Studies on Fascioliasis and Ostertagiasis in Cattle and Sheep

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

The work described in this thesis is concerned with two parasitic helminths, Fasciola hepatica and Ostertagia ostertagi. Although the major part of the thesis describes investigation into fascioliasis in both cattle and sheep, the first section records a hitherto undescribed condition in cattle where both F. hepatica and O. ostertagi coexisted in significant numbers. Single experimental infections of cattle and sheep with metacercariae of F. hepatica were studied, and these were followed by observations on the sequential development of an outbreak of fascioliasis in sheep grazing under natural conditions, a subject investigated in detail for the first time. The final section describes a method of control by use of a fasciolicide.

The thesis is divided into five sections as follows:

Section I Field Studies on Clinical Parasitism in Young Dairy Cattle in South-west Scotland

The first part of this section reviews a series of outbreaks of ostertagiasis occurring during the course of the winter (Type II). This is followed by the description of a further series of outbreaks of parasitic disease in which both O. ostertagi and F. hepatica were present in significant numbers (the fascioliasis/ostertagiasis complex). The two conditions had different background histories, seasonal incidences, clinical signs and necropsy findings, and these differences are discussed in detail.

Section II Experimental *Fasciola hepatica* Infections in Calves

Single oral inoculations of susceptible calves with 1,000 or 2,000 metacercariae of *F. hepatica* resulted in the establishment of an adult fluke burden capable of producing clinical signs and death. The haematological and blood biochemical values recorded at weekly intervals demonstrated the presence of a macrocytic, normochromic anaemia and a hypoalbuminaemia. At post-mortem all but two of the infected calves showed a percentage take (i.e. the percentage of the inoculation which became established) of between 16% and 37%; the two remaining calves, given the higher level infection, showed very low percentage takes at necropsy.

Section III Experimental *Fasciola hepatica* Infections in Sheep

Inoculation of susceptible lambs with single oral doses of 1,000 metacercariae of *F. hepatica* each resulted in the development of clinical fascioliasis followed by death between 12 and 23 weeks after infection. A severe, macrocytic, hypochromic anaemia was recorded commencing 5 to 6 weeks post-infection, the degree of anaemia being proportional to the number of flukes recovered at necropsy. Reticulocytes appeared in the peripheral circulation and a hypoalbuminaemia was also present. The percentage of the inoculum established ranged from 10.8% to 81.5%.

Section IV Field Studies on Fascioliasis in Sheep

An outbreak of clinical fascioliasis, in lambs grazing permanent pasture known to have been responsible for outbreaks of fascioliasis in sheep during the previous two years, commenced during the first part of October. The major clinical signs were weight loss and pallor of visible mucous membranes; a macrocytic, hypochromic anaemia developed with reticulocytes present in the peripheral circulation. A hypoalbuminaemia and in many cases a frank hypoproteinaemia was recorded in these lambs. The total number of F. hepatica recovered from individual animals at necropsy ranged from 110 to 1628.

Concurrent studies on the changes in pasture populations of metacercariae of F. hepatica revealed that the maximum number of metacercariae was available between mid-August and mid-September although metacercariae were available to a varying degree at all times of the year.

Section V The Use of Nitroxynil in Ovine Fascioliasis

The efficiency of nitroxynil administered in the course of an outbreak of fascioliasis was studied. At a dosage rate of 10 mg. per kg. bodyweight, the drug arrested mortality and resulted in rapid improvement of the animals' general condition with the anaemia disappearing within 3 weeks of treatment. F. hepatica eggs disappeared from the animals' faeces within 5 days of treatment and they were not present again until 9 weeks post-treatment although it was 13 weeks after treatment before the majority of the lambs had positive faecal egg counts.

STUDIES ON FASCIOLIASIS AND OSTERTAGIASIS IN CATTLE AND SHEEP

A Thesis

submitted for

The Degree of Doctor of Philosophy

in

The Faculty of Medicine

of

The University of Glasgow

by

James Fraser Smith Reid, B.V.M.S., M.R.C.V.S.

Department of Veterinary Medicine,

University of Glasgow.

June 1968.

Acknowledgements

The author wishes to express his gratitude for the assistance received in the course of completing this thesis. He is particularly grateful to Professor W.I.M. McIntyre and Dr. G.M. Urquhart under whose supervision the work was carried out.

Also gratefully acknowledged is the assistance of Dr. J. Armour who assisted with the parasitological examinations and Dr. F.W. Jennings who carried out the biochemical estimations. Both these persons receive a special thanks for the many helpful suggestions offered in the course of this work.

Thanks are also due to Dr. C. Watts for the biochemical results contained in Section III and to Mr. A. Finnie for the preparation of the photographs and the original prints of the graphs. The author is also grateful to Mr. J. Murphy, Miss E. Morrison and Mr. S. Brown for technical assistance in the post-mortem room and laboratory; and to his wife for typing the text.

Finally, the author wishes to express his thanks to the Agricultural Research Council, Allen and Hanburys Ltd., and Merck Sharp and Dohme, with whose financial support this work was carried out.

CONTENTS

CONTENTS: GENERAL MATERIALS AND METHODS

CONTENTS: SECTION I

FIELD STUDIES ON CLINICAL PARASITISM IN YOUNG DAIRY CATTLE IN SOUTH-WEST SCOTLAND

CONTENTS: SECTION II

EXPERIMENTAL FASCIOLA HEPATICA INFECTIONS IN CALVES

CONTENTS: SECTION III

EXPERIMENTAL FASCIOLA HEPATICA INFECTIONS IN SHEEP

CONTENTS: SECTION IV

FIELD STUDIES ON FASCIOLIASIS IN SHEEP

CONTENTS: SECTION V

THE USE OF NITROXYNIL IN OVINE FASCIOLIASIS

GENERAL INTRODUCTION

The initial plan behind this thesis was to investigate the general syndrome of weight loss and diarrhoea in young dairy cattle and sheep in the west of Scotland. It became apparent during the course of this work that the cause of this type of morbidity was, in cattle, infection with the abomasal parasite Ostertagia ostertagi and, in sheep, parasitic gastro-enteritis and fascioliasis.

Published reports on the importance of O. ostertagi as a pathogen for cattle in Great Britain go back almost to the beginning of this century (Gardner, 1911) and it was recorded more recently in reports by Stewart and Crofton (1941) and Bruford and Fincham (1945). The condition as it exists in south-west Scotland was first described by Martin, Thomas and Urquhart (1957) and this was followed by a description of a field study of parasitic gastritis in cattle by Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965) in which they confirmed that O. ostertagi was the only parasite present in significant numbers. As will be seen later this situation is now somewhat more complex. Anderson et al. (1965) classified clinical ostertagiasis in the bovine into two forms, Type I and Type II, both of which were characterised by diarrhoea and weight loss, although they differed in seasonal occurrence and clinical history. In investigating cases of this condition it became apparent that a syndrome existed which was similar to Type II ostertagiasis but which was associated at autopsy with relatively large numbers of mature

Fasciola hepatica accompanied by variable, but usually significant, numbers of O. ostertagi.

Since bovine ostertagiasis has been recorded, described and discussed extensively in several recent publications (Ross and Dow, 1964, 1965 a & b; Anderson et al. 1965; Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart, 1966; Jennings, Armour, Lawson and Roberts, 1966; Anderson, Armour, Eadie, Jarrett, Jennings, Ritchie and Urquhart, 1966; Armour, 1967; Anderson, 1968) it is not reviewed in detail here and attention has been focussed on fasciolíasis in cattle, both as an uncomplicated disease and in association with ostertagiasis. The initial investigations into infection of cattle with F. hepatica stimulated further interest in this parasite and led to an examination of fasciolíasis in sheep. Thus the major part of this thesis is concerned with studies on fasciolíasis in both these species.

The existence of the liver fluke, F. hepatica, as a parasite of sheep was first recorded by Jean de Brie in 1379 (cited by Reinhard, 1957). Since then it has been found in the livers of many domestic and wild mammals. One of the first reports of its presence in cattle was recorded by Fromann in 1663 (cited by Taylor, 1964) who concluded wrongly that it only attacked calves and heifers up to two years of age, but not oxen or cows. That F. hepatica not only infects domestic animals but also kills them was adequately described by Thomas (1882) who reported that during the winter of 1879/80 in Great Britain losses were estimated at 3 million sheep or about one tenth of the total sheep population. He also noted that although the disease was

widespread throughout the country, ten counties of England were unaffected. In more recent years, Peters and Clapham (1942) investigating infestation in 73,000 cattle slaughtered in Great Britain in June, 1942 estimated an annual loss of 600 tons of liver valued at about £100,000. Ross (1966 c) reported that in Northern Ireland a slaughterhouse survey demonstrated that 88% of the cattle had some degree of fascioliasis. Other reports on the incidence of infection of cattle and sheep with F. hepatica are worldwide. Enger and Karbo (1956) reported that in Norway 4910 (i.e. 80%) of 6137 cattle examined were infected with liver fluke and that infection was so severe in 3101 of these that the whole liver was condemned. Colombo de la Villa (1955) records liver condemnations at four slaughterhouses in Spain where on average 27.4% of cattle livers and 25.6% of sheep livers were infected. In Australia, Gordon (1955 b) records that at a Sydney abattoir out of a total of 27,098 sheep slaughtered 12,071 or 44.6% of the livers were condemned. In Japan it is reported (Ono, 1958) that about 1,300,000 cattle are believed infected i.e. about 40% of the total bovine population. Popov and Kalitina (1962) working in Russia report that in the central Caucausus out of 3483 sheep 1197 (34.4%) were affected with F. hepatica and 250 (38.7%) of 646 cattle. Carrara and Recalcati (1961) found 35.5% of 32,660 cattle infected at Milan slaughterhouse and in Czechoslovakia, Oxoschaft (1963) records that 17.18% of 5,905 sheep and 53.3% of 337 cattle were affected. In France Doby and Chicho (1965) found that of 5,000 animals slaughtered 48.6% of cattle and 12.8% of sheep had fascioliasis whilst in

Germany Hiepe and Grunwaldt (1965) found an incidence of 37.14% in cattle at slaughter and Neuhaus and Six (1965) also in Germany found an incidence of 33.9% also in cattle.

The existence of a high incidence of both bovine and ovine fascioliasis in the west of Scotland can be appreciated by examination of the records at the Glasgow slaughterhouse for 1965, 1966 and 1967 (Nicolson, 1968). In each of these years approximately 130,000 cattle and 500,000 sheep were slaughtered and the annual percentage of livers affected with liver fluke ranged from 30 per cent to 42 per cent in cattle and 12 per cent to 14 per cent in sheep. The apparently low incidence in sheep compared to cattle can be readily explained as the majority of sheep slaughtered were lambs which would not have grazed long enough to have acquired the infection before they were killed.

That the infection of cattle and sheep with the liver fluke Fasciola hepatica is a problem of monumental importance cannot be denied but the incidence of liver condemnations is not the only parameter by which financial loss can be judged. Other factors which must be taken into account are loss of condition, poor growth rate and mortality on the farm and these may be difficult to estimate due to concurrent infections with other parasitic helminths. One other major financial item is the cost of anthelmintic treatment and this may be extremely high particularly in an endemic area.

A large amount of research has been applied to the problem of

fascioliasis in general but large gaps in our knowledge still exist particularly where an understanding of the disease process in cattle and sheep is concerned. Although liver fluke disease of sheep was first recorded in the fourteenth century it was not until the nineteenth century that a serious study of Fasciola hepatica infection was undertaken when the life-cycle of the parasite was described (Leuckart, 1881; Thomas, 1881). Since then many aspects of the life cycle have been studied both within and without the definitive host.

A knowledge of the influence of the environment on the life-cycle of the parasite is of major importance in order to predict the level of pasture infection to which stock may be exposed. The earliest report on the importance of temperature in the development of the egg of F. hepatica was by Thomas (1883) and some time later Ross and McKay (1929) noted that fluke eggs would not develop below a temperature of 10°C. Krull (1934) recorded that development did not take place below 5°C but was taken up again when the temperature reached 13°C. Since then a number of publications have appeared describing the time taken for fluke eggs to hatch under various circumstances (Tagle, 1944; Roberts, 1950; Ono and Isoda, 1951; Rowan, 1956; Ollerenshaw, 1959). The environmental conditions necessary for hatching are summarised by Rowcliffe and Ollerenshaw (1960) who state that (a) the eggs must be freed from the faecal mass, (b) they must be covered by a film of moisture and (c) the temperature must be in excess of 9.5°C. These latter authors also point out that fluke eggs will only remain viable and hatch where the environmental

pH is between 4.2 and 9.0. A high oxygen content of water is also beneficial but only at normal temperatures. A similar situation regards temperature, moisture and pH exists for the free-living miracidium to survive long enough to infect the intermediate host in Great Britain, the mud-snail, Lymnaea truncatula.

In order that development of F. hepatica might continue it is necessary that eggs are deposited in the vicinity of the intermediate host and in general the environmental conditions necessary for the survival and development of Lymnaea truncatula are essentially similar to those required by the parasite. The snail needs moist conditions and does not move very far from water (Peters, 1938; Taylor and Mosley, 1943); hatching of snail eggs takes about 1 month at 9 - 11°C (Roberts, 1950; Taylor, 1964) but only two weeks at room temperature. The pH of the soil also plays an important part in snail distribution and development and is in the range 6.2 - 7.2 (Bryant, 1935). Peters (1938) examining snail habitats in England and Wales concluded that the snail prefers clay soils. Snails can survive a period of drought for some time and Kendall (1953) found that adult snails can remain alive for over a year in a very dry environment.

The pathogenesis of fasciolianis has been studied in a number of species but initial investigations were concerned with the migratory route of the parasite from the alimentary tract to the liver. There were originally three hypotheses as to which route the young flukes followed (a) by migrating across the peritoneal cavity, (b) by transport in the blood stream, or (c)

by travelling up the common bile duct. The first report on this subject was by Sinitsin (1914) who infected rabbits orally and found flukes in the peritoneal cavity before there was any evidence of liver damage. These findings were supported by Shirai (1927) and Schumacher (1938) who got similar results after infecting rabbits, guinea-pigs and mice. The hypothesis that young flukes were transported in the blood stream seems to have originated with Lutz (1892) and this was later supported by Fuggo (1928, 1935) and accepted by Hwytra, Marek and Manninger (1946). Fuggo found the parasite in unusual sites at autopsy and assumed they had arrived there in the circulation. Cohrs (1967) maintains young flukes normally travel to the liver in the portal blood stream but in heavy infestations or under certain conditions, as yet unknown, they migrate across the peritoneal cavity. However, the ability of young flukes to penetrate the liver capsule was demonstrated by Shaw (1932) in rabbits, goats and sheep and Morrill and Shaw (1942) in cattle, by injecting young flukes directly into the peritoneal cavity. Other workers who confirmed that this was the common migratory route were Krull and Jackson (1943) using rabbits and sheep, Urquhart (1956) using rabbits, Schumacher (1956) using guinea-pigs and Dawes (1961) using mice as experimental animals.

The clinical condition produced by infection with F. hepatica is variable and "the pathogenicity of the infection depends essentially upon the number of organisms which invade the liver and mature in the bile ducts." (Soulsby, 1965). As a result clinical disease is divided into two basic categories, i.e. acute and chronic infections. Acute fascioliasis is primarily

a result of damage caused by large numbers of immature flukes migrating through the liver parenchyma (Lapage, 1956; Taylor, 1964; Soulsby, 1965; Ross, Dow and Todd, 1967) whereas the chronic form of the disease, which is much more common, results from lower level infections which do not produce a marked effect until the flukes become adult in the bile ducts (Taylor, 1964; Soulsby, 1965 & 1968; Ross, Dow and Todd, 1967). A third form, subacute fascioliasis, has been described in sheep (Soulsby, 1965 and 1968; Ross, Dow and Todd, 1967) but clinically at least this appears to be indistinguishable from the acute form. Although these three forms occur in sheep, Taylor (1964) states that the acute disease is rare in cattle; Ross and Dow (1966) describe an acute syndrome in calves associated with pneumonia.

The main clinical signs of acute fascioliasis in sheep are depression, inappetence and weakness; the abdomen is swollen and there may be evidence of pain on palpation of the liver area (Lapage, 1956; Taylor, 1964). Ross, Dow and Todd (1967) observed pallor of mucous membranes and dyspnoea. The chronic form of the disease is similar in cattle and sheep and the main features are progressive weight loss, pallor of visible mucous membranes and, in a proportion of cases, submandibular oedema. Diarrhoea is recognised as being occasionally present (Lapage, 1956; Smith and Jones, 1957; Taylor, 1964; Soulsby, 1968) but Ross (1966 b) states that it does not occur. Jaundice is reported as being occasionally seen (Smith and Jones, 1957; Jubb and Kennedy, 1963).

The occurrence of anaemia in clinical cases of fascioliasis in ruminants has been recognised for some time and reference to this feature is made in many of the standard veterinary textbooks (Udall, 1943; Mönnig, 1956; Lapage, 1956; Smith and Jones, 1957; Jubb and Kennedy, 1963; Nieberle and Cohrs, 1967; Soulsby, 1968). Although anaemia is universally accepted as being present particularly in the chronic phase of the disease differences of opinion exist as to its aetiology and character. Most of the earlier research workers believed that the anaemia was due to circulating toxins produced by the parasite. Fleury and Leeb (1926) claimed that metabolic products and extracts of flukes haemolysed red blood cells and this was supported by Rose and Gordon (1936). Although these claims were founded on very slender evidence they are accepted by Cameron (1951), Smith and Jones (1957) and Lapage (1962). Another toxic effect was put forward by Barbieri (1935) and Balfen (1940 b) who considered that bone marrow activity became depressed. The latter author also suggested that there may be a toxic effect on the liver which might lead to a deficiency of an anti-anaemic factor and this point was supported by Holman (1945). Sewell (1965) discussing the effects of Fasciola gigantica in cattle, also believes that the anaemia is of the dyshaemopoietic type due to either the action of a toxin produced by the parasite or preferential absorption of Vitamin B 12. Sinclair (1962) considered the anaemia to be of the dyshaemopoietic type also but due to a toxin acting directly on the bone marrow or indirectly by affecting liver function. The theory that adult Fasciola hepatica is a blood feeder was put forward by Weinland and von Brand (1926) who considered that flukes ingested blood in the smaller bile ducts

and both these authors and Stephenson (1947) observed flukes feeding on clotted blood *in vitro*. Van Grembergen (1950) supported the view that the adult fluke was haematophageous. Urquhart (1955) produced a similar anaemia to that of fascioliasis by repeated bleedings in rabbits. Further support to the theory that the adult liver fluke is a blood feeder is provided by the use of radioactive red cell labels (Jennings, Mulligan and Urquhart, 1956; Jennings, 1962; Pearson, 1963; Todd and Ross, 1966; Holmes, Maclean, Dargie, Jennings and Mulligan, 1967 a and b; Sewell, 1967; Symons and Boray, 1967). Todd and Ross (1966) demonstrated that the caecal contents of adult *F. hepatica* contained breakdown products of haemoglobin and confirmed the results of Stephenson (1947) and van Grembergen (1950). Other authors in favour of the blood feeding theory are Jubb and Kennedy (1963) and Cohrs (1967). Dawes (1963, a & b) and Dawes and Hughes (1964) disagree with the theory that the adult fluke is haematophageous and are in no doubt that it feeds on hyperplastic biliary epithelium; they make no attempt to suggest the origin of the anaemia. Gresham and Jennings (1962) state that the cause of the anaemia is unknown but postulate that the effect of *F. hepatica* may be threefold, and due to blood feeding, production of a haemolysin or toxic metabolic products.

There is also some difference of opinion as to the type of anaemia which occurs in fascioliasis. Urquhart (1955) found that in the rabbit it was associated with a macrocytosis, hypochromasia and the presence of reticulocytes in the peripheral circulation. Sinclair (1962), describing

experimental infections in sheep, stated that the anaemia was normochromic and normocytic but omitted the special staining procedure for reticulocytes. Sewell (1965) working with F. gigantica in white Fulani Zebu cattle also reported a normochromic, normocytic anaemia but this was accompanied by a reticulocytosis. Recently Ross (1967 a & b) recorded the anaemia in sheep as being macrocytic and normochromic but in acute fascioliasis Ross, Dow and Todd (1967) found that the macrocytic, normochromic anaemia was accompanied by circulating erythroblasts.

Haematological changes have also been observed with regard to the white cell series. Ballen (1940 a & b) reports the occurrence of an eosinophilia which was accompanied by a marked leucocytosis and although the former was confirmed by Wirth (1950) this author did not note a rise in the total white cell count. Urquhart (1955), Secretan and Bickel (1960) and Sinclair (1962) all recorded a rise in total white cell count, a progressive leucocytosis becoming apparent soon after the administration of metacercariae. Sinclair (1962) noted that the leucocytosis was due to an absolute eosinophilia.

It is of interest that an eosinophilia is a marked feature of fascioliasis in man (Facey and Marsden, 1960; Kirk, 1961; Taylor, 1961; Sagar, 1962) but an anaemia is not a prominent feature (Facey and Marsden, 1960).

Considering the amount of liver damage which must occur in the course

of the disease there are surprisingly few changes reported in blood chemistry and the main alterations are in the plasma proteins. There seems to be general agreement that albumin levels are reduced and this has been demonstrated in sheep (Ibrovic and Gall-Palla, 1959; Nikolic, Nikolic, Nevenic, Bugarski, Pavlovic, Ciric, Mladenovic and Polic, 1962; Sinclair, 1962; Ross, 1967 a & b; Ross, Dow and Todd, 1967) in cattle, (Nikolic et al. 1962; Hankiewicz, 1965; Ross, Todd and Dow, 1967), in rabbits (Secreton and Bickel, 1960; Dargie, Holmes, Maclean and Mulligan, 1967, 1968), and in rats (Thorpe, 1963). Balian (1940 a & b) and Haiba and Solim (1960) recorded a fall in total protein levels in ruminants whilst Ibrovic and Gall-Palla (1959) noted a rise and Secretan and Bickel (1960) and Nikolic et al. (1962) did not find any significant alteration in total protein. Sinclair (1962), however, noted an initial elevation followed by a subsequent fall in total protein levels in sheep. The level of globulin increases in fascioliasis, the increase resulting mainly from an elevation of the gamma fraction and this has been recorded in sheep (Ibrovic and Gall-Palla, 1959; Sinclair, 1962); in cattle (Nikolic et al., 1962; Hankiewicz, 1965; Ross, Todd and Dow, 1967) and in rabbits (Secretan and Bickel, 1960).

Although there are important alterations in the plasma proteins in fascioliasis it would appear that none of the other so-called liver function tests are of value. Urquhart (1955) described a temporary bilirubinaemia in the rabbit but there were no changes in serum alkaline phosphatase levels or in bromsulphthalein clearance. Thorpe (1963) reporting on experimental

infection in the albino rat noted no alteration in serum bilirubin and only marginal changes in alkaline phosphatase and serum glutamic oxaloacetic transaminase (S.G.O.T.) during the migratory stage of the parasite. Valcarenghi and Molinari (1959) recorded no change in S.G.O.T. or serum glutamic pyruvic transaminase (S.G.P.T.). Ross, Todd and Dow (1966) found significant alterations in S.G.O.T. in calves but they did not note any change in bromsulphthalein clearance although they did record an alteration in the iodine liver function test but only in one calf; Hankiewicz (1965) found a positive iodine test in 80% of infected cattle. Although this test has been used to detect liver dysfunction in a number of conditions in cattle (Woolf, 1951; Delli & Chini, 1954; Lloyd, 1957; Coulson, Davies and Evans, 1960; Hindson, 1965) its use has a major drawback because the mechanism is not known.

Attempts to control fascioliasis in sheep and cattle have been predominantly based on the administration of anthelmintics which are used either strategically or during outbreaks of disease. The first effective anthelmintic against Fasciola hepatica, carbon tetrachloride, was described by several workers in Germany in 1925 and independently in Britain by Montgomerie (1926). This was followed within a short period of time by the introduction of a second fasciolicide, hexachloroethane (Thienel, 1926; Noller and Schmidt, 1927). Since then several other anthelmintics have been described; freon - 112 (Demidov, 1955); hexachlorophene (Dorsman, 1959; Federmann, 1959); hetol (Lämmler, 1960). Unfortunately the efficiency of these anthelmintics extends only to the adult bile duct stages of F. hepatica at normal therapeutic levels. Many of these anthelmintics can be

toxic at the recommended therapeutic dose and those which are not are readily toxic when the dose is increased in an attempt to eliminate immature forms of the parasite.

In more recent years several new anthelmintics with activity against F. hepatica have been described. These are Bayer 9015A (Buttler, Matthews and Marble, 1963; Knapp, Hyberg, Dutson and Shaw, 1963; Lee, O'Sullivan and Power, 1966); Hilomid (Boray, Happich and Andrews, 1965; Boray and Happich, 1966); oxyclozanide (Valley, 1966); disophenol (Boray, Happich and Andrews, 1967); nitroxynil (Lucas, 1967). All these anthelmintics are efficient and non toxic at the normal therapeutic dose against the adult parasite and all can be shown to have an irregular effect against immature flukes at increased dose levels whilst toxicity is not marked, especially in the case of oxyclozanide and nitroxynil.

The control of fascioliasis has also been attempted by the use of molluscicides. Copper sulphate is the most widely used compound and its use against the snail was first described by Chandler (1920). The main problems of using copper sulphate are due to the difficulty of finding effective ways of applying the compound. Mosley (1952) sprayed the area to be treated with washing soda before applying the copper sulphate whilst Tolgyesi (1958) mixed the copper sulphate with 10 per cent hydrochloric acid.

A substance with more killing power than copper sulphate is sodium pentachlorophenate and the use of this compound has been described by

Enigk (1950). Unfortunately this substance is very toxic to those who handle it (Taylor, 1964). A large number of compounds were tested for activity against snails by Fatto, Swanson and Murphy (1951 a & b) and Fatto and Swanson (1952) but very few showed any promise.

Taylor (1964) considered the approach with molluscicides ineffective because even if 95% of the snails were removed so great is the rate of recovery of the snail population that large numbers will again be present only weeks after treatment of the pasture. Gordon (1955 a) had also found that the rapid reproductive rate of the snails offset the effects of molluscicides.

The first part of this thesis describes the clinical, haematological, biochemical, parasitological and pathological changes in two series of 10 outbreaks of parasitic disease in the bovine. In one series the only parasite present in significant numbers was the abomasal parasite, Ostertagia ostertagi whilst in the other series relatively large numbers of Fasciola hepatica were found together with variable but usually significant numbers of O. ostertagi. The differential diagnosis of these two series is then discussed in detail (Section I).

Secondly, the development of uncomplicated fascioliasis in young cattle was investigated following the administration of single oral inocula of metacercariae of F. hepatica at two different dose levels designed to establish an adult fluke burden similar to that which might be acquired on natural grazing. The clinical, haematological, biochemical, parasitological

and pathological changes taking place in the course of the disease were then recorded (Section II).

Thirdly, using a single oral inoculation of metacercariae of F. hepatica, a group of lambs was infected and the resulting clinical, haematological, biochemical and parasitological changes observed. Again the infective dose was such that it was likely to produce a fluke burden which could be readily acquired by natural infection (Section III).

Fourthly, in order to study the clinical, haematological and biochemical changes taking place during the course of an outbreak of naturally acquired infection, a group of lambs was allowed to graze pasture known to be infected with metacercariae of F. hepatica. This study was supplemented by including observations on the availability and infectivity of pasture populations of metacercariae of F. hepatica at different times of the year (Section IV).

Finally, the use of the fasciolicide, nitroxynil, given during the course of a natural outbreak of fascioliasis in sheep is described with particular reference to clinical improvement and recovery of the haematological and biochemical indices. These results are then applied to the control of ovine fascioliasis in the field (Section V).

THE LIFE CYCLE OF OSTERTAGIA OSTERTAGI

The adult parasite is normally found on the mucosal surface of the bovine abomasum. Eggs are laid in the abomasal liquor and are passed on to pasture in the faeces where development to the infective third stage larva takes place in the dung pad. If ideal conditions exist, i.e. a temperature of 20°C and humidity over 80 per cent, then development will take place in about 7 days. When climatic conditions are not optimal then development will be less rapid. The infective larva, measuring 850 - 900 microns (μ), and still within its second stage sheath migrates, in the presence of rainfall, on to the pasture where it is ingested by the grazing calf.

Once ingested the infective larval stage loses its extra sheath and passes to the abomasum where it enters a gastric gland and moults to become the early fourth larval stage about 4 days later. The fourth stage larva grows in the gastric gland where it reaches the fourth moult stage about 10 days after ingestion and 2 - 3 days later it has become the fifth larval stage. This fifth stage larva grows and matures to become an adult within a further 4 to 7 days i.e. 16 to 21 days after ingestion.

The adult O. ostertagi males are 6.5 - 7.5 mm. long and the females are 8.3 - 9.2 mm. in length. Because they are now 7 or 8 times as big as the early fourth stage larva, the gastric gland has become stretched and distended. Now that it is adult the parasite leaves the gland to lie on the surface of the mucosa.

THE LIFE CYCLE OF FASCIOLA HEPATICA

The adult parasite (Figure 1) is normally found in the bile ducts of cattle and sheep but it has also been recorded in the livers of many domestic and wild animals and man. It is flattened and leaf-shaped being broader anteriorly than posteriorly; in size it may reach 35 mm. in length and 13 mm. in breadth across its widest part. At the anterior end there is a conical projection with an anterior or oral sucker at its extremity. A second or ventral sucker is situated a little further back about the level of the widest part. The surface of the parasite is covered with small sharp spines whilst the alimentary tract consists of long, branched caeca which give off primary, secondary and tertiary branches. The parasite is hermaphrodite and the reproductive organs which consist of one ovary and two testes occupy most of the body; the ovary lies anterior to both testes and both reproductive tracts meet at the genital pore which is situated in ventral midline midway between the two suckers.

The eggs are oval operculate about 140 μ by 70 μ in size and are yellowish in colour. They pass into the intestine via the bile and are passed out in the faeces. Once on the pasture the eggs will develop as long as the temperature is above 9°C. As the temperature rises above this point, development takes place at an over-increasing rate up to 30°C. At the optimum temperature of 22 - 26°C development takes 9 - 15 days, however under natural conditions in Great Britain between June and August development takes about 3 weeks. Development will not take place to the hatching stage unless the egg becomes detached from the faeces.

Fig. 1. Adult liver flukes (Fasciola hepatica) about twice actual size.

When the egg hatches a small, "carrot-shaped" miracidium emerges. The anterior extremity is the broadest part and the surface, apart from the anterior extremity, is covered with cilia which propel the miracidium through the water. The aim of the miracidium is to penetrate a mud-snail of Lymnaea spp., commonly Lymnaea truncatula in Britain. Penetration is achieved by use of the papillary protrusion on the anterior end.

Once in the snail the miracidium loses its cilia and is then known as a sporocyst which is virtually a bag of tissue with walls which contain germ cells; these cells multiply producing larvae known as rediae. The rediae grow, rupture the sporocyst and are liberated into the snail tissues. A fully-grown redia is cylindrical in shape with a raised collar toward the anterior end and two projections toward the posterior end. This stage has a mouth leading to a pharynx and simple unbranched intestine. Under certain adverse conditions a second generation of rediae are produced.

The next stage of the parasite, the cercaria, is produced from germinal cells within the redia and emerges through a birth pore situated just behind the collar at the anterior end. The cercaria then leaves the snail and can be seen with the naked eye as a small, free swimming, tadpole-like structure. The two suckers of the adult parasite can be recognised, also a primitive intestine. After leaving the snail the cercaria must find a suitable object on which to encyst within the space of one hour. The cercaria usually encysts on a blade of grass and loses its tail.

Under optimal conditions the time between infection of the snail by the miracidium and the shedding of the cercariae takes about 6 - 7 weeks.

The encysted cercaria is not infective for 2 - 3 days after encystment when it has developed rudimentary genital organs and is termed a metacercaria. This encysted metacercaria can remain infective for several months and when ingested by a suitable host will excyst in the intestine and penetrate the gut wall and make its way through the visceral peritoneum to the liver. Thus the young flukes may be found in the liver within 48 hours of infection. During the following weeks the young flukes wander in the liver parenchyma eventually to end up in the bile ducts and grow to adults about 8 - 9 weeks after infection.

THE LIFE CYCLE OF LYMNAEA TRUNCATULA

The eggs of Lymnaea truncatula are laid in groups surrounded by a large amount of gelatinous material. The number of eggs in each group is dependent on the nutritional status of the snail and ranges from 2 or 3 to 31 (Taylor, 1964) and they are laid on the surface of mud adjacent to a pool of water. Incubation of the eggs takes place over a variable period of time and depends on the environmental temperature and under optimum conditions the incubation period is about 2 weeks. After hatching, the young snail grows and reaches maturity in about 3 weeks although growth continues after this time. Several adult snails are illustrated in Figure 2.

Although development of the snail from the egg to maturity only takes 3 weeks under optimum conditions, its development under field conditions is not so rapid. Snail activity is largely dominated by temperature and moisture and is almost entirely absent at temperatures below 9°C. This means that breeding activity only occurs between the months of May and October, and young snails hatching during the late summer and surviving the winter will not produce eggs until the following spring and even then only if the moisture content of the environment is adequate. It is this latter generation which, if infected by miracidiae of F. hepatica, will produce cercariae, under field conditions, about 3 months later. Therefore, snails becoming infected in May will produce cercariae in August and this results in the "summer infection" of farm stock and leads to outbreaks of acute fascioliasis in sheep from September onwards. Snails becoming infected in the late autumn may survive the winter and hence cercariae could be released from May onwards and this can lead to acute fascioliasis in sheep occurring from July.

Fig. 2. Lymnaea truncatula on the surface of a mud slope x 2.

GENERAL MATERIALS AND METHODS

A. Experimental Animals

(1) The rearing and maintenance of parasite free animals

Calves: Ayrshire bull calves, purchased when three to seven days old were housed in individual galvanised iron huts. These were cleaned out weekly and the calves bedded daily with oat straw. Whole milk was fed for the first four weeks at the rate of one pint of milk per 10 lb. live weight per day (divided into two feeds). During the third and fourth weeks, hay and calf weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) were introduced and milk feeding was stopped at the end of the fourth week. From this point until the commencement of the experiment the animals were given ½ lb. of the pelleted ration daily with hay and water available ad lib.

Lambs: Male Blackface lambs purchased when one week of age were housed on concrete floors with oatstraw bedding. The animals received whole milk for the first four weeks at the rate of half a pint per 5 lb. live weight daily divided into two feeds. During the third and fourth weeks, hay and lamb weaner pellets (British Oil and Cake Mills Ltd., Renfrew, Scotland) were introduced and milk feeding was discontinued by the end of the fourth week. From then until the end of an indoor experiment or until the commencement of an outdoors experiment the lambs were given ½ lb. of the pelleted ration per 15 lbs. bodyweight daily, with hay and water available ad lib. The lambs were castrated and docked when 8 weeks of age when they were inoculated with a combined clostridial sheep vaccine ("Covexin", Burroughs Wellcome and Co., Beckenham, Kent, or "Clostrin", Glaxo Laboratories Ltd., Greenford, Middlesex). The inoculation was repeated when the lambs were 12 weeks old.

(2) Weighing Procedure

Calves: The calves were weighed on Avery cattle scales which were accurate to 1 lb. Weighings of experimental calves were carried out in the morning, two to three hours after the morning feed had been given. This procedure was not possible with field cases when animals were weighed on arrival at the Veterinary Hospital.

Lambs: The lambs were weighed on an Avery spring-balance pig weigher which was accurate to 1 lb. All weighings were carried out in mid-morning, i.e. two to three hours after feeding in the case of housed animals.

D. Blood Analysis

(1) Collection and storage of samples

All blood samples were taken from the jugular vein and collected into glass bottles prepared in three different ways. The first bottle contained the anticoagulant, ethylenediaminetetraacetate (E.D.T.A.) and was prepared by putting 0.1 ml. of an 11 per cent solution of the anticoagulant into a Bijou bottle and allowing it to evaporate to dryness at room temperature. About 2 ml. of blood was collected in this way, the bottle being shaken gently to allow the anticoagulant to dissolve. This sample was for haematological examination which was carried out within a few hours of collection.

Secondly, for estimations involving plasma, 15 ml. of blood were collected into a Universal bottle containing two or three drops of a 1:1,000 solution of heparin. The heparinised sample once thoroughly mixed, was

centrifuged at room temperature for 20 minutes at 2,000 r.p.m. in a M.S.E. centrifuge (Measuring Scientific Equipment, London, England). The plasma was then transferred, by means of a pipette, into plastic tubes (Metal Box Co., Portslade, Sussex, England), immediately frozen and stored at -5°C.

Thirdly, for estimations involving serum, 15 ml. of blood were collected into a Universal bottle containing no anticoagulent. This sample was left standing overnight at room temperature and then the serum which had separated was removed and stored as described above for plasma.

(2) Packed Cell Volume (P.C.V.)

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

(3) Haemoglobin Concentration (Hb)

Haemoglobin concentration expressed as grams per 100 ml. was estimated by the oxyhaemoglobin method of Dacie and Lewis (1966). A one in 200 dilution of blood was prepared in 0.04 per cent solution of ammonium hydroxide and, after thorough mixing, the resulting solution of oxyhaemo-globin was read in a colorimeter (Evans Electroselenium Ltd., Harlow, England) using a yellow-green filter (Ilford, No. 625). The colorimeter was calibrated using a cyanmethaemoglobin standard solution (cyanmethaemo-globin standard solution - C. Davis Keeler, Ltd., London, England).

(4) Total Red and White Blood Cell Counts (R.B.Cs.)(W.B.Cs.)

Total counts of the circulating red and white blood cells were made on an electronic particle counter (Coulter Model "D", Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.) by the methods described for feline blood cells by Crighton (1965).

(5) Differential White Cell Counts

Differential white cell counts were made using the technique described by Dacie and Lewis (1966). Blood smears were made and stained with Leishman's stain (British Drug Houses, Ltd., Laboratory Chemicals Division, Poole, England). The counts were performed by selecting a thin strip of cells down the centre of the smear and counting all white cells along this line until a total of 200 cells had been counted. The numbers of eosinophils, neutrophils and lymphocytes counted in this manner were then expressed as a percentage of the total number of cells counted.

(6) Reticulocyte Counts

Reticulocytes were stained and counted by the methods described by Dacie and Lewis (1966). The staining solution was prepared by dissolving 1.0 g. of brilliant cresyl-blue (water soluble, Gurr - G.T. Gurr Ltd., London, England) in 100 ml. of citrate-saline solution (1 part of 3% sodium citrate to 4 parts of 0.85% sodium chloride) and the resulting mixture filtered before use. Two to three drops of this cresyl-blue solution were pipetted into a small glass tube of 8 mm. internal diameter and to this was added two volumes of E.D.T.A. blood. After mixing, the tube and its contents were incubated at 37°C for 15 minutes when the cells

were resuspended by gentle mixing and films were made on a glass slide. (The reticular material was stained deep blue and the non-reticulated cells shades of pale greenish blue.).

For counting, an area of the film was chosen where cells were undistorted and the staining was good. The cells were counted using a 2 mm. oil-immersion lens and for counts of less than 10 per cent successive fields were examined until at least 100 reticulocytes had been counted, the total cells in every tenth field being counted until at least ten fields were counted in this way. The reticulocytes present were expressed as a percentage of the total cells in the following manner:

Number of reticulocytes in 120 fields = 100

Total cells present in 12 fields = 300

Therefore, approximate number of cells (all types) in 120 fields = 3000

Therefore reticulocyte percentage = $\frac{100}{3000} \times 100$

= 3.3

For counts of more than 10 per cent a greater number of complete fields were counted.

(7) Mean Corpuscular Volume (M.C.V.)

M.C.V. was calculated from the formula

$$\frac{\text{P.C.V.\%} \times 10}{\text{R.B.C.} \times 10^{6} \text{ per cu.mm.}}$$

and expressed as cubic microns (c.μ)

(8) Mean Corpuscular Haemoglobin Concentration (M.C.H.C.)

M.C.H.C. was calculated from the formula

$$\frac{\text{Hb gms. per 100 ml.}}{\text{P.C.V.\%}} \times 100$$

the result being expressed as a percentage.

(9) Plasma pepsinogen

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

(10) Total Protein Concentration

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

(11) Serum Protein Fractionation

Separation of the serum protein fractions was carried out by electrophoresis. Cellulose acetate strips (Oxoid Ltd., London, England) were saturated with barbitone buffer (pH 8.6) and placed in a horizontal electrophoresis tank (Shandon Scientific Co. Ltd., London, England). Serum (0.003 ml.) was pipetted on to the strips and a voltage of 150 volts

was applied for one hour from a Vokam power pack (Shandon Scientific Co. Ltd.). The strips were then removed and dried in an oven at 80 to 100°C for ten minutes and developed by staining with 0.2 per cent Ponceau S (for electrophoresis) (G.T. Gurr Ltd., London, England), in 3 per cent aqueous trichloracetic acid for five minutes. After staining, the strips were evaluated automatically as described by Neill (1963) using a Chromoscan recording densitometer (Joyce Loebl and Co. Ltd., Gateshead, England). The results were expressed as grams per 100 ml. of serum albumin, total globulin, alpha/beta globulin and gamma globulin.

(12) Serum Glutamic Oxaloacetic Transaminase (S.G.O.T.) and Serum Glutamic Pyruvic Transaminase (S.G.P.T.)

S.G.O.T. and S.G.P.T. were measured by the colorimetric method described by the Sigma Chemical Co., Technical Bulletin No. 505 (1964). The optical densities were determined on a Unicam SP600 spectrophotometer and the results were expressed in Sigma-Frankel (S-F) units.

(13) Serum Alkaline Phosphatase

Serum alkaline phosphatase was estimated by the method of Kind and King (1954). In this method phenol is released from disodiumphenyl phosphate by the action of alkaline phosphatase. The liberated phenol is then estimated colorimetrically using the reagent 4-amino-antipyrine. The results were expressed in King-Armstrong (K.A.) units.

(14) Serum Bilirubin

Serum bilirubin was assayed by a colorimetric method using

Boehringer test combinations (C.F. Boehringer and Soehne, GMBH, Mannheim, Germany).

(15) pH of Abomasal Contents

The pH of abomasal contents was determined using a pH meter (Beckman Instruments Ltd., Glenrothes, Scotland) with micro-electrodes, the results being periodically tested on larger samples using standard electrodes. All samples were examined within one half hour of collection.

C. Autopsy Procedures

(1) Details of Slaughter

All animals were slaughtered by captive bolt pistol after which the carcase was bled out. The abdomen was then incised along the ventral midline and the whole gastro-intestinal tract removed. A gross pathological examination was then made of the rest of the carcase. Where animals had been confined indoors prior to slaughter they were starved during the previous 24 hours.

(2) Liver

The liver was removed intact as rapidly as possible, photographed, its gross appearance noted and, in some instances, sections taken for histological examination. The gall bladder was removed and the bile poured into a glass container when any flukes present were retained. The organ was then sliced into strips about half an inch thick and each strip thus obtained was squeezed and any flukes present removed and placed in a jar of water. After each slice of liver had been treated in this fashion it

was placed in a polythene bucket containing warm normal saline for about one hour in an attempt to release any flukes which had not been detected on the first occasion. After one hour the strips of liver were removed from the saline, squeezed once again, and then discarded. The sediment left at the bottom of the saline solution was collected and examined for the presence of flukes. After collection the flukes were counted on a white enamel tray and measured individually in millimetres.

(3) Abomasum

The abomasum and omasum were separated from the rest of the intestinal tract, care being taken not to lose any of the contents. The abomasum was incised along its greater curvature and the contents collected in a polythene bucket; the organ was washed under a steady stream of water and the washings collected in the same bucket. The abomasal contents and washings were then made up, in a graduated polythene bucket, to four litres (cattle) or two litres (sheep) and, after thorough mixing, two samples of 200 ml. each were taken in a graduated scoop, for subsequent microscopic enumeration of the worm population; to each sample 10 ml. of 40 per cent formalin was added as preservative.

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

and two samples of 200 ml. each were removed as described above. The pepsin-HCl mixture was adapted from that described by Herlich (1956): 10 gm. of 1:2,500 pepsin powder (British Drug Houses, Poole, Dorset, England) was dissolved in 600 ml. of water and acidified by adding 30 ml. of concentrated HCl.

(4) Small Intestine

The first 30 feet of the small intestine only was examined as this area contains almost all the small intestinal parasite burden (Armour, 1966). The intestine was separated from its mesenteric attachments, opened and washed under running water into a graduated polythene bucket. The volume was made up to four litres (cattle) or two litres (sheep) and a single sample of 200 ml. was taken and formalinised as above.

(5) Large Intestine and Caecum

The large intestine and caecum were opened and examined by the naked eye for the presence of worms or worm nodules.

D. Parasitological Techniques

(1) Culture and Harvesting Metacercariae of F. hepatica

Maintenance of Snails. Lymnaea truncatula were reared on mud slopes prepared in the following manner. The mud was collected from known snail habitats, allowed to air dry, and was then broken up and all large particulate matter removed by passing the materials through a sieve of 1 cm. mesh. When required the dried mud was mixed into a paste with

deionised water in a mechanical mixer ("Kenwood-Peerless", Kenwood Manufacturing (Woking) Ltd., Hampshire, England) and then layered into polythene-lined boxes as shown in Figure 3. The boxes were kept at a slope and a constant drip of deionised water was allowed to flow through them entering at the higher end of the slope. The mud was removed every two or three months.

As snails often climbed up the sides of the boxes they were washed back down to the mud by a jet of water each day. The snails were given food once per day, care being taken not to overfeed as excess food promotes the growth of moulds and destroys the mud habitat. The supplementary food provided was prepared as follows: 4 parts by weight Cow and Gate milk powder (Cow and Gate Ltd., Guildford, Surrey, England), 3 parts by weight Farex (Glaxo Laboratories Ltd., Greenford, Middlesex, England), 1 part by weight Bemax (Vitamins Ltd., London, England). To this was added powdered calcium carbonate to about one quarter of the total volume and the ingredients were ground thoroughly in a pestle and mortar.

Infection of Snails. The snails were infected when they measured between three and five millimetres. F. hepatica eggs of ovine origin, collected at the Glasgow abattoir, were suspended in water in a plastic container and allowed to hatch under the stimulus of light; the liberated miracidiae were diluted so that 1 drop (0.025 ml.) contained 4 - 5 miracidiae. One drop was placed in each well of a perspex haemagglutination plate and a representative number of wells were checked under a dissecting microscope (Model M.5, Wild, Heerbrugg, Switzerland) to ensure that the required number of miracidiae

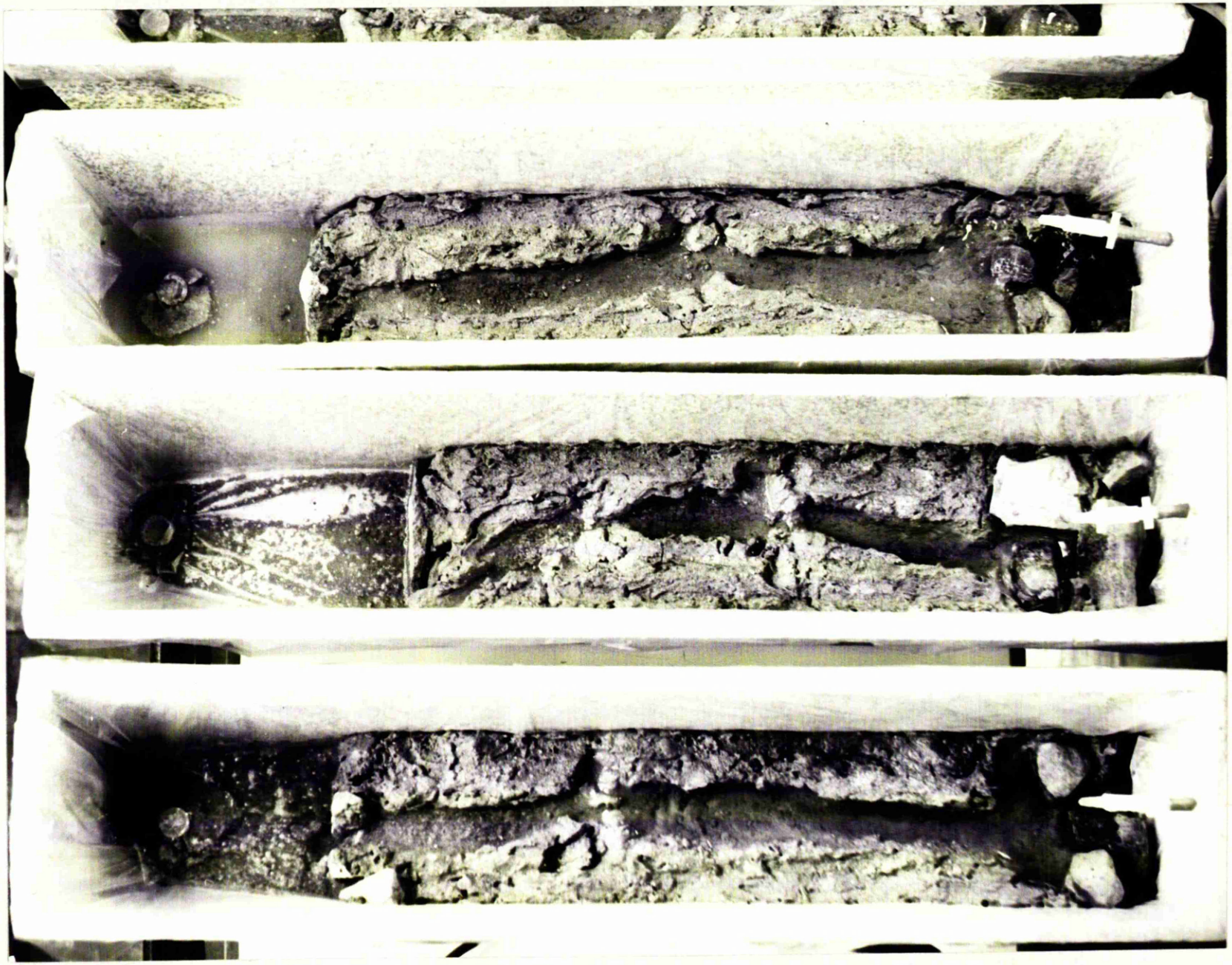

Fig. 3. Method of rearing Lymnaea truncatula; three polythene-lined boxes containing mud mixture and central channel of constantly running de-ionised water.

were present; then a washed snail was placed in each well, (Fig. 4). Each snail was confined to its respective well for 2 hours when the wells were again checked under the microscope to ensure all the miracidia had penetrated the snail host. The infected snails were then placed on a freshly prepared mud slope where it was found that it requires almost 8 weeks for the cercariae to develop in the snail.

