A GENERAL SUMMARY OF THE FIELD AND EXPERIMENTAL RESULTS

Following a study of nineteen outbreaks of naturally occurring bovine parasitic gastroenteritis, in which 45.5 per cent of 337 young dairy cattle were clinically affected, O.ostertagi was the predominant nematode species found. The fields which were used year after year for the raising of replacement calves, together with the practice of grazing spring born and autumn born calves in the same field, were factors which were considered to be major predisposing causes of outbreaks of this economically important disease.

Three phases of bovine ostertagiasis could be dinstinguished on the basis of clinical history and laboratory findings.

TYPE I 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 which occurred at any time from late July until the end of the grazing season. The vast majority of the ingested larvae developed to maturity within the expected period of three weeks.

PRE-TYPE II was clinically not apparent though large populations of O.ostertagi were present, of which over 80 per cent were inhibited at the early 4th stage of

larval development. These animals had grazed infected pasture until the late autumn, but had no history of diarrhoea and usually appeared healthy to the farmer.

The second clinical phase, Type II, was different to the first in that calves, which had no history of diarrhoea or weight loss during the grazing season and which were well grown and in excellent condition, were taken indoors about the beginning of November. After a variable period of time, ranging from 3 weeks to 4 months, these animals started to lose weight and to show a profuse, watery diarrhoea. The appearance of the clinical signs coincided with the development to maturity and emergence from the abomasal mucosa of large numbers of inhibited O.ostertagi larvae, which were ingested during the late autumn grazing period.

In the diagnosis of bovine ostertagiasis the results of faecal worm egg counts must be interpreted cautiously. All calves with egg counts of 1,000 e.p.g. and over were clinically affected animals. Because of the gross variation in worm egg counts which occurred in individual cases, low egg counts could not exclude bovine ostertagiasis from the differential diagnosis.

In contrast to the effect seen in Type I cases, the response of Type II affected animals to anthelminthic treatment was poor; consequently, the prognosis was grave

in the latter category.

Anaemia, hypoproteinaemia and hypoalbuminaemia were not detected in cases of Type I ostertagiasis. In cases of Type II there occurred a moderate, normocytic, normochromic anaemia and a marked hypoalbuminaemia. The plasma pepsinogen concentration of infected calves was increased, markedly so in clinical cases, and was correlated with the severity of the abomasal lesions and the numbers of O.ostertagi found at autopsy. The pH and sodium ion concentration of the abomasal contents was markedly increased in Type I and Type II cases, but was only slightly elevated in Pre-Type II affected animals. The physiological abnormalities were associated with marked histopathological changes within the abomasum. These were described as a loss of cellular differentiation of the specialised cells, hyperplasia of the mucous epithelium and infiltration by reticulo-endothelial cells. Further study would be required to establish how the parasites caused the abnormalities observed.

A detailed study of the naturally occurring disease revealed that the numbers of larvae available on the pasture of the calf rearing fields increased progressively from May to July and reached a peak at the end of August. Type I disease occurred after 9 to 16 weeks grazing on these pastures and some 50,000 to 60,000 O.ostertagi were required to precipitate clinical signs in calves of 4 to 6 months of

age. In contrast to the worm counts from calves autopsied prior to October, a marked increase in the numbers of early 4th stage parasites was found in both previously infected and worm free calves which had grazed the heavily contaminated pasture, for as short a period as 14 days, during the late autumn period. This phenomenon was caused by inhibition of development at the early 4th larval stage and was attributed to an unspecified physiological change within the host or the larvae at this time of the year.

The pathogenesis of a single infection of 100,000 O.ostertagi larvae was studied experimentally in parasite free calves. Eighteen calves were autopsied in pairs from 2 to 90 days post infection. The larvae were found within the gastric glands 2 days after infection and emergence from the gland into the lumen, which coincided with patency and the development of clinical signs, occurred between days 16 and 21 after infection. Inhibition of development was not observed, but a marked loss of adult worms occurred between 16 and 28 days after infection. All the lesions seen in the typical field case were reproduced.

With the growth of the larva, the gastric gland dilated and became lined with undifferentiated, tall mucous cells to produce the characteristic white mucosal nodule, which was readily seen in the fundic region of the abomasum. Prior to the 4th larval moult at day 8 of the infection, the mesenchymal reaction was slight. Towards the end of the

growth phase this reaction increased and glands adjacent to that containing the larva lost their differentiated epithelium and became lined with cuboidal cells. Coalescence of hyperplastic nodules produced the "morocco leather" appearance of the mucosa. When the worms emerged from the gastric glands, they lay close to the surface epithelium at which site cytolysis and superficial sloughing occurred. Plasma cells were numerous at this time. Replacement and differentiation of the glandular epithelium took place very slowly.

In an experimental attempt to reproduce massive inhibition of development (a necessary pre-requisite for the appearance of the Type II syndrome) three regimes of infection of calves with O.ostertagi larvae were carried out. Firstly, groups of five calves were given single doses of 50,000; 100,000; 200,000; 400,000 and 800,000 larvae; secondly, calves were given four doses of 100,000 larvae at one week intervals, and thirdly, calves were given 20 doses of 1,000 larvae over 28 days and this was followed 21 days later by a single dose of either 400,000 or 800,000 larvae. When the calves were autopsied, usually 21 days after the last infection, a significant degree of inhibition of larval development was not found in any of the O.ostertagi worm populations. Clinical signs of Type I ostertagiasis occurred in the majority of these calves and at autopsy the

characteristic biochemical and pathological alterations associated with the Type I disease were found. From these experiments it was concluded that inhibition of development of O.ostertagi larvae was not related to the size of the larval infection nor was it an inevitable outcome of previous multiple infections.

However, before the pathogenesis of the Type II syndrome can be adequately studied, inhibition of development must be induced in calves. An assessment of the environmental factors, or the possible changes in the physiological makeup of host and parasite associated with the pre-Type II and Type II syndromes, would, most likely, reveal the mechanism of the inhibition phenomenon.

BOVINE OSTERTAGIASIS

A DISSERTATION FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

NORMAN ANDERSON

DECEMBER, 1967.

BOVINE OSTERTAGIASIS

Field studies, into the nature and cause of bovine parasitic gastritis caused by the Trichostrongyle nematode Ostertagia ostertagi, were carried out in Renfrewshire, Ayrshire, Lanarkshire and Wigtonshire, Scotland.

The experimental work presented in this dissertation was carried out in the Department of Veterinary Medicine, Glasgow University.

The results given herein were obtained during the course of an investigation embracing the study of parasitic gastroenteritis affecting sheep and cattle in this part of the British Isles.

My colleagues associated with this research programme were Mr. J. Armour, Mr. J.D.S. Ritchie and Dr.F.W.Jennings, under the supervision and direction of Professors W.I.McIntyre and W.F.N. Jarrett and Dr. G.M. Urquhart.

The pathological changes associated with infections of Ostertagia ostertagi will form the subject of a separate communication by Mr.J.D.S.Ritchie. However, to facilitate the understanding of certain physiological and parasitological observations, permission has been obtained to include in this dissertation, a short description of the relevant histopathological changes.

The field and experimental data, presented herein, are original and have not been submitted in any form to any other University.

BOVINE OSTERTAGIASIS

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BOVINE OSTERTAGIASIS

INTRODUCTION

A LITERATURE REVIEW OF OSTERTAGIA OSTERTAGI: A PARASITIC NEMATODE OF THE BOVINE ABOMASUM.

A LITERATURE REVIEW OF OSTERTAGIA OSTERTAGI; A PARASITIC NEMATODE OF THE BOVINE ABOMASUM.

1. Classification of Genera and Species.

Ostertagia ostertagi, commonly known as the Medium Stomach Worm or the Small Brown Stomach Worm, was first recorded from the bovine in 1890 by Ostertag, who gave it the name of Strongylus convolutus. Stiles (1892) renamed the parasite Strongylus ostertagi but it was Ransom (1907) who gave the first detailed description of this abomasal nematode of bovines, naming it Ostertagia ostertagi, the type species of the genus Ostertagia.

Skrjabin (1954) within the superfamily Trichostrongyloidea, established the tribe Ostertagica containing twelve genera. The type genus remained Ostertagia but it was divided into three subgenera:- Costarcuata, Grosspiculagia and Ostertagia. The subgenus Ostertagia contains fifteen species three of which, O. ostertagi, O. circumcincta and O. trifurcata have been recovered from the abomasum and small intestine of domestic cattle. Ostertagia lyrata (subgenus Grosspiculagia) has also been reported from cattle.

An excellent description of the subgenus Ostertagia and a diagnostic key to species differentiation is given

by Skrjabin (1954).

2. Geographical Distribution

The geographical distribution of the species found in cattle is a wide one and includes Western Europe, North and South America, Asia, Australia, New Zealand and U.S.S.R. (Ostertag, 1890; Stadelman, 1891; Stiles, 1892; Gilruth 1910; Moras, 1907; Ransom, 1907; Gardiner, 1911; Skrjabin, 1927; Rose, 1961.)

3. The Life Cycle of Ostertagia ostertagi.

The life cycle of Ostertagia ostertagi has been described by various workers, (Stadelman 1891; Stodter 1901; Threlkeld 1946; and Douvres 1956) and the description given below is taken from their work.

The Preinfective Phase

O. ostertagi eggs when laid are usually in the 2 to 8 cell stage. They are passed in the faeces and in warm weather they hatch in 21 to 24 hours releasing motile, first stage larvae. Two days later, after a period of lethargy prior to moulting, the second stage larvae develop. Third stage or infective larvae, encased in the sheath of the second stage larvae are found 5 to 6 days after oviposition under optimal conditions. During this free living phase of development the first and second stage larvae are believed to feed on bacteria.

The Infective or Parasitic Phase

The Infective or Parasitic Phase

Exsheathment of Larvae

When ingested by suitable ruminants the third stage or parasitic larvae exsheathe in the rumen. Rogers and Somerville (1964) state that the chief stimulus for exsheathment is carbon dioxide although other factors such as the hydrogen ion concentration, reducing agents and the presence of salts may be important under certain conditions. Given favourable circumstances the stimulus has its maximum effect within 30 minutes.

Morphogenesis of Larvae

Osborne, Batte and Bell (1960) found third stage larvae in the gastric glands of the abomasum as early as 6 hours after ingestion.

Douvres (1956) found that two days following infection the parasites were exsheathed third stage but on day three the majority of the larvae were undergoing their third moult.

By day four of the infection early fourth stage larvae predominated by which time sex differentiation was possible.

At the seventh day the larvae were still in the fourth stage, but were considerably larger, measuring 2.52 to 3.08mms. in length. The fourth moult commenced on day eight after infection and was complete by day twelve, all parasites were then in the fifth or immature adult stage. Larvae which have completed their last moult may be found free in the abomasal contents. They usually emerge from the gastric

glands after the fifteenth day. Immature adults, 15 to 17 days of age, were found deep in the lumen of the glands, AND in histological sections were seen to occupy spaces ranging from 400 to 500 microns in diameter. Rarely, and then only in heavy infections did the larvae penetrate the muscularis mucosae. Larval development in the gastric glands is often termed the histotrophic phase and Threlkeld (1958) gave evidence that this phase may terminate at any time between four days and thirteen weeks after post infection for individual larvae. Threlkeld gave no explanation why this occurred. However, delayed development or inhibition of development was commonly found in cattle clinically affected with ostertagiasis (Martin, Thomas and Urquhart, 1957).

The Adult Stage

Threlkeld (1946) noted that mature male and female worms were recovered on the twenty-first day post infection; then the uteri of the females contained 20 to 30 eggs. Eggs appeared in the faeces two days later. Porter and Cauthen (1946) showed that patency can vary from 19 to 31 days and that egg laying may continue for a period of 7½ months following a single infection.

Threlkeld (1958) in a series of 27 postmortem examinations, found that mature adults were usually present on the surface of the mucosa in the pyloric region of the abomasum.

TABLE I

MEASUREMENTS OF LENGTH OF THE VARIOUS DEVELOPMENTAL STAGES OF OSTERTAGIA OSTERTAGI.

A SUMMARY OF PUBLISHED REPORTS

Morphogenic Stage	Measurements in mms.	Author
Embryonated Eggs (in faeces)	Length 0.071 - 0.085 Width 0.035 - 0.045	Skarbilovich (1935)
	Length 0.070 - 0.084 Width 0.040 - 0.050	Threlkeld (1946)
First Stage Larvae	Length 0.348 - 0.372	Skarbilovich (1935)
	Length 0.300 - 0.500	Threlkeld (1946)
Second Stage Larvae	Length 0.547	Skarbilovich (1935)
	Length 0.750	Threlkeld (1946)
Third Stage or Infective Larvae	Length 0.850 - 0.900	Threlkeld (1946)
	Length 0.825 - 0.925	Keith (1953)
	Length 0.920	Douvres (1956)
Early Fourth Stage Larvae	Length 1.00	Threlkeld (1946)
	Length 1.40	
	Length 1.18	Douvres (1956)
	Length 1.29	
Fourth Moult Larvae	Length 1.7 - 2.0	Threlkeld (1946)
	Length 2.7 - 3.0	
	Length 3.97	Douvres (1956)
	Length 4.73	
Fifth Stage or Immature adults	Length 3.37 - 4.00	Threlkeld (1946)
	Length 4.1 - 6.7	
	Length 4.87	Douvres (1956)
	Length 6.12	
Adult Stage	Length 6.0 - 7.0	Threlkeld (1946)
	8.0 - 9.0	

Douvres (1956 and 1957) in his study of the morphogenesis of the parasitic phase, gives an excellent description with measurements of the various developmental and adult stages and his results agree with the work of Threlkeld (1946) and Keith (1953). Table I summarises the measurements made by several workers of the various developmental stages in the life cycle of Ostertagia ostertagi.

4. The Bionomics of Free Living Ostertagia Larvae. Larval Development and Survival under Laboratory Conditions. Larval Development in Faecal Culture.

Ciordia and Bizzell (1963) noted that considerable variation occurred in the rate of larval development when cultures were maintained at constant temperatures. They tried, unsuccessfully, to reduce this variation. Their results for the survival of larvae were expressed by minimal and median percentages, at several different constant temperature readings. The developmental temperature was optimum at 25°C. Seven to nine days' incubation at this temperature were needed before infective larvae could be recovered. However, at 25°C only 30 per cent of the eggs had developed to the third larval stage. Temperatures above 25°C hastened development but increased mortality. The percentage of eggs which developed to 3rd stage larvae at 15°C was 13; at 8°C was 3.5 percent and at temperatures

below 6°C development did not proceed beyond the gastrula stage.

Survival of Eggs and Larvae Isolated from Faecal Culture

Furman (1944), working with O. circumcincta, and using a controlled humidity and temperature box, found that non-embryonated and embryonated eggs kept in 5mm. of water were killed in 1 to 2 days by a temperature of 45°C. In ice at -6 C° they survived 10 to 22 days. Embryonated eggs resisted temperatures up to 37°C.

Preinfective larvae were more sensitive than eggs to the effects of environmental temperature. Freezing or high temperatures killed them quickly; whilst those in shallow water, at 5°C, could survive for 1 to 2 months.

Of the free living stages, infective larvae were the most resistant to changes in temperatures and humidity. Freezing usually killed infective larvae within 14 days. Temperatures just above freezing increased survival which could be greater than 270 days. The most favourable of the temperature tested was 27°C; generally the mortality of infective larvae increased as temperatures rose above this.

Resistance to desiccation was greatest in infective larvae and embryonated eggs and least in preinfective larvae and non-embryonated eggs. Furman (1944) noted that infective larvae withstood a saturation deficiency of

19.8 mm. Hg at 27°C for 43 days. The Animal Health Services Report of Great Britain for 1958, records that the majority of larvae, when dried on microscope slides and stored at a relative humidity of 75 to 95 per cent, were still alive after seven days; further desiccation resulted in high mortality, but a few larvae survived for seven weeks: in contrast to this, it was found, that eggs and preinfective larvae, were killed by short periods of desiccation.

Larval Development and Survival under Field Conditions

Survival of Isolated Third Stage Larvae on Pasture.

Bell, Galvin and Turk (1960) estimated the survival of O.ostertagi larvae by placing 5,000 fresh infective larvae at the base of Bermuda grass grown in areas six inches square. Larvae were recovered from the grass and topsoil by the Baermann method. Considerable variation was noted but more larvae were recovered from long grass than short grass. This experiment, carried out in Texas, showed there was little or no correlation between survival time and average maximum temperature, relative humidity or rainfall. The maximum survival time for O.ostertagi larvae was 144 days; 50 percent however, were dead from exposure by the 36th day.

Development and Survival of Larvae in the Faecal Pat.

Furman (1944) placed fresh sheep faeces containing a known number of eggs on grass plots and recovered O.circumcinta larvae from the surrounding grass and soil. He found that

large numbers of 3rd stage larvae had developed during the summer months on irrigated Californian pastures and those had survived throughout the whole summer period. In contrast larvae were killed rapidly on non-irrigated pastures in summer. During autumn and winter a much greater percentage of eggs developed to the infective stage than in summer. This was thought to account for the greater incidence of parasitic gastroenteritis in sheep during the warmer months.

Threlkeld and Johnston (1948) in Virginia, found that O.ostertagi remained viable on pastures following 4½ to 6½ months' exposure to environmental effects, including those of winter.

Rose (1961, 1962) carried out an intensive study of the bionomics of O.ostertagi in the South-west of England. Faeces which were spread thin on the pasture dried out completely in 4 to 5 days and no larvae were recovered either from the faeces or the surrounding herbage and soil.

The faecal pat was found to be an ideal environment for the development of infective larvae. The pat dried out slowly and although a hard crust a quarter of an inch thick formed in 4 to 5 days, it took almost a month for the entire mass to dry out. The minimum and maximum time taken for eggs to develop into 3rd stage larvae was 11 and 19 days respectively during April and May; 6 and 14 days respectively

in June and July. Larval development in the faecal pat took place all the year round except winter; eggs which had remained viable resumed development in the spring, when the temperatures began to rise.

Third stage larvae could survive on herbage for up to 2 years and large numbers were found to survive for 8 months. It was also found that some larvae could withstand winter conditions but the percentage mortality during this period was not estimated.

Rose concluded that in the South-west of England, the average range of climatic conditions did not appear to have any significantly deleterious effect on the longevity of 3rd stage larvae.

Migration from the Faecal Pat

The studies of Rose (1961) showed that the larvae of O.ostertagi tended to congregate in and just beneath the hard crust of the faecal pat, even when deeper parts of the pat contained more moisture. Larval migration to the pastures was not possible until this hard crust was softened by rain. Migration from the faecal pat was found to be gradual and it took the form of either a continuous process or a series of migration waves depending upon the pattern of soaking rain. Infective larvae could be recovered from the faecal pat until it disintegrated; even though the majority had migrated within 4 months.

Lateral migration was limited; the majority of larvae were found within a radius of two inches from the faecal pat and only a fewwere found 4 or 6 inches away.

Rose (1961) reported that most larvae were found less than 2" above the soil surface. Knapp (1963) showed that humidity had a marked effect on the vertical migration of Trichostrongylid larvae onto pasture plants; relative humidity of 30 per cent completely inhibited migration, while 90 per cent began it immediately.

Since the faecal pat had been shown to serve as a reservoir for larvae and pasture contamination, the effect of pasture harrowing on larval survival was investigated by Rose (1961) During dry periods harrowing increased the mortality of larvae as the spread faeces dried out rapidly. Under suitable rainy periods mechanical spreading of faeces only facilitated the dissemination of larvae and mortality was not increased.

The question of dissemination of infective larvae by fungae of Pilobilus sp. such as occurs with Dictyocaulus viviparus (Robinson, 1962) has not been investigated.

It has been demonstrated, (Report on the Animal Health Services in Great Britain (1960)), that during the summer in Southern England, infective larvae from eggs passed in faeces onto the pasture were first recovered from the herbage some 8 to 10 weeks after faecal deposition. However, the

reasons for this delay do not appear to have been investigated.

The Limitations of Bionomic Studies

Rogers and Sommerville (1963) have drawn attention to the difficulties associated with larval survival studies. A failure to measure such parameters as temperature, humidity and rainfall, in the immediate environment of the parasite, could limit the value of the results to the place in which the experiment was carried out. Also, Durie (1959) has pointed out that the Baermann method of recovering larvae from pasture and soil was not satisfactory. A criticism of the application of this method is that there is no information on the infectivity of the larvae recovered; Durie (1962) working with H.contortus, has given some evidence which suggested that aged larvae were less infective and that morphology and motility might be unreliable criteria for assessing infectivity.

Quantitative Studies on the Availability of Infective Larvae on Pasture.

To overcome the difficulties outlined above, Goldberg and Rubin (1956) and Goldberg and Tucker (1956, 1963) in Maryland, U.S.A. used calves as indicators of the availability of trichostrongylid larvae.

Faeces, containing large numbers of eggs of the common bovine gastrointestinal nematodes, including O.ostertagi, were first spread over four or five equivalent areas of

pasture. One calf was grazed on each area at set intervals after plot contamination. After 14 or 28 days of grazing these test calves were taken indoors for 3 weeks prior to autopsy. The number of worms recovered from the test calves was used as the main index of the survival and infectivity of the Trichostrongylid nematodes tested. The results of spring, summer and autumn pasture contamination of the plots indicated that:-

(a) the numbers of larvae which developed following summer contamination declined sharply two months after the last contamination date. Four of the ten nematode species tested, including O.ostertagi, survived, in rather small numbers, both the heat and dryness of summer and the cold of the following winter.

(b) the numbers of larvae which survived 2 and 4 months following spring contamination were considerably higher than those recorded following summer contamination. O.ostertagi was the one species which survived the longest period of time.

(c) the numbers of larvae which developed following autumn contamination were maximal 2 to 4 months after contamination. O.ostertagi survived well until April of the following year.

In general terms, the rate of decline of available larvae was lower in the experiments which were commenced

in October than those begun in May or August.

Throughout their series of experiments, the authors were unable to demonstrate a correlation between the numbers of larvae present on the pasture and the number of worms which became established in the test calves.

Tallis and Donald (1964) have discussed the theoretical aspects of larval distribution on pasture and have presented a mathematical model for future study.

SUMMARY

A literature review of the bovine nematode O.ostertagi, has revealed that this parasite has a wide geographical distribution and that it is well adapted to a wide range of environments within the temperate zones of the world.

The life cycle of O.ostertagi has been shown to be direct and it can be completed within 4 to 5 weeks. The moist faecal pat provides an ideal medium for the hatching of eggs and the development of the free living first and second stage larvae. The infective third stage larvae were found to be more resistant to heat, cold and dryness than the preinfective stages. Migration of infective larvae from the faecal pat onto the pasture was dependent upon an adequate amount of moisture.

The degree of heat and amount of moisture were the major determinants which controlled larval development and survival. However, these factors have not been thoroughly studied under field conditions so that the factors controlling the size of larval populations on pasture are not well understood.

Once ingested by the bovine, larvae undergo two further moults in the gastric glands of the abomasum and reach maturity within 19 to 34 days.

A prolonged histotrophic phase has been noted but

the reasons for the delay in larval development of O.ostertagi have not been studied.

SECTION ONE

MATERIALS AND METHODS

SECTION ONE

MATERIALS AND METHODS

1. EXPERIMENTAL ANIMALS
2. FIELD INVESTIGATION PROCEDURE
3. HAEMATOLOGICAL METHODS AND MATERIALS
4. BIOCHEMICAL METHODS AND MATERIALS
5. AUTOPSY PROCEDURE
6. PARASITOLOGICAL METHODS AND MATERIALS

MATERIALS AND METHODS

1. EXPERIMENTAL ANIMALS

The rearing and maintenance of parasite free calves.

Ayrshire bull calves, a few days old, were purchased from local markets and were individually house in galvanised iron huts. The calves were fed whole milk for the first four weeks at a rate of 1 pint of milk per 10 lbs. live weight. From two weeks of age, small amounts of Calf Weaner Pellets, (British Oil and Cake Mills Ltd., Renfrew, Scotland) and hay were made available. At four weeks of age, the calves were weaned on to Calf Weaner Pellets with hay and water fed ad lib. Weekly weighing showed that a mean growth rate of 1 lb. per day per calf was attained on these rations. The iron huts were cleaned out weekly and the calves bedded daily with clean straw. A regular examination failed to show the presence of any nematode eggs in the faeces of these animals. The calves ranged from 8 to 18 weeks of age prior to their inclusion in an experiment.

Weighing procedure

Experimental and hospitalised field cases were weighed at intervals of one week. Food and water were not restricted prior to weighing which was carried out between 9 and 11 a.m. on each occasion.

FIELD INVESTIGATION PROCEDURE

Outbreaks of parasitic gastroenteritis in young cattle were referred by practising veterinary surgeons to the Veterinary Hospital, Bearsden Road, Glasgow, for investigation. A visit was made to each farm and a farm history was obtained. Blood and faecal samples were collected for laboratory examination. Where possible, one or more of the affected animals were purchased from each outbreak and transported to the hospital for further clinical and subsequent post-mortem examination. Follow up visits were made to each farm on various occasions.

3. HAEMATOLOGICAL METHODS AND MATERIALS

Blood samples

Calves were bled from the jugular vein and all animals were handled as quietly as possible to avoid excitement. About 2 ml. of blood was collected into a 7 ml. Bijou bottle containing a few crystals of the anti-coagulant, disodium ethylenodiaminetetracetic acid (E.D.T.A.). The bottle was gently shaken to dissolve the crystals.

Generally, the haematological examinations were carried out on the same day as the blood sample was taken. When this was not possible, the blood samples were stored in a refrigerator at 6°C overnight and the estimations were carried out the following morning.

The packed cell volume (P.C.V.) 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 & Sons Ltd., London, England). The percentage packed cell volume was determined from a Hawksley Micro-haematocrit Reader.

Haemoglobin concentration (Hb conc.) was estimated by the oxy-haemoglobin method, Dacie (1958). A one in 200 dilution of blood was prepared in a 0.04 per cent solution of ammonium hydroxide. After thorough mixing, the amount of light absorption of the solution was determined at 540 mu in an EEL colorimeter (Evans Electroselenium Ltd., Harlow, Essex, England) using a 625 green filter.

Total red blood cell counts (R.B.C.) were made using an electronic Coulter Counter (Coulter Electronics Ltd. Dunstable, Beds., England) by the method of Weide, Trapp, Weaver and Lagace (1962). Results were expressed as millions of cells per cubic millimeter and the repeatability of this method was found to be 5 per cent or less.

The Mean cell volume (M.C.V.) was calculated from the formula:

$$\frac{\text{P.C.V.}}{\text{R.B.C.}} \times 10 \text{ cubic microns}$$

Likewise the Mean cell haemoglobin concentration (M.C.H.C.) was calculated from the formula:

$$\frac{\text{Hb conc. in GMs per 100 ml blood}}{\text{P.C.V.}} \text{ per cent.}$$

4. BIOCHEMICAL METHODS AND MATERIALS

Serum and plasma samples

Two samples, each of about 15 ml. of jugular blood were collected into two Universal bottles, one of which ontained 2 to 3 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 a super minor centrifuge (Measuring and Scientific Equipment Ltd. London, England). The plasma was then pipetted into 7 ml Bijou bottles which were stored at -5°C.

Serum was collected from the clotted sample which had separated overnight at room temperature.

Plasma pepsinogen estimation

The method of Edwards, Jepson and Wood (1960) was used except that Bovine Crystalline Albumin (Armour Pharmaceutical Co.Ltd., Eastbourne, England) was substituted for the human plasma substrate. A phenol standard was prepared by dissolving 1 gm crystalline phenol in 1 litre distilled water. Phenol standards were set up with each estimation and by calculation the peptic activity was expressed, as micro gms of phenol per ml plasma per 24 hours at 37°C.

Total serum protein concentration

The total serum protein concentration was estimated by the Biuret method, Weichselbaum (1946). Readings from the EEL colorimeter were read off a graph which had been

calibrated with a standard serum of known total nitrogen content. The results were expressed as gm per 100 ml serum (gm per cent).

Determination of the albumin/globulin ratio

Electrophoresis of the serum was carried out using barbitone buffer (veronal buffer) at pH 8.6 for 12 hours and Whatman 3MM 3cm electrophoresis paper (W. and R.Balston Ltd., London, England). The volume of serum used was 0.006 ml, applied with a standard applicator. After drying at 100°C for 10 minutes the strips were stained in 1 percent bromophenol blue and then washed clear with 2 percent acetic acid. The albumin-globulin ratios were determined by elution of the bound dye according to the method described by Jennings and Mulligan (1953).

Determination of gastric pH

As soon as a sample of abomasal contents was collected, the pH was determined at room temperature using an Expanded Scale pH meter (Beckman Instruments Ltd., Glenrothes, Scotland) fitted with micro glass electrodes. The sample was then centrifuged at 20,000 g for 20 minutes. The pH of the supernatant was determined again but no significant difference from the initial measurement was noted.

Estimation of gastric electrolytes, Na, K, Cl

Sodium and potassium concentration.

Sodium and potassium concentrations were determined on the

centrifuged samples and were estimated by the flame photometer method ~~set out in~~ DESCRIBED BY Varley (1958), using an EEL flame photometer (Evans Electroselenium Ltd., Harlow, Essex, England).

Chloride concentration

Chloride ion concentration of the centrifuged samples was estimated by the mercuric nitrate titration method of Schales and Schales (1941).

5. AUTOPSY PROCEDURE

Slaughter

All animals to be autopsied were starved for 24 hours prior to slaughter although, during this time, they had free access to water. The animals were shot with a captive bolt and bled out. After reflecting the abdominal skin, the abdomen was opened and the whole gastrointestinal tract was removed. A full pathological examination of each animal was carried out in collaboration with Mr. J.D.S. Ritchie.

Abomasum

The abomasum, omasum and 12 to 18 inches of the duodenum were separated from the rest of the viscera, care being taken not to lose their contents. After separation from the omasum, the abomasum was opened immediately along the greater curvature and a sample of contents was taken into 1 or 2 Universal bottles for pH and electrolyte determinations. The abomasal contents were collected into a

bucket and the abomasal mucosa was gently hosed to remove the larger pieces of ingesta. The mucosa was not handled until histological blocks had been taken. A description of the gross lesions was noted then the abomasal mucosa was thoroughly washed; the total washings were made up to 4 litres and, using a 200 ml measure, two samples were taken after a thorough figure of eight mixing. Each sample was preserved with 2 to 3 ml of undiluted formalin. For histological examination several blocks of tissue were selected from typical lesions in the fundic, pyloric and duodenal areas. These were fixed in 10 per cent formalin or corrosive formol; processed through a routine paraffin embedding procedure and stained with haematoxylin AND eosin.

Small Intestines

The small intestines of the field cases were separated from the omentum and divided into approximately three equal lengths. Each length was opened and washed under running water into a bucket. The volume was made up to 4 litres and a single sample of 200 ml was taken as above.

Digestion technique

The abomasum was divided transversely at the pyloric fundic junction and, together with a 2 foot length of duodenum, the whole mucosa of each part was scraped off, chopped finely and put into separate Kilner or 1-lb honey jars. The weight of the mucosa was recorded. The mucosa was digested by the

method described by Herlich (1956). A pepsin solution was prepared by adding 10 gm of 1:2500 pepsin powder (British Drug Houses Ltd., Poole, Dorset, England) to 600 ml. of water and acidifying with 12 ml of concentrated HCl acid. This pepsin solution was added to the mucosa at a rate of 2 to 5 ml per gm of mucosa.

Six hours of digestion at 41°C was sufficient to reduce the mucosa to a fine granular state. This degree of digestion had little effect upon the larval stages. The reaction was stopped by the addition of a few ml of undiluted formalin. The digests were made up to 4 litres, mixed thoroughly and a 200 ml sample was taken for microscopic examination.

6. PARASITOLOGICAL METHODS AND MATERIALS

Larval culture

The original culture of *O.ostertagi* was supplied by Mr.J. Armour, formerly of Cooper, McDougall and Robertson, Berkhamstead, England. Two parasite free calves were used as culture calves and these were dosed with 100,000 motile, 3rd stage *O.ostertagi* larvae. Eggs appeared in the faeces 18 and 21 days after dosing and, when the faecal egg count reached 500 eggs per gm or greater, a harness and collecting bag was fitted to each calf and a 24 hour collection of faeces was made.

The faeces were mixed with dry, sterile, sphagnum moss

until a moist, crumbly mixture was obtained. This mixture was loosely packed into 1-lb. honey jars and the lid screwed down. The jars were stored in the dark, ~~either~~ in a cupboard at room temperature AND AFTER 21 TO 25 DAYS ~~at which times~~ 3rd stage O.ostertagi larvae were present.

A modified Baermann technique was used to recover the larvae. The jars were filled with lukewarm tap water and allowed to stand without lids for one hour in diffuse light. The jars were then inverted into Petri dishes and after standing for 4 to 6 hours all the fluid was collected into buckets. Larvae remaining on the bottom of the Petri dish were washed off with clean water. The culture medium was discarded. The fluid was passed through two sieves, one placed on top of the other. The top sieve (100 meshes per inch) was covered with a single layer of milk filter pad (Cloverleaf Ltd.) to retain the coarse material. A small number of larvae passed through the top sieve but were trapped in the double layer of milk filter placed on the bottom sieve (400 meshes per inch). The colouring matter was washed out with clean tap water. The two bottom milk filters were removed and inverted over the milk filter on the 100 mesh sieve, which was then set up in a Baermann apparatus. The larvae collected were all motile and relatively free from foreign matter.

It was possible to recover large numbers of larvae,

often millions, by this technique. All larvae were stored in Roux flasks at about 6°C and viability, as judged by good motility, was maintained for at least 5 months at this temperature.

Larval counting technique

Larval collections were bulked, put into flasks of 2 or 5 litre capacity and the volume adjusted to give a larval concentration of about 1,000 per ml. O.ostertagi larvae tended to form clumps, so that it was necessary to thoroughly mix by shaking to keep the suspension agitated throughout the sampling procedure.

Samples of 0.025 ml were dropped on to microscope slides by means of a 0.025 autozero pipette (H.J.Elliott Ltd. Glamorganshire, Wales), and were counted under the 20 times objective of a projection microscope (Projectina Ltd., Heerugberg, Switzerland).

Preparation of doses and administration to calves

Doses for infection were measured out by means of measuring cylinders and pipettes. The doses were sedimented overnight in the refrigerator at 6°C; excess fluid was carefully removed with a suction pump and then the small volume remaining was filtered through a 5 cm Whatman's No. 1 filter paper.

The filter papers were folded and calves were dosed with the aid of a balling tube, making sure that the animal

swallowed the filter paper.

Faecal egg counting technique

All faeces examined were freshly taken rectal samples. A modified McMaster technique was used, Gordon and Whitlock (1939). Three grams of faeces were weighed out and mixed with 42 ml water by means of a top drive macerator. The homogenised sample was then passed through a sieve (60 meshes per inch) and a 15 ml sample of the filtrate was centrifuged for 2 minutes at 2,000 R.P.M. The supernatant was poured off and remaining material re-suspended in saturated NaCl solution. Both chambers of the special McMaster Worm Egg Counting Slide (Allen and Hanbury Ltd., Ware, England) were filled. The average number of eggs per chamber was multiplied by 100 to give the number of eggs per gram (e.p.g.)

In some field cases where Fascioliasis was suspected, saturated $ZnSO_4$ replaced saturated NaCl to detect the eggs of Fasciola hepatica.

Worm counting technique

To the 200 ml samples of abomasal and intestinal contents, a few ml of strong iodine solution was added. After thoroughly mixing the 200 ml. sample, by bubbling air through it, a 10 ml straight pipette, which had been sawn off at the 8 ml mark, was used to measure out 5 ml samples into a long counting dish.

The counting dish, made of perspex, measured 19 cm long by $2\frac{1}{2}$ cm wide by $\frac{1}{2}$ cm deep. Counts of worms were made

with the 10 times objective of the projection microscope. A few drops of saturdated sodium thiosulphate solution decolourised the excess iodine and only the worms remained stained. This was an aid to identification and facilitated counting.

From the 200 ml. sample 5 ml. samples were taken until 100 worms had been counted. When very low numbers of worms were present, ten counts of 5 ml. were made from each 200 ml. sample. The average number of worms per 5 ml. sample was calculated and multiplied by 800 to give the total number of worms in the original 4 litre volume.

Identification and differentiation

The descriptions of Ransom (1911); Douvres (1956,1957) were used to identify species and differentiate the morphogenic stages of the several nematode species found.

Measurement of O.ostertagi

Third and fourth stage O.ostertagi larvae tend to remain straight when formalised and these were measured directly on the graduated glass window of the projection microscope. The 20 times objective was used and the measurement in millimeters or microns was calculated.

Fifth stage larvae and adult O.ostertagi, especially males, were very often coiled and this made direct measurement impossible. A planimetric method was used. An outline of the image of the worm on the glass window of the projection microscope was traced onto thin white paper. Using a road

map mileage calculator and a calibrated scale, the measurement of worms at 20 times magnification was determined and these measurements were converted into millimeters.

SECTION 2

A FIELD STUDY OF PARASITIC GASTRITIS IN CATTLE.

SECTION TWO

A FIELD STUDY OF PARASITIC GASTRITIS IN CATTLE

PART 1 A REVIEW OF PREVIOUSLY REPORTED OUTBREAKS OF BOVINE OSTERTAGIASIS

PART 2 BOVINE PARASITIC GASTROENTERITIS IN SOUTH-WEST SCOTLAND

PART 3 A DETAILED STUDY OF NATURALLY OCCURRING BOVINE OSTERTAGIASIS

PART 1 - A REVIEW OF PREVIOUSLY REPORTED OUTBREAKS OF BOVINE OSTERTAGIASIS

Abomasal parasites of the genera Haemonchus, Ostertagia and Trichostrongylus and the intestinal parasites, Cooperia and Oesophagostomum are considered to be major pathogens associated with clinical bovine parasitic gastro-enteritis. Despite the fact that parasitic gastroenteritis of young bovines is common in the experience of veterinarians and farmers alike, it is surprising that so little information on this subject is available.

Incidence of the parasite O.ostertagi

The genus Ostertagia has a world wide distribution Skrabjin (1954) and in some areas the incidence of the parasite in cattle populations has been determined. Roberts (1939) found that 72.2 per cent of 237 apparently healthy cattle of varying age, slaughtered in a Queensland abattoir were infected with O. ostertagi. Of these the majority, 72.8 per cent, had less than 1,000 worms, a further 25 per cent had 1,000 to 5,000 and only 2.2 per cent were infected with numbers between 5,000 and 7,500. A similar type of study was carried out in South Eastern U.S.A. by Porter (1942): of 74 young bovines aged between 1 and 18 months he found that O.ostertagi was present in small to moderate numbers (5 to 4,000), in 74 per cent of these animals. Bell (1957), in a slaughterhouse survey of 180 cattle from

three areas in North Carolina, showed that O.ostertagi infected 70 to 80 per cent of all cattle, in numbers averaging about 2,000. More yearlings than adults or calves were infected with heavy populations.

Another abattoir survey of 29 cattle, 6 to 18 months of age, was carried out in the coastal plain region of Georgia, U.S.A., by Becklund (1959); the results were similar to those of Porter (1942) in that 72 per cent of the animals were infected with O.ostertagi, the numbers of worms ranging between 6 and 880.

Results from a sample of 30 abomasa examined at an Ayrshire knackery (Martin, Thomas and Urquhart (1957)) showed that neither worms nor lesions were present in 13 of these abomasa. From this investigation it was suggested that from 5,000 to 25,000 Ostertagia were required to produce mild abomasal change, whereas a number greater than 25,000 produced severe abomasal damage.

The seasonal incidence of O.ostertagi infection, between March 1963 and February, 1964, was assessed by Ross (1965) who collected abomasa and abomasal contents from 189 cattle aged 6 months to 2 years at a knackery in Northern Ireland. The cause of death of these animals was not determined but 34 per cent of them had abomasal lesions; 16 per cent had high worm burdens which, according to the author, were likely to be associated with clinical ostertagiasis. Peak numbers of O.ostertagi (10,000 to 40,000) were observed twice during the year, the worm population of the autumn peak

showed a preponderance of adult papasites whereas that of the winter peak, was mainly composed of immature stages. Before the winter peak and in the early spring, worm burdens consisted of equal numbers of immature and mature stages. Parasite numbers were low during the summer (2,000 to 9,000) and very few were immature.

The occurrence of clinical bovine ostertagiasis

The occurrence of clinical parasitic gastroenteritis affecting bovines has not been determined in any geographical area. Epizootics of clinical disease primarily caused by O.ostertagi have been reported sporadically, and most of those occurred either in North America or in Britain. It is proposed to discuss separately the disease as it has been observed in these two regions.

Ostertagiasis in North America

Reports of epizootic gastrointestinal parasitism caused by Ostertagia spp. affecting cattle in the U.S.A., have been produced by Stiles (1900); Ackert and Muldoon (1920); Baker (1937); Threlkeld and Bell (1950); Andrews, Sippel and Jones (1953); Bailey (1953 and 1954); Becklund (1962) and in Canada by Smith and Jones (1962).

Andrews et al. (1953), who worked in Georgia, U.S.A., recorded that clinical parasitism had increased during the period 1950 to 1953 and that it accounted for 17 per cent of the post mortem material presented to them. More recent examinations made in Georgia showed that between 1956 and

1957 approximately 25 per cent of the cattle received for autopsy had suffered from clinical parasitism, Becklund (1959). No attempt was made to determine accurately the species or numbers of parasites involved, but the above authors stressed the absence of large numbers of nematode eggs in the faeces of the majority of these cattle and concluded that in 80 per cent of cases the faecal egg count was of no diagnostic value.

A clinical and economic study of helminthiasis in cattle from Georgia was made by Becklund (1962). O.ostertagi was considered to be the most pathogenic parasite encountered. The economic loss was calculated from the morbidity, mortality and the number of anthelmintic medications, which occurred on 24 farms involving 8,000 cattle, and this loss ranged from 56 to 5,240 dollars per farm over a relatively short period of a few months. A 9 per cent morbidity accounted for a little over a third of the entire cost of clinical parasitism; a 3.4 per cent mortality almost two thirds, while the cost of treatment was between 7 per cent and 8 per cent of the total cost. This assessment was considered very conservative because further losses occurred after the survey period ended: therefore costs for special feeding, involving additional labour during the outbreak, were not included.

After considering the American reports, it appears that under their systems of cattle management, outbreaks of disease fall into two main categories, namely as:

(i) endemic outbreaks which affect young cattle 4 to 12 months of age; and

(ii) outbreaks affecting mature cattle, which have been brought into endemic areas from regions where O.ostertagi infection appears to be negligible or absent.

In either case, the clinical signs of the disease follow the same pattern.

Signs of the disease first became apparent during the spring and continued well into the summer. Losses, presumably by death, of up to 40 per cent were recorded from some outbreaks. The reports available, were from outbreaks of disease in animals, which were not housed during the winter. Unthriftiness, caused by loss of weight, severe diarrhoea (often with signs of dehydration), emaciation and anaemia in some cases, were the most frequently observed clinical characteristics of the disease. Submandibular and brisket oedema were noted in very severely affected animals. Temperature, pulse rate and respiratory rate were not altered in the majority of cases. Even seriously ill animals continued eating until they died, but inappetence was common among affected cattle. Thirst was evident in those cases suffering from severe diarrhoea, affected animals survived approximately a week after the onset of diarrhoea. Weakness, emaciation, anaemia and diarrhoea were terminal features of the disease.

Autopsies were marked by emaciation, anaemia and dehydration were evident. The abomasal mucosa was pale, sometimes oedematous and often completely covered with greyish white nodules, varying in size from a pinhead to a pea. These nodules or pustules were superficial and easily ruptured when squeezed. Erosion of the mucosa was another constant feature.

Bailey (1953) gives a brief description of the histopathology. Many of the glandular crypts were dilated and tortuous and these contained developing stages of the parasite. Other glands were empty or contained debris which consisted principally of polynuclear and mononuclear leucocytes and necrotic cells. Scattered glands were lined by unspecialised epithelial cells, which were columnar, cuboidal or flattened in form. The small pustules noted grossly, appeared as micro-abscesses in the lamina propria and in some sections the remains of dead parasites could be seen. There was a moderate amount of cell infiltration which consisted mainly of heterophil granulocytes and large mononuclear cells. Marked oedema of the submucosa was also present in some cases.

Very large numbers of O.ostertagi were found at post mortem examination; where estimates of these numbers were given, figures of 30,000 to 62,000 and of 100,000 to 1,000,000 O.ostertagi were said to have been recovered from yearlings and mature cattle respectively. Most of the parasites were

found free in the luminal contents: some were seen protruding from the nodules. In adult cattle it was frequently found that high percentages of the total worm burden were immature parasites. These immature stages were recovered from pepsin - hydrochloric acid digests of the abomasal mucosa.

Osterbagiasis in Britain

Gardener (1911) reported parasitic gastritis in young cattle from different localities in South Eastern England during the years 1907 and 1909. In the North of England parasitic gastritis in bovines was exceedingly prevalent during 1940 and 1941 (Stewart and Crofton 1941). These outbreaks were most commonly in winter and early spring when young cattle, 1 to 2 years old, showed diarrhoea, wasting and anaemia. In some districts up to 50 per cent of the young cattle were affected and of that figure up to 90 per cent died. The greatest number of losses were seen during the winter months when the cattle were stall fed on a very low plane of nutrition. Several species of parasite were found but the authors attributed the pathogenicity to Trichostrongylus axei which measured 2.5 to 4.2 mm. in length. The abomasal mucosa was markedly thickend, roughened and inflamed. Worm counts, mucosal digests and histopathological examinations were not carried out on these cases.

Bruford and Fincham (1945) were of the opinion that, in 1945, advanced clinical parasitic gastroenteritis was not widespread among calves or young cattle in Britain, although

many farms had a proportion of unthrifty animals. The low nematode egg counts found by these workers were attributed to subclinical infection and they considered counts over 600 eggs per gram to be associated with heavy infection. These workers carried out a phenothiazine trial involving forty 9 to 12 months old beef cattle, and during this trial, clinical parasitic gastroenteritis was first seen 5 weeks after being put on to old permanent pasture. Seven of the animals died between August and November and worm counts revealed 10,000 to 390,000 O.ostertagi and Trichostrongylus spp., up to 50 per cent of which were in the immature stages of development.

A survey of livestock diseases was carried out in Northern Ireland during 1954 and 1955 by Gracey (1960). Of the 2 per cent total mortality in heifers and steers, parasitic gastroenteritis accounted for 5 per cent of these deaths with another 5 per cent due to enteritis of unspecified aetiology. Most losses occurred during the months of July, August, October and November.

During the spring and early summer of 1956, ten outbreaks of severe chronic diarrhoea and emaciation, affecting 12-18 months old dairy cattle on farms in the West and South of Scotland, were investigated by Martin, Thomas and Urquhart (1957). The outstanding feature of those outbreaks was that the animals were apparently healthy when housed in the November to December period and that 3 to 10 weeks later,

severe diarrhoea and weight loss was observed. Mild anaemia and marked hypoproteinaemia was noted. Post mortem revealed that large numbers, from 20,000 to 250,000 of O.ostertagi were recovered from the abomasum: up to 60 per cent of the total worm burden was present in the immature stages of development. A detailed description of the gross and histopathological change of the abomasal mucosa was given. The authors stated that the disease was not confined to the South West of Scotland, because similar clinical histories and abomasal specimens were received from other parts of Scotland and England during the period.

Generally, these reports indicate that, in Britain, parasitic gastroenteritis affects only young cattle up to 2 years of age, that losses from the clinical disease are more commonly associated with housed cattle and that the predominant species of parasite is O.ostertagi.

Factors considered important in the epizootiology of O. Ostertagi Infections

Case histories taken by the American authors, while investigating outbreaks of clinical disease, and facts gained from epizootiological studies in Georgia indicate that certain management practices favour the development of the clinical disease.

Overcrowding and overgrazing of cattle on small areas of pasture were two such factors which have received specific attention.

On 10 farms, investigated by Andrews et al (1953), the sole source of drinking water was ponds, and pastures around those had a high degree of faecal contamination. On farms investigated by Becklund (1962), the majority of the cattle had been maintained on the same pasture for many months or several years at a stocking rate of one animal to two acres. Both of these authors considered that overstocking and overgrazing were important in the development of clinical disease.

In Georgia, U.S.A., a series of investigations was carried out over a period of 11 years by Ciordia and his co-workers, with the aim of understanding the interrelationship between grazing management, nutritional level, climate and degree of parasitism resulting from these factors. Ciordia, Bizzell, Vegors, Baird, McCampbell and Sell (1962a); Ciordia, Bizzell, Baird, McCampbell, Vegors and Sell (1962b) and Ciordia, Bizzell, Baird, McCampbell and White (1964).

