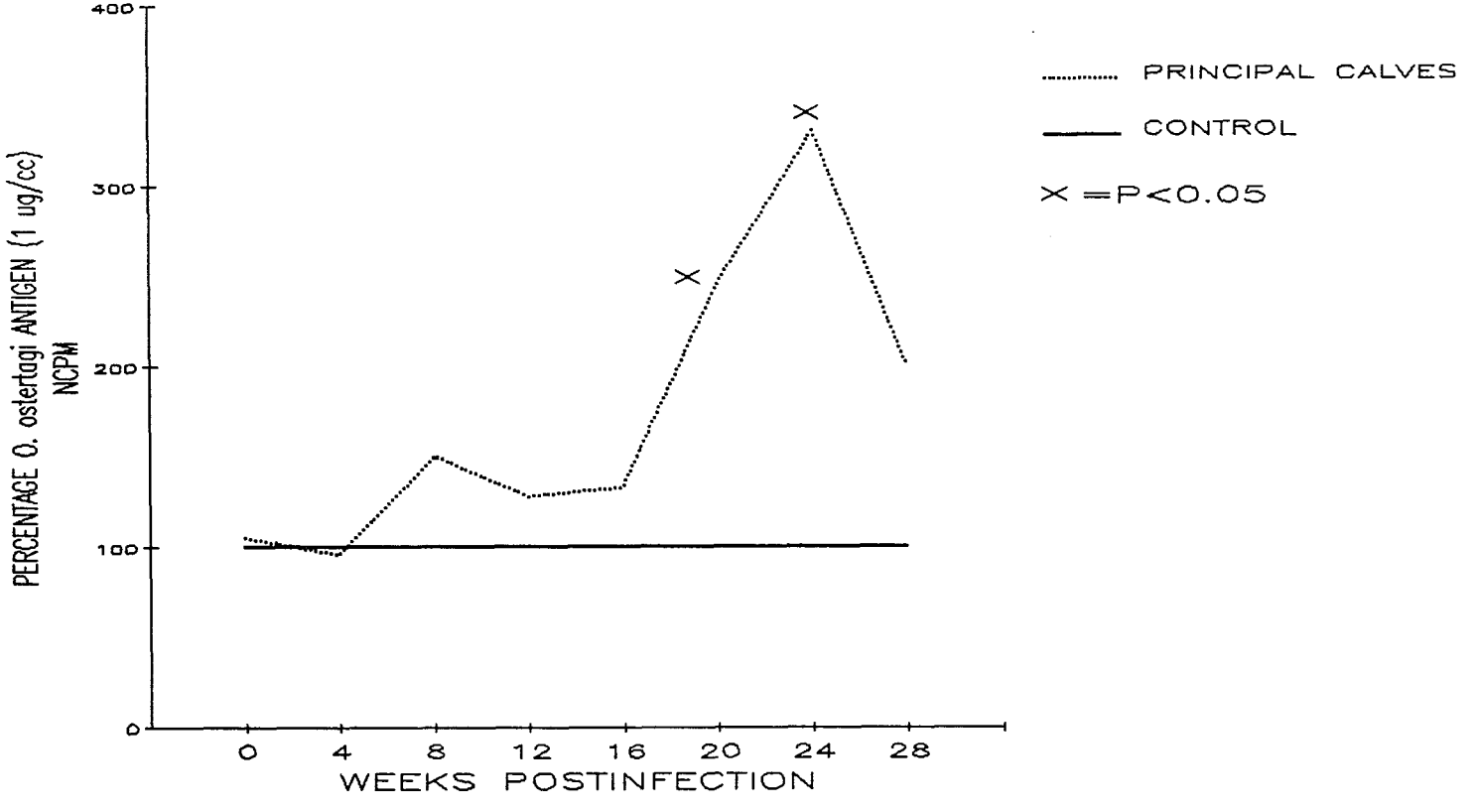

Figure 18: O. ostertagi antigen (1 ug/cc) stimulation of peripheral blood lymphocytes from principal calves grazed on pasture, expressed as mean percentage of stimulation in control calves (n=2)

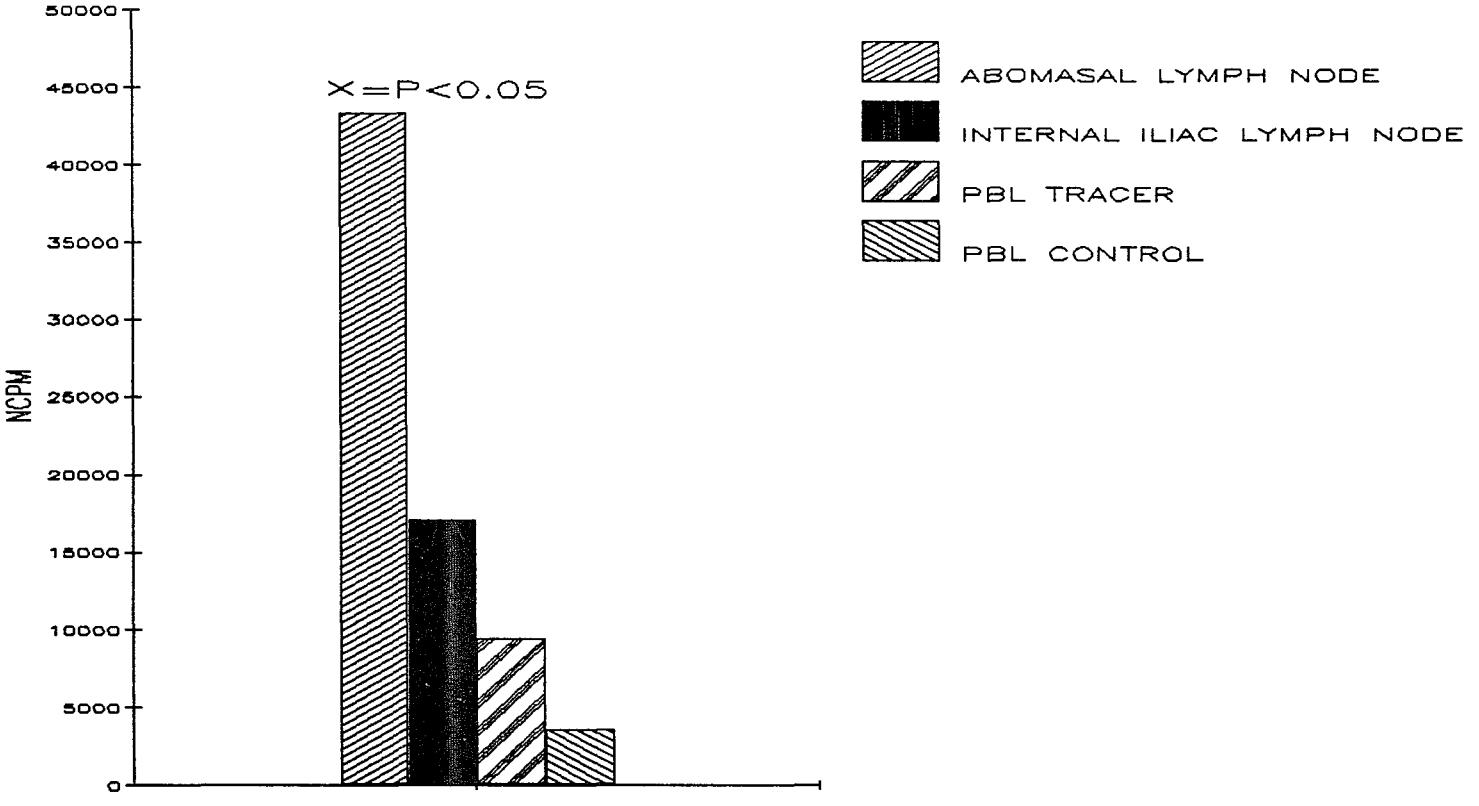

Figure 19: Phytohemagglutinin stimulation of mononuclear cells from abomasal and internal iliac lymph nodes and from peripheral blood of tracer calves (n=2) and uninfected control calves (n=20)

