Ostertagia ostertagi Infections in the Bovine: Field and Experimental Studies

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GENERAL INTRODUCTION

Though disease caused by gastro-intestinal parasites in cattle is sometimes due to the combined effects of several species, there is a greater tendency than in sheep for monospecific infections to be of importance (Soulsby, 1965a). Of all these species the most pathogenic and economically important in temperate areas of the world is undoubtedly the abomasal nematode Ostertagia ostertagi. This parasite was first described in 1890 by Ostertag and named Strongylus convolutus. It was later renamed by Stiles (1892) as Strongylus ostertagi. The present name of the nematode, i.e. Ostertagia ostertagi was assigned by Ransom in 1907. Published reports on the significance of O. ostertagi as a pathogen for cattle are available from several countries and date back to the beginning of the 20th century, e.g., in Great Britain (Gardner, 1911; Stewart and Crofton, 1941; Bruford and Fincham, 1945; Martin, Thomas and Urquhart, 1957; Gracey, 1960); in U.S.A. (Stiles, 1901; Ackert and Muldoon, 1920; Barger, 1927; Baker, 1937; Threlkeld and Bell, 1952; Andrews, Jones and Siffell, 1953; Bailey and Monlish, 1953; Bailey and Thorson, 1954; Bell, 1957; Becklund, 1962; Smith and Jones 1962); in Canada (Johnston and Macpherson, 1956); in Australia (Gilruth and Sweet, 1910; Benko and Hitton, 1960); and recently in Poland and Russia (Malczewski, 1965) and in Denmark (Nielsen, 1966). These reports are principally concerned with the clinical description of outbreaks of bovine ostertagiasis and the gross pathological changes seen at the autopsy of affected animals. A comprehensive account is not available of the possible effects of sub-clinical levels of parasitism in cattle, although the detrimental effects on the host of low burdens of gastro-intestinal nematodes in sheep has been adequately reviewed by Spedding (1955).

Considering the economic importance of bovine ostertagiasis and its world-wide occurrence, it is strange that comparatively little work has been carried out on the epidemiology and pathogenesis of this disease. The majority of publications relating to experimental studies on ostertagiasis have been orientated towards taxonomic details of various species belonging to the genus Ostertagia or a study of the bionomics of larval populations on the pasture.

Thus in Great Britain apart from O. ostertagi, three other species have been described from cattle, namely, Ostertagia lyrata (Rose, 1959b), Ostertagia extensis (Rose, 1963) and Grossspiculagia podjapolskyi (Rose, 1963). Extensive reviews of the genus Ostertagia have been produced by workers in Russia and Poland (Skrjabin, Schikhobalova and Schulz, 1954; Sarwar, 1956; Andreeva, 1957; Dwożak, 1965).

The bionomics of the larval stages of O. ostertagi on pasture have been studied in some detail (Baker, 1939; Goldberg and Rubin, 1956; Drudge, Leland, Wyant and Rust, 1958; Goldberg and Lucker, 1959, 1963; Bell, Galvin and Turk, 1960; Rose, 1961, 1962; Schwink, 1963). While these results indicated the numbers of O. ostertagi larvae available on the pasture at various seasons of the year, their significance is limited in that no attempt was made to link these studies with observations on the development of clinical ostertagiasis.

"Immunity to gastro-intestinal nematodes in cattle is thought to develop at a relatively early age and to persist throughout the animal's life" (Soulsby, 1965b). Despite this, clinical outbreaks of parasitic gastritis occur in

many parts of the world and can involve both young stock (Martin et al, 1957) and adults (Bailey and Herlich, 1953). Following field investigations into outbreaks of parasitic gastritis in which O. ostertagi were involved but Haemonchus placei predominated, Roberts, O'Sullivan and Rick (1952) predicated two main factors as being responsible for the onset of disease. First, exposure to excessively large numbers of larvae and secondly, a lowered nutritional state.

When heavy infections occur early in life there may be a delay in the onset of immunity and an animal may die before it has a chance to become resistant, or if not, it survives in a state of chronic parasitism. In certain instances, a sudden intake of very large numbers of larvae due to increase in pasture contamination with larvae may break down an existing immunity and lead to clinical parasitism; the ability of cattle strongyle larvae to develop and persist in the dung pad for periods of up to five months, even under conditions of drought (Roberts et al, 1952; Durie, 1961; Rose, 1962) has an important bearing on such outbreaks. Thus, the onset of rains following a dry period allows the migration of large numbers of infective larvae on to the pasture, at a time when the host immunity may be waning after a period of minimal stimulation. This is apparently different from the situation occurring with sheep nematodes where a continuous period of dry weather is thought to lead to a substantial reduction in larval populations on pasture (Soulsby, 1965c).

The mechanism of immunity to gastro-intestinal nematodes in cattle is

as yet unknown and attempts to artificially immunise (using irradiated H. placei larvae) were only partially successful (Ross, Armour, Hart and Lee, 1959). However, from the results of daily experimental infections of calves with O. ostertagi over a period of 318 days, Michel (1963) postulated that where calves grazing infected pasture ingest regular numbers of O. ostertagi larvae the resulting worm burden is not the product of the daily uptake of larvae and the number of days' grazing; rather the worm burden is maintained initially at a level where the rate of loss balances the daily uptake and at any given rate of uptake there will be a worm burden in the animal corresponding to this uptake. This initial relationship is disturbed eventually by resistance; first, the rate of replacement by adult worms is reduced and a large population of larvae inhibited at a particular stage develops (in O. ostertagi this is the fourth larval stage). Subsequently, a 'resistance to the establishment' of worms causes the reservoir of inhibited forms to decrease so that the replacement of lost adults declines until neither larvae nor adults remain.

Michel's elegant theory is dependent on cattle being constantly exposed to a steady rate of challenge and among other things does not take into account the effects on the immune status of the host when the rate of challenge is suddenly increased or decreased. The former may happen in Britain where calves are frequently unwittingly moved to contaminated grazing in the summer, or the latter when calves are removed completely from pasture and housed during winter. Indeed, a clinical entity in young housed cattle associated

with large populations of adult and larval O. ostertagi has been reported by Martin et al (1957); this condition was seen in cattle five months after removal from pasture. A likely explanation offered at the time for this situation was that during the grazing season large numbers of larval stages are accumulated in the abomasal mucosa as the host acquires immunity; upon being housed the immune status of the host falls and reaches the point when large numbers of larval stages recommence development. If sufficient numbers are present clinical parasitic gastritis is produced.

The relationship of host nutrition to the level of parasitism in ruminants has been emphasised by Whitlock (1949) and by Roberts et al (1952) and there can be little doubt that the diet profoundly influences the debilitating effects of parasitic disease in animals. Experimental studies have demonstrated that cattle on a high protein diet show less effects from a similar number of parasites (including O. ostertagi) than cattle on a low protein diet (Goldberg, 1965) and that parasitic disease frequently occurs when cattle graze pastures of poor quality (Vegors, Sell, Baird and Stewart, 1955; Giordin, Bizzell, Vegors, Baird, McCampbell and Sell, 1962). In beef cattle, disease tends to appear after the animals have been weaned (Roberts et al, 1952).

Since no successful method of vaccination against parasitic gastritis in cattle has been described, control measures have been aimed at reducing the intake of infective larvae to a level compatible with health and yet sufficient to allow a satisfactory immunity to develop. To do this, use is customarily made of systems of rotational grazing combined with the

the strategic application of anthelmintics. Since pasture may remain infective for much longer periods due to the protection afforded to cattle nematode larvae in dung pads, control of parasitic gastritis in the bovine by rotational systems is difficult to apply and farmers and veterinarians tend to rely on the use of anthelmintics. Unfortunately, these drugs are expensive and in the past have seldom been used prophylactically being reserved for use in outbreaks. In the last few years several new anthelmintics have been developed and claimed to be efficient against a wide range of cattle nematodes, including O. ostertagi, e.g., metriphonate (Rick and Keith, 1958); methyridine (Malley, 1961); thiabendazole (Bailey, Diamond and Walker, 1961); haloxon (Armour, 1964). However, the methods used for testing these anthelmintics frequently did not take into account efficiency against specific larval or adult stages and certainly not against larval stages inhibited in their development. These trials on anthelmintic efficiency of a drug have also seldom been supported by field trials against clinical outbreaks or studies on their application in prophylactic regimens of dosing.

The work described in this thesis is concerned firstly with a study of the parasitological findings from 30 outbreaks of parasitic gastritis in young bovines in which the abomasal nematode O. ostertagi predominated. These investigations are supplemented by studies on the accumulation, infectivity and longevity of pasture populations of O. ostertagi at different seasons of the year (Section I).

Secondly, using a standardised inoculum of O. ostertagi infective larvae, an attempt was made to determine the essential structural changes which occurred in the abomasum following the establishment of the experimental infection and what the biochemical and physiological consequences of these were; these studies were aided by the use of abomasal cannulae (Section II).

Thirdly, a detailed investigation into the cause of the massive inhibition of larval development of O. ostertagi which occurs at the early fourth larval stage was carried out. These investigations were pursued under natural conditions. The involvement of the relationships host/environment, host/nutrition, parasite/environment, host/parasite in the occurrence of inhibition of larval development was studied; the possibility of different strains or mutants of O. ostertagi occurring was also considered (Section III).

Fourthly, immunological studies on bovine ostertagiasis with particular reference to age immunity, acquired immunity and attempts to produce artificial acquired immunity using irradiated O. ostertagi larvae were conducted (Section IV).

Finally, a description of experiments with the anthelmintic thiabendazole is given. These experiments were designed to assess anthelmintic efficiency against adult, larval and inhibited larval stages of O. ostertagi and to provide information on alterations in the abomasal biochemistry before and after successful removal of the worm population. The application of these results to the control of bovine ostertagiasis in the field are discussed (Section V).

A fuller resume of the relevant literature is given in the introduction of each section. Results of work completed and published by other writers while the research described in this thesis was in progress are considered in the discussion after each section.

MATERIALS & METHODS

A. Experimental Animals

(1) The rearing and maintenance of parasite free calves

Ayrshire bull calves, purchased when three to seven days old, were housed in individual galvanised iron huts. These were cleaned out weekly, and the calves bedded daily with oat straw. Whole milk was fed for the first four weeks at the rate of one pint of milk per 10 lb. live weight per day (divided into two feeds). During the third and fourth weeks, hay and calf weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) were introduced and milk feeding was stopped by the end of the fourth week. From then until the end of each experiment the calves were given 3 lb. of the pelleted ration daily with hay and water available ad lib. In certain experiments it was necessary to weigh the amount of concentrates fed and the amount remaining in the bucket at the next feed. These weighings were carried out on a Mettler balance (E. Mettler, Zurich, Switzerland). Where experiments were carried out at pasture, water was constantly available but no supplementary feeding was given unless the grass became scarce, in which case some hay was fed.

Calves reared in the above manner had a mean growth rate of 1 lb. per day. Regular examination of their faeces for nematode eggs gave negative results.

(2) Weighing procedure

The calves were weighed on Avery cattle scales which were accurate to 1 lb. Weighings of experimental calves were carried out in the morning, two to three hours after the morning feed had been given; this procedure was not possible with field cases of ostertagiasis brought to the Veterinary Hospital for autopsy and these were weighed immediately on arrival.

B. Autopsy Procedure

(1) Details of Slaughter

All animals to be autopsied were starved for 24 hours prior to slaughter. A captive bolt pistol was used to shoot the calves after which they were bled out. The abdomen was opened and the whole gastro-intestinal tract removed; a gross pathological examination of the rest of the carcass was then made.

(2) Abomasum

The abomasum and omasum were separated from the rest of the intestinal tract, care being exercised not to lose any of the contents. The abomasum was then opened along the greater curvature and a sample of fluid contents taken for estimation of pH and electrolyte concentrations. Histological blocks were prepared and then the omasum was removed and the abomasum washed under moderate pressure (retention of the omasum until this point facilitated handling of the abomasum). The abomasal washings and contents were made up to four litres in a graduated polythene bucket and after thorough mixing, two samples of 200 ml. each were taken in a graduated scoop, for subsequent microscopic enumeration of the worm population; to each sample, 10 ml. of 40 per cent formalin was added as a preservative.

The entire abomasal mucosa was then scraped off with a butcher's knife, chopped finely with a cleaver and put in 200 gm. lots into separate Kilner jars. The weight of the mucosa was recorded. The Kilner jars were then filled with a pepsin-hydrochloric acid (HCl) mixture and incubated for six hours at 42°C; the digests were then formalinised and made up to four litres and two samples of 200 ml. each were removed as described above. The pepsin-HCl

mixture was adapted from that described by Herlich (1956); 10 gm. of 1:2500 pepsin powder (British Drug Houses, Poole, Dorset, England) was dissolved in 600 ml. of water and acidified with 30 ml. of concentrated HCl.

(3) Small Intestine

The small intestines were separated from their mesenteric attachments and divided into three equal lengths. Each length was opened and washed under running water into a graduated bucket. The volume was made up to four litres and a single sample of 200 ml. was taken and formalinised as above.

(4) Large Intestine and Caecum

The large bowel was opened and examined by the naked eye for the presence of worms or worm nodules. In most instances no worms or lesions were detectable though occasionally a few Trichuris species were noticed.

(5) Liver

If hepatic fibrosis was present the liver was examined for the presence of Fasciola hepatica; this was done by alternatively slicing ($\frac{1}{8}$-inch thicknesses) and squeezing the liver to express the F. hepatica onto a white tray where they could be easily counted.

C. Parasitological Techniques

(1) Culture and Harvesting of O. ostertagi

The original culture of O. ostertagi was supplied in 1961 by Dr. F.E. Gibson of the Ministry of Agriculture Laboratories at Weybridge to the Cooper Technical Bureau, Berkhamsted, where the author was then employed. The

culture was passaged in parasite-free calves four times at Berkhamsted before it was brought to Glasgow by the author in 1963. The techniques used for larval culture at Berkhamsted and Glasgow were essentially similar and were as follows: calves reared worm-free and eight to twelve weeks old, were inoculated orally with 100,000 motile, third stage O. ostertagi larvae, suspended in water. O. ostertagi eggs usually appeared in the faeces 18 to 21 days after inoculation. When the faecal egg count reached 500 eggs per gram (e.p.g.) or greater as estimated by the McMaster technique (see later), a harness and collecting bag were fitted to each calf and 24 hour collections of the faeces made until the faecal egg count dropped below 500 e.p.g., usually five to seven days later. The faeces were mixed with vermiculite (Horticultural vermiculite, No. 2 size) until a moist crumbly mixture was obtained. This mixture was loosely packed into 1 lb. honey jars and the lid lightly screwed down. The jars were stored above trays of water in a dark cupboard at 20 - 22°C and a humidity of 70 to 80 per cent; the temperature and humidity were checked daily on a thermograph and hygrograph respectively. Incubation of the cultures was continued for 10 days by which time many third stage O. ostertagi larvae could be observed in clumps on the jars.

The infective third stage larvae were recovered by a method essentially similar to that of Roberts and O'Sullivan (1950). The jars were three-quarters filled with lukewarm tap water and allowed to stand without lids for one hour in diffuse light; water was then added until it reached the brim of the jars which were then inverted into Petri dishes and after standing for three hours all the fluid was collected into polythene buckets. Larvae adhering to the bottoms of the Petri dishes were washed off with water and the

culture medium discarded. The fluid in the buckets was passed through a sieve (60 meshes per inch) to remove coarse material and then poured onto a double layer of gauze strengthened, milk filter mediums (Cloverleaf No. 9, Johnson & Johnson, Slough, Buckinghamshire, England) placed on top of a Buchner funnel. The larvae were trapped on the milk filter pads which were placed without being inverted in a Baermann apparatus. The larvae collected one to two hours later at the neck of the filter funnel were motile and free from foreign material. Using these techniques of culture and harvesting it was possible to recover up to two million larvae from one calf five weeks after a single inoculum of 100,000 O. ostertagi infective larvae had been given.

The larval collections in tap water were bulked in 2- or 5-litre flasks, the volume being adjusted to give a concentration of approximately 1,000 larvae per ml. Storage of the larvae was in a refrigerator at 6°C. The viability of larvae stored in this manner declined rapidly after the first month and up to 50 per cent were frequently dead at the end of three months. In the experiments described in this thesis, only larvae harvested less than one month previously were used.

(2) Preparation of Larval Inoculum

O. ostertagi larvae suspended in water have a tendency to form clumps so it is necessary to mix thoroughly and keep the suspension agitated while sampling. The concentration of the larval suspension was determined by examining at least twenty 0.025 ml. portions of the suspension until a total of 400 larvae was recorded. Doses for inoculation were measured by pipetting

the calculated volumes of the original larval suspension and several checks made to ensure that the desired number of larvae ± 10 per cent were present.

The individual doses, after preliminary sedimentation and removal of excess supernatant fluid, were administered to each calf from a narrow necked bottle.

(3) Faecal Egg Counting Techniques

Faecal samples collected from the rectum were examined by the zinc sulphate (saturated zinc sulphate solution) flotation method. In this technique 3 gm. of faeces were mixed with 20 ml. of water and passed through a sieve (60 meshes per inch); a 15 ml. sample of the filtrate was centrifuged in a flat bottomed test tube for two minutes at 2,000 revolutions per minute (r.p.m.) and the supernatant then poured off. The sediment was resuspended in saturated zinc sulphate solution and centrifuged again at 2,000 r.p.m. for two minutes. A platinum loop was used to remove the upper layer of the centrifuged fluid and smear it on a clean glass slide. Fasciola eggs, Dictyocaulus larvae, cestode eggs, coccidia oocysts and strongyle eggs are readily detected by this method and when the latter were present a modified McMaster technique (Gordon and Whitlock, 1939) was used to count the eggs. In this method 3 gm. of faeces were mixed with 42 ml. of water and passed through a sieve (60 meshes per inch); a 15 ml. sample of the filtrate was centrifuged as before and the supernatant discarded. The sediment was re-suspended in saturated salt (NaCl) solution, the test tube inverted several times, then, using a pipette, both chambers (volume 0.15 ml.) of a McMaster Worm Egg Counting Slide (Hawksley & Sons, London, England) were filled with

the suspension. The average number of eggs per chamber was multiplied by 100 to give the numbers of eggs per gram of faeces.

(4) Collection of Worms from Faeces

The total faecal output was collected over each 24-hour period. The 24-hour samples were weighed, spread on a tray, and fifty 2-gm. portions were taken. This 100-gm. sample, on a 75-mesh sieve, was washed with a moderate-force jet of water until the filtrate was clear. The total contents of the sieve were transferred to a Kilner jar (capacity 800 ml.) containing 80 ml. formalin and then examined microscopically for O. ostertagi. The total number of O. ostertagi in the faeces on each day was then calculated using a factor based on the weight of faeces per 24-hour period.

(5) Worm Counting and Identification

After thorough mixing the 200 ml. samples of abomasal washings, abomasal digests and intestinal contents collected at autopsy were treated as follows: using a 10 ml. straight pipette sawn off at the 8 ml. mark, 5 ml. aliquots were withdrawn and pipetted into Petri dishes, stained for a few minutes with a few drops of a 45 per cent iodine solution (to 16 lb. potassium iodide in five litres of warm distilled water, 10 lb. iodine crystals were added and made up to ten litres with distilled water), then decolourised with a 5 per cent sodium thiosulphate solution and counted and identified under a Wild dissection microscope (Model M.5, Wild, Heerbrugg, Switzerland). It was found that after staining with iodine and decolourising with sodium thio-sulphate, only the worms retained the stain and counting and identification were facilitated. From the 200-ml. samples, aliquots of 5 ml. were screened

until at least 100 worms and a minimum of five aliquots had been examined. When very low numbers of worms were present, only ten counts of 5 ml. aliquots were made. The average number of worms per 5 ml. aliquot was calculated and multiplied by 800 to give the total number of worms in the original four litre volume. The number of worms present was expressed to the nearest hundred. This counting technique gave an estimate which varied within ± 20 per cent of the mean.

The Ostertagia spp. of worms present at autopsy were identified using the descriptions of Ransom (1911), Douvres (1956, 1957) and Rose (1959b, 1963). They were classified into adult stages, developing stages and early fourth larval stages. The worms were considered to be adult when development of the male spicules was complete or the females contained eggs in their uteri. Developing stages were classed as all stages up to adult, except the early fourth larval stages, which were classified separately. Differentiation of O. ostertagi and other species of Ostertagia was based on the morphology of the male spicules; to examine these, the male worms were cleared in lacto-phenol and examined on a slide under a coverslip. This rather lengthy process was not undertaken at all autopsies but samples taken from the different types of field outbreaks were combined and 100 male worms cleared and examined.

The other worms present, namely Trichostrongylus axei in the abomasum, Cooperia species and Nematodirus species in the small intestine were identified using the descriptions and criteria of Ransom (1911), Douvres (1956, 1957), Gordon (1932) and Rose (1959a).

(6) Worm Measurement

Measurements of fourth stage larvae of O. ostertagi were made directly on the graduated glass screen of a Projectina microscope (Projectina Co. Ltd., Heerbrugg, Switzerland) using the 20 x objective. The minimum number of worms measured at each observation was 30.

D. Blood Analysis

(1) Collection and Storage of Samples

Blood samples were collected from the jugular vein into three bottles prepared in different ways. First, for haematological examinations, 2 ml. of blood were collected in a Bijou bottle containing a few crystals of the anticoagulant, ethylenediaminetetraacetate (E.D.T.A.) and the bottle gently shaken to dissolve the crystals. The haematological examinations were made within a few hours of collecting the samples.

Secondly, for estimations involving plasma, 15 ml. of blood were collected into a Universal glass bottle containing two to three drops of a 1:1,000 solution of heparin. The heparinised sample was centrifuged at room temperature for 20 minutes at 2,000 r.p.m. in an M.S.E. centrifuge (Measuring Scientific Equipment, London, England). The plasma was then pipetted off into plastic tubes (Metal Box Co., Portslade, Sussex, England), immediately frozen and stored at -5°C.

Thirdly, for estimations involving serum, 15 ml. of blood were collected into a bottle containing no anticoagulant and the serum collected from the clotted sample which had separated overnight at room temperature. The serum was stored as described above for plasma.

(2) Packed Cell Volume (PCV)

The packed cell volume percentage was determined by the micro-haematocrit method used by Fisher (1962). Capillary tubes containing the blood sample were sealed at one end and were centrifuged at 12,000 g. for 6 minutes in a micro-haematocrit centrifuge (Hawksley & Son Ltd., London, England). The percentage PCV was determined from the scale on a Hawksley Micro-Haematocrit reader.

(3) Haemoglobin concentration (Hb)

Haemoglobin concentration expressed as grams per 100 ml. was estimated by the oxyhaemoglobin method of Davis and Lewis (1963a). A one in 200 dilution of blood was prepared in 0.04 per cent solution of ammonium hydroxide and after thorough mixing the resulting solution of oxyhaemoglobin was read in a colorimeter (Evans Electroselenium Ltd., Harlow, England) using a yellow green filter (Ilford, No. 625). The colorimeter was calibrated using a cyanmethaemoglobin standard solution (cyanmethaemoglobin standard solution - C. Davis Keeler, Ltd., London, England).

(4) Total Red and White Blood Cell Counts (R.b.c.'s) (W.b.c.'s)

Total counts of circulating red and white blood cells were made on an electronic particle counter (Coulter Model 'D', Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.) by the methods described for feline blood cells by Crighton (1965). This instrument was later calibrated and evaluated for bovine blood cells by Crighton and Gore (1965).

(5) Total Eosinophil Counts

Total counts of circulating eosinophils were made using the technique

described by Dacie and Lewis (1963). The cells were counted in a chamber with a Fuchs-Rosenthal ruling.

(6) Mean Cell Volume (MCV)

MCV was calculated from the formula $\frac{\text{PCV\%} \times 10}{\text{R.B.C.} \times 10^6 \text{ per cu. mm.}}$ expressed as cubic microns (μ).

(7) Mean Cell Haemoglobin Concentration (MCHC)

MCHC was calculated from the formula $\frac{\text{Hb gms. per 100 ml.}}{\text{PCV\%}} \times 100.$

(8) Plasma Pepsinogen

Plasma pepsinogen was estimated by a method essentially similar to that described by Edwards, Jepson and Wood (1960) in that plasma was incubated at a pH of 2.0 with a bovine serum albumin substrate (Armour's Fraction V, Armour Pharmaceutical Co. Ltd., Eastbourne, England) for 24 hours at 37°C. The liberated tyrosine, non-precipitable with trichloroacetic acid was estimated with Folin-Ciocalteau reagent (British Drug Houses, Poole, England) and read in a spectrophotometer (Unicam, Cambridge, England). The enzyme activity was expressed as milli-units (mU) tyrosine (μ mols tyrosine per litre of plasma per minute x 100).

(9) Total Serum Protein Concentration

Total serum protein concentration was estimated by the biuret method of Weichselbaum (1946).

(10) Serum Protein Fractionation

Separation of serum protein fractions were carried out by electrophoresis.

Cellulose acetate strips (Oxoid Ltd., London, England) were saturated with barbitone buffer (pH 8.6) and placed in a horizontal electrophoresis tank (Shandon Scientific Co., London, England). Serum (0.003 ml.) was pipetted onto the strips and a voltage of 150 volts was applied for one hour from a Vokam power pack (Shandon Scientific Co.). The strips were then removed and dried in an oven at 80 to 100°C. for ten minutes and developed by staining with 0.2% Ponceau S (for electrophoresis) (G.T. Gurr Ltd., London, England) in 3% aqueous trichloroacetic acid for five minutes. After staining the strips were washed in 5% acetic acid until the background was white. The strips were evaluated automatically as described by Neill (1963), using a Chromoscan recording densitometer (Joyce Loebl and Co. Ltd., Gateshead, England). The results were expressed as grams per 100 ml. of serum albumin, total globulin and gamma globulin.

(11) Plasma Sodium and Potassium

Plasma sodium and potassium were estimated using the E.E.L. flame photometer (Evans Electroselenium Ltd.). The plasma samples were diluted 1:100 or 1:200 with deionised water.

(12) Plasma Chloride

Plasma chloride was estimated using an E.E.L. Chloride Meter or by the method of Schales and Schales (1941).

(13) Plasma Bicarbonate

Plasma bicarbonate was estimated by the method of Astrup, Jorgensen, Andersen and Engel (1960).

E. Abomasal Cannulae

(1) Insertion of cannula

The abomasal cannula was like that described by Phillipson and Innes (1939) and is depicted in Figures 1(a) and 1(b). While the calf was under cyclopropane anaesthesia, the cannula was surgically inserted as close as possible to the pyloric-fundic junction and exteriorised to the right of the ventral mid-line of the calf. The calves were treated with an antibiotic, procaine penicillin G (Mylipen, Glaxo Laboratories, Greenford, England) for at least three days after the cannulae were inserted. Since an inflammatory reaction tended to occur around the cannulae, the cannula and the surrounding area was syringed and washed with cetrimide (Cetavlon, Imperial Chemical Industries, Wilmslow, England), twice weekly.

(2) Collection of Samples

Samples of abomasal fluid were taken twice daily from each cannula using a length of perforated rubber tubing. One end of the rubber tubing was inserted through the cannula into the abomasum while the other projected into a Universal glass bottle. Care had to be exercised to avoid excessive loss of abomasal fluid during the sampling.

F. Biochemical Analysis of Abomasal Contents

(1) pH of Abomasal Contents

The pH of abomasal contents was determined using a pH meter (Beckman Instruments Ltd., Glenrothes, Scotland) with micro-electrodes, the results

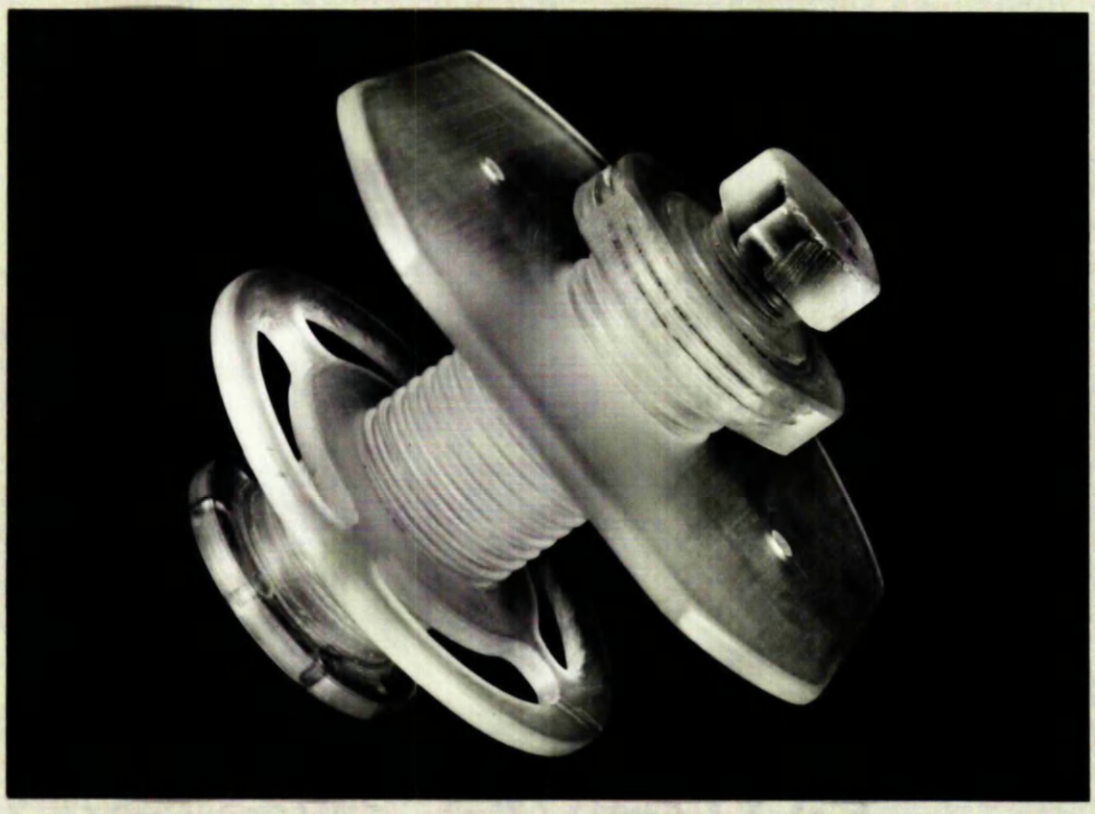


Fig. 1(a). Assembled perspex cannula.

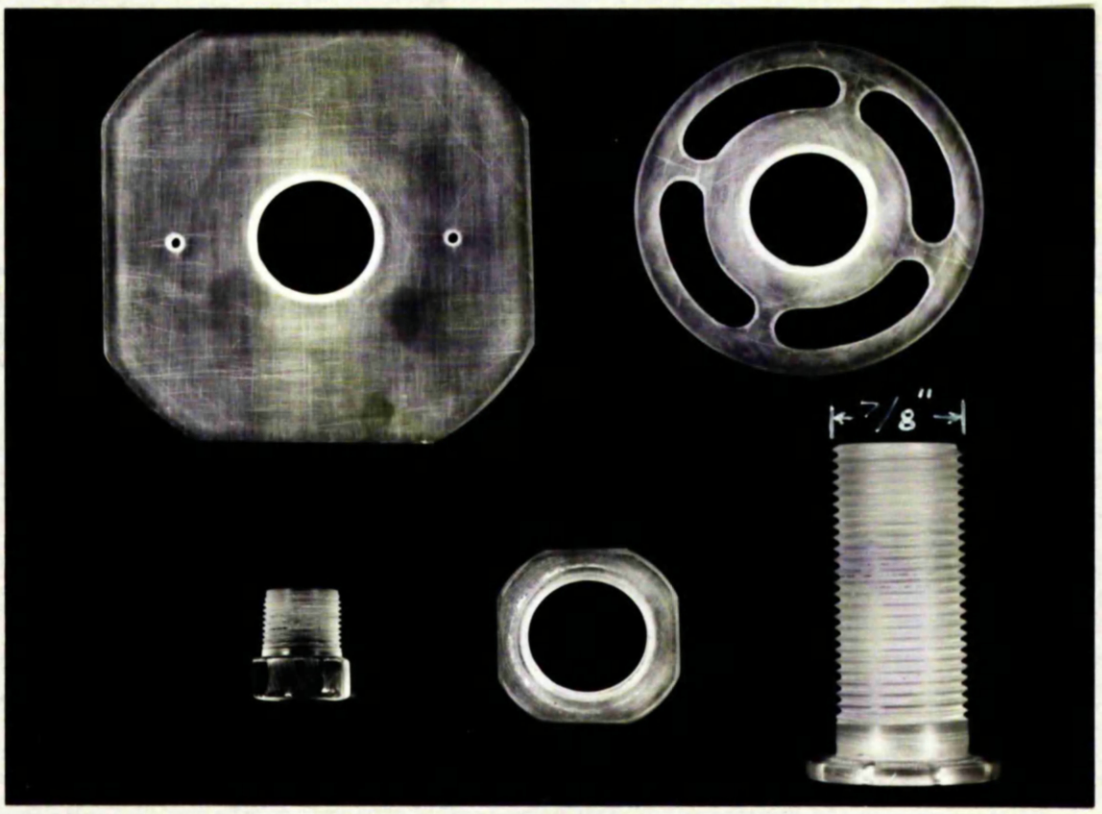


Fig. 1(b). Components of perspex cannula.

being periodically tested on larger samples using standard electrodes. All samples were examined within one half hour of collection.

(2) Sodium, Potassium and Chloride Ion Concentrations

Each sample of abomasal contents, after estimation of the pH, was centrifuged for 15 minutes at 15,000 g. and the supernatant fluid removed and analysed for sodium, potassium and chloride ions. The methods used were the same as for plasma but the dilutions of the sample were appropriate for the concentrations of the different ions.

(3) Pepsin Concentration

Pepsin concentration was estimated by incubation for exactly one hour(37°C) of 0.25 ml. centrifuged abomasal contents with 5.0 ml. of a 2% bovine serum albumin substrate (Armour's Fraction V) previously adjusted to pH 2.0 with 2N HCl. After precipitation with 2.0 ml. 40% trichloroacetic acid, the liberated tyrosine was developed with Folin-Ciocalteau's Reagent and estimated with a spectrophotometer (Unicam SP 600) at 680 mμ. Results were expressed as Units tyrosine (μ mols tyrosine per litre per minute).

C. Bacteriological Observations

(1) Preparation of Samples for Culture

Samples of abomasal fluid were filtered through sterilised coarse gauze, and then diluted to 1:1,000, 1:10,000 and 1:100,000 with sterilised, distilled water. Portions (0.2 ml.) of each dilution were set up in quintuplicate with both Nutrient agar and MacConkey agar; 10 ml. of the melted agar was poured

over the diluted sample in a sterilised Petri dish. The plates were incubated at 37°C for 48 hours.

(2) Enumeration of Viable Bacteria

Following incubation the numbers of viable bacteria were counted by the method of Miles and Misra (1938).

H. Histological Methods

(1) Preparation of Sections

Histological blocks were fixed by formol-sublimate; the blocks were dehydrated and cleared in an alcohol-amyl acetate chloroform series under reduced pressure. Paraffin embedding under reduced pressure was employed.

(2) Staining Methods

Routine staining was by haemalum and eosin; other methods used for special purposes were picro-Mallory, periodic acid-Schiff, methylene blue and Southgate's mucicarmine.

I. Irradiation Procedure

I. Irradiation Procedure

(1) Preparation of Larvae

Larvae which had been harvested from culture less than seven days previously were counted as described above and diluted in tap water to a concentration of 10,000 to 50,000 per ml. During storage and irradiation the larvae were maintained as far as possible at room temperature (i.e. in 15 to 20°C).

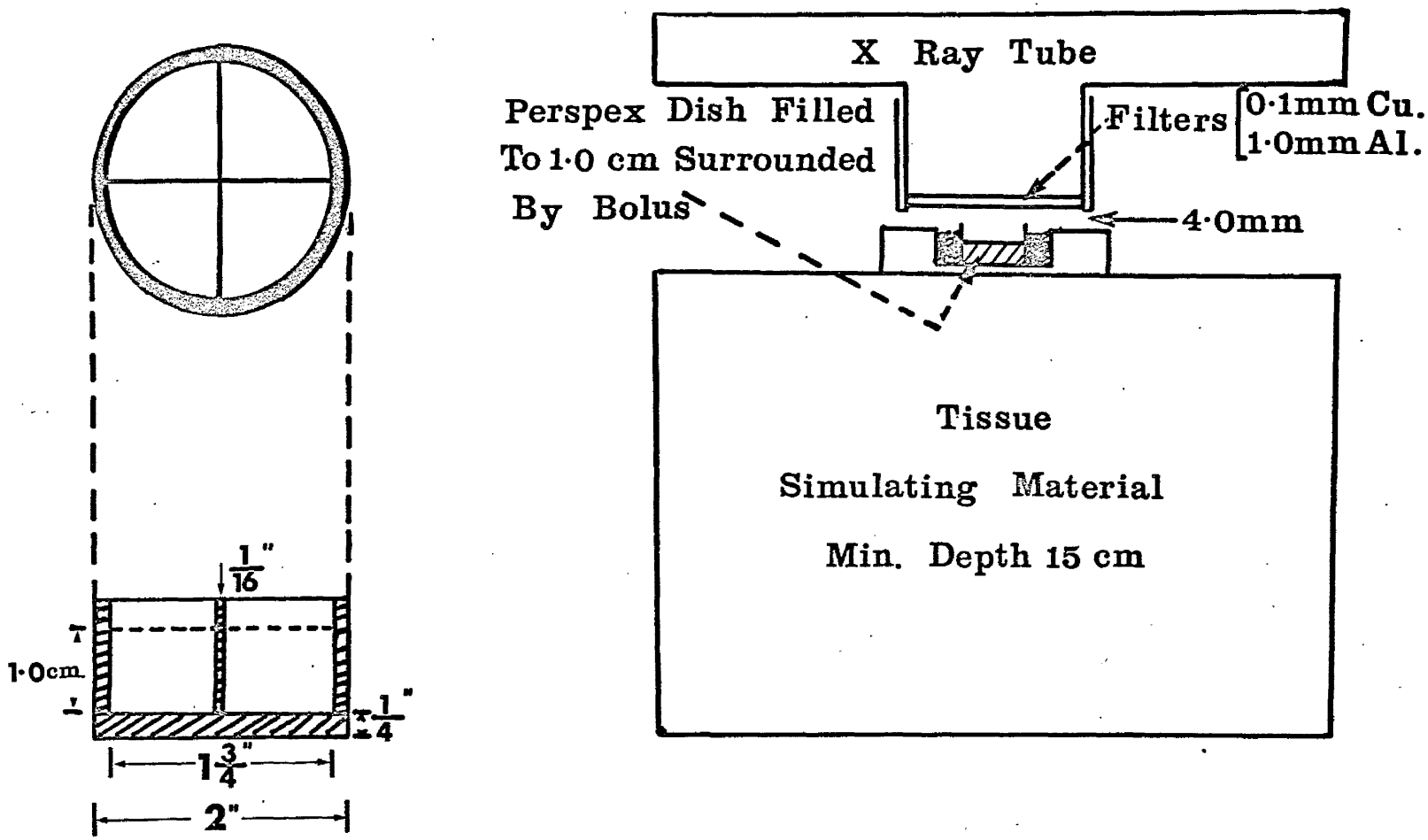


Fig. 2 Arrangement for irradiating larval suspensions

(2) Technique of Irradiation

The irradiation procedure was as described by Jennings, Mulligan and Urquhart (1963). The larval suspensions were pipetted into the quadrants of a perspex dish and placed under the X-ray machine (Siemens Stabilipan, Erlangen, West Germany) as illustrated diagrammatically in Figure 2. Calibration of the X-ray machine was carried out with a perspex 'Phantom' which permitted the ionisation chamber of the dosimeter (Baldwin Farmer Sub-standard Dosimeter, Baldwin Instrument Co. Ltd., Dartford, England) to be placed at a point corresponding to the bottom of the irradiation dish, i.e. the doses recorded are those delivered to the larvae not those delivered to the surface. For irradiation the 'perspex' dish was placed on a minimum of 15 cm. of tissue simulating material to standardise back-scatter.

J. Statistical Methods

The statistical methods employed were those described by Snedecor (1956).

THE LEFT SIDE OF COPENHAGEN CEMETERY 17

A description of the life cycle of the trichostrongyle nematode O. ostertagi at this stage will facilitate the understanding of the material presented at a later stage in this thesis. The facts about the life cycle have been garnered from the many published papers on the subject and from the results of the experimental infections described later.

The progression of the life cycle is outlined on the left hand side of the page while the salient features of each stage are listed under the right hand column.

		Remarks
EXTRA HOST STAGE	Eggs in faeces (dung pad) Eggs in faeces (dung pad)	Development from egg to the infective larval stage takes place within the dung pad. Under ideal conditions (temperature 20°C and humidity over 60 per cent) this development will take about 7 days. As temperature and humidity decline, so the period of development increases. Migration of infective larvae onto pasture requires the presence of rainfall. The infective larvae measure 850-900 microns (μ) in length. Figure 3 shows infective third stage O. ostertagi larvae.
	1st stage larvae	
	2nd stage larvae	
	3rd stage larvae This is the infective stage and possesses an extra sheath (retained from 2nd stage).	

Larva Ingested and Swallowed by Grazing Calf

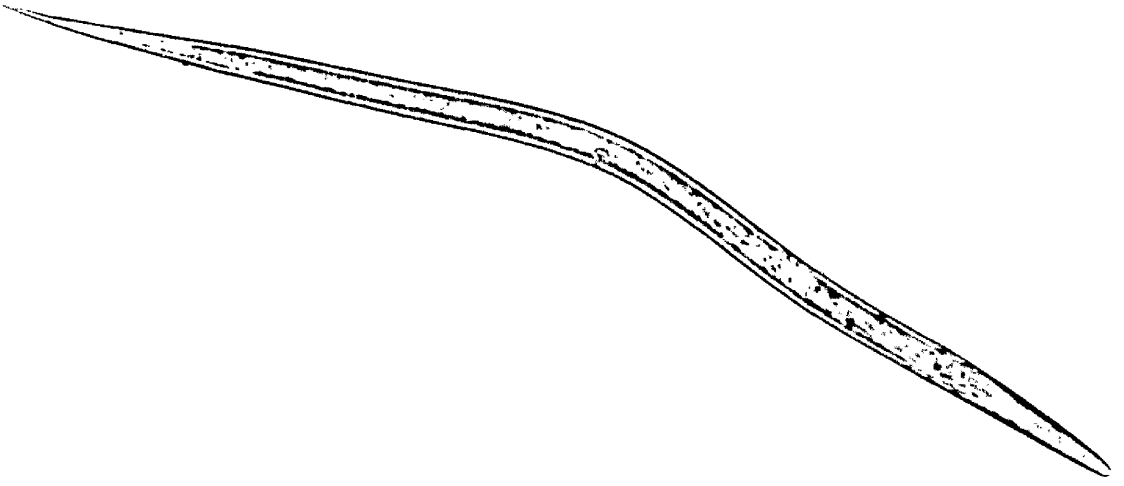


Fig. 3. Ostertagia ostertagi. Third or infective stage larva x 160. Killed in hot 70% alcohol, mounted in lactophenol containing 0.01% cotton blue. Note extra sheath.

	Life Cycle (contd.)	Day of Infection	Remarks
	3rd stage larvae exsheath	0	The infective larval stage loses its extra sheath, passes to the abomasum, enters the gastric glands and moults to become the early fourth larval stage, which is 1.3 mm. in length (Fig. 4). The gastric glands in which the larvae develop are important in the digestive processes of the calf. They are lined by two types of secretory epithelial cells, (a) zymogen cells which produce pepsinogen, the inactive precursor of pepsin, the main gastric digestive enzyme, and (b) the parietal cells which produce hydrochloric acid which in turn activates pepsinogen to pepsin. The fourth stage larva grows in the gastric gland reaching the fourth moult stage (Fig. 5) about ten days after ingestion. After completion of the moult, the fifth larval stage grows and matures to become the adult egg producing stages (Figs. 6, 7 and 8). By the time the adult stage is reached 16 to 21 days after ingestion, the O. ostertagi males and females are 6.5 - 7.5 mm.
I	4 days		
N			
T			
R	4th stage larvae	4	
A			
H			
O			
S			
T	6-8 days		
S			
T			
A			
G			
E	4th moult	10-12	
S			
	2-3 days		
	5th larval stages	12-14	
	4-7 days		
	Adult worms	16-21	

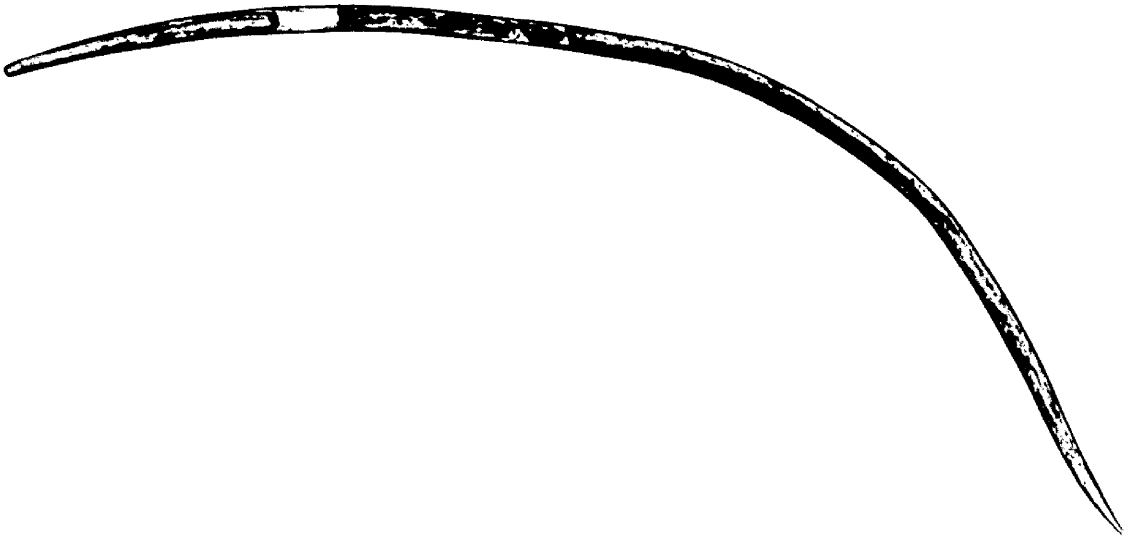


Fig. 4. Ostertagia ostertagi. Early fourth stage larva x 130. Killed in hot 70% alcohol and stained with 4% iodine solution. Note clear area representing lightly striated posterior oesophagus.

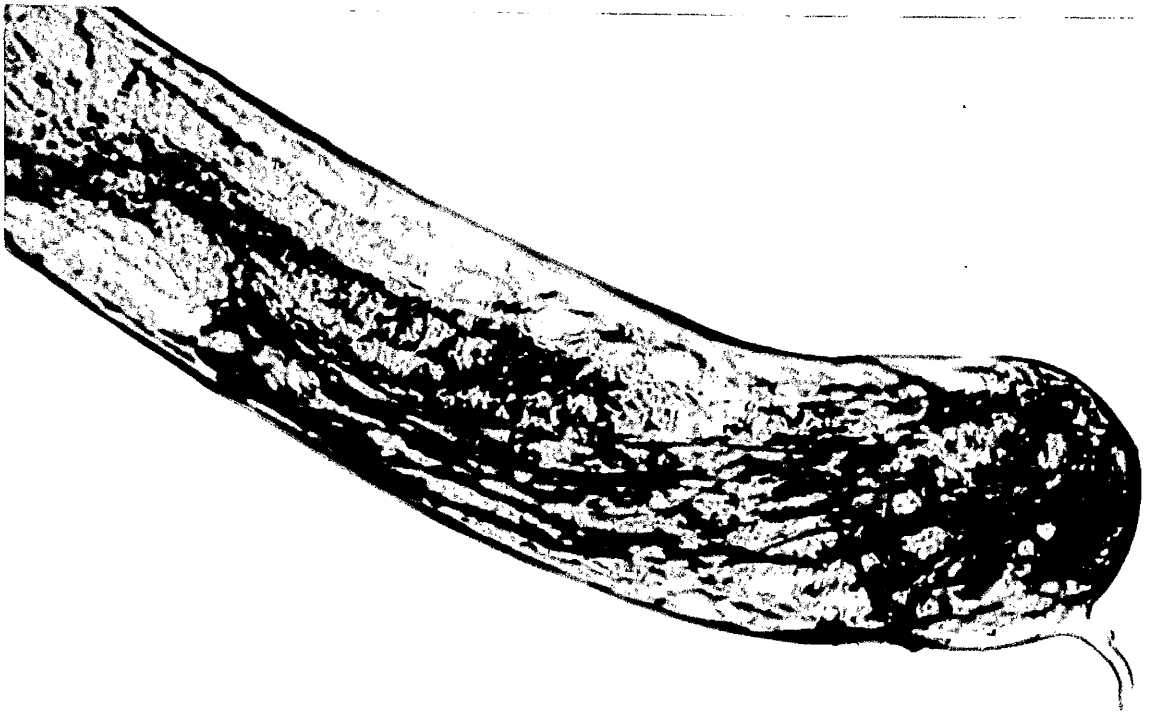


Fig. 5. Ostertagia ostertagi. Fourth moult (male) x 1000. Killed in hot 70% alcohol, mounted in lactophenol containing 0.01% cotton blue. Note sheath spike at end of sheath and developing genital system.

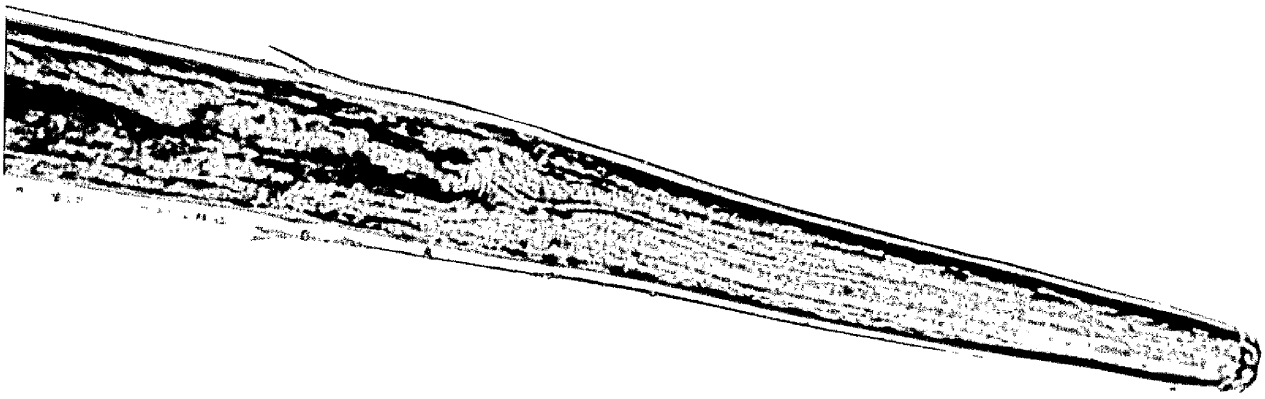


Fig. 6. Ostertagia ostertagi. Head of adult worm x 450. Killed in hot 70% alcohol, mounted in lactophenol containing 0.01% cotton blue. Note cervical papillae.

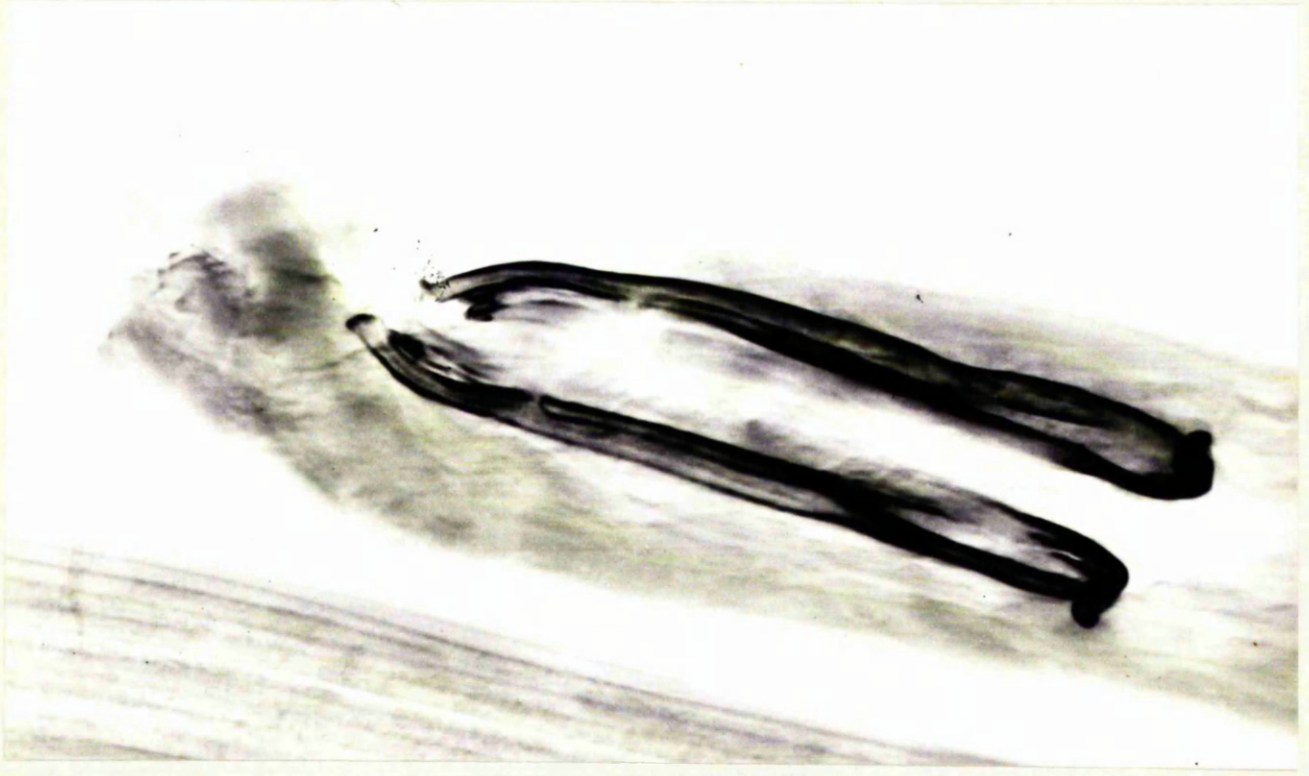


Fig. 7. Ostertagia ostertagi. Tail of adult male x 450. Killed in hot 70% alcohol, mounted in lactophenol containing 0.01% cotton blue. Note slender nature of spicules which are divided distally into three branches with enlarged ends.

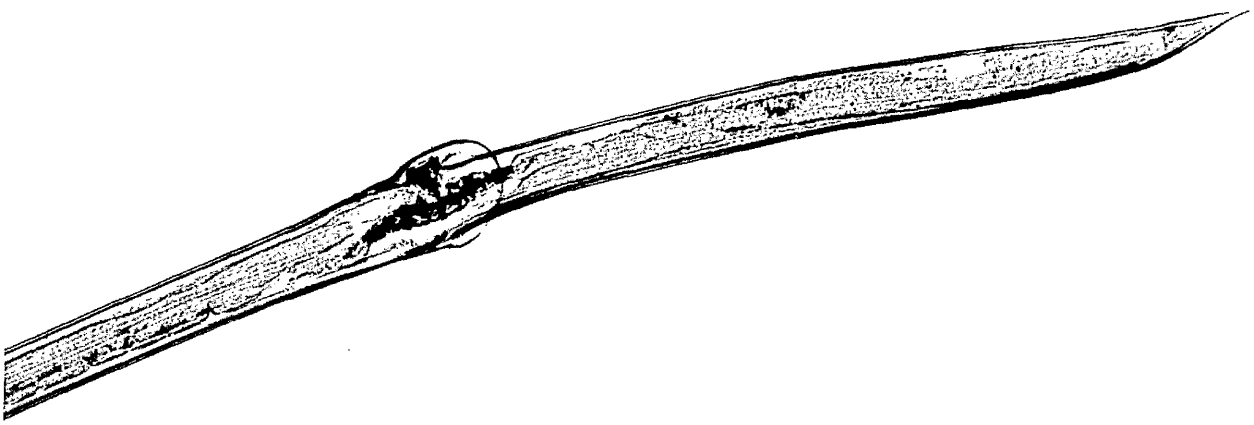


Fig. 8. Ostertagia ostertagi. Tail of young adult female x 130. Killed in hot 70% alcohol, mounted in lactophenol containing 0.01% cotton blue. Note presence of vulvar flap, and lack of striations on tail.

Life Cycle (contd.)	Day of Infection	Remarks
		and 8.3 - 9.2 mm. long respectively, i.e. 7 or 8 times as big as the early fourth stage larvae. This means the gastric glands become stretched and distended. Once the worms are adult, they emerge from the glands and lie on the mucosal surface.
Adult worms	> 21	

SECTION I

FIELD STUDIES ON PARASITIC GASTRITIS IN YOUNG DAIRY CATTLE IN SOUTH-WEST SCOTLAND

A. Parasitological Findings in 30 Outbreaks of Parasitic Gastro-Enteritis in Young Cattle, 1963 - 1965

B. The Availability and Infectivity of Ostertagia ostertagi Larvae on Pasture between May and November

C. Observations on the Development and Longevity of Ostertagia ostertagi Eggs and Larvae on Pasture during the Winter Months

General Introduction

In Great Britain outbreaks of bovine parasitic gastritis have been reported from several different parts of the country, e.g. in South-east England (Gardner, 1911); in central England (Urquhart and Pinchan, 1945); in North-east England (Stewart and Crofton, 1941); in Northern Ireland (Gracey, 1960); in South-west Scotland (Martin et al, 1957); and in central Scotland (Watt, Nicholson and McLeod, 1961). In the majority of these investigations the abomasal parasite O. ostertagi was the predominant parasite present.

Since 1957, when Martin et al described outbreaks of atypical parasitic gastritis in housed cattle in South-west Scotland, many similar outbreaks of disease characterised by diarrhoea and weight loss in young dairy cattle have been referred to the clinical teaching departments at Glasgow University Veterinary Hospital. Autopsy of these animals confirmed that both mature and immature O. ostertagi were invariably present in large numbers and it became obvious that a detailed field study of bovine parasitic gastritis combined with experimental work in the laboratory was necessary.

The field work was designed with the following objects in view: (a) the investigation and classification of outbreaks of parasitic gastritis in bovines, (b) a study of the availability and infectivity of populations of O. ostertagi larvae on pasture prior to outbreaks of ostertagiosis in calves at grass in summer and before cattle are housed for the winter, and (c) to observe the development and longevity of O. ostertagi eggs and larvae

during the winter in South-west Scotland and establish how the disease persists from year to year.

A. Parasitological Findings in 30 Outbreaks of Parasitic Gastro-Enteritis in Young Cattle, 1963 - 1965

Procedure in Outbreaks

Outbreaks reported by practising veterinary surgeons were visited and a detailed history obtained from farmers. In almost every instance, affected animals were purchased and brought to the laboratory for examination prior to autopsy. At some farms, animals which were not clinically affected and which had a grazing history similar to affected animals were also purchased and slaughtered.

Observations

Apart from clinical examination, the following collections and examinations were made prior to autopsy.

Faeces	-	Faecal egg count
Whole blood	-	Packed cell volume and haemoglobin estimations; red blood cell counts
Plasma	-	Plasma pepsinogen
Serum	-	Total serum protein, serum albumin and serum globulin.

At autopsy, samples of abomasal contents were collected for pH determination and worm counts, worm measurements and pathological observations made as described previously.

Meteorological Data

The data referred to in this thesis was recorded by the Meteorological Section, Prestwick Airport, which is situated within 30 miles of most outbreaks investigated. The mean monthly maximum and minimum temperature and monthly rainfall in the period 1963 - 1966 are shown in Figure 9.

Calf Husbandry in Dairy Farms in South-west Scotland

Calves aged four to six months are first put out early in May, usually onto a permanent calf paddock or paddocks, close to the farm-house to facilitate management, such as supplementary feeding. The time spent in these fields varies from six weeks to six months, depending on the size of the field, availability of grass and farm management. A common practice is to move the calves onto hay aftermath in August. Sometimes young calves born in the spring, i.e. February and March, are added to the group of older calves at the time they are moved to aftermath. On some farms, the calves are returned to the permanent calf paddock in late autumn and supplementary feeding may be given when grass becomes scarce. Calves are finally taken indoors for winter feeding in October or November; some farmers continue to allow their stock access to grazing during the winter days.

Results

Thirty outbreaks of bovine parasitic gastritis in which the predominant parasite was O. ostertagi were investigated. The clinical, haematological, biochemical, parasitological and pathological findings have been described by

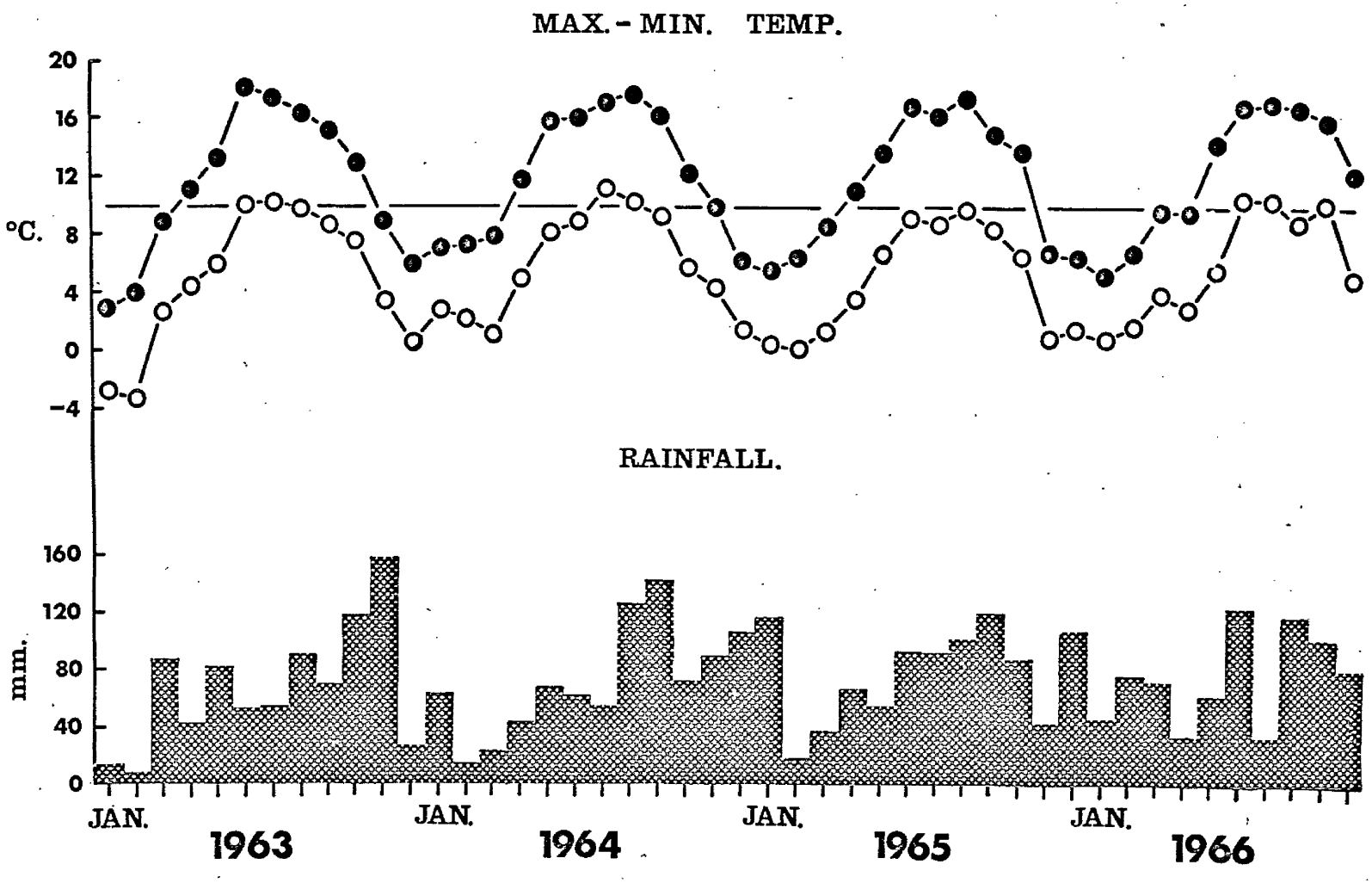


Fig. 9 Mean monthly maximum and minimum temperature (^{o}C) and monthly rainfall from January 1963 to October 1966.

Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965) and will be discussed in detail in theses by Anderson (1967) and Ritchie (1967). However, as the results pertaining to this field investigation are also relevant to this thesis, they will be summarised below. The parasitological findings for which the author was directly responsible will be discussed and presented in detail.

From the results of the investigation of the 30 outbreaks, the disease was classified into three types.

Type I. - This corresponded to the classical description of clinical parasitic gastritis in which calves, at grass for the first time, showed a loss of weight and diarrhoea; it occurred at any time from late July to the end of the autumn grazing season, i.e. October. The vast majority of the larvae ingested at that time develop to maturity in the expected period of three weeks.