Earlier, Vegors, Sell, Baird and Stewart (1955) grazed groups of naturally infected yearling Hereford Steers on three different types of winter pasture. This pasture had not been grazed for the previous two years and was considered to be Worm free. The main conclusions of this study were:

(a) that the better nutritive value of some pastures protected the yearlings against the effects of

parasitism but did not reduce the parasite populations as judged by post mortem worm counts;

(b) that supplementary feeding of yearlings with bruised maize resulted in the establishment of fewer parasites when compared with yearlings whose diet was not supplemented. Similar findings were reported by Ciordia et al (1962b); and

(c) that the difference between the maize fed and non-maize fed groups was associated with a specific effect of maize feeding rather than with a higher plane of nutrition.

With regard to the latter point, it is interesting to note the studies of Ciordia and Bizzell (1963) in which it was shown that grain feeding had a marked depressive effect on the number of Trichostrongylus axei eggs which developed in faecal culture.

In the experience of Vegors et al (1955), O.ostertagi was the parasite most commonly found at post mortem examination and diarrhoea and emaciation were recorded in some groups.

Their reliance on monthly nematode egg counts from an unspecified number of animals and the meagre post mortem information, 2 or 3 animals from each group, detracts from the value of these authors' conclusions.

A similar experimental design was used by Ciordia and his co-workers to evaluate the specific effect on the degree of parasitism which resulted in groups of Hereford steers maintained:

(a) at various stocking rates:

(b) on pastures of different composition and nutritive value; and

(c) under a system of rotational grazing.

No definite conclusions resulted from this work because the gross variation between seasons in different years, together with the small group size used, tended to obscure any real effect between groups given different treatments.

However, an interesting discussion of the problems associated with this type of investigation has been presented by Ciordia et al (1962a). In particular, the observations of Smith (1959) are cited as being relevant in that, when herbage is short and scarce, cattle spend more time grazing and graze over a larger area to consume the required amount of feed. On pastures which have a high larval contamination increased numbers of larvae would be ingested. On the other hand, it has been shown that short pasture exposes larvae to adverse climatic conditions which result in a higher larval mortality (Bell, Galvin and Turk, 1960; Durie, 1962).

PART 2

BOVINE PARASITIC GASTROENTERITIS

IN

SOUTH-WEST SCOTLAND

BOVINE PARASITIC GASTROENTERITIS IN SOUTH-WEST SCOTLAND.

INTRODUCTION

Following a general consideration of the reports which describe enzootic bovine ostertagiasis in Britain and North America, it can be concluded that in these regions at least a large percentage of the cattle population are infected with Ostertagia ostertagi and that in some years this parasite causes disease which seriously affects beef and dairy production.

The reports describe the clinical syndrome, record the autopsy appearance of diseased animals and draw attention to the difficulty in making a diagnosis and in applying effective treatment. Diagnostically the difficulties arise from three main sources, namely, (1) that the clinical signs of diarrhoea, loss of weight and illthrift are not characteristic and are shared by a number of disease syndromes, (2) that clinically affected animals frequently show low nematode egg counts in their faeces despite the presence of large numbers of parasites and (3) that the disease can appear in housed cattle at a time when larval ingestion is unlikely. As yet improved diagnostic procedures are not available.

During the study of the outbreaks reported, little attention had been directed towards making an estimate of the minimum number of O.ostertagi necessary to produce clinical

disease in cattle or towards assessing the effects of clinical infections on the host.

Large numbers of immature parasites have been recorded from animals autopsied during outbreaks of bovine ostertagiasis but, apart from speculation, the evidence is meagre regarding the significance of these stages in relation to the epizootiology and pathogenesis of the disease.

In this section of the thesis, the results are given of an investigation into the nature and causes of bovine parasitic gastroenteritis in the South-west of Scotland. The object of this field study was to define more clearly the disease syndrome as seen in the field and to assess the significance of the inhibited larval stages.

MATERIALS AND METHODS

Procedure in outbreaks

Outbreaks of parasitic gastroenteritis in young dairy cattle were reported to the Medicine Department, University of Glasgow Veterinary Hospital by veterinary surgeons practising in South-west Scotland. All affected farms were visited and a detailed history obtained from the farmers. Where possible, affected animals were purchased and hospitalised for detailed examination prior to autopsy. In some outbreaks, animals which were not clinically affected and which had a grazing history similar to affected ones were also purchased.

A summary of calf husbandry in South-West Scotland

In the South-west of Scotland calves are born from Autumn onwards to the early Spring, reared indoors until they are 4 to 6 months old and are first put out to graze early in May, usually into a permanent calf field close to the house to facilitate supplementary feeding. The quality of the pasture in this calf field varies from very old weed infested grass to newly sown rye grass clover mixtures. The time spent in the field ranges from 6 weeks to 6 months depending on the size of the field, availability of grass and general farm management. A common practice is to move the young calves onto hay aftermath in July or August and often signs of parasitism appear 2 to 3 weeks following this move. Sometimes the calves are returned to the permanent calf field in the late autumn and supplementary feeding, usually inadequate in amounts, may be given when grass becomes scarce. When the weather is mild this period of grazing is frequently prolonged. Towards the end of November or the beginning of December the calves are taken indoors for winter feeding. A general tendency of dairy farmers is to allocate the minimum of grazing area to the rearing of their replacement stock and this practice may lead to overstocking and over-grazing. Spring born calves are often put out with older, autumn born calves and are frequently either the first or the most severely affected calves. Older animals, calving heifers and cows, are not necessarily excluded from the permanent calf field.

A few farmers allow their stock to graze during the day throughout the winter period. Generally all animals are housed at night during winter.

Laboratory Techniques

Together with clinical examinations, the following observations were made prior to autopsy:
Faecal egg counts; packed cell volume; haemoglobin concentration; red blood cell counts; total serum protein; albumin/globulin ratio and plasma pepsinogen concentration.

The methods used to make these observations have been set out in Section 1.

At autopsy a full pathological and parasitological examination was carried out and the abomasal contents were collected for pH and electrolyte determinations. The procedure and methods have been detailed in Section 1.

RESULTS

Classification of the Disease

It soon became apparent from the autopsy data that O.ostertagi was the predominating nematode found and for all practical purposes bovine parasitic gastroenteritis in this region of Scotland could be considered synonymous with bovine Ostertagiasis.

From the observations made in this study, bovine ostertagiasis could be classified into three categories, two of which (Type I and Type II) are clinically apparent. The terminology (Type I and Type II) is preferable to the words acute, chronic, typical, atypical and so on, since, for example, both Type I and Type II can show acute clinical phases.

The three phases of ostertagiasis are defined as follows:

Type I

Type I corresponds to the classical description of parasitic gastroenteritis in which calves show a loss of weight and diarrhoea during their first summer of grazing; this may occur from late July until the end of the grazing season in October or early November. The vast majority of the ingested larvae develop to maturity after a normal pre-patent period of 17 to 21 days.

Pre Type II or the stage of inhibition

In this phase, which precedes Type II, large populations

of O.ostertagi are present, of which over 80 per cent are inhibited at the early 4th stage of development in the gastric mucosa. These calves have grazed infected pasture in the late autumn but have no history of diarrhoea and usually appear healthy to the farmer.

Type II

The second clinical phase is different to the first (Type I) in that calves, which have no history of diarrhoea or weight loss during the grazing season and which are well grown and in excellent condition, are taken indoors about the beginning of November. After a variable period of time, ranging from 3 weeks to 4 months, these animals start to lose weight and show a profuse, watery diarrhoea. The appearance of the clinical signs coincides with the development to maturity and emergence from the mucosa of large numbers of inhibited larvae. This syndrome was first described by Martin, Thomas and Urquhart (1957) who regarded it as "atypical" but it is now recognised to be a common occurrence. The clinical signs of weight loss and diarrhoea, the latter being either continuous or intermittent, are present in both syndromes so the main epizootiological difference between the two types is the time of the year when the disease is first seen.

The Type I Syndrome

On nine farms where Type I disease occurred 72.2 per cent of 119 calves at risk were affected, i.e. showing clinical signs and 24.3 per cent, i.e. 29 calves died of the

disease. This high figure for mortality is due to two farms which had a 50 per cent and 100 per cent mortality respectively. The exclusion of these two farms lowers the mortality rate to the less dramatic value of 7.5 per cent.

Two important epizootiological factors associated with the outbreaks were (a) overstocking and (b) the permanent calf paddock. Grass was scarce in calf paddocks during August and calves were frequently moved onto aftermath at this time. This more often coincided with or immediately preceded the onset of clinical signs. The youngest calves, i.e. those born in the spring were usually the first and the most severely affected of the group.

Cases presented as dead animals on farms were usually too decomposed to provide useful pathological or parasitological material. In addition, owing to the widespread use of apparently effective anthelminthics, both curatively and prophylactically, no firm conclusions could be reached regarding the correlation between numbers and stages of parasites causing the clinical signs or the associated physiological and pathological changes. Most of the deaths occurred prior to the use of anthelminthics, but farmers who had losses were very reluctant to sell the calves which were clinically affected.

For these reasons it was decided to make a separate study of the disease under natural conditions. The details of this study are reported later in Part 3, but it is

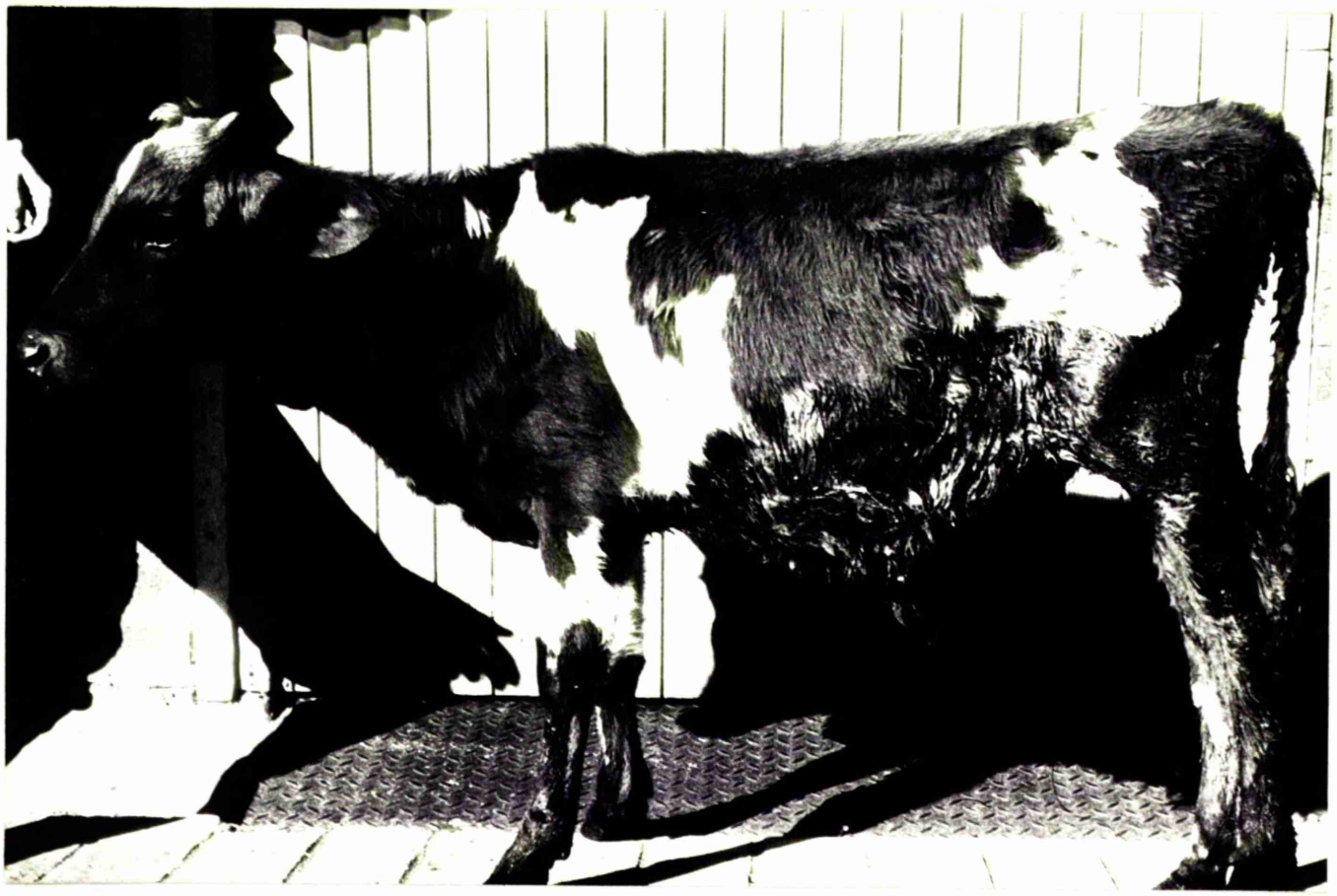


Fig. 1. A photograph of a 4 to 5 months old calf severely affected with Type I Ostertagiasis.

considered pertinent to include here a description of the Type I disease.

Ten parasite free Ayrshire calves, 2½ months old were grazed in the calf paddocks on each of two farms, which were mentioned above to have had a calf mortality of 50 and 100 per cent during the previous year.

Clinical disease first occurred on Farm A in the 3rd week of July i.e. after 9 weeks of grazing and on Farm B during the 1st week of September i.e. after 16 weeks of grazing. On Farm A the 10 calves had been killed ~~in extremis~~ by the end of the grazing season in late October, while only 2 calves survived until late October on Farm B.

Twice weekly visits were made to both farms and severely affected calves were removed for autopsy. Clinically affected animals were dull, had harsh staring coats, were stunted in size and characteristically showed a bright green diarrhoea which stained the tail, hocks and perineal region. (see figure 1). Weight loss, inappetence and less frequently, increased thirst and anorexia, were prominent clinical signs. As the duration of diarrhoea increased the eyes became sunken and weight loss was marked. These animals became progressively weaker until they were unable to stand.

From the analysis of biweekly faecal egg counts three points emerged:

a) Egg counts of less than 1,000 e.p.g. were commonly seen in calves suffering from mild to severe Type I disease

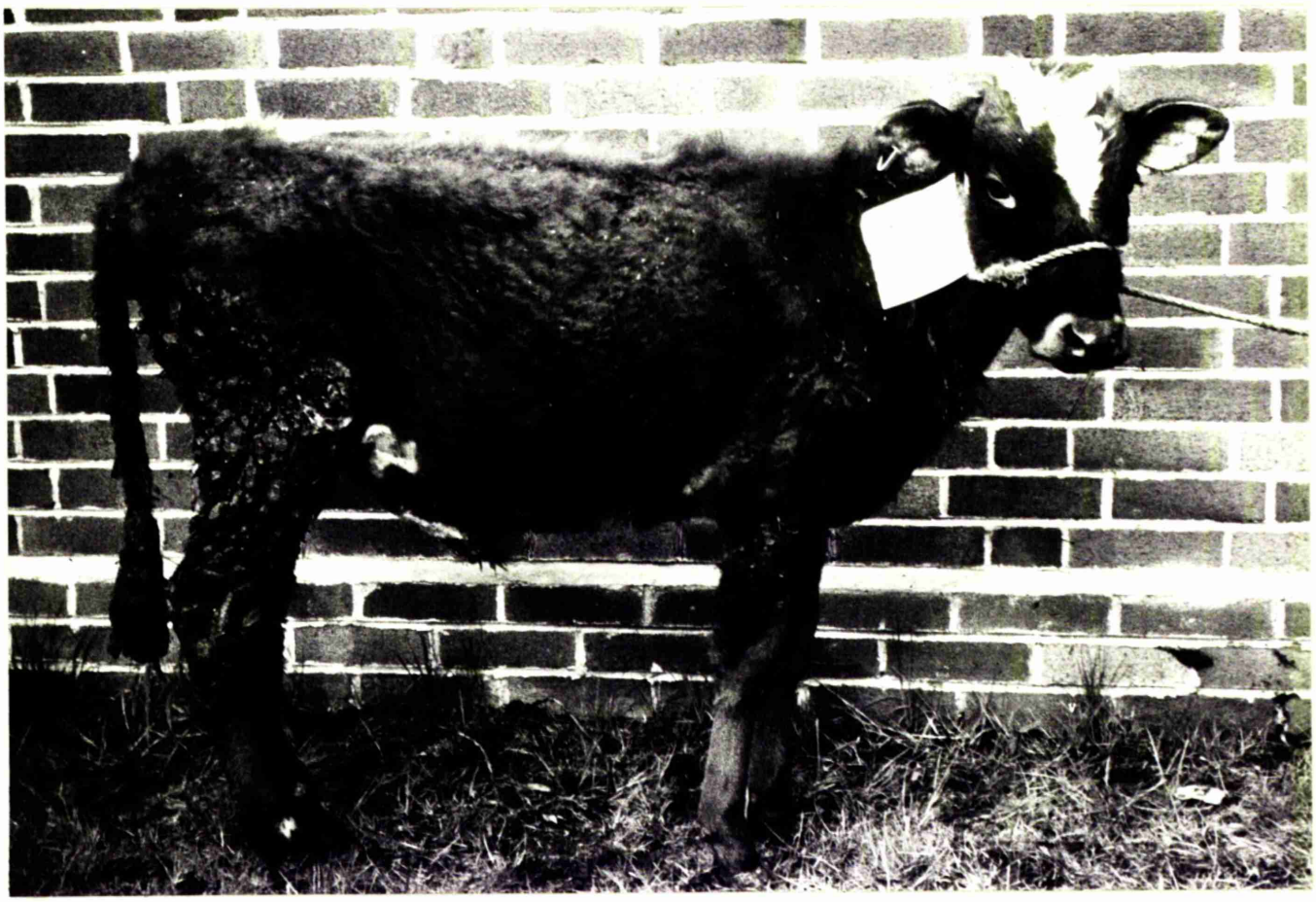


Fig. 2. A photograph of a 12 months old calf affected with Type II Ostertagiasis.

TABLE 2

The Grazing History, Weight Gains and Parasitological Data from Ten Typical Cases of Type I Ostertagiasis

	Calf No.	Initial Weight	No. of Days Grazed	Weight Gain Lb/Day[x]	Post-Mortem Worm Counts: O.Ostertagi Total	% early 4th stage	T.axei	C.oncophera and C.Momasteri	N.helvetianus and N.spathiger
FARM A	A1	144	71	0.7	64,000	4	1,000	9,300	10,400
	A2	112	71	0.2	51,500	6	5,600	2,500	2,200
	A3	168	71	0.6	43,100	5	300	3,400	1,500
	A4	124	98	0.2	84,300	9	0	5,200	4,400
	A5	140	98	0.8	84,900	9	2,400	7,900	2,800
FARM B	B1	126	122	0.8	61,800	33	0	26,200	3,100
	B2	126	122	0.8	111,200	18	0	1,300	0
	B3	109	122	0.8	81,100	12	0	27,500	3,800
	B4	100	122	0.7	89,600	16	0	22,500	3,300
	B5	116	122	1.0	79,700	6	0	18,700	600

x At the West of Scotland Agricultural College, Ayrshire, heifer calves at grass, aged 3 to 6 months, gain 1.33 lb. per day (Walker-Love, personal communication.)

The Cooperia species were C.oncophora and C.McMasteri. A few Trichostrongylus axei, Nematodirus helvetianus and N.spathiger were also found in these post mortems.

TABLE 3

Biochemical Data From Type I, Pre Type II and Type II Cases of Bovine Ostertaglasis

Mean Values and Standard Errors.

	Plasma Pepsinogen ug phenol/ml *	Abomasal Contents at Autopsy			
		pH	Na^+	K^+	Cl^-
			milli equivalents/litre		
Type 1	76.5 ± 4.5	6.2 ±0.3	107 ±4.2	15.1 ±1.3	112 ±2.0
Inhibition or Pre-Type II	29.40 ± 1.7	3.3 ±1.0	70 ±3.2	17.8 ±0.9	122 ±2.1
Type II	106.60 ±11.4	5.7 ±0.2	99 ±8.0	16.3 ±2.4	107 ±3.6
Uninfected Controls	8.60 ± .2	2.4 ±0.1	66 ±3.3	19.0 ±9.9	120 ±2.0

* ug phenol per ml per 24 hours at 37°C.

and also from apparently healthy calves grazing the same pasture.

b) All calves with egg counts of over 1,000 e.p.g. had shown clinical signs. Egg counts in clinically affected calves fluctuated markedly both above and below the 1,000 e.p.g. level.

c) At autopsy there was no correlation between the faecal egg count and the number of female worms present.

The differential worm counts at the post mortem examination of 10 calves affected with Type I disease are shown in Table 2, together with details of weight gains during the grazing period.

Anaemia was not detected in calves which developed the Type I syndrome. Increases of up to 30 per cent in packed cell volume, with corresponding increases in haemoglobin concentration and red blood cell counts were observed in calves which had severe diarrhoea over a period of 3 to 4 days. No significant changes in total serum proteins, albumin concentration or A/G ratios were noted.

Calves severely affected with the Type I syndrome had, at autopsy, marked increases in the pH of the abomasal contents together with an increase in the Na^+ ion and a decrease in K^+ and Cl^- ion concentrations.

The concentration of plasma pepsinogen was significantly increased in calves with Type I ostertagiasis. A summary of these biochemical changes is given in Table 3.

Fig. 3. Macroscopic appearance of the primary nodule.

Fig. 4. Microscopic appearance of the primary nodule.

A description of the main pathological lesions of Type I O.ostertagi infections.

Five main types of lesion were seen in Type I cases of bovine ostertagiasis. These lesions have all been produced experimentally and their sequential development will be described in a later section.

The five types of lesion are:

1. Nodules.
2. Morocco leather like appearance of mucosal thickening.
3. Submucosal and omental oedema.
4. Epithelial cytolysis.
5. Congestion.

Nodules

The mucosal nodules are raised, circumscribed, umbilicated, translucent areas of 1 to 4 mms in diameter each containing a central orifice. The majority of nodules are about 2 mms. in diameter (Fig. 3). This is the primary lesion and the central orifice is the gland originally dilated by the presence of a larva (Fig. 4). The distribution of nodules in the abomasum follows no clear cut pattern but almost invariably the fundic region contains more nodules per square inch than the pyloric region. In the fundic area nodule aggregates are often seen along the greater curvature towards the junction of the fundic and pyloric regions. Sometimes the nodule concentration is so

Fig. 5. Hyperplastic mucous membrane showing the "morocco leather" like appearance.

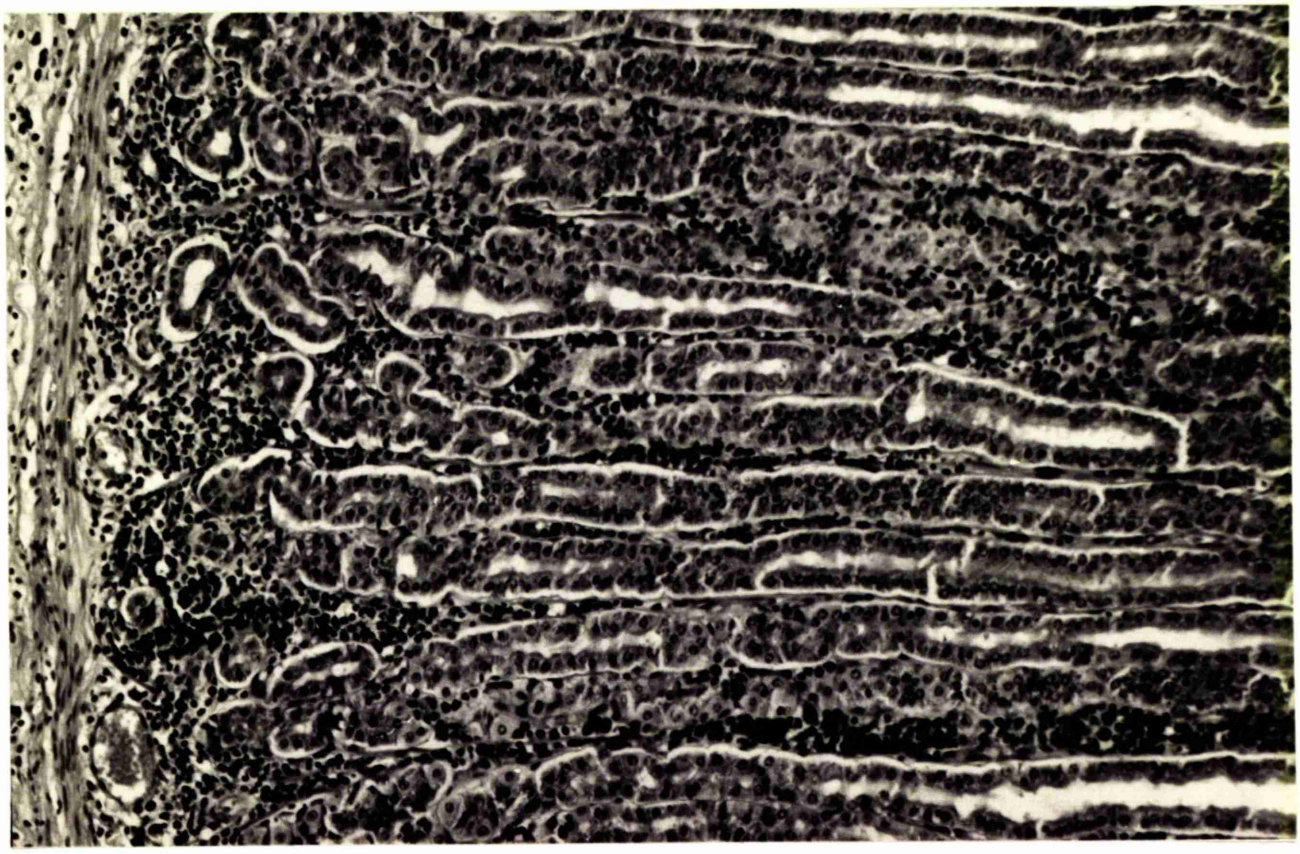


Fig. 6. Microscopic appearance of Fig. 5. showing hyperplastic glands lined by undifferentiated cells.

great that the distinct nodular appearance is obscured, and differentiation from the morocco leather-like lesion becomes difficult.

Nodally, the nodule is a dilated, but intact, gastric gland lined by cuboidal or mucus secreting epithelium instead of the usual differentiated cell types. In the normal prepatent stage it contains a developing larva; the lesion persists after the emergence of the young adult.

Morocco Leather-like Appearance of the Thickened Mucosa.

The morocco leather-like appearance is a feature of heavy infections (Fig. 5). During the development of the lesion after the emergence of the young adult has taken place many of the glands around the one which had contained a larva, become lined with a hyperplastic undifferentiated cell sheet (Fig. 6). When the level of infection is high each nodule component of the lesion is closely approximate to one another and this produced the thickened and fissured mucosa which looks like morocco leather. The distribution of this lesion is also irregular in that in some cases it may be confined to localised areas on the abomasal folds while in other cases the entire fundic and pyloric mucosa may be involved.

Submucosal and Omental Oedema

Oedema, when present, is found in two sites; (a) between the layers of the omentum supporting the abomasum, (b) in the submucosa of all parts of the abomasal

Fig. 7. "Morocco leather" appearance of the abomasal folds and the gross submucosal oedema of the folds.

Fig. 8. The "thumbprint" lesion caused by localised severe superficial erosion of the mucosa.

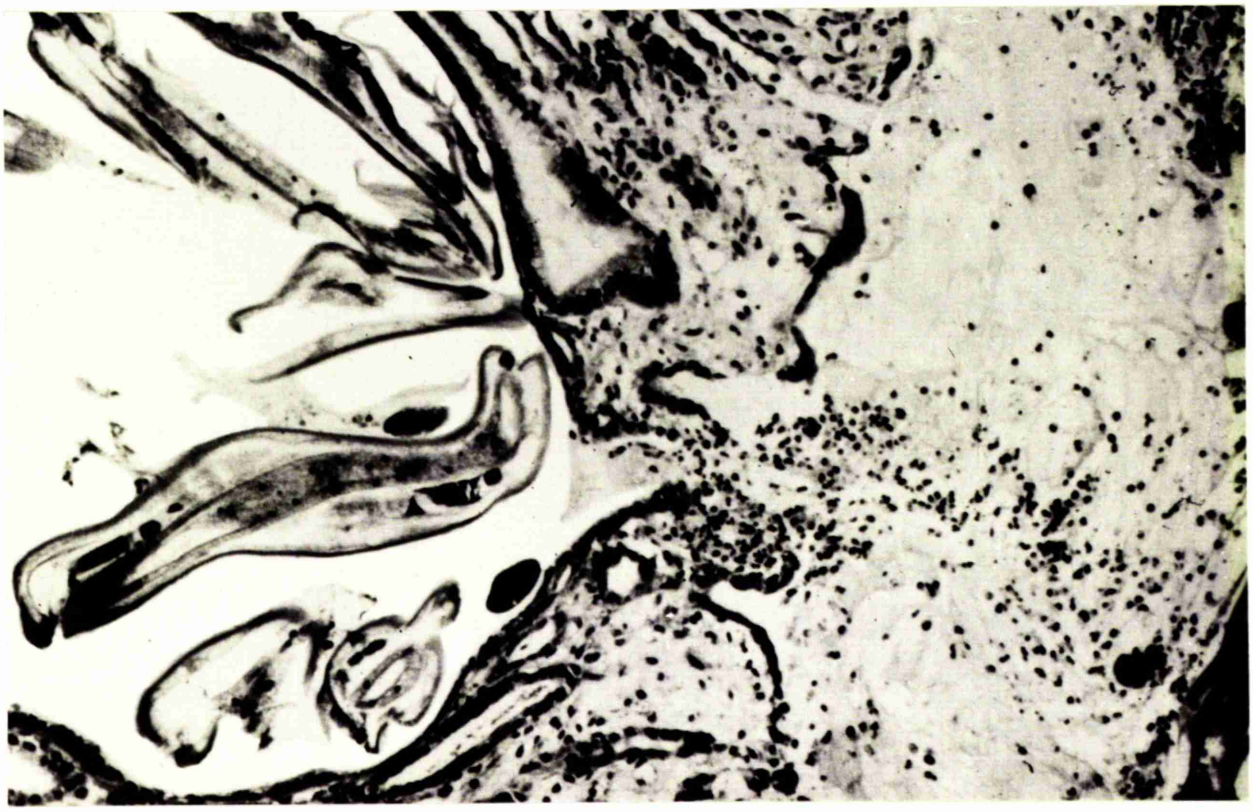


Fig. 9. Worms emerging from the gland and worms in the overlying inflammatory exudate; severe damage to the surface epithelium.

mucous membrane. Occasionally the oedema within the omentum is so severe that 300 to 600 mls. of oedema fluid can be expressed. The degree of oedema in the abomasal folds is such that at times these are enlarged up to 5 times normal size (Fig. 7). Localised oedema occurs involving a part or a whole abomasal fold; the remaining folds show no evidence of oedema. Generalised oedema of the whole abomasal mucosa also occurs.

Epithelial Cytolysis

Circumscribed depressed, congested, irregular areas in the mucous membrane, rather resembling "thumb prints", are common in field cases (Fig. 8). These areas are associated with an increased degree of epithelial sloughing, which if severe results in a gross diptheritic appearance of the abomasal mucosa (Fig. 9).

Congestion

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

Pre Type II: The Stage of Inhibition

This phase of bovine ostertagiasis is not clinically apparent, but is essential for the subsequent development of the Type II Syndrome.

The term inhibition describes the arrested development of O.ostertagi in the early 4th stage. Total worm counts from cases of Type II ostertagiasis often had large percentages (40 to 90) of early 4th stage parasites whereas in cases of Type I these percentages were small (2 to 30).

From a consideration of field cases, it became evident that the presence of large numbers of inhibited larvae were important in the understanding of the Type II syndrome. During the course of an investigation of the Type II syndrome in two affected herds two apparently healthy calves were autopsied.

Both of these calves had been indoors, for a period of 140 days in one case and 28 days in the other. Neither calf had been given anthelminthic treatment.

The total worm burdens of these calves were 67,000 and 55,000 with 89 and 96 per cent respectively, of the population present as inhibited larvae.

To obtain further information on the significance of these large numbers of inhibited larvae a total of 18 calves were purchased from four farms where outbreaks of Type II ostertagiasis had occurred. None of these calves had a history of diarrhoea and their general condition was

TABLE 4

The Clinical History and Parasitological Data from Ten Typical Cases of Pre Type II Ostertagiasis

Case No.	Age in Months	Month Housed	Month Killed	History of Diarrhoea	Anthelmintics since Housing	Weight at P.M. in Kg.	Faecal Egg Count at P.M.	Post-Mortem Data: O.Ostertagi Total	Post-Mortem Data: % Early 4th Stage	Post-Mortem Data: Cooperia Species	Post-Mortem Data: % Early 4th Sta
D1	12	Nov	March	Nil	Thibenzole & Promintic	410	250	66,900	89	5,400	44
D2	9	Oct.	Jan.	Nil	Mintic	234	250	103,400	95	5,100	39
D3	12	Oct.	Feb.	Nil	Mintic	314	-ve	128,700	85	1,400	57
D4	12	Oct.	Feb.	Nil	Mintic	327	50	140,800	98	8,600	46
D5	12	Oct.	Feb.	Nil	Mintic	263	50	142,900	89	12,800	49
D6	12	Oct.	Feb.	Nil	Mintic	267	250	87,500	93	8,600	53
D7	12	Oct.	Feb.	Nil	Mintic	225	250	150,200	90	6,300	50
D8	12	Oct.	Feb.	Nil.	Mintic	266	50	117,100	97	5,100	21
D9	14	Oct.	March	Nil	Mintic & Thibenzole	344	-ve	159,400	98	0	0
D10	14	Oct.	March	Nil	Mintic & Thibenzole	293	-ve	141,100	93	6,000	30

Thibenzole = Thiabendazole (Merck Sharp & Dohme)

Promintic & Mintic = Methyridine (I.C.I.)

N.B. The *Cooperia* spp. were *C.oncophera* and *C.Mcmasteri*

A few *T.axei*, *N.helvetianus* & *N.spathiger* were found in some calves.

TABLE 5

A Comparison of the Length of Inhibited and Early Fourth Stage O.ostertagi Larvae from Naturally and Experimentally Infected Calves.

Source	Number Measured	Length in mm Mean & SD	Students' "t" Test *
Knockendale	50	1.28 ± 0.08	P = NS
Laight Mains	30	1.32 ± 0.08	P = NS
Bracken Hill	30	1.30 ± 0.03	P = NS
Auchnamoch	30	1.34 ± 0.07	P = NS
Experiment I	49	1.39 ± 0.07	
Douvres (1956)	10	1.18 to 1.29	

* The values obtained from animals on each of the farms were compared with 4 day old early 4th stage larvae from calves which had been experimentally infected with 100,000 O.ostertagi larvae.

P = Probability
NS = Not significant
SD = Standard deviation

representative of young Ayrshire cattle in late autumn and winter. Some calves were well grown while others were up to 100 lbs below the normal weight for their age. These calves had been grazing the same fields as the calves which developed severe Type II ostertagiasis and all calves had been treated with anthelminthics during the preceding two months. The association of inhibition and late autumn grazing will be shown in a later section. The clinical history and parasitological data of ten of these calves, summarised in Table 4, show that the total worm counts were high, the numbers ranging from 67,000 to 159,000 and the percentage of early 4th stages varied from 89 to 98 per cent. Also it will be noted that a high percentage of Cooperia spp. larval stages were present at this time.

Generally, the faecal worm egg counts were low, less than 500 e.p.g.; many samples were negative and gave no indication of the large numbers of parasites present.

The early fourth stages found in the abomasal digests of several calves from different farms were measured and compared with measurements of early fourth stage larvae recovered 4 days after a single experimental infection of 100,000 O.ostertagi. The results of these measurements are shown in Table 5. It is apparent that there is no difference between 4 day old early 4th stage larvae and those which may have been present in the abomasal mucosa for as long as 6 months. The measurements shown in Table 5 compare well with results obtained

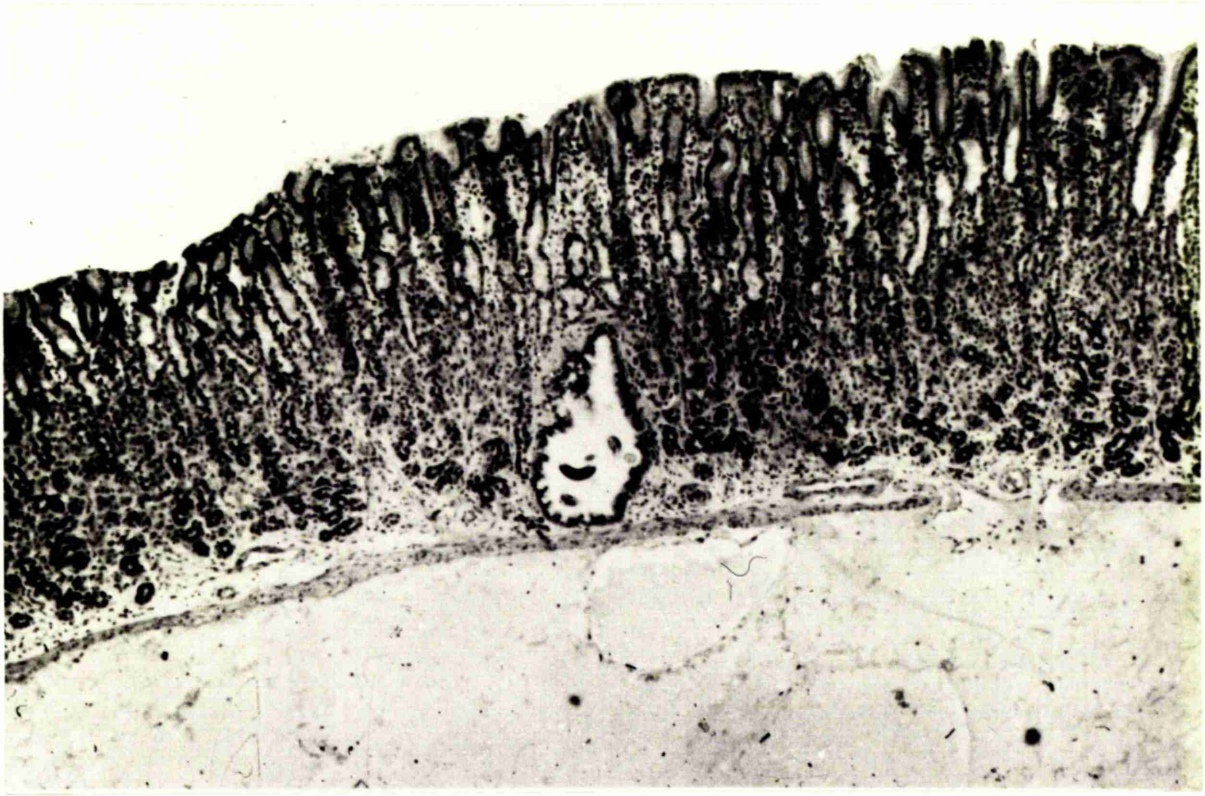


Fig.10. Inhibited larva in gland; surrounding mucosa virtually normal.

by Douvres (1956). However, the larvae are longer than the 4 day old larvae examined by Threlkeld (1946), which measured 0.95 mm but they are of similar length to the 5 day old larvae (1.4mm) measured by Threlkeld, who recognised only two larval ecdyses in O.ostertagi. Douvres (1956) reported three larval ecdyses in the parasitic phase of O.ostertagi.

The results of the biochemical determinations have been recorded in Table 3. The pH of the abomasal contents was relatively normal in all cases and the ionic concentrations of sodium, potassium and chloride in the gastric contents were not markedly altered. In all cases the plasma pepsinogen levels were moderately elevated.

The striking pathological feature of cases which showed virtually complete inhibition was the minimal nature of the host reaction to the larvae and the absence of gross damage to the mucosa. The inhibited larvae were situated in gastric glands which had become lined with mucous type epithelium. There was almost no reaction in the surrounding glands and mesenchymal reaction was usually slight (Fig.10). There was no cytolysis or umbilication of the area superficial to the larvae.

In some cases lymphoreticular foci were found specifically related to inhibited larvae; these appeared grossly as white nodules. However, the significance of this association could not be determined. In some cases lesions which consisted

of empty mucous cell lined glands associated with a reaction in the lamina propria could be found. The mesenchymal reaction consisted of a lymphoreticular hyperplasia of varying degree, plasma cell aggregates, eosinophil infiltration and the presence of globular leucocytes.

The Type II Syndrome

Ten outbreaks of Type II bovine ostertagiasis were investigated between January and June 1964; the latter occurred within a week of the animals being put out to graze for their second summer. On these 10 farms 218 young dairy cattle were housed. Some 30.7 per cent of those were clinically affected and 9.6 per cent i.e. 21 cases died or were autopsied in the terminal stages of the disease. Although this syndrome might take as long as 6 months to become clinically evident it must be stressed that Type II can be an acute disease with death supervening within 1 to 2 weeks of the onset of clinical signs.

Animals affected with the Type II disease were sometimes well grown, but were usually dull and in poor condition compared with others of the same group which did not show clinical signs. A rough, harsh, scurfy coat, often lighter in colour than usual, and caked with faeces around the tail and perineum made the appearance of these poorly thriven beasts worse (see Fig. 2). Submandibular oedema was noted in a few cases: this oedema was not constant, appearing and disappearing irregularly in the same animal. Submandibular oedema was most marked in the group of calves, referred to above, which had been turned out to graze for a few days in June; in some of the cases, the oedema took from 2 to 3 weeks to disappear completely.

TABLE 6

A Summary of the Clinical History of Twelve Typical Cases of Type II Ostertagiasis

Animal No.	Hospitalised Age Month	Hospitalised Month	Post Mortem No. Days	Post Mortem Condition	Weight lb.	Anthelmintic Treatment	Faeces Consist	Faeces Diarrhoea
1	12	January	5	Very poor	400	Thiabendazole 2X	D+++	Contin.
2	12	March	2	Poor	400	Thiabendazole Hexaphen	N	Inter-mitt.
3	12	March	4	Good	585*	"	D+++	Contin.
4	14	April	8	Poor	218	Minel, Prom-intic	D+++	Contin.
5	14	April	8	Poor	248	"	D+++	Contin.
6	14	April	8	Poor	308	"	N	Normal
7	15	April	8	Poor	363	Promintic 3X	D+++	Inter-mitt.
8	15	May	23	Poor	275	Promintic, Haloxon	N	Inter-mitt.
9	15	May	36	Poor	296	"	N	Inter-mitt.
10	15	May	20	Poor	319	"	D+++	Contin.
11	16	April	12	Poor	328	Thiabendazole	N	Inter-mitt.
12	16	April	12	Poor	384	Not dosed	D+++	Contin.

* Friesian

D+++ = Fluid Faeces

N. = Normal

2X = Twice

3X = Three times

The loss of weight in Type II cases was a marked clinical feature and even when hospitalised for as long as a month and kept on a high plane of nutrition, these animals were slow to gain weight.

At clinical examination, no other constant abnormality was noted other than decreased appetite, thirst, associated with a diarrhoea which was either intermittent or continuous. In a few cases a systolic murmur was heard, loudest over the cardiac area on the left side and which appeared and disappeared irregularly from day to day. The significance of this finding was not understood.

When the period of continuous diarrhoea lasted 7 to 10 days, the majority of Type II cases became extremely weak, were unable to stand and had sunken eyes. Some of these animals continued eating and drinking small amounts up till death while others ceased eating and drinking 2 or 3 days prior to death.

The prognosis of severe Type II cases was grave, even when multiple anthelminthic treatments were given; weak animals which ceased to eat and drink usually died within a week; cases which continued to eat and drink may die with equal rapidity or may linger for some weeks. Less severely affected cases which survived until the following spring seem to recover quickly when turned out to good pasture. A summary of the clinical history of 12 typical cases of type II ostertagias has been recorded in Table 6.

TABLE 7

The Parasitological Data from Twelve Typical Cases of Type II Ostertagiasis

Animal No.	Worm Egg Count per GM Faeces Mean	Post Mortem	Ostertagia	4th Stage +	Sex Ratio M/F	Trich.	Coop	Nem.
1	-ve	-ve	23,320*	76	1/1	-	-	-
2	550	550	174,660	93	1/44	-	9,240	-
3	550	800	239,100	62	1/2	-	19,000	-
4	2,500	4,400	69,170	19	1/1	-	200	-
5	200	100	93,280	15	1/1	-	28,000	-
6	400	400	114,100	44	1/1	-	2,880	-
7	800	700	56,900	19	1/1	240	-	-
8	250	50	18,240	28	1/1	160	-	-
9	250	100	23,320	38	1/1	-	100	-
10	1,500	3,100	30,470	0	1/3	-	300	40
11	50	50	40,220	15	1/2	-	100	-
12	800	350	133,100	15	2/1	-	1,520	-

* All 5th Stage

\+ The percentage of the total O.ostertagi count present as early 4th stage larvae.

M/F = Male to Female ratio.

The Cooperia spp. were C.oncophora and C.Momasteri

T.axei, Nematodirus helvetianus and N.spathiger

TABLE 8

A Comparison of the Haematological Data From Twelve Typical Cases of Type II Ostertagiasis and Twenty Unaffected Heifers of Similar Age

Parameter	Type II Cases	Unaffected Animals	Students' "t" Test *
P.C.V. %	26.9 ± 3.9	33.6 ± 2.6	P 0.001
HB Gms.%	9.3 ± 0.5	11.1 ± 0.9	P 0.001
RBC $10^6_{mm^3}$	5.7 ± 0.7	6.9 ± 0.9	P 0.05
MCV cu	47.0 ± 5.5	50.9 ± 5.7	NS
MCHC %	34.3 ± 2.1	33.1 ± 1.8	NS
Total Serum Proteins gm %	5.30 ± 0.93	5.93 ± 0.4	NS
A/G Ratio	0.38 ± 0.14	0.9 ± 0.2	P 0.1
Albumin Conc gm %	1.38 ± 0.41	2.88 ± 0.2	P 0.001

* The statistical significance between Type II cases and unaffected animals

P 0.05 significantly different.

N.S. = Not signigicant.

From a comparison of the weights of Type II cases (see Table 6) with the values for average Ayrshire heifers aged 12 to 16 months at the West of Scotland Agricultural College, namely 495 to 603 lbs. liveweight (Walker-Love, personal communication), it can be appreciated that the majority of Type II cases were at least 100 to 150 lbs underweight.

Generally the mean daily faecal egg count, taken over a number of days while hospitalised, was less than 1,000 e.p.g., but during diarrhoeic phases it was common to record egg counts of 1,000 to 3,000 e.p.g. There was no correlation between the post mortem egg count or a mean egg count and the number of adult females present.

Despite the use of repeated anthelminthic treatments the numbers of worms, particularly of O.ostertagi, present at autopsy were frequently high. Compared with Type I, the total numbers of worms were double in some cases and the percentage of immature stages from all species present was much higher. The results of the parasitological examination of the 12 cases of Type II ostertagiasis have been set out in Table 7.

A comparison of various haematological measurements of 12 Type II cases with those from 20 clinically unaffected animals of about the same age is shown in Table 8. The unaffected animals were a group of calves 10 months of age and judged to be free from inter-current disease. However, these animals were grazing whereas Type II cases were indoors on a

varying dry diet. Although dietary effects have been thought to exert an influence on haematological criteria the specific effects of nutrition, if any, have not been made clear (Schalm, 1961).

The data shown in Table 8 indicates that there was a ~~satisfactory~~ STATISTICALLY significant decrease in the packed cell volume, the haemoglobin concentration and the total red blood cell counts, of the affected animals. Values for the mean cell volume and the mean cell haemoglobin concentration were within the normal range so that the mild to moderate anaemia evident in these Type II cases, was of a normocytic, normochromic type.

While individual animals did show a decrease in their total serum protein concentration to 4.0 gms per cent, on a group basis there was no significant difference from the unaffected controls. Nevertheless, a decrease in the A/G ratio did occur in the affected group and the values for serum albumin concentration were markedly reduced.