Shedding of Cercariae. Infected snails were removed from the mud slopes and thoroughly washed in tap water. They were then placed in cellophane-lined plastic boxes containing a small quantity of deionised water and a few deionised ice-cubes (Fig. 5); the cellophane had previously been washed for several days in running tap water and finally in deionised water. As the ice-cubes melt and the temperature rises to room temperature, the cercariae leave the snail and encyst on the cellophane lining the box. When shedding is complete the snails are returned to the mud slopes and the encysted cercariae are stored at $4^{o}C$ in containers containing deionised water. Infected snails were treated several times in this way and they will continue to shed cercariae for a considerable period.

Preparation of Inoculum. When required the sheets of cellophane and their adherent encysted metacercariae were cut up into strips (Fig. 6) and the strips were examined under a dissecting microscope when the metacercariae were counted. In this way various infective doses could be prepared and the metacercariae, complete with cellophane, were given as a drench.

The above method of culturing and harvesting metacercariae is a

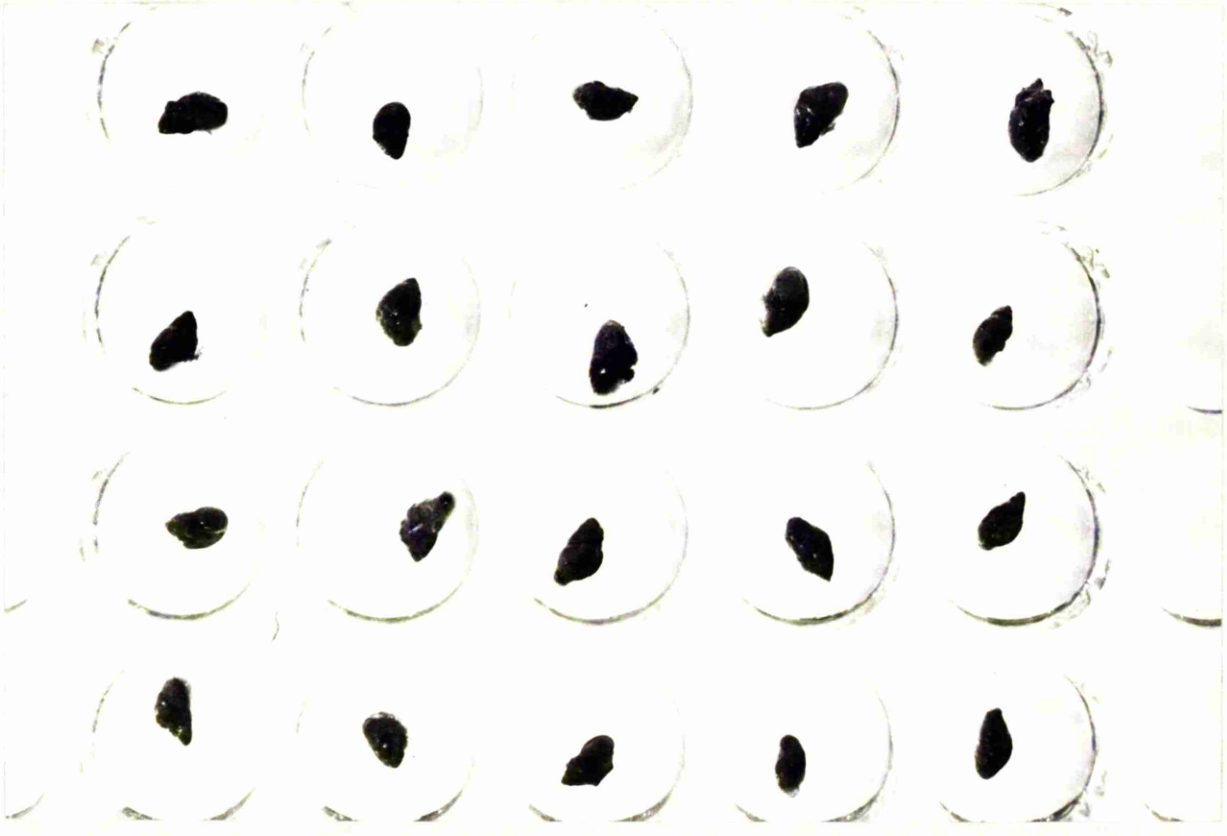

Fig. 4. Method of infecting Lymnaea truncatula with miracidia of F. hepatica; Snails are contained in the wells of a haemagglutination plate x 2.

Fig. 5. Method of shedding cercariae of F. hepatica. Infected mud-snails (Lymnaea truncatula) on the surface of ice cubes x 2.

Fig. 6. Metacercariae of Fasciola hepatica encysted on strips of cellophane x 2.

modification of that used by Jaggers (1966). The original snail population was provided by Dr. Jaggers, I.C.I. Ltd., Alderley Edge, Cheshire, as was the mud which was prepared by mixing several muds found in Somerset. Local muds obtained from snail habitats in the west of Scotland were also tried but these were not suitable for the artificial rearing of snails.

(2) Faecal Egg Counting Techniques

All faecal samples were collected from the rectum and two 3 gm. amounts were weighed out each to be treated in different ways. The first 3 gm. of faeces was mixed with 20 ml. of water and passed through a sieve (60 meshes per inch); a 15 ml. sample of the filtrate was centrifuged in a flat bottomed test tube for two minutes at 2,000 revolutions per minute (r.p.m.) and the supernatant then poured off. The sediment was resuspended in saturated zinc sulphate solution and centrifuged again at 2,000 r.p.m. for two minutes. From the top of the supernatant, enough fluid was removed to fill both chambers (volume 0.15 ml.) of a McMaster Worm Egg Counting Slide (Hawksley & Sons, London, England). The average number of eggs per chamber was multiplied by 100 to give the number of eggs per gram of faeces. This method of counting eggs was essentially similar to that of Gordon and Whitlock (1939).

The second 3 gm. of faeces were mixed with 42 ml. of water and passed through a sieve (60 meshes per inch); a 15 ml. sample of the filtrate was centrifuged as before and the supernatant discarded. The sediment was resuspended in saturated salt (NaCl) solution, the test tube inverted

several times and then using a pipette, enough of the suspension to fill both chambers of a McMaster Worm Egg Counting Slide was removed, the number of eggs present being estimated as above.

(3) Counting and Identification of Gastro-Intestinal Nematodes

After thorough mixing, the 200 ml. samples of abomasal washings, abomasal digests and intestinal contents collected at autopsy were treated as follows: using a 10 ml. straight pipette sawn off at the 8 ml. mark, 5 ml. aliquots were withdrawn and pipetted into Petri dishes, stained for a few minutes with a few drops of a 45 per cent iodine solution (to 16 lb. potassium iodide in five litres of warm distilled water, 10 lb. iodine crystals were added and the mixture was made up to ten litres with distilled water), then decolourised with a 5 per cent sodium thiosulphate solution and counted and identified under a Wild dissection microscope (Model M.5, Wild, Heerbrugg, Switzerland). After staining with iodine and decolourising with sodium thiosulphate, only the worms retained the stain. From the 200 ml. samples, aliquots of 5 ml. were screened until at least 100 worms and a minimum of five aliquots had been examined. When very low numbers of worms were present, only ten counts of 5 ml. aliquots were made. The average number of worms per 5 ml. aliquot was calculated and multiplied by 800, in the case of cattle samples, or 400, in the case of sheep samples, to give the total number of worms in the original four or two litres respectively. The number of worms present was expressed to the nearest hundred. This counting technique gave an estimate which varied within ± 20 per cent of the mean.

The Ostertagia spp. of worms present at autopsy were identified using the descriptions of Ransom (1911), Douvres (1956, 1957) and Rose (1959b, 1963). They were classified into adult stages, developing stages and early fourth larval stages. The worms were considered to be adult when development of the male spicules was complete or the females contained eggs in their uteri. Developing stages were classed as all stages up to adult, except the early fourth larval stages which were classified separately.

E. Statistical Methods

The statistical methods employed were those described by Snedecor (1956) and Bishop (1966). Throughout this thesis all deviations of the means are expressed as the Standard Error (S.E.) of the means.

SECTION I

FIELD STUDIES ON CLINICAL PARASITISM IN YOUNG DAIRY CATTLE IN SOUTH-WEST SCOTLAND

A. Clinical, Haematological, Biochemical, Parasitological and Pathological Findings in 10 Outbreaks of Ostertagiasis in Young Cattle, 1965 - 1967

B. Clinical, Haematological, Biochemical, Parasitological and Pathological Findings in 10 Outbreaks of the Fascioliasis/Ostertagiasis Complex in Young Cattle, 1965 - 1967

General Introduction

A specific syndrome associated with diarrhoea and weight loss in young calves during or following their first grazing season is now well recognised in the British Isles having been reported over a number of years from many different areas of Great Britain. The aetiology of this syndrome is gastro-enteritis due to nematode parasitism. Gardner (1911) recorded its existence in south-east England, Bruford and Fincham (1945) in central England, Stewart and Crofton (1941) in north-east England, Gracey (1960) in Northern Ireland, Watt, Nicolson and Macleod (1961) in central Scotland and Martin, Thomas and Urquhart (1957) and Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965) in south-west Scotland. Most of these reports showed that the specific pathogen present was the abomasal worm, Ostertagia ostertagi.

The field study carried out by Anderson et al., (1965) on the incidence of bovine ostertagiasis in the west of Scotland from 1963 to 1965 was continued into 1966 and 1967 by the author. In the course of this extended survey it became apparent that a syndrome existed which was similar to the Type II ostertagiasis described by Anderson et al.(1965) and the "winter" ostertagiasis reported from Northern Ireland by Ross and Todd (1965) and Ross (1966); this syndrome was associated, at autopsy, with relatively large numbers of Fasciola hepatica, as well as variable but usually significant numbers of adult O. ostertagi. It soon became apparent that the recognition and differential diagnosis of this fascioliasis/ostertagiasis complex was important, since morbidity will continue unchecked unless anthelmintic treatment against both parasites is initiated.

The object of the work reported in this section was to describe this fascioliasis/ostertagiasis complex in detail and to establish criteria on which differential diagnosis between this condition and uncomplicated ostertagiasis could be based. Towards this end the results are presented in two parts.

a) A description of the clinical, haematological, biochemical, parasitological and pathological findings in ten field cases of ostertagiasis in the bovine.

b) A description of the clinical, haematological, biochemical, parasitological and pathological findings in ten field cases of the fascioliasis/ostertagiasis complex in the bovine.

The significance of this fascioliasis/ostertagiasis complex and the differential diagnosis between it and uncomplicated ostertagiasis are then fully dealt with in the general discussion at the end of the section.

Materials and Methods

Procedure in Outbreaks

Outbreaks reported by practising veterinary surgeons were visited and a detailed history obtained in each case. At each farm visited, affected animals were purchased and conveyed to Glasgow University Veterinary Hospital where they were subjected to a detailed clinical and post mortem examination.

Clinical Examination

A detailed clinical examination was conducted paying particular attention to three points; (a) the appearance of the animals' faeces, (b) the appearance of visible mucous membranes, and (c) the presence or absence of submandibular oedema.

Blood Analysis

Blood samples were collected for both haematological and biochemical estimation. The haematological values recorded were packed cell volume, haemoglobin concentration and total red cell count from which the mean corpuscular volume and mean corpuscular haemoglobin concentration were calculated. The biochemical values recorded were total serum protein, serum albumin, serum globulin, S.G.O.T., S.G.P.T., serum bilirubin, alkaline phosphatase and plasma pepsinogen. All these estimations were conducted using techniques identical to those described in materials and methods.

Weighing Procedure

All the animals were weighed prior to autopsy on Avery cattle scales accurate to 1 lb.

Autopsy Procedure

The procedure at post mortem was as described in the section on materials and methods. This included detailed examination of the liver for the presence of F. hepatica, digestion of the abomasal mucosa and subsequent examination of the digest and the abomasal contents, and examination of the first 30 feet of the small intestine.

Samples of abomasal contents were collected and the pH determined using a glass electrode.

Parasitological Estimation

The faeces were examined by both the techniques described in the section on materials and methods and the population of O. ostertagi and F. hepatica present in each animal were counted.

Meteorological Data

The data referred to in this section was supplied by the Superintendent, Meteorological Office, 26 Palmerston Place, Edinburgh 12. The figures for rainfall were recorded at Darvel burgh yard, and temperatures were recorded at Kilmarnock. These towns are situated 9 miles apart and are within 30 miles of the majority of outbreaks investigated. The mean monthly maximum and minimum temperature and monthly rainfall in the period 1965 to 1967 are shown in Figure 7.

The method of calf rearing on dairy farms in south-west Scotland is described in Appendix 1.

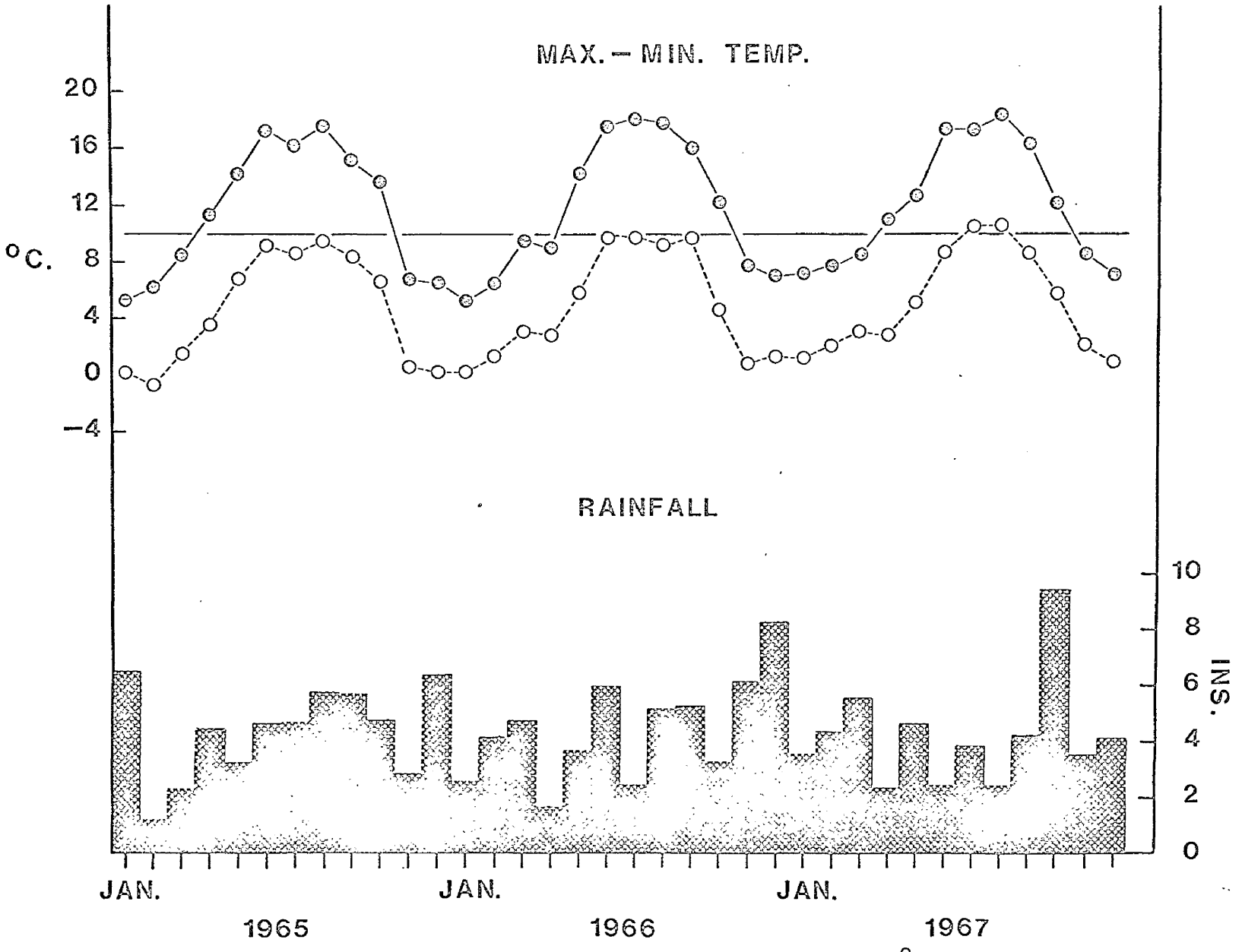

Fig. 7 Mean monthly maximum and minimum temperature (°C) and monthly rainfall from January 1965 to December 1967

A. The Clinical, Haematological, Biochemical, Parasitological and Pathological Findings in 10 Outbreaks of Ostertagiasis in Young Cattle, 1965 - 1967

Introduction

Anderson et al., (1965) describing field cases of bovine ostertagiasis classified the disease into three phases, two of which (Type I and Type II) are clinically obvious. These three phases are briefly as follows:

Type I This corresponded to the classical description of clinical parasitic gastritis in which calves, at grass for their first grazing season, show a loss of weight and diarrhoea; this may occur any time from late July until the end of the autumn grazing season. The vast majority of the ingested larvae develop to maturity in the expected period of three weeks.

Pre-Type II or Stage of Inhibition In this stage which precedes Type II, large populations of O. ostertagi (over 100,000 in many cases) are present of which over 80% are inhibited in the early fourth stage. These animals have grazed infected pasture in the late autumn but have no history of diarrhoea and usually appear healthy to the farmer although to the veterinarian a proportion appear ill-thriven.

Type II The usual history of this type is that calves in which diarrhoea or weight loss had not been prominent during the grazing season have been housed in early November. After some time which may range between 3 weeks to 6 months, the animals start losing weight and show profuse watery diarrhoea. The appearance of these clinical signs coincides with the development to maturity of large numbers of inhibited larvae. This syndrome may also occur

in outwintered animals.

A series of outbreaks of Type II ostertagiasis was investigated subsequent to the survey of Anderson et al. (1965). Although these authors described the clinical, haematological, biochemical, parasitological and pathological findings of this condition and more detailed information can be found in theses by Armour (1967) and Anderson (1968), the degree of similarity between this condition and the fascioliasis/ostertagiasis complex, to be described in the second part of this section, is so great that the inclusion of a series of ten outbreaks of Type II ostertagiasis investigated by the author is relevant here.

Results

General Background and Clinical History

The total number of calves on the ten farms was 174 of which 57 (33%) were clinically affected and 15 (9%) died. The majority of cases occurred between March and May and involved both housed and outwintered stock. Stocking rates on the farms were between two and three animals per acre. The main features are summarised in Table 1.

Clinical Signs

All the animals affected were thin although frequently well-grown. Their coats were dull and the tails, perineal regions and hocks were soiled with faeces which had become adherent to these areas. The demeanour of the animals was variable in that a proportion were bright and alert, and eating

Table 1

Clinical History of 10 Field Cases of Type II Ostertagiasis in Dairy Calves Aged 10 to 18 Months

Case No.	Month of Farm Visit	Autumn Stocking Rates	Housed/ Outwintered	Morbidity Rate(%)	Mortality Rate(%)
A1	January	3/2 acres	Outwintered	30	5
A2	February	2/acre	Housed	11	4
A3	February	2/acre	Housed	40	20
A4	March	3/acre	Housed	61	15
A5	April	4/acre	Housed	50	13
A6	April	3/acre	Outwintered	25	15
A7	April	2/acre	Housed	56	11
A8	April	5/2 acres	Housed	6	6
A9	April	1/acre	Housed	53	Nil
A10	May	1/acre	Housed	27	Nil
Mean		2.2		36	9
Standard Error		0.30		6.0	2.2

whilst others were very dull, lethargic, anorexic and occasionally recumbent.

The majority of animals developed a profuse, watery diarrhoea which was predominantly continuous although in some cases was of an intermittent nature. Less frequently a calf with soft, semi-liquid faeces was observed. In 60% of the cases visible mucous membranes were pink in colour but in the remainder of the animals a slight degree of pallor was observed. Submandibular oedema was also a feature of the clinical syndrome and in 40% of the calves this was very marked and in general occurred in those calves with some pallor of mucous membranes. The weight loss was a particularly marked feature and bodyweights were substantially lower than the average values for dairy calves at this age. Values for average Ayrshire heifers aged 12 - 15 months at the West of Scotland Agriculture College are 495 to 603 lbs. (Walker-Love, 1965), so that affected animals are at least 150 to 200 lbs. underweight. The salient clinical features are summarised in Table 2.

Haematological Observations

Packed cell volume percentages, haemoglobin levels and total red cell counts were reduced to a moderate degree in 80% of cases. The range of packed cell volume values was 21.0 per cent to 36.5 per cent with a mean of 25.0 ± 1.49 per cent. Haemoglobin concentrations ranged from 6.6 to 11.1 gms. per 100 ml. with a mean of 8.0 ± 0.43 gms. per 100 ml.

Table 2

Major Clinical Signs in 10 Field Cases of Type II Ostertagiasis in Dairy Calves Aged 10 to 18 Months

Case No.	General Appearance	Type of Faeces	Mucous Membranes	Submandibular Oedema	Weight (lbs)
A1	Dull	Profuse Continuous Diarrhoea	Pale +	Very Marked	324
A2	Dull	Profuse Continuous Diarrhoea	Pink	Very Marked	319
A3	Bright	Profuse Intermittent Diarrhoea	Pale +	Absent	300
A4	Bright	Soft Intermittent	Pink	Absent	275
A5	Bright	Profuse Continuous Diarrhoea	Pink	Slight	298
A6	Dull	Profuse Continuous Diarrhoea	Pale +	Very Marked	271
A7	Bright	Soft Intermittent	Pink	Absent	307
A8	Very Dull	Profuse Continuous Diarrhoea	Pink	Absent	298
A9	Bright	Soft Continuous	Pink	Very Marked	286
A10	Dull	Profuse Continuous Diarrhoea	Pale +	Absent	179

+ = slight degree of pallor

Total red cell counts ranged from 4.05 to 6.72 million per cu.mm. with a mean of 5.33 ± 0.26 million per cu.mm. The anaemia was of the normochromic and normocytic type, the mean values for M.C.V. and M.C.H.C. being 48 cu.u and 32 per cent respectively. The individual results are summarised in Table 3.

Biochemical Observations

A significant drop in total serum protein levels was not constant and occurred in only 30% of cases. A more marked and constant feature was a reduction in serum albumin concentrations and albumin:globulin ratios. The mean total serum protein and serum albumin concentrations were 5.2 ± 0.30 gms. per 100 ml. and 1.6 ± 0.22 gms. per 100 ml. respectively, and the mean albumin:globulin ratio was 0.5.

There were no significant changes in serum alkaline phosphatase or serum bilirubin levels where mean values were 6 ± 1.0 K.A. units and 0.4 ± 0.04 mg. per 100 ml. respectively. A similar position was recorded regarding serum glutamic oxaloacetic transaminase (S.G.O.T.) and serum glutamic pyruvic transaminase (S.G.P.T.) where the mean values were 113 ± 17.3 S-F units and 13 ± 2.0 S-F units respectively.

Plasma pepsinogen was elevated to a marked degree (mean 4,000 ± 1134 mU tyrosine) and in some cases was in the region of 15 to 20 times the value in uninfected control animals of the same age (300 ± 8).

Table 3

Haematological Observations on 10 Field Cases of Type II Ostertagiasis in Dairy Calves Aged 10 to 18 Months

Case No.	Packed Cell Volume (%)	Haemoglobin (gm/100 ml)	Erythrocytes (x 10^6/cu.mm.)	M.C.V. (cu.u)	M.C.H.C. (%)
A1	22.0	7.0	5.24	42	32
A2	21.5	6.6	4.05	53	31
A3	24.0	7.8	5.71	42	33
A4	30.0	9.2	6.30	53	31
A5	24.0	7.5	4.67	51	31
A6	21.0	6.9	4.61	46	33
A7	23.5	7.2	5.03	47	31
A8	36.5	11.1	6.72	54	30
A9	25.5	8.8	5.10	50	35
A10	24.0	8.1	5.89	41	34
Mean	25.2	8.0	5.33	47.9	32.1
Standard Error	1.49	0.43	0.26	1.56	0.50

Individual biochemical observations are summarised in Table 4.

Autopsy Findings

The mean pH of abomasal contents was 6.30 ± 0.33 and individual values for abomasal pH are given in Table 5.

Pathological Data

The lesions observed on the abomasal mucosa were variable and several types of lesions were present in any one animal at the same time. Sometimes small circular, greyish-white umbilicated nodules characteristic of the inhibited stage and "morocco-leather" and sloughing lesions due to diffuse irregular epithelial hyperplasia and epithelial cytolysis of the emergent stage were seen. In some cases the mucosa was congested whilst in others it was oedematous, the oedema being most marked in the abomasal folds, the surface of which had a smooth, grey, shiny appearance. A gelatinous oedema was frequently seen elsewhere particularly in the folds of mesentery around the large intestine.

Parasitological Data

The faecal egg counts were low being less than 900 o.p.g. and there was no correlation between the faecal egg counts and the number of adult female worms present. The total number of Ostertagia spp. present ranged from 13,400 to 545,300 and the proportion of early 4th stages from 1 to 97% with a mean of 49%. Ninety-eight per cent of the Ostertagia spp. males present were O. ostertagi, the remaining two per cent being O. lyrata.

Table 4

Biochemical Data on 10 Field Cases of Type II Ostertagiasis in Dairy Calves Aged 10 to 18 Months

Case No.	Total Protein (gms/100 ml.)	Albumin (gms/100ml)	Globulin (gms/100 ml)	Alk.Phos. (K.A.units)	SGOT (S-F units)	SGPT (S-F units)	Bilirubin (mgs/100 ml)	Pepsinogen (mU tyrosine)
A1	3.0	0.9	2.1	7	82	6	0.3	1500
A2	7.5	2.5	5.0	6	64	4	0.5	4000
A3	5.4	0.5	4.9	9	160	21	0.3	2400
A4	5.5	2.3	3.2	6	105	10	0.2	3900
A5	5.8	1.9	3.9	8	93	15	0.4	5700
A6	4.6	1.1	3.5	2	157	7	0.4	4000
A7	5.2	1.7	3.5	5	79	11	0.1	5300
A8	5.0	1.7	3.3	11	235	22	0.8	4000
A9	4.2	1.2	3.0	1	87	16	0.3	5600
A10	6.2	2.6	3.6	9	66	17	0.2	5500
Mean	5.2	1.6	3.6	6	113	13	0.4	4000
Standard Error	0.38	0.22	0.27	1.0	17.3	2.0	0.04	1134

Table 5

The Abomasal pH of 10 Cases of Type II Ostertagiosis in Dairy Calves Aged 10 to 18 Months

Case Number	Abomasal pH
A1	7.00
A2	6.45
A3	5.00
A4	6.55
A5	6.50
A6	6.85
A7	4.30
A8	7.65
A9	7.15
A10	5.50
Mean	6.30
Standard Error	0.33

A few adult Fasciola hepatica were occasionally found but these did not exceed a total of 75 in any one animal.

The relevant parasitological findings are summarised in Table 6.

Discussion

The ten outbreaks of severe weight loss and diarrhoea in young dairy cattle which have just been described fit into the category of Type II ostertagiasis as reported by Anderson et al., (1965). The condition occurred in late winter or early spring and involved both housed and outwintered stock. It was associated at autopsy with the presence of large numbers of the abomasal parasite, O. ostertagi, and with severe lesions of the abomasal mucosa. This syndrome appears to be identical to the atypical parasitic gastritis in housed cattle described by Martin et al., (1957) and a similar syndrome has been reported from Northern Ireland, Ross and Todd (1965) and Ross (1966).

The main clinical signs of Type II ostertagiasis were weight loss and diarrhoea which were the most constant features of the disease; other less constant features were pallor of visible mucous membranes and submandibular oedema which were only present in a proportion of cases. Although the submandibular oedema was very marked in some animals, pallor of visible mucous membranes was never more than slight.

Table 6

Parasitological Data at Autopsy of 10 Cases of Type II Ostertagiasis in Dairy Calves Aged 10 to 18 Months

Case No.	Faecal Egg Count e.p.g.		Worm Counts		
	Strongyle	Fluke	Ostertagia Spp.*		Fasciola hepatica
			Total	% Early 4th Larval Stages	
A1	550	Nil	545,300	97	Nil
A2	500	Nil	118,400	83	Nil
A3	700	Nil	124,800	73	75
A4	150	Nil	79,200	43	Nil
A5	850	Nil	132,000	39	50
A6	650	Nil	109,600	38	Nil
A7	300	Nil	52,300	57	Nil
A8	25	Nil	150,200	35	Nil
A9	860	Nil	60,500	21	Nil
A10	50	Nil	13,400	1	Nil
Mean	464	-	138,600	48.7	13
Standard Error	100	-	47,200	9.2	9

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

Data on blood analysis demonstrated the presence of a mild to moderate anaemia in the majority of animals, this being of the normochromic, normocytic type and in all cases the packed cell volume was above 20 per cent. This anaemia was accompanied by a hypo-albuminaemia and lowered albumin:globulin ratio and, in a minority of cases, a reduced total serum protein level. No significant alterations were observed in serum transaminase (S.G.O.T. and S.G.P.T.), alkaline phosphatase, or bilirubin levels. Although the mean figure for S.G.O.T. was 113 S-F units this is considered to be the result of generalised tissue loss, particularly muscle, and is observed in a variety of chronic wasting diseases in the bovine.

The total number of Ostertagia spp. present at autopsy ranged from 13,400 to 543,300 and the proportion of early fourth stage larvae ranged from 1 per cent to 97 per cent. In general the number of early fourth stages diminished as the year progressed hence the figure of 1 per cent inhibition seen in one animal in May. This particular animal had been diarrhoeic for several weeks and subsequently would lose a large proportion of the original worm burden.

The numbers of Fasciola hepatica, if present, did not exceed 75 in any animal and it is unlikely that this number is of any significance.

D. The Clinical, Haematological, Biochemical, Parasitological and Pathological Findings in 10 Outbreaks of the Fascioliasis/Ostertagiasis Complex in Young Cattle, 1965 - 1967

Introduction

Although the existence of a syndrome of weight loss and diarrhoea in young cattle due to bovine ostertagiasis is now well established, information on comparable aspects of fascioliasis in cattle is negligible; this, despite the fact that the disease has been reported from most countries in the world. There are no detailed descriptions of bovine fascioliasis as it occurs under field conditions and the few accounts of experimental infection have dealt with certain aspects of the disease only. For instance, Morrill and Shaw (1942) were mainly interested in the pathological changes produced in the liver, Sazanov (1961) only recorded the effects of infection on weight gain, whilst Moroshkin, Kostina, Ivanskii and Sutyagin (1964) observed changes in the blood and bone marrow.

A number of the standard textbooks (Morgan and Hawkins, 1953; Lapage, 1956; Smith and Jones, 1957) describe fascioliasis in cattle as a chronic wasting disease eventually producing pallor of mucous membranes and in some cases submandibular oedema. These textbooks also refer to diarrhoea being present in a proportion of cases. Thus, it would appear from the above description that there is apparently a marked degree of similarity between ostertagiasis and fascioliasis in the bovine.

The following section of this thesis describes a field syndrome where both Ostertagia ostertagi and Fasciola hepatica co-existed in significant numbers. This fascioliasis/ostertagiasis is described in detail for two reasons. Firstly, it is of considerable practical significance in the field and was previously unrecognised as a specific entity. Secondly, it provided a stimulus for the subsequent study of the clinical significance of uncomplicated fascioliasis in the bovine.

Results

General Background and Clinical History

The total number of calves on the ten farms was 230 of which 92 (40%) were clinically affected and 19 (8%) died. The majority of cases occurred between January and March and involved both housed and outwintered stock. Stocking rates on the farms were always less than one animal per acre. The main features are summarised in Table 7.

Clinical Signs

The affected animals were all very thin although usually well-grown. In appearance the majority of the animals were dull and lethargic, anorexic and frequently recumbent. These clinical signs appeared slowly and the farmer usually complained that the calves had not been thriving for some time. The calves' coats were dull and the tails, perineal regions and hocks in some, but not in all cases, were soiled with faeces which had become adherent to these areas.

Table 7

Clinical History of 10 Field Cases of the Fascioliasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months.

Case No.	Month of Farm Visit	Autumn Stocking Rates	Housed/ Outwintered	Morbidity Rate(%)	Mortality Rate(%)
B1	January	1/acre	Housed	52	26
B2	February	1/2 acres	Outwintered	40	15
B3	February	1/2 acres	Outwintered	36	12
B4	February	1/3 acres	Outwintered	55	Nil
B5	February	1/2 acres	Housed	100	23
B6	February	1/3 acres	Housed	5	2
B7	February	1/3 acres	Housed	3	2
B8	March	1/8 acres	Housed	32	12
B9	March	1/7 acres	Housed	28	8
B10	April	1/acre	Housed	100	5
Mean		0.5		42.9	10.5
Standard Error		0.10		10.6	2.8

In 30% of cases the calves' faeces were firm and formed, whilst in the remaining 70% of cases the faeces were intermittently soft in character. A continuous profuse watery diarrhoea was never observed. The visible mucous membranes all exhibited a variable degree of pallor which ranged from a pale pink colour to almost pure white. Submandibular oedema was present to a marked degree in 50% of the cases but its presence bore no relation to the degree of pallor of mucous membranes. In 20% of the cases the liver was palpable, projecting up to 2 inches behind the last rib on the right side. The organ appeared firm to the touch, had a rounded edge, a smooth surface and was freely movable. Palpation of the organ did not appear to cause the animal any pain or discomfort.

Another clinical feature of only minor importance was that a soft systolic murmur of the haemic type was detected on auscultation in 70% of cases. This murmur although not constantly present was most frequently localised to the pulmonic area.

The weight loss was very marked in all the calves and their bodyweights were much lower than the average values for dairy calves at this age. Affected animals were at least 100 to 150 lbs. underweight.

The main clinical features are summarised in Table 8.

Table 8

Major Clinical Signs in 10 Field Cases of the Fascioliasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months

Case No.	General Appearance	Type of Faeces	Mucous Membranes	Submandibular Oedema	Weight (lbs)
B1	Dull	Intermittently Soft	Pale ++	Absent	355
B2	Very Dull	Intermittently Soft	Pale ++	Slight	320
B3	Bright	Intermittently Soft	Pale +++	Very Marked	310
B4	Dull	Intermittently Soft	Pale +++	Marked	296
B5	Dull	Intermittently Soft	Pale +++	Very Marked	321
B6	Dull	Intermittently Soft	Pale +++	Very Marked	287
B7	Dull	Intermittently Soft	Pale +++	Very Marked	298
B8	Dull	Firm	Pale ++	Absent	317
B9	Bright	Firm	Pale +++	Absent	326
B10	Dull	Intermittently Soft	Pale ++	Absent	425

\+ = slight degree of pallor
++ = moderate degree of pallor
+++ = marked degree of pallor

Haematological Estimations

Packed cell volume percentages, haemoglobin levels and total red cell counts were markedly reduced in all cases. The range of packed cell volume values was 12.5 per cent to 20.5 per cent with a mean of 16.5 ± 0.30 per cent. Haemoglobin concentrations ranged from 3.1 to 7 gms. per 100 ml. with a mean of 5.3 ± 0.30 gms. per 100 ml. Total red cell counts ranged from 2.45 to 4.99 million per cu.mm. with a mean of 3.48 ± 0.24 million per cu.mm. The anaemia was of the normochromic and normocytic type; the mean values for M.C.V. and M.C.H.C. being 48 cu.μ and 32 per cent respectively. Individual results are summarised in Table 9.

Biochemical Observations

Total serum protein levels were reduced in only 40% of cases but a more constant feature was a fall in albumin concentrations and albumin: globulin ratios. The mean total serum protein and serum albumin concentrations were 5.0 ± 0.30 gms. per 100 ml. and 1.5 ± 0.17 gms. per 100 ml. respectively, and the mean albumin:globulin ratio was 0.4.

There were no significant changes in serum alkaline phosphatase or serum bilirubin levels where mean values were 6 ± 0.7 K.A. units and 0.3 ± 0.02 mg. per 100 ml. respectively. A similar situation was observed regarding serum glutamic oxaloacetic transaminase (S.G.O.T.) and serum glutamic pyruvic transaminase (S.G.P.T.) where the mean values were 100 ± 6.4 S-F units and 14 ± 2.2 S-F units respectively.

Table 9

Haematological Observations on 10 Field Cases of the Fascioliasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months

Case No.	Packed Cell Volume (%)	Haemoglobin (gm/100 ml)	Erythrocytes (x 10^6/cu.mm.)	M.C.V. (cu.μ)	M.C.H.C. (%)
B1	20.5	7.0	4.99	41	34
B2	20.5	6.6	4.30	48	32
B3	15.0	4.5	3.11	48	30
B4	18.5	6.0	3.80	49	32
B5	17.0	5.4	3.29	52	32
B6	12.5	3.1	2.45	51	25
B7	17.0	6.4	3.53	48	38
B8	13.0	4.2	2.67	49	32
B9	16.5	5.1	3.42	48	31
B10	14.0	5.0	3.24	46	36
Mean	16.5	5.3	3.48	48	32.2
Standard Error	0.30	0.38	0.24	0.94	1.10

Plasma pepsinogen was only moderately elevated with a mean value of 2,000 ± 236 n Units tyrosine.

Individual biochemical results are summarised in Table 10.

Autopsy Findings

The mean pH of abomasal contents was 4.6 ± 0.39 and individual results for abomasal pH are given in Table 11.

Pathological Data

In 80% of cases there was marked oedema of the abomasal wall and folds. The mucosa over the folds was smooth, shiny and grey in colour whilst in the areas between the folds large numbers of small, circular, greyish-white, umbilicated nodules were present. In the remaining cases although the organ was not oedematous the main lesions were the small nodules and there were no areas of sloughing or "morocco-leather" appearance.

The liver was enlarged to a variable degree in most cases and had a firm, rounded edge. The most noticeable feature was the enlargement and thickening of the bile ducts. In each case the bile duct wall was irregularly thickened with a variable amount of fibrous tissue. Frequently very hard, white areas were present in the bile duct wall and these areas were difficult to cut. When sectioned the bile duct mucosa was studded with small, dark-coloured, lentil shaped areas which were very hard and gritty

Table 10

Biochemical Data on 10 Field Cases of the Fasciolíasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months

Case No.	Total Protein (gms/100 ml)	Albumin (gms/100ml)	Globulin (gms/100 ml)	Alk.Phos. (K.A. units)	SGOT (S-F units)	SGPT (S-F units)	Bilirubin (mgs/100 ml)	Pepsinogen (ml tyrosine)
B1	6.2	2.7	3.5	5	133	9	0.4	1000
B2	5.0	1.4	3.6	6	88	7	0.2	2700
B3	3.9	0.7	3.2	7	123	24	0.5	1400
B4	5.2	1.7	3.5	5	87	10	0.2	2300
B5	4.3	1.0	3.3	7	63	6	0.3	2500
B6	4.6	1.6	3.0	5	112	10	0.1	1300
B7	3.5	1.0	2.5	10	92	15	0.4	1000
B8	5.5	1.3	4.2	6	110	25	0.1	2200
B9	6.0	1.4	4.6	5	100	19	0.2	2700
B10	6.0	1.7	4.3	1	95	16	0.3	2900
Mean	5.0	1.5	3.6	6	100	14	0.3	2000
Standard Error	0.30	0.17	0.20	0.7	6.4	2.2	0.02	237

Table 11

The Abomasal pH of 10 Cases of Fascioliasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months

Case Number	Abomasal pH
B1	3.05
B2	2.65
B3	5.95
B4	3.95
B5	4.95
B6	5.35
B7	2.40
B8	4.10
B9	3.90
B10	5.30
Mean	4.16
Standard Error	0.38

to the touch. The bile duct lumen contained a variable number of adult flukes which in some cases were difficult to remove as they became impaled on the small, gritty areas which lined the mucosa. As well as flukes being present in the bile duct lumen an amount of viscid, dark green bile, containing an amount of tissue debris was present. The liver parenchyma did not appear to be markedly damaged but the ventual lobe of the organ was much paler and firmer than areas adjacent to it, and in some cases it appeared to be atrophied.

Parasitological Data

The faecal egg counts in the case of strongyle eggs was frequently low being less than 700 e.p.g. and on several occasions negative. In the case of Fasciola eggs there was a maximum of 1,300 e.p.g. and although a positive faecal count was present in every case there was no correlation between the number of eggs and the number of adult flukes recovered.

The total number of Ostertagia spp. recovered at autopsy ranged from 4,800 to 181,700 and the proportion of early 4th stages from 34% to 100% with a mean of 66%. Ninety-eight per cent of the Ostertagia spp. males present were O. ostertagi, the remaining two per cent being O. lyrata. The total number of Fasciola hepatica (all adults) present ranged from 238 to 650 with a mean of 372 ± 127.

The parasitological findings in individual animals are summarised in Table 12.

Table 12

Parasitological Data at Autopsy of 10 Cases of the Fascioliasis/Ostertagiasis Complex in Dairy Calves Aged 10 to 18 Months

Case No.	Faecal Egg Count e.p.g.		Worm Counts			
	Strongyle	Fluke	Ostertagia Spp.*		Fasciola hepatica	
			Total	% Early 4th Larval Stages	Adult	Immature
B1	Nil	50	81,500	91	320	Nil
B2	Nil	50	127,800	97	330	Nil
B3	50	1,300	181,800	92	650	Nil
B4	275	100	33,700	47	238	Nil
B5	200	50	150,800	57	295	Nil
B6	Nil	125	22,600	50	408	Nil
B7	Nil	150	4,800	100	337	Nil
B8	50	500	7,800	34	510	Nil
B9	Nil	50	7,300	40	240	Nil
B10	700	120	40,500	50	393	Nil
Mean	128	250	65,800	65.8	372	-
Standard Error	71	125	20,600	26.0	127	-

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

Discussion

The syndrome described in the foregoing ten outbreaks does not appear to have been described before. It occurs in winter, particularly in the months of January, February and March and involved both housed and outwintered stock and at autopsy it is associated with the presence of large numbers of *Fasciola hepatica* as well as variable but usually significant numbers of *Ostertagia ostertagi* of which the majority are in the inhibited early fourth stage.

The clinical significance of *F. hepatica* infections in cattle is largely unexplored and this makes it difficult to assess the respective parts played by *O. ostertagi* and *F. hepatica* in this syndrome. It would appear that *F. hepatica* was primarily responsible for the severe anaemia as *O. ostertagi* alone does not produce an anaemia of this magnitude. That the former parasite is capable of producing a marked anaemia in young calves has recently been demonstrated by Ross, Todd and Dow (1966) and by the author (see Section II) in contemporaneous studies. The former found that an adult fluke burden of 250 or more will produce an anaemia of the haemorrhagic type and this is in agreement with the results described in Section II where an adult fluke burden of 226 or more was also capable of producing an anaemia. Both these figures compare favourably with the minimum adult fluke burden of 238 found at autopsy in cases of the fascioliasis/ostertagiasis complex, and indicates that the severe anaemia

found in this syndrome is mainly a result of the F. hepatica component. Adult fluke burdens of 250 or more (Ross et al., 1966) and 226 or more (this thesis, Section II) will also produce a marked hypoalbuminaemia in calves.

The lack of profuse diarrhoea in the fascioliasis/ostertagiasis complex would appear to contradict those publications which describe diarrhoea as a major, though inconstant, clinical sign in bovine fascioliasis (Morgan and Hawkins, 1953; Lapage, 1956; Smith and Jones, 1957; Taylor, 1964). Subsequent experimental and field studies by the author (see Section II) and by Ross (1966 a & b) indicates that diarrhoea does not occur in cases of bovine fascioliasis. Since it has been shown in experimental ostertagiasis in young cattle that the onset of diarrhoea is always associated with the emergence of adults from the abomasal glands (Jennings, Armour, Lawson and Roberts, 1966), it is probable that the intermittent fluid faeces observed in the fascioliasis complex is caused by the periodic maturation and emergence of moderate numbers of previously inhibited O. ostertagi from those glands.

The sudden appearance of this syndrome in the west of Scotland is attributable to several factors. The first of these is that the summer and autumn of 1965 and 1966 were particularly suitable for the development of the intermediate host, the mud-snail Lymnaea truncatula, which likes

wet conditions. Secondly, for economic reasons farmers are currently maintaining greater numbers of stock on their farms than before; this often means that poor quality and inadequately drained grazing must be used where the chances of the stock coming into contact with the parasite are greatly increased. The third factor involved is again an economic one in that the high cost of foodstuffs and lack of accommodation, mainly due to the increased stocking rates, forces many farmers to overwinter young stock, or at least leave them outside until the end of the year; this increases the chance of their being exposed to a heavy challenge of cercariae.

General Discussion

An examination of the results described in both parts of this section indicates that two similar syndromes exist in young cattle in the west of Scotland. The first condition, bovine parasitic gastritis, is identical to the Type II ostertagiasis described by Anderson et al. (1965) and is associated at autopsy with the presence of large numbers of O. ostertagi, a variable number of which are in the inhibited early fourth stage. This condition is also similar to the atypical parasitic gastritis in housed cattle described by Martin et al. (1957) and the "winter" ostertagiasis reported by Ross and Todd (1965) and Ross (1966). In the second syndrome, the fascioliasis/ostertagiasis complex, two parasites are present and at autopsy this is associated with relatively large numbers of mature Fasciola hepatica as well as variable but usually significant numbers of O. ostertagi. Although very similar, there are some striking differences between the two conditions on closer inspection.

The first point of difference is with regard to the grazing history where in outbreaks of Type II ostertagiasis stocking rates in the late autumn averaged 2 - 3 animals per acre and the small, permanent, calf paddock was generally in use, whilst in outbreaks of the fascioliasis/ostertagiasis complex stocking rates were always less than one animal per acre and larger areas of predominantly wet, permanent grazing were used. This latter condition occurred more frequently on farms where young stock

were left to graze outside until the end of the year or even later. Although both conditions involved housed and outwintered stock following their first grazing season cases of the fascioliasis/ostertagiasis complex appeared predominantly between January and March with a peak in February, whilst cases of Type II ostertagiasis generally appeared between March and May with a peak in April.

Although the background information described above allows a broad division between the two conditions to be drawn, examination of the clinical signs is even more helpful. The profuse diarrhoea of Type II ostertagiasis is not a feature of the fascioliasis/ostertagiasis complex where faeces are often quite firm or intermittently soft in character. Another sign of value in helping to differentiate the two conditions is the appearance of the mucous membranes; in Type II ostertagiasis mucous membranes are frequently normal or show a slight degree of pallor often not easily observed even by the experienced clinician, whilst in the case of the fascioliasis/ostertagiasis complex mucous membranes are always pale. Other clinical features such as submandibular oedema, cardiac murmurs and palpable livers are not of much assistance in differential diagnosis as both the former are found in each condition to a similar degree and the latter although present only in the fascioliasis/ostertagiasis complex are detectable only in a minority of cases. Morbidity and mortality rates were similar in both conditions, being in the region of 30% and 10% respectively.

An examination of the laboratory findings demonstrates striking differences between the two conditions particularly in the case of the haematology. A severe anaemia of the normochromic, normocytic type is present in every case of the fascioliasis/ostertagiasis complex with mean figures for packed cell volume, haemoglobin concentration and total erythrocyte counts being 16.5 ± 0.30 per cent, 5.3 ± 0.38 gms. per cent, and 3.48 ± 0.24 million per cu.mm. respectively. In Type II ostertagiasis although the anaemia was not constant it was generally present to a moderate degree with mean figures for packed cell volume, haemoglobin concentration and total erythrocyte count being 25.0 ± 1.49 per cent, 8.0 ± 0.43 gm. per 100 ml., and 5.33 ± 0.26 million per cu.mm. respectively.

As a result of the more severe abomasal damage the plasma pepsinogen values were higher in Type II ostertagiasis (4,000 ± 1134 m Units tyrosine) than in the fascioliasis/ostertagiasis complex (2,000 ± 237 m Units tyrosine). Although total serum proteins and albumin:globulin ratios were reduced in both conditions there was no significant difference between the results. Similarly there were no differences in serum transaminase, alkaline phosphatase or bilirubin levels in either condition, these levels being not significantly altered.

The final difference between Type II ostertagiasis and the fascioliasis/ostertagiasis complex was seen at autopsy, where in the former condition the pH of abomasal contents was usually in excess of 5, the total F. hepatica

count was a maximum of 75 with little or no hepatic fibrosis present; the abomasal mucosa showed severe hyperplasia and oedema, and unless the animals had been recently treated over 50,000 adult O. ostertagi and large numbers of 4th larval stages were present. In cases of the fasciolinosis/ostertagiosis complex the pH of abomasal contents was usually less than 3, the total F. hepatica count ranged from 238 - 650 with accompanying severe hepatic fibrosis; the abomasal mucosa showed mild to severe hyperplasia and oedema, and unless recently treated upward of 20,000 adult O. ostertagi and large numbers of 4th larval stages were present.

The essential differences between the two syndromes are summarised in Table 13.

Table 13

The Differential Findings of the Fasciolíasis/Ostertagiasis Complex and Type II Ostertagiasis

	Fasciolíasis/Ostertagiasis Complex	Type II Ostertagiasis
Seasonal Incidence	December to early April	Predominantly April and May
History	Grazed at average stocking rates during autumn on poorly drained permanent pasture previously grazed by calves during spring and summer	Usually overstocked during autumn on permanent calf paddock previously grazed by calves during spring and summer
	Involved both housed and outwintered stock following first grazing season	
Clinical Signs	Usually intermittent soft faeces, occasionally profuse diarrhoea	Continuous or intermittent profuse diarrhoea
	Progressive loss of bodyweight	Rapid loss of bodyweight
	Liver may be palpable	
	Submandibular oedema, pale mucous membranes and cardiac murmurs sometimes present Morbidity and mortality rate similar, i.e. 30 per cent and 10 per cent respectively	

Laboratory Findings	Severe anaemia always present	Mild to moderate anaemia common
	PCV % 16.5 ± 0.30	PCV % 25.2 ± 1.49
	Rbc x 10^6/cu.mm. 3.48 ± 0.24	Rbc x 10^6/cu.mm. 5.33 ± 0.26
	Hb gm/100 ml. 5.5 ± 0.38	Hb gm/100 ml. 8.0 ± 0.43
	Plasma pepsinogen slightly elevated (2000 ± 237 mU tyrosine)	Plasma pepsinogen markedly elevated (4000 ± 1134 mU tyrosine)
	Total serum proteins and A/G ratios low (5.1 gm/100 ml. and 0.4)	
	Serum transaminase, bilirubin and alkaline phosphatase levels not significantly altered	
Autopsy	pH of abomasal contents usually less than 5.0	pH of abomasal contents usually greater than 5.0
	238 - 650 adult F. hepatica with accompanying hepatic fibrosis	0 - 75 adult F. hepatica. Hepatic fibrosis absent or mild
	Abomasal mucosa shows mild to severe hyperplasia and oedema	Abomasal mucosa shows severe hyperplasia and is frequently oedematous
	Unless recently treated with an anthelmintic, upward of 20,000 adult O. ostertagi and large numbers of 4th larval stages	Unless recently treated with an anthelmintic, over 50,000 adult O. ostertagi and large numbers of 4th larval stages (the latter inversely proportional to the duration of diarrhoea)

± Standard Error

Summary

1. Ten outbreaks of parasitic gastritis in the young bovine are described in which O. ostertagi was the predominant parasite present. All the outbreaks were reported during the winter and early spring, the majority occurring in April and May. The main clinical signs were rapid loss of bodyweight accompanied by a profuse diarrhoea. An anaemia developed in a proportion of cases and this was always mild to moderate in severity and frequently not suspected on clinical examination. The main changes in blood chemistry involved the plasma proteins where a hypoalbuminaemia was recorded with a marked increase in plasma pepsinogen levels.