Pre-Type II or the stage of inhibition. - In this stage, which precedes Type II, large populations of O. ostertagi (over 100,000 in many cases) were present, of which over 80% were inhibited at the early fourth stage. These animals had grazed infected pasture in the late autumn, i.e. October onwards, but had no history of diarrhoea and usually appeared healthy to the farmer although to the veterinarian a proportion appeared ill-thriven.

Type II. - The usual history of this type was that calves in which diarrhoea and weight loss had not been prominent during the grazing season were housed about the beginning of November. After a lapse of time which

ranged between three weeks to six months, the animals started losing weight and developed severe diarrhoea. The appearance of these clinical signs coincided with the development to maturity of large numbers of inhibited fourth stage larvae. This syndrome occurred in both housed and outwintered stock.

The Type I Syndrome

On ten farms where Type I occurred, 70.4% of 135 calves at risk, were affected, i.e. showed clinical signs, and 25.2%, i.e. 34 calves, died of the disease. This high figure for mortality is due to two farms which had a 50% and 100% mortality respectively. Exclusion of these two farms lowered the mortality rate to 11.4%. Two important epidemiological factors in the outbreaks were:- (a) overstocking and (b) the permanent calf paddock. In most of the outbreaks investigated, the stocking rate was in excess of three calves per acre. The youngest calves, i.e. those born in the late spring, were usually the first and most severely affected of the group. Owing to the widespread and apparently successful use of anthelmintics in the Type I disease, no firm conclusions could be reached or correlations made between the numbers and stages of parasites causing the clinical signs and the associated biochemical and pathological changes.

To collect more data on these points it was decided to follow the natural development of the disease during 1964 and 1965 on each of two local dairy farms with a previous history of Type I ostertagiasis. One of these farms, Laight Mains Farm, New Cumnock, Ayrshire (Farm A), used a

single calf rearing paddock, while the other, Knockadale Farm, Symington, Ayrshire (Farm B), used a two-paddock rotational system. At both farms in 1964, groups of ten male parasite-free Ayrshire calves, ten weeks old, were introduced in May. Individual calves were subsequently removed for autopsy at the onset of clinical ostertagiasis; surviving calves were removed from pasture and autopsied at the end of October. A similar procedure was adopted in 1965, except that groups of eight calves, twelve to fourteen weeks old, were used and the grazing period was from late June until the first week of October. In both years vaccination of calves against Dictyocaulus viviparus was carried out (Motol, Allen & Hanburys, Ware, England) as both farms also had a previous history of parasitic bronchitis. No supplementary feeding was given and the grazing routine adopted was a replica of the management used in previous years. In Farm B the paddocks were rotated as indicated by the farm manager. In 1965 a weighing scale was available on Farm A and weekly weighings were undertaken. Twice weekly visits were made to both farms in 1964 and once weekly in 1965 to make clinical examinations and remove calves severely affected with ostertagiasis for autopsy at the laboratory.

Clinical Signs. - In 1964, clinical signs characterized by a cessation of growth, bright green diarrhoea and sometimes anorexia and thirst first occurred at Farm A at the end of July and Farm B in early September. All of the 10 calves at Farm A had been killed *in extremis* by the end of October and only two calves survived at Farm B.

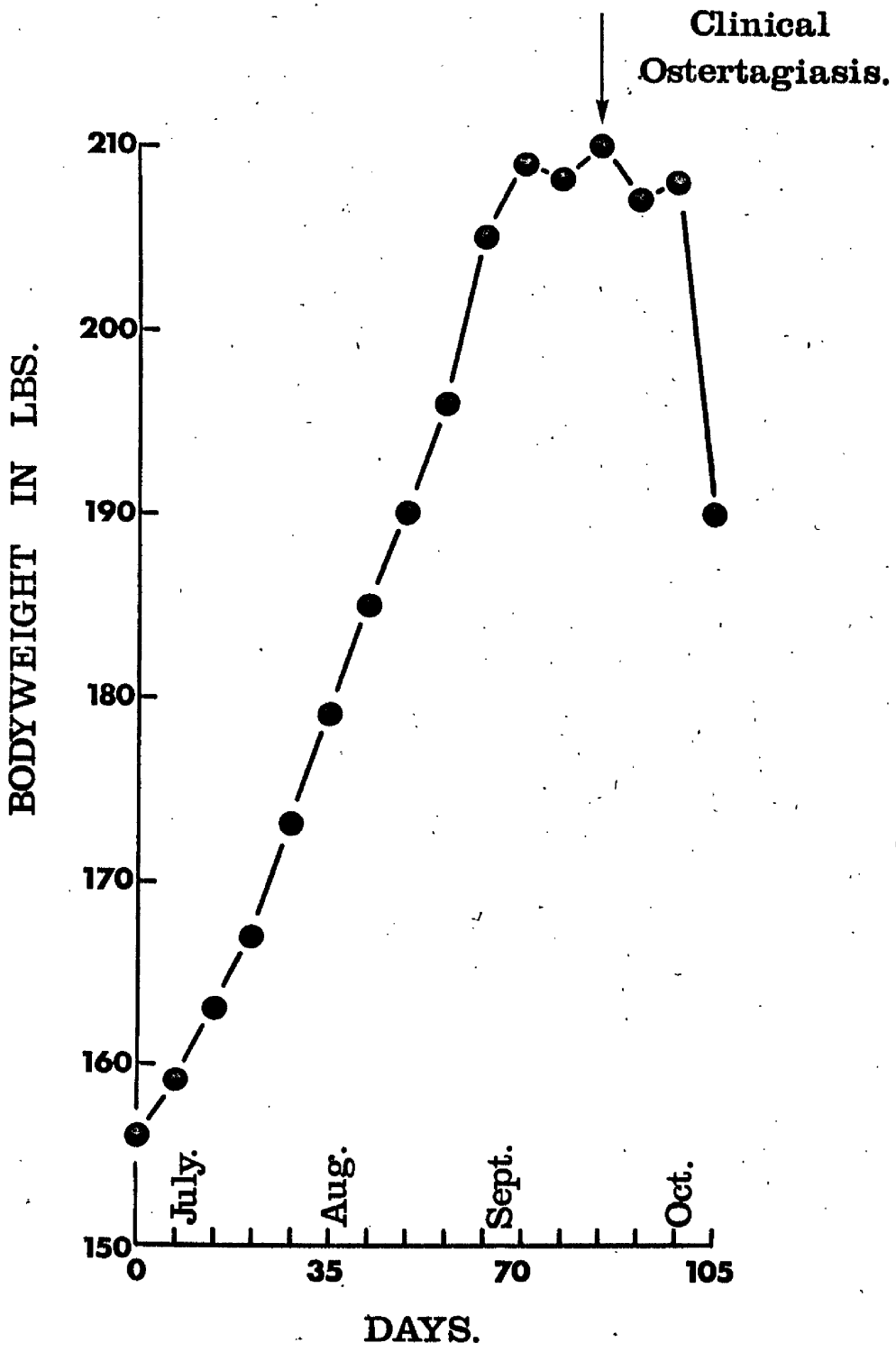


Fig. 10 Mean bodyweights of calves which grazed at Farm A from June until October 1965.

In 1965, similar clinical signs were noticed and first occurred at Farm A in mid-September and in Farm B in late September. The mean bodyweights of calves at Farm A in 1965 are shown in Figure 10 and demonstrate the marked weight loss which occurred following the onset of clinical disease. (Individual bodyweights are given in Appendix 1, Table 1.) By the end of September, five calves at Farm A and one calf at Farm B had to be killed in extremis.

Blood Analysis .- No significant changes in haematological indices, total serum protein, albumin or globulin were noticed. The plasma pepsinogen levels in calves at each farm, in both years, increased during the grazing season and were significantly raised in calves clinically affected with Type I disease. The mean plasma pepsinogen at autopsy of Type I cases was 2900 ± standard error (s.e.) 170; this level is five times that present in uninfected calves.

Autopsy Findings .- In all of the calves autopsied, mild lesions of parasitic bronchitis were present, except for one calf at Farm A in 1964, which had a severe patent infection at autopsy in August. A few *F. hepatica* (less than 50) were present in calves from Farm A in 1965 but were not found in any of the other calves. The mean pH of the abomasal contents at autopsy of Type I cases was increased to 6.2 ± s.e. 0.3. No other abnormalities were noticed at autopsy.

Pathological Data .- Five main types of abomasal lesions were seen at autopsy of calves from Type I outbreaks.

(1) Nodules.

These are oval or circular greyish-white umbilicated lesions which protrude to a varying degree from the abomasal mucosal surface depending upon their stage of development. The central orifice of the nodule is the gastric gland originally dilated by the presence of a larva. There is replacement of the specialised secretory epithelium of the gland (i.e. parietal and zymogen cells) by a mucus secreting type.

(2) Diffuse irregular epithelial hyperplasia.

This gives rise to the so-called 'morocco leather' or 'cobblestone' effect. It is caused by the hyperplasia and loss of differentiation of the secretory epithelium of the gastric glands surrounding that in which a larva has lain and becomes most marked at the time of emergence of adult worms from the glands.

(3) Epithelial Cytolysis.

At the time of emergence of the parasite from the gland there is often a marked local sloughing of superficial cells particularly in the centre of nodules. This enhances the central depression of the nodule. When, in heavy infections, nodules are closely packed together, this change produces a larger area of surface erosion, which sometimes resembles the mark produced in an epithelium by pressing a finger into it; hence the descriptive term 'thumb-print' lesion may be applied to this lesion. Severe diffuse epithelial cytolysis results in a gross diptheritic appearance of the abomasal mucosa.

(4) Oedema.

Often, but not always in severe cases, oedema of the abomasal folds is found. Histologically, the greater part of the microscopic effect is seen to be caused by diffuse oedema of the loose connective tissue forming the core of the folds.

(5) Congestion.

Naturally, when all the above lesions are present to a severe degree, there is a variable amount of congestion of the abomasal mucosa.

These lesions were all reproduced following the experimental inoculations of O. ostertagi described in Section II and are depicted in Figures 19 to 26.

Parasitological Data. - The course of the mean strongyle faecal egg counts at both farms during both 1964 and 1965 are shown in Figure 11. Details of individual faecal egg counts at weekly intervals are given in Appendix 1, Tables 2, 3, 4 and 5. The eggs in the faecal egg counts were predominantly Ostertagia type eggs, though some Cooperia species eggs were also seen. No attempt was made to differentiate these eggs at routine faecal examinations. A few Nematodirus spp. eggs were also seen but have not been included in the graphed figures. From the analysis of faecal egg counts, three points emerged: (a) the majority of individual calves with faecal egg counts of over 1,000 e.p.g. were clinically affected; (b) faecal egg counts of under 1,000 e.p.g. were seen in calves suffering from mild to severe ostertagiasis and from apparently healthy calves; (c) at autopsy there was no correlation between the faecal egg counts and the number of female worms present. The

Fig. 11 Mean strongyle faecal egg counts from calves grazing at Farms A and B in 1964 and 1965.

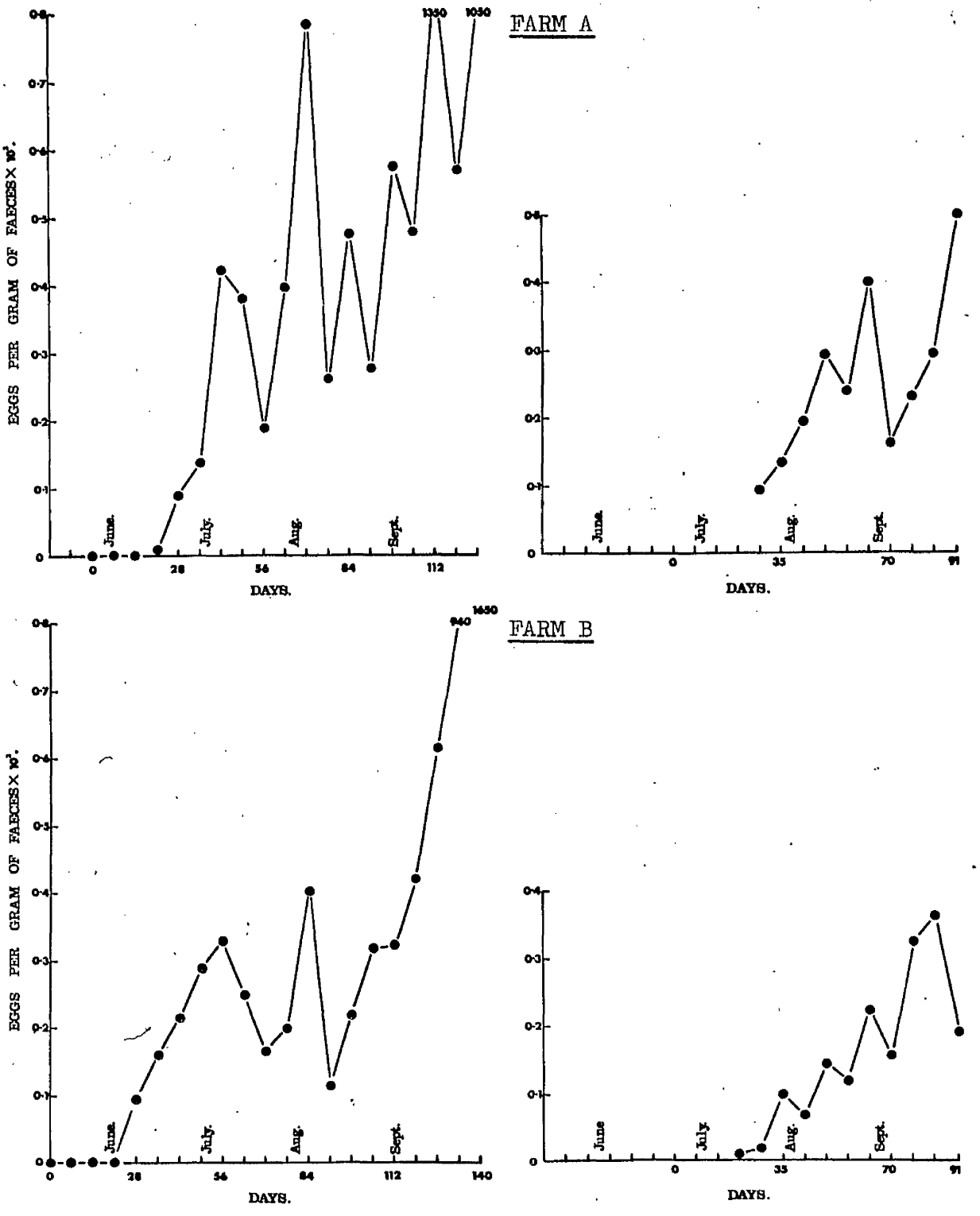


Table 1

Bodyweights and Parasitological Data at Autopsy of 15 Cases of Type I Ostertagiasis in Ayrshire Male Calves Aged 4 to 6 Months

Farm & Case No.	Body Weight in lbs. at Autopsy	Weight of Abomasal Mucosa in gms.	Worm Counts			
			Ostertagia spp.*		Total** Other Species	Per cent of *Ostertagia* spp. in total worm population
			Total	Per Cent Early 4th Larval Stages		
1964						
A1	195	570	64,000	4	20,700	76
A2	182	618	51,500	6	10,300	83
A3	210	454	43,100	5	5,900	88
A4	138	320	24,200	15	4,400	85
A5	144	330	23,100	4	3,900	86
1964						
B1	228	602	61,800	34	29,300	68
B2	218	570	111,200	18	1,300	99
B3	202	550	81,100	12	31,300	72
B4	186	432	89,600	16	22,700	80
B5	236	574	79,700	6	19,300	81
1965						
A11	189	320	84,700	3	4,000	95
A12	190	390	84,500	31	11,100	88
A13	180	320	48,000	48	14,200	77
A14	192	500	200,300	67	28,700	87
A15	192	310	37,000	4	23,300	61

* Adult male *Ostertagia* spp. were 98% *O. ostertagi*, 2% *O. lyrata*

** Other species = *Trichostrongylus axei*, *Cooperia* spp., *Nematodirus* spp. (see Appendix 1, Table 6)

bodyweights, abomasal mucosal weights and differential total worm counts at autopsy of the 15 calves severely affected with Type I disease at the onset of clinical ostertagiasis at both farms in 1964 and 1965 are shown in Table 1. Further details of the worm stages present at autopsy are given in Appendix 1, Table 6. The percentage of Ostertagia spp. present in the total worm population ranged from 60 to 99 with a mean of 79. The total Ostertagia spp. present ranged from 23,100 to 200,300 and, with the exception of two calves, the proportion of early fourth stages was low.

Ninety-eight per cent of the Ostertagia spp. males, examined in lacto-phenol, were O. ostertagi and two per cent were O. lyrata.

Pre-Type II or Stage of Inhibition

This stage of the disease is not clinically evident but is an essential prelude to the Type II disease. It appears that the majority of Ostertagia spp. larvae ingested by calves grazing in the late autumn do not develop to adults (presence of adults is necessary before clinical signs occur) in the normal period of three weeks but remain for weeks or months as early fourth stage larvae in the abomasal mucosa. Eventually, if a sufficiently large number of these larvae resume their development to maturity, the clinically obvious Type II disease will occur. Since this stage only becomes apparent in retrospect, i.e. when Type II occurs, it is difficult to establish the precise numbers of inhibited fourth stage larvae which will eventually produce disease. To obtain information on the latter point, 23 apparently normal calves were purchased from nine farms on all of which outbreaks of

Type II ostertagiasis had recently occurred. None of the 23 calves had a history of diarrhoea, some were well grown and others were up to 100 lbs. below the normal weight for their age. All had a history of autumn grazing shared with others which had severe Type II disease and the majority had been treated with anthelmintics during the preceding two months.

Blood Analysis. - Analysis of whole blood samples showed that a microcytic normochromic anaemia was present. There were no significant changes in serum protein values. Plasma pepsinogen values were only slightly elevated (1100 ± s.e. 60 mU), being twice the level in uninfected controls.

Autopsy Findings. - In 18 of the 23 calves autopsied, mild lesions of post patent parasitic bronchitis were present. In nine calves less than 75 adult F. hepatica were found. The mean pH of the abomasal contents was not markedly increased (mean 3.3 ± s.e. 1.0) from the normal range of 2.0 to 3.0. No other abnormalities were noticed at autopsy.

Pathological Data. - The main pathological features of calves in the stage of inhibition are the minimal nature of the reaction to the larva and the absence of gross damage to the mucosa. The inhibited fourth stage larvae lie in the gastric glands which become lined by mucous type epithelium. There is almost no reaction in the surrounding glands and cytolysis or umbilication of the area superficial to the larvae is not present. In some instances lymphoreticular foci are found specifically related to inhibited larvae; grossly these appear as white nodules.

Table 2

Clinical History of 15 Cases of Pre-Typo II Osteoplasiosis in Ayrshire Heifer Calves Aged 9 to 14 Months

Case No.	Month Housed	Month Killed	Anthelmintics since Housing	Weight at Autopsy in lb.
D1	Oct.	Jan.	Mintic	234
D2	Nov.	Jan.	Nil	227
D3	Nov.	Jan.	Nil	347
D4	Nov.	Jan.	Nil	340
D5	Nov.	Jan.	Nil	327
D6	Oct.	Feb.	Mintic	314
D7	Oct.	Feb.	Mintic	327
D8	Oct.	Feb.	Mintic	263
D9	Oct.	Feb.	Mintic	267
D10	Oct.	Feb.	Mintic	225
D11	Oct.	Feb.	Mintic	266
D12	Oct.	March	Mintic & T.b.s.	344
D13	Oct.	March	Mintic & T.b.s.	295
D14	Nov.	March	T.b.s. & Promintic	410
D15	Oct.	May	T.b.s.	313

Mintic & Promintic = Methyridine (Imperial Chemical Industries Ltd., Wilmslow, England).

T.b.s. = Thiabendazole (Merck Sharp & Dohme Ltd., Haddendon, England).

Table 3

The Parasitological Data at Autopsy of 15 Cases of Pre-Type II Ostertagiasis in Ayrshire Female Calves Aged 9 to 14 Months

Case No.	Faecal Egg Count in e.p.g.	Worm Counts			
		Total	*Ostertagia* spp.* Per cent Early 4th Larval Stage	Total** Other Species	Per cent. of *Ostertagia* spp. in total worm population
D1	250	103,400	95	5,100	95
D2	100	93,000	91	4,700	95
D3	250	130,700	87	17,100	89
D4	150	96,700	94	5,700	94
D5	50	72,200	97	200	99.7
D6	-ve	120,700	85	1,400	99
D7	50	140,800	98	8,600	94
D8	50	142,900	89	13,600	91
D9	250	87,500	93	9,200	90
D10	250	150,200	90	6,600	96
D11	50	117,100	97	5,100	96
D12	-ve	159,400	98	0	100
D13	-ve	141,100	92	6,000	96
D14	250	66,900	81	5,400	93
D15	100	101,700	91	0	100

*Adult male *Ostertagia* spp. were 90% *O. ostertagi*, 8% *O. lyrata*

** Other species = *Trichostrongylus axei*, *Cooperia* spp., *Nematodirus* spp. (see Appendix 1, Table 7)

Parasitological Data. - The faecal egg counts of calves D3, D4, D5, D6, D7 and D8 were followed thrice weekly over a five week period. These faecal egg counts were consistently low, rarely exceeded 200 e.p.g., and bore no relation to the size of the total worm burdens at subsequent autopsy. The history and differential total worm counts at autopsy of 15 cases of pre-Type II ostertagiasis are shown in Tables 2 and 3. (The remaining eight calves purchased were used in other studies on inhibition of development and efficiency of anthelmintics). Further details of the stages of worms present are given in Appendix 1, Table 7. The percentage of Ostertagia spp. present in the total worm population was 89 or greater in all cases. The number of Ostertagia spp. found ranged from 66,900 to 181,700 and the proportion of early fourth stages always exceeded 80%.

Ninety-eight per cent of the Ostertagia spp. males present were O. ostertagi, the remaining two per cent being O. lyrata.

The mean length and standard error of 30 inhibited early fourth larval stages from four different autopsies were 1.3 mm. ± s.e. 0.03, 1.3 mm. ± s.e. 0.50, 1.3 mm. ± s.e. 0.07, 1.3 mm. ± s.e. 0.06.

The Type II Syndrome

The Type II disease was found on 20 farms where a total of 385 young cattle were housed or outwintered. One hundred and thirty eight (36%) of these were clinically affected and 44 (11%) died. Although Type II disease may take as long as six months to become clinically evident, it must be stressed that it is usually, but not always, an acute disease and death may

supervene within one or two weeks of onset of clinical signs. Of the 20 outbreaks investigated, the first occurred as early as December and the last as late as June; the latter occurred within a week of the young cattle being put out for their second summer. The majority of the outbreaks were concentrated into the months of April and May.

Clinical Signs .- Animals suffering from Type II disease were sometimes well grown but more often were in poor condition compared with those of the same group which did not show the characteristic clinical signs of weight loss and diarrhoea. The coat was usually rough and the tail, perineal region and hocks were coated with faeces. Submandibular oedema was noted in a few animals but was not a constant feature.

A loss of weight in Type II cases was a marked feature. Comparing the weight of Type II cases with the values for average Ayrshire heifers aged 12 to 15 months at the West of Scotland Agricultural College, i.e. 495 to 603 lbs. (Walker Love, 1965), it can be appreciated that the majority of animals are at least 100 to 150 lbs. underweight (Table 4). The prognosis of severe Type II ostertagiasis cases, even with repeated anthelmintic treatment was poor. A proportion of calves (11%) died despite treatment while at least 50% required two treatments. Less severely affected animals which survived until the following spring seemed to recover quickly when turned out to grass.

Blood Analysis .- A moderate anaemia of the normocytic normochromic type was present. There were no significant changes in total serum protein

levels when examined on a group basis. However, a significant decrease in serum albumin concentration and albumin:globulin ratio was present in all affected animals. The mean serum albumin concentration and albumin:globulin ratios of uninfected control calves of the same age were 2.9 ± s.e. 0.04 and 0.9 ± s.e. 0.04 respectively, whereas in the Type II cases they were 1.4 ± s.e. 0.1 and 0.4 ± s.e. 0.03 respectively. Plasma pepsinogen was increased to a marked degree (mean 4,000 ± s.e. 430) in some instances to 20 times the value in uninfected controls of the same age (300 ± s.e. 8).

Autopsy Findings. - In the majority of the 24 calves autopsied, mild lesions of postpatent parasitic bronchitis were present. In a few animals, hepatic fibrosis was noticed and up to 75 F. hepatica were collected from each of these livers. No other abnormalities were noticed. The mean pH of the abomasal contents was markedly increased to a level of 5.7 ± s.e. 0.2.

Pathological Data. - The main pathological features of Type II disease are the sequential development and the emergence from the gastric glands of large numbers of previously inhibited larvae. Thus one finds some lesions of the inhibited stage (pre-Type II) plus the simultaneous presence of all lesions due to larval development and emergence from the glands. Nodules are produced as in Type I and surrounding these after emergence of the parasites from the glands, are areas of epithelial hyperplasia and glands lined by undifferentiated cells. Confluence of these leads to large areas which present a 'morocco leather' appearance and which are devoid of functionally differentiated cells. Epithelial cell sloughing over such areas

can be marked and if extreme may cause 'thumb-print' lesions, diptheroeis, superficial inflammation and congestion.

Parasitological Data .- Generally the faecal egg counts were low, i.e. less than 1,000 e.p.g., but during severe diarrhoeic phases it was sometimes possible to record egg counts as high as 4,000 e.p.g. There was no correlation between the faecal egg counts at autopsy and the number of adult female worms present.

Despite the fact that most of the Type II cases had been recently treated with anthelmintics, the numbers of O. ostertagi present at autopsy were frequently high. Compared with Type I disease, the total numbers of worms were double in some instances and the percentage of immature forms from all species present was much higher. Details of the clinical history and total numbers of worms present in 15 cases of Type II disease selected at random from the 24 calves autopsied are given in Tables 4 and 5. Further details of the particular stages of the worms present are given in Appendix 1, Table 8. Except in one calf (C8), Ostertagia spp. were responsible for over 90 per cent of the worm population present. The total Ostertagia spp. found ranged from 23,000 to 390,000 and the proportion of early fourth larval stages from 15 to 87%.

A few O. lyrata males were found among the Ostertagia spp. males examined but the proportion did not exceed three per cent.

Table 4

Clinical History from 15 Cases of Type II Osterotagiasis in Ayrshire Heifer Calves Aged 9 to 16 Months

Case No.	Month of Autopsy	Pattern of Diarrhoea	Anthelmintic Treatment	Weight in lbs.
C1	Dec.	Continuous	Mintic 2x	216
C2	Jan.	Continuous	T.b.z. 2x	400
C3	Feb.	Intermittent	Nil	230
C4	Feb.	Continuous	T.b.z.	262
C5	March	Continuous	T.b.z.	305
C6	April	Intermittent	Promintic 3x	365
C7	April	Continuous	Promintic	210
C8	April	Continuous	Promintic	260
C9	April	Intermittent	Promintic	308
C10	April	Continuous	Nil	328
C11	April	Intermittent	Mintic 2x	352
C12	April	Intermittent	Mintic	310
C13	May	Continuous	Mintic	272
C14	May	Continuous	Mintic	266
C15	June	Intermittent	Promintic & Lexon 2x	296

Mintic & Promintic = Methyridine (Imperial Chemical Industries Ltd., Wilmslow, England).

T.b.z. = Thiabendazole (Merck Sharp & Dohme Ltd., Hoddesdon, England).

Table 5

The Parasitological Data at Autopsy of 15 Cases of Type II Ostertagiasis in Ayrshire Female Calves Aged 9 to 16 Months

Case No.	Faecal Count in e.p.g.	Worm Counts: Ostertagia spp.* Total	Ostertagia spp.* Per cent Early 4th Larval Stage	Total** Other Species	Per cent of Ostertagia spp. in total worm population
C1	350	390,800	87	10,200	97
C2	-ve	23,000	60	0	100
C3	350	82,900	22	4,300	95
C4	750	150,800	57	0	100
C5	800	239,100	62	19,000	93
C6	550	56,900	16	240	99.5
C7	4,400	69,200	19	160	99.7
C8	100	93,300	16	28,000	77
C9	400	114,100	45	4,500	96
C10	350	153,100	15	1,500	99
C11	400	40,500	50	3,200	93
C12	500	39,900	79	2,400	94
C13	1,100	237,600	23	3,400	99
C14	50	24,000	70	0	100
C15	100	23,300	38	100	99.5

* Adult male Ostertagia spp. were 97% O. ostertagi, 3% O. lyrata

** Other Species = Trichostrongylus axei, Cooperia spp., Nematodirus spp. (See Appendix I Table 8)

Discussion

In the 30 outbreaks of severe weight loss and diarrhoea in young dairy cattle which have been described above, the only significant abnormalities found at autopsy were the presence of large numbers of stomach worms, almost entirely *O. ostertagi*, together with severe lesions of the abomasal mucosa. It is therefore apparent that parasitic gastro-enteritis is an important disease of young dairy cattle in the South-west of Scotland and that *O. ostertagi* is the primary cause of this disease.

The disease can be clearly divided into two clinically apparent types, both characterised by weight loss and diarrhoea. The first, Type I, develops in calves from larvae ingested during their first summer at grass and becomes clinically apparent from late July until early October. Larvae ingested during late autumn, *i.e.* September onwards, are thought to be responsible for Type II. The development of these larvae is arrested at the early fourth larval stage, deep in the gastric glands, for several weeks or months and this phase (pre-Type II) is not clinically apparent. When sufficient numbers of larvae resume their development and have emerged from the gastric glands, clinical signs of Type II appear. This usually occurs in late winter or early spring and is seen mainly in housed stock, but may occur in outwintered animals.

Type II ostertagiasis has been recognised annually in the South-west of Scotland since the winter of 1956 when Martin *et al.* (1957) described their outbreaks of atypical parasitic gastritis in housed cattle.

In Northern Ireland, Ross and Todd (1965) and Ross (1966) described syndromes similar to Type I and Type II ostertagiasis and designated these 'summer' and 'winter' ostertagiasis respectively. Working in Denmark, Nielson (1966) has reported outbreaks of parasitic gastritis in housed cattle which resemble Type II both clinically and on gross pathological examination.

The above pattern of outbreaks is different from that described by Michel (1966), who observed that O. ostertagi produced two types of outbreaks of parasitic gastritis in Southern England. The first, developed in calves three to four weeks after going to grass in the spring, i.e. May/June, and the second from mid-July onwards. Michel ascribed the first form to large populations of overwintered larvae and the second to larvae hatching from overwintered eggs and from eggs laid by worm populations developed from overwintered larvae. In the present investigation, the first form described by Michel did not occur but the second appears synonymous with Type I ostertagiasis.

The total number of Ostertagia spp. found at autopsy of clinical cases of Type I ostertagiasis ranged from 23,100 to 201,300 and the total number of adults from 20,000 to 75,700. With the exception of two calves at Farm A autopsied in late September, 1965, the proportion of early fourth stage larvae present was low. It has been shown experimentally that there is an exponential loss of adult worms beginning during the diarrhoeic phase which occurs three to four weeks after a large single inoculation of O. ostertagi larvae (see Section II); since the calves from Type I outbreaks were autopsied, as far as possible, at the onset of clinical signs, it is unlikely that any marked loss of worm

population would have occurred prior to autopsy. From the results of these autopsies it therefore appeared that upwards of 20,000 to 30,000 adult O. ostertagi are necessary to produce clinical Type I ostertagiasis in Ayrshire calves aged four to six months. These results agree with those obtained following experimental infections with O. ostertagi (Armour, Anderson, Ritchie and Jennings, 1964; Anderson, Armour, Endie, Jarrett, Jennings, Ritchie and Urquhart, 1966), in which calves aged 15 to 17 weeks developed clinical ostertagiasis when upwards of 40,000 adult worms were present. The actual number of adult worms required to produce clinical ostertagiasis may be related to the size of the abomasum; thus in the Type I calves autopsied (Table 1) the lower worm burdens were present in the calves whose abomasal mucosa weighed the least.

At autopsy of pre-Type II animals, the total numbers of Ostertagia spp. present ranged from 66,900 to 181,700. The proportion of early fourth larval stages present was high and ranged from 81 to 98%; these stages were apparently unaffected by anthelmintic treatment given since housing. The absence of clinical signs in these animals is attributable to the fact that only a few adult stages were present. It is clear, however, that if the numbers of inhibited fourth stage larvae found were to resume their development clinical ostertagiasis would occur.

The total numbers of Ostertagia spp. present at autopsy of cases of Type II ostertagiasis ranged from 390,800 down to 23,000. The proportion of early fourth larval stages present in these animals was much higher than that found at autopsy of cases of Type I ostertagiasis but less than that

present at autopsy of pre-Type II cases. Any reduction in adult worm numbers was usually associated with recent anthelmintic treatment or prolonged diarrhoea and the percentage of early fourth larval stages apparently unaffected by anthelmintics (see Section V) was inversely proportional to the period of clinical illness, i.e. the longer the animal had shown clinical signs, the fewer the inhibited forms in the mucosa.

Although several other nematode species, mainly Cooperia spp., were found in the gastro-intestinal tract of most of the autopsied Type I calves, it is unlikely that they played a significant role in the production of clinical disease since they were present in relatively small numbers and on only five occasions exceeded 25 per cent of the total worm population; preliminary observations by the author (unpublished data) in experimental infections with Cooperia oncophora have indicated that calves harbouring a worm burden of 75,000 adult C. oncophora do not show any obvious clinical signs up to 28 days after infection. Herlich (1965) also failed to demonstrate any ill effect in calves following experimental inoculations of 300,000 C. oncophora larvae. Fewer numbers of gastro-intestinal nematode species other than Ostertagia spp. were found at autopsies of animals in the pre-Type II or Type II stage of ostertagiasis; since most of these animals had been recently treated with modern wide-spectrum anthelmintics, this may indicate that these drugs are more efficient against gastro-intestinal nematode species other than Ostertagia spp. An alternative explanation is that immunity is developed more rapidly against these other species, than against Ostertagia spp. The numbers of F. hepatica found

never exceeded 75 and it is unlikely that a burden of this magnitude has any significance.

The clinical cases observed in this investigation were all obviously affected by severe diarrhoea and loss of weight. Both Type I and Type II are acute forms of ostertagiasis and the accompanying diarrhoea produces a rapid loss of weight (Fig. 10). The figures for loss of bodyweight may be an underestimate as Halliday, Dalton, Anderson and Mulligan (1965) have studied the body composition of animals suffering from Type II ostertagiasis, and found that body solids, as a percentage of bodyweight, was greatly reduced. This means the tissue loss associated with Type II disease cannot be assessed solely from the loss in bodyweight because of the change in body composition which occurs. Presumably, there are many calves whose worm burdens lie below the clinically apparent threshhold but these are sufficient to cause retardation of growth in otherwise apparently normal calves.

With one exception, Type I and Type II disease did not occur on the same farm within one year. Presumably this was due to calves being treated with an anthelmintic and/or removed from the heavily contaminated pasture when Type I disease occurred and therefore the possibility of these calves ingesting large numbers of larvae in late autumn was reduced. In the single farm where both types were seen in the one year, the calves were treated, moved to another pasture, and then returned to the original pasture in late autumn.

Finally, it is interesting that no outbreaks of parasitic gastritis in adult animals were reported by practising veterinary surgeons. The possible reasons for this will be discussed in the section dealing with immunity (Section IV).

B. The Availability and Infectivity of O. ostertagi Larvae on Pasture between May and November, 1964

Introduction

The fluctuations of nematode larval populations on pasture and their relationship to outbreaks of clinical parasitism have been studied in three different ways. First, by turning susceptible animals onto known infected pasture and subsequently counting, at regular intervals, the numbers of infective stage larvae present in weighed samples of the herbage. Secondly, experimentally infected animals are grazed on parasite-free pasture and the resulting pasture populations of larvae estimated from samples of herbage. These two techniques have been employed in studies on pasture populations of sheep gastro-intestinal nematode larvae (Crofton, 1949, 1952) and more specifically Nematodirus species larvae (Thomas and Stevens, 1956; Gibson, 1959, 1963). Recently, they have also been applied to the investigation of seasonal variations in pasture populations of Trichostrongylus colubriformis infective larvae (Gibson, 1966) and O. ostertagi infective larvae (Michel, 1966). These approaches require few facilities but have three main disadvantages; they give no indication of the infectivity of the larvae (as measured by their ability to establish themselves in a susceptible host) and take no account of the grazing pattern of the calves nor of variations in the length of herbage.

Thirdly, by introducing a group of susceptible animals to graze infected pasture for a prolonged period ('permanent' calves) and then adding further

susceptible 'tracer' or 'indicator' animals at regular intervals to graze for restricted short periods. These 'tracer' calves are then autopsied a few days after removal from the pasture and the numbers of worms present used to calculate the population of infective larvae available on the herbage during the period grazed. Thus, an index is obtained of the numbers of larvae ingested by the 'permanent' calves. This technique was pioneered in sheep by Tetley (1959) and in cattle by Durie (1962), who studied the fluctuations of cattle strongyle larvae on pasture in Queensland, Australia. It has the advantage of providing information on the 'infectivity' (as previously defined on page 49) of the larvae available on the herbage irrespective of the nutritional state of the pasture, but does not provide information on the actual numbers of larvae available. A further disadvantage is the cost in terms of experimental animals. Durie (1962) overcame the first of these disadvantages by combining this technique with larval counts from herbage samples. Southcott (1966) further improved on Tetley's method by inserting oesophageal fistulae in some of his test grazing lambs and collecting and counting the numbers of third stage larvae ingested.

The results from these three methods are extrapolated to provide a relationship between the magnitude of pasture populations of nematode infective larval stages, meteorological data and outbreaks of clinical parasitism.

The present experiments were designed to study the availability and infectivity of O. ostertagi larval populations on permanent calf pasture in South-west Scotland between the months of May and November, i.e. during the recognised grazing season. The method adopted for this study was similar to that of

Tetley (1959) and Durie (1962) described above, and involved the use of two systems of calves, i.e. 'permanent' and 'tracer' calves. These experiments were carried out on fields rented at the two Ayrshire farms described in the previous section where outbreaks of Type I ostertagiasis had occurred in the previous year.

Experimental Design

Farm A (Laight Mains, New Cumnock, Ayrshire)

At this dairy farm of 200 acres, a single paddock system was used to rear replacement heifer calves each year. This paddock, which was wooded, measured two and a half acres in area. It had been grazed by the farmer's calves in 1963 and a severe outbreak of Type I ostertagiasis occurred in August and September of that year. No stock had access to this field between September, 1963, and the commencement of the present trial in May, 1964.

Farm B (Knockendale Farm, Symington, Ayrshire)

This dairy farm had a total acreage of 300 acres and used a two paddock rotational system to rear replacement heifer calves each year. The two paddocks were of unequal size, the smaller being one half acre, the larger four acres. The procedure usually adopted by the farmer was to graze the smaller field initially in the spring and then rotate the two fields as dictated by the availability of grass. This procedure was followed in the present trial. In late June, 1963, an outbreak of Type I ostertagiasis had

occurred shortly after the farmer had moved his calves from the smaller field to the larger one. No stock had grazed these fields from October, 1963, until the present experiment began in May, 1964.

Animals. - At each farm ten 'permanent' calves, ten weeks old, were turned out in May, 1964 (25th May at Farm A; 11th May at Farm B), to graze until November, unless the onset of severe clinical ostertagiasis necessitated their earlier removal. As five 'permanent' calves* at each farm developed clinical ostertagiasis and had to be removed by the first week in September, five 'replacement' calves, ten weeks old, were added at that time in an attempt to keep the stocking rate even throughout the grazing season.

All of these calves were males of the Ayrshire breed and were reared worm free. As both farms had a previous history of parasitic bronchitis, the 'permanent' and 'replacement' calves were given two doses of lungworm vaccine (Dictol, Allen & Hanburys Ltd., Ware, England).

In addition to the above, two 'tracer' calves were put out at each farm at the beginning of the experiment to graze the same pasture as the 'permanent' calves. These calves were replaced by another four calves each fortnight during the entire period of the trial. The 'tracer' calves were all Ayrshire males aged between eight and twelve weeks at the time of going to pasture. All were reared worm free and were acclimatised by grazing on worm free pasture for at least two weeks prior to being transferred to Farms A and B.

Except for a few animals which had to be killed in extremis, the calves were autopsied four to seven days after removal from pasture.

* These were the calves described in Section I (Table 1).

Observations

Twice weekly visits were made to both farms. A clinical examination of all calves was carried out and faecal samples collected from each individual calf. Faecal egg counts were made by the flotation and modified McMaster techniques and trichostrongyle type eggs and Nematodirus spp. eggs counted. Differential worm counts and worm measurements of larval stages were made at autopsy as described previously. Meteorological data was collected from the records of the Ministry of Aviation at Prestwick Airport, which was situated within 20 miles of both farms.

Results

Clinical Data. - Clinical Type I ostertagiasis occurred at both farms. At Farm A, clinical signs of ostertagiasis were first noticed in the 'permanent' calves at the end of July and five calves severely affected were removed for autopsy. The remaining five 'permanent' calves had all died or were killed in extremis by the end of October. The five 'replacement' calves turned out at the beginning of September all developed Type I ostertagiasis by the end of September and died or were killed in extremis by the end of October.

On Farm B, clinical signs of ostertagiasis were first noticed at the end of August and five calves severely affected were removed for autopsy. Three of the remaining five 'permanent' calves had to be killed by the end of October, the other two calves surviving until the end of the experiment on November 18th. The five 'replacement' calves, which grazed from the beginning of September, all developed clinical ostertagiasis by the end of

September and had to be autopsied before the end of October.

Clinical ostertagiasis was noticed in some of the 'tracer' calves in August and September; these signs developed at the end of the 14 day grazing period.

The mean maximum and minimum temperatures and rainfall for 1964 are recorded in Figure 9.

Parasitological Data. - The mean faecal egg counts of the 'permanent' calves at both farms are shown in Figure 12. Low Nematodirus egg counts were also recorded but have not been included. At Farm A, an increase in individual and mean faecal egg counts occurred at the end of July and coincided with the onset of clinical ostertagiasis. At Farm B, an increase in individual and mean faecal egg counts also coincided with the outbreak of clinical ostertagiasis. Individual faecal egg counts are recorded in Appendix 1, Tables 2 and 3.

Since O. ostertagi was the predominant parasite present at autopsy, and for the sake of brevity and clarity, only parasitological results pertaining to this parasite are presented here. Details of the differential worm counts at the autopsy of 'permanent', 'replacement' and 'tracer' calves at both farms are given in Appendix 2, Tables 1, 2, 3 and 4. Where calves died and their autopsy was inadvertedly delayed for a few hours, the worms recovered were fragmented and difficult to identify and enumerate; these results have not been included.

Autopsies up to outbreaks of Type I ostertagiasis

The mean numbers of O. ostertagi and the percentage of early fourth

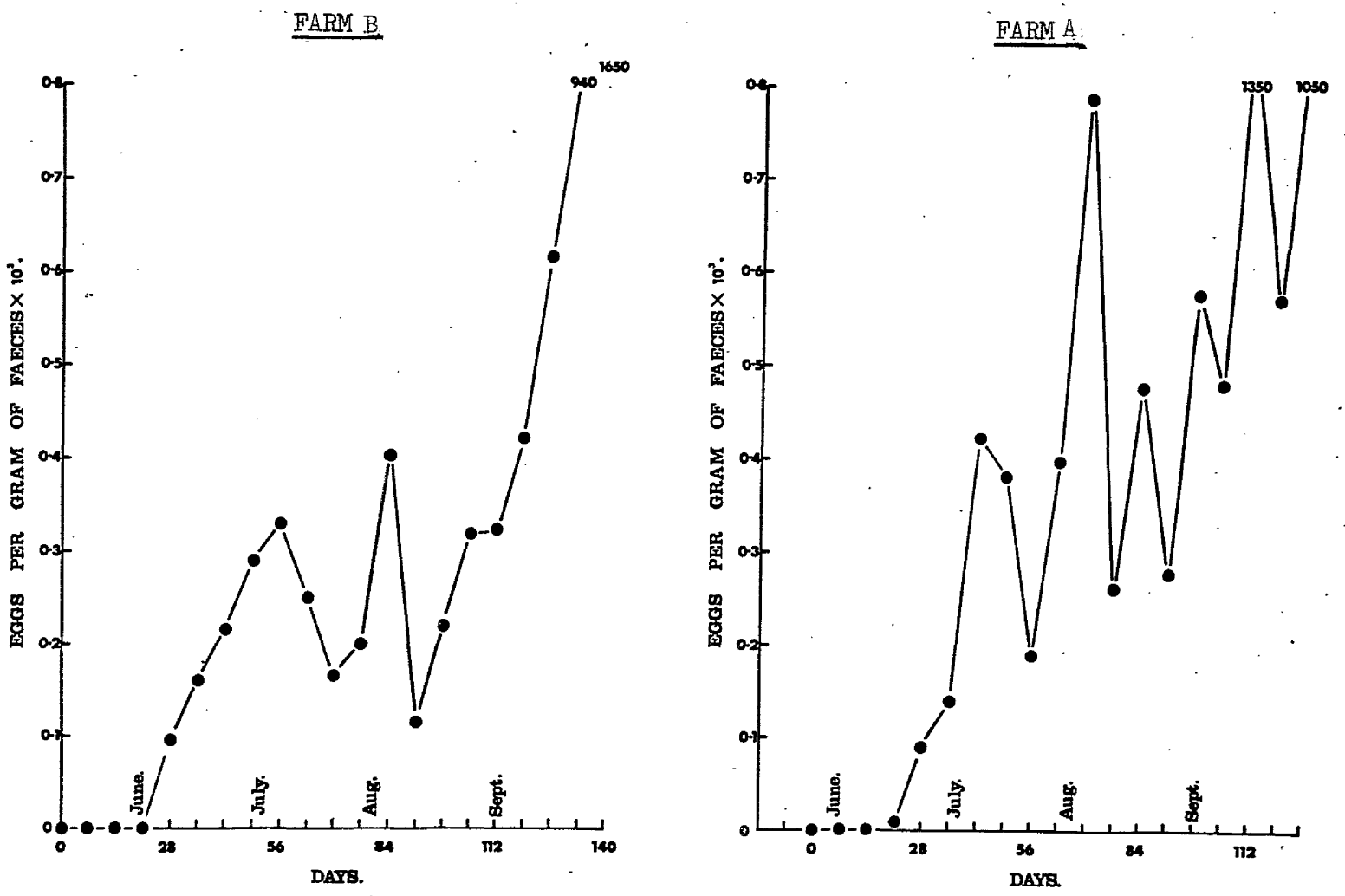


Fig. 12 Mean strongyle faecal egg counts of calves grazed at Farms A and B in 1964.

larval stages present in the 'tracer' calves up to the onset of clinical ostertagiasis are given in Table 6. The mean worm burdens of the five 'permanent' calves autopsied from each farm at the onset of clinical ostertagiasis are also included in this table along with the summated mean worm burdens from the 'tracer' calves. It is of interest that at both farms the sum of the mean worm burdens of the 'tracer' calves up to the onset of clinical ostertagiasis in the 'permanent' calves is approximately the same as the mean worm burdens in the 'permanent' calves autopsied at this time.

Autopsies after outbreaks of Type I ostertagiasis

The mean total numbers of O. ostertagi, the mean numbers of adult, developing and early fourth larval stages and the percentage of early fourth larval stages present in 'permanent' calves at both farms severely affected with ostertagiasis and autopsied before and after October 1st are shown separately in Table 7. The mean total O. ostertagi burdens in both groups of calves are essentially similar but the mean numbers of adult and developing stages are decreased and both the numbers and proportions of early fourth larval stages are increased in the calves autopsied after October 1st. These changes in population were accentuated in the calves autopsied towards the end of October (Appendix 2, Table 1).

The mean total numbers of O. ostertagi, the mean numbers of adult, developing and early fourth larval stages and the percentage of early fourth larval stages present at autopsy of 'replacement' calves from both farms are also given in Table 7. The mean total numbers of O. ostertagi in the 'replacement' calves were greater than in both groups of 'permanent' calves.

Table 6

The Mean Numbers and Percentage of Early Fourth Larval Stages of Ostertagia ostertagi at Autopsy of Pairs of 14 Day Tracer* Calves up to the Onset of Clinical Ostertagiasis in Permanent** Grazing Calves, and the Mean Numbers of O. ostertagi at Autopsy of 5 Permanent Calves with Clinical Ostertagiasis

	PAIR A		PAIR B	
Date 1964	Mean Numbers O. ostertagi	Mean % Early 4th Larval Stages	Mean Numbers O. ostertagi	Mean % Early 4th Larval Stages
11/5 - 25/5	-	-	1,700	19
25/5 - 8/6	1,500	21	700	29
8/6 - 22/6	3,200	34	400	9
22/6 - 6/7	16,200	26	300	0
6/7 - 20/7	13,700	22	300	0
20/7 - 3/8	10,400	13	800	27
3/8 - 17/8	1,300*	15	10,800	17
17/8 - 31/8	Clinical ostertagiasis in permanent calves		48,500	7
31/8 - 14/9			36,400**	14
			Clinical ostertagiasis in permanent calves	
Total	46,300		99,900	
Mean of 5 Permanent Calves	42,200 ±s.e. 7,900 (removed from pasture 4.9.64, autopsied 5.9.64)		84,700 ±s.e. 9,000 (removed from pasture 10.9.64, autopsied 11.9.64)	

*Mean for single days grazing 3 - 4.9.64

**Mean for ten days grazing 31.8 - 10.9.64

*Tracer Calves - Parasite Free Calves Grazed for 14 days and autopsied 4 days later

**Permanent Calves - Calves Reared Worm Free and Grazed from May onwards

s.e. - standard error

Table 7

The Mean and Standard Error of Total Numbers, Numbers of Adult, Developing and Early Fourth Larval Stages and the Mean Percentage and Range of Early Fourth Larval Stages of *Ostertagia ostertagi* at Autopsy of Permanent* Calves Autopsied Before and After October 1st and at Autopsy of Replacement** Calves in Late September and October, 1964

Group	No. of Calves	*O. ostertagi* Worm Burdens					
		Total	Adults	Developing Stages	Early Fourth Larval Stages		
					Total	Mean Percentage	Range
Permanent Calves Autopsied Before Oct. 1st 1964	13	69,600 ±7,800	40,500 ±5,200	20,700 ±4,300	8,400 ±1,900	12	4 - 34
Permanent Calves Autopsied After Oct. 1st 1964	7	65,500 ±14,500	26,500 ±6,300	5,500 ±2,500	33,500 ±11,400	50	28 - 81
Replacement Calves Autopsied in Late Sept./Oct. 1964	6	89,200 ±13,200	46,900 ±7,800	20,700 ±6,300	21,600 ±5,100	24	8 - 46

*Permanent Calves = Calves Reared Worm Free and Grazed from May onwards

**Replacement Calves = Calves Reared Worm Free and Grazed from September onwards

Table 8

The Mean Numbers and Percentage of Early Fourth Larval Stages of Ostertagia ostertagi in 14 Day Tracer* Calves from Farms A and B and Autopsied from August to November, 1964

Period Grazed	No. of calves	Mean Nos. of O. ostertagi	Percentage Early 4th Larval Stages	
			Mean	Range
17/8 - 31/8	4	45,600	7	3 - 12
31/8 - 14/9	2	53,400	13	12 - 14
14/9 - 28/9	3	43,300	23	15 - 37
28/9 - 12/10	2	10,900	28	24 - 32
12/10 - 26/10	3	17,400	54	44 - 70
26/10 - 9/11	2	55,300	86	84 - 88
26/10 - 10/11*	3	21,700	94	91 - 96

*3 calves grazed for 23 days

*Tracer Calves - Reared Worm Free and Grazed for 14 days, then autopsied 4 days later

The mean numbers of adult and developing stages in the 'replacement' calves were similar to those present in the 'permanent' calves autopsied prior to October 1st but greater than in those autopsied after October 1st. The mean numbers and percentage of early fourth larval stages present at autopsy of the 'replacement' calves were greater than in the 'permanent' calves autopsied before October 1st but less than in those autopsied after October 1st.

Finally, the mean numbers of O. ostertagi and the percentage of early fourth larval stages present in 'tracer' calves autopsied from both farms after mid-August are shown in Table 8 and Appendix 2, Tables 3 and 4. The mean percentage of early fourth stage larvae and the mean numbers of O. ostertagi at autopsy per days grazed are also shown graphically in Figure 15. These results show that the numbers of worms established in the 'tracer' calves increased during the grazing season until a peak was reached in late August and early September; thereafter, a decline in numbers of worms present occurred, except where the 'tracer' calves were concentrated on the small field at Farm B in November, in which case a further peak occurred. At both farms the proportion of early fourth larval stages increased during the season and reached a maximum in November. The mean lengths of 30 early fourth stage larvae from each of two 'tracer' calves in the autumn at both farms were 1.3 mm. ± s.e. 0.04, 1.3 mm. ± s.e. 0.03, 1.3 mm. ± s.e. 0.03, 1.3 mm. ± s.e. 0.04.

Discussion

The results of the field investigation into outbreaks of parasitic gastritis had suggested that massive inhibition of development of O. ostertagi only occurred in calves which had grazed during late autumn on pasture known to be contaminated

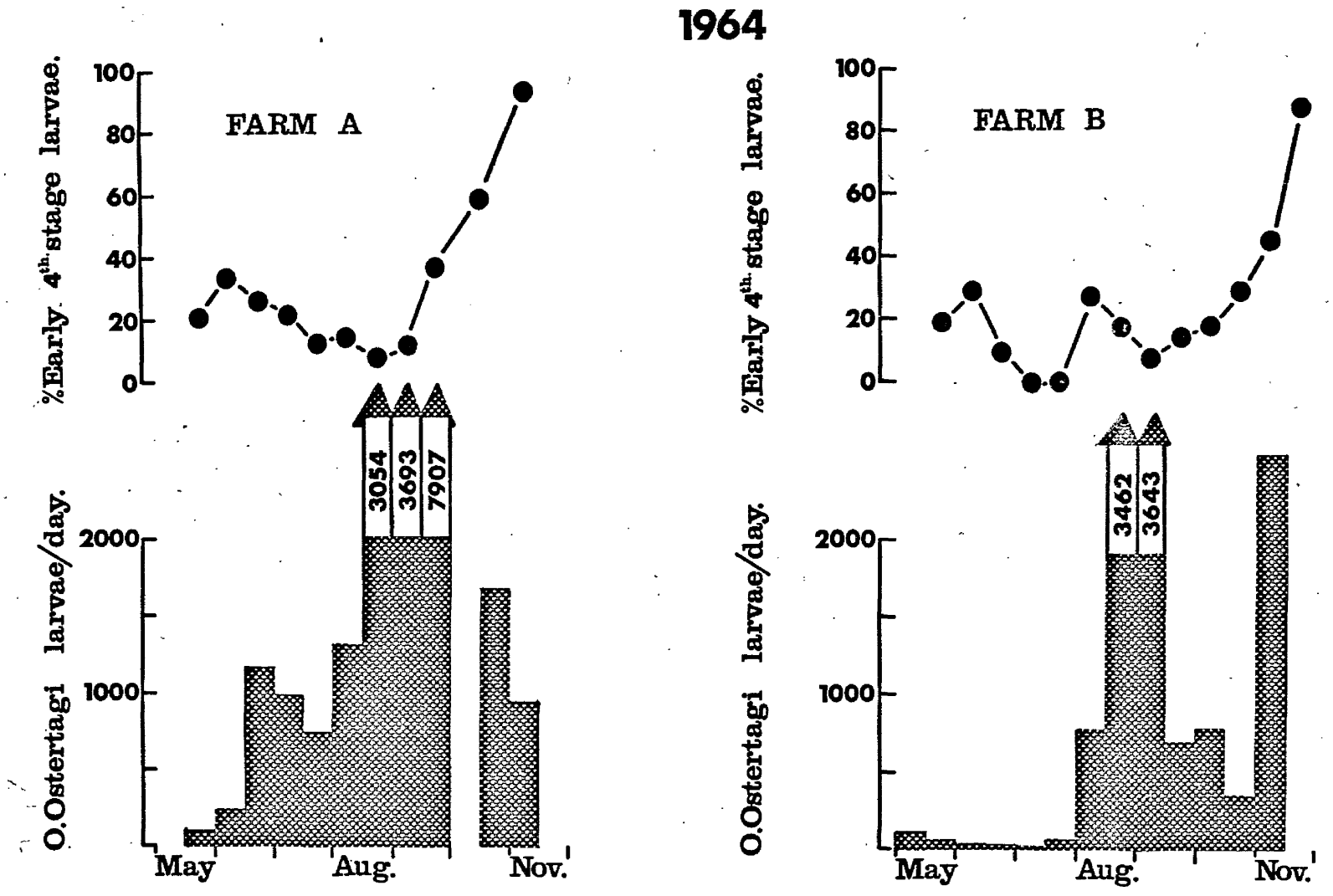


Fig. 13 Mean numbers of Ostertagia ostertagi established per day of grazing and mean percentages of early 4th larval stages in pairs of calves grazing for 14 days at Farms A and B in 1964.

with O. ostertagi larvae. Ross (1965) had also noticed during a knackery survey of cattle abomasums over a period of twelve months, that the numbers and proportion of larval stages of O. ostertagi were markedly increased in late autumn and early winter. It was therefore necessary that any study of O. ostertagi larval populations on pasture, and their relationship to outbreaks of disease, should employ a technique which measured the infectivity of the larvae available rather than the actual numbers of larvae available. The method adopted in the experiments in this section of examining worm burdens from pairs of 'tracer' calves of comparable age and grazed for restricted set periods, provided both a guide to the numbers of larvae available for the grazing season and an index of their infectivity. In certain instances (see Appendix 2) the variation in O. ostertagi populations between the two 'tracer' calves was considerable and it would have been preferable to use four 'tracer' calves rather than two at each farm.

The numbers of O. ostertagi present in the 'tracer' calves autopsied during May and early June were low, suggesting that either relatively few infective larvae had overwintered or were still available on the pasture. The subsequent increase in numbers of O. ostertagi found at autopsy of 'tracer' calves was initially more rapid at Farm A than at Farm B; it is likely that this was due to a more rapid build up of larval populations on the single paddock used at Farm A in contrast to the two paddock rotation employed at Farm B. The rapid increase in worm burdens found in 'tracer' calves at both farms in July may also be accounted for by the delayed hatching until June of O. ostertagi eggs which had overwintered; a similar lag in development of O. ostertagi eggs at this

time of the year has been reported by Michel (1966).

It is interesting that at both farms the sum of the mean worm burdens of the 'tracer' calves up to the onset of clinical ostertagiasis in the 'permanent' calves was approximately the same as the mean worm burdens in the 'permanent' calves autopsied at this time (Table 6). Even allowing for differences in size and appetite of the 'tracer' and 'permanent' calves, these results indicate that little loss of adult worms has occurred in the 'permanent' calves up to the occurrence of clinical ostertagiasis. It would appear therefore that up to the middle of September calves acquiring a light infection of O. ostertagi in the spring, do not develop a significant degree of immunity but continue to re-infect themselves until clinical disease occurs.

The fact that the mean total O. ostertagi burdens of the 'permanent' calves autopsied later in the grazing season, i.e. after October 1st, are essentially similar to those of 'permanent' calves autopsied earlier although many infective larval stages were still available on the pasture (see worm burdens of 'tracer' calves) suggests that some restriction of the O. ostertagi population was developing towards the end of September. Further evidence for this is provided from a study of the results of the 'replacement' calves which first grazed at the beginning of September. These calves proved particularly susceptible and developed clinical ostertagiasis within three weeks of going to grass and the mean numbers of total, adult and developing stages of O. ostertagi were considerably higher than in the 'permanent' calves which grazed at the same time and were autopsied after October 1st (Table 7; Appendix 2, Tables 1 and 2). The apparent restriction of worm population in

these 'permanent' calves in late autumn was not borne out by the results of their faecal egg counts (Fig. 12; Appendix 1, Tables 2 and 3) from mid-September onwards; these counts fluctuated considerably and in some instances were higher than earlier in the grazing season.

Since most of the 'permanent' calves which survived until late autumn were showing clinical signs of ostertagiasis, it is possible that the reduction in the worm burdens at that time could be attributed to either a reduction in appetite resulting in few larvae being ingested or the altered environment in the abomasum (elevated pH) proving unsuitable for the existing adult worms or the establishment of the incoming larvae. A third and perhaps more plausible reason is that the calves were acquiring an immunity to ostertagiasis after several months of constant exposure to the parasite. Analysis of the worm burdens at autopsy of the 'permanent' calves autopsied after October 1st (Table 7) suggests that this immunity could be acting in two ways. First, by causing a loss of the existing adult worm burden and secondly by inhibiting the development of newly established larvae.

Inhibited development of O. ostertagi occurs at the early fourth stage, immediately after the third moult has taken place in the gastric glands, three to four days after infection. At this time the larvae are about 1.3 mm. in length and it is interesting that the inhibited larvae found in Pre-Type II field cases were of a similar length to those measured from autopsies of 14 day 'tracer' calves. The proportion of early fourth stage larvae of O. ostertagi found in the 'permanent' and 'tracer' calves from May until early October (Tables 6 and 7) was low and could largely be accounted for by the

development of infections acquired during the last two days of grazing, i.e. four or five days previously. In the 'permanent' calves autopsied after October 1st, there was a marked increase in the percentage of early fourth larval stages of O. ostertagi (Table 7). This latter finding agrees with the results of Ross (1965) obtained from a parasitological survey of abomasums at a knackery over one year and in which a marked increase in numbers of O. ostertagi larval stages was noticed in late autumn/early winter. Michel (1963) found that massive inhibition of development of O. ostertagi first occurred following daily experimental inoculations of O. ostertagi over a prolonged period (120 days). Therefore, it might have been postulated that the 'permanent' calves in the present experiment were acquiring immunity as the grazing season progressed and this was reflected in a delayed development of a proportion of the larvae ingested.

However, the results in Table 8 and Figure 13 show that the mean percentage of early fourth stage O. ostertagi also increased in the 14 day 'tracer' calves towards the late autumn; this increase was not accompanied by an increase in total worm burdens. In late October, over 84% of the worm population acquired in 14 days grazing was inhibited in development. This proportion was such that it could not be attributed to the development of larvae ingested in the last day or two of grazing. It was also noted that the small populations of Cooperia oncophora present in calves autopsied in October and November contained a high proportion of fourth larval stages, whereas relatively low proportions were found in calves examined in previous months (Appendix 2, Tables 3 and 4).

The fact that massive inhibition of development of O. ostertagi was found only in calves at pasture in late autumn and occurred within as short a time interval as 14 days suggests that inhibition of the parasite may not necessarily be a manifestation of acquired immunity but may be associated with 'physiological' changes in either the host or the larvae in late autumn. A fuller investigation of this problem is given in Section III.

C. Observations on the Development and Longevity of O. ostertagi Eggs and Larvae on Pasture during the Winter

Introduction

Working in South-east England, Rose (1961) demonstrated that eggs of O. ostertagi survived the winter months but that little or no development took place during this period. In this region the temperature seldom rises above 10°C during the winter months (November to February) and may be below 5°C over restricted periods. Rose (1961) also found that the survival rate of O. ostertagi eggs was high when they were situated in the dung pad but decreased when faecal pads were broken up and disseminated. The eggs which successfully overwinter can continue their development in the spring months as the temperature rises. In South-west Russia, Bessonov (1958) found that O. ostertagi eggs were killed by the extremely cold winter prevalent in that area (temperatures below freezing for prolonged periods). In U.S.A., Clordia and Bizzell (1960b, 1963) conducted experiments on the development of O. ostertagi eggs; these observations were made at various controlled temperatures and it was found that development and hatching of O. ostertagi eggs was minimal under 5°C and took at least 41 days at 10°C.

Various workers in the U.S.A., including Baker (1939) in New York State, Goldberg and Rubin (1956) in Maryland and Drudge et al (1958) in Kentucky have demonstrated in field experiments that O. ostertagi infective larval stages (third stage) successfully overwinter and were still capable of infecting susceptible calves, after nine months on the herbage.

Boosenov (1958) also found that infective larval stages of O. ostertagi survived the extreme winters of South-west Russia. In South-east England, Rose (1961) established that many infective larval stages of O. ostertagi could survive the winter months and remain viable for at least eight months on the herbage. Unfortunately, the infectivity of these aged infective larval stages in Rose's experiments was not ascertained. According to Rose (1961) the first and second stage larvae of O. ostertagi are readily killed by cold weather unlike the more resistant third or infective larval stages.

Since the ambient temperature seldom exceeds 10^{0}C during the winter months (mid-October to mid-March) in South-west Scotland (Fig. 9), it seems unlikely that O. ostertagi eggs deposited on the pasture from October onwards will develop and hatch until the following spring. The present experiments were designed to confirm that O. ostertagi eggs will survive on the herbage over the winter and also to study the longevity of O. ostertagi third stage larvae in the same environment. The experiments were extended to include observations on the development of ostertagiasis from separate overwintered populations of O. ostertagi eggs and infective larval stages.

Experimental Design

Two small fields or paddocks at the Glasgow University Veterinary School, each 1500 square yards in area and not grazed by livestock for at least four years previously were used.