The results of plasma pepsinogen, abomasal pH and gastric electrolyte determinations have been included in Table 9 for ready comparison with the other phases of bovine ostertagiasis. The abomasal pH of typical cases of Type II was markedly raised. The sodium ion concentration of the gastric contents was also increased while decreases in potassium and chloride ion concentrations were noted. Plasma pepsinogen concentration was increased to a marked

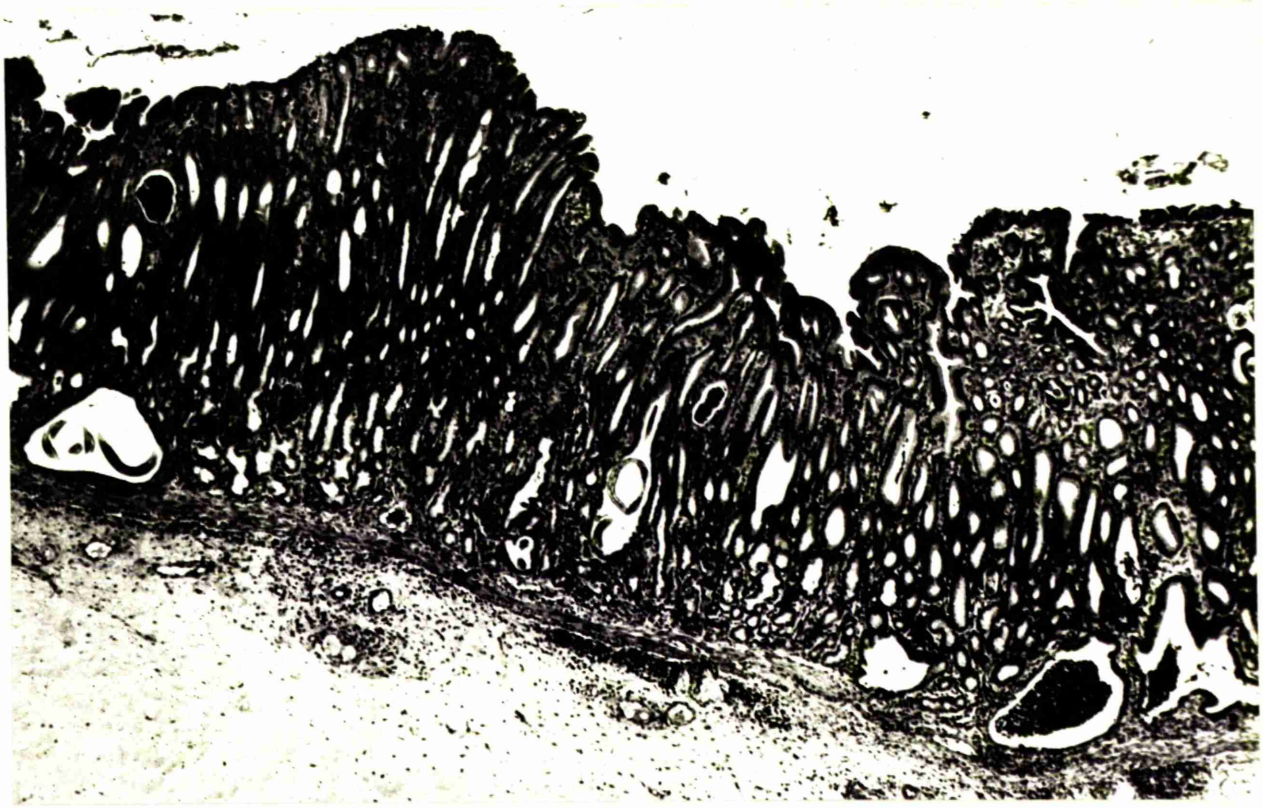


Fig.11. Severe Type II reaction showing gross destruction of the gastric glands and marked submucosal oedema.

degree, in some cases, 20 times the uninfected value.

The main pathological features of this stage were the sequential development and emergence of large numbers of previously inhibited larvae. Lesions typical of the stage of inhibition were found together with lesions produced by larval development and emergence. Nodules were evident as in Type I cases and surrounding these, after emergence, were areas of epithelial hypertasis and absomasal glands lined with undifferentiated cells. The confluence of these lead to the formation of large areas of "morocco leather" like mucosa. Epithelial sloughing in some of these areas was marked and if extreme caused "thumbprint" lesions, diptheresis, superficial inflamation and congestion (Fig. 11). Globular leucocytes were numerous and lymphoreticular and plasma cell activity was marked in some areas.

DISCUSSION

Following an investigation of 19 outbreaks of parasitic gastroenteritis in young Aryshire cattle it is apparent that O.ostertagi is the primary pathogen in this part of Scotland. A morbidity of 45.4 per cent and a mortality of 14.8 per cent found during this study, which involved some 337 dairy calves, indicates that at least on some farms bovine ostertagiasis is a serious cause of economic loss to the farmer. The figures quoted above are much higher than the 9 and 3.4 per cent respectively, reported by Becklund (1962), who concluded that mortality accounted for two thirds of the total financial

loss involved.

Bovine ostertagiasis can be clearly divided into two clinically apparent types separated by a phase which is not clinically evident.

The Type I syndrome appears in calves after they have ingested O.ostertagi larvae picked up during their first summer at grass. The clinical signs of diarrhoea and weight loss usually appear in these calves from late July to September. Larvae ingested during the late autumn, i.e. September to early November, are responsible for the subsequent development of the Type II syndrome, which is also characterised by diarrhoea and loss of weight. These larvae are inhibited in development at the early 4th stage following the 3rd moult and may remain so, within the gastric glands, for as long as 6 months. Morphologically these inhibited larvae appear to be identical to early 4th stage larvae derived from a 4 day infection of O.ostertagi. During this period of inhibition clinical signs are not observed. When large numbers of inhibited larvae complete their development to adult stages, the clinical signs of the Type II syndrome appear. This usually occurs in housed stock from December until the following June, but may occur in out wintered animals as well.

The Type I syndrome was probably responsible for the outbreaks reported by Ackert and Muldoon (1920); Bruford and Fincham (1945); Threlkeld and Bell (1952); Bailey and Thorson

(1954) and others whereas the Type II syndrome was specifically described by Martin, Thomas and Urquhart (1957). It is likely that the reports of outbreaks by Stewart and Crofton (1941) and Bailey and Herlich (1953) were also Type II ostertagiasis.

In the diagnosis of bovine ostertagiasis the results of faecal worm egg counts must be interpreted cautiously. In Type I ostertagiasis, counts of less than 1,000 e.p.g. were encountered both in apparently healthy calves and from clinical cases. All calves with egg counts of over 1,000 e.p.g. were clinically affected animals although a third of these were not diarrhoeic at the time when the sample was taken. In cases of Type II ostertagiasis faecal egg counts were of little value (see Table 4). The variation in worm egg counts of individual cases of either Type I or Type II is such that low egg counts cannot exclude bovine ostertagiasis from the differential diagnosis. The conclusion, that faecal worm egg counts are of little diagnostic value in outbreaks of bovine ostertagiasis, is in agreement with the findings of Roberts, O'Sullivan and Riek (1951); Andrews, Sippel and Jones (1953); Ross and Armour (1960); Banks and Mitton (1960).

The occurrence of the Type I syndrome in July can be explained by two factors: climatic conditions in June are suitable for the development and migration of larvae, Rose (1961 and 1962); apparently about 50,000 to 60,000 adult

O.ostertagi are necessary to produce typical clinical signs in calves 3 to 5 months old. During the summer months larvae are continually ingested, develop to the adult stage and precipitate clinical disease when the tolerated or threshold number of parasites is exceeded. The difference between spring and autumn born calves in their susceptibility to the disease may be due to relationship between animal size and threshold number of worms i.e. the smaller the calf the lower the threshold. Banks and Mitton (1960) put forward a similar concept.

The total numbers of O.ostertagi found in cases of Type II ranged from 239,000 down to 18,000 and the number of early 4th stages were much higher than in Type I cases. The percentage, of inhibited larvae, apparently unaffected by the anthelminthics used, was inversely proportional to the period of clinical illness i.e. the longer the case had shown clinical signs, the fewer the inhibited forms in the mucosa. In this regard Dunsmore (1964), found that removal of adult O.circumcincta in sheep by anthelminthic treatment allowed inhibited larvae to resume development. The possibility of successive waves of maturing larvae would account for the periods of intermittent diarrhoea commonly associated with Type II cases and also the failure to effect a clinical cure following anthelminthic administration. In cases of Pre Type II or inhibition the stimulus causing the synchronous development of large numbers

of inhibited larvae is not known.

There must be some dubiety as the origin of the low numbers of inhibited larvae found in cases of Type I since they either may have been acquired a few days prior to autopsy or may have been inhibited earlier. This reservation does not hold for the larger populations found in Type II cases or in cases of Pre Type II, since all of these animals had been kept indoors for at least 4 weeks prior to autopsy.

The mechanism of inhibition is unknown, but, Taylor and Michel (1953) thought that the function of inhibition is to enable a parasite to maintain itself in an environment unsuited to development e.g. in a resistant host. Inhibition of development has been induced experimentally with infections of other nematodes affecting ruminants, for example, Oesophagostomum columbianum Gordon (1949); Dictyocaulus filaria Taylor and Michel (1952); Haemonchus placei Bremner (1956); Roberts (1957); H.contortus Silverman & Patterson (1960); Ostertagia circumcinta, Sommerville (1963); Dunsmore (1963); O.ostertagi Michel (1963); and Nematodirus spp. Gibson (1959).

In some of these infections, for example O.circumcinta in sheep, larval development can be inhibited by the administration of a large single dose of larvae, while in others e.g. Haemonchus spp., O.ostertagi, it would appear that inhibition primarily depends either on the presence of a

pre-existing adult population or previous infection by that parasite. From the present study it is not clear which of these methods of producing inhibition, if any, would apply to O.ostertagi. It is interesting to note that results from this study indicate that the inhibition of larval development occurs simultaneously in both O.ostertagi and Cooperia spp. larvae, and this fact may be associated with an alteration in the physiology of the host.

Haematological changes may be noted in individual animals affected with either clinical syndrome. Increases in packed cell volume, haemoglobin concentration and total red blood cell counts can be accounted for by the decrease in total body water which occurs in the Type I (Mulligan et al 1965). The significance of the mild to moderate normocytic, normochromic anaemia found in cases of Type II is not clear. An increase in total body water does occur in Type II cases (Mulligan et al., 1965) so the haematological changes noted may be apparent rather than real.

The marked changes in the abomasal pH, sodium, potassium and chloride ion concentrations are similar in both Type I and Type II syndromes and are associated with the presence of large numbers of adult parasites or severe abomasal lesions. The changes from normal values noted in cases of Inhibition or Pre Type II are small in spite of the presence of large numbers of early 4th stage parasites. The early 4th stage larva produces little pathological change

apart from that affecting the gland in which it is lying. This gland becomes lined by a layer of mucous cell epithelium and when large numbers of glands are affected in this way a small degree of functional disturbance becomes evident, e.g. slight increase in pH.

The main pathogenic effect comes with the emergence of the young adult stages from the glands when epithelial cell sloughing occurs and there is conversion of surrounding functionally differentiated glands ~~by~~ to hyperplastic pits lined by undifferentiated cells. Confluence of the circular hyperplastic lesion surrounding the nodule occurs, the morocco leather appearance is produced and large areas of mucous membrane lost their specialised secretory functions.

It is at this time that severe changes in abomasal ionic concentrations occur and these changes must have significant physiological consequences. For instance, the autocatalytic conversion of inactive pepsinogen to active pepsin is almost instantaneous at pH 2.0 but, as the pH rises the conversion time becomes longer taking many hours at pH 4.6, Hirschowitz (1957). Furthermore, the optimum activity of pepsin occurs at pH 1.5 to 2.0 and with a progressive increase in the pH of the medium peptic activity decreases until at pH 5.4 all proteolytic activity is inhibited. At pH 7.0 and above pepsin is destroyed, Wilkinson (1962). Exactly how the pH change affects protein metabolism remains to be found out. Significant changes in the serum proteins

of Type I cases were not recorded but a marked hypoalbuminaemia was noted in Type II cases. A marked loss and decreased rate of weight gain were features of both Type I and Type II.

Under normal physiological conditions the acid secretion destroys the ruminal micro flora and fauna which enter the abomasum and probably assists in the release of their intracellular contents. It would be of interest to learn the fate of these micro-organisms in clinical cases of ostertagiasis and to determine their role in the development of diarrhoea in these cases, presuming that they were not destroyed.

Increased concentrations of sodium ion in the abomasal contents of a diarrhoeic animal may lead to a sodium deficit via the faeces. Combined with losses of body ~~fluids~~ FLUIDS, all the clinical signs of dullness, anorexia, thirst, weight loss and general weakness can be accounted for, since these symptoms are associated with fluid and sodium loss in man (Black 1964).

Plasma pepsinogen concentrations are significantly increased in all phases of bovine ostertagiasis. This test may prove to be of useful diagnostic value if it is specific for the lesions produced by O.ostertagi. Type II cases have concentrations twice that of Type I cases. ~~Reid et al.,~~ SPIRO AND SCHWARTZ (1958) explained the increased plasma pepsinogen levels found in certain cases of superficial gastritis in man, by suggesting

that the surrounding inflammatory reaction produced a functional blockage of the gastric gland and pepsinogen leaked back into the blood stream. Alternately, the higher values noted in Type II cases may result from further back diffusion through damaged cell junctions in the epithelial sheet, Jarrett (personal communications). Leakage of macromolecules through the abomasal mucosa has been shown in Type II cases (Mulligan, Dalton and Anderson, 1963) who demonstrated an increased plasma albumin loss into the gastrointestinal tract.

It is evident from this study that the onset of diarrhoea in clinically affected animals is associated with the adult stage of O.ostertagi; it is absent in the inhibited phase and appears in the Type II case, its severity and duration depending on the numbers of larvae resuming development. Two factors in the initiation of diarrhoea appear to be of importance:

a) the direct effect of the emergent adult per se or through its cytolytic action on gastric epithelium. Large amounts of pharmacologically active polypeptides, belonging to the family of kinins, are known to be released from sites of inflammation and tissue damage, Lewis (1960): and

b) the indirect effect of the hyperplasia and loss of differentiation of the gland epithelium which produces loss of parietal cells and a subsequent rise in abomasal pH.

The former explanation seems more likely as achlorhydria in man is not always associated with diarrhoea.

SUMMARY

Field investigations of bovine parasitic gastro-enteritis in the Southwest of Scotland showed that Ostertagia ostertagi was the predominant parasite. The disease produced by this parasite was classified into three phases, two of which (Type I and Type II) were clinically apparent.

Type I corresponded to the classical description of clinical parasitic gastritis in which calves, at grass for the first time showed a loss of weight and severe diarrhoea, this occurred at any time from late July until the end of the grazing season. The vast majority of ingested larvae had developed to maturity within the expected period of three weeks.

Pre Type II was clinically inapparent although large populations of O.ostertagi were present, of which over 80 per cent were inhibited in the early 4th stage. These animals had grazed infected pasture in the late autumn but had no history of diarrhoea and usually appear healthy to the farmer.

Type II resulted from the maturation, up to 6 months later, of sufficient numbers of these inhibited larvae to cause a clinical condition characterised by weight loss and diarrhoea. Usually it occurred in housed stock, following

the first grazing season, during which no clinical signs of parasitic gastritis were detected.

A description of the clinical signs, parasitological data, and haematological changes and pathological lesions from each type have been given.

PART 3

A DETAILED STUDY OF NATURALLY OCCURRING BOVINE OSTERTAGIASIS

INTRODUCTION

During the investigation of 19 outbreaks of bovine ostertagiasis in young Aryshire cattle it was possible to classify the disease into three phases, Type I, Pre Type II or the stage of Inhibition and Type II. The characteristics of these phases have been described in an earlier section.

However, definite conclusions could not be reached about the relationships between the numbers and stages of the parasite, the observed clinical signs and the associated pathological and physiological changes in animals affected with naturally acquired ostertagiasis. Moreover, the widespread use of various anthelminthics together with the difficulty of purchasing valuable replacement calves, made the study of Type I and Type II outbreaks of limited value.

In order to gather more specific data a field experiment was designed to study the factors leading up to the development of the clinical disease. Laboratory experiments have been unsuccessful in attempts to reproduce the non clinical stage of inhibition, i.e. Pre Type II, so observations on the association between inhibition and late autumn grazing were included in the experimental design.

MATERIALS AND METHODS

Farms

Two Ayrshire farms, which had experienced heavy losses from parasitic disease in previous years, were chosen for the field experiment. The permanent calf rearing fields were

leased from the farmers for the grazing season May to November 1964.

A short description and history of the two Ayrshire farms is given in Appendix C.

Animals

Ayrshire bull calves were reared parasite free at the Veterinary Hospital as previously described (see Section 1.) At 6 weeks of age these calves were grazed for 2 or 4 weeks on a newly sown cocksfoot and white clover pasture at the Veterinary Hospital. This pasture, 7 acres in size, was considered to be free of parasitic larvae as no grazing animal had access to this field since 1961 and during the previous two years it had been ploughed and resown. This assumption proved to be valid as frequent faecal examinations from calves grazing this field failed to detect the presence of nematode eggs.

Ten calves, called the "permanent" calves, 9 to 11 weeks of age and weighing in excess of 100 lbs. were taken to each farm where they grazed the fields, associated with outbreaks in previous years, until clinical signs of ostertagiasis became apparent. Affected calves were returned to the Veterinary Hospital for further study and subsequent autopsy examination. In order to prevent bovine dictyocaulidiasis from complicating the clinical syndromes all "permanent" calves were vaccinated with Dictol (Allen and Hanburys Ltd., Ware, England).

In addition 2 calves of similar age, called the "tracer" calves were grazed together with the "permanent" calves on each farm. These four calves were replaced by four calves every fortnight during the entire period of the experiment, and following removal from the infected pasture they were returned to the Veterinary Hospital where, after 4 days maintenance under parasite free conditions, they were autopsied.

The age and weight of the "tracer" calves were kept approximately constant throughout the experiment and this necessitated a regular routine for the rearing of parasite free calves.

Another ten calves of similar age called "replacement" calves, were reared parasite free during the summer and 5 of these calves supplemented the "permanent" calves on each farm during the late autumn grazing period to simulate the usual agricultural practice of grazing spring born with autumn born calves. The "replacement" calves were also vaccinated with Dictol.

Clinical Observations

All calves were weighed before leaving and immediately on returning to the Veterinary Hospital.

Each Monday and Thursday, from May 11th to November 9th 1964, all calves at both farms were examined and the consistency of the faeces was noted as previously described.

Parasitological Observations

Fresh rectal samples of faeces were examined twice weekly and daily during hospitalisation, for the presence of nematode eggs. At autopsy a full parasitological examination was carried out as described in Section 1.

At autopsy the small intestines of the "permanent" calves were divided into three, approximately equal, lengths. The contents and mucosal washings were prepared and examined as described earlier. The mucous membrane of each length was scraped off the underlying muscle and separately digested with a pepsin-HCl solution. Examination was carried out in the same manner as abomasal digest. The results of these examinations have been included in Appendix C.

Haematological Observations

A 2 to 5 ml sample of jugular blood was taken from all calves at weekly intervals and determinations of packed cell volume, haemoglobin concentration and total red blood cell counts were carried out. EDTA was the anticoagulant used.

Biochemical Observations

About 25 to 30 ml of heparinised blood was collected at weekly intervals from all calves. Plasma pepsinogen concentration, total protein concentrations and albumin to globulin ratios were estimated using the methods set out in Section 2.

At autopsy a sample of abomasal contents was collected from each calf. The pH, sodium, potassium, chloride ion and

gastric pepsin concentrations were estimated as described previously.

Metereological Observations

Recordings of temperature and relative humidity were made at each farm on thermographic and hydrographic recorders supplied by the Department of Metereology, Edinburgh. These recordings were made at ground level inside a standard metereological weather screen and were continuous from June to November, 1964. The paper on the recording drum was changed every Monday morning and from the data collected, the monthly mean amximum and mean minimum temperature and mean relative humidity were calculated.

Measurements of daily rainfall were made at the local metereological station which in each instance was less than one mile from the farms studied.

RESULTS

For the sake of convenience it is proposed to record the results of these observations in three parts, namely:

Part 1 "Tracer Calves"

Part 2 "Permanent Calves"

Part 3 "Replacement Calves"

"Tracer Calves"

Clinical Data

Diarrhoea, D+++, was recorded from only one calf on Farm A; calf No. 2 on day 14. On Farm B six calves were observed to have D+++ diarrhoea but in each case fluid faeces was noted on one observation day only. In no case was the diarrhoea continuous. The presence of diarrhoea was not related to the degree of parasitic infection and was considered to be non-specific and of unknown aetiology.

Weight gains showed extreme variability on both farms; values from +1.0 to -3.4 lbs. per day were recorded. There was no relationship between weight gain and parasite burden.

In general, the majority of the "tracer" calves appeared bright and well contented when grazing on the calf rearing fields of both farms. However, during cold and wet periods the "tracer" calves did not thrive particularly well.

Haematological Data

Estimation of the packed cell volume, the haemoglobin concentration, the total serum protein and the serum albumin concentrations were carried out on weekly blood samples taken

TABLE 9

The Parasitological Data from the "tracer" Calves Grazed on Farm A

Calf No.	O.ostertagi Total	E4th Stage*	O.ostertagi per day	Coopria Total	Nematodirus Total	Total Burden	Worms per da
1	1,600	10	114	80	160	1,840	131
2	1,460	33	104	160	160	1,780	127
3	2,640	33	189	0	2,400	5,040	360
4	3,680	34	263	80	2,400	6,160	440
5	25,280	33	1,806	560	240	26,080	1,863
6	7,040	18	503	480	1,600	9,120	651
7	14,280	27	1,020	560	1,200	16,040	1,145
8	13,090	15	935	160	720	13,970	998
9	15,140	1	1,081	2,080	3,680	20,900	1,493
10	5,740	25	410	960	1,280	7,980	570
11	14,520	13	1,037	3,040	2,240	19,800	1,414
12	22,120	16	1,580	5,120	480	27,720	1,980
13	45,980	3	3,284	6,560	3,360	55,900	3,993
14	39,520	12	2,822	2,560	5,280	47,360	3,383
15	3,840	8	274	1,440	1,840	7,120	509
16	51,680	12	3,691	8,820	1,400	60,900	4,350
17	16,810	40	1,401	4,800	3,200	24,810	2,001
18	110,730	37	7,909	12,850	460	24,040	8,860
19	9,760	48	697	880	800	11,440	817
20	37,440	70	2,674	4,080	3,520	45,040	3,217
21	18,000	94	783	4,800	480	23,280	1,011
22	19,400	95	844	1,960	3,800	25,160	1,097
23	27,600	95	1,200	4,800	480	32,880	1,429

* The percentage of the total O.ostertagi count present as early 4th stage larvae.

TABLE 10

The Parasitological Data from the "tracer" Calves, Grazed on Farm B.

Calf No.	O.ostertagi Total	E 4th Stage*	O.Ostertagi per day	Cooperia Total	Nematodirus Total	Total Burden	Worms per day
1	2,080	27	149	240	1,440	3,760	269
2	1,160	7	83	640	760	1,920	137
3	0	0	0	0	0	0	0
4	720	0	6	0	0	720	6
5	240	0	17	0	0	240	17
6	560	14	40	80	560	1,200	86
7	160	0	11	480	80	720	51
8	440	0	31	160	240	840	60
9	240	0	17	0	480	720	51
10	320	0	23	160	160	640	48
11	800	20	57	320	240	1,360	97
12	720	33	51	320	560	1,600	114
13	11,200	15	800	3,600	4,640	19,440	1,388
14	10,360	18	740	3,120	1,040	14,520	1,037
15	64,320	9	4,594	26,160	80	80,560	5,754
16	32,660	4	2,969	12,340	0	45,000	4,091
17	51,760	14	3,697	19,600	0	71,360	5,097
18	4,560	18	351	0	0	4,560	351
19	7,800	9	709	10,880	0	18,680	1,698
20	11,400	18	1,036	2,320	160	13,880	1,262
21	13,630	23	974	12,340	650	26,620	1,901
22	8,080	31	577	4,640	480	13,200	943
23	2,000	70	154	1,360	1,200	4,560	351
24	4,960	45	354	7,360	2,740	15,060	1,114
25	41,760	85	2,983	8,000	480	50,240	3,865
26	28,750	88	2,054	21,220	660	50,630	3,895

* The percentage of the total O.ostertagi count present as early 4th stage larvae.

from the "tracer" calves on Farm A and Farm B.

A students' "t" test analysis of the difference between paired readings taken on day 0 and on day 14 of the grazing period, was carried out. All values of "t" were found to be not significant at the 5 per cent level.

Parasitological Data

Faecal Egg Counts

Two calves, Nos. 3 and 8, from Farm A and calf No.19 from Farm B showed positive egg counts on day 18, i.e. autopsy day. In each case the count was 50 Nematodirus eggs per gram.

Worm Counts

The autopsy worm counts of the "tracer" calves from Farm A and from Farm B have been recorded in Tables 9 and 10 respectively. In every case O.ostertagi was the predominant nematode species found at autopsy. An estimate of the number of O.ostertagi and total nematode larvae which became established per day of grazing was calculated by dividing the respective totals by the number of grazing days. These figures are shown in the appropriate columns of the above tables.

Calf No. 3 from Farm B was the only calf in which nematodes were not detected at autopsy. As a small number of lesions, typical of O.ostertagi infection, were found in the abomasum it is likely that the number of abomasal nematodes was less than 160. The minimum number of worms which

TABLE 11

The Combined Worm Count Data from the "tracer" Calves which Grazed Farms A and B

The Mean O. ostertagi Count and the Percentage of the Count Present as Early 4th Stage Larvae

Period Grazed	No. of Calves	O.ostertagi Mean Count	Percentage Early 4th Stage	
			Mean	Range
11/5 - 25/5	2	1,770	17	7 - 27
25/5 - 8/6	4	940	12	0 - 33
8/6 - 22/6	4	1,750	20	0 - 34
22/6 - 6/7	4	8,230	13	0 - 33
6/7 - 10/7	4	6,980	10	0 - 27
20/7 - 3/8	4	5,490	11	0 - 26
3/8 - 17/8	4	14,600	15	13 - 19
17/8 - 31/8	4	45,700	8	3 - 12
31/8 - 14/9	4	26,000	19	8 - 42
14/9 - 28/9	4	36,700	26	9 - 40
28/9 - 12/10	2	10,900	27	23 - 31
12/10 - 26/10	4	13,600	58	48 - 70
26/10 - 9/11	2	35,300	87	86 - 88
26/10 - 18/11	3	21,700	95	94 - 95

can be detected by the counting technique used is 80.

The results of the worm counts from "tracer" calves on both farms have been combined in Table 11 which shows the mean O.ostertagi count and the mean and range of the percentages of early 4th stage O.ostertagi for each grazing period. The numbers of O.ostertagi recovered increased sharply during the second half of August and remained at a high level until the end of September. A second rise in parasite numbers occurred in November. The percentage of the total number of O.ostertagi present as early 4th stage parasites increased directly with time after the end of September.

Biochemical Data

Calves infected with 10,000 (OR MORE) O.ostertagi showed higher concentrations of plasma pepsinogen on day 14 than on day 0, but this increase exceeded the normal limits only in those calves with the heaviest infections.

Six calves from each farm had abomasal pH values greater than the normal range of 2.4 ± 0.4. A similar comparison of abomasal electrolyte concentrations showed that the samples examined were still within the normal range. None of the "Trader" calves developed the degree of abomasal change characteristic of clinical ostertagiasis.

TABLE 12

The Ages, Grazing Periods and Bodyweight Data of the "permanent" Calves on Farms A and B

Calf No.	Age Days	Dates Grazed	No. Days Grazing	Weight lbs Day 0	Weight lbs P.M. Day	Gain per Day lb.
1	77	25/5 - 4/8	71	144	195	+ 0.7
2	77	25/5 - 4/8	71	112	138	+ 0.2
3	77	25/5 - 4/8	71	136	182	+ 0.6
4	67	25/5 - 4/8	71	120	144	+ 0.3
5	77	25/5 - 4/8	71	168	210	+ 0.6
6	77	25/5 - 31/8	98	124	140	+ 0.2
7	77	25/5 - 31/8	98	140	216	+ 0.8
8	77	25/5 - 20/9	118	134	-	-
9	68	25/5 - 5/10	133	105	150	+ 0.4
10	77	25/5 - 16/10	144	120	161	+ 0.3

Farm B.

1	63	11/5 - 10/9	122	126	228	+ 0.8
2	63	11/5 - 10/9	122	126	218	+ 0.8
3	63	11/5 - 10/9	122	109	202	+ 0.8
4	48	11/5 - 10/9	122	100	186	+ 0.7
5	61	11/5 - 10/9	122	116	236	+ 1.0
6	55	25/5 - 28/9	126	118	192	+ 0.6
7	63	11/5 - 2/10	144	104	135	+ 0.2
8	54	11/5 - 2/10	144	117	150	+ 0.2
9	63	11/5 - 19/10	161	138	249	+ 0.7
10	74	6/7 - 19/10	105	143	166	+ 0.2

"Permanent" Calves

Clinical Data

The details of age, grazing time, initial weight and daily weight gain of each permanent calf used in the study are given in Table 12.

Four weeks after being put out to graze on Farm B, one calf was found to be not thriving and was autopsied on June 29th 1964. A severe diptheretic lesion typical of Corynebacterium pyogenes infection was found involving the pharynx and oesophagus. Calf No. 10 replaced the autopsied calf on July 6th 1964.

Five of the ten calves grazing on Farm A showed severe diarrhoea (D+++) on one or two observation days during the period 3 to 5 weeks from the commencement of the experiment. This period of diarrhoea proved transitory and no noticeable change in growth rate of general appearance occurred at this time. All ten calves continued to grow satisfactorily and were in good condition up to July 27th, i.e. the 9th week of the investigation. During the 9th and 10th weeks four of the permanent calves, No.s 1, 2, 5 and 9 showed a severe, bright green coloured diarrhoea and in calves Nos. 1, 2 and 5 this was accompanied by a noticeable loss of weight and a depressed disposition. The clinical signs continued for 9 days and at this time i.e. 7 days after the onset of clinical signs, the three affected calves were autopsied together with calves Nos. 3 and 4 which were

not clinically affected. The severe diarrhoea shown by calf No. 9 ceased after a few days and together with the remaining calves no sign of ill health could be determined at this time, although in retrospect, their growth rates from the 11th week onwards were noticeably reduced.

Between the 14th and 16th weeks of the study, four of the five remaining calves showed varying periods of intermittent, severe, watery diarrhoea. In each case, when the diarrhoea was continuous for two observation days, the affected calf was taken back to the Veterinary Hospital for autopsy examination.

Calf No.10 differed from the 9 other "permanent" calves in that no change in faecal consistency or wellbeing was noted until day 140 of the study at which time severe continuous diarrhoea was recorded. A moderately reduced rate of growth was noted in this calf from the 10th week onwards. Clinically affected calves were typical of those already described earlier on connection with the Type I syndrome, and the usual procedure was to autopsy the calves within one week after clinical signs had appeared.

In general a similar pattern of clinical signs developed in the "permanent" calves on Farm B except that the onset of signs was not as sharply demarcated as those seen in calves from Farm A.

Between the 4th and 7th weeks of grazing eight of

the ten calves showed a transitory period of moderate diarrhoea (D++) which appeared to have little if any effect on their general well-being.

During the period between the 9th and 11th weeks five calves, Nos. 1, 2, 4, 6 and 8 developed severe but intermittent diarrhoea. A marked check in growth and weight gain occurred in these calves. The harsh, staring coats and faeces caked tails and hocks contributed greatly to the ill thriven appearance of this group of calves. By the end of the 15th week another three calves, Nos. 3, 5 and 10 had become similarly affected and during the 17th week the former five calves were autopsied. Intermittent diarrhoea, severe at times, and a progressive loss of weight developed in each of the five remaining calves which were autopsied at varying intervals. The last autopsy was of calf No. 3 which survived a period of 161 days of grazing.

Throughout the experimental period the weight gain of all calves on both farms was unsatisfactory, compared with calves reared at the West of Scotland Agricultural College. There, Ayrshire calves at grass aged between 3 and 6 months gained 1.33 lbs. per day. (Walker - Love, personal communication).

Haematological Data

Data collected from both farms were analysed separately by the analysis of variance method.

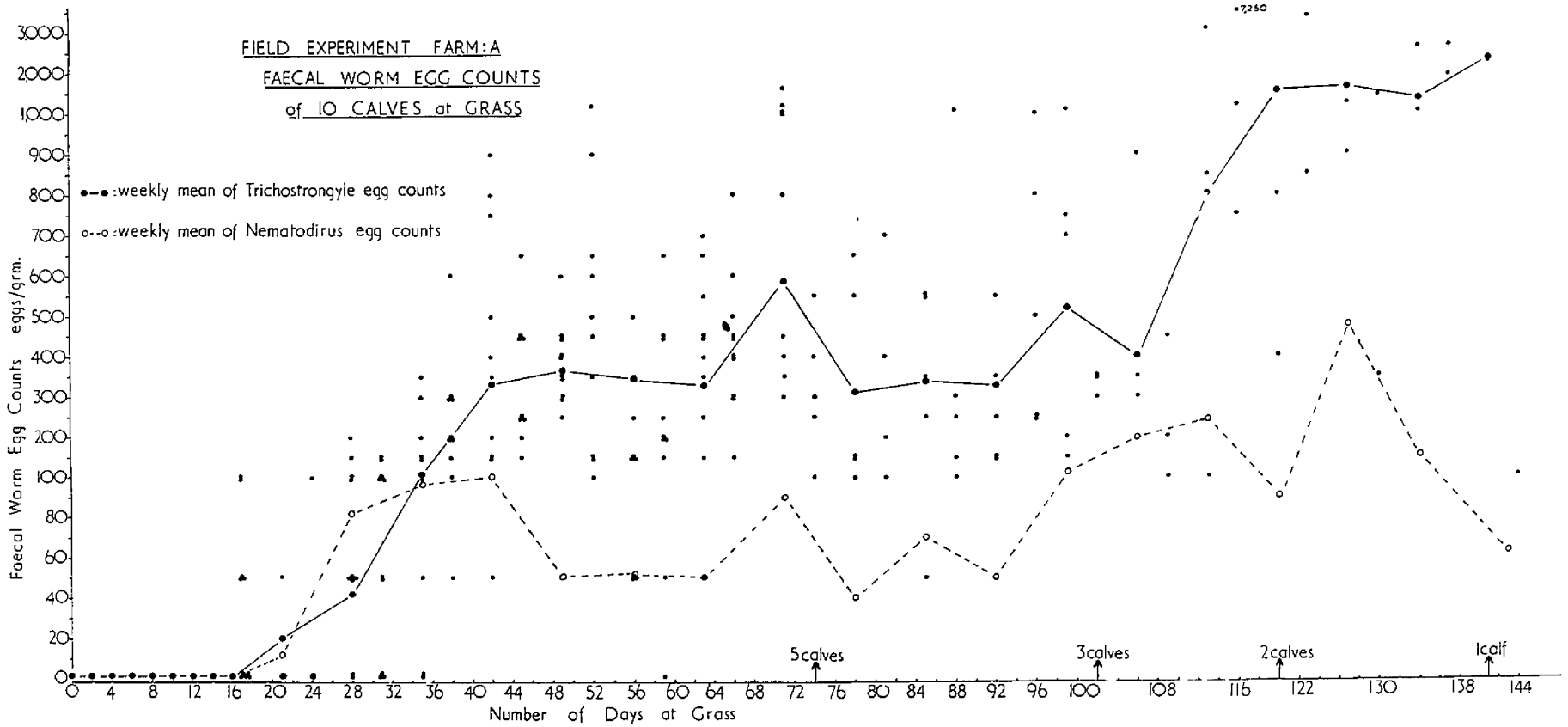


Fig. 12 A graph of the individual and group mean faecal egg counts, at each sampling period, from the "permanent" calves grazing Farm A.

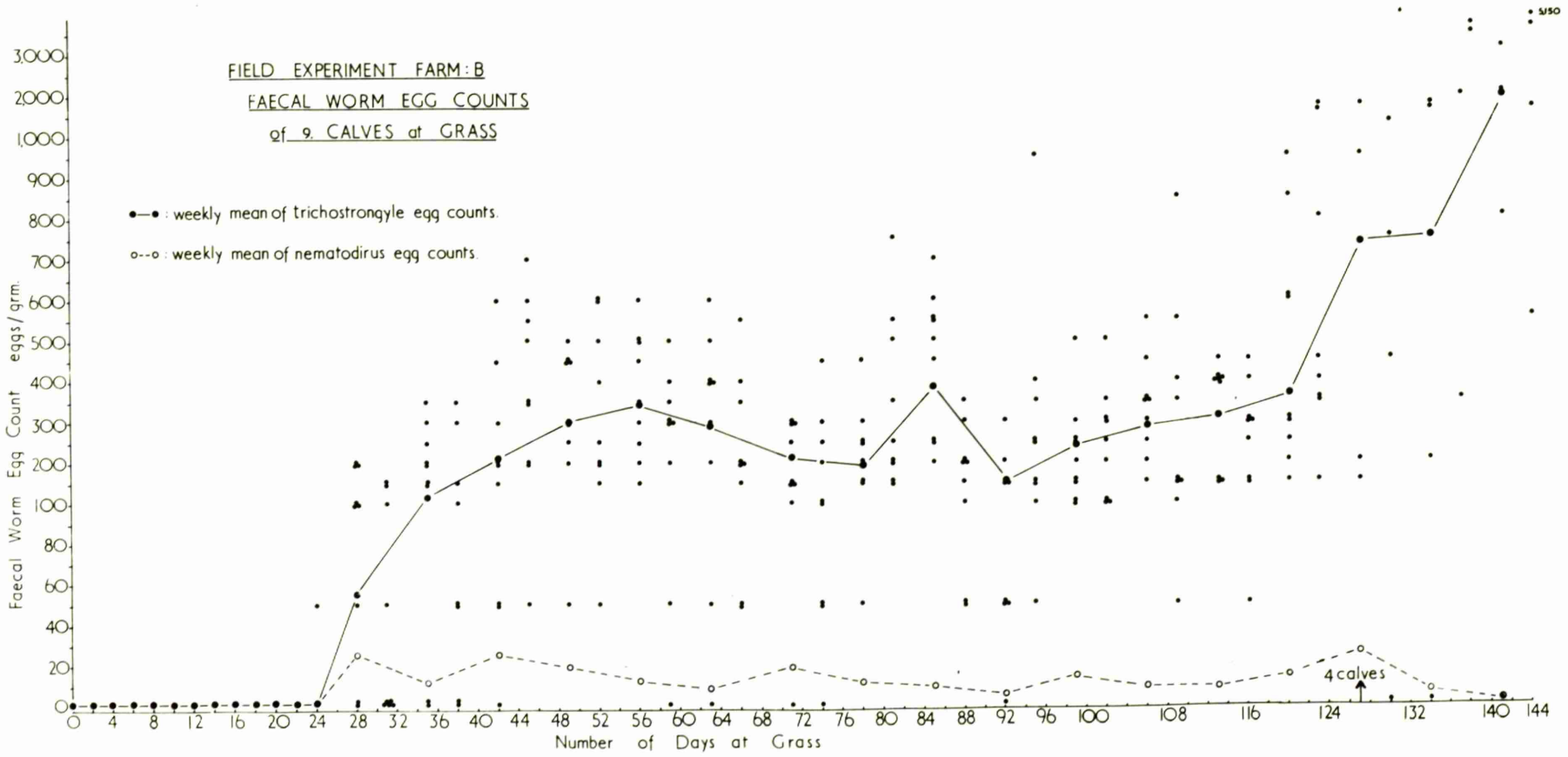


Fig. 13 A graph of the individual and group mean faecal egg counts, at each sampling period, from the "Permanent" calves grazing Farm B.

There was a significant difference between calves on each farm at different sampling periods. Each one of the parameters measured showed this difference.

However, as the trend with time was non linear it was decided to compare the overall group mean (i.e. the group mean averaged over all times included in the analysis) with a similar group mean calculated from groups of non affected calves from various farms. (see Appendix A.)

The overall group mean values for packed cell volume, red blood cell counts and haemoglobin concentration of unaffected animals of similar age were 34.8, 8.13 and 11.4 respectively. When these values are compared with the corresponding values of 33.4, 7.88 and 11.2 from the clinically affected calves it can be appreciated that there is no significant difference between the mean values from affected and the non affected animals.

Parasitological Data

Faecal EggCounts

The individual results of the Trichostrongyle spp. egg counts together with the weekly mean Trichostrongyle spp. and Nematodirus spp. egg counts are shown in Figures 12 and 13 for calves on Farm A and Farm B respectively. The mean Nematodirus spp. egg counts were significantly different on all sampling dates; counts of calves from Farm A were 2 to 2 times higher than those from Farm B.

Because calf No. 10 from Farm B did not commence grazing with the other 9 calves, the data from this calf has been excluded from Figure 12.

The shape and amplitude of the curves for the mean Trichostrongyle spp. egg counts are remarkably similar on both farms. The essential difference between the two sets of data lies in the fact that events on Farm A occurred earlier and that a greater degree of variation between individual calves was shown by the calves on Farm A compared with those on Farm B.

Calves on Farm A had patent infections by day 17 while on Farm B this did not occur until day 24 of the experiment. The mean egg counts of calves on Farm A rose to 350 e.p.g. on day 42 and it was maintained at this level for the next 60 days of the experiment except for two observation periods around day 70 when the mean count increased to 550 e.p.g. At this time 5 calves were removed from the group and the mean egg count fell to 350 e.p.g. once again. Towards the end of the experiment, when only two calves remained alive the mean egg count was maintained above 1,000 e.p.g.

The mean egg count of calves from Farm B varied between 200 and 400 e.p.g. but when the mean reached this level on day 42 of the experiment it remained constant up to day 122 at which time 5 calves were removed from the

TABLE 13

The Autopsy Worm Count Data from the "permanent" Calves Grazing Farms A and B

Calf No.	Ostertagi Total	4th Stage Ostertagi	Ostertagi per day	Cooperia Total	Nematodirus Total	Total Worm Burden	Worms per day
1	64,100	4	903	9,300	10,400	84,800	1,194
2	51,500	6	725	2,500	2,200	61,800	870
3	24,200	15	341	1,100	1,400	27,500	387
4	23,120	4	326	800	2,000	26,640	375
5	43,100	5	607	3,400	1,500	48,320	680
6	84,320	9	860	5,200	4,400	93,920	958
7	84,900	9	866	7,900	2,800	96,000	1,000
8	106,600	12	903	0	4,800	111,400	944
9	86,200	25	647	28,200	48,100	163,200	1,220
10	139,000	72	965	12,000	7,600	148,600	1,031

Farm B

1	61,800	33	506	26,200	3,100	91,100	747
2	111,200	18	911	1,300	-	112,300	920
3	81,100	12	665	27,500	3,800	112,400	921
4	89,600	16	734	22,500	3,300	115,400	946
5	79,700	6	653	18,700	600	99,000	811
6	90,800	44	720	15,600	400	106,800	848
7	25,600	40	247	20,000	4,200	59,800	415
8	42,000	36	291	35,300	6,100	83,720	581
9	40,700	80	253	9,000	200	49,900	310
10	24,000	40	229	20,000	10,000	54,900	523

TABLE 14

The Cumulative Mean Total of Nematodes Recovered from the "tracer" Calves Compared with the Mean Total Worm Counts Found at Autopsy of the "permanent" Calves

FARM A.

Grazing Period	"Tracer" Calves		"Permanent" Calves	
	Cumulative Count		Mean Autopsy Count	
	No. of Calves	Worm Count	No. of Calves	Worm Count
25/5 - 4/8	10	54,450	5	49,810
25/5 - 31/8	14	129,840	2	95,960
25/5 - 20/9	16	162,850	1	111,400
25/5 - 5/10	18	188,275	1	163,200
25/5 - 16/10	20	216,515	1	148,600

FARM B.

11/5 - 10/9	18	114,580	5	106,040
11/5 - 28/9	20	140,860	1	106,800
11/5 - 2/10	22	146,770	2	71,760
11/5 - 19/10	24	165,470	2	52,400

group. From day 122 onwards the mean egg count increased progressively until it exceeded 1,000 e.p.g. by day 140.

Worm Counts at Autopsy

The worm counts of the "permanent" calves from both farms have been set out in Table 13. An estimate of the number of O.ostertagi and total nematodes which became established per day was calculated by dividing the respective total counts by the number of grazing days.

The relationship between the numbers of worms which became established in the "Tracer" calves and those established in the "permanent" calves was derived by adding the mean total worm count of each successive pair of "tracer" calves for the period prior to the last day of grazing of the "permanent" calves. As succeeding "permanent" calves were autopsied a cumulative total worm count for "tracer" calves was calculated.

The results of these calculations, for both Farm A and Farm B are shown in Table 14.

At the time when clinical signs first became apparent there was good agreement between the estimates of established worm populations in both "tracer" and "permanent" calves. However, after this time the numbers of worms becoming established in the "permanent" calves was less than the cumulative total found in "tracer" calves.

Differential worm counts were carried out on the

TABLE 15

The Biochemical Data from Autopsy Samples of Abomasal Contents Collected from the "permanent" Calves of Farms A and B.

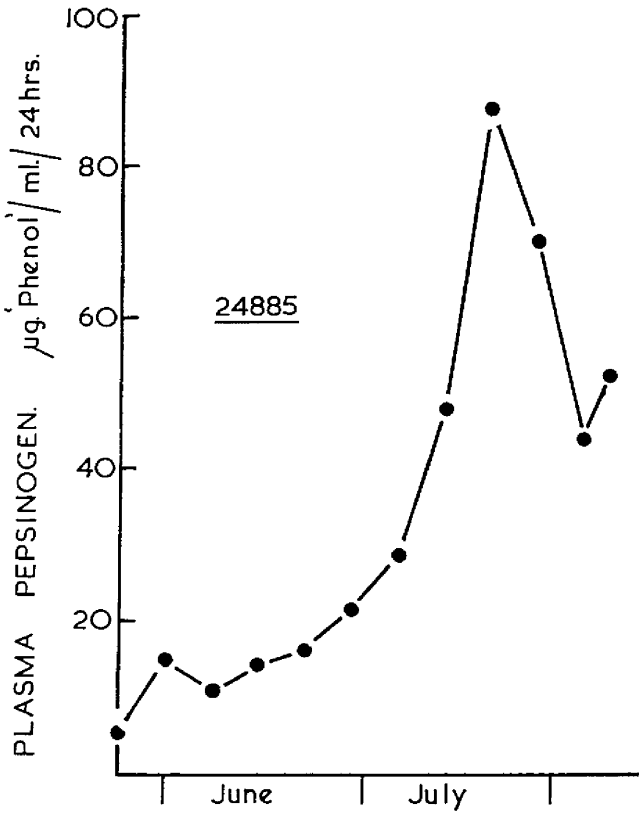
FARM A

Calf No.	Abomasal pH	Electrolyte Conc.m.e.q/l			Gastric Pepsin mg phenol/ml/hour
		Na^+	K^+	Cl^-	
1	6.25	106	19.2	123	0.125
2	6.05	106	16.0	119	0.135
3	4.90	104	11.8	120	0.157
4	2.95	92	13.8	127	0.070
5	6.65	104	23.8	104	0.076
6	7.05	124	12.4	114	0.093
7	7.15	128	7.0	101	0.069
8	N.S.	N.S.	N.S.	N.S.	N.S.
9	6.49	94	18	93	0.039
10	5.2	82	19.0	112	0.120

FARM B

1	6.70	113	17.0	114	0.075
2	5.90	104	15.2	116	0.104
3	6.35	115	8.4	132	0.055
4	7.05	121	9.4	110	0.060
5	7.10	124	10.8	117	0.064
6	6.90	124	15.2	112	0.065
7	6.80	130	29.6	104	0.011
8	3.60	66	6.8	118	0.108
9	3.00	68	18.8	108	0.046
10	3.85	92	16.0	118	0.095

N.S. = No Sample
m.e.q/l = milli equivalents per litre



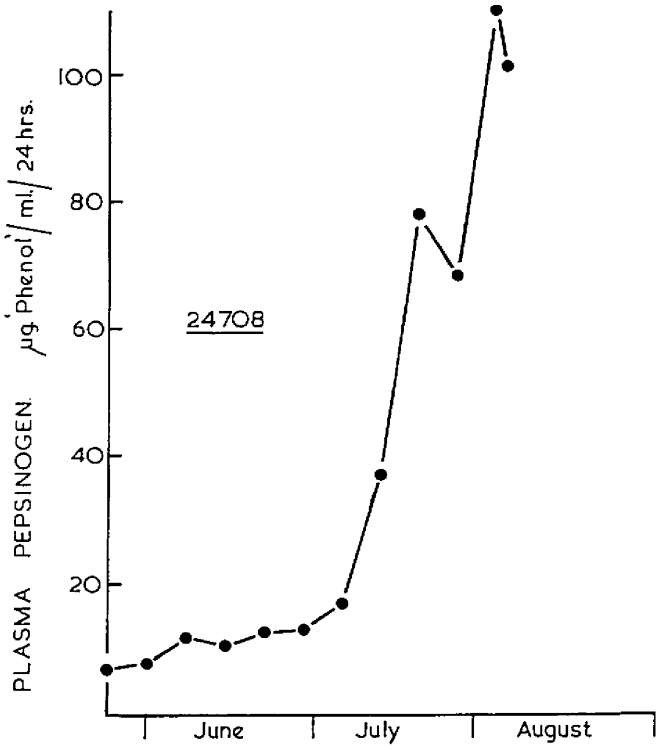
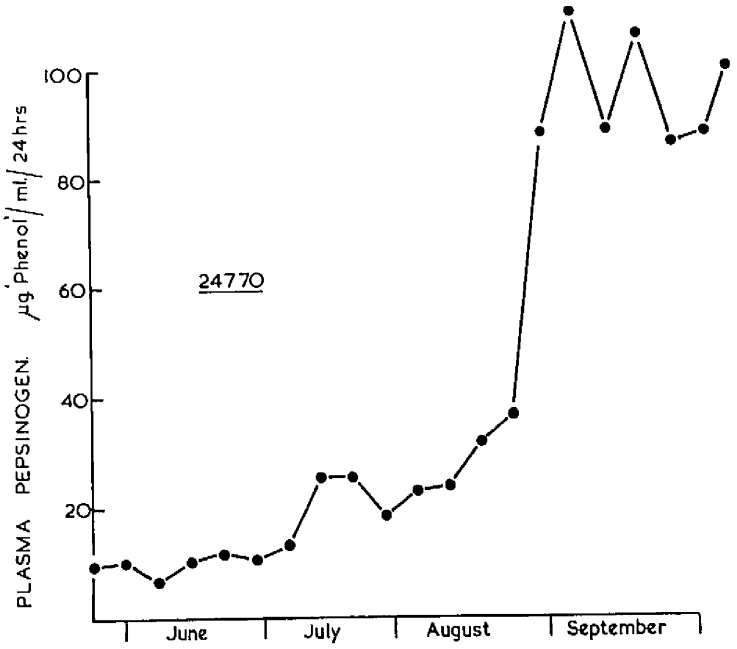


Fig. 14 Graphs of the change in plasma pepsinogen concentration of two "permanent" calves which were autopsied after 72 days grazing on Farm A.