2. In a further ten outbreaks of clinical parasitism in the young bovine the presence of variable but usually significant numbers of O. ostertagi was accompanied by relatively large numbers of mature F. hepatica. This condition called the fascioliasis/ostertagiasis complex also occurred during the winter, the majority of cases being reported between January and March. The main features of clinical significance were loss of bodyweight and the relative or complete absence of diarrhoea. A severe anaemia was present in all cases and readily appreciated on clinical examination. A hypo-albuminaemia was noted but plasma pepsinogen levels were normal or only slightly elevated.

3. The essential differences between Type II ostertagiasis and the fascioliasis/ostertagiasis complex are discussed.

SECTION II

EXPERIMENTAL *FASCIOLA HEPATICA* INFECTIONS IN CALVES

The Results of Single Infections at Two Dose Levels

Introduction

Although it has long been recognised that F. hepatica is responsible for considerable condemnation of bovine livers at meat inspection in the slaughter-house there is little information on the importance of fasciolíasis as a cause of clinical disease in cattle. A few reports record the subclinical effects of the disease including reduction in milk yield in dairy cows (Gebauer, 1939; Doeksen, Heringa and Swierstra, 1949), reduction in live-weight gain (Frederick, 1943) and poor quality flesh in animals at slaughter (Goncharuk, 1959 a & b). The majority of reports on clinical fasciolíasis in the bovine are based on observations of field cases where the condition is described as being of a chronic nature producing progressive loss of bodyweight, and pallor of visible mucous membranes with diarrhoea commonly present. This latter feature has been the subject of some controversy and the majority of reports describe it as being present (Ruzeby, 1955; Morgan and Hawkins, 1953; Ershov, 1956; Lapage, 1956; Smith and Jones, 1957; Ono, 1958; Taylor, 1964). A similar syndrome has been described in this thesis (Section I) where large numbers of mature F. hepatica co-existed with variable but usually significant numbers of the abomasal parasite O. ostertagi. This fasciolíasis/ostertagiasis complex occurs frequently in the field and the diarrhoea so commonly referred to by many writers could be readily explained by the presence of the latter parasite; it is, therefore, possible that many of the field cases described as chronic fasciolíasis may result from the combined effects of both F. hepatica and O. ostertagi. Ross (1966 a & b) has also recently stated that diarrhoea is not seen in clinical

cases of fascioliasis in the bovine. These observations introduce an element of doubt as to whether F. hepatica alone is capable of producing clinical disease in cattle.

A series of 18 field cases of fascioliasis in cattle was reviewed by Balian (1940 a) in which adult cattle ranging from four to fourteen years of age were examined. These cases were divided into two groups based on post mortem findings, i.e. those animals recently infected and those with chronic infections. This author was mainly interested in investigating haematological and biochemical changes and as a result no reference is made to clinical signs although he noted that in almost half of these cases the animals were in poor bodily condition. Balian described a moderate to severe anaemia as a feature of the disease with marked reductions in both total red cell counts and haemoglobin levels. The degree of severity of the anaemia was a function of the number of adult flukes recovered at autopsy. Eichler (quoted by Balian, 1940 a) examined fifty field cases of bovine fascioliasis in animals of unspecified age and also found an anaemia with similar reductions in total erythrocyte and haemoglobin levels.

Although there is now an extensive literature on experimental infections with F. hepatica in small laboratory animals, namely, in rabbits (Montgomerie, 1931; Shaw, 1932; Urquhart, Mulligan and Jennings, 1954; Urquhart, 1954, 1955, 1956), in rats (Thorpe, 1963, 1965 a & b) and in mice (Lagrange and Gutmann, 1961; Dawes, 1961, 1962, 1963 a & b; Hughes, 1963), there are very few descriptions of experimental infections with this parasite in cattle. The first

report on experimental fascioliasis in the bovine was made by Morrill and Shaw (1942) who infected two 2½ year old cattle with 1,000 metacercariae of F. hepatica given orally. One of these animals received a further 40 metacercariae five months later and both animals were slaughtered nine months after the initial infection. These authors detected no clinical signs of fascioliasis but observed a mild anaemia and concluded that the detrimental effect of F. hepatica in the bovine must be small, despite the presence of 554 adult flukes in the liver of one animal at autopsy. However, Ershov (1956) states that a fluke burden of 250 will produce clinical disease in cattle. Sazanov (1961) infected eight calves with 400 to 13,000 metacercariae of F. hepatica but in this case only the effect on liveweight gain was studied, a decrease in weight gain being recorded as the infection rate was increased. None of the animals was slaughtered and no clinical pathology was recorded. Moroshkin, Kostina, Ivanskii and Sutyagin (1964) infected four month old calves and examined blood and bone marrow over a period of five months. They recorded a marked eosinophilia, a gradual fall in total erythrocyte counts and haemoglobin levels and the calves became weak and thin. Dixon (1964) used low level infections of 50 to 100 metacercariae in calves but was mainly interested in the ability of F. hepatica to become established in cattle and sheep and compared the infectivity of the parasite between these two hosts. The most recent reports on experimental infections in cattle are by Ross (1966 b) and Ross, Todd and Dow (1966) who infected two and a half to three month old calves with single oral doses of either 200, 300 or 1,300 metacercariae of F. hepatica and found that an anaemia developed only in those animals which received 1,300 metacercariae;

unfortunately they did not describe the clinical signs at this dose level. They suggested that the anaemia was proportional to the number of adult flukes in the bile ducts and concluded that 300 to 400 flukes are required to produce anaemia in the young calf. This is similar to the findings of Reid, Armour, Jennings, Kirkpatrick and Urquhart (1967) who, describing a condition in which there was co-existence of both O. ostertagi and F. hepatica, concluded that the severe anaemia seen in this condition was attributable to a burden of between 175 and 510 adult flukes.

The object of the present experiment was to attempt to establish an adult fluke burden in calves at a level which could readily be acquired by calves grazing under natural conditions. Two groups of calves were given single oral inoculations of 1,000 and 2,000 metacercariae of F. hepatica and the clinical, haematological, biochemical, parasitological and pathological findings were recorded.

Materials and Methods

Animals

Parasite-free calves were reared by a method identical to that described in general materials and methods.

Blood Analysis

Blood samples were collected for both haematological and biochemical estimation. The haematological values recorded were packed cell volume, haemoglobin concentration, total red and white cell counts and differential white cell counts; blood smears were stained and examined for reticulocytes and mean corpuscular volume (M.C.V.) and mean corpuscular haemoglobin concentration (M.C.H.C.) were calculated. The biochemical values recorded were total serum protein, serum albumin, alpha/beta globulin and gamma-globulin. All these values were obtained using techniques identical to those described in general materials and methods.

Parasitological Data

Faecal egg counts were performed by the zinc sulphate flotation method already described.

Weighing Procedure

The calves were weighed on Avery cattle scales.

Autopsy Procedure

All the animals, whether killed *in extremis* or at the termination of the experiment were slaughtered with a captive bolt pistol and the abdomen was

incised along the ventral midline. The liver was removed intact as rapidly after death as possible, photographed and its gross appearance recorded. The organ was then sliced into strips by the method already described and the flukes present were collected and counted.

Experimental Design

Animals

When two and a half months of age four calves were each given a single oral dose of 1,000 metacercariae of F. hepatica (Group 1) and four calves were each given a single oral dose of 2,000 metacercariae of F. hepatica (Group 2). The metacercariae were of ovine origin and produced by the method already described in detail. Both groups of calves, accompanied by the same group of three uninfected control animals, were put outside on permanent fluke-free grass in August where they were kept until late in the year, thus simulating field conditions as they occur in the west of Scotland. All the calves were castrated when three months of age. When grazing became scarce towards the end of October hay and cake was provided at the rate of 10 lbs. and 1 lb. per head per day respectively, given in two feeds. In mid-December when weather conditions deteriorated the calves were housed.

Observations

The calves were visually inspected daily and weekly detailed clinical examinations were carried out when samples for haematological, biochemical and parasitological estimations were collected. The calves were weighed weekly on

Avery cattle scales which were accurate to 1 lb. All weighings and collection of samples were undertaken in the morning, two to three hours after the morning feed had been given.

Results

Clinical Data

The main clinical features observed were loss of bodyweight and pallor of visible mucous membranes in the infected groups.

At the time of infection all three group mean weights were similar and weight gains increased at a comparable rate until 8 weeks post-infection when growth in the two infected groups was arrested whilst weight increase in the control group continued unchecked (Figure 8). The mean weights of the two infected groups remained stationary until 13 weeks post-infection when they began to fall; this feature is also illustrated in Figure 8. The mean weight increase in the three control calves continued. Details of the individual bodyweights of all three groups are given in Appendix 2, Tables 1, 2 and 3. All the calves in Group 1 died between week 15 and week 17 post-infection. Two of the calves in Group 2 died during week 12 and the remaining two animals in this group survived and by week 20 were showing a mean weight gain per week which was similar to the mean weight gain of the control animals. At about this time a sharp increase in weight gain was observed in all surviving animals due to housing.

Apart from changes in bodyweight the other main clinical finding in the infected groups was pallor of visible mucous membranes occurring earliest in

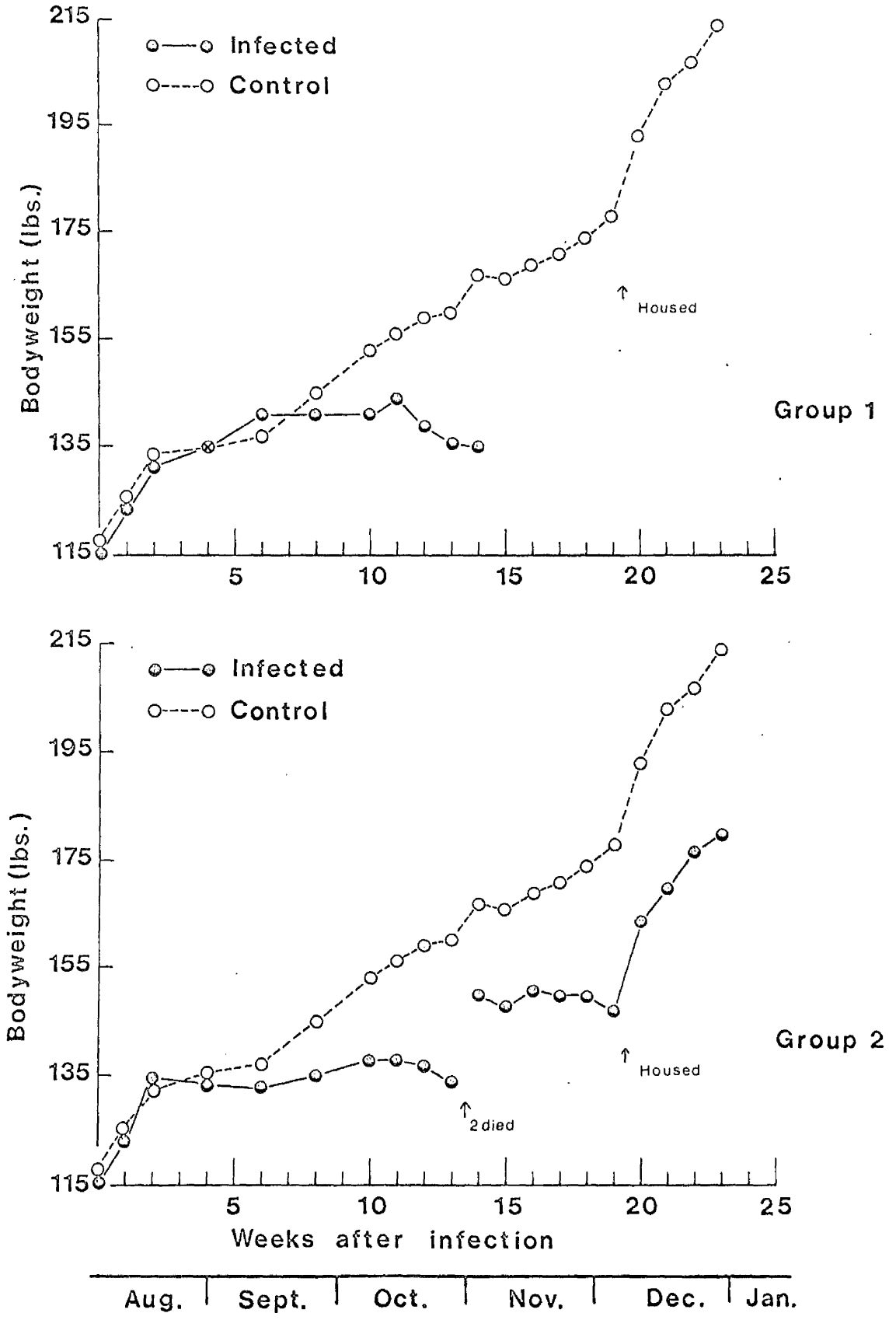

Fig. 8. The mean bodyweights of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

two calves in Group 2 at 10 and 12 weeks after infection respectively. Pallor of visible mucous membranes was also a feature in Group 1 but occurred later, between weeks 12 and 15. The appearance of pale mucous membranes in the calves was accompanied by a change of demeanour. Whereas up to this point infected calves had been bright and alert and interested in their surroundings those animals with pale mucous membranes became dull and lethargic.

At no stage during the course of the experiment was the liver palpable nor was there any evidence of pain in this region or clinically detectable ascites.

One other feature of major clinical significance was the complete lack of diarrhoea at any stage post-infection. The perineal region, tail and hocks were always clean and dry and never soiled by faeces.

During the latter part of October, 10 weeks after infection, occasional calves were observed to cough. These calves were scattered throughout all three groups and although the coughing was noticeable it was not accompanied by marked tachypnoea or other signs of respiratory embarrassment. An Dictyocaulus viviparus larvae were demonstrated in the faeces of one calf about this same time all the animals were treated with diethylcarbamazine acid citrate. This treatment resulted in rapid reduction in the frequency of the coughing.

Haematological Data

The animals in both infected groups developed a marked anaemia. On initial examination it appeared that the anaemia was approximately proportional to the number of adult flukes found at autopsy but when the correlation between terminal

haemoglobin levels and number of flukes recovered was measured the correlation coefficient was only -0.391, which for 7 degrees of freedom is not significant.

The mean total red cell count before infection for Group 1 and Group 2 calves was 8.15 ± 0.34 million per cu.mm. and 7.89 ± 0.37 million per cu.mm. respectively and this remained largely unaltered until about 10 weeks after infection when it began to fall. Once the fall in red cell count commenced it increased rapidly and by 15 to 17 weeks post-infection, when all the calves in Group 1 died, individual values ranged from 3.49 to 5.80 million per cu.mm. with a mean of 4.60 ± 0.48 million. A similar situation was recorded in 2 calves in Group 2 which died 12 and 13 weeks post-infection with red cell counts of 4.99 and 3.52 million respectively. The remaining 2 calves in this latter group did not die but their red cell counts fell to a minimum between 20 and 21 weeks post-infection after which time they gradually increased and by 24 weeks post-infection their mean total red cell count was similar to that of the controls. Changes in mean total red cell counts for both groups of calves are illustrated in Figure 9 and individual values for both infected groups and controls are given in Appendix 2, Tables 4, 5 and 6.

Alterations were also observed in the packed cell volume and haemoglobin concentration of both groups of infected animals and these were approximately proportional to the changes already described for total red cell count. These alterations are illustrated in Figures 10 and 11 and individual values for haematocrit and haemoglobin concentration are given in Appendix 2, Tables 7, 8, 9, 10, 11 and 12.

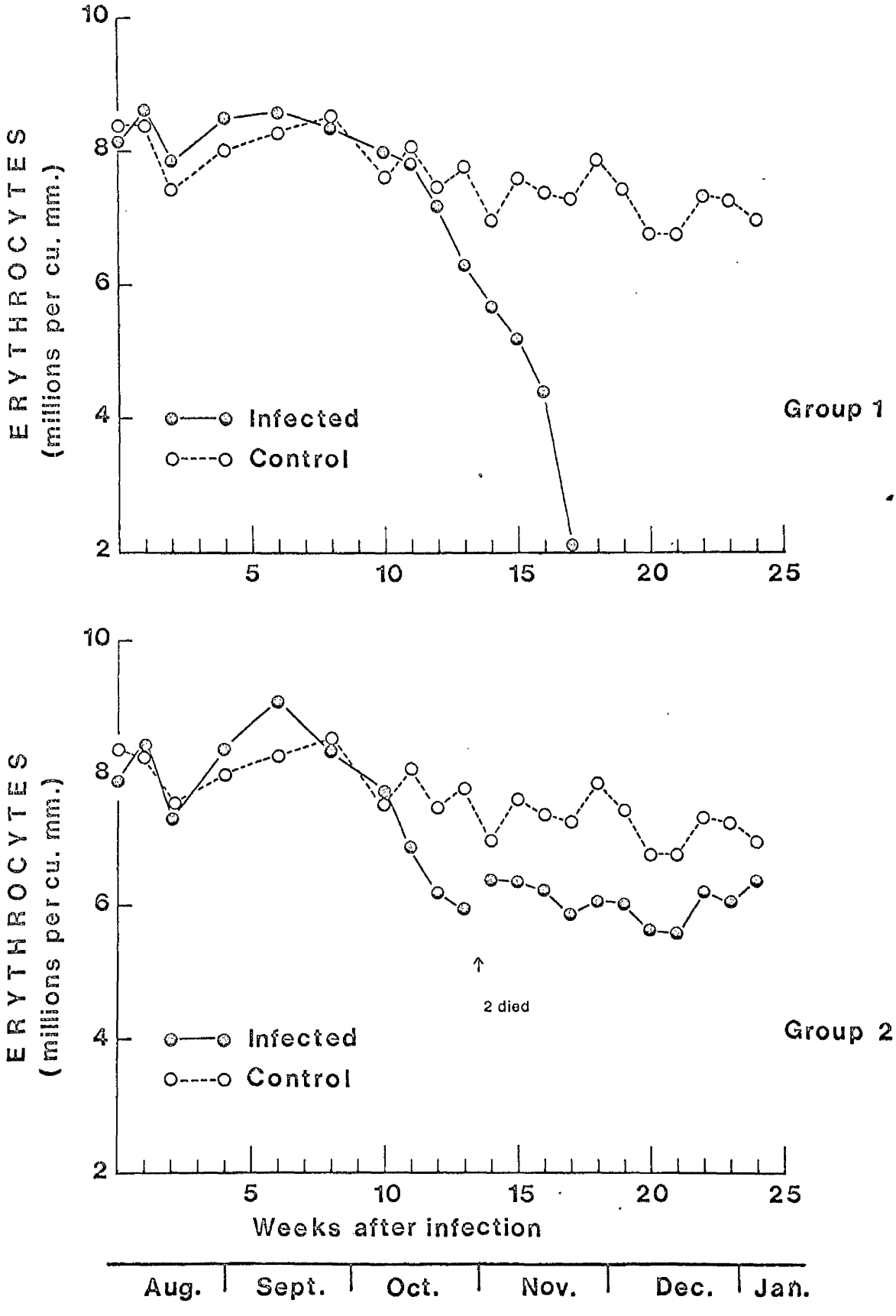

Fig. 9. The mean total red cell counts of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

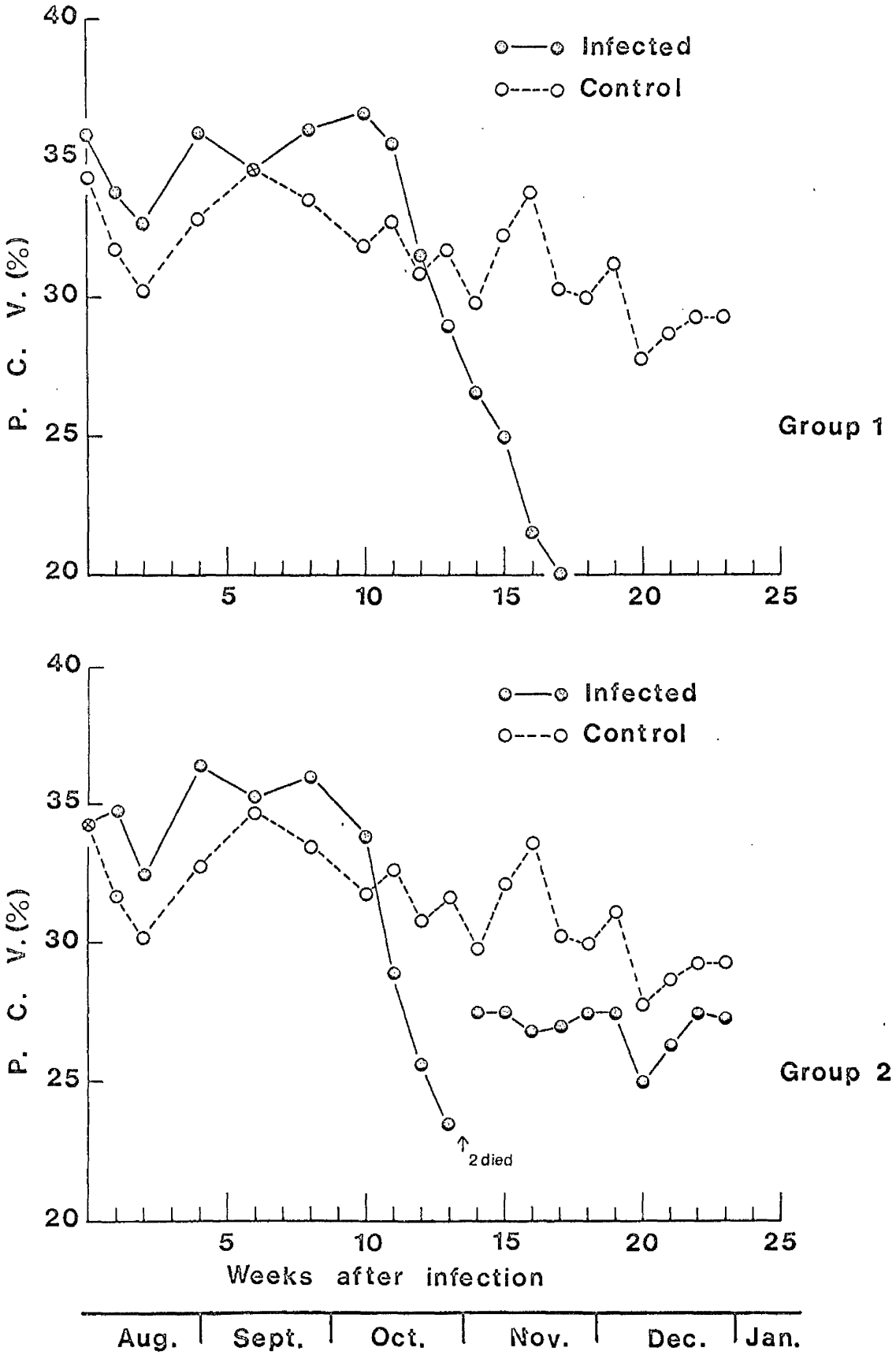

Fig. 10. The mean packed cell volume percentages of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

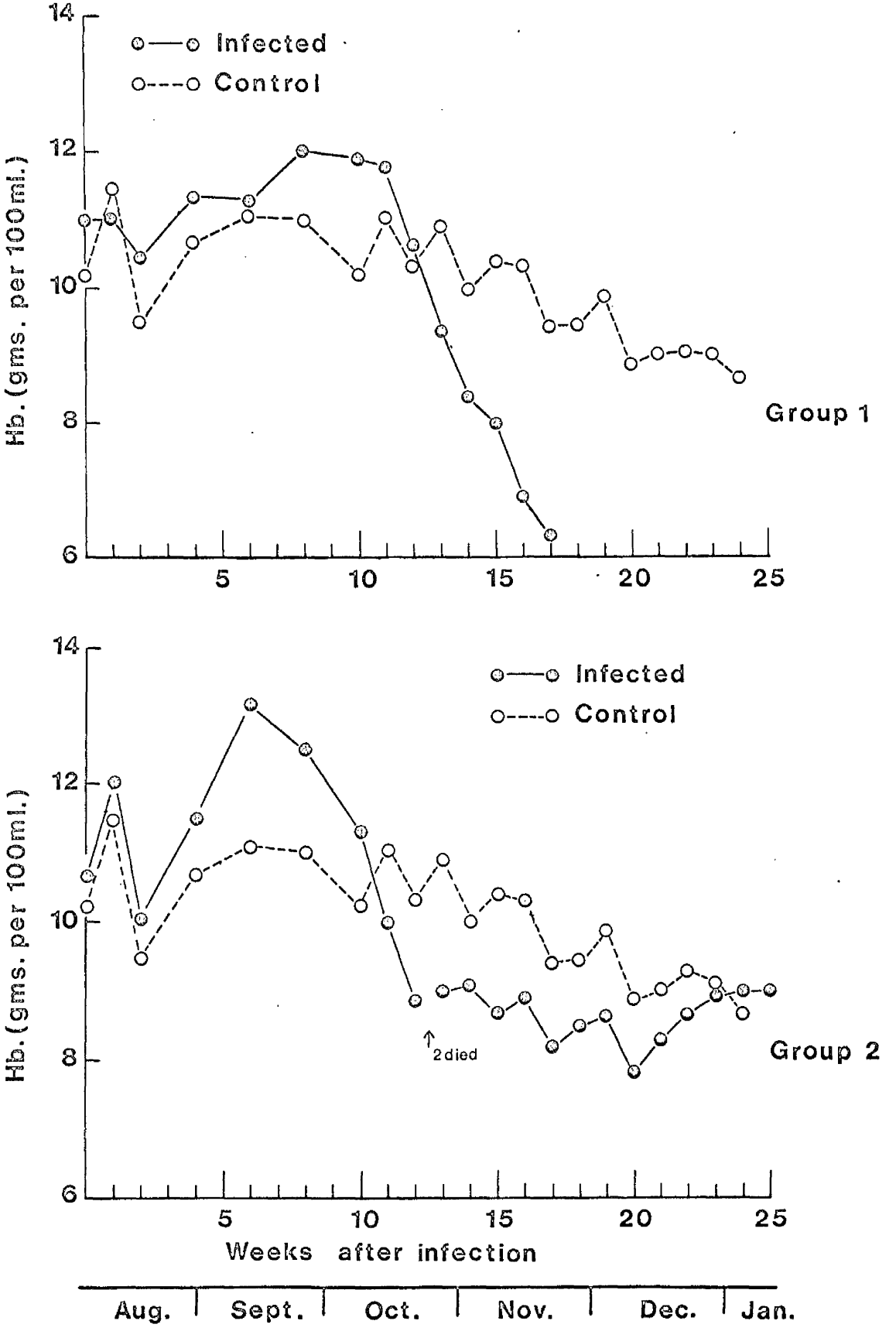

Fig. 11. The mean haemoglobin concentrations of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

The mean corpuscular volume (M.C.V.) prior to infection was 44 ± 1.75 cu.μ in the Group 1 calves and 44 ± 2.33 cu.μ in the calves of Group 2. This value remained unaltered until 10 weeks after infection when it began to rise in the Group 1 calves and at the time of death the mean value for this group was significantly higher than that of the controls. This feature is illustrated in Figure 12. The values for M.C.V. at the death of these calves from 46 to 50 cu.μ with a mean of 48.5 ± 1.05 cu.μ.

The mean change in M.C.V. for the Group 2 calves is also illustrated in Figure 12 but in this case variations from the control value were less dramatic although the M.C.V. of the two surviving calves remained consistently higher than that of the controls. The individual results for M.C.V. are given in Appendix 2, Tables 13, 14 and 15.

There were no significant alterations in M.C.H.C. and the mean values for all three groups were very similar. The only variation from this was observed 13 weeks post-infection in the Group 2 calves when the mean value rose to 41 per cent due entirely to one terminal sample from one of the calves. Individual values for M.C.H.C. are given in Appendix 2, Tables 16, 17, and 18.

Reticulocytes were not present in the systemic circulation at any stage post-infection.

There were no significant differences in total white cell count between the infected groups and the control animals although a neutrophilia developed, in the animals which died, a variable period before death. An eosinophilia was

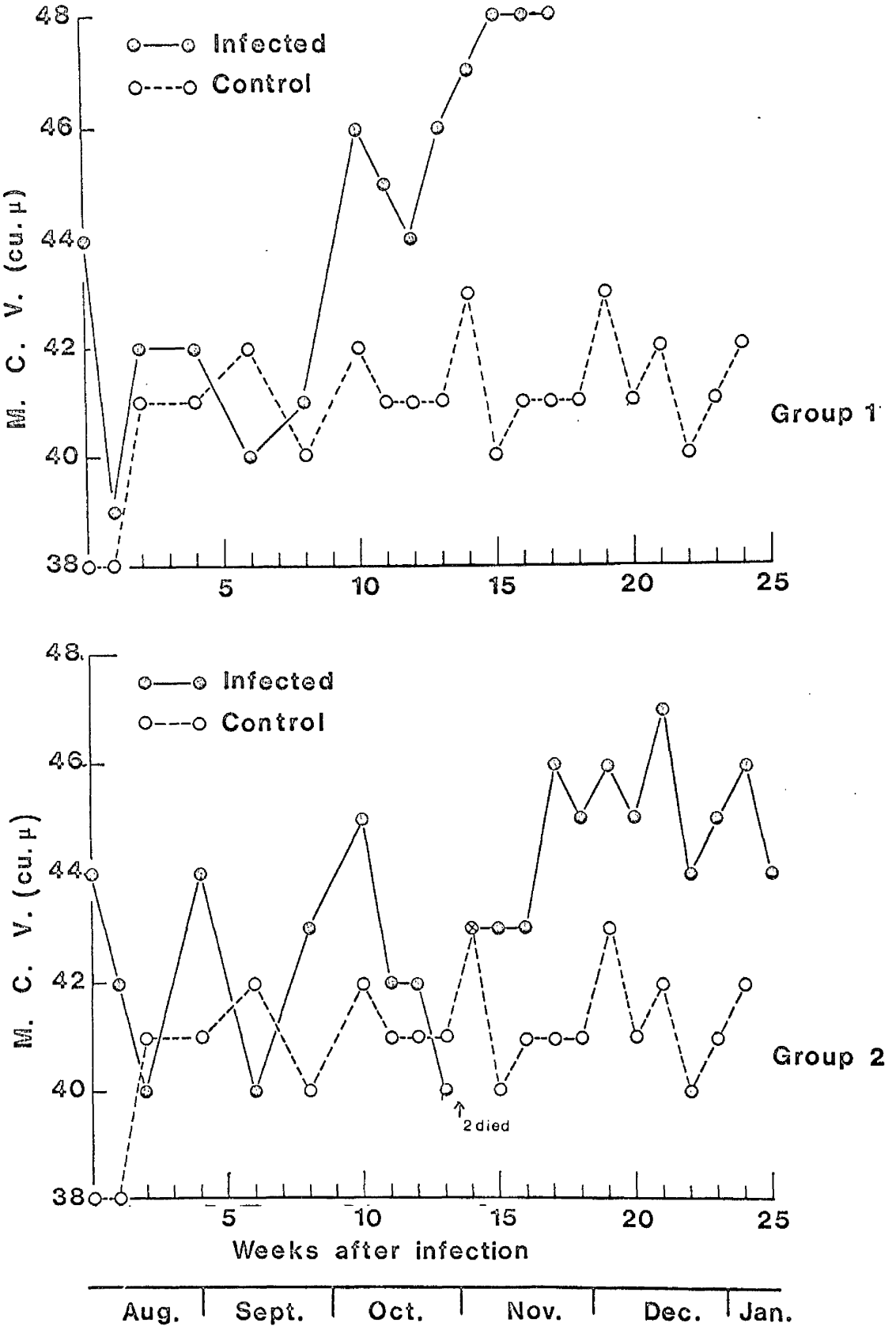

Fig. 12. The mean alterations in the mean corpuscular volumes of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

observed commencing about 6 weeks post-infection but this was present in all animals whether infected with F. hepatica or not and thus was probably primarily due to infection with Dictyocaulus viviparus. Individual values for total white cell count and differential leucocyte counts are given in Appendix 2, Tables 19 to 30 inclusive.

Biochemical Data

The mean level of total protein in both infected groups increased up till 10 weeks post-infection but this was followed by a gradual decrease although very low levels were not observed even at the time of death. A similar increase in mean total protein level was recorded in the control groups. The alterations in mean total protein levels are illustrated in Figure 13, and individual values are given in Appendix 2, Tables 31, 32 and 33.

Alterations in the mean total globulin levels for each group followed a similar pattern to the changes in total protein. The changes resulted in an initial increase in the alpha/beta and gamma fractions followed by a progressive decrease in both infected groups although the higher level was maintained in the control group. Individual values for alpha/beta and gamma-globulin are given in Appendix 2, Tables 34 to 39 inclusive.

Serum albumin levels commenced to fall 6 weeks post-infection in the Group 1 calves and 2 weeks later in the Group 2 calves. The lowest levels of serum albumin were observed in those two animals in Group 2 which died between

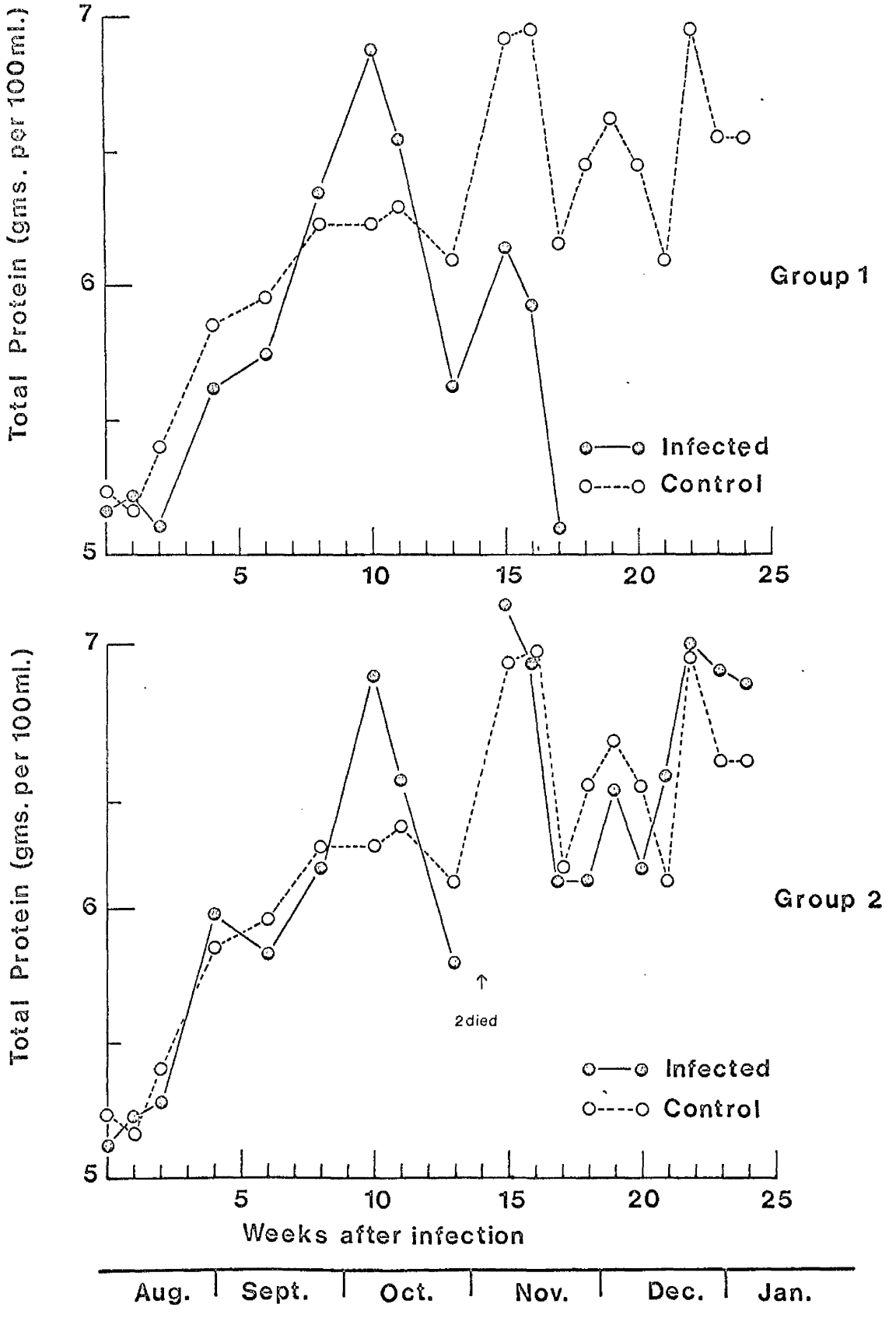

Fig. 13. The mean total serum protein levels of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

weeks 11 and 13 post-infection when the values were 1.10 and 1.15 gms. per 100 ml. but albumin levels in the remaining two calves in this group were depressed for only a short period. In the case of the Group 1 calves serum albumin levels were also reduced and at death ranged from 1.38 to 1.64 gms. per 100 ml. with a mean value of 1.50 ± 0.07 gms. per 100 ml. The alterations in the mean level of serum albumin for each infected group compared to the controls are illustrated in Figure 14 and individual values for each infected group are given in Appendix 2, Tables 40, 41 and 42.

The changes in serum albumin and serum globulin are reflected in the alterations in the albumin:globulin ratio and the ratio decreased in both infected groups; from 6 weeks post-infection in Group 1 calves and from 8 weeks post-infection in the Group 2 calves. The alterations in the mean albumin: globulin ratios are illustrated in Figure 15 and individual values are given in Appendix 2, Tables 43, 44 and 45.

Parasitological Observations

Fluke eggs were first observed in the faeces at week 10 post-infection in one calf out of each infected group. After this time the faeces of all infected calves showed positive egg counts and on only one occasion was this faecal egg count in excess of 250 eggs per gram. Fluke eggs, however, were not constantly present in the faeces. The fluctuations in mean faecal egg count for each infected group are illustrated in Figure 16 and individual details are given in Appendix 2, Table 46.

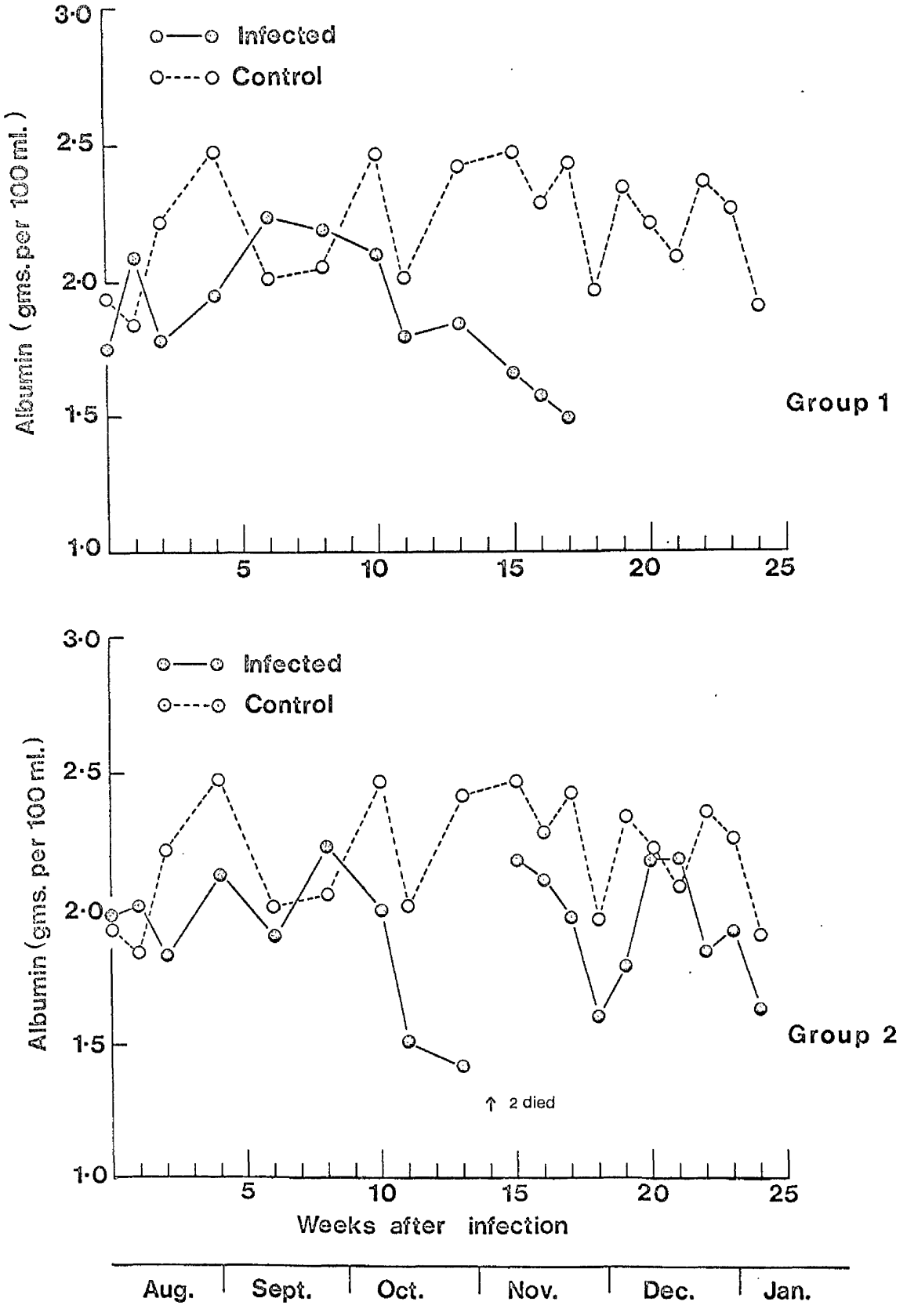

Fig. 14. The mean serum albumin levels of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica.

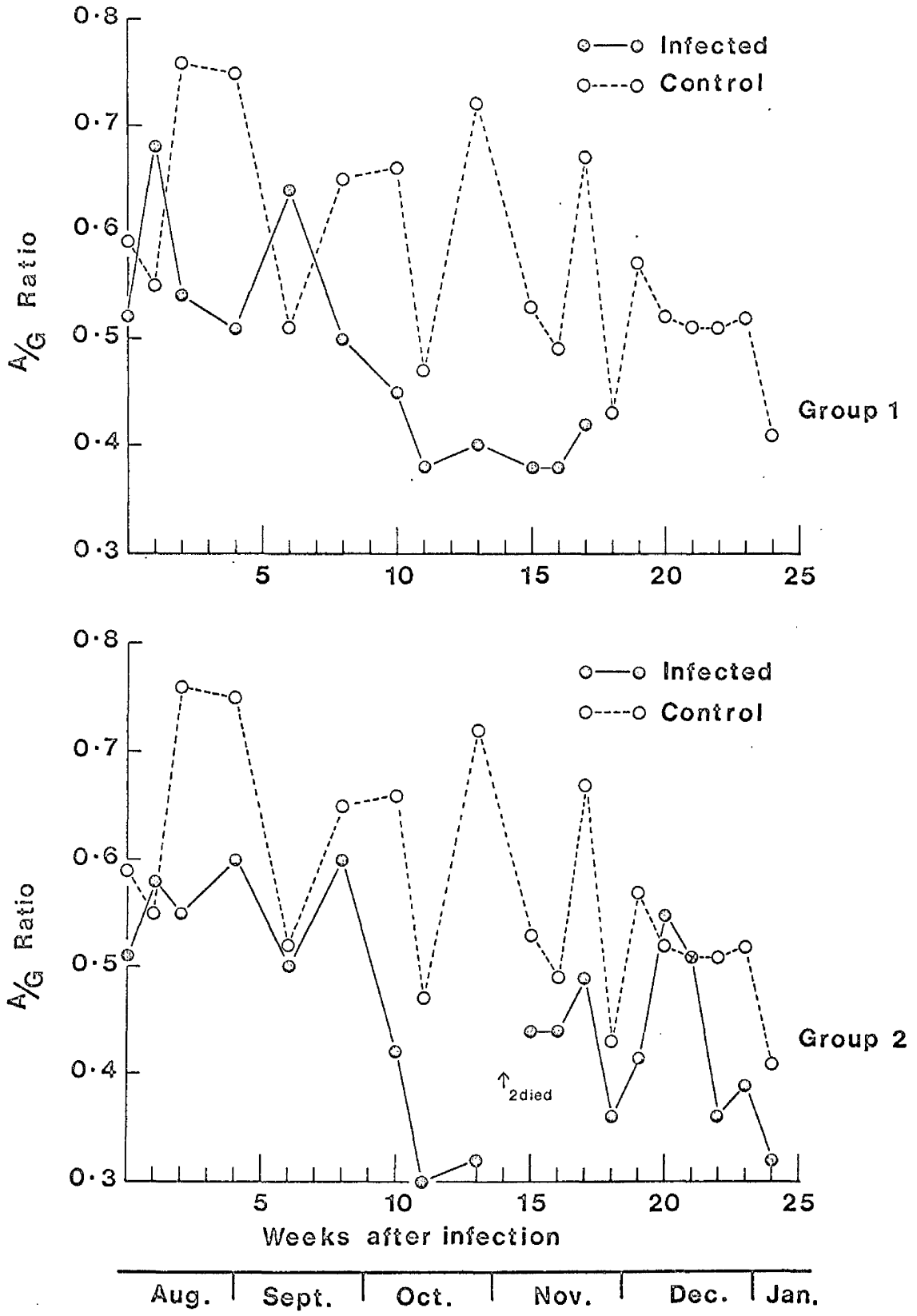

Fig. 15 The mean albumin:globulin ratios of calves following a single oral inoculation of 1,000 (Group 1) and 2,000 (Group 2) metacercariae of F. hepatica

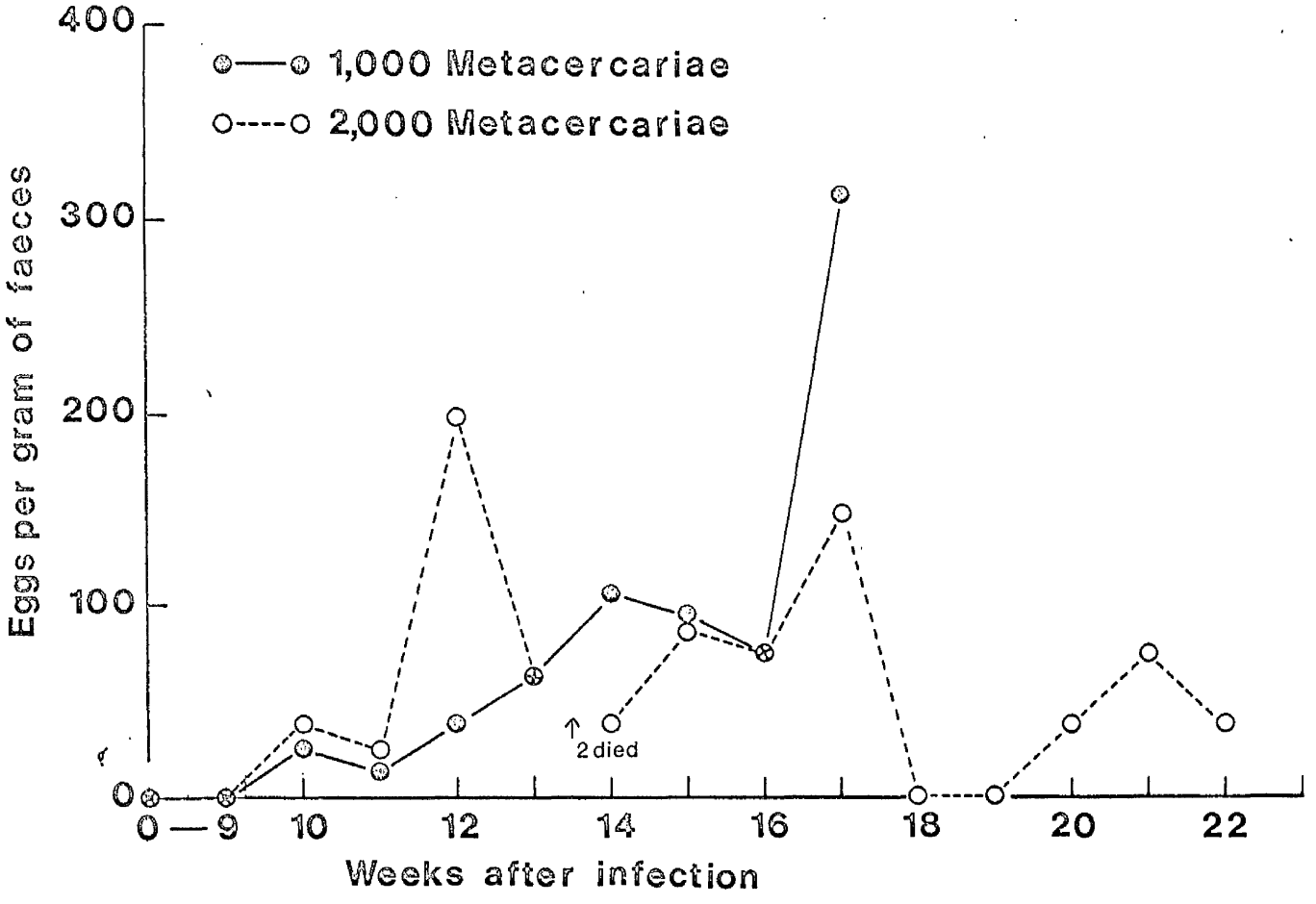

Fig. 16. The mean fluke faecal egg counts of calves following a single oral inoculation of 1,000 and 2,000 metacercariae of F. hepatica.

The numbers of F. hepatica recovered from the livers of the calves in Group 1 at autopsy expressed as a percentage of the number of metacercariae administered (percentage take), the number of flukes recovered from each liver, their mean length and breadth and duration of infection are shown in Table 14.

In Group 1 where the infective dose was 1,000 metacercariae between 164 and 358 F. hepatica were recovered at post mortem i.e. a percentage take of 16 - 36% with a mean of 26.8%. At the time of autopsy all the parasites were in the bile ducts and although all four calves died between weeks 15 and 17 post-infection there was some variation in fluke size which bore no relation to the duration of infection. The mean lengths of the flukes ranged from 14.40 mm. to 20.19 mm. with a mean of 17.36 mm.

The picture in Group 2 where only two of the calves died was not so uniform and here the number of F. hepatica recovered at autopsy of those two calves was 353 and 654, i.e. a percentage take of 18% and 33% with a mean of 25.5%. This percentage take is very similar to that obtained in the calves in Group 1. However, the position regards the other two calves is different because they did not die and were slaughtered 25 weeks post-infection when they were, clinically, virtually normal. In these two animals the number of F. hepatica recovered was 15 and 93, i.e. a percentage take of 1% and 5% respectively. The percentage take in Group 2, the number of flukes recovered, their mean length and breadth and duration of infection are shown in Table 15.