Paddock E was employed to study the survival and development of O. ostertagi

Table 9

Plan of Experiments Designed to Study Development and Longevity of Overwintered Eggs and Larvae of Ostertagia ostertagi

Date of Grazing Period	Paddock 1	Paddock 2
July 1 - 27 1965	Group 1	Group 2
	3 calves with patent O. ostertagi infections (Goodore)*	Nil
Sept. 27 - Oct. 21 1965	Nil	3 calves with patent O. ostertagi infections (Goodore)
Oct. 21 - 29 1965	Group 3	Group 4
	4 p.f. calves (autumn tracers)	4 p.f. calves (autumn tracers)**
Nov. 7 1965	Killed	Killed
May 17 - 25 1966	Group 5	Group 6
	3 p.f. calves (spring tracers)	3 p.f. calves (spring tracers)
June 1st 1966	Killed	Killed
May 27 - Sept. 13 1966	Group 7	Group 8
	3 p.f. calves (permanents)	3 p.f. calves (permanents)***
Sept. 20 1966	Killed	Killed

***Permanents = Calves Reared Worm Free and Grazed from May onwards

**Tracers = Calves Reared Worm Free, Grazed for short periods and autopsied at least 7 days later

*Goodore = Calves experimentally infected with O. ostertagi and turned out to graze.

eggs during the winter and ensuing months and was first contaminated by grazing with three calves for 26 days in late September and October, 1965; these calves ('seeders') had been inoculated orally at 21 and ten days prior to grazing with 100,000 O. ostertagi third stage larvae. At the time of this 'seeding' the temperature hardly ever rose above 10°C and it was unlikely that the eggs deposited by the calves onto the pasture would develop to the infective larval stage until the following spring.

Paddock L was used to observe if O. ostertagi infective larval stages could successfully overwinter and was first contaminated by grazing three calves ('seeders'), inoculated as on Paddock E, for 26 days in July, 1965. The majority of eggs deposited at this time should have developed to the infective larval stage before the winter.

The development and longevity of these herbage infections was followed by grazing groups of three or four 'tracer' parasite free calves on both paddocks, for periods of eight days in the autumn of 1965 and spring of 1966, and examining their abomasal worm counts at autopsy seven days after removal from pasture. Two further groups, each consisting of three 'permanent' parasite free calves, were grazed on the experimental paddocks from May until September, 1966, to observe the build up of infections from each source.

The calves used in these experiments were Ayrshire male calves, reared worm free and aged between nine and fourteen weeks at the time of first going to pasture.

The experimental design is summarised in Table 9.

Observations

All the calves were given a daily clinical examination. Faecal samples were collected weekly from the calves in groups 1,2,7 and 8, and faecal egg counts made by the flotation and modified McMaster methods, as described previously. Abomasal worm counts were carried out at the autopsy of the 'tracer' calves in groups 3,4,5 and 6, and from groups 7 and 8.

Meteorological data was collected from the records of the Ministry of Aviation, Prestwick Airport.

Results

The mean monthly maximum and minimum temperatures and monthly rainfall are given in Table 9. It can be seen that the mean temperatures did not rise above $10^{o}C$ during the months of November through March. In addition to the information given in Table 9, two light falls of snow were recorded, one in December, 1965, one in February, 1966.

The mean weekly faecal egg counts of group 1 (grazed July, 1965) ranged between 550 and 1,650 e.p.g. while the mean weekly faecal egg counts of group 2 (grazed September/October, 1965) ranged between 880 and 1,390 e.p.g.; both plots were therefore seeded with approximately similar numbers of O. ostertagi eggs.

The mean weekly faecal egg counts of the 'permanent' calves in groups 7 and 8 are shown in Figure 14. These results show that the faecal egg counts of calves in group 8 (Paddock E) increased sharply in mid-August reaching a

Fig. 14 Mean Ostertagia ostertagi faecal egg counts from calves grazing paddocks E and L from 17th June, 1966.

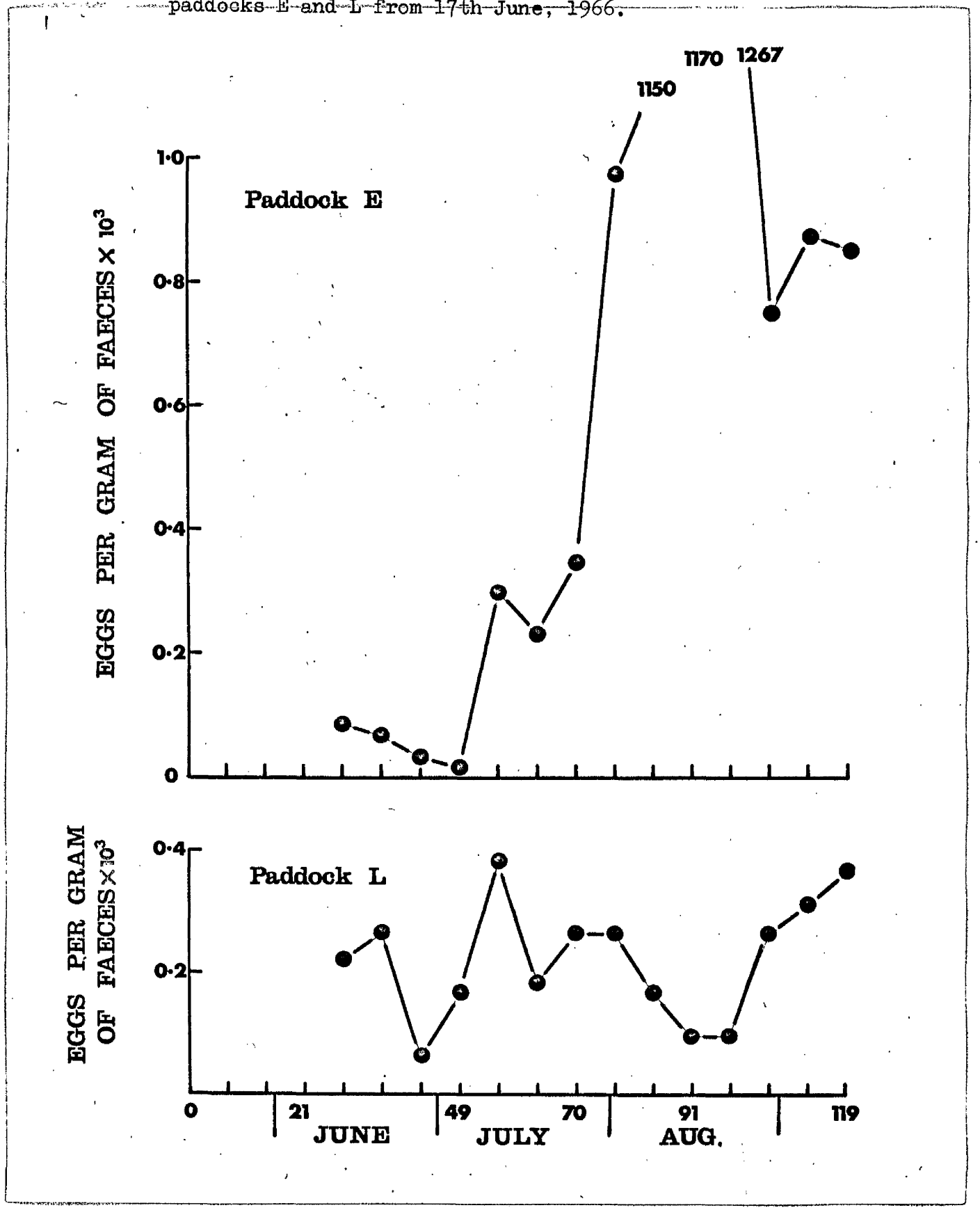


Table 10

Worm burdens at autopsy of calves which grazed Paddocks 'E' and 'L' in October, 1965, May, 1966 and from May - September, 1966.

Total Nos. of Ostertagia ostertagi

	Paddock L		Paddock E
October 1965			
Group 5 (tracers)	27,000	Group 4 (tracers)	0
	12,000		60
	14,000		0
	11,000		200
May 1966			
Group 5 (tracers)	5,300	Group 6 (tracers)	400
	2,300		0
	1,900		80
September 1966			
Group 7 (permanents)	32,600	Group 8 (permanents)	45,400[X]
	63,200		105,300*
	26,900		21,300

[X]Died from Type I ostertagiasis 27.9.66

*Died from Type I ostertagiasis 13.9.66

Tracers and permanent calves as in Table 9

maximum of 1,700 e.p.g., by which time clinical signs of Type I ostertagiasis, i.e. loss of condition and diarrhoea, were apparent in all three calves, and two calves died; in group 7 (Paddock L), the mean faecal egg counts did not exceed 380 e.p.g. and only one of the three calves developed clinical signs of Type I ostertagiasis.

The O. ostertagi worm burdens at autopsy of the 'tracer' calves in groups 3 through 6 are shown in Table 10. The numbers of O. ostertagi in the 'tracer' calves from paddock E (groups 4 and 6) in October, 1965 and May, 1966, were negligible, suggesting that only a few eggs deposited on the pasture in October, 1965, had developed to infective larvae by the following May, 1966. On the other hand, the numbers of O. ostertagi in the 'tracer' calves from paddock L in October, 1965 (Group 3), were high, ranging from 11,800 to 27,000 and indicating that many of the eggs deposited in July, 1965, had developed into infective larvae by October, 1965. A proportion of these larvae had successfully overwintered as evidenced by the numbers of O. ostertagi in the 'tracer' calves from paddock L in May, 1966 (Group 5); these ranged from 1,900 to 5,300. None of the 'tracer' calves displayed any clinical signs of ostertagiasis.

The total numbers of O. ostertagi at autopsy of the 'permanent' calves in groups 7 and 8, which grazed paddocks E and L respectively from May to September, 1966, are also shown in Table 10. The numbers present ranged from 21,300 to 105,300 and are similar to those found in field outbreaks of Type I ostertagiasis.

Discussion

The results of these experiments show: first, that many of the O. ostertagi

eggs deposited on the herbage in July, 1965, had hatched and developed into the third or infective larval stage by October, 1965. Evidence for this is provided by the considerable worm burdens in the 'tracer' calves of group 3 (Table 10). A comparison of the worm burdens of group 3 with those of the 'tracer' calves grazed in May, 1966 (group 5), suggests that less than 25 per cent of the infective larval stages on the pasture in the autumn of 1965 had successfully overwintered. However, this low figure for survival rate may be due to large numbers of third stage larvae, potentially capable of overwintering, being removed by the autumn 'tracer' calves in 1965 (group 3). The larvae which overwintered survived on the herbage for a period of at least eight months (October, 1965 to May, 1966), i.e. a similar duration to that noted by Rose (1961) in South-east England, and workers in the U.S.A. (Baker, 1939; Goldberg and Rubin, 1956; Drudge et al, 1958; Schwink, 1963). It is significant that the numbers of infective larval stages which overwintered, as distinct from the O. ostertagi eggs, were sufficient to lead to the build up of larval populations on the pasture of a magnitude capable of producing Type I ostertagiasis in the group of 'permanent' calves (group 7) by mid-September, 1966.

Secondly, that many of the O. ostertagi eggs laid on the pasture in late September and early October, 1965, survived the winter but had not hatched and developed into infective stage larvae by May, 1966. This is evident from the negligible numbers of worms found at autopsy of the autumn and spring 'tracer' calves (groups 4 and 6, Table 10). However, patent O. ostertagi infections developed by early July, 1966 (Fig. 14) in the 'permanent' calves grazing

paddock E (group 8) and since the pre-patent period of O. ostertagi infections at this time of the year is usually about 21 days, it appeared that the over-wintered eggs had hatched and developed in June, 1966, i.e. eight to nine months after being deposited on the pasture. The numbers of O. ostertagi infective stage larvae available on the pasture by the end of July, 1966, must have been considerable as an outbreak of Type I ostertagiasis occurred in the calves of group 8 by the end of August. Since clinical ostertagiasis did not occur in the calves grazing paddock L (group 7 - overwintered larvae) until September, 1966, although the faecal egg counts of this group (Fig. 14) were higher early in July than those from the calves grazing paddock E (group 8 - overwintered eggs), the increase in numbers of infective larval stages on paddock E in late July could not be attributed solely to the development of eggs deposited earlier in that month. A more likely explanation is that a mass hatching of overwintered eggs took place during the month of July. The survival of O. ostertagi eggs over the winter and their subsequent development in the next year, is in agreement with the findings of Rose (1961) but disagrees with those of Besenov (1958).

The climatic conditions in the autumn, winter and spring of 1965-66 were not markedly different from other years in South-west Scotland (Fig. 9). In some years the snowfalls are heavier than those recorded in 1965-66, but it is improbable that these would have an adverse effect on the survival of O. ostertagi eggs or infective larval stages. Thus, Gibson (1966) demonstrated that the presence of snow cover on the ground prolonged the survival of T. colubriformis larvae; he reported that although the ambient temperature was frequently as low as -10°C, the soil temperatures under the snow rarely fell

below $0^{o}C$. It therefore seems likely that both O. ostertagi eggs and infective larval stages are capable of successfully overwintering on pastures in South-west Scotland and that the development of the overwintered eggs can continue during the following spring and summer months. In years where the spring months are particularly mild, the development of these overwintered eggs may be accelerated.

General Discussion

From a consideration of the results presented in this section, the occurrence in South-west Scotland dairy farms of two types of clinical ostertagiasis (Type I, Type II) and a quiescent form (pre-Type II) may be explained as follows: calves born indoors in the previous autumn are turned out for the first time about mid-May onto grazing usually reserved solely for the use of calves. They ingest O. ostertagi larvae which developed in the previous summer or autumn and then successfully overwintered. These infections become patent three weeks later and eggs are deposited on the pasture. These eggs, together with eggs deposited the previous autumn and which have overwintered, will develop into infective larvae by the end of June provided normal summer weather prevails. The grazing calves therefore ingest a considerable number of infective larvae at this time and the resulting adult population gives rise to high numbers of eggs on the pasture by mid-July. The temperature is particularly suitable for development of O. ostertagi eggs in July (Rose, 1961) and provided sufficient rainfall occurs to permit migration of the larvae from the dung pad (Rose, 1962) the number of infective larvae on the pasture will reach a level capable of producing heavy adult infections in calves from the end of July onwards. It was also shown in the study using pairs of 14 day 'tracer' calves to quantitate the numbers of larvae available on the pasture, that calves which acquire a relatively light infection in spring, do not develop a significant degree of immunity but continue to re-infect themselves; the resulting accumulation of adult O. ostertagi eventually produces clinical disease. Thus, by the end of July or early

August, or in some years September, clinical Type I ostertagiasis occurs.

The time of onset of Type I ostertagiasis is dependent on various factors such as density of stocking, rotation of pasture and temperature and moisture. Thus lower density of stocking and rotation of pasture at Farm B delayed the onset in 1964 and 1965 when compared to Farm A. The lower temperatures in the spring of 1965 (April and May) delayed the hatch of overwintered eggs at both Farm A and Farm B and Type I disease did not occur until September, whereas it was seen in July and August in 1964.

These events and the occurrence of clinical ostertagiasis in August have also been described by Michel (1966) in Southern England. If calves born in the spring of the same year are turned out in August to join the older calves, they prove particularly susceptible and develop ostertagiasis within three to four weeks. The vast majority of the infective larvae on the pasture at this time are capable of developing to maturity in the calf in 21 days. As the autumn progresses, the numbers of ingested larvae which mature in 21 days are reduced and many larvae are inhibited in their development at the early fourth larval stage in the gastric glands. By early November, over 80 per cent of the larvae ingested and established will become inhibited in their development at the early fourth larval stage (Fig. 13). As clinical signs of Type I ostertagiasis in young calves only occur with adult worm populations in excess of 20,000 to 30,000, the situation is reached in late autumn and early winter where calves harbour thousands of inhibited larval stages of O. ostertagi but show no obvious clinical signs; this is the pre-Type II stage.

The specific mechanism of inhibition of larval development in O. ostertagi is not known but it is now thought to be a function of 'physiological' changes in either the larvae on the pasture or in the host in late autumn and occurs in both Ostertagia spp. and Cooperia spp. larvae (Section III). The majority of these inhibited larvae remain in the gastric glands until late winter or early spring; if sufficient numbers of inhibited larvae are present and resume their development en masse, then clinical Type II ostertagiasis will result when the adult stages are reached. If only a few larvae resume their development at one time, obvious clinical signs will not develop. Although Type II ostertagiasis results from the maturation of inhibited fourth stage larvae, this should not necessarily be interpreted as indicating a different type of pathogenesis from Type I.

Two factors, one management and the other climatic, appear to increase the incidence of pre-Type II and Type II ostertagiasis. First, the management practice where calves are grazed from May until late July on a permanent calf paddock, then removed to aftermath, only to return to the original paddock in late autumn. In this system, the build up of infection on the original paddock will be occurring at the time the calves are moved to aftermath. The original pasture will still be heavily infected when the calves return in late autumn and massive inhibition of development of ingested and established larvae will occur.

Secondly, in dry summers, i.e. July and August, the infective larvae capable of producing Type I disease do not migrate from the dung pad onto the pasture (Rose, 1962). When sufficient rainfall occurs in September, these

larvae migrate onto pasture within the next few weeks but by this time a high proportion of the larvae will become inhibited in development if ingested by a grazing calf.

A high incidence of winter outbreaks of ostertagiasis (Type II) following dry summers has also been noticed by Martin et al (1957) and Ross (1966).

Summary

1. Field investigations into 30 outbreaks of parasitic gastro-enteritis in South-west Scotland showed that O. ostertagi was the predominant parasite. The disease caused by the parasite was classified into three forms, two of which (Type I, Type II) were clinically apparent. Type I occurred in calves at pasture for the first time, was characterised by weight loss and diarrhoea and occurred at any time between late July and early October. Pre-Type II was not clinically obvious but the calves harboured large populations of O. ostertagi, of which over 80 per cent were inhibited at the early fourth stage. Type II resulted from the maturation, up to six months later, of sufficient numbers of these inhibited larvae to cause the clinical condition characterised by weight loss and diarrhoea.

2. A study on the fluctuations of pasture populations of O. ostertagi infective larvae during the grazing season was made using the worm burdens at autopsy of 'tracer' calves, which grazed for only 14 days, to quantitate the level of herbage infections. The numbers of O. ostertagi infective larvae increased during the grazing season and reached a maximum in August and September; thereafter they declined slightly. The proportion of worms inhibited in their development in the 'tracer' calves also increased as the season progressed, reaching a maximum of over 90 per cent early in November.

3. Observations on the development and longevity of O. ostertagi eggs and larvae during the winter months were carried out. Both eggs and infective

larval stages successfully overwintered but the subsequent development of the eggs to infective larvae was not completed until mid-summer.

SECTION II

EXPERIMENTAL OSTERTAGIA OSTERTAGI INFECTIONS IN CALVES

A. The Pathogenesis and Parasitology of a Single Large Infection

B. Studies Using Abomasal Cannulae

Introduction

The early publications relating to outbreaks of bovine ostertagiasis were primarily concerned with descriptions of the clinical signs (Stiles, 1901), the gross pathology (Ackert and Muldoon, 1920) and enthelmintic treatments of infected animals (Bruford and Pinchum, 1945). The first adequate description of the gross and histopathological abomasal changes present at autopsy of cattle clinically affected with ostertagiasis was made by Bailey (1956). Subsequently, Martin et al (1957) described the abomasal lesions found at the autopsy of young housed cattle dying from atypical parasitic gastritis (now designated Type II ostertagiasis). The principle gross abomasal changes reported by those workers were the presence of greyish white nodules varying in size from a pen to a pinhead, marked thickening and granularity of the mucosa and extensive submucosal oedema. Histologically, changes were seen in the specialised secretory cells lining the glands occupied by O. ostertagi larvae; the parietal and zymogen cells were replaced by unspecialised epithelial cells. A normocytic, normochromic anaemia and a hypoalbuminaemia were present in the calves from outbreaks investigated by Martin et al (1957). None of the above publications made any reference to physiological or biochemical changes in the abomasum nor discussed the pathogenesis of ostertagiasis. The first reference to biochemical changes in ostertagiasis was made by Ross, Todd and Dow (1963), who observed an elevation of the pH of the abomasal contents at autopsy of cattle severely affected with ostertagiasis.

In the same year, Mulligan, Dalton and Anderson (1963) reported experiments in which labelled albumin was injected into parasite free calves and calves suffering from ostertagiasis. In the infected calves, the half life of the albumin was significantly shortened.

Anderson et al (1965a) gave a detailed description of the abomasal lesions found at autopsy of field outbreaks of ostertagiasis. These outbreaks were subdivided into two clinically apparent types, Type I and Type II, and a third and clinically inapparent form, pre-Type II, and the distinctive features of the pathology of each type were described. In Type I and Type II, the principal gross lesions were umbilicated nodules, diffuse irregular epithelial hyperplasia, epithelial cytolysis, congestion and oedema. Histologically, the most significant changes occurred in the specialised cells lining the abomasal glands, these being replaced by rapidly multiplying undifferentiated epithelial cells. These cellular changes applied to both parasitised and surrounding non-parasitised glands. In the pre-Type II disease, the cellular changes were confined to the parasitised glands and the general mesenchymal reaction was minimal. These authors also noticed a marked elevation of the pH of abomasal contents at autopsy of Type I and Type II cases (but not pre-Type II). Increased plasma pepsinogen levels were also present in Type I and Type II ostertagiasis. A significant degree of anaemia and hypoalbuminaemia occurred in Type II ostertagiasis.

Abomasal lesions have also been described at autopsy following single experimental inoculations of O. ostertagi to susceptible calves by Threlkeld and Johnson (1948), Osborne, Datto and Dell (1960) and Mahrt, Hammond and

Miner (1964). Threlkeld and Johnson (1948) noticed an erosion of the gastric mucosa, a round cell infiltration and decrease in haemoglobin levels and neutrophil leucocytes. Mahr et al (1964) noted a chronic abomasitis, cloudy swelling of the liver and a hypoalbuminaemia. Osborne et al (1960) observed that a tissue reaction occurred shortly after the infective larvae penetrate the gastric gland. This tissue reaction took the form of a round cell infiltration and after 19 days oedema of the submucosa became marked. As the parasitised gland enlarged due to the increase in size of the developing larvae the adjacent glands became compressed. It was postulated that when this occurs the glands are non-functional. By 25 days post inoculation, the O. ostertagi had emerged from the glands. Unfortunately in these experiments a standard dose of inoculum was not employed and the autopsies were carried out at long intervals after inoculation; no mention was made of changes in abomasal biochemistry.

More recently, Ross and Dow (1965b) and Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart (1966) have studied the sequential development of the abomasal lesions following experimental inoculations with 100,000 third stage O. ostertagi larvae. The calves in these experiments were reared worm free and were eight to ten weeks old at inoculation. They were subsequently autopsied in pairs at selected intervals up to 90 days post inoculation. Ross and Dow (1965b) found that discrete white nodules containing larvae covered the mucosal surface at one week post inoculation. By the second week these nodules had

coalesced and the gastric glands occupied by the larvae showed mucoid metaplasia with loss of parietal and zymogen cells. In a separate communication, Ross and Todd (1965) reported that the pH of the abomasal contents was elevated at autopsy of calves in the experiments described by Ross and Dow (1965b). The same authors state that a moderate anaemia and a significant degree of hypoalbuminaemia are constant features of clinical ostertagiasis. Ross and Todd (1965) further postulated that the lowered acidity of the abomasum together with the lesions present and the nutritional requirements of thousands of parasites, must reduce the functional efficiency of the gastric activity to a very low level.

Ritchie et al (1966) found that the inoculum of 100,000 O. ostertagi larvae produced all the abomasal lesions seen in typical field cases of Type I ostertagiasis by Anderson et al (1965b) but that these lesions were not severe enough to cause marked clinical signs, haematological or biochemical changes in blood or abomasum. The relationship with the stages of the life cycle of the parasite to the structural alterations in the mucosa were also discussed.

In further experiments (Anderson et al, 1966) groups of young parasite free calves were given a range of doses of O. ostertagi larvae and all were killed on a single day to give quantitative dose-response data; doses of 400,000 larvae and upwards produced severe abomasal lesions, marked elevation of the pH of abomasal contents at autopsy on day 21 and severe clinical signs of diarrhoea, loss of weight and anorexia.

Another interesting feature of the experiments described by Ross and Dow (1965b), Ross and Todd (1965) and Ritchie et al (1966) is the apparent loss of adult worm population which sometimes occurs following emergence of the adult

O. ostertagi from the gastric glands. Ross and Dow (1965b) suggest this loss takes place gradually from the fourth week onwards, while Ritchie et al (1966) postulated it occurs between 16 and 28 days after inoculation.

The present experiments were undertaken using a single inoculum of 300,000 O. ostertagi larvae, i.e. a dose large enough to produce clinical signs of ostertagiasis and severe abomasal changes. The object of the two experiments described below was fourfold:

(1) to observe the clinical, haematological, biochemical, parasitological and pathological changes in calves killed serially following inoculation with 300,000 O. ostertagi larvae;

(2) to follow, using abomasal cannulae, the daily biochemical changes in the abomasal fluid of two calves after inoculation with a similar dose of O. ostertagi larvae;

(3) to study more closely the loss of adult worm population that occurs three to five weeks after large inoculations of O. ostertagi larvae;

(4) to collate these results and discuss their relationship to the pathogenesis of ostertagiasis.

A. The Pathogenesis and Parasitology of a Single Large Infection

Experimental Design

Twenty-two Ayrshire male calves, reared parasite free from birth, were given a single oral inoculum of 300,000 O. ostertagi larvae. At the time of inoculation (day 0) the calves were ten weeks old and their bodyweights ranged between 122 and 135 lbs. The calves were autopsied singly on days 4, 10, 17 and then daily until day 30. Thereafter single calves were autopsied at weekly intervals from day 35 to day 70.

Observations

A daily clinical examination was made of all calves. The five calves due to be autopsied from day 42 onwards were weighed thrice weekly and the quantity of concentrates consumed at each feed measured. Blood samples were also collected thrice weekly from these five calves and the following estimations carried out using the techniques previously described: packed cell volume percentages, haemoglobin concentration, red cell counts, total serum protein, serum albumin, total serum globulin and gamma globulin concentrations and plasma pepsinogen levels.

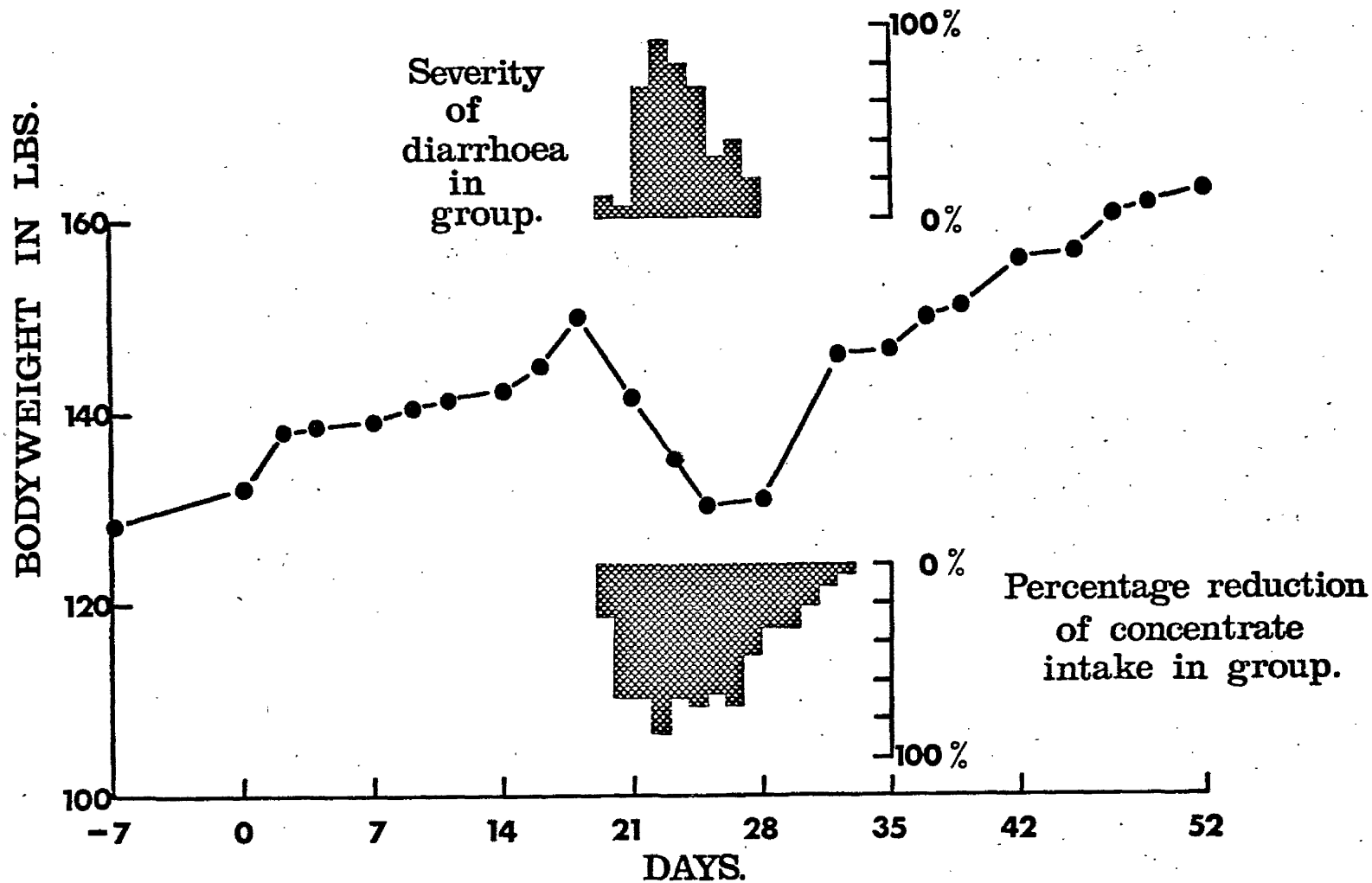
Faecal samples were collected daily and examined for consistency; faecal egg counts were made on days 0 and 17 and thereafter daily until the end of the experiment. At autopsy, the pH of the abomasal contents was recorded, a parasitological examination of the abomasal contents and mucosa was performed and blocks of abomasal mucosa removed for histological examination.

Results

Clinical Observations. - The mean bodyweights of the group of five calves examined and weighed thrice weekly are shown in Figure 15, together with a classification of the diarrhoea and reduction in appetite for concentrates displayed by the calves in the group. Softening of the faeces did not occur prior to 18 days post inoculation but 16 of the 18 surviving calves were diarrhoeic by day 19. In the selected group of five calves, severe diarrhoea was apparent by day 22; this period of severe diarrhoea coincided with a marked reduction in the intake of concentrates and a mean weight loss of 12 lbs. per calf. Although it was not practical to weigh the hay consumed, a reduction in intake of hay was also noticed during the period of severe diarrhoea. Details of the individual bodyweights and concentrate intake and diarrhoea classification are given in Appendix 3, Tables 1, 2 and 3. One of the five calves due to be autopsied from day 42 onwards lost 30 lbs. bodyweight and had to be killed *in extremis* on day 28; the calf due to be killed on day 28 was then autopsied on day 42. At the end of the diarrhoeic phase (day 28) the four surviving calves gradually regained normal appetite.

Blood Analysis. - In the group of five calves bled regularly, no significant anaemia occurred during the course of the experiment, though some haemoconcentration was apparent during the period of severe diarrhoea (Appendix 3, Tables 6, 7 and 8). Serum protein values were not significantly altered (Appendix 3, Tables 9, 10, 11 and 12). An increase in plasma pepsinogen levels first occurred by day 4 (mean 1100 mU) and this value increased gradually

Fig. 15 The mean bodyweights, classification of diarrhoea and reduction of concentrate intake in a group of five calves inoculated with 300,000 Ostertagia ostertagi larvae. (Classification of diarrhoea as in Fig. 28A.)



until day 14 (mean 2200 mU) after which it increased sharply to a maximum mean value of 7400 mU by day 23. The mean plasma pepsinogen levels from the group of five calves are shown in Figure 16. Details of individual plasma pepsinogens are given in Appendix 3, Table 5.

Abomasal pH. - The pH of the abomasal contents in calves autopsied up to day 21 was not significantly altered; thereafter a marked increase in the pH occurred and this increase was maintained in calves autopsied until day 30 (except day 27). From day 35, the pH gradually returned to pre-infection levels. The mean pH of abomasal contents up to day 20, between days 21 and 30 and from day 35 are given in Table 11.

Parasitological Observations. - The mean faecal egg counts from day 18, i.e. when egg counts first occurred, are shown in Figure 17 and details of the individual faecal egg counts are given in Appendix 3, Table 4. The mean egg counts increased to a maximum of 1,000 e.p.g. on day 24, and thereafter decreased to zero by day 65.

Details of the numbers, sex ratios and stage of development of O. ostertagi present at autopsy are given in Table 12. A plot of the log number of O. ostertagi against time is drawn on Figure 18. Regression analysis of this data shows that through days 17 to 35 there is an inverse linear functional relationship between log worm numbers and time, i.e. the loss of worms is exponential.

The development of the O. ostertagi found here accords well with previous descriptions of the life cycle (Threlkeld, 1946; Porter and Cauthen, 1946; Douvres, 1956; Ritchie et al, 1966). At day 4, the infective third stage

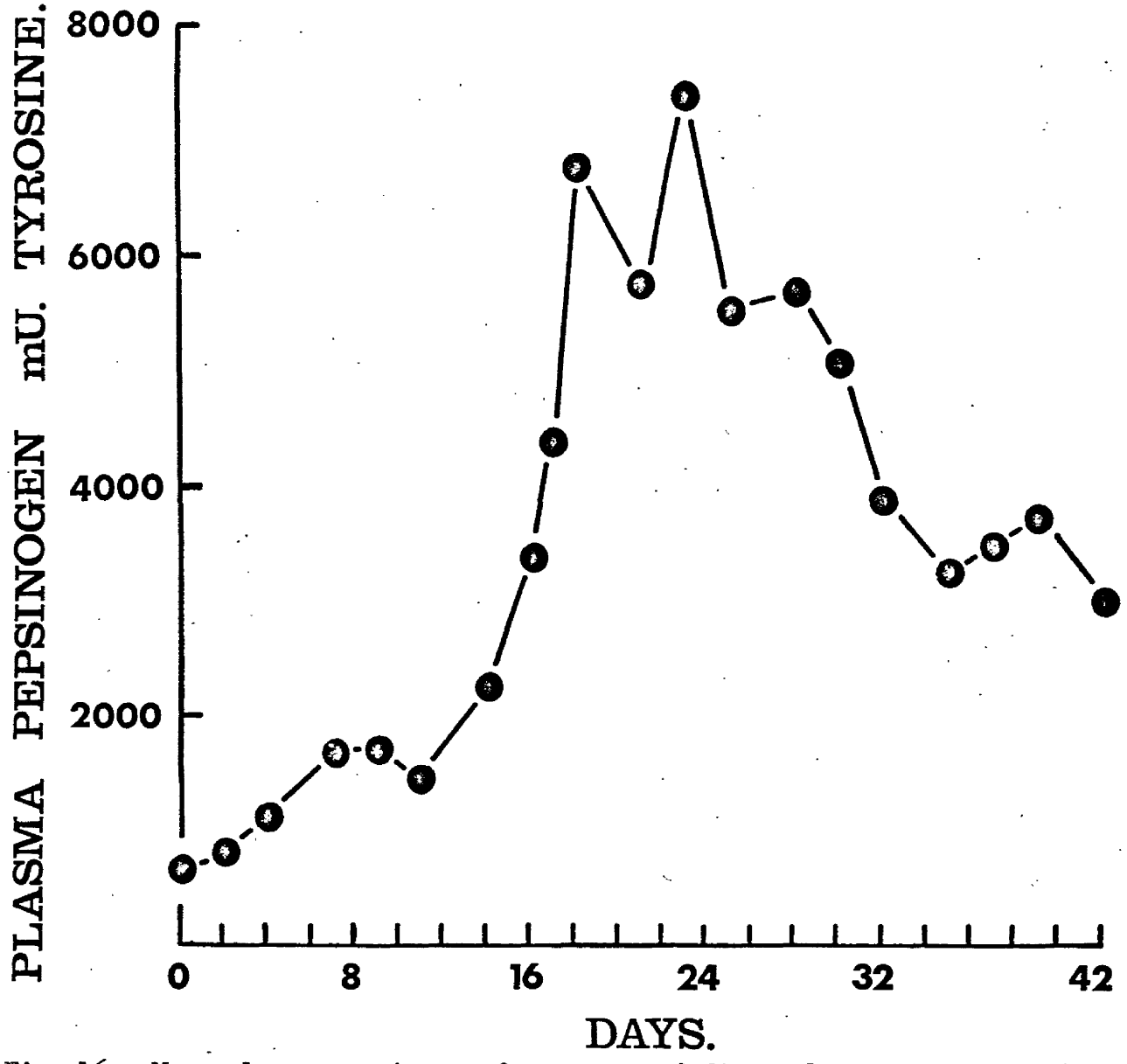


Fig. 16 Mean plasma pepsinogen from group of five calves inoculated with 300,000 Ostertagia ostertagi on day 0.

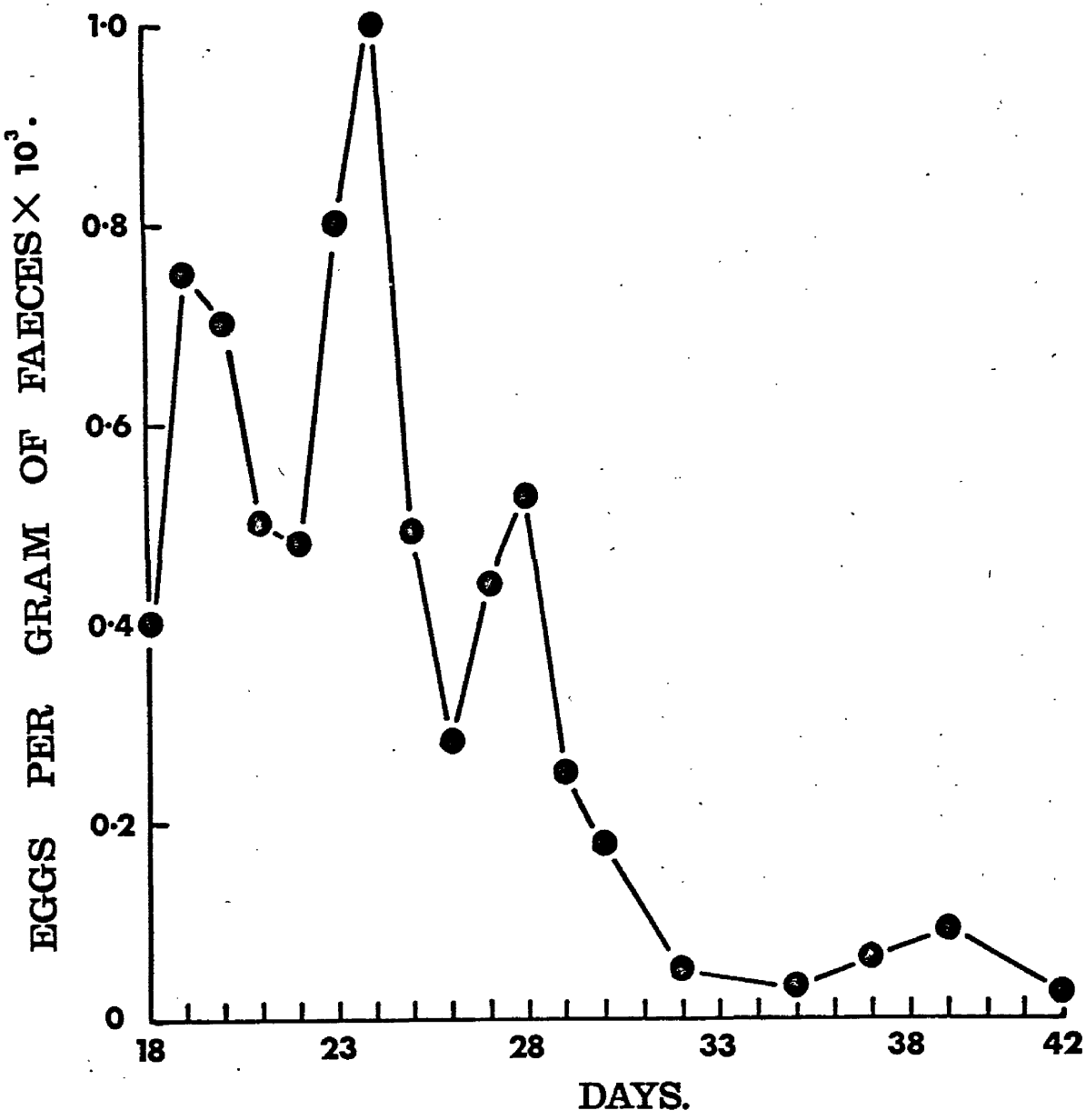


Fig. 17 Mean faecal egg count for group of five calves inoculated with 300,000 Ostertagia ostertagi on day 0.

Table 11

Mean pH of Abomasal Contents from Calves Autopsied during Three Different Periods of Infection Following Inoculation with 300,000 Ostertagia ostertagi

	Day of Autopsy		
	4 - 20	21 - 30	35 - 70
No. of calves	6	10	6
Mean pH	3.27	6.42	3.56
	± s.e. 0.39	± s.e. 0.32	± s.e. 0.21

s.e. = standard error

Table 12

Worm Counts at Autopsy of Calves Given a Single Dose of 300,000 Ostertagia ostertagi Larvae

Day Killed	No. of Adult Worms	No. of developing stages i.e. late 4th, 4th moult & 5th larval stages	No. of Early 4th Larval Stages	Percentage of Inoculum Established	Sex Ratio M:F
4	"	"	50,000	17	...
10	"	34,000	"	11	...
17	12,200	56,000	"	23	1:1
18	5,600	38,700	"	15	1:1
19	36,600	40,000	"	26	1:1
20	33,600	8,000	"	14	1:1
21	28,000	24,200	"	18	1:1
22	50,000	10,500	"	20	1:1
23	24,000	1,000	"	9	2:1
24	33,000	2,200	"	12	3:2
25	12,600	"	100	4	9:4
26	10,000	500	"	4	5:4
27	9,600	"	"	3	3:2
28	21,000	900	"	7	2:1
29	15,000	200	"	5	2:1
30	28,000	200	"	9	3:1
35	10,600	"	200	4	4:1
42	10,000	"	"	3	7:3
49	2,000	"	200	1	5:4
56	6,700	"	"	3	5:1
63	200	"	"	< 1	0:1*
70	600	"	"	< 1	1:7

*No Male Worms Found

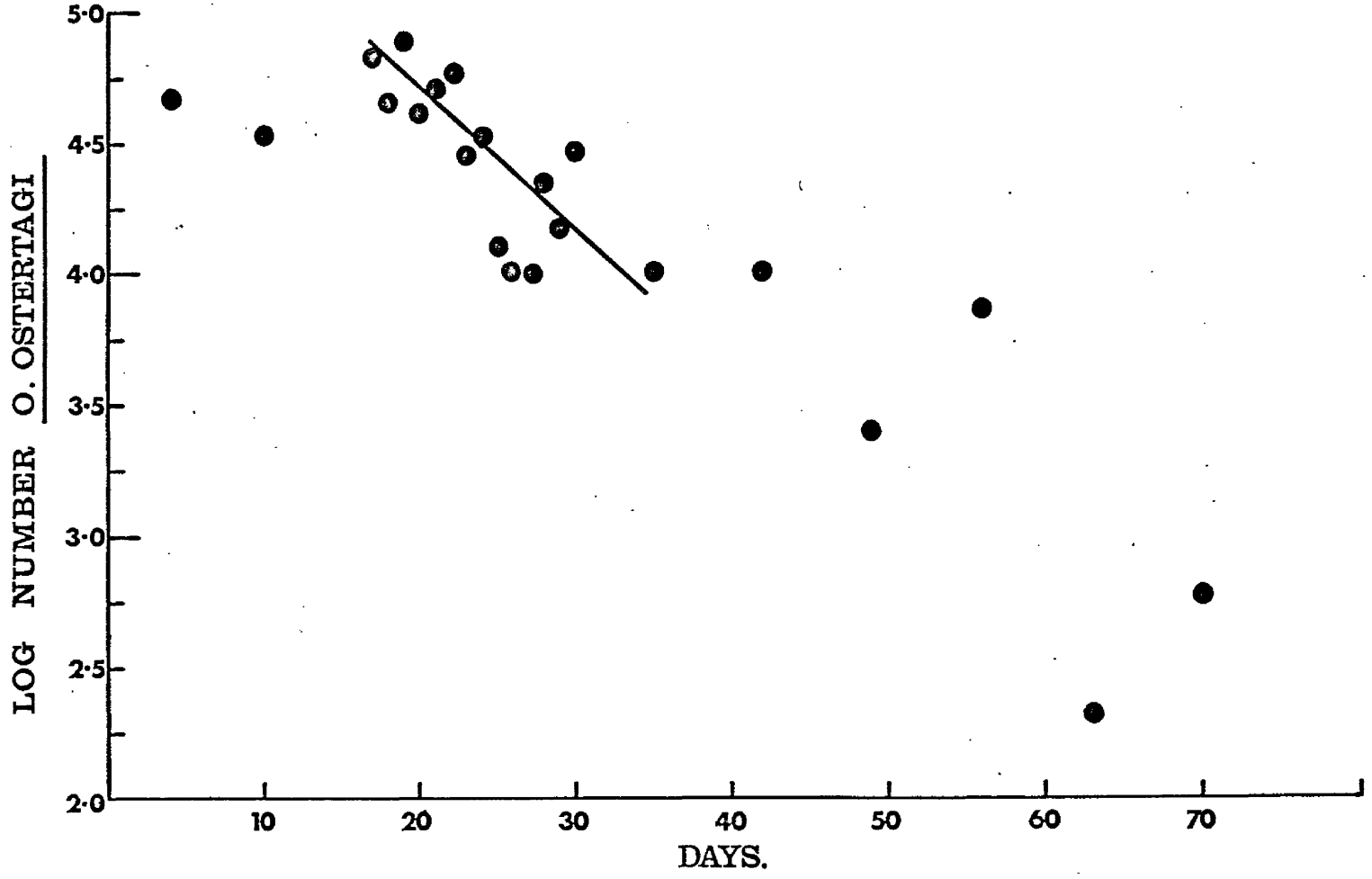


Fig. 18. Logarithm of worm numbers found at different times after infection.
The slope of the line between days 17 and 35 (b) is 1 in 20.
Each calf was inoculated on day 0 with 500,000 Ostertagia ostertagi larvae.

larvae had exsheathed, entered the gastric glands and moulted to become early fourth stage larvae; by day 10, the whole worm population was in the fourth moult stage; by day 17, the final moult was completed and the majority of the worm population was in the fifth larval stage though a proportion had matured and eggs were detectable in some female worms; by day 19, the whole worm population was adult and the females had many eggs in their uteri (this coincided with an increase in faecal egg counts); by day 21, the majority of the O. ostertagi adults had left the gastric glands to lie on the mucosal surface. The numbers of male and female worms were approximately equal until day 23; thereafter the number of male worms constantly exceeded the number of females, suggesting that female worms were being eliminated first when the loss of adult worms occurred from day 17 onwards.

Pathological Data .- The sequential development of the abomasal lesions following a single inoculation of 100,000 O. ostertagi was studied in this experiment. The lesions were severe and resembled those observed in severe field cases of Type I ostertagiasis as described by Anderson et al (1965) and in experimental cases described by Ritchie et al (1966).

The sequential changes may be summarised as follows:

The primary nodule .- Within four days of inoculation, the larva has penetrated a gland of the gastric mucosa and this becomes distended to form the primary nodule. The cells lining the gland are of the high mucus secreting type. The specific differential features of the cell types of the gland are lost. As the larva grows, the primary nodules enlarge until just prior to the emergence of the parasite from the gland (about days 17 to 20).

Figure 19 shows a primary nodule 16 days after infection.

The secondary nodule. - Figure 20 shows the O. ostertagi emerging from the gland (day 18) and the next change, the development of the secondary nodule, is becoming evident at this stage (Fig. 21). The worm can be seen in the gland, the latter being lined by high mucous cells; in the glands surrounding the primary nodule, the changes characteristic of the secondary nodule are starting. The nearest gland is stretched and has become lined by a low cuboidal epithelium. Other surrounding glands are slightly less stretched and it can be seen that some parietal cells are still present. Within two or three days this change becomes very marked. Figure 22 shows the fully developed hyperplastic lesion; here the lining cells are low cuboidal, they are multiplying rapidly and they have lost their morphological and functional differentiation (Fig. 22). It has been suggested by Jarrott (1966) that these rapidly dividing cells do not attach together and fail to form an effective seal against the inter-cellular passage of macromolecules, e.g. pepsinogen or albumin.

Post-emergent phase. - In heavy infections, a lesion which follows emergence of the worms from the gastric glands is death of the superficial cells and sloughing of the epithelium (Fig. 23). Here the worms lie on top of the hyperplastic mucosa embedded in an exudate of protein and cells. Figure 23 also shows the merging of the lamina propria with the exudate, the close approximation of the worms to the mucous membrane and the loss of superficial epithelium. There is also a marked increase in plasmablasts and plasmacytes suggesting a local production of antibody.

Ostertagia ostertagi Infections in the Bovine: Field and Experimental Studies

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Summary of a thesis submitted for the degree of Doctor of Philosophy of the University of Glasgow by James Armour M.R.C.V.S.

The work described in this thesis is concerned with studies on bovine parasitic gastritis with particular reference to the abomasal nematode, *Ostertagia ostertagi*. Two factors which influence the natural disease, namely, inhibited development of the parasitic fourth stage larvae and the immune response of the host were investigated in detail for the first time. Studies on the epidemiology, pathogenesis and control of the disease are also described.

The thesis is divided into five sections as follows:

Section I. Field Studies on Parasitic Gastritis in Young Dairy Cattle in South-West Scotland

1. In this section the outbreaks of parasitic gastritis known to occur in young cattle in summer (Type I) and in winter (Type II) are reviewed. Studies on the changes in pasture populations of *O. ostertagi* infective larvae which precipitate these outbreaks were made. The number of infective larvae available on the pasture reached a maximum in late summer and thereafter declined slightly though considerable numbers of larvae

successfully overwintered. O. ostertagi eggs also survived the winter and completed their development by the following summer.

Section II. Experimental Ostertagia ostertagi Infections in Calves

2. Inoculation of susceptible calves with 300,000 O. ostertagi larvae each resulted in the occurrence of weight loss and diarrhoea 19 days later; these symptoms persisted for seven days and then abated. The daily changes in abomasal biochemistry were followed with the aid of abomasal cannulae. During the clinical phase the pH of the abomasal contents was elevated and an increase in the population of viable bacteria occurred; plasma pepsinogen levels were also increased.

An exponential loss of worm population occurred between 17 and 35 days after inoculation.

Section III. Studies on Inhibited Larval Development of Ostertagia ostertagi

3. Seasonal analyses of the abomasal worm counts at autopsy of calves with ostertagiasis revealed that high proportions of inhibited fourth stage larvae were present only from late autumn until early spring. It was also found that when susceptible calves were grazed for short periods

(1 or 14 days) during different seasons that an increase in the proportion of inhibited fourth stage larvae occurred only in calves autopsied in late autumn. The proportion of inhibited larvae present in these calves was independent of the number of days grazed or the magnitude of the total worm burden. The administration of immunodepressant drugs (cortisone, methotrexate) did not result in the resumption of development of significant numbers of inhibited larvae.

4. From the results of experiments with susceptible grazing calves in late autumn it was found (a) that a high proportion of the natural strain of O. ostertagi became inhibited at the fourth larval stage following ingestion whereas (b) a low proportion of a laboratory strain developed at pasture became inhibited and (c) negligible numbers of larvae were inhibited following inoculation of laboratory cultured larvae. Furthermore, when laboratory cultured larvae were inoculated to calves harbouring inhibited larvae the challenge inoculum matured in 21 days and apparently 'leapfrogged' the inhibited larvae in the gastric glands.

It was therefore concluded that inhibition in ostertagiasis is dependent on two factors (1) an innate physiological susceptibility of a particular strain of larvae (2) the environmental circumstances of late autumn.

Section IV. Immunity to Ostertagiasis

5. Experiments were carried out to observe the effect that age and previous exposure to O. ostertagi infection had on immunity. It was found that in adult animals the numbers of worms established were fewer and the pre-patent period extended compared to young animals.

In calves previously exposed and challenged experimentally the challenge inoculum became established but in the subsequent loss of worm population the rate of loss was increased compared to previously uninfected controls.

6. Prior immunisation of calves with 2 x 100,000 O. ostertagi larvae irradiated with X-ray doses of 60 or 80 kiloroentgens did not result in a high degree of immunity to an experimental challenge with 300,000 larvae. Immunisation of calves with 2 x 100,000 larvae irradiated with 60 kiloroentgens resulted in the development of some resistance when subjected to natural field challenge.

Section V. The Use of Thiabendazole in Bovine Ostertagiasis

7. The anthelmintic efficiency of thiabendazole against larval and adult stages of O. ostertagi was studied. At a dosage level of 110 mg. per kg. bodyweight the drug was efficient against normally developing

fourth stage larvae but inefficient against inhibited larvae. Following treatment at 220 mg. per kg. bodyweight a complete cessation of clinical signs occurred in affected calves within 48 hours. The adult worm burdens of the treated calves were reduced by over 90 per cent compared to those of untreated calves.

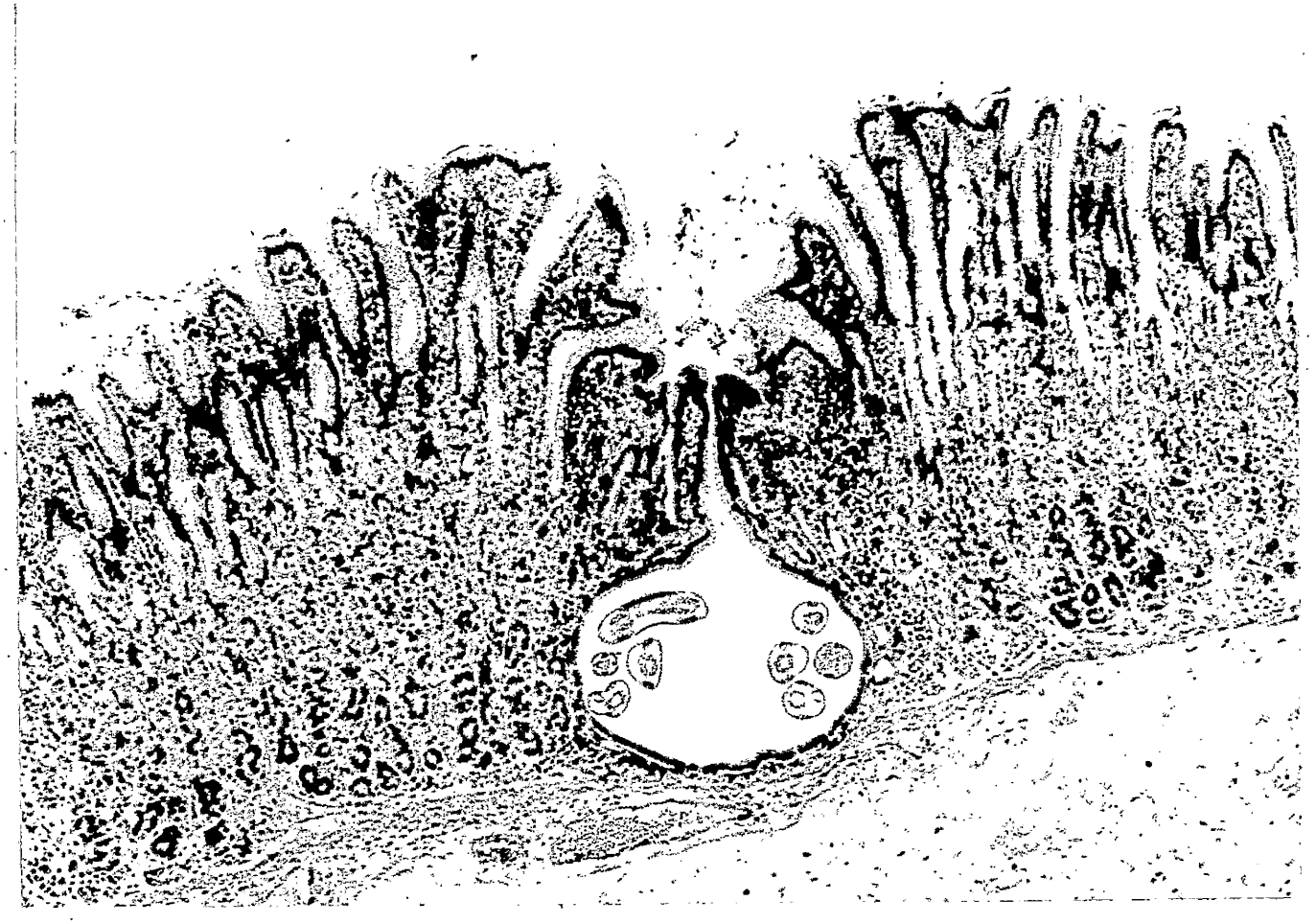


Fig. 19. Primary nodule: mucosa of calf abomasum 16 days after inoculation with 300,000 Ostertagia ostertagi larvae. The worm lies in a dilated gastric gland lined by columnar mucous secreting cells. The functional differentiated cells lining the surrounding gastric glands are normal (x 180).

Fig. 28. An adult Ostertagia ostertagi emerging from a gastric gland in calf abomasum (x 180).

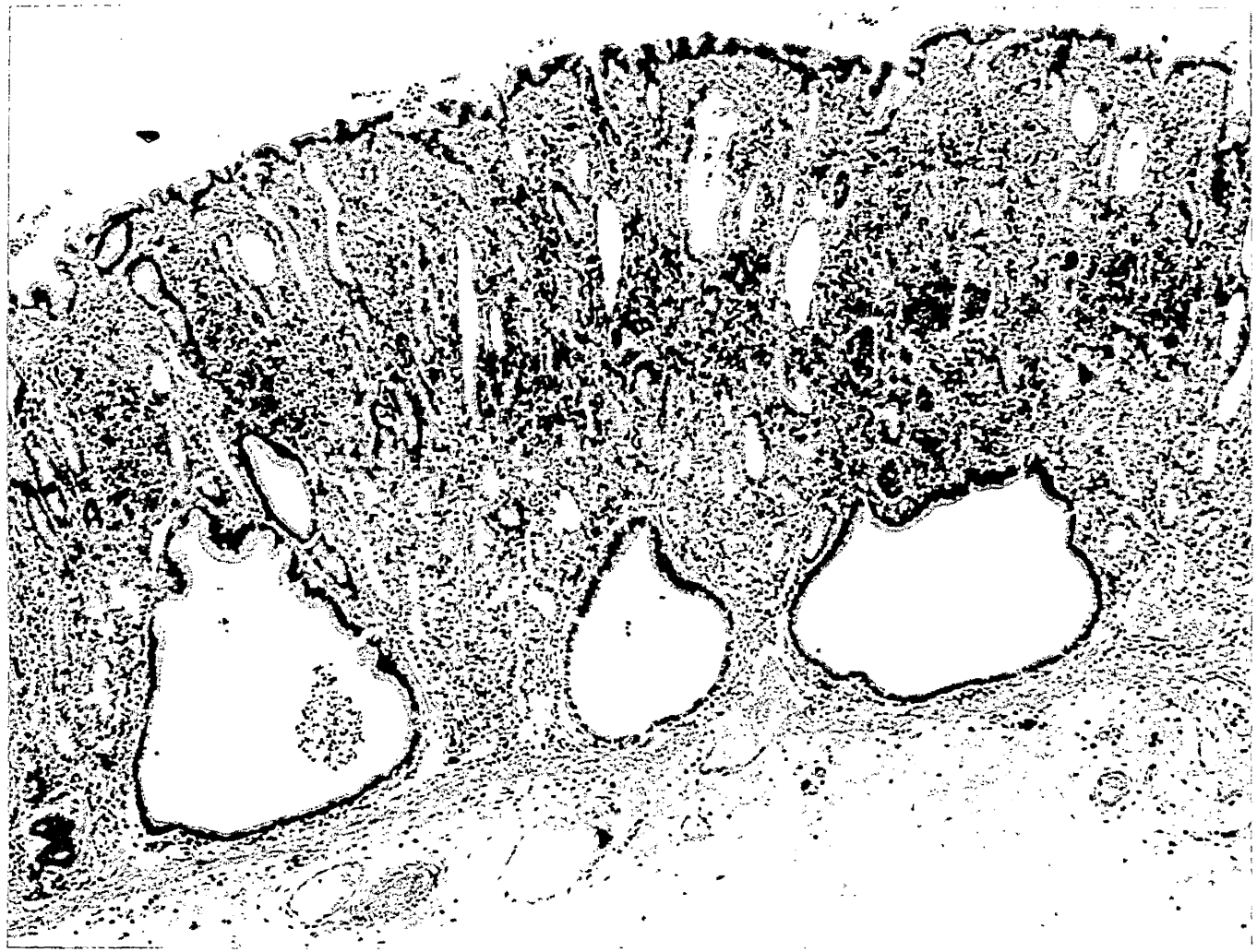


Fig. 2. Secondary nodule: mucosa of calf duodenum 18 days after inoculation with 200,000 Ostertagia ostertagi. The post-parasitical glands are lined by columnar mucus secreting cells while the surrounding glands are lined by non-functional cuboidal epithelium (x 160).

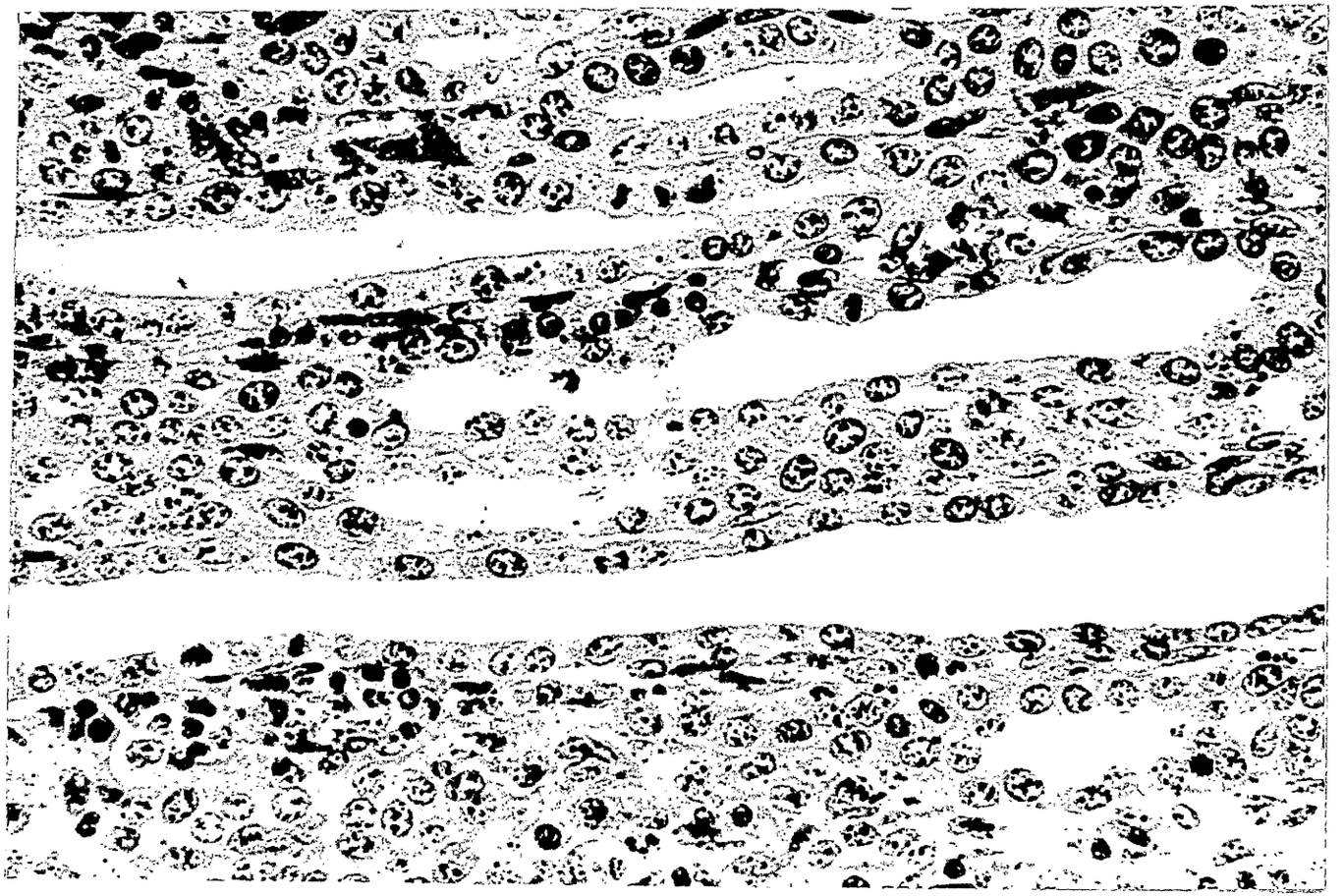


Fig. 22. Secondary nodule: high power of Fig.21 showing low cuboidal non-functional epithelium with a high mitotic index in glands surrounding post-parasitised gland (x 1250).

Fig. 23. Post-emergent phase: mucosa of calf abomasum 24 days after inoculation with 300,000 Ostertagia ostertagi. There is cytolysis of the superficial epithelium on which adult worms are closely applied. Plasmablasts and plasmacytes are present immediately below the epithelial cytolysis (x 160).