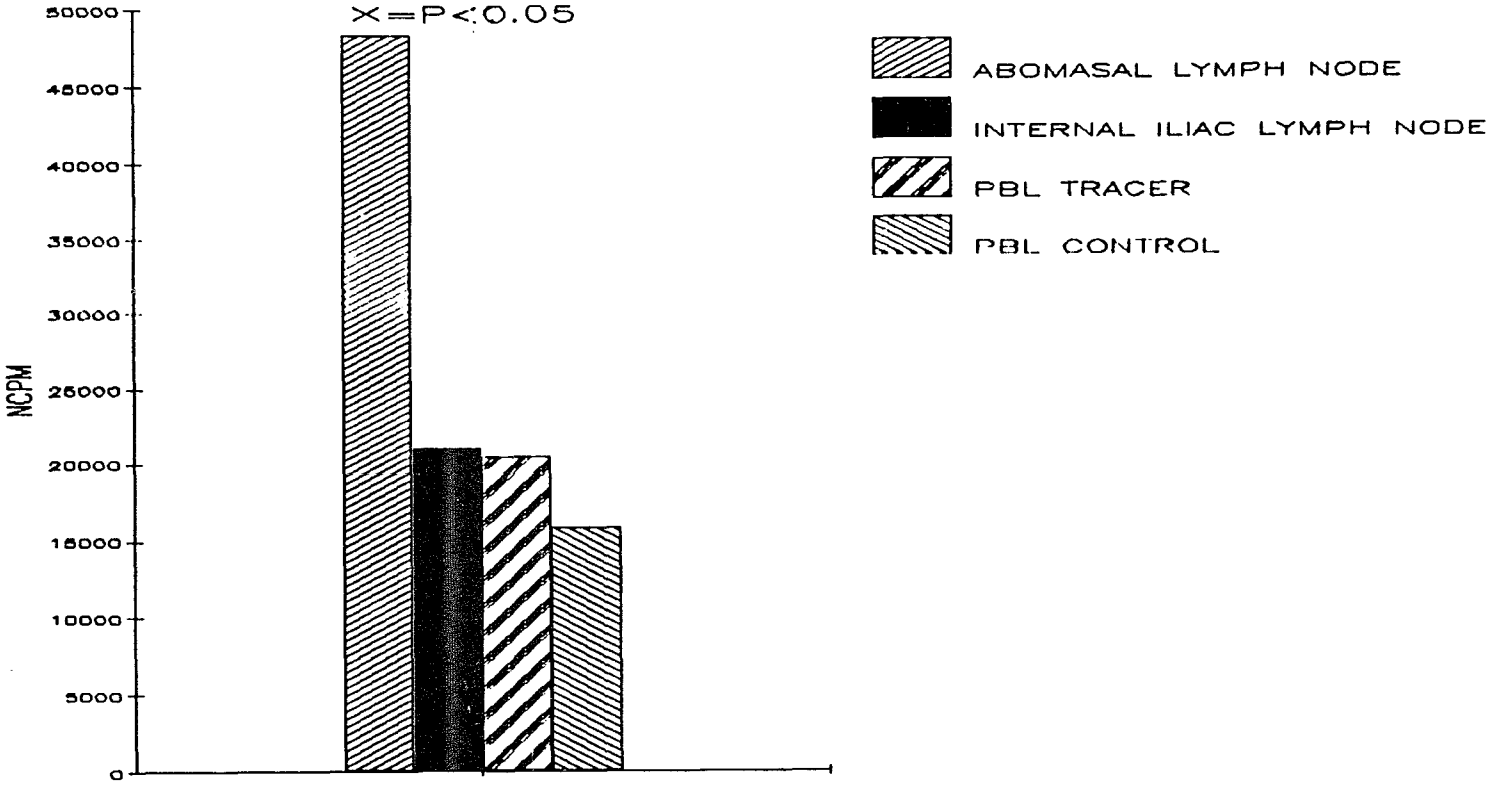

Figure 20: Pokeweed mitogen stimulation of mononuclear cells from abomasal and internal iliac lymph nodes and from peripheral blood of tracer calves (n=2) and uninfected control calves (n=2)

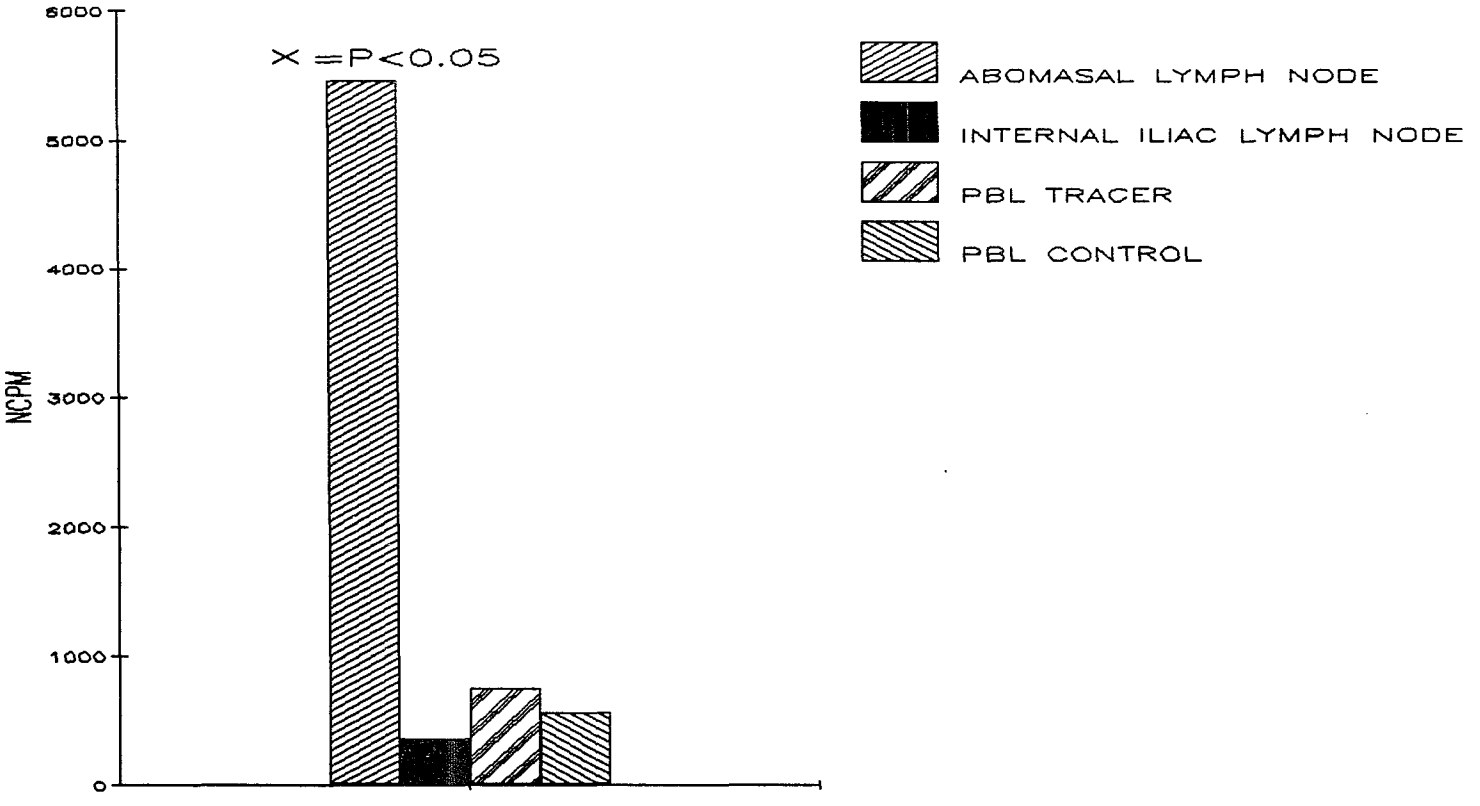

Figure 21: O. ostertagi antigen (0.5 ug/cc) stimulation of mononuclear cells from abomasal and internal iliac lymph nodes and from peripheral blood of tracer calves (n=20) and uninfected control calves (n=2)

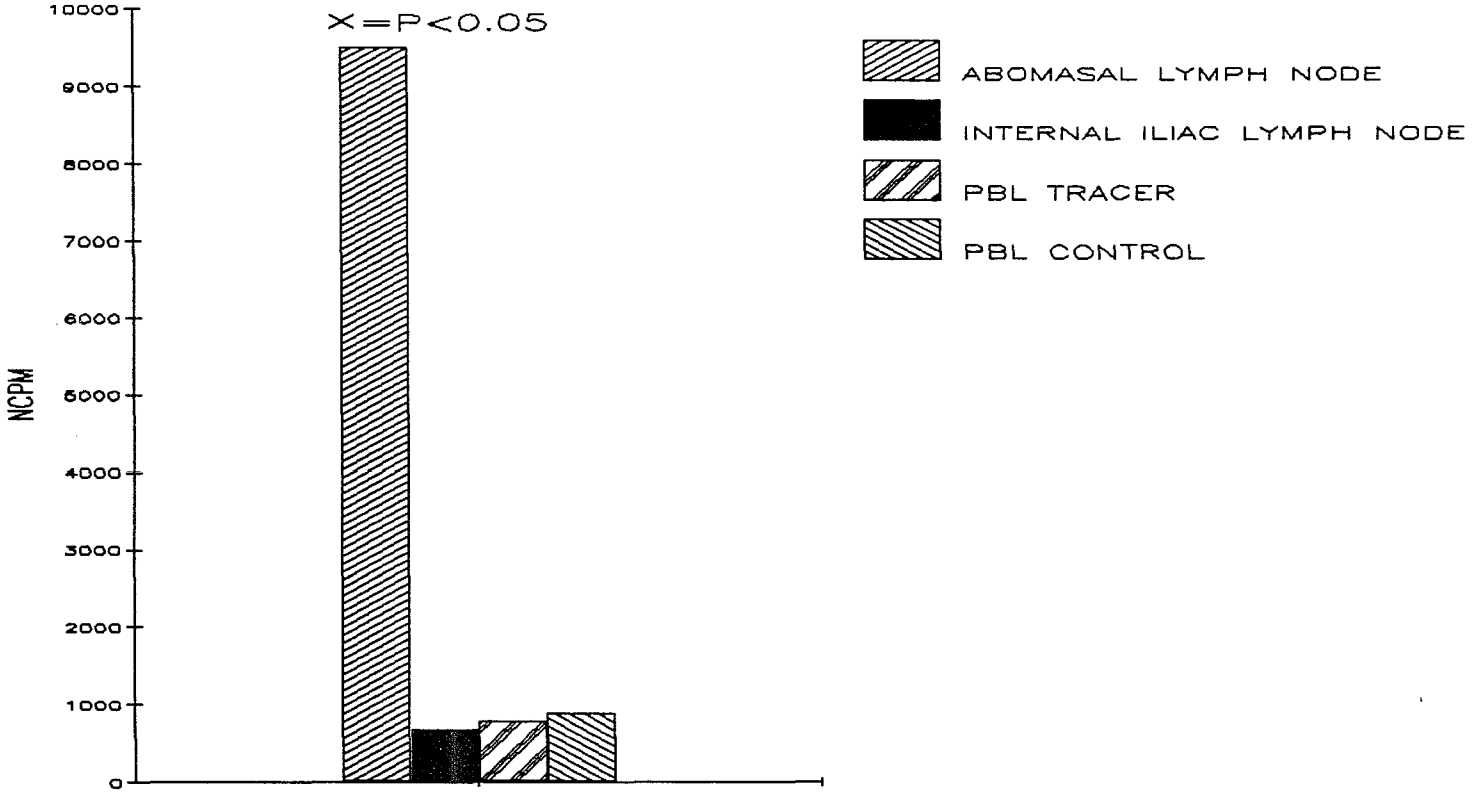

Figure 22: O. ostertagi antigen (1 ug/cc) stimulation of mononuclear cells from abomasal and internal iliac lymph nodes and from peripheral blood of tracer calves (n=2) and uninfected control calves (n=2)

BIBLIOGRAPHY

Al Saqur, I.M., Armour, J., Bairden, K., Dunn, A.M., and Jennings F.W. 1982. Observation on the infectivity and pathogenicity of three isolates of Ostertagia spp. sensu lato in calves. Res. Vet. Sci. 32:106-112.