Pathological Data

The livers of all calves infected with Fasciola hepatica were similar.

Table 14

The Numbers of Fasciola hepatica Recovered at Post Mortem from Calves given Single Oral Inoculations of 1,000 Metacercariae

Calf No.	Number of F. hepatica	Mean Length and Standard Error	Mean Breadth and Standard Error	Percentage Take	Duration of Infection (weeks)
1	260	20.19 mm. ± 0.11	10.77 mm. ± 0.48	26	17
2	164	18.53 mm. ± 0.063	8.68 mm. ± 0.03	16.4	15
3	288	16.30 mm. ± 0.098	9.10 mm. ± 0.054	28.8	17
4	358	14.40 mm. ± 0.057	-	36.6	16

Table 15

The Numbers of Fasciola hepatica Recovered at Post Mortem from Calves given Single Oral Inoculations of 2,000 Metacercariae

Calf No.	Number of F. hepatica	Mean Length and Standard Error	Percentage Take	Duration of Infection (weeks)
1	353	17.30 mm. ± 0.034	17.7	13
2	93	> 20.00 mm. ± -	4.7	25
3	654	14.60 mm. ± 0.068	32.7	12
4	15	> 20.00 mm. -	0.75	25

There was marked bile duct thickening particularly in the case of the major bile ducts. On sectioning these ducts with a knife the tissue was very firm and brittle and the mucosa of the duct was studded with small, dark brown, lentil-shaped, raised areas. The most severe thickening and calcification of the bile duct wall was observed in the two animals which survived the higher level of infection. The lumen of the duct contained a very viscid, predominantly dark green material. Most of the area of liver parenchyma appeared normal but that area known as the ventral lobe was very firm to touch and paler in colour than the surrounding tissue. On incision it was tougher to cut than the adjacent liver parenchyma.

Discussion

This experiment demonstrates that infection with the liver fluke, Fasciola hepatica, is pathogenic for the young bovine and infective doses of 1,000 and 2,000 metacercariae are capable of producing marked clinical signs and even death. The severity of clinical signs and the rate at which they develop does not appear to be a function of the infective dose but is proportional to the number of parasites becoming established in the liver. There were only two constant clinical signs observed, weight loss and pallor of visible mucous membranes. The fall in body weight was preceded by a period of reduced weight gain. Sazanov (1961) however, using doses of 400, 1,500 and 5,500 metacercariae recorded only reductions in weight gain compared to the control animals of 92.5%, 78.3% and 76.5% respectively over a 17 week period. This author

did not report any frank loss of weight. At no time was diarrhoea observed in any of the infected animals and this is in disagreement with most reports on this subject (Morgan and Hawkins, 1953; Dunsby, 1955; Ershov, 1956; Lapage, 1956; Smith and Jones, 1957; Ono, 1958; Taylor, 1964) but supports the view of Ross (1966 b). In the present experiment neither submandibular oedema or clinically detectable ascites was present, nor was jaundice observed. Although clinical signs were recorded in infections with 1,000 metacercariae in two and a half month old calves it is interesting that a similar infective dose, resulting in the establishment of 554 adult flukes, failed to produce marked clinical signs in two and a half year old cattle (Morrill and Shaw, 1942). Adult cattle may harbour several hundred flukes without this being suspected and so may act as a source of infection for other stock.

An anaemia was initially recorded in the calves between the 11th and 14th weeks of the infection and this is in agreement with other reports on experimental fascioliasis in cattle. Morrill and Shaw (1942) suggested that the anaemia appeared in the 4th week post-infection but examination of their results indicates that fluctuations in the erythrocyte counts were similar both in the controls and the infected animals at this time and the presence of an anaemia is only conclusive about 13 weeks post-infection. Moroshkin _et al._, (1964) observed that the anaemia was first apparent 2 - 3 months after infection. While the work of this thesis was in progress Ross, Todd and Dow (1967) reported that the anaemia produced by infection with 1,300 metacercariae of _F. hepatica_ occurred about 12 weeks after infection. From the results described in this

thesis and the report of Ross, Todd and Dow (1967) it would appear that the anaemia is associated with the adult bile duct stages of the parasite because it is about 10 to 12 weeks post-infection that faecal egg counts first become positive. These findings are in agreement with the results in other species. In the rabbit, Urquhart (1955) observed that flukes first appeared in the bile ducts 5 weeks after infection, patency being established 6 - 8 weeks post-infection, whilst the anaemia developed about the 6 weeks stage. Thorpe (1963) records a similar situation in the rat 6 weeks post-infection. Sinclair (1962, 1964) also observed that the appearance of the anaemia was closely linked with patency as he describes the anaemia being first apparent 9 weeks after infection whilst eggs were present in the faeces one week later.

In the present experiment the anaemia was normochromic and essentially macrocytic particularly in the case of those animals infected with 1,000 metacercariae where the mean values for M.C.V. were significantly higher than the controls whilst the situation was more variable in those calves infected with 2,000 metacercariae. A macrocytic, normochromic anaemia was also found by Ross (1966 a) and Ross, Todd and Dow (1966) in calves infected with 1,300 metacercariae. Although Morrill and Shaw (1942) recorded an anaemia it is not possible to determine its character as they omitted haematocrit estimations. Sinclair (1962, 1964) in his experimental infections in sheep also described the anaemia as normocytic although examination of his data on two sheep which died of fascioliasis indicates that in one case a macrocytosis was present. Urquhart (1955) in his experimental infection in the rabbit states that the

anaemia is characterised by oligocytosis, macrocytosis, hypochromia and reticulocytosis. There would appear to be a striking difference in the character of the anaemia between the rabbit and the bovine. This can be explained by the findings of Brenner (1966) and Schnappauf, Stein, Sipe and Cronkite (1967) who demonstrated that a reticulocytosis only appears in the calf when 30 to 40 per cent of the animal's total blood volume is removed within 12 hours and does not appear when only 5 to 10 per cent of the initial total blood volume is removed daily for 10 days. As a result of these observations a reticulocytosis cannot develop in an anaemia of a chronic nature in the bovine and due to the absence of immature red cells in the circulation a hypochromic anaemia is unlikely to occur. Ross (1967 a & b) has described field cases in sheep where erythroblasts were present in the circulation and the anaemia was macrocytic and normochromic and this situation will be discussed in more detail later (Section IV).

The anaemia was accompanied by a hypoalbuminaemia in both infected groups with a consequent reduction in the albumin/globulin ratio. This finding is in agreement with other authors reporting biochemical changes in cattle infected with *T. hemitica* (Nikolic *et al.*, 1962; Hankiewicz, 1965; Ross, 1966 a; Ross, Todd and Dow, 1967) and is also similar to reports on other species, for example, in sheep (Ebrovic and Gall-Palla, 1959; Nikolic *et al.*, 1962; Sinclair, 1962; Ross, 1967 a & b; Ross, Dow and Todd, 1967), in rabbits (Secreton and Bickel, 1960; Dargie *et al.*, 1967, 1968) and in rats (Thorpe, 1963). The remaining haematological and biochemical findings are more difficult to interpret due to

the presence of Dictyocaulus viviparus in all the experimental animals. Thus the eosinophilia and hypergammaglobulinaemia which were recorded may result from infection with this latter parasite or perhaps the dual infection with this parasite and F. hepatica. The presence of D. viviparus was considered to be of only minor significance and because it was detected at an early stage it contributed little to the clinical syndrome. The appearance of this second species of parasite does serve to remind us that more than a single species of parasite is usually detected in the animal grazing under natural conditions.

The preferential migration of the young fluke to the ventral or left lobe of the liver is a constant feature in the bovine and has been recorded in a number of publications (Balian, 1940 a; Morrill and Shaw, 1942; Supperer, 1964; Ross, 1966; Ross, Todd and Dow, 1966). Morrill and Shaw (1942) and Supperer, (1964) suggest that the ventral lobe of the liver was in close proximity to the duodenum and hence is the first point of contact by young flukes migrating through the duodenal wall. Ross (1966 a) offers the hypothesis that pressure changes within the anterior part of the abdominal cavity cause the flukes to be drawn to the ventral area of the liver. This author also suggests that there may be some difference in the blood supply between dorsal and ventral areas of the liver; although this is certainly true (Simon, 1958) it is strange that the young fluke, a tissue-feeder, should find it necessary to invade the organ at its most vascular area. It would appear that the close proximity of duodenum and ventral lobe of liver provide the best reason for a simple migration between these two areas.

There was a marked difference between the calves infected with 1,000 metacercariae and those infected with 2,000 metacercariae. All the animals in the lower level infection died between weeks 15 and 17 post-infection whilst in the higher level infection although two calves died before this time the remaining two survived and only showed slight clinical signs and a mild to moderate anaemia. At autopsy 25 weeks after infection these latter animals had percentage takes of only 1 per cent and 5 per cent. The bile ducts were markedly thickened with areas of calcification. A similar situation is described by Morrill and Shaw (1942) in older animals where they recorded percentage takes of 55 per cent and 5 per cent 37 weeks after administration of 1,000 cercariae, the animal with the low percentage take exhibiting more marked bile duct calcification. This situation is similar to the condition in man where an acquired self-cure is thought to occur and this is believed to result from calcification (Facey & Marsden, 1960; Taylor, 1961). Ross (1966a) also refers to an acquired self-cure in calves.

The cause of death was obscure in several of the calves, particularly those which did not develop a severe anaemia. All the animals which did die, however, developed a neutrophilia a variable period before death and this may indicate that secondary infection is a contributory factor.

The results of this experiment demonstrate that single infections of F. hepatica, particularly at the level of 1,000 metacercariae, will produce an adult fluke burden in the young bovine of between 164 and 358 flukes. This number is capable of producing weight loss, anaemia and death in calves five to six months of age and is also similar to the range of adult F. hepatica

recovered from field cases of the fascioliasis/ostertagiosis complex described by Reid et al. (1967) and would suggest that the fluke component of this complex was playing a major part in the production of that syndrome.

Summary

Infection of two and a half month old male Ayrshire calves with a single oral inoculation of 1,000 and 2,000 metacercariae of *F. hepatica* results in the establishment of an adult fluke burden capable of producing clinically recognisable signs. All the infected animals developed clinical signs but whilst every animal given a single dose of 1,000 metacercariae died only 2 animals given 2,000 metacercariae died whilst the others survived.

The major clinical signs observed were loss of bodyweight and pallor of visible mucous membranes. Diarrhoea was never present and submandibular oedema and ascites were not recorded.

The main haematological finding was the presence of a moderate to severe anaemia of the macrocytic, normochromic type and this was not accompanied by a reticulocytosis. Although an eosinophilia was observed it was not possible to determine whether this resulted from infection with *F. hepatica* as a concurrent infection with *Dictyocaulus viviparus* was present.

Alterations in the serum protein fractions occurred and took the form of an increase in the globulins, particularly gamma-globulin, and a decrease in albumin levels. Although the hypoalbuminaemia occurred only in those animals infected with *F. hepatica*, the hypergammaglobulinaemia occurred in both infected animals and controls and would appear to result from infection with *D. viviparus*.

At autopsy the percentage take was between 16 per cent and 37 per cent in all but two animals which were killed at the termination of the experiment.

These latter calves had a percentage take of only 0.75 per cent and 4.7 per cent and examination of their liver revealed thickening of the bile ducts which was very much more severe than was the case in any of the other animals.

SECTION III

EXPERIMENTAL FASCIOLA HEPATICA INFECTIONS IN SHEEP

The Results of a Single Infection

Introduction

The clinical syndrome produced by infection of sheep with Fasciola hepatica is generally observed to occur in two forms, acute and chronic, although a third subacute form is sometimes described. Acute fascioliasis results from ingestion of large numbers of metacercariae and death frequently takes place suddenly as a result of the migrations of the immature flukes in the liver parenchyma whilst chronic fascioliasis which is associated with the adult bile duct stages of the parasite results in the affected animal becoming thin, extremely weak, anaemic and death is a common sequel (Cluniss Ross and Gordon, 1936; Morgan and Hawkins, 1953; Lapage, 1956; Hönnig, 1956; Oxenham and Jennings, 1962; Taylor, 1964; Soulsby, 1965). Diarrhoea has also been recorded in animals suffering from chronic infections (Morgan and Hawkins, 1953; Smith and Jones, 1957; Taylor, 1964) whilst occasional jaundice has been described resulting from obstruction of the major bile ducts (Morgan and Hawkins, 1953; Smith and Jones, 1957; Jubb and Kennedy, 1963).

Apart from descriptions of clinical signs and gross pathology of ovine fascioliasis recorded in a large number of veterinary textbooks there is a surprising lack of detailed information on naturally occurring infections. There does not appear to be any recent data on field cases although Fallon (1940 b) has described the haematological and biochemical changes in a few naturally occurring cases; unfortunately many of these animals had dual infections with F. hepatica and Dicrocoelium dendriticum.

Despite the widespread occurrence of fascioliasis in sheep in many parts

of the world there are few publications on experimental infections in this species. Taylor (1949) made an attempt to reproduce acute fascioliasis in sheep by administering up to as many as 10,000 cercariae both in repeated doses and as a single dose but was unable to produce the characteristic enlarged and friable liver. Sinclair (1962) has described the clinical pathology of chronic fascioliasis in sheep given oral infections of 600 metacercariae administered either as a single dose or in four doses of 150 metacercariae given at weekly intervals. He records the clinical signs which appeared and followed the changes in haematology which led him to conclude that the anaemia which developed was normochromic and normocytic. The biochemical changes which took place were a fall in serum albumin with a progressive rise in the globulins. The total serum protein levels initially rose and then fell. Sinclair (1964) subsequently carried out a modified version of his previous experiment using four groups of lambs, three groups receiving 600 metacercariae given either as a single dose or in several doses whilst the fourth group was subjected to a daily removal of 60 ml. of blood. He confirmed his previous findings that the anaemia in sheep resulting from fascioliasis is of the normocytic, normochromic type and demonstrated that the anaemia produced by daily bleeding was of a similar type but not so severe. Dixon (1964) infected sheep with 50 to 100 metacercariae of F. hepatica using metacercariae of both ovine and bovine origin but was mainly interested in the infectivity in sheep compared to cattle and recorded only the prepatent period, faecal egg counts and the number of parasites recovered at autopsy in each species. Boray, Happich and Andrews (1965), Boray and

Happich (1966) and Boray, Happich and Andrews (1967) also used single oral infections of F. hepatica in sheep in their studies on the efficiency of the anthelmintic, Hilomid. These authors recorded faecal egg counts and were able to demonstrate the percentage of the initial infection which became established in the sheep by virtue of the numbers of parasites recovered at autopsy of their uninfected control animals.

The following experiment records the clinical, haematological, biochemical and parasitological findings in lambs given single oral inoculations of 1,000 metacercariae of F. hepatica.

Materials and Methods

Animals

Parasite-free lambs of the Scottish Blackface breed were reared and maintained by a method identical to that described in general materials and methods.

Blood Analysis

Blood samples were collected for both haematological and biochemical estimation. The haematological values recorded were packed cell volume, haemoglobin concentration, total red and white cell counts and differential white cell counts; blood smears were stained and examined for reticulocytes, and mean corpuscular volume and mean corpuscular haemoglobin concentration were calculated. The biochemical values recorded were total serum protein, serum albumin, alpha/beta globulin, gamma-globulin, serum glutamic oxaloacetic transaminase (S.G.O.T.), serum glutamic pyruvic transaminase (S.G.P.T.), serum alkaline phosphatase and serum bilirubin. The serum enzymes and bilirubin levels were only recorded during the initial 17 weeks of the experiment. All these values were obtained using techniques identical to those already described in general materials and methods.

Parasitological Data

Faecal egg counts were performed by the zinc sulphate flotation method already described.

Autopsy Procedure

At post mortem the liver was examined in the manner described in general

materials and methods. The flukes recovered were measured with both length and greatest breadth, in millimetres, being recorded.

Experimental Design

Animals

When six months of age eight lambs were given a single oral inoculation of 1,000 metacercariae of F. hepatica. The metacercariae were of ovine origin and produced by the method described in general materials and methods. After infection the lambs were housed on concrete floors.

Observations

The animals were examined once weekly at which time blood samples were collected for haematological and biochemical estimations and faecal samples were also obtained. The initial blood samples were collected prior to the lambs receiving the infective dose.

Results

All the lambs developed clinical fascioliasis and died or were slaughtered in extremis. The animals died at various intervals, the earliest death occurring 12 weeks after infection and the final death taking place 23 weeks post-infection. In the later stages of the disease the lambs became dull, anorexic and visible mucous membranes were pale.

Haematological Data

A degree of anaemia developed in each of the infected lambs. This

commenced during the 5th week after infection although it was not well established until 6 weeks after infection, a time when flukes were arriving in the bile ducts. The degree of anaemia was approximately proportional to the number of flukes recovered and Figure 17 demonstrates the correlation between the terminal haemoglobin levels and the number of flukes present. The statistical significance of this relationship was $p < 0.02$. The haematology of each lamb is presented graphically and is so arranged that those animals dying at similar intervals after infection can be compared.

The mean total red cell count of the eight lambs prior to infection was 11.98 ± 0.22 million per cubic millimetre and this remained fairly constant until about 4 weeks after infection when it began to fall rapidly, due mainly to one animal, Y 87, which was carrying the largest fluke burden. The pattern of this reduction in red cell count is illustrated in Figure 16; the only exception was lamb number Y 84 in which the drop did not commence until about 6 weeks post-infection even although this animal was carrying one of the larger fluke burdens. This sharp drop in the red cell count was interrupted about 10 weeks after infection and thereafter the fall was less steep although still very marked. The data from the lambs did not show any evidence that this fall in total red cell count might be arrested completely and the red cells be maintained at a low level. All the animals died and at death their total red cell counts were below 5 million in each case. The lowest value recorded was 1.82 million which was observed in Y 84, the animal which exhibited a delayed drop initially but died 18 weeks after infection.

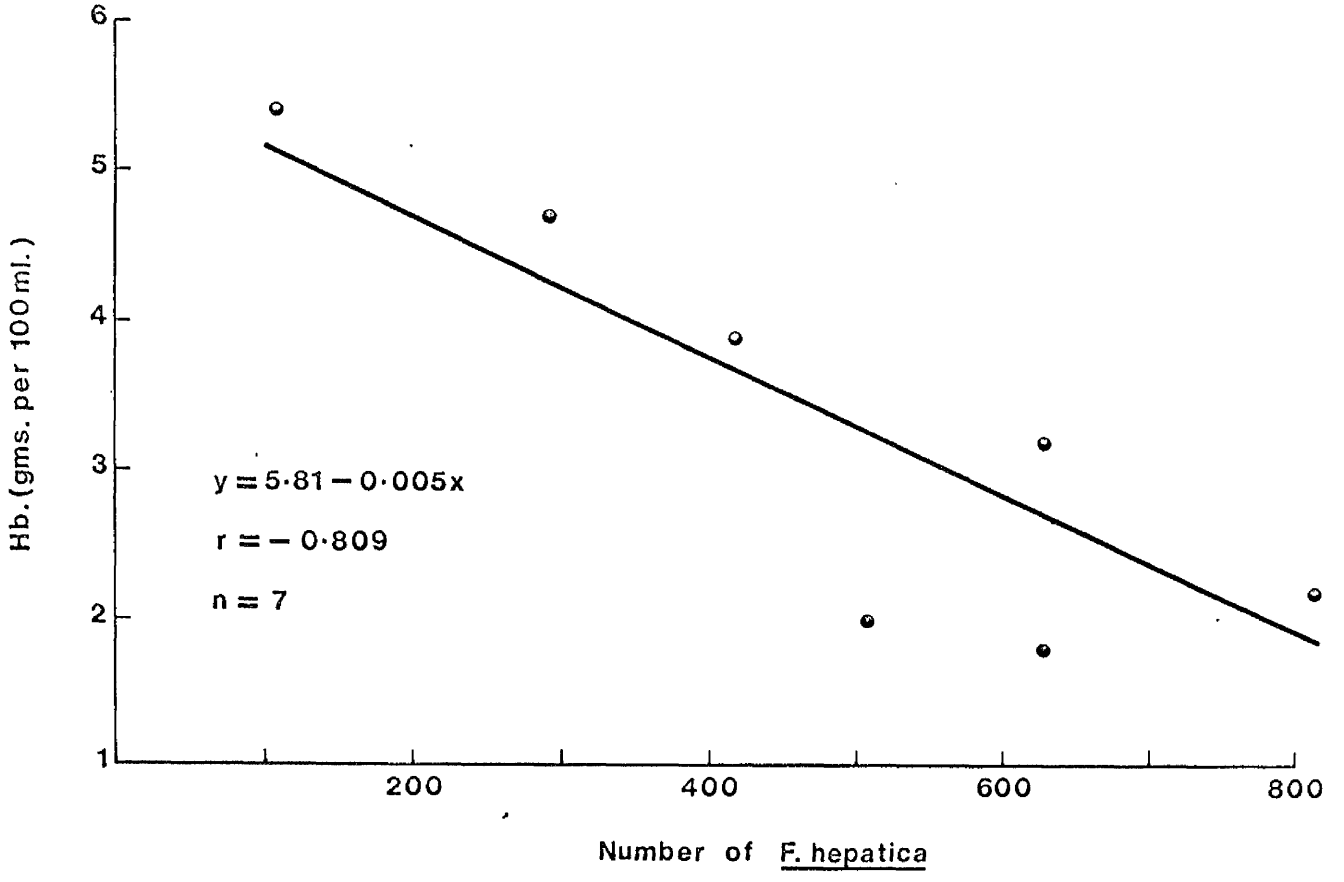

Fig. 17. The relationship between terminal haemoglobin levels and the number of F. hepatica recovered at autopsy of lambs given a single oral inoculation of 1,000 metacercariae. ($p<0.02>0.01$).

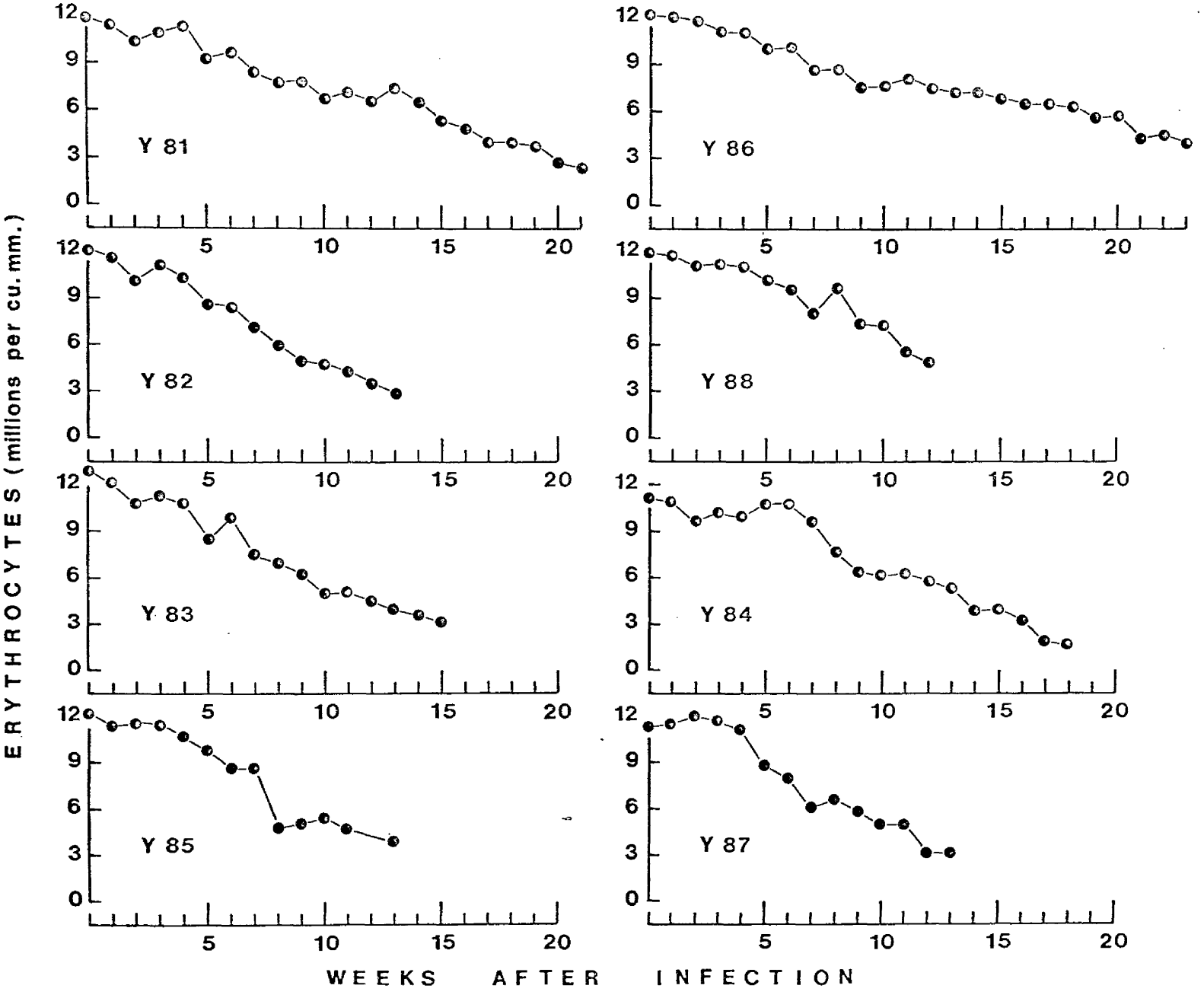

Fig. 18 Total red cell counts of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

Before infection the mean value for haemoglobin concentration and packed cell volume was 12.3 ± 0.32 gms. per 100 ml. and 36.3 ± 0.54 per cent respectively. Reductions in both these levels occurred after infection, the pattern being essentially similar to that of the red cell count and this is illustrated for each animal in Figures 19 and 20. At death all the animals had haemoglobin levels of 5.4 gm. per 100 ml. or less, the lowest figure being 1.8 gms. per 100 ml. The packed cell volume fell until it was 16.5 per cent or less, the lowest figure being 8.0 per cent.

The mean values for total red cell count, packed cell volume and haemoglobin concentration are shown in Table 16, whilst individual values are given in Appendix 3, Tables 1, 2 and 3.

The mean corpuscular volume (M.C.V.) of the lambs before infection was 30.4 ± 0.37 c.μ and this increased as the anaemia developed, the increase being first apparent about 11 to 12 weeks after infection. Figure 21 demonstrates that the increase in M.C.V. was most marked in those animals which survived the greatest length of time, although it was not the animal which survived longest which had the highest value of 53 cu.μ. In two of the earliest deaths, Y 87 and Y 88 there was no significant rise in the M.C.V.

Before infection the mean corpuscular haemoglobin concentration (M.C.H.C.) of the lambs was 33.9 ± 0.34 per cent and again (Figure 22) it was only those animals which survived for the longest period which showed any marked reduction in M.C.H.C. as the anaemia developed. The lowest values recorded

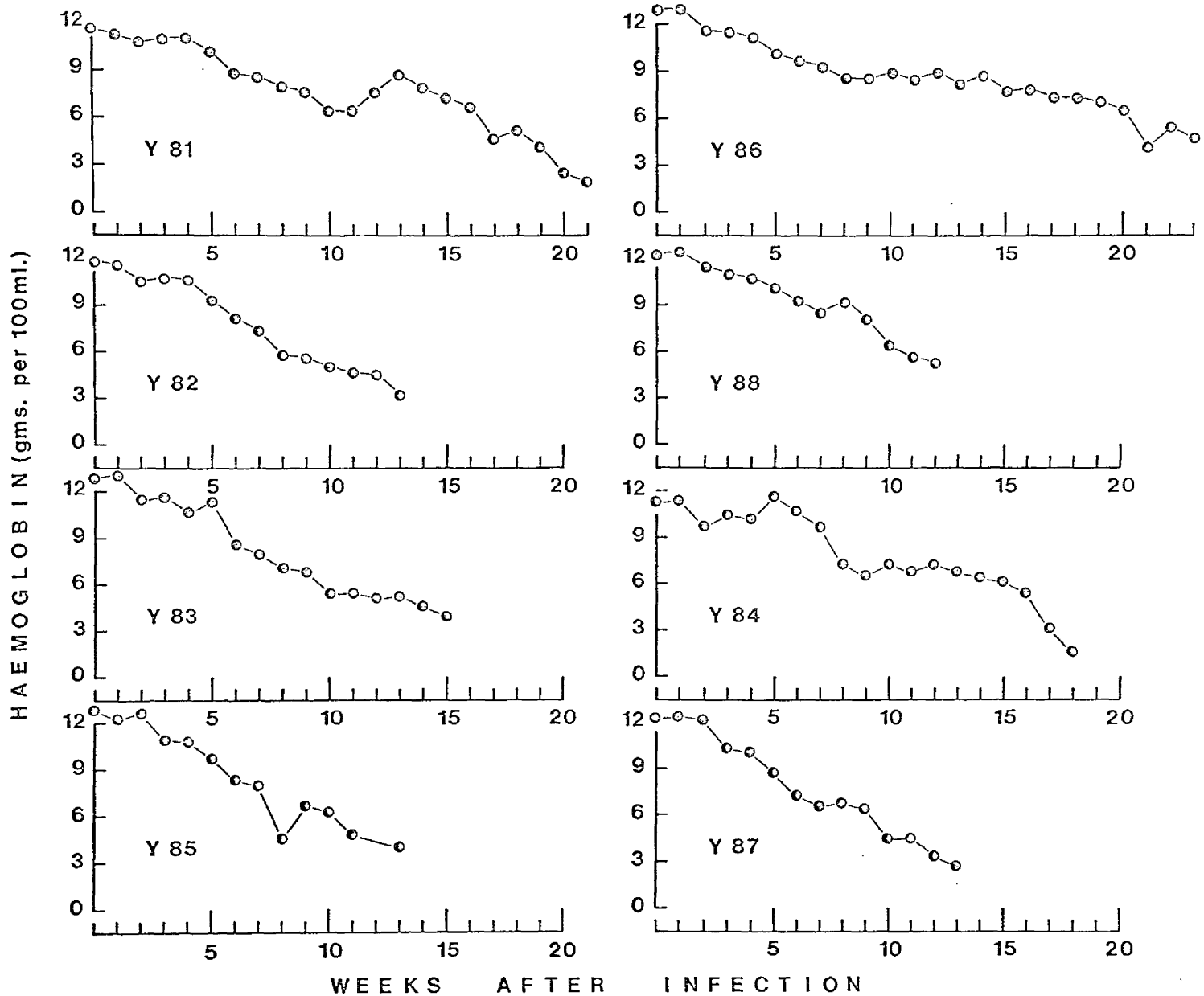

Fig. 19 Haemoglobin concentrations of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

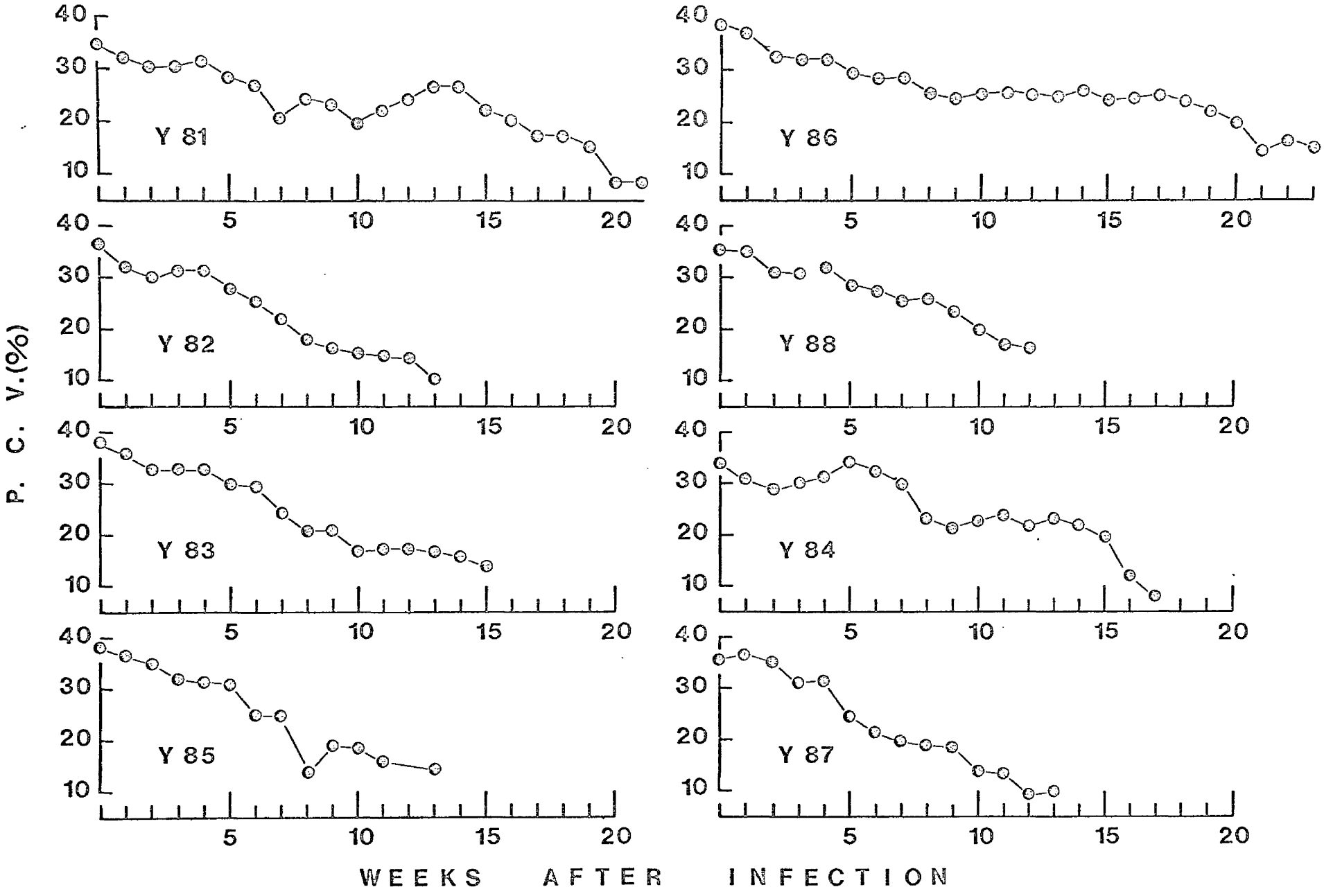

Fig. 20. Packed cell volumes of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica.

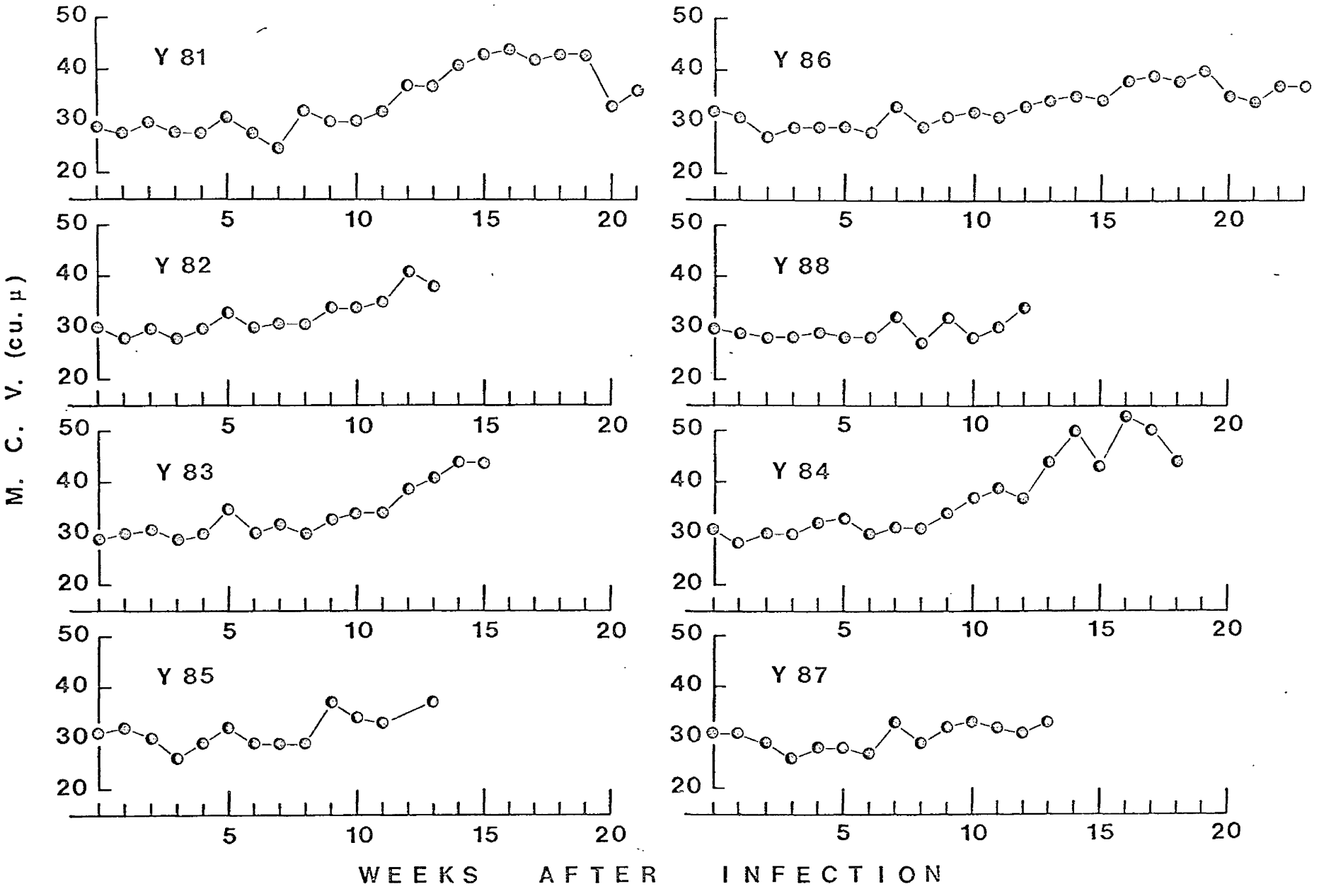

Fig. 21 Mean corpuscular volumes of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

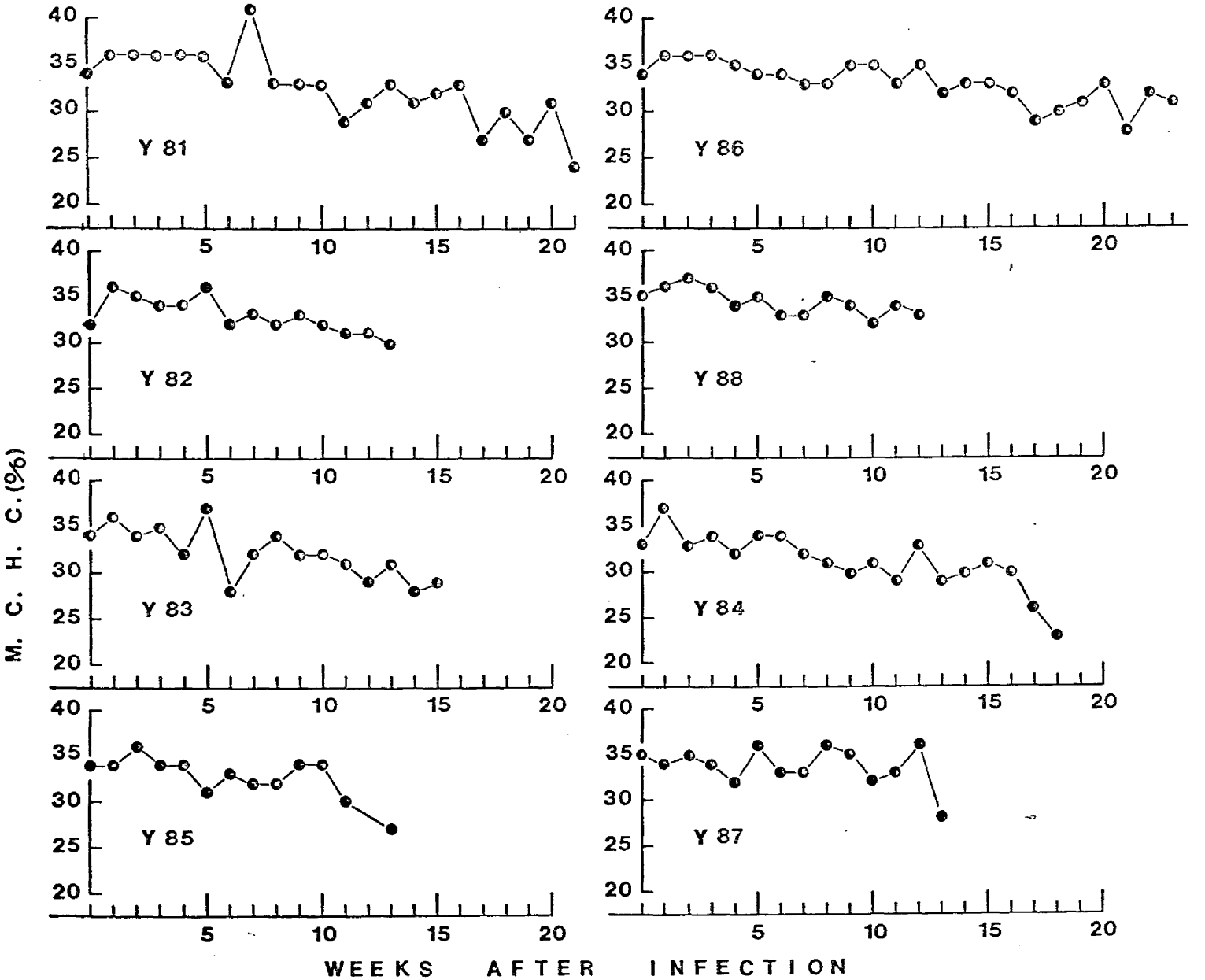

Fig. 22 Mean corpuscular haemoglobin concentrations of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

Table 16

The Alterations in Mean Packed Cell Volume, Haemoglobin Concentration and Total Red Cell Count of Lambs (Y 81 to Y 88 inclusive) infected with 1,000 Metacercariae of *Fasciola hepatica*

Weeks after infection	No. of lambs remaining in group	P.C.V. (%) Mean	S.E.	Hb (grs/100 ml) Mean	S.E.	R.B.Cs. (10^6/cu.mm.) Mean	S.E.
0	8	36.3	0.54	12.3	0.32	11.98	0.22
1	8	34.5	0.86	12.3	0.26	11.60	0.15
2	8	32.0	0.80	11.3	0.33	10.89	0.32
3	8	31.4	0.31	11.0	0.14	11.13	0.19
4	8	31.8	0.91	10.7	0.12	10.83	0.16
5	8	29.3	1.01	10.1	0.32	9.50	0.28
6	8	27.1	1.16	8.8	0.39	9.35	0.32
7	8	24.6	1.24	8.2	0.36	8.00	0.39
8	8	21.4	1.49	7.1	0.52	7.25	0.55
9	8	21.0	1.00	7.0	0.35	6.43	0.41
10	8	19.2	1.35	6.3	0.48	5.90	0.47
11	8	18.9	1.59	5.9	0.46	5.69	0.48
12	8	18.6	2.18	6.0	0.74	5.18	0.64
13	7	18.2	2.64	5.5	0.98	5.18	0.74
14	4	22.8	2.50	7.0	0.93	5.49	1.02
15	4	20.1	2.20	6.3	0.83	5.01	0.80
16	3	20.8	-	6.6	-	4.78	-
17	3	18.2	-	5.0	-	4.31	-
18	3	16.5	-	4.8	-	4.06	-
19	2	18.8	-	5.6	-	4.64	-
20	2	14.3	-	4.6	-	4.18	-
21	2	11.5	-	3.2	-	3.31	-
22	1	16.5	-	5.3	-	4.44	-
23	1	15.0	-	4.7	-	4.07	-

were from Y 81 and Y 84 with terminal values of 24 and 23 per cent respectively.

The mean values for M.C.V. and M.C.H.C. are shown in Table 17 whilst the individual results are given in Appendix 3, Tables 4 and 5.

There were no circulating reticulocytes present in any of the lambs before infection. About 10 weeks after infection reticulocytes began to appear in the circulation in significant numbers (Figure 23) with the exception of Y 86, and this reticulocytosis, once established, persisted until the death of the animal. Once present the degree of reticulocytosis increased but fluctuations in the weekly levels were marked. The reticulocyte counts of individual animals are given in Appendix 3, Table 6.

The mean total white cell count before infection was 14.1 ± 0.64 thousand per cu.mm., and despite variations in this level from week to week there were few significant changes in most animals. In those animals which survived the greatest length of time there was a progressive reduction in the total white cell count from the 12th week onwards.

Although there was little change in the total leucocyte count significant changes were seen in the differential white cell count.

A marked eosinophilia was apparent from the second week of infection onwards and this reached a maximum between weeks 8 and 12 post-infection, when the mean differential eosinophil count was in the region of 25 per cent.

Table 17

The Alterations in Group Mean Values of Mean Corpuscular Volume and Mean Corpuscular Haemoglobin Concentration of Lambs (Y 01 to Y 80 inclusive) Infected with 1,000 Metacercariae of Fasciola hepatica

Weeks after infection	No. of lambs remaining in group	M.C.V. (cu.μ) Mean	S.E.	M.C.H.C. (%) Mean	S.E.
0	8	30.4	0.37	33.9	0.32
1	8	29.6	0.57	35.6	0.35
2	8	29.4	0.46	35.3	0.45
3	8	28.0	0.52	34.9	0.32
4	8	29.4	0.46	33.6	0.53
5	8	31.1	0.92	34.9	0.67
6	8	28.8	0.41	32.5	0.64
7	8	30.8	0.94	33.6	1.07
8	8	29.8	0.56	33.3	0.60
9	8	32.9	0.76	33.3	0.59
10	8	32.8	0.98	32.6	0.46
11	8	33.3	1.00	31.3	0.67
12	8	36.0	1.33	32.6	0.95
13	7	37.7	1.44	30.0	0.81
14	4	42.5	1.32	30.5	1.04
15	4	41.0	2.35	31.3	0.86
16	3	45.0	-	31.7	-
17	3	43.7	-	27.3	-
18	3	41.7	-	27.7	-
19	2	41.5	-	29.0	-
20	2	34.0	-	32.0	-
21	2	35.0	-	26.0	-
22	1	37.0	-	32.0	-
23	1	37.0	-	31.0	-

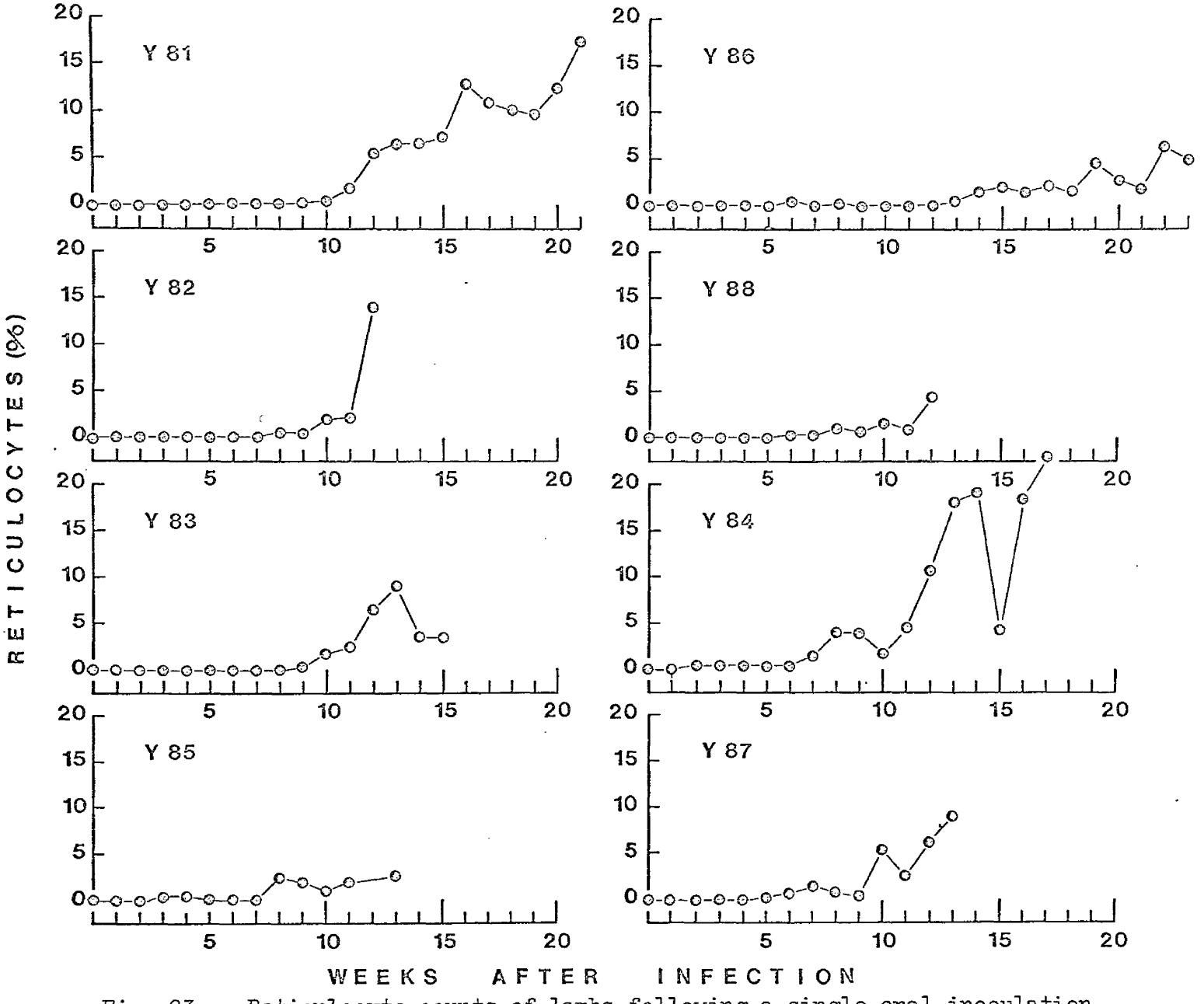

Fig. 23 Reticulocyte counts of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

After this time the degree of eosinophilia was sharply reduced although in many animals the eosinophil counts did not return to the original level. From weeks 2 to 5 the eosinophilia was accompanied by a reduction in the percentage of neutrophils and when the neutrophil level began to increase from weeks 5 to 10 post-infection a reduction in the numbers of lymphocytes occurred, hence the reason for only slight changes being recorded in the total leucocyte count. The alterations in mean values for total white cell count and differential leucocyte counts until the occurrence of the first deaths (i.e. 12 weeks post-infection) are illustrated in Figure 24, and individual results are given in Appendix 3, Tables 7, 8, 9 and 10.