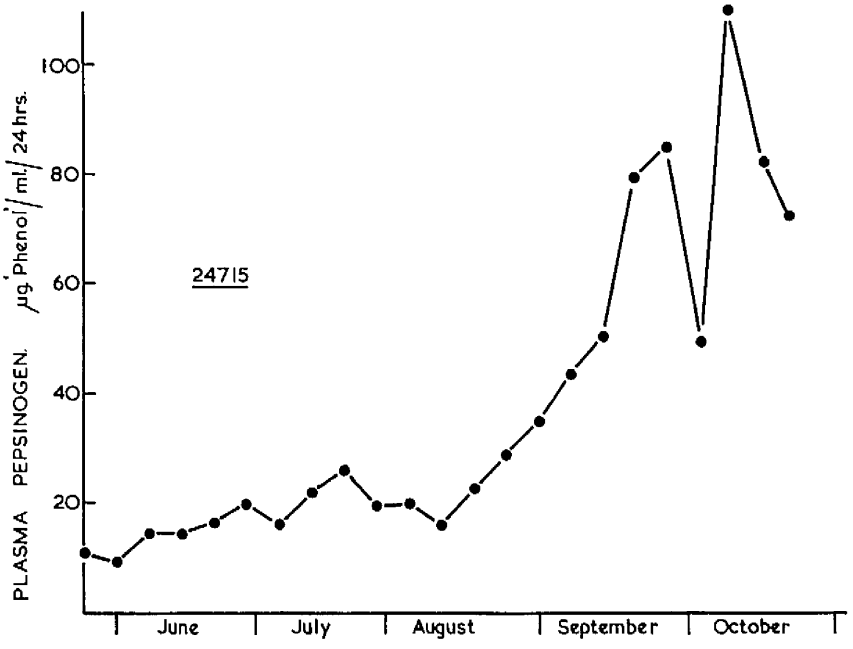
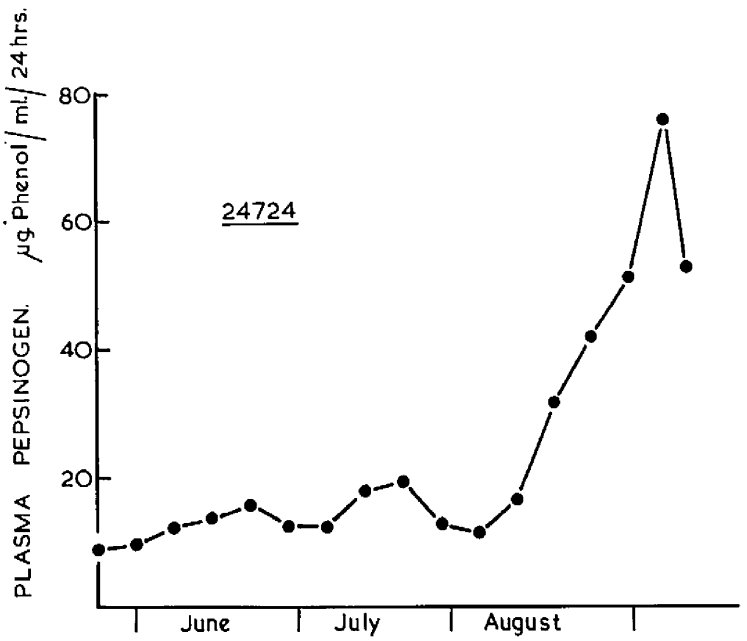


Fig. 15 Graphs of the change in plasma pepsinogen concentration of two "permanent" calves which were autopsied after 144 days grazing on Farm A.



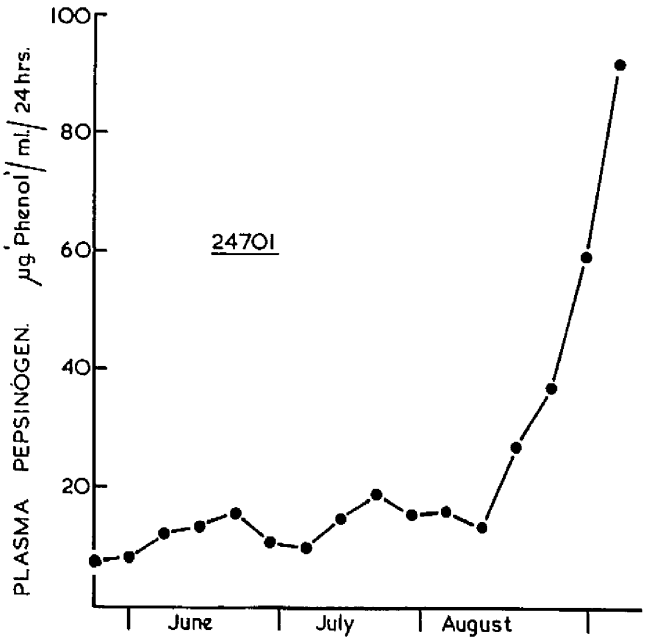


Fig. 16 Graphs in the change in plasma pepsinogen concentration of two "permanent" calves which were autopsied after 122 days grazing on Area B.

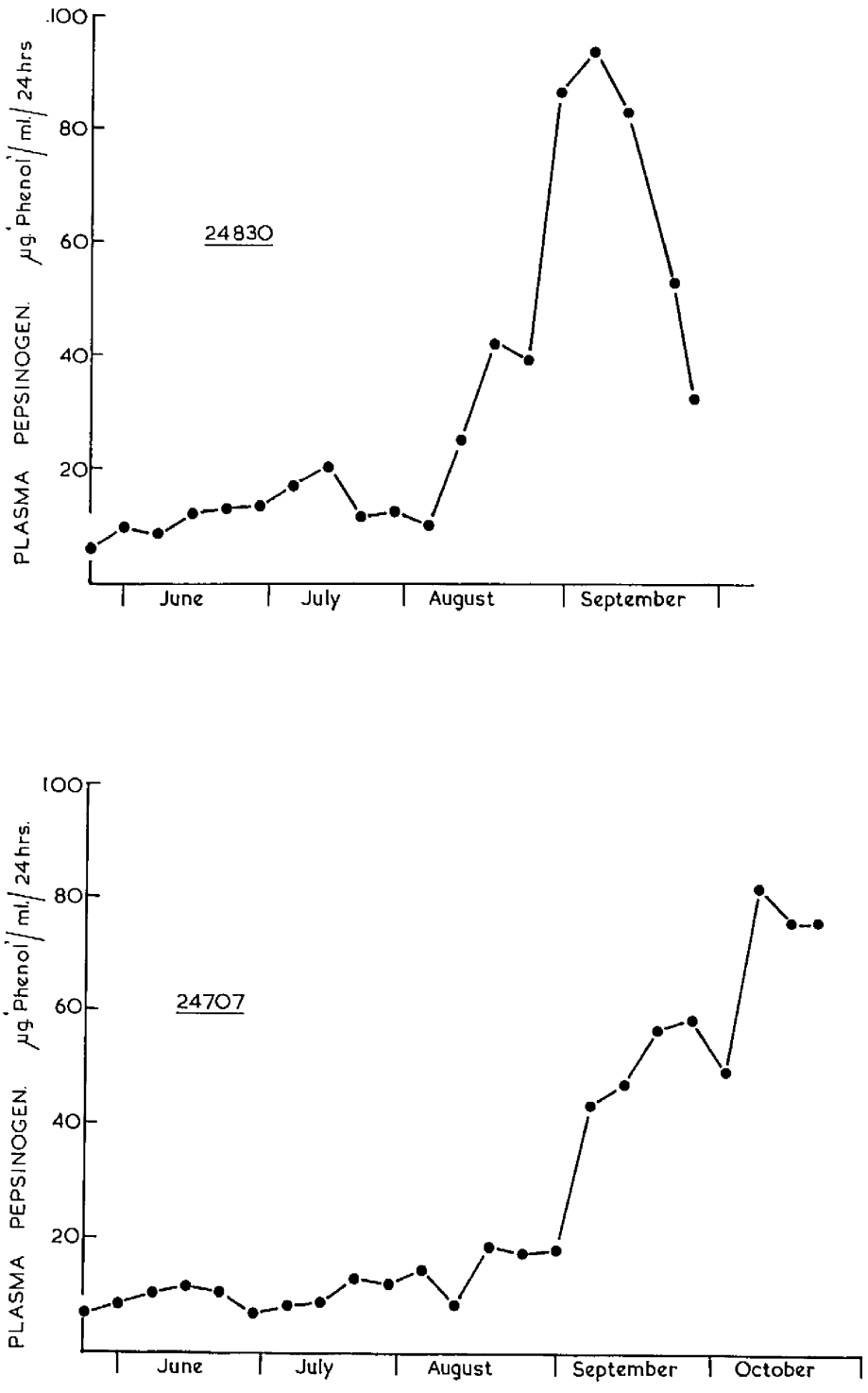


Fig. 17 Graphs of the change in plasma pepsinogen concentration of two "permanent" calves which were autopsied after 126 and 161 days grazing on Farm D.

contents plus washings and the mucosal digest of each third of the small intestine and these results have been included in Appendix C. Both Cooperia and Nematodirus spp. were more numerous in the first third of the intestine but in heavy infections substantial numbers were found in the second region. Only small numbers were recovered from the distal third of the small intestine. Up to 15 per cent of the total worm burden was recovered from the mucosal digests.

Biochemical Data

The results of determinations made at autopsy on abomasal pH, gastric electrolyte and gastric pepsin concentration are recorded in Table 15. These results were indistinguishable from those obtained from other outbreaks of Type I bovine ostertagiasis.

Figures 14 and 15 show the graphs of the change with time in the plasma pepsinogen concentration of individual calves from Farm A. Similarly, figures 16 and 17 depict graphs drawn from data collected from calves which grazed Farm B. There was considerable variation in the rate and degree of change in plasma pepsinogen concentration between individual calves on both farms. The rapid increase of the plasma pepsinogen concentration in individual calves was directly related to the onset of severe clinical signs and also to the number of O.ostertagi present at autopsy. Calves which developed the clinical disease later in the

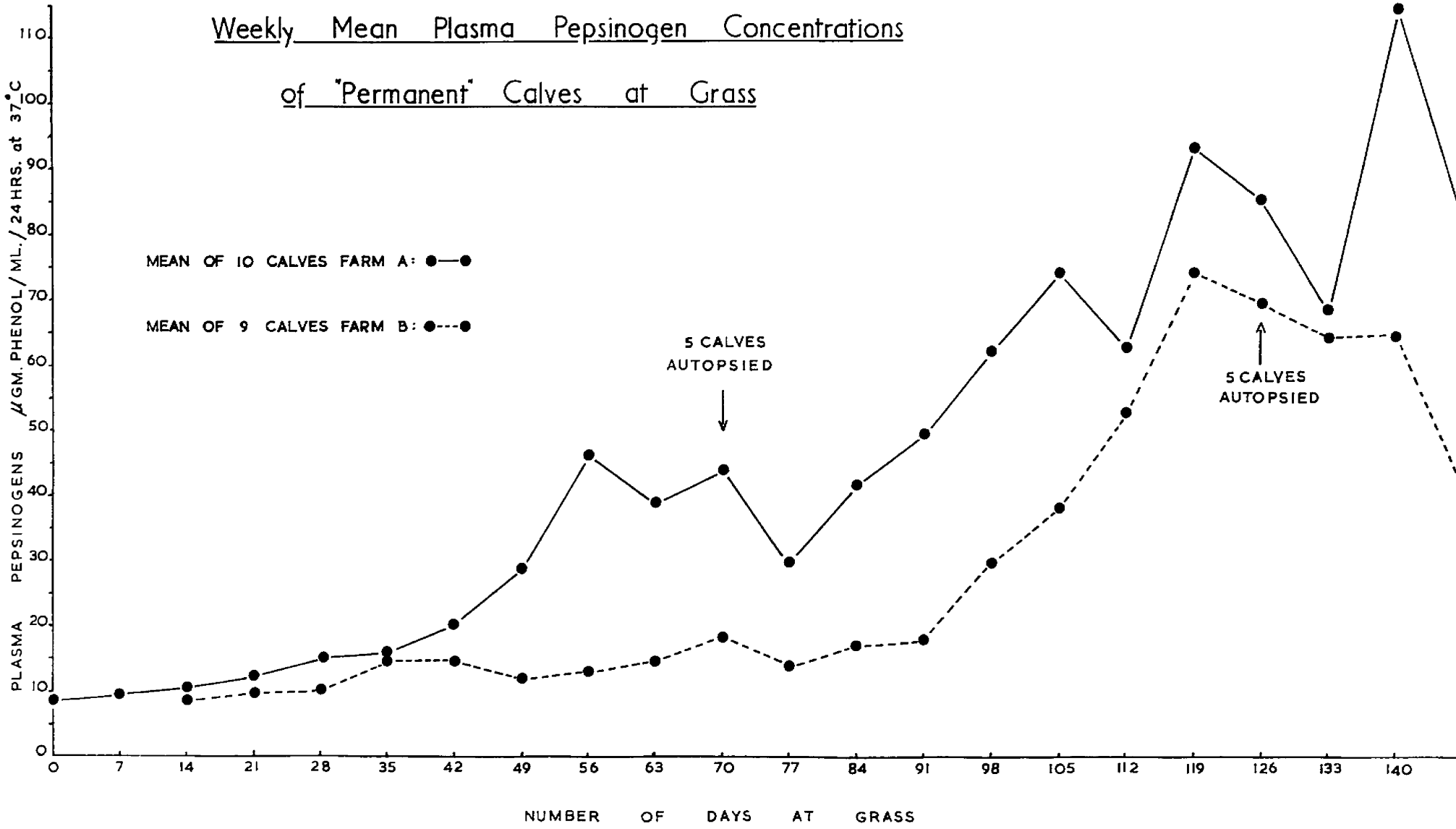
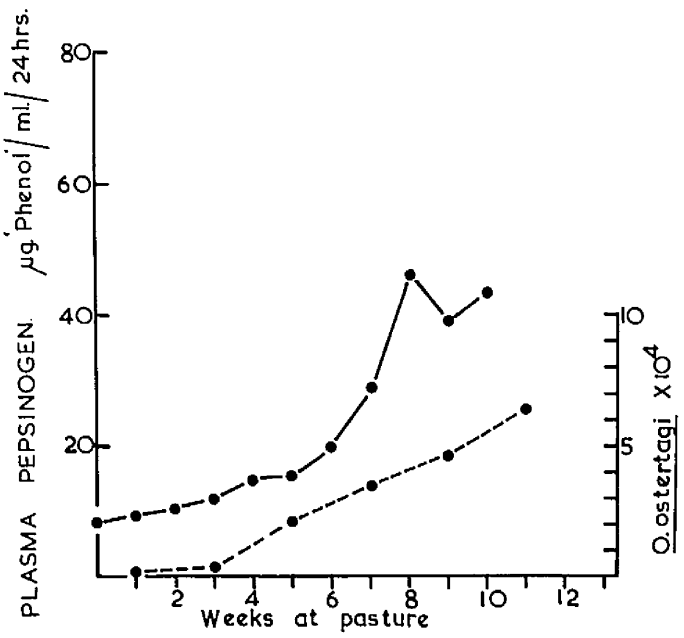


Fig. 18 A graph of the weekly change in mean plasma pepsinogen concentration of the "permanent" calves which grazed Farms A and B.



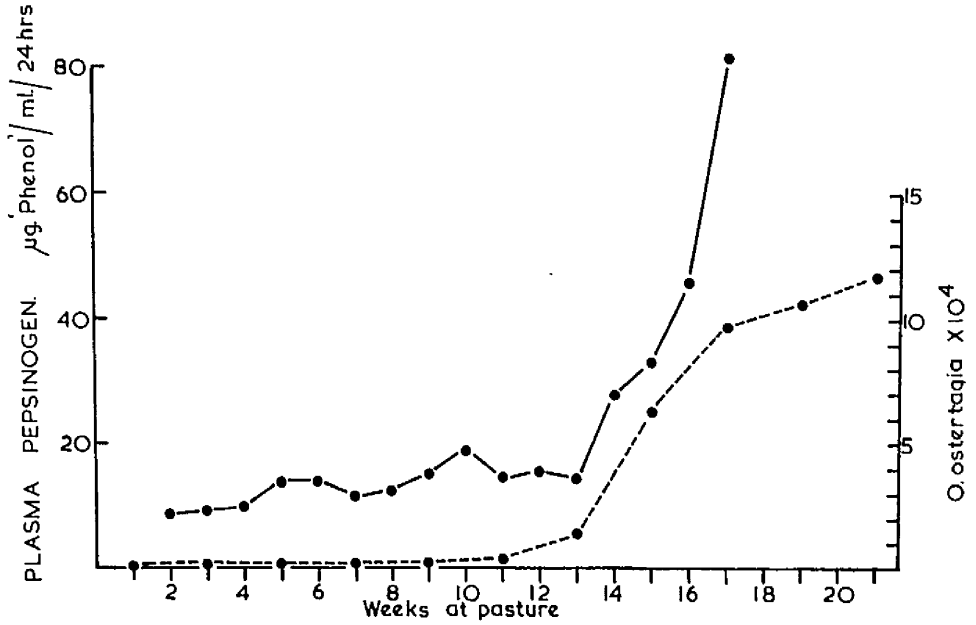


Fig. 19 Graphs of the relationship between the mean change in plasma pepsinogen concentration of the "permanent" calves and the mean number of O. ostertagi recovered at autopsy of the "tracer" calves. Above, data from Farm A. Below, data from Farm B.

experiment had low plasma pepsinogen concentrations during the earlier period.

A graph of the group mean change in pepsinogen concentration of calves from each farm is shown in Figure 18. The relationship between the plasma pepsinogen concentrations of the "permanent" calves and the mean number of O.ostertagi found at autopsy in the "tracer" calves is shown in Figure 19. The difference in response between calves on Farm A and those on Farm B was directly related to the numbers of O.ostertagi which became established.

Pathological Data

A full pathological examination was made on each calf and the lesions found were indistinguishable from those associated with the outbreaks of bovine ostertagiasis. A description of the main pathological findings has been given in Part 1. Only the relevant pathological conclusions will be discussed here.

Meteorological Data

Data relating to the mean weekly maximum and minimum temperature and relative humidity together with the total weekly rainfall are shown for both Farm A and Farm B in Figure 20.

No consistent relationship could be found between the meteorological observations made and the degree of

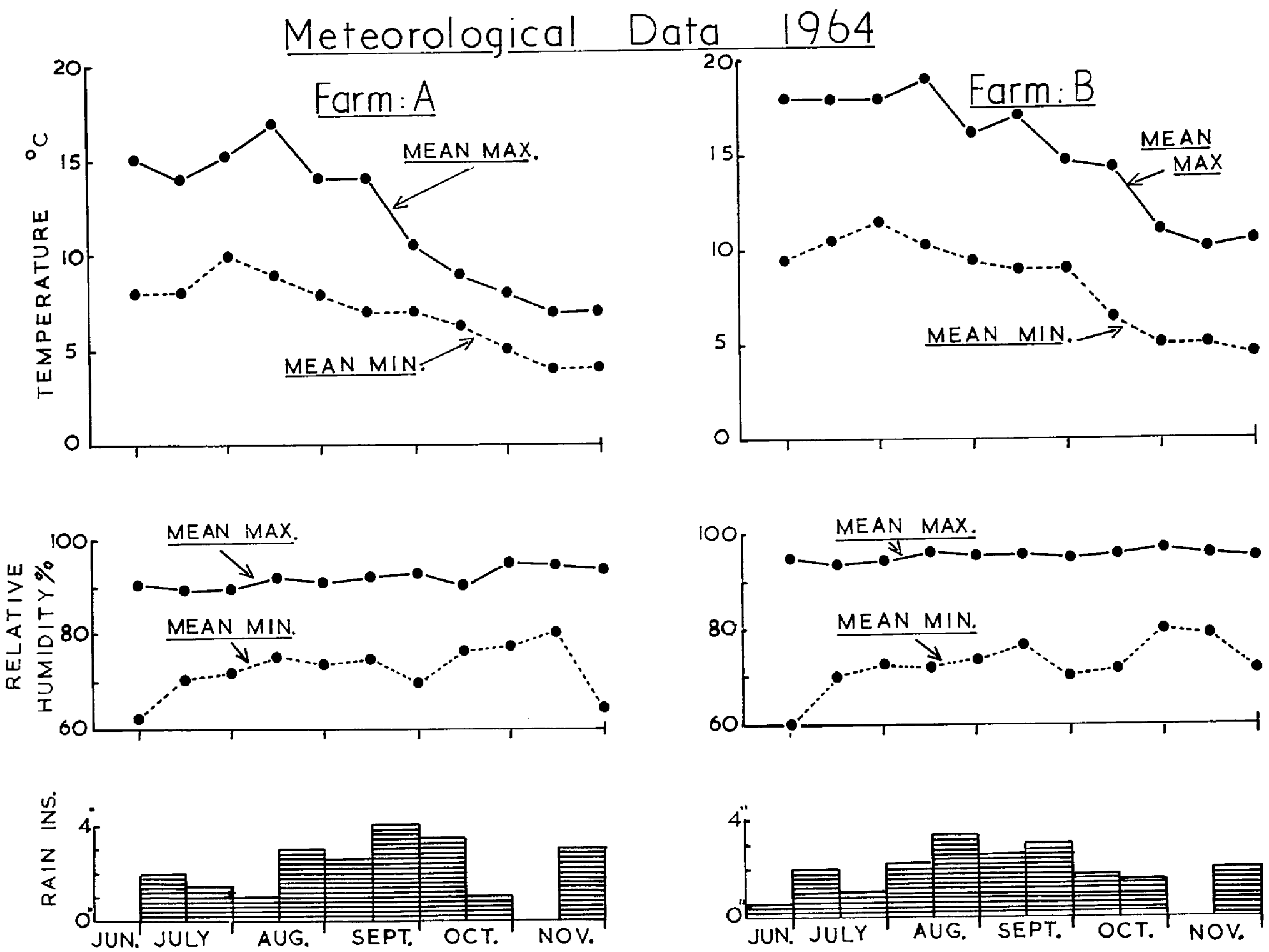


Fig. 20 Graphs of the meteorological data collected at Farm A and Farm B in 1964.

TABLE 16

The Ages, Grazing Periods, Bodyweight Data and Autopsy Details of the "replacement" Calves on Farms A and B

FARM A

Calf No.	Age Days	Dates Grazed	No. Days Grazed	Initial Weight	Autopsy Day	Autopsy Remarks	Autopsy Abomasal pH
1	115	8/9 - 21/9	12	128 lbs	12	Died	N.S.
2	115	8/9 - 29/9	21	164 lbs	21	Died	N.S.
3	115	8/9 - 1/10	23	142 lbs	24	In extremis	7.0
4	115	8/9 - 5/10	27	170 lbs	28	Died	N.S.
5	115	8/9 - 5/10	27	217 lbs	28	Died	N.S.

FARM B

1	115	8/9 - 14/9	6	108 lbs	10	Died	N.S.
2	115	8/9 - 7/10	29	165 lbs	31	In extremis	7.05
3	115	8/9 - 7/10	29	175 lbs	31	In extremis	7.02
4	115	8/9 - 7/10	29	154 lbs	31	Died	N.S.
5	115	8/9 - 14/10	36	148 lbs	37	Fair	N.S.

N.S. = No Sample

TABLE 17

The Autopsy Worm Count Data from the "replacement" Calves Grazing Farms A and B

FARM A

Calf No.	O.ostertagi Total	E4th Stage*	O.ostertagi per day	Cooperia Total	Nematodirus Total	Total Worms Burden	Worms per day
1	18,080	16	1,506	N.E.	N.E.	-	-
2	123,080	16	5,860	N.E.	N.E.	-	-
3	129,800	22	5,643	14,240	7,040	151,080	6,568
4	21,000	9	777	5,280	160	26,440	979
5	5,100	3	188	4,000	4,400	13,500	500

FARM B

1	16,000	42	2,666	8,000	80	24,080	4,013
2	87,180	46	3,006	20,720	2,320	110.220	3,455
3	87,560	23	3,019	60,720	3,280	151,560	5,226
4	53,940	8	1,860	N.E.	N.E.	-	-
5	53,520	27	1,480	32,000	4,480	90,000	2,500

N.E. = Not examined

* The percentage of the total O.ostertagi count present as early 4th stage larvae

parasitism which was found in calves grazing heavily infected pasture.

"Replacement" Calves

The prime object of the five "replacement" calves on each farm was to simulate the practice of turning out spring born calves with older autumn born calves because it appeared that the former animals were more susceptible to the disease. In addition it was likely, on the basis of previous experience, that at least half of the "permanent" group would have succumbed to the disease by September and it was thought necessary to maintain a constant stocking rate throughout the autumn grazing period.

The "replacement" calves were taken to the farms on September 8th and the details of their age, grazing period, initial weight and autopsy weight are given in Table 16.

Each one of the "replacement" calves developed severe Type I ostertagiasis within 4 weeks of being put on to the contaminated pastures. In all, six of the ten calves died on the farms and a further three were *in extremis* when brought back to the Veterinary Hospital. On Farm A, the clinical picture was complicated by a moderate degree of dictyocauliasis in four of the five calves. This disease did not occur in Farm B.

The results of the autopsy worm counts are shown in Table 17 from which it can be seen that heavy infections

of O.ostertagi resulted and accounted for the clinical signs observed. Inhibition was not a feature of the worm population present in these calves.

DISCUSSION

The use of parasite free calves to determine whether or not a given pasture was contaminated with species of bovine nematode larvae, has been reported by Goldberg and Rubin (1956); Goldberg and Tucker (1959) and Durie (1962). Each of these papers record that a single calf, 3 to 5 months of age, was used to estimate the availability of of larvae on artificially infected pasture plots over a short period of time at various intervals throughout the year. This is the first report of an attempt to relate the availability of larvae, assessed from the autopsy worm counts of "tracer" calves and the occurrence of outbreaks of bovine gastroenteritis under natural grazing conditions.

A significant feature of the "tracer" calf technique was the choice of a grazing period of 14 days and an autopsy interval of 4 days post grazing. These times were chosen because it had been shown earlier that, under experimental conditions, the adult O.ostertagi population decreased between the 3rd and 4th week post infection and that this decrease was marked following the onset of the clinical signs of bovine ostertagiasis. None of the "tracer" calves developed clinical signs during this study and in

addition at autopsy, marked changes in the pH or electrolyte concentrations of the abomasal contents were not recorded. It is likely, therefore, that the numbers of O.ostertagi and probably of all the nematode species found at autopsy were a true estimate of the parasites which became established in these calves.

Because the mean autopsy worm count of calves slaughtered at the onset of clinical disease is of the same order as the mean cumulative worm count of the "Tracer" calves, it follows that, despite the variability between calves of a tracer pair, the "tracer" calf technique provides a good estimate of the numbers of infective larvae which are available to the other young calves grazing the same field. It demonstrates also that O.ostertagi larvae are available throughout the entire grazing season, a finding which confirms the ecological studies made by Rose (1961) in the south of England. Calves had not grazed the calf rearing fields on either of the two farms since the previous autumn yet moderate numbers of worms are present in the first "tracer" calves. The number of larvae which survived the winter was not sufficient to cause an immediate outbreak of disease but readily infected the young calves, established a patent infection, and initiated the accumulation of infective larvae on the pasture. On both farms the mean faecal egg counts of the "permanent" calves quickly rose to

300 eggs per gram.

In 1964, some 9 to 16 weeks of continual larval intake were required before the characteristic clinical signs of bovine ostertagiasis appeared. Two factors which influenced the onset of clinical disease were the rate of larval intake and establishment of the clinical threshhold number. The rate of larval intake and establishment was initially lower on Farm B than on Farm A and this accounts for the delayed appearance of the onset of clinical signs on this farm. The clinical threshhold number of parasites, defined as that number which precipitates the appearance of clinical signs, appears to be related to the bodyweight of the calf during the infection phase, since the threshhold was shown to be some 50,000 in calves of about 170 lbs. weight and 100,000 in heavier calves of 210 lbs. weight.

When young "replacement" calves were introduced into the fields in September, pasture contamination was heavy, larval intake and establishment was high, and because the calves were smaller than the previous batch their clinical threshold number of parasites was quickly exceeded and they succumbed to typical Type I ostertagiasis. It had been noted in Part I that spring born calves were either the first or the worst affected calves of a group suffering from the Type I disease. The rapid onset and severity of the disease in the "replacement" calves confirms this observation and

clearly demonstrates that this management practice is unsound.

The common farm practice of rearing calves in the same small field year after year must also be condemned, because it readily facilitates the rapid accumulation of large populations of nematode larvae, many of which survive the winter to initiate the parasitic cycle in subsequent groups of susceptible calves.

The clinical syndrome which resulted, in the calves grazing the calf rearing fields on both farms, was indistinguishable from the Type I syndrome described in Part I. Affected calves were dull, miserable animals which lost weight quickly and characteristically showed a profuse, watery diarrhoea. Those animals which succumbed died shortly after the onset of severe diarrhoea, presumably as a result of the rapid fluid and electrolyte loss.

Anaemia and hypoproteinaemia, commonly associated with the Type II syndrome, were not observed in any of the affected calves but of the animals autopsied, the majority showed severe physiological and pathological changes within the abomasum.

Once the clinical disease became evident in the "permanent" calves, the agreement between the mean cumulative worm count of the "tracer" calves and the mean worm count of the "permanent" calves did not continue, Table 14. Also, some of the "replacement" calves had as great or greater a

number of parasites as the "permanent" calves which had grazed the fields throughout the experiment. Both the "tracer" and "replacement" calves were fully susceptible to the establishment of ingested larvae by virtue of their being worm free whereas the "permanent" calves had developed a measure of resistance. Reasons for this observed phenomenon may be many but the likely possibilities are considered. Firstly, the "permanent" calves, grazing throughout the summer, were heavily infected by September and these worm burdens may have caused a degree of inappetence which resulted in the ingestion of fewer larvae. An alternative explanation may be that the ingested larvae were unable to become established because of the damaged abomasal mucosa and the associated physiological changes in the host or that an immune mechanism caused a rejection of the ingested larvae.

From the results it can be seen that the mean O.ostertagi worm burden of the 13 "permanent" calves autopsied prior to October 1st was of the same order as the mean burden of 7 "permanent" calves autopsied after October 1st. However, the composition of the population in each case was markedly different in that in the former group only 4 to 33 per cent of the total population were early 4th stage parasites whereas some 28 to 80 per cent were inhibited in the latter group. The lower percentages of early 4th stages

in the first group could be largely accounted for by the development of recently acquired infection. The higher proportion of inhibited larvae in calves autopsied after October 1st was such that it could not be attributed to the development of larvae ingested on the last day of grazing. It was also noted that populations of Cooperia oncophora found in calves autopsied during October and November contained a high proportion of early 4th larval stages. The percentage of inhibited O.ostertagi larvae in calves autopsied at the end of the grazing season were comparable with those noted earlier in cases of Pre Type II and Type II ostertagiasis. This phenomenon can be explained in broad terms by either of two mechanisms.

Firstly, those animals autopsied after October 1st had developed a degree of immunity which either accelerated the loss of adult worms or inhibited the larval development at the early fourth stage. Support of this argument derives from the experimental work of Michel (1963) who showed that inhibition of larval development occurred in calves after 80 days of a daily infection of O.ostertagi larvae.

Secondly, that a change had occurred in the physiological makeup of the host and this change inhibited or arrested the larvae at the early 4th stage. Supportfor this suggestion arises from the post mortem worm counts of the

previously uninfected "tracer" calves, Table 11, which grazed the same fields as the "permanent" calves and which showed the same phenomenon at the same time of year, that is after October 1st or during the late autumn period. It would seem unlikely that the "tracer" calves grazed in autumn were immunologically more effective than those grazed at other times of the year when the rates of parasite establishment were equivalent. Thus the striking feature of inhibition, or Pre Type II, is that it appeared during a limited period of the grazing season and was present in calves which had grazed infected pasture for six months as well as in calves which had grazed the same fields for only 14 days.

During the late autumn grazing period several factors occur coincidentally which may alter host physiological mechanisms. It can be seen from Fig. 20 that the mean maximum and the mean minimum temperatures were declining during this period. Also, the hours of daylight were decreasing with the approach of winter. It can be assumed therefore that the rate of pasture growth declined during autumn and this may have resulted in a decreased nutritional plane of the immature bovines grazing this pasture. Under similar conditions Blaxter and Wainman (1961 and 1964) and Blaxter (1964) have demonstrated significant changes in the metabolic rate of Ayrshire cattle subjected to cold and

and windy environments. Post (1965) has shown that a decrease in nutritional plane of yearly cattle results in a decrease of circulating thyroxine. It would be of value to know whether inhibition of Larval development was associated with either the thyroid or the pituitary hormone status of the host.

Finally, the possibility exists that the altered climatic environment effected a change in the infective larvae which developed during this period.

From a diagnostic and prognostic point of view, the faecal egg counts of infected animals are difficult to interpret. Counts from the same individual fluctuated considerably on different examination days. At the onset of the outbreak calves with 1,000 eggs per gram were clinically affected; however, later in the season severely affected calves often had low faecal egg counts. A negative egg count does not remove bovine ostertagiasis from the differential diagnosis.

Plasma pepsinogen concentration on the other hand provided a sensitive indication of the degree of abomasal damage present in infected calves and therefore gave an indirect assessment of the numbers of O.ostertagi present in the living bovine. The mean change in plasma pepsinogen concentration, shown in Figure 18, was closely related to the larval intake and establishment of O.ostertagi which

was assessed by autopsy worm counts (see Figure 19 also). Furthermore, the mean concentration of plasma pepsinogen clearly distinguished the course of the outbreak on each of the two farms studied and in this regard proved to be of greater predictive value than was the mean faecal egg count. The figures 14 to 17 inclusive show that differences between calves within a group can be usefully interpreted and therefore it is concluded that the plasma pepsinogen concentration would be a valuable aid in epizootiological studies of bovine ostertagiasis.

SUMMARY

1. A field experiment was carried out in which ten parasite free calves 2½ months of age, were put out to graze the calf rearing fields of each of two Ayrshire farms. These "permanent" calves grazed from May to November and were autopsied when they developed clinical signs characteristic of Type I bovine ostertagiasis. A series of "tracer" calves grazed the same fields as the "permanent" calves for 14 days and were autopsied 4 days after removal from the pasture, in order to assess the parasite intake of the "permanent" calves.

2. Clinical disease developed in each of the 20 "permanent" calves; during the grazing period O.ostertagi was the predominant pathogen and between 50,000 and 60,000 adult O.ostertagi were required to produce Type I ostertagiasis in calves 4 to 6 months of age. Older calves acquired heavier infections and young calves, put out in September, developed severe Type I ostertagiasis in less than one month.

3. The disease first appeared on Farm A 9 weeks and on Farm B 16 weeks after commencement of grazing. The rate of parasite establishment in the "tracer" calves did account for the difference between farms and it proved to be a good estimate of the number of parasites established in the "permanent" calves prior to development of clinical signs in the latter. After this time this relationship did not hold

true and reasons for this are discussed.

4. Plasma pepsinogen concentrations were reliable but faecal egg counts were unreliable indicators of the degree of infection in individuals and groups of calves.

5. Anaemia and hypoproteinaemia were not observed in calves affected with Type I ostertagiasis but severe changes in abomasal physiology were noted and they were directly related to the degree of pathological change.

6. Inhibition of larval development occurred in both "tracer" and "permanent" calves autopsied from October to November but not at other times of the grazing period. Possible explanations for this phenomenon are given.

SECTION THREE

EXPERIMENTAL INFECTIONS OF

OSTERTAGIA OSTERTAGI IN CALVES

SECTION THREE

EXPERIMENTAL INFECTIONS OF OSTERTAGIA OSTERTAGI IN CALVES

PART 1 INTRODUCTION

PART 2 EXPERIMENT 1 - THE COURSE OF A SINGLE INFECTION OF 100,000 O.OSTERTAGI LARVAE

PART 3 EXPERIMENT 2 - SINGLE INFECTIONS USING FIVE GRADED DOSE LEVELS OF O.OSTERTAGI

PART 4 THE RESULTS OF TWO RE-INFECTION REGIMES WITH O.OSTERTAGI LARVAE.

PART I INTRODUCTION

A Review of Reported Experimental Infections of Ostertagia ostertagi in Calves

The literature which concerns the experimental infections of O.ostertagi has been reviewed with the purpose of establishing the types of experiments carried out, the conclusions reached and to provide background for a series of experiments designed to produce, in the laboratory, all of the characteristics of naturally occurring bovine ostertagiasis. The review deals with work published up till 1963 and subsequent papers are dealt with in the relevant discussions.

Experimental infections with pure cultures of O.ostertagi larvae have been carried out by various workers to study:

The morphogenesis of the parasitic stages.

The host reaction to the parasite.

The efficiency of anthelminthic drugs.

Morphogenesis of the Parasitic Stage

Threkeld (1946); Porter and Cauthen (1946); Douvres (1956) and Threkeld (1958) have studied the development of the parasitic stage within the abomasum and have given good descriptions of the growth and differentiation of O.ostertagi A brief account of this has been given in the Introduction,

"A Literature Review of Ostertagia ostertagi; A Parasitic Nematode of the Bovine Abomasum."

Host reaction to the Parasite

Threlkeld and Johnston (1948) failed to produce clinical parasitism in 5 calves, 2 to 6 months old, when they administered, over a period of 3 to 5 months, a total of 12,408, 49,390, 129,400, 216,373 and 14,800 O.ostertagi respectively.

Cauthen and Landram (1958) dosed each of seven 9 month old Jersey calves with 118,000 to 224,000 O.ostertagi, which were given over a period of a month. The degree of parasitism which resulted did not affect weight gain, food consumption or food utilisation during an eight week post infection period. Four calves were autopsied 63 days after initial infection and a mean count of 23,000 O.ostertagi was recovered. It is clear that with respect to the age of calf used in the above experiments, either the size of the larval dose administered or the percentage of parasites which became established was too small for the development of clinical parasitism.

Herlich (1959) used groups of 3 Jersey calves 4 to 5 months old and compared the effects of single doses of 250,000 O.ostertagi larvae with a similar number of Trichostrongylus axei larvae and a mixed O.ostertagi - T.axei infection. All infected calves showed anorexia, diarrhoea and weight loss when compared with uninfected controls.

However, there were no changes in the haematological or biochemical criteria measured throughout the 6 weeks of the experiment. Calves with the mixed infection of 125,000 O.ostertagi and 125,000 T. axei showed a greater degree of change than either of the other two groups and this was accounted for by the greater total number of worms which became established. In the mixed infection group 48 per cent of the larval dose became established compared with 17 per cent and 10 per cent for T. axei and O.ostertagi respectively; 99 per cent of the worm populations were adults. The weight changes noted occurred with the onset of diarrhoea, which was observed as early as the second week of the infection. In a similar experiment three calves were given 500,000 O.ostertagi, 500,000 T. axei and 250,000 larvae of each species respectively; the percentage of worms which became established were 46, 16 and 62 per cent respectively. On the basis of one calf which died 23 days after dosing, Herlich concluded that a larval dose of 500,000 O.ostertagi is lethal for calves whereas a similar number of T. axei is not; therefore, O.ostertagi is more pathogenic than T. axei. Because of the small group size, the variation in the numbers of worms established within and between groups was such that it is doubtful whether the author was justified in reaching this conclusion.

Davis (1959) infected calves less than one month of age with O.ostertagi larvae, with Eimeria spp. oocysts, and

with both parasites. He found that clinical signs of parasitism were more evident in the Eimeria infected than the O.ostertagi infected calves. Mixed infections did not enhance the development of either parasite. Because of the small group size and the age of the calves, little significance can be attached to the findings.

Andersen, Graff, Hammond, Fitzgerald and Miner(1960) compared the haematological changes induced in 3 to 4 month old calves by separate infections of O.ostertagi and Haemonchus placei. Four calves given 300,000 O.ostertagi larvae showed clinical signs of parasitism with a reduction in weight gain and a marked decrease in serum albumin concentration. Unfortunately this abstract makes no mention of the haematological findings in the O.ostertagi infected groups or of the numbers of parasites which became established. A full account of this experiment has not been reported in the available literature.

Ross (1963) studied the effects on calves of single infections of 2,000 and 100,000 O.ostertagi larvae and compared these with infections of 2,000 followed by 100,000 and 100,000 followed by 100,000 at three week intervals. One calf died three weeks after a single infection of 100,000 and one calf showed intermittent diarrhoea following a second dose of 100,000 larvae while the other 21 calves showed no adverse effects. No significant changes were

observed in the haematological criteria or serum albumin levels. Despite the presence of large numbers of mature worms the faecal egg counts were low and fluctuated erratically egg counts fell to 100 to 400 eggs per gram, 2 to 3 weeks post patency. As the initial dose of larvae was increased from 2,000 to 100,000 the number of worms becoming established decreased from 50 to 19.6 per cent of the larval dose: only a very small percentage were present in the 4th stage at 4 to 6 weeks after infection. A loss of the adult population appeared to occur between the fifth and eighth week post infection.

Michel (1963) studied the host resistance and the course of infection of O.ostertagi in calves. A daily dose of 1,500 larvae was given to each of 20 calves for up to 300 days. Calves were killed and autopsied at intervals during this period. Only two calves failed to develop resistance to the parasite and these succumbed 85 and 158 days after the initial infection: these calves had a total worm burden of 37,000 and 31,200 respectively. The results of this regime of daily dosing were interpreted by Michel to indicate the development of hose resistance in that :

(1) A constant loss of adult worms apparently occurred and appeared to depend on the number of adults present, a greater loss occurring with a large adult population.

(2) An inhibition of development occurred at the early 4th stage and this seemed to depend partly on the presence of adults. He considered that the inhibition of development was reversible, that is, when adults were lost inhibited larvae resumed development.

(3) Stunting of adult worms occurred. Those parasites developing late in the infection failed to attain the length of those which developed earlier.

(4) Mature female worms, which resulted from later infections, produced a smaller number of eggs but this was independent of the reduction in size which occurred.

(5) A resistance to the establishment of newly acquired worms began to operate after prolonged exposure to infection.

The pathological changes noted following the experimental infections made by Porter and Cauthen (1946); Threkeld and Johnston (1948); Osborne, Batte and Bell (1960) and Ross (1963) have been described by these workers in general terms. In each case the lesions, usually small raised areas of mucosa, were confined to the abomasum and duodenum of infected calves.

Grossly, small white plaques or nodules, 1 to 2 mm. diameter, situated on or between the abomasal folds, were

noted as early as 6 hours after infection by Osborne, Batte and Bell (1960), and at 24 hours post infection the gastric folds were erythematous. Lesions in the pyloric area were less numerous than in the fundic area. At 4 days these circumscribed, slightly raised plaques appeared to have a clear centre. No marked change occurred in the lesion type after this time but at 35 days lesions were reduced in size and 46 days after a single infection of 30,000 larvae the characteristic plaques were indistinct.

As a result of two infections of 100,000 O.ostertagi larvae 3 weeks apart, Ross (1963), one out of 6 calves died with an acute abomasitis which was particularly marked in the fundic area. Associated with the inflammation was a number of raised pin point lesions surrounded by an intense haemorrhagic zone. Submucosal oedema was not present in the dead calf but was observed at autopsy in other calves of the same group. In these latter cases the fundic mucosa was wrinkled, had an intense red appearance and an oedematous thickening of the folds was evident. Inflammation of the pyloric area was generally less marked and in two cases a severe reaction was noted in the first 6 to 8 inches of the duodenum. Abomasitis was milder in calves receiving fewer larvae. Ross concluded that the severity of the lesion primarily depended on a superimposed challenge after a previous experience of infection but it was also influenced

by the size of the larval dose.

Threkeld and Johnston (1948), investigating the effects of multiple infections of O.ostertagi, noted that the histological lesions were characterised by a round cell infiltration surrounding the larvae, a localised eosinophilia, oedema and erosion of the mucosa which sometimes involved the muscularis mucosa. Osborne et al. (1960) dosed calves of varying age with 10,000 to 175,000 O.ostertagi larvae and autopsied the calves at regular intervals. The general conclusion of these authors was that the tissue reaction was less than might have been anticipated. In their descriptions they noted that early in the infection focal capillary dilatation with very slight oedema was seen. Four days after infection a slight leucocytic infiltration was observed together with a moderate hyperplasia of the lining epithelium of parasitised glands. As larvae developed the dilatation of the gland increased, reaching a maximum at about 19 to 21 days. Concurrently there was a marked leucocytic infiltration consisting of many eosinophils, large and small lymphocytes. Marked submucosal oedema was noted in one calf. Most larvae had left the gland by day 25 and many empty glands were involuting, a process which was almost complete by day 46 post infection.

THE EFFICIENCY OF ANTHELMINTHIC DRUGS

The controlled anthelmintic trial is becoming recognised as a better method of assessing potential anthelmintic drugs and consequently is gaining wider use. In this test two suitably sized groups of animals are dosed with equal numbers of infective larvae. One group is treated with the test anthelminthic, then a few days later, both groups are autopsied at the same time. From a comparison of the total worm burden from animals in each group, the percentage efficiency of the drug can be calculated. Mosky and Harwood (1941); Steward (1955); Reinecke (1963).

Banks and Michel (1960) assessed the value of the drug, O,O-dimethyl 2,2,2-trichloro-1-hydroxyl methyl phosphonate (Neguvon) as an anthelmintic drug against O.ostertagi. Jersey calves 4 months old in groups of 2 were given 42,000 larvae and an oral dose of Neguvon was administered on the 7th, 15th, 21st and 28th day of infection. Two dose levels of 66 and 110 mg per kg body weight were used. The results show that efficiency was high 96 to 98.5 per cent against 28 day adults and 64 to 65 per cent against 21 day adults. There was little effect on mature worms. Both dose rates gave comparable results. Toxic symptoms were observed and appeared to be due to individual idiosyncrasy.

Armour (1964) used a similar experimental design but with 5 animals per group to test the efficiency of O, O di-

(2-chloroethyl)O-(3-chloro-4 methcoumarin-7-yl) Phosphate (Haloxon) at the dose rates of 40 mg per kg and 100 mg per kg. The conclusions were that this drug was highly effective (97 per cent) against fully mature adult worms (28 days), moderately effective (64 per cent) against young adults (21 days), and almost ineffective (28 per cent) against immature stages (7 and 14 days). Increasing the dose rate to 100 mg per kg did not improve the efficiency against the 7 day immature stages. No signs of toxicity were observed.