Anderson, N. 1971. Ostertagiasis in beef cattle. Victorian Vet. Proc. 30:36-38.

Anderson, N. 1980. The epidemiology and control of bovine ostertagiasis in a winter rainfall region of south-eastern Australia. In: Gordon, H. (ed.). Ostertagia Symposium 80'. Roseworthy Agricultural College, Australia, pp. 63-69.

Anderson, N., Armour, J., Jarrett, W.F., Jennings, F.W., Ritchie, J.S. and Urquhart, G.M.1965. A field study of parasitic gastritis in cattle. Vet. Rec. 77:1196-1204.

Anderson, N., Armour, J., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. 1969. The sequential development of naturally occurring ostertagiasis in calves. Res. Vet. Sci. 10:18-28.

Anderson, N., Hansky, J. and Titchen, D.A. 1976. Hypergastrinaemia during parasitic gastritis in sheep. J. Physiol. (London), 256:51-52.

Anderson, N., Hansky, J. and Titchen, D.A. 1985. Effects on plasma pepsinogen, gastrin and pancreatic polypeptide of Ostertagia spp. transferred directly into the abomasum of sheep. Int. J. Parasitol. 15:159-165.

Anderson, R.C., Chabaud, A.G., Willmont, S. 1974. C.I.H. Keys to the Nematode Parasites of Vertebrates. No. 1, General Introduction, Comm. Agr. Bureaux, Farnham Royal, UK. pp. 17.

Armour, J. 1967. Ostertagia ostertagi infections in the bovine: Field and experimental studies. Ph.D. Thesis, University of Glasgow, U.K.

Armour, J. 1970. Bovine Ostertagiasis: A review. Vet. Rec. 86:184-189.

Armour, J. 1974. Parasitic gastroenteritis in cattle. Vet. Rec. 8:391-395.

Armour, J., Jennings, F.W. and Urquhart, G.M. 1967a. The possible existence of two strains of Ostertagia ostertagi. [Correspondence] Vet. Rec. 80:208-209.

Armour, J., Jennings, F.W. and Urquhart, G.M. 1967b. The possible existence of two strains of Ostertagia ostertagi. [Correspondence] Vet. Rec. 80:605-606.

Armour, J., Jennings, F.W. and Urquhart, G.M. 1969. Inhibition of Ostertagia ostertagi at the early fourth larval stage. I. The seasonal incidence. Res. Vet. Sci. 10:232-237.

Armour, J., Jennings, F.W., Murray, M. and Selman, I. 1973. Bovine Ostertagiasis. In: Urquhart, G.M. and Armour J. (eds.), Helminth Parasites of Cattle, Sheep and Horses in Europe, University Press, UK, pp. 11-23.

Armour, J. and Bruce, R.G. 1974. Inhibited development in Ostertagia ostertagi infections - a diapause phenomenon in a nematode. Parasitol. 69:161-174.

Armour, J., Jennings, F.W., Reid, J.F.S. and Selman, I.E. 1975. Treatment of bovine ostertagiasis. Vet. Rec. 97:188.

Armour, J., Bairden, K., Duncan, J.L., Jennings, F.W. and Parkins, J.J. 1979. Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. Vet. Rec. 105:500-503

Armour, J., Al Saqur, I.M., Bairden, K., Duncan, J.L. and Urquhart, G.M. 1980. Parasitic bronchitis and ostertagiasis on aftermath grazing. Vet. Rec. 106:184-185.

Armour, J., Bairden, K, Al Saqur, I.M., Duncan, J.L. 1981. The role of the soil as a potential reservoir of infective larvae of Ostertagia ostertagi. In: Nansen, P., Jorgensen, R.J., Soulsby, E.J.L. (eds.) Epidemiology and Control of Nematodiasis in Cattle. Martinus N hoff Publishers, The Hague, The Netherlands, pp. 277-282.

Armour, J. and Ogbourne, C.P. 1982. Bovine Ostertagiasis: a review and annotated bibliography. Miscellaneous publication No. 7 of the Commonwealth Inst. of Parasitol., St. Albans, pp.5-6.

Armour, J. and Duncan, M. 1987. Arrested larval development in cattle nematodes. Parasitology Today, 3:171-176.

Armour, J., Bairden, K. and Ryan, W.G. 1988. Immunity of ivermectin treated cattle to challenge from helminth parasites in the following season. Vet. Rec. 122:223-225.

Bairden, K., Parkins, J.J. and Armour, J. 1979. Bovine Ostertagiasis: a changing epidemiological pattern ?. Vet. Rec. 105:33-35.

Baker, N.F., Fisk, R.A., Bushnell, R.B. and Oliver, M.N. 1981. Seasonal occurrence of infective nematode larvae on irrigated pasture grazed by cattle in California. Am. J. Vet. Res. 42:1188-1191.

Barger, I.A., Bremner, K.C. and Waller, P.J. 1986. Factors influencing worm populations in cattle. In: The Epidemiology and Control of Gastrointestinal Parasites of Cattle in Australia. Comm. Sci. and Ind. Res. Org. (C.S.I.R.O.), Div. of An. Hlth, Australia. pp. 35-46.

Bayly, W.M. 1970. Type II bovine ostertagiasis - a review. Southwest. Vet. 30:171-175.

Becklund, W.W. 1962. Helminthiasis in Georgia cattle: A clinical and economic study. Am. J. Vet. Res. 23:510-515.

Bessanov, A.S. 1959. Study on the development of Ostertagia ostertagi in sheep. Helminthologica, 1:159-161.

Bisset, S.A. 1980. Goats and sheep as hosts for some common cattle trichostrongylids. Vet. Parasitol., 7:363-368.

Bizzell, W.E. and Ciordia, H. 1965. Dissemination of infective larvae of trichostrongylid parasites of ruminants from feces to pasture by the fungus Pilobolus spp. J. Parasitol. 51:184.

Borgsteede, F.H. and Hendriks, J. 1979. Experimental infections with Cooperia oncophora (Railliet, 1918) in calves. Results of single infections with two graded dose levels of larvae. Parasitol. 78:331-342.

Brauer, M.A. 1983. Epidemiology and bionomics of Ostertagia ostertagi in beef cattle in Missouri. Ph.D. thesis, University of Missouri, Columbia, pp. 52-61.

Bruce, R.G., Armour, J. 1974. Studies on the induction and duration of inhibition in Ostertagia ostertagi. Parasitol. 69:13-14.

Brunsdon, R.V. 1971. Trichostrongyle worm infections in cattle: further studies on problems of diagnosis and seasonal patterns of occurrence. N. Z. Vet. J. 19:203-212.

Brunsdon, R.V. 1972. Inhibited development of Ostertagia spp. and Cooperia spp. in naturally acquired infections in calves. New Z. Vet. J. 20:183-189.

Brunsdon, R.V. 1972. The potential role of pasture management in the control of trichostrongyle worm infection in calves with observations on the diagnostic value of plasma pepsinogen determinations. N. Z. Vet. J. 20:214-220.

Bürger, H.J. and Hartig, L. 1978. Some cases of winter ostertagiosis in cattle. In: Borgsteede, F.H., Armour, J. and Jansen, J. (eds.). Workshop on

"Arrested development of nematodes in sheep and cattle", Lelystad, The Netherlands, pp. 121-124.

Cabaret, J., Raynaud, J.P. and Le Stang, J.P. 1982. Comparison between tracer calves and herbage samplings for the assessment of pasture infectivity in trichostrongylosis of cattle. Vet. Parasitol. 10:65-71.

Cawley, G.D. and Lewis, C.J. 1975. Some observations on the treatment of ostertagiasis. Vet. Rec. 97:107-108.

Chalmers, K. 1983. Serum pepsinogen levels and Ostertagia ostertagi populations in clinically normal adult beef cattle. N. Z. Vet. J. 31:189-191.

Chiejina, S.N. 1977. Plasma pepsinogen levels in relation to ostertagiasis in adult cattle. Vet. Rec. 100:120.

Chiejina, S.N. 1978. Field observations on the blood pepsinogen levels in clinically normal cows and calves and in diarrhoeic adult cattle. Vet. Rec. 103:278-281.

Christie, P.R., Wakelin, D. and Wilson, M.M. 1979. The effect of the expulsion phase of Trichinella spiralis on Hymenolepis diminuta infection in rats. Parasitol. 78:323:330.

Ciordia, H., Bizzel, W.E. 1963. The effects of various constant temperatures on the development of the free-living stages of some nematode parasites of cattle. J. Parasitol. 49:60-63.

Coop, R.L., Smith, W.D., Angus, K.W., Graham, R.B., Wright, S.E., and Jackson, F. 1985. Effect of Ostertagia ostertagi on lamb performance and cross resistance to O. circumcincta. Res. in Vet. Sci. 39:200-206.