Biochemical observations

There was little change in the level of total serum protein during the first 5 weeks after infection but from the 5th week onwards a gradual increase, frequently in a stepwise fashion, took place reaching a maximum between weeks 8 and 11 post-infection. From 11 weeks onwards there was a progressive decline in total protein levels but very low terminal values were only recorded in those animals which survived beyond 15 weeks. A notable exception was Y 88 which followed a course exactly the reverse of all the other animals and in this case the level of total protein fell between weeks 6 and 8 and increased sharply from week 8 until death four weeks later. The pattern of increase and decrease in total protein was almost entirely due to alterations in the gamma-globulin fraction. Similar but less marked changes occurred in the alpha/beta fraction. The decrease in total protein level from its maximum was more rapid than that of gamma

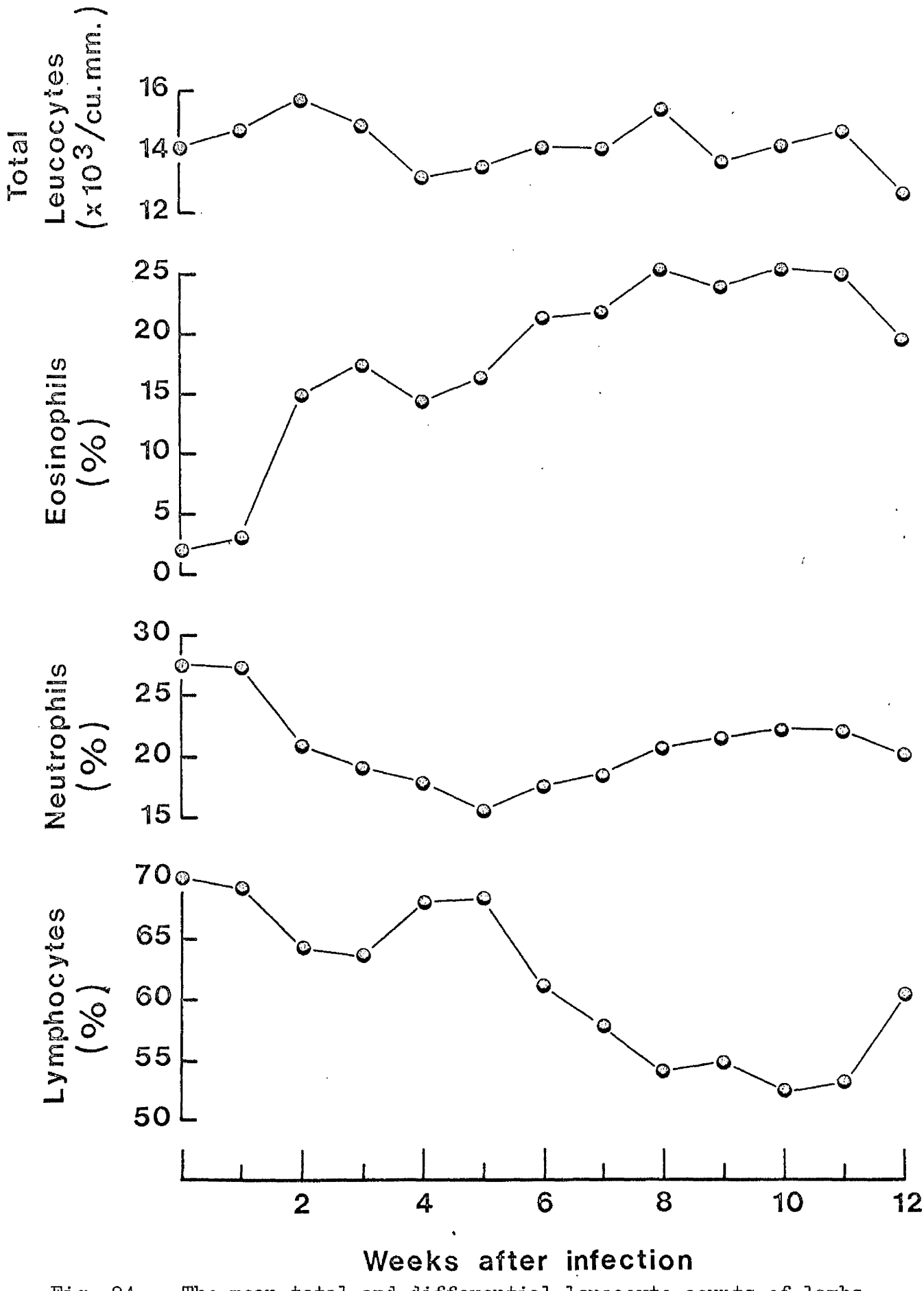

Fig. 24 The mean total and differential leucocyte counts of lambs (Y 81 to Y 88 inc.) following a single oral inoculation of 1,000 metacercariae of F. hepatica

globulin because at this point albumin levels were also falling. Albumin levels started to fall between the 4th and 6th weeks after infection but it was the 10th week before levels fell below 2 gms. per 100 ml. Albumin/globulin ratios fell in a similar fashion.

The serum proteins of the individual animals are illustrated in graphic form to compare the levels of total protein (Figure 25), serum albumin (Figure 26), alpha/beta globulin (Figure 27) and gamma-globulin (Figure 28). Mean results are shown in Table 18 whilst individual values together with the albumin:globulin ratios, are given in Appendix 3, Tables 11,12,13,14 and 15.

Of the three serum enzymes estimated during the course of the infection changes of any significance were only observed in two of them. These changes occurred in serum glutamic oxaloacetic transaminase (S.G.O.T.) and serum alkaline phosphatase, and were similar. Increase in the activity of these enzymes occurred between the 2nd and 6th week after infection and had returned to the original level by the 12th week. Although there was very marked variation in the level of serum glutamic pyruvic transaminase (S.G.P.T.) there was no particular pattern involved. Serum bilirubin levels were never increased at any stage of the infection.

The mean values of S.G.O.T., S.G.P.T., alkaline phosphatase and bilirubin for the first 12 weeks after infection are illustrated in Figure 29, whilst individual values are given in Appendix 3, Tables 16, 17, 18 and 19.

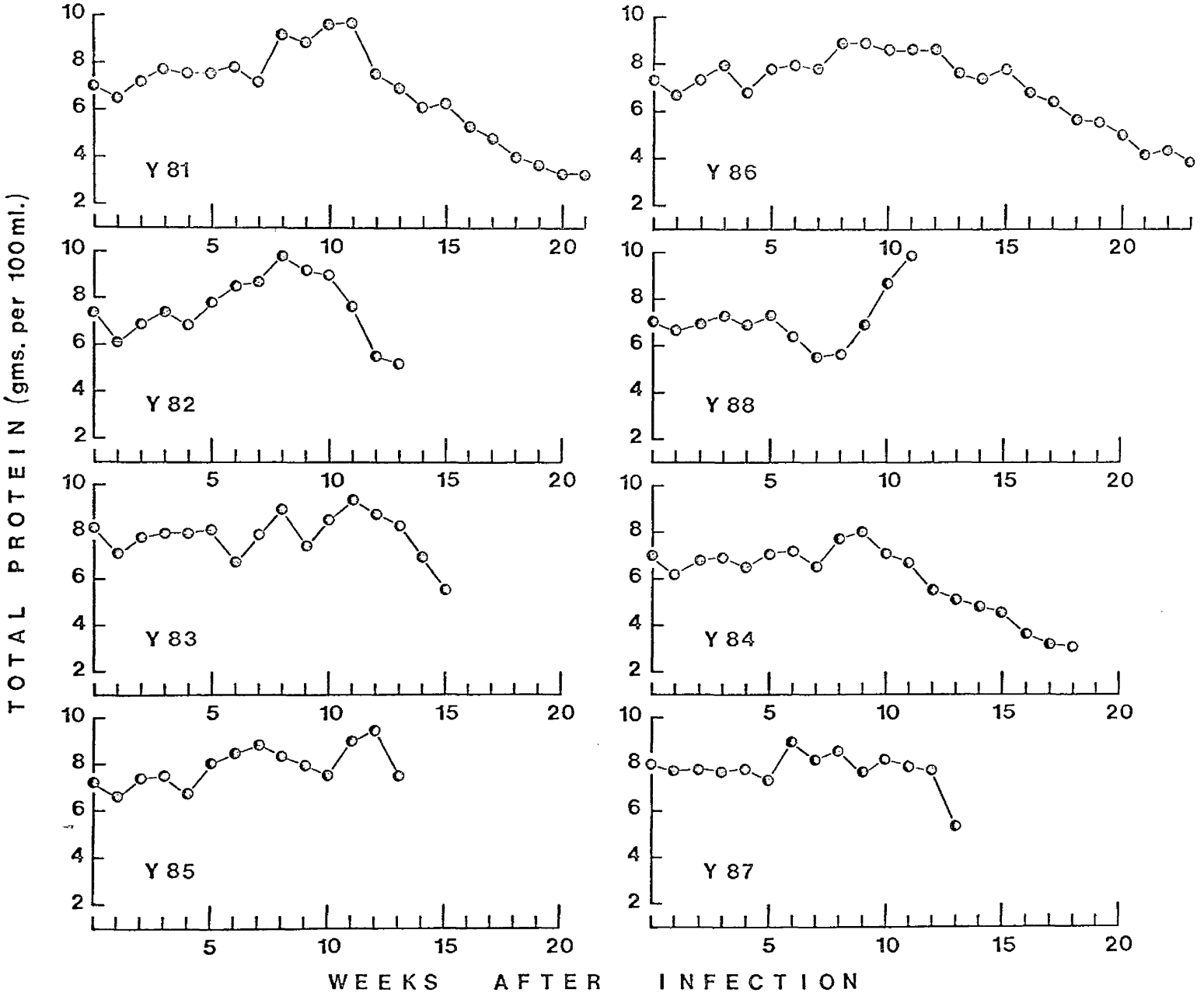

Fig. 25 Total serum protein levels of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

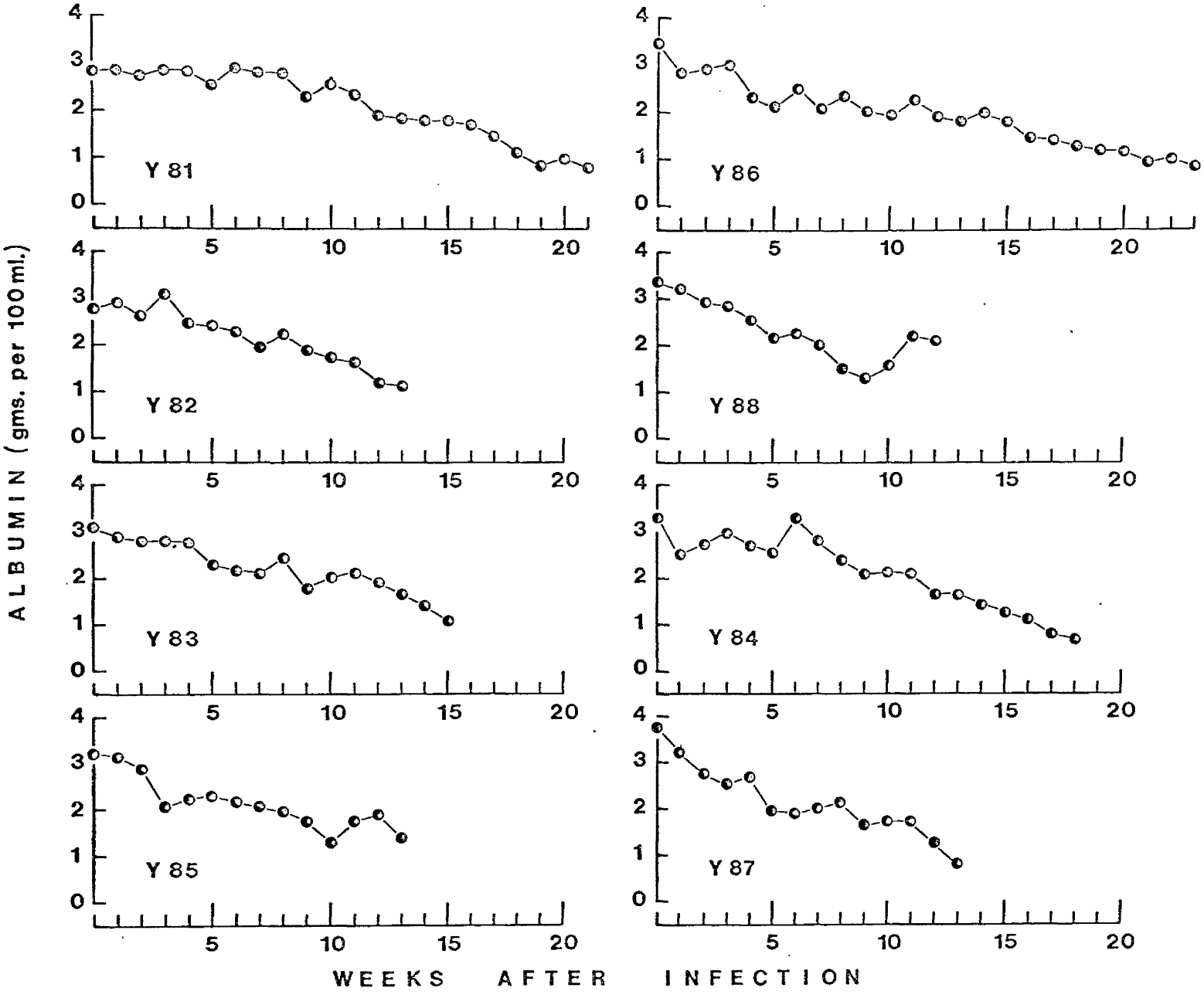

Fig. 26 Serum albumin levels of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

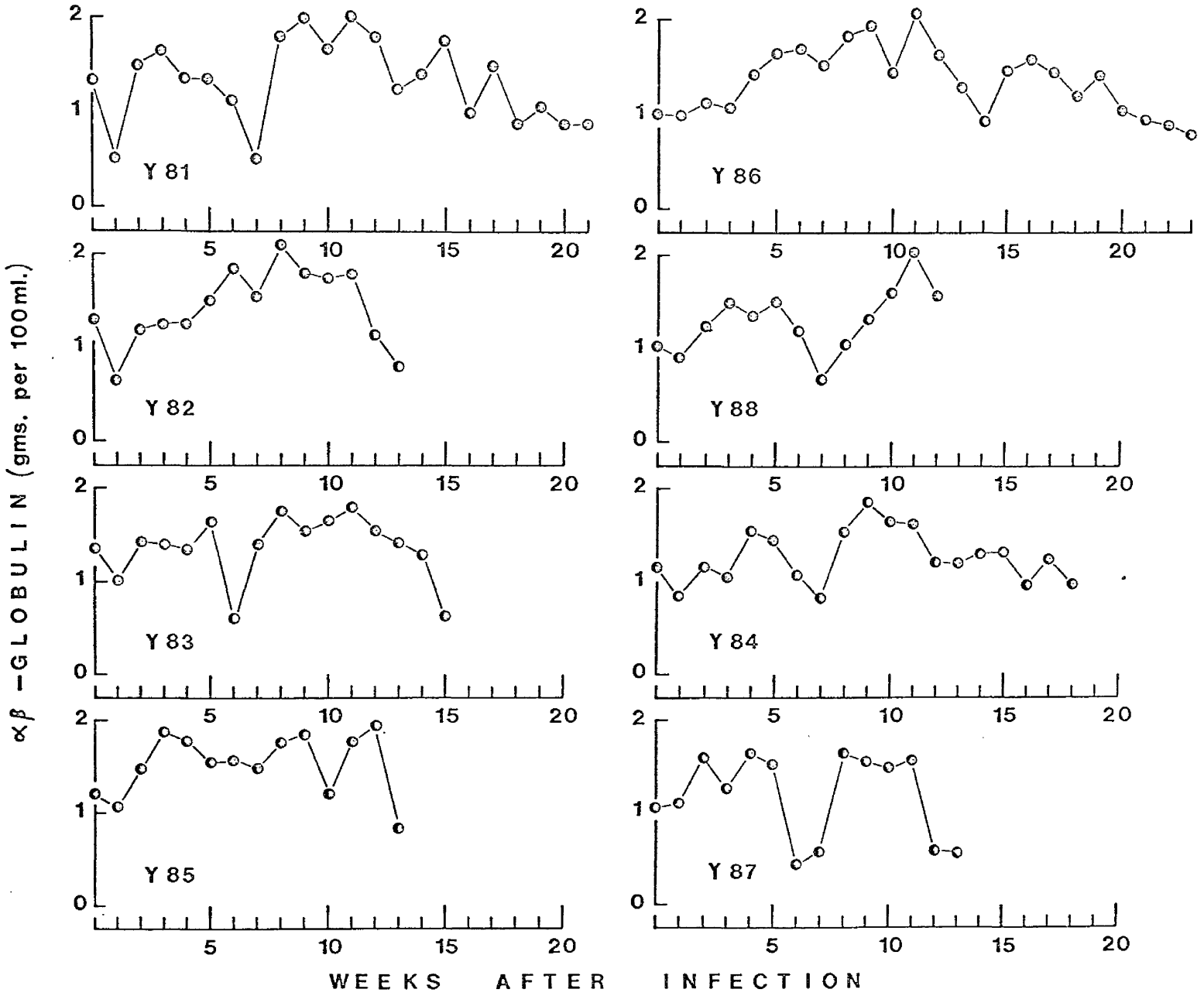

Fig. 27 Serum alpha/beta globulin levels of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

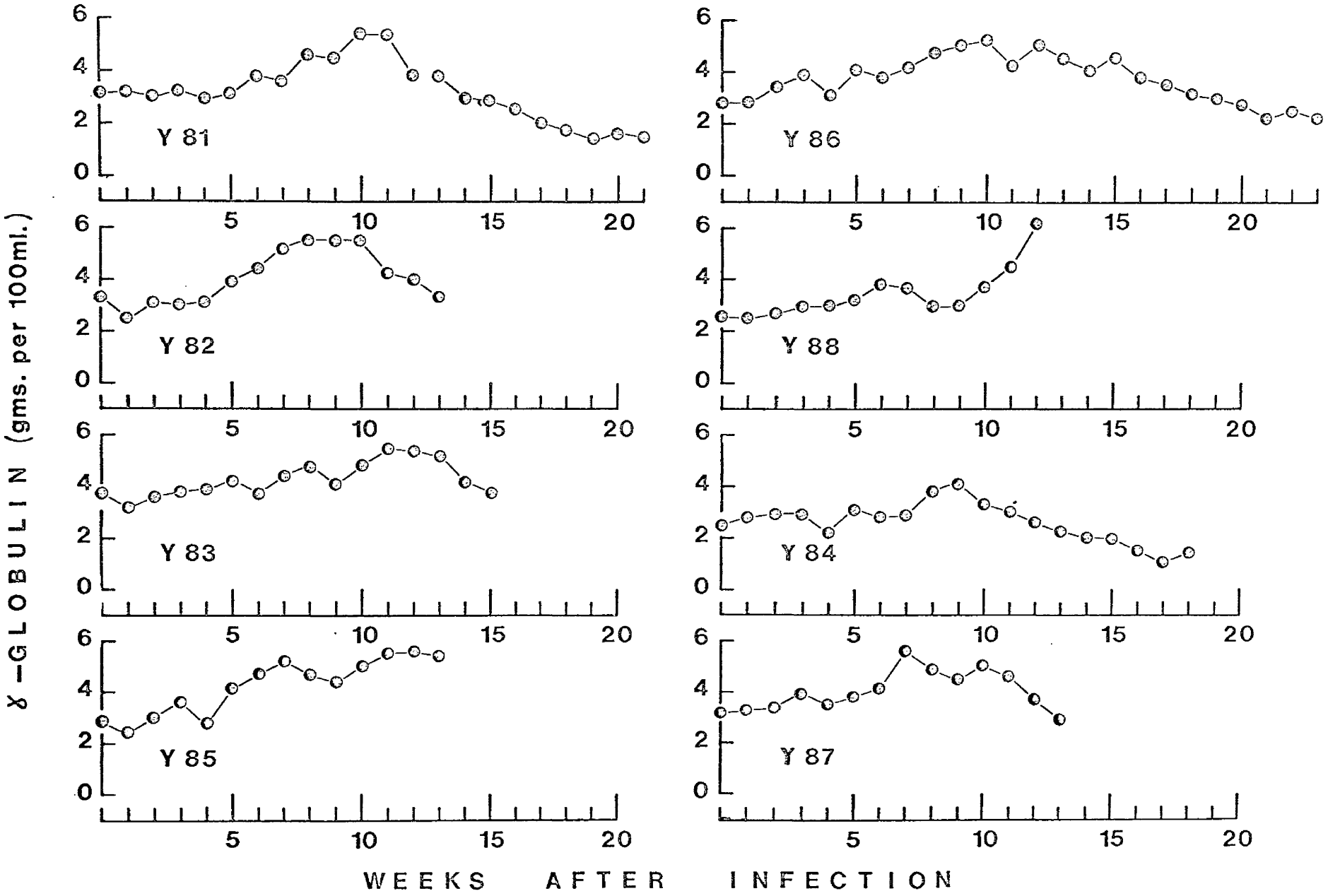

Fig. 28 Serum gamma-globulin levels of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica

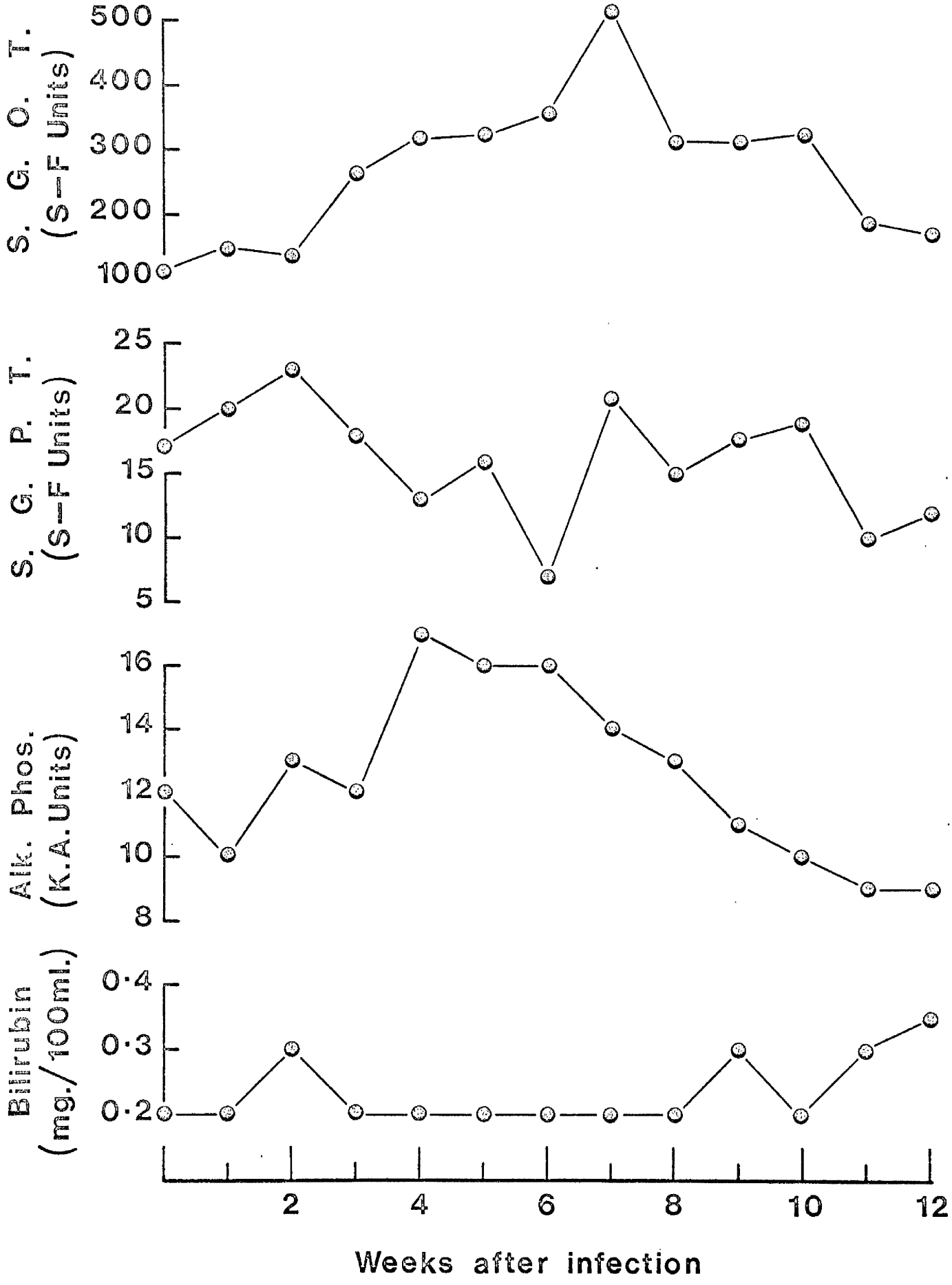

Fig. 29. The mean alterations in S.G.O.T., S.G.P.T., serum alkaline phosphatase and serum bilirubin of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica.

Table 18

The Alterations in Mean Values of Total Serum Protein, Serum Albumin, Alpha/beta-globulin and gamma-globulin of Lambs (Y 81 to Y 88 inclusive) infected with 1,000 Metacercariae of F. hepatica

Weeks after infection	No. of lambs remaining in group	Total Protein (gms/100 ml)		Albumin (gms/100 ml)		α&β-Globulin (gms/100 ml)		γ-Globulin (gms/100 ml)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
0	8	7.4	0.14	3.22	0.44	1.18	0.20	2.91	0.14
1	8	6.7	0.18	2.95	0.09	0.89	0.08	2.84	0.13
2	8	7.3	0.14	2.81	0.04	1.35	0.06	3.12	0.10
3	8	7.6	0.13	2.78	0.36	1.38	0.10	3.40	0.15
4	8	7.1	0.19	2.57	0.08	1.47	0.06	3.02	0.17
5	8	7.6	0.15	2.30	0.08	1.52	0.03	3.67	0.16
6	8	7.7	0.32	2.43	0.16	1.23	0.16	3.88	0.19
7	8	7.6	0.40	2.23	0.13	1.10	0.15	4.36	0.33
8	8	8.4	0.45	2.23	0.13	1.69	0.12	4.47	0.27
9	8	8.1	0.28	1.84	0.10	1.73	0.08	4.36	0.26
10	8	8.2	0.45	1.87	0.13	1.56	0.06	4.49	0.35
11	8	8.6	0.40	2.03	0.10	1.83	0.07	4.59	0.30
12	8	7.4	0.65	1.74	0.11	1.46	0.13	4.53	0.43
13	7	6.6	0.55	1.48	0.16	1.09	0.10	3.90	0.65
14	4	6.3	0.55	1.66	0.15	1.25	0.10	3.27	0.50
15	4	6.1	0.68	1.51	0.20	1.30	0.25	3.24	0.55
16	3	5.2	"	1.44	"	1.19	"	2.56	"
17	3	4.8	"	1.24	"	1.41	"	2.21	"
18	3	4.3	"	1.05	"	1.03	"	2.12	"
19	2	4.7	"	1.03	"	1.26	"	2.20	"
20	2	4.2	"	1.10	"	0.97	"	2.16	"
21	2	3.8	"	0.90	"	0.94	"	1.90	"
22	1	4.4	"	1.01	"	0.90	"	2.49	"
23	1	3.9	"	0.86	"	0.80	"	2.24	"

Parasitological Data

The mean faecal egg count is shown in Figure 30 and details of individual faecal egg counts are given in Appendix 3, Table 20. Fluke eggs were first observed in the faeces 10 weeks after infection but in one animal only and it was the 13th week before eggs appeared in the faeces of the majority of the lambs. The faecal egg count was very variable and was not proportional to the number of parasites recovered at autopsy.

Details of the numbers of flukes, their size and percentage take in relation to the duration of the infection are given in Table 19. The percentage take was extremely variable and ranged from 10.8 per cent to 81.5 per cent with a mean of 48.5 per cent. Table 19 also demonstrates that, in general, those animals dying earliest had a mean fluke size smaller than those animals dying later. There was no correlation between fluke size and percentage take.

Discussion

Experimental infection of six-month old lambs with 1,000 metacercariae of F. hepatica produces clinical signs, substantial changes in haematology and blood biochemistry and eventually death. The degree of anaemia was approximately proportional to the numbers of flukes recovered at autopsy but there was no relationship between the fluke burden and the terminal blood biochemistry values.

The anaemia commenced five weeks after infection when a sharp fall in

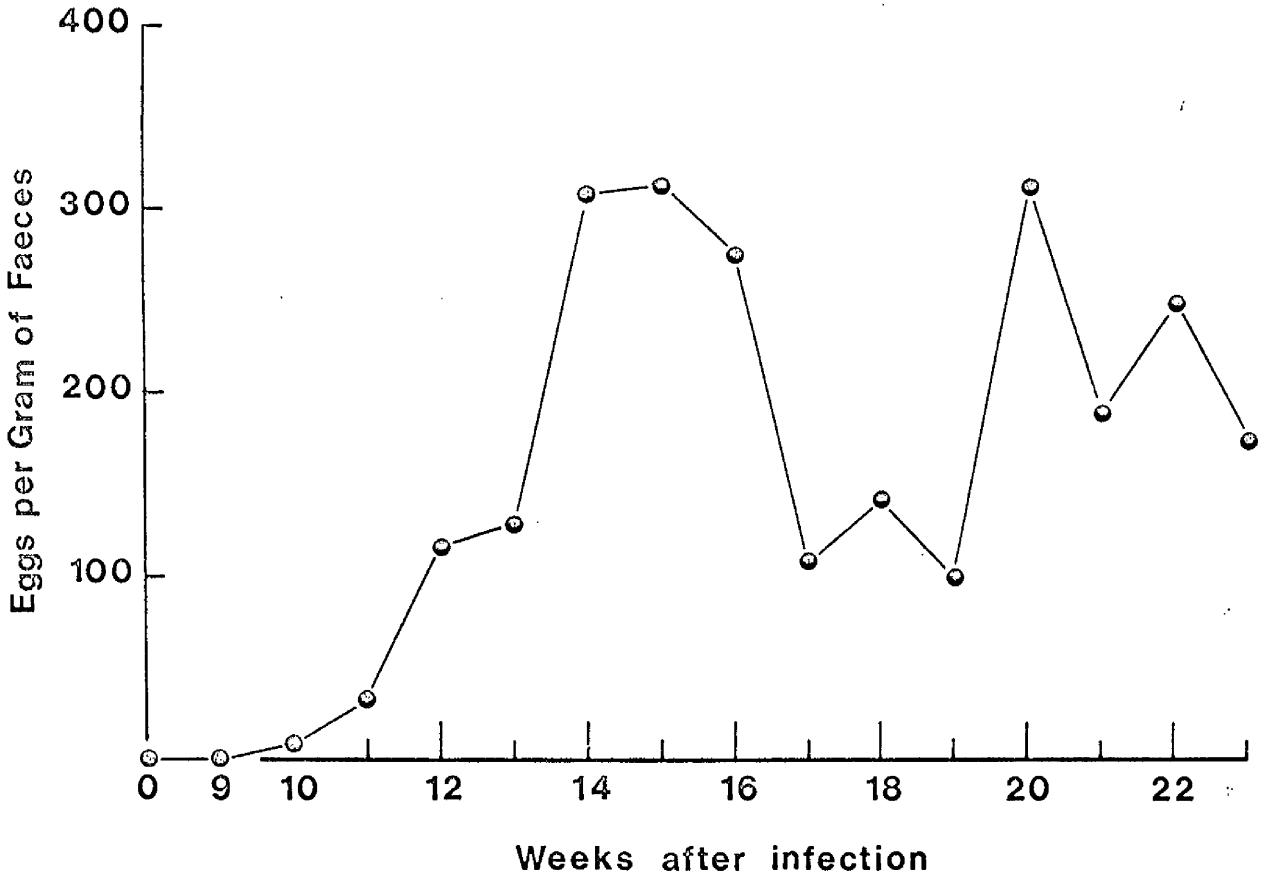

Fig. 30. The mean fluke faecal egg counts of lambs following a single oral inoculation of 1,000 metacercariae of F. hepatica.

Table 19

The Numbers, Size Distribution and Percentage Take of *Fasciola hepatica* Recovered at Autopsy from Lambs (Y 81 to Y 88 inclusive) Infected with 1,000 Metacercariae

Lamb No.	Number of *F. hepatica*	Mean Length and Standard Error(mm)	Percentage Take	Duration of Infection (weeks)
Y81	507	16 ± 0.504	50.7	23
Y82	627	15 ± 0.47	62.7	12
Y83*				
Y84	626	16 ± 0.39	62.6	18
Y85	417	12 ± 0.65	41.7	13
Y86	291	21 ± 0.76	29.1	23
Y87	815	9 ± 1.10	81.5	13
Y88	108	9 ± 0.84	10.8	12

* Recovery of flukes was impossible due to post mortem change

total red cell count, haemoglobin level and packed cell volume was observed in one animal. However, it was 7 weeks post infection before the anaemia was well established in most animals. The animal which developed the anaemia earliest was the one which had the largest fluke burden at autopsy and in this case the anaemia appeared before the flukes were in the bile ducts. Kendall and Parfitt (1962) state that it takes 40 days for flukes to reach the bile duct in sheep and so in all other cases the anaemia did not develop until the flukes were arriving in the bile ducts. Initially the anaemia was of the normochromic, normocytic type but as the disease progressed reticulocytes appeared in the circulation of all the lambs. This reticulocytosis was accompanied by a variable macrocytosis and an occasional terminal hypochromia which developed particularly in those animals which survived the greatest length of time. This latter type of anaemia is similar to that described in experimental fascioliasis in the rabbit (Urquhart 1955) and the rat (Thorpe, 1963) but it appears much earlier than that described in sheep by Sinclair (1962, 1964) using lower doses of metacercariae. Sinclair (1962) states that the anaemia is normochromic and normocytic but omitted to carry out reticulocyte counts. This author, however, shows in his results that the terminal mean corpuscular volume in one animal dying with a gross anaemia was 43 c.μ and this would appear to demonstrate the occurrence of a macrocytosis. In a repeat experiment (Sinclair, 1964) the normochromic, normocytic anaemia was confirmed commencing nine weeks after infection; again reticulocyte counts were omitted but bone marrow studies led him to conclude that although the bone marrow in infected sheep was

functional the production of erythrocytes was retarded.

There are several differences between the present experiment and those of Sinclair (1962, 1964); firstly, the animals were of different ages when infected, in the former case 6 months old, in the latter 10 to 12 months old; secondly, the infective doses of metacercariae were not identical and thirdly, there was a marked difference in the percentage take where in the present experiment there was a mean percentage take of 48.5% of 1,000 metacercariae as against a mean percentage take of 32.5% of 600 metacercariae in Sinclair's experiments. This means that in the present experiment substantially younger animals were carrying mean fluke burdens two and a half times greater than Sinclair's animals. This may be the reason for the different descriptions of the character of the anaemia in ovine fascioliasis.

While the present work was in progress two reports by Ross (1967 a & b) have appeared describing the anaemia of natural infections in lambs where fluke burdens at autopsy were similar to those produced by us; this anaemia was macrocytic, normochromic and accompanied by the appearance of erythroblasts in the circulation. Although Ross records results which are in general agreement with the present experiment, erythroblasts, defined as "spherical cells with spherical nuclei" (Maximov and Bloom, 1957) were not observed in the peripheral circulation in the present experiment. As Ross (1967 b) did not record a reticulocytosis one wonders if there had been some confusion in identifying immature stages of red cells.

Even more recently Furmaga and Gundlach (1967 a) have described single

experimental infections in sheep using 800 to 1100 metacercariae of F. hepatica. Although these authors confirmed that an anaemia developed in sheep given these infective doses of metacercariae they made no attempt to define the character of this anaemia. Unfortunately, Furmaga and Gundlach (1967) only record mean values for their haematological indices but it would appear from examination of their data that a macrocytosis was present.

There is a striking difference between the anaemia described in the calf (Section II) and that observed in lambs, in that while a reticulocytosis was a constant and marked feature of the disease in sheep this was not the case in cattle. Although Brenner (1966) and Schnappauf et al.,(1967) failed to produce a reticulocytosis in calves with the daily removal of 5 to 10% of the blood volume over a 10 day period, Granzell (1955) has shown that daily removal of a similar amount of blood from a sheep will produce a reticulocytosis within 4 days of the commencement of bleeding. This latter author also demonstrated in another sheep that removal of larger quantities of blood over a short period will produce a hypochromic, macrocytic anaemia. Sinclair (1964) removing small amounts of blood from sheep only succeeded in producing a normochromic normocytic anaemia similar to the type found in his experimental ovine fascioliasis. It would appear that just as the character of the anaemia resulting from blood-letting varies with the volume of blood removed and the duration over which it is removed, so does the anaemia of fascioliasis in sheep vary with the fluke burden and the duration of the infection.

As the liver is the source of production of the serum proteins it is not surprising that alterations take place in the various protein fractions. The results of the present experiment show that initially an increase in total serum protein occurs which is entirely due to an elevation of the globulins, particularly gamma-globulin. A maximum is reached about 10 weeks after infection and as the globulins fall so does the level of total protein although the reduction in total protein took place at a greater rate than that of the globulins due to concurrent reduction in serum albumin levels. These findings are similar to those of Sinclair (1962). Investigators of field cases of fascioliasis record results which fit into the pattern observed in experimental infections. Variations do occur and these presumably depend on at which stage of the disease any sample was taken, hence Ibrovic and Gall-Pallin (1959) recorded an increase in total protein, Nikolic et al., (1962) no change in total protein level whilst Kalian (1940 b) and Haiba and Selim (1960) observed reduced levels of total protein. All these authors, however, record a hypoalbuminaemia.

The serum enzyme estimations showed varied results with a significant result being observed only in S.G.O.T. and alkaline phosphatase. Although the values of both enzymes increased during the first few weeks of infection in several cases these increases were only minimal. Unfortunately, measurement of S.G.O.T. activity as an aid to diagnosis of field cases is of little value for two reasons. Firstly, all major tissues contain a high concentration of this enzyme (Cornelius and Kaneko, 1963) and so increased

levels could result from cell necrosis of a number of organs. Secondly, the accepted range of normal values is so wide that accurate interpretation of any one result is impossible. Again with serum alkaline phosphatase there is a wide range of serum activity in normal sheep (Allcroft and Folley, 1941; Ford, 1958) and this enzyme has also elevated serum activity in various forms of bone disease and so its activity in relation to the liver would presuppose that these other conditions did not exist. The activity of S.G.P.T. was very variable and although this is a much more specific liver enzyme it suffers the disadvantage that the livers, particularly of mature sheep, do not contain very significant levels of this enzyme (Connelius, Bishop, Switzer and Rhode, 1959).

Despite similar changes taking place in each of the lambs deaths occurred at different times after infection and the reason for this requires further investigation as it can have an important effect on the prognosis of field cases. There is no relationship between the number of flukes recovered at autopsy and the survival time of each lamb so the fluke burden itself is not the sole cause of death. The alterations in serum enzymes were not of any value in predicting the more severe liver damage which might lead to earlier deaths. Similarly at the time of death the degree of anaemia present varied from lamb to lamb and it would appear that this alone was not the cause of death because in some cases the terminal haematocrit was twice as high as in others. However, on closer examination it is apparent that there is a marked difference in the rate of development of the anaemia and this appears to be the major factor in determining at what degree of anaemia

death occurs. As a result single samples will be of no value in prognosis.

Summary

Eight six-month old lambs were given a single oral dose of 1,000 metacercariae of F. hepatica. All the lambs developed clinical fascioliasis and died between 12 and 23 weeks after infection.

An anaemia developed which was first apparent 5 to 6 weeks post infection. Initially, this anaemia was of normochromic and normocytic type but as the disease progressed it became hypochromic and macrocytic in several cases and was accompanied by a reticulocytosis in every case. The degree of anaemia was approximately proportional to the number of flukes recovered at autopsy.

A relative eosinophilia was present first appearing during the second week of infection.

At the same time as the anaemia appeared a hypoalbuminaemia developed and in those animals which survived the greatest length of time a frank hypoproteinaemia was present. Serum enzyme activity was estimated and apart from slight elevations in S.G.O.T. and alkaline phosphatase commencing during the migratory phase of the parasite no other abnormalities were detected.

The number of parasites from the original infection which became established in the liver was variable, ranging from 10.8 per cent to 81.5 per cent with a mean percentage take of 48.5 per cent.

SECTION IV

FIELD STUDIES ON FASCIOLIASIS IN SHEEP

A. Observations on the Sequential Development of an Outbreak of Fascioliasis in Sheep Grazing under Natural Conditions

B. Studies on the Availability and Infectivity of Metacercariae of *Fasciola hepatica* on pasture Over a Period of One Year

General Introduction

The epidemiology of fascioliasis has to be studied from several different angles as a result of the complex life-cycle of Fasciola hepatica. Essentially this is composed of four phases; 1) Passage of the egg in the faeces of the definitive host and the hatching of the miracidium on the pasture; 2) invasion of the intermediate host, Lymnaea truncatula, by the miracidium and subsequent development of cercariae within this host; 3) transfer of the cercariae to the final host via the ground; 4) development to the adult stage within the final host. All these phases have been investigated but unfortunately certain aspects of the life-cycle are more readily examined than others and so the former tend to be studied in greater detail and hence may appear more important. As a result a study of the development of the disease in the final host, particularly as it occurs under field conditions, has been largely neglected.

Observations on the resistance and viability of metacercariae of F. hepatica under natural conditions have been described by Olsen (1945) and Taylor (1949) whilst the ecology of Lymnaea truncatula has been described by Taylor (1949, 1964) and Mozley (1957) also under natural conditions. A number of publications describe the relationship between the size of snail population, climate and outbreaks of fascioliasis (Ollerenshaw, 1958, 1959; Ollerenshaw and Rowlands, 1959). Ollerenshaw (1959) stresses the importance of moisture and temperature; both these factors must be favourable or the life-cycle will not be accomplished. Because temperatures below 10°C limit the development of the parasite and its intermediate host optimal conditions

can only occur between May and October. As temperature is fairly constant from year to year between these months it is primarily the variation in moisture conditions which decides whether fascioliasis will be a problem or not. If wet conditions are present between May and October snail populations will increase in size due to increased breeding activity and so more snails are likely to become infected thus leading to a higher number of metacercariae on the grass. Ollerenshaw (1958) points out that different areas of the country have different climatic patterns and this leads to varying incidence and geographical distribution of the disease throughout the country. The importance of climatic factors in the life-cycle of F. hepatica enabled Ollerenshaw and Rowlands (1959) to evolve a system of forecasting the incidence of fascioliasis based on meteorological data.

Although fluke eggs are deposited on pasture all the year round there will not be continuous production of cercariae because of climatic factors and large numbers of metacercariae are available at specific times of the year only. The major source of infection according to Ollerenshaw (1959) is the "summer infection" which develops from eggs put out in the late spring and early summer and, to a very minor extent, from eggs overwintered on the pasture. Thus the infection in the snail will develop throughout the summer and early autumn producing metacercariae on the herbage during the late summer, autumn and early winter resulting in outbreaks of acute disease commencing from October onwards. Those eggs deposited on the pasture in the late summer and early autumn may infect snails during the autumn but, because of the onset of low temperatures, further development is inhibited.

Thus the parasite over-winters in the snail and infective stages will not be present on the pasture until the following spring. Ingestion of cercariae from this source together with cercariae which have overwintered on the grass results in the "winter infection" which produces outbreaks of acute disease in the late summer and early autumn.

The results of the previous section (Section III) confirmed that a single oral dose of 1,000 metacercariae of F. hepatica is capable of producing clinical disease in six-month old lambs with marked alterations in haematological and biochemical values. Unfortunately the design of this experiment is far removed from the conditions of natural grazing in the field and the disease process might not, therefore, be identical in each case. The major disadvantage of the single experimental infection is that it does not take into account the sequential uptake of metacercariae by the sheep during the grazing period; the situation identical to the natural field infection can only be reproduced by allowing sheep to graze a known infected area. Even under these latter conditions, however, it may be difficult to reproduce the so-called "typical disease" of textbook description as variations in the pasture population of metacercariae will occur from year to year depending on climate and each grazing animal may show an individual variation as a result of its grazing pattern.

Although single experimental infections of sheep with metacercariae of F. hepatica, using a wide range of infective doses, have been reported in the literature (Montgomerie, 1920; Schumacher, 1938; Taylor, 1949; Sinclair,

1962, 1964; Hughes, 1963; Fumaga and Gundlach, 1967 a & b) and a few reports record some aspects of natural infection (Naiba and Selim, 1960; Hankiewicz, 1965) there are no reports on the sequential development of the disease under natural conditions, except that recently described by Ross (1967 a & b). Ross recorded the results of an epidemiological study of fascioliasis in sheep in which parasite-free lambs were turned out to pasture known to be infected with metacercariae of F. hepatica. The lambs were introduced to the infected area at various times of the year and were allowed to graze for specified periods after which time they were removed from the infected field and subsequently killed and autopsied after a variable period of time. As a result of grazing infected pasture, a number of lambs developed marked clinical signs of fascioliasis and many died. Ross (1967 a) reports that the principal infection period occurred between August and February when 96% of the total infection was picked up; in the following year (Ross 1967 b) 97% of the infection took place between August and December.

The only blood changes described by Ross (1967 a & b) were observed immediately prior to autopsy when those animals dying in extremis had a severe anaemia and a hypoalbuminaemia. In the initial publication (Ross 1967 a) the anaemia was macrocytic and normochromic whilst in the following paper (Ross 1967 b) a large number of circulating erythroblasts were observed in one animal dying with a severe anaemia. Unfortunately the total number of lambs used was relatively small and they were weighed, blood and faecal

sampled at infrequent intervals; as a result there is no indication of when clinical signs appeared or when the anaemia and biochemical changes were first apparent nor was there any indication of the rate of change of the haematological and biochemical values. Other inadequacies in Ross's experiments were that parasitic gastroenteritis was a problem in the initial report (Ross 1967 a) whilst in both publications (Ross 1967 a & b) the lambs were not put out to grass until the summer (i.e. July and August), hence normal sheep management procedure was not adopted.

The work to be described in this section had the following objects in view.

A. To observe the sequential development of an outbreak of fascioliasis in sheep over one year's grazing under natural conditions.

B. To study the availability and infectivity of metacercariae of F. hepatica over a similar period.

Materials and Methods

Experimental Field

The field used in this experiment was sited at Brocklees Farm, Darvel, Ayrshire. This is a hill farm of 2,000 acres and is situated at a height of 900 feet above sea level (Figure 31). The total stock carried is approximately 300 ewes of the Scottish Blackface breed and 70 hill cows. The management policy is such that ewes up to the age of 5 years graze on the hill whilst cast ewes and hoggs remain at a lower level and graze fenced, upland pasture of permanent grass. In each year the cast ewes and lambs are brought off the hill in August and are moved down to the fenced grazing where they are accompanied by cows and calves which also have access to this area. Occasional cases of fascioliasis were observed in the ewes remaining on the hill from October onwards but serious losses were incurred in the cast ewes and lambs grazing at the lower level; these losses commenced in October also and continued until late February. The outbreaks of fascioliasis which occurred during the winters of 1965/66 and 1966/67 resulted in the deaths of 40% of the sheep grazing the fenced area in each of these winters; this despite the fact that fasciolicides were used at monthly intervals between August and the following March during each of these seasons. These losses predominantly occurred on the field chosen for this experiment and on another adjacent field. In an unsuccessful attempt to reduce the incidence of the disease in sheep these fields were grazed by cattle only during the autumn of 1966 although sheep were moved on to it in November of that year.

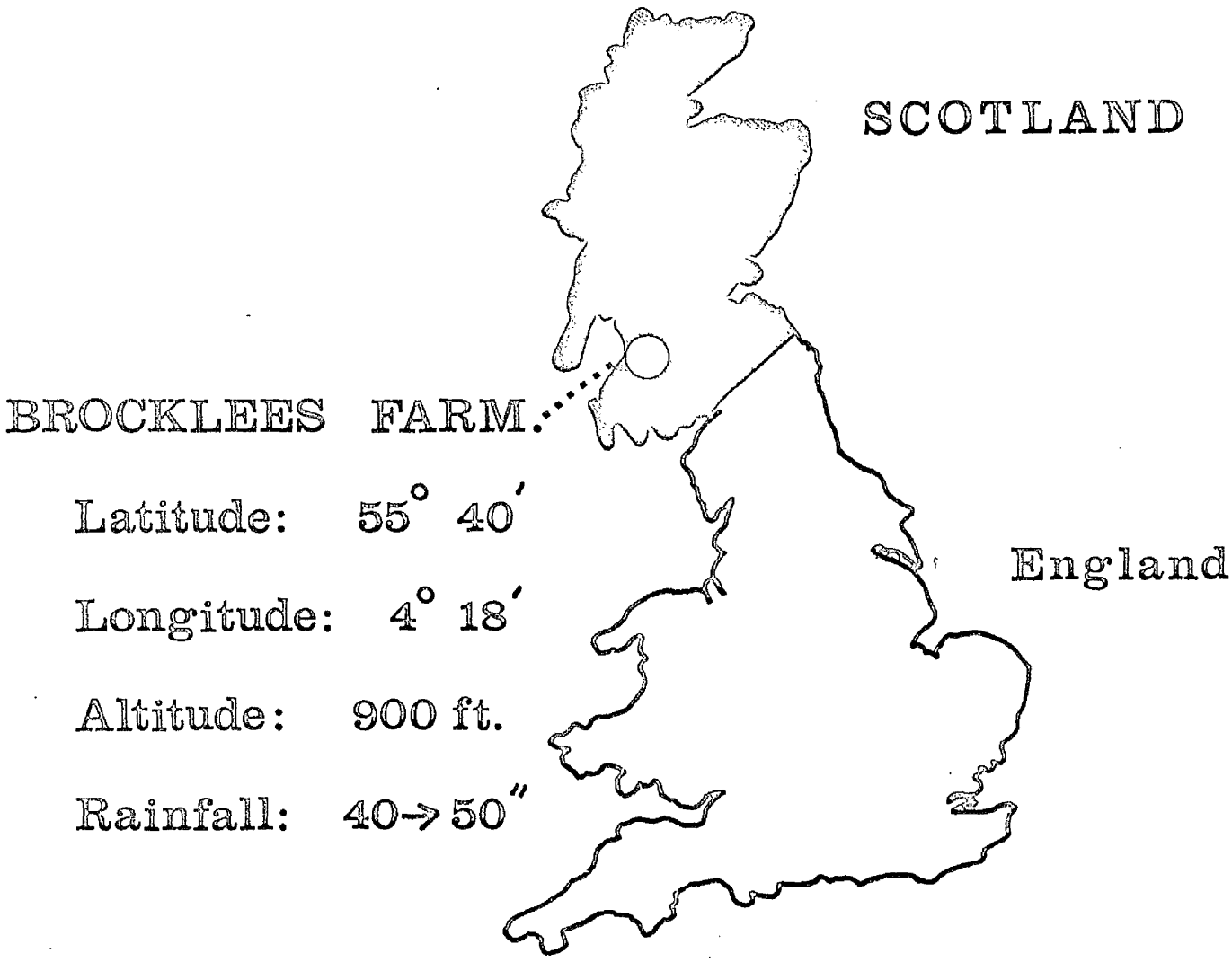

Fig. 31. The position of Brocklees Farm, Darvel, Ayrshire.