SUMMARY

1. The morphogenesis of the parasitic phase of the life cycle of O.ostertagi has been adequately defined. However, a clear picture of the successive pathological change related to the morphogenic stage is not available in the current literature.

2. Regimes of multiple infections of O.ostertagi have been reported by four authors. Generally, these regimes have not been successful in producing clinical disease. The authors do not state on what basis their choice of dose rate or infection regime was made.

3. Clinical disease was observed following the administration of single infection of between 100,000 and 300,000 infective larvae. Within the first six weeks post infection, changes in various haematological and serum protein criteria were not noted in the diseased animals. A study of the relationships between results obtained from experimental infections and field cases of ostertagiasis, on the one hand, and the development of the abomasal lesions on the other, has not been undertaken.

4. Resistance to the establishment of newly acquired worms has been shown to operate after prolonged exposure to infection and inhibition of development was noted to occur after eleven weeks of daily administration of 1,500 infective larvae. Again, the significance of these events to field

conditions, where the intake of larvae may fluctuate both in quantity and regularity, is not known.

5. These anthelmintics which have been adequately tested have shown a high degree of efficiency in removing adult populations of O.ostertagi but they have a more erratic and less efficient effect against the immature stages.

EXPERIMENT 1

THE COURSE OF A SINGLE INFECTION OF

100,000 O. OSTERTAGI LARVAE

IN AYRSHIRE CALVES

INTRODUCTION

From a consideration of the review, which relates to previous reports on experimental infections of O.ostertagi in calves, it is apparent that many important features of the disease are, as yet, unexplained.

It would seem that the first experiments should be directed towards establishing worm populations in calves characteristic of those which have been recorded from the naturally occurring disease. Once the different phases of bovine ostertagiasis can be readily reproduced under laboratory conditions then other techniques can beemployed to assess the nature and degree of the pathogenic effects which O.ostertagi inflicts upon the young bovine.

More specifically, information relating to the following three aspects of bovine ostertagiasis are considered essential for a better understanding of the disease and consequently the purpose of this series of experimental infections is to determine:

1. The minimum worm burdens necessary to produce the clinical syndrome characteristic of the natural disease.
2. The correlation between the pathological changes in the abomasal mucosa, the physiological changes of the host and the parasite population within the abomasum.
3. The course of inhibition of larval development

and the relationship of the immature stages to clinical disease.

The Course of a Single Infection of 100,000 Ostertagia ostertagi Larvae in Dairy Calves

The first experiment was an attempt to assess the relationship between a single dose of 100,000 3rd stage O.ostertagi larvae and the associated abomasal changes; pilot experiments had shown that a single dose of 100,000 larvae produced pathological changes without severe clinical disease.

MATERIALS AND METHODS

Animals

Eighteen male Ayrshire calves were reared parasite free to 8½ weeks of age, paired on a weight basis and each calf was then given a single dose of 100,000 3rd stage O.ostertagi larvae.

Observations

The calves were weighed and samples of blood were collected at weekly intervals. Daily samples of rectal faeces were examined by a modified McMaster egg counting technique and the faecal consistency on each occasion was graded as (a) normal, (b) soft D+, (c) Semi-fluid D++ (d) fluid D+++.

Autopsy

Calves were autopsied in pairs at 2, 4, 8, 12, 16,

21, 28, 60 and 90 days post infection. Details of the materials and methods used in this experiment have been given in Section 1.

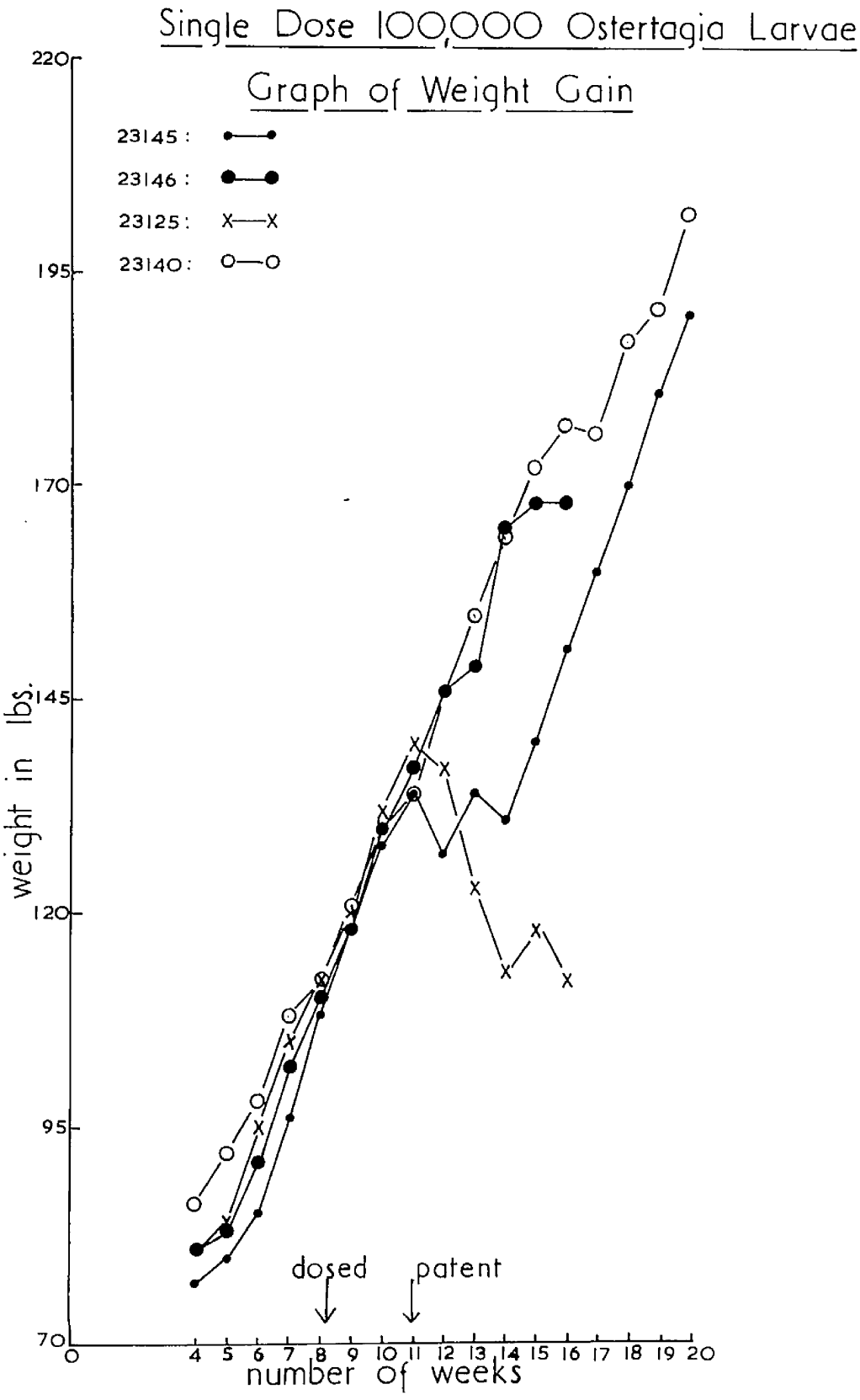


Fig. 21 Graph of weight gain of calves infected with a single dose of 100,000 O. ostertagi larvae

RESULTS

Clinical Observations

No change in faecal consistency was noted in any calf during the first 19 days of infection. Softening of the faeces occurred in 19 to 21 days post infection and of the six calves remaining alive after day 21, four developed an intermittent diarrhoea, the faeces being semifluid or fluid. Three of the affected calves had normal faeces by day 28 but the remaining calf, No.15, showed an intermittent diarrhoea up to day 44. A decrease in appetite occurred in all calves during the diarrhoeic phases and some calves were anorexic.

Weight gains were normal until day 21 post infection and the graph (Fig. 1) shows the weights of the four calves kept to day 60 and 90. Two of these calves maintained a growth rate of 1 lb. per day throughout the experimental period. Of the remaining two, one calf, No.18, showed a check in growth rate over a three week period and then continued to grow at the same rate as before; 6 weeks later at day 90 this calf was 12 lbs. lighter than the other of the pair. The other calf, No.15, continued losing weight until it was killed in extremis on day 59. At this point it was 50 lbs. less than its fellow. These two calves which lost weight were clinically the worst affected.

TABLE 18

Calves Given a Single Dose of 100,000 O.ostertagi Larvae

Post Mortem Worm Counts and Abomasal pH

Day Killed	Calf No.	No. of Adults	No. of Immatures	Percentage Established	Sex Ratio M/F	Abomasal pH
2	1	0	12,980	12.9	-	2.95
	2	0	9,480	9.4	-	3.00
4	3	0	19,920	19.9	-	3.95
	4	0	13,108	13.1	-	1.90
8	5	0	35,573	35.5	-	3.45
	6	0	31,253	31.2	-	1.55
12	7	0	16,440	16.4	1/1	4.45
	8	0	54,550	54.4	1/1	2.30
16	9	48,400	0	48.4	1/1	2.90
	10	38,800	0	38.8	1/2	2.85
21	11	6,640	0	6.6	5/2	2.70
	12	10,000	160	10.1	2/1	4.35
28	13	3,680	280	3.88	1/1	3.65
	14	2,280	200	2.48	1/2	2.45
60	15	3,480	0	3.4	1/1	-
	16	720	0	0.7	5/1	5.0
90	17	160	0	0.16	1/1	2.10
	18	80	0	0.08	0/1	1.95

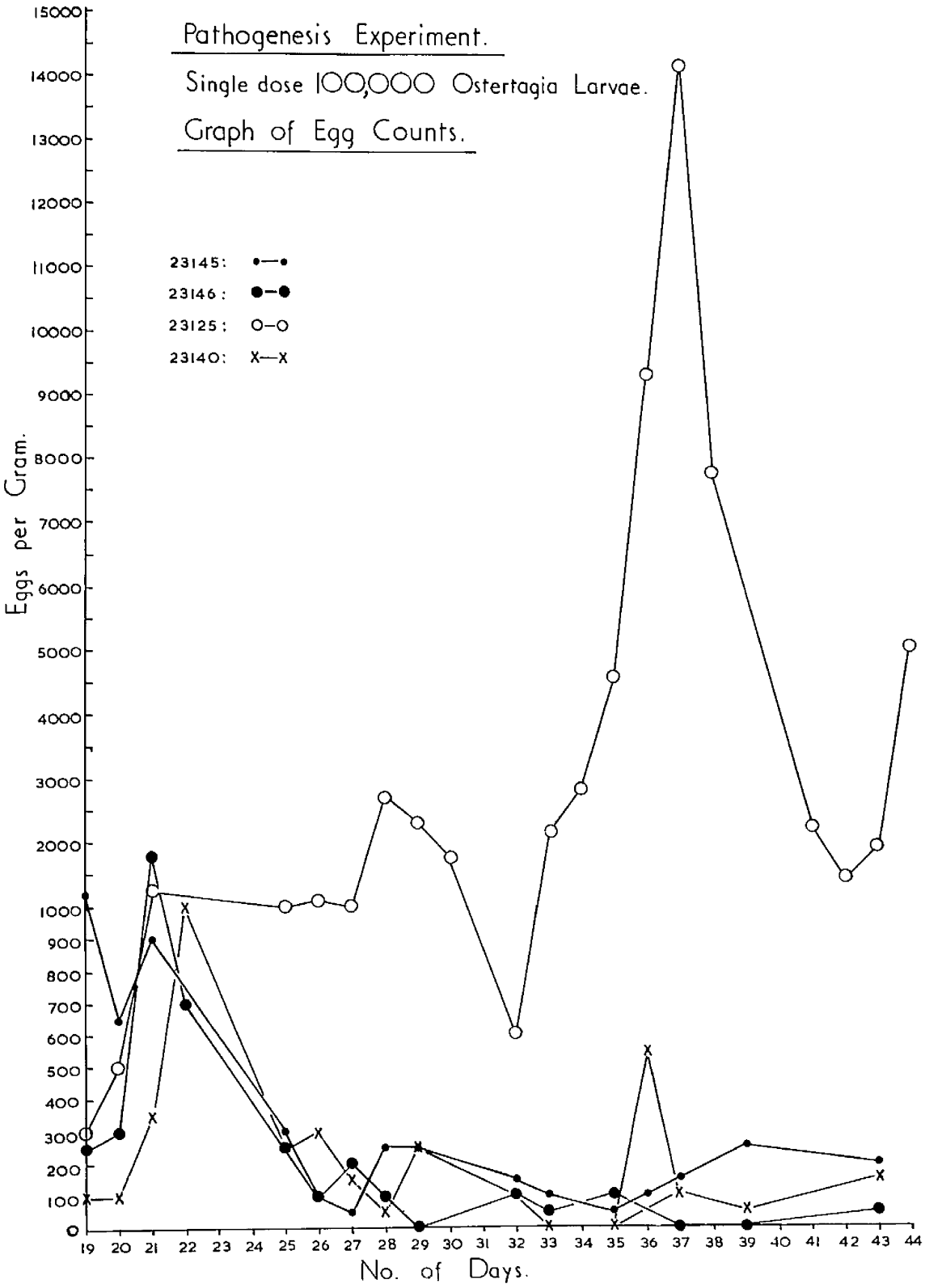


Fig. 22 Graph of faecal egg counts of calves infected with a single dose of 100,000 O.ostertagi larvae

Haematological Data

There was no change in packed cell volume, haemoglobin concentration, total serum proteins or albumin concentration during the experimental period.

Abomasal pH

Samples from calves Nos. 8, 12 and 16, which had pH values of 4.4, 4.3 and 5.0 respectively, were the only ones significantly different from the normal value of 2.1$\frac{4}{}$ 0.4 (see Table 18).

Parasitological Data.

Worm Egg Counts

The graph (Fig.22) traces the faecal egg counts of the calves with infections lasting 60 and 90 days. In three of the four calves there was a sharp rise to a peak shortly after patency and an equally sharp decline one week later. Subsequently, egg counts of low values persisted for 90 days. The egg count of the fourth calf remained high for 47 days, when it fell abruptly to the level of 250 e.p.g.; during a period of inappetance 5 days prior to autopsy the egg count again rose to 1,000 e.p.g.

Worm Counts at Autopsy

Table 18 shows the results of the worm counts from the calves which were autopsied at different intervals post infection.

Day 2. From an examination of the worm counts from the pair

of calves autopsied 2 days post infection it was found that all the parasites present were exsheathed third stages and they were present in the mucosal digest.

Day 4. 80 per cent of the larvae recovered from the calves autopsied on this day were in the early 4th stage; the remaining 20 per cent were exsheathed 3rd stage. Figure 30 is a photograph of a 3rd stage infective O.ostertagi while figure 31 shows an early 4th stage larva which was recovered from the abomasal digest.

Day 8. All larvae were late 4th stages. Figure 32 depicts the posterior end of a late 4th stage male O.ostertagi and shows the characteristic prebursal swelling.

Day 12. 50 per cent of the larvae were in the 4th moult and 50 per cent had moulted to the 5th stage. Figures 33 and 34 respectively are photographs of the posterior end of male and female 5th stage parasites.

Day 16. All worms present were 5th stage or adults and about 20 percent of the females had eggs present in their uteri. Very few 4th or 5th stage larvae were found in the abomasal lumen contents of calves autopsied prior to Day 21 post infection.

Day 21. 40 per cent of the males and 75 per cent of the females were found in the lumen contents and 60 per cent and 25 per cent respectively were found in the mucosal digest. All females were producing eggs. Figures 35 and 36 respectively are photographs of the posterior ends of adult male

TABLE 19

Calves Given a Single Dose of 100,000 O.ostertagi Larvae

The Distribution of Developing Larvae in the Fundic and Pyloric Parts of the Abomasum

Day	Calf No.	Larvae/gm in Fundus	No.Larvae/gm. in Pyloric Area	Ratio. Larvae/gm. Fundic / Larvae/gm. Pyloric areas
2	1	67	16	4/1
	2	98	29	3/1
4	3	140	67	2/1
	4	109	73	1.5/1
8	5	253	113	2/1
	6	185	23	8/1

and female O.ostertagi parasites.

Day 28. The total number of worms was reduced (Table 18) and were in equal proportions in lumen and digest.

Days 60 and 90. Small numbers of adults were found in the lumen contents of the calves autopsied on days 60 and 90 post infection. The percentage of the 100,000 larvae which became established in each of the calves has been included in Table 18.

Inhibition of development or stunting of adults was not observed.

Digests of the total fundic and pyloric areas were carried out separately. The numbers and distribution of larvae in each part are shown in Table 19 for calves autopsied at 2, 4 and 8 days post infection. The number of larvae per gram of fundic mucosa was, with one exception, more than double the number of larvae per gram of pyloric mucosa.

Pathology

Day 2.

Lesions could be seen in all parts of the abomasum and occasionally in the first foot of the duodenum, but as in subsequent days the largest number was in the fold area of the fundus. Small, whitish nodules of about 1 mm diameter were found just raised above the surface of the mucosa. The central pit containing the larvae was dilated giving the umbilicated appearance which was to become more

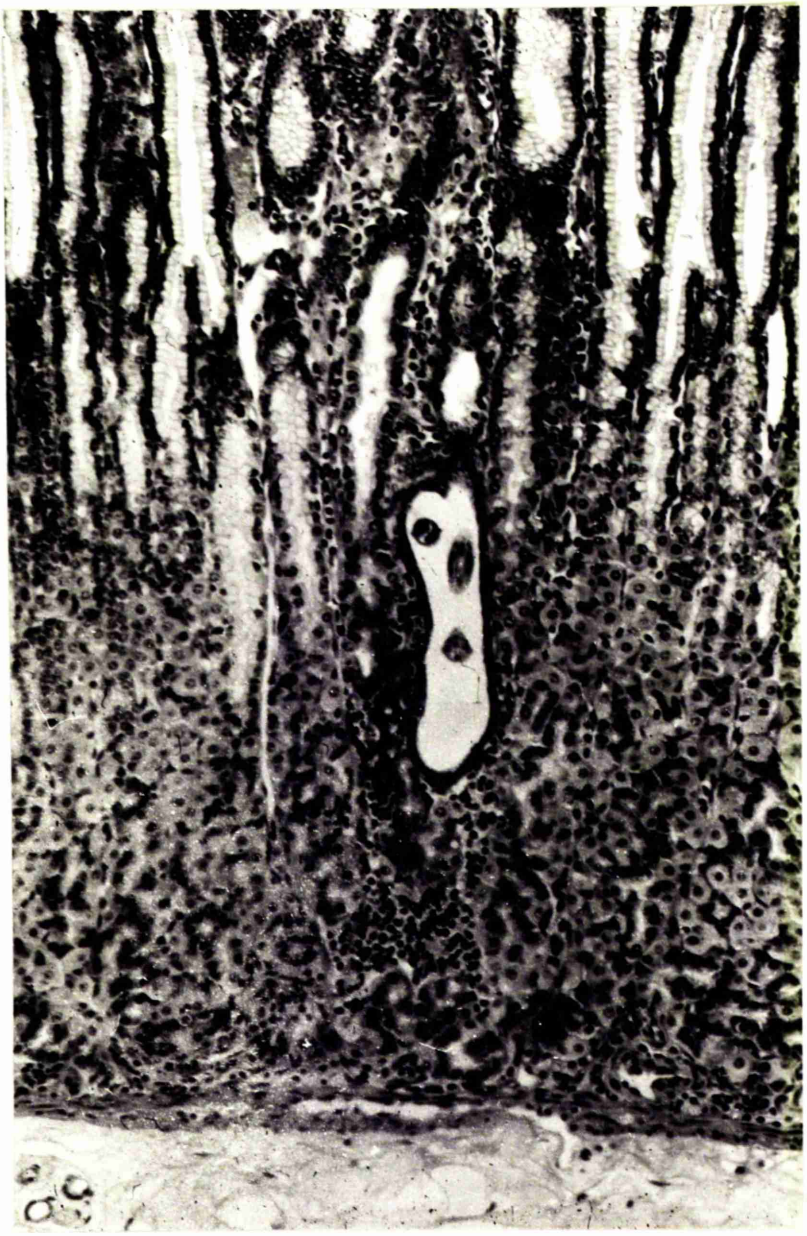


Fig. 23 Four day O.ostertagi infection in a calf. The early 4th stage larva lies in a gland lined by cuboidal epithelium.

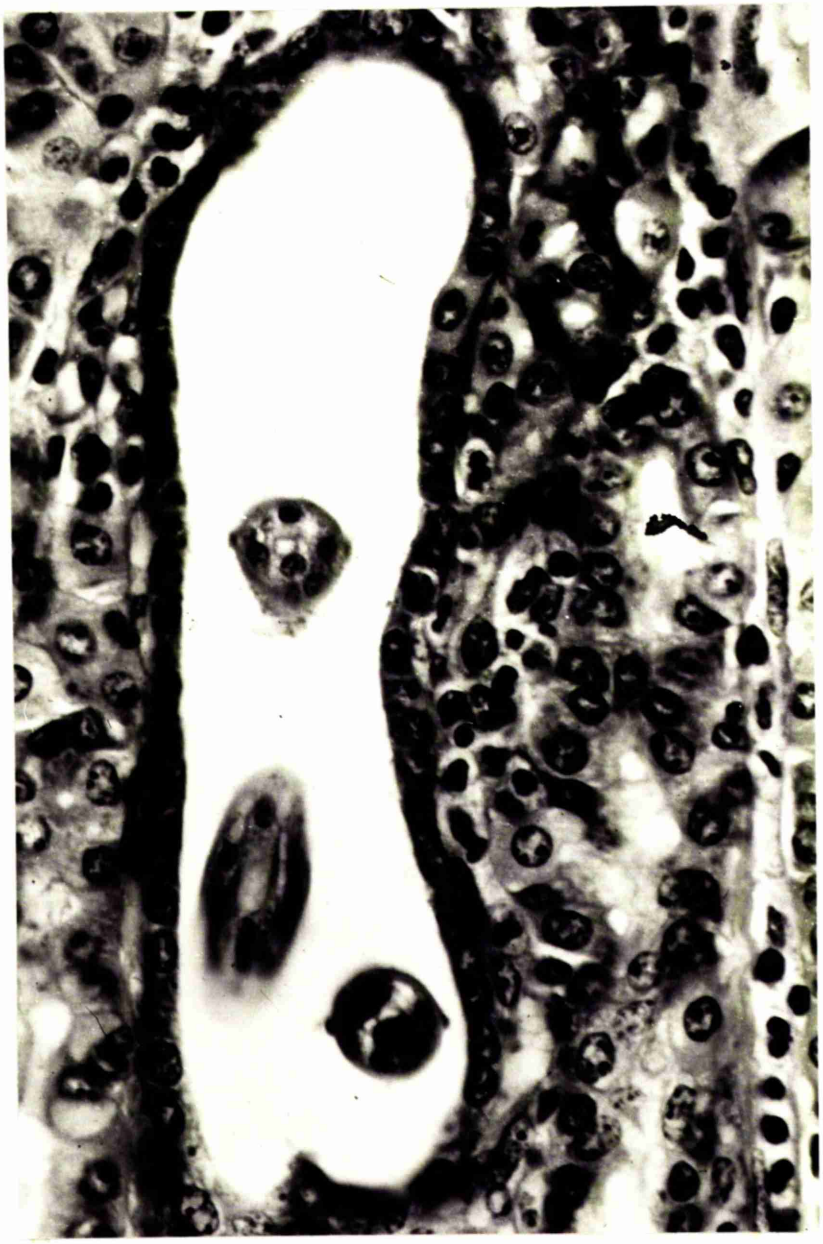


Fig. 24 Four day O.ostertagi infection in a calf. A high power view of Fig. 23.

marked in longer standing infections. Histologically these nodules were formed by the slight dilatation of a gland by a larva. The epithelium of the infected gland had already lost its clarity of delineation of the mucous, parietal and zymogen cell layers but surrounding glands were normal. A few granulocytes and mononuclear cells were infiltrating between the cells of the occupied glands (~~Fig. 23~~).

Day 4

The abomasal nodules were by now 1 to 2 mm in diameter and could be seen easily. The larvae in the glands had grown so that the pits were extended and protruded above the general surface of the mucosa. The epithelium of glands occupied by larvae was now of a cuboidal type, i.e. relatively undifferentiated, although the eosinophilic staining of some of these indicated their former parietal cell identity. Where the larvae did not touch the epithelium it was sometimes hyperplastic and was differentiating into mucous cells. Again a few neutrophil and eosinophil cells were migrating into the occupied pits, but there was no general lamina proprial reaction (Figs 23/24).

Days 8, 12, and 16

Material examined from the abomasa of calves autopsied on days 8, 12 and 16 post infection basically had a similar appearance, the pattern being one of larval growth, lengthening of the pit, conversion of the lining cells of the pit

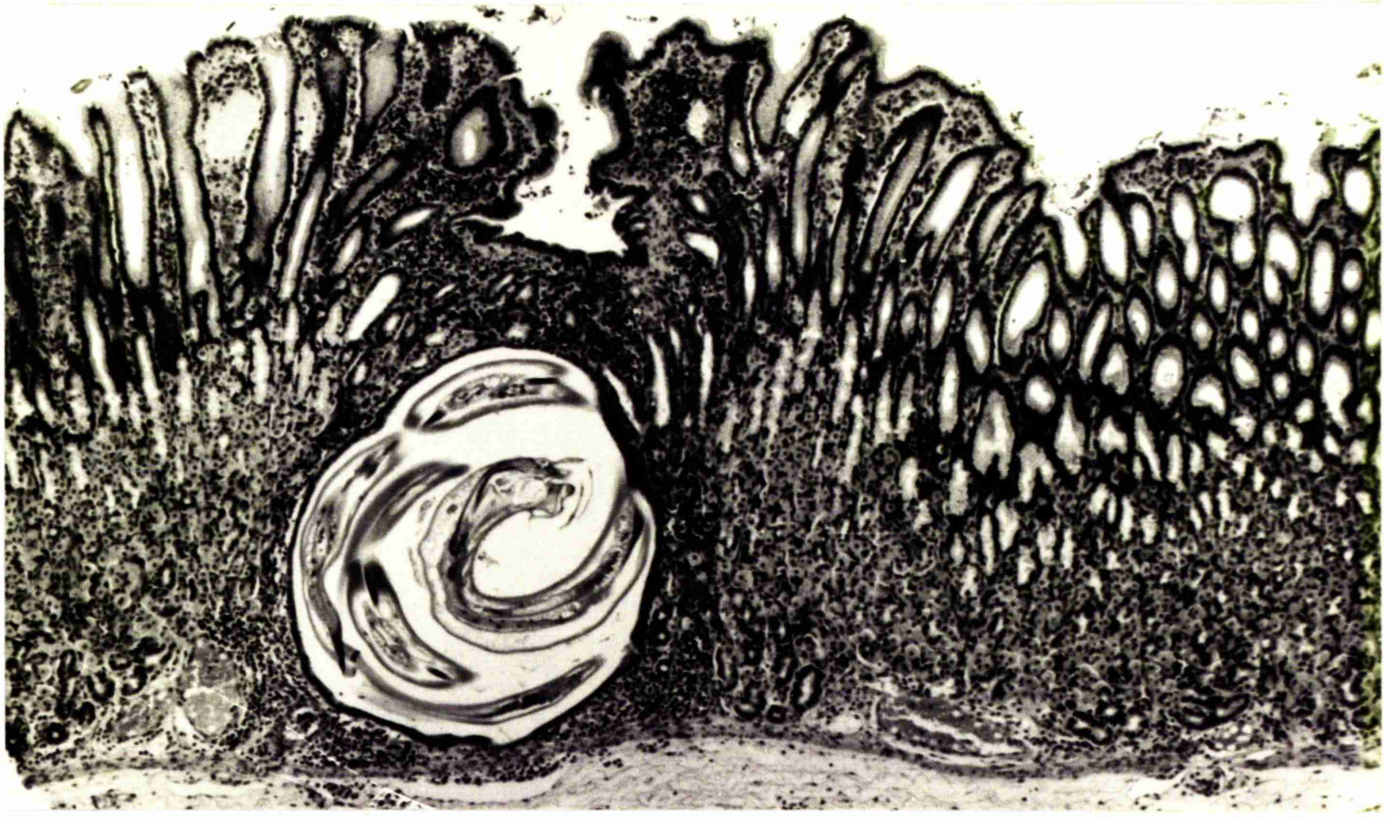


Fig. 25 A general view of the mucosal nodule at the pre-emergent stage of the infection.

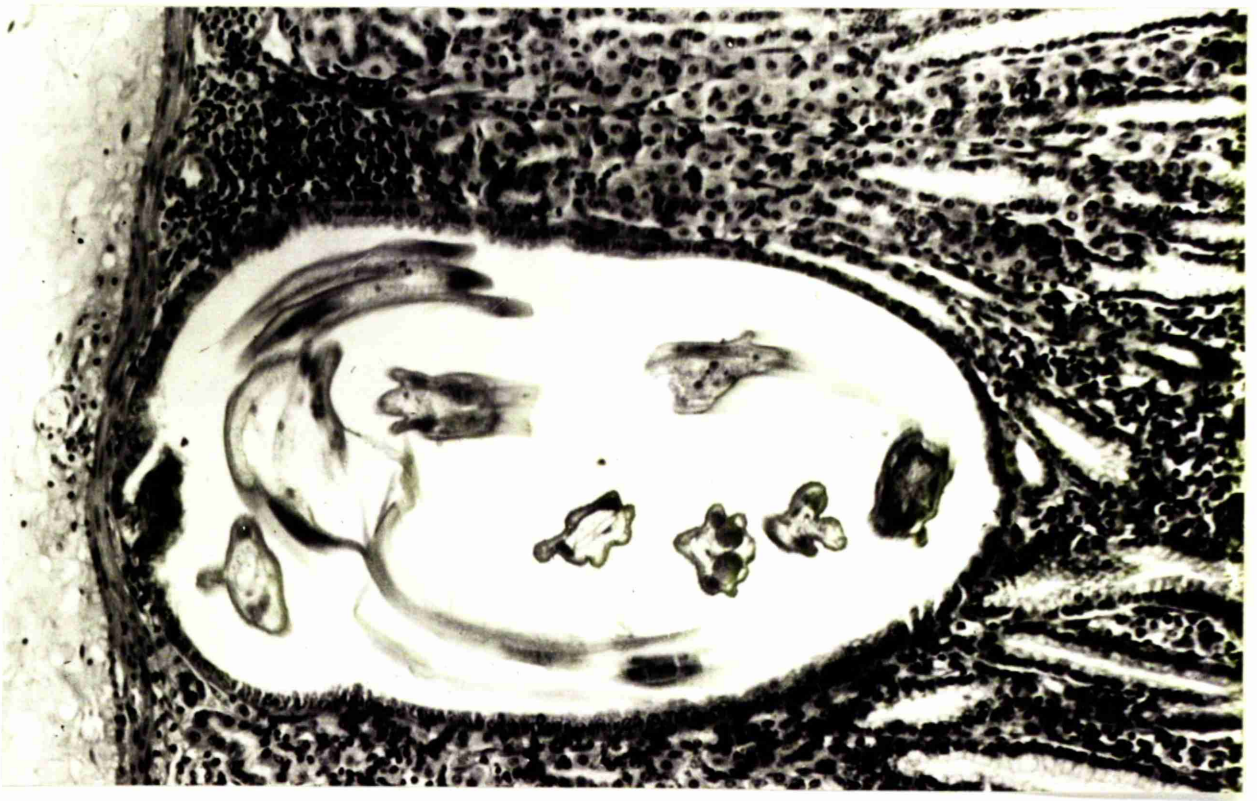


Fig.26 Sixteen day O.ostertagi infection in a calf. Cellularity of the lamina propria is increased while surrounding glands appear normal.

Fig. 27 Sixteen day O.ostertagi infection in a calf. The parasitised gland is lined by mucous epithelium. Most of the surrounding glands are normal; some show conversion to undifferentiated cells.

completely to mucous cell type and some stromal oedema around the neck of the pit. Granulocyte and mononuclear cell infiltration was evident between the epithelial cells but was not marked. Foci of plasma cells gradually appeared at all levels of the mucosa. All of these dilated glands with converted epithelium contained larvae (Figs. 25, 26 and 27).

Day 21.

At this time, that is, when the young adult O.ostertagi were emerging from the gastric gland, there was a change in the lesion pattern. Many of the affected crypts contained no larvae but were easily recognised by their high mucous cell lining and often contained cell debris. The glands immediately adjacent to these now showed a marked loss of differentiation, i.e. most of the epithelium of the glands forming the visible nodules were lined by undifferentiated cuboidal epithelium. Where two or more such nodules coalesced this epithelial hyperplasia was the underlying cause of the naked eye "morocco leather" or "crazy paving" effect. Associated with these changes was a cytolysis and sloughing of the superficial epithelial cells particularly in the centre of nodules, enhancing the umbilication. Occasional larvae had broken through into the lamina propria causing a foreign body reaction in which eosinophils were prominent. Plasma cell aggregates and radial streaks were prominent in the lamina propria of both the fundic and pyloric parts of

Fig. 28 Twenty-one day O.ostertagi infection in a calf. Post-emergent lesion showing the empty glands and hyperplastic mucus cells.

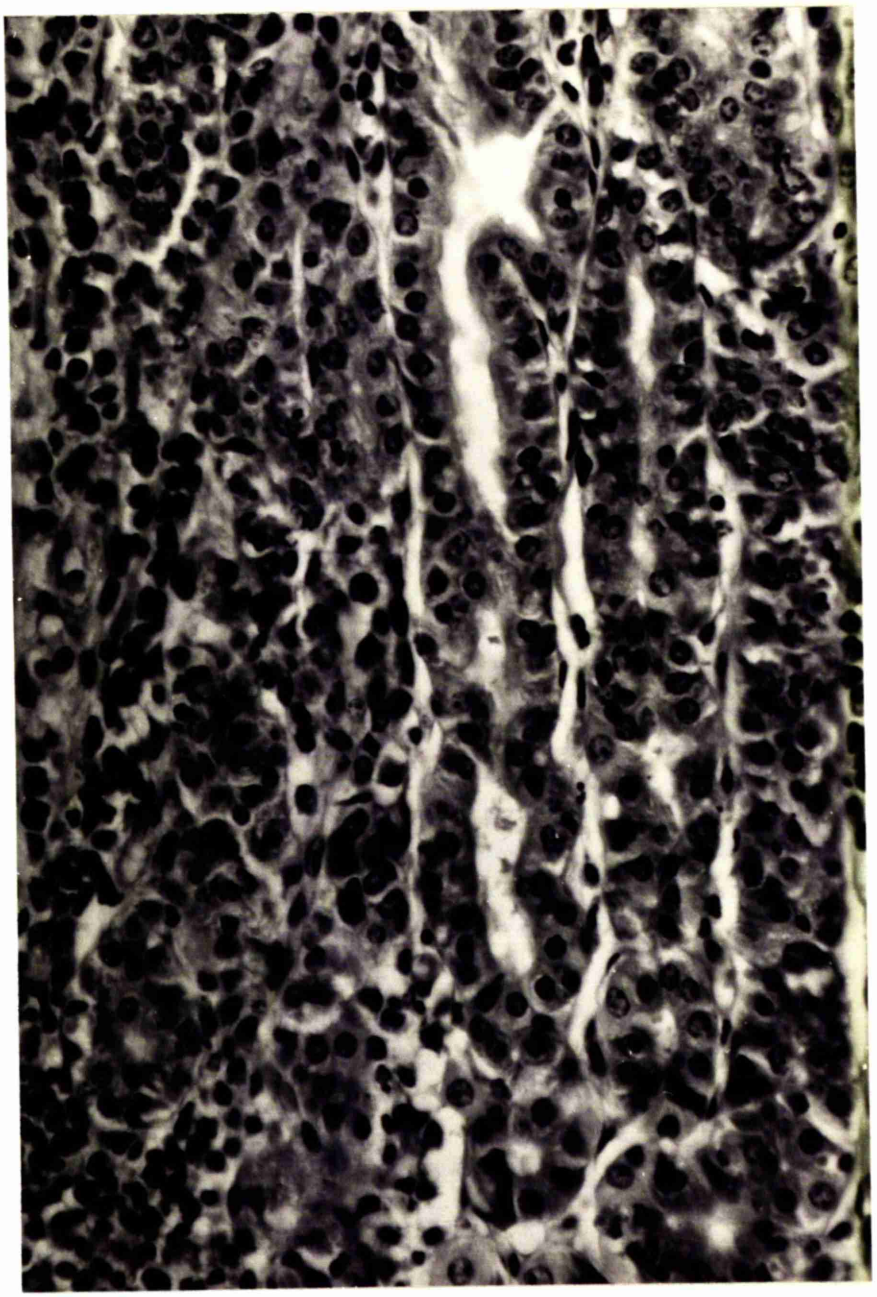


Fig. 29 Twenty-one day O.ostertagi infection in a calf. Gastric glands surrounding those previously containing larvae are lined by hyperplastic undifferentiated cells. Foci of plasma cells are prominent.

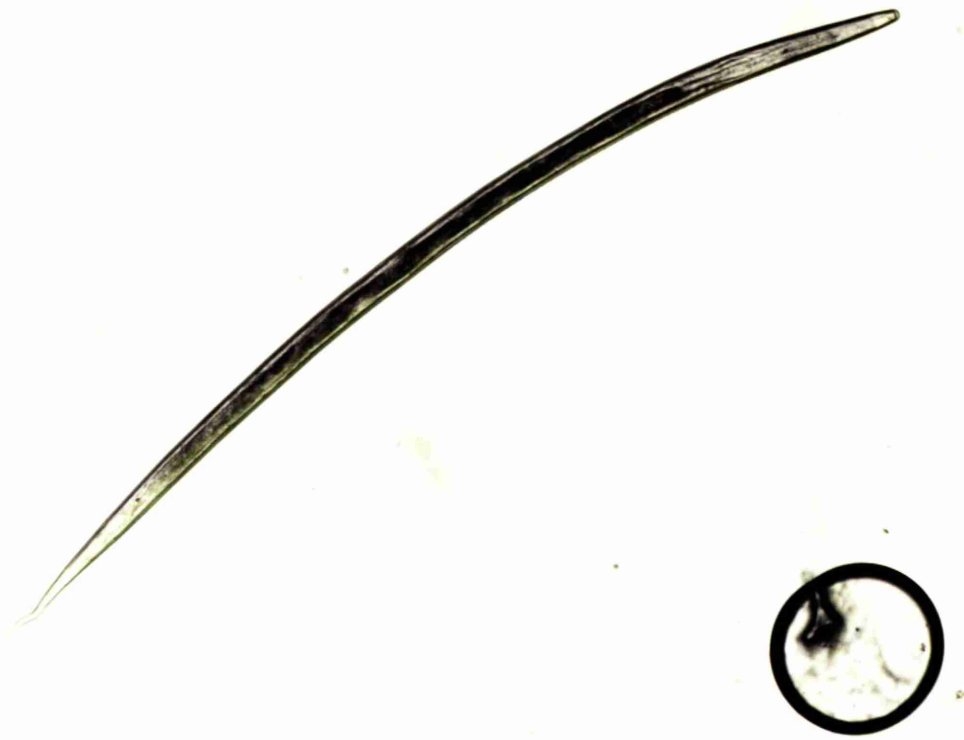


Fig. 30 O.ostertagi 3rd stage infective larva. Note the long tail with its characteristic kink.

Fig. 31 O.ostertagi early 4th stage parasite recovered from an abomasal digest on day 4 post infection. Note the clear area just behind the oesophagus; an artefact of the digestion technique.

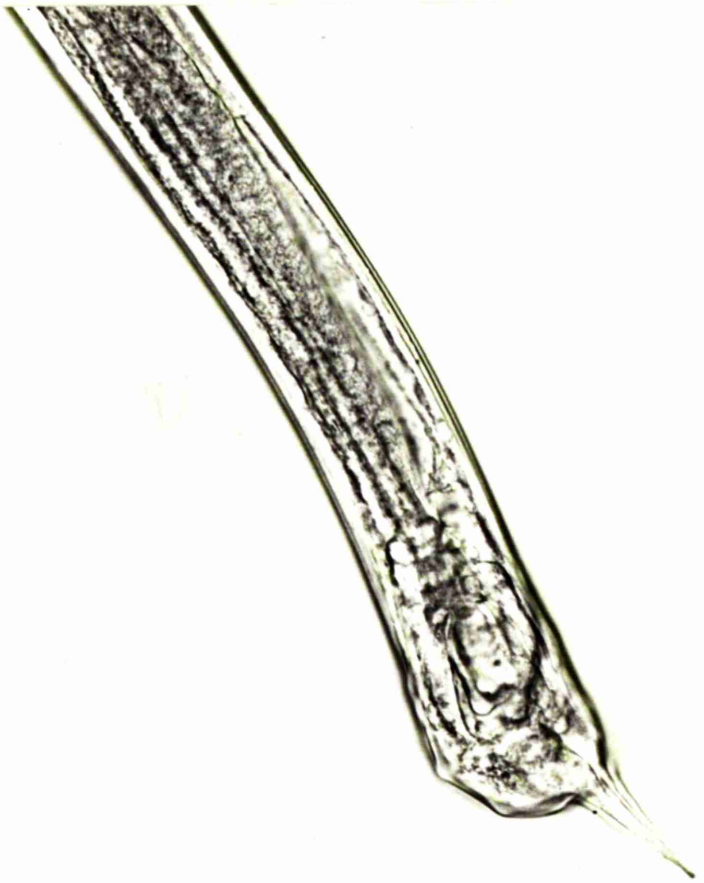


Fig. 32 O.ostertagi posterior end of a late 4th stage male larva, 8 days after infection, showing characteristic prebursal swelling.

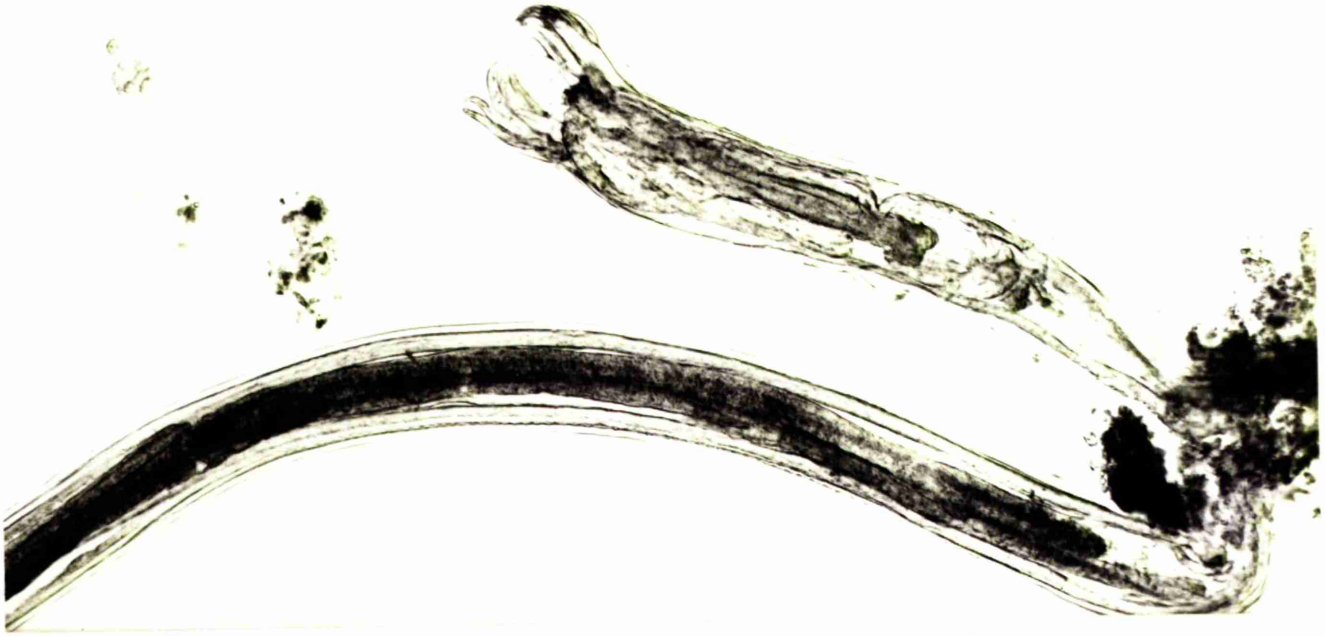
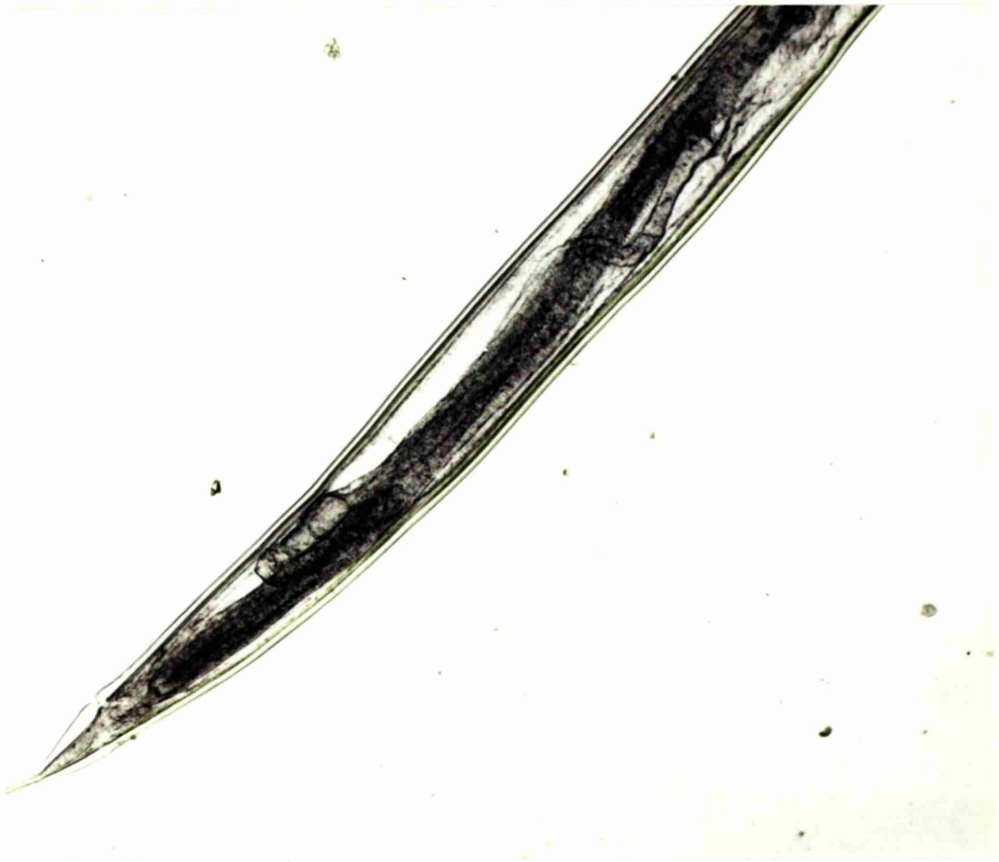


Fig.33 O.ostertagi. Posterior end of a 5th stage male recovered from the mucosal digest of a calf killed 16 days after infection.



ig.34 O.ostertagi. Posterior end of a 5th stage female. Note that the ampulla and utorus contain no eggs.

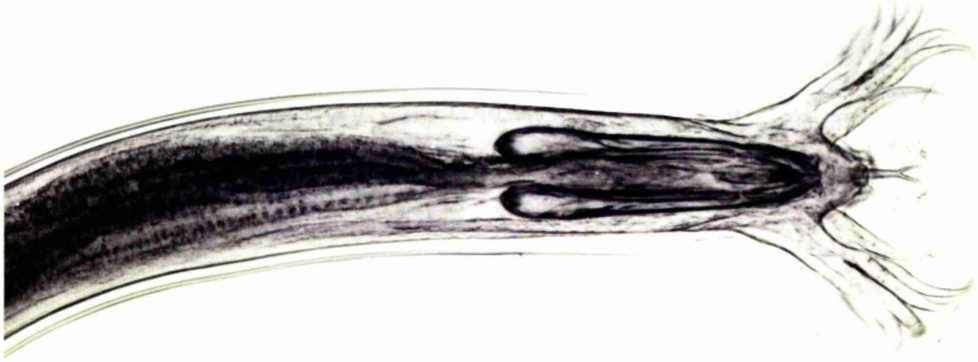


Fig. 35 O.ostertagi. Posterior end of adult male showing bursal structure and spicules.