Craig, T.M. 1979. Seasonal transmission of bovine gastrointestinal nematodes in the Texas Gulf coast. J. Amer. Vet. Med. Assoc. 174:844-847.

Crofton, H.D. 1954. The vertical migration of infective larvae of strongyloid nematodes. J. Helminthol. 28:35-32.

Cross, D.A., Klesius, P.H. and Haynes, T.B. 1986. Lymphocyte blastogenesis responses of calves experimentally infected with Ostertagia ostertagi. Vet. Parasitol. 22:49-55.

Cross, D.A., Klesius, P.H., Hanrahan, L.A. and Haynes, T.B. 1987. Dermal cellular responses of helminth-free and Ostertagia ostertagi infected calves to intradermal infections of soluble extracts from O. ostertagi L3 larvae. Vet. Parasitol. 23:257-264.

Cross, D.A., Klesius, P.H. and Williams, J.C. 1988. Preliminary report: immunodiagnosis of Pre-Type II ostertagiasis. Vet. Parasitol. 27:151-158.

Cummins, L.J. and Callinan, A.P.L. 1979. Effects of levamisole and human chorionic gonadotrophin on ostertagiasis in western Victoria, Australia. Vet. Rec. 104:77-78.

Cunliffe, G. and Crofton, H.D. 1953. Egg sizes and differential egg counts in relation to sheep nematodes. Parasitol. 43:275-286.

Dineen, J.K., Donald, A.D., Wagland, B.M. and Offner, J. 1965. The dynamics of the host-parasite relationship. III. The response of sheep to primary infection with Haemonchus contortus. Parasitol. 55:515-525.

Donald, A.D., Dineen, J.K., Turner, J.H. and Wagland, B.M. 1964. The dynamics of the host-parasite relationship. I. Nematodirus spathiger infection in sheep. Parasitol. 54:527-544.

Douvres, F.W. 1956. Morphogenesis of the parasitic stages of Ostertagia ostertagi, a nematode parasite in cattle. J. Parasitol. 42:626-635.

Drozdz, J. 1965. Studies on helminths and helminthiases in Cervidae. I. Revision of the subfamily Ostertagiinae Sarwar 1956 and an attempt to

explain the phylogenesis of its representatives. Acta Parasitol. Pol. 13:445-481.

Drudge, J.H., Leland, S.E., Wyant, Z.N. and Rust, J.W. 1958. Winter survival of some cattle parasites on a Kentucky pasture with observations on the effects of low-level phenothiazine treatment. J. Parasitol. 44:434-438.

Eddi C.S. and Williams, J.C. 1988. Epidemiology of Dictyocauliasis in Louisiana, USA. Vet. Parasitol. (in press).

Entrocasso, C.M. 1987. Economic impact of gastrointestinal verminosis in the temperate climate areas of South America, with special reference to Argentina. In: Leaning, W.H. and Guerrero, J. (eds.). The economic impact of Parasitism in cattle. Proc. MSD AGVET Symposium Merck and Co., Montreal, Canada, pp. 53-58.

Entrocasso, C.M., McKellar, Q., Parkins, J.J., Bairden, K., Armour, J. and Kloostermann, A. 1986. The sequential development of Type I and Type II ostertagiasis in young cattle with special reference to biochemical and serological changes. Vet. Parasitol. 21:173-188.

Fiel, C.A., Steffan, P.E., Ambrustolo, R.R. and Biondani, C.A. 1985. Seasonal variations of internal parasitism in cattle, in the southeast of the province of Buenos Aires (Argentina) with special references to hypobiosis. W.A.A.V.P., 11th Conference, Rio de Janeiro, Brazil, pp. 23.

Fife, E.H. 1971. Advances in methodology for immunodiagnosis of parasitic diseases. Exp. Parasitol. 30:132-163.

Fincher, G.T. and Stewart, T.B. 1979. Vertical migration by nematode larvae of cattle parasites through soil. Proc. Helminthol. Soc. Wash. 46:43-46.

Fox, M.T., Pitt, S.R., Gerrelli, D., and Jacobs, D.E. 1988. Effect of diet on gastrin response to

Ostertagia ostertagi infection in the calf. Int. J. Parasitol. 18:547-548.

Frank, G.R., Herd, R.P., Marbury, K.S. and Williams, J.C. 1986. Effects of transfer of Ostertagia ostertagi between northern and southern U.S.A. on the pattern and frequency of hypobiosis. Int. J. Parasitol. 16:391-398.

Frankena, K. 1987. The interaction between Cooperia spp. and Ostertagia spp. (Nematoda: Trichostrongylidae) in cattle. PhD Thesis, Agricultural University Wageningen, The Netherlands, pp. 40-53.

Gardener, R.C. 1911. Parasitic gastritis in sheep and cattle. J. of the South Eastern Agr. Coll., Wye, Kent, 20:482-493.

Gasbarre, L.C. 1986. Limiting dilution analyses for the quantification of cellular immune responses in bovine ostertagiasis. Vet. Parasitol. 20:133-147.

Gibbs, H.C. 1986. Hypobiosis in parasitic nematodes - an update. Adv. in Parasitol. 25:129-174.

Gibbs, H.C. 1979. Relative importance of winter survival of larval nematodes in pasture and infected carrier calves in a study of parasitic gastroenteritis in calves. Am. J. Vet. Res. 40:227-231.

Goldberg, A. 1968. Development and survival on pasture of gastrointestinal nematode parasites of cattle. J. Parasitol. 54:856-862.

Goldberg, A. 1970. Development, migration and survival on pasture of gastrointestinal nematodes of cattle: summer contamination. Proc. Helminthol. Soc. Wash. 37:166-169.

Goldberg, A. and Rubin, R. 1956. Survival on pasture of gastrointestinal nematodes of cattle. Proc. Helminthol. Soc. Wash. 23:65-68.

Gronvold, J. 1979. On the possible role of earthworms in the transmission of Ostertagia ostertagi

third-stage larvae from feces to soil. J. Parasitol. 65:831-832.

Hay, J.B., Cahill, R.N., and Trnka, Z. 1974. The kinetics of antigen-reactive cells during lymphocyte recruitment. Cell. Immunol. 10:145-153.

Herd, R. 1980. Animal health and public health aspects of bovine parasitism. J. Am. Vet. Med. Assoc. 176:737-743.

Herlich, H. 1958. Experimental infections of sheep with three species of gastrointestinal nematodes of cattle. Trans. Am. Microsc. Soc. 77:373-379.

Herlich, H. 1976. Attempts to produce protection against Ostertagia ostertagi in cattle. Am. J. Vet. Res. 37:61-64.

Herlich, H. and Douvres, F.W. 1979. Gastrointestinal nematode immunization trials in cattle. Am. J. Vet. Res. 40:1781-1782.

Herlich, H. and Tromba, F.G. 1980. Effect of ultraviolet radiation on some gastrointestinal nematode parasites of cattle. J. Parasitol. 66:692-694.

Hirschowitz, B.I. 1965. Pepsinogen in the blood. J. Lab. Clin. Med. 46:568-569.

Holtenius, P., Uggla, A. and Olson, G. 1983. Epidemiological studies on Ostertagia ostertagi infections in cattle during their 1st, 2nd, and 3rd summer on pasture. Nord. Vet. Med., 8:233-238.

Inderbitzin, F. 1980. Serum pepsinogen levels in cattle with trichostrongylid infections. Proc. 2nd International Symposium of Veterinary Laboratory Diagnosticians, Lucerne, Switzerland, Vol. II, pp. 185-188.

Jacobs, D.E., Todd, M.E., Dunn, A.M. and Walker, J. 1968. Farm-to-farm transmission of porcine oesophagostomiasis. Vet. Rec. 82:57.

Jarrett, W.F., Jennings, F.W., McIntyre, W.L., Mulligan,

W. and Urquhart, G.M. 1960. Immunological studies on Dictyocaulus viviparus infection: immunity produced by the administration of irradiated larvae. Immunol. 3:145.

Jennings, F.W., Armour, J., Lawson, D.D. and Roberts, R. 1966. Experimental Ostertagia ostertagi infections in calves: studies with abomasal cannulas. Am. J. Vet. Res. 27:1249-1257.

Jensen, P.T. and Nansen, P. 1978. Immunodiagnosis in bovine ostertagiasis. Acta Vet. Scand. 19:601-603.

Kennedy, M.W. 1980. Immunological mediated, non-specific interactions between the intestinal phases of Trichinella spiralis and Nippostrongylus brasiliensis. Parasitol. 80:61-72.

Klei, T.R., Chapman, M.R., Fulton, R.W. and Downing, M.M. 1981. Enzyme-Linked Immunosorbent Assay. In: Barta, O. (ed.) Manual of Veterinary Clinical Immunology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, pp. 171-175.

Klesius, P.H., Washburn, S.M., Ciordia, H., Haynes, T.B. and Snider III, T.G. 1984. Lymphocyte reactivity to Ostertagia ostertagi L3 antigen in type I ostertagiasis. Am. J. Vet. Res. 45:230-233.

Klesius, P.H., Haynes, T.B. and Cross, D.A. 1985. Chemotactic factors for eosinophils in soluble extracts of L3 stages of Ostertagia ostertagi. Int. J. Parasitol. 15:517-522.

Klesius, P.H., Haynes, T.B., Cross, D.A. and Ciordia, H. 1986. Ostertagia ostertagi: Excretory secretory chemotactic substance from infective larvae of eosinophil locomotion. Exp. Parasitol. 61:120-125.

Klesius, P.H. 1988. Immunity to Ostertagia ostertagi. Vet. Parasitol. 27:159-167.

Kloosterman, A., Borgsteede, F.H. and Eysker, M. 1984.

The effect of experimental Ostertagia ostertagi infections in started milking cows on egg output, serum pepsinogen levels, antibody titers and milk production. Vet. Parasitol. 17:299-308.