Recovery phase. - As the infection subsides and the worm population is gradually lost, replacement and differentiation of epitholial cells takes place. The mucosa then returns to its normally functionally differentiated state except for some of the glands which had contained larvae and which remain lined by tall mucus secreting epithelium.

To the naked eye the abomasal changes are quite dramatic. Individual discrete nodules (Fig. 24) develop in a moderate infection and show a visible central orifice surrounded by a raised area of hyperplastic cells. In a severe infection, these individual lesions coalesce to form an intensely hyperplastic mucosa with virtual complete loss of function; macroscopically this has a characteristic 'morocco leather' appearance (Fig. 25). Where cytolysis of the surface epithelium and upper glands occurs, small areas of superficial mucosal erosion are seen, i.e. the so-called 'thumbprint' lesion (Fig. 26).

Discussion

It is now known that bovine ostertagiasis in parts of the British Isles can occur naturally in three distinct forms (Anderson et al, 1965). In the first form (Type I), the worms develop to the adult stage in about 21 days; thus the clinical signs become apparent three to four weeks after ingestion of a large dose of larvae. The second form (pre-Type II) is characterised

Fig. 24. Macroscopic appearance of primary nodules.

Fig. 25. Hyperplastic mucous membrane showing morocco leather appearance.

Fig. 26. The "thumbprint lesion" caused by localised severe superficial erosion.

by massive inhibition of development at the early fourth larval stage; the subsequent development of these larvae up to six months later produces Type II disease. Since practically all the larvae in this experiment matured within 21 days (Table 12), the results can only be compared directly with Type I disease.

At the dose level used in this experiment, one calf had to be killed *in extremis* on day 28, and 16 of the 18 calves allowed to survive beyond day 19 had diarrhoea starting at the same time as faecal egg counts became positive. In the seven calves allowed to survive to day 30, the diarrhoea terminated within seven days of onset. A significant degree of weight loss was observed during the period of diarrhoea in the group of five calves weighed regularly and this was paralleled by a marked reduction in appetite for concentrates (Fig. 15).

The mean number of *O. ostertagi* recovered from the calves autopsied through days 4 to 20 was 53,000; from the calves autopsied through days 21 to 30, it was 26,000 and from those autopsied from day 35 onwards, it was 5,000 (see Table 12). This apparent loss in worm burden coincided with a decrease in faecal egg counts (see Fig. 17). Analysis of the semi-log plot shown in Figure 18 suggests that the functional relationship between log worm numbers and time through days 4 to 70 is not linear but approaches linearity through days 17 to 35 (*i.e.* after emergence of the worms from the glands). The probable pattern of worm expulsion is therefore that a plateau exists in mucosal worm burdens prior to day 17, followed by a period of exponential loss at a given rate ($\frac{1}{20}$) from day 17 to 35; the rate of expulsion (if present at all) then slows markedly. It is possible that the loss of adult worm population is associated with an immune reaction similar to the 'self-cure' observed following single inoculations of *Nippostrongylus brasiliensis* (Africa, 1931; Mulligan, Urquhart, Jennings and Neilson, 1965). Thus

the appearance (post-emergent phase) in the mucosa of cells associated with antibody production, namely plasmablasts and plasmacytes, heralded the onset of the expulsion of adult O. ostertagi. On the other hand, the altered environment of the abomasum (elevated pH) may be unsuitable for the survival of O. ostertagi and this may trigger off their expulsion.

Irrespective of the cause, it appears that a loss of adult worm population is a characteristic feature of experimental infection with O. ostertagi. Thus, Ross (1963) using single inoculations of 2,000 and 100,000 O. ostertagi larvae concluded that a loss of adult worms occurred between the fifth and eighth week of infection and that the loss was greater at the higher dose level. Ross and Dow (1965) later suggested that the loss takes place gradually after the end of the fourth week and Ritchie et al (1966) postulated it occurred between 16 to 20 days after inoculation. An exponential loss of both adult and larval stages of Ostertagia circumcincta has been reported by Armour, Jarrett and Jennings (1966) following experimental infections of sheep with this parasite.

In calves autopsied through days 21 to 30, the mean pH of the abomasal contents was increased to 6.42 ± s.e. 0.32, i.e. a level similar to that observed at autopsy of field cases of ostertagiasis (Ross, 1963; Anderson et al, 1965a; Ross and Todd, 1965). This increase coincided with the development of the secondary nodule lesion and therefore the loss of parietal cell function.

During experimental studies on the pathogenesis of O. circumcincta infection in sheep, Horak, Clark and Botha (1965) and Armour et al (1966) observed that the pH of the abomasal contents became markedly elevated (> 6.0) during the third week of infection. This loss of acid conditions in the abomasum persisted for up to two weeks during which time (Horak and Clark, 1964) the

sheep were found to be in negative nitrogen balance. Though no balanced metabolic studies were made on the calves in the present experiment, it seems likely that a similar situation existed.

The results of this experiment show an increase in plasma pepsinogen levels occurred from day 4 onwards. This increase was gradual until the development of the secondary nodule lesions began about day 17. Thereafter, plasma pepsinogen levels increased sharply, reaching a mean maximum of 7400 mU on day 23 (i.e. 12 times the pre-infection level). These high levels were maintained for approximately seven days and then gradually decreased to pre-infection levels in the calves allowed to survive until day 70.

Plasma pepsinogen is produced in the zymogen cells at the bottom of the gastric gland and travels up the gland lumen where it is normally activated by the hydrochloric acid secreted by the parietal cells and is discharged into the lumen as pepsin. It is normally found in very small amounts in the blood because it is secreted directly from the cells into the stomach. The increase of pepsinogen detected in the plasma between days 4 and 17 may be explained by a moderate amount of pepsinogen leaking through the rapidly dividing cells of the parasitised gland. The marked increase which occurred from day 17 can be explained by the non-activation of pepsinogen due to the absence of acid conditions (increased pH) and an increased leakage of pepsinogen between the rapidly dividing cells of the secondary nodule. The maximum plasma pepsinogen levels coincided with the period of severe clinical disease and abomasal dysfunction and confirmed the observation of Anderson (1967) that plasma pepsinogen levels are a good indicator of abomasal damage and of considerable promise as a diagnostic aid for ostertagiasis.

A significant degree of anaemia did not occur in any of the calves. Since the results of this experiment are only directly applicable to the Type I syndrome, the absence of an anaemia agrees with the findings of Anderson et al (1965a) in the field and the observations of Ross (1963), Mahrt et al (1964) and Ritchie et al (1966) following experimental inoculations of calves with O. ostertagi; they differ from those reported by Threlkeld and Johnson (1948) in which a drop in haemoglobin concentration occurred in calves with experimental infections of O. ostertagi.

There has often been speculation on the aetiology of the anaemia noticed in some outbreaks of ostertagiasis (Martin et al, 1957; Anderson et al, 1965a). Thus both Morgan and Hawkins (1953) and Lapage (1956) cite anaemia as being a feature of ostertagiasis in cattle and refer to the parasite as being a blood sucker. Ross and Todd (1965) state that anaemia is a common feature of ostertagiasis but suggest it is attributable to the poor nutritional level of the host and not to any blood sucking activity of the parasite. This would explain the presence of anaemia in pre-Type II and Type II ostertagiasis (Martin et al, 1957; Anderson et al, 1965a) which occur during winter and early spring when cattle are usually on a low plane of nutrition. On the other hand, O. ostertagi may not be a voracious blood sucker and the effect of any blood sucking activity may become apparent only after a prolonged period in the host, perhaps aggravated by a declining plane of nutrition; this hypothesis could also explain the occurrence of anaemia in pre-Type II and Type II ostertagiasis.

In the present experiment, no significant changes occurred in serum protein levels following the inoculation of 300,000 O. ostertagi larvae.

Ritchie et al (1966) using a dose of 100,000 larvae also failed to produce changes in serum protein levels following inoculation of calves aged eight to ten weeks. However, Mahrt et al (1964) observed significant decreases in serum albumin and significant increases in serum gamma globulin levels, three weeks after experimental inoculation of calves aged 14 weeks, with 300,000 O. ostertagi larvae. Ross (1963) and Ross and Todd (1965) also observed a fall in serum albumin and a rise in the serum gamma globulin concentration, at a non-specified time, after inoculation of calves eight weeks old with 100,000 O. ostertagi infective larvae. It is possible that the differences in these results may be ascribable to variations in the electrophoretic techniques employed in the serum protein fractionation; the calves in all of these experiments were of a similar age and were fed a balanced ration, therefore neither age nor nutrition are likely to have influenced the serum protein levels.

In the field, Martin et al (1957), Mulligan et al (1963) and Anderson et al (1965a) found hypoalbuminaemia to be a constant feature of Type II ostertagiasis. Ross and Todd (1965) also found a significant hypoalbuminaemia in summer outbreaks of ostertagiasis (i.e. Type I) but Anderson et al (1965a) state that hypoalbuminaemia is not a feature of Type I ostertagiasis. Mulligan et al (1963) have suggested that the hypoalbuminaemia of Type II ostertagiasis is associated with an increased loss of albumin into the gastro-intestinal tract. Halliday, Dalton and Mulligan (1967) specified this loss as a leakage from the hyperplastic abomasal mucosa associated with infections of O. ostertagi. Ross and Todd (1965) disagreed with this hypothesis and could find no indication

in their experiments that albumin was lost preferentially through the abomasum. Nielson (1966) postulated that although an increased loss of albumin does occur in the gastro-intestinal tract in ostertagiasis, it is the accompanying diarrhoea which is primarily responsible for this and not the lesions caused by the parasite. Although further research is required to clarify these points, it is clear that hypoalbuminaemia, though not a constant feature of Type I ostertagiasis, is important in the pathogenesis of Type II ostertagiasis.

All of the Type I ostertagiasis lesions seen by Anderson et al (1965) were reproduced and the sequential development of these lesions may be divided into four stages:-

(1) The primary nodule, where the larva enters and grows in the gastric gland. This gland becomes stretched and protuberant and the specialised cells lining the gland lose their differentiation. This stage covers the period up to approximately 17 to 18 days post-inoculation.

(2) The secondary nodule, where the specialised secretory cells in the glands surrounding the parasitised gland become hyperplastic and lose their functional differentiation. This first occurs at the period where the worms seem to be emerging from the gastric glands, i.e. 18 to 23 days after inoculation.

(3) The post-emergent phase, where sloughing of the abomasal mucosa occurs and oedema becomes marked, i.e. 23 to 30 days post-inoculation.

(4) The recovery phase in which the mucosa gradually returns to its normally functionally differentiated state. This occurs after day 30.

It appears that a single inoculum of 300,000 O. ostertagi larvae is a suitable dose for studying the pathogenesis of Type I ostertagiasis in Ayrshire calves ten to twelve weeks old. Thus, in the present experiment, the chief clinical signs of ostertagiasis, i.e. diarrhoea, weight loss and anorexia, and severe abomasal lesions and abomasal biochemical changes were produced in the majority of calves without severe mortality occurring.

B. Studies Using Abomasal Cannulae

Experimental Design

Two male Ayrshire calves, designated A and B, were reared parasite free from birth, and were each inoculated orally with 300,000 third stage O. ostertagi. At the time of inoculation, the calves were twelve weeks old and weighed 150 and 159 lbs. respectively. Abomasal cannulae were inserted by surgical technique, four days prior to inoculation of calf A, and 12 days prior to inoculation of calf B. It was intended to autopsy both calves after the biochemical values in the abomasal fluid had returned to pre-infection levels but, due to the occlusion of the cannula, calf A had to be autopsied at day 32. Calf B was autopsied on day 62.

To study the possible effect of cannulation, abomasal cannulae were inserted into two parasite free calves, designated C and D, and the biochemical values in the abomasal fluid were studied for the next 30 days; these calves were of comparable age and weight to calves A and B.

Observations

Clinical examination of the calves was made daily and they were weighed twice weekly. Samples of blood were collected daily from the jugular vein into bottles containing heparin. Plasma was analysed for pepsinogen, sodium, potassium and chloride. Plasma bicarbonate was estimated during the period of clinical ostertagiasis in calves A and B. Samples of abomasal fluid were taken twice daily for pH determination and following centrifugation, the supernatant was analysed for sodium, potassium, chloride ions and for peptic

activity. Abomasal fluid samples were also collected daily (in sterilised bottles) on days 18 through 25 for bacteriological examination.

Faecal samples were obtained daily for egg count examination. After day 18 a faecal collecting bag was kept on each infected calf and the faeces collected over each 24 hour period and samples examined microscopically for the presence of O. ostertagi. At autopsy a parasitological examination of the abomasal contents and mucosa was performed as previously described.

Results

Clinical Signs. - Inappetence occurred in all four calves after the cannulae were inserted but, at the time of inoculation with O. ostertagi larvae in calves A and B (day 0), appetite had been regained.

Further inappetence was noticed in the two infected calves on day 18 when some hay, but no concentrates, was eaten. On day 22, the two infected calves had softening of the faeces and, three to four days later, there was a profuse watery diarrhoea. During the period of severe diarrhoea, the two infected calves refused to take concentrates but continued to eat a little hay. At the end of the severe diarrhoea, the calves gradually regained normal appetite. The diarrhoea was classified in histogram form (Fig. 28A, B; Fig. 30A, B). Diarrhoea did not occur in the two uninfected calves (C and D).

A temporary stoppage of body weight gain occurred in the four calves after the cannulae were inserted but, by the day of inoculation of calves A and B (day 0) all calves had resumed their previous rates of weight gain.

A further stoppage in weight gain did not occur in calves C and D, but calves A and B lost 6 and 5 lbs. respectively during the period of diarrhoea.

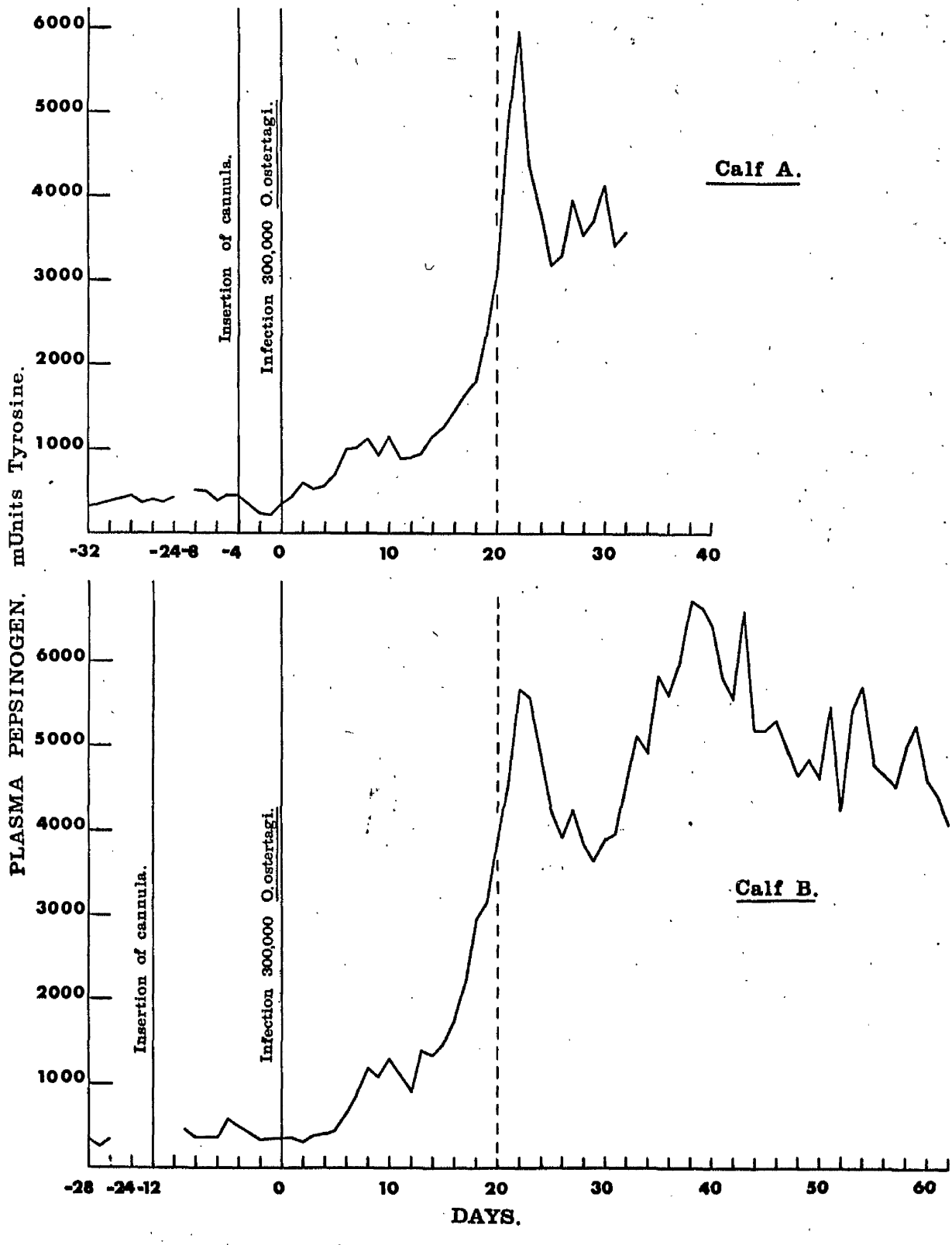
Biochemical Results" - Plasma pepsinogens" - Plasma pepsinogens were determined before cannulae were inserted and before calves A and B were inoculated with 300,000 O. ostertagi larvae. During this period, there was little fluctuation in plasma pepsinogen levels, and the mean values expressed as tyrosine up to day 0, i.e. day of infection, were 400 ± s.e. 18 and 400 ± s.e. 18 mU for calves A and B respectively.

After the inoculations on day 0 were made, little change occurred in calves A and B until day 5 when plasma pepsinogen values began to rise to approximately 1,000 mU; the pepsinogen values stayed at this level with minor fluctuations between days 5 and 15 (Fig. 27). Plasma pepsinogen values increased steadily and rapidly after day 15, reaching a maximum in the region of 6,000 mU at day 22. Values then decreased to approximately 3,500 mU at days 25 to 29. In calf B, where the cannula remained functional for the longer period, plasma pepsinogen values again increased after day 29, reached a maximum of 6,700 mU at day 38, and then began to decrease with variations until the end of the experiment (day 62).

Plasma pepsinogen levels in calves C and D during the 30-day period after insertion of the abomasal cannulae were 300 ± s.e. 62 and 400 ± s.e. 62 mU respectively.

Plasma Electrolytes" - There were no significant alterations of plasma sodium, potassium and chloride concentrations during the experiment

Fig. 27 Pepsinogen levels in the plasma of 2 calves (A and B) inoculated with 300,000 Ostertagia ostertagi larvae.



despite the presence of profuse, watery diarrhoea in calf A for four days and in calf B for three days.

Plasma pH and bicarbonate values were determined during the period of diarrhoea and significant variations from the normal did not occur.

Abomasal pH and Electrolytes. - In calf A, the pH of the abomasal fluid remained unchanged after the cannula was inserted until day 20 of the infection, the mean being 2.67 (s.e. 0.06) as is shown in Figure 28A. In calf B, a slight increase of pH occurred during the 5 days after the cannula was inserted, but the overall mean pH of the abomasal fluid up to day 20 of the infection was 2.64 (s.e. 0.05) as is shown in Figure 28B.

Between days 20 and 22 in both calves, there was a rapid rise in the pH of the abomasal fluid to more than 7.0 where it remained for three days in calf A and four days in calf B. In calf A, after day 25, the pH of the abomasal fluid rapidly decreased and was 4.8 at autopsy on day 32. In calf B, the return was slower, and the pH remained between 6.0 and 7.0 from days 26 to 45 and between 5.0 and 6.0 from days 45 to 55. Thereafter, the rate of return was accelerated and on day 61 (just prior to autopsy), the pH was nearly normal (pH 3.0).

In both inoculated calves, there was significant, positive correlation (r) between the pH of the abomasal fluid and plasma pepsinogen throughout the experiment (calf A, $r = +0.7779$; calf B, $r = +0.8002$). ($P < 0.001$ for both calves.)

There was an increase in the concentration of sodium ions in the abomasal fluid at days 20 to 23 in both calves, paralleling the increase in the pH of

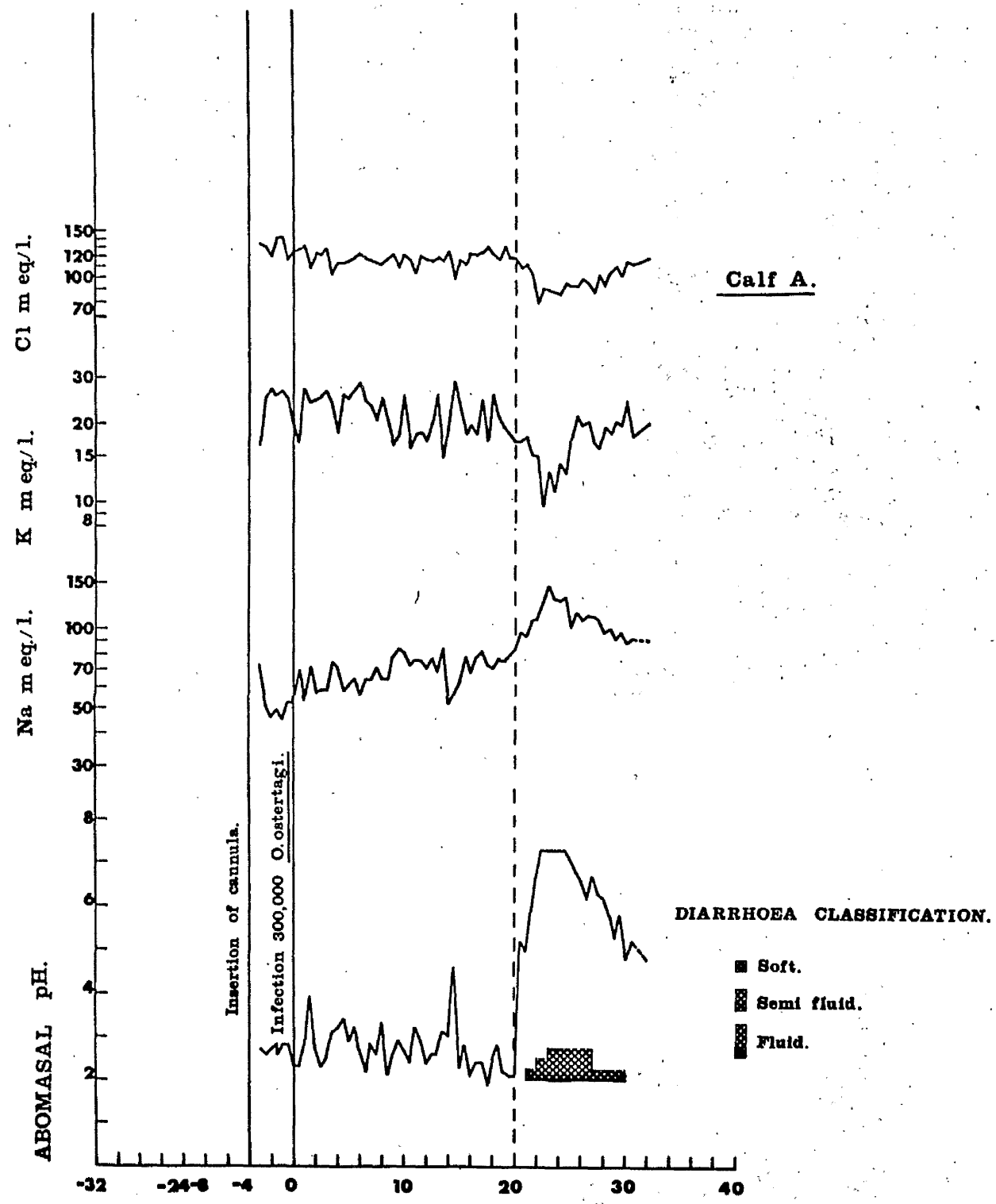


Fig. 28A The pH and electrolyte values of the abomasal fluid and diarrhoea in Calf A following inoculation with 300,000 Ostertagia ostertagi larvae.

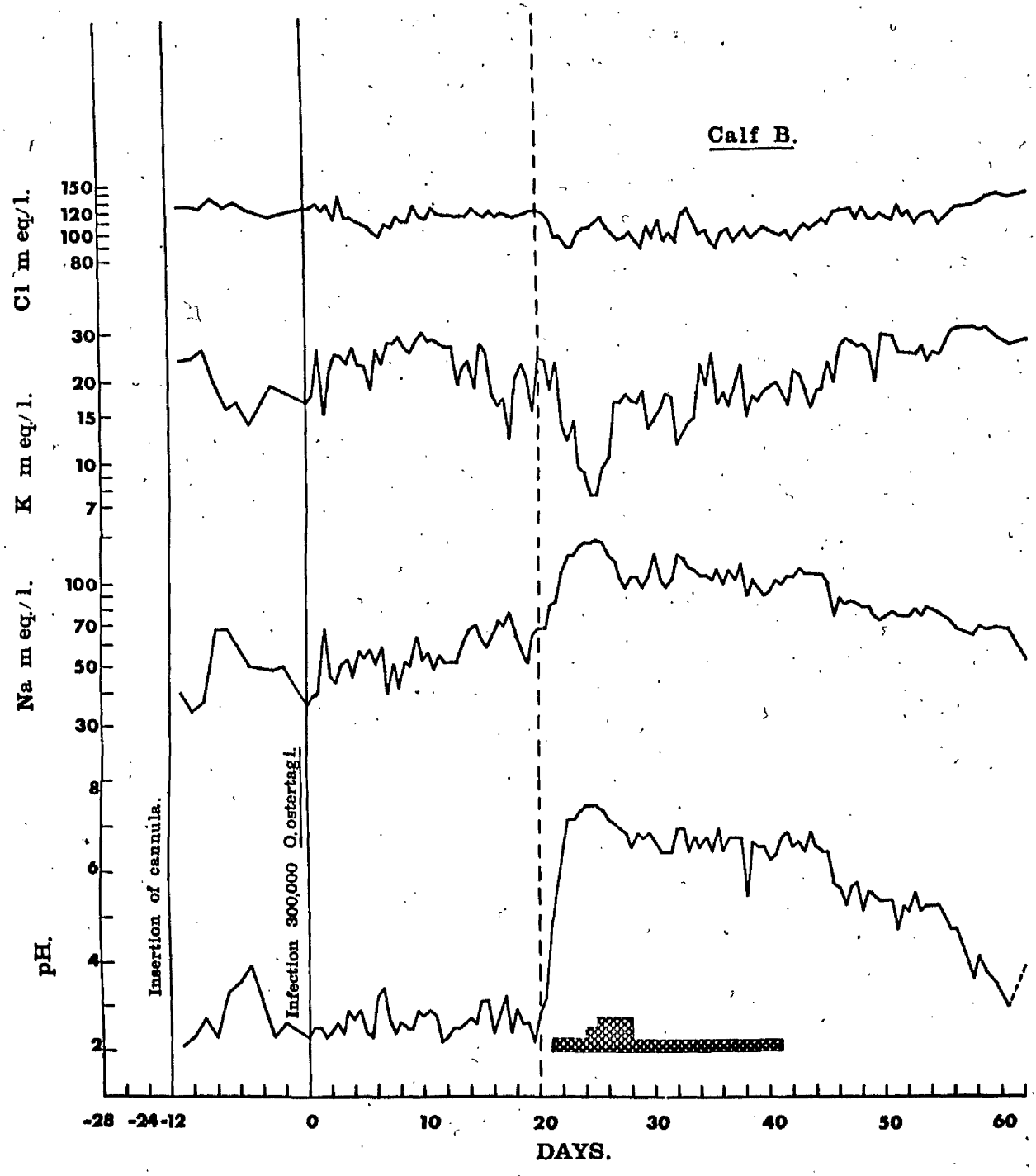


Fig. 28B The pH and electrolyte values of the abomasal fluid and diarrhoea in Calf B following inoculation with 300,000 Ostertagia ostertagi larvae. (Classification of diarrhoea as in Fig. 28A.)

the abomasal fluid and, at the same time, there was a decrease in potassium and chloride concentrations (Fig. 28B). The correlations between these values are tabulated (Table 13).

The bicarbonate concentrations in the abomasal fluid were estimated periodically throughout the experiment, and, although there was no bicarbonate value while the pH of the abomasal fluid remained below 6.0, it increased proportionally as the pH rose above this value, and a maximum of 46.3 μM/L. was recorded when the pH was 7.4.

The mean values for pH and electrolytes of abomasal fluid of the two uninfected calves (C and D) are also tabulated (Table 14).

Abomasal Pepsins. - In calf A, peptic activity of the abomasal fluid was unchanged until the pH changed on day 21. Values varied between days 22 and 28 and, during this period, the mean was much lower than between days 0 and 21. After day 28, when the pH of the abomasal fluid returned to less than 6.0, the peptic activity rose markedly to greater than the preinfection level.

In calf B, the values for peptic activity in the abomasal fluid remained stable for 5 days prior to infection until the pH changed at day 21. Between days 21 and 45, when the pH was greater than 6.0, the variations became marked, the peptic activity nearly disappearing in some samples and recovering to preinfection levels in others. After day 40, when the pH decreased to less than 6.0, the variations between samples decreased and the general level of peptic activity was greater than the preinfection values (Fig. 29; Table 15).

Table 13

Coefficients of Correlation (r) of pH and Electrolyte Values of the Abomasal Fluid from 2 Calves (A and B) Inoculated with 500,000 *Ostertagia ostertagi* Larvae

Abomasal fluid pH and electrolytes	Calf	Na	K	Cl
pH	A	+0.8677	-0.5173	-0.8665
	B	+0.9225	-0.5190	-0.7790
Na	A	***	-0.7521	-0.8215
	B	***	-0.6660	-0.6935
K	A	***	***	+0.5212
	B	***	***	+0.3857

P values were <0.001 in each instance

Table 14

Mean pH and Electrolytes, and Pepsin Values of Abomasal Fluid from 2 Calves (C and D) for 30 days after Abomasal Cannulae were Inserted*

Calf	pH	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	Pepsin (units of tyrosine)
C	2.5 ± 0.10	55 ± 2	25.4 ± 0.9	124 ± 0.0	81 ± 12
D	2.4 ± 0.07	57 ± 2	22.9 ± 1.2	128 ± 1.7	109 ± 10

± = Standard error of means

*These calves were not inoculated with Ostertagia ostertagi larvae

Table 15

Mean Pepsin Values (Units of Tyrosine) in Abomasal Fluid from 2 Calves (A and B) Inoculated with 300,000 Ostertagia ostertagi Larvae, During 3 Different Periods of Infection as Indicated by the pH of the Abomasal Fluid

Calf	pH of abomasal fluid		
	2.0 to 3.0	> 6.0	< 6.0
A	75 ± 2	35 ± 7	113 ± 11
B	74 ± 5	61 ± 4	137 ± 2

± = Standard error of means

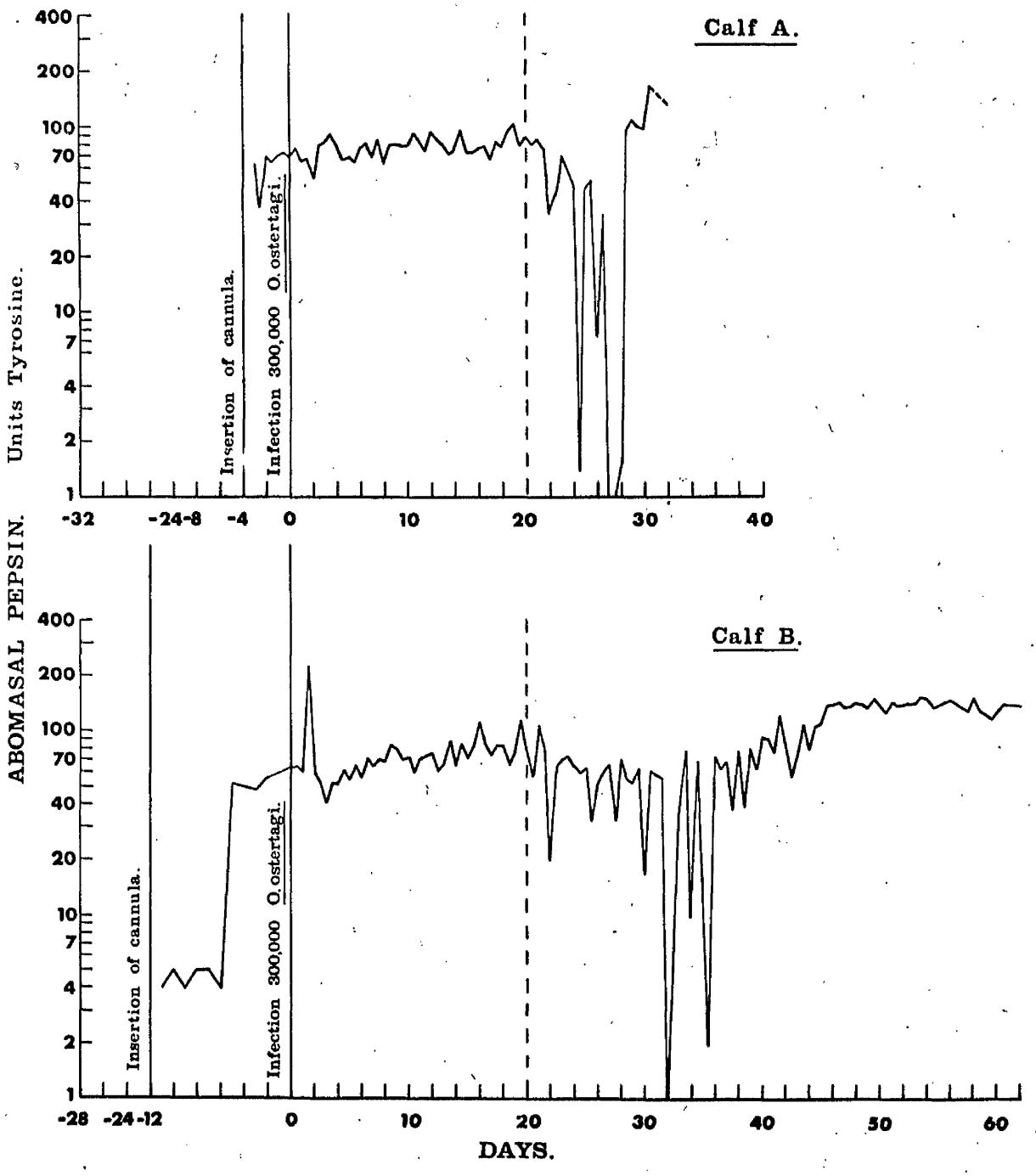


Fig. 29 Peptic activity of the abomasal fluid from 2 calves (A and B) inoculated with 300,000 Ostertagia ostertagi larvae.

The mean values of pepsin in the abomasal fluid of the two uninfected calves (C and D) are tabulated (Table 14).

Bacteriological Results. - Samples of abomasal fluid examined aerobically for viable bacteria with nutrient and MacConkey agar showed a marked increase in bacterial counts just before the calves had profuse watery diarrhoea. With these mediums there was an increase from 7.3×10^4 to 12.7×10^5 (calf A) and 7.5×10^4 to 39.0×10^5 (calf B) viable bacteria per millilitre of abomasal fluid during the period the calves had diarrhoea; both gram-positive and gram-negative organisms were present.

Parasitological Results. - Eggs in Faeces. - Ostertagia ostertagi eggs first appeared in the faeces of the infected calves (A and B) on day 19, and the eggs per gram (e.p.g.) were greatest on days 23 to 27, i.e. approximately the time of severe diarrhoea; thereafter, the e.p.g. decreased. The faecal egg counts are presented graphically (Fig. 30A, B). Faecal egg counts for calves C and D were negative throughout the experiment.

Recovery of Worms from Faeces. - Adult O. ostertagi were recovered from the faeces of calves A and B. In calf A, O. ostertagi were first recovered on day 19, the number increasing to a maximum on day 26 and then decreasing to 0 on day 29. During this period 21,200 worms were recovered.

In calf B, a small number of adult O. ostertagi was first recovered on day 20; the number increased to a maximum on day 28 and then declined to 0 on day 36. During this period a total of 18,700 worms was recovered.

The numbers of worms recovered from the faeces are presented graphically (Fig. 30A, B).

Fig. 30A Eggs per gram of faeces, number of adult worms recovered from the faeces, and consistency of faeces of Calf A following inoculation with 300,000 Ostertagia ostertagi larvae. (Classification of diarrhoea as in Fig. 28A.)

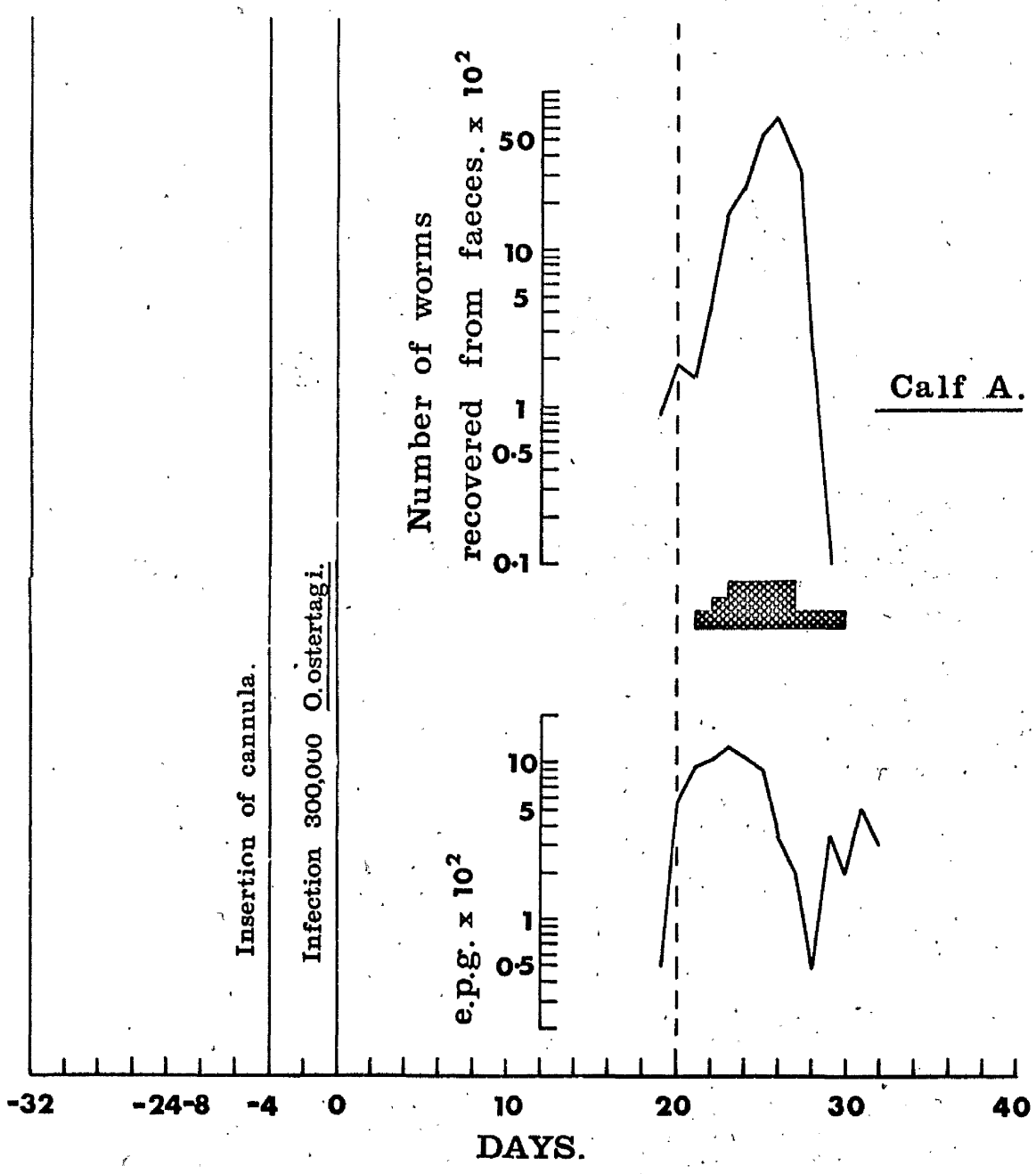
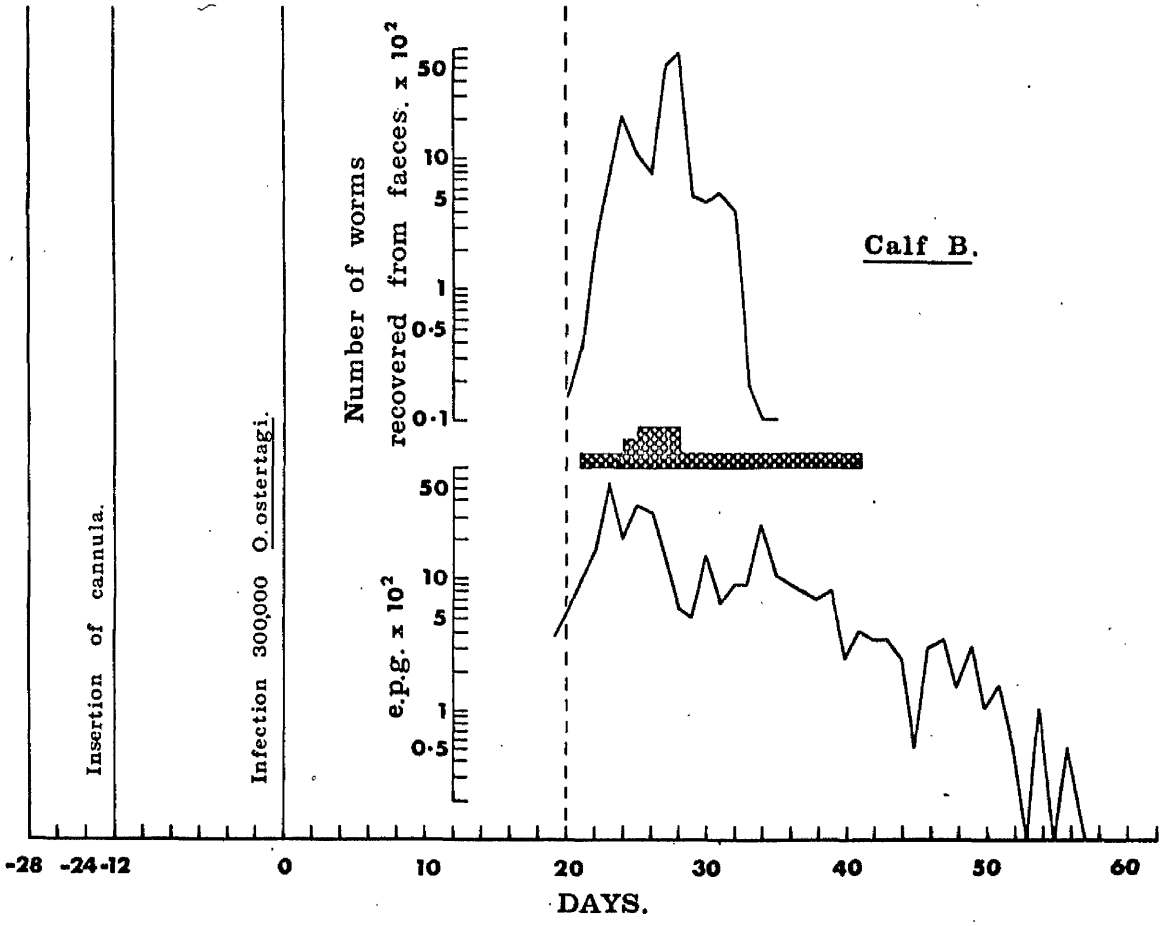


Fig. 30B Eggs per gram of faeces, number of adult worms recovered from the faeces and consistency of faeces of Calf B following inoculation with 300,000 Ostertagia ostertagi larvae. (Classification of diarrhoea as in Fig. 28A.)



Worms at Autopsy of Infected Calves - At autopsy of calf A (day 32), there were 9,000 adult O. ostertagi in the abomasum, whereas calf B, autopsied on day 62, had 1,200 adult O. ostertagi.

Discussion

From the previous experiment, it is clear that a single inoculation of 300,000 O. ostertagi larvae produces clinical disease identical to that of Type I ostertagiasis in the field. It is therefore reasonable to assume that the biochemical changes in the abomasal fluid described in the present experiment are similar to those occurring in the field cases of Type I ostertagiasis.

Although the period between the insertion of the cannula and inoculation was four days in calf A and 12 days in calf B, the sequential development of biochemical changes was almost identical. These changes could not be attributed to the effects of the cannulae, since the two uninfected calves (C and D) did not have significant biochemical changes in either blood or abomasal fluid for as long as 30 days after cannulation.

The principal changes in the calves after inoculation of O. ostertagi larvae, occurred first between days 15 and 22. Thus, the plasma pepsinogen levels increased markedly after day 15, and the abomasal fluid pH rose sharply on day 20. Results of the previous experiment had indicated that following a single inoculation of calves with 300,000 O. ostertagi, larvae remained in the gastric glands until about day 16, when the young adults emerged. This phase of emergence is associated with an apparent replacement of the differentiated cells in glands immediately adjacent to parasitised glands by hyperplastic,

undifferentiated cells. It is thought (Jarrett, 1966) that these rapidly dividing cells do not appear to attach together to form an effective seal against the intercellular passage of protein and this probably produces an area of increased permeability. This would allow an increased backflow of pepsinogen into the blood and account for the elevated levels obtained between days 15 and 22 in the present study. The rapid replacement of parietal cells by undifferentiated cells results in a cessation of hydrogen ion production and the consequent sharp increase in the pH of abomasal fluid noticed on day 20.

There was an inverse correlation between the concentrations of hydrogen ions and sodium ions and a direct correlation between the concentrations of hydrogen ions and potassium and chloride ions. Although the latter correlations were not as marked as the former, they were highly significant.

The period of emergence of the worms from the gastric glands was also associated with the first clinical abnormality (i.e. anorexia) and with the appearance of O. ostertagi eggs in the faeces on day 19. Profuse, watery diarrhoea occurred approximately 24 hours after the pH of the abomasal fluid increased to more than 7.0 in both calves and ceased 40 hours after the pH had returned to less than 7.0.

Results of the bacteriological examination of the abomasal fluid performed at this period indicated that the profuse, watery diarrhoea was accompanied by, or was slightly preceded by, a large increase in viable bacteria, as indicated by aerobic growth on nutrient agar or MacConkey agar; this increase is possibly a consequence of the increased pH of 7.0 to 7.4 which may be more suitable than the normal pH of 2.0 to 3.0 for the maintenance

and multiplication of ruminal bacteria.

After day 22, the two calves differed. Calf A had a moderately rapid decrease in pH of abomasal fluid, indicating a recovery of parietal cell function. At day 34, the pH had returned to 4.8, whereas in calf B, at the same period after inoculation, the pH was 6.5 and did not return to 4.8 until day 55 of the infection. Pepsinogen is not activated above pH 6.0 and peptic activity is negligible above pH 4.5 so that, in calf A, peptic digestion had not taken place for 13 days, whereas in calf B there was no peptic digestion for 35 days.

Results of pepsin determinations also indicated that, although marked daily variations occurred over the whole period of the experiment, maximal variations of pepsin occurred during the period following worm emergence, when the pH of the abomasal fluid was higher than 6.0; thus pepsin could be absent in one sample and equal to preinfection levels in the next. When the pH returned to less than 6.0, the mean estimate of pepsin in the abomasal fluid was higher than the preinfection levels; this might indicate that differentiation of the hyperplastic mucosa was gradually progressing and producing an overall increase in the zymogen cell mass.

The recovery of adult worms in the faeces confirmed the results of the previous experiment that a loss of worms took place between days 17 and 35. This loss in calf A began on day 19 (i.e. as soon as the calf was passing eggs in the faeces) and continued for 12 days; maximal worm loss occurred one day preceding and during the period of profuse, watery diarrhoea. In calf B, worm loss continued for 15 days, and the maximal worm loss was one

day preceding and during the period of profuse, watery diarrhoea. The data for worm recoveries from both calves are probably low, since it was impossible (due to the severe diarrhoea) to collect all the faeces during the period of maximal worm loss.

In the previous experiment, calves of a similar age inoculated with 300,000 O. ostertagi larvae and autopsied up to day 21 had a mean worm burden of 53,000; recovery of 20,000 worms from the faeces of both calves in the present experiment would represent a loss of 40% of the established infection between days 19 and 36 when compared to the previous experiment.

General Discussion

From the results of these two experiments, the pathogenesis of Type I bovine ostertagiasis can be postulated. The period immediately after infection, when the larvae are developing to the adult stage in the gastric glands, is associated with cellular changes which are confined to the parasitised glands; during this period, significant changes do not occur in the biochemical values of either the abomasal fluid or the blood, and clinical signs are not detectable. By day 16 after infection, adult O. ostertagi begin to emerge from the gastric glands and marked cellular changes, i.e. an apparent loss of differentiation and hyperplasia, occur in the glands surrounding the parasitised glands, and this leads to a loss of parietal cell function. When the areas affected become confluent, giving the 'morocco leather' appearance to the mucosa, loss of parietal cell function is complete, and the pH of the abomasal fluid rises markedly. This undifferentiated and hyperplastic mucosa is abnormally permeable to macromolecules, which is reflected in the marked increase in plasma pepsinogen. The loss of parietal cell function and the increased permeability of macromolecules develop concurrently, as indicated by the significant positive correlation between pH of abomasal fluid and plasma pepsinogen values. The principal clinical signs, i.e. severe diarrhoea, anorexia and weight loss, also occur after emergence of the adult O. ostertagi from the glands and coincide with the increase in the pH of the abomasal fluid to more than 7.0.

Until recently it was unknown why the parietal cells of the glands

ringing the parasitised gland should be largely replaced by undifferentiated and hyperplastic cells. However, as a result of current studies on the electron microscope by Murray and Jarrett (1967), the following explanation is now available. The distended parasitised gland stretches the surrounding glands and this stimulates the multiplication of new cells in order to maintain the continuity of the epithelium. At the same time, the parietal cells become incorporated in this stretched epithelium. In this circumstance they cannot be distinguished by light microscopy although electron microscopy shows them still to be present but to have lost their microvillary structure, which is necessary for the ability to secrete hydrochloric acid. This situation is different from that in the parasitised gland where the parietal cells are permanently lost, the gland being lined by mucous neck cells.

On emergence of the parasite from the glands, the changes are intensified due to the loss of the superficial epithelial cells which further stimulates cell division for replacement purposes.

Consequently on removal of the worms, the glands which contained the larvae do not readily resume normal fundic glandular function (since they no longer contain parietal cells); the surrounding glands of the secondary nodule are, however, able to resume normal function on the re-establishment of their original ultrastructural characteristics.

There has often been speculation on the direct cause of diarrhoea in bovine ostertagiasis. It appears that two factors may be of importance (a) the direct effect of the emergent parasite per se or through its cytolytic action on the gastric epithelium and (b) the indirect effect, i.e. the hyperplasia and loss of differentiation of the gastric gland epithelium producing

loss of parietal cells, a subsequent rise in abomasal pH and an increase in numbers of viable bacteria. A third factor suggested by Ross and Todd (1965), namely congestion in the intestine due to the passage of partially digested material into the duodenum, was not noticed in these experiments.

Although the results of these experiments are only directly referable to Type I ostertagiasis, it seems likely that the pathogenesis of Type II is essentially similar. Thus, Anderson et al (1965) have described how the abomasal lesions of Type II, though more severe, are similar to those of Type I. The increased loss of plasma albumin into the abomasum demonstrated by Mulligan et al (1963) and the anaemia, albeit of unknown origin, are further factors which contribute to the pathogenesis of Type II disease. The poorer prognosis which is associated with Type II ostertagiasis is probably related to the repeated assaults on the abomasal mucosa caused by the maturation of successive waves of inhibited larvae, which are insusceptible to anthelmintics.

Summary

1. Twenty two ten-week-old parasite free calves were given a single dose of 300,000 O. ostertagi larvae. The calves were autopsied singly on days 4, 10, 17 and then daily until day 30; from day 35 to day 70 the remaining calves were autopsied at weekly intervals.

The principal clinical signs of ostertagiasis, i.e. diarrhoea, loss of weight and anorexia, first occurred on day 19 and coincided with a marked rise in plasma pepsinogen and the appearance of eggs in the faeces. In nine out of the ten calves autopsied between days 21 and 30 there was a marked increase in the pH of abomasal contents.

Since clinical signs were observed in 16 out of the 18 calves allowed to survive until day 19 and only one calf died, it appears that 300,000 O. ostertagi is a suitable dose for the study of the pathogenesis of ostertagiasis in Ayrshire calves.

The parasitological data showed that the fourth stage larvae in the gastric glands had developed through the fourth moult stage and the fifth larval stage to become adults by day 17; the adults then emerged to be on the mucosal surface. These adult worms were lost exponentially from about day 17 onwards. Since the vast majority of the larvae developed to maturity within 21 days, the disease produced was Type I ostertagiasis.

All of the lesions seen at autopsy of typical field outbreaks were produced. The sequential development of the lesions is: (1) the primary nodule in which the larva grows within a gland, (2) the secondary nodule in which the neighbouring glands to the parasitised gland become hyperplastic and the secretory epithelial cells become non-functional, (3) the post-emergent phase where epithelial

cytolysis of the surface mucosa may occur, (4) the recovery phase in which a functional gastric mucosa is gradually restored.

2. An experiment with abomasal cannulae was designed to study the pathogenesis of O. ostertagi infections in calves. Samples of abomasal fluid were removed daily from two twelve-week-old calves inoculated orally with a single dose of 300,000 O. ostertagi larvae and from two uninoculated calves.

Biochemical changes in the blood and abomasal fluid occurred only in the infected calves. The period of maximal change was from 19 to 27 days after inoculation, i.e. after the adult O. ostertagi had emerged from the glands. The principal change in the abomasal fluid was a decreased concentration of hydrogen ions (increased pH). At the same time there was an increase in plasma pepsinogen levels. When the pH of the abomasal fluid increased to more than 7.0, there was an increase in the number of viable bacteria which coincided with a bodyweight loss and diarrhoea. A loss of adult worm population occurred between days 19 and 36 of the infection.

SECTION III

STUDIES ON INHIBITED LARVAL DEVELOPMENT OF *OSTERTAGIA OSTERTAGI*

A. The Association Between Season and the Occurrence and Duration of Inhibited Larval Development

B. The Relationship Between Acquired Immunity and Inhibited Larval Development

C. Studies on the Association Between Physiological Changes in the Host or the Parasite and Inhibited Larval Development

General Introduction

Inhibition of larval development of a nematode parasite in its final host is a common phenomenon in many host/parasite relationships, e.g. Oesophagostomum dentatum and Hyostrongylus rubidus in the pig (Kotlan, 1949), Trichonema spp. in the horse (Gibson, 1953), Trichostrongylus retortaeformis in rabbits (Michel, 1952), Ostertagia circumcincta in sheep (Sommerville, 1954), Haemonchus placei in cattle (Roberts, 1957), Ostertagia ostertagi in cattle (Vegors, 1958; Michel, 1963; Anderson, Armour, Jennings, Ritchie and Urquhart, 1965b), Nematodirus spathiger in sheep (Donald, Dineen, Turner and Wagland, 1964) and Cooperia oncophora in cattle (Anderson et al, 1965b). Its occurrence in naturally acquired O. ostertagi infections of cattle in Britain (Anderson et al, 1965a; Ross, 1965) has been frequently reported and is of considerable practical importance as the subsequent maturation of inhibited larval stages, when present in sufficient numbers in the abomasal mucosa, will precipitate clinical disease (Martin et al, 1957; Anderson et al, 1965a).

Until recently, inhibition of larval development in field infections of bovine ostertagiasis was generally ascribed to the acquisition of immunity by the host (Martin et al, 1957; Ross, 1963). This was generally supported by the results of experimental infections (Michel, 1963) in which calves, which had been given repeated doses of O. ostertagi larvae for at least 127 days, had large numbers of inhibited fourth larval stages at autopsy.

A second explanation for the mechanism of inhibition was recently advanced by Anderson et al (1965b) who postulated that inhibition of O. ostertagi was associated with physiological changes in the parasite or possibly in the

host, in the late autumn, and that these changes were independent of the immunological status of the host. These conclusions were based on the results of experiments in 1964 (see Section I E) in which parasite-free calves, which had grazed pasture contaminated with O. ostertagi for periods of 14 days during the summer and autumn, were autopsied four days after removal from pasture. While some inhibited larval stages were found at autopsy in nearly all of the calves, the proportion of these increased significantly in the late autumn.

Ross (1965) in a survey of cattle abomasums and their contents from a knackery in Northern Ireland also noticed that there was a definite increase in the numbers and proportions of larval stages of O. ostertagi present in late autumn and early winter; he suggested that nutrition and husbandry in the late autumn may play a major role in the increased proportion of O. ostertagi larvae inhibited in the host at that time. Ross pointed out that in Northern Ireland in the late autumn, pastures were frequently overgrazed, overstocked and of poor nutritive value and that Ciordia et al (1962) had demonstrated that cattle in Georgia, U.S.A., on over-grazed pastures and at high stocking rates had a significantly higher proportion of fourth larval stages of O. ostertagi than cattle on undergrazed pastures and at low stocking rates.

The objects of the experiments described below were threefold: first to confirm the 1964 observations that a significant proportion of O. ostertagi larvae become inhibited in their development in calves grazing for a short period in the autumn, and also to find out if this inhibition existed for a reasonable period of time.

Secondly, to study the association between acquired immunity and inhibited development of O. ostertagi (a) by treating calves harbouring large numbers of inhibited larval stages (pre-Type II) with drugs known to interfere with the immune cellular response and subsequently observing if re-development of inhibited larval stages took place; (b) by finding out if calves in the pre-Type II stage were immune to challenge with an experimental inoculum of O. ostertagi.

Thirdly, to study the association between 'physiological' changes in the host or parasite and inhibited larval development of O. ostertagi.

A. The Association Between Season and the Occurrence and Duration of Inhibited Larval Development

Introduction

The results of a knackery survey of cattle abomasums and their contents, which Ross (1965) carried out in Northern Ireland, indicated there was a distinct seasonal variation in the proportions of adult and larval stages of O. ostertagi present. Thus, from late spring through summer until early autumn adult stages predominated; in late autumn, the proportion of larval stages had increased and by mid-winter accounted for over 80% of the total O. ostertagi population; by early spring, the proportion of larval stages present had declined and equal numbers of adults and larval stages were present.

A similar seasonal trend in the proportion of adult and larval stages of O. ostertagi present in calves has also been recorded by the author (Section I, Part B). In experimental field studies in bovine ostertagiasis during the summer and autumn of 1964, it was found that a high proportion of early fourth larval stages were present only in calves autopsied during the late autumn. It was also noticed that an equally high proportion of early fourth larval stages was present in calves which had grazed for several weeks or for 14 days. These contemporaneous findings by Ross (1965) and the author suggested that, in the northern parts of the British Isles at least, the majority of O. ostertagi infective larvae ingested by calves grazing in the late autumn do not mature in the accepted period of 21 days; instead, they are delayed in their development at the early fourth larval stage for an unknown period of time.

The experiments described below are an extension of those reported in Section I and were designed (1) to confirm the results of the 1964 experiments, (2) to extend these observations to the spring months, and (3) to find out if the cessation in larval development extended over a period of at least four weeks.

Experimental Design

These experiments were carried out at the same farms (A and B) as described in Section I, and utilised the same fields. The calves used were all Ayrshire males and were reared worm-free.

In order to establish if inhibition of development was not merely transient, six 'tracer' calves were grazed at Farm A in 1964 from October 27th until November 18th, i.e. a period of 22 days, and were then housed. Three of the calves were autopsied four days after housing, while the remaining three were autopsied 27 days after removal from pasture.

To ascertain if a significant proportion of larvae ingested in the spring were inhibited in their development, two 'tracer' calves, ten weeks old, were turned out at each farm on April 16th, 1965, to graze for two weeks. At the end of that period they were replaced by another four 'tracer' calves, which also grazed for two weeks. After both fortnightly periods of grazing, the calves were housed for seven days, then autopsied.

The experiments conducted to confirm the 1964 findings were designed as follows: at each farm from June 24th, 1965, eight 'permanent' calves grazed until the first week in October, unless the development of clinical ostertagiasis

necessitated their earlier removal. On October 7th, 1965, another eight 'permanent' calves aged twelve weeks were turned out to graze for a period of four weeks. The surviving calves from the June to October and the October to November grazing periods were housed and used for other experiments.

In addition to the above, two 'tracer' calves, ten to twelve weeks old, were put out at each of the two farms on June 24th to graze the same pasture as the 'permanent' calves for two weeks. On August 26th, 1965, a further two 'tracer' calves were turned out at both farms to graze for a fortnight. From that date until the termination of the experiment on November 4th, 1965, i.e. ten weeks, the 'tracer' calves were replaced by another four calves every fortnight. Two 'tracer' calves were also grazed at each farm for periods of only 24 hours in late summer (September 9th) and late autumn (November 3rd). After removal from pasture, the 'tracer' calves were housed for seven to nine days prior to being autopsied.

Observations

Weekly visits were made to both farms to make clinical examinations and remove calves severely affected with ostertagiasis for subsequent autopsy at the laboratory. A weighing scale was available at Farm A and weekly weighings were undertaken. Faecal samples were collected at weekly intervals and faecal egg counts examined by the zinc sulphate flotation and by the McMaster technique. At autopsy of the 'permanent' calves, differential worm counts were made as previously described. Since O. ostertagi had been the

predominant parasite present at calf autopsies from both farms in 1964, only the abomasums of the 'tracer' calves were examined. Meteorological data was collected, as before, from the records of the Ministry of Aviation at Prestwick Airport.

Results

Details of the clinical and parasitological observations on the 'permanent' calves autopsied during the grazing season at both farms have already been presented and discussed in Section I and Appendix 1, Tables 1, 4, 5 and 6. Only the results pertaining to the autopsy of 'tracer' calves will be given here.

The O. ostertagi worm burdens of the six 'tracer' calves which grazed from October 27th until November 18th, 1964, are shown in Table 16. The total numbers of early fourth larval stages present in the calves, whether autopsied four or 27 days after removal from pasture, were similar, suggesting that little development of inhibited early fourth larval stages had taken place since housing.

The mean O. ostertagi worm burdens of the calves which grazed for 24 hours or 14 days during spring, summer and autumn of 1965 are given in Tables 17 and 18. Further details of the individual worm counts and grazing history are given in Appendix 4, Tables 1 and 2. It is apparent from these results that although a proportion of early fourth larval stages was present in nearly all of the 'tracer' calves autopsied, only those autopsied in the late autumn contained a high proportion of early fourth larval stages.

Table 16

The Ostertagia ostertagi Worm Burdens at Autopsy of Six Tracer*Calves Grazed at Farm A for a Period of 23 Days Between October 27th and November 10th, 1964.

No. of Calves	No. of days between removal from pasture & autopsy	O. Ostertagi Worm Counts			
		Total	Adults & Developing Stages	Early 4th Larval Stages	Per cent of early 4th larval stages
3	4	18,000	1,700	16,300	91
		19,400	800	18,600	96
		27,600	1,700	25,900	94
3	27	17,600	1,300	16,300	93
		27,600	9,300	18,300	66
		40,900	2,500	38,400	94

*Tracer Calves were reared worm free

Table 17

The Mean Numbers and Percentage of Early Fourth Larval Stages of *Ostertagia ostertagi* at Autopsy of Pairs of 14 Day Tracer Calves* Grazed at Farms A and B in 1965

Date Grazed 1965	FARM A		FARM B	
	Mean Numbers *O. ostertagi*	Mean % Early 4th Larval Stages	Mean Numbers *O. ostertagi*	Mean % Early 4th Larval Stages
16/4 - 30/4	13,000	0	24,400	10
30/4 - 14/5	17,600	16	26,600	7
24/6 - 8/7	300	0	400	0
26/8 - 9/9	4,900	6	5,000	5
9/9 - 23/9	14,200	30	5,900	15
23/9 - 7/10	10,100	36	5,200	54
7/10 - 21/10	15,300	64	5,000	70
21/10 - 4/11	15,100	64	3,700	64

* 14 Day Tracer Calves = Calves reared worm free and grazed for 14 days

Table 18

The Total Numbers and Percentage of Early Fourth Larval Stages of Ostertagia ostertagi at Autopsy of 24 Hour Fasted* Calves Grazed at Farm A in 1965

Date	No. of Grazed Calves	O. ostertagi Worm Counts			
		Total	Developing Stages	Early 4th Larval Stages	Per cent of Early 4th Larval Stages
9 - 10/9	4	5,500	2,300	1,200	34
		2,300	1,600	700	30
		1,500	1,200	300	20
		1,100	900	200	18
3 - 4/11	3	1,500	300	1,200	80
		1,400	200	1,200	86
		1,800	800	1,000	56

*Fasted Calves were reared worm free

Table 19

Ostertagia ostertagi Worm Burdens and Percentage of Early Fourth Larval Stages at Autopsy of Calves Grazed for Short Periods on Naturally Contaminated Pasture in Spring, Summer and Autumn of 1964 and 1965

	1964			1965					
Length of Grazing Period	14 days			14 days			1 day		
Period Grazed	No. of Calves	Mean Nos. of worms & Range	Mean Per Cent Early 4th Larval Stages & Range	No. of Calves	Mean Nos. of Worms & Range	Mean Per Cent Early 4th Larval Stages & Range	No. of Calves	Mean Nos. of Worms & Range	Mean Per Cent Early 4th Larval Stages & Range
Spring April/ May	-	-	-	8	20,600 (8,400-40,400)	10 (3-21)	-	-	-
Summer Aug./ Sep.	6	47,500 (32,700-64,500)	9 (3-14)	8	7,300 (3,400-17,300)	14 (4-35)	4	2,100 (1,000-3,500)	26 (18-34)
Autumn Oct./ Nov.	8	21,000 (5,000-41,800)	77 (48-95)	8	9,800 (2,000-24,600)	65 (48-79)	3	1,600 (1,400-1,800)	74 (56-86)

The worm burdens at autopsy of 'tracer' calves grazing in spring, summer and late autumn, are summarised in Table 19 together with those from 'tracer' calves which grazed at similar seasons in 1964. The mean percentages of inhibited or early fourth larval stages present in the calves which grazed for only 14 days in 1964 and 1965 are shown graphically in Figure 31. These results show that in both years the percentage of early fourth larval stages increased markedly in the late autumn.