Prior to the start of the present experiment in April 1967 these fields had been left free of stock for 5 months. The experimental field (Figures 32 and 33) was of permanent grass and over at least half of its area the surface was almost continuously soft and wet; although attempts had been made to lay shallow drains in many places the drainage tiles had become damaged and broken, the pieces lying exposed on the surface. As a result of poor drainage water tended to lie and saturate the surface of the field (Figures 34 and 35). Over the past 5 years the field was treated with lime on alternate years.

Meteorological Data

The meteorological data discussed in this section was supplied by the Superintendent, Meteorological Office, 26 Palmerston Place, Edinburgh 12. The figures for rainfall were recorded at Darvel burgh yard which is about 2 miles away from the experimental farm and 347 feet above sea level. Temperatures were recorded at Kilmarnock which is 10 miles from the farm and the figures for transpiration are estimates of potential transpiration for the county of Ayr. Due to the more exposed position of the experimental farm the rainfall figures there are likely to be in excess of those quoted.

Analysis of the above data was carried out by the method of Ollerenshaw and Rowlands (1959) who devised a formula for monthly moisture value derived from the difference between rainfall and transpiration and the number of raindays. The formula $M = N(R - P + 5)$ is explained as follows:

Fig. 32. General view of the experimental field at Brocklees Farm, showing rough, permanent grazing.

Fig. 33. General view of part of the experimental field at Brocklees Farm, showing a snail habitat in the foreground.

Fig. 34. A close-up view of part of the surface of the experimental field at Brocklees Farm, showing a soft, muddy area disturbed by hoof-prints.

Fig. 35. A further close-up view of part of the experimental field at Brocklees Farm also demonstrating the soft, moist consistency of the grazing surface.

M = monthly moisture value.

N = the number of raindays per month, i.e. days when more than 0.1 ins. of rain fell in 24 hours.

R = monthly rainfall in inches.

P = potential transpiration.

The figure 5 is a constant arbitrarily fixed so that the difference between rainfall and transpiration always has a positive value.

Values for M are corrected to a maximum of 100 to prevent distortion of the final figure due to one very wet month. In May and October development of the parasite is reduced by half and so values for M are halved. The value of M for October is always 50 as transpiration is negligible during this month. These corrected values for M are designated Mt.

Blood Analysis

Blood samples were collected for both haematological and biochemical estimation. The packed cell volume percentage, haemoglobin concentration, total red cell count, total white cell count, differential white cell count and reticulocyte count were performed by the methods described in detail in general materials and methods and mean corpuscular volume (M.C.V.) and mean corpuscular haemoglobin concentration (M.C.H.C.) were calculated from the formulae also described earlier.

Total serum protein concentration was estimated by the biuret method of Weichselbaum (1946) and serum protein fractionation was performed by the

electrophoresis method described in general materials and methods. The serum enzymes and bilirubin levels were not estimated as changes of any real significance do not appear to take place (see Section III).

Weighing Procedure

The sheep were weighed on an Avery, spring-balance, pig weigher accurate to 1 lb.

Parasitological Observations

All faecal samples were examined by both the zinc sulphate flotation method and the saturated salt solution method, both of these procedures being identical to those described in general materials and methods.

Autopsy Procedure

Where possible animals which became recumbent were taken to the Veterinary Hospital where they were shot with a captive bolt pistol, bled out and the abdomen incised along midline. After removal of the liver and entire gastro-intestinal tract the carcase was examined for any gross abnormality. In some cases animals had to be slaughtered on the farm and again this was accomplished by use of captive bolt pistol and the liver and gastro-intestinal tract were removed as above, placed in polythene bags and conveyed to the Veterinary Hospital for further examination.

After removal the liver was photographed, its gross appearance noted and sections taken for histological examination. The organ was then treated as described under general materials and methods and the flukes collected

were counted and divided into three broad groups according to their size (i.e. $<$ 6 mm., 6 - 12 mm. and $>$ 12 mm.).

The abomasum was examined by the method described for sheep under general materials and methods and included digestion of the mucosa and subsequent enumeration of the nematode population. The contents of the first 30 feet of the small intestine were examined by the method also already described.

A. Observations on the Sequential Development of an Outbreak of Fascioliasis in Sheep Grazing under Natural Conditions

Introduction

The object of the experiment reported in this part of the section was to study the development of a naturally acquired infection with Fasciola hepatica in lambs. This involved allowing lambs to graze permanent pasture known to have been responsible for clinical fascioliasis in sheep in each of the two years prior to the commencement of the present experiment.

Experimental Design

Animals

In April, 1967, forty ewes and fifty lambs (i.e. thirty single lambs and ten pairs of twins) were turned out to graze the fourteen acre field on Brooklees Farm. The ewes were east hill ewes of the Scottish Blackface breed and they had spent most of the previous winter indoors at the Veterinary Hospital where the lambs were born in late March and early April. Prior to housing the ewes were treated with thiabendazole at a dosage rate of 110 mg. per Kg. to eliminate any gastro-intestinal nematodes present but no attempt was made to remove any existing fluke burden. This latter procedure was adopted to ensure that fluke eggs would be available on the grass when snail activity commenced and so repeat the normal management routine followed on this farm.

The lambs were cross lambs and when put out to graze on the experimental field were about two weeks old. When the lambs were six weeks old they were docked, castrated and given 2 ml. of a combined clostridial vaccine ("Clostrin", Glaxo Laboratories, Greenford, Middlesex); this vaccination was repeated four weeks later when the lambs were ten weeks old.

In July, the lambs were weaned and the ewes were removed from the field. At the same time the lambs were inoculated with Black Disease Vaccine (Glaxo Laboratories). When the lambs originally arrived on the farm they were given pink ear tags numbered from P 31 to P 81; as the regular examination of individual samples from 50 lambs was not practical, twenty lambs were selected at random from the fifty and given a second red ear tag numbered R 81 to R 100.

All lambs were dosed with thiabendazole at the rate of 110 mg. per Kg. every fourth week to keep gastro-intestinal nematode infestation to a minimum.

Observations.

The farm was visited weekly when all animals were visually inspected from a distance. After weaning, i.e. from July onwards, a clinical examination was carried out fortnightly when the group of twenty lambs with red tags was weighed, bled and faecal sampled. When clinical signs appeared, the clinical examination and collection of samples was carried out at weekly intervals and as deaths began to take place and the mortality rate increased

rapidly the remaining animals were included in the weekly routine of weighing and sampling. Whether an animal was having samples taken at regular intervals or not, all animals killed *in extremis* had terminal samples collected. Unfortunately, as the experimental farm was forty miles distant from the Veterinary Hospital and a number of animals died unexpectedly, terminal samples were not always available.

Results

Meteorological Data

The monthly minimum and maximum temperatures for 1965, 1966 and 1967 together with the mean monthly rainfall are illustrated in Figure 36. The predicted incidence of fascioliasis was estimated from the formula M = N(R-P+5) and was based on the values recorded for monthly rainfall, number of raindays and potential transpiration; these values are given in Table 20. The sum of Mt values predicting winter infection (i.e. the values for August, September and October, 1966, and May and June, 1967) was 351 and the sum of Mt values predicting summer infection (i.e. the values for May to October 1967 inclusive) was 451. Since the latter was above the critical figure of 400 it indicated that a significant incidence of fascioliasis could be anticipated to arise from "summer infection" (Ollerenshaw, 1959).

Clinical Data

Clinical signs were first observed during the first week of October following a spell of continuous wet weather, and by the end of that month

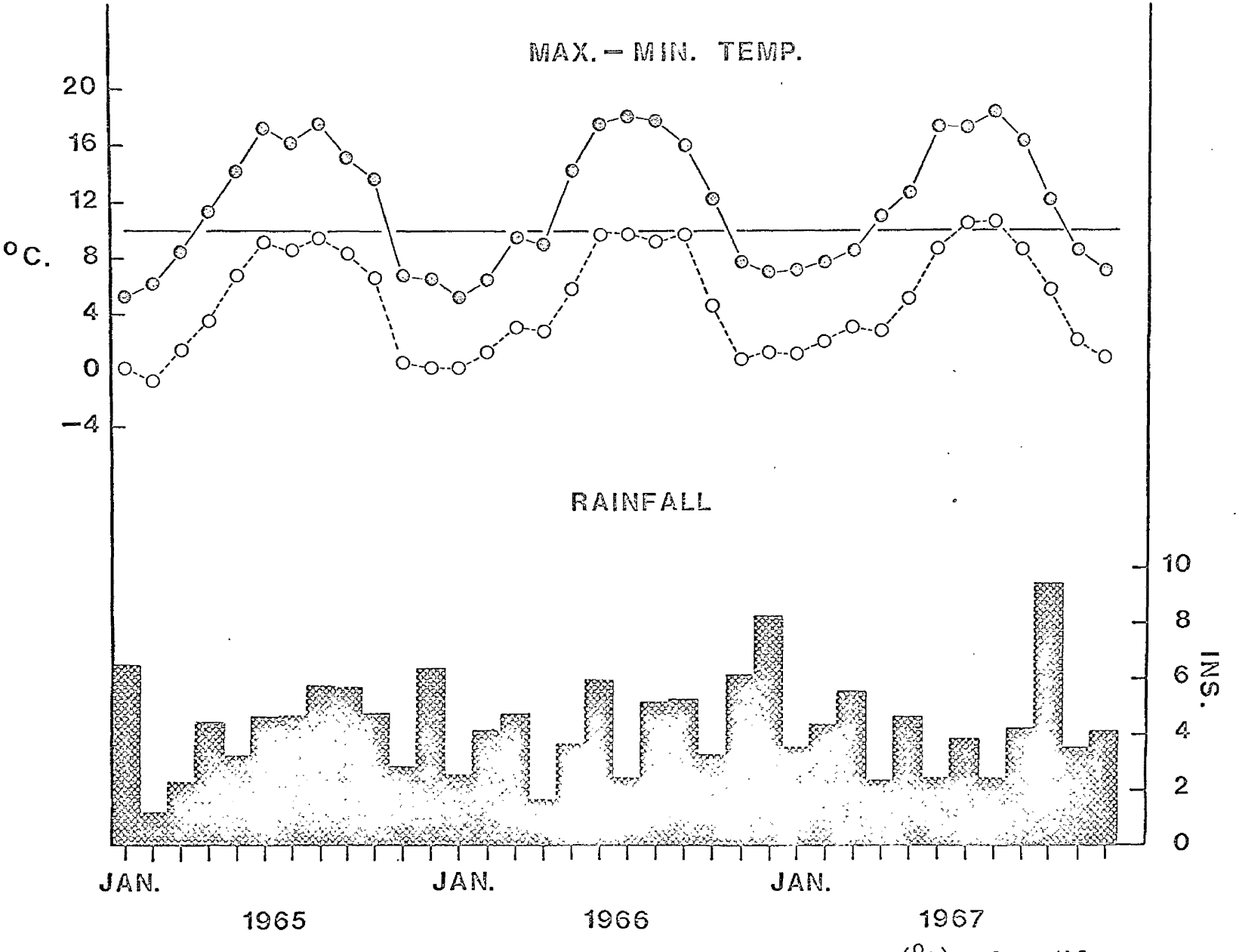

Fig. 36 Mean monthly maximum and minimum temperature (°C) and monthly rainfall from January 1965 to December 1967

Table 20

Method of Calculating Mt for Meteorological Station at Darvel (after the method of Ollerenshaw and Rowlands, 1959)

Year	Month					
	May	June	July	August	September	October
			Rainfall in Inches (R)			
1966	3.59	5.91	2.40	5.06	5.16	3.22
1967	4.60	2.35	3.76	2.35	4.23	9.37
			Rain-days per Month (N)			
1966	18	21	17	18	15	17
1967	25	16	25	22	20	26
			Transpiration (P)			
1966	3.24	3.00	3.62	2.66	1.40	0.82
1967	3.11	4.15	3.33	2.62	1.59	0.84
			$M = N(R - P + 5)$			
1966	96	166	68	132	131	126
1967	162	51	136	104	173	252
			Mt (subject to 100 Maximum)			
1966	48	100	68	100	100	50
1967	50	51	100	100	100	50

Sum of Mt values, "winter infection" 1967 = 351
Sum of Mt values, "summer infection" 1967 = 451

all but two lambs were affected. Four lambs died during the second week of October and two were slaughtered *in extremis*. This was followed by a further five lambs dying or requiring to be killed *in extremis* by the end of that month. The trend continued and it was necessary to kill nine more lambs in November, eleven in December and three in January. Another four lambs which had all shown clinical signs of fasciolinosis died unexpectedly during November and December. Two animals survived beyond January and these lambs died during the last week of March. The remaining ten lambs had been treated with an anthelmintic and these results will be reported elsewhere.

The main clinical signs recorded were loss of weight, a marked weakness, increasing pallor of visible mucous membranes and resentment of palpation of the anterior abdomen. These signs were accompanied by submandibular oedema, ascites and a palpable liver in a small proportion of cases. In general the weight loss took place gradually over a period of weeks but this was most marked over the three weeks prior to death during which time the lambs lost between 1 and 19 lbs. bodyweight with an average of 8.5 lbs. Mean bodyweights are shown in Table 21, whilst individual bodyweights recorded during the whole grazing season can be seen in Appendix 4, Table 1. The weight loss was accompanied by a progressive dullness and weakness and lambs gradually became more lethargic in their movements and frequently had difficulty in rising from a recumbent position. When driven, severely ill animals would lag behind the rest of the flock and, when forced to move more rapidly, the back legs would collapse and the lamb would fall on its haunches.

Table 21

The Alterations in Mean Bodyweight of Lambs (R 81 to R 100 inclusive) Grazing at Brooklees Farm, July 1967 to March 1968

Date	No. of lambs remaining in group	Bodyweight (lbs.) Mean	S.E.
19/7	19	59.2	2.31
1/8	19	61.6	2.22
16/8	20	70.7	2.29
29/8	20	73.1	2.08
12/9	19	77.3	2.47
26/9	19	80.1	2.30
11/10	20	80.7	2.86
25/10	15	83.5	2.88
9/11	12	85.3	4.06
16/11	10	88.0	4.21
29/11	11	81.5	4.87
6/12	10	79.0	4.86
13/12	9	78.1	5.60
20/12	6	78.0	7.17
27/12	6	82.4	6.79
3/1	5	80.6	5.73
10/1	2	90.5	-
24/1	2	88.0	-
7/2	2	91.0	-
21/2	2	87.0	-
6/3	2	82.5	-
28/3	2	82.5	-

Eventually these animals became so weak they were unable to rise and blood samples could be removed without any restraint being applied. A further marked feature was a change in the colour of the visible mucous membranes, particularly the conjunctiva, which varied from pale pink to chalk white. In some animals the change was gradual, in others extremely rapid and the time from first appearance of pallor until death ranged from 5 to 71 days.

About 75% of animals examined clinically between mid-October and mid-November appeared to resent palpation of the anterior abdomen because when light pressure was applied in this area with the fingers the abdominal muscles were tensed and occasional animals emitted a grunt. This was thought to be due to pain in this area and this theory received more support when it was also observed that many of these animals did not like to lie down; when they did so the movement was accomplished very rapidly and the animal would drop on to its sternum and then proceed to lie in a semi-upright position taking most of the weight on the left side. As a result of the tension of the abdominal muscles it was difficult to palpate the area behind the last rib on the right side where an enlarged liver would be located and in only 7% of cases was the organ palpable. In each case the posterior border of the liver extended one to two inches behind the last rib, it was firm to the touch, had a rounded edge and was not readily moved about; palpation was resented. From mid-November onwards tension of the abdominal muscles decreased and by the end of that month this feature was recorded in only 36% of animals examined. The presence of clinically detectable ascites was not a prominent

finding and in only two lambs (i.e. 4% of cases) was it recorded. In only three lambs (i.e. 6.6% of cases) was submandibular oedema observed; this was inconstant and was present on some days but not on others. Diarrhoea was not recorded and jaundice was not a feature of the clinical syndrome.

Haematological Data

Apart from the animals which died suddenly in mid-October all the lambs developed a severe anaemia. The latter was first apparent during the first part of October. The rate of development of the anaemia was variable and at the time of death there was a difference of degree. The degree of anaemia was not proportional to the number of flukes found in the bile ducts at autopsy.

At the commencement of routine sampling in mid-July the mean total red cell count was 12.65 ± 0.18 million per cu.mm. From mid-September onwards a sharp fall in this level was recorded until by early December a mean value of 5.00 ± 0.81 million per cu.mm. was observed. Regression analysis of the individual red cell values over this period demonstrates the existence of a highly significant ($p < 0.001$) linear relationship between reduction in total erythrocyte count and time (Figure 37).

The mean packed cell volume and haemoglobin concentration recorded in mid-September was 33.8 ± 0.48 per cent and 11.5 ± 0.23 gms. per 100 ml. and both these values fell by early December to 16.8 ± 1.80 per cent and 4.3 ± 0.60 gms. per 100 ml. respectively. Regression analysis of individual

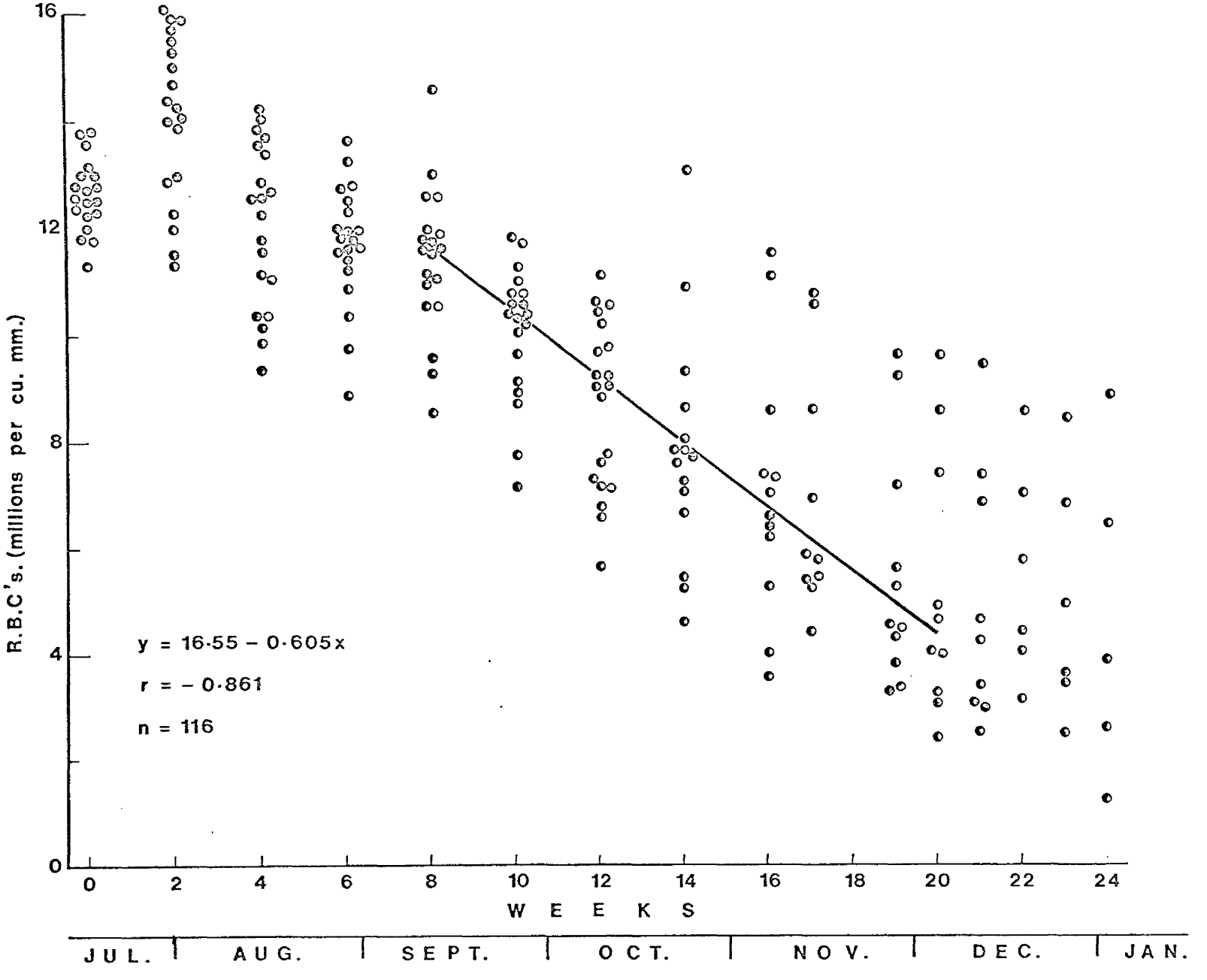

Fig. 37. The alterations in the total red cell counts of lambs during the course of a severe outbreak of fascioliasis.

values for both these parameters over this period (Figures 38 and 39) revealed a significant linear relationship similar to that observed with total red cell count ($p < 0.001$).

The alterations in mean packed cell volume, haemoglobin concentration and total red cell count are summarised in Table 22, whilst individual values are given in Appendix 4, Tables 2, 3 and 4.

As the severity of the anaemia increased a macrocytosis developed in 35.7% of cases when the mean corpuscular volume (M.C.V.) rose to between 40 c.μ and 52 cu.μ at death. The macrocytosis occurred primarily in those animals which died towards the end of the year and 80% of those animals which died after the beginning of December had elevated M.C.V. values. However, there was no apparent relationship between rate of development of the anaemia and increase in M.C.V. Regression analysis of the M.C.V. against time of all samples collected between early November 1967, and the first week of January 1968, demonstrates a significant linear relationship ($p < 0.001$)(Figure 40).

The mean corpuscular haemoglobin concentration (M.C.H.C.) of the lambs in the last week of September had an average value of 33.7 ± 0.79 per cent and by mid-December the mean value had fallen to 23.4 ± 1.04 per cent. Regression analysis of the individual values over this period (Figure 41) demonstrated a highly significant ($p < 0.001$) linear relationship between reduction in M.C.H.C. values and the passage of time and at death the M.C.H.C. in these animals ranged from 13.3 per cent to 25.0 per cent. All

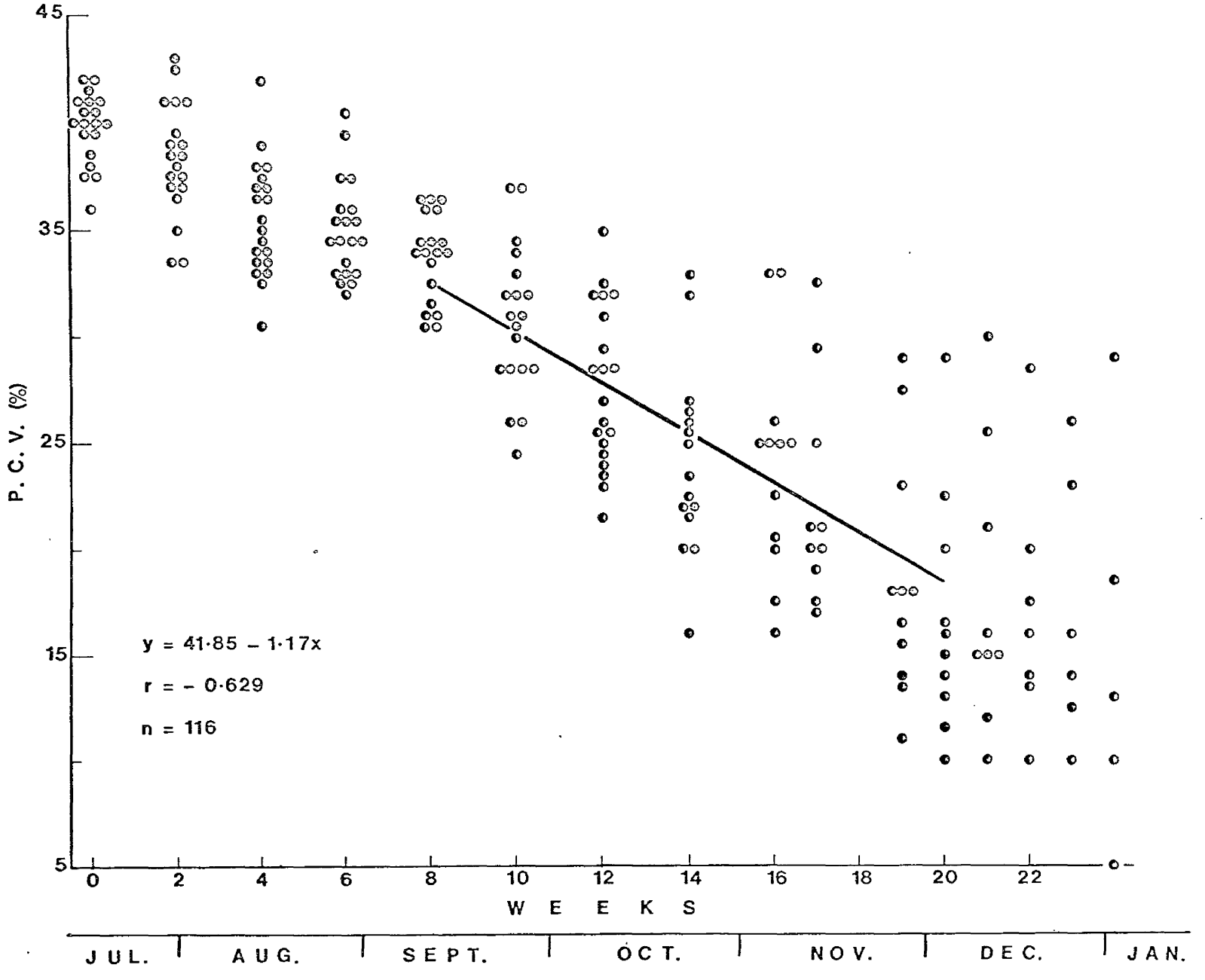

Fig. 38. The alterations in the packed cell volumes of lambs during the course of a severe outbreak of fascioliasis.

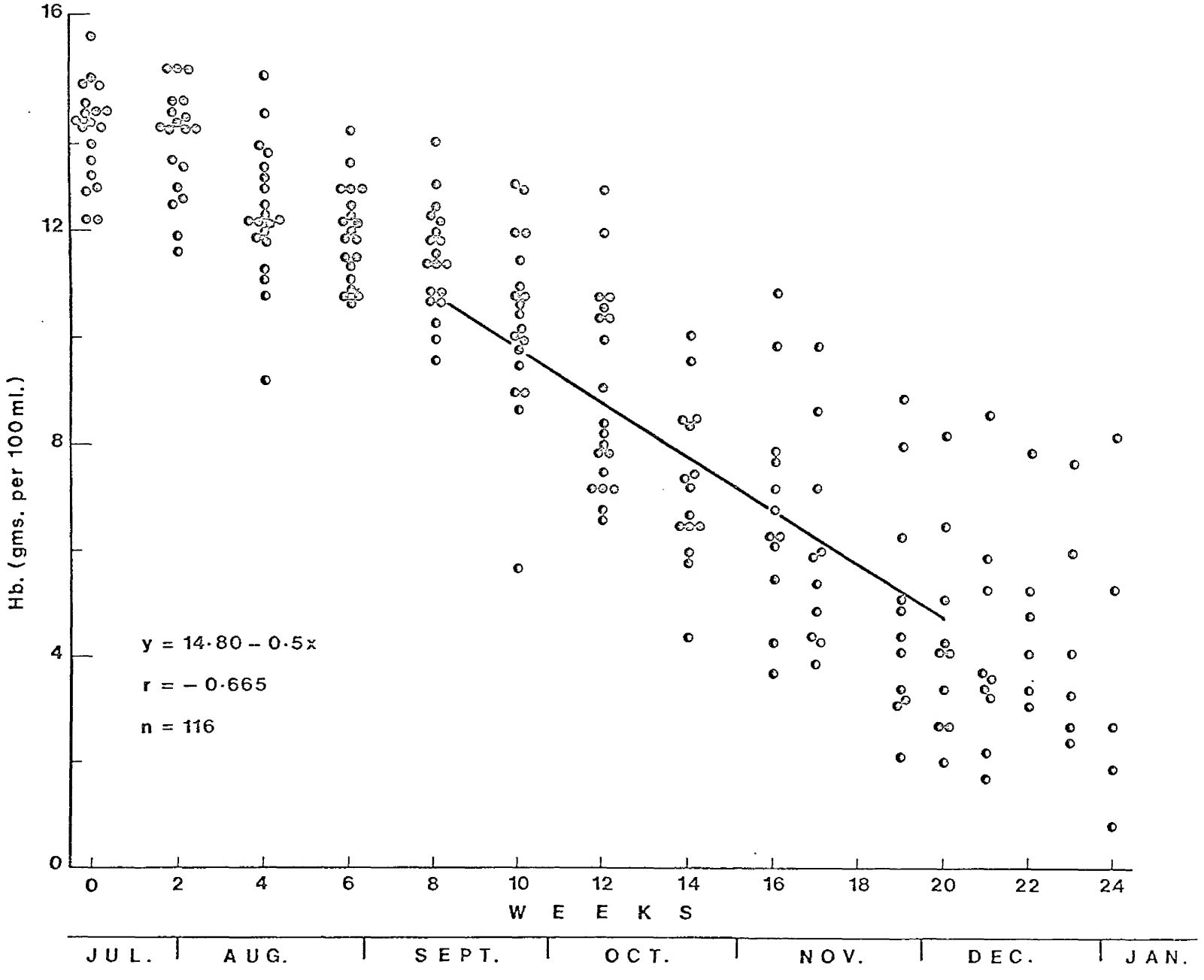

Fig. 39. The alterations in the haemoglobin concentrations of lambs during the course of a severe outbreak of fascioliasis.

Table 22

The Alterations in Mean Packed Cell Volume, Haemoglobin Concentration and Total Red Cell Count of Lambs (R 81 to R 100 inclusive) Grazing at Brockless Farm, July 1967 to March 1968

Date	No. of lambs remaining in group	P.C.V. (%)		Hb (gms/100 ml)		R.B.Co. (x10⁶/cu.mm)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
19/7	19	39.8	0.38	13.8	0.21	12.65	0.18
1/8	19	38.3	0.60	13.7	0.23	14.09	0.36
16/8	20	35.6	0.61	12.5	0.23	11.95	0.51
29/8	20	35.1	0.52	12.0	0.20	11.78	0.22
12/9	19	33.8	0.48	11.5	0.23	11.44	0.44
26/9	19	30.8	0.80	10.4	0.38	10.14	0.29
11/10	20	27.0	0.84	9.0	0.43	8.77	0.36
25/10	15	24.2	1.16	7.3	0.39	7.90	0.56
9/11	12	23.9	1.55	6.9	0.60	7.17	0.71
16/11	10	22.1	1.60	6.1	0.63	6.99	0.73
29/11	11	18.0	1.84	4.9	0.64	5.59	0.65
6/12	10	16.8	1.80	4.3	0.60	5.25	0.79
13/12	9	17.3	2.17	4.2	0.71	5.00	0.81
20/12	6	18.3	2.27	4.8	0.66	5.56	0.84
27/12	6	16.9	2.56	4.4	0.85	5.04	0.94
3/1	5	15.1	4.11	3.8	1.33	4.66	1.38
10/1	2	25.3	-	7.1	-	7.62	-
24/1	2	22.8	-	6.4	-	6.30	-
7/2	2	22.5	-	6.2	-	6.13	-
21/2	2	19.0	-	5.2	-	5.35	-
6/3	2	18.5	-	4.3	-	4.80	-
28/3	2	15.0	-	4.0	-	4.91	-

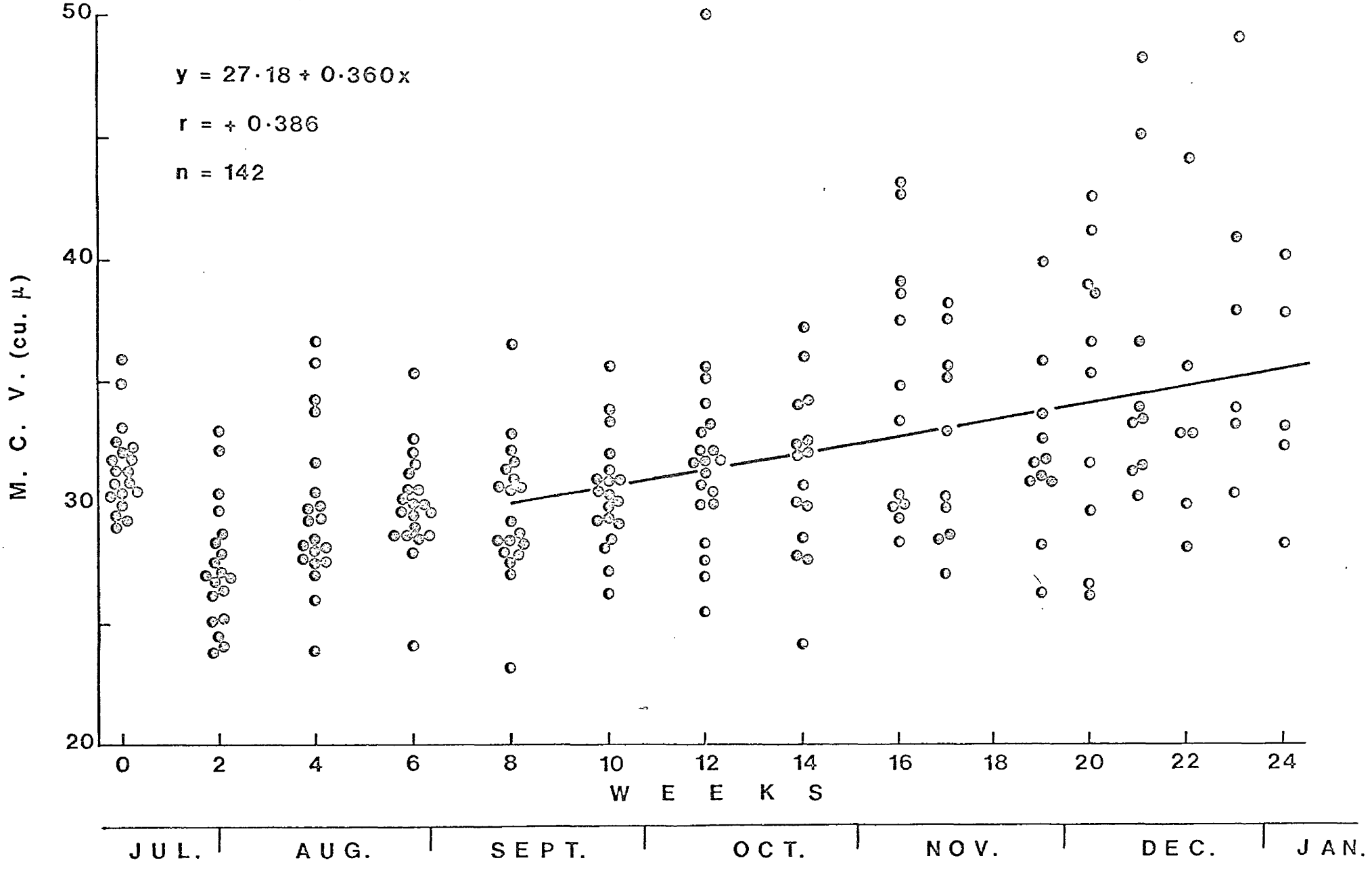

Fig. 40. The alterations in the mean corpuscular volumes of lambs during the course of a severe outbreak of fascioliasis.

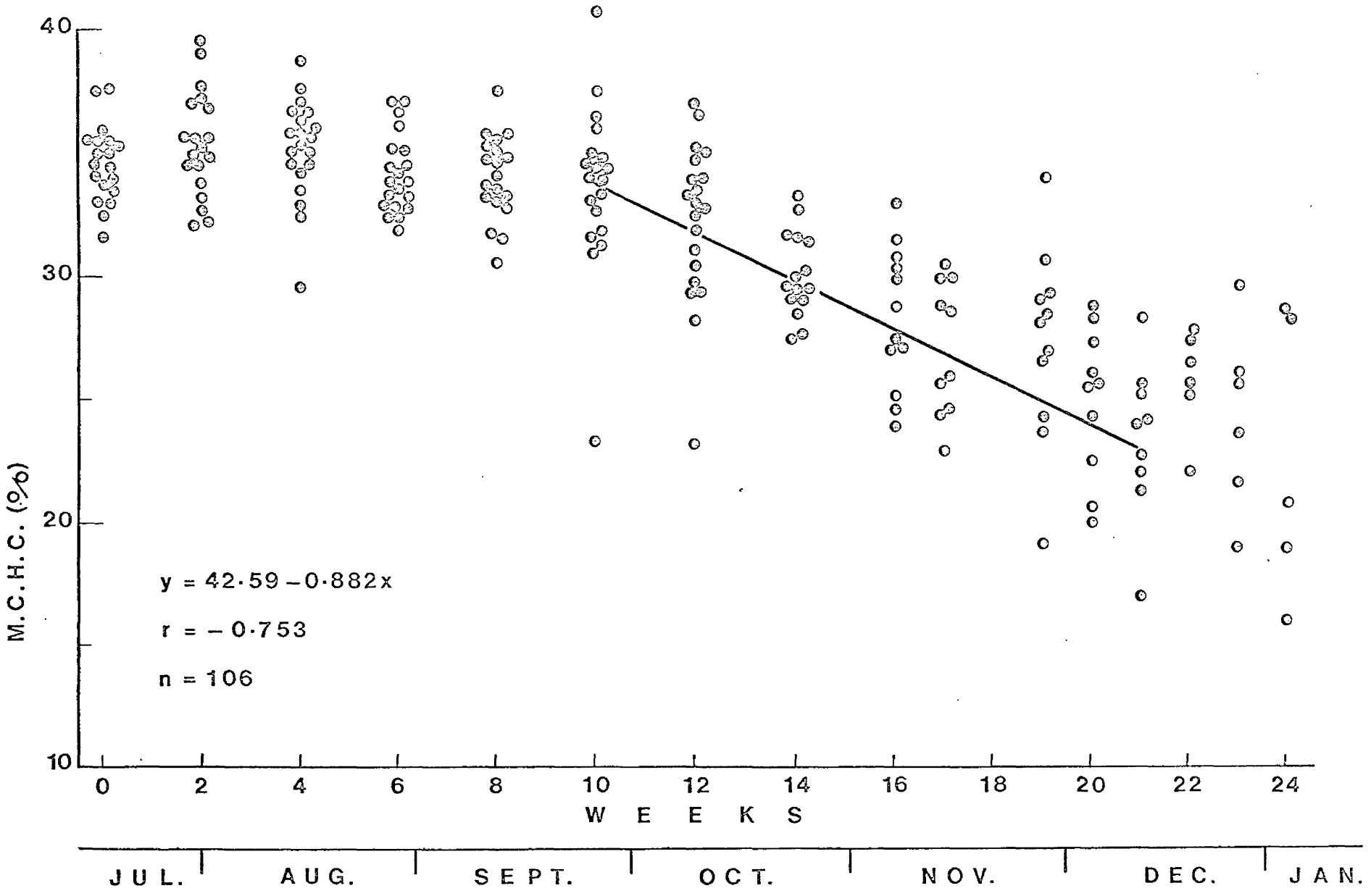

Fig. 41. The alterations in the mean corpuscular haemoglobin concentrations of lambs during the course of a severe outbreak of fascioliasis.

the animals dying after the third week of November developed a hypochromic anaemia and those animals with the most prolonged anaemia tended to have the lowest M.C.H.C. values.

When routine sampling was commenced in mid-July there were no reticulocytes present in the peripheral circulation of the lambs but during the first week of November reticulocytes were observed in the circulation of several animals and after this time almost all of the lambs dying exhibited a moderate to marked reticulocytosis. The reticulocytes appeared in the circulation between 2 and 8 weeks prior to death and once present were constantly observed although the numbers varied in an irregular pattern from week to week. Reticulocytes were only noticed in animals with a haematocrit of 25 per cent or less and at death the range of reticulocytes was 8 per cent to 30 per cent.

The mean values for M.C.V., M.C.H.C. and reticulocyte counts recorded between July 1967 and March 1968 are summarised in Table 23, whilst individual values are given in Appendix 4, Tables 5, 6 and 7.

The mean terminal values of packed cell volume, haemoglobin concentration, total red cell count, mean corpuscular volume, mean corpuscular haemoglobin concentration and reticulocyte count of lambs dying in each of the months between October 1967 and March 1968 are summarised in Table 24, whilst individual terminal values are given in Appendix 4, Table 8.

The interval of time between the first appearance of the anaemia and

Table 23

The Alterations in Mean M.C.V., M.C.H.C. and Reticulocyte Counts of Lambs (R 81 to R 100 inclusive) Grazing at Brooklees Farm, July 1967 to March 1968

Date	No. of lambs remaining in group	M.C.V. (cu.u) Mean	S.E.	M.C.H.C. (%) Mean	S.E.	Reticulocytes (%) Mean	S.E.
19/7	19	31.5	0.41	34.7	0.33	0	-
1/8	19	27.2	0.64	35.7	0.49	0	-
16/8	20	29.7	0.73	35.3	0.47	0	-
29/8	20	29.9	0.51	34.2	0.36	0	-
12/9	19	29.8	0.66	34.0	0.42	0	-
26/9	19	30.4	0.52	33.7	0.79	0	-
11/10	20	32.1	0.80	32.2	0.73	0	-
25/10	15	31.3	0.89	30.1	0.46	1.0	0.64
9/11	12	34.8	1.55	28.3	0.84	4.2	1.63
16/11	10	32.4	1.28	27.0	0.84	2.0	1.03
29/11	11	32.1	1.10	27.1	1.18	6.5	2.47
6/12	10	34.8	1.88	24.9	0.94	5.4	1.81
13/12	9	36.1	2.15	23.4	1.04	6.3	1.85
20/12	6	34.0	2.32	25.8	0.83	8.3	2.29
27/12	6	37.7	2.77	24.2	1.52	7.0	1.75
3/1	5	34.4	2.10	22.5	2.53	6.6	3.20
10/1	2	33.3	-	28.0	-	3.0	-
24/1	2	36.4	-	28.1	-	4.5	-
7/2	2	36.9	-	27.5	-	0	-
21/2	2	35.7	-	27.1	-	8.0	-
6/3	2	30.7	-	22.9	-	7.5	-
20/3	2	29.8	-	22.2	-	14.5	-

Table 24

The Mean Terminal Haematological Values of Lambs Dying in Each Month between October 1967 and March 1968 as a Result of Naturally Acquired Fascioliasis

		No. of Lambs	P.C.V. (%)	Hb (gms/100 ml)	R.B.Cs. (x10⁶/cu.mm.)	M.C.V. (cu.μ)	M.C.H.C. (%)	Reticulocytes (%)
October 1967	Mean	6	22.5	6.2	6.50	35.9	28.3	0.2
	S.E.		2.77	0.73	0.98	3.24	1.14	-
November 1967	Mean	7	13.9	3.2	4.00	35.2	22.7	11.6
	S.E.		1.62	0.50	0.59	1.79	1.17	3.32
December 1967	Mean	11	11.4	2.3	2.81	41.7	19.8	16.2
	S.E.		0.62	0.19	0.19	2.24	0.80	2.74
January 1968	Mean	3	7.7	1.2	1.81	42.1	15.3	15.0
	S.E.		1.33	0.20	0.30	1.70	1.04	1.15
March 1968	Mean	2	13.5	3.5	3.98	33.6	24.6	12.0
	S.E.		5.90	1.80	1.54	0.80	3.30	8.00

death was variable and ranged from 4 to 15 weeks. Similarly, there was variation in the time between onset of the anaemia and a reticulocytosis becoming apparent; this time interval ranged from 2 to 10 weeks.

Biochemical Data

At the commencement of routine sampling in mid-July the mean level of total serum protein was 6.5 ± 0.07 gms. per 100 ml. This value increased gradually from mid-August and reached a maximum of 7.8 ± 0.14 gms. per 100 ml. by the last week of September, but thereafter total protein levels fell gradually although very low levels were not recorded in those animals dying early in the course of the disease. The individual values for the twenty lambs, R 81 to R 100 are illustrated in graphic form in Figure 42, where regression analysis of the results between mid-August and late September demonstrates a positive correlation ($p < 0.001$); a second regression line of negative correlation ($p < 0.001$) was calculated from results recorded between late September and early December. Individual values for total serum protein recorded throughout the experiment are given in Appendix 4, Table 9. The increase in total protein levels between mid-August and late September was due to an elevation of both albumin and the globulins but the major component of this increase was gamma-globulin.

Serum albumin levels commenced to fall during the second week of September when they had a mean value of 2.41 ± 0.05 gms. per 100 ml. and by early December the mean figure was 0.98 ± 0.10 gms. per 100 ml. This

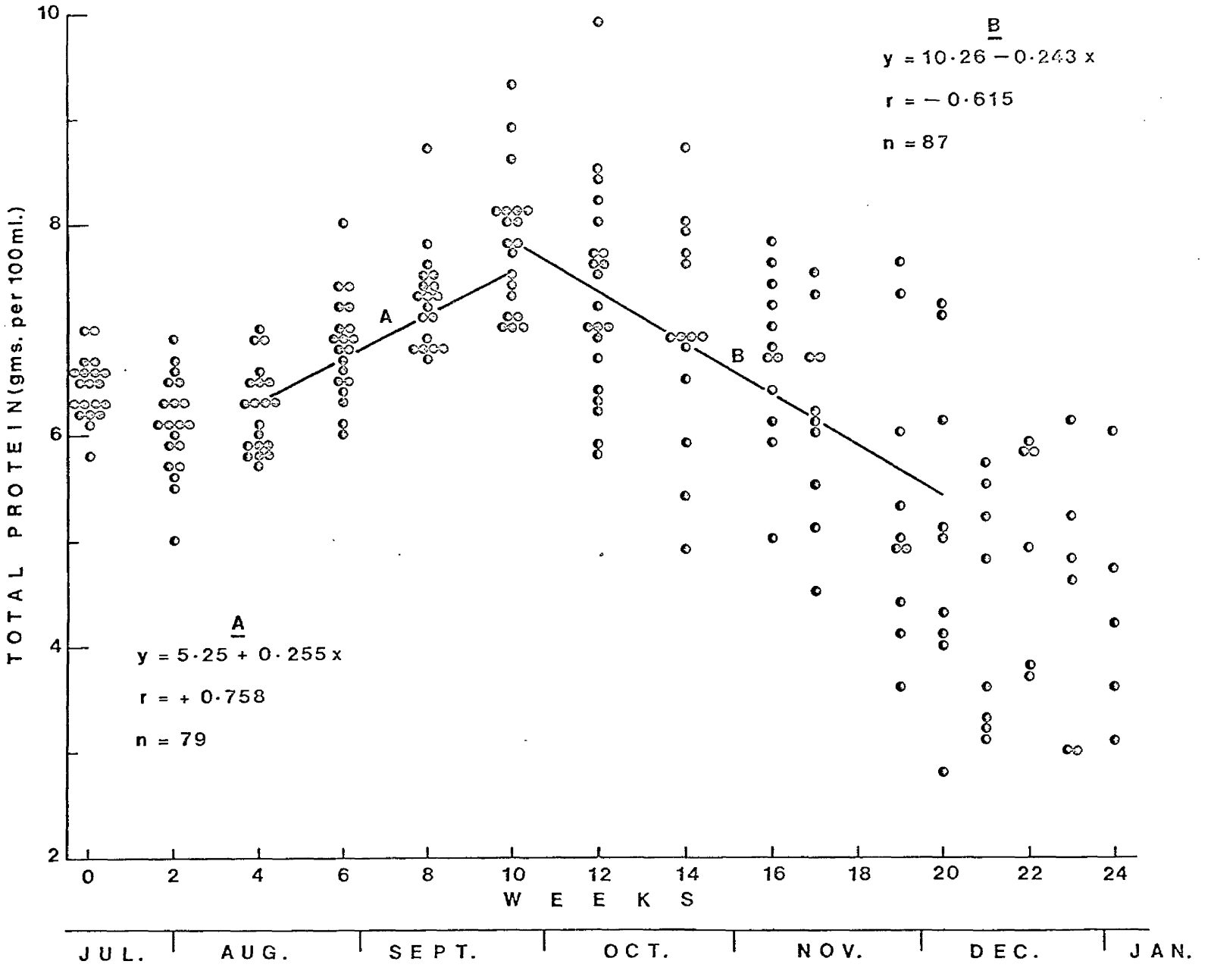

Fig. 42. The alterations in the total serum protein levels of lambs during the course of a severe outbreak of fascioliasis.

reduction in serum albumin level took place in a linear fashion and Figure 43 shows the regression line for values recorded between early September and early December which has a statistical significance $p < 0.001$. Terminal values for serum albumin ranged from 0.55 to 2.63 gms. per 100 ml. Individual values are given in Appendix 4, Table 10.

An increase in the globulins, particularly gamma-globulin occurred during August and September when, in the course of 6 weeks, the level of gamma-globulin increased from a mean value of 2.18 ± 0.09 gms. per 100 ml. to a mean figure of 3.63 ± 0.16 gms. per 100 ml. This increase in the globulin fractions did not follow a linear or exponential pattern. After this initial elevation a reduction in the serum globulins was observed the fall commencing in early September with a reduction in the alpha/beta fraction and this was followed about two weeks later by a decrease in the gamma fraction. The reduction in the globulins, as in the case of serum albumin, followed a linear pattern and this is demonstrated by regression analysis of the results obtained between September and December which has a statistical significance, $p < 0.001$ for both alpha/beta and gamma-globulin. This relationship over this period between reduction in globulins with time is illustrated in Figures 44 and 45, whilst individual values for alpha/beta, gamma-globulin and total globulin are given in Appendix 4, Tables 11, 12 and 13.