Kloosterman, A., Albers, G.J. and van den Brink, R. 1984. Negative interactions between Ostertagia ostertagi and Cooperia oncophora in calves. Vet. Parasitol. 15:135-150.

Korot'ko, G.F. and Islyamova, M.E. 1963. Determination of the proteolytic activity of gastric juice, urine and serum. Sbornik Nauchmykh Trudor. Andizahnskii Gosudorstrennyi Meditsinskii Institute (Collection of Scientific Works) 4:114-126.

Kristensen, F., Kristensen, B. and Lazary, S. 1982. The lymphocyte stimulation test in veterinary medicine. Vet. Imm. Immunopath. 3:203-277.

Leland, S.E. 1963. Studies on the in vitro growth of parasitic nematodes. I. Complete or partial parasitic development of some gastrointestinal nematodes of sheep and cattle. J. of Parasitol. 49:600-611.

Levine, N.D. 1978. Veterinary Parasitology. Burgess Publishing Co. Minneapolis, Mn pp. 93-100.

Levine, N.D. 1980. Nematode Parasites of Domestic Animals and of Man. 2nd. Ed., Burgess Publishing Co., Minneapolis, Mn, pp. 125-221.

Lichtenfels, J.R., Pilitt, P.A., and Lancaster, M.B. 1988. Systematics of the nematodes that cause ostertagiasis in cattle, sheep and goats in North America. Vet. Parasitol. 27:3-12.

Lowry, D.H., Rosenbrough, N.V., Farr, A.L. and Randall, R.J. 1951. Protein measurements with the Folin Phenol reagent. J. Biol. Chem. 193:265-274.

Malczewski, A., Wescott, R.B., Spratling, B.M. and Gorham, J.R. 1975. Internal parasites of Washington cattle. Am. J. Vet. Res. 36:1671-

1675.

Manual of Veterinary Parasitology Laboratory Techniques. 1977. Technical Bulletin No. 18, Ministry of Agriculture, Fisheries and Food. Agricultural Development and Advisory Service. pp. 38-51.

Martin, W.B., Thomas, B.A. and Urquhart, G.M. 1957. Chronic diarrhoea in housed cattle due to atypical parasitic gastritis. Vet. Rec. 69:736-740.

Martin, B. 1977. The control of bovine ostertagiasis. Vet. Rec. 101:334-335.

McKellar, Q.A. 1984. Studies on the pathophysiology and immune response to Ostertagia ostertagi in cattle. PhD Thesis submitted to the Faculty of Veterinary Medicine of the University of Glasgow, Scotland, pp. 4-68.

McKellar, Q., Duncan, J.L. and Armour, J. 1986. Response to transplanted adult Ostertagia ostertagi in calves. Res. Vet. Sci. 40:367:371.

McKellar, Q., Duncan, J.L., Armour, J., and Lindsay, F.E. 1987. Further studies on the response to transplanted adult Ostertagia ostertagi in calves. Res. Vet. Sc. 42:29-34.

McKellar, Q., Eckersall, P.D., Duncan, J.L. and Armour, J. 1988. Forms of bovine pepsinogen in plasma from cattle in three different "syndromes" of Ostertagia ostertagi infection. Res. Vet. Sci. 44:29-32.

Michel, J.F. 1963. The phenomenon of host resistance and course of infection of Ostertagia ostertagi in calves. Parasitology, 53:63-84.

Michel, J.F. 1967. The possible existence of two strains of Ostertagia ostertagi. Vet. Rec. 80:316-317.

Michel, J.F. 1969a. The epidemiology and control of some nematode infections of grazing animals. Adv. Parasitol. 7:211-282.

Michel, J.F. 1969b. The regulation of egg output by

Ostertagia ostertagi in calves infected once only. Parasitol. 59:829-835.

Michel, J.F. 1969c. Some observations on the worm burdens of calves infected daily with Ostertagia ostertagi. Parasitol. 59:575-595.

Michel, J.F. 1971. Adult worms as a factor in the inhibition of development of Ostertagia ostertagi in the host. Int. J. Parasitol. 11:31-36.

Michel, J.F. 1974. Arrested development of nematodes and some related phenomena. Adv. Parasitol. 12:279-366.

Michel, J.F. 1978. Topical themes in the study of arrested development. In: Borgsteede, F.H. (ed.). Facts and Reflections. III. Workshop on Arrested Development of Nematodes of Sheep and Cattle. Central Veterinary Institute, Netherlands, Lelystad, pp. 7-17.

Michel, J.F. and Sinclair, I.J. 1969. The effect of cortisone on the worm burdens of calves infected daily with Ostertagia ostertagi. Parasitol. 59:691-708.

Michel, J.F., Lancaster, M.B. and Hong, C. 1971. Host-induced effects on the size and body proportions of Ostertagia ostertagi. J. Parasitol. 57:1185-1189.

Michel, J.F., Lancaster, M.B. and Hong, C. 1972a. The development and symmetry of the vulval flap of Ostertagia ostertagi. Int. J. for Parasitol., 2:297-304.

Michel, J.F., Lancaster, M.B. and Hong, C. 1972b. Host induced effects on the vulval flap of Ostertagia ostertagi. Int. J. Parasitol. 2:305-317.

Michel, J.F., Lancaster, M.B. and Hong, C. 1973. Ostertagia ostertagi: protective immunity in calves. The development in calves of a protective immunity to infection with

Ostertagia ostertagi. Exp. Parasitol. 33:179-186.

Michel, J.F., Lancaster, M.B. and Hong, C. 1976a. Observations on the resumed development of arrested Ostertagia ostertagi in naturally infected yearling cattle. J. Comp. Path. 86:73-80.

Michel, J.F., Lancaster, M.B. and Hong, C. 1976b. The resumed development of arrested Ostertagia ostertagi in experimentally infected calves. J. Comp. Path. 86:615-619.

Michel, J.F., Lancaster, M.B. and Hong, C. 1979. The effect of age, acquired resistance, pregnancy and lactation on some reactions of cattle to infection with Ostertagia ostertagi. Parasitol. 79:157-168.

Miller, T.A. 1965. Persistence of immunity following double vaccination with x-irradiated Ancylostoma caninum larvae. J. Am. Vet. Med. Assoc. 150:508.

Moqbel, R. and Wakelin, D. 1979. Trichinella spiralis and Strongyloides ratti: immune interaction in adult rats. Exp. Parasitol. 47:65-72.

Murray, M. 1969. Structural changes in bovine ostertagiasis associated with increased permeability of the bowel wall to macromolecules. Gastroenterol. 56:763-772.

Murray, M., Jennings, F.W. and Armour, J. 1970. Bovine ostertagiasis: structure, function and mode of differentiation of bovine gastric mucosa and kinetics of the worm loss. Res. Vet. Sci. 11:417-427.

Mylrea, P.J., and Hotson, I.K. 1969. Serum pepsinogen activity and the diagnosis of bovine ostertagiasis. Br. Vet. J. 125: 379-387.

Nansen, P., Jorgensen, R.J., Henriksen, F. and Foldager, J. 1987. The effects of late turnout on the epidemiology and control of ostertagiasis in calves. Vet. Parasitol. 24:139-147.

Niec, R. 1979. Control of bovine ostertagiasis. Revista de Medicina Veterinaria. 60:196-197.

Nilsson, O. and Sorelius, L. 1973. Trichostrongyle infections of cattle in Sweden. Nord. Vet. Med. 25:65-78.

O'Sullivan, B.M. and Donald, A.D. 1970. A field study of nematode parasite populations in the lactating ewe. Parasitol. 61:301.

Osborne, J.C., Batte, E.C. and Bell, R.R. 1960. The pathology following single infections of Ostertagia ostertagi in calves. Cornell Vet. 50:223-235.

Pandey, V.S. 1972. Effect of temperature on development of the free-living stages of Ostertagia ostertagi. J. Parasitol. 58:1037-1041.

Prichard, R.K., Donald, A.D. and Hennessy, D.R. 1974. The effect of corticosteroid treatment on the development of inhibited Ostertagia ostertagi larvae. Res. Vet. Sci. 16:267-269.

Raynaud, J.P. and Bouchet, A. 1976a. Bovine ostertagiosis: a review. Analysis of types and syndromes found in France by post mortem examinations and total worm counts. Ann. Rech. Vét. 7:253-280.

Raynaud, J.P. and Bouchet, A. 1976b. Results of post-mortem examinations and total worm counts on cattle with Ostertagia infection in France. In: 9° Congrés International sur les maladies du bétail, 6-9 Septembre, 1976, Paris. Rapportes et Résumés, Tome 2. Boigneville. France: Société Française de Buiatrie, pp. 1083-1089.

Raynaud, J.P. and Bouchet, A. 1977. Bovine ostertagiasis. General review and analysis of types and syndromes found in France according to total worm counts. Rev. Med. Vet. 128:155-156, 159-178, 183-189.

Raynaud, J.P. 1978a. Facts and reflections on cattle

ostertagiosis in France. In: Facts and reflections III. Borgsteede, F.H., Armour, J. and Jansen, J. (eds.). Centr. Vet. Inst. Lelystad, The Netherlands, pp. 143-155.

Raynaud, J.P. 1978b. Facts and reflections on cattle ostertagiosis in France. In: Borgsteede, F.H., Armour, J. and Jansen, J. (eds.). Workshop on "Arrested development of nematodes in sheep and cattle", Lelystad, The Netherlands, pp. 157-162.

Reynolds, G.W., Anderson, N., Stiffe, G., Hansky, J. and Titchen, D.A. 1979. Ovine tissue and serum gastrin in ostertagiasis. Ann. Rech. Vet. 10:341-343.