Discussion

The results of these experiments clearly indicate that only at autopsies of calves grazing during the late autumn, i.e. October onwards, were a significant proportion of early fourth larval stages of O. ostertagi found. Although both the total numbers of O. ostertagi and the proportion of early fourth larval stages present at autopsy in the 14-day 'tracer' calves were less in 1965 than in 1964, this should not be interpreted as indicating a possible relationship between the established worm population and the proportion of inhibited fourth larval stages present. Thus, in both summer and autumn, the same degree of inhibition of larval development occurred in calves which grazed for 24 hours or for 14 days and inhibition is therefore unlikely to be a function of the quantity of larval intake or the size of the worm population established. A more likely explanation for the increased proportion of early fourth larval stages in the 1964 'tracer' calves is the fact that they were autopsied only four days after removal from pasture; the numbers of early fourth larval stages recorded would therefore

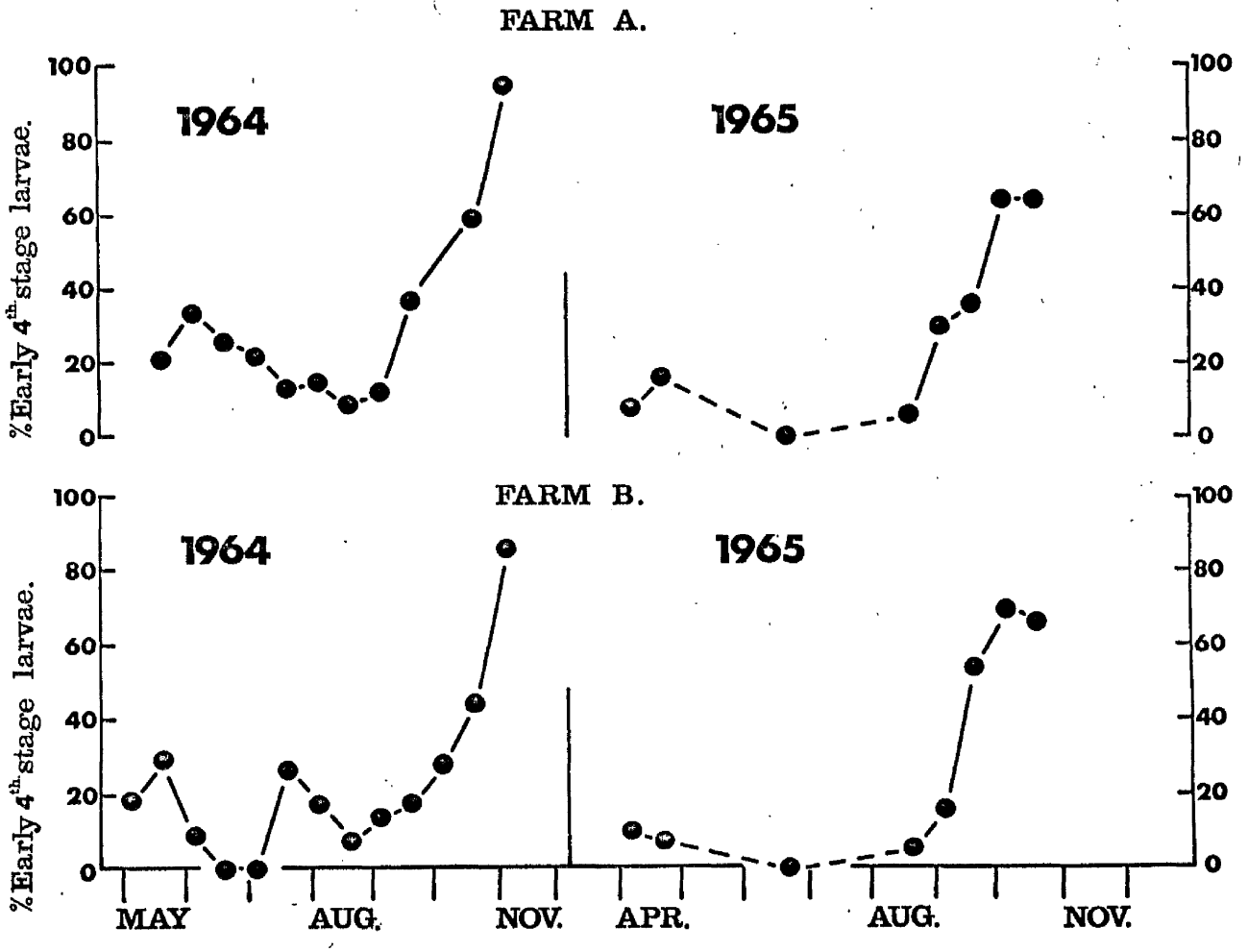


Fig. 31 The mean percentage of early fourth larval stages of O. ostertagi present at autopsy of 14 day 'tracer' calves at Farms A and B in 1964 and 1965.

include a number of larvae ingested on the last day of grazing and which were developing normally. In the 1965 'tracer' calves, this would not be applicable as these calves were autopsied seven days after removal from pasture.

It is more difficult to explain why the total worm burdens of the 'tracer' calves were lower in 1965 than in 1964. The apparently low numbers of larvae available on the pasture was also reflected in the later onset of clinical ostertagiasis in the 'permanent' calves in 1965 than in 1964 (see Section I A). It is possible that the 'tracer' calves which grazed in the spring removed some of the infective larvae which had over-wintered or alternatively that a heavy mortality in the remaining larvae occurred between the spring and the introduction of the 'permanent' and 'tracer' calves in mid-summer. It is interesting that Michel (1966) states that a heavy mortality of O. ostertagi larvae on pasture occurs during the late spring and early summer. Another possibility is that an abnormally early hatch and development of overwintered eggs had occurred and that a heavy mortality in these larvae took place before the 'permanent' calves were introduced in mid-summer.

The numbers of O. ostertagi present in some of the calves which grazed for only 14 days in the spring would have been sufficient to cause Type I ostertagiasis had they been allowed to complete their development to adult stages. Clinical ostertagiasis has not been recorded in South-west Scotland at this time of the year (Section I A) but it is probable that outbreaks can occur on occasion (Michel, 1966).

Unless an early development and hatch of overwintered eggs took place in April and May, 1965, which is unlikely in view of the results of Michel (1966) and those reported in Section I of this thesis, the larvae which were present on the pasture in the spring of 1965 were the survivors of the same population of larvae which was present on the pasture in the late autumn of 1964. When these larvae were ingested by calves grazing in the late autumn, a high proportion of those established were inhibited in their development, whereas in the spring of 1965 only a low proportion of larvae ingested and subsequently established became inhibited in their development. In the 'tracer' calves from late autumn, 1964, and the spring of 1965, the mean and range of total worm burdens were similar (see Table 19); the difference in the proportion of larvae inhibited in development could not therefore be attributed to differences in magnitude of total worm burdens.

These findings augment the hypothesis made in Section I B that inhibited development of O. ostertagi associated with 'physiological' changes in either the larvae or the host, is a particular characteristic of the late autumn period and is independent of the size of the established worm burden.

Finally, the inhibition of larval development noticed in calves which grazed for a short period in late autumn does not appear to be transient as approximately the same numbers of inhibited larvae were present in the calves grazed for 21 days in late autumn whether they were autopsied four or 27 days after removal from pasture.

B. The Relationship Between Acquired Immunity and Inhibited Larval Development

Introduction

Prolonged inhibition of larval development in nematodes associated with acquired immunity has been recorded by several workers. Scott (1928) showed that, in dogs previously infected with Ancylostoma caninum, inhibited larvae could be recovered from the small intestine for up to six weeks after challenge; these larvae were found to be capable of development if transferred to a susceptible host. Monnig (1949) has stated, apparently on the basis of field observations, that the developing stages of O. columbianum may remain viable within the intestinal mucosa of previously infected sheep for several months. Gordon (1950) considered that such larvae may emerge and develop to maturity as long as 12 months afterwards. Michel (1952) has reported that when a massive number of T. retortaeformis is administered to a previously infected rabbit, a large proportion remains in the intestinal mucosa as late third larval stages and that these larvae may recommence development together or in relays in decreasing numbers up to 98 days post-inoculation. Dunsmore (1960) made a similar observation in Ostertagia spp. in susceptible sheep; fourteen days after the administration of 1000 larvae more than 98% of the worms recovered were over 6 mm. in length, whereas following administration of 100,000 larvae more than 75% were less than 2 mm. in length. Mulligan, Gordon, Stewart and Wagland (1961) recovered from the abomasums of sheep immunised with X-irradiated H. contortus larvae and killed 40 days after challenge, a number of inhibited fourth stage larvae. Michel

(1963) found a significant proportion of inhibited O. ostertagi fourth stage larvae present in the worm burdens of calves autopsied following daily larval inoculations with 1,500 larvae for a period of over 100 days; he tentatively ascribed the cause of inhibition in this experiment to the presence of adults and speculated on the association between the development of inhibited populations and acquired resistance. Taylor and Michel (1953) have described inhibition of Dictyocaulus filaria in the bronchi of sheep for up to 100 days and D. viviparus in adult cattle for 150 days; it was not stated whether these sheep or cattle had previous exposure to lungworms. Donald et al (1964) found that in sheep a higher proportion of inhibited N. spathiger larvae resulted from repeated inoculations of larvae than from a single large inoculation; they postulated that immunological control of a nematode infection by the host is mediated about threshhold levels of immunological responsiveness and manifested in several ways including inhibition of larval development.

The present experiments were designed to test the hypothesis that inhibition of O. ostertagi is associated with acquired immunity: first, by studying the effect of cortisone and methotrexate on inhibited O. ostertagi in calves in the pre-Type II stage of ostertagiasis. Both these drugs are known to interfere with the availability or multiplication of blood cellular elements associated with immunity.

Secondly, by observing the fate of experimental challenge inoculations of normal or irradiated O. ostertagi larvae given to animals in the pre-Type II stage of ostertagiasis.

A third experiment was included to confirm the observations of Michel (1963) that a high proportion of the O. ostertagi present in calves following a prolonged period of daily inoculations, was inhibited at the fourth larval stage.

Table 20

Design of Experiment where Pre-Type XI Cases of Osteoclastoma were given Large Doses of Cortisone or Methotrexate

Group	No. of Calves	Treatment	Day Killed
A	3	140 mg. betamethasone* between day 0 - 7	2 calves on Day 14 1 calf on Day 28
B	2	140 mg. methotrexate** between day 0 - 7	1 calf on Day 14 1 calf on Day 28
C	3	Nil	2 calves on Day 14 1 calf on Day 28
D	3	Nil	Day 0

*Betnelan, Glaxo Laboratories Ltd., Greenford, England

**Methotrexate, Lederle Laboratories Division, Pearl River, New York.

Experiment 1

Experimental Design

Eleven cross-Hereford calves, aged 12 to 15 months, were purchased in January, 1965, from a farm where one animal had recently died from Type II ostertagiasis. The group of twelve calves had been housed in October, following their first season at grass, and with the exception of the one calf which died, none of the other calves had shown any obvious clinical signs of ostertagiasis. It was therefore considered that these animals were in the pre-Type II stage and this was confirmed by slaughter of three calves at the beginning of this experiment (Group D - Table 21). Three of the remaining calves (Group A) were treated with cortisone daily for seven days while another two calves (Group B) received an injection of methotrexate daily for seven days. The other three calves (Group C) acted as untreated controls. Some calves from each group were autopsied 14 days and some at 28 days after treatment. The experimental design is outlined in Table 20.

The cortisone derivative used was betamethasone (Betsolan, Glaxo Ltd., Greenford, Middlesex, England) and 20 mg. was given by the intramuscular route daily for seven days. The methotrexate used was methotrexate sodium (Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York) and was also given intramuscularly daily for seven days. An antibiotic, namely oxytetracycline hydrochloride (Terramycin, Pfizer Ltd., Sandwich, Kent, England) was given at 400 mg. daily to each of the calves receiving cortisone

and methotrexate and also to one of the controls.

Observations

A daily clinical examination of all calves was made and faecal samples and whole blood samples were collected twice weekly. Faecal egg counts and white blood cell counts were made twice weekly and a total eosinophil count was carried out on days 7, 9, 14 after commencement of treatment. At autopsy a parasitological examination of each abomasum was carried out as previously described.

Results

Blood Analysis. - In the cortisone treated group (A) the total circulating white cell count increased threefold in the week following dosage while the total eosinophil count decreased to negligible proportions. Both of these parameters returned to levels equivalent to those in the control group following cessation of cortisone treatment.

Parasitological Data .- Initially, the faecal egg counts were low as previously noted in pre-Type II cases of ostertagiasis (Section I A). Following treatment of the calves in group A with betamethasone, the faecal egg counts increased sharply and then fell abruptly at cessation of treatment. Figure 32 shows the mean faecal egg count of groups A, B and D.

The numbers and stages of O. ostertagi present at autopsy are given in

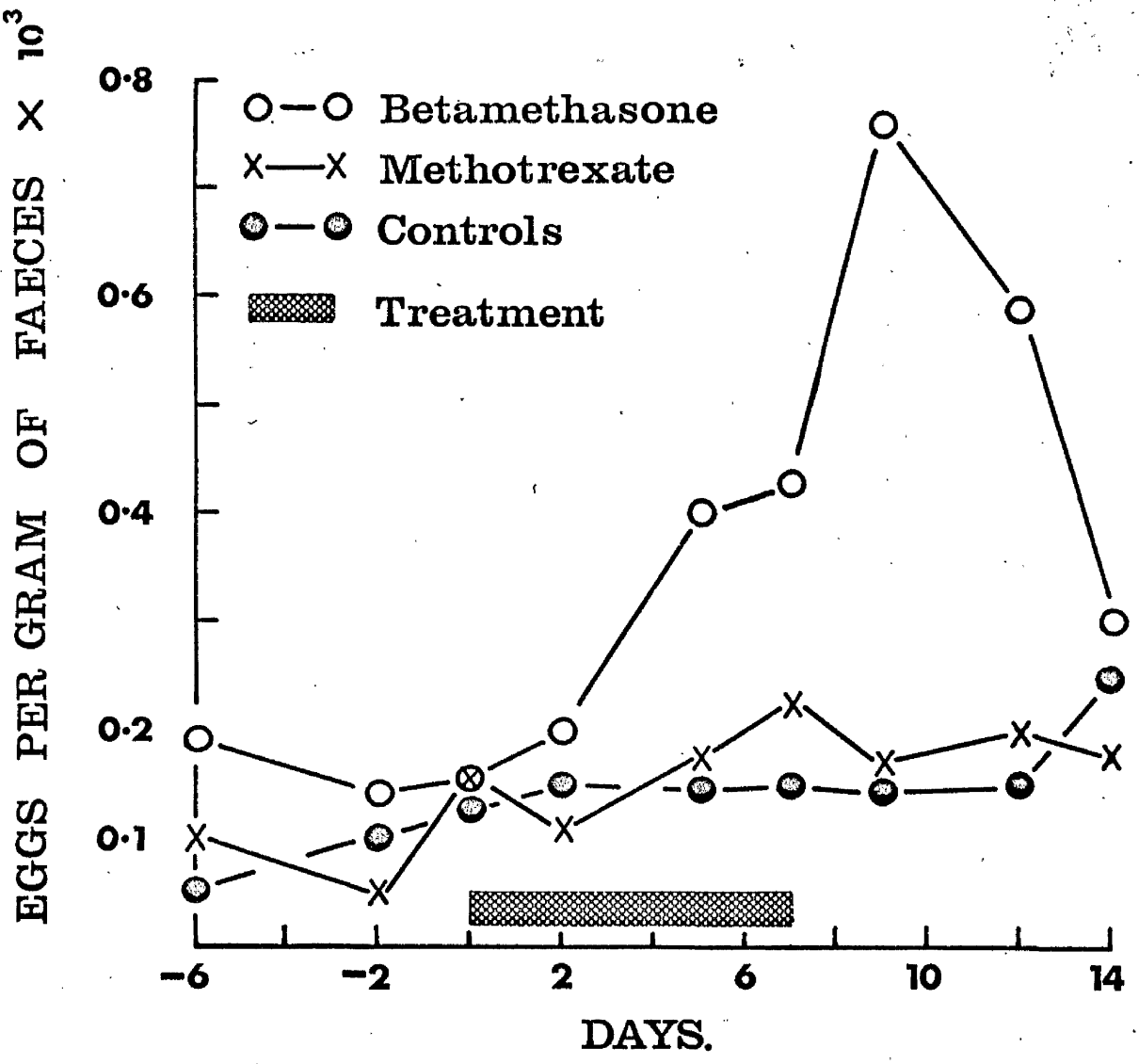


Fig. 32 Mean faecal egg counts in groups of pre-Type II ostertagiasis calves following treatment with betamethasone and methotrexate.

Table 21

Ostertagia ostertagi Worm Counts at Autopsy of Untreated Pre-Type II Cases and Those Treated with Cortisone and Methotrexate

Group	Day Killed	O. ostertagi Worm Counts				
		Total	Adults	Developing Stages	Early 4th Larval Stages	
					Total	Per cent
A (cortisone treated)	14	129,000	5,000	15,000	109,000	84
	14	148,000	4,000	11,000	133,000	90
	28	145,000	7,000	14,000	124,000	86
B (methotrexate treated)	14	98,000	3,000	4,000	91,000	93
	28	141,000	6,000	5,000	130,000	92
C	14	87,500	6,000	200	81,300	93
	14	150,200	12,700	2,700	134,800	90
	28	117,100	3,500	600	113,000	96
D	0	128,700	18,000	1,700	109,000	85
	0	140,800	3,700	0	137,100	97
	0	142,900	13,500	1,000	128,400	90

Table 21 and have been divided into three populations, namely, mature adults, developing stages (which includes third, late fourth, fourth moult and early fifth larval stages), and early fourth or inhibited larval stages. In the cortisone treated group (A) the numbers of developing stages were increased compared with the control groups, although massive resumption of development of inhibited stages had not materialised. There was no significant increase in the numbers of developing stages in group B, treated with methotrexate.

Experiment 2

Experimental Design

In 1965, at the two farms described previously, i.e. Farms A and B, groups of eight parasite-free Ayrshire male calves, twelve weeks old, were turned out at both farms to graze from October 7th until November 4th, i.e. a period of four weeks. It was assumed that calves which had grazed these pastures in the late autumn would be harbouring fairly large burdens of inhibited O. ostertagi at the end of four weeks grazing; this assumption was proved correct when some of the calves were autopsied seven days after removal from pasture (Table 23).

The original design included four groups of four calves each in this experiment, but unfortunately one of the calves at Farm A and three of the calves at Farm B died during the course of the grazing period; the number of calves per group had therefore to be reduced to three.

Table 22

Design of Experiment where Four Groups of Pre-Type II Cases of Osteriagiasis were Housed and Two Groups Given an Experimental Challenge Inoculum of Normal or Irradiated 3rd Stage Ostertagia ostertagi Larvae

Group	No. of Calves	Days Grazed	Treatment Following Housing	Day of Killing Following Housing
1	3	28	Nil	7
2	3	28	Nil	49
3	3	28	100,000 normal 3rd stage O. ostertagi larvae on day 28	49
4	3	28	100,000 X-irradiated O. ostertagi larvae on day 28	49
5	1	Nil	100,000 normal 3rd stage O. ostertagi larvae on day 28	49
6	1	Nil	100,000 X-irradiated* 3rd stage O. ostertagi larvae on day 28	49

*Irradiated at 60 kilorocntgens

The twelve calves surviving at the time of housing, i.e. November 4th, were randomised into four groups of three calves each. The calves in Group 1 were autopsied seven days after housing to ascertain the numbers of O. ostertagi and proportion of larval stages present at that time. The calves in Group 3 and Group 4 were each inoculated with 100,000 normal or 100,000 X-irradiated O. ostertagi larvae respectively, 28 days after housing. These groups of calves together with Group 2 were autopsied 21 days after inoculation of the larvae, i.e. 49 days after removal from pasture. Two groups of single susceptible calves (Groups 5 and 6) were also inoculated with 100,000 normal or X-irradiated O. ostertagi larvae to establish the infectivity of the larvae given to Groups 3 and 4. The experimental design is summarised in Table 22.

Observations

The calves were clinically examined twice weekly at pasture and daily after housing. Samples of faeces were collected twice weekly throughout the experiment for faecal egg counts. At autopsy each abomasal mucosa and its contents was examined for the presence of O. ostertagi as previously described. Prior to, and on days 7, 15 and 21 after the challenge inocula of larvae were given, blood samples were collected for estimation of plasma pepsinogen levels.

Results

Clinical Data .- While the calves were at pasture, the weather conditions

were particularly severe and grass was scarce. The cause of death in the four calves which died is unknown, but may have been due to exposure since they died following a week of particularly severe weather. Although no clinical signs of ostertagiasis were noticed, the calves were in poor condition at the time of housing but from then on steadily improved. Softening of the faeces of two of the three calves challenged with 100,000 normal O. ostertagi larvae occurred 19 days after challenge.

Parasitological Data .- The faecal egg counts of all calves remained below 300 e.p.g. with the exception of two of the three calves challenged with normal O. ostertagi larvae; in these calves, the faecal egg count increased 18 days after challenge, exceeding 1,000 e.p.g. on the day of slaughter, i.e. day 21.

The numbers, stages, percentages of inhibited forms and sex ratios of the O. ostertagi worms present in groups 1 to 6 at autopsy are shown in Table 23 together with details of the grazing history. The numbers of worms present in the calves grazing at Farm A were greater than from those grazed at Farm B. This trend, was also reflected in the worm burden of the 'tracer' calves grazing at the same time and described in Table 17. There was also a marked increase in the number of adult O. ostertagi stages in the group challenged with 100,000 normal larvae and a material increase in the number of sterile adult female stages in the group receiving irradiated larvae. In the latter group the sex ratio was altered in favour of female worms.

Plasma pepsinogen .- The plasma pepsinogen levels in the two challenged groups

Table 23

Worm Counts at Autopsy of Cases of Pre-Type II Ostertagiasis Following Experimental Challenge with Laboratory Cultured *Ostertagia ostertagi* Larvae

Group	Form	Day Killed after Housing	Challenge Inoculum	Total	Adults	*O. ostertagi* Worm Counts: Developing Stages	Early 4th Larvae: Total	Early 4th Larvae: Per Cent	Sex Ratio Male/Female
1	A	7	Nil	39,000	13,000	5,000	21,000	54	1:1
	B			23,500	8,300	1,300	13,900	59	1:1
	B			4,500	1,800	0	2,700	60	4:5
2	A	49	Nil	17,400	5,700	0	11,700	67	1:1
	A			7,400	2,800	900	3,700	50	1:1
	B			8,600	2,400	1,000	5,200	60	4:5
3	A	49	200,000 3rd stage *O. ostertagi* larvae on day 28	54,300	23,600	27,000	3,700	7	1:1
	B			57,400	14,200	38,900	4,400	8	2:3
	A			18,300	1,000	13,100	3,400	19	1:1
4	B	49	100,000 X-irradiated 3rd stage *O. ostertagi* larvae on day 28	35,300	100	*30,200	3,000	9	1:4
	A			42,600	300	*38,300	4,000	9	3:4
	A			28,700	3,700	*14,900	10,100	35	2:9
5	Normal larvae infectivity control. Killed day 21			30,900	9,900	1,000	0	0	1:1
6	X-irradiated larvae infectivity control. Killed Day 21			4,400	100	x4,300	0	0	1:50

*75-80 per cent female fifth larval stages
x 90 per cent female fifth larval stages

increased sharply following challenge being more marked in the group receiving the normal O. ostertagi larvae. The mean plasma pepsinogen levels are shown in Figure 33.

Experiment 3

Experimental Design

Five male Ayrshire calves, reared worm-free and ten to twelve weeks old, were inoculated orally with 250,000 O. ostertagi larvae. The faecal egg counts had increased above 500 e.p.g. at 19 days post-inoculation and by day 30 had decreased to below 500 e.p.g. The calves were then treated with thiabendazole (Thibenzole, Merck Sharp & Dohme Ltd., Hoddesdon, Herts., England) at a dosage level (220 mg. per kg. bodyweight) known to remove the existing adult worm population (see Section V). Twenty-one days later each calf was given an oral inoculation of 1,500 O. ostertagi larvae daily for 100 days. The calves were autopsied seven days after the final inoculation of 1,500 larvae.

Observations

Each calf was clinically examined daily. Faecal samples were collected daily from day 19 to day 30 and thereafter twice weekly. At autopsy, each abomasum and its contents was examined for the presence of O. ostertagi as previously described.

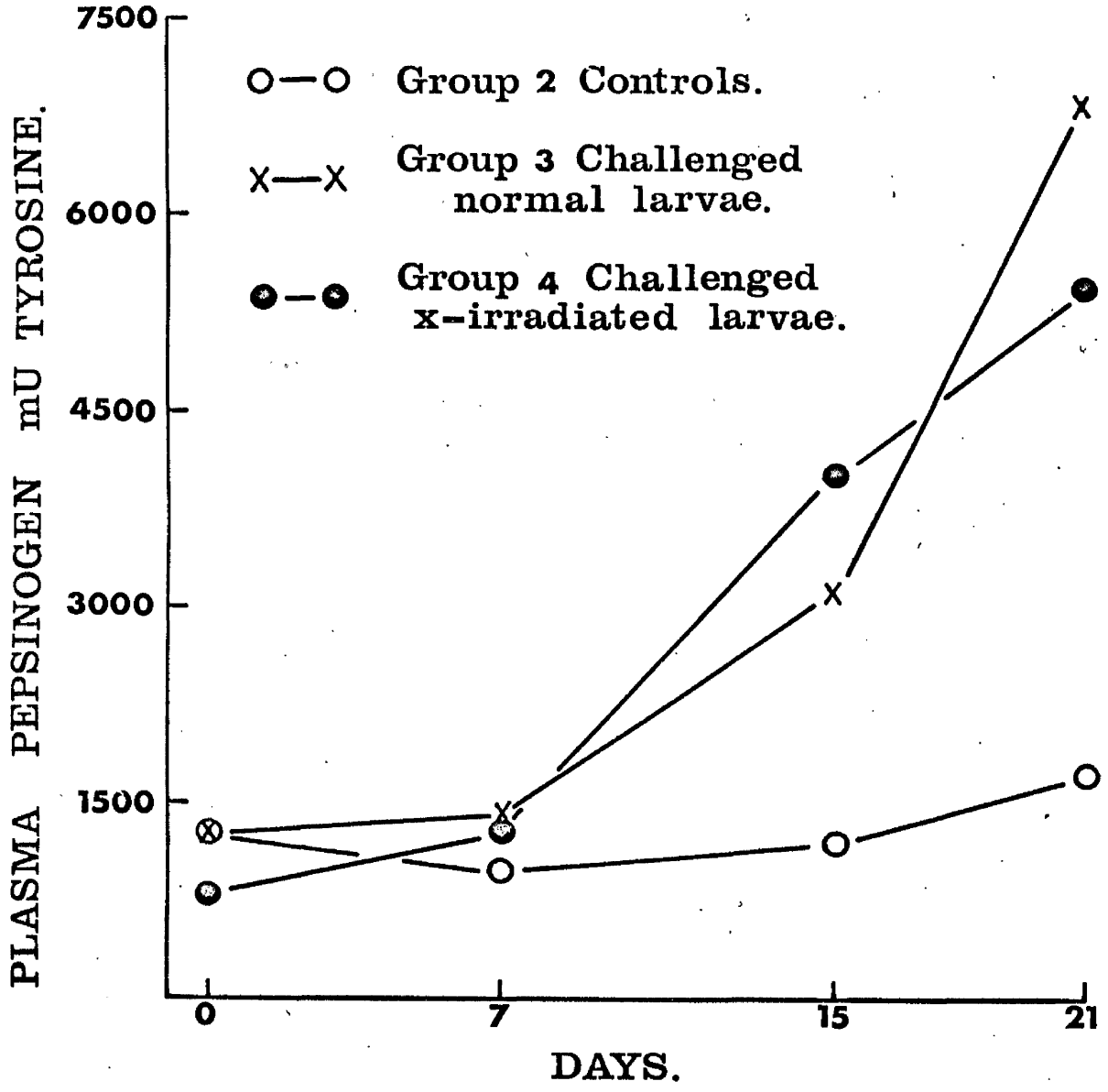


Fig. 33 Mean plasma pepsinogen levels in groups of pre-Type II ostertagiasis calves following administration of normal or X-irradiated larvae.

Results

Softening of the faeces was apparent in each of the five calves 19 to 20 days following inoculation of the 250,000 larvae. By day 22, all five calves were diarrhoeic and this sign persisted until day 25 and then gradually abated. No other clinical signs were detected except in one calf, which showed softening of the faeces for eight days after 60 daily doses of 1,500 larvae had been given. Faecal egg counts exceeded 1,000 e.p.g. between the 21st and 25th day after the primary inoculation of 250,000 larvae and then decreased to near zero by day 30. Following treatment with thiabendazole at 220 mg. per kg. bodyweight, the faecal egg counts remained at zero until 17 days after the commencement of daily dosing with 1,500 larvae, confirming the anthelmintic efficiency of the drug at this dosage level; thereafter the faecal egg counts fluctuated considerably. The faecal egg counts are given in Appendix 4, Table 3.

The total O. ostertagi worm counts and the percentage of early fourth or inhibited larval stages present are given in Table 24. The proportion of these stages are expressed as a percentage of the total O. ostertagi worm population and range from 7 to 37%.

Discussion

In considering the results of the first experiment, it is apparent that although some maturation of inhibited O. ostertagi (10 to 15%, see Table 21) occurred following administration of cortisone, this was not of a magnitude comparable to the massive redevelopment of inhibited stages which gives rise

Table 24

Ostertagia ostertagi Worm Burdens at Autopsy* of Calves Given 1,500 Larvae Daily for 100 Days Following an Initial Primary Infection of 250,000 Larvae, which had been Removed by Thiabendazole

Calf No.	O. ostertagi Worm Burdens		
	Total	Early 4th Larval Stages	
		Total	Per Cent
12	7,900	2,900	37
21	8,600	1,600	19
23	6,200	2,200	35
49	15,000	1,300	7
50	5,300	500	9

*Calves were Autopsied 7 Days after Last Dose of Larvae

to the clinical signs of Type II ostertagiasis. Possibly, the dosage rate of cortisone employed in the present experiment was too low, as Dunsmore (1961) found that the percentage of inhibited larval stages present in sheep following a single inoculation of O. circumcincta larvae was reduced, compared to control sheep, when prior treatment with hydrocortisone was given. Dunsmore used a dosage rate of 4 mg. per kg. bodyweight of hydrocortisone daily for five days which is double the daily dosage rate employed in the present experiment (0.25 mg. per kg. bodyweight of betamethasone is equivalent to 2 mg. per kg. bodyweight of hydrocortisone - Uvarov, 1965).

Another possibility is that more than one mechanism may operate in producing inhibition of larval development and that only a proportion of larvae, which become inhibited in their development, do so because of the host's immune response; this would explain why only a proportion (10 to 15%) of the total population of inhibited larval stages in Experiment 1 were able to mature following the administration of betamethasone. Certainly the dosage rate of betamethasone employed in Experiment 1 was sufficient to cause a reduction in blood cellular elements (eosinophils), normally seen following cortisone therapy. The increase in faecal egg counts (Fig. 32) during the period of cortisone therapy is also of interest; the reason for this increase is not known but two tentative hypotheses are (a) a direct effect on the metabolism of the worm increasing its egg production or (b) an inhibiting effect on immune mechanisms which depress egg production; cortisone is thought to reduce capillary permeability (Rose, 1958) and it is possible that the concentration of antibody is therefore reduced around the worm.

The methotrexate-treated calves did not show a marked depletion of blood cellular elements, although a transient fall of circulating white cells did occur during the period of therapy. The effect of methotrexate treatment on the subsequent development of inhibited O. ostertagi is apparently negligible as a relatively small increase in numbers of developing stages was present when compared with untreated controls. There was also no significant increase in faecal egg counts (Fig. 32) in this group.

The low proportion of adults and developing stages present in both groups of untreated calves (Groups C and D; Table 21) autopsied on days 0, 14 and 28 indicated that little or no natural resumption of development of inhibited O. ostertagi had taken place during the period of the experiment.

From the results of the second experiment several points of interest emerge. First, that the proportions of inhibited larval stages present in the calves autopsied at seven or 49 days after removal from pasture are the same. Secondly, that the total number of inhibited stages in these calves and also in the two groups challenged experimentally are not significantly different. These facts suggest that little or no maturation of O. ostertagi larvae had taken place in the seven weeks after housing. The third and perhaps most significant finding is that the challenge inoculum of normal larvae has become established and matured in the accepted period of 21 days and had apparently 'leap-frogged' the inhibited fourth stage larvae in the gastric mucosa. This is evident from the increase in adult worm population (Table 23) and the increase in faecal egg counts which occurred 18 to 21 days after challenge. It may be argued that some of the

challenge infection could have become inhibited in the early fourth stage and the adult population could have resulted from the development of the existing inhibited population. This hypothesis is unlikely and is not borne out by the parasitological results from the calves challenged with X-irradiated larvae. Here, the developing worm population (sex ratio 1:4) consisted mainly of immature adult females, i.e. the worms contained no eggs. These worms were certainly derived from the X-irradiated larvae since male nematode larvae are particularly susceptible to the effect of ionising radiation (Giordia and Mazzoll, 1960a) and the population established following the administration of suitably irradiated larvae therefore consists of sterile females. Another fact which confirms the establishment of the challenge infection is the course of the plasma pepsinogens (Fig. 33) which follows a similar pattern to that obtained following single inoculations of O. ostertagi larvae and described in Section II. The rather low numbers of worms found in infectivity control calves in Groups 5 and 6 are difficult to explain, but may be associated with the difference in age of these calves (8 weeks) and the challenge calves (22 weeks).

The results of these experiments, namely (1) the failure of immunodepressant drugs to initiate massive resumption of development of inhibited O. ostertagi and (2) the successful establishment of a superimposed infection over the existing inhibited population of O. ostertagi suggest that inhibition of O. ostertagi in the field is not necessarily associated with acquired immunity. The fact that the challenge infection of the laboratory larvae developed without apparently being affected by the host immune response

or disturbing the existing inhibited larvae may be due to two reasons. Either (a) that the laboratory and field strains of O. ostertagi, though morphologically similar, are antigenically different or (b) that inhibition of the larvae ingested at pasture was associated with changes which occurred in the larvae prior to ingestion by the host.

It is interesting that Ross and Dow (1964) describe an experiment in which calves grazed from spring until the early autumn on pasture known to be contaminated with O. ostertagi larvae were treated with an anthelmintic (haloxan) to remove the adult worm population; some of these calves were subsequently given a single inoculum of 2,000 O. ostertagi larvae followed 70 days later by another single inoculum of 50,000 to 500,000 larvae. At autopsy 42 days after the second inoculation the numbers of inhibited stages in the inoculated and non-inoculated calves were similar. It is probable that in this experiment the experimental infections may have also 'leap-frogged' the inhibited population of O. ostertagi larvae which resulted from the previous natural infections.

The results of the third experiment show that a fairly high percentage of early fourth stage larvae (19-37%, Table 25) were present in the worm burdens at autopsy of three out of five calves inoculated previously with 100 daily doses of 1,500 O. ostertagi larvae. In the other two calves less than ten per cent of the total worm burden at autopsy consisted of early fourth larval stages.

These findings agree with the results obtained by Michel (1963) following prolonged daily dosing of 1,500 O. ostertagi larvae to calves of a

Table 25

The Proportion of Early Fourth Larval Stages of Ostertagia ostertagi at Autopsy of Calves Following Prolonged Daily Inoculations of 1,500 Larvae Expressed as a Percentage of the Total Inoculum or Total Worm Population

Calf No.	Age at start of experiment (days)	Day Killed	Total Larvae Administered	Total O. ostertagi	Early 4th Larval Stages: Total	Early 4th Larval Stages: as % of Total Inoculum	Early 4th Larval Stages: as % of Total O. ostertagi
				Michel's Experiments*			
1193	147	42	63,000	34,000	3,000	5	9
1259	116	79	118,500	39,400	11,220	9	28
1296	96	85	127,500	37,520	5,570	4	15
1275	109	112	168,000	32,650	14,260	8	44
				Present Experiment			
12	135	107	150,000	7,900	2,900	2	37
21	135	107	150,000	8,600	1,600	1	19
23	135	107	150,000	6,200	2,200	1	35
49	121	107	150,000	15,000	1,300	< 1	7
56	121	107	150,000	5,300	500	< 1	9

*See Parasitology (1963) 53, p. 65, Table 1 (Groups A_1, A_2).

similar age to those in the present experiment. In Michel's experiment the calves killed on days 79, 85 and 112 had a similar proportion of their worm burdens inhibited at the early fourth larval stage as the calves in the present experiment (Table 25).

Although the proportions of early fourth stage larvae in the total worm burdens obtained in the present experiment and by Michel were similar, the absolute numbers of adult and larval stages were much increased in the latter. Indeed, the number of adults established in Michel's experiment were sufficient to cause the death of one calf after 85 doses of larvae. It seems logical to ascribe the increased worm burdens in Michel's experiment to the fact that his calves were fully susceptible whereas in the present experiment the calves had been previously exposed to O. ostertagi.

The present results largely confirm those of Michel (1963), i.e. that daily doses of O. ostertagi larvae over a prolonged period result in a high proportion of the established worm population becoming inhibited at the fourth larval stage. It cannot be concluded, however, that the cause of the inhibition was an immune response by the host or the presence of adult worms as postulated by Michel (1963). Although his conclusions may be correct, it is possible to propose an alternative hypothesis. This is based on the finding that in both experiments the proportion of larvae inhibited is a relatively small percentage of the total number of larvae administered (<2% in this experiment, 4-9% in Michel's - see Table 25). It may be that a small proportion of each dose of 1,500 larvae does not develop beyond the early fourth larval stage and that the total numbers of

larval stages found at subsequent autopsies are due to the accumulation of larvae inhibited after each dose, rather than to a larger proportion of larvae from later doses becoming inhibited by an immune response. The apparent increase in the proportion of early fourth stage larvae, when expressed as a percentage of the total worm burden, could be accentuated by an exponential loss of adult worms such as occurred in the experiments described in Section II.

C. Studies on the Association Between Physiological Changes in the Host or the Parasite and Inhibited Larval Development

Introduction

The dog ascarid, Toxocara canis, is probably the best known example of a nematode which largely depends on the normal physiological activities of its host animal for its own propagation. Prenatal infection of puppies is common and is probably the principal method by which this parasite is transferred to a new host (Sprent, 1958). Infective eggs ingested by an adult dog, usually hatch and the resulting larvae enter the systemic circulation and encyst in the tissues. If the host is a bitch, and subsequently becomes pregnant, the larvae are then activated, re-enter the circulation during late pregnancy, and cross the placental barrier to the foetal puppies in which they mature after birth. The physiological mechanisms leading to the release of the encysted larvae is not known but is thought to be under hormonal control. Thus, Oshima (1961) studied the migrations of T. canis in mice, and showed marked alterations in the behaviour of the larvae in mice approaching and after parturition and also alterations depending on whether the mouse was allowed to suckle her litter. He also showed a marked fall in the numbers of larvae recovered from the tissues of the mice after six days or more of prolactin injections. It would appear that this pituitary hormone triggered the release of many larvae from the mouse's tissues into the circulation. It is not unlikely that a similar host hormone, released during

late pregnancy, initiates the entry of T. canis larvae to the blood stream of the bitch.

Bull (1959) showed that in nature, female rabbits are more heavily infested with adult T. retortaeformis during the spring, when reproductive activity of the rabbit is at its peak. This observation is particularly interesting when it is remembered that T. retortaeformis is a parasite in which inhibited larval development has been reported (Michel, 1952) and it may be that inhibition and subsequent maturation of this parasite is allied to the reproductive activities of its host.

Leucocytozoon simondi, a haemosporidian parasite of ducks, is rarely found in circulating blood during the winter months but shows a 'relapse phenomenon' in spring associated with the duck's breeding season (Huff, 1942). Chernin (1952) also studied this phenomenon in housed ducks in which the probability of reinfection was prevented. He was able to control the time of onset of egg laying by altering the daily light stimulus to the ducks and showed that the relapse phenomenon remained chronologically associated with the onset of egg laying and occurred at the same time in the male ducks. Since the onset of egg laying is controlled by a neurosecretory mechanism acting by way of the anterior pituitary gland, he postulated that this release of gondotrophins also caused the sudden increase in L. simondi and gametocytes being released into the circulation.

Dunsmore (1965) studied the faecal egg output of ewes in New South Wales, Australia, and showed that a marked increase in the Ostertagia spp. faecal egg count occurred in pregnant ewes at or closely associated with parturition.

The course of these egg counts showed a double peak, a minor pre-parturient rise and a major one at or around parturition. It was also shown that it was unlikely that these ewes had themselves ingested many Ostertagia spp. larvae during the latter part of pregnancy and that the parturient rise in faecal egg count resulted mainly from the maturation of larvae ingested early in pregnancy and subsequently inhibited in their development. Concurrent observations on housed ewes which received repeated experimental inoculations of Ostertagia spp. during mid-pregnancy also showed that the peak egg output was delayed until parturition.

Furthermore speculated that pituitary hormones influenced the resumption of development of inhibited larvae thus resulting in a rise in faecal egg counts in sheep at the time of parturition. He suggested that pituitary hormones possibly acted as a trigger to the release of other hormones with a more direct action on the parasite.

The proportion of O. ostertagi larvae inhibited in their development following ingestion by the host is known to be increased in the late autumn and many of these inhibited larvae resume their development in the following spring (Andersen et al, 1965a). Since, in sheep, variations in the secretions of hormones controlled by the anterior pituitary are known to be associated with seasonal factors (Roberts, 1961), it is possible that both inhibition of O. ostertagi larvae in the late autumn and the subsequent maturation of these larvae in the spring could be associated with changes in the activity of the bovine anterior pituitary gland. If this is so, the mechanism influenced by the pituitary must be common to both sexes since inhibited development of O. ostertagi occurs in both male and female calves.

It is therefore interesting that Post (1965) in Australia, observed a positive relationship between secretion rates of the thyroid gland (under pituitary control) of grazing bovines and quality of pasture; and that Dobson (1966b) recorded stunting of Amblyomma roberts, in thiouracil (thyroid inhibitor) treated mice.

The possibility that nutrition of the host may influence the development of larval inhibition of O. ostertagi has been suggested by Ross (1965). He based his suggestion on the fact that an increase in the number of early fourth larval stages of O. ostertagi had been noted in cattle on overgrazed pasture of poor nutritional value in South-east U.S.A. (Gordia et al, 1962) and in Northern Ireland (Ross, 1965).

The combination of poor nutrition and severe climatic conditions in the late autumn may result in calves, grazing at that time, being subjected to severe stress. There are several published examples of the relationship between stress and parasitism (Noble, 1961, 1962; Mathies, 1962; Oliver, 1962; Vermberg and Vermberg, 1963). Of particular interest is the study of stress induced by cold and its effect on populations of Syphacia citelli in the ground squirrel (Noble, 1966); squirrels stressed by exposure to cold harboured significantly higher worm burdens than control animals.

The first suggestion that inhibited development of a nematode in a susceptible host could be associated with physiological changes in the nematode itself was made by Anderson et al (1965b), (see Section I, B). In these experiments, high proportions of inhibited O. ostertagi fourth stage larvae were found at autopsies of calves which grazed in the late autumn for short periods of one to 14 days. Prolonged inhibition of larval

development in the egg prior to hatching has, of course, been demonstrated in Nematodirus battus (Thomas, 1959). It is generally considered that this delay is due to the non-hatching of N. battus eggs and is thought to be associated with the absence of the essential stimulus to hatching, at the time the eggs are deposited on the pasture, i.e. in the late spring, and will only occur after exposure to winter temperatures (Thomas and Stevens, 1960). It may be that the non-hatching of N. battus eggs in the summer and autumn and inhibited development of O. ostertagi larvae ingested in the autumn are part of the same biological phenomenon.

The actual mechanism by which such a phenomenon operates is not known but it is possible that a similar process to that operating in diapause in insects is involved. Thus, many of the cyclical diapause phenomena of insects are in fact induced by seasonal changes of diet, temperature and length of day; the occurrence of diapause in other instances may be determined genetically and is often associated with a fixed number of generations, i.e. voltinism (Wigglesworth, 1965).

So far four possible mechanisms have been suggested to account for inhibition of larval development as a consequence of physiological changes in the host or parasite. Three of these (hormonal, nutritional or stress) involve alterations in the physiology of the host; the fourth incriminates physiological changes in the free living stages of the parasite, particularly during the late autumn.

On reflection, two other theories may be suggested; both are hypothetical but do not conflict with the evidence available on the mechanism of inhibition.

The first is that inhibition of larval development is caused by an immune response of the host acting on the developing eggs in the ovaries or uterus of the female worm; the effect of this immune response is to inhibit the subsequent development of these eggs when they become fourth stage larvae in the host. Although this is basically an immune effect it would appear in calves grazed for only short periods on ground contaminated by 'permanent' calves, and could be interpreted as a 'physiological' effect.

The second possibility is that only a proportion of larvae in the pasture population are susceptible to the factors that produce inhibition. The magnitude of this proportion may vary greatly, either in laboratory passaged cultures or in naturally occurring populations.

The experiments described in this section extend over two years and were designed to investigate the significance of the factors reviewed above, in the production of inhibited larval development of O. ostertagi.

Experimental Design

1965 Experiments

These were carried out on paddocks, 1,500 square yards in area, in the grounds of the Glasgow University Veterinary Hospital. The pastures had not been grazed for over four years and were therefore considered for all practical purposes to be parasite free. Some of the paddocks were contaminated at various times of the year by grazing with 'seeder' calves, inoculated previously with O. ostertagi larvae cultured in the laboratory;

the other paddocks were grazed with parasite free calves. This was done in an attempt to keep the nutritional status of all the paddocks similar.

At the end of October, groups of four parasite free calves aged ten weeks were introduced to each of the paddocks and treated as outlined in Table 26. Four other calves were grazed for 14 days at Farms A and D in Ayrshire as previously described and four calves remained housed during the entire period of the experiment. At the end of the 8-day grazing period, calves were housed for ten days prior to autopsy. The experimental design is summarised in Table 26.

These experiments were designed to find if the proportion of inhibited larval stages found at autopsy in calves grazed in late October was a function of

(a) seasonal changes in the physiology of the host in which case a high proportion of inhibited stages would be present at autopsy of all the 'tracer' calves, including the housed calves,

(b) an environmental influence on the host at pasture, e.g. nutritional or weather, by comparing the proportion of inhibited larvae in the worm burdens of housed calves and calves at pasture, both of which were given oral inoculations of O. ostertagi larvae in the late autumn,

(c) an environmental influence on the developing larval stages or infective larval stages by comparing the proportion of inhibited stages present at autopsy of calves from paddocks A, B, C and D, which had each been contaminated at a different time during the summer or autumn by calves grazing for up to 28 days, i.e. less than the generation time of O. ostertagi.

Table 27

Plan of 1966 Experiments Designed to Investigate the Cause of Inhibited Larval Development of Ostertagia ostertagi

Paddock	Source of Infection Grazing History	Treatment of Grazer Calves Grazed from 20.10.66 to 1.11.66
A	Overwintered Larvae from July infection in 1965. Grazed by 3 'seeders' from 17.5 to 6.9.66	Nil
D	Overwintered Eggs from October infection in 1965. Grazed by 3 'seeders' from 17.5 - 6.9.66	Nil
E	Overwintered Eggs & Larvae from Plot grazed July-Sept.(1965). Grazed by 3 'seeders' from 17.5-6.9.66	Nil
G	Grazed by 3 'seeder' calves Inoculated at 10 & 21 days prior to grazing with 100,000 O. ostertagi. Grazed 17.5-6.9.66	Nil
H	Grazed by 3 'seeder' calves Inoculated at 10 & 21 days prior to grazing with 100,000 O. ostertagi. Grazed 21.4-10.5.66	Nil
I	Grazed by 2 'seeder' calves which grazed at Farm A in Ayrshire from 3.5-17.5.66, then transferred to this plot to graze from 17.5-6.9.66	Nil
J	Grazed by 3 Parasite Free calves 17.5-6.9.66	Orally inoculated with 5,000 O. ostertagi 3rd stage larvae daily for 12 days.

(d) a diapause effect, or an immune effect mediated through the developing egg to the larvae, by comparing the proportion of inhibited stages present at autopsy of calves from paddock E (which had been grazed for nearly three months and presumably had second or third generation eggs deposited on it) with those from other paddocks,

(e) a strain difference by comparing the proportion of inhibited stages present in the worm burdens of calves which grazed paddocks contaminated with the laboratory strain, i.e. A, B, C, D, E, with that present in calves which grazed in Ayrshire.

1966 Experiments

The experiments in 1966 were a continuation of the 1965 experiments and utilised certain of the 1965 paddocks as well as other parasite free paddocks. The experimental design, which was based on the 1965 results, is outlined in Table 27.

These experiments were designed first to confirm the 1965 findings, namely that the natural strain of O. ostertagi in Ayrshire gave rise to a higher proportion of inhibited larval stages in calves grazing in the late autumn than the laboratory strain used at Glasgow Veterinary School; this was done by comparing the proportion of early fourth larval stages present at autopsy of 'tracer' calves grazing paddocks G and I. Secondly, to see if the proportion of inhibited stages in 'tracer' calves would increase when the laboratory strain of O. ostertagi was allowed to develop under natural conditions and to observe if overwintering of eggs or larvae, or age of the

larvae, increased inhibition potential of this strain; this was done by comparing the proportion of early fourth larval stages present at autopsy of naturally infected groups (A, D, E and H) with those of group J, which was given an experimental oral inoculum of larvae prepared in the laboratory.

Observations

The 'seeder' calves and autumn 'tracer' calves were observed clinically on each day of the experiment. Faecal samples were collected weekly from the 'seeder' calves for faecal egg count examination by zinc sulphate, flotation and McMaster methods. At autopsy the abomasums and contents of 'seeder' and 'tracer' calves were examined for the presence of O. ostertagi as previously described.

Results

In both 1965 and 1966 the experimental paddocks were heavily contaminated with O. ostertagi eggs. Thus, the 'seeder' calves in 1965 and 1966 all developed clinical Type I ostertagiasis, with the exception of two calves in paddock A in 1966. In 1965, the mean faecal egg counts of the groups of three 'seeder' calves which grazed at different seasons, ranged from 500 e.p.g. to 2,500 e.p.g. In 1966, the mean faecal egg counts of the calves inoculated prior to grazing, ranged from 400 e.p.g. to over 3,000 e.p.g. In paddocks A, D, E and I, where the source of infection was natural, the faecal egg counts of the calves remained low until July, after which time they steadily increased reaching a maximum at the end of August

and, except for the two calves which did not develop clinical signs, exceeded 1,000 e.p.g.

The O. ostertagi worm counts at autopsy of the 'seeder' calves ranged from 26,000 to 110,000 and the percentage of early fourth larval stages present from one to 15. As these worm burdens are not particularly relevant to the object of these experiments, they have not been tabulated. The total O. ostertagi worm counts at autopsy of the 'tracer' calves in 1965 and 1966 and the percentages of early fourth larval stages are given in Tables 28 and 29.

The results of the 1965 experiments showed that a high proportion of O. ostertagi early fourth larval stages was present at autopsy only in the calves grazed at the Ayrshire farms (60 - 67%). On the paddocks contaminated with the laboratory strain of O. ostertagi the proportion of larval stages was higher in the 'tracer' calves which grazed the paddocks infected in July (17 - 25%) than in the calves which grazed the paddocks contaminated in August and September (4 -10%). Only negligible numbers of O. ostertagi were present in the paddock seeded in October, indicating that little or no development of eggs had taken place at that time. A few early fourth larval stages were present in the calves infected orally (1 - 3%).

The results of the 1966 experiments again demonstrated that a high proportion of early fourth larval stages was only present at autopsy of calves which had grazed the paddock infected with the Ayrshire field strain of O. ostertagi. The calves from paddocks originally contaminated with O. ostertagi larvae cultured in the laboratory (paddocks A, D, E, G and H) had

Table 26

Mean Total and Range of Ostertagia ostertagi Worm Burdens and Percentage of Early Fourth Larval Stages at Autopsy of Groups of Four 'Tracer' Calves which Grazed from 21 to 29 October, 1965.

Paddock	Date Infected with O. ostertagi by 'Seeder' Calves	O. ostertagi Worm Burdens: Total Mean	Total Range	Percentage Early 4th Larval Stages Mean	Percentage Early 4th Larval Stages Range
A	July 1-25	16,200	11,800-27,000	22	17 - 25
B	August 6-31	5,400	4,700- 6,600	7	4 - 10
C	September 2-27	3,200	800- 4,400	6	2 - 13
D	October 1-21	< 100	0- 200	0	0
E	July 1 - September 27	10,700	4,100-21,000	12	7 - 15
F	Tracer calves given orally 5,000 O. ostertagi larvae per day while at pasture 21-29.10.65	6,100	5,600- 6,900	2	1 - 3
	Housed calves given orally 5,000 O. ostertagi larvae per day between 21-29.10.65	6,100	3,200- 6,700	2	1 - 3
	Group of 4 Tracer calves which grazed Farms A & B from 21.10-4.11.65	9,400	2,000-20,400	64	60 - 67

*Tracer = Calves reared worm free, grazed for short periods and autopsied at least 7 days later

Table 29

Mean Total and Range of Ostertagia ostertagi Worm Burdens and Percentage of Early Fourth Larval Stages of Groups of Four Tracer Calves Grazed from 20th October, 1966, to 1st November, 1966.

Paddock	Source of O. ostertagi infection	O. ostertagi Worm Burdens			
		Total		Early 4th Larval Stages	
		Mean	Range	Mean	Range
A	Overwintered laboratory larvae from 1965. Cycled by 'Seeders' from 17.5 - 6.9.66	9,900	8,000-10,900	11	10 - 11
D	Overwintered laboratory Eggs from 1965. Cycled by 'Seeders' from 17.5 - 6.9.66	6,000	3,300- 8,000	13	9 - 17
E	Overwintered laboratory Eggs & larvae from 1965. Cycled by 'Seeders' from 17.5 - 6.9.66	4,700	3,400- 6,100	21	16 - 28
G	First Infected on 17.5.66 by 'Seeders' inoculated with laboratory strain of larvae. Cycled until 6.9.66	6,000	5,400- 7,000	16	13 - 19
H	First Infected on 21.4.66 by 'Seeders' inoculated with laboratory larvae. Grazed until 10.5.66	1,600	1,000- 2,600	18	8 - 23
I	First Infected 17.5.66 by 'Seeders' grazed at Ayrshire from 3.5.66. Cycled until 6.9.66	9,500	6,300-11,400	77	73 - 85
J	Tracer Calves Given Inoculum of 5,000 O. ostertagi Daily from 20.10.66 - 1.11.66	17,500	13,000-22,400	< 1	0 - 2

*Tracer = Calves reared worm free, grazed for short periods and autopsied at least 7 days later

a low proportion of early fourth larval stages (8-20%) at autopsy while in the calves at grass which were given 12 daily doses of 5,000 O. ostertagi larvae less than 1% of the worm population was in the early fourth larval stage.

Examination of adult male O. ostertagi from the calves which had grazed paddocks contaminated with either the Ayrshire or laboratory strain and from the calves which had been given larvae cultured in the laboratory showed that there were no morphological differences between these populations.

Discussion

A high proportion of inhibited fourth stage larvae was only present in autumn 'tracer' calves which had ingested larvae developed under natural conditions (see Tables 28 & 29); in those calves given larvae cultured in the laboratory and administered orally the proportion of larvae inhibited was negligible. There was no correlation between the total numbers of O. ostertagi and the percentages of inhibited fourth stage larvae found at autopsy of the 'tracer' calves (see Tables 28 & 29). It therefore appears that, under natural conditions, inhibition of development of O. ostertagi does not depend solely on factors involving the host, e.g. nutrition, environment or hormonal status. More likely it is associated with physiological changes in the larvae themselves or a combination of these changes and alterations in the host physiology in late autumn.

A comparison of the low degree of inhibition obtained from infections with the laboratory strain of O. ostertagi which developed at pasture, with the high

degree shown by the field strain both in calves at Farms A and B in 1965 and on the paddocks at the Veterinary Hospital in 1966, suggests two possibilities. First, that two strains of O. ostertagi exist, one susceptible and one relatively insusceptible to the factors which produce inhibition. Alternatively, that the laboratory culture techniques or repeated passage through housed experimental stock has largely eliminated the original proportion of the Ostertagia population susceptible to inhibition. It is interesting that Whitlock (1966) has emphasised the need for studying host/parasite relationships in their natural environment and suggested that removal of the host or parasite to the laboratory environment may remove at least some of the signals for population control mechanisms, such as inhibited larval development.

The precise nature of this type of larval inhibition has yet to be elucidated, but the results of the present experiment suggest that it is particularly associated with changes in the infective larvae in the late autumn and possibly also a host mechanism involving recognition of these changes in the larvae. One is tempted to speculate that this situation may be akin to the diapause mechanism which operates in insects. According to Wigglesworth (1965) the main factors inducing diapause are diet, temperature and length of day. The actual physiological mechanisms are unknown but there are two theories; first, the temporary absence of hormones necessary to maintain growth or secondly the accumulation of some chemical constituents in the body which inhibit growth. These two hypotheses are not, of course, mutually exclusive, and it is possible to imagine a diapause factor, chemical or otherwise, inhibiting the secretion of growth hormones. It is known

(Harvey, 1962) that the cuticle of diapausing insects is impermeable to many substances and it has been suggested that the substitution of permeability barriers in place of active regulation is an energy conserving device (Telfer and Williams, 1960) that contributes to the low metabolic rate of diapausing insects. As will be seen in Section V, normally developing early fourth stage O. ostertagi larvae are susceptible to the anthelmintic thiabendazole but inhibited early fourth stage larvae are not so; this may be another indication that inhibited larvae are 'in diapause' and unable to absorb the thiabendazole through their cuticles.

General Discussion

The results in this section have shown that the massive inhibition of larval development of O. ostertagi, which occurs in the field (Martin et al, 1957; Anderson et al, 1965a; Ross, 1965), is a complicated phenomenon and that more than one mechanism may be responsible for its occurrence.

Irrespective of the mechanism involved, it is clear that under natural field conditions, at least in the West of Scotland, the proportion of larval stages which become inhibited in their development at the early fourth stage increases in the late autumn and early winter. This increase is not apparently dependent on a large larval intake or a prolonged grazing period.

Thus, inhibition of larval development was not a feature of the worm burdens of 'tracer' calves which grazed during early autumn at a time when 'permanent' calves were dying from Type I ostertagiasis and the larval intake from the pasture was obviously high; on the other hand, inhibition of larval development was a feature of worm burdens of 'tracer' calves which grazed in the late autumn when the larval intake from the pasture was apparently low, as susceptible 'permanent' calves which also grazed at that time, showed no clinical signs of ostertagiasis.

With regard to the length of the grazing period, the proportion of early fourth stage larvae found in the worm burdens of 'tracer' calves which grazed for either one or fourteen days in the late summer was similar, as was the proportion found in calves which grazed for either one or fourteen days in the late autumn.

The cessation of development at the fourth larval stage of O. ostertagi found in calves which had grazed for short periods was observed to persist for

at least 27 days in one experiment (Table 16) and 49 days in another (Table 23). Although no absolute proof was obtained that these larvae would eventually have matured, there is little doubt that a proportion would, from an analogy with the situation in the field (see Table 5, Section I).

In view of the results obtained in this section, it is necessary to reconsider whether the immune response of the host plays the major role in the production of larval inhibition in bovine ostertagiasis; this was first suggested by Martin et al (1957) on the basis of results obtained from natural infections and later substantiated by the results from experimental infections (Michel, 1963). In the latter experiments, daily doses of 1,500 O. ostertagi larvae were administered over a prolonged period (318 days) and in those calves autopsied after 100 days there was a marked increase in the proportion of the worm burden inhibited at the early fourth larval stage. Michel postulated that this increase was due to a higher proportion of the later doses of larvae becoming inhibited in their development due to acquired resistance of the host. An alternative hypothesis is that a fixed, but relatively low proportion of larvae ingested at any one time become inhibited in their development. If the latter theory is correct, it is interesting that in Michel's experiments the proportion of inhibited fourth stage larvae present at autopsy and expressed as a percentage of the inoculum was 4 to 9%, whereas in the author's experiments the percentage of inhibited stages calculated in a similar manner was less than 2% (see Table 25). This difference in the proportions of inhibited stages present may be due to two reasons.

First, that the infectivity of the larvae used by Michel and the author differed. Madsen (1962) discussed inhibition of larval development in

nematodes and postulated that inhibition is dependent on an interplay of factors involving both worms and host, including the viability and infectivity of the ingested larvae. It is therefore interesting that Michel (1963) used a technique for preparing his larval inoculum of O. ostertagi which involved a process of double passage through a Baermann apparatus whereas the author used only a single passage (see Materials & Methods, p.12). Conceivably, the larvae used by Michel were partially exhausted by the extra passage through the Baermann apparatus and were more easily inhibited by the host's immune response.

Secondly, it may be that only a low proportion of each larval inoculum becomes inhibited due to immune processes and since Michel (1963) was working with a recently isolated field strain the capacity for inhibition still existed in a higher proportion of the larval inoculum used. The fact that some resumption of development of inhibited stages occurred in calves treated with cortisone (Table 21) could be interpreted as supporting this theory.

The establishment and subsequent 'leap-frogging' of an experimental challenge inoculum of O. ostertagi larvae over an existing population of inhibited fourth stage larvae (Experiment 2, Part B) appeared, at first, to be further evidence that larval inhibition occurs independently of the host's immune status. However, the results of this experiment must be interpreted with caution as the challenge inoculum of O. ostertagi used (laboratory strain) may have been antigenically different from the inhibited larvae which had resulted from a natural infection.

Before finally rejecting the hypothesis that the immune response of the host plays the major role in inhibited larval development in the host/parasite relationship under discussion, one other possibility should be considered. Namely, that since little or no development of O. ostertagi eggs to the infective larval stage takes place after September (Section I, Part C; Michel, 1966) the population of infective larvae on the pasture in late autumn is an 'ageing' one that can be more easily inhibited by the host's immune response. These aged larvae are 'weak' and unable to climb blades of grass but due to the short nature of the pasture at the late autumn are taken in by the grazing calves. In the spring, however, when growth of grass has recommenced, only the stronger larvae will climb the blades of grass to be ingested by the calves and the weaker 'inhibition-prone' larvae will remain in the mat at the bottom of the grass blades. This could explain why a high proportion of the larvae ingested in the autumn become inhibited whereas the survivors of the same population of larvae do not become significantly inhibited when ingested in the spring. Some evidence for the first part of this hypothesis is provided in the results of the 1965 'paddock' experiments; in these the highest proportions of inhibited larvae were found in calves which had grazed on the paddocks contaminated for the longest time (Table 28), and presumably had ingested the oldest larvae.

The demonstration of two different strains of O. ostertagi which are apparently morphologically identical, is particularly interesting. The larval population of one (a naturally occurring strain) is extremely susceptible to the factors producing inhibition, particularly in the late autumn, whereas the larvae of the other (a laboratory passaged strain) are relatively insusceptible.

It is perhaps worth recording that, using the laboratory strain of O. ostertagi, Armour et al (1964) and Andersen et al (1966) found extremely few inhibited fourth stage larvae at autopsy of calves 21 days after a single inoculation of 800,000 larvae or four weekly inoculations of 100,000 larvae. The question of whether the strains have always been different or whether the population susceptible to inhibition has been eliminated following repeated passage of the one strain in the laboratory, remains to be elucidated.

Strain differences in other gastro-intestinal nematodes, namely, H. contortus and O. circumcincta, in terms of cultural requirements, have previously been demonstrated by Crofton, Whitlock and Gaasar (1965) and Crofton and Whitlock (1965).

Although the mechanism of massive inhibition of O. ostertagi at the early fourth larval stage in the late autumn has not been solved, it appears to have many superficial analogies to the phenomenon of diapause in insects (Wigglesworth, 1965). Thus, diapause, a phase of arrested development, may affect the larval stages of insects; in some species it characteristically affects a particular stage. The inclination to diapause is frequently present in small proportions of the population even when the environment is optimal. On exposure to adverse environmental circumstances, e.g. falling temperature, this proportion is greatly increased. Once diapause has occurred it is not necessarily terminated by the restoration of a suitable environment for development, e.g. the host. Finally, although the occurrence of diapause is regulated in large measure by environmental factors, the capacity to respond seems to be dependent on the genetic constitution of a particular strain.