The albumin:globulin ratio decreased in an irregular fashion. The reduction commenced in July and apart from a temporary increase in late August there was a gradual fall in the ratio until mid-October after which

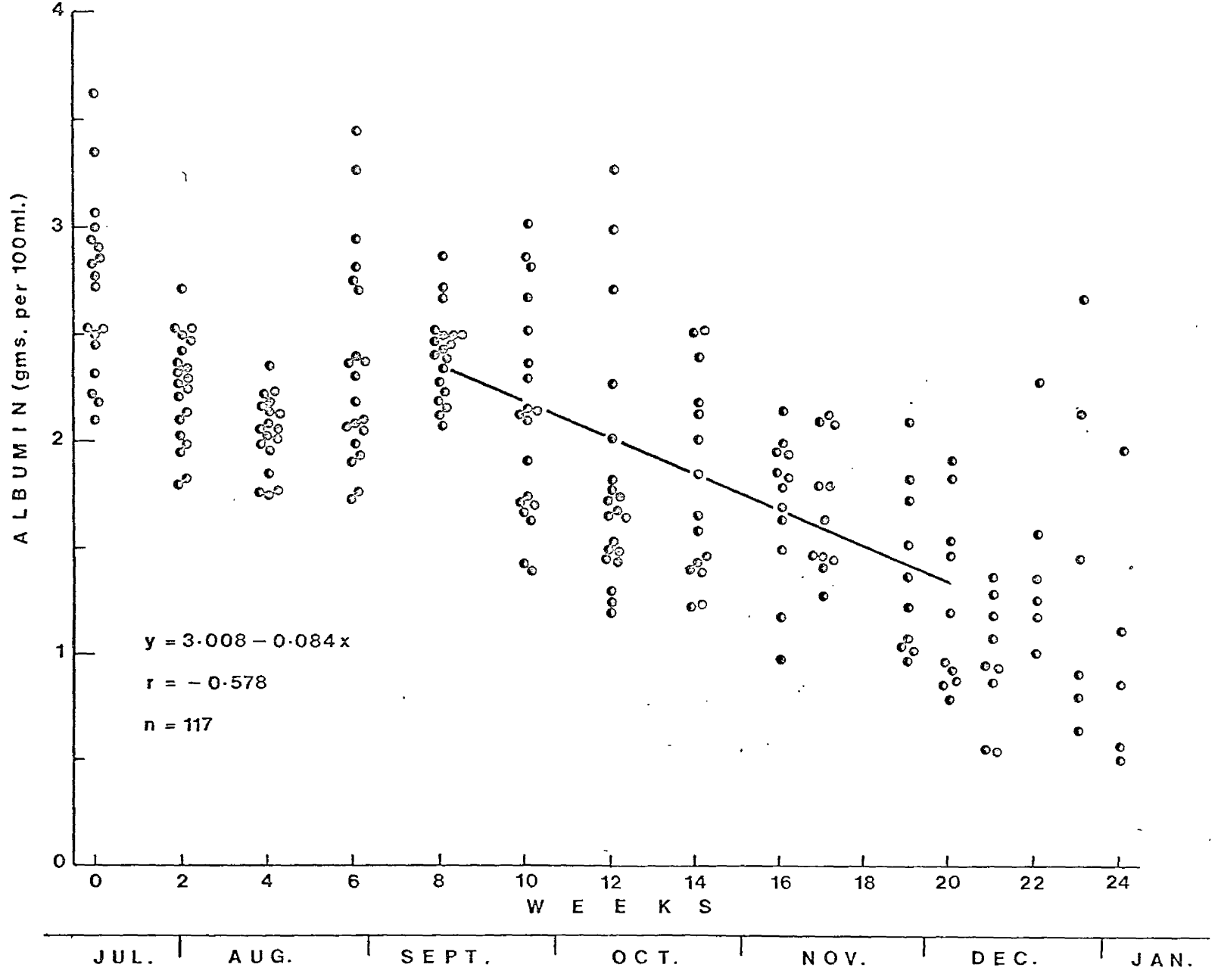

Fig. 43. The alterations in serum albumin levels of lambs during the course of a severe outbreak of fascioliasis.

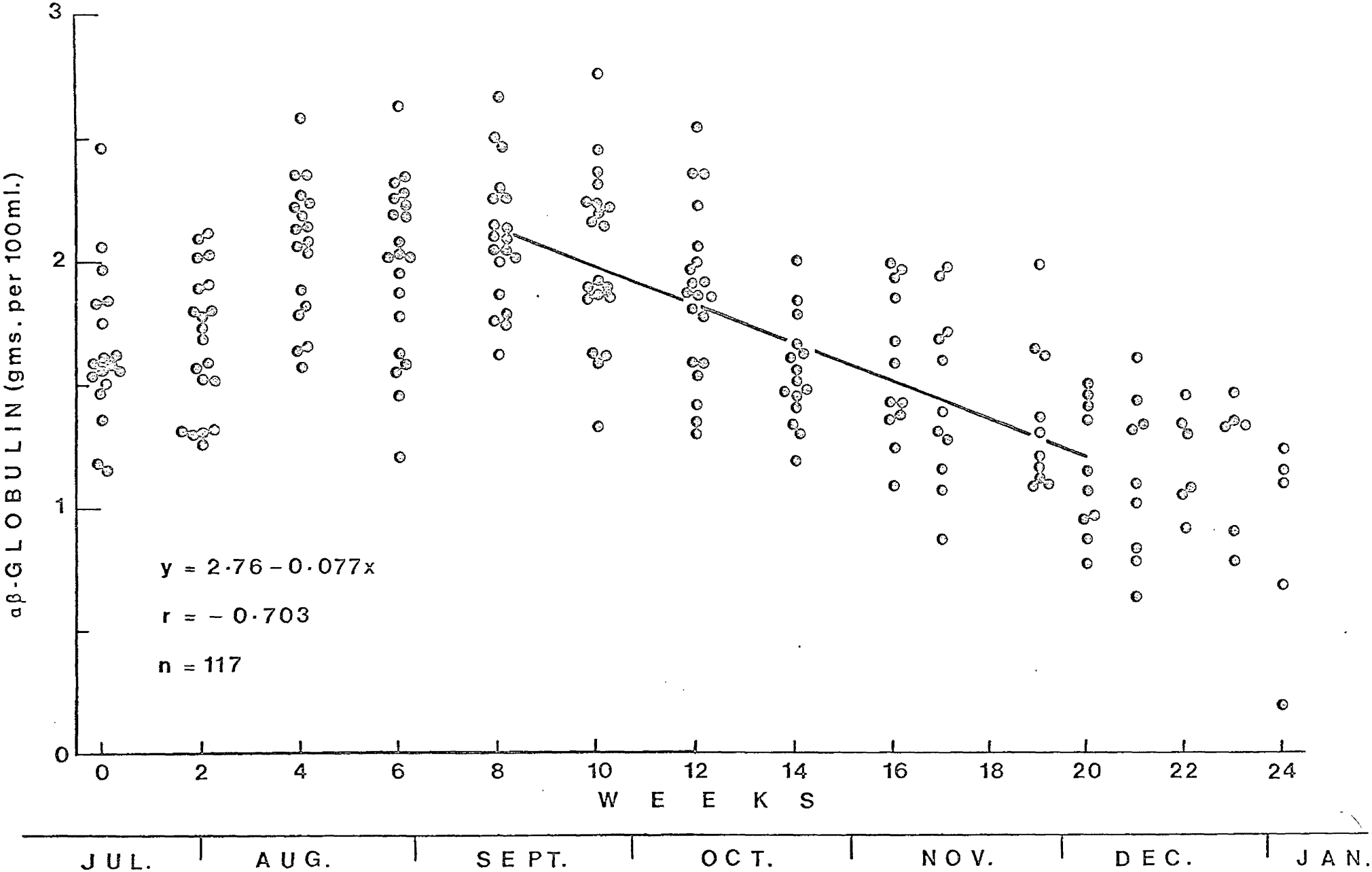

Fig. 44. The alterations in the serum alpha/beta globulin levels of lambs during the course of a severe outbreak of fascioliasis.

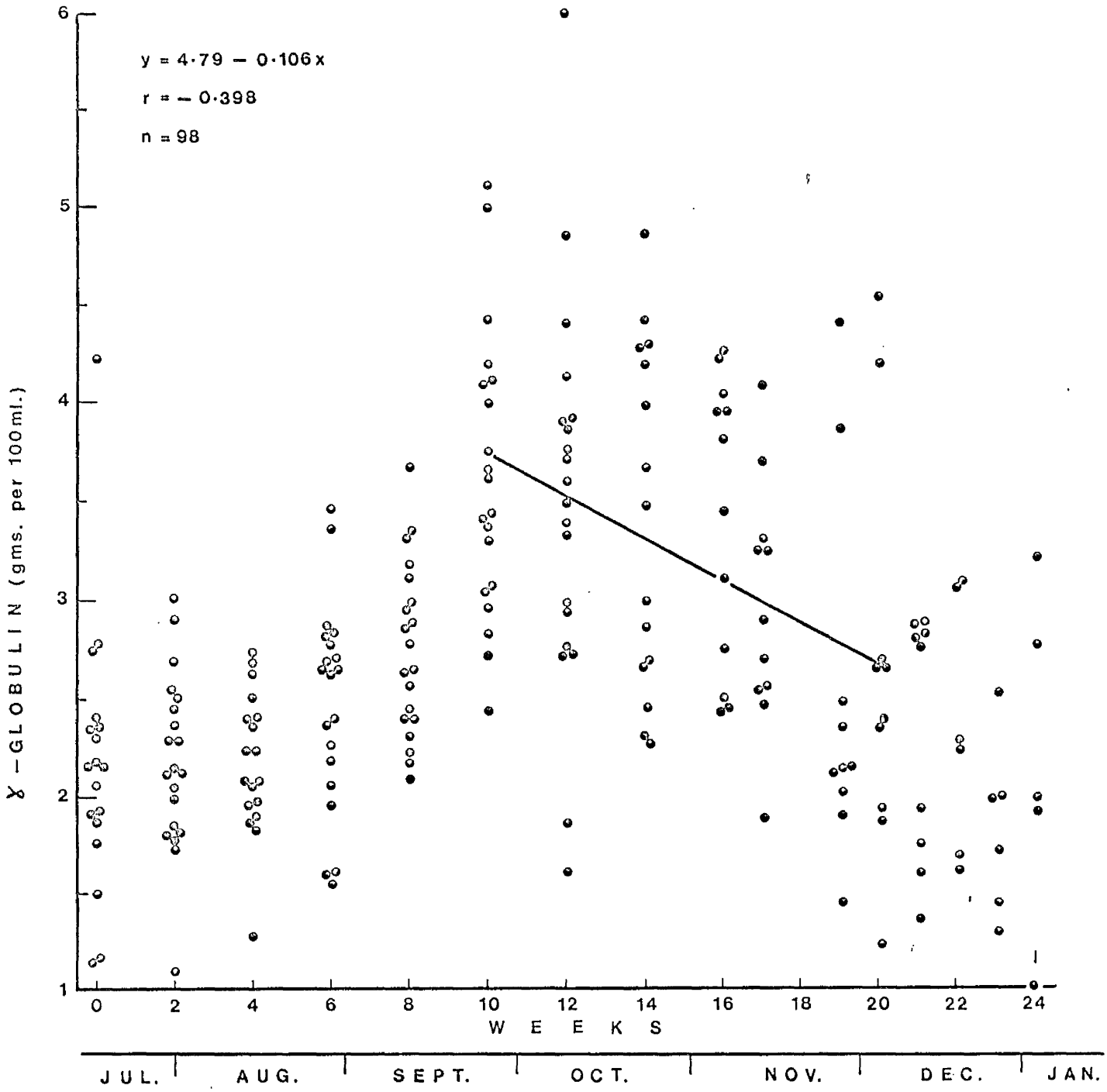

Fig. 45. The alterations in serum gamma-globulin levels of lambs during the course of a severe outbreak of fascioliasis.

time the albumin:globulin ratio remained low although slight variations occurred from week to week. Individual values are given in Appendix 4, Table 14.

The mean values for total serum protein, serum albumin, albumin:globulin ratio, alpha/beta globulin, gamma globulin and total globulin recorded between July 1967 and March 1968 are given in Tables 25 and 26, whilst the mean terminal values for lambs dying in each month between October 1967 and March 1968 are summarised in Table 27, and individual terminal values are recorded in Appendix 4, Table 15.

Parasitological Data

Fluke eggs were first recorded in the faeces of one lamb during the last week of September. This initial appearance of eggs in the faeces was followed by a rapid increase in the faecal egg count which was positive in all lambs by the last week of October. The increase in faecal egg count was variable in degree and not progressive as faecal egg counts in any one animal increased or decreased in an irregular pattern from week to week. The faecal egg counts were generally of a low order and seventy per cent of the counts recorded were of 500 e.p.g. or less although on one occasion a count of 5,900 e.p.g. was recorded. There was no correlation between the faecal egg count and the number of bile duct stages of F. hepatica present. Details of individual faecal egg counts are given in Appendix 4, Table 16, whilst the course of the mean fluke faecal egg count is shown in Figure 46.

The total numbers of F. hepatica recovered from each lamb at autopsy

Table 25

The Alterations in Mean Total Serum Protein, Serum Albumin and Albumin: Globulin Ratio of Lambs (R 81 to R 100 inclusive) Grazing at Brocklees Farm, July 1967 to March 1968

Date	No. of lambs remaining in group	Tot.Protein (gms/100 ml.) Mean	S.E.	Albumin (gms/100 ml.) Mean	S.E.	A/G Ratio Mean	S.E.
19/7	19	6.5	0.07	2.72	0.10	0.75	0.05
1/8	19	6.1	0.10	2.22	0.06	0.59	0.03
16/8	20	6.3	0.09	2.03	0.04	0.48	0.01
29/8	20	6.8	0.10	2.37	0.11	0.55	0.05
12/9	19	7.3	0.11	2.41	0.05	0.50	0.02
26/9	19	7.8	0.14	2.13	0.11	0.39	0.03
11/10	20	7.2	0.23	1.83	0.13	0.35	0.04
25/10	15	7.0	0.27	1.94	0.19	0.41	0.05
9/11	12	6.7	0.23	1.71	0.09	0.35	0.03
16/11	10	6.2	0.30	1.69	0.09	0.40	0.03
29/11	11	5.2	0.38	1.39	0.13	0.40	0.04
6/12	10	5.0	0.84	1.24	0.13	0.31	0.03
13/12	9	4.4	0.39	0.98	0.10	0.30	0.03
20/12	6	5.0	0.42	1.45	0.19	0.42	0.05
27/12	6	4.5	0.51	1.42	0.34	0.47	0.10
3/1	5	4.3	0.50	1.11	0.30	0.34	0.08
10/1	2	5.3	-	1.72	-	0.48	-
24/1	2	5.6	-	1.28	-	0.30	-
7/2	2	4.9	-	1.19	-	0.32	-
21/2	2	5.2	-	1.27	-	0.33	-
6/3	2	4.7	-	1.16	-	0.29	-
28/3	2	5.0	-	1.14	-	0.28	-

Table 26

The Alterations in Mean Serum Alpha/Beta Globulin, Gamma-Globulin and Total Globulin of Lambs (R 61 to R 100 inclusive) Grazing at Brockleas Farm, July 1967 to March 1968

Date	No. of lambs remaining in group	αβ-Globulin (gms/100 ml.) Mean	S.E.	g-Globulin (gms/100 ml.) Mean	S.E.	Tot. Glob. (gms/100 ml.) Mean	S.E.
19/7	19	1.65	0.07	2.17	0.16	3.82	0.17
1/8	19	1.68	0.06	2.18	0.10	3.86	0.12
16/8	20	2.06	0.07	2.18	0.09	4.24	0.09
29/8	20	1.99	0.08	2.47	0.14	4.46	0.13
12/9	19	2.11	0.06	2.74	0.10	4.85	0.10
26/9	19	2.04	0.08	3.63	0.16	5.68	0.19
11/10	20	1.88	0.08	3.50	0.22	5.38	0.27
25/10	15	1.56	0.06	3.43	0.22	5.01	0.21
9/11	12	1.59	0.09	3.41	0.21	4.94	0.21
16/11	10	1.46	0.11	2.97	0.19	4.44	0.26
29/11	11	1.38	0.10	2.50	0.29	3.67	0.54
6/12	10	1.16	0.09	2.66	0.32	3.80	0.38
13/12	9	1.13	0.11	2.32	0.21	3.45	0.31
20/12	6	1.20	0.08	2.33	0.26	3.54	0.34
27/12	6	1.20	0.11	1.83	0.18	3.04	0.52
3/1	5	0.88	0.20	2.18	0.38	3.06	0.71
10/1	2	1.15	-	2.43	-	3.58	-
24/1	2	1.44	-	2.83	-	4.34	-
7/2	2	1.18	-	2.50	-	3.62	-
21/2	2	1.33	-	2.56	-	3.89	-
6/3	2	1.30	-	2.25	-	3.54	-
28/3	2	1.38	-	2.48	-	3.86	-

Table 27

The Terminal Blood Biochemistry Values of Lambs Dying in Each Month between October 1967 and March 1968 as a Result of Naturally Acquired Fascioliasis

		No. of Lambs	Total Protein (gms/100 ml.)	A/G Ratio (gms/100 ml.)	Albumin (gms/100 ml.)	α6 Globulin (gms/100 ml.)	α-Globulin (gms/100 ml.)	Tot. Globulin (gms/100 ml.)
October 1967	Mean	6	8.1	0.30	1.73	1.75	4.49	6.24
	S.E.		0.75	0.05	0.19	0.12	0.79	2.79
November 1967	Mean	7	5.7	0.23	1.05	1.33	3.29	4.62
	S.E.		0.61	0.02	0.14	0.14	0.46	0.50
December 1967	Mean	11	4.5	0.30	0.94	1.07	2.45	3.52
	S.E.		0.37	0.03	0.10	0.07	0.30	0.30
January 1968	Mean	3	4.0	0.23	0.62	1.01	2.09	3.10
	S.E.		0.46	0.06	0.08	0.17	0.54	0.70
March 1968	Mean	2	4.6	0.26	0.99	1.23	2.39	3.61
	S.E.		1.40	0.07	0.47	0.23	0.70	0.93

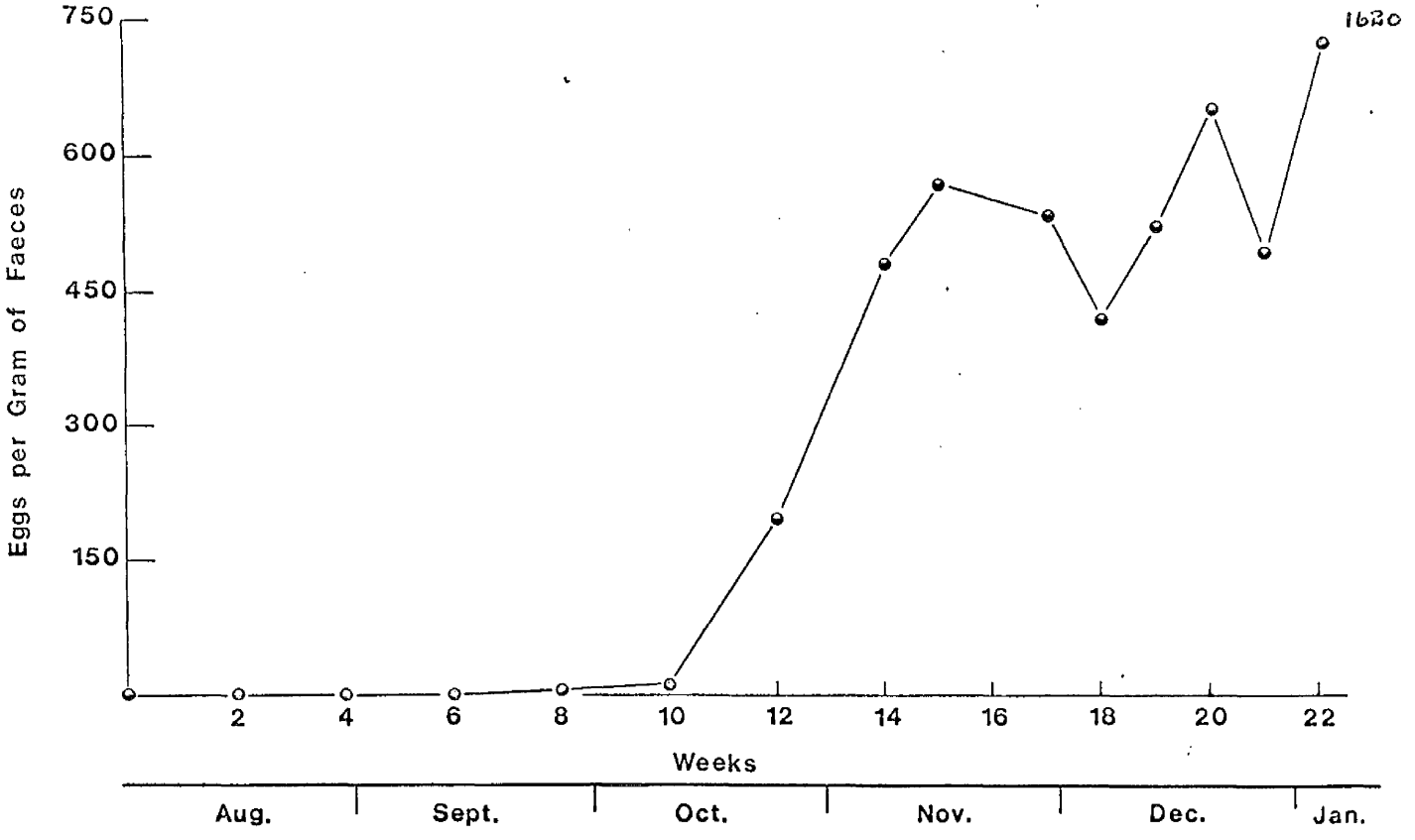

Fig. 46. The mean fluke faecal egg counts of lambs grazing at Brocklees Farm.

was variable and ranged from 110 to 1628. From October through to December there was an increase in the mean total of flukes recovered from the lambs. Although in several cases very low numbers of flukes were recovered, particularly from those animals dying earliest, it must be remembered that, due to the predominantly immature burden present, recovery of very small flukes proved very difficult and that the low numbers recorded may be considerably underestimated. There was not any significant alteration in the proportions of flukes less than 6 mm. in length or between 6 mm. and 12 mm. in length over the period October 1967 to January 1968 but there was a marked increase in the proportion of flukes over 12 mm. in length in lambs autopsied during December and January. Thus, the mean percentage of flukes over 12 mm. in length recovered in October and November was 10.4% and 11.8% respectively whilst the mean percentage for December and January was 25.1% and 19.3% respectively. The mean percentage of flukes over 12 mm. in length recovered in March was 87.0%.

Individual liver weights, terminal faecal egg counts and details of the numbers and sizes of F. hepatica recovered at autopsy are shown in Appendix 4, Table 17, whilst mean values for lambs dying in each of the months between October 1967 and March 1968 are summarised in Table 28.

Although thiabendazole was administered to the lambs every fourth week a few parasitic nematodes were recovered at autopsy. In every case strongyle faecal egg counts remained negative for three weeks following

Table 28

The Mean Liver Weights (gms.) of, and the Mean Numbers and Size Distribution of F. hepatica recovered from, Lambs Dying in Each Month between October 1967 and March 1968, as a Result of Naturally Acquired Fascioliasis

		No. of Lambs	Fluke e.p.g. at P.M.	Liver Weight (gms.)	Number of F. hepatica				
					Total	<6 mm.	6 - 12 mm.	>12 mm.	%>12 mm.
October 1967	Mean	9	121	-	440	266	145	51	10.4
	S.E.		93	-	109	79	43	24	2.8
November 1967	Mean	9	581	1229	768	308	371	89	11.8
	S.E.		173	85	127	54	75	23	2.5
December 1967	Mean	11	850	1247	913	333	351	229	25.1
	S.E.		313	37	77	52	26	38	2.9
January 1968	Mean	3	2400	1222	634	341	193	125	19.3
	S.E.		418	91	111	51	51	47	4.8
March 1968	Mean	2	1425	875	414	0	54	360	87.0
	S.E.		725	35	36	-	0.5	36	1.3

treatment and although a few eggs were found in the faeces four weeks after treatment the lambs were immediately given another dose of anthelmintic.

Discussion

The outbreak of fascioliasis described was adequately predicted by an analysis of the prevailing climatic factors after the method of Ollerenshaw and Rowlands (1959). These authors selected the sum of Mt values of 300 and 400 as being significant and where the sum of Mt values was less than 300 little or no disease should occur, whilst the sum of Mt values in excess of 400 indicated that the disease should be prevalent. In the present experiment the sum of Mt values for August, September and October, 1966 and May and June 1967 was 351, thus indicating that only occasional losses from "winter infection" might be expected. "Winter infection" is derived from metacercariae overwintered in the snail or on the grass resulting in animals becoming infected in late spring and early summer. The sum of Mt values from May to October 1967, however, was 451 thus indicating that a high incidence of the disease resulting from "summer infection" was likely to occur. "Summer infection" develops from eggs put out in the late spring and early summer which result in infection developing in the snail throughout the summer and producing metacercariae on the grass during the late summer and autumn. The value of 451 is not too far removed from the value of 474 recorded in Wales over a similar period in 1954 (Ollerenshaw and Rowlands, 1959), when the disease assumed epidemic proportions. It is interesting that the incidence of

fascioliasis over the winter of 1967/68 as predicted by the Ministry of Agriculture, was estimated as low and this illustrates the difficulty in forecasting the incidence of the disease over Great Britain as a whole where climatic conditions can vary markedly from one district to another.

In the present experiment the outbreak of fascioliasis commenced in October when clinical signs were first observed. This was preceded by the appearance of fluke eggs in the lambs' faeces during the last week of September and the outbreak is compatible with a "summer infection" picked up from late July onwards. The majority of animals, after becoming clinically affected, survived for several weeks and the main clinical signs observed were weight loss and pallor of visible mucous membranes. The occurrence of submandibular oedema and ascites was very infrequent and the clinical syndrome observed in these animals was very similar to the subacute fascioliasis described by Soulsby (1965) and Ross, Dow and Todd (1967). These authors state that submandibular oedema and ascites are mainly features of acute or chronic fascioliasis. The lack of success in palpating an enlarged liver in the present experiment was a result of the tense abdominal musculature together with frequently extensive adhesions between the liver and surrounding organs.

The severe anaemia which developed in the lambs was characterised by hypochromia, a macrocytosis and a marked reticulocytosis. In this respect, therefore, it is very similar to the anaemia observed in the rabbit (Urquhart, 1955) and the rat (Thorpe, 1963) but the character differs from previous accounts in sheep. Sinclair (1962, 1964) in his studies on the anaemia in

sheep concluded that it was of the normochromic, normocytic type but examination of his data in the first of these two papers suggests a macrocytosis in one animal. In both his investigations Sinclair omitted to stain for reticulocytes. Ross (1967 a) recorded a macrocytic, normochromic anaemia in sheep but he also omitted to stain immature red blood cells although in a later publication (Ross 1967 b) he included this technique and Giemsa stained smears revealed large numbers of erythroblasts in one animal dying of subacute fascioliasis. Ross, Dow and Todd (1967) also described a macrocytic, normochromic anaemia in sheep but large numbers of circulating erythroblasts were observed in animals dying from the acute disease. In the present experiment erythroblasts defined as "spherical cells with spherical nuclei" (Maximov and Bloom, 1957) were not observed and the reticulocytes described were non-nucleated cells which, when supravitally stained with brilliant cresyl-blue, contained one or more granules or a diffuse network of fibrils. A further investigation of the anaemia in sheep was carried out by Furmaga and Gundlach (1967 a) but these authors did not calculate individual H.C.V. and M.C.H.C. levels and so an accurate appreciation of the character of the anaemia cannot be achieved. However, examination of their mean values of haematocrit, haemoglobin concentration and erythrocyte counts suggests that the anaemia is of the normochromic, macrocytic type. Furmaga and Gundlach (1967 a) also omitted the staining procedure for reticulocytes.

The main disparity in the character of the anaemia of ovine fascioliasis between the present experiment and other publications involves the changes

in the mean corpuscular haemoglobin concentration. That M.C.H.C. values fell to a very marked degree was an outstanding feature in the present experiment and it is important to eliminate factors other than fascioliasis which may alter this value e.g. nutrition, other helminth parasites. Although grass was becoming scarce from the end of October onwards the lambs were still on an adequate diet and as grazing conditions deteriorated further supplementary feeding was commenced. Gruneell (1955) has shown, however, that worm-free sheep receiving a half maintenance ration show no alteration in M.C.H.C. A small nematode burden was recorded in most of the lambs in the present experiment despite the routine use of anthelmintics but their numbers were not sufficient to account for the haematological changes which occurred, particularly in the case of the M.C.H.C. (Gruneell, 1955).

Biochemical changes observed were essentially similar to those described for single experimental infections (Sinclair, 1962, 1964; this thesis). An initial increase in total protein particularly resulting from elevation of gamma-globulin levels was recorded during the migratory phase of the parasite in the single experimental infection (Section III). In the present experiment a similar rise was observed between mid-August and mid-September; this was succeeded by a reduction in all protein fractions commencing in mid and late September. It is interesting that in the present experiment a reduction in mean haematocrit was apparent only 4 to 5 weeks following the initial rise in gamma-globulin and would, therefore, infer that the anaemia commenced about the time the parasites were arriving

in the bile ducts, a similar situation to that observed in the single experimental infection. The pattern of reduction in albumin levels was essentially similar to the rate of development of the anaemia and terminal albumin values were approximately proportional to the duration of the anaemia.

The number of parasites recovered from each lamb would be expected to vary with the individual's grazing pattern and the length of time grazed. Thus there was a wide range of fluke-burden in each animal, 110 to 1628 flukes being recovered, and an increase in the mean total of flukes recovered from lambs dying from October through to December. Low fluke burdens present in lambs dying at the beginning of the outbreak may be underestimated due to the difficulty of recovering immature forms. The total fluke burdens recovered from the lambs in the present experiment were similar to those recorded by Ross (1967 a & b) also grazing lambs on infected pasture, but the latter author observed an infection which was predominantly mature whereas the present results indicate that adult forms are in the minority.

Ovine fascioliasis has been divided into four categories by Ross, Dow and Todd (1967) according to fluke burden, mean fluke length, clinical signs and haematological and biochemical findings. These categories, acute Type I, acute Type 2, subacute and chronic fascioliasis were compiled from both natural and experimental infections. The most interesting feature is that the subacute form described by these authors which clinically is similar to the present outbreak resulted in the recovery of fluke populations with a

mean length in excess of 12 mm. whilst in the present experiment at least 75% of the fluke burden measured less than 12 mm. in length. Ross, Dow and Todd (1967) make no distinction between the natural and experimental infection when describing their four types of the disease and one can only conclude that in the case of the subacute infection at least they are describing the results of single experimental infections which would produce a burden of similar age and more uniform size. It would appear that under field conditions fluke burdens are likely to be composed of a wide range of parasite size.

Only two animals survived beyond the early part of January and progressed into chronic fasciolinsis. In these lambs the anaemia was also essentially macrocytic and hypochromic with a reticulocytosis being recorded. The main difference between these animals and the majority of the lambs was recorded at post-mortem where liver weights were less than those of the lambs dying earlier and the fluke burdens present consisted almost entirely of adult parasites.

B. The Availability and Infectivity of Metacercariae of Fasciola hepatica between April 1967 and April 1968

Introduction

The results described in the first part of this section demonstrated that when susceptible sheep were allowed to graze pasture infected with metacercariae of F. hepatica and an outbreak of fascioliasis permitted to develop, serious losses occurred from October onwards. It is, therefore, apparent that, prior to October, large numbers of metacercariae must have been available on the pasture. These metacercariae could have been ingested in large numbers over a relatively short period. It was important to clarify this point because a knowledge of the relative availability of metacercariae over the grazing period before October might facilitate an appreciation of the pathogenesis of the disease and provide a basis for the elaboration of control measures. The present experiment was designed to investigate the availability and infectivity of metacercariae of F. hepatica on permanent sheep pasture on a hill farm in south-west Scotland over a period of one year from April 1967 to April 1968.

The majority of studies on the fluctuations of the infective stages of helminth parasites on pasture have involved nematode larval populations. The methods adopted have been a) to turn susceptible animals on to known infected pasture and subsequently count, at regular intervals, the numbers of infective larvae in known amounts of herbage or b) to infect animals experi-

mentally, graze them on parasite-free ground, and estimate pasture populations of larvae from samples of herbage. These techniques were used by Crofton (1949, 1952), who studied pasture populations of sheep gastro-intestinal nematodes and Thomas and Stevens (1956) and Gibson (1959, 1963) who applied similar methods to a study of Haematodirus species larvae. Gibson (1966) and Michel (1966) have also used these methods to study seasonal variations in pasture populations of infective larvae of Trichostrongylus colubriformis and Ostertagia ostertagi respectively. There are almost no reports on the use of a similar technique with reference to metacercariae of Fasciola hepatica although Ross (1967 a) describes a method where he recorded the number of mud snails, Lymnaea truncatula present in a known area of pasture. These snails were then examined and the percentage infected noted.

All the above techniques are of limited value because they only give an estimate of the numbers of infective stages available. They give no information regarding the infectivity of the larvae (i.e. their ability to become established in a susceptible host) nor do they take into account the grazing pattern of the host or variations in the length of the herbage. Taylor and Parfitt (1957) have described a method of measuring infectivity of metacercariae of F. hepatica in snail faeces by administering them to mice and Ross and O'Hagan (1966 a & b) have described a guinea-pig biological test in an attempt to assess the viability and numbers of metacercariae of F. hepatica on pasture. This latter method involves collection of grass

samples from infected pasture and feeding known amounts to guinea-pigs and although it provides an index of infectivity it suffers from the disadvantage that the sampling procedure will not necessarily replicate the grazing pattern of the host.

A third technique for measuring the pasture fluctuations of nematode larvae has been described by Tetley (1959) in sheep and Durie (1962) in cattle. A similar method was used by Armour (1967) in investigating the availability and infectivity of O. ostertagi larvae on pasture. This technique permits a group of susceptible animals to graze infected pasture for an extensive period (usually a normal grazing season) and these are accompanied by further susceptible animals which are introduced at regular intervals to graze for specified short periods. The former animals are called "permanents" whilst the latter are called "tracers" and these serve the purpose of indicating the level of infection that the "permanents" are exposed to during the period each successive group of "tracers" are at grass. A few days after removal from pasture the "tracers" are autopsied, the number of worms present counted, this figure reflecting the population of infective larvae available on the herbage during the period grazed. Ross (1967 a & b) has recently described a modified version of the above method with regard to metacercariae of F. hepatica but it involves a complex system of grazing sheep on infected pasture for various periods none of which was particularly short. As a result interpretation of results is difficult.

The general plan adopted in the present experiment was similar to

that of Tetley (1959), Durie (1962) and Armour (1967) and involved the use of both "permanent" and "tracer" sheep.

Experimental Design

Farm

The field used in this experiment was the same one described in detail in the first part of this section (Part A) and was located at Brocklees Farm, Darvel, Ayrshire.

Animals

Forty ewes and fifty lambs were put out to graze the above field in April 1967. The ewes were cast hill ewes of the Scottish Blackface breed and the lambs were cross lambs about two weeks of age. These lambs henceforth referred to as the permanent lambs were weaned in mid-July when the ewes were removed to parasite-free grazing in the grounds of the Veterinary Hospital. At the same time four of the "permanent" lambs were removed and housed to allow any flukes already established in the liver to grow to a size which would enable them to be readily seen six weeks later when they were autopsied along with five of the ewes.

The remaining forty-six "permanent" lambs were joined at weaning time (i.e. mid-July) by four "tracer" lambs (Group A), thus bringing the total number of animals on the field back to fifty. The "tracer" lambs were castrated males of the Scottish Blackface breed which had been reared indoors on concrete floored pens from one week of age. They had been

vaccinated when eight and twelve weeks of age with a combined clostridial sheep vaccine ("Clostrin", Glaxo Laboratories, Greenford, Middlesex) and had received a booster dose of black disease vaccine (Black Disease Vaccine, Glaxo Laboratories, Greenford, Middlesex) when they were fourteen weeks of age. During the entire period of the trial these "tracer" lambs were replaced monthly by further groups of four lambs (Groups B to H inclusive) which were of a similar age to the "permanent" flock. One group, Group G, remained on the field for eight weeks as snow lay on the ground for four weeks of this period. The first two groups of "tracers" were put out to graze directly from indoors whilst the others were allowed to graze on parasite-free ground for two weeks before transfer to the farm. After being brought back from the farm each group of "tracer" lambs was housed for six weeks prior to autopsy. It was also intended to remove four "permanent" lambs at the end of each third month of the trial but between October and December almost all of the "permanent" lambs developed clinical fascioliasis and many died so that this scheme had to be abandoned.

Observations

The animals were examined clinically, weighed, faecal and blood sampled before being put on to the infected field. Whilst grazing the infected field they were inspected once weekly and examined in detail, weighed and blood sampled every two weeks. After removal from the infected field the "tracers" were examined, blood and faecal sampled every two weeks until autopsy.

Results

Clinical Signs

Clinical fascioliasis was first observed in the "permanent" lambs during the first part of October and by the end of that month all but two of them were affected. The details of this outbreak have already been described in Section IV, Part A.

Clinical signs of fascioliasis were not observed in any of the "tracer" lambs whilst at grass. One lamb put outside with the first group of "tracers" in July was found dead by the farmer but nothing significant was demonstrated at autopsy. Clinical signs became apparent in the lambs which grazed from mid-August to mid-September (Group B), just prior to autopsy six weeks after leaving the farm. These animals showed a degree of weight loss and two of them developed pale mucous membranes. At no time was submandibular oedema, ascites or a palpable liver present in any of the groups of "tracer" animals. The individual bodyweights for each "tracer" animal are recorded in Appendix 4, Table 10 whilst the mean bodyweights for each group are shown in Table 29. In most cases there was little change in mean bodyweight for each "tracer" group over the period of observation apart from a marked weight increase in the lambs grazing between mid-July and mid-August.

No clinical signs of fascioliasis were observed in the "permanent" lambs prior to weaning when four of these lambs were removed and housed for 6 weeks prior to autopsy but a mild outbreak of nematodiriasis had occurred in the "permanent" animals in late May and early June.

Table 29

Mean Bodyweights (lbs.) of Tracer Lambs at Brooklees Farm, July 1967 to March 1968

	Date	Mean	S.E.		Date	Mean	S.E.
GROUP A	18/7	38.6	0.9	GROUP E	16/11	57.8	4.5
	1/8	40.0	0.6		29/11	59.5	4.7
Grazed 19/7/67	16/8	42.0	1.2	Grazed 16/11/67	13/12	60.0	6.1
to 16/8/67	31/8	49.3	0.7	to 13/12/67	29/12	56.5	4.0
Housed	14/9	58.0	1.5	Housed	12/1	61.5	3.4
thereafter	27/9	62.3	1.2	thereafter	26/1	66.5	3.7
GROUP B	16/8	54.5	1.2	GROUP F	14/12	70.3	2.3
	29/8	51.0	2.7		27/12	72.5	2.3
Grazed 16/8/67	12/9	51.3	2.0	Grazed 13/12/67	10/1	67.0	0.9
to 20/9/67	28/9	53.3	3.4	to 18/1/68	19/1	69.3	0.4
	12/10	53.3	3.0		2/2	69.3	0.6
Housed	23/10	49.8	3.4	Housed	16/2	68.8	0.6
thereafter	30/10	48.0	3.4	thereafter	1 /3	67.3	1.7
GROUP C	20/9	67.8	1.1	GROUP G	18/1	68.5	4.0
	4/10	67.8	1.3		2/2	67.0	3.1
Grazed 20/9/67	18/10	67.8	1.7	Grazed 18/1/68	21/2	67.5	3.5
to 16/11/67	30/10	69.8	1.3	to 13/3/68 *	13/3	66.0	2.2
	13/11	71.8	1.3		28/3	64.5	2.3
Housed	27/11	72.5	1.6	Housed	10/4	65.3	1.9
thereafter				thereafter	25/4	65.5	1.8
GROUP D	18/10	72.3	2.5	GROUP H	11/3	68.0	0.7
	1/11	72.0	2.8		25/3	66.5	1.7
Grazed 18/10/67	15/11	74.3	3.8	Grazed 13/3/68	8/4	67.0	1.9
to 16/11/67	27/11	73.5	3.6	to 8/4/68	22/4	73.3	2.7
Housed	11/12	68.3	4.2	Housed	10/5	79.3	2.3
thereafter	28/12	68.0	5.1	thereafter	21/5	85.3	3.0

* Snow on ground for 4 weeks of this period

Haematological Data

There was little significant alteration in the haematology values of the majority of groups of "tracer" lambs. The only group to show any marked change was Group B which grazed from mid-August to mid-September. These animals developed a mild to moderate anaemia which only became apparent after they were housed. The mean packed cell volume of this group fell from 36.4 ± 1.36 per cent at the commencement of grazing to 23.0 ± 1.50 per cent at autopsy some 10 weeks later. A drop in the mean packed cell volume also occurred in Groups C & D, i.e. in those groups grazing between mid-September to mid-October and mid-October to mid-November where in the former case the P.C.V. fell from 39.6 ± 1.51 per cent at the commencement of grazing to 32.5 ± 1.04 per cent at autopsy whilst in the latter case P.C.V. fell from 44.4 ± 1.68 per cent to 34.5 ± 2.25 per cent over a similar period. Individual values of P.C.V. for each "tracer" group are given in Appendix 4, Table 19.

The alterations in total erythrocyte count and haemoglobin concentration for each "tracer" group were approximately proportional to the changes in packed cell volume. As a result the mean total red cell count of Group B fell from 12.68 ± 0.66 million per cu.mm. at the commencement of grazing to 8.07 ± 0.31 million per cu.mm. at autopsy 10 weeks later, whilst the haemoglobin concentration fell from a mean value of 12.4 ± 0.56 gms. per 100 ml. to 7.3 ± 0.45 gms. per 100 ml. over a similar period. The Group C "tracers" showed a drop in mean total red cell count from a pre-infection figure of

12.71 ± 0.65 million per cu.mm. to 11.15 ± 0.49 million per cu.mm. at autopsy and a fall in mean haemoglobin concentration from 14.6 ± 0.87 gm. per 100 ml. to 10.6 ± 0.42 gm. per 100 ml. over the same period. The "tracers" in Group D showed a fall in mean red cell count from 16.61 ± 1.27 million per cu.mm. to 12.59 ± 0.31 million per cu.mm. given pre-infection to autopsy and a drop in mean haemoglobin concentration from 16.0 ± 0.32 gm. per 100 ml. to 10.3 ± 0.96 gm. per 100 ml. over this period.

Individual values for haemoglobin concentration and total red cell count for each group of "tracer" lambs are given in Appendix 4, Tables 20 and 21.

There were no significant changes in mean corpuscular volume or mean corpuscular haemoglobin concentration in any of the "tracer" groups and individual values are given in Appendix 4, Tables 22 and 23.

Reticulocytes did not appear in the peripheral circulation of any of the "tracer" animals nor were there any significant alterations in total white cell counts.

The four "permanent" lambs removed at weaning time in mid-July showed no significant changes in any of the haematological indices.

Biochemical Data

In all groups of "tracers" there was an increase in the mean level of total protein commencing during the first two weeks of grazing. This

initial increase was followed by a decrease in the mean total protein level in Groups B, C and D, whilst the increase continued in the other groups. This fall in mean level was only apparent 4 weeks after housing. The alterations in both individual and mean total protein values for each "tracer" group are given in Appendix 4, Table 24.

Serum albumin levels remained fairly constant and in only Groups B and D was there a slight fall in mean values and then only commencing 6 to 8 weeks after the start of grazing. Groups B and D were to prove to be carrying the largest mean fluke burden. Individual serum albumin levels are given in Appendix 4, Table 25.

In all groups the albumin:globulin ratio fell during the grazing period but on housing the ratio increased only to fall again during the ensuing 2 to 6 weeks. These changes are recorded in Appendix 4, Table 26.

An increase in group mean gamma-globulin levels was observed in all "tracer" groups commencing during the first two weeks after introduction to the infected field and these levels remained elevated until autopsy although in a few cases they were beginning to fall. The pattern of change of both the group mean and individual gamma-globulin levels is given in Appendix 4, Table 27. The changes in alpha/beta globulin were not significant and are recorded in Appendix 4, Table 28.

The four "permanent" lambs removed at weaning time in mid-July showed no significant changes in biochemical values.

Parasitological Data

The mean faecal egg counts of the "permanent" lambs are shown in Figure 47 and they are accompanied by a histogram illustrating the mean numbers of F. hepatica picked up by each "tracer" group per week of the grazing period. It is apparent that metacercariae were ingested throughout the observation period although the majority were picked up between July and the following January, and by far the greatest number were ingested by Group B grazing from mid-August to mid-September. It is also apparent that the marked increase in faecal egg count seen in the "permanent" lambs during the latter part of October resulted from infection picked up between mid-August and mid-September. Figure 48 illustrates the relationship between the numbers of F. hepatica established in the "tracer" animals and the mean monthly burdens and size distribution of F. hepatica recovered from the "permanent" lambs dying as a result of fascioliasis.

The numbers of F. hepatica recovered from members of the same "tracer" group showed a large variation. For instance, the Group B "tracers", which picked up the largest number of flukes, had individual totals ranging from 91 to 379 flukes and examination of the other groups reveals that the animal with the greatest fluke burden in each group is carrying 2½ to 4 times the number of parasites present in the animal carrying the lightest infection in that same group. The numbers and size distribution of F. hepatica recovered from individual "tracer" animals at post-mortem are given in Appendix 4, Table 29.

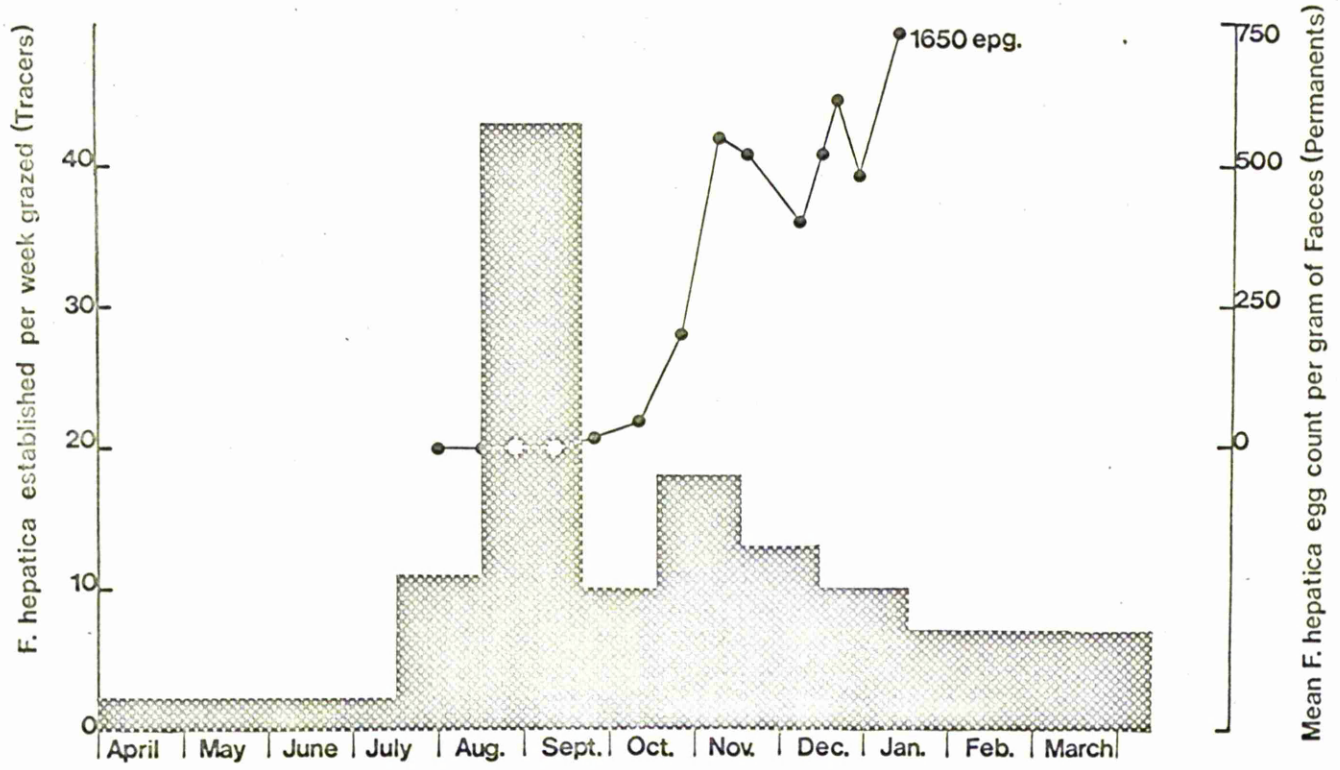

Fig. 47. The mean numbers of Fasciola hepatica established per week of grazing by "tracer" lambs and the mean fluke faecal egg counts of the "permanent" lambs.

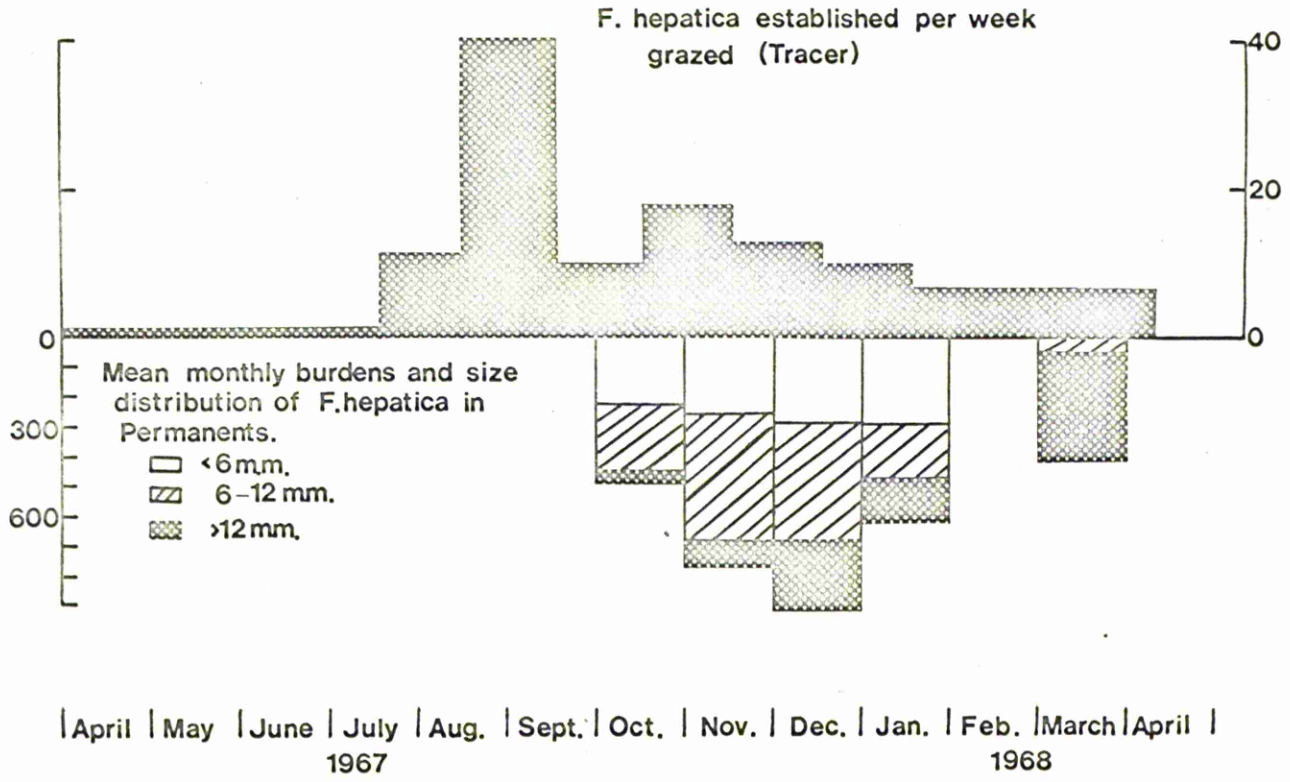

Fig. 48. The mean numbers of Fasciola hepatica established per week of grazing by "tracer" lambs and the mean number and size distribution of F. hepatica recovered from the "permanent" lambs dying in each month between October and the following April.