Ritchie, J.S., Anderson, N., Armour, J., Jarrett, W.F., Jennings, F.W. and Urquhart, G.M. 1966. Experimental Ostertagia ostertagi infections in calves: parasitology and pathogenesis of a single infection. Am. J. Vet. Res. 27:659-667.

Rogers, W.P. and Sommerville, R.I. 1960. The physiology of the second ecdysis of parasitic nematodes. Parasitol. 50:329-348.

Rogers, W.P. 1966. Exsheathment and hatching mechanisms in helminths. In: Biology of Parasites, Soulsby, E.J.L. (ed.) Academic Press, New York, London, pp. 33-39.

Rose, J.H. 1961. Some observations on the free-living stages of Ostertagia ostertagi, a stomach worm of cattle. Parasitol. 51:295-307.

Rose, J.H. 1969. The development of the parasitic stages of Ostertagia ostertagi. J. Helminthol. 2:173-184.

Rose, J.H. 1970. Parasitic gastro-enteritis in cattle. Factors influencing the time of the increase in the worm population on pastures. Res. Vet. Sci. 11:199-208.

Ross, J.G. and Dow, C. 1964. Further experimental infections of calves with the nematode parasite Ostertagia ostertagi. Brt. Vet. J.

120:279-285.

SAS User's Guide: Statistics 1985. 6th Edition. SAS Institute Inc., Cary, NC, USA.

SAS System for Elementary Statistical Analysis 1987. SAS Institute Inc., Cary, NC, USA, pp. 113-125.

Schad, G.A. 1977. Regulation of Parasite Populations. Esch, G.W. eds., Academic Press, New York, pp. 18-21.

Schillhorn-Van Veen, T.W., Nachreiner, R.F., and Williams, J.F. 1980. Serum gastrin levels in cattle. In: 61 st Conference of Research Workers in Animal Disease, CRWAD, Ohio Agric. Res. Dev. Cent., Wooster, OH, p. 61.

Schillhorn-Van Veen, T.W. and Melancon, J.J. 1984. The trichostrongylid egg output during spring in feedlot cattle. Vet. Parasitol. 14:159-163.

Schillhorn-Van Veen, T.W. 1988. Evaluation of abomasal enzyme and hormone levels in the diagnosis of ostertagiasis. Vet. Parasitol. 27:139-149.

Schwink, T.M. 1963. Development and survival of some roundworm larvae of cattle at high altitudes in Wyoming. Proc. Helminthol. Soc. Wash. 30:15-18.

Selman, I.E., Reid, J.F., Armour, J. and Jennings, F.W. 1976. Type II ostertagiasis in adult cattle. Vet. Rec. 99:141-143.

Skrjabin, K.I., Shikhobalova, N.P., Schultz, R.S. 1954. Essentials of Nematology. Vol. III. Trichostrongylids of Animals and Man. Academy of Sciences, Moscow, USSR,

Slocombe, J.O.D. 1974. Overwintering of bovine gastrointestinal nematodes in southwestern Ontario. Can. J. Comp. Med. 38:90-93.

Smeal, M.G., Hotson, I.K., Mylrea, P.J., Jackson, A.R., Campbell, and N., Kirton, H.C. 1977. Studies on nematode infections of beef cattle in New South Wales. Australian Vet. J., 53:566-573.

Smeal, M.G. and Donald, A.D. 1981. Effects on inhibition of development of the transfer of Ostertagia ostertagi between geographical regions of Australia. Parasitol. 82:389-399.

Smith, H.J. 1970. On the development of gastrointestinal parasitism in bovine yearlings. Can. J. Com. Med. 34:303-308.

Smith, H.J. 1972. On the persistence of infective Ostertagia ostertagi, Cooperia oncophora and Nematodirus helvetianus larvae on pastures. Can. J. Com. Med. 36:333-338.

Smith, J.D. 1976. Physiology of Nematodes. Introduction to animal parasitology, 2nd edition, Hodder and Stoughton, London, pp. 355.

Smith, H.J. and Perreault, J.P. 1972. A type II ostertagiasis outbreak in cattle in New Brunswick. Can. Vet. J. 13:114-117.

Smith W.D., Jackson, F., Jackson, E., Graham R., Williams, J., Willadsen, S.M. and Fehilly, C.B. 1986. Transfer of immunity to Ostertagia circumcincta and Ig A memory between identical sheep by lymphocytes collected from gastric lymph. Res. Vet. Sci. 41:300-306.

Snider, T.G., Williams, J.C., Sheehan, D.S. and Fuselier, R.H. 1981. Plasma pepsinogen, inhibited larval development and abomasal lesions in experimental infections of calves with Ostertagia ostertagi. Vet. Parasitol. 8:173-184.

Snider III, T.G., Klesius, P.H. and Haynes, T.B. 1985. Dermal responses to Ostertagia ostertagi and Cooperia punctata-infected calves. Am. J. Vet. Res. 46:887-890.

Snider III, T.G., Williams, J.C., Karns, P.A., Romaire, T.L., Trammel, H.E. and Kearney, M.T. 1986. Immunosuppression of lymphocyte blastogenesis in cattle infected with Ostertagia ostertagi and/or Trichostrongylus axei. Vet. Imm. and Immunopathol. 11:251-264.

Snider III, T.G., Williams, J.C., Knox, J.W., Marbury, K.S., Crowder, C.H. and Willis, E.R. 1988. Sequential histopathologic changes of type I, pre-type II and type II ostertagiasis in cattle. Vet. Parasitol. 27:169-179.

Snider III, T.G., Williams, J.C., Karns, P.A., Markovitz, J.E., Romaire, T.L. 1988. High Concentration of serum gastrin immunoreactivity and abomasal mucosal hyperplasia in calves infected with Ostertagia ostertagi and/or Trichostrongylus axei. Am.J. Vet. Res. 49:2101-2104.

Sokolic, A., Javanovic, M., Cuperlovic, K. and Movsesijan, M. 1965. Vaccination against Dictyocaulus filaria with irradiated larvae. Brit. Vet. J. 121:212.

Sollod, A.E. 1967. The possible existence of two strains of Ostertagia ostertagi. [Correspondence] Vet. Rec. 81:547-548.

Soulsby, E.J.L. 1982. Helminths, Arthropods and Protozoa of Domestic Animals, 7th Ed., Lea and Febiger, Philadelphia, pp. 212-258.

Steel, R.G.D. and Torrie, J.H. 1985. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed., McGraw-Hill Book Company, New York. pp. 592-593.

Steffan, P.E., Fiel, C.A., Ambrustolo, R.R. and Biondani, C.A. 1985. Use of ivermectin in the control of endo and ectoparasites in cattle: epidemiological implications and effect on weigh gains. W.A.A.V.P. 11th Conference, Rio de Janeiro, Brazil, p. 23.

Stewart, W.L. and Crofton, H.D. 1954. Parasitic gastritis in cattle. Vet. Rec. 53:619-621.

Stringfellow, F. and Madden, P.A. 1979. Effects of Ostertagia ostertagi on pepsinogen granules of chief cells from calf abomasa correlated with selected plasma and abomasal proteins. Proc. Helminthol. Soc. Wash. 46:223-239.

Suarez, V.H. 1985. Seasonal variation of nematodes in beef production on grazing systems in the pampean semiarid region of Argentina. W.A.A.V.P. 11th Conference, Rio de Janeiro, Brazil, p. 41.

Suderman, M.T. 1979. Seasonal Hypobiosis of Ostertagia ostertagi (Stiles, 1892) Ransom, 1907 in cattle in Brazos County, Texas. M.S. Thesis, Texas A & M University, College Station, pp. 1-79.

Taylor, E.L. 1930. Some factors concerning the occurrence of disease due to Strongyloid worms in grazing animals - verminous gastritis, Hoose and Equine Strongylidosis. Vet. Rec. 10:602-606.

Taylor, S.M., Cawthorne, R.J., Kenny, J. and Regan, M. 1973. Acute parasitic gastro-enteritis in calves in spring. Vet. Rec. 93:603-604.

Terry, R.J. 1968. The application of immunology to helminth diseases. In: Taylor, A.E. (ed.), Immunity to parasites. Blackwell, Oxford, pp. 91-103.

Threlkeld, W.L. 1946. The life history of Ostertagia ostertagi. Va. Agric. Exp. Sta. Bull. No. 100, p. 27.

Threlkeld, W.L. and Johnson, E.P. 1948. Observations on the pathogenicity and viability of Ostertagia ostertagi. Vet. Med. 43:446.

Threlkeld, W.L. 1958. The histotrophic phase and other biological aspects of Ostertagia ostertagi. J. Parasitol. 44:342-343.

Uete, T., Wasa, M. and Shimogani, A. 1969. A simplified method for the detection of pepsinogen in blood and urine. Clin. Chem. 15:42-55.

Vegors, H.H. 1958. Observations on inhibited development of cattle nematodes. Proc. of the Helminthol. Soc. of Wash. 25:86-90.

Washburn, S.M. and Klesius, P.H. 1984. Leukokinesis in bovine ostertagiasis: stimulation of leukocyte migration by Ostertagia. Am. J. Vet. Res. 45:1095-1098.

Wedderburn, J.F. 1970. Ostertagiasis in adult cattle: a clinical report of an outbreak in the field. N. Z. Vet. J. 18:168-170.

Wedderburn, J.F. 1972. Clinical observations on ostertagiasis in yearling cattle. N. Z. Vet. J. 20:58-59.

Whitlock, J.H. 1960. Diagnosis of Veterinary Parasitism. Lea and Febiger, Philadelphia, p. 167.

Williams, J.C. 1983. Ecology and control of gastrointestinal nematodes of beef cattle. Vet. Clin. N. Am.: (Large Animal Practice). 5:183-205.

Williams, J.C. 1987. Infection with Ostertagia ostertagi in goats and calves. Vet. Parasitol. 23:51-57.