Possibly the initial 'trigger' to diapause affects the infective larvae

prior to ingestion in the late autumn, while the 'trigger' to the inhibited larvae in the gastric glands to recommence development in the spring is associated with seasonal hormonal changes in the host. This theory also offers an explanation for the occurrence of the spring rise in faecal egg counts of sheep and is not inconsistent with the hypothesis of Dunsmore (1965).

Although some evidence is available that arrested development of Cooperia spp. in cattle (Section I, p.60) and Ostertagia spp. in sheep (unpublished data) may also result from physiological changes in the larvae in late autumn, in other host/parasite relationships inhibition due to such 'physiological' changes in the pre-parasitic stages has not been incriminated. Indeed there are several published experiments in which the evidence points strongly to the immune response of the host as the principal factor in the production of larval inhibition (Donald et al, 1964; Dineen, Donald, Wagland and Offner, 1965). Thus, Dineen et al (1965) have recently described a situation in which inhibition of H. contortus at the fourth larval stage occurred in sheep following repeated dosing with larvae. Since inhibition, for all practical purposes, did not occur in sheep which received the same total number of larvae in a single dose, it was concluded that inhibition in this case was caused by an immune response.

However, it is clear that the two factors necessary in nature for the production of large populations of inhibited O. ostertagi are (1) a strain of larvae susceptible to inhibition and (2) the environmental circumstances of late autumn which appear to produce the optimal changes in the larvae for their subsequent inhibition when ingested by the host. The exact part played by the immune response of the host remains to be elucidated.

Summary

1. Susceptible worm free calves were grazed during spring, summer and autumn for periods of either one day or 14 days on pasture known to be contaminated with O. ostertagi larvae. At autopsy, seven days after removal from pasture, the proportion of the worm burden which consisted of inhibited fourth stage larvae was significantly higher in calves which had grazed during autumn; the proportion was the same in calves which had grazed for either one day or 14 days. The duration of inhibited larval development in calves grazed for short periods was shown to be at least 27 days.

2. Calves known to be harbouring large numbers of inhibited fourth stage O. ostertagi larvae were (a) treated with large doses of cortisone or methotrexate or (b) given a challenge inoculum of 100,000 normal or X-irradiated O. ostertagi third stage larvae. The administration of the drugs did not result in the resumption of development of significant numbers of inhibited larval stages, though some increase in numbers of developing stages was noticed following cortisone therapy. The challenge inocula of either normal or irradiated larvae developed to the adult stage in 21 days and apparently 'leap-frogged' the inhibited larvae in the gastric gland.

3. A daily oral inoculation of 1,500 O. ostertagi infective larvae was given for 100 days to each of five calves previously exposed to an experimental infection of O. ostertagi. At autopsy, seven days after the last dose, a high proportion of the worm burdens present in three out of the five calves

was inhibited in development at the fourth larval stage. It was suggested that this was due to the accumulation of a low and fixed proportion of each dose becoming inhibited and that the high proportion of early fourth stage larvae found at autopsy was accentuated by the exponential loss of adult worms.

4. Susceptible calves were grazed in late autumn on paddocks contaminated at various times of the year with either a laboratory strain of O. ostertagi eggs or a strain obtained from a farm in the West of Scotland. Further susceptible calves were grazed on a worm free paddock and inoculated orally with O. ostertagi larvae cultured in the laboratory. Inhibition of larval development did not occur in the calves inoculated orally but was present in all of the calves which ingested larvae developed at pasture. A significantly higher proportion of inhibited fourth stage larvae was found in the worm burdens of calves which grazed on the paddock contaminated with the field strain.

5. It was concluded that larval inhibition in naturally occurring bovine ostertagiasis is probably dependent on two factors (a) an innate physiological susceptibility of a particular strain of larvae (b) the environmental circumstances of late autumn which, acting on developing or infective larvae, produce an optimal stimulus for the subsequent inhibition of ingested larvae.

SECTION IV

IMMUNITY TO OSTERTAGIASIS

A. Age Immunity

B. Acquired Immunity - the Immunity Resulting from Naturally Acquired Infections

C. Acquired Immunity - the Immunity Resulting from the Administration of Irradiated Larvae

General Introduction

Over the past two decades there has been a steadily increasing recognition of the importance in the epidemiology of helminthic disease of the immune reactions of the host. Prior to 1957, there was only one comprehensive review of helminth immunity of direct interest to the veterinarian, namely, that by Culbertson (1941). Since 1957, numerous excellent reviews of work on helminth immunity have been published, notably those by Soulsby (1957, 1958, 1960, 1961), Stewart (1959) and Urquhart, Jarrett and Mulligan (1963). The bulk of the work reviewed by these authors may conveniently be divided into three sections.

First, that dealing with the studies on antigen-antibody reactions. These have involved the use of not only the conventional quantitative serological techniques but also observations on the *in vitro* effect of immune serum on parasites and the more sophisticated qualitative techniques of gel diffusion and immuno-electrophoresis. Although the ultimate aim of this type of work is to determine which of the parasite antigens give rise to protective antibodies, progress towards this goal has been slow as the antigenic structure of the helminths is extremely complex.

Secondly, observations on the cellular manifestations of the host's immune reaction to the invading parasite. There is no doubt that helminths in tissues can be directly attacked by cells, the reaction often being similar to that seen in a foreign body reaction with the appearance of multinucleated giant cells. Eosinophil leucocytes are also frequently present in large numbers and are thought to be closely involved with the cellular

reactions to invading parasites. Perhaps the most significant observations were those of Jarrett and Sharp (1963), who noticed the development of foci of antibody producing cells (haemocytoblasts, immature and mature plasma cells) in the lungs of calves within five days of re-infection with Dictyocaulus viviparus. The exact function of the antibody produced by these foci has not been determined but their presence shows that invading larvae will stimulate the production of antibody producing cells.

Thirdly, the effect of immune reactions on the parasite. This effect has been demonstrated in one or more of four ways: (a) by the reduction in the total numbers of adult worms, (b) a decrease in size or biotic potential (egg-laying) of mature worms, (c) inhibition of larval development, (d) the self-cure phenomenon. The first three of these ways are frequently used to demonstrate the acquisition of acquired immunity of a host to a parasite, e.g. O. ostertagi in the calf (Michel, 1963) or N. spathiger in the sheep (Donald et al, 1964).

The fourth, the self-cure reaction, has been demonstrated in several nematode infections but is best known in the case of H. contortus in the sheep (Stoll, 1929; Stewart, 1950) and N. brasiliensis in the rat (Africa, 1931; Mulligan et al, 1965). The self-cure reaction in H.contortus infection occurs when a challenge dose of infective larvae is superimposed on an existing infection in a sensitised sheep. This results in elimination of the existing burden of adult worms and is associated with a rise in titre of complement fixing antibody and in the blood levels of histamine. This is different from the situation with N. brasiliensis where the self-cure reaction occurs following a primary infection, spontaneous elimination of

the worm burden taking place between the tenth and twentieth day of infection. A similar loss of O. ostertagi adult worm population was shown to occur between the 17th and 35th day of the primary infection (Section II). It may be that the mechanism of immunity in ostertagiasis is associated with a self-cure type of reaction similar to that described in N. brasiliensis by Barth, Jarrett and Urquhart (1966).

Although much of the work on helminth immunity described above is of an academic nature involving basic immune mechanisms, two points of prime practical importance have emerged. First, that immunity to helminths does develop under natural conditions, e.g. in cattle to the stomach worm, H. placei (Roberts et al, 1952), the intestinal worm, Cooperia punctata (Bailey, 1949) and the lungworm, D. vivipurus (Taylor, 1951; Jarrett, McIntyre, Urquhart and Bell, 1955). Secondly, in order to produce a good degree of immunity it is necessary for the host to have had some experience of the live parasite; this has become apparent from repeated failures to immunise animals with dead materials.

Jarrett, Jennings, McIntyre, Mulligan and Urquhart (1957) therefore investigated the possibilities of attenuating D. vivipurus larvae so that they would develop for long enough to exert an antigenic effect unaccompanied by any significant pathogenic effect. This was achieved by irradiating the larvae with X-rays. It is now known that administration to calves of two doses of irradiated D. vivipurus larvae at an interval of one month confers a high degree of immunity against parasitic bronchitis and this form of immunisation is available commercially (Dictol, Allen & Hanburys Ltd., Ware, Hertfordshire, England).

Attempts to produce similar vaccines against gastro-intestinal nematodes in sheep have met with mixed success. Thus Jarrett, Jennings, McIntyre, Mulligan and Sharp (1959, 1961) obtained a high degree of protection against a single challenge dose of H. contortus following vaccination with irradiated larvae of this species. The sheep used in these experiments were over 7 months old. When young lambs (2-3 months old) were similarly immunised, no protection was obtained (Urquhart, Jarrett, Jennings, McIntyre, Mulligan and Sharp, 1966). Using normal larvae, Menton, Peacock, Poynter, Silverman and Terry (1962) also demonstrated an inability of lambs two to four months old to become immune to H. contortus, whereas those over nine months did so readily. It is possible, therefore, that age of the host may play an important part in the development of immunity to gastro-intestinal nematodes.

The objects of the present experiments with O. ostertagi in calves were (a) to see if age of the host per se influences the degree of immunity, (b) to test, using an experimental challenge, the immune status of calves which grazed pasture contaminated with O. ostertagi and were then housed for several months, (c) to examine the part, if any, played by the self-cure reaction in the immune response of the calves in (b), and finally (d) to attempt to immunise calves against ostertagiasis using irradiated O. ostertagi larval vaccines.

A. Age Immunity in Ostertagiasis

Introduction

During studies in Queensland, Australia, on the epidemiology of parasitic gastro-enteritis of cattle, Roberts et al (1952) observed that cattle developed a strong acquired resistance to gastro-intestinal nematode infections during the first 18 months of life. These authors also stated that the age at which such a resistance developed varied with different nematode species, e.g. most animals became resistant to infestation with Cooperia spp. at about five months of age whereas resistance to O. ostertagi did not develop until several months later. The likelihood of the age of the host influencing the development of resistance to O. ostertagi is strengthened by the observations made in Section I of this thesis that clinical ostertagiasis had not been recorded in animals over two years old during a three year study of field outbreaks of parasitic gastro-enteritis. Whether resistance to bovine ostertagiasis is associated with the age of the host per se or is dependent on a combination of acquired and age immunity, has yet to be determined. In U.S.A. outbreaks of clinical bovine ostertagiasis have been recorded when adult stock are moved from areas where O. ostertagi is not endemic to areas where outbreaks are frequently recorded (Bailey and Henrich, 1953; Andrews et al, 1953; Bailey and Thorson, 1954), and these authors are convinced that immunity to ostertagiasis is dependent on previous exposure to the parasite rather than to age.

Henrich (1960) carried out laboratory and field studies on age resistance

of cattle to nematodes of the gastro-intestinal tract (including O. ostertagi) and made the following observations. First, that adult cattle were generally more resistant to the debilitating effects of nematodes than calves. Secondly, that age per se did not influence either the pre-patent period, the size of the mature worms or the egg production of the worms. Thirdly, that age of the animals did not affect rate of larval development, i.e. there was no significant inhibition of larval development. The adult cattle used by Herlich were 18 to 25 months old.

The present experiment was designed to study the influence of the age of the host on the numbers established and the development of O. ostertagi following a single large experimental inoculation.

Experimental Design

Seven Ayrshire bullocks (castrated males) aged two and a half years and housed permanently from birth were obtained from an experimental farm in the West of Scotland. Four of the bullocks (Group 1) were inoculated orally with 300,000 O. ostertagi third stage larvae, the remaining three bullocks being used as uninfected controls (Group 2). It was intended to autopsy all seven animals 21 days after inoculation but as the faecal egg counts of the inoculated animals remained negative up to this date, only two of the inoculated group were autopsied on that date along with the uninfected bullocks. The remaining two animals were autopsied on day 28.

At the same time, five Ayrshire male calves (Group 3) reared worm free and four months old were inoculated orally with 300,000 O. ostertagi larvae

and autopsied 21 days later. (These were the same calves as in Group G1 of Experiment 2 in Part C of this Section.)

Observations

The calves were examined clinically on each day of the experiment. Faecal samples were collected on day 0 and day 16 and thereafter daily for faecal egg count examination. At autopsy, each abomasum and its contents was examined for O. ostertagi as previously described and the pH of the abomasal contents was estimated.

Results

The adult bovines in Groups 1 and 2 showed no clinical signs during the course of the experiment, whereas the young calves in Group 3 developed signs of clinical ostertagiosis, i.e. weight loss, diarrhoea, anorexia, from day 17 onwards.

At autopsy, the pH of the abomasal contents of the bullocks in Groups 1 and 2 were within the normal range, i.e. between 2.0 and 3.5, whereas those of the calves in Group 3 were markedly elevated and ranged from 6.5 to 7.2.

Faecal egg counts from Groups 1 and 2 remained negative throughout the experiment. In Group 3, the faecal egg counts first became positive on day 17 and increased to over 2,000 o.p.g. by day 20.

The O. ostertagi worm counts at autopsy of the three groups are shown in Table 30. Neither worms nor abomasal parasitic lesions were present in

Table 30

Worm burdens at Autopsy of Adult and Young Male Bovines Following Inoculation of 300,000 Ostertagia ostertagi

	Group	Day Killed	Total	O. ostertagi Worm Burdens			
				Mature Adults	Immature Adults	Developing Stages	Early Fourth Larval Stages
1	Adult Males	21	0	-	-	-	-
	Not Inoculated	21	0	-	-	-	-
		21	100	100	0	0	0
2	Adult Males	21	46,600	2,000	44,500	0	100
	Inoculated	21	46,200	5,000	40,300	300	600
		28	56,400	1,400	54,600	200	200
		28	50,400	5,100	45,200	0	100
3	Young Male Calves	21	78,600	36,600	41,000	0	400
	Inoculated	21	105,000	35,200	64,000	0	5,600
		21	125,600	114,300	11,000	0	300
		21	49,400	46,100	3,000	0	300
		21	157,000	150,000	7,000	0	0

two of the uninfected bullocks of Group 2, but 100 O. ostertagi and a few parasitic nodules were found in the abomasum of the other bullock. It seems therefore that despite being housed throughout their lives, some of the bullocks had acquired light nematode infections.

The O. ostertagi worm burdens in Group 1 ranged from 46,200 to 56,400 and consisted almost entirely of fifth larval stages still located in the gastric glands. Few adult males or females were present, even in the bullocks autopsied on day 28.

In Group 3, the worm burdens at autopsy ranged from 49,400 to 157,000 and consisted mainly of mature adult O. ostertagi which had emerged from the gastric glands.

Discussion

It is apparent from the results of this experiment, that following inoculation of 300,000 O. ostertagi infective larvae there are considerable differences in both clinical response and parasitological findings between young male parasite free calves and adult males with minimal previous experience of the parasite. These results are contrary to the findings of Herlich (1960) though it should be remembered that the adult animals used in the present experiment were about a year older than Herlich's animals.

The difference in clinical response between the groups may be explained partially by the disparity in numbers of O. ostertagi established and size of the abomasums, but mainly by the difference in rate of development of this parasite. It has been shown previously (Section II) that clinical

signs of ostertagiasis will only develop after considerable numbers of worms have emerged from the gastric glands and the pH of the abomasal contents has become elevated. This series of events took place in the young calves (Group 3) but emergence of O. ostertagi from the glands had not commenced by day 28 in the adult animals (Group 2).

The retardation of development of O. ostertagi at the fifth larval stage in the adult animals is really a 'retardation of maturation' as the final larval moult had taken place. Thus, retardation should not be con-

The retardation of development of O. ostertagi at the fifth larval stage in the adult animals is really a 'retardation of maturation' as the

Whether the delay in maturation is permanent or simply part of a prolonged pre-patent period remains to be established. However, it is clear that such a retardation of O. ostertagi occurs in adult animals and if prolonged could explain why adult animals grazing on pasture known to be contaminated with O. ostertagi larvae seldom develop clinical ostertagiasis.

There are few reports in the literature on age immunity per se to gastro-intestinal nematodes of domestic animals. Soulsby (1929a, 1929b) observed age immunity to Ancylostoma braziliense in adult dogs and cats and to Ancylostoma caninum in adult dogs. More recently, Gibson and Everett (1963) noted that lambs over four months old became resistant to N. battus infections. Miller (1965) has also noted an age immunity to infections with A. caninum in adult dogs. It may be that age immunity to nematodes is quite common but the expense and labour involved in rearing large numbers of animals, free from parasitic infections, until adulthood has precluded thorough experimental investigation of this problem.

D. Acquired Immunity - the Immunity Resulting from Naturally Acquired Infection

Introduction

The apparent absence of outbreaks of clinical ostertagiasis in adult cattle in an endemic area, such as the West of Scotland, has caused speculation as to why this situation should exist. It is possible that it is merely a reflection of the management practised in delaying areas where productive adult cows are grazed on the best and frequently new pastures, which are not contaminated with O. ostertagi eggs or larvae. Young stock on the other hand are grazed on permanent pasture usually contaminated with O. ostertagi eggs or larvae. Alternatively, it may be associated with the greater size of the abomasal mucosa in adult animals; since the latter is up to six times as great as that found in young calves, it would require six times as many infective larvae to produce the same degree of abomasal damage in adult stock.

A third and more plausible reason is that by the time cattle become adult they will have acquired an immunity to O. ostertagi through exposure to this nematode during their first and possibly their second grazing season. Certainly, Roberts (1951) and Roberts et al (1952) showed that cattle developed a strong resistance to infestation with gastro-intestinal nematodes during the first 18 months of life and stated that this depended on exposure to infection with those nematodes. Ross and Dow (1964, 1965a) also noticed that administration in the autumn of large numbers of O.

ostertagi infective larvae to calves grazing naturally infected pastures failed to produce clinical signs or severe abomasal lesions; these authors suggested that this was due to the immune status of the calves against ostertagiasis being high following prolonged exposure to a natural infection during the summer and early autumn.

In many parts of Britain young stock at the end of the autumn grazing season (i.e. October/November) are housed until the following spring (i.e. April/May) when they are put out to graze again. The pasture used for the second year of grazing is invariably different from that grazed during the first year. Although Ross and Dow (1964, 1965) found that young cattle became resistant to the effects of ostertagiasis by the end of the first grazing season, it is equally important to know the immune status of such young cattle prior to their grazing again in the following spring after being housed for several months.

Accordingly, the present experiment was designed to test the resistance to a large single dose of O. ostertagi infective larvae of young cattle in the spring, prior to their second year of grazing.

Experimental Design

Thirteen Ayrshire male calves which were survivors from outbreaks of Type I ostertagiasis at three different farms were housed at the beginning of October, 1965. Four of these calves were from the Farm A described previously and five from Farm B. The remaining four calves had grazed on Paddock E at the Veterinary Hospital, described in Section III, Part C.

Table 31

Design of Experiment where Groups of 15 Month Old Parasite Free Calves and Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965 were given an Experimental Inoculum of 800,000 O. ostertagi Larvae in the Spring of 1966

Group	No. of Calves	Treatment
1	3	G - E_2
2	3	G — H — E_2
3A	4	G — H — G — E_2
3B	4	G — H — G — E_3
4A	3	N.G. — H — G — E_2
4B	2	N.G. — H — G — E_3

G = Grazed in Summer of 1965 (June-October)
N.G. = Did not Graze
H = Housed October 1965 until April 1966
C = Challenge Inoculum of 800,000 O. ostertagi
E_1 = Autopsied at end of Grazing Period
E_2 = Autopsied 21 Days After Challenge Inoculum
E_3 = Autopsied 28 Days After Challenge Inoculum

Three of these animals (Group 1 - one calf from each farm) were autopsied at the time of housing to establish the approximate numbers of O. ostertagi present in the calves at this time.

In the late spring of 1966 (i.e., April/May) seven of the housed calves were inoculated orally with 800,000 O. ostertagi infective larvae. At the same time five Ayrshire calves (one male, four female) of similar age, i.e. 15 to 18 months, and reared under worm free conditions were also inoculated with 800,000 O. ostertagi larvae. Twenty one days later (day 21), the three uninoculated calves (Group 2), together with three of the Type I survivors which had been inoculated (Group 3A) and three of the parasite free calves (Group 4A) also inoculated, were autopsied. The remaining six calves were autopsied on day 28 (Groups 3B, 4B).

The experimental design is summarised in Table 31.

Observations

Each calf was examined clinically and faecal samples collected twice weekly from the time of housing until the experimental inoculum of O. ostertagi larvae was given. Thereafter, clinical examinations and collections of faecal samples were made daily. The faecal samples were assessed for consistency and examined by zinc sulphate flotation and McMaster methods for the presence of strongyle eggs.

Blood samples were collected for plasma pepsinogen estimations at the time the calves were housed and at monthly intervals prior to inoculation of the larvae. Thereafter, samples were collected on days 14, 17, 21 and 28.

At autopsy, the numbers of O. ostertagi present in the abomasum and contents were counted and the pH of the abomasal contents noted. In the calves autopsied on days 21 and 28, blocks of abomasal mucosa were prepared for subsequent histological studies.

Results

Clinical Data. - Softening of the faeces was observed in all the calves at the time of housing, i.e. early October, but by the end of October the faeces from these animals were of normal consistency. No further clinical signs were noticed in these animals nor in the parasite free calves until after the experimental inoculum of O. ostertagi was given. Diarrhoea first occurred on the 19th day of the experimental infection and continued in some calves up to day 25, i.e. for 7 days. One calf in Group 4A, not previously exposed to O. ostertagi infection, was killed in extremis on day 21. The calves in this group (4B) which were allowed to survive to day 28 were severely diarrhoeic for five days and lost condition badly. In contrast, the calves of group 3B, which had grazed on contaminated pasture in the previous summer and autumn, had a short period of diarrhoea lasting two or three days. The pattern of diarrhoea in the different groups is given in Appendix 5, Table 1.

Blood Analysis .- The plasma pepsinogen levels of individual calves at housing and following inoculation of 800,000 O. ostertagi infective larvae are shown in Appendix 5, Table 2. The mean plasma pepsinogen levels of

groups 2, 3 and 4 on days 0, 14, 17, 21 and 28 after experimental infection are shown in Figure 34. There was a marked decrease in plasma pepsinogen levels of calves from the time of housing in October until the experimental inoculation was given in April. Thereafter in the groups given 800,000 O. ostertagi infective larvae, the levels of plasma pepsinogen increased markedly.

pH of Abomasal Contents .- The pH of the abomasal contents at autopsy of groups 2, 3 and 4 are given in Table 32. The pH was markedly elevated in all of the animals given the challenge inoculum and autopsied on days 21 and 28. In group 2, which did not receive the challenge inoculum, the pH of the abomasal contents was within normal range.

Parasitological Findings .- The faecal egg counts of the calves housed at the beginning of October, 1965, were low, ranging from 50 e.p.g. to 450 e.p.g. Following housing, there was an increase in the faecal egg counts but during the winter they gradually declined and by the time the calves were given the experimental inoculum in the spring had decreased to negligible proportions. Following inoculation of the 800,000 O. ostertagi larvae, there was a slight increase in the faecal egg counts of the majority of inoculated calves between day 17 and autopsy on day 21 or day 28. The faecal egg counts of two calves in Group 3 (previously exposed) and one calf in Group 4 (reared worm free) increased to over 2,000 e.p.g. between days 19 and 22. The faecal egg counts of individual calves are recorded in Appendix 5, Table 3.

The mean O. ostertagi worm burdens present at autopsy of calves in Groups 1 through 4 are given in Table 33 and the individual calf worm

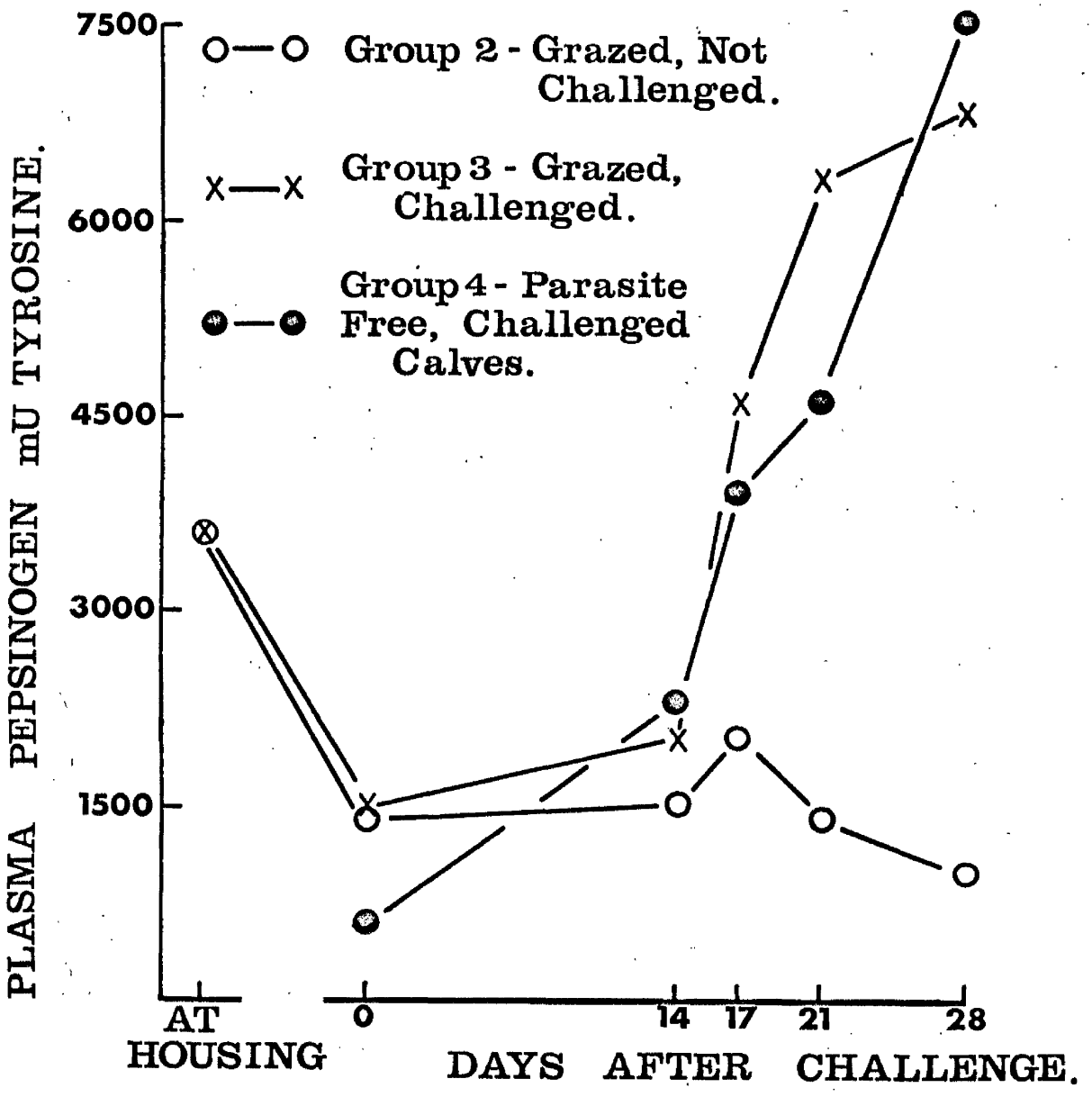


Fig. 34 Mean plasma pepsinogen levels in three groups of calves following inoculation of 800,000 Ostertagia ostertagi larvae. Two groups (2 and 3) had previously grazed on pasture contaminated with O. ostertagi larvae.

Table 32

The pH of Abomasal Contents at Autopsy of Groups of 15 Month Old Parasite Free Calves and Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965, 21 to 28 Days after some Groups were given an Experimental Inoculum of 800,000 O. ostertagi larvae in the Spring of 1966

Group 2		Group 3		Group 4	
Grazed June - October 1965 Housed Thereafter Not Challenged Killed Day 21		Grazed June - October 1965 Housed Thereafter Challenged April 1966 Killed Day 21	Killed Day 28	Parasite Free Calves Challenged April 1966 Killed Day 21	Killed Day 28
3.3		7.3	6.6	7.3	6.9
2.6		7.3	6.9	6.2	6.9
2.4		7.4	7.4	7.2	
		7.1	5.7		
Mean	2.7	Mean 7.3	Mean 6.7	Mean 6.9	Mean 6.9

Table 33

Mean Worm Counts at Autopsy of Groups of 15 Month Old Parasite Free Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965, Some of which were Subsequently Given an Experimental Inoculum of 800,000 O. ostertagi Larvae in the Spring of 1966, Together with Previously Uninfected Controls

Group	Inoculation of O. ostertagi Larvae	Day of Killing after Experimental Inoculation	Mean O. ostertagi Worm Counts			
			Total	Adults	Developing Stages	Early Fourth Larval Stages
1	Nil	0*	54,600	23,300	14,000	17,500
2	Nil	21	11,500	10,000	400	1,100
3(A)	800,000	21	180,500	171,500	800	8,000
3(B)	800,000	28	23,400	19,600	600	3,200
4(A)**	800,000	21	173,200	169,000	1,100	3,100
4(B)**	800,000	28	43,000	40,300	300	2,400

*Group 1 was killed at end of grazing period in early October 1965

**Groups 4(A) & 4(B) were previously uninfected control groups

burdens in Appendix 5, Table 4. The mean numbers of worms present in the uninoculated calves autopsied in April, 1966 (Group 2) were much lower (11,500) than those recorded (54,800) in the calves autopsied at housing in October, 1965 (Group 1). This decrease in numbers of O. ostertagi applied to both adult and larval stages. In the calves of Groups 3A and 4A autopsied 21 days after the challenge inoculum of 800,000 larvae was given, the mean worm burdens were 180,300 and 173,200 respectively, indicating that the challenge inoculum had become established. The majority of these worms were in the adult stage but many of the female worms from calves in both groups did not contain eggs. In the calves of Groups 3B and 4B, which were autopsied 28 days after challenge, the mean worm burdens were much lower, being 23,400 and 43,000 respectively and consisted mainly of mature adults.

Pathological Findings .- The main features of the post-emergent lesion described in Section II, Part A, were again present in calves from all groups. These included many plasmablasts and plasmacytes, probably producing antibody, and situated in the superficial mucosa, while in the lower part of the mucosa and sub-mucosa lympho-reticular follicles with active germ centres were observed (Fig. 35). The significance of the presence of these cell types is the subject of a separate investigation.

Discussion

It is clear from the results of this experiment that in calves housed in early October and harbouring considerable burdens of O. ostertagi, a significant reduction in these burdens had taken place by the following

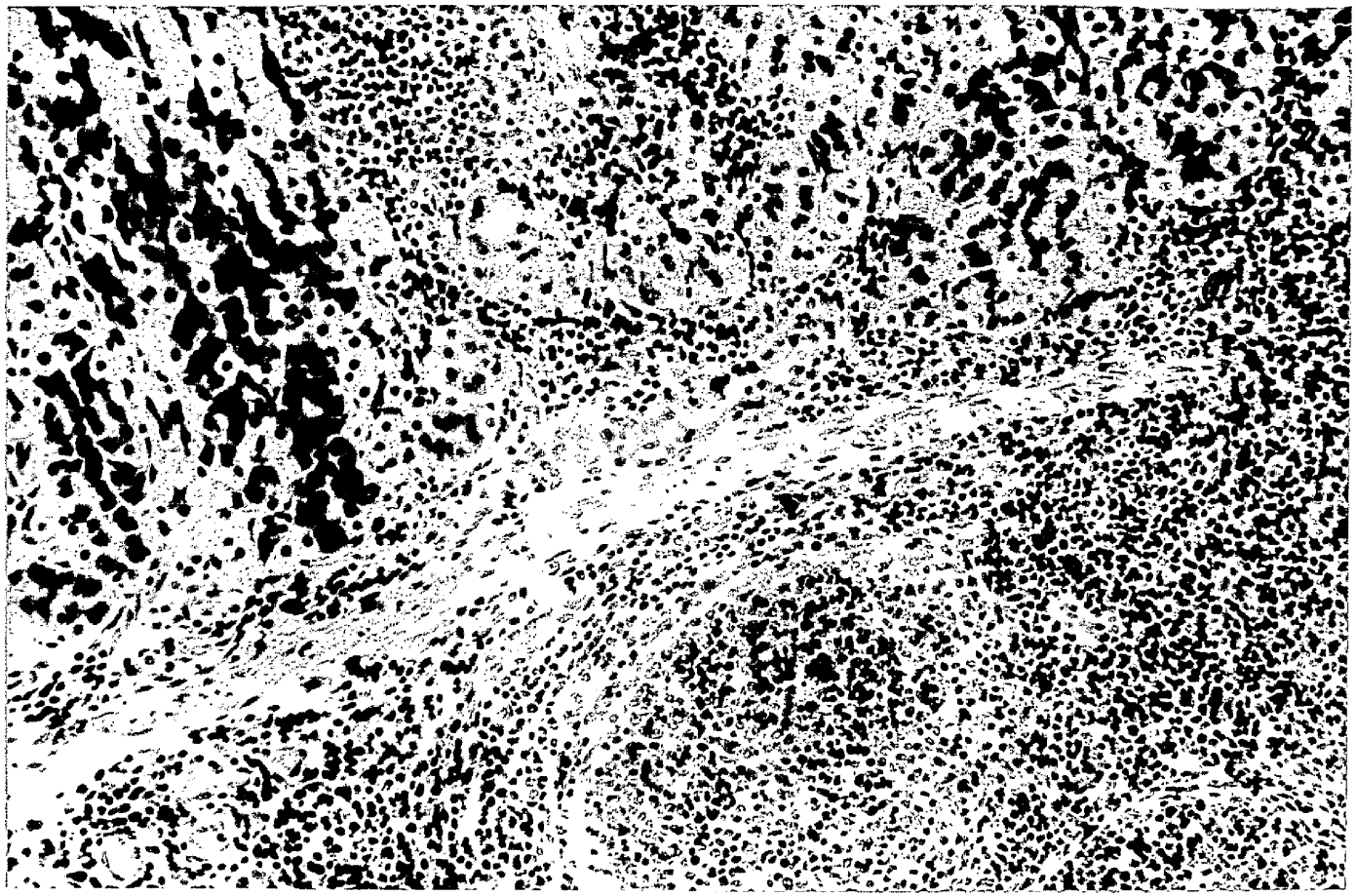


Fig. 35. Mucosa of abomasum from calf previously exposed to Ostertagia ostertagi infection and challenged with 800,000 larvae. Lymphoreticular follicles are present in the lower mucosa and in the submucosa (x 450).

spring. This is evident from a comparison of the worm burdens in calves autopsied at the time of housing (Group 1) and the uninoculated calves of Group 2 autopsied in the spring (see Table 33). Further evidence is provided by the gradual disappearance of clinical signs in the calves after housing, the decrease in levels of plasma pepsinogen and the reduction in faecal egg counts between housing in the autumn and the following spring (see Appendix 5, Tables 2 and 3). The reduction in worm burdens involved both adult and inhibited fourth stage larvae and occurred in calves from the three different farms (Appendix 5, Table 4).

Following administration of the challenge inoculum of 800,000 O. ostertagi larvae, large populations of adult worms were established by day 21 as shown by the worm counts of the calves autopsied on that day (Appendix 5, Table 4) and by comparison with the negligible worm burdens existing in the uninoculated calves (Group 2). There was no significant difference in the numbers of worms present at autopsy of the calves previously exposed to O. ostertagi infection (Group 3) and the group of calves maintained worm free until challenge (Group 4) and inhibition of larval development was not a feature of the worm populations present in either group. The development of the O. ostertagi resulting from the challenge inoculum was accompanied by a marked rise in plasma pepsinogen levels (Fig. 34), the onset of severe diarrhoea beginning on day 18 and an increase in faecal egg counts, though in the latter considerable variation in the magnitude of the increase was observed between individual animals.

In the calves autopsied on day 28, a significant decrease in worm burden had occurred when compared to those found in calves autopsied on day 21.

This apparent loss of worm burden took place in the calves of both Group 3 and Group 4 (Table 33; Appendix 5, Table 4) and was accompanied by the abatement of clinical signs and in certain instances, a decrease in faecal egg counts.

On first examination of the above findings there appeared to be no significant differences between the groups in terms of response to the challenge inoculum and within the parameters measured. If, however, the pattern of diarrhoea in the individual calves of the different groups retained until day 28 is studied (Appendix 5, Table 1) closely, it can be seen that the duration of diarrhoea in the calves maintained worm free until challenge (Group 4) is twice as long as in the calves previously exposed to natural O. ostertagi infection (Group 3). Since the period of diarrhoea is associated with a period of maximal worm loss or self-cure (Experiment 1, Section II), it is possible that calves previously exposed to infection with O. ostertagi will self-cure more rapidly than calves not previously exposed, when given a large challenge inoculum of O. ostertagi larvae.

The period of diarrhoea and the expulsion of O. ostertagi coincide with the sloughing of the superficial mucosa, the so-called cytolytic reaction. Jarrett (1966) has suggested that these phenomena may be linked to an immunological reaction and evidence for this is provided by the histological findings that, at autopsy of calves on day 21, there are antibody producing cells (plasmablasts, plasmacytes) in the lamina propria. It seems possible that the situation existing with O. ostertagi infections at the time of self-cure is analogous to that described by Jarrett (1966) and Barth et al (1966)

with N. brasiliensis infections in the rat.

The increase in faecal egg count following administration of the challenge inoculum varied considerably in individual calves both in time of onset and magnitude (see Appendix 5, Table 3). In the calves of Group 3, previously exposed to natural O. ostertagi infections, no marked increase in faecal egg count occurred before day 21, whereas in young worm free male calves given this size of inoculum it usually occurs by day 10 (Anderson et al, 1966). Two of the calves in Group 3 did show a marked increase in faecal egg count between days 22 and 25 and while this may have been associated with a genuine increase in numbers of adult worms, it could also be attributed to fragmentation of expelled female worms en route through the intestines.

In Group 4, the pattern of faecal egg counts following challenge was particularly interesting. The solitary male animal in the group developed a high faecal egg count (1,800 e.p.g.) by day 10 and followed a similar course (up to 7,000 e.p.g.) to that described by Anderson et al (1966) in young male calves inoculated with 800,000 O. ostertagi larvae. However, the four female calves did not develop egg counts until day 21 and in the calves allowed to survive beyond this point, did not reach particularly high levels (up to 350 e.p.g.). Since the male and female calves were of similar age, it is possible that these differences may be associated with the hormonal status of the female calves at the time of inoculation. Recent work in laboratory animals (Dobson, 1966a) and in sows (Jacobs, 1966) have suggested that the development and maturation of helminth worm burdens may be influenced by the hormonal status of the host. Corticelli and Lai (1960) have also reported an increase in faecal egg counts of cows about the time of parturition.

In the present experiment, the calves were housed in early October, before large populations of inhibited early fourth stage larvae had accumulated and therefore the part played by these stages in maintaining the immune status of the host during the winter cannot be assessed; however, as it has already been shown by Martin et al (1957) and in Section I, that development of inhibited stages can give rise to clinical disease in the spring, it is unlikely that the presence of these stages greatly influences the maintenance of immunity in the animals during the winter.

Since the results of Section III of this thesis have demonstrated a difference in the proportion of inhibited fourth stage O. ostertagi larvae which developed from infections with a laboratory strain and a naturally occurring strain of O. ostertagi, it is possible that these strains also possess antigenic differences. This was not borne out, however, in the present experiment where calves which grazed on Paddock E, which was contaminated with a laboratory strain of O. ostertagi eggs, responded in a similar manner following challenge with laboratory cultured O. ostertagi larvae, to the calves which grazed at Farms A and B and were initially infected with a naturally occurring strain.

From the results obtained in the present experiment, it would appear that the immune status of the calves which grazed in 1965 and were then housed until the spring of 1966, was clearly different from the calves used in the two experiments described by Ross and Dow (1964, 1965a). In the first of these experiments, six month old calves which had grazed during the summer and autumn on pastures known to be contaminated with O. ostertagi and were subsequently challenged with up to 500,000 O. ostertagi, did not

develop clinical signs of ostertagiasis and had low worm burdens and few abomasal lesions at autopsy 42 days later. In the second experiment, calves grazed on known contaminated pasture from May until October resisted an experimental challenge of 200,000 O. ostertagi in the late autumn, whereas some of the calves given a similar challenge inoculum earlier in the grazing season, succumbed to the infection. It would therefore appear that while many calves acquire a strong degree of immunity by the end of the autumn grazing season, this immunity has partially waned by the following spring and allows the development of the challenge inoculum, although the pre-patent period may be extended. The degree of immunity present is still sufficient, however, for the self-cure reaction to be accelerated and also to limit egg production by the female worms.

C. Acquired Immunity to Ostertagia ostertagi Infection - the Immunity Resulting from the Administration of X-Irradiated Larvae

Introduction

Infective cattle nematode larvae, partially inactivated by X-irradiation have been used to stimulate immunity to subsequent infection with normal larvae of the same species; this technique of immunisation has been successfully applied experimentally in two cattle nematodes, viz. Dictyocaulus viviparus (Jarrett et al, 1957), Haemonchus placei (Ross et al, 1960), and in the field in the control of bovine parasitic bronchitis (Jarrett, Jennings, Martin, McIntyre, Mulligan and Urquhart, 1958).

The use of radiation vaccines against helminthic diseases of other livestock has also been studied and their possibilities are reviewed in Technical Report No. 30 of the International Atomic Energy Agency, Vienna (1964).

The apparent absence of clinical bovine ostertagiasis in adult cattle in the West of Scotland, where this disease is endemic in cattle under two years of age (Anderson et al, 1965a), suggests that some form of immunity to ostertagiasis operates under natural conditions.

It was therefore decided to study the acquired immunity developed following double vaccination of calves with irradiated O. ostertagi larvae and subsequently challenged with experimental and natural infections of O. ostertagi.

Three experiments were carried out (a) to find the radiation dose which

would significantly reduce the pathogenic effect of the larvae, (b) to study the immunity to experimental challenge which resulted from the administration of two doses of irradiated O. ostertagi larvae at monthly intervals, (c) to study the immunity to natural challenge following the administration of two doses of irradiated larvae at monthly intervals and also to see if populations of inhibited fourth stage developed in the challenged animals.

Experimental Design

Experiment 1

This experiment was designed to find the radiation dose which would significantly attenuate O. ostertagi larvae.

Twenty calves reared parasite free and aged ten weeks were allotted to five groups of four each. The four calves in Group 1 were inoculated orally with 100,000 normal O. ostertagi infective larvae. Each of the calves in Groups 2 to 5 were inoculated with 100,000 irradiated O. ostertagi larvae, these larvae being prepared from the same batch as the normal larvae. The larvae given to these groups were irradiated as follows: Group 2 at 20 kr, Group 3 at 40 kr, Group 4 at 60 kr, Group 5 at 80 kr. All the calves were killed on day 21. The design of this experiment is summarised in Table 34.

Experiment 2

The object of this experiment was to test the immunity which developed following double vaccination of calves with X-irradiated O. ostertagi larvae, by the administration of a single large experimental challenge with normal O. ostertagi third stage larvae.

Table 14

Plan of Experiment Designed to Study Effect of Various Levels of X-Irradiation upon the Subsequent Development of Ostertagia ostertagi Larvae

Group	No. of Calves	Irradiation (kr)	Day Killed
1	4	0	
2	4	20	
3	4	40	21
4	4	60	
5	4	80	

kr = kiloroentgen

Twenty-seven ten week old calves reared free from parasites were allotted to three principal groups of nine each (Groups A, B and C). These groups were each divided into three sub-groups containing five, two and two calves respectively (Groups A1, A2, A3), (B1, B2, B3) and (C1, C2, C3). On day 0, the calves in Groups A and B were inoculated with 100,000 third stage larvae which had been irradiated at 60 kr and 80 kr respectively; these radiation doses were selected on the basis of results from Experiment 1. The calves in Group C2 were inoculated orally with 100,000 normal third stage O. ostertagi larvae prepared from the same culture as the irradiated larvae. On day 30, the calves in Groups A2, B2 and C2 were killed and the calves in groups A1, A3, B1, B3 were given a second dose of vaccine prepared similarly to that received on day 0 and the calves in Group C3 were inoculated orally with 100,000 normal third stage O. ostertagi larvae. On day 60, the calves in Group C3 were killed and Groups A1, B1, C1 were challenged orally with 300,000 normal third stage O. ostertagi larvae and together with Groups A3 and B3 were killed on day 61. The design of this experiment is summarised in Table 35.

Experiment 3

The object of this experiment was to test the immunity developed following double vaccination of calves with X-irradiated O. ostertagi larvae against a natural challenge with O. ostertagi larvae.

Ten Ayrshire calves, ten weeks old and reared under parasite free conditions, were allotted to two groups of five each. One group was inoculated

Table 35

Experimental Plan of Vaccination of Calves with X-Irradiated Ostertagia ostertagi larvae followed by Experimental Challenge with Normal Larvae

			Immunizing Phase		Challenge Phase	
Group	No. Calves	X-ray dose kilo-roentgens	Day 0	Day 30	Day 60	Day 61
A1	5	60	100,000 x-irradiated larvae	100,000 x-irradiated larvae	300,000 larvae	Killed
A2	2	60	100,000 x-irradiated larvae	Killed	"	"
A3	2	60	100,000 x-irradiated larvae	100,000 x-irradiated larvae	0	Killed
B1	5	80	100,000 x-irradiated larvae	100,000 x-irradiated larvae	300,000 larvae	Killed
B2	2	80	100,000 x-irradiated larvae	Killed	"	"
B3	2	80	100,000 x-irradiated larvae	100,000 x-irradiated larvae	0	Killed
C1	5	0	0	0	300,000 larvae	Killed
C2	2	0	100,000 larvae	Killed	"	"
C3	2	0	0	100,000 larvae	Killed	"

with two doses of 100,000 irradiated O. ostertagi third stage larvae at 30 days interval. Each larval dose had been subjected to 60 kr irradiation by the method previously described. The other group served as controls. Both groups were put out to pasture 23 days after the second vaccination. The pasture used for the trials was two acres of permanent pasture which had been grazed during the month of May, 1966, by five calves inoculated in the first week of April, 1966, with 100,000 O. ostertagi larvae; all these calves had patent faecal egg counts during the month of May. The vaccinated and control groups of calves were turned onto the pasture two weeks after it was vacated by the five 'seeder' calves, i.e. 15th June, 1966.

Observations

In Experiment 1, the calves were examined clinically each day. Faecal samples were collected three times weekly until day 16, thereafter they were collected daily; zinc sulphate flotation and McMaster techniques were used to detect the presence of eggs and count the number of eggs per gram of faeces. At autopsy, worm counts were made from the abomasal contents and following peptic digestion of the abomasal mucosa as previously described. The worms were counted, sexed and 30 females examined at a magnification of at least 20 times to detect the presence or absence of eggs in their uteri.

The irradiation procedure was as described under Materials and Methods.

In Experiment 2, a daily clinical examination was made of all calves and bodyweights were obtained at weekly intervals. Blood was collected in heparinised bottles at weekly intervals for plasma pepsinogen estimations.

Faecal egg counts by the zinc sulphate flotation and McMaster methods were carried out also at weekly intervals until day 76; thereafter, faecal egg counts were made daily until the calves were killed on day 81. At autopsy, the pH of the abomasal contents was recorded and worm counts were made from the abomasal contents and following peptic digestion of the abomasal mucosa.

In Experiment 3, a clinical examination was made of the ten calves at weekly intervals and samples of blood were collected in heparinised bottles for plasma pepsinogen estimations; faecal samples were also collected at weekly intervals for faecal egg counts by the McMaster method. It was unfortunately not possible to weigh the ten calves during the course of this experiment. At autopsy ,the pH of abomasal contents was recorded and worm counts were made from the abomasal contents and peptic digests of the abomasal mucosa.

Results

Experiment 1

Clinical and Parasitological Data. - Clinical signs of ostertagiasis, i.e. anorexia, weight loss and diarrhoea, did not occur in any of the calves. Table 36 lists the numbers, sex ratios, and percentage of sterile female worms at autopsy of the different groups.

The numbers of O. ostertagi present in Group 1, which each received 100,000 normal infective larvae, were lower than usually obtained following inoculations of this magnitude. However, as the level of irradiation was increased, even allowing for the apparent low infectivity of the larvae used,

Table 36

The Effect of Various Levels of Larval X-Irradiation upon the Numbers, Sex Ratio and Development of *Ostertagia ostertagi* in Calves Aged 10 Weeks. All Calves were Inoculated with 100,000 *O. ostertagi* on Day 0 and were Autopsied on Day 21

Group	Mean Faecal Egg Count (e.p.g.) day 17-21	Nos. of *O. ostertagi*	Sex Ratio Male:Female	Percentage of 30 Females with Eggs
1	100	6,900	1:1	93
	150	5,300	1:1	90
Normal larvae	350	6,300	1:1	90
	150	4,700	1:1	87
2	0	2,600	1:2	40
	0	4,000	1:2	40
Larvae irradiated	0	4,000	1:2	33
at 20 kr	0	3,600	1:5	50
3	50	3,400	1:5	27
	0	2,600	1:5	20
Larvae irradiated	0	300	1:3	0
at 40 kr	0	2,100	1:3	20
	0	1,400	0:1	4
4	0	1,100	1:10	0
Larvae irradiated	0	3,300	0:1	4
at 60 kr	0	2,600	1:10	0
5	0	400	1:10	0
	0	200	0:1	0
Larvae irradiated	0	300	0:1	0
at 80 kr	0	200	0:1	0

kr = kiloroentgen

it is clear that infectivity, as measured by adult worm burdens at autopsy, was progressively reduced. Male larvae were more sensitive to the effects of irradiation than were the female larvae and this disparity between sexes was more marked at higher levels of irradiation. At radiation dose levels of 60 kr and greater, the female worms in the resulting populations were invariably sterile.

The results of the faecal egg counts confirmed the findings that irradiation of infective O. ostertagi larvae at levels of 60 kr or greater resulted in the development of sterile female O. ostertagi following inoculation of these larvae to calves. Despite the presence of a considerable proportion of fertile female worms (i.e. eggs in the uteri) at autopsy of the 20 kr and 40 kr groups, only one calf from these two groups had a positive faecal egg count; reproductive capacity of the worms irradiated at these levels also appears to have been considerably reduced.

Experiment 2

Clinical Data. - The pattern of diarrhoea between days 77 to 81 in the three groups of calves (A1, B1, C1) which received the challenge inoculum on day 60 is tabulated (Appendix 5, Table 5) and the mean and individual bodyweights of these three groups of calves are shown in Figure 36 and Appendix 5, Table 6, respectively. The calves in control group C1 became diarrhoeic 19 days after challenge, i.e. day 79, whereas the vaccinated calves in Groups A1 and B1 did not show diarrhoea until 20 days after challenge, i.e. day 81. The earlier occurrence of diarrhoea in Group C1 is reflected in the more severe decrease in bodyweight of this group (Fig. 36) between days 75 to 81 when compared to the mean bodyweights of Groups A1 and B1.

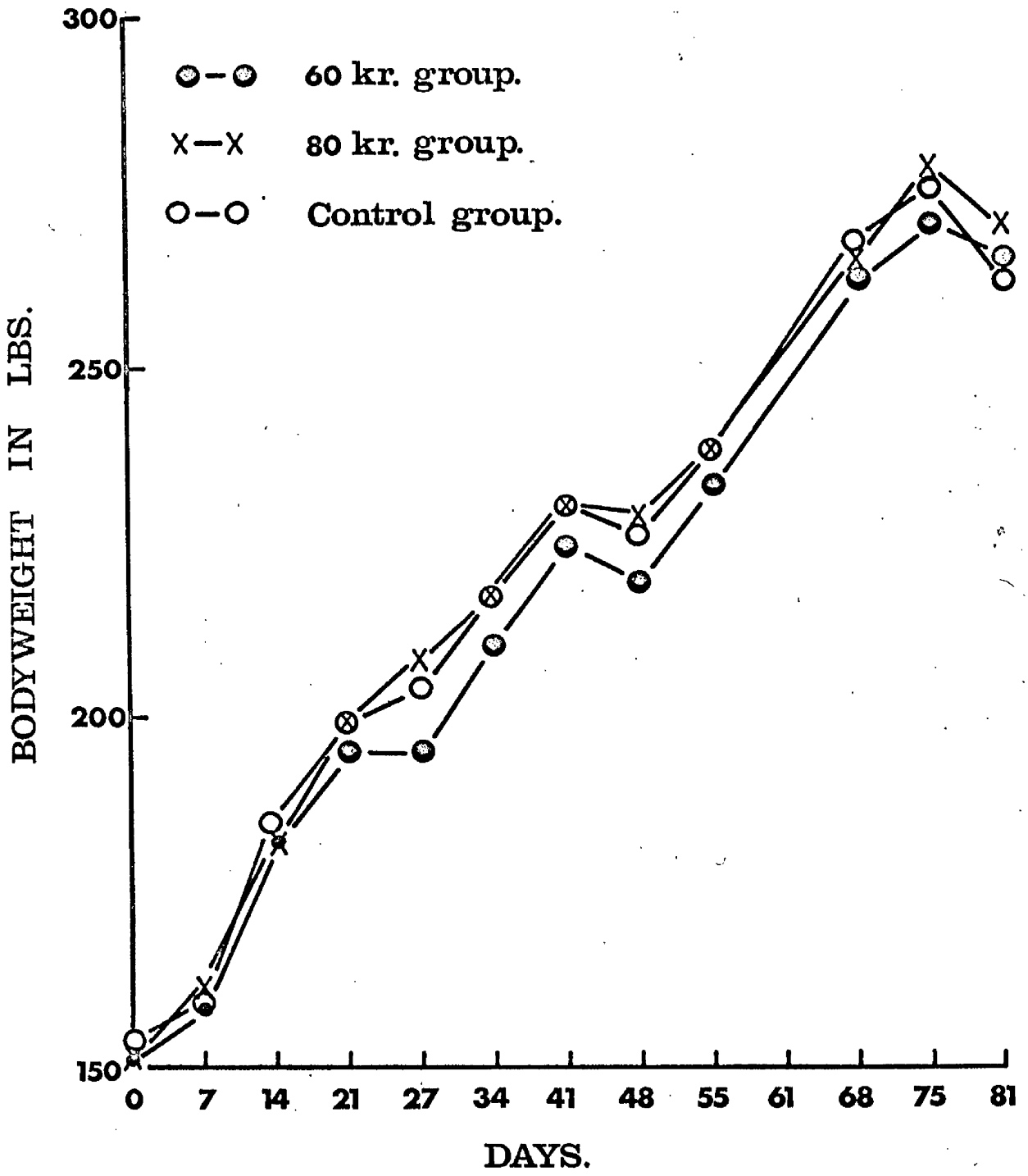


Fig. 36 Mean bodyweights of three groups of five calves each, two groups being immunised (60 kr and 80 kr) with irradiated Ostertagia ostertagi larvae on days 0 and 30 and all three groups given a challenge inoculum of 300,000 normal larvae on day 60.

Biochemical Results. - The mean plasma pepsinogen levels of calves in Groups A1, B1 and C1 are plotted in Figure 37 and the individual values for each calf are given in Appendix 5, Table 9. Following administration of irradiated larvae to Groups A1 and B1, an increase occurred in levels of plasma pepsinogen; this increase was more marked in Group A1 which received larvae irradiated at 60 kr. Two weeks after Groups A1, B1 and C1 were challenged with 300,000 normal third stage larvae, a marked increase in plasma pepsinogen occurred in all three groups; there was no significant difference between groups.

The pH of the abomasal contents at autopsy of calves in Groups A1, B1 and C1 are shown in Table 37. The pH of the abomasal contents was elevated in all calves, except for one calf in Group A1, in which it was 3.6.

Parasitological Findings. - The mean faecal egg counts of Groups A1, B1 and C1 are graphed in Figure 38 and show that the numbers of O. ostertagi eggs in the faeces of the control group C1 increased between day 76 and day 81 to a higher level (1,960 e.p.g.) than that noticed in the vaccinated groups A1 (390 e.p.g.) and B1 (720 e.p.g.). Individual faecal egg counts are recorded in Appendix 5, Table 7.

The numbers, stages and sex ratios of O. ostertagi present at autopsy in calves from Groups C2 and C3 which were given 100,000 normal larvae prepared from the same culture as the irradiated larvae were within the range usually obtained following inoculations of this magnitude. This indicates that the larvae used in the experiment were of good infectivity. The numbers of O. ostertagi present at autopsy in the irradiation control groups, A2, A3, B2, and B3, were low and consisted mainly of sterile adult female worms. This shows a good degree of attenuation was achieved following irradiation of the

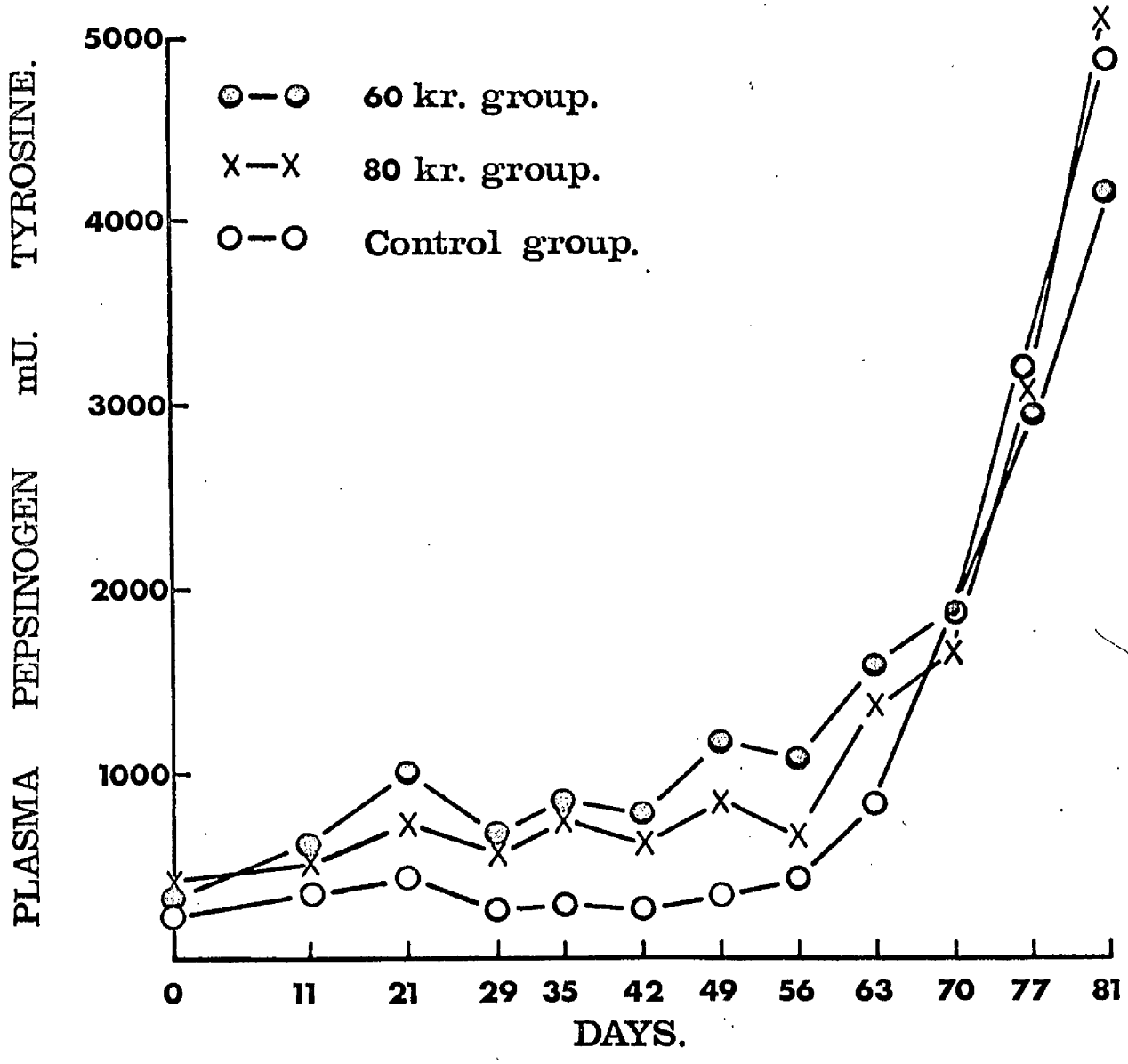


Fig. 37 Mean plasma pepsinogens of three groups of five calves each, two groups being immunised (60 kr and 80 kr) with irradiated Ostertagia ostertagi larvae on days 0 and 30 and all three groups given a challenge inoculum of 300,000 normal larvae on day 60.

Table 37

The pH of Abomasal Contents at Autopsy (Day 81) of Calves 21 Days After an Experimental Challenge Inoculum was given (Day 60) to Calves Previously Immunized with X-Irradiated Ostertagia ostertagi Larvae on Days 0 and 30 (Groups A1, B1) and Non Immunized Calves (C1)

Group	A1 (60 kr)	B1 (60 kr)	C1
pH of Abomasal Contents	6.7	7.0	7.2
	3.6	7.3	7.0
	7.3	6.9	6.5
	7.2	7.0	6.9
	6.8	7.1	7.0
Mean & Standard Error	6.3 ± 0.7	7.1 ± 0.1	6.9±0.1

kr = kiloroentgen

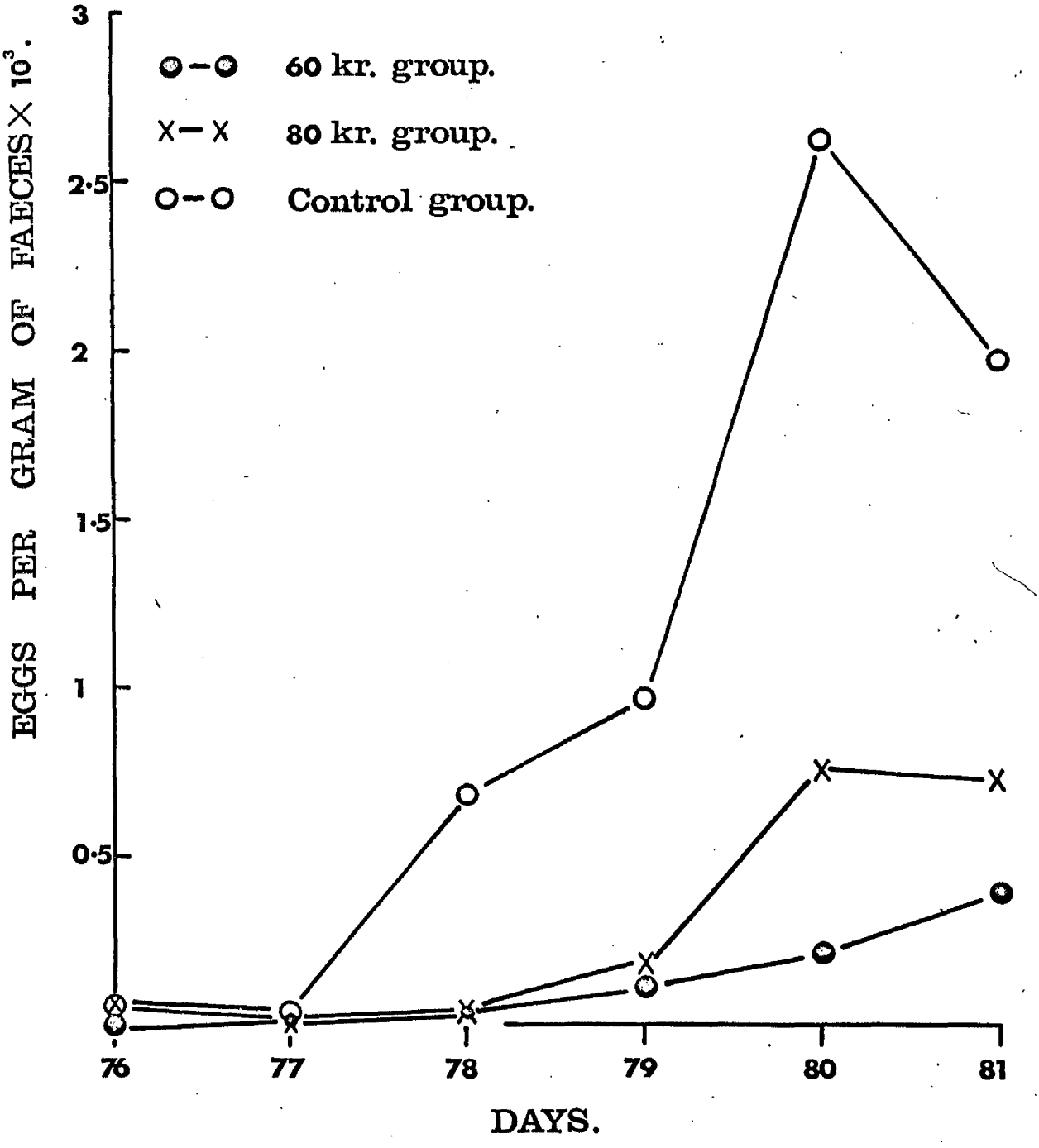


Fig. 38 Mean faecal egg counts of three groups of five calves each, two groups being immunised (60 kr and 80 kr) with irradiated Ostertagia ostertagi larvae on days 0 and 30 and all three groups given a challenge inoculum of 300,000 normal larvae on day 60.

larvae and that the vast majority of worms found at autopsy after challenge are likely to have originated from the challenge inoculum. Details of the abomasal worm counts from Groups A2, A3, B2, B3, C2 and C3 are given in Table 38.