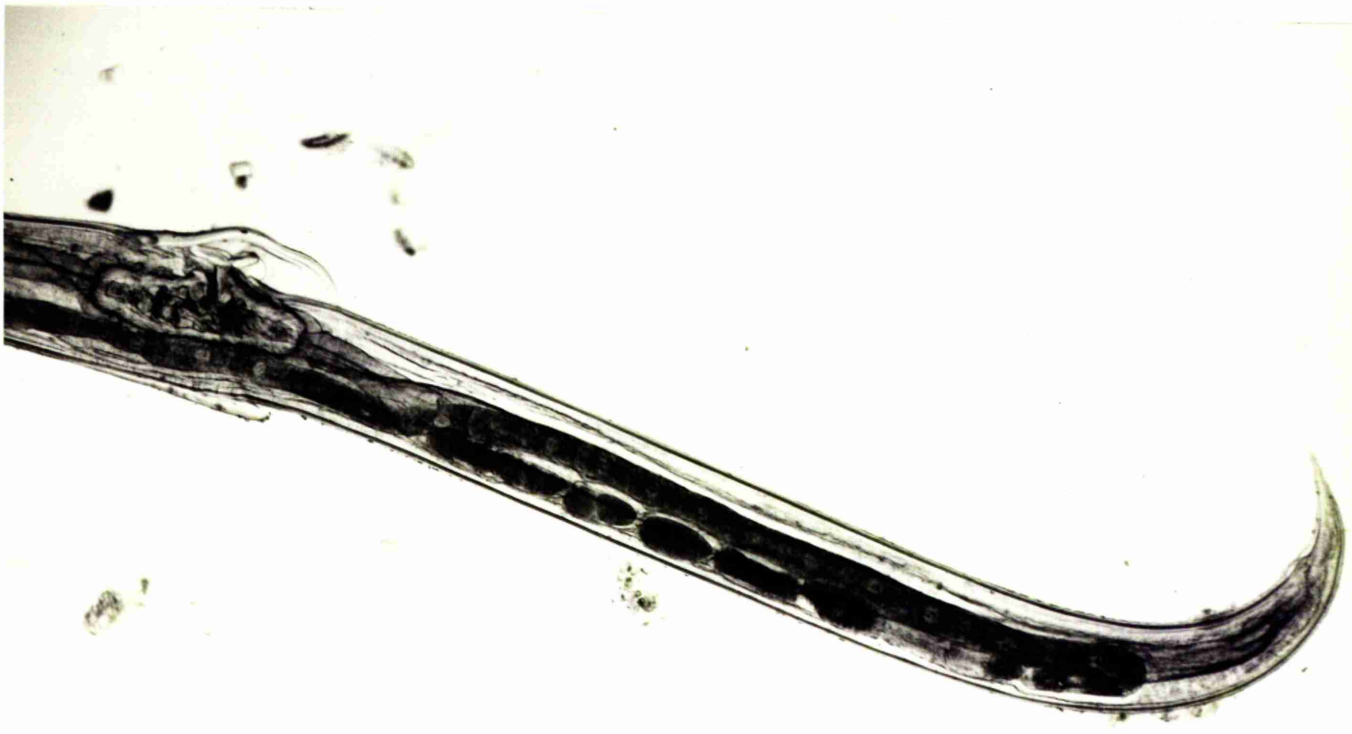


Fig. 36 O.ostertagi. Posterior end of adult female showing the cuticular vulval flap and egg filled uterus.

the infected abomasum (Figs. 28 and 29).

Day 28.

By this time the emergence phase was almost complete; very few glands contained larvae although all of the changes noted at day 21 were still present. Globular leucocytes were now prominent, particularly between the epithelial cells in the superficial areas of the pits.

Day 60.

Most of the epithelium had returned to its normal differentiation, although glands which had formerly contained larvae were obvious because of their mucous cell lining.

Day 90.

The mucosa was almost normal apart from some pits as described above. Globular leucocytes still persisted and plasma cell foci remained.

The abomasal lymph nodes showed the expected changes throughout the course of the infection. After the first week there were reactive changes in the germinal centres and this had become very marked by day 21, when many plasmacytes could be seen packing the medullary cords. By day 90 the nodes looked normal once more.

DISCUSSION

It has been shown earlier in this thesis that bovine ostertagiasis in South West Scotland occurs naturally in

three distinct phases. In the first phase, Type I, the worms develop to the adult stage in about 21 days; thus clinical signs become apparent 3 to 4 weeks after ingestion of a large dose of larvae. The second phase, Pre Type II, is characterised by massive inhibition of development at the early 4th larval stage. The subsequent maturation and emergence of these inhibited larvae up to 6 months later produce the third phase or Type II syndrome.

Since all the larvae in this experiment matured within 21 days the results can only be compared with the Type I syndrome.

At the dose level used in this experiment, four of the six calves allowed to survive beyond 21 days showed diarrhoea which commenced at the time of patency. The diarrhoea terminated within 7 days in three calves, but the fourth scoured intermittently for three weeks. Two of these calves showed significant weight loss during this period. Since severe diarrhoea and weight loss were the chief signs of the natural disease it would appear that a single dose of 100,000 larvae is about the minimal number of larvae required to produce the clinical disease in Ayrshire calves 2 to 3 months of age. Ross (1963) reported that during a period of 49 to 63 days following a single infection of 100,000 O.ostertagi larvae, only one out of 8 calves 2½ months of age developed an intermittent diarrhoea.

It would appear that observations were made at weekly intervals in the latter experiment, consequently diarrhoeic episodes of shorter duration may have been overlooked.

The low number of larvae recovered from the 2 and 4 day infections is probably an underestimate. First, the digestion technique is severe on the 3rd and early 4th stages; second, a comparison of the nodule counts from the 2 and 4 day, with the 12 and 16 day infections suggested that a similar number of parasites was present. Histological sections demonstrated that in almost every case only one larva was present in each nodule.

The proportion of larvae and adults found in the abomasal lumen and mucosal digest respectively (Table 18) may not represent their absolute distribution at these sites since the mucosa was not washed vigorously and some free adults or larvae may have remained adherent to the mucosa. Nevertheless, at day 16 the entire worm population - all immature adults - were found in the digest and by day 21, at least 40 per cent of the male and 75 per cent of the female worms at least, were free in the lumen. This suggests that the emergence from the gastric glands of the majority of the worms occurred between the 16th and 28th day.

The observations made on the different morphogenic stages found at autopsy in calves killed 2, 4, 8, 12 and 16 days post infection agree well with the findings of Douvres (1956).

The results in Table 19 show that a greater proportion of parasites were found in the fundic area of the abomasal mucosa when the larvae were in the tissue phases of development. This distribution of larvae differs from that observed in O.circumcinta infections of parasite-free sheep for Sommerville (1953 and 1954) and Dunsmore (1963) describe the greatest concentration of lesions and larvae in the pyloric area with a smaller aggregation around the cardia; very few were found in the fundic area. This distribution was relatively constant at the doses used.

From Table 18, it is apparent that the mean number of worms recovered from the 8-12-16 day infections was 35,000 and from the 21-28-60 day infections the number was only 3,000, that is a percentage establishment of 35 and 3 per cent respectively. This indicates that a marked loss of adults had occurred between day 16 and day 28 in this experiment. Whether or not this is characteristic feature of O.ostertagi infection is calves requires further confirmation. Ross (1963) who used single infections of 2,000 and 100,000 larvae, concluded that a loss of adult worms occurred between the 5th and 8th week of the infection and that the loss was greater at the higher dose level.

The daily faecal worm egg counts of 3 out of the 4 calves indicate that the peak egg production occurs during the week following patency and peaks of 900 to

1,800 eggs per gram were recorded. It is interesting to note that the fall in egg count occurs at about the same time as the loss of adult worms. This pattern of egg output has been confirmed in other experiments (unpublished information) following single doses of 100,000 larvae. The cause of the atypical pattern recorded in the fourth calf is not known; this calf was the most severely affected and was eventually killed *in extremis*.

Inhibition of larvae described in natural outbreaks of bovine ostertagiasis, Bruford and Finchem (1945); Bailey (1953); Martin *et al*. (1957); Smith and Jones (1962) and stunting of adults observed in a reinfection regime Michel (1963) was not found in this experiment.

All of the lesions seen in the typical field case were reproduced in this experiment. The process can be divided into three parts.

1. Each larvae enters a gland and grows within it. The gland dilates and the pit becomes stretched and protuberant. It then loses its cellular differentiation and comes to be lined by tall mucous cells. There is little mesenchymal reaction at this stage. Either towards the end of the growth phase or around the time of larval emergence the glands, adjacent to that containing the larva, lose their differentiated epithelium and become lined with cuboidal cells. This spreads the apparent area of the nodule and if

coalescence of these areas occurs, the "morocco leather" type of mucosa is produced.

2. The larvae emerge from the crypts and lie closely opposed to the surface epithelium. At this point cytolysis of the superficial lining of crypts and surface may occur. If confluence of the lesions is present, the so-called 'thumb-print' or superficial mucosal erosion is seen. Plasma cells are present in fairly large numbers in the lamina propria at this time. Sub-epithelial oedema, separating the epithelium from the blood vessels of the lamina propria, may also be found.

3. As the number of parasites decrease, replacement and differentiation of epithelial cells takes place and the mucosa returns to its normal~~ly~~, functionally differentiated state except for some of the pits which contained larvae and which remained lined by tall mucous secreting cells.

The infecting dose was too low in this experiment to produce enough lesions to cause widespread confluence. Hence the main features of the severely affected clinical cases, i.e. large areas of mucosa which had obviously lost most of its ~~functional~~ differentiated cells and hence its function, were not present.

SUMMARY

Eighteen, 8 week old Aryshire calves were given a single dose of 100,000 O.ostertagi larvae and the calves were autopsied in pairs from 2 to 90 days post infection.

Diarrhoea and weight loss were observed in a proportion of the calves after day 21 and it was considered that 100,000 O.ostertagi larvae was the minimal number necessary to produce clinical disease.

The parasitological findings revealed (a) that the larvae were located in the gastric GLAND by day 2 of the infection and that the majority of larvae did not emerge into the abomasal lumen until between days 16 and 21; and (b) that a comparison of the percentage establishment between calves autopsied before and after day 16 indicated that a marked loss of worms occurred between days 16 and 28 post infection. Inhibition of larval development was not observed.

All of the lesions seen in the typical clinical case of naturally acquired ostertagiasis were reproduced. The sequential development of the lesions is: (1) the larva grows within a gastric gland, the epithelium of which becomes stretched and undifferentiated; (2) at the time of emergence of the larvae, the neighbouring glands also become undifferentiated and hyperplastic which, if extensive, produces a morocco leather appearance; and (3) after emergence, cytolysis of the surface mucosa may occur which, if confluent, produces focal mucosal sloughing.

EXPERIMENT 2

SINGLE INFECTIONS USING FIVE GRADED DOSE LEVELS OF O.OSTERTAGI LARVAE

SINGLE INFECTIONS USING FIVE GRADED DOSE LEVELS OF O.OSTERTAGI LARVAE

The results obtained from the first experiment showed that a single dose of 100,000 O.ostertagi larvae was close to the minimum number necessary to produce Type I ostertagiasis in young calves. Larval inhibition or stunting of adult worms was not observed at this dose level.

The objectives in this experiment were to study the clinical and pathological aspects of ostertagiasis of graded severity and to determine whether inhibition of larval development or stunting of adult worms was a function of the larval dose size at several dose levels.

MATERIALS AND METHODS

Animals

Twenty-five male Ayrshire calves were reared parasite free to 15 weeks of age, weighed and then allotted to five groups of five calves so that the mean weight of each group was approximately equal. Each calf in Group 1 received a single oral infection of 50,000 infective O.ostertagi larvae; similarly in the remaining groups each calf received a dose of 100,000; 200,000; 400,000 and 800,000 larvae respectively. All calves were autopsied 21 days post infection, except one calf which died on day 18 post infection.

Observations

Faecal examinations were carried out daily and the

calves were weighed and blood samples were collected at weekly intervals. Plasma pepsinogen estimations were carried out. The methods and materials used have been described in Section I.

TABLE 20

Calves Given Five Graded Doses of O.ostertagi Larvae

A summary of the Severity and Duration of Diarrhoea

Group	Larval Dose	Diarrhoea			
		No.Calves Affected	Day of Onset	Severity	No.of Calf Diarrhoea Days
1	50,000	0/5	-	-	0/45
2	100,000	2/5	13 19	Continuous ++ Continuous ++	6/45
3	200,000	2/5	14 16	Intermittent ++ Intermittent ++	10/45
4	400,000	3/5	13	Continuous +++ Intermittent +++ Continuous +++	17/45
5	800,000	5/5	12 * 13 13 14 21	Continuous +++ Continuous +++ Intermittent +++ Continuous +++ - +++	29/41 *

* One calf died on day 18.

RESULTS

Clinical observations

Diarrhoea, which includes only the semifluid, D++, and the fluid D+++ gradings, was first recorded on day 12 post infection. Group 1 calves showed no evidence of diarrhoea throughout the experiment, whereas calves in groups 2 to 5 had a variable degree of diarrhoea which was continuous in some calves and intermittent in others. The number of calves showing D++ and D+++ diarrhoea on each day between day 12 and day 21 has been summed and the resulting number of "calf diarrhoea days" has been included in the summary given in Table 20.

The clinical severity of the disease was very apparent in the calves of groups 4 and 5: these calves were dull, anorexic, tucked up and reluctant to move, giving the impression of abdominal pain. Calves in group 5 were particularly affected, one died on day 18 post infection: had the experiment not terminated at day 21 the remainder probably would have died a few days later.

At the time of infection all group mean weights lay between 160 and 162 lb and the growth rate over a period of 10 weeks prior to infection had averaged 1 lb per calf per day. This average weight gain continued unaltered in calves of groups 1 to 4 and by day 20 thegroup mean weights were within the range of 179 to 184 lbs. The calves of

TABLE 21

Calves Given Five Graded Doses of O.ostertagi Larvae

Group Mean Faecal Worm Egg Counts +

DAY POST INFECTION

	Day 17	Day 18	Day 19	Day 20	Day 21	Five day Mean
Group 1	120	150	160	520	560	286
Group 2	150	110	240	370	380	220
Group 3	510	440	560	750	580	550
Group 4	570	320	270	490	620	454
Group 5	1330	1220	1670*	2430*	2700*	1750*

+ Counts = No. of Eggs per gram of Faeces.

* 4 calves only

group 5 had a mean weight of 166 lbs.at day 20 and this was significantly lower than the others. The loss of weight occurred during the week prior to the infection becoming patent.

Haematological Data

No significant changes were recorded in packed cell volume, haemoglobin concentration or total red blood cell counts throughout this experiment except in the calves of group 5 when during the diarrhoeic phase, these indices increased, e.g. the mean packed cell volume increased from 33 to 40 per cent. This may have been due to haemoconcentration caused by the diarrhoea.

The total serum protein levels and the albumin to globulin ratios did not alter significantly throughout the experiment.

Parasitological Data

Faecal Egg Counts

Most calves had patent infections on day 17 post infection and all calves reached this stage by day 19. The group mean faecal egg counts for each day from day 17 to day 21, together with a 5 day group mean egg count, have been shown in Table 21. The differences in the mean faecal egg counts of calves in groups 1 to 4 were not significant and averaged 380 eggs per gram from day 17 to day 21. Group 5 had a significantly different group mean of 1,750 eggs.

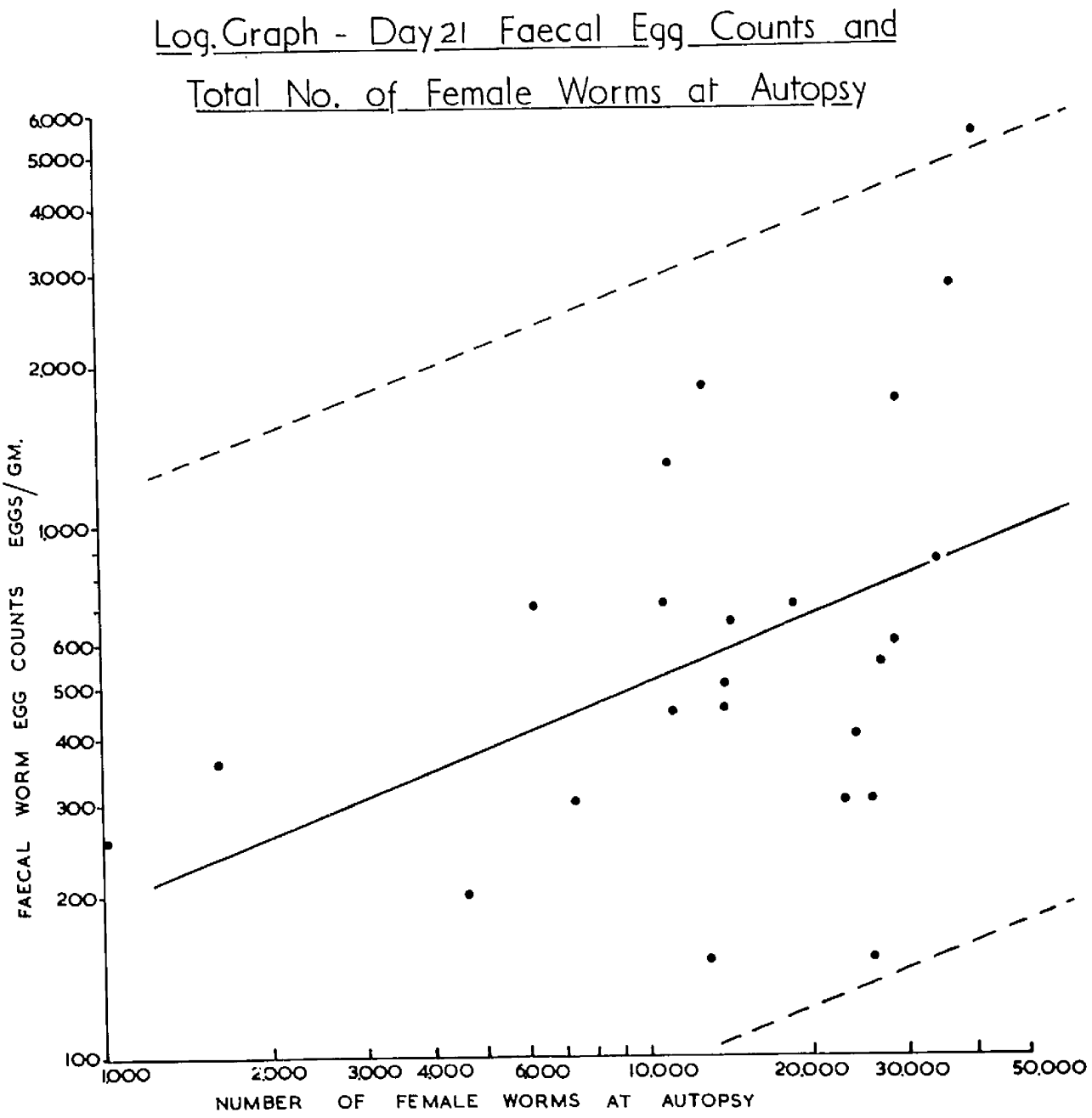


Fig. 97 A log graph of the faecal egg count of 25 calves on day 21 post infection and the total number of female worms present at autopsy.

---- Regression line r = 0.6 - - - Approximate 95 per cent tolerance limits

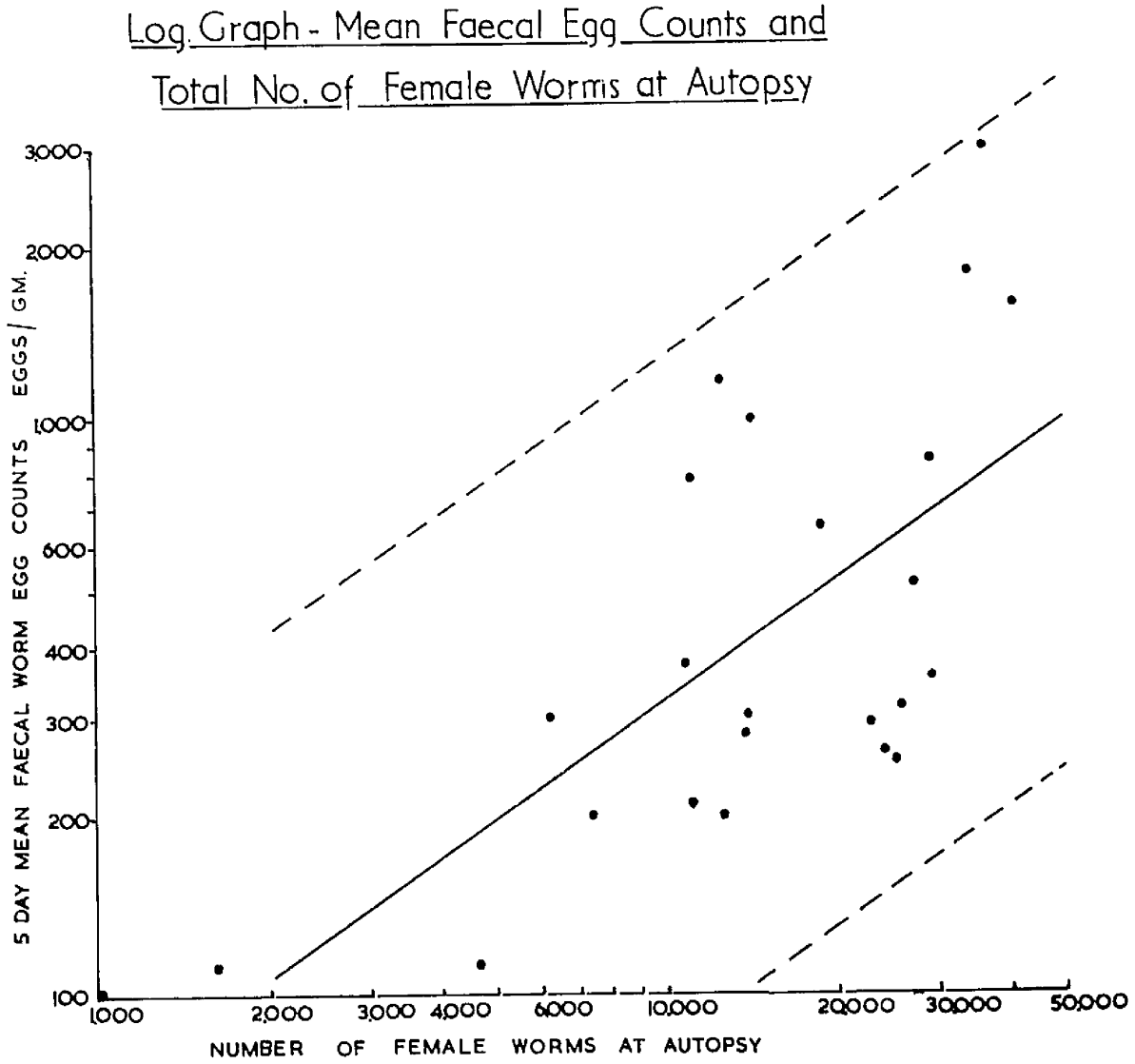


Fig. 38 A log graph of the five day mean faecal egg count and the total number of female worms present at autopsy.
---- Regression line r = 0.7 - - - Approximate 95 per cent tolerance limits.

To determine the relationship between the faecal worm egg count and female parasite population present in the abomasum two linear regressions were calculated in which the faecal egg count was taken as the dependent variable and the number of female worms present at autopsy was the independent variable. In the first of these regressions a single faecal worm egg count taken on day 21 post infection was used as the dependent variable and in the second case a 5 day mean egg count became the dependent variable. In order to establish a linear relationship and to make the variability of the data more homogeneous it was necessary to transform to logarithmic values both the independent and dependent variables.

In the first case a significant, ($P<0.05$), linear regression was found to exist between the two variables. However, only 18 per cent of the variation in log single egg count was accounted for by the variation in log number of female worms present at autopsy. Similarly, using the 5 day mean egg count as dependent variable, a significant ($P<0.01$), linear regression was found between the log 5 day mean egg count and the log number of female worms found at autopsy. In this case 49 per cent of the variation in log 5 day mean egg count was accounted for by the log number of female worms.

Both of these regressions have been drawn graphically in Figs.37 and 38 respectively and in each case the approximate 95 per cent tolerance limits as defined by Williams (1959) are shown also.

TABLE 22

Calves Given Five Graded Doses of O.ostertagi Larvae

WORM COUNTS AT AUTOPSY

Group and Dose	Abomasum Lumen & Digest	Duodenum Lumen & Digest	Grand Total	Sex Ratio M/F	Percentage Established
Group 1 50,000	2,760	280	3,040	1 : 1	6.1
	14,460	440	14,906	3 : 2	29.8
	16,920	-	16,920	1 : 1	33.8
	3,600	-	3,600	3 : 1	7.2
	21,580	440	22,020	1 : 1	44.0
	Mean and S.D. 12,000± 8420				
Group 2 100,000	11,360	440	11,800	1 : 1	11.8
	21,840	720	22,560	1 : 1	22.6
	30,680	480	31,160	1 : 1	31.2
	44,960	760	45,720	1 : 1	45.7
	29,480	1,200	30,680	1 : 1	30.7
	Mean and S.D. 28,380± 12,470				
Group 3 200,000	46,640	80	46,720	1 : 1	23.4
	58,120	320	58,440	2 : 3	29.2
	39,320	120	39,440	2 : 1	19.7
	58,750	440	59,190	1 : 1	29.6
	59,160	320	59,480	2 : 1	29.7
	Mean and S.D. 50,720± 8,590				
Group 4 400,000	58,860	1,360	60.220	3 : 2	15.1
	21,080	1,640	22,720	1 : 1	5.7
	71,540	2,400	73.940	2 : 1	18.5
	31,940	680	32,620	2 : 1	8.2
	24,720	880	25,600	3 : 2	6.4
	Mean and S.D. 43,637± 22,310				
Group 5 800,000	86,600	Not done	86,600	3 : 2	10.8
	68,460	1,880	70,340	3 : 2	8.8
	91,360	10,700	102,060	3 : 2	12.8
	86,760	920	87,680	3 : 2	10.9
	82,610*	26,040	108,610	2 : 1	13.6
	Mean and S.D. 88,780± 12,460				

* Died on Day 18

SD = Standard Deviation

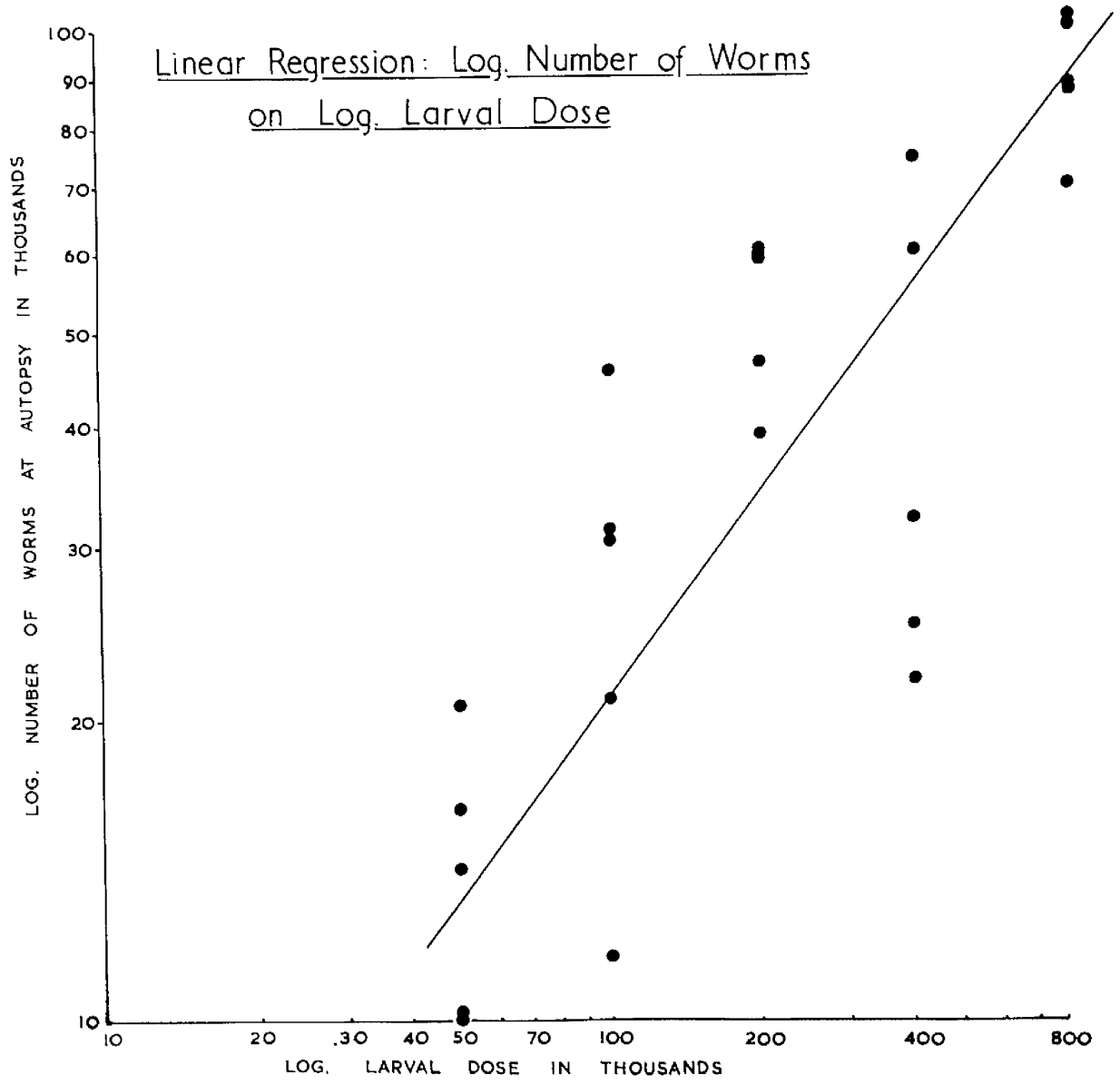


Fig.39 A log graph of larval dose and the number of worms found at autopsy 21 days post infection.
---- Regression line r = 0·79 - - - Approximate 95 per cent tolerance limits

TABLE 23

Calves Given Five Graded Doses of O.ostertagi Larvae

Mean Faecal Egg Count, Female Length, No. of Eggs per Female and No. of Female Worms at Autopsy

Calf No.	No. Females Autopsy	Faecal Egg Count/gm 5 Day Mean	Female Length in mm Mean ± S.D.	No.Eggs per Female Mean ± S.D.
802	1,600	110	8.97 ± 0.30	16.50 ± 5.89
805	6,160	300	8.07 ± 0.39	16.40 ± 4.87
807	7,280	200	8.70 ± 0.42	16.96 ± 4.53
808	960	80	8.64 ± 0.71	16.80 ± 4.37
963	11,000	780	8.61 ± 0.37	17.03 ± 6.11
798	4,600	110	No sample	No sample
799	10,960	210	No sample	No sample
800	13,680	280	7.95 ± 0.44	12.83 ± 4.08
821	22,920	290	7.89 ± 0.45	10.76 ± 2.57
038	13,900	300	8.48 ± 0.49	14.20 ± 4.99
801	24,080	260	8.68 ± 0.36	13.83 ± 4.68
804	29,160	350	8.68 ± 0.64	12.20 ± 4.60
810	14,200	990	7.89 ± 0.55	8.56 ± 3.56
813	27,450	510	7.77 ± 0.39	10.20 ± 3.00
819	18,360	640	7.85 ± 0.51	10.60 ± 4.64
812	25,200	250	8.13 ± 0.45	10.96 ± 3.04
820	12,400	200	No sample	No sample
822	25,870	310	8.39 ± 0.32	13.30 ± 3.60
826	12,410	1,140	8.04 ± 0.52	6.60 ± 3.07
831	10,900	370	8.14 ± 0.55	16.96 ± 5.16
806	33,960	1,790	8.00 ± 0.43	10.86 ± 5.70
815	29,140	840	8.12 ± 0.50	12.80 ± 4.15
816	40,120	1,590	7.64 ± 0.62	11.30 ± 4.29
818	36,840	2,940	8.33 ± 0.66	13.26 ± 3.35

SD = Standard deviation.

Worm Counts

The results of the post mortem worm counts are shown in Table 22. In all calves the percentage of 4th and 5th stages was less than 2 per cent. The number of worms recovered from the duodenum was significantly larger in groups 4 and 5 than in groups 1 to 3.

A significant linear regression was established between the log number of adult worms and the log larval dose in which 60 per cent of the variation in log number of adult worms was accounted for by the variation in log larval dose. The approximate 95 per cent tolerance limits were calculated and have been drawn on the regression in Fig. 39.

The sex ratios of males to females were approximately 1:1 and there was no significant difference between groups.

Measurements of the Length of Female Worms and Counts of the Number of Eggs for Female Worm.

Thirty female O.ostertagi adults were measured from each calf and the number of eggs present in the uterus of each female was noted. Table 23 sets out the mean values and standard deviations of the observations together with the number of female worms found at autopsy.

The linear regression of the number of eggs per female on the number of female worms found at autopsy is significant at the 5 per cent level but only 24 per cent of the variation in eggs per female is accounted for by the regression on the number of female worms. In other words,

TABLE 24

Calves Given Five Graded Doses of O.ostertagi Larvae

Plasma Pepsinogen Concentration and the pH and Electrolyte Concentrations of Abomasal Contents

Group and Infecting Dose	Plasma Pepsinogen ug/phenol/per ml.	Abomasal pH	Abomasal electrolytes		
			Na^+	K^+	Cl^-
1	5.4	2.8	-	-	-
	47.0	3.3	88	15.2	116
	56.5	2.4	80	14.6	108
50,000 Larvae	13.0	2.1	69	19.2	123
	48.0	4.2	84	17.0	102
Mean	33.9	3.0	80	16.5	112
Standard Error	10.3	0.4	4	2.1	9
2	39.7	-	-	-	-
	70.4	4.1	104	17.0	114
	97.0	7.1	130	13.4	81
100,000 Larvae	115.0	5.7	104	14.4	103
	72.0	7.0	133	16.2	98
Mean	78.8	6.0	118	15.3	99
Standard Error	12.8	0.7	8	0.8	7
3	102.9	6.7	130	12.4	105
	86.9	7.2	134	15.0	97
	111.1	7.0	142	10.2	97
200,000 Larvae	92.9	6.9	136	11.2	99
	94.8	7.2	130	12.8	100
Mean	97.7	7.0	134	12.3	100
Standard Error	4.2	0.2	2	0.8	2
4	114.5	7.1	129	16.0	100
	39.7	3.2	93	12.8	116
	117.4	6.7	132	11.9	98
400,000 Larvae	57.8	7.1	135	16.0	89
	77.1	4.4	97	14.7	103
Mean	81.2	5.7	117	14.3	101
Standard Error	15.3	0.8	9	0.8	4
5	95.0	7.7	147	10.2	103
	91.2	7.3	144	10.6	95
	94.0	7.3	138	7.0	97
80,000 Larvae	64.0	7.4	130	9.2	83
	-	7.3	-	-	-
Mean	86.0	7.4	140	9.3	94
Standard Error	12.6	0.1	3	1.6	4

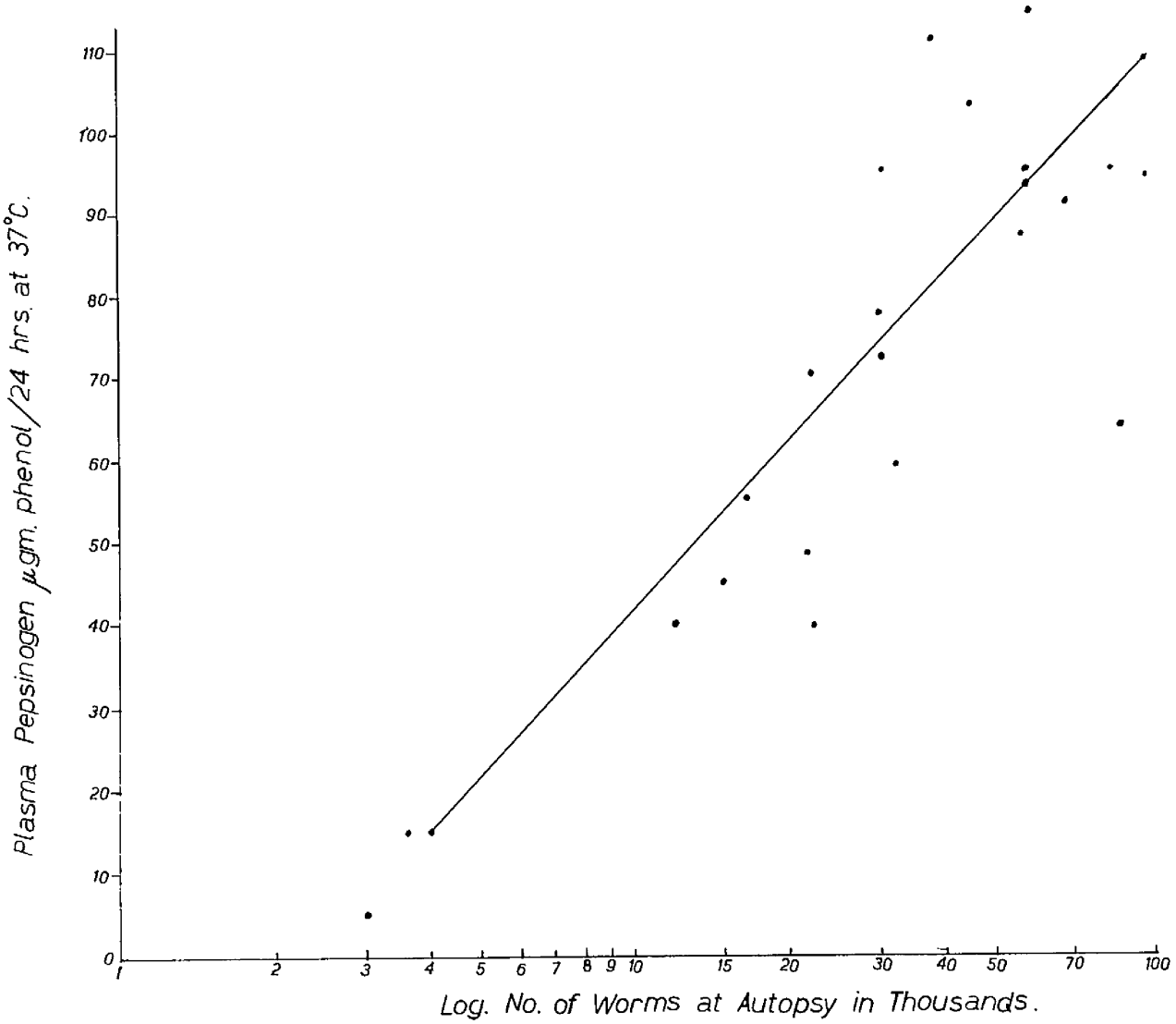


Fig. 40 A graph of the plasma concentration of pepsinogen and the log. numbers of O. ostertagi found at autopsy.

while there is a tendency for the number of eggs per female to decrease as the number of female worms increase, the relationship between these two variables is poor.

The linear regression of length of female worms on the number of female worms at autopsy was not significant at the 5 per cent level. It was found that there was a linear relationship between the number of eggs per female and the length of the female worm. This regression was significant at the 1 per cent level although only 43 per cent of the variation in eggs per female was accounted for by the length of female worm.

Biochemical Data

The results of the determinations on the pH and electrolyte (sodium, potassium and chloride) concentrations of abomasal contents and the plasma pepsinogen concentration have been shown together in Table 24. A highly significant relationship was found between the plasma pepsinogen concentration and the total number of worms found at autopsy. The linear regression depicting this relationship has been graphed in Fig.40.

Pathological Data

In calves given 50,000 larvae the basic change was identical with that in experiment 1. The unit lesion was the nodule which comprised the dilated central gland containing a larva. This gland was lined with high columnar

mucus secreting cells. Surrounding this gland were non-parasitized glands, which had lost their specific differentiated cells and were lined by low columnar stem cell types. When the dose of larvae was low, namely, 50,000 or 100,000, these nodules appeared as discrete foci; with higher doses, coalescence and overlap of the nodule lesion led to the morocco leather appearance of a thickened hyperplastic, non differentiated and non functional mucosa. Cytolysis similar to that in experiment 1 was seen. The epithelial sloughing of the superficial areas of this converted mucosa took place shortly after the emergence of larvae from the mucosal glands. The degree of this change was found to be directly proportional to the dose of larvae given. Hence, diphtheresis and congestion of the mucosa was mild in the 100,000 larvae group and severe in the 800,000 group.

Plasma cell aggregates were found in the lamina propria in all groups and were very intense in the two highest levels of infection. Small numbers of eosinophils were found in the lamina propria except where larvae had penetrated into it from the glands; then a severe, localised, lymphoid, giant cell and eosinophilic granuloma occurred around the aberrant parasite. Globular leucocytes were present in all cases, but their numbers were not markedly increased at the higher doses. In all cases, marked reactive changes were present in the abomasal lymph nodes. Where gross diphtheresis and

ulceration were present in the abomasal mucosa, i.e. in 400,000 and 800,000 larval doses, there was a superimposed purulent lymphadenitis.

DISCUSSION

Twelve of the twenty-five calves developed clinical signs and, in nine of these, diarrhoea was evident before the infections became patent at 17 days post-infection. Diarrhoea coincided with the time when most of the parasite population were in the 4th moult stage of development; the severity of the diarrhoea increased as the infection progressed. Clinical signs were absent during the period prior to the 4th moult stage, and this supports the field observation that large numbers of early 4th stage larvae can be present in the abomasal mucosa with no apparent effect on the well-being of the calf.

Confirmation was obtained of results obtained in the first experiment in which it was concluded, that a single dose of 100,000 larvae was about the minimum number necessary to produce the syndrome of Type I ostertagiasis in young calves. A single larval dose of 50,000 O.ostertagi larvae, which resulted in a mean establishment of 12,000 worms, produced no detectable change in the well-being of 4 month old Ayrshire calves during the first 3 weeks of the infection. Increasing the dose level about 100,000 larvae produced a corresponding increase in the clinical severity. At the two

higher dose levels of 400,000 and 800,000 larvae, all of the signs of naturally induced Type I ostertagiasis were produced; the latter dose level appears to be lethal within four weeks in calves of this age.

Since one of the objects of this experiment was to determine the relationship between dose size and parasite population and the first experiment had indicated that a marked loss of adult O.ostertagi occurred during the third week of an infection, all calves were autopsied 21 days post infection.

There was a positive correlation between the log larval dose and the total number of worms found at autopsy, such that with an increase in the number of larvae administered an increased number of adult worms became established. Based on the relative numbers of worms found in the abomasum and duodenum respectively, it appears that there is an earlier loss of the O.ostertagi population in those groups which received the 400,000 and 800,000 larvae. In these groups about 10 per cent of the total population was recovered from the duodenum, whereas in the other groups, the duodenal population was only 2 per cent. It is likely that the severe abomasal lesions, together with the severe diarrhoea, caused the accelerated loss of adult worm population.

Inhibition of larval development did not occur in any of the calves. Therefore, it seems unlikely that massive

inhibition of larval development, (a pre-requisite of the Type II syndrome of ostertagiasis), is solely a function of a high level of larval intake, a suggestion put forward by Martin, Thomas and Urquhart (1957). The above finding is in contrast with that found in Merino sheep, whereby single infections of 100,000 O.circumcinta resulted in the establishment of large numbers of early 4th stage parasites in the abomasal mucosa (Dunsmore, 1963).

There was no significant relationship between the length of female worms and the number of worms present in the abomasum; thus it can be concluded that the stunting of adult O.ostertagi it not a density dependent function as defined by Solomon (1958). Michel (1963) stated that there was no correlation between the length of the female worm and the number of eggs it contained. In this experiment, a significant linear relationship was found between these two variables such that longer females were found to contain a greater number of eggs; however, the relationship was not a strong one because of the variable response found in indivnal calves. The number of eggs per female worm decreased with an increase in the number of female parasites in the abomasum, although once again the relationship was not strong. Rather than attributing the above relationships to the size of the parasite population in the host, it would be more reasonable to relate the differences in length and

content of eggs to the response which each larval dose elicited in the calf, because it was the variability of this response which made the other relationships poor.

Following a consideration of Figs. 37 and 38, it can be appreciated that when the number of female worms increases an increase in the faecal worm egg count results. However, the graphs clearly show that faecal worm egg counts, determined during the clinical phase of the disease, are not useful criteria for the accurate prediction of the numbers of worms present in individual or in groups of calves. Under field conditions it has been noted that a high proportion of clinically affected calves had egg counts which were less than 1,000 eggs per gram.

The pH of the abomasal contents, collected from the calves which received 50,000 larvae, was not very different from the values obtained from uninfected calves. By way of contrast, a marked change was observed in the pH of the abomasal fluid from calves infected with larger doses of larvae. This pH change was associated with widespread loss of parietal cells which occurred when the adult parasites were emerging from the gastric glands in the abomasal mucosa. Peptic digestion is negligible at pH values above 4.5 so that it is likely that serious impairment of protein digestion does occur in these cases. This increase in the pH of abomasal contents has been shown earlier to occur in cases

of the natural disease. The pH values were correlated with changes in the electrolyte concentration of the abomasal fluid, Table 24.

The marked elevation in plasma pepsinogen concentration is thought to be due to an increased permeability of the severely damaged abomasal epithelium, since large molecules, such as albumin and polyvinylpyrrolidone, have been shown to pass from the blood into the gastrointestinal contents in greatly increased quantities in Type II bovine ostertagiasis, Mulligan, Dalton and Anderson (1963).

The plasma pepsinogen level was directly correlated with the severity of the abomasal lesion and also with the numbers of O.ostertagi present at autopsy 21 days post infection. These facts indicate that plasma pepsinogen concentration might be a useful diagnostic and epidemiological aid.

The lesions were identical in type to those described in the first experiment and differed only in their increased severity. At dose levels of 400,000 and 800,000 larvae, widespread confluence of the nodule lesions produced the "morocco leather" appearance and cytolysis usually found at autopsy of clinical field cases. This is contrary to the observations of Ross & Dow (1964), who stated that the production of clinical disease at normal patency was probably dependent on partial immunity induced by previous infection.

SUMMARY

Five groups of five 15 week old calves each were given single infections of O.ostertagi larvae, the group individual doses being 50,000, 100,000, 200,000, 400,000 and 800,000. All calves were autopsied on day 21 post infection.

Diarrhoea and weight loss were observed just prior to patency at day 17 in calves given 100,000 larvae or more. In the calves in groups given infections of 400,000 and 800,000 larvae, the severity of the signs and lesions increased to a degree typical of Type I ostertagiasis in the field.

Marked increases in the pH of abomasal contents and of plasma pepsinogen concentration were recorded at autopsy in all calves except those given 50,000 larvae.

There was a direct correlation between the log numbers of worms found at autopsy and the log larval dose. Although correlated, the single sample and 5 day mean faecal egg counts were not useful criteria to predict the number of female worms at autopsy.

Only a few inhibited larval stages were found at autopsy, so it is concluded, that inhibition of development is unlikely to be a function of the larval dose size. Likewise the stunting of adults was not a feature of this infection regime.

EXPERIMENTS 3 and 4

TWO REGIMES OF REINFECTION WITH O. OSTERTAGI LARVAE

TWO REGIMES OF REINFECTION WITH O.OSTERTAGI LARVAE

INTRODUCTION

The two previous experiments described the experimental production of a disease indistinguishable from Type I bovine ostertagiasis.

To facilitate the study of the pathogenesis of the Type II syndrome it was necessary to produce a state of inhibition of larval development in experimental calves, whereby large numbers of early 4th stage parasites would be present in the abomasal mucosa. Two explanations have been advanced as to the cause of inhibition in O.ostertagi infections;

(a) the inhibition of larval development results from a massive intake of larvae over a short period of time, Martin et al (1957);

(b) inhibition is a consequence of immunity derived from a previous infection, Ross (1963), or in the later stages of a current infection, Michel (1963).

Experiments 1 and 2 showed that when calves, aged between 8 and 15 weeks, were given single infections of between 50,000 and 800,000 O.ostertagi larvae some 95 to 100 per cent of the established worms developed to maturity in 17 to 21 days; the remaining 5 per cent were present as early 4th stage parasites. Therefore, it seems unlikely that gross inhibition of larval development (i.e. over 50 per cent of the total O.ostertagi burden) is associated

with a massive intake of larvae over a short period of time.