Williams, J.C. and Mayhew, R.L. 1967. Survival of infective larvae of the cattle nematodes, Cooperia punctata, Trichostrongylus axei, and Oesophagostomum radiatum. Am. J. Vet. Res. 5:629-640.

Williams, J.C. and Bilkovich, F.R. 1971. Development and survival of infective larvae of the cattle nematode Ostertagia ostertagi. J. of Parasitol. 57:327-338.

Williams, J.C. and Bilkovich, F.R., 1973. Distribution of Ostertagia ostertagi infective larvae on pasture herbage. Am. J. Vet. Res. 34:1337-1344.

Williams, J.C., Roberts, D.E. and Todd, J.M. 1974. Ostertagia ostertagi: establishment of patent infections in calves by intravenous inoculation. Int. J. Parasitol. 4:55-61.

Williams, J.C. and Knox, J.W. 1976. Effect of nematode parasite infection on the performance of

stocker cattle at high stocking rates on coastal bermudagrass pastures. Am. J. of Vet. Res. 37:453-464.

Williams, J.C., Knox, J.W., Sheehan, D.S. and Fuselier, R.H. 1977. Efficacy of albendazole against inhibited early fourth- stage larvae of Ostertagia ostertagi. Vet. Rec. 101:484-486.

Williams, J.C., Knox, J.W., Sheehan, D.S., Fuselier, R.H. and Shields, C.R. 1978. Problems of controlling stomach worms in cattle. La. Agric. 22:14-15.

Williams, J.C., Knox, J.W., Sheehan, D.S. and Fuselier, R.H. 1979a. Activity of fenbendazole against inhibited early fourth-stage larvae of Ostertagia ostertagi. Am. J. Vet. Res. 40:1087-1090.

Williams, J.C., Knox, J.W., Sheehan, D. and Fuselier, R.H. 1979b. Further evaluation of the activity of albendazole against inhibited larvae of Ostertagia ostertagi. Vet. Rec. 105:98-100.

Williams, J.C., Knox, J.W., Baumann, B.A., Snider, T.G. and Hoerner, T.J. 1981a. Anthelmintic efficacy of albendazole against inhibited larvae of Ostertagia ostertagi. Am. J. Vet. Res. 42:318-321.

Williams, J.C., Knox, J.W., Baumann, B.A., Snider, T.G. and Hoerner, T.J. 1981b. Further studies on the efficacy of fenbendazole against inhibited larvae of Ostertagia ostertagi. Vet. Rec. 108:228-230.

Williams, J.C., Knox, J.W., Baumann, B.A., Snider, T.G., Kimball, M.D. and Hoerner, T.J. 1983. Seasonal changes of gastrointestinal nematode populations in yearling beef cattle in Louisiana with emphasis on prevalence of inhibition in Ostertagia ostertagi. Int. J. Parasitol. 13:133-143.

Williams, J., Knox, J., Marbury, K. and Kimball, M.D. 1985. Ostertagia ostertagi. A continuing problem of recognition and control. Anim.

Health and Nutr. 40:42-45.

Williams, J.C. 1986a. Epidemiological patterns of Nematodiasis in Cattle. In: Gibbs, H., Herd, R. and Murrell, D. (eds.), Parasites: Epidemiology and Control. Vet. Clin. N. Am., W.B. Saunders, Co., Philadelphia, PA, pp. 235-246.

Williams, J.C., Corwin, R.M., Craig, T.M. and Wescott, R.B. 1986b. Control strategies for Nematodiasis in cattle. In: Parasites: Epidemiology and Control. Vol. 2, No.2. Gibbs, H., Herd, R.P. and Murrell D.K. (eds.), Vet. Clin. N. Am., W.B. Saunders Company, Philadelphia. pp. 247-260.

Williams, J.C., Knox, J.W., Marbury, K.S., Kimball, M.D., Baumann, B.A. and Snider, T.G. 1987. The epidemiology of Ostertagia ostertagi and other gastrointestinal nematodes of cattle in Louisiana. Parasitol. 95:135-153.

Williams, J.C. and Knox, J.W. 1988. Epidemiology of Ostertagia ostertagi in warm temperate regions of the United States. Vet. Parasitol. 27:23-28.

Worley, D.E., Sharman, G.A. 1966. Gastritis associated with Ostertagia bisonis in Montana range cattle. J. Am. Vet. Med. Assoc. 149:1291-1294.

Yamaguti, S. 1961. Systema Helminthum. Vol. III The Nematodes of Vertebrates. Intersciences Publ., Inc. New York. pp. 433-436.

APPENDICES

Table A-1. Test for normality of variables measured in the experiment

Variable	Skewness	Kurtosis	W: Normal	Prob < W
Pepsinogen	2.3224	6.1877	0.7292	0.0002*
Weight	0.193	0.2347	0.9809	0.9504
TWC[1]	2.6360	6.9609	0.4961	0.0001*
EPG[2]	2.3113	6.5886	0.7562	0.0005*
$\%EL_4$[3]	-0.8800	-1.0739	0.8015	0.0062*
ELISA[4]	1.7943	2.5138	0.7084	0.0001*
MPS[5]	-0.4197	0.4540	0.9638	0.7816
Blastogenesis	0.9565	0.1351	0.9309	0.5969*
NEF[6]	-0.4187	-1.2060	0.9071	0.0584*
LAF[7]	0.0795	-0.5106	0.9676	0.6996
LAM[8]	0.8062	-0.0971	0.9299	0.1627
LEL_4F[9]	0.3790	-1.1048	0.9355	0.2082
LEL_4M[10]	-0.6907	-0.3260	0.9047	0.0526
Vulvalflap	-0.2894	-1.0859	0.8978	0.0384*
Spicules	1.1860	1.8275	0.9093	0.0646*

1 = Total worm counts;
2 = Eggs per gram of feces
3 = Percentage of early 4th stage larvae
4 = Enzyme Linked Immunoabsorbert Assay
5 = Mucosal pathology score
6 = Number of eggs in females
7 = Length of adult females
8 = Length of adult males
9 = Length of early 4th stage larvae (female)
10 = Length of early 4th stage larvae (male)
* = Distribution not normal

Table A-2. Percentage and (Arcsin transformations) of Ostertagia ostertagi early 4th stage larvae recovered at necropsy of principal calves (n=2/group) killed from June to September 1988.

	June	July	August	September
Group 1				
Grazing	69 (56.17) 72 (58.05)	81 (64.16) 82 (58.05)	77 (61.34) 74 (59.34)	39 (38.65) 49 (44.43)
Confinement	78 (62.03) 81 (64.16)	87 (80.02) 84 (66.42)	67 (54.94) 69 (56.17)	30.8 (33.71) 39 (38.65)
Group 2				
Grazing	63 (52.53) 62 (51.94)	15 (22.79) 78 (62.03)	60 (50.77) 59 (50.18)	27.6 (31.69) 32.6 (34.82)
Confinement	76 (60.67) 67 (54.94)	70 (56.79) 66 (54.33)	58 (49.60) 31 (33.83)	1 (5.74) 4.3 (11.97)
Group 3				
Grazing	20 (26.56) 24 (29.33)	50 (45) 42 (40.40)	26 (30.66) 40 (39.23)	3.3 (10.47) 10.5 (18.91)
Confinement*	11 (19.37) 7 (15.34) 69 (56.17)	29 (32.58) 6 (14.18)	0 (0) 72 (58.05)	0 (0)

*A third or extra group 3 (confinement) calf was inadvertently killed in June; this left only 1 calf in the subgroup for slaughter in September.

Table A-3. Gross pathology scores* of abomasal mucosa from individual principal animals killed from June to September, 1988.

	June	July	August	September
Group 1				
Grazing	2.5	4.0	2.0	5.0
	2.5	4.5	2.5	5.0
Confinement	4.5	2.0	5.0	5.0
	2.0	4.0	4.5	5.0
Group 2				
Grazing	3.5	3.0	2.5	3.5
	3.5	1.0	4.0	4.0
Confinement	3.0	3.0	2.5	3.0
	3.5	2.0	4.0	3.0
Group 3				
Grazing	0.0	2.0	4.0	1.5
	1.5	2.0	2.0	2.0
Confinement**	1.5	2.0	1.5	2.5
	1.5	3.0	1.5	
	2.0			

*Observed gross pathology was scored from 0 to 5 with 0 being normal and 5 being severe pathology.

**A third or extra group 3 (confinement) calf was inadvertently killed in June; this left only 1 calf in the subgroup for slaughter in September.

Table A-4. Actual numbers (Log_{10} transformation) of total Ostertagia ostertagi recovered from principal calves (n=2/group) killed from June to September, 1988

	June	July	August	September
Group 1				
Grazing	9,420(3.974)	118,790(5.075)	154,800(5.190)	139,887(5.146)
	107,138(5.030)	102,680(5.011)	211,070(5.324)	229,253(5.360)
Confinement	16,360(4.214)	2,085(3.319)	2,490(3.396)	12,492(4.097)
	164,291(5.216)	3,091(3.490)	2,077(3.317)	4,752(3.677)
Group 2				
Grazing	38,786(4.589)	11,732(4.069)	9,168(3.962)	21,001(4.322)
	13,556(4.132)	8,960(3.952)	3,026(3.481)	24,533(4.390)
Confinement	16,360(4.214)	2,085(3.319)	2,490(3.396)	12,492(4.097)
	164,291(5.216)	3,091(3.490)	2,077(3.317)	4,752(3.677)
Group 3				
Grazing	2,387(3.378)	23,273(4.367)	3,323(3.522)	2,113(3.325)
	2,497(3.397)	16,376(4.214)	946(2.976)	2,670(3.423)
Confinement*	645(2.810)	850(2.929)	1,500(3.179)	1,200(3.079)
	2,334(3.368)	2,175(3.337)	6,350(3.803)	
	1,916(3.282)			

*A third or extra group 3 (confinement) calf was inadvertently killed in June; this left only 1 calf in the subgroup for slaughter in September.