The abomasal worm counts at autopsy on day 81 of calves which received the challenge inoculum of 300,000 larvae on day 60 (Groups A1, B1, C1) are shown in Table 39; there was no significant difference between the total numbers of O. ostertagi present at autopsy of calves from groups. However, a considerable variation in the numbers and distribution of parasitic stages was observed; in control group C1, three out of the five calves had over 90% of their total worm burden as mature adults, whereas in Group A1, immunised with larvae irradiated at 60 kr, less than 50% of the total worm burden were mature adults. In Group B1, which was immunised with larvae irradiated at 80 kr, the percentage of mature worms was over 50% but less than 90%. In Calf 2 of Group A1, which had a normal pH of the abomasal contents of 3.6, the fewest number of adult parasites had emerged from the gastric glands (10,000); this number is below that usually associated with marked pH changes in the abomasal contents.

Experiment 3

Clinical Data. - Clinical signs of ostertagiasis (loss of condition and diarrhoea) occurred in four of the control and one of the immunised group of calves early in September, i.e. eleven weeks after going on to the pasture. These clinical signs continued to be present intermittently until the calves were housed on October 4th; by this date two of the affected control calves and one immunised calf were severely diarrhoeic and had to be killed in extremis. The surviving calves were killed seven days after housing, i.e. on October 11th.

Table 30

Worm Counts at Autopsy of Groups of Calves Inoculated Orally with Irradiated or Normal Ostertagia ostertagi Larvae

Group	Inoculum	Day Killed	Nos. of O. ostertagi at Autopsy		Sex Ratio
			Adults	Larval Stages	Males : Female
A2	On day 0, 100,000 larvae irradiated at 60 kr	30	11,400	0	1:6
			6,700	0	1:100
A3	On days 0 and 30, 2 x 100,000 larvae irradiated at 60 kr	31	1,300	0	1:20
			1,400	0	1:14
B2	On day 0, 100,000 larvae irradiated at 60 kr	30	4,200	0	1:25
			4,400	0	1:9
B3	On days 0 and 30, 2 x 100,000 larvae irradiated at 60 kr	31	200	0	0:1*
			1,300	0	0:1
C2	On day 0, 100,000 normal larvae	30	26,400	0	1:1
			16,000	0	5:6
C3	On day 30, 100,000 normal larvae	60	20,200	0	4:5
			20,000	0	1:1

*No male worms present

kr = kiloroentgen

Table 39

Worm Counts at Autopsy (day 81) of Calves 21 Days after an Experimental Challenge Inoculum of *Ostertagia ostertagi* was Given (Day 60) to Calves Previously Immunised with X-irradiated *O. ostertagi* Larvae on Days 0 and 30 (Groups A1, B1) and Non Immunised Calves (C1)

Group	Immunisation Procedure	Challenge dose of *O. ostertagi* Larvae on Day 60	Nos. of *O. ostertagi* at Autopsy			
			Total	Emergent Adults	Pre-Emergent Adults	4th Larval Stages
A1	2 x 100,000 larvae irradiated at 60 kr on day 0 - 30	300,000	63,000	31,000	32,000	0
			51,000	10,500	40,000	500
			85,000	47,500	37,000	500
			62,000	26,500	32,000	3,500
			128,400	49,600	75,000	3,000
B1	2 x 100,000 larvae irradiated at 80 kr on day 0 - 30	300,000	102,400	76,000	26,000	400
			57,600	42,200	15,000	400
			105,000	71,800	32,000	1,200
			90,500	29,900	59,000	1,600
			95,700	65,500	30,000	200
C1	-	300,000	78,000	36,600	41,000	400
			105,000	35,200	64,000	5,000
			125,600	114,300	11,000	300
			49,400	46,100	3,000	300
			157,000	150,000	7,000	0

kr = kiloroentgen

Biochemical Results. - Mean plasma pepsinogen levels of both groups from the day of first vaccination until autopsy are shown in Figure 39. These levels increased following each vaccination then decreased again indicating the initial development of the parasite and its subsequent attenuation. After several weeks at pasture the plasma pepsinogens of both groups increased markedly reaching a peak at a period following the onset of severe clinical signs. Plasma pepsinogens from individual calves are recorded in Appendix 5, Table 10.

The pH of abomasal contents are given in Table 40 and although the mean pH from the immunised group was lower than that from the non-immunised group, this difference was not significant.

Parasitological Data. - Faecal egg counts of both groups were first positive 28 days after going to pasture; the egg counts then remained at a low level until the onset of clinical signs of weight loss and diarrhoea early in September when they increased in both groups. This increase was greater in the control group than in the immunised group. Mean faecal egg counts for both groups are graphed in Figure 40 and individual counts given in Appendix 5, Table 8.

The abomasal worm counts at autopsy of both groups are given in Table 41. Two of the calves in the immunised group had worm burdens ($< 10,000$) markedly lower than those in the non-immunised group.

Discussion

The results shown in Tables 35 and 36 indicate that as the level of inoculation of *O. ostertagi* is increased there is a reduction in the numbers of worms present at autopsy 21 days after inoculation. The preponderance

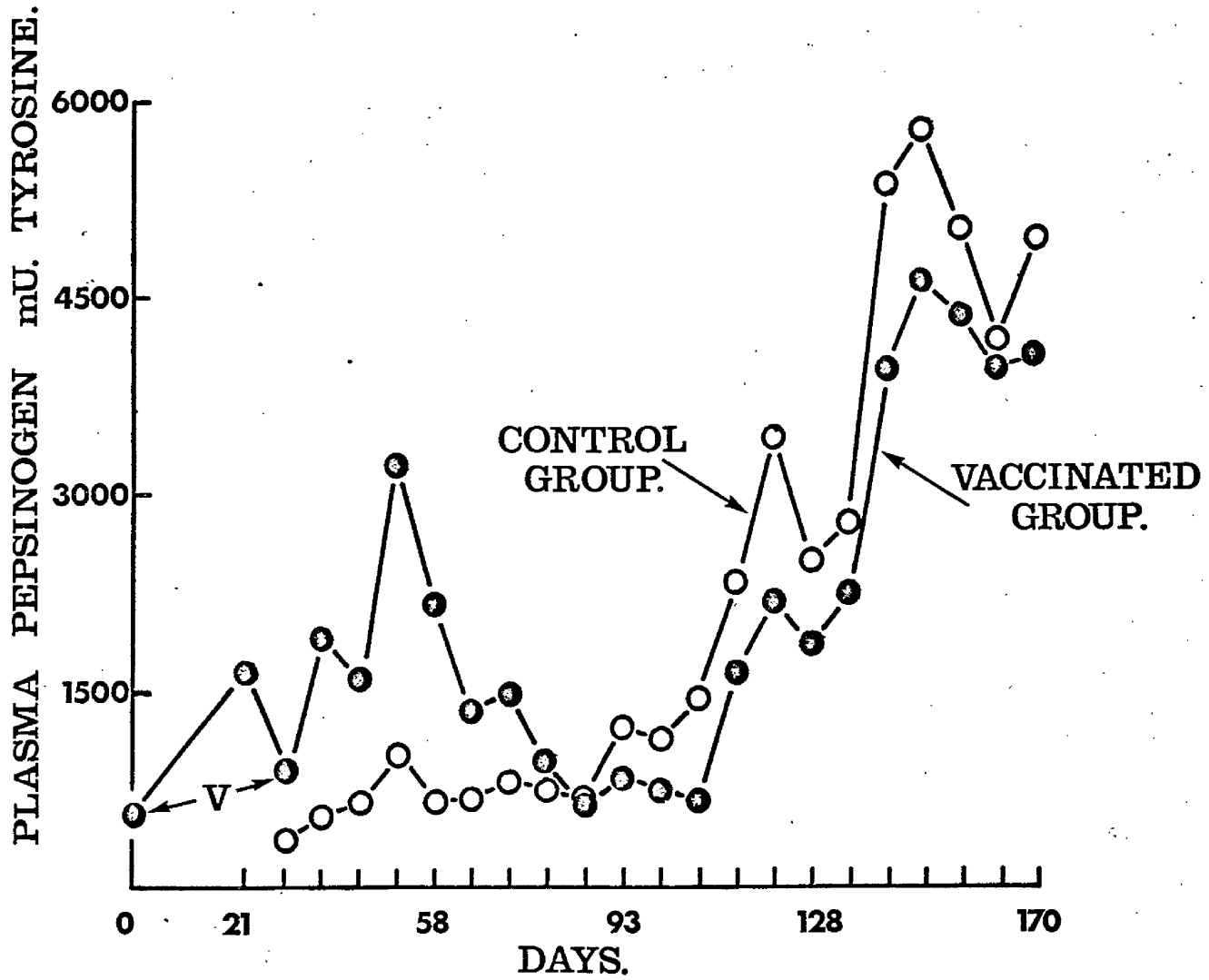


Fig. 39 Mean plasma pepsinogens of two groups of five calves each, one group being vaccinated with irradiated Ostertagia ostertagi larvae (60 kr) on days 0 and 30 and both groups subsequently grazed (from day 53) on pasture known to be contaminated with O. ostertagi larvae.

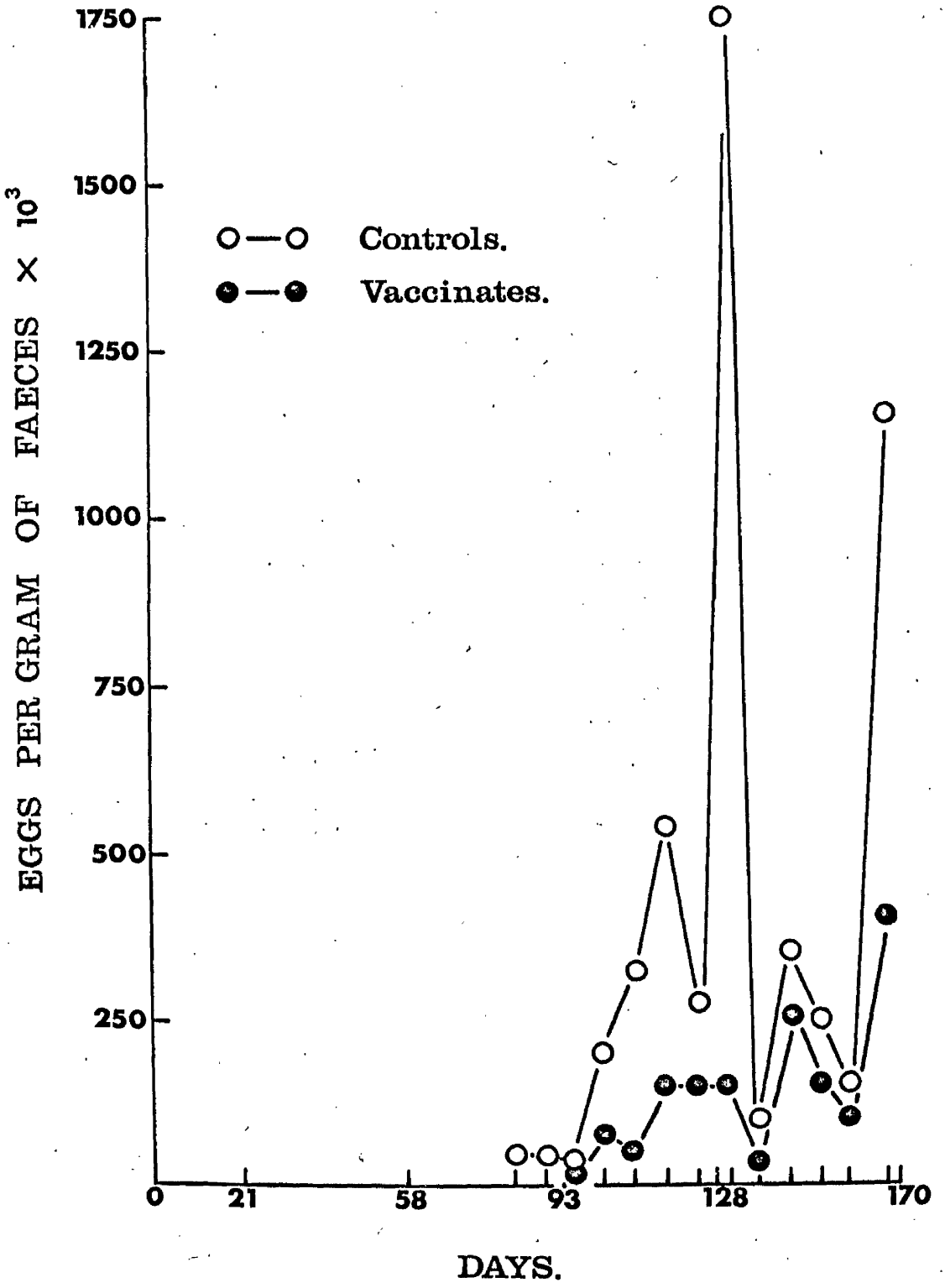


Fig. 40 Mean faecal egg counts of two groups of five calves each, one group being vaccinated with irradiated Ostertagia ostertagi larvae (60 kr) on days 0 and 30 and both groups subsequently grazed (from day 53) on pasture known to be contaminated with O. ostertagi larvae.

Table 40

The pH of the Abomasal Contents at Autopsy of Two Groups of Five Calves Which Grazed from June until October, 1966, on Pasture known to be Contaminated with Ostertagia ostertagi Larvae. One Group of Calves being Immunized with Irradiated O. ostertagi Larvae Prior to Grazing

	pH of Abomasal Contents	
	Immunized	Non-Immunized
	2.7	4.7
	4.9	5.7*
	4.5	5.9
	6.5*	6.8*
	4.3	5.5
Mean & Standard Error	4.5 ± 0.6	5.3 ± 0.5

*Killed in Extremis

Table 41

Worm Counts at Autopsy of Two Groups of Five Calves which Grazed from June until October, 1966, on Pasture known to be Contaminated with Ostertagia ostertagi Larvae, One Group of Calves being Immunized with Irradiated O. ostertagi Larvae prior to Grazing

Group	Total	Adults & Developing Stages	Early Fourth Larval Stages	
			Number	Per Cent
Immunized	8,200	7,400	800	10
	36,400	29,500	6,900	19
	20,700	10,300	10,400	36
	86,600**	81,600	4,800	6
	8,300	7,500	800	10
Non-Immunized	30,900	25,700	5,200	17
	52,200**	43,000	9,200	18
	25,400	19,200	5,200	22
	54,100*	54,100	0	0
	32,700	27,000	5,700	17

*Killed in Extremis First Week in September

**Killed in Extremis First Week in October

of females in these worm populations suggests that the male O. ostertagi larvae are more susceptible to the effects of irradiation than are the female larvae. This phenomenon of altered sex ratio of the worm population following irradiation of larvae was first described by Ciordia and Bizzell (1960a) as a result of their work on T. axei and has since been noticed in other nematodes subjected to X-irradiation (Mulligen, 1963). At irradiation levels of 60 kr and greater, the female O. ostertagi present at autopsy were invariably sterile.

The administration to calves aged eight to ten weeks of a single inoculum of 100,000 O. ostertagi third stage larvae usually results in a softening of the faeces, loss of bodyweight, some inappetence and occasionally severe diarrhoea (Ritchie et al, 1966). In Experiment 1, neither the calves given 100,000 normal nor those given 100,000 irradiated O. ostertagi third stage larvae showed the clinical signs of ostertagiasis described above. This result must be interpreted with caution as the infectivity of these larvae, judged by the worm burdens at autopsy of the calves given normal larvae, was rather low. However, in Experiment 2 the infectivity control calves (Groups C2, C3) given 100,000 normal O. ostertagi third stage larvae showed clinical signs of ostertagiasis (softening of faeces and decreased appetite) whereas the calves given irradiated larvae (Groups A2, A3, B2, B3) showed no clinical signs. The absence of clinical signs in the latter calves indicated that irradiation at 60 kr or 80 kr sufficiently attenuated the O. ostertagi larvae by reducing the adult worm burden which finally developed to a non-pathogenic level. The increased plasma pepsinogen levels (Fig. 37) following inoculation of irradiated larvae suggests that sufficient numbers of O. ostertagi developed

initially to cause cellular changes in the gastric glands but these either died off prior to emergence from these glands or were insufficient in number to produce the marked cellular changes associated with emergence from the glands (see Section II).

On first appraisal of the worm burdens and the pH of abomasal contents (Tables 37 and 39) present at autopsy following experimental challenge of the calves in Experiment 2, there appeared to be no differences between those of previously immunised calves and non-immunised calves. If, however, the results in the tables showing the pattern of diarrhoea (Appendix 5, Table 6) and the faecal egg counts (Fig. 38; Appendix 5, Table 7) are examined, it is apparent that in the immunised groups (A1, B1) the onset of diarrhoea was delayed, the faecal egg counts were lower and the emergence of adult worms from the gastric glands had been delayed when compared to the calves in the non-immunised group (C1). In retrospect, it would have been advantageous to autopsy the calves serially following the experimental challenge inoculum rather than at one fixed point. It could then have been established if the accelerated 'self-cure' observed following experimental challenge of naturally infected calves (Part B, Section IV) operated in the calves immunised with irradiated larvae.

However, it was hoped that this information could be obtained from the results of Experiment 3. In this experiment, calves immunised with irradiated O. ostertagi larvae and non-immunised calves were subjected to a natural and presumably aerial challenge by grazing for 16 weeks on pasture known to be contaminated with O. ostertagi. Unfortunately, the numbers of O. ostertagi larvae initially available on the pasture, as judged by plasma

pepsinogen levels and faecal egg counts in non-immunised calves (Appendix 5, Tables 8 and 10), were low until the middle of August. It is therefore possible that the lack of antigenic stimulation to the immunised calves up to mid-August resulted in some waning of the immunity developed following administration of the irradiated O. ostertagi larvae. Thus, one immunised calf had to be killed in extremis as well as two non-immunised calves. However, it appears that a degree of immunity was present in the calves given the irradiated larvae as the four surviving calves in this group were never diarrhoeic, had low faecal egg counts and at autopsy two of the four calves had worm burdens of less than 10,000 O. ostertagi. On the other hand, only one of the surviving calves in the non-immunised groups did not develop diarrhoea and at autopsy only this calf had a worm burden of less than 30,000 O. ostertagi.

There was no significant differences between the mean pH of the abomasal contents of both groups.

It is interesting that in the non-immunised calf killed early in September, no early fourth stage larvae of O. ostertagi were present whereas up to 10,000 of these stages were present in the calves autopsied in early October. This finding confirmed the association between late autumn grazing and inhibition of larval development.

The results of these experiments suggest that immunisation of calves with irradiated O. ostertagi larvae results in the development of some resistance to further infection with this nematode but that this resistance is not absolute and varies considerably in individual animals. It is unlikely that this form of immunisation will prove a practical proposition in

the control of Type I ostertagiasis and since inhibited early fourth larval stages were present in both immunised and non-immunised calves, will certainly not prevent the development of pre-Type II or Type II ostertagiasis.

General Discussion

From the results of these experiments, it is apparent that the immunity which operates in bovine ostertagiasis is associated with a previous exposure of the host to the parasite and to a lesser extent with the age of the host.

This immunity is mediated by the host principally in two ways (a) by reducing the egg output of the parasite either, due to an interference with egg laying by the parasite, or by delaying its final maturation (b) through an accelerated expulsion of the adult worm burden, this period being associated with the presence of severe diarrhoea. Though the evidence for an accelerated self-cure reaction in calves previously exposed to infection is relatively slender, the situation may be akin to that occurring in N. brasiliensis in the rat where it has been demonstrated experimentally that previous infection with the parasite results in an accelerated self-cure of secondary infections (Jarrett, Jarrett and Urquhart, 1967). Clearly, a similar type of experiment where animals are killed serially following a primary and secondary infection of O. ostertagi is merited.

It may be that when animals are exposed for a more prolonged period to constant infection by O. ostertagi that immunity will become more absolute as suggested by Michel (1963, 1966). However, this theory depends on a steady rate of infection occurring over a period of over 200 days and as shown in Section I the build up of larval populations on pasture does not present such a steady situation. Furthermore, under the conditions of husbandry existing in many dairying areas of Britain, animals are usually

housed during the winter months and are therefore seldom exposed to infection with O. ostertagi for periods exceeding six months.

A tentative hypothesis of the situation occurring in the field is as follows: calves aged four to six months are turned out to graze for the first time in the spring and ingest O. ostertagi larvae which have overwintered. In late June, overwintered eggs of O. ostertagi hatch and the calves ingest more larvae until eventually clinical disease occurs if the numbers ingested are sufficient. Up to this point, it would appear that the calves have developed little or no immunity to ostertagiasis. Later in the season, immunity begins to develop and a restriction of adult worm burdens takes place and in some instances a self-cure reaction occurs. Since little development of O. ostertagi eggs takes place in the late autumn, it may be that the population of larvae on the pasture are 'ageing' and it becomes easier for the host's immunity to operate successfully. At this time, inhibition of larval development takes place with most larvae ingested and although this phenomenon may be partially associated with immunity, other factors are also involved. If challenged experimentally at this time, calves which have grazed all season are immune (Ross and Dow, 1964, 1965a). On the other hand, spring-born calves which have only been put out to graze in the autumn (and acquire mainly inhibited worm burdens) are not immune (Section III, Part B). In the normal course of events, calves are then housed until the following spring. During the winter and early spring there is a gradual loss of adult worm burden and a resumption of development of inhibited larval stages. If the latter is synchronous, Type II disease may occur. If an experimental challenge is given at this time, the challenge

infection becomes established and matures but a self-cure reaction then occurs. It seems likely in Type II ostertagiasis, which is frequently associated with intermittent diarrhoea, that a series of 'self-cures' occur following emergence of successive waves of developing stages from the gastric glands. Each of these waves makes the animals diarrhoeic and more debilitated; hence the poor prognosis in severe cases of Type II disease.

When the animals are turned out in the spring for the second year of grazing they seldom graze in the same pasture as in the first year and infections may be light. If, however, they do become infected, faecal egg counts do not become high and severe contamination of the pasture will not occur. Should the pasture be severely contaminated, and large numbers of O. ostertagi larvae be ingested, a rapid self-cure will occur. As the animals become older, the age immunity will begin to operate and larvae ingested will take a longer time to mature and indeed may never become egg laying. The possible influence of sex hormones on the egg laying of such worms should be borne in mind.

From such a hypothesis it is difficult to envisage how outbreaks of ostertagiasis in adults could occur as described by Bailey and Herlich (1953), Andrews et al (1955) and Bailey and Thorson (1954). Presumably, no immunity is absolute and if the challenge infection is particularly high, the resistance of an animal can break down. Since the animals in the American outbreaks referred to above had come from a non-endemic ostertagiasis area to an endemic one, just prior to the outbreak occurring, it is conceivable that a break down in resistance could occur.

Finally, the possibility of successfully artificially immunising calves against Type I and Type II ostertagiasis with irradiated larvae does not seem to be a practical proposition and other methods of control such as the judicious use of anthelmintics are more likely to prove efficacious.

Summary

1. A preliminary experiment on possible age immunity to O. ostertagi was carried out. The numbers of worms developing from a single oral inoculation of adult bovines with 300,000 O. ostertagi third stage larvae were fewer than those developing in young bovines inoculated with the same dose. The prepatent period of the O. ostertagi which became established in the adult animals was extended by at least seven days.

2. Calves which had been exposed to a heavy natural infection with O. ostertagi during the summer and early autumn were housed in late autumn. One group of calves was autopsied shortly after housing while the remainder were retained until the spring when they and worm free bovines of the same age were inoculated orally with 800,000 O. ostertagi infective larvae.

There was a reduction in the total worm burdens of the calves autopsied in the spring compared to those from the calves autopsied in the autumn. The worm burdens which developed from the challenge inoculum of 800,000 larvae were similar in the group of calves previously exposed to infection and the worm free group; there was an indication, however, that the self-cure reaction had been accelerated in the former group.

3. Prior immunisation of calves with two doses of 100,000 O. ostertagi third stage larvae irradiated at 60,000 or 80,000 roentgens did not result in a high degree of immunity to a challenge inoculum of 300,000 normal larvae.

4. Prior immunisation of calves with two doses of 100,000 O. ostertagi third stage larvae irradiated at 60,000 roentgens resulted in the development of none

resistance to further infection when grazed on a field known to be contaminated with O. ostertagi eggs and larvae. There was considerable individual variation between calves in the degree of resistance developed; it was therefore concluded that immunisation with X-irradiated larvae was unlikely to prove a practical proposition in the control of bovine ostertagiasis.

SECTION V

THE USE OF THIABENDAZOLE IN BOVINE OSTERTAGIASIS

A. The Anthelmintic Efficiency of Thiabendazole Against Developing and Inhibited Early Fourth Stage Larvae of *Ostertagia ostertagi*

B. The Treatment of Experimental Type I Disease

Introduction

The treatment of naturally occurring and experimental bovine ostertagiasis with thiabendazole (Thibenzole, Merck Sharp & Dohme, Rahway, New York, U.S.A. and Hoddesdon, Herts., England) at 25 to 330 mg. per kg. bodyweight has been reported by several workers (Bailey, Diamond and Walker, 1961, 1962; Baker and Douglas, 1962; Leiper and Crowley, 1963; Rubin, Ames and Cheyney, 1964). In these trials the criteria employed were clinical response, faecal egg counts and abomasal worm counts, and the general conclusion was that thiabendazole at the dosage rate of 110 mg. per kg. bodyweight was effective in the treatment of clinical ostertagiasis. The anthelmintic efficiency of the drug was high against adult stages of the worm at 110 mg. per kg. but variable against the larval stages in the mucosa, even at 330 mg. per kg. bodyweight (Bailey *et al*, 1962).

Within the last few years, the status of ostertagiasis as an important disease of cattle in the British Isles has been clearly established (Anderson *et al*, 1965a; Ross, 1966; Section I, Part A). The disease presents characteristics undescribed elsewhere and since thiabendazole is widely used in its treatment and prophylaxis it was thought desirable to study in more precise detail some aspects of the use of this drug in bovine ostertagiasis.

First, an attempt was made to establish a dosage level which would be effective against developing or inhibited early fourth stage larvae of *O. ostertagi*. It was shown earlier in this thesis (Section I) that the early fourth stage larvae are particularly important under field conditions.

The majority of larvae ingested in the late autumn do not develop beyond the early fourth larval stage but are delayed at this point for periods of up to six months; subsequent development of large numbers of these inhibited stages produces clinical signs of ostertagiasis.

Secondly, a study was made of the biochemical and pathological changes in the abomasum before and after the successful treatment of clinical ostertagiasis. It was shown earlier in this thesis (Section II) that clinical ostertagiasis is associated with a loss of parietal cell function resulting in a rise of the pH of abomasal fluid and a failure of peptic digestion. Consequently it is important to ascertain if removal of the O. ostertagi worm burdens accelerates the resolution of the pathological changes and a more rapid return to normal gastric function.

It was also hoped to study the treatment of the different types of ostertagiasis as seen in the field (Anderson et al, 1965a; Ross, 1966) using dosage levels which had proved effective against experimental infections. However, this work is not yet complete and results have not been included.

A. The Anthelmintic Efficiency of Thiabendazole Against Developing and Inhibited Early Fourth Stage Larvae of Ostertagia ostertagi

Experimental Design

Experiment 1

This experiment was designed to test the anthelmintic efficiency of thiabendazole against developing early fourth stage larvae of O.ostertagi. Fifteen parasite free calves, aged eight weeks, were allotted to three groups of five each, inoculated with 110,000 O. ostertagi third stage larvae and treated as follows four days after inoculation.

Group 1 - No treatment.

Group 2 - Thiabendazole orally at a dosage rate of 110 mg. per kg. bodyweight.

Group 3 - Thiabendazole orally at a dosage rate of 220 mg. per kg. bodyweight.

All calves were autopsied 11 days after inoculation.

Experiment 2

This experiment was designed to test the anthelmintic efficiency of thiabendazole against inhibited early fourth stage larvae of O. ostertagi. Two calves in the pre-Type II stage of ostertagiasis were obtained from each of three farms where Type II disease had occurred within the previous few weeks. It was assumed that these calves would be harbouring large numbers of inhibited early fourth stage larvae plus a few adult worms; this was later confirmed at autopsy of these calves. One calf from each

pair was treated orally with thiabendazole at a dosage rate of 110 mg. per kg. bodyweight and the other acted as an untreated control. The calves were autopsied seven days after treatment.

Observations

In both experiments daily clinical examinations of all calves were made prior to and following treatment. At autopsy, each abomasum and its contents and a peptic digest of the abomasal mucosa were examined for the presence of O. ostertagi.

Results

Experiment 1

The calves in this experiment, killed on day 11, did not show clinical signs and faecal consistency was unaltered. The numbers and stages of worms found at autopsy in treated and untreated groups of calves are shown in Table 42. The percentage efficiency of thiabendazole against developing early fourth stage O. ostertagi when used at 110 mg. per kg. and 220 mg. per kg. was 64% (range 26 to 78%) and 90% (range 80 to 96%) respectively.

Experiment 2

The calves in this experiment, which were in the pre-Type II stage, did not show any obvious clinical signs. The numbers and stages of O. ostertagi found at autopsy in treated and untreated calves are shown in Table 43. The percentage efficiency of thiabendazole against the few adults present was

Table 42

Worm Counts at Autopsy on Day 11 of Three Groups of Five Calves Inoculated with 110,000 *Ostertagia ostertagi* Larvae on Day 0, Two Groups Being Treated Orally with Thiabendazole on Day 4

		GROUP 1	GROUP 2	GROUP 3
Dosage Rate of Thiabendazole		Nil	110 mg/Kg	220 mg/Kg
Nos. of *O. ostertagi*		33,100	8,600	1,800
		40,000	28,000	7,500
		49,000	8,200	5,900
		36,600	15,500	3,700
		31,100	9,800	1,600
	Mean	37,960	13,620	3,700
Percentage Reduction Compared with Controls	Mean	-	64	90
	Range		26 - 73	80 - 96

Table 45

Worm Counts at Autopsy of Pairs of Pre-Type II Ostertagiasis Cases, One Calf in each Pair being Treated with Thiabendazole Seven Days prior to Autopsy

Dosage Rate in mg/Kg	Numbers of *Ostertagia ostertagi* at Autopsy						Estimate of Efficiency Against 4th Larval Stages
	Treated			Untreated			
	Total	Adults & Developing Stages	Early Fourth Larval Stages	Total	Adults & Developing Stages	Early Fourth Larval Stages	
110	141,000	3,200	137,800	* 67,500	6,200	61,300	Nil
	156,000	5,000	151,000	*150,200	15,400	134,800	Nil
	23,200	5,300	18,500	40,000	18,200	21,800	15

*Also used as Controls in Experiment 1, Section 3B

high, while the efficiency against inhibited fourth stage larvae was nil in two calves and 15% in the other.

Discussion

The results from these experiments, designed specifically to test the anthelmintic efficiency of thiabendazole against both normally developing and inhibited early fourth stage O. ostertagi larvae, show that although a dosage level of 110 mg. per kg. bodyweight will give a reasonable efficiency (64%) against normally developing early fourth stage larvae, the same dosage rate was completely ineffective against inhibited early fourth stage larvae. By increasing the dosage rate to 220 mg. per kg. bodyweight, the efficiency against developing fourth stage larvae was increased to 90%.

It is interesting that Dunsmore (1962, 1965) working with experimental infections in sheep, found thiabendazole used at 62.5 mg. per kg. bodyweight to be highly effective against fourth stage Ostertagia spp. larvae, but using the same dosage rate found the drug much less efficient against inhibited fourth stage larvae in naturally occurring infections of Ostertagia spp. It is possible that the lack of anthelmintic efficiency also noted by Bailey et al (1962) against fourth stage O. ostertagi in field infections may have been due to the fact that these larvae were inhibited in their development.

These results strengthen the suggestion put forward in Section III, that inhibited O. ostertagi larvae are not metabolising normally and might therefore be unable to absorb thiabendazole in the manner of normally developing larvae.

B. The Treatment of Experimental Type I Disease

Experimental Design

This experiment was designed to test the anthelmintic efficiency of thiabendazole against adult O. ostertagi at 220 mg. per kg. and to study the biochemical and pathological changes in the abomasum before and after treatment of clinical ostertagiasis. Ten parasite free calves, aged eight to ten weeks, were allotted to two groups of five each (Groups 1 and 2) and inoculated on day 0 with 400,000 O. ostertagi third stage larvae. Group 1 remained untreated, while each calf in Group 2 was treated orally with thiabendazole at 220 mg. per kg. bodyweight on the day of onset of clinical signs (i.e. anorexia, weight loss and diarrhoea). One calf in each group had an abomasal cannula inserted to enable samples of abomasal fluid to be withdrawn, so that daily changes in the pH of abomasal contents could be followed. All calves in Groups 1 and 2 were autopsied 28 days after inoculation. A further two parasite free calves (Group 3) were also inoculated on day 0 with 400,000 O. ostertagi larvae and were autopsied 14 days later to establish the infectivity of the inoculum; in experimental infections of this magnitude a large proportion of the worm population is usually expelled between the 17th and 35th day (see Section II, Experiment 1).

Observations

A clinical examination of all calves was made daily and faecal samples were inspected for an assessment of their consistency. The calves in Groups

1 and 2 were weighed prior to inoculation on day 0 and thereafter on day 7, 14, 20, 25 and 28 and faecal samples were collected on day 0 and daily thereafter from day 17 to 28. Faecal egg counts were made using the zinc sulphate flotation and McMaster methods. Samples of abomasal fluid for pH estimation were collected daily from the two calves with abomasal cannulae. These cannulae were inserted as previously described. At autopsy, the pH of abomasal contents of the calves were determined and parasitological and pathological examinations of the abomasum carried out as described earlier. The calculated dose of thiabendazole was weighed out, mixed with water in a bottle and administered orally.

Results

Clinical Data .- All the calves in Groups 1 and 2 developed clinical signs of ostertagiasis, i.e. anorexia, weight loss and diarrhoea, within 17 to 28 days of inoculation; these signs persisted in the calves of the untreated Group 1 until autopsy on day 28, by which time three of these calves were in extremis. Following the treatment of calves in Group 2, there was a complete cessation of diarrhoea and return of appetite within 48 hours; these changes were reflected in the resumption of weight gains three to four days after treatment. The mean bodyweights of the calves in both groups during the experiment are shown in Figure 41.

Biochemical Results .- In the treated calves (Group 2) the pH of their abomasal contents (see Table 44) had returned to normal levels (mean 3.2) whereas in the untreated calves (Group 1) they remained elevated (mean 7.0).

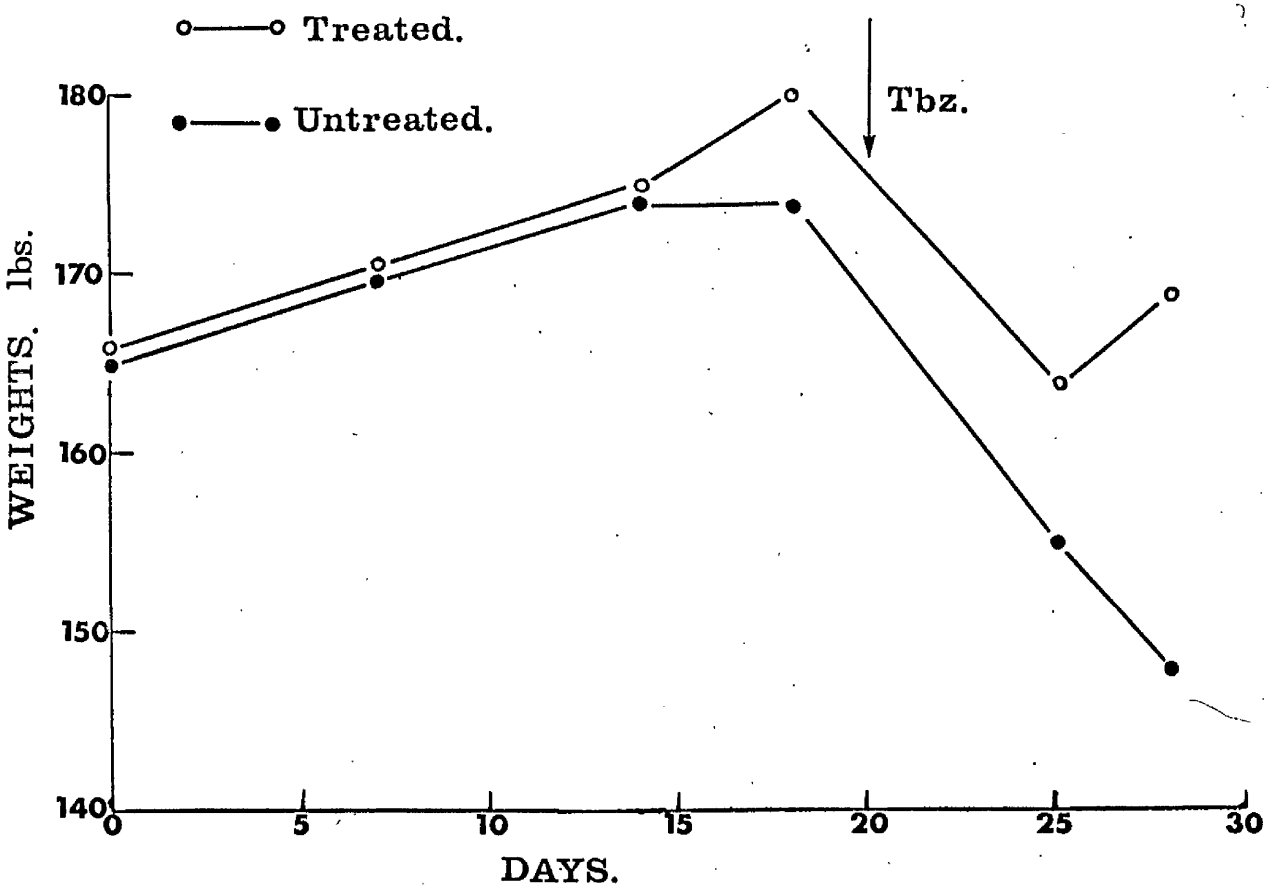


Fig. 41 Mean bodyweights from two groups of five calves each, following inoculation of 400,000 Ostertagia ostertagi on day 0, one group being treated with thiabendazole (Tbz.) on day 21 or 22.

Daily changes in the pH of the abomasal contents of the two calves with abomasal cannulae are outlined in Figure 42. These results demonstrate that the pH of the abomasal contents became markedly elevated in both calves after day 19 and remained elevated in the untreated calf until autopsy on day 28; in the treated calf the pH of the abomasal contents decreased steadily until autopsy on day 28.

Parasitological Findings. - Faecal egg counts were first positive in calves of both groups on day 17 and increased in magnitude until day 23 in the untreated calves and thereafter decreased steadily; in the treated calves, faecal egg counts decreased to zero within 24 hours of treatment. The course of the mean faecal egg counts in both groups are shown in Figure 43.

The worm counts at autopsy of the calves in Group 1 and Group 2 on day 28 are given in Table 44. In the treated group, the mean numbers of O. ostertagi were reduced by 94% compared to the numbers present in the untreated group. The numbers of O. ostertagi found at autopsy on day 14 of the two calves in Group 3 were 138,000 and 110,000 respectively; this indicated that the larvae used in this experiment were of high infectivity.

Pathological Data. - The gross lesions found at autopsy of the calves in Groups 1 and 2, at 28 days after inoculation, were similar to the post-emergent lesions described in Section II and by Jarrott (1966). Thus, the unit lesions, i.e. the nodules, had coalesced to give rise to a 'morocco leather' like appearance. There were also areas of superficial necrosis and the abomasal lymph nodes were highly reactive. The abomasal mucosa in the untreated calves was thickened and more oedematous than in

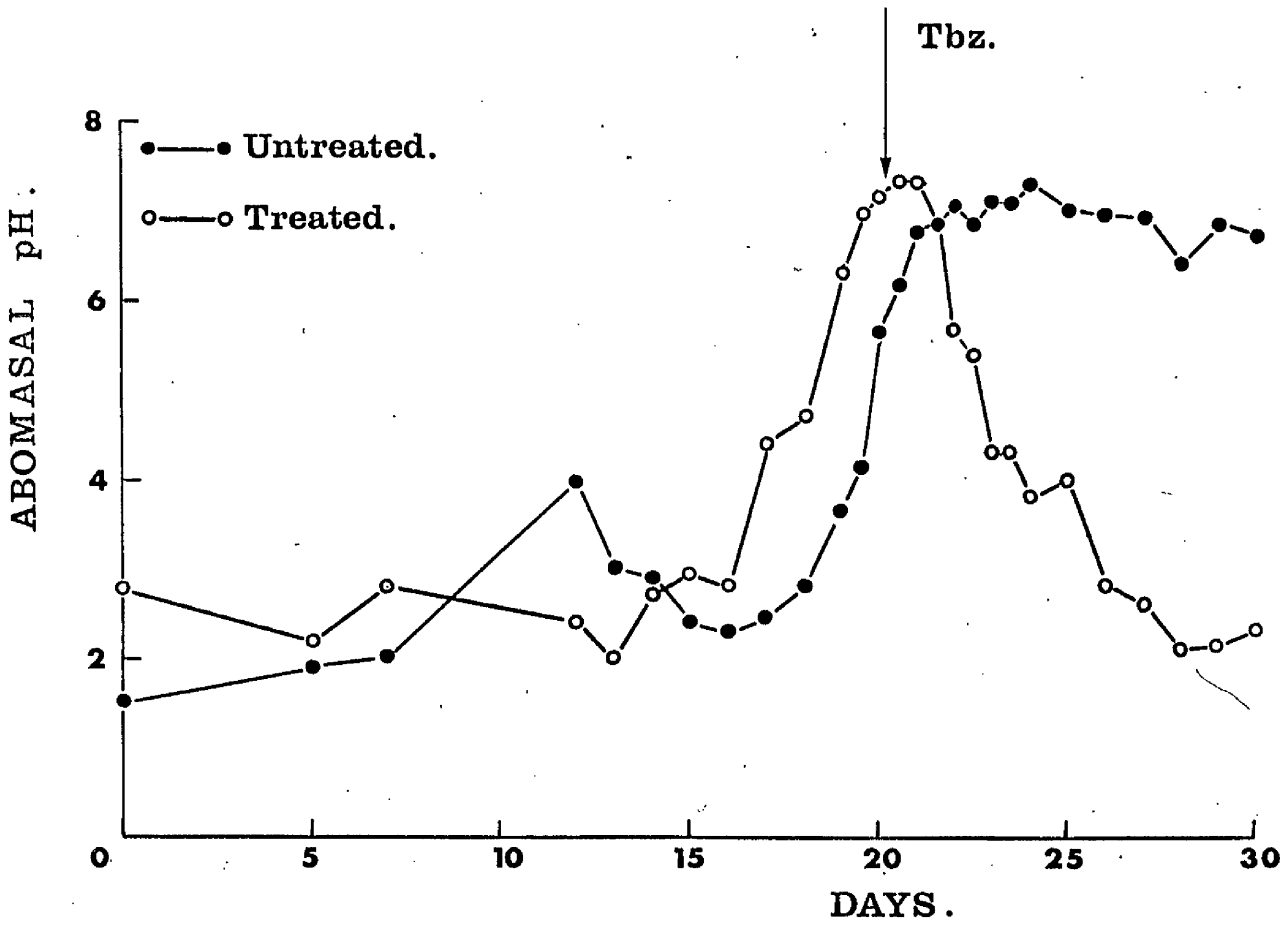


Fig. 42 The pH of abomasal contents from two calves (with abomasal cannulae) following inoculation of 400,000 Ostertagia ostertagi on day 0, one calf being treated with thiabendazole (Tbz.) on day 21.

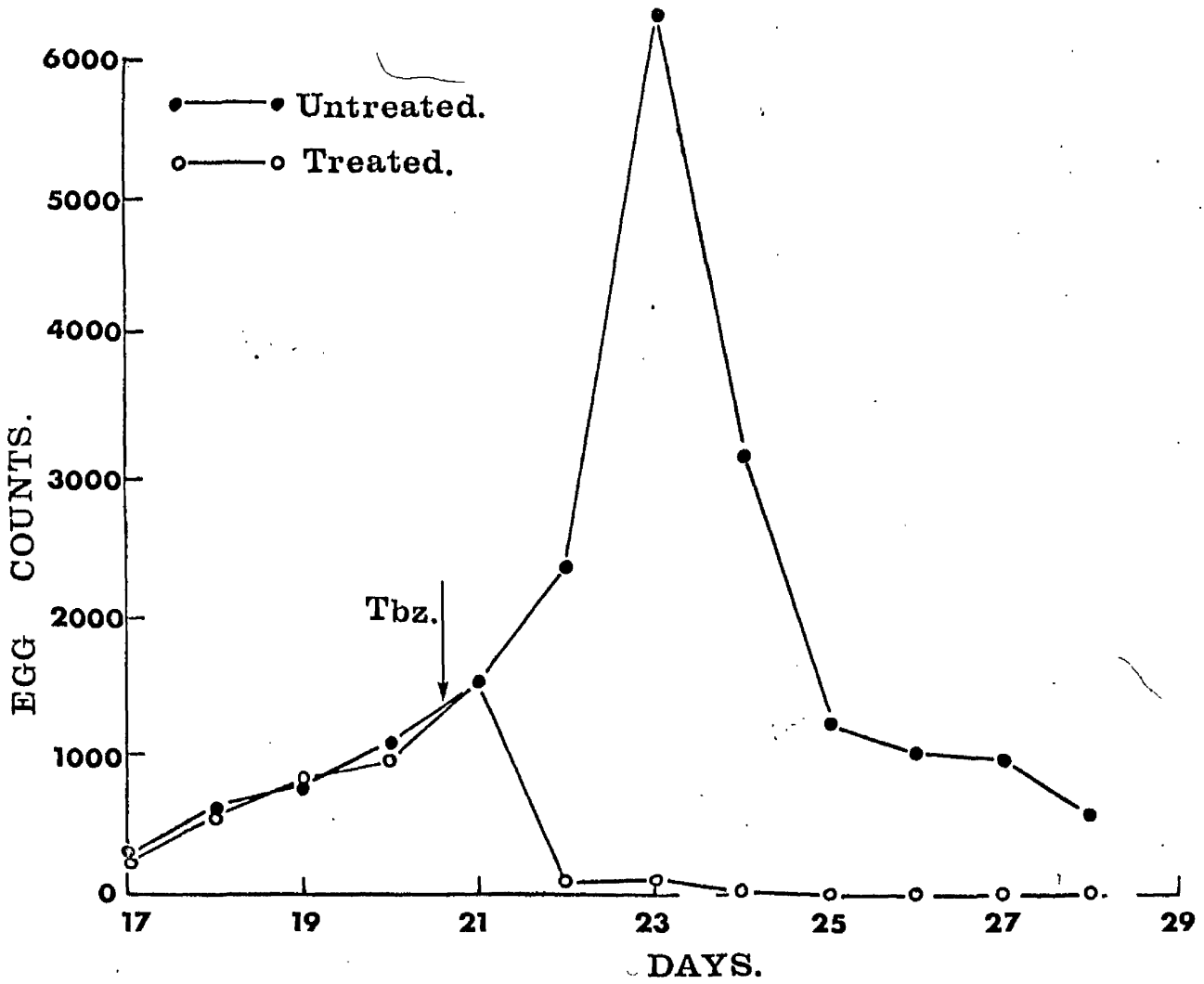


Fig. 43 Mean faecal egg counts from two groups of five calves each following inoculation of 400,000 Ostertagia ostertagi on day 0, one group being treated with thiabendazole (Tbz.) on day 21 or 22.

Table 44

Worm Counts and pH of Abomasal Fluid at Autopsy on Day 28 of Two Groups of Five Calves Inoculated with 400,000 *Ostertagia ostertagi* on Day 0, One Group being Treated Orally with Thiabendazole on the Day of Onset of Clinical Signs of Ostertagiasis.

Group	Day Treated with Thiabendazole		Enc. of *O. ostertagi*	pH of Abomasal Fluid
1	-		12,500	6.7
			15,900*	7.0
			19,800	6.9
Untreated			14,600*	7.2
			11,500*	7.1
		Mean	14,460	7.0
	20		300	2.3
2	20		100	4.9
Thiabendazole	22		1,700	2.0
@	20		700	2.8
220 mg/Kg	21		1,700	4.1
		Mean	900	3.2
Percentage reduction compared with controls		Mean	94	
		Range	88 - 99	

*Killed *in extremis*

the treated ones.

Histologically, in both treated and untreated calves emergence of the O. ostertagi from the gastric glands was complete and there were numerous dilated post-infection glands lined by columnar mucous secreting cells. The glands surrounding these in the untreated calves of Group 1 showed a marked apparent loss of functional differentiation (see Fig. 22 in Section II). In some instances these were lined by cuboidal epithelium and in other instances by columnar mucous secreting cells.

By contrast, in the treated calves, many of the surrounding glands showed a return of functional differentiation (Fig. 44) and were lined by zymogen cells, parietal cells and mucous neck cells, although some hyperplasia of undifferentiated cuboidal cells and mucous secreting cells was still present.

There was a mononuclear cell response with numerous plasma cells in the lamina propria of the superficial part of the gastric mucosa in both groups of calves, but the reaction was much more intense in the untreated calves. A moderate eosinophil response was present in the lamina propria in both treated and untreated animals. Cytolysis and necrosis of the superficial gastric mucosa were extensive in the untreated animals.

Discussion

It has previously been shown that single large experimental inoculations of O. ostertagi of a similar magnitude to that used here, do not produce inhibition of larval development but mature in 21 days and produce clinical ostertagiasis identical to Type I disease in the field (Anderson et al, 1966;

Fig. 44. Mucosa of abomasum from calf inoculated with 400,000 *Ostertagia ostertagi* on day 0, treated with thiabendazole on day 21 and killed on day 28. The hyperplastic mucosa contains post-parasitised glands and shows the return of the parietal cell mass (x 180).

Section II, Part A). Since inhibition of larval development did not occur in this experiment and clinical signs of ostertagiasis were noticed 17 to 21 days after inoculation, the results are primarily applicable to the Type I syndrome.

The results of this experiment are interesting for two reasons. First, the high efficiency of thiabendazole at 220 mg. per kg. bodyweight in the treatment of clinical ostertagiasis was confirmed, in that a dramatic cessation of diarrhoea and resumption of appetite in the treated calves occurred within 48 hours and at autopsy the mean number of adult worms present was reduced by 94% when compared to the untreated group. The relatively low O. ostertagi worm burdens in the untreated calves in Group 1, which were autopsied 28 days after inoculation of 400,000 O. ostertagi larvae, is a reflection of the normal elimination of worm burdens which usually takes place 17 to 35 days following inoculations approaching this magnitude with O. ostertagi (Section II). The decrease in faecal egg counts in the untreated calves (Group 1) between days 23 and 28 is also attributable to this loss of worm burden (Fig. 43).

Secondly, the treatment of clinical Type I ostertagiasis with thiabendazole facilitated the rapid return of parietal cell function as indicated by the comparative figures for the pH of abomasal contents of calves in Groups 1 and 2 (Table 44; Fig. 42). Previous experiments (Section II) have demonstrated that 21 days after inoculation with 300,000 O. ostertagi larvae, the pH of abomasal fluid rises and remains elevated for 30 days during which peptic digestion is not possible. The return of normal peptic digestion and appetite in the treated calves in Group 2 explains the

resumption in body weight gains (Fig. 41). These biochemical changes are reflected in the comparative pathological findings, in that the abomasal lesions of treated calves have begun to regress five to seven days after treatment and histologically showed a reappearance of parietal cells (Fig. 44).

General Discussion

The results of these experiments suggest that 220 mg. per kg. bodyweight is likely to be the optimal dosage rate of thiabendazole for the treatment of severe cases of naturally occurring Type I ostertagiasis, where the larvae ingested develop to adults in 21 days. However, it is still not known if this dose level will be efficacious in the treatment of the pre-Type II and Type II syndromes, where larvae do not develop to the adult stage within 21 days but may remain inhibited at the early fourth stage for an indefinite period. A dosage level of 110 mg. per kg. bodyweight of thiabendazole may also give good control of outbreaks of Type I ostertagiasis but in particularly severe outbreaks the reduced efficiency of the drug at this dosage rate against fourth stage larvae (64%) and also described by Rubin et al (1964), may necessitate a second treatment being given. This dosage level is inefficient in the treatment of pre-Type II ostertagiasis and is therefore unlikely to be efficacious in the treatment of Type II disease.

However, 110 mg. per kg. bodyweight is probably an adequate dosage level of thiabendazole for routine prophylactic treatments of calves during the summer grazing season. The number and timing of such treatments has been discussed in detail by Armour and Urquhart (1965) and Reid, Armour, Jennings, Kirkpatrick and Urquhart (1967). By extrapolation of the studies described in Section I, Part B of this thesis, these authors suggested that a single treatment in mid-July at the time when pasture levels of infective larvae were increasing, followed by a move to clean pasture, would suffice

to control both the Type I and Type II disease. However, in many dairying areas where it is current practice to graze calves annually on the same paddocks, the pastures are heavily infected, even after severe winters, and a single treatment in mid-July might not be sufficient to prevent clinical ostertagiasis developing. In these circumstances, two treatments may be necessary, firstly, three to four weeks after going to grass (i.e. about early June) to remove burdens resulting from overwintered larvae, and secondly, six to eight weeks later (i.e. about early August) to remove the burdens resulting from ingestion of larvae hatched from overwintered eggs. The second treatment should, if possible, be accompanied by a move to clean pasture. Provided this procedure is adopted, Type I and Type II disease will not occur and it will be safe to graze young spring-born calves beside the older calves. However, if clean ground is not available and calves cannot be moved, a third treatment in mid-September may be necessary. Following this treatment, calves must be moved to clean pasture or housed, otherwise they may become reinfected with larvae which do not develop immediately, are insusceptible to anthelmintics, and could give rise to Type II disease in the following spring.

Based on observations on faecal egg counts of calves and the annual patterns of numbers of O. ostertagi larvae on herbage, Michel (1966) made similar recommendations for controlling Type I ostertagiasis, namely, a single treatment with thiabendazole in mid-July, followed by a move to clean pasture. He did not, however, consider the need to control Type II ostertagiasis.

Summary

1. The anthelmintic efficiency of thiabendazole against both normally developing and inhibited O. ostertagi fourth stage larvae was studied. At a dosage level of 110 mg. per kg. bodyweight the drug had an efficiency of 64% against normally developing larvae and nil against inhibited larvae. When the dose level was increased to 220 mg. per kg. bodyweight the anthelmintic efficiency against normally developing larvae was increased to a mean of 90%.

2. The treatment of experimentally produced clinical ostertagiasis with thiabendazole was studied. Administration of the drug at a dose level of 220 mg. per kg. bodyweight resulted in a complete cessation of clinical signs within 48 hours. In a group of five calves autopsied after the abatement of clinical signs, the mean O. ostertagi worm burden was reduced by 94% when compared to that from an untreated group of five calves autopsied at the same time. Histological examination of the abomasal mucosa revealed that thiabendazole treatment had facilitated the rapid return of parietal cell function and acid conditions in the abomasum.

GENERAL SUMMARY

Section I

Field Studies on Parasitic Gastritis in Young Dairy Cattle in South-West Scotland

1. Field investigations into 30 outbreaks of parasitic gastro-enteritis in young cattle in South-west Scotland showed that O. ostertagi was the predominant parasite. The disease caused by this parasite was classified into three forms, two of which (Type I, Type II) were clinically apparent and characterised by weight loss and diarrhoea. Type I occurred at any time during the summer and early autumn, i.e. July until October. Pre-Type II was not clinically apparent although the calves harboured large populations of O. ostertagi, of which over 80% were inhibited in their development at the fourth larval stage. Type II occurred at any time during late winter and early spring, i.e. January until May, and resulted from the maturation of large numbers of inhibited larval stages.

2. A study on the fluctuations in pasture populations of O. ostertagi larvae during the grazing season, i.e. May until November, was made using the worm burdens at autopsy of susceptible 'test' calves which grazed for only 14 days to quantitate the level of herbage infections. The numbers of O. ostertagi larvae on the pasture increased during the season and reached a maximum in August and September; thereafter they

declined slightly. The proportion of worms inhibited in their development in the 'test' calves reached a maximum of over 90% in November.

3. Observations on the development and longevity of O. ostertagi eggs and larvae on pasture during the winter months were carried out. Both the eggs and infective larvae successfully overwintered but the development of the eggs to infective larvae was not completed until the following summer.

Section IX

Experimental Ostertagia ostertagi Infections in Calves

4. Inoculation of calves with 300,000 O. ostertagi larvae resulted in the occurrence of clinical signs of ostertagiasis 19 days later. These symptoms persisted for a period of one week and then abated. In the calves autopsied during the period of severe diarrhoea, the pH of the abomasal contents was markedly elevated as were the levels of plasma pepsinogen. Abomasal lesions seen at autopsy of severe field outbreaks of ostertagiasis were produced. An exponential loss of adult worms occurred from day 17 onwards.

5. The daily changes in abomasal biochemistry following inoculation with 300,000 O. ostertagi were followed in calves with abomasal cannulae. The period of maximal changes was between 19 and 27 days after inoculation, when there was an increased pH and an increased

number of viable bacteria present. During this period, considerable numbers of adult O. ostertagi were found in the faeces.

Section XII

Studies on Inhibited Larval Development of Ostertagia ostertagi

6. Susceptible worm free calves were grazed during spring, summer and autumn for periods of one day or 14 days on pasture known to be contaminated with O. ostertagi larvae. At autopsy seven days after removal from pasture the proportion of inhibited fourth stage larvae found was significantly higher in calves which had grazed during autumn; the proportion was the same in calves which grazed for one day or 14 days.

7. Calves known to be harbouring large numbers of inhibited larval stages of O. ostertagi (Pre-Type II) were treated with large doses of cortisone or methotrexate. The administration of these drugs did not result in the resumption of development of significant numbers of inhibited larval stages.

8. Calves known to be harbouring large numbers of inhibited larval stages of O. ostertagi (Pre-Type II) were given a challenge inoculum of 100,000 normal or 100,000 X-irradiated O. ostertagi infective larvae. The challenge inoculum developed to maturity in the expected period of 21 days and apparently 'leap-frogged' the inhibited larvae in the gastric glands.

9. A daily oral inoculation of 1,500 O. ostertagi infective larvae was given for 100 days to each of five calves previously exposed to an experimental infection of O. ostertagi. At autopsy seven days after the last dose, a high proportion of the worm burdens present in three of the five calves was inhibited in development at the fourth larval stage.

10. Experiments were carried out to elucidate the mechanism whereby infective larvae of O. ostertagi ingested in the late autumn became inhibited in their development at the fourth larval stage. Susceptible calves were grazed in late autumn on paddocks contaminated at various times of the year with either a laboratory strain of O. ostertagi eggs or a strain obtained from a farm in the West of Scotland. Further susceptible calves were grazed on a worm free paddock and inoculated orally with O. ostertagi larvae cultured in the laboratory. Negligible numbers of inhibited fourth stage larvae were present in the calves inoculated orally whereas considerable numbers were present in the calves which ingested larvae developed at pasture. There was no correlation between the size of the total worm burdens and the percentages of inhibited fourth stage larvae. A significantly higher proportion of inhibited larval stages was found in the calves which grazed on the paddock contaminated with the field strain. It was concluded that larval inhibition in bovine ostertagiasis is probably dependent on two factors (a) an innate physiological susceptibility of a particular strain of larvae and (b) the environmental circumstances of late autumn which, acting on developing or infective larvae, provide an optimal stimulus for the subsequent inhibition of ingested larvae.

Section IV

Immunity to Ostertagiasis

11. A preliminary experiment on possible age immunity to O. ostertagi was carried out. The numbers of worms developing from a single oral inoculation of adult animals with 300 000 O. ostertagi infective larvae were fewer than in young calves and the pre-patent period was extended.

12. Calves which had been exposed to a heavy field infection with O. ostertagi during the summer were housed in late autumn. One group of calves was autopsied shortly after housing while the remainder were retained until the spring when they and worm free calves of the same age were inoculated orally with 600,000 O. ostertagi infective larvae. There was a reduction in the worm burdens of calves autopsied in the spring compared to those from calves autopsied in the autumn. Although the worm burdens which developed from the challenge inoculum were similar in both the calves previously exposed to infection and the worm free calves there was an indication that the self-cure reaction was accelerated in the former.

13. Prior immunisation of calves with two doses of 100,000 O. ostertagi larvae irradiated at 60,000 or 80,000 roentgens did not result in a high degree of immunity to a challenge inoculum of 300,000 normal larvae.

14. Prior immunisation of calves with two doses of 100,000 O. ostertagi larvae irradiated at 60,000 roentgens resulted in the development of some resistance to further infection when grazed on a field known to be contaminated with O. ostertagi larvae. There was considerable individual variation between calves in the degree of resistance developed.

Section V

The Use of Thiabendazole in Bovine Ostertagiasis

15. The anthelmintic efficiency of thiabendazole against normally developing and inhibited O. ostertagi fourth stage larvae was studied. At a dosage level of 110 mg. per kg. bodyweight the drug had an efficiency of 64% against normally developing larvae and nil against inhibited larvae. When the dose level was increased to 220 mg. per kg. bodyweight the efficiency against normally developing larvae was increased to a mean of 90%.

16. The treatment of experimentally produced clinical ostertagiasis with thiabendazole was studied. Administration of the drug at a dose level of 220 mg. per kg. bodyweight resulted in a complete cessation of clinical signs within 48 hours and in a group of five calves autopsied at that time the mean worm burden was reduced by 94% when compared to that from an untreated group of five calves. The thiabendazole treatment also facilitated the rapid return of parietal cell function and acid conditions in the abomasum.

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A P P E N D I C E S 1 to 5

APPENDIX I – Table 1

Bodyweights of Calves Grazed at Farm A in 1965

Date	CALF NO. A11	A12	A13	A14	A15	A16	A17	A18	Mean & Standard Error
24.6	150	143	146	170	178	175	148	138	156 ± 5.6
1.7	148	150	148	172	170	181	155	139	159 ± 5.6
8.7	154	152	147	183	180	184	157	139	162 ± 6.3
15.7	160	157	151	189	185	188	162	140	167 ± 6.6
22.7	168	168	194	200	193	188	171	142	173 ± 7.0
29.7	174	177	162	200	202	183	177	150	179 ± 6.0
5.8	181	178	172	210	206	191	187	156	185 ± 6.2
12.8	186	185	177	220	210	191	192	158	190 ± 6.7
19.8	194	187	180	230	216	205	192	167	196 ± 7.1
26.8	203	198	192	237	223	215	201	174	205 ± 6.9
2.9	208	189	194	249	230	216	205	184	209 ± 7.7
9.9	210	181	194	251	227	217	205	180	208 ± 8.5
16.9	211	187	199	255	230	215	204	180	210 ± 8.5
23.9	189 $^{+}_{A}$	183	203	254	224	214	203	183	207 ± 8.5
30.9	-	155 $^{+}_{A}$	199	232	219	214	203	182	201 ± 9.7
7.10	-	-	180 $^{+}_{A}$	192 $^{+}_{A}$	192 $^{+}_{A}$	205	192	181	190 ± 3.7

A = Autopsied + = severe diarrhoea

N.B. Weights for calves at autopsy not included in mean shown in Fig. 10.

APPENDIX 1 - Table 2

Weekly Strongyle Faecal Egg Counts of Calves Grazed at Farm A in 1964

Date	CALF NO. A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Mean
25.5-15.6	All negative										
22.6	100	50	0	200	50	0	100	200	50	150	90
29.6	150	350	150	100	0	200	0	50	100	300	140
6.7	900	750	400	200	150	500	150	50	500	350	425
13.7	300	450	600	350	300	400	450	250	400	350	385
20.7	250	150	50	N.S.	500	350	150	50	50	150	189
27.7	350+	400+	300	650	500	700	450	50	450	150	400
3.8	1000+	1600+	1200+	800	N.S.	350	450	300	950	400	783
10.8*	1700+ A	3400+ A	2150+ A	850+ A	2050+ A	N.S.	150	100	650	250	1144
17.8	-	-	-	-	-	3100	50	250	500	500	480
24.8	-	-	-	-	-	150	350	150	500	250	280
31.8	-	-	-	-	-	700+	750	150	1100+	200	580
7.9	-	-	-	-	-	1900+ A	2100+ A	900+	250	300	1090
At autopsy	-	-	-	-	-	-	-	850+ (12.9.64)	800+ (9.10.64)	1750+ (26.10.64)	1133

A = Autopsied + = severe diarrhoea

* = 5 calves autopsied and sampled 5.8.64

N.S. = No sample

N.B. Counts from calves at autopsy not included in means shown in Figs. 11 and 12.