The present experiments were designed to study the alternative explanation, i.e. to find out if inhibition of development could be produced experimentally, by two different regimes of multiple reinfection, with large numbers of O.ostertagi larvae.

EXPERIMENT 3

CALVES GIVEN FOUR DOSES OF 100,000 O.OSTERTAGI LARVAE AT INTERVALS OF ONE WEEK

CALVES GIVEN FOUR DOSES OF 100,000 O.OSTERTAGI AT INTERVALS OF ONE WEEK

MATERIALS AND METHODS

Animals

Nine male Ayrshire calves, were reared parasite free, to 9 weeks of age, and were then divided into two groups of 4 and 5 calves respectively so that the mean weight of each group was the same. Each calf in both groups was dosed with 100,000 motile 3rd stage O.ostertagi larvae on days 0,7,14, and 21 of the experiment: a total of 400,000 larvae in 4 equally divided doses was given at intervals of one week.

Observations

A daily faecal examination was carried out in the manner described previously. The calves were weighed and samples of blood were collected at weekly intervals.

Autopsy

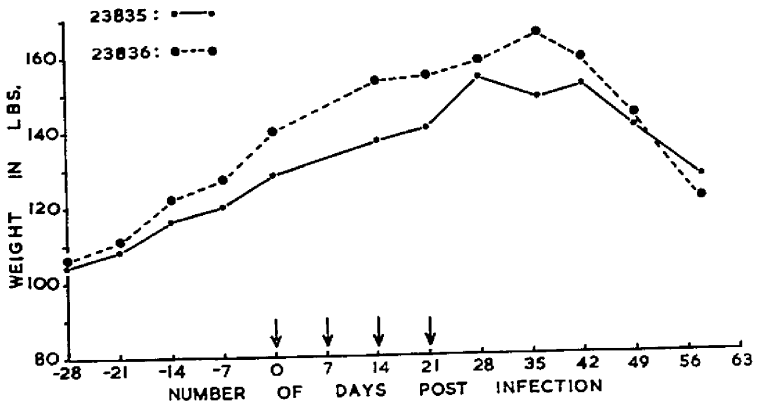
Group 1, which comprised four calves, was scheduled to be autopsied 21 days after the last dose of larvae was given, i.e. day 42 of the experiment, and the remaining 5 calves in Group 2 were to be allowed to survive for an additional period to find out if the inhibited population of larvae, if present, would mature and produce clinical signs of Type II ostertagiasis.

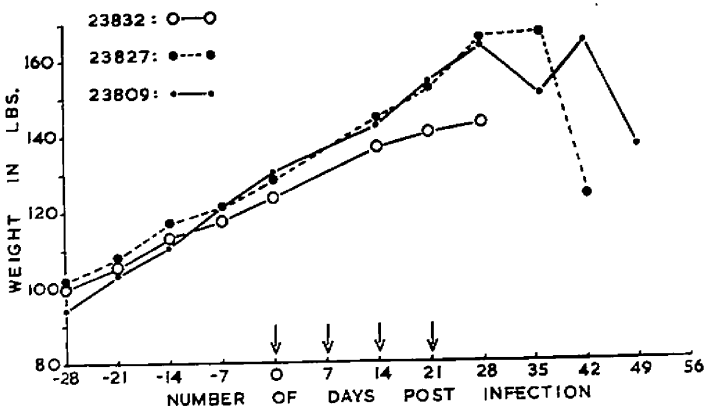
Because of the severity of the disease, which resulted, it was not possible to follow the original plan, for a calf in Group 1 died on day 25 and 3 calves from Group 2 died or

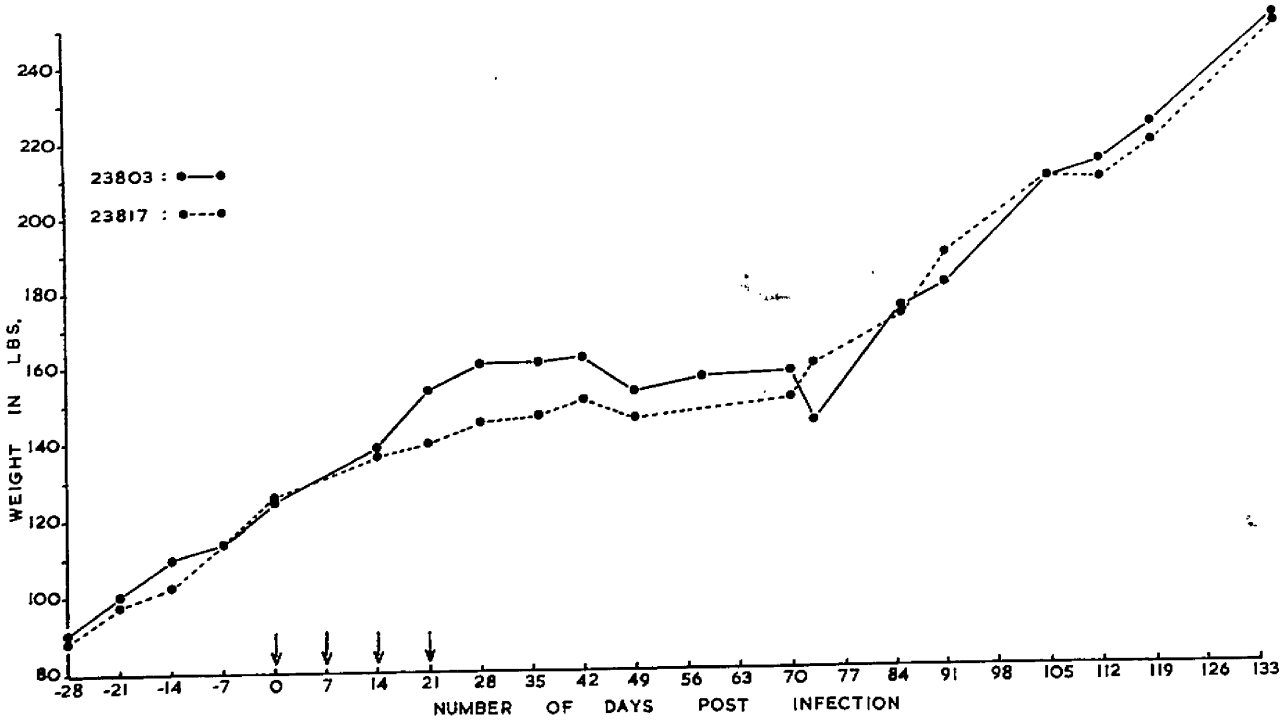
EXPERIMENT : 3

Graphs of Weight Changes

↓ : calves dosed with 100,000 O. ostertagi larvae







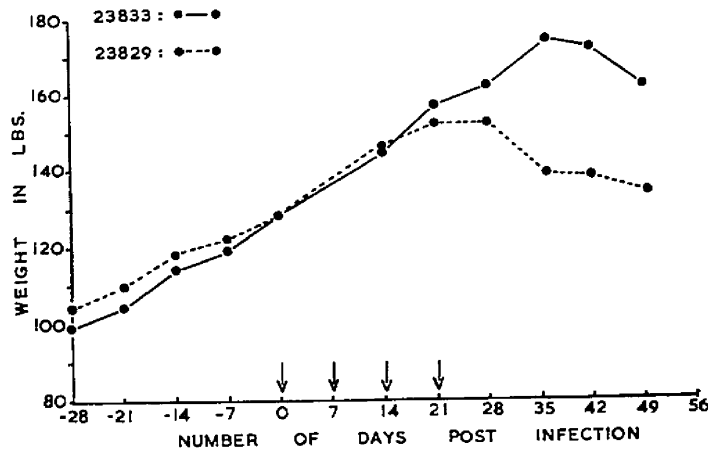


Fig. 41 Graphs of the weight changes of individual calves which were infected with 100,000 O. ostertagi larvae at weekly intervals.

TABLE 25

Calves Given Four Doses of 100,000 O.ostertagi Larvae at Intervals of One Week

A Summary of the Severity and Duration of Diarrhoea and the Condition of Calves at Autopsy

Calf No.	Mean Weight Day 0	Diarrhoea		Egg-counts of 1000+ No.of days*	Condition at Autopsy	Autopsy Day
		No.of Days	Day of Onset			
Group 1						
1	127 lb	8/8	16	3/8	In extremis	25
2		16/24	23	9/24	In extremis	42
3		2/24	35	4/24	Moderate	42
4		7/24	26	10/24	Fair	42
Group 2						
1	129 lb	8/20	27	9/18	In extremis	38
2		15/37	25	20/37	In extremis	55
3		14/36	32	25/36	In extremis	54
4		12/72	32	10/72	Fair	136
5		9/72	39	5/72	Fair	136

* Number of days when faecal worm egg counts of over 1,000 eggs per gram.

were autopsied *in extremis* on days 38, 54 and 58 of the experiment. Only two calves survived until the scheduled autopsy time of 136 days.

RESULTS

Clinical Observations

Severe diarrhoea and a loss of bodyweight, which were characteristics of the naturally occurring disease, was noticed in all 9 infected calves. Diarrhoea first occurred on day 16 and continued, sometimes intermittently, for 21 days. The loss of bodyweight, shown graphically in Fig. 41, was first recorded on day 20 and was greatest in those calves which showed prolonged, severe diarrhoea; some of the severely affected calves continued to eat their normal ration while others showed inappetance.

Two calves became so weak that they were unable to stand and these were autopsied *in extremis* on days 25 and 38 of the experiment. One of the three calves autopsied on day 42 was also killed *in extremis*. Of the four calves intended for autopsy on day 136, two were killed *in extremis* on days 54 and 55 respectively. The clinical signs shown by the two surviving calves were less severe than in the others, and from day 70 onwards they appeared bright and gained weight at a rate similar to that observed prior to infection. A summary of the clinical observations has been given in Table 25.

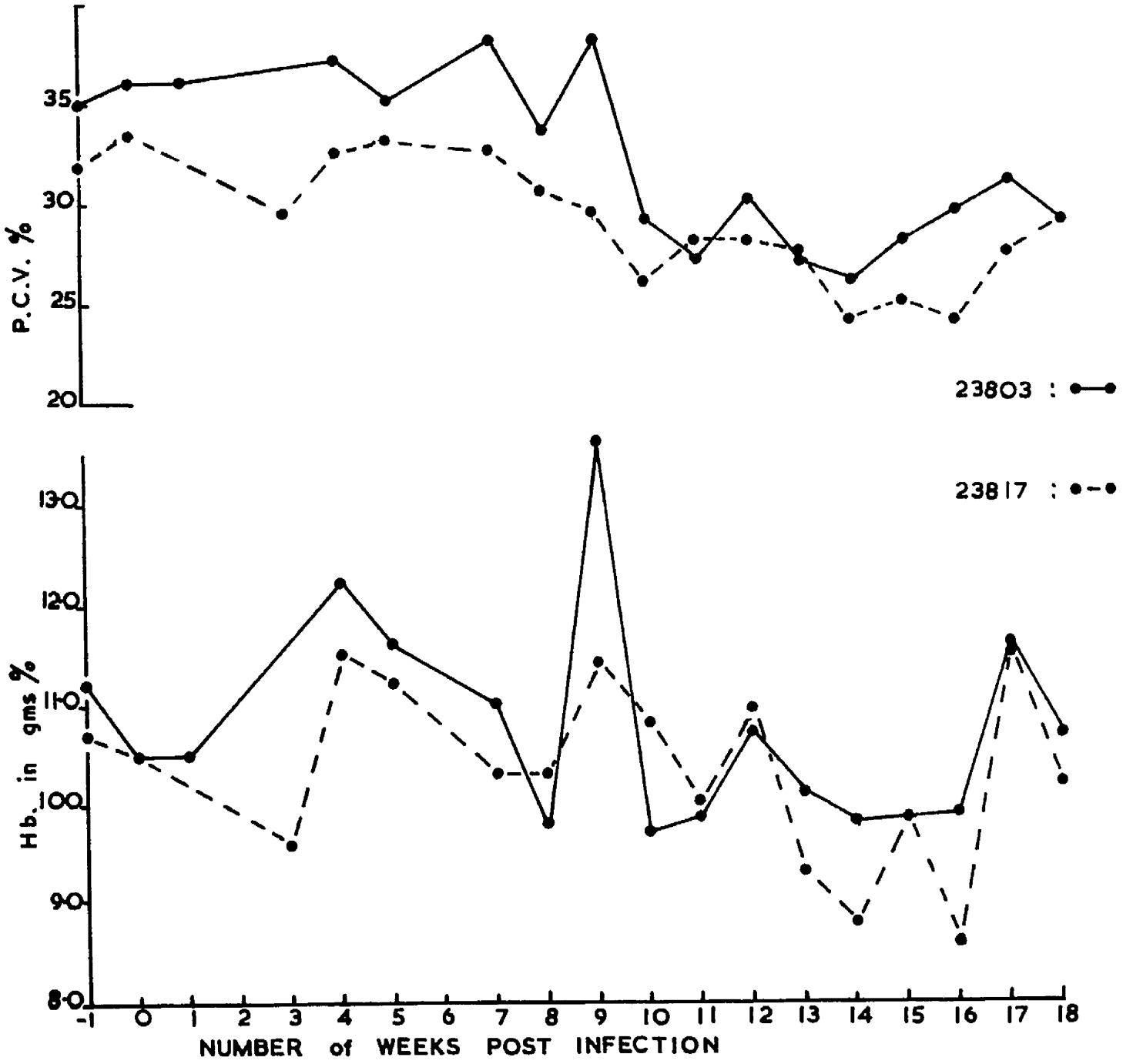


Fig. 12 A graph of the change in packed cell volume (P.C.V.) and haemoglobin concentration (Hb.) in two calves given 100,000 O.osteopheri larvae at weekly intervals.

Haematological Data

Fluctuations in packed cell volume, P.C.V., did occur in all calves autopsied on or before day 55, but as a group these calves did not differ from uninfected calves of the same age. During the diarrhoeic phases of the disease the P.C.V. of individual calves increased by amounts of up to 12 per cent; the largest increase was associated with the effects of four or more days of continuous diarrhoea. Haemoglobin concentration and red blood cell counts, paralleled the P.C.V. changes, but were of a smaller magnitude. The P.C.V. and haemoglobin concentration determinations of the two calves Nos. 4 and 5, which survived to day 136, have been graphed in Fig. 42. At day 79 the packed cell volume of these two calves decreased to 29 and 26 per cent respectively and remained at this lower level until day 128 when an upward trend towards preinfection levels was noted. During this period the haemoglobin concentration also decreased. No change was observed in the total serum protein or albumin concentration throughout the experimental period.

pH of Abomasal Contents

Gross changes in the pH of the abomasal contents were noted at autopsy in all calves, except the two which survived until day 136 of the experiment. In these latter calves the abomasal acidity was similar to that found in non parasitised animals. The pH values have been included in Table 26.

TABLE 26

CALVES GIVEN FOUR DOSES OF 100,000 O.OSTERTAGI LARVAE AT INTERVALS OF ONE WEEK

POST MORTEM WORM COUNTS AND ABOMASAL pH

Calf No.	Abomasal ph	Total O.ostertagi	E 4th Stage *	Sex Ratio M/F	Percentage Established
Group 1					
1	7.1	97,930	3	1/1	25
2	7.1	31.400	3	3/4	7
3	7.0	28,200	2	6/7	7
4	7.1	21,770	1	1/1	5
Group 2					
1	7.1	52,840	2	1/1	13
2	6.1	15,020	3	8/7	4
3	6.8	43,960	1	3/4	11
4	3.3	1,080	8	1/2	1
5	2.6	960	0	2/3	1

* The percentage of the total O.ostertagi present as early 4th stage larvae.

Parasitological Data

Faecal Egg Counts

All calves had patent infections by day 19. Subsequently, marked day to day fluctuations in the faecal egg counts were recorded from all calves; these ranged from 500 to 6,000 eggs per gram with a mean peak of 1,000 e.p.g. which occurred between days 19 and 55. In the two calves which survived beyond day 55 of the experiment, it was found that the faecal egg counts gradually decreased to zero 70 and 90 days after their last infection.

Worm Counts

The results of post morten worm counts have been set out in Table 26. The percentage of worms which had become established was 25 per cent of the larvae given, in the calf which was autopsied on day 25 i.e. only 4 days after the last dose of larvae. Calves which were killed later in the experiment showed a progressive reduction in the number of worms found at post morten.

The number of early 4th stage parasites varied between 0 and 8 per cent of the total worm population recovered from the abomasum.

Pathological Data

Lesions which were found, were generally similar to those described previously; coalescence of nodules was not achieved but was almost so in some areas; also there was

widespread conversion of the glandular epithelium around the distended glands, lined with mucous cells, in which the larvae had developed. In most cases, all the glands were empty, the larvae having previously emerged. The prominent lesions were, the presence of large numbers of plasma cells, eosinophils and mast cells around the necks of the glands; and the dilation of the blood vessels and oedema of the sub-epithelial spaces beneath the superficial epithelium. A feature not seen in experimental calves, given single infections, was the presence of fairly large numbers of globular leucocytes which were emigrating into the epithelium, or through it, the deep and the superficial epithelia were affected. Abomasal sections from calves autopsied on day 136 showed that the mucosal epithelium had reverted to normal.

DISCUSSION

The stage of inhibition or the pre-Type II phase of bovine ostertagiasis, described in naturally acquired infections, did not develop in these calves since the percentages of early 4th stage parasites recovered ranged from 0 to 3 per cent. Ross (1963) gave two oral infections of 100,000 O.ostertagi three weeks apart to each of four 11 week old calves and, at autopsy 28 to 42 days after the second infection, found that between 2.5 and 6.5 per cent of the established populations were inhibited at the early

bth stage. At autopsy of the four control calves which were given the second dose only, between 1.00 and 2.5 per cent of the established worm burdens were inhibited. Rose concluded that, compared with a primary infection, a significant part of the second infection was inhibited at autopsy. However, it has been shown earlier that up to 8 per cent of the worm burden was inhibited following single infections of between 100,000 and 400,000 O.ostertagi larvae to calves of this age.

In the present experiment the last dose of 100,000 larvae was given 21 days after the first when a large number of worms had completed their parasitic phase of development. Inhibition of larvae from the last dose, similar to that found in field cases of inhibition, did not occur. Therefore, it would appear that the presence of a previous infection or the presence of an adult population is not the main factor which induces the inhibition of larval development. However, it is possible that because of the gross physiological and pathological changes which were present in the calves at autopsy, conditions within the abomasum were not favourable for the establishment of an inhibited population of O.ostertagi larvae. In naturally occurring cases of inhibition the abomasal change may be classified as mild to moderate.

Severe clinical disease developed in seven of the nine calves and five of them had to be destroyed in extremis. The clinical signs were indistinguishable from those shown

by calves affected with Type I bovine ostertagiasis. The onset and severity of the diarrhoea was similar to that seen in calves given single doses of 400,000 and 800,000 O.ostertagi larvae.

It can be seen from Table 26 that the populations of O.ostertagi which remained up to day 55 post infection were sufficient to maintain a severe abomasal lesion and the accompanying marked alteration in abomasal pH.

Clinically, the two calves which survived to day 136 were markedly affected. Both of these animals sustained a severe check in weight gain which persisted for eight weeks. Following this check, the calves gained weight at a rate similar to their pre-infection rate. Concurrent with the decreased worm burden found in these calves, there was a decrease in the severity of the abomasal lesion which allowed a return to normal gastric function. This was indicated by the pH values of 3.3 and 2.6 respectively. It is clear that the lesion and the associated physiological changes can persist for a considerable time following severe clinical disease, but if animals are not further infected, they can recover.

SUMMARY

Nine Ayrshire calves, 9 weeks of age, were each infected with four doses of 100,000 O.ostertagi larvae at intervals of one week.

Five of the nine calves were killed in extremis and all developed clinical signs typical of those shown by cases of Type I ostertagiasis. The pathological and biochemical findings were indistinguishable from those associated with naturally occurring Type I ostertagiasis.

An attempt was made, to experimentally reproduce, the massive inhibition of development in O.ostertagi larvae. However, a significant degree of inhibition was not found in any of the calves, since the number of early 4th stage parasites, recovered at autopsy, varied between 0 and 8 per cent of the total worm burden; a reason for this failure could have been associated with the gross physiological and pathological changes which were present in the abomasa of these calves.

The two calves which survived until day 136 post infection showed a decrease in packed cell volume and haemoglobin concentration between days 79 and 128 post infection. This decrease was similar to that noted in some field cases of Type II ostertagiasis, but the reason for the occurrence was not determined.

EXPERIMENT 4

REPEATED SMALL DOSES FOLLOWED BY A SINGLE LARGE DOSE OF O. OSTERTAGI LARVAE

REPEATED SMALL DOSES FOLLOWED BY A SINGLE LARGE DOSE OF O.OSTERTAGI LARVAE

INTRODUCTION

The aim of the present experiment was to reproduce pre-Type II in the laboratory as a pre-requisite for the study of the Type II syndrome.

In the previous experiment it had been concluded that the presence of the severe abomasal lesion which occurred, was one explanation for the failure to induce inhibition of larval development. Also, the possibility existed that the abomasal mucosa had to be sensitised to O.ostertagi before inhibition of development could be induced. Therefore, repeated small doses of larvae were given in the initial part of the experiment.

Judging from the numbers of early fourth stage larvae recovered in the digest material, from cases of pre-Type II and Type II, it was thought that relatively large numbers of larvae were ingested during the short, late autumn grazing period, consequently a large single dose of larvae was thought appropriate to simulate this type of larval intake.

MATERIAL AND METHODS

Animals and Dosing Regime

Seventeen male Ayrshire calves were reared parasite-free to 8 weeks of age, weighed, allotted to 5 groups and dosed according to the schedule set out in Table 27.

TABLE 27

Repeated Small Doses Followed by a Single Large Dose of O.ostertagi larvae

The Mean Weights, Dosing Schedule and Autopsy Day of the Five Groups of Calves

Group No.	No. of Calves	Mean Weight Day 0	Day Post Infection and Larval Dose given			Autopsy Day
			0-27	28-45	46	
1	5	120lbs	20x1,000	Nil	800,000	67
2	5	105lbs	20x1,000	Nil	400,000	67
3	5	104lbs	20x1,000	Nil	400,000	134
4	2	168lbs	20x1,000	Nil	Nil	46
5	2	93lbs	Nil	Nil	400,000	67

Observations

A faecal examination of each calf was made every alternate day from day 15 onwards. The calves were weighed regularly and samples of jugular blood were taken each week. In addition, extra blood samples were taken from Groups 1 and 2 on days 53 and 57 of the experiment.

Autopsy

Group 4 was autopsied on day 46 post infection. Groups 1, 2, 3 were autopsied on day 67, that is 21 days after the challenge dose. It was planned that group 3 would be maintained for a further 67 days, i.e. to day 134 of the experiment. However, this proved to be impossible and calves of group 3 were autopsied between days 67 and 72. A full pathological and parasitological examination was made on each calf at autopsy.

TABLE 28

Repeated Small Doses Followed by a Single Large Dose of O.Ostertagi Larvae

The Severity and Duration of Diarrhoea and Inappetence of Infected Calves

Calf No.	Days 0-45 Post Infection	Days 46-72 Post Infection				Autopsy Day
		Diarrhoea		Inappetence		
		Onset Day	No. of Days	Onset Day	No. of Days	
1	-	60	6	54	7	67
2	-	64	3	-	-	67
3	-	54	10	54	10	64
4	-	54	9	54	10	64
5	-	65	3	-	-	67
6	-	56	4	58	6	67
7	-	67	1	54	12	67
8	-	64	1	-	-	67
9	-	60	8	-	-	67
10	-	64	3	-	-	67
11	-	56	6	70	1	70
12	-	68	4	56	12	70
13	-	60	5	69	3	71
14	-	56	9	70	2	71
15	-	67	1	67	1	67
16	-	-	-	-	-	46
17	-	-	-	-	-	46
18	-	62	6	54	8	67
19	-	56	12	60	4	67

in Pounds

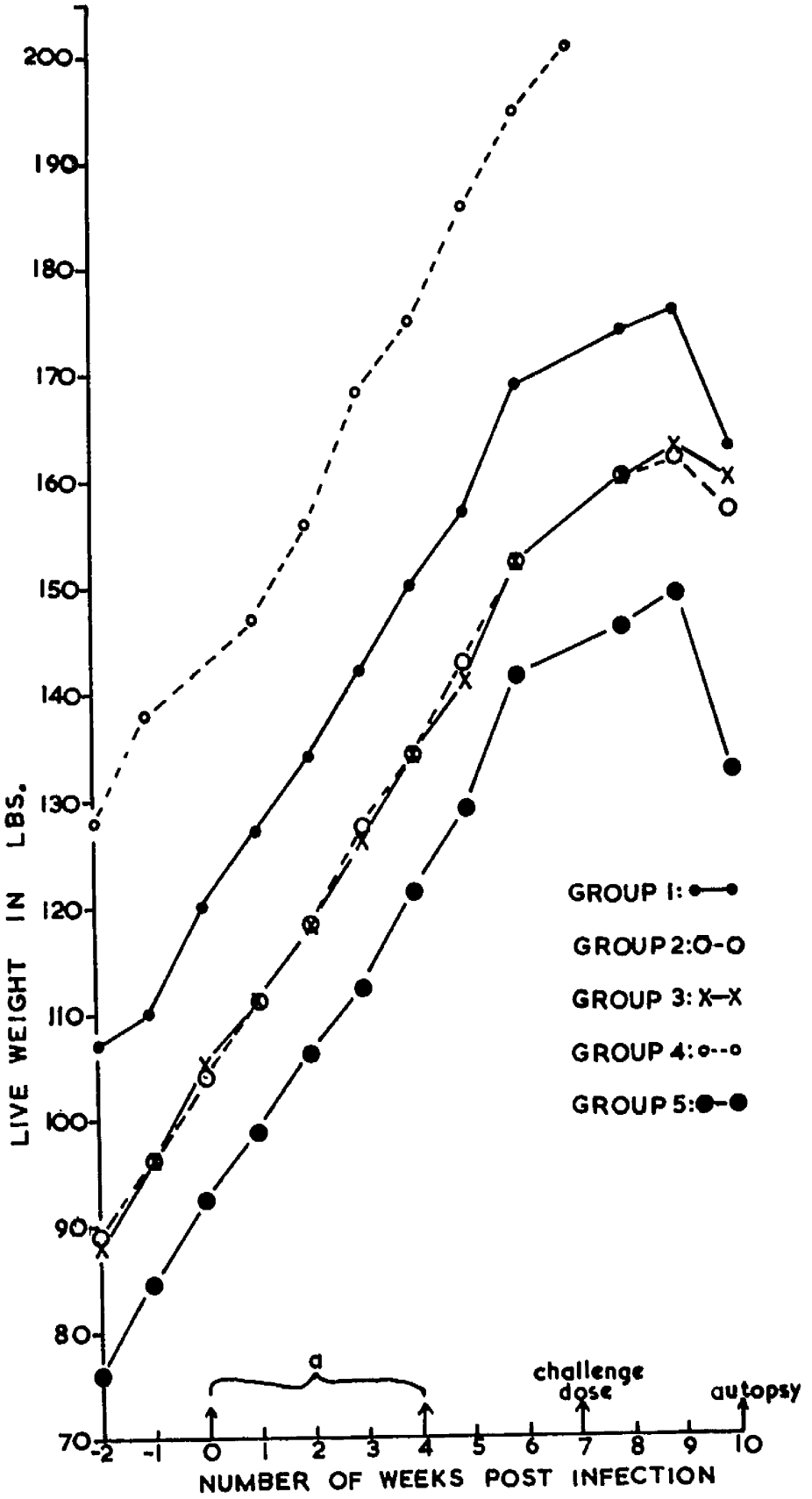


Fig. 43 A graph of the group mean weight.
a. During this period calves in groups 1 to 4 were given 20 doses of 1,000 O.ostertagi larvae.

RESULTS

Clinical Observations

Days 0 to 45 of the Experiment.

The faeces of calf No.11 were semi-fluid in consistency on days 15 to 18 inclusive, but this appeared to have no effect on the general well-being of the calf and produced no detectable change in weight gain. All other infected calves were bright, in good condition, eating and drinking readily, and their faecal consistency and weight gain showed no departure from normal.

Days 46 to 72 of the experiment

Severe clinical signs were shown by all calves in Groups 1, 2, 3 and 5. The clinical signs were identical in type and severity to those described in the second and third experiments. A summary of the clinical findings has been given in Table 28. A graph of the group mean weight change has been depicted in Fig. 43.

Inappetence was a marked feature of the experiment and it occurred in 12 out of the 17 calves which received the high doses of larvae. It was first noted on day 54, i.e. 8 days following the challenge dose, and in affected calves the duration of inappetence varied from 1 to 12 days in the period 12 days prior to autopsy.

Diarrhoea occurred in all 17 calves prior to autopsy and it was first recorded on day 54: some calves had

continuous diarrhoea for 8 to 10 days while others showed this abnormality for only 1 or 2 days prior to autopsy. There did not appear to be a clear relationship between the occurrence of inappetence and diarrhoea in individual calves.

The severity of the clinical signs was similar in all groups which received the challenge doses of larvae and the severity was of such a degree that five calves were autopsied in extremis prior to the day scheduled for their autopsy.

Haematological and Biochemical Data

Anaemia and alteration of the total serum protein concentrations or change in the albumin to globulin ratios did not occur within the experimental period.

Gross changes in abomasal pH and electrolyte concentrations (sodium, potassium and chloride) were recorded at autopsy in all calves except the two calves of Group 4, which were given 20 doses of 1,000 larvae but not subsequently challenged. There were no significant differences between the values from calves of Groups 1, 2, 3 and 5.

By day 46 post infection, the plasma pepsinogen concentration of calves from each group varied between 7.5 and 17.8 ug phenol. The plasma pepsinogen concentration of uninfected calves of this age is 8.0 ± 2.0 ug phenol/ml/2½ hours at 37°C; however, plasma pepsinogen levels increased

TABLE 29

Repeated Small Doses Followed by a Single Large Dose of O.ostertagi Larvae

The pH and Electrolyte Concentrations of Abomasal Contents at Autopsy and the Plasma Pepsinogen Concentration

Calf No.	Abomasal Contents				Plasma Pepsinogen ug phenol		
	pH	Na^+ *	K^+ *	Cl^- *	Day 46	Day 53	Day 60
1	7.20	118	4.8	88	13.5	60.2	82.6
2	7.20	145	9.6	106	17.1	60.9	81.3
3	N.S.	N.S.	N.S.	N.S.	14.2	48.6	54.4
4	7.10	130	8.2	100	16.0	39.8	46.5
5	7.55	139	5.6	96	13.5	64.8	119.3
Mean	7.26	133	7.05	97	14.86	54.86	76.82
6	7.40	133	7.6	99	17.2	36.5	59.4
7	7.70	136	11.8	99	10.8	34.4	69.0
8	6.30	112	14.2	117	7.5	28.2	61.8
9	7.35	136	8.4	108	14.8	44.7	84.3
10	7.20	133	10.4	110	12.1	49.0	46.3
Mean	7.19	130	10.48	106	12.48	38.56	64.16
11	7.10	127	9.8	81	14.3	47.3	113.0
12	7.10	118	9.4	76	12.2	43.5	87.2
13	6.90	115	15.4	88	13.4	51.5	86.8
14	6.85	118	13.8	86	9.4	29.7	55.0
15	N.S.	N.S.	N.S.	N.S.	12.8	41.7	66.6
Mean	6.99	119	12.1	82	12.42	42.74	81.72
16	3.38	78	19.6	122	11.5	-	-
17	2.92	68	23.5	119	14.8	-	-
Mean	3.15	73	21.5	120	13.1	-	-
18	7.30	130	9.4	106	N.S.	37.9	48.3
19	7.40	124	5.6	95	N.S.	18.5	51.1
Mean	7.35	127	7.5	100	-	28.2	49.7

N.S. = No Sample

* ion concentrations in milli equivalents per litre.

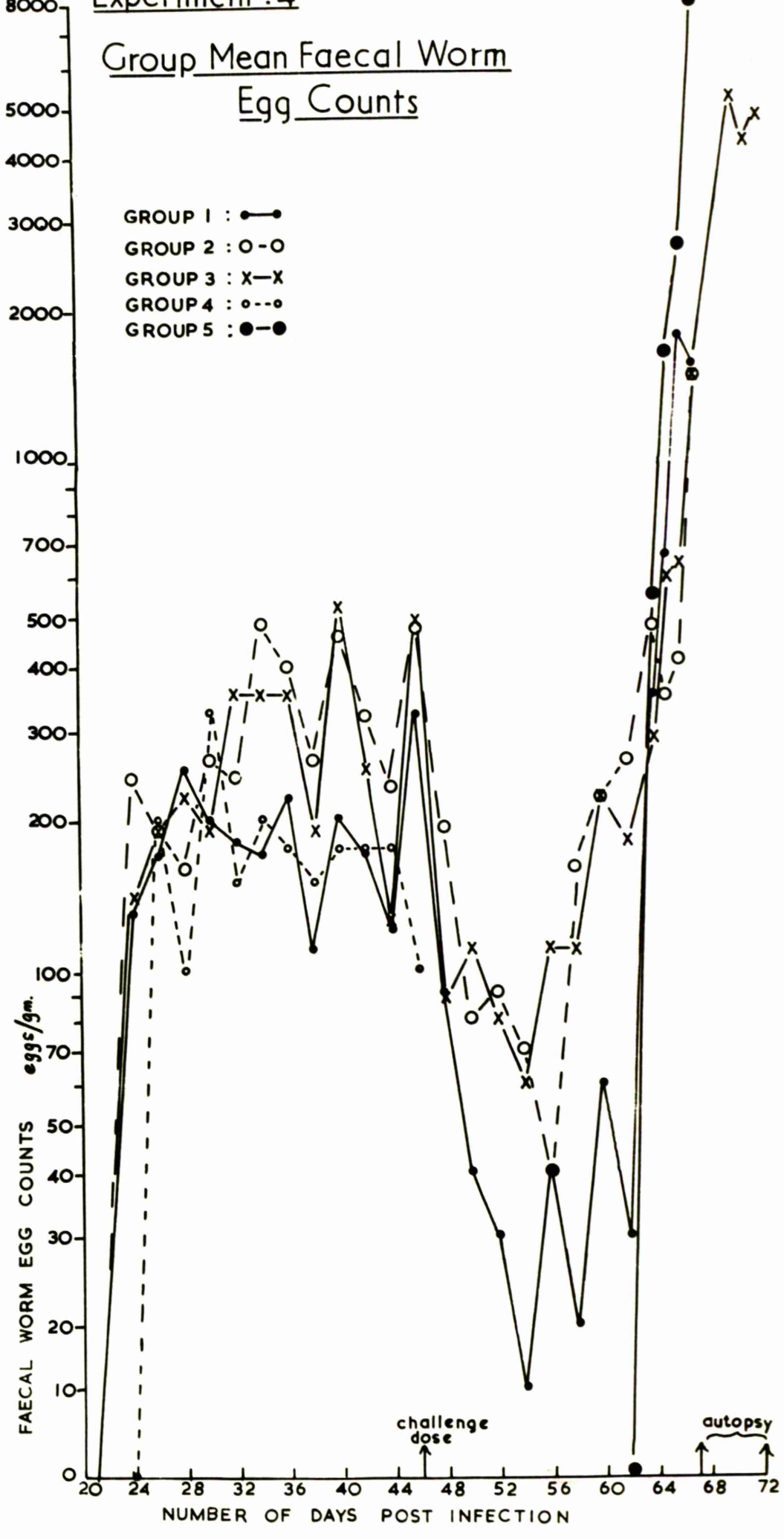


Fig. 44 A graph of the group mean faecal egg counts of calves given repeated small doses followed by a large single dose of *O. ostertagi* larvae.

TABLE 30

Repeated Small Doses Followed by a Single Large Dose of O.ostertagi Larvae

Post Mortem Worm Counts and Measurements

Group Challenge Dose	Calf No.	O.ostertagi Total	Sex Ratio M/F	E 4th Stage ✓	Percentage Established	Female Length (mm) Mean ± SD
1	1	137,600	1/1	4	17	8.02 ± 0.46
	2	146,100	3/4	4	18	7.53 ± 0.50
20,000 +	3	133,600	3/4	1	16	7.47 ± 0.82
800,000	4	106,800	3/4	7	13	6.34 ± 0.88
	5	102,400	5/4	2	13	7.79 ± 0.73
Mean		125,300	-	3.6	15.4	7.43 ± 0.67
2	6	130,000	5/4	1	31	7.36 ± 0.44
	7	103,200	4/5	3	25	7.75 ± 0.46
20,000 +	8	94,400	2/1	4	23	8.19 ± 0.44
400,000	9	119,600	6/5	2	29	8.04 ± 0.53
	10	143,200	7/6	1	34	7.96 ± 0.43
Mean		118,080	-	2.6	28.4	7.86 ± 0.46
3	11	79,700	1/1	2	19	8.01 ± 0.39
	12	77,200	6/5	5	18	7.88 ± 1.08
20,000 +	13	36,200	2/3	1	9	7.42 ± 0.46
400,000	14	12,700	2/3	7	3	7.56 ± 0.51
	15	81,800	1/1	1	20	7.15 ± 0.63
Mean		57,520	-	3.2	13.8	7.60 ± 0.61
4	16	7,400	3/4	0	37	8.81 ± 0.57*
	17	3,500	1/1	0	18	
20,000						
Mean		5,450	-	0	27	8.81 ± 0.57
5	18	176,600	4/1	0	44	8.03 ± 0.48
	19	127,200	5/6	1	32	8.45 ± 0.56
400,000						
Mean		151,900	-	0.5	38	8.24 ± 0.52

✓ The percentage of the total O.ostertagi count present as early 4th stage larvae.

* Combined sample from both calves.

markedly 7 days post challenge and remained high until autopsy.

Individual and group mean values for the biochemical determinations are shown in Table 29.

Parasitological Data

Faecal Egg Counts

Twenty-one days after the first dose of larvae the faecal egg count of all calves was negative, but by day 24 the majority of infections were patent.

The mean faecal worm egg counts from calves in each group have been graphed in figure 44. Initially, egg counts from individual calves greater than 1,000 e.p.g. were not recorded and the group mean egg counts did not exceed 500 e.p.g.

Following the administration of the challenge doses of O.ostertagi larvae on day 46 a significant decrease occurred in the group mean egg counts of Groups 1 to 3. The mean counts decreased to less than 100 e.p.g. From day 58 onwards, that is, 12 days post challenge, the mean egg counts of Groups 2 and 3 began to increase and were well above 1,000 e.p.g. by day 67. The mean egg count of Group 1 did not increase until day 64 but it continued to rise thereafter in a similar fashion to Groups 2 and 3.

Worm Counts

The results of the post mortem worm counts are shown in Table 30.

The total worm counts of Groups 1 and 2 were very

similar but the percentage of the dose established in Group 1 was approximately half that of Group 2. The worm counts from calves in Group 3 were significantly different from those in Group 2; the numbers of worms found at autopsy decreased directly with the number of days prior to autopsy.

The percentage of early 4th stage larvae present in the abomasal mucosa was small; the maximum number was 7 per cent of the total worm burden.

Worm sex ratios, approximately 1 : 1, were of the same order as those found in other experiments.

The Measurement of Female Worms

The length of each of about 20 female worms was measured from every calf in the Groups 1, 2, 3 and 5. Measurements were made from a pooled sample of female worms obtained from both calves in Group 4; these measurements are shown in Table 30. An analysis of variance was carried out, and it was found that there was no difference in the length of female worms present in the calves from Groups 1, 2, 3 and 5.

Pathological Data

The lesions were similar to those described earlier. In calves from the challenged groups the lesions were severe with coalescence of the nodules, marked thickening of the mucosa, eosinophile and plasma cell infiltration and subepithelial oedema. There was also a marked

lymphoreticular immunological type reaction on both sides of the muscularis mucosa, particularly in the submucosal region. Those cases which received 800,000 larvae also showed severe diphtheresis and cytolysis associated with marked sub-epithelial oedema.

In all of the cases the local lymph nodes were grossly reactive and showed both numerous active germinal centres and also packing of the medullary cords with plasmablasts and plasma cells.

DISCUSSION

Inhibition of development of O.ostertagi at the early 4th larval stage has been observed at autopsy of field cases of bovine ostertagiasis.

The mechanism for this phenomenon is unknown but two separate factors have been postulated as being implicated in its aetiology. The first is associated with repeated infections of O.ostertagi larvae and this has been described by Michel (1963) and Ross (1963).

Throughout his entire experimental period, Michel (1963) administered daily oral infections of 1,500 O.ostertagi larvae to each of 15 parasite-free calves, which were subsequently autopsied singly between days 42 and 318 of the experiment; a high percentage of the established worm population, in excess of 50 per cent, was inhibited at the early 4th larval stage in the calves autopsied after day 127.

By comparison, the regime of infection described in the present experiment did not result in the inhibition of a significant proportion of the established worm burden.

Two reasons may be suggested to explain this different result. These are:

(i) that the regime of infection used was neither as great nor as prolonged as in Michel's experiment;

(ii) that inhibition of larval development may be associated with the age of the host.

The calves in Michel's experiment were between 105 and 273 days old at the time of their first infection, whereas the calves used in the present study were 63 days old when first infected with O.ostertagi. ~~The youngest of the calves in the pilot field study was 105 days when first put out to graze infected pasture.~~

The second factor thought to be responsible for the inhibition of O.ostertagi development is associated with physiological changes in the host, or in the infective larvae found on pasture in late autumn, and these changes have been discussed earlier.

The lengths of the female worms collected from calves in Groups 1, 2 and 3 did not differ significantly and they were not different from the calves in Group 5, which received only a single infection of 400,000

O.ostertagi larvae. Despite the fact that there were only two calves in Group 5, it seems more likely that stunting of adult female worms is a function associated with the individual calf response to infection rather than an expression of the numbers of parasites present per se. These results confirm a similar finding described earlier in the second experiment. The loss of the adult worm population observed between the 21st and 28th day after large infections of O.ostertagi was again confirmed in this experiment; the mean worm burden decreased from 118,000 to 57,000 between day 21 and day 28 following an infection of 400,000 larvae (Table 30, Groups 2 and 3).

During the period of 0 to 45 days post infection, when each calf in Groups 1, 2, 3 and 4 received daily infections of 1,000 O.ostertagi, no clinical abnormality was detected. It can be concluded, therefore, that worm burdens of up to at least 7,000 adult O.ostertagi produce no detectable change in the well being or physiological state of calves, 63 days old, over a period of seven weeks following the first dose of larvae. In addition, these calves proved to be equally susceptible to the effects of a single large dose of larvae as did those calves which did not receive the daily dose regime.

The result of the challenge infections was the development of a syndrome indistinguishable from severe

cases of naturally occurring Type I bovine ostertagiasis. Clinically, calves in Group I, which received a challenge infection of 800,000 larvae, were affected earlier and more severely than those calves which received 400,000 larvae. Two of the five calves in Group 1 died before day 21 following the challenge infection, thus confirming an earlier conclusion that a single infection of 800,000 O.ostertagi larvae approaches the lethal dose for calves of this age. In the experimental design, it was planned that the calves of Group3 would be autopsied on day 134 of the experiment, but the severity of the clinical signs was such that all of those calves would have died before day 77 so the two surviving calves were autopsied on day 71.

The increased abomasal pH values and the changes noted in gastric ionic concentrations of sodium, potassium and chloride were similar to those already described.

The plasma pepsinogen concentration increased dramatically ~~after seven days~~, following the challenge infections, and continued to increase until autopsy day, then, it provided a good indication of the severity of the pathological changes in the abomasal mucosa.

The lesions resembled those found in severe field cases of Type I ostertagiasis, that, is coalescence of the mucosal nodules, thickening of the mucosa, marked cellular infiltrates, both of eosinophils and plasma cells, development of lymphoreticular foci deep in the mucosa, and a marked vascular dilatation with oedema evident beneath the superficial epithelium.

SUMMARY

Fifteen Ayrshire calves, 63 days old, were infected with 20 doses of 1,000 O.ostertagi larvae over a period of one month. Three weeks after the last dose these calves were given a single challenge infection of either 400,000 or 800,000 O.ostertagi larvae. All calves were autopsied within 28 days of receiving the challenge infection.

The experiment was designed to produce a state whereby a large proportion of the established worm burden would be present as inhibited early 4th stage parasites. The maximum percentage of inhibited larvae recovered was seven. The possible reasons for this failure, to produce inhibition experimentally, have been discussed.

The resulting clinical syndromes, the pathological and physiological changes present at autopsy, were indistinguishable from those found in severe cases of naturally occurring Type I bovine ostertagiasis.

Confirmation has been obtained about the loss of adult worms between days 21 and 28 post infection, also the association between stunting of worms and the individual host's response.

A GENERAL SUMMARY OF THE FIELD AND EXPERIMENTAL RESULTS.

A GENERAL SUMMARY OF THE FIELD AND EXPERIMENTAL RESULTS

Following a study of nineteen outbreaks of naturally occurring bovine parasitic gastroenteritis, in which 45.5 per cent of 337 young dairy cattle were clinically affected, O.ostertagi was the predominant nematode species found. The fields which were used year after year for the raising of replacement calves, together with the practice of grazing spring born and autumn born calves in the same field, were factors which were considered to be major predisposing causes of outbreaks of this economically important disease.

Three phases of bovine ostertagiasis could be distinguished on the basis of clinical history and laboratory findings.

TYPE I 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 which occurred at any time from late July until the end of the grazing season. The vast majority of the ingested larvae developed to maturity within the expected period of three weeks.

PRE-TYPE II was clinically not apparent though large populations of O.ostertagi were present, of which over 80 per cent were inhibited at the early 4th stage of

larval development. Those animals had grazed infected pasture until the late autumn, but had no history of diarrhoea and usually appeared healthy to the farmer.

The second clinical phase, Type II, was different to the first in that calves, which had no history of diarrhoea or weight loss during the grazing season and which were well grown and in excellent condition, were taken indoors about the beginning of November. After a variable period of time, ranging from 3 weeks to 4 months, these animals started to lose weight and to show a profuse, watery diarrhoea. The appearance of the clinical signs coincided with the development to maturity and emergence from the abomasal mucosa of large numbers of inhibited O.ostertagi larvae, which were ingested during the late autumn grazing period.

In the diagnosis of bovine ostertagiasis the results of faecal worm egg counts must be interpreted cautiously. All calves with egg counts of 1,000 e.p.g. and over were clinically affected animals. Because of the gross variation in worm egg counts which occurred in individual cases, low egg counts could not exclude bovine ostertagiasis from the differential diagnosis.

In contrast to the effect seen in Type I cases, the response of Type II affected animals to anthelmintic treatment was poor; consequently, the prognosis was grave

in the latter category.

Anaemia, hypoproteinaemia and hypoalbuminaemia were not detected in cases of Type I ostertagiasis. In cases of Type II there occurred a moderate, normocytic, normochromic anaemia and a marked hypoalbuminaemia. The plasma pepsinogen concentration of infected calves was increased, markedly so in clinical cases, and was correlated with the severity of the abomasal lesions and the numbers of O.ostertagi found at autopsy. The pH and sodium ion concentration of the abomasal contents was markedly increased in Type I and Type II cases, but was only slightly elevated in Pre-Type II affected animals. The physiological abnormalities were associated with marked histopathological changes within the abomasum. These were described as a loss of cellular differentiation of the specialised cells, hyperplasia of the mucous epithelium and infiltration by reticulo-endothelial cells. Further study would be required to establish how the parasites caused the abnormalities observed.

A detailed study of the naturally occurring disease revealed that the numbers of larvae available on the pasture of the calf rearing fields increased progressively from May to July and reached a peak at the end of August. Type I disease occurred after 9 to 16 weeks grazing on these pastures and some 50,000 to 60,000 O.ostertagi were required to precipitate clinical signs in calves of 4 to 6 months of

age. In contrast to the worm counts from calves autopsied prior to October, a marked increase in the numbers of early 4th stage parasites was found in both previously infected and worm free calves which had grazed the heavily contaminated pasture, for as short a period as 14 days, during the late autumn period. This phenomenon was caused by inhibition of development at the early 4th larval stage and was attributed to an unspecified physiological change within the host or the larvae at this time of the year.

The pathogenesis of a single infection of 100,000 O.ostertagi larvae was studied experimentally in parasite free calves. Eighteen calves were autopsied in pairs from 2 to 90 days post infection. The larvae were found within the gastric glands 2 days after infection and emergence from the gland into the lumen, which coincided with patency and the development of clinical signs, occurred between days 16 and 21 after infection. Inhibition of development was not observed, but a marked loss of adult worms occurred between 16 and 28 days after infection. All the lesions seen in the typical field case were reproduced.