Table A-5. Actual numbers (and Log_{10} transformation) of total worms recovered from principal calves (n=2/group) killed from June to September 1988

	June	July	August	September
Group 1				
Grazing	13,120(4.118)	160,132(5.204)	341,446(5.533)	196,749(5.294)
	143,733(5.158)	128,384(5.109)	258,481(5.412)	291,380(5.464)
Confinement	120,183(5.080)	88,518(4.947)	115,978(5.064)	81,825(4.913)
	84,304(4.926)	115,103(5.061)	67,784(4.831)	120,869(5.082)
Group 2				
Grazing	52,075(4.717)	47,592(4.678)	75,706(4.879)	212,528(5.327)
	65,355(4.815)	17,234(4.236)	20,944(4.321)	116,818(5.068)
Confinement	22,070(4.344)	3,385(3.530)	4,390(3.642)	14,122(4.150)
	185,468(5.268)	4,291(3.633)	8,176(3.913)	5,752(3.760)
Group 3				
Grazing	18,370(4.264)	228,954(5.630)	10,793(4.033)	30,343(4.482)
	9,719(3.988)	68,588(4.836)	111,333(5.047)	54,149(4.734)
Confinement*	8,645(3.937)	2,651(3.423)	2,943(3.469)	2,235(3.349)
	9,172(3.962)	6,175(3.791)	13,383(4.127)	
	10,843(4.035)			

*A third or extra group 3 (confinement) calf was inadvertently killed in June; this left only 1 calf in the subgroup for slaughter in September.

Table A-6. Mean numbers of O. ostertagi and total numbers of all worm genera (TNW) recovered from principal calves that were grazed continuously or grazed for 1 month and then kept in pens

	June		July		August		September	
	O. ostertagi	TNW	O. ostertagi	TNW	O. ostertagi	TNW	O. ostertagi	TNW
Group 1								
Grazing	58,279 (4.765)	78,426 (4.894)	110,734 (5.044)	144,237 (5.159)	182,934 (5.262)	299,961 (5.477)	184,569 (5.266)	244,064 (5.387)
Confinement	99,102 (4.996)	102,243 (5.009)	100,410 (5.002)	101,809 (5.008)	90,580 (4.957)	91,881 (4.963)	99,797 (4.999)	101,347 (5.006)
Group 2								
Grazing	26,520 (4.419)	58,713 (4.768)	10,344 (4.015)	32,410 (4.511)	6,097 (3.785)	48,325 (4.684)	22,767 (4.357)	164,708 (5.217)
Confinement	90,325 (4.955)	103,769 (5.016)	2,587 (3.412)	3,837 (3.584)	2,283 (3.358)	6,283 (3.798)	8,621 (3.935)	9,935 (3.997)
Group 3								
Grazing	2,441 (3.387)	14,043 (4.147)	19,823 (4.297)	148,769 (5.172)	2,134 (3.329)	61,013 (4.785)	2,391 (3.378)	42,246 (4.626)
Confinement	1,630 (3.212)	9,553 (3.980)	1,512 (3.179)	4,412 (3.645)	3,929 (3.594)	8,163 (3.912)	1,200 (3.079)	2,235 (3.349)

Numbers in brackets are Log_{10} transformations used in statistical analyses.

Table A-7. Analysis of variance of mean net cell count per minute (NCPM) from lymphocyte blastogenesis assays carried out with lymph nodes and blood of tracer and control calves using pokeweed mitogen (PWM)

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	3	3924275390	1308091797	2.94	0.0582
Error	20	8905741834	445287092		
Corrected Total	23	12830017224			

Table A-8. Analysis of variance of mean net cell count per minute (NCPM) from lymphocyte blastogenesis assays carried out with lymph nodes and blood of tracer and control calves using phytohaemagglutinin (PHA)

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	3	7526221563	2508740521	8.09	0.0010
Error	20	6199556426	309977821		
Corrected Total	23	13725777988			

Table A-9. Analysis of variance of mean percentage of Ostertagia ostertagi EL_4 recovered from principal calves

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	23	13897.996	604.260	3.59	0.0014
Error	24	4038.634	168.276		
Corrected Total	47	17936.631			

Table A-10. Analysis of variance of mean numbers of all worm genera (TNW) recovered from principal calves which grazed continously or grazed for 1 month and then were kept in pens

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	23	15.94409	0.69322	6.53	0.0001
Error	23	2.44059	0.10611		
Corrected Total	46	18.38469			

Table A-11. Analysis of variance of mean total numbers of Ostertagia ostertagi recovered from principal calves that were grazed continuously or grazed for 1 month and then were kept in pens.

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	23	27.58687	1.19942	13.09	0.0001
Error	23	2.10783	0.09164		
Corrected Total	46	29.69470			

Table A-12. Analysis of variance of mean gross pathology scores of abomasa from principal calves killed monthly

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	23	54.74479	2.38020	3.87	0.0008
Error	24	14.75000	0.61458		
Corrected Total	47	69.49479			

Table A-13. Analysis of variance of mean lengths of Ostertagia ostertagi early 4th stage female larvae recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	0.63865	0.15965	3.25	0.0152
Error	95	4.66903	0.04914		
Corrected Total	99	5.30760			

Table A-14. Analysis of variance of mean lengths of Ostertagia ostertagi early 4th-stage male larvae recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	0.31741	0.07935	1.72	0.1524
Error	95	4.38851	0.04619		
Corrected Total	99	4.70593			

Table A-15. Analysis of variance of mean length of Ostertagia ostertagi adult males recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	18.97913	4.74491	4.87	0.0013
Error	95	92.49850	0.97363		
Corrected Total	99	111.47763			

Table A-16. Analysis of variance of mean length of Ostertagia ostertagi adult females recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	148.22440	37.05610	31.23	0.0001
Error	95	112.73751	1.18671		
Corrected Total	99	260.96193			

Table A-17. Analysis of variance of mean spicule length of Ostertagia ostertagi adult males recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	0.00388	0.00097	0.48	0.7482
Error	95	0.19096	0.00201		
Corrected Total	99	0.19485			

Table A-18. Analysis of variance of mean numbers of eggs in utero of Ostertagia ostertagi adult females recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	0.72505	0.18126	8.09	0.0001
Error	95	2.12756	0.02239		
Corrected Total	99	2.85262			

Table A-19. Analysis of variance of mean vulval flap development scores of Ostertagia ostertagi adult females recovered from the first set of tracer calves (n=2) grazed during March and from group 1 principal calves which were grazed on pasture continuously from March and killed monthly (n=2) in June through September

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	4	0.12761	0.03190	2.13	0.0831
Error	95	1.42334	0.01498		
Corrected Total	99	1.55096			

Table A-20. Analysis of variance of mean fecal egg counts (EPG) measured in principal calves

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	112	290.86	2.596	29.18	0.0001
Error	292	25.98	0.0889		
Corrected Total	404	316.84			

Table A-21. Analysis of variance of mean antibody titers (ELISA) measured in principal calves

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	83	335372243.60	4040629.44	4.38	0.0001
Error	171	157666261.27	922024.91		
Corrected Total	254	493038504.88			

Table A-22. Analysis of variance of mean plasma pepsinogen units measured in principal calves

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	111	127710302.12	1150543.26	10.19	0.0001
Error	295	33321705.99	112954.93		
Corrected Total	406	161032008.11			

Table A-23. Analysis of variance of mean net cell count per minute (NCPM) from lymphocyte blastogenesis assays carried out with lymph nodes and blood of tracer and control calves using 0.5 µg/cc. of Ostertagia ostertagi L_3 antigen

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	3	108542246.8	36180748.9	3.53	0.0335
Error	20	204776334.4	10238816.7		
Corrected Total	23	313318581.2			

Table A-24. Analysis of variance of mean net cell count per minute (NCPM) from lymphocyte blastogenesis assays carried out with lymph nodes and blood of tracer and control calves using 1.0 µg/cc of Ostertagia ostertagi L_3 antigen

Source	DF	Sum of Squares	Mean	F value	Pr > F
Model	3	342480922.8	114160307.6	2.45	0.0932
Error	20	931439525.5	46571976.3		
Corrected Total	23	1273920448.3			

VITA

Carlos S. Eddi was born November, 23 1945, in Rio Negro, Argentina, the son of Julio Eddi and Teresa Tasat.

He earned a D.V.M. degree from the University of Buenos Aires in April 1975 and the Master of Science at Louisiana State University in 1987. He taught normal histology and embryology in the College of Veterinary Medicine, University of Buenos Aires from 1969 to 1975. Following a year's involvement in veterinary rural practice, he became Assistant Professor of Parasitology and Parasitic Diseases at the University of La Pampa (1977-1978). He was a research fellow in the Pan-American Center of Zoonoses-WHO (1978), and in the Parasitology Department, University of Rio Grande do Sul, Brazil (1983).

He is currently on leave as a chief scientific investigator in charge of the Laboratory of Helminthology, Research Center of Veterinary Science, The National Institute of Agricultural Technology (I.N.T.A.), where he has served in various other capacities since 1978.

Carlos S. Eddi has a number of publications in

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Carlos S. Eddi is married to the former Elisa Mourelle and they have two sons, Pedro and Felipe and a daughter, Adriana Maria.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Carlos S. Eddi

Major Field: Veterinary Medical Sciences

Title of Dissertation: Ostertagia ostertagi: Population dynamics under pasture and confinement conditions with particular reference to the inhibition phenomenon.

Approved:

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March 6, 1989