APPENDIX 1 - Table 3

Weekly Strongyle Faecal Egg Counts of Calves Grazed at Farm B in 1964

Date & Field		CALF NO. B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	Mean
	S		11.5 - 1.6 All negative					7.6 All	100 o.p.g.			
15.6	L	250	150	200	300	150	0	200	350	0	0	160
22.6	L	300	200	200	600	150	200	200	450	50	50	240
29.6	L	300	200	250	500	450	50	450	450	300	50	300
6.7	L	250	500	450	300	350	150	500	600	150	50	330
13.7	L	450	350	250	150	350	150	550	350	0	50	265
20.7	L	250	0	300	300	100	250	150	300	100	50	180
27.7	S	300	250	450	250	200	200	150	250	50	100	220
3.8	S	700	550	500	450	200	200	550	600	250	50	405
10.8	L	50	300	0	150	50	350	150	50	150	50	130
17.8	L	250	150	300	150	250	150	100	300	100	200	215
24.8	S	450	550	350	200	300	250	250	350	150	250	310
31.8	S	400	400	400	400	150	200	150	450	400	350	330
7.9	L	600+	800+	600+	150	200+	200	950	300	350	50	420
x 14.9	L	3400+ A	1250+ A	3400+ A	3150+ A	600+ A	3150+	1650+	950+	150	150	1785
Δt Autopsy		-	-	-	-	-	5150+ (1.10.64)	1650+ (2.10.64)	3450+ (2.10.64)	250 (26.10.64)	850 (26.10.64)	2270

A = Autopsied + = severe diarrhoea

x = 5 calves autopsied and sampled 11.9.64

S = Small Field L = Large Field

N.B. Counts from calves at autopsy not included in means shown in Figs. 11 and 12.

APPENDIX 1 - Table 4

Weekly Strongyle Faecal Egg Counts of Calves Grazed at Farm A in 1965

	CALF NO.								
Date	A11	A12	A13	A14	A15	A16	A17	A18	Mean
24.6-15.7				A11 negative					
22.7	50	0	100	300	150	100	50	0	94
29.7	50	300	0	50	300	200	100	100	130
5.8	250	150	150	100	250	400	100	150	194
12.8	500	300	50	650	150	250	150	300	294
19.8	150	500	100	400	100	150	200	300	250
26.8	550	200	400	750	200	250	400	550	413
2.9	250	150	50	550	50	100	150	200	163
9.9	200	200	300	400	50	100	200	400	251
16.9	1000*	200	100	500	0	250	150	150	294
23.9	1000*A	1000*	150	900	50	100	50	50	513
30.9	"	" A	0	250	50	50	0	200	92
7.10	"	" A	950*A	1000*A	1250*A	100	150	150	733

A = Autopsied * = Severe diarrhoea

N.B. Counts from calves at autopsy not included in means shown in Fig. 11.

APPENDIX 1 - Table 5

Weekly Strongyle Faecal Egg Counts of Calves Grazed at Farm B in 1965

Date & Field		CALF NO. B11	B12	B13	B14	B15	B16	B17	B18	Mean
24.6-15.7	S	All negative								
22.7	S	0	0	0	0	50	0	50	50	19
29.7	S	0	100	100	50	100	50	250	50	68
5.8	S	50	50	0	50	100	250	0	100	75
12.8	L	100	100	450	150	200	100	0	50	144
19.8	L	0	100	200	150	100	50	100	250	119
26.8	S	150	300	200	50	350	550	150	50	225
2.9	L	100	50	250	250	100	250	100	150	156
9.9	S	300	300	350	350	300	400	500	100	325
16.9	L	200	400	400	50	200	100	300	500	269
23.9	S	50	50	100	100	100	250	300	600	194
30.9	L	50	200	200	50	150	450	350	350	225
7.10	S	50	50	650^{+}_{A}	200	500	350	500	550	301

A = Autopsied

S = Small Field

$^{+}$ = Severe diarrhoea

L = Large Field

N.B. Counts from calves at autopsy not included in means shown in Fig. 11.

APPENDIX 1 - Table 6

Differential Worm Counts at Autopsy of 15 Cases of Type I Ostertagiasis

Farm & Case No.	Date Autopsied	Total	Adults	Ostertagia spp.* Developing Stages	Ostertagia spp.* Early 4th Larval Stages		Sex Ratio	Cooperia spp. (C. oncophora & C. mcmasteri) 4th		Nematodirus spp. (N. helvetianus & N. spathiger) 4th	
					Total	%	M:F	Adults	Larval Stages	Adults	Larval Stages
1964											
A1		64,000	22,000	39,600	2,400	4	1:1	9,100	200	8,700	1,700
A2		51,500	45,700	2,800	3,000	6	1:1	2,500	0	2,200	0
A3	5.8.64	43,100	21,200	19,900	2,000	5	1:1	3,200	200	1,200	300
A4		24,200	20,000	600	3,600	15	3:4	1,000	100	1,300	1,000
A5		23,100	20,200	2,000	900	4	3:4	900	0	1,800	200
1964											
B1		61,800	28,500	12,500	20,800	34	1:1	19,700	6,500	2,300	800
B2		111,200	65,400	25,800	20,000	18	3:4	1,000	300	0	0
B3	11.9.64	81,100	44,000	27,800	9,300	12	1:1	24,700	2,800	2,300	1,500
B4		89,600	75,700	0	13,900	16	1:1	21,000	1,500	0	200
B5		79,700	47,700	27,200	4,800	6	1:1	16,000	2,700	400	200
1965											
A11	23.9.65	84,700	46,500	35,900	2,700	3	1:1	1,100	200	2,400	300
A12	30.9.65	84,500	33,600	24,500	26,400	31	3:4	2,900	600	4,800	2,800
A13	7.10.65	48,000	24,000	1,000	23,000	48	1:1	1,100	500	10,700	1,900
A14	7.10.65	200,300	36,700	30,100	133,500	67	1:2	4,800	4,600	15,100	4,200
A15	7.10.65	37,000	29,500	6,900	1,500	4	1:1	4,500	300	18,200	300

Up to 1,000 Trichostrongylus axei present in A1, A3, A4, A5

Up to 5,600 T. axei present in A2

*98 per cent adult males Ostertagia ostertagi, 2 per cent Ostertagia lyrata

APPENDIX 1 - Table 7

Differential Worm Counts at Autopsy of 15 Cases of Pre-Type II Ostertagiasis

Case No.	Month Autopsied	Ostertagia spp.* Total	Adults	Developing Stages	Early 4th Larval Stages Total	%	Sex Ratio	Cooperia spp. C. oncophora & C. mcmasteri Adults	4th Larval Stages	Nematodirus spp. N. helvetianus & N. spathiger Adults	4th Larval Stages
D1	Jan.	103,400	5,300	0	98,100	95	1:1	3,200	1,900	0	0
D2	Jan.	85,000	6,400	1,200	77,400	91	1:1	3,400	1,500	0	0
D3	Jan.	138,700	18,100	0	120,600	87	3:4	700	700	0	0
D4	Jan.	86,700	5,800	1,600	81,300	94	3:4	2,600	3,100	0	0
D5	Jan.	72,200	2,400	0	69,800	97	2:3	100	0	0	0
D6	Feb.	128,700	18,000	1,700	109,000	85	1:1	600	600	0	0
D7	Feb.	140,800	3,700	0	137,100	98	2:3	4,700	3,900	0	0
D8	Feb.	142,900	13,500	1,000	128,400	89	1:1	6,500	6,300	400	400
D9	Feb.	87,500	6,000	200	81,300	93	1:4	4,200	4,400	300	300
D10	Feb.	150,200	12,700	2,700	134,800	90	1:1	3,200	3,100	200	100
D11	Feb.	117,100	3,500	600	113,000	97	3:2	4,100	1,000	0	0
D12	March	159,400	4,000	0	155,400	98	2:3	0	0	0	0
D13	March	141,100	11,000	0	130,100	92	1:2	4,200	1,800	0	0
D14	March	66,900	9,200	3,600	54,100	81	1:1	2,600	2,800	0	0
D15	May	181,700	8,500	7,400	165,800	91	1:1	0	0	0	0

15,700 Trichostrongylus axei in D3 100 T. axei in D5

*98 per cent Adult Males Ostertagia ostertagi, 2 per cent Ostertagia lyrata

APPENDIX 1 - Table 3

Differential Worm Counts at Autopsy of 15 Cases of Type II Osterbagiasis

Case No.	Month Autopsied	Ostertagia spp.* Total	Adults	Developing Stages	Early 4th Larval Stages Total	%	Sex Ratio	Cooperia spp. C. oncophora & C. monasticci Adults	4th Larval Stages	Nematodirus spp. N. helvetianus & N. spathiger Adults	4th Larval Stages
C1	Dec.	390,800	45,000	5,000	340,800	87	2:3	3,500	3,300	1,400	0
C2	Jan.	23,000	0	9,300	13,700	60	1:1	0	0	0	0
C3	Feb.	62,900	42,000	22,700	18,200	22	3:4	4,100	0	200	0
C4	Feb.	150,000	45,700	20,000	85,100	57	1:1	0	0	0	0
C5	March	239,100	61,000	29,800	148,300	62	1:1	18,100	900	0	0
C6	April	56,900	19,600	28,000	9,300	16	1:1	0	0	0	0
C7	April	69,200	56,100	0	13,100	19	1:1	160	0	0	0
C8	April	93,300	10,000	68,700	14,600	16	3:4	27,600	400	0	0
C9	April	114,300	49,200	13,100	51,800	45	1:1	2,900	0	0	0
C10	April	133,100	65,500	47,600	20,000	15	1:1	1,500	0	0	0
C11	April	40,500	13,900	6,400	20,200	50	2:3	2,900	300	0	0
C12	April	39,900	8,200	300	31,400	79	4:3	1,200	1,200	0	0
C13	May	237,600	159,900	24,300	53,400	23	1:1	3,200	200	0	0
C14	May	24,000	2,600	4,400	17,000	70	1:2	0	0	0	0
C15	June	25,300	2,000	12,400	8,900	38	1:1	100	0	0	0

240 Trichostrongylus axei in C6. 1,600 T. axei in C9.

*97 per cent Adult Males Ostertagia ostertagi, 3 per cent Ostertagia lyrata

APPENDIX 2 - Table 1

Worm Counts at Autopsy of Permanent Calves at Farms A & B in 1964

Calf No. & Farm	Date of Autopsy	Ostertagia ostertagi					Cooperia spp. C. oncophora & C. mcmasteri		Haematodirus spp. H. helvetianus & H. snathiges	
		Total	Adults	Developing Stages	Early 4th Larval Stages		Adults	4th Larval Stages	Adults	4th Larval Stages
					Total	%				
A1	5. 8.64	64,000	22,000	39,600	2,400	4	9,100	200	6,700	1,700
A2	5. 8.64	51,500	45,700	2,800	3,000	6	2,500	0	2,200	0
A3	5. 8.64	43,100	21,200	19,900	2,000	5	5,200	200	1,200	300
A4	5. 8.64	24,200	20,000	600	3,600	15	1,000	100	1,300	1,000
A5	5. 8.64	23,100	20,200	2,000	900	4	900	0	1,800	200
A6	2. 9.64	84,300	35,700	41,100	7,500	9	4,200	1,000	2,900	1,500
A7	2. 9.64	84,900	36,000	41,300	7,600	9	6,600	1,300	1,900	900
B1	11. 9.64	61,800	28,500	12,500	20,800	34	19,700	6,500	2,300	800
B2	11. 9.64	111,200	65,400	25,800	20,000	18	1,000	300	0	0
B5	11. 9.64	81,100	44,000	27,800	9,300	11	24,700	2,800	2,300	1,500
B4	11. 9.64	89,600	75,700	0	13,900	16	21,000	1,500	0	200
B5	11. 9.64	79,700	47,700	27,200	4,800	6	16,000	2,700	400	200
A8	12. 9.64	106,600	65,000	20,900	12,700	12	6,100	5,900	7,000	600
B6	1.10.64	90,800	48,000	2,200	40,600	45	6,700	8,900	400	0
B7	2.10.64	55,600	20,500	500	14,600	41	18,000	2,000	4,000	200
B8	2.10.64	42,000	26,000	800	15,200	36	22,200	13,100	5,500	600
A9	9.10.64	86,200	49,300	12,900	24,000	28	20,400	7,800	40,400	7,800
A10	26.10.64	139,000	25,100	16,500	97,400	70	6,100	5,900	7,000	600
B9	26.10.64	40,700	7,400	500	32,800	81	4,100	4,900	160	0
B10	26.10.64	24,000	9,100	5,100	9,800	41	13,700	6,200	10,000	900

N.B. Up to 1,000 Trichostrongylus axei present in most calves
Up to 5,600 T. axei in A2

APPENDIX 2 - Table 2

Worm Counts at Autopsy of Replacement Calves at Farms A & B in 1964

Calf No.	Date of Autopsy	Ostertagia ostertagi					Cooperia spp. (C. oncophora & C. punctata)		Nematodirus spp. (N. helvetianus & N. spathiger)	
		Total	Adults	Developing Stages	Early 4th Larval Stages		Adults	4th Larval Stages	Adults	4th Larval Stages
					Total	%				
FARM A										
A2	29.9.64	125,100	75,900	27,800	19,400	16	N.E.		N.E.	
A3	2.10.64	129,300	53,000	47,900	28,900	22	11,800	2,400	5,800	1,200
FARM B										
B7	9.10.64	87,200	31,300	16,100	39,800	46	3,000	17,000	300	2,000
B8	9.10.64	87,600	53,000	11,700	22,900	26	15,600	45,100	2,800	500
B9	15.10.64	53,900	45,600	4,100	4,200	8	N.E.		N.E.	
B10	15.10.64	53,500	22,400	16,000	14,300	27	9,900	22,100	3,500	1,000

At Farm A, A1, A4, A5 died several hours before autopsy
At Farm B, B6 died several hours before autopsy
} These results are not included

N.E. = Not examined

APPENDIX 2 - Table 3

Worm Counts at Autopsy of Tracer Calves at Farm A in 1964

Period Grazed	Ostertagia ostertagi Total	Ostertagia ostertagi Adults & Developing Stages	Ostertagia ostertagi Early 4th Larval Stages Total	Ostertagia ostertagi Early 4th Larval Stages %	Cooperia oncophora & C. mcmasteri 4th Larval Stages Adults	Cooperia oncophora & C. mcmasteri 4th Larval Stages Stages	Nematodirus helvetianus & N. spathiger 4th Larval Stages Adults	Nematodirus helvetianus & N. spathiger 4th Larval Stages Stages	Nos. of O. ostertagi per day during grazing period Individual	Nos. of O. ostertagi per day during grazing period Mean
25/5	1,600	1,400	200	13	100	0	100	100	114	107
-8/6	1,400	1,000	400	29	100	100	100	100	100	
8/6	2,800	1,900	900	32	0	0	1,800	600	200	232
-22/6	3,700	2,400	1,300	35	100	0	2,200	200	264	
22/6	25,300	16,900	8,400	33	500	100	200	0	1,809	1,154
-6/7	7,000	5,700	1,300	19	400	100	1,500	300	500	
6/7	14,300	10,300	4,000	28	400	200	400	800	1,021	979
-20/7	13,100	11,200	1,900	15	100	100	300	400	936	
20/7	15,100	15,100	0	0	1,800	300	2,200	1,500	1,079	743
-3/8	5,700	4,300	1,400	25	800	200	1,100	200	407	
3/8	14,500	12,600	1,900	13	2,000	1,000	1,200	1,000	1,036	1,308
-27/8	22,100	18,500	3,600	16	4,000	1,100	400	100	1,579	

APPENDIX 2 - Table 3 (Continued)

Period Grazed	Ostertagia ostertagi Total	Ostertagia ostertagi Adults & Developing Stages	Ostertagia ostertagi Early 4th Larval Stages Total	Ostertagia ostertagi Early 4th Larval Stages %	Cooperia oncophora & C. mcmasteri 4th Larval Stages Adults	Cooperia oncophora & C. mcmasteri 4th Larval Stages 4th Larval Stages	Nematodirus helvetianus & N. spathiger 4th Larval Stages Adults	Nematodirus helvetianus & N. spathiger 4th Larval Stages 4th Larval Stages	Nos. of O. ostertagi per day during grazing period Individual	Nos. of O. ostertagi per day during grazing period Mean
17/8	46,000	44,700	1,300	3	6,400	2,200	2,400	1,000	3,296	
-31/8	39,500	34,600	4,900	12	2,100	500	5,200	1,200	2,821	3,054
31/8	[3]	"	"	"	"	"	"	"	"	
-14/9	51,700	45,500	6,200	12	5,200	2,900	1,300	200	3,693	3,693
14/9	[3]	"	"	"	"	"	"	"	"	
-28/9	110,700	69,200	41,500	37	2,200	10,700	300	200	7,907	7,907
28/9 -12/10	No traces available									
12/10	9,800	5,100	4,700	48	200	700	800	0	700	
-26/10	37,400	11,300	26,100	70	700	3,400	2,900	600	2,672	1,686
26/10	18,000	1,700	16,300	91	1,000	3,800	500	0	783	
	19,400	800	18,600	96	1,000	1,000	3,200	600	843	942
-18/11	27,600	1,700	25,900	94	1,900	2,900	500	0	1,200	

[3] Calf died several hours before autopsy

A few Trichostrongylus axei recorded are not included in this table

APPENDIX 2 - Table 4

Worm Counts at Autopsy of Tracer Calves at Farm D in 1964

Period & Field Grazed		Ostertagia ostertagi Total	Ostertagia ostertagi Adults & Developing Stages	Ostertagia ostertagi Early 4th Larval Stages Total	Ostertagia ostertagi Early 4th Larval Stages %	Cooperia oncophora & C. punctata 4th Larval Stages Adults	Cooperia oncophora & C. punctata 4th Larval Stages Larval Stages	Nematodirus helvetianus & N. spathiger 4th Larval Stages Adults	Nematodirus helvetianus & N. spathiger 4th Larval Stages Larval Stages	Nos. of O. ostertagi per day during grazing period Individual	Nos. of O. ostertagi per day during grazing period Mean
11/5	S	2,100	1,500	600	29	200	0	1,400	400	150	118
-25/5		1,200	1,100	100	8	600	0	600	200	86	
25/5	S	-	-	-	-	-	-	-	-	-	50
-8/6		700	500	200	29	0	0	0	0	50	
8/6	L	200	200	0	0	0	0	0	0	14	29
-22/6		600	500	100	17	100	0	500	100	43	
22/6	L	200	200	0	0	400	100	0	100	14	21
-6/7		400	400	0	0	100	200	100	100	28	
6/7	L	200	200	0	0	0	0	200	200	14	18
-20/7		300	300	0	0	200	0	100	100	21	
20/7	S	800	600	200	25	200	100	200	100	57	54
-3/8		700	500	200	29	100	200	200	400	50	
3/8	L	11,200	9,500	1,700	15	2,200	1,400	3,100	1,500	800	772
-17/8		10,400	8,500	1,900	18	2,200	900	800	200	743	

S = Small field L = Large field

* Calf died several hours before autopsy

APPENDIX 2 - Table 4 (Continued)

Period & Field Grazed		Ostertagia ostertagi Total	Ostertagia ostertagi Adults & Developing Stages	Ostertagia ostertagi Early 4th Larval Stages Total	Ostertagia ostertagi Early 4th Larval Stages ♀	Cooperia oncophora & C. Hamasteri Adults	Cooperia oncophora & C. Hamasteri 4th Larval Stages	Nematodirus helvetianus & N. spathiger Adults	Nematodirus helvetianus & N. spathiger 4th Larval Stages	Nos. of O. ostertagi per day during grazing period Individual	Nos. of O. ostertagi per day during grazing period Mean
17/8 -31/8	S	64,300	58,500	5,800	9	22,400	3,800	100	0	4,592	3,462
		32,700	31,400	1,300	4	10,600	1,700	0	0	2,334	
31/8 -14/9	L	51,000	44,000	7,000	14	17,600	2,000	0	0	3,643	3,643
		-[2]	-	-	-	-	-	-	-	-	
14/9 -28/9	S & L	7,800	6,600	1,200	15	2,300	8,600	0	0	557	686
		11,400	9,300	2,100	18	1,800	1,500	160	0	814	
28/9 -12/10	S & L	13,600	10,400	3,200	24	6,900	5,400	700	0	971	775
		8,100	5,500	2,600	32	1,400	3,200	400	100	578	
12/10 -26/10	S & L	-[2]	-	-	-	-	-	-	-	-	357
		5,000	2,800	2,200	44	800	6,600	2,700	-	357	
26/10 -9/11	S	41,800	6,300	35,000	84	1,400	6,600	500	0	2,986	2,522
		28,800	3,500	25,300	88	1,800	19,400	700	0	2,057	

S = Small field

L = Large field

S & L = Grazed alternate weeks

[2] Calf died several hours before autopsy

A few Trichostrongylus axei recorded, not included in this table

APPENDIX 3 - Table 1

Bodyweights of Calves following a Single Oral Inoculation with 300,000 *Ostertagia ostertagi*

Day	-7	0	2	4	7	9	11	14	16	18	21	23	25	28
Calf No.														
1	128	133	135	135	130	141	142	145	146	153	147	137	131	139
2	126	135	139	139	138	143	144	147	147	155	147	136	131	133
3	122	129	131	132	133	133	137	136	137	142	133	117	109	102
4	135	138	138	141	139	147	148	147	145	156	149	145	145	152
5	128	134	135	137	144	145	145	143	146	150	141	134	134	134
Mean	128	134	136	137	138	142	143	144	144	151	143	134	130	132
S.E.	2.1	1.5	1.4	1.6	1.8	2.5	1.9	2.1	1.9	2.6	2.9	4.7	5.9	6.3

Day	30	32	35	37	39	42	45	47	50	52	59	63	70
Calf No.													
1	148	150	144	150	154	162	164	168	179	176	184	189	198
2	135	132	134	139	147	148	149	156	156	165	171	179	A
3	dead	-	-	-	-	-	-	-	-	-	-	-	-
4	160	160	160	164	165	169	168	176	A	-			
5	138	138	141	140	142	143	143	150	155	160	A		
Mean	145	145	145	148	152	156	156	163	163	167			
S.E.	5.7	6.3	5.5	5.8	4.9	6.1	5.9	5.8	6.0	4.8			

A = Autopsied S.E. = Standard Error

APPENDIX 3 - Table 2

Intake of Concentrates (gms) by Calves Following a Single Oral Inoculation of 300,000 *Ostertagia ostertagi* (700 gm fed morning and evening)

Day	CALF NO. 1		2		3		4		5		Total
	M	E	M	E	M	E	M	E	M	E	
0 to 18	700	700	700	700	700	700	700	700	700	700	7000
19	700	700	700	361	454	290	700	700	439	235	5279
20	500	310	75	374	35	115	115	125	114	195	1958
21	0	35	275	700	73	0	100	730	0	75	1988
22	113	10	49	0	33	13	477	160	43	0	898
23	58	480	0	195	0	40	165	630	160	445	2173
24	0	46	90	5	0	0	400	106	260	0	987
25	168	310	12	0	0	47	300	410	450	473	2250
26	446	356	57	0	0	0	312	561	353	150	2235
27	468	670	78	680	0	0	513	537	593	475	3614
28	344	700	700	120	0	A	139	645	550	450	3640
29	700	700	145	179		-	700	700	670	466	4260
30	700	700	93	284		-	700	700	514	547	4238
31	700	700	575	568		-	700	700	700	434	4877
32	700	700	550	521		-	700	700	700	607	5186
33 to 43	700	700	700	700		-	700	700	700	700	5600

M = Morning E = Evening A = Autopsied

APPENDIX 3 - Table 3

Classification of Diarrhoea in Calves Following a Single Oral Inoculation with 300,000 Ostertagia ostertagi larvae

Day	CALF NO. 1	2	3	4	5	Total +
0 - 18	0	0	0	0	0	0
19	+	0	0	+	0	2
20	0	0	0	+	0	1
21	+	+	+++	+	++	10
22	+++	++	+++	++	+++	14
23	+++	++	+++	++	+	12
24	+++	+	+++	+	+	10
25	+	0	+++	0	0	5
26	+	++	++	+	0	6
27	0	0	+++	0	0	3
28	0	0	Δ	0	0	0
29	0	0	-	0	0	0
30-Autopsy	0	0	-	0	0	0

Δ = Autopsied

\+ = soft faeces

++ = semi-fluid faeces

+++ = fluid faeces

APPENDIX 3 - Table 4

Faecal Egg Counts (e.p.g.) from Calves Following a Single Oral Inoculation of 500,000 Ostertagia ostertagi

	CALF NO.					
Day	1	2	3	4	5	Mean
0-17	0	0	0	0	0	0
18	350	50	400	450	750	400
19	100	850	750	1350	700	750
20	300	750	1350	350	750	700
21	250	650	250	600	750	500
22	200	350	1100	250	500	480
23	250	600	1900	50	1200	800
24	700	350	250	2450	1250	1000
25	50	400	1300	250	450	490
26	150	350	100	500	300	280
27	N.S.	900	400	0	450	430
28	200	1550	N.S.	150	400	525
29	100	550	A	100	250	250
30	0	300	"	50	300	163
32	0	250	"	0	50	75
35	50	0	"	100	0	38
37	0	0	"	250	0	63
39-Autopsy	<100	<100	"	<100	<100	<100

A = Autopsied

APPENDIX 3 - Table 5

Plasma Pepsinogen Levels (m.U. of Tyrosine) in Calves Following a Single Oral Inoculation of 300,000 *Ostertagia ostertagi* Larvae

Day	0	2	4	7	9	11	14	16	17	18	21	23
Calf No.												
1	600	800	1,200	1,800	2,200	1,700	3,000	3,600	4,500	7,600	5,400	7,900
2	1,200	1,200	1,500	1,900	1,600	1,500	1,900	2,900	4,000	5,800	6,700	9,700
3	800	N.S.	1,000	1,700	1,300	1,900	3,100	4,600	5,200	7,300	4,400	5,000
4	400	800	900	1,300	2,300	1,200	1,800	3,000	4,400	7,600	7,900	9,300
5	400	400	1,000	1,400	1,600	1,100	1,500	2,800	2,800	5,700	4,500	5,100
Mean	700	800	1,100	1,600	1,800	1,500	2,300	3,400	4,200	6,800	5,800	7,400
Standard Error	100	170	140	160	80	80	330	340	400	450	660	1,010

Day	25	28	30	32	35	37	39	42	49	56	63	70
Calf No.												
1	4,500	6,700	4,100	2,600	1,700	1,800	1,600	1,500	800	800	1,100	400
2	8,200	7,500	7,500	5,700	5,300	6,300	7,700	5,600	2,700	2,300	1,800	A
3	3,200	2,400	A	...	...	...	...	...	...	...	...	...
4	7,700	6,100	4,300	3,400	2,000	2,600	2,400	2,000	1,100	A	...	...
5	4,100	5,600	4,300	3,700	2,900	3,100	3,100	2,900	2,100	2,000	A	...
Mean	5,500	5,700	5,100	3,900	3,000	3,500	3,700	3,000	1,700	1,700	1,400	400
Standard Error	1,010	870	820	660	820	980	1,370	910	440	460	...	...

A = Autopsied

N.B. All Plasma Pepsinogen Values to Nearest 100
Standard Errors to Nearest 10

APPENDIX 3 - Table 6

Red Blood Cell Counts (10^6 per cu. mm) of Calves Following a Single Oral Inoculation of 500,000 *Ostertagia ostertagi*

	CALF NO.					Group Mean	
Day	1	2	3	4	5		Standard Error
0	9.5	7.1	9.4	9.1	8.1	8.6	0.46
2	9.9	7.4	9.3	8.9	7.6	8.6	0.48
4	9.7	7.4	9.5	8.7	7.4	8.5	0.49
7	9.6	7.2	9.9	9.2	7.4	8.7	0.57
9	9.5	7.0	9.0	8.6	7.5	8.3	0.46
11	8.9	7.0	8.5	8.3	7.0	7.9	0.39
14	8.6	7.0	8.6	8.1	7.0	7.8	0.36
16	9.1	7.3	8.7	8.0	7.0	8.1	0.30
18	9.1	6.9	8.7	8.0	7.2	8.0	0.42
21	11.1	7.6	10.3	8.4	7.5	9.0	0.73
23	11.6	7.7	11.1	8.5	7.5	9.3	0.86
25	11.3	8.0	11.4	8.3	7.5	9.3	0.83
28	9.2	8.9	A	8.9	8.0	8.8	0.26
30	10.5	8.4	-	8.1	7.7	8.7	0.63
35	10.1	8.3	-	8.1	7.0	8.4	0.65
42	8.9	7.7	-	7.9	7.3	7.9	0.34
49	9.0	6.9	-	8.1	6.4	7.6	0.59
56	8.9	7.0	-	A	6.1	7.3	0.83
Mean	9.7	7.5	9.5	8.4	7.3		
Standard Error	0.21	0.14	0.28	0.10	0.12		

A = Autopsied

APPENDIX 3 - Table 7

Haemoglobin Concentration (gms/100 ml) in Calves Following a Single Oral Inoculation of 300,000 Ostertagia ostertagi

	CALF NO.						
Day	1	2	3	4	5	Group Mean &	Standard Error
0	10.9	8.6	10.9	10.0	9.8	10.2	0.45
2	10.0	9.0	10.8	10.0	9.4	10.2	0.39
4	10.6	9.7	10.7	10.6	8.7	10.1	0.37
7	10.7	9.2	10.7	10.9	8.9	10.1	0.42
9	10.0	8.0	10.2	10.0	9.4	9.7	0.26
11	10.0	8.7	10.2	10.2	8.7	9.6	0.35
14	9.6	9.2	10.0	10.4	8.9	9.6	0.35
16	10.2	10.0	10.2	10.2	9.0	10.1	0.08
18	9.9	8.9	10.8	10.7	8.9	9.0	0.41
21	11.4	10.0	11.8	10.8	9.4	10.7	0.44
23	12.2	10.2	13.3	11.2	9.7	11.3	0.66
25	11.4	10.7	13.4	11.1	9.7	11.5	0.60
28	12.2	10.2	Δ	11.2	9.7	10.8	0.56
30	10.0	11.1	"	10.6	10.0	10.4	0.27
35	10.0	10.8	"	9.7	9.4	10.0	0.30
42	9.4	10.6	"	10.0	9.2	9.7	0.21
49	10.0	8.6	"	10.1	8.9	9.4	0.38
56	9.7	8.8	"	Δ	8.9	9.1	0.29
Mean	10.5	9.6	11.1	10.5	9.3		
Standard Error	0.20	0.19	0.34	0.11	0.10		

Δ = Autopsied

APPENDIX 3 - Table 8

Packed Cell Volume Percentages of Calves Following a Single Oral Inoculation of 300,000 Ostertagia ostertagi

Day	CALF NO. 1	2	3	4	5	Group Mean & Standard Error	
0	35.0	27.0	33.0	33.5	31.5	32.0	1.30
2	35.0	27.5	34.0	33.5	29.5	31.9	1.44
4	34.0	27.5	34.0	33.0	28.0	31.3	1.46
7	33.5	27.0	33.5	33.5	29.0	31.3	1.30
9	32.0	29.0	32.0	32.0	30.0	31.0	0.63
11	32.0	26.5	32.0	33.0	29.0	30.5	1.20
14	33.0	27.5	31.5	33.5	29.5	31.0	1.10
16	33.0	30.5	31.5	31.5	30.5	31.4	0.44
18	32.5	27.0	32.0	33.0	28.0	30.5	1.24
21	36.5	31.5	36.0	34.0	30.0	33.6	1.26
23	38.5	32.5	43.0	35.0	31.5	36.1	2.09
25	37.5	32.5	42.5	36.0	30.5	35.6	2.08
28	35.5	31.5	A	35.0	31.5	33.4	1.09
30	33.0	33.0	"	31.5	30.0	31.9	0.72
35	32.0	33.5	"	31.0	28.0	31.1	1.16
42	30.5	30.0	"	30.5	27.5	29.6	0.72
49	30.5	27.5	"	32.0	28.0	29.5	1.06
56	30.5	27.0	"	A	28.0	28.5	1.12
Mean	33.6	29.4	34.6	33.0	29.5		
Standard Error	0.55	0.59	1.17	0.36	0.32		

A = Autopsied

APPENDIX 3 - Table 9

Total Serum Proteins (gms/100 ml) of Calves Following a Single Oral Inoculation of 300,000 Ostertagia ostertagi

Day	CALF NO. 1	2	3	4	5	Group Mean & Standard Error	
0	6.2	6.4	6.0	6.0	6.1	6.1	0.07
4	6.3	6.6	6.2	6.1	5.8	6.2	0.11
7	6.5	6.5	6.5	6.4	5.7	6.3	0.15
9	5.7	6.0	6.7	5.9	5.3	5.9	0.23
11	5.9	6.0	6.1	6.0	5.4	5.9	0.12
14	6.6	6.3	6.1	6.0	5.1	6.0	0.25
16	6.4	6.5	6.2	6.2	5.2	6.1	0.23
18	6.2	6.7	6.4	6.2	5.2	6.1	0.25
21	6.4	6.6	5.9	6.2	5.4	6.1	0.19
23	6.0	6.9	5.8	6.1	5.5	6.1	0.23
25	6.9	7.0	6.2	6.4	5.6	6.4	0.26
28	5.8	5.5	A	6.0	5.8	6.0	0.29
35	6.1	6.4	"	6.5	5.6	6.2	0.20
42	6.5	6.4	"	6.7	5.9	6.3	0.17
49	6.2	6.3	"	6.3	5.0	6.2	0.12
56	6.7	6.4	"	A	6.0	6.4	0.20
Mean	6.3	6.4	6.2	6.3	5.6		
Standard Error	0.32	0.36	0.26	0.27	0.30		

A = Autopsied

APPENDIX 5 - Table 10

Serum Albumin Levels (gms/100 ml) of Calves Following a Single Oral Inoculation of 300,000 Ostertagia ostertagi

Day	CALF NO. 1	2	3	4	5	Group Mean & Standard Error	
0	2.2	2.9	2.3	2.7	2.7	2.6	0.13
4	2.2	2.4	2.5	2.6	2.4	2.4	0.07
7	2.4	2.4	2.7	2.6	2.3	2.5	0.07
9	2.4	2.5	2.8	2.6	2.4	2.5	0.07
11	2.1	2.3	2.7	2.6	2.3	2.4	0.11
14	2.3	2.5	2.9	2.4	2.2	2.5	0.12
16	2.6	2.3	2.2	2.9	2.5	2.5	0.12
18	2.6	2.8	2.8	2.6	2.2	2.6	0.11
21	2.1	2.3	2.4	2.3	2.5	2.3	0.07
23	2.4	2.1	2.4	2.3	2.2	2.3	0.06
25	2.0	2.4	2.4	2.3	2.3	2.3	0.07
28	2.4	1.9	A	2.5	2.1	2.2	0.14
35	2.1	2.3	"	2.7	2.3	2.4	0.13
42	2.2	2.2	"	2.5	2.1	2.3	0.09
49	2.3	2.3	"	2.3	2.5	2.4	0.05
56	2.4	2.2	"	A	2.5	2.4	0.09
Mean	2.3	2.4	2.6	2.5	2.3		
Standard Error	0.05	0.06	0.07	0.05	0.04		

A = Autopsied

APPENDIX 3 - Table 11

Serum Globulin Levels (gm/100 ml) of Calves Following a Single Oral Inoculation of 300,000 Ostertagia ostertagi

Day	CALF NO. 1	2	3	4	5	Group Mean & Standard Error	
0	4.0	3.5	3.7	3.3	3.4	3.6	0.12
4	4.1	4.2	3.7	3.5	3.4	3.8	0.16
7	3.9	4.1	3.8	3.8	3.4	3.8	0.13
9	3.3	3.5	4.0	3.3	2.9	3.4	0.18
11	3.8	3.7	3.4	3.4	3.1	3.5	0.12
14	4.3	3.8	3.2	3.6	2.9	3.6	0.24
16	3.8	3.2	4.0	3.3	2.7	3.4	0.23
18	3.6	3.9	3.6	3.6	3.0	3.5	0.15
21	4.1	4.3	3.5	3.9	2.9	3.8	0.27
23	3.6	3.8	3.4	3.8	3.3	3.6	0.10
25	4.9	4.6	3.8	4.1	3.3	4.1	0.26
28	3.4	3.6	A	4.3	3.7	3.8	0.19
35	4.0	4.1	-	3.8	3.3	3.8	0.18
42	4.1	4.2	-	4.2	3.8	4.1	0.10
49	3.9	4.0	-	4.0	3.3	3.8	0.17
56	4.3	4.2	-	A	3.5	4.0	0.25
Mean	3.9	3.9	3.6	3.7	3.2		
Standard Error	0.10	0.09	0.08	0.09	0.08		

A = Autopsied

APPENDIX I -- Table 12

Serum Serum Globulin Levels (gms/100 ml) of Calves Following a Single Oral Inoculation of 300,000 Salmonella enteritidis

Day	Group No.					Group Mean & Standard Error	
	1	2	3	4	5		
0	2.0	2.3	1.6	1.5	1.6	1.8	0.14
4	2.1	2.3	1.7	1.5	1.3	1.7	0.17
7	2.0	1.9	1.7	1.7	1.5	1.8	0.09
9	1.8	2.1	1.8	1.5	1.3	1.6	0.16
11	1.7	1.7	1.4	1.6	1.1	1.5	0.11
14	1.6	D.S.	1.5	1.6	1.2	1.5	0.06
16	2.0	1.8	1.8	1.5	1.2	1.6	0.16
18	1.8	2.2	1.5	D.S.	1.2	1.7	0.24
21	2.5	2.3	1.8	1.5	1.3	1.6	0.27
23	2.1	2.5	1.5	1.4	1.4	1.7	0.20
25	1.9	N.S.	1.4	N.S.	1.7	1.7	0.15
28	2.2	2.3	A	1.6	1.7	1.9	0.19
35	1.9	2.0	"	2.0	1.6	1.9	0.10
42	1.5	2.1	"	1.9	1.7	1.8	0.13
49	2.1	2.0	"	2.0	1.8	2.0	0.07
56	1.9	2.1	"	A	1.5	1.8	0.18
Mean	1.9	2.1	1.5	1.6	1.4		
Standard Error	0.06	0.05	0.06	0.07	0.06		

A = Antequated

D.S. = Do sample

APPENDIX 4 - Table 1

Worm Counts at Autopsy of 14 Day Zwoon Calves at Farm A in 1965

Period Grazed	Ostertagia ostertagi				Nos. of O. ostertagi per day during Grazing Period	
	Total	Adults & Developing Stages	Early 4th Larval Stages			
			Total	Per Cent	Individual	Mean
16/4 - 30/4	16,000 10,000	15,600 9,900	1,200 900	7 8	1,200 771	986
30/4 - 14/5	19,700 15,400	17,500 12,200	2,200 3,200	11 22	1,407 1,100	1,254
24/6 - 8/7	200 400	200 400	0 0	0 0	14 29	22
26/8 - 9/9	5,400 4,400	5,000 4,200	400 200	7 4	396 314	350
9/9 - 23/9	11,100 17,300	9,300 11,200	2,000 6,100	25 55	793 1,236	1,015
23/9 - 7/10	16,800 3,300	10,400 2,200	6,400 1,100	38 33	1,200 236	718
7/10 - 21/10	24,800 5,000	5,300 3,000	19,500 2,000	79 40	1,771 414	1,093
21/10 - 4/11	9,800 20,400	3,500 7,300	6,300 13,100	64 64	700 1,457	1,079

APPENDIX 4 - Table 2

Worm Counts at Autopsy of 14 Day Tracer Calves at Farm B in 1965

Period Grazed	Field Grazed	Ostertagia ostertagi				Nos. of O. ostertagi per day during Grazing Period	
		Total	Adults & Developing Stages	Early 4th Larval Stages			
				Total	Per Cent	Individual	Mean
16/4 - 30/4	S	8,400 40,400	7,300 37,600	1,100 2,800	13 7	600 2,886	1,743
30/4 - 14/5	S	27,200 25,900	26,400 23,300	800 2,600	3 10	1,943 1,850	1,897
24/6 - 8/7	S	300 500	300 500	0 0	0 0	21 36	29
26/8 - 9/9	S & L	5,900 4,000	5,600 3,800	300 200	5 5	421 286	354
9/9 - 23/9	S & L	3,400 8,400	3,200 6,500	200 1,900	6 23	243 600	422
23/9 - 7/10	S & L	5,900 4,400	2,800 2,100	3,100 2,300	54 53	421 314	368
7/10 - 21/10	S & L	5,400 4,600	1,700 1,300	3,700 3,300	69 72	386 329	358
21/10 - 4/11	S & L	5,400 2,000	1,800 800	3,600 1,200	67 60	386 143	265

S = Small Field L = Large Field S & L = Grazed alternate weeks

APPENDIX 4 - Table 3

Twice Weekly Faecal Egg Counts of Calves During Daily Inoculations of 1,500 *Ostertagia ostertagi* Larvae over a Period of 100 Days

Calf No.	DAY 19	17	21	24	28	32	35	39	42	45	49
12	-ve	150	150	100	-ve	-ve	50	100	50	100	300
21	-ve	100	450	1050	450	100	250	-ve	-ve	750	200
23	-ve	-ve	-ve	150	-ve	-ve	-ve	150	50	-ve	50
49	-ve	50	250	250	200	400	250	300	600	350	500
50	-ve	50	-ve	-ve	-ve	200	50	50	150	150	150

	52	56	60	64	66	70	73	77	80	84	87
12	100	200	50	200	150	-ve	-ve	-ve	-ve	-ve	-ve
21	50	50	-ve	200	100	400	100	100	50	150	150
23	300	50	50	100	150	50	50	100	50	50	50
49	350	450	350	250	350	500	150	150	50	400	-ve
50	-ve	200	150	50	250	50	50	-ve	50	-ve	50

	91	94	101	107
12	50	100	50	50
21	50	50	-ve	50
23	-ve	50	50	-ve
49	-ve	50	50	50
50	50	50	50	50

APPENDIX 4 - Table 4

Total *Ostertagia ostertagi* Worm Burdens, Numbers and Percentage of Early Fourth Larval Stages at Autopsy of Groups of Tracer Calves which Grazed from 21 to 29 October 1965 and Slaughtered 10 days later

Plot Grazed	Date Plot Infected with *O. ostertagi* Eggs by Seeders	*O. ostertagi* Worm Burdens		
		Total	Early Fourth Larval Stages	
			Number	Percentage
A	July 1 - 25	27,000	6,800	25
		12,100	2,100	17
		14,000	3,200	23
		11,800	2,200	19
	Mean	16,200	3,600	21
B	August 6 - 31	5,300	200	4
		5,000	500	10
		6,600	600	9
		4,700	300	6
	Mean	5,400	400	7
C	September 2 - 27	3,400	300	9
		4,100	100	2
		800	100	13
		4,400	100	2
	Mean	3,200	200	6
D	October 1 - 21	0	0	0
		100	0	0
		0	0	0
		200	0	0
	Mean	< 100	0	0

APPENDIX 4 - Table 4 (Continued)

Plot Grazed	Date Plot Infected with O. ostertagi Eggs by Goodens		O. ostertagi Worm Burdens Early Fourth Larval Stages		
			Total	Number	Percentage
E	July 1- September 27		21,000	3,100	15
			7,000	1,000	14
			4,100	300	7
			4,000*	1,300	33
		Mean	10,700	1,400	12
F	Not Infected by Goodens.		5,900	100	2
	Tracer calves given 0 x		5,900	200	3
	5,000 O. ostertagi 3rd		5,600	100	2
	stage larvae orally		6,900	100	1
	between 21 - 29.10.65				
		Mean	6,100	100	2
	Housed Tracer Calves		8,700	100	1
	given 0 x 5,000 O. ostertagi		6,500	100	2
	3rd stage larvae orally		3,200	100	3
	between 21 - 29.10.65		**	-	-
		Mean	6,100	100	2
Farm A & B	2 Tracer Calves at Farm A & B from 21.10 - 4.11.65	A	9,000	6,300	64
		A	20,400	13,100	64
		B	5,400	3,600	67
		B	2,000	1,200	60
		Mean	9,400	6,100	64

* - Killed in extremis. Not included in mean.

** - Died overnight. No Worm Count made.

APPENDIX 4 - Table 5

Total Ostertagia ostertagi Worm Burdens, Numbers and Percentage of Early Fourth Larval Stages at Autopsy of Groups of Fraser Calves Grazed from 20th October, 1966, to 1st November, 1966, and Slaughtered 9 days later.

Plot Grazed	Source of O. ostertagi Infection	O. ostertagi Worm Burdens		
		Total	Early Fourth Larval Stages	
			Number	Per Cent
A	Overwintered Larvae from Plot Seeded in July 1965 (Laboratory Strain) Infection Cycled	10,000	1,100	10
		10,900	1,200	11
		8,000	900	11
		-*	-	-
	Mean	9,900	1,100	11
D	Overwintered Eggs from Plot Seeded in October 1965. (Laboratory Strain) Infection Cycled.	3,300	300	9
		8,000	1,200	15
		6,900	1,200	17
		5,000	700	12
	Mean	6,000	800	13
E	Overwintered Eggs & Larvae from Plot Seeded July - Sept., 1965 (Laboratory Strain) Infection Cycled.	6,100	1,000	16
		3,400	600	18
		4,400	1,000	23
		4,700	1,300	28
	Mean	4,700	1,000	21
G	Seeded with O. ostertagi Eggs 17.5 - 9.6.66. (Laboratory Strain)	5,600	900	16
		5,400	1,000	19
		7,000	900	13
	Mean	6,000	900	16

*Died overnight. No worm count made.

APPENDIX 4 - Table 5 (Continued)

Plot Grazed	Source of O. ostertagi Infection	O. ostertagi Worm Burdens Early Fourth Larval Stages		
		Total	Number	Per Cent
H	Seeded with O. ostertagi Eggs between 21.4 - 10.5.66 (Laboratory Strain)	1,300	300	23
		1,000	200	20
		2,600	200	8
		1,500	300	20
	Mean	1,600	300	18
I	Seeded with O. ostertagi Eggs between 17.5 - 9.6.66 (Field Strain)	10,200	8,600	85
		6,300	4,600	73
		11,400	8,300	75
		10,100	7,600	75
	Mean	9,900	7,300	77
J	Pasture not Infected. Tracers given 12 x 5,000 O. ostertagi 3rd Stage Larvae Orally between 20.10 - 1.11.66 (Laboratory Strain)	17,000	0	0
		15,000	0	0
		22,400	500	2
	Mean	17,500	200	< 1

APPENDIX 5 - Table 1

Patterns of Diarrhoea in Groups of 15 Month Old Parasite Free Calves and Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965, 17-28 Days Following an Experimental Inoculum of 800,000 O. ostertagi Larvae had been given

	Days After Challenge Inoculum											
	17	18	19	20	21	22	23	24	25	26	27	28
Group 3 Grazed June - October, 1965. Housed. Thereafter challenged April 1966	-	-	+	+++	+++	K						
	-	-	-	-	++	K						
	-	-	-	-	++	K						
	-	-	-	-	++	K						
	-	-	-	-	++	+++	-	-	-	-	-	-
	-	-	-	-	++	++	-	-	-	-	-	-
	-	-	-	++	++	++	-	-	-	-	-	-
	-	-	-	-	+++	+++	++	-	-	-	-	-
Group 4 Parasite Free Calves Challenged April, 1966.	+	+++	+++	+++	+++	K						
	-	-	-	-	+++	K						
	-	-	-	+	+++	K						
	-	-	-	-	+++	+++	+++	+++	++	-	-	-
	-	-	-	-	+++	+++	+++	+++	++	-	-	-

\+ = soft faeces

++ = semi-fluid faeces

+++ = fluid faeces

APPENDIX 5 - Table 2

Plasma Pepsinogen Levels in Groups of 15 Month Old Parasite Free Calves and Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965, some of which were subsequently given an Experimental Inoculum of 500,000 O. ostertagi larvae in the Spring of 1966

Group	Plasma Pepsinogen Levels in ml Tyrosine					
	Days After Challenge Inoculum					
	At Housing	0	14	17	21	28
2						
Grazed June-October, 1965.	2,400	1,400	1,700	3,000	1,400	1,400
Housed thereafter. Not	4,000	1,400	1,700	1,700	1,600	800
challenged.	4,400	1,400	1,000	1,200	1,100	600
Mean	3,600	1,400	1,500	2,000	1,400	1,000
3						
Grazed June-October, 1965.	5,600	1,800	2,100	1,800	6,200	K
Housed thereafter.	4,000	1,900	2,500	5,300	5,400	K
Challenged April 1966.	4,500	2,300	2,100	6,400	6,300	K
	3,300	600	N.S.	3,300	3,900	K
	4,400	2,300	2,400	5,400	5,500	8,500
	1,500	1,100	3,400	4,900	6,700	6,100
	2,100	1,000	1,600	5,900	5,900	6,700
	3,500	1,200	2,000	4,000	6,300	5,800
Mean	3,600	1,500	2,000	4,600	6,300	6,800
4						
Parasite Free Calves	N.S.	600	2,200	5,200	6,100	K
Challenged April 1966	N.S.	700	2,000	3,700	3,600	K
	N.S.	400	2,800	3,300	3,500	K
	N.S.	700	2,500	4,100	4,000	6,300
	N.S.	600	2,100	3,100	6,000	8,700
Mean		600	2,300	3,900	4,600	7,500

K = Killed

APPENDIX 5 - Table 3

Faecal Egg Counts from Groups of 15 Month Old Parasite Free Calves and Calves Exposed to Natural *Ostertagia ostertagi* Infection in the Summer of 1965, some of which were subsequently given an Experimental Inoculum of 800,000 *O. ostertagi* in the Spring of 1966

Group	Sex of Calf	At Housing	Faecal Egg Counts in Eggs Per Gram of Faeces — Days After Challenge Inoculum													
			0	7	14	18	19	20	21	22	23	24	25	26	27	28
2																
Grazed June -	M	400	-ve	-ve	-ve	-ve	-ve	-ve	-ve	K						
October, 1965.	M	400	50	100	50	100	50	50	100	K						
Housed thereafter	M	350	50	50	50	-ve	50	-ve	50	K						
Not Challenged																
	Mean	380	30	50	30	30	30	20	50							
	M	150	150	150	200	-ve	50	150	250	K						
3	M	350	150	150	-ve	200	200	200	200	K						
Grazed June -	M	450	100	100	50	100	150	500	600	K						
October, 1965.	M	50	250	-ve	50	50	50	50	100	K						
Housed thereafter	M	150	100	200	250	300	300	500	250	50	350	9500	4900	2000	300	750
Challenged April	M	200	-ve	150	100	200	400	500	600	600	1100	2750	300	250	100	950
1966	M	350	50	-ve	50	50	100	100	200	500	50	400	100	250	300	350
	M	250	-ve	50	50	-ve	50	100	50	100	50	200	50	100	250	250
	Mean	240	100	100	90	110	160	260	280	310	390	3210	1330	650	240	570
4	M	-	-ve	-ve	-ve	1800	1850	3150	7000	K						
Parasite Free	F	-	-ve	-ve	-ve	-ve	-ve	-ve	150	K						
Calves.	F	-	-ve	-ve	-ve	-ve	-ve	-ve	200	K						
Challenged April	F	-	-ve	-ve	-ve	-ve	-ve	-ve	100	250	200	100	150	300	150	300
1966	F	-	-ve	-ve	-ve	-ve	-ve	-ve	100	200	150	100	50	100	250	250
	Mean	-	-	-	-	360	370	630	1510	220	170	100	100	200	200	270

K = Killed M = Male F = Female

APPENDIX 5 - Table 4

Worm Counts at Autopsy of Groups of 15 month old Parasite Free Calves and Calves Exposed to Natural Ostertagia ostertagi Infection in the Summer of 1965, some of which were subsequently given an Experimental Inoculum of 800,000 O. ostertagi larvae in the Spring of 1966

Group	Day Killed after Experimental Inoculation	Grazed at	Sex	O. ostertagi Worm Counts			
				Total	Adults	Developing Stages	Early Fourth Larval Stages
1	End of Grazing Period, i.e. early October, 1965	Farm A	M	48,000	24,000	1,000	23,000
		Farm B	M	35,300	23,000	0	12,100
		Plot E	M	81,400	23,000	40,900	17,500
			Mean	54,600	23,300	14,000	17,500
2	21	Farm A	M	7,000	5,300	1,000	700
		Farm B	M	5,100	4,300	200	600
		Plot E	M	22,400	20,500	0	1,900
			Mean	11,500	10,000	400	1,100
3 (A)	21	Farm A	M	175,800	172,600	0	3,200
		Farm B	M	184,100	169,700	0	14,400
		Farm B	M	261,400	243,800	3,200	14,400
		Plot E	M	99,900	99,700	0	200
			Mean	180,300	171,500	800	8,000

APPENDIX 5 - Table 4 (Continued)

Group	Day Killed after Experimental Inoculation	Grazed at	Sex	Geometric Worm Counts			
				Total	Adults	Developing Stages	Early Fourth Larval Stages
3 (B)	28	Farm A	M	28,000	17,400	2,600	8,000
		Farm B	M	6,200	5,600	0	600
		Farm B	M	35,400	31,900	0	3,500
		Plot E	M	24,100	23,300	0	800
			Mean	23,400	19,600	600	3,200
4 (A)	28	-x	M	321,300*	321,300	0	0
		-x	F	101,600	95,600	1,400	4,600
		-x	F	96,600	90,200	1,800	4,600
			Mean	173,200	169,000	1,100	3,100
4 (B)	28	-x	F	26,500	25,000	200	1,300
		-x	F	59,400	55,600	300	3,500
			Mean	43,000	40,300	300	2,400

*Killed in extremis

M = Male F = Female

Plot = Paddock

x = Not Grazed - Reared Worm Free

APPENDIX 5 - Table 5

Patterns of Diarrhoea 16 to 21 Days After an Experimental Challenge Inoculum of 300,000 Cryptosporidia oocysts/calf Larvae was given (Day 60) to Calves previously Immunised with X-irradiated C. parvum Larvae on Days 0 and 30 (Groups A1 and B1) and Non-Immunised Calves (C1)

	DAY					
	76	77	78	79	80	81
GROUP A1 (60 kr)	-	-	-	-	+	+-
	-	-	-	-	-	-
	-	+	+	+	+	+++
	-	-	-	-	-	+++
	-	-	-	-	-	-
TOTAL +	-	1	1	1	2	3
GROUP B1 (60 kr)	-	-	-	-	-	-
	-	-	-	+	+	+++
	-	-	-	-	-	-
	-	-	-	-	-	+
	-	-	-	-	+	++
TOTAL +	-	-	-	1	2	6
GROUP C1	+	+	+	+++	+++	+++
	+	+	-	+	+	++
	-	-	+	+	+	++
	+	+	+	+	++	++
	-	-	+	+	++	++
TOTAL +	3	3	4	7	9	13

kr = kilorogatory

\+ = soft faeces

++ = semi-fluid faeces

+++ = fluid faeces

APPENDIX 5 - Table 6

Weekly Bodyweights in lbs. of Calves Immunised with X-irradiated Ostertagia ostertagi Larvae (Groups A1, B1) and Non-Immunised Calves (Group C1). Before and After an Experimental Challenge Inoculum of 300,000 O. ostertagi Third Stage Larvae was given

Group	Immunised ↓				Immunised ↓				Challenged ↓				
Day	0	7	14	21	27	34	41	48	55	61	68	75	81
A1 (60 kr)	146	150	178	188	195	206	224	218	236	-	256	271	269
	126	136	164	178	184	192	208	200	214	-	266	259	255
	151	164	190	202	206	220	238	226	240	-	275	282	272
	160	158	170	160	182	190	200	200	212	-	215	224	225
	170	182	208	225	220	242	250	250	264	-	297	314	305
Mean & Standard Error	151 ±7.4	158 ±7.6	182 ±7.8	195 ±8.7	197 ±8.2	210 ±9.7	224 ±9.2	219 ±9.5	233 ±9.5	- -	262 ±13.5	270 ±14.7	265 ±12.9
B1 (60 kr)	150	158	185	198	206	214	222	220	230	-	261	270	266
	130	146	164	185	187	204	222	210	232	-	261	273	262
	162	167	182	200	209	222	238	230	240	-	268	276	257
	150	160	188	200	204	218	240	238	242	-	267	280	283
	164	174	206	212	211	230	230	232	246	-	271	288	282
Mean & Standard Error	151 ±6.1	161 ±4.7	185 ±6.7	199 ±4.6	205 ±4.2	218 ±4.3	230 ±3.8	228 ±3.6	238 ±3.1	- -	266 ±2.0	277 ±3.1	270 ±5.3
C1	130	135	158	170	178	188	203	205	218	-	240	246	231
	164	170	200	211	222	238	249	244	258	-	286	296	280
	152	160	185	200	230	222	240	225	242	-	271	287	265
	178	170	200	225	221	244	260	246	250	-	296	297	302
	144	158	176	191	191	192	204	211	220	-	240	252	232
Mean & Standard Error	154 ±8.2	159 ±6.4	184 ±7.9	199 ±9.0	204 ±8.6	217 ±11.5	231 ±11.7	226 ±8.3	238 ±8.0	- -	267 ±11.6	276 ±11.0	262 ±13.9

kr = Kilorontgen

APPENDIX 5 - Table 7

Faecal Egg Counts 16-21 Days After an Experimental Challenge Inoculum of 300,000 *Ostertagia ostertagi* Larvae was Given (Day 60) to Calves Previously Immunised with X-irradiated *O. ostertagi* Larvae on Days 0 and 30 (Groups A1, B1) and Non Immunised Calves (C1)

	DAY					
	76	77	78	79	80	81
GROUP A1 (60 kr)	-	-	-	50	100	200
	-	-	-	300	250	650
	-	50	150	100	550	600
	+	+	-	100	-	-
	-	-	-	-	150	500
MEAN	-	10	30	110	210	390
GROUP B1 (60 kr)	-	-	-	300	700	750
	50	-	-	100	150	400
	50	-	-	-	750	450
	-	-	150	350	750	300
	150	-	-	100	1400	1700
MEAN	50	-	30	170	750	720
GROUP C1	50	50	1000	2350	850	150
	-	-	-	1000	1600	2000
	50	-	700	450	2000	3700
	100	50	350	300	7000	2000
	50	50	550	700	000	1950
MEAN	50	30	600	960	2610	1960

\+ = Positive on zinc sulphate flotation

kr = kilorecntgens

APPENDIX 5 - Table 8

Weekly Faecal Egg Counts in Two Groups of Five Calves which Grazed from June until October, 1966, on Pasture known to be Contaminated with Ostertagia ostertagi Larvae, One Group being Immunised with X-irradiated O. ostertagi Larvae Prior to Grazing

Group	June 13th-July 12th	Faecal Egg Counts in Eggs per Gram and Date Sampled												
		12.7	19.7	26.7	2.8	9.8	16.8	23.8	30.8	6.9	13.9	20.9	27.9	4.10
Immunised	-ve	-	-	-	50	50	200	-ve	50	-ve	250	150	-ve	300
		-	-	-	100	50	-ve	50	50	-ve	50	150	100	500
		-	-	50	50	50	-ve	50	150	-ve	50	100	-ve	x450
		-	-	50	100	50	500	400	400	150	750	400	400	x500
		-	-	-	-	50	50	-ve	100	-ve	100	-ve	-ve	200
	Mean	-	-	20	60	50	150	100	150	30	240	150	100	390
Non-immunised	-ve	-	-	-	-	100	-ve	200	200	150	100	150	100	x200
		100	50	50	500	500	700	750	700	300	600	400	150	x2400
		-	100	-	50	350	150	150	-ve	-ve	-ve	100	150	550
		100	-	100	400	800	700	x7200						
		-	50	-	50	50	1150	400	550	-ve	300	250	250	1400
	Mean	40	40	30	200	320	540	1750	360	110	250	220	160	1140

x = Killed in Extremis

APPENDIX 5 - Table 9

Plasma Pepsinogen Levels in Calves Immunised with X-irradiated *Ostertagia ostertagi* (Groups A1 and B1) and Non-immunised Controls (Group C1) Before and After an Experimental Challenge Inoculum of 300,000 *O. ostertagi* Third Stage Larvae was given

	immunised ↓			immunised ↓				challenged ↓				
Day	0	11	21	29	35	42	49	56	63	70	77	81
A1 (60 kr)	500	600	1,300	800	1,000	800	1,400	1,400	2,000	2,300	3,800	3,500
	200	700	900	500	700	800	1,200	1,200	1,900	1,800	2,200	4,900
	500	500	800	300	900	800	1,200	1,000	1,500	1,500	2,600	4,300
	200	600	1,000	400	500	600	900	800	1,200	1,600	2,500	2,300
	300	500	1,000	800	1,100	900	1,200	1,000	1,800	2,300	3,700	5,600
Mean	300	600	1,000	600	800	800	1,200	1,100	1,600	1,900	3,000	4,100
& Standard Error	50	40	90	80	130	50	70	100	140	170	330	570
B1 (80 kr)	300	700	800	700	700	800	1,000	800	1,800	2,100	3,700	5,200
	300	400	800	400	700	600	700	500	1,100	1,800	2,800	3,900
	400	700	500	800	900	600	800	700	1,600	1,500	2,800	6,500
	400	400	1,000	400	700	600	800	700	1,100	1,300	2,700	3,700
	400	500	500	500	800	500	800	700	1,100	1,600	3,100	6,100
Mean	400	500	700	600	800	600	800	700	1,300	1,700	3,000	5,100
& Standard Error	20	70	100	80	40	60	50	50	150	140	180	570

kr = kilorcentgen

N.B. All Plasma Pepsinogen Values to Nearest 100
Standard Errors to Nearest 10

APPENDIX 5 - Table 9 (Continued)

	immunised ↓			immunised ↓				challenged ↓				
Day	0	11	21	29	35	42	49	56	63	70	77	81
	200	300	200	200	300	300	300	400	600	1,200	3,900	4,400
G1	300	300	500	200	500	200	200	300	900	1,500	1,500	3,800
non-	300	500	600	400	400	300	400	400	700	1,400	3,300	6,800
immunised	200	300	500	300	300	200	500	500	1,100	3,300	2,500	3,600
	300	400	300	300	300	300	300	400	800	2,000	4,800	5,900
Mean & Standard Error	300	400	400	300	300	300	300	400	800	1,900	3,200	4,900
	20	40	70	40	20	20	50	30	90	400	600	600

kr = kilorecmtgen

N.B. All Plasma Pepsinogen Values to Nearest 100
Standard Errors to Nearest 10

APPENDIX 5 - Table 10

Weekly Plasma Pepsinogens in Two Groups of Five Calves which Grazed from June until October, 1966, on Pasture known to be Contaminated with *Ostertagia ostertagi* Larvae, One Group being Immunised with X-irradiated *O. ostertagi* Larvae Prior to Grazing

	Immunised Calves						
Calf No.	1	2	3	4	5	Mean and	Standard Error
Date							
21.4	700	400	700	400	400	500	70
21.5	1,600	800	1,000	800	900	1,000	150
11.6	3,200	2,900	3,800	2,900	3,400	3,200	170
15.6	2,200	2,000	2,300	2,000	2,400	2,200	80
21.6	1,500	1,300	1,300	1,400	1,400	1,400	40
28.6	1,200	1,500	1,600	1,400	1,500	1,400	70
5.7	800	900	1,300	1,500	800	1,100	140
12.7	500	500	800	800	500	600	70
19.7	800	800	1,100	1,500	300	900	200
26.7	500	600	1,100	1,300	100	700	220
2.8	400	800	1,000	1,100	100	700	190
9.8	900	800	1,300	3,100	200	1,300	490
16.8	1,800	2,000	2,000	4,200	700	2,100	570
23.8	1,500	1,400	1,500	4,700	700	2,000	700
30.8	1,700	1,800	2,100	5,700	800	2,400	850
6.9	2,000	2,800	3,800	9,400	1,500	3,900	1,430
13.9	2,700	5,100	4,300	7,400	2,300	4,400	920
20.9	2,600	4,100	4,000	3,300	3,200	3,400	280
27.9	2,100	4,000	2,900	8,300	2,400	3,900	1,140
4.10	1,500	3,900	3,100	8,800*	2,900	4,000	1,250

* = Killed in extremis

N.B. Plasma Pepsinogen Values to Nearest 100
Standard Errors to Nearest 10

APPENDIX 5 - Table 10 (Continued)

Non Immunised Calves

Calf No.	6	7	8	9	10	Mean and	Standard Error
Date							
21.4	500	200	600	200	200	300	90
21.5	600	200	600	300	200	400	90
11.6	1,000	900	700	1,000	800	900	60
15.6	700	700	700	400	700	600	60
21.6	700	700	800	500	600	700	50
28.6	1,000	600	900	700	1,100	900	90
5.7	900	600	700	700	600	700	50
12.7	900	700	600	400	600	600	80
19.7	1,200	1,000	900	1,600	1,500	1,200	120
26.7	1,400	800	900	1,500	1,200	1,200	170
2.8	1,600	1,400	700	1,400	1,700	1,400	170
9.8	1,800	1,000	900	1,700	2,000	1,000	300
16.8	NS	3,100	1,800	3,000	3,800	2,900	400
23.8	2,300	2,900	1,500	5,600	3,000	3,100	690
30.8	2,600	3,700	2,000	2,800	3,800	3,000	340
6.9	4,800	8,000	2,700	1,800*	6,400	4,700	1,150
13.9	5,000	9,500	4,200		4,500	5,800	1,240
20.9	3,800	6,300	3,400		6,600	5,000	860
27.9	3,700	6,500	3,500		2,900	4,200	800
4.10	3,400	6,200*	3,700		6,600	5,000	840

* = Killed in extremis

N.B. Plasma Fopolnogen Values to Nearest 100
Standard Errors to Nearest 10