With the growth of the larva, the gastric gland dilated and became lined with undifferentiated, tall mucous cells to produce the characteristic white mucosal nodule, which was readily seen in the fundic region of the abomasum. Prior to the 4th larval moult at day 8 of the infection, the mesenchymal reaction was slight. Towards the end of the -

growth phase this reaction increased and glands adjacent to that containing the larva lost their differentiated epithelium and became lined with cuboidal cells. Coalescence of hyperplastic nodules produced the "morocco leather" appearance of the mucosa. When the worms emerged from the gastric glands, they lay close to the surface epithelium at which site cytolysis and superficial sloughing occurred. Plasma cells were numerous at this time. Replacement and differentiation of the glandular epithelium took place very slowly.

In an experimental attempt to reproduce massive inhibition of development (a necessary pre-requisite for the appearance of the Type II syndrome) three regimes of infection of calves with O.ostertagi larvae were carried out. Firstly, groups of five calves were given single doses of 50,000; 100,000; 200,000; 400,000 and 800,000 larvae; secondly, calves were given four doses of 100,000 larvae at one week intervals, and thirdly, calves were given 20 doses of 1,000 larvae over 28 days and this was followed 21 days later by a single dose of either 400,000 or 800,000 larvae. When the calves were autopsied, usually 21 days after the last infection, a significant degree of inhibition of larval development was not found in any of the O.ostertagi worm populations. Clinical signs of Type I ostertagiasis occurred in the majority of these calves and at autopsy the

characteristic biochemical and pathological alterations associated with the Type I disease were found. From those experiments it was concluded that inhibition of development of O.ostertagi larvae was not related to the size of the larval infection nor was it an inevitable outcome of previous multiple infections.

However, before the pathogenesis of the Type II syndrome can be adequately studied, inhibition of development must be induced in calves. An assessment of the environmental factors, or the possible changes in the physiological makeup of host and parasite associated with the pre-Type II and Type II syndromes, would, most likely, reveal the mechanism of the inhibition phenomenon.

APPENDICES

A TO D

INTRODUCTION

During this investigation into the causes and nature of parasitic gastroenteritis of young dairy calves it was necessary to introduce new techniques, establish normal criteria and to consider the sources of variation noted in some of the methods used. The need to carry out such determinations arose from the fact that the information required was not present in the available literature.

The results of studies carried out on some of the methods used in this investigation have been set out in the following appendices which are grouped together for easy reference. A brief introduction relevant to each topic under consideration has been included.

During the investigation ~~and~~ INTO the nature of naturally occurring bovine ostertagiasis a considerable amount of data was collected. After perusal, some of this data was considered to be of only indirect value and has not been discussed in the body of the thesis. Nevertheless, for the sake of completeness and to assist other workers with special interests, two aspects of the study have been included in Appendix C.

APPENDIX A

NORMAL HAEMATOLOGICAL CRITERIA FOR AYRSHIRE CATTLE

NORMAL HAEMATOLOGICAL CRITERIA FOR AYRSHIRE CATTLE

INTRODUCTION

Schalm (1961) reviews the reports of many investigations relating to the normal haematological criteria for bovines. Close agreement has been established with regard to estimations of blood haemoglobin concentration, but a considerable degree of variation has been noted in measurements of packed cell volume and total red and white blood cell counts. Much of this variation was undoubtedly due to the different methods employed, the several technical errors associated with these methods and Schalm made a plea for uniformity and standardisation of procedure.

Among the specific sources of variation noted in normal haematological criteria, were the effects of breed difference, age, climate and nutritional status. Under British conditions of management, age appears to exert the greatest effect, Holman (1955 and 1956); Greatorex (1954 and 1957). Greatorex (1957) showed that the total red blood cell counts of dairy cattle were highest in calves, and these values decreased gradually until the adult level was attained; this occurred between 18 and 36 months of age. Holman (1956) stated that the most definite change in Ayrshire cattle occurred in the mean cell volume which decreased from 44.9 cubic microns at birth to 30.8 cubic microns at 2 months of age, after which time it increased slowly to 57.0 cubic microns;

TABLE 31

Some Normal Haematological Values of Dairy Cattle in Britain

Breed and Age of Cattle, Methods, Mean Values and Range

Author	No. of Animals	Age	Breed	P.C.V. per cent	Method	R.B.C. 10^6 per cm^3	Hb.conc gms per cent	Method
Greatorex (1954)	233	0 to 12 months	Various 98 Ayrshires	38.6±6.6	Wintrobe tube	7.2± 1.9 4.0 to 10.0	11.3± 2.0 9.0 to 14.5	Haldane
Holman (1956)	22	0 to 24 months	Ayrshire females	32 to 35	Wintrobe tube	6.0 to 8.0	10.5 to 11.0	Levibond
Greatorex (1957)	49	Adults	Various	37.0±4.0	Wintrobe tube	5.7± 1.3	12.0± 1.5	Haldane
Holman (1955)	81	Adults	Ayrshire females	33.7±4.1	Wintrobe tube	5.9± 0.8	11.3± 1.5	Levibond
Fisher (1962)	20	Adults	Aryshire females	30.1±2.8 25.6 to 34.1	Micro-haemato-crit	-	-	-

P.C.V. = Packed cell volume.
R.B.C. = Red blood cell count.
Hb.conc = Haemoglobin concentration.

the normal value for adult cattle. A similar but less marked trend was noted, by this author, in the packed cell volume and blood haemoglobin concentration.

Fisher (1962) used the micro-haematocrit method to determine the packed cell volume of Ayrshire cattle. The results of this investigation showed that the micro-haematocrit centrifuge gave better packing of red cells, in a very much shorter time, than other types of centrifuge. Variations in the measurement of packed cell volume were observed and these were partly accounted for by differences in sampling technique, by the choice of the blood vessel used and by diurnal effects.

Table 31 summarises the findings of Holman (1955, 1956) and Fisher (1962) who studied Ayrshire cattle and Greatorex (1954, 1957) who studied various breeds of dairy cattle. This table illustrates the wide range of normal values which have been recorded.

Because of the variety of techniques employed by different workers, Schalm (1961) is of the opinion that normal data collected by one worker is unlikely to be valid for clinical interpretation by others. For this reason, it was considered necessary to establish the normal haematological picture of parasite free Ayrshire cattle of various ages which were reared under the management practices currently used in the South-western area of Scotland.

TABLE 32

Normal Haematological Values of Aryshire Cattle in South-west Scotland

Origin and Age of the Cattle; Means and Standard Deviations of the Parameters measured

Origin and No. of Calves	Age	P.C.V.	R.B.C.	Hb.Conc.	M.C.V.	M.C.H.C
	Weeks	Per Cent	10^6 per cmm.	gms per cent	Cubic microns	per cent
Parasite Free 10	7	34.8 ± 2.52	8.39 ± 0.60	10.82 ± 1.60	41.48 ± 1.98	31.46 ± 1.90
Parasite Free 10	10	35.55 ± 1.72	7.55 ± 0.68	11.03 ± 0.76	47.33 ± 4.01	31.06 ± 2.12
Chapelarroch Farm 12	24	32.04 ± 3.66	6.76 ± 0.60	10.63 ± 1.53	47.41 ± 3.86	33.18 ± 2.60
Auchmannoch Hill Farm 12	24	34.58 ± 2.56	6.64 ± 0.56	11.24 ± 1.00	50.65 ± 6.37	32.38 ± 1.24
Chapelarroch Farm 12	36	33.62 ± 2.63	6.91 ± 0.92	11.16 ± 0.93	50.92 ± 5.76	33.17 ± 1.80
Auchmannoch Hill Farm 12	40	36.13 ± 2.45	7.43 ± 0.85	11.49 ± 1.14	48.93 ± 3.08	31.83 ± 2.40

P.C.V. = Packed cell volume
R.B.C. = Red blood cell count
Hb conc = Haemoglobin concentration
M.C.V = Mean cell volume
M.C.H.C. = Mean cell haemoglobin concentration

ANIMALS

Experimental animals were Ayrshire bull calves reared and maintained parasite free and judged to be free from intercurrent disease. Values for older calves, Ayrshire heifers, were obtained from two farms with different management practices. These animals were well grown, healthy and apparently minimally affected with gastrointestinal nematodes, for regular faecal examinations showed that the majority of samples were negative for nematode eggs.

METHODS

The methods used in this study have been described earlier in Section I.

RESULTS AND DISCUSSION

The results have been set out in Table 32 and in general they agree well with those values set out in Table 31. An analysis of variance was carried out on the data shown in Table 32.

PACKED CELL VOLUME

The analysis of variance revealed that the mean values of the groups shown in Table 32, differed significantly, $p < 0.01$, but there was no significant difference between younger and older animals. The cause of the difference between the means was not investigated.

HAEMOGLOBIN CONCENTRATION

The group mean values for haemoglobin concentration were homogeneous for all age groups and calf origin.

RED BLOOD CELL COUNTS

A significant difference, $P < 0.05$, was found between the mean values from young calves as compared with those from older calves. The trend suggested that the mean value decreased with age, and this finding agreed with the reports cited by Schalm (1961).

CONCLUSION

The results indicated that when the presence of anaemia is suspected, the values from affected animals should be compared with those from parasite free animals of similar age. From the practical viewpoint, this was not always possible when field cases of osbortagiasis were being investigated, because the age of affected animals varies from 3 to 18 months. Suitable worm-free control animals were not available.

It was considered that for the purposes of this investigation, the overall group mean value for each of the parameters studied would adequately define the normal haematological values. Animals which had values differing by more than two standard deviations from these mean values would be classed as abnormal.

APPENDIX B

AN EXAMINATION OF THE LARVAL AND WORM COUNTING TECHNIQUES

1. LARVAL COUNTING TECHNIQUE

INTRODUCTION

The volume which each larva occupies is small when compared with the volume of the suspending fluid. Under such circumstances it is most likely that counts of larvae per unit volume would be distributed according to the Poisson series, in which case the mean count should be numerically equal to the variance, (Snedecor (1963)). It follows that for a dose of larvae to lie between ± 5 or ± 10 per cent of the mean, 95 times out of a hundred, the required number of counts to estimate the mean count of the dose will vary for each value of the mean, according to the following equations:

(i) For ± 10 per cent of mean count

$$0.1\bar{x} = 2\bar{x}/n \quad \text{or} \quad n = 4/0.01\bar{x}$$

(ii) For ± 5 per cent of mean count

$$0.05\bar{x} = 2\bar{x}/n \quad \text{or} \quad n = 4/0.0025\bar{x}$$

in which $\bar{x}$ = mean count of larvae

and n = number of counts.

A mean count of about 20 larvae per 0.025 ml sample was found to be ideal for counting under the 20 times objective of the projection microscope. The minimum number of counts required to give a value of ± 10 or ± 5 per cent of the desired number 95 times out of a hundred for several mean values, has been calculated from the aforesaid formulae and set out in Table 39.

TABLE 33

(a) The Mean Count, Standard Deviation, Variance and Limits of 50 Counts Taken from Five Flasks Containing Five Litres of Larval Suspension

Parameter	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5
No. of Counts	50	50	50	50	50
Mean Count	19.64	24.44	28.70	29.28	38.08
± Standard Deviation	4.17	4.11	4.88	5.22	4.86
Variance	17.42	16.88	23.89	27.29	23.59
95 per cent Limits as percentage of Mean	±6	±5	±5	±5	±4

(b) The Calculated Minimum No. of Counts Required to Give ± 10 and ± 5 per cent of Various Mean Values, 95 Times Out of a Hundred

Mean	No. of Counts Required	
Count	±10percent of Mean	± 5 percent of Mean
16	25	100
20	20	80
25	16	64
36	11	44

THE RESULTS OF COUNTS TAKEN FROM A BULK SAMPLE OF LARVAE

Fifty samples of 0.025 ml each were taken from a five litre flask which contained a suspension of O.asteriarni larvae. The number of larvae in each sample was counted under the projection microscope and the results from five flasks, each of which contained a different concentration of larvae, have been set out in Table 33(a). The mean count, standard deviation and variance were calculated. The approximate 95 per cent confidence limits were calculated from the equation:

$\bar{x} = \pm 2SD/\sqrt{n}$ where $\bar{x}$ = mean, SD = standard deviation and n = the number of counts.

The confidence limits have been expressed as a percentage of the mean count in Table 33 (a).

A comparison of the observed values in Table 33(a) with the calculated values in Table 33(b) shows that the results obtained agree well with the expected values. Therefore, it can be stated that larval counts from a sufficient number of samples, taken in the manner described, will yield a mean count which lies within ± 10 per cent of the true mean count, 95 times out of a hundred.

TABLE 34

The Mean and Standard Deviation of Larval Counts Carried Carried out atDifferent Times

	DAY 28				DAY 42				DAY 67			
Bottle	1	2	3	4	1	2	3	4	1	2	3	4
n	20	20	20	20	20	20	20	20	20	20	20	20
Σx	384	363	367	361	354	380	367	358	441	418	417	410
$\bar{x}$	19.2	18.15	18.35	18.05	17.7	19.0	18.35	17.90	22.05	20.9	20.85	20.5
Σx^2	7646	6711	6897	6671	6792	7544	7125	6604	10,107	9186	9147	8730
SD	3.78	2.55	2.92	2.85	5.26	4.12	4.54	3.20	4.48	4.86	4.88	4.13

DAY 99				DAY 141			
1	2	3	4	1	2	3	4
20	20	20	20	20	20	20	20
330	353	341	385	330	401	392	393
16.5	17.65	17.05	19.25	16.5	20.05	19.6	19.65
5612	6517	6009	7705	5676	8365	7898	7915
2.96	3.88	3.20	3.93	3.48	4.13	3.42	3.18

THE REPEATABILITY OF THE LARVAL COUNTING TECHNIQUE

In addition to knowing the accuracy of the technique it is desirable to know whether or not estimates made at different times from the same suspension of larvae, provide a repeatable estimate of the true mean. The question of repeatability was examined in the following manner.

From a bulk sample of 5 litres containing about 800 larvae per ml, 20 samples of 15 ml each were pipetted into separate bottles and stored at +6°C. Twenty-eight days later four bottles were warmed to room temperature and, after thorough mixing by inversion, 20 counts of about 20 larvae per 0.025 ml were made. This procedure was repeated at 42, 67, 99 and 141 days after the initial count. The percentage of motile larvae exceeded 95 on each occasion, but for the present consideration, the total number of motile and non-motile larvae has been considered.

The data obtained from this study has been set out in Table 34 and were analysed by the analysis of variance method (Snedecor (1969)). Because it was likely that counts of larvae follow a Poisson distribution, it was necessary to carry out a square root transformation of the data before the analysis could be calculated.

The analysis revealed that there was no significant difference between counts made on the bottles sampled on the same day, but counts made on different days varied

significantly in an unpredictable manner. The reason for this variation was not sought, but it was considered likely that the mixing method of inversion or the different 0.025 ml pipettes used on different occasions, contributed to the variation noted.

In order to overcome the effects of these technical errors, all doses of larvae used in a particular experiment were prepared on the same day with the same pipette and several check counts were carried out to ensure that the numbers of larvae per dose were, in fact, between ± 10 per cent of the desired number. Doses of larvae were then stored at +6°C until required.

2. AN EXAMINATION OF THE WORM COUNTING TECHNIQUE

A worm counting technique has been described by Taylor (1934) and by Porter (1942). Other authors, for example, Michel (1963) and Ross (1963), simply state that a dilution worm count method was used. However, in none of these reports was an attempt made to evaluate the method or to standardise the procedure.

A standard procedure for the determination of the total worm count has been detailed earlier in Section I. Once this procedure was adopted as routine it was desirable to learn how many counts should be made from a given volume of suspended material and what accuracy could be expected after counting a minimum number of samples. The small size of the

TABLE 35

The Mean and Standard Deviation of Worm Counts of Abomasal Lumen Contents

Ten Counts of 5 ml each per 200 ml Pot

Calf No.	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Grand Mean
1	0.8± 1.40	1.1± 0.99	1.6± 1.35	1.1 ± 1.29	1.6± 1.84	1.24± 1.3
2	1.5± 0.97	1.4± 1.26	1.3± 0.82	1.5± 1.27	1.2± 1.13	1.38± 1.07
3	5.8± 3.05	3.0± 1.41	4.3± 0.95	3.1± 1.59	3.9± 1.97	4.02± 2.11
4	7.3± 3.06	9.1± 2.18	6.6± 1.90	7.7± 4.14	7.1± 2.02	7.56± 2.81
5	10.5± 1.90	9.3± 2.16	11.3± 3.83	9.5± 3.21	9.0± 2.71	9.92± 2.86
6	15.7± 4.11	10.7± 3.47	12.3± 3.50	10.7± 2.11	8.1± 3.28	11.50± 4.09
7	21.2± 3.99	18.5± 3.53	18.4± 3.10	20.5 ± 3.37	21.6± 2.88	20.04± 3.53
8	25.5± 4.90	27.6± 5.62	21.0± 2.49	19.6± 4.43	24.3± 5.27	23.60± 5.39
9	84.5± 10.39	80.1± 9.36	72.7± 14.79	59.4± 5.04	73.8± 15.78	74.1 ±14.16

TABLE 36

The Mean and Standard Deviation of Worm Counts of Abomasal Digest Material

Ten Counts of 5 ml Each per 200 ml Pot

Calf No.	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Grand Mean
1	10.1 ± 2.96	9.4 ± 2.55	11.3 ± 2.51	9.7 ± 3.53	10.6 ± 3.13	10.22 ± 2.92
2	11.3 ± 3.8	11.8 ± 2.7	9.8 ± 2.9	11.0 ± 2.26	11.8 ± 3.43	11.14 ± 3.03
3	11.8 ± 3.43	11.6 ± 2.95	10.9 ± 2.73	11.1 ± 2.77	12.2 ± 3.43	11.52 ± 2.85
4	12.6 ± 2.17	11.2 ± 3.49	10.9 ± 3.14	12.6 ± 3.53	16.2 ± 4.39	12.70 ± 3.79
5	11.4 ± 4.17	16.1 ± 3.51	12.4 ± 2.37	13.10 ± 2.77	13.4 ± 3.13	13.28 ± 3.49
6	21.5 ± 4.55	22.2 ± 5.22	22.6 ± 4.53	18.3 ± 4.47	17.08 ± 5.05	20.48 ± 5.01
7	21.7 ± 5.54	23.1 ± 4.28	16.8 ± 4.52	19.1 ± 5.63	25.6 ± 6.69	21.26 ± 6.03
8	22.8 ± 3.39	30.0 ± 3.02	25.9 ± 5.85	29.2 ± 5.65	28.4 ± 6.17	27.24 ± 5.48
9	51.9 ± 10.2	35.4 ± 7.28	50.6 ± 13.83	36.0 ± 7.45	57.7 ± 12.5	46.32 ± 13.62

TABLE 37

Analysis of Variation of the Worm Count Data

Presented in Tables 35 and 36

Square Root Transformation

Analysis of Variation of the Data in Table 35

Calf No.	Sums of Squares	Mean Square	F Statistic	P	95 per cent limits as Percentage of Mean *	
	45df	45df			2 Pots,5/Pot	1 Pot,10/Pot
1	24.3583	0.5414	1.009	N.S.	72	72
2	16.7646	0.3725	0.1512	N.S.	50	50
3	10.8368	0.2408	2.866	N.S.	44	56
4	10.8885	0.2419	1.225	N.S.	24	26
5	10.5447	0.2342	0.9506	N.S.	19	19
6	11.3079	0.2514	6.782	0.01	13	15
7	6.6811	0.1485	1.971	N.S.	22	28
8	10.0263	0.2236	5.046	0.01	19	26

Analysis of Variation of the Data in Table 36

1	9.6379	0.2142	0.697	N.S.	18-	18
2	9.6457	0.2144	0.737	N.S.	17	17
3	8.3164	0.1848	0.261	N.S.	16	16
4	10.3611	0.2302	3.530	N.S,	26	34
5	8.7159	0.1936	3.059	N.S.	22	26
6	12.4282	0.2763	2.396	N.S.	19	22
7	16.2698	0.3616	3.948	N.S.	26	32
8	10.5764	0.2351	3.510	N.S.	18	22
9	26.6132	0.5914	9.311	0.01	32	44

P = Probability
N.S. = Not significant
df = Degrees of freedom
* see Text

mature and immature nematodes from the genera Ostertagia, Trichostrongylus, Cooperia and Nematodirus necessitated the use of a dissecting or projection microscope for counting. A sample size of either 2.5 or 5.0 ml was found to be suitable for examination. The smaller volume was chosen when the digests contained a large amount of particulate matter.

An examination of the accuracy and repeatability of the method, was carried out on the abomasal contents of nine calves selected from the range of expected worm burdens in Experiment 2. The lumen contents and the digest material have been considered separately and, in each case, 5 samples of 200 ml each were taken from the 4 litre total suspension. Ten counts, each of 5 ml, were carried out on each 200 ml sample.

The mean and standard deviation of 10 counts for each 200 ml sample pot is given in Tables 35 and 36. The distribution of the counts was assumed to follow the Poisson series. Following a square root transformation of the data, an analysis of variance was carried out to examine the components of variation within and between pots. The results of this analysis have been set out in Table 37. From the analysis, it was concluded that the distribution of the counts was probably Poisson but, in some cases, there was significant variation between pots as compared with the variation within pots from the same animal. This indicated

that mixing or sampling techniques were at fault. In the light of this conclusion, when subsequent worm counts were carried out special attention was directed towards thorough mixing and to the accurate measurement of samples. A reduction in the degree of variation, between counts taken from animals, which had received the same experimental treatment was noted, and as a result of this the between animal variation was also reduced (see Experiment 4, Table 3).

It was stated earlier that, if the distribution is Poisson, the number of observations required to give estimates of the mean count with the same percentage precision will depend on the magnitude of the mean; i.e. for a small mean a large number of counts is required.

Because there was significant variation between sample pots in the examination detailed above, estimates have been made of variance components within and between pots, and from these it would appear that if only 10 counts are to be made per calf, 5 counts from each of 2 sample pots per calf will give more precise estimates of means than 10 counts from a single sample pot.

The approximate 95 per cent confidence limits for mean counts from the test calves assuming 5 counts from each of 2 sample pots and 10 counts from one sample pot have been calculated, and the results have been included in Table 37.

From these calculations, it can be appreciated that the counting technique detailed herein, on the majority of occasions, will give an estimate within ± 20 per cent of the true mean. With small mean values, (e.g. those shown by calves 1 and 2 in the abomasal lumen data), the precision of estimating the mean is very poor. However, this is not a serious drawback, because with the small gastroenterostimal nematodes encountered in this investigation, total counts of 5,000 nematodes or less are unlikely to be clinically significant in calves 3 months and older.

APPENDIX C.

ADDITIONAL DATA COLLECTED DURING THE DETAILED STUDY OF NATURALLY OCCURRING BOVINE OSTERTAGIASIS

A SHORT DESCRIPTION AND HISTORY OF TWO AYRSHIRE FARMS

The following is a short description and history of the two Ayrshire farms on which the detailed field studies were carried out:

FARM A.

Laight Mains, a dairy farm of some 165 acres, owned by Mr. James Purdie, is situated 1,500 feet above sea level a mile to the Southeast of the town of New Cumnock, Ayrshire. The general situation and condition of the farm could be described as marginal and was made worse by the deterioration of the pastures allowed by the previous owners.

The calf field, 4.8 acres in area and situated close to the house on a rocky, partially wooded knoll was used each year for rearing calves. Two low lying depressions in the field retained water for some weeks after heavy rain; a permanent spring helped to maintain one of these areas in a boggy condition. The pasture was of poor quality and consisted mainly of weeds and natural grasses. Because of its aspect, the field was cold, windy and damp with no shelter other than the tall Beech trees and the stone fences.

Mr. Purdie took over Laight Mains in 1962 and in that year milked 67 cows; the total number of cattle was 106. Seventeen replacement heifer calves, 3 to 5 months of age,

were put into the field reserved for the rearing of calves in May, 1962, and by the end of August, all of these calves had shown signs of diarrhoea and severe weight loss. Ten samples of diarrhoeic faeces revealed worm egg counts of from 300 to 4,200 e.p.g; 6 of these counts were in excess of 1,000 e.p.g. Despite several doses of anthelmintics (Methyridine and Phenothiazine) given at the end of August and during September, a total of 9 calves died in the period August to December 1962. Post mortem examinations of some of these calves revealed heavy infections of O.ostertagi. In 1963 only 6 replacement heifers were reared. These calves were first put out to graze in the permanent calf field on the 1st of May and three weeks later they were noticed to be losing weight. Two calves died during the next month and a further 2 calves died prior to August. None of these 4 calves were available for autopsy. The two remaining calves were very severely affected, and one calf was admitted to the Veterinary Hospital early in August for post mortem examination. The several administrations of anthelmintic given to this calf would explain the presence of only a few hundred parasites. However, the abomasal lesions were very severe, the fundic folds were grossly oedematous and the whole mucosal surface had the appearance of thickened morocco leather. The pH of the abomasal contents was 6.0. The sole survivor of the 1963 replacement calves died before the end of October.

Fodder conservation in the form of hay on Laight Mains was meagre in both quantity and quality. Supplementary feeding of the young calves during the summer grazing period was not a usual practice.

Clearly, Laight Mains had a history of severe Type I ostertagiasis, and mortality, which ranged from 50 to 100 per cent of the replacement calves, occurred during the two years prior to the start of the field experiment.

FARM B

Mr. James Paton is the owner of Knockendale Farm, the second farm studied during 1964. Knockendale is situated on gently undulating coastal plain country, less than one mile North-west of Symington village, Ayrshire. It is a farm of 165 acres, of which 20 or so acres are sown to barley and root crops each year. The total number of cattle in the herd was 110 in 1963; 75 of these were milking cows. Knockendale is a well managed and productive commercial dairy farm.

The calf rearing area on this farm comprised two fields, which were considered less productive for other purposes. The smaller field of 0.8 acres was situated across the road from the house, but was well situated and produced a good rye grass clover pasture, which had been sown down in 1962. The size and position, being bounded on two sides by roads, precluded its use for grazing by the milking herd.

The larger field was a rectangular strip 4.5 acres in size also close to the house, but in the main body of the farm. Most of the latter field was low lying and poorly drained so that the runoff from the elevated fields on three sides collected and lay there for some time. Consequently, the pasture was of poor quality and was largely composed of weeds. Apart from a light annual top dressing with superphosphate this field had remained untouched for the past 20 years, and each year had been used for the rearing of replacement calves. There were no trees in either field but some shelter was afforded by the low hedges, which grew along most of the fences.

The usual practice on Knockendale was to alternate the 15 or so replacement calves reared annually, from the smaller to the larger field at roughly fortnightly intervals, depending on the availability of grass in the appropriate field. Supplementary feeding during the summer grazing period was not usual; bruised barley at about 1 lb. per calf per day was given when it was considered the calves were not thriving. Because of the proximity of the larger field to the house, other cattle which included calving cows and heifers had intermittent use of this field.

In 1963, 9 replacement calves were first put out to grass in these fields in May. One of these calves died in June, 2 more in August and another 2 in October; all

calves showed a syndrome characterised by severe diarrhoea and loss of weight. A diagnosis of parasitic gastro-enteritis was made at the beginning of October, following a postmortem examination of one calf, which had recently died; 18,000 adult O.ostertagi were found in the abomasum. The four surviving calves were given 2 doses of Methyridine, 14 days apart, and in addition they were taken indoors and fed on a diet of hay and concentrates. Subsequent samples of faeces were collected from these 4 calves at intervals of about one month. Diarrhoea was not observed during the winter period; the faecal worm egg counts were less than 400 e.p.g. and were frequently negative. These calves ceased growing in October and it was not until the end of January they began to show an increase in weight and size. One of the 4 calves was purchased early in December, 6 weeks after housing and 4 weeks after the last dose of anthelmintic. This calf had had no access to grass for the previous month. An autopsy of this calf revealed a total worm burden of 55,300 O.ostertagi, 96 per cent of which were present in the mucosal digest as early 4th stages. The abomasal pH and electrolyte concentrations were within the normal range.

Mr. Paton described a syndrome, similar to Type II ostertagiasis, which had caused the death of 4 of his calves during the winter of 1961-1962.

It would appear likely that on Knockondale Farm all three phases of bovine ostertagiasis have occurred in

TABLE 38

The Numbers and Distribution of the Nematode Genera, Cooperia and Nematodirus in the Small Intestine of Ayrshire Calves

FARM A

Calf No.	Cooperia Spp.				Nematodirus Spp.				Per cent in Digest*
	SI 1	SI 2	SI 3	Digest	SO 1	SI 2	SI 3	Digest	
1	7,580	1,520	80	160	9,600	320	80	160	1.64
2	3,200	1,360	400	320	3,440	640	240	80	4.13
3	2,080	160	0	300	1,680	80	0	400	14.89
4	1,040	0	0	0	3,200	0	0	240	5.35
5	9,600	320	160	1,920	6,300	640	0	480	12.35
6	2,160	4,320	480	960	2,080	560	0	160	10.44
7	17,800	9,760	560	240	35,800	15,360	4,480	1,680	2.24
8	880	0	0	0	1,840	0	0	160	5.55
9	2,640	480	160	80	1,360	160	0	0	1.63

FARM B

1	22,240	3,200	480	240	3,040	80	0	0	0.81
2	480	640	0	160	0	0	0	0	12.50
3	8,000	640	80	240	160	0	0	0	2.63
4	20,120	5,570	1,040	720	2,530	960	80	240	3.07
5	15,520	2,140	2,000	320	4,000	160	0	80	1.65
6	16,640	720	160	480	320	0	0	240	3.89
7	19,360	13,730	1,600	480	1,280	1,730	1,560	640	2.77
8	12,240	8,160	640	1,120	2,080	960	0	240	5.34
9	10,600	4,400	560	0	400	0	0	0	0
10	17,600	880	160	2,000	6,240	3,440	80	80	6.82

* Percent of the total intestinal worm burden present in the mucosal digest.

previous years and that losses from both Type I and Type II syndrome occur frequently, the magnitude varying from year to year; in 1963, 5 out of 9 calves died of Type I disease.

THE DISTRIBUTION OF NEMATODES IN THE SMALL INTESTINE OF THE CALF

A limited study of the percentage recovery and distribution of the small intestinal nematodes was carried out on the autopsy material which was available from the detailed study of naturally occurring bovine ostertagiasis.

METHOD

The small intestine of each calf was separated from the mesentery and was divided into 3 approximately equal lengths. Beginning at the pyloric sphincter the first section was labelled SI 1; the second SI 2; and the last length SI 3. Each length was opened and the contents were washed into a bucket; the contents and the washings were made up to 4 litres; sub samples were collected. The mucosa of each length was then scraped off the muscle layer and was digested separately with pepsin-hydrochloric acid solution. Sub samples were taken, preserved and counted in the manner described earlier.

RESULTS

The numbers of worms from the lumen contents and washings of each of the sections have been set out in Table 38. The number of worms found in the digest material of each section was small so these counts have been summed

for each calf; the digest total has been included in Table 38. Nematodes of the genera Cooperia and Nematodirus were found in the same section, namely, the first third of the small intestine. In general large populations of worms were distributed throughout the 3 sections of the small intestine.

If the digestion procedure was omitted, up to 15 per cent of the intestinal burden was not included in the total count. The number of worms found in the digests was not related to the total worm burden.

APPENDIX D

THE PLASMA PEPSINOGEN CONCENTRATION, pH AND ELECTROLYTE CONCENTRATION OF BOVINE ABOMASAL CONTENTS

INTRODUCTION

Because the nematode O.ostertagi parasitises the bovine abomasum, it was considered relevant to investigate the nature of the probable changes in abomasal function.

Previous reports have indicated that the parasite produced severe morphological changes within the gastric mucosa and the first experiment in the current series describes in detail these changes, which affect all of the specialised secretory cells.

However, up to the present time, attention has not been directed towards the relationship between the structure and function of the parasitised mucosa. Plasma pepsinogen concentration has, in certain cases, provided an indirect assessment of the gastric secretory function in man, but it would appear that similar studies have not been undertaken in the ruminant. The first part of the appendix is given over to a consideration of the normal plasma pepsinogen concentration of Ayrshire calves.

Similarly, a conclusive definition of the pH and electrolyte concentrations of abomasal contents from uninfected calves has not been found despite an extensive search of the available literature. In the second part of this appendix an attempt has been made to define these parameters in worm free calves.

A comprehensive review by Hill (1961) collates much

useful background data concerning the development, anatomy and some of the physiological characteristics of the abomasum, the true digestive stomach of the ruminant, and serves as a suitable introduction to the study of abomasal function.

THE PLASMA PEPSINOGEN CONCENTRATION OF AYRSHIRE CALVES

INTRODUCTION

Pepsinogen, the precursor of the proteolytic enzyme pepsin, is produced in the zymogen cells of the gastric mucosa and is secreted largely into the gastric lumen, where it is activated to pepsin by hydrochloric acid. A small amount is normally found in the blood and is distributed ubiquitously throughout the body tissues and fluids, and is finally excreted in the urine. Pepsinogens purified from several species, including man, the dog, the ox and the sheep, have been shown to have similar chemical compositions. The origin, secretion, characteristics and excretion of pepsinogen have been reviewed by Hirschowitz (1957).

Ever since 1952, when Mirsky and his colleagues introduced a simple digestion technique to estimate the concentration of plasma, serum or urine pepsinogen in man, it was hoped that these estimations would provide an indirect, but more convenient, assessment of gastric secretory function. That plasma or serum pepsinogen has a gastric origin has been shown by Hirschowitz (1955), and Edwards, Jepson and Wood (1960), both of whom found that plasma levels disappear on total gastrectomy and are markedly reduced in patients with partial gastrectomy.

Edwards et al (1960) found that repeated estimations on an individual were within 10 per cent of one another,

that the daily fluctuations over one month were between 12 and 18 per cent, and that the variation between individuals was within 30 per cent of the mean value. These authors observed that the variations bore no relationship to diurnal effects, diet, food intake or exercise. Goldsmithova, Valkhoff and Kirsner (1958) reported that serum pepsinogen indicates the concentration at a given moment but does not measure the total output of pepsinogen because gastric stimulants, which caused an increased output of gastric pepsin, failed to increase the serum pepsinogen concentration.

Spiro, Ryan and Jones (1956) were able to establish a good correlation between serum pepsinogen levels and gastric pepsin output after histamine stimulation. Goldsmithova et al (1958) also established a good relationship between the two under basal conditions, but they emphasised the need for meticulous methods. Hirschowitz (1955), Palmer and Spiro (1958), and other workers, on the other hand, failed to establish a constant relationship between gastric peptic secretion and plasma pepsinogen levels. This led Hirschowitz (1955) to postulate, that plasma or serum pepsinogen provided a measure of the zymogen cell mass rather than the total pepsin output, and the relative constancy of plasma pepsinogen levels in any individual would support this suggestion.

In clinical cases of gastric disease in man, there is

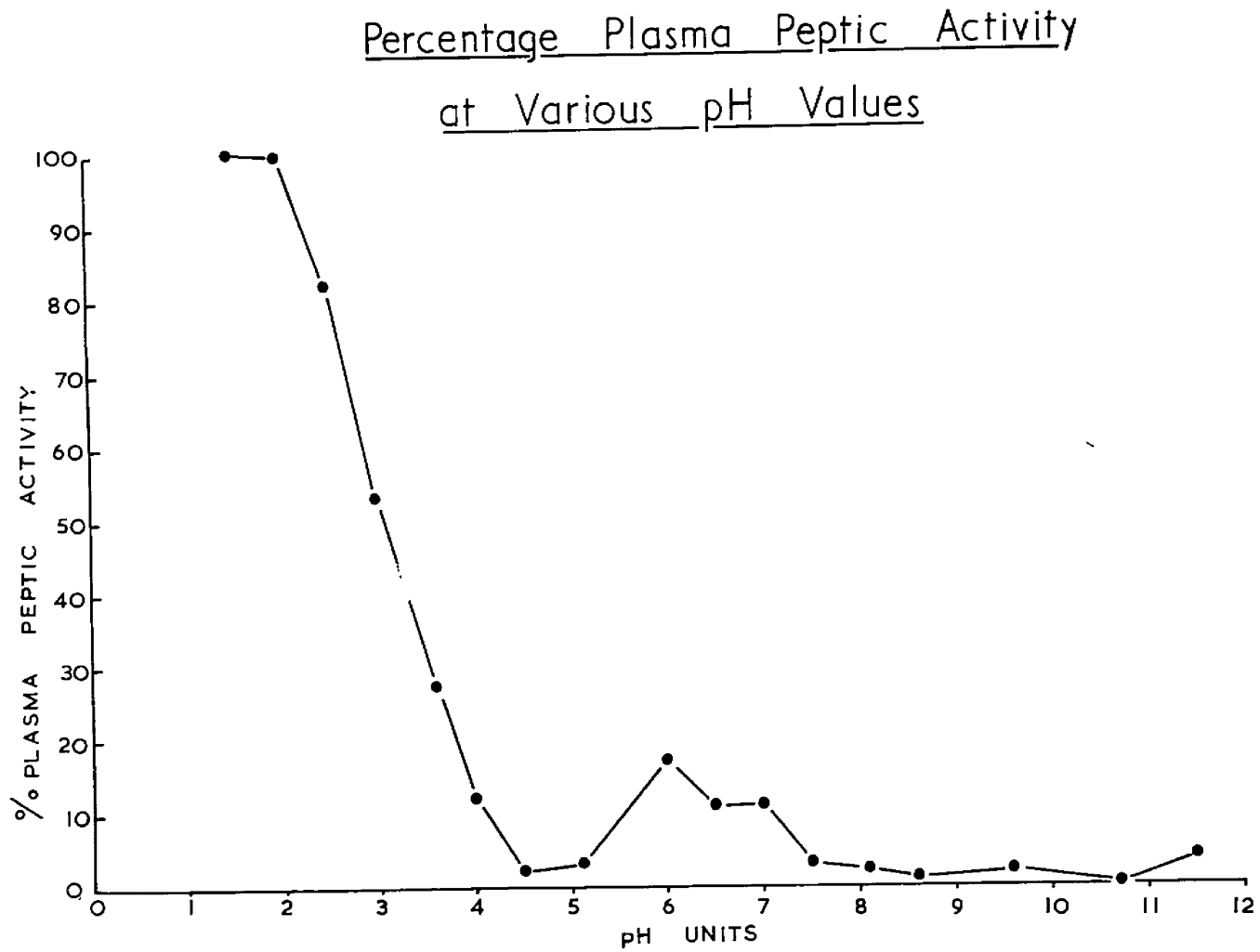


Fig. 45 A graph of the effect of pH on the peptic activity of bovine plasma.

a relationship between the serum pepsinogen level and gastric histological change determined from gastric biopsy. (Spiro and Schwartz (1958); Nolan (1958); Pollner and Spiro (1958); Bock, Arapakis, Witts and Richards (1963)). The serum popsinogen concentration in patients suffering from pernicious anaemia was very low, and therefore the serum pepsinogen test was likely to prove a useful screening test for patients with severe atrophic gastritis and gastric atrophy. High or high normal serum pepsinogen levels have been associated with superficial gastritis, and affected patients show little or no pepsin in the gastric juice. Spiro and Schwartz (1958) suggested that the necrobiosis of mucous neck cells and the inflammation, noted in the biopsies, could produce functional blocking of the gastric gland, so causing pepsinogen to leak back into the blood stream.

Histological examination of sections from cases of naturally acquired and experimentally produced infections of O.ostertagi have shown that, at times, large areas of the zymogen cell mass were destroyed. The degree of damage to the zymogen cells was not constant and varied considerably between sections from individual cases. To assess the functional significance of this damage, it was decided to estimate the concentration of plasma pepsinogen. Following the initial determinations, see experiment 2, it became clear, that the plasma pepsinogen concentration would be a

useful diagnostic and epizootiological test for the study of natural and experimental bovine ostertagiasis.

Studies on bovine plasma pepsinogen, similar to those in man, have not been undertaken in cattle, so at the outset it was necessary to examine the variations which occur in different samples of bovine plasma. The materials and methods used have been described in Section I.

THE OPTIMUM pH FOR BOVINE PLASMA PEPTIC ACTIVITY

A bulk sample of albumin substrate and bovine plasma, in the ratio of 10 to 2.5 respectively, was adjusted to pH 1.5 with 2N hydrochloric acid to activate the pepsinogen. Step by step the pH was raised with N sodium hydroxide and samples were taken at pH intervals of 0.5 units between pH 1.5 and pH 11.5. Each sample was then estimated according to the method of Edwards, Jepson and Wood (1960).

It was decided that the highest level of peptic activity would equal 100 per cent and the activity of all other samples was expressed as a percentage of this figure. The results obtained from this procedure have been graphed in Fig. 45.

It is clear from these results that the optimum pH for the determination of plasma peptic activity lies between pH 1.5 and pH 2.0. All subsequent plasma pepsinogen determinations were carried out at a pH reading between these values.

The peptic activity of bovine plasma decreases

rapidly as the pH of the substrate increases from pH 2 to pH 4, by which time the activity has decreased to about 10 per cent of the optimal value. Between pH 6 and pH 7 there is a slight increase in peptic activity, but this increase soon disappears once the pH rises above 7.5

THE REPEATABILITY OF ESTIMATION ON A SINGLE SAMPLE

From a single sample of bovine plasma twenty replicate pepsinogen estimations were carried out simultaneously. The mean and standard deviation of these estimations was found to be 91.5 ± 2.08 ug phenol/ml/24 hrs at 37°C. If two standard deviations are expressed as a percentage of the mean value, it can be stated, that 95 times out of 100, the estimation of a single sample will lie within ± 4.5 per cent of the true mean.

DIURNAL VARIATION IN PLASMA PEPSINOGEN CONCENTRATION

From four Ayrshire heifers, which were affected with Type II ostertagiasis, ten blood samples were collected at approximately hourly intervals throughout the period between 7 a.m. and 8 p.m. A single estimation from each sample period was carried simultaneously on the day following the collection of the samples.

The mean, standard deviation and the 95 per cent confidence limits (expressed as a percentage of the mean) for the four animals studied were respectively 174 ± 14.84 and 8.52 per cent; 126.3 ± 10.49 and 8.31 per cent;

106.5 ± 9.90 and 9.29 per cent, and 79.5 ± 1.70 and 2.13 per cent. It can be appreciated that very little variation, i.e. less than 10 per cent of the mean value, occurred throughout the day. Therefore it can be assumed that estimations on blood samples collected during any part of the day accurately reflect the circulating level of pepsinogen in the individual animal.

THE VARIATION IN PLASMA PEPSINOGEN CONCENTRATION AMONG A POPULATION OF AYRSHIRE CALVES.

A single estimation of plasma pepsinogen was carried out on a total of 35 parasite free Ayrshire calves whose mean ageuas 76 days. The determinations were not carried out on a single occasion but were done at intervals over a period of five months.

The mean and standard deviation for these determinations were 8.79 ± 2.46 respectively.

THE pH AND ELECTROLYTE CONCENTRATIONS OF ABOMASAL CONTENTS

INTRODUCTION

Quantitatively the main sources of the electrolytes found in abomasal contents are derived from the diet and the digestive secretions.

Denton, Goding, McDonald, Sabine and Wright (1961), and Bett, Denton and Goding (1964) have shown that large variations in the concentrations of sodium, potassium and chloride ions do occur in the pastures available to ruminants. In general, the sodium ion concentration of pasture decreases with increasing distance from the sea.

By far the greatest contribution to the sodium concentration of the reticulorumen contents is made by the salivary secretions, which have a high sodium content relative to other ions, and which are secreted in considerable quantities, 6 to 16 litres per day in sheep (Dellharn and Kay (1963)); 98 to 190 litres per day in adult cows (Dalley (1961)). However, other ions, namely potassium and chloride, are present in ample quantities in pasture.

From a study of omasal function, Dyaert and Boucknert (1961) observed that part of the fluid leaving the reticulum passed directly into abomasum and another part was retained in the omasum. The pH and chloride concentrations of omasal liquid were higher than those of reticulorumenal fluid but the carbon dioxide, sodium, potassium and ammonia

concentrations were lower (Mason and Phillipson (1952) and Gyaert and Bouckaert (1961). The quantity of water and electrolytes absorbed by the omasum depended on the solute concentration in the reticulorumen; a greater absorption took place at higher concentrations than at lower ones. The outflow of digesta from the omasum appears to be continuous throughout the 24 hours period.

The pH of the abomasal contents is usually between 2.0 and 3.0 pH units and at these values conditions are optimal for pepsinogen activation and peptic digestion, Mierschowitz (1957) and Hill (1961). According to Ash (1959) the concentration of sodium and chloride ions increased and those of potassium and calcium decreased when the acidity of the abomasal secretion was increased. However, these changes were found to be independent of secretory rate.

THE pH AND ELECTROLYTE CONCENTRATIONS OF ABOMASAL CONTENTS FROM HEALTHY CALVES

Since the normal pH values and electrolyte concentrations of abomasal contents from non-parasitised calves has not been found in the available literature determinations were carried out to define the normal limits of these parameters in bovines less than 18 months of age. These animals originated from a wide area of south-west Scotland and would have experienced varying regimes of management before going for slaughter at the Glasgow Corporation abattoirs.

TABLE 39

The pH and Electrolyte Concentration of Abomasal Contents from Normal Calves

Mean Value and Standard Deviation

Animal source	No. of Samples	pH units	Na^+ meq/l	K^+ meq/l	Cl^- meq/l
Glasgow Abattoirs	20	2.78 ±0.56	90.6 ± 6.7	15.3 ± 2.8	116 ± 5.5
Experimental Calf 1	11 (hourly)	2.30 ±0.2	51.0 ± 5.0	25.0 ± 1.0	127 ± 6.0
Experimental Calf 1	15 (daily)	2.50 ±0.4	55.0 ± 6.0	25.4 ± 3.6	128 ± 6.0
Experimental Calf 2	13 (daily)	2.40 ±0.3	57.0 ± 7.0	22.9 ± 4.5	128 ± 6.0

meq/l = milli equivalents per litre.

Samples of abomasal contents were collected within 10 minutes after slaughter and were prepared and estimated according to the procedure set down in Section I.

RESULTS

Only samples from abomasa which showed no macroscopic lesions were included in the final 20 determinations, the mean and standard deviation of which are shown in Table 39.

In addition, each of two 4-month old Ayrshire calves, which had been reared parasite free, were surgically prepared with a simple abomasal cannula. Two weeks after the operation, at which time both calves appeared healthy, a series of hourly and daily collections of abomasal contents was made. Determinations of pH, sodium, potassium and chloride ion concentrations were carried out and the results have been included in Table 39.

The results were found to be similar to those obtained in sheep by Ash (1959).

The values appear to be relatively constant, with little diurnal or daily variation, in any one calf maintained on a fixed diet. The most likely cause of the higher values for sodium concentration of the abattoir samples, compared with those from the experimental calves, lies in the nature of the diet fed to each group. The experimental calves were maintained, almost wholly, on a diet of meadow hay.

The values set out in Table 39 were considered to be normal values for healthy parasite free cattle under the age of 18 months and were used as a basis for comparison with values derived from cases of bovine ostertagiasis.

ACKNOWLEDGMENTS

It was Professor W.I. McIntyre who introduced me to this problem and to him I wish to express my gratitude for his continued encouragement, for the facilities and technical assistance, which he has provided at Glasgow.

I appreciate the unstinted interest which Dr. G.M. Urquhart has given throughout the course of the investigation. In particular, I wish to thank him for his advice on the presentation of the data.

I wish to thank my colleagues Dr. F.W. Jennings, Mr. J. Armour, Mr. J.D.S. Ritchie and Professors W.F.H.Jarrett and W. Mulligan, for the many lively and stimulating discussions on various aspects of host parasite relationships.

Mr. A. Finnie was responsible for the photomicrographs.

I am indebted to Dr. R.A. Robb, of the Department of Mathematics, Glasgow University, and to Miss. N. Ditchburne, of the Division of Mathematical Statistics, C.S.I.R.O., Melbourne, Australia, for their assistance with the statistical techniques and interpretation.

I express my thanks to a number of practising Veterinary Surgeons whose co-operation made this investigation possible. These include: Messrs. Allison, C.J.; Barr, G.L.; McMillan, I.A.; Begg, H.; Beswick, W.; Botcherby, W.C.; Love J.; Martin, B.; Moodie, D.; Flemming, T.B.; Nimmo, I.S.; Sim, L.S.; and Wood, C.J.

Acknowledgments (continued)

The Agricultural Research Council financed the work with the exception of the field experiment in which Type I disease was produced. This was supported by Messrs. Allen and Hanbury Ltd.

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