The four "permanent" lambs removed at weaning time had very low fluke burdens and the numbers recovered ranged from 2 to 22 with a mean of 14 ± 4 flukes. This represents a mean uptake of less than 2 flukes per week during the period mid-April to mid-July.

The fluke burdens found in the 5 ewes which were removed from the infected field in mid-July are recorded in Table 30.

Discussion

The period of observation stretched from April 1967 to April 1968, and between these dates variable numbers of metacercariae were picked up by the grazing lambs. Although those "tracer" lambs which grazed the infected field from mid-August to mid-September ingested the greatest number of metacercariae significant numbers were also picked up by all "tracer" groups grazing between mid-July 1967 and mid-January 1968. The position prior to July is not so readily appreciated. Although the five ewes removed from the field at weaning in mid-July were carrying a fluke burden when subsequently autopsied it is unlikely that the majority of these parasites originated from the infected field. Since almost all the ewes had positive faecal egg counts at weaning time it is possible that the adult fluke burden at least was likely to have been acquired prior to grazing the experimental field. The autopsy results of the "permanent" lambs removed in mid-July support this. As these animals had developed mild, though clinically apparent, nematodirosis in late May and early June it

Table 30

The Numbers and Size Distribution of F. hepatica Recovered from Five Ewes Removed from Brocklees Farm in July 1967

Ewe No.	c.p.g. at P.M.	Number of F. hepatica			
		Total	< 6 mm.	6 - 12 mm.	> 12 mm.
1	50	74	25	23	26
2	50	129	61	27	41
3	100	212	105	74	33
4	50	100	32	26	42
5	50	36	18	0	18

can be inferred that they must have been ingesting significant quantities of grass from late May onwards and this would indicate that the fluke burden observed at autopsy of these animals resulted predominantly from the period grazed between late May and mid-July. Since the mean number of flukes recovered at autopsy was 14 it is likely that only small numbers of metacercariae were available over this period.

Since it has been established that metacercariae are present on the pasture at all times between May and the following January, with the largest number available in August and September, it is necessary to discuss the possible origin of these metacercariae. It is generally agreed that metacercariae can arrive on pasture from four sources. First, they can develop from eggs which are passed on to the pasture in the spring and whose hatch allows snails to become infected in the late spring and early summer; subsequent development within the snail permits cercariae to appear on the grass some two months later, i.e. from August onwards: Secondly, encysted metacercariae could overwinter on the grass and so survive until the following year: Thirdly, development of the parasite may be arrested within the snail allowing rediae and/or cercariae to overwinter in the intermediate host. The fourth source of infection might be the overwintered egg. According to Ollerenshaw (1959) the last source of infection may be virtually eliminated as many eggs die due to the varied conditions they are exposed to during the winter; in a later publication, however, he tended to contradict this statement (Ollerenshaw, 1964); It is most likely that the

large numbers of metacercariae available from August onwards in the present experiment developed from snails infected that same year indicating that the major source of infection was the ewes which were passing eggs during the spring; this would constitute the typical "summer infection" of Ollerenshaw (1959). A less likely explanation is that these metacercariae were derived from an overwintered infection in the snail. It is possible that large numbers of snails carrying early developmental stages overwintered successfully; development was resumed in the spring and early summer resulting in heavy pasture infection in August. Further experiments are necessary to elucidate this point.

Metacercariae ingested prior to August could have come from either overwintered cercariae on the pasture or infection which has overwintered in the snail. Ross (1967 a) states that there is very little carry-over of infection in the winter months either in the snail or on the pasture. However, Oshanova (1959) found that overwintering in the snail might be significant if the winter is mild and several authors have shown that encysted metacercariae can survive considerable changes in the environment. For instance, Ollerenshaw (1959) found they remain infective for 4 months in winter whilst Shaw (1932) found some cysts still infective after 11 months in a refrigerator and Pantelouris (1965) indicates that cysts will survive for several months in water. Both the above sources could, therefore, be responsible for infection picked up in May and June, but as encysted metacercariae prove most resistant to changes in environment, this may be the major source. It is interesting that, although prior to the start of the

experiment sheep had not grazed the experimental field since the spring of 1966, cattle had grazed this area in the autumn of that year. Although the cattle were a possible source of overwintered infections in the snail it is also probable that cattle might leave a higher proportion of metacercariae, derived from the "summer infection" of 1966, to overwinter as they do not graze the field as closely as sheep.

The numbers of F. hepatica recovered at autopsy from lambs out of the same "tracer" group was very variable and in every group the greatest number recovered from any one animal was two and a half to four times the number recovered from the animal with the lowest worm burden. This difference occurs despite the fact that, as well as following the grazing pattern of the "permanent" lambs, the "tracer" animals tend to graze close together. These observations indicate that either variation in the numbers of metacercariae exists over relatively small areas or that natural resistance varies greatly in individual animals. This latter suggestion may be the reason for the difference in percentage takes recorded in the animals described in Section III. If the former is the case it would appear that a guinea-pig biological test such as that described by Ross and O'Hagan (1966 a & b) as well as being of limited value in estimating numbers of metacercariae on pasture may not necessarily give an accurate estimate of the uptake by grazing animals.

General Discussion

The results presented in this section demonstrate the relationship between pasture populations of metacercariae of F. hepatica and an outbreak of clinical fasciolíasis in sheep. There is a clear indication of a period of maximum uptake of metacercariae i.e. mid-August to mid-September, resulting in the occurrence of clinical disease from October onwards.

One of the most striking features of the clinical syndrome observed in the "permanent" lambs was the complete absence of the acute fasciolíasis of textbook description; this despite the fact that there was 100 per cent morbidity in the group of "permanent" lambs and also 100 per cent mortality if one excludes the treated animals. Acute fasciolíasis is of sudden onset and usually results in the death of the animal often without prior clinical signs, although these may be present for a few days before death (Clunies, Ross and Gordon, 1936; Morgan and Hawkins, 1953 Lapage, 1956; Ross, Dow and Todd, 1967). Gordon (1955 a) points out there may be some confusion with black disease. In the present experiment all the animals survived for several weeks after the onset of clinical signs and sudden, unexpected deaths were not observed.

It has already been shown that the high incidence of clinical disease which occurred was predicted by calculating Mt values using the formulae of Ollerenshaw (1959). The Mt value for the "summer infection" of 1967 was 451, a substantial way above the critical figure of 400, indicating that outbreaks

of disease would be prevalent. Calculation of the Mt values at Brockloes Farm for the previous two years (Table 31) illustrates that climatic factors were even more favourable for the development of the disease in 1965 and 1966. Thus it is interesting that although there was a high incidence of fascioliasis in the present experiment under ideal climatic conditions, acute fascioliasis did not occur. As a result one wonders just how often acute fascioliasis, of an uncomplicated nature, appears under natural conditions.

Most of the textbook reports on fascioliasis refer to its occurrence in "sheep", no reference being made as to whether the term "sheep" means lambs or adults. However, examination of these reports leads to the conclusion that the clinical descriptions apply to adult animals. Thus, there is possibly a difference in the clinical disease depending on the age of the animal and whether it has been exposed to infection with F. hepatica in its earlier life.

During October 1967 a group of rams and cast ewes of the Scottish Blackface breed, grazing a field contiguous to the experimental field, developed fascioliasis. These animals were being treated monthly with various anthelmintics including carbon tetrachloride, hilomid and oxyclozanide but despite this several animals suddenly became anorexic and developed marked ascites and in some cases submandibular oedema; death occurred within a few days of the onset of clinical signs. None of these animals had a gross anaemia and only a few developed a moderately severe anaemia.

Table 31

Mt* Values from Brockleas Farm, Darvel, Ayrshire

	1965	1966	1967
Winter infection Aug., Sept., Oct., May - June	378	398	351
Summer infection May - October	500	466	451

* Critical Mt = 400

At post-mortem no adult flukes were found and only a small number of immature stages were recovered.

It was thus apparent that clinical fascioliasis in sheep grazing on the same farm under almost identical environmental conditions was different in adult sheep and lambs. The adult animals had been exposed to metacercariae of F. hepatica over several years and one wonders if this provides the main reason for the different clinical syndromes observed. Thus the acute syndrome recorded in adult sheep may be the result of a severe infection superimposed on a liver already cirrhotic from previous infection or alternatively may be associated with an immune or hypersensitive response (Lang, 1967). Another possibility is that regular anthelmintic treatment of the adult sheep had produced an altered pathogenesis.

Summary

1. In April, a group of fifty lambs and their dams were put out to graze permanent pasture known to have been responsible for outbreaks of fascioliasis during the previous two years. The lambs were weaned in July when the ewes were removed. Clinical fascioliasis developed in the lambs, being first apparent during the early part of October. Between October and the following March, forty of the lambs died, the remaining ten animals having been treated. None of the lambs died suddenly and all the animals dying between October and early January presented a subacute syndrome whilst two lambs dying at the end of March did so as a result of chronic fascioliasis.

The principal clinical signs recorded were weight loss, dullness, pallor of visible mucous membranes and, in many cases, resentment of palpation of the anterior abdomen. In only a minority of cases was submandibular oedema or ascites present.

An anaemia developed in almost all the lambs; this was of the macrocytic, hypochromic type and reticulocytes were observed in the peripheral circulation. The degree of anaemia at death was variable and appeared to be associated with the rate of development of the anaemia, i.e. the P.C.Vs. of lambs which died soon after exposure fell very rapidly but their terminal values were higher than lambs which survived longer and whose P.C.V. fell more slowly to a lower terminal value. Following an initial elevation in total serum protein levels, reduced levels were

recorded; the elevation resulted from an increase in the globulins, particularly gamma-globulin, whilst the subsequent reduction was due to a fall in both serum albumin and globulin levels. At death a hypo-albuminaemia was always present and in many cases there was a frank hypoproteinaemia.

The total number of flukes recovered from the lambs ranged from 110 to 1628. The percentage of immature stages present became progressively reduced in each succeeding month from October onwards until by March the majority of flukes present were adult.

2. Observations on the pasture populations of metacercariae of F. hepatica over one year from April to April were carried out using the fluke burden at necropsy of "tracer" lambs, which grazed for 4 or 5 weeks, to quantitate the level of herbage infections. Metacercariae were picked up throughout the period of observation but the maximum number was recovered from those "tracer" lambs grazing between mid-August and mid-September.

SECTION V

THE USE OF NITROXYNIL IN OVINE FASCIOLIASIS

The Treatment of Naturally Occurring Disease

Introduction

Since the discovery of the anthelmintic efficiency of carbon tetrachloride in 1926, many drugs have been described with activity against Fasciola hepatica. The majority of these suffer from two main disadvantages; first, they may prove toxic at the recommended therapeutic dose and secondly, their activity against immature stages of the parasite is very poor. It is not intended to discuss these various anthelmintics here as there is a vast literature on this subject and reviews have been published by Gibson (1965) and Pugh (1965) and comparative therapeutic tests in sheep with a number of anthelmintics have been reported by Doxey, Happich and Andrews (1967).

The activity of 4-cyano-2-iodo-6-nitrophenol, M & B 10,755 - nitroxynil (Trodax, May and Baker Ltd., Dagenham, England) against Fasciola hepatica was first demonstrated by Davis, Lucas, Rosenbaum and Wright (1966) and controlled trials with this compound have been described in experimental fascioliasis in rabbits, sheep and calves (Lucas, 1967). The latter author administered the drug by both oral and parenteral routes and found that, in sheep and calves, it was six times more potent by injection than by mouth and as the results of oral dosing showed that the ratio between the minimum effective and maximum tolerated dose was close, further use of this route in these species was not recommended. Lucas (1967) also found that, in sheep, a single subcutaneous injection of nitroxynil at a dosage rate of 10 mg. per kg. completely removed 10 week-old fluke infections, 89% of

seven-week old infections and 70% of six-week old infections, whilst the maximum tolerated dose in sheep carrying a natural infection was 40 mg. per kg. Further tolerance studies on nitroxynil in naturally infected sheep were conducted by Colegrave (1968a) who confirmed the findings of Lucas (1967) and found high efficiency and good tolerance of subcutaneous administration dose at 10 mg. per kg. and 20 mg. per kg. Colegrave (1968a) reported that nitroxynil at 10 mg. per kg. virtually arrested mortality in a naturally occurring outbreak of fascioliasis and he pointed out that although only a small proportion of immature flukes would be killed at this level it nevertheless provided a useful therapeutic effect. The efficiency of nitroxynil at the dose level of 10 mg. per kg. was also described in calves by Colegrave (1968b).

Although Lucas (1967) based his evaluation of the drug's efficiency on its ability to reduce parasite burdens as judged by reduction of fluke numbers at autopsy, Colegrave (1968a) based his interpretation solely on absence of or reduced mortality and elimination of faecal egg output. Neither of these authors recorded details of clinical improvement post-treatment with particular reference to the persistence of this improvement and to how rapidly the haematological and biochemical indices return to normal.

This section of the thesis describes the efficiency of nitroxynil in the treatment of an outbreak of fascioliasis in sheep grazing under natural conditions and records the changes taking place in clinical condition, haematological, biochemical and parasitological findings.

Materials and Methods

Animals

The animals used in this experiment were cross Scottish Blackface lambs approximately seven months of age, and were the survivors of those animals described in Section IV, Part A. These lambs had grazed a fourteen acre field since April 1967; this was known to be infected with metacercariae of F. hepatica. They were accompanied by their dams from that date until July, 1967. From July onwards the lambs were left grazing by themselves and during the second week of October deaths due to fasciolíasis began to occur. Between this point and the first week of December a total of 30 animals in a group of 50 died all showing clinical, haematological, biochemical and pathological changes consistent with severe F. hepatica infection. At post mortem all of the thirty lambs had a liver which was grossly enlarged and exhibited many fluke tracts and haemorrhagic areas throughout its substance. In most cases adhesions existed between the liver and surrounding organs. A fluke burden ranging from 110 to 1628 parasites, predominantly in an immature form, was present.

Anthelmintic

The anthelmintic used in this trial was 4-cyano-2-iodo-6-nitrophenol, M & B 10,755-nitroxynil (Trodax, May & Baker Ltd , Dagenham, England). It was used as the N-methylglucamine salt in a solution containing 20 per cent W/V active ingredient and was administered once only by the intramuscular route at a dosage rate of 10 mg. per kg.

Blood Analysis

Blood samples were collected for both haematological and biochemical estimation. The haematological values recorded were packed cell volume, haemoglobin concentration and total red cell counts; blood smears were stained and examined for reticulocytes and mean corpuscular volume and mean corpuscular haemoglobin concentration were calculated. The biochemical values recorded were total serum protein, serum albumin, alpha/beta globulin and gamma-globulin. All these values were obtained using techniques identical to those already described in general materials and methods.

Parasitological Data

Faecal egg counts were performed by the zinc sulphate flotation method already described.

Weighing Procedure

The lambs were weighed on an Avery, spring-balance, pig weigher accurate to 1 lb.

Experimental Design

Animals

Twenty lambs, the survivors of those animals described in Section IV, Part A, were divided into two groups of ten (Group A and Group B). The Group A lambs received treatment whilst the animals in Group B remained untreated and acted as controls.

The twenty lambs were observed for a period of approximately four weeks prior to Group A receiving treatment. Observations commenced on the 9th of November from which time clinical examination was carried out, blood and faecal samples were collected and the animals were weighed. These procedures took place at weekly intervals until 5 weeks post treatment when further sampling was continued at fortnightly intervals. All the animals in Group A were treated on the 8th of December.

Results

Clinical Signs

The clinical appearance of the lambs has already been described in detail (Section IV, Part A). Immediately prior to treatment the animals were weak and thin; they were unwilling or unable to move with any rapidity and when approached in the field were generally easily caught. The majority of the lambs were dull and spent little time grazing and on clinical examination visible mucous membranes, in particular the conjunctiva, showed a variable but usually marked degree of pallor. Submandibular oedema and ascites were not detected on clinical examination.

Apart from two lambs which died within twenty-four hours of treatment there was marked improvement in the remaining eight lambs in Group A within 5 days of treatment. These lambs were less lethargic and could not be approached so readily in the field and when caught they were capable of putting up some resistance. There was no appreciable change in the

appearance of mucous membranes which were still very pale. This improvement in the treated animals continued and the degree of pallor of visible mucous membranes had greatly diminished by 12 days post treatment and in the succeeding two weeks mucous membranes returned completely to normal. The clinical appearance of this group of lambs remained satisfactory for a period of approximately 13 weeks after treatment. There was only a temporary arrest of weight loss in the lambs following treatment after which time weights were either maintained or fell slightly. Individual bodyweights are given in Appendix 5, Table 1.

The untreated group (Group B) showed a progressive deterioration in condition with the majority of animals becoming weaker and duller. Mucous membranes already pale at the commencement of the experiment became even paler and at the time of death were almost white. By the end of the observation period 8 of the 10 animals in this group had died and the remaining two animals were in poor condition both dying eventually. One of these lambs developed submandibular oedema which was inconstantly present.

Haematological Data

At the commencement of the experiment the mean total red cell counts of the Group A and Group B lambs was 6.94 ± 0.52 and 7.71 ± 0.72 million per cu.mm. respectively. In the ensuing four weeks the mean red cell count of Group A fell to 4.66 ± 0.46 million and that of Group B fell to 5.25 ± 0.80 million per cu.mm. After treatment there was a rapid

increase in the total red cell count of the Group A lambs and 5 days after treatment the mean figure had risen to 6.53 ± 0.34 million per cu.mm. This trend continued and 5 weeks post-treatment the mean total erythrocyte count had increased to 10.42 ± 0.34 per cu.mm. and subsequently despite fluctuations from week to week the mean red cell count was 8.31 ± 0.40 million per cu.mm. 13 weeks after treatment. The Group B lambs showed a persistently falling red cell count and 8 weeks after the start of the experiment, i.e. approximately 4 weeks after Group A received treatment, there were only 5 lambs still surviving and they had a mean total red cell count of only 4.66 ± 1.38 million per cu.mm.

The alterations in mean total red cell count for each group are illustrated in Figure 49, and individual values are given in Appendix 5, Table 2.

The changes in packed cell volume and haemoglobin concentration in each group of lambs followed a similar pattern to those observed with the red cell count and these changes are also illustrated in Figure 49. At the start of the experiment the mean packed cell volume of Group A was 22.6 ± 1.27 per cent and that of Group B was 25.3 ± 1.55 per cent and in the course of the following 4 weeks this level fell to 16.4 ± 1.43 per cent and 16.8 ± 1.79 per cent respectively. Five days after treatment the mean P.C.V. of Group A had risen to 21.0 ± 1.15 per cent and by 5 weeks post-treatment the mean P.C.V. was 28.9 ± 1.03 per cent whilst even 13 weeks following treatment the mean P.C.V. of this group was 27.1 ± 1.26

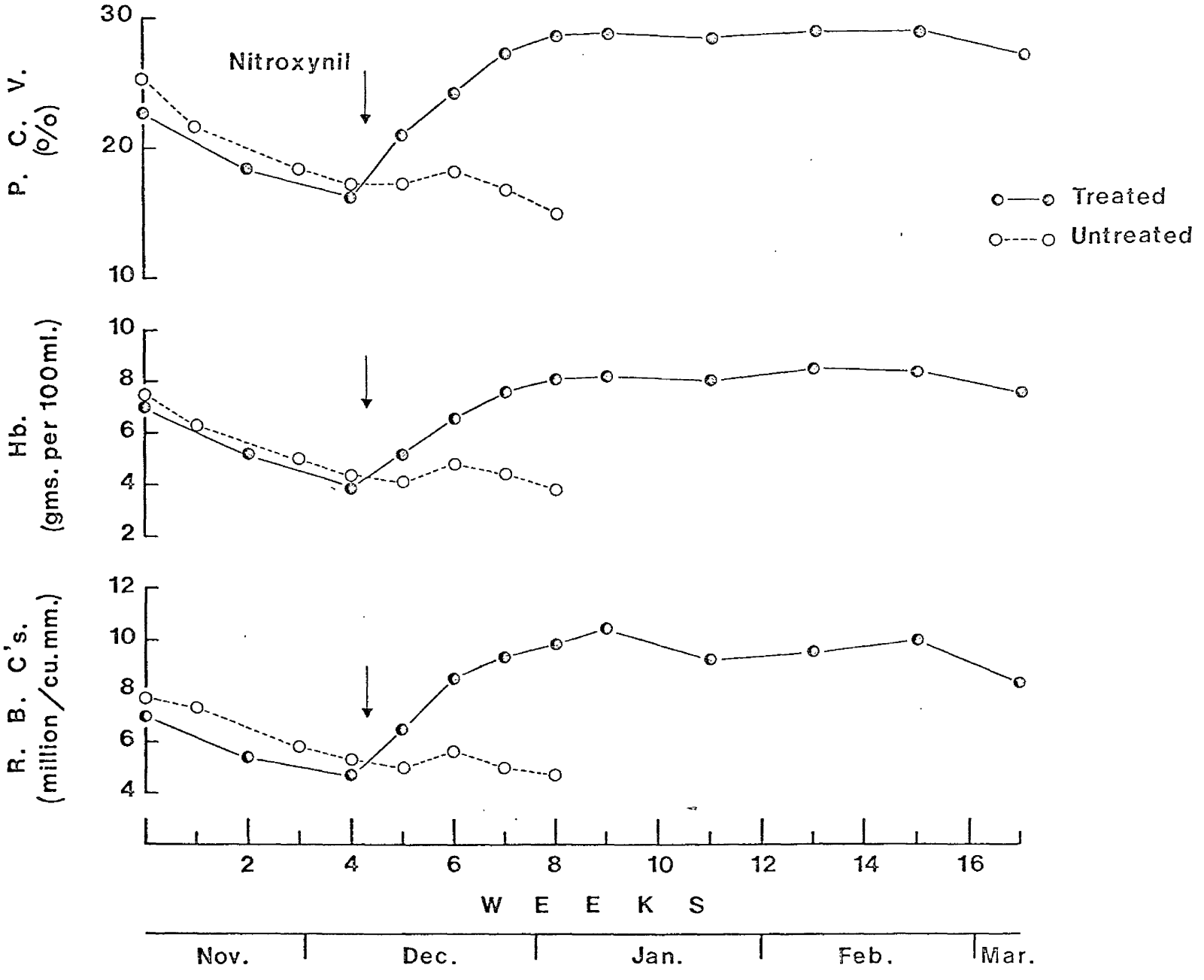

Fig. 49. The mean packed cell volumes, haemoglobin concentrations and total red cell counts of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

per cent. The haematocrit of the untreated group Group B continued to fall and 8 weeks after the commencement of the experiment when only five lambs remained alive the mean P.C.V. was 15.1 ± 4.11 per cent.

The mean haemoglobin concentration for Group A at the beginning of the experiment was 7.0 ± 0.41 gms. per 100 ml. and that of Group B was 7.3 ± 0.60 gms. per 100 ml. and this fell in the course of the following 4 weeks to 4.0 ± 0.47 and 4.3 ± 0.60 gms. per 100 ml. respectively. Group A showing a response to treatment had a mean haemoglobin concentration 5 days post-treatment of 5.2 ± 0.36 gms. per 100 ml. and this increase continued until at 5 weeks post-treatment the mean value was 8.2 ± 0.38 gms. per 100 ml. and a further 6 weeks later the level was still 7.8 ± 0.42 gms. per 100 ml. The mean haemoglobin concentration in Group B continued to fall and 8 weeks after the commencement of the experiment the level was 3.8 ± 1.33 gms. per 100 ml.

Individual values recorded for packed cell volume and haemoglobin concentration for both groups are given in Appendix 5, Tables 3 and 4.

The mean corpuscular volume (M.C.V.) of both groups of lambs was increasing during the first four weeks of the experiment. Five days after treatment Group A showed a marked reduction in the mean value for M.C.V. whilst the M.C.V. of Group B continued to increase. This relationship is illustrated in Figure 50. During the 13 weeks following treatment the mean M.C.V. of Group A lambs remained substantially below the pre-treatment level. The individual values of M.C.V. recorded for both groups

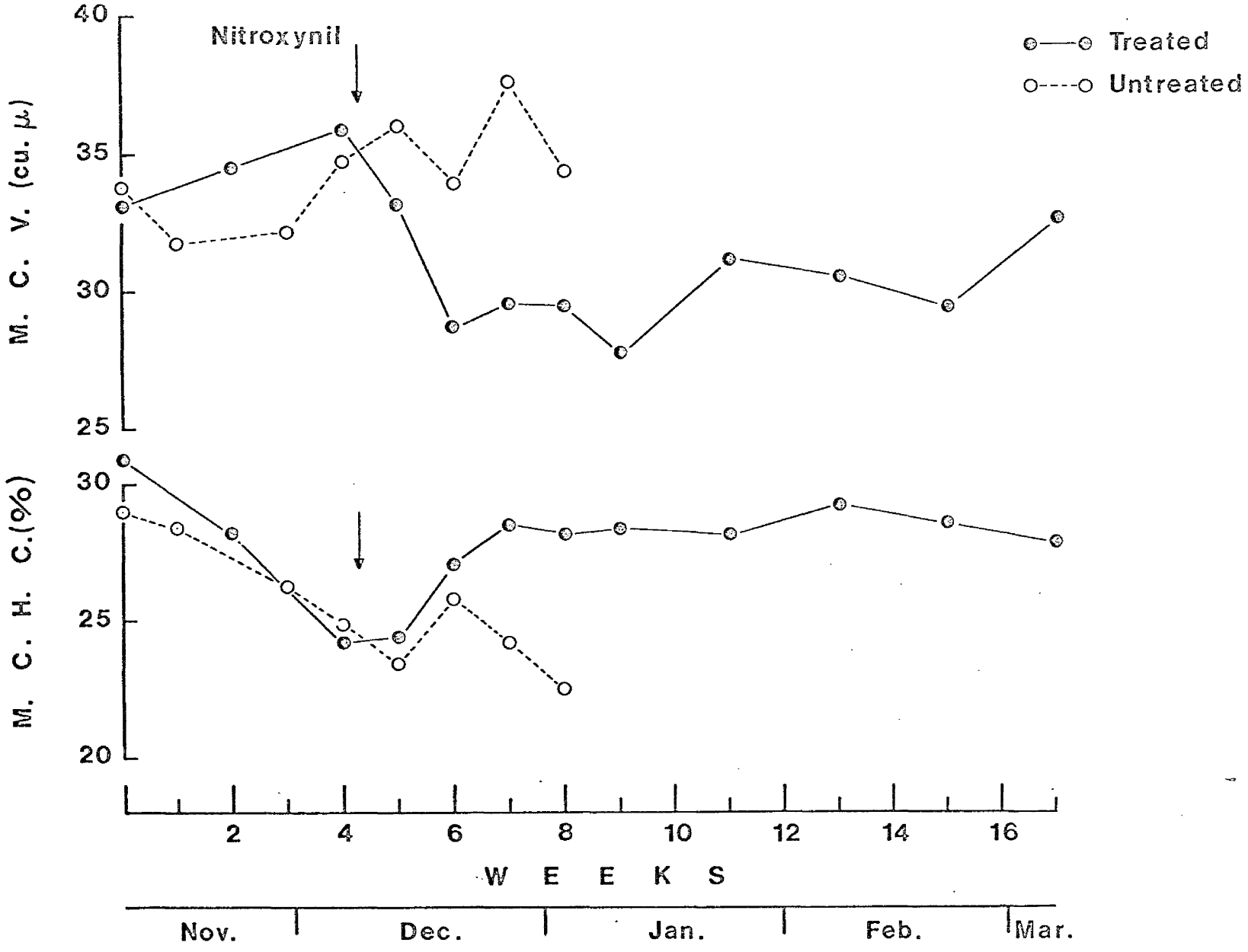

Fig. 50. The mean alterations in the mean corpuscular volumes and mean corpuscular haemoglobin concentrations of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

are given in Appendix 5, Table 5.

The mean corpuscular haemoglobin concentration (M.C.H.C.) was falling rapidly in the first 4 weeks of the experiment but in Group A, following treatment, there was a less rapid increase in this mean value than with all the other haematological values. (Figure 50). At 5 days post-treatment the mean M.C.H.C. in this group was similar to the pre-treatment level and it was not until the following week that a significant increase was observed. Within 3 weeks of treatment the mean M.C.H.C. for Group A had reached a level at which it persisted until the end of the observation period 10 weeks later. The mean level in Group B fell steadily. The individual values of M.C.H.C. for each group are given in Appendix 5, Table 6.

Reticulocytes were present in the peripheral circulation of the majority of the animals in both groups at the beginning of the experiment and in the ensuing 4 weeks the mean reticulocyte count rose in Group A to 5.4 per cent and in Group B to 5.8 per cent. Following treatment the reticulocyte count fell rapidly but by 5 days post-treatment the mean level of Group A was still 2.5 % although after a further 7 days reticulocyte counts were negative and thus they remained for at least another 11 weeks. The variations in mean reticulocyte count for each group are illustrated in Figure 51 and individual values are given in Appendix 5, Table 7.

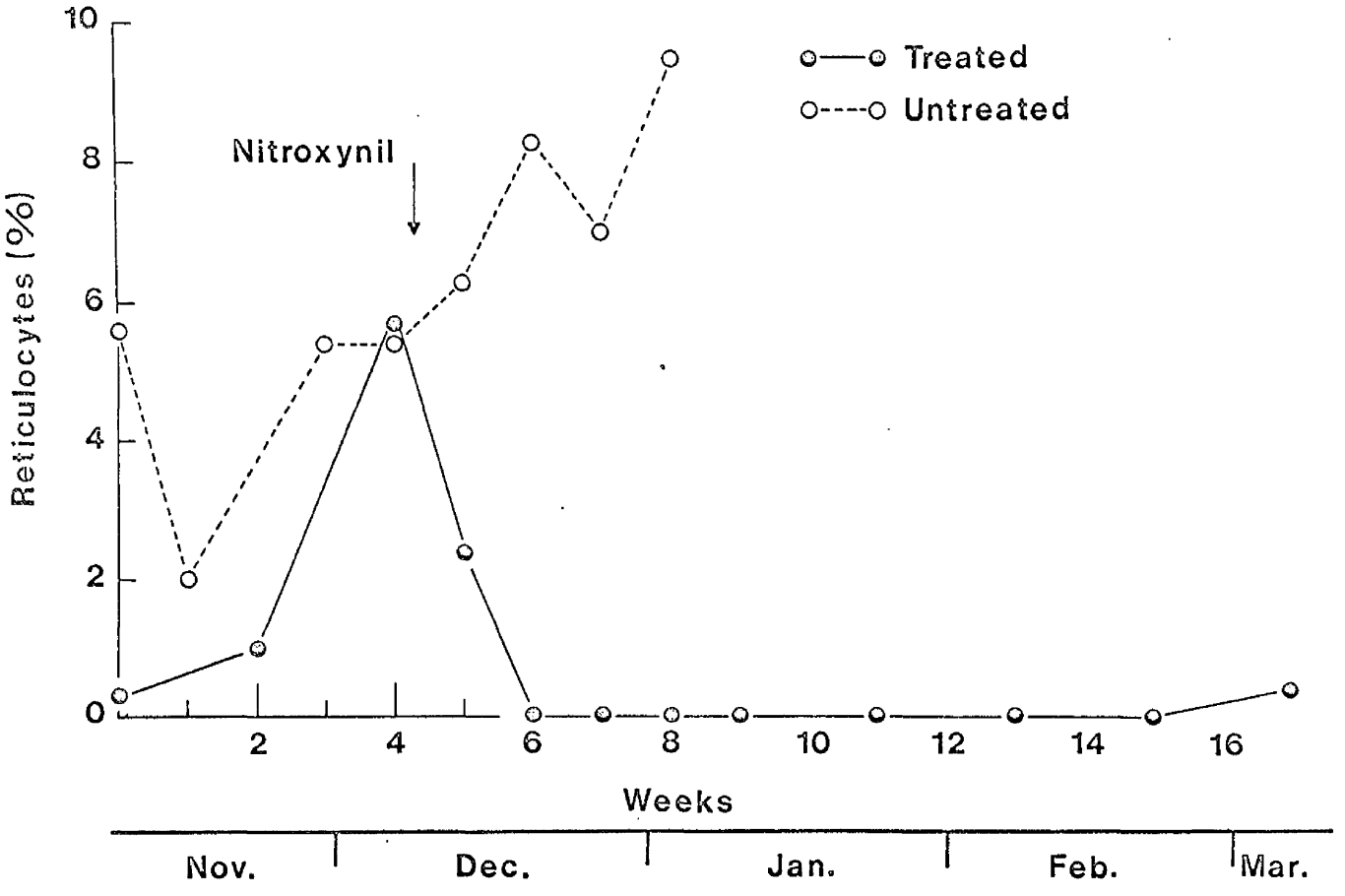

Fig. 51. The mean reticulocyte counts of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

Biochemical Data

During the initial 4 weeks of the experiment the mean total protein level for each of the two groups of lambs was falling steadily (Figure 52). In the course of these 4 weeks the mean level of total protein of the Group A lambs fell from 6.7 ± 0.26 gms. per 100 ml. to 5.0 ± 0.30 gms. per 100 ml. and that of the Group B lambs fell from 6.7 ± 0.27 gms. per 100 ml. to 5.0 ± 0.04 gms. per 100 ml. Following treatment a rapid increase in mean total protein level was observed in Group A whilst Group B continued to show a steady drop. These changes are apparent in Figure 52 and reference to the individual results in Appendix 5, Table 8, demonstrates that the mean total protein level of Group A increased by 41 per cent in the 3 weeks following treatment.

A similar pattern was observed with regard to serum albumin levels where in the first 4 weeks of the experiment the mean level in Group A fell from 1.79 ± 0.12 gms. per 100 ml. to 1.20 ± 0.12 gms. per 100 ml. After treatment the mean serum albumin level of Group A increased and by 3 weeks post-treatment was 2.50 ± 0.15 gms. per 100 ml. (i.e. an increase of more than 100 per cent of the pre-treatment figure) whilst that of Group B continued to fall. These changes are illustrated in Figure 53 and individual values of serum albumin for both groups are given in Appendix 5, Table 9.

An increase in serum globulin levels also took place following treatment and the alterations in mean value for the alpha/beta and gamma

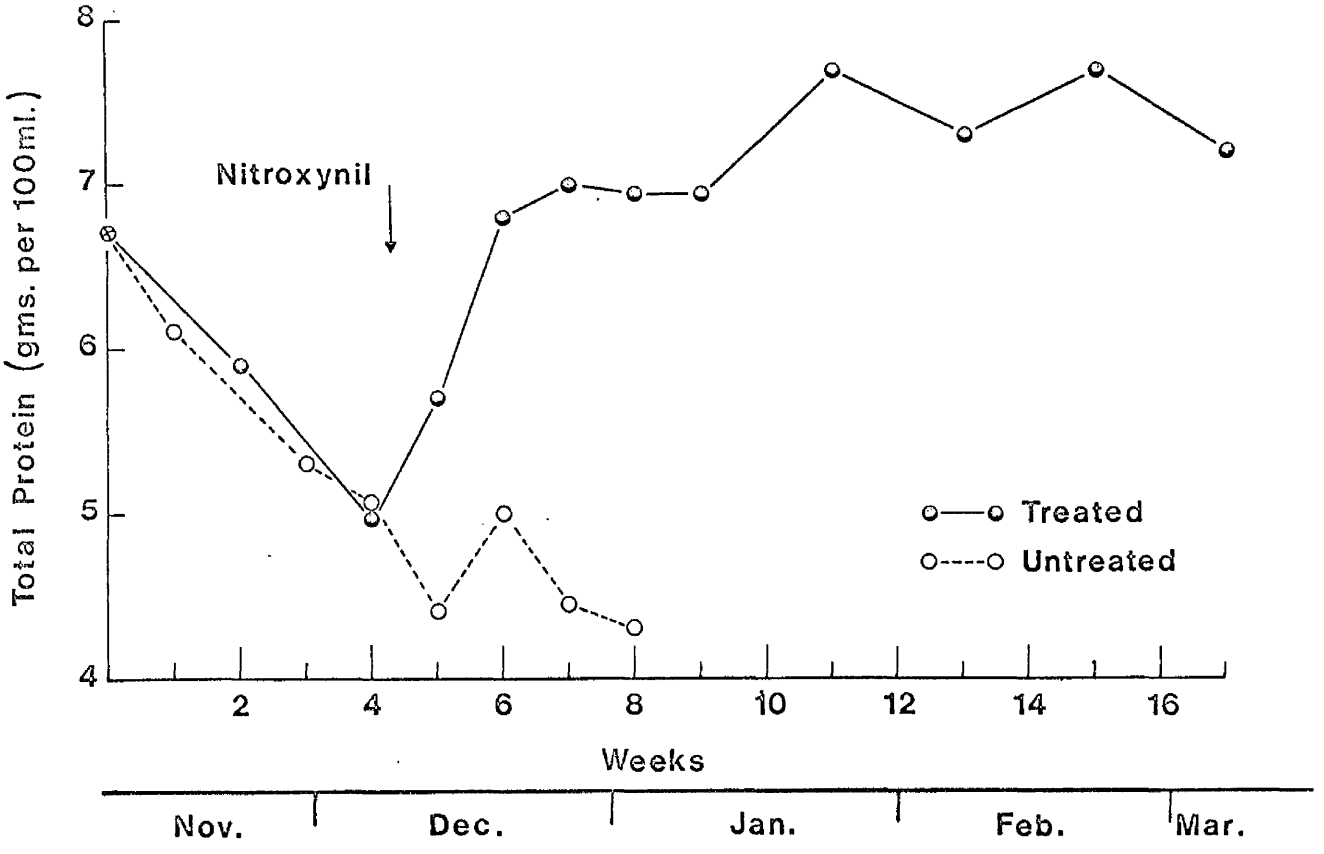

Fig. 52. The mean total serum protein levels of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

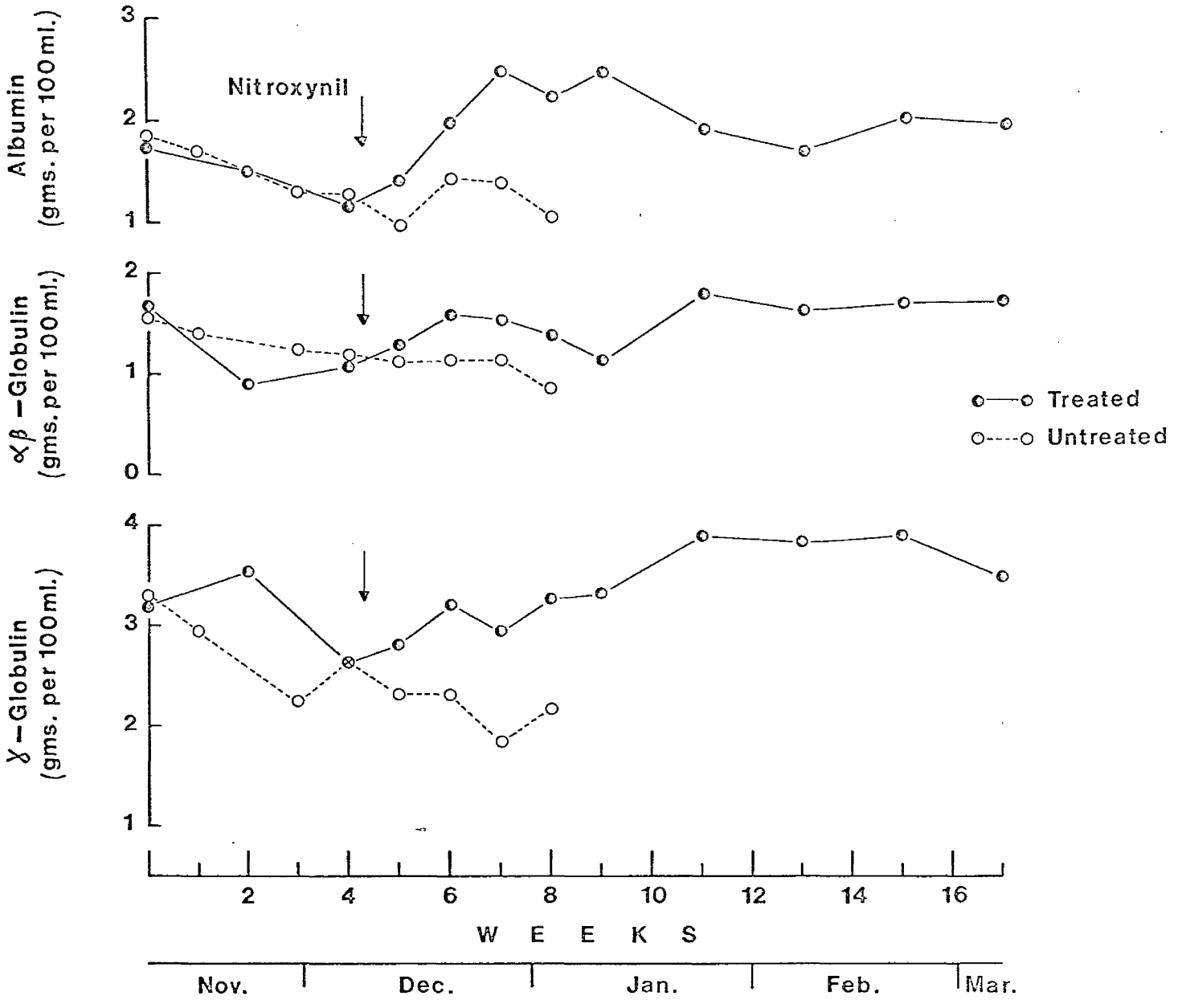

Fig. 53. The mean serum albumin, alpha/beta globulin and gamma-globulin levels of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

fractions are also illustrated in Figure 53. In both cases levels were falling during the first 4 weeks of the experiment but after treatment Group A showed an increase which although not so rapid as that observed in the case of total protein and serum albumin was nevertheless marked. Individual values for alpha/beta and gamma-globulin for both groups are given in Appendix 5, Tables 10 and 11 respectively.

The albumin-globulin ratio reflected the changes described above and so the ratio was decreasing during the first 4 weeks of the experiment in both groups. Following treatment the ratio increased rapidly in Group A and at 3 weeks post-treatment the mean pre-treatment level had increased by 78 per cent. Individual values for the A/G ratio in both groups are given in Appendix 5, Table 12.

Parasitological Data

Immediately prior to treatment the mean faecal egg output of the Group A lambs was 550 ± 84 fluke eggs per gram of faeces whilst that of Group B was 406 ± 81 fluke eggs per gram of faeces. Five days after treatment the faecal egg count of all the lambs in Group A was negative whilst the mean count for the Group B animals was 522 ± 71 fluke eggs per gram. The faeces of the animals in Group A remained negative for fluke eggs for 9 weeks post-treatment after which time fluke eggs were detected in the faeces of one lamb and in the course of the following 4 weeks virtually all the animals had positive egg counts. The alterations in faecal egg count are illustrated in Figure 54, whilst individual values

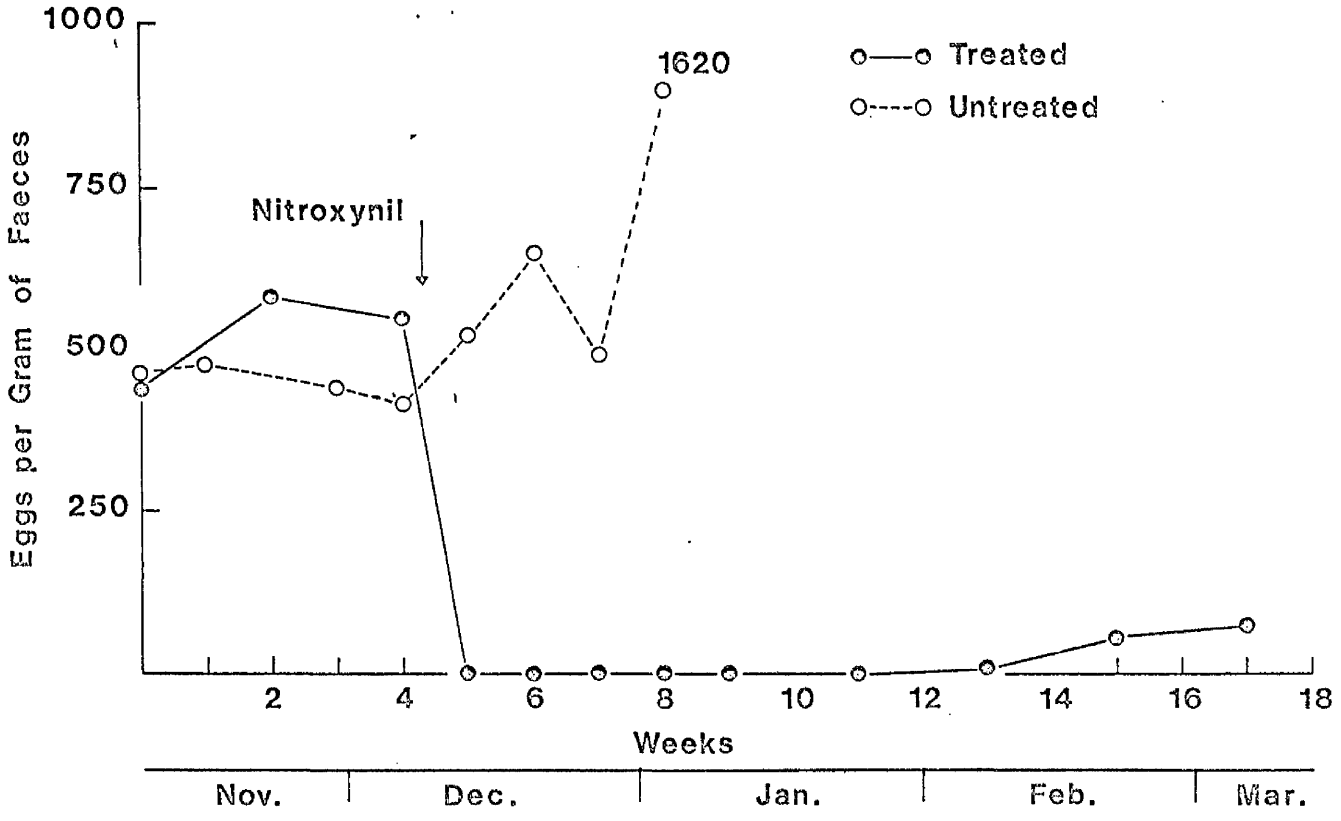

Fig. 54. The mean fluke faecal egg counts of two groups of lambs during a severe outbreak of fascioliasis, one group being treated with nitroxynil.

for each group are given in Appendix 5, Table 13.

Discussion

The administration of nitroxynil at a dosage rate of 10 mg. per kg. produces marked improvement in sheep suffering from subacute fascioliasis within a short period of treatment. The efficiency of the drug as judged by reduced mortality demonstrates that 80% of the treated animals survived whilst all the untreated animals died eventually. Both the lambs which died in the treated group did so within 24 hours of treatment and these lambs were amongst the poorest in the group and at autopsy both had a grossly enlarged liver and carried a total fluke burden of 584 and 886. Occasional deaths within a short time of treatment were also recorded at the 10 mg. per kg. dose level (Colegrave, 1968 a) and these were always seen in sheep carrying heavy fluke burdens with associated severe liver damage.

The gross anaemia which was apparent prior to treatment disappeared rapidly after treatment although the rate of improvement varied from one sheep to another. For instance in sheep number P 72 it was about 10 weeks before the haematological indices returned to normal limits whilst in sheep number P 61 with a similar degree of anaemia at treatment normal limits were achieved in 3 to 4 weeks. The character of the anaemia was confirmed as being of a macrocytic and hypochromic nature when on treatment a marked reduction in mean corpuscular volume took place and

an even more obvious increase in mean corpuscular haemoglobin concentration was observed. The reticulocytosis also disappeared after treatment but only after approximately 2 weeks had elapsed. Sinclair (1962) also found that on treatment of infected sheep with carbon tetrachloride a fall in mean corpuscular volume occurred although he did not consider that M.C.V. levels were elevated prior to treatment. This latter author makes no reference to changes in M.C.H.C.

Accompanying the changes in the red cell picture were alterations in serum protein levels. Whilst before treatment the levels of total protein and serum albumin were falling steadily and the levels of alpha/beta globulin and gamma-globulin were commencing to fall treatment brought about a reversal of the trend and a marked increase in all these values was recorded. This increase was particularly noticeable in the case of serum albumin where, after treatment, the group mean pre-treatment level more than doubled in the course of 3 weeks.

The changes in haematological values observed after treatment would appear to support the views of those workers who considered that the adult liver fluke is a blood feeder (i.e. Weinland and von Brand, 1926; Stephenson, 1947; van Grembergen, 1950; Urquhart, 1955; Jennings et al., 1956; Jennings, 1962; Pearson, 1963; Todd and Ross, 1966; Holmes et al., 1967 a & b; Sewell, 1967; Symons and Boray, 1967). If the source of haemorrhage, i.e. the fluke, is removed by the use of anthelmintic then all blood values return rapidly to normal. The rapidity with which these

changes take place would tend to eliminate the suggestion that a toxic effect on bone marrow plays the major part in the production of the anaemia (Barbieri, 1935; Dalian, 1940 a; Sinclair, 1962, 1964). Similarly the alterations in biochemical values particularly in the case of serum albumin support the whole blood loss theory. Dargie et al., (1967 & 1968) have demonstrated that hypercatabolism of plasma albumin occurs in fluke infected rabbits and hence this would adequately explain the very sharp rise in albumin levels in sheep following treatment.

Following treatment fluke eggs disappeared rapidly from the faeces of the treated lambs and this situation persisted for a period of 9 weeks post-treatment. These findings are similar to those of Colegrave (1968a) who, also using nitroxynil at 10 mg. per kg. in naturally infected sheep, found that fluke egg counts virtually remained negative for 58 days after treatment. Although Lucas (1967) only used the 10 mg. per kg. dose of nitroxynil on immature infections down to the age of six weeks he did use the drug at 20 mg. per kg. on four week infections with a percentage reduction in fluke burden of 66.5%. The results of the present experiment suggest that the drug shows marked efficiency against a large part of the immature burden as judged by the time required for fluke eggs to re-appear in the faeces. As the prepatent period in sheep given single infective doses of metacercariae of F. hepatica is in the region of 9 to 12 weeks (Sinclair, 1962; Furmaga and Gundlach, 1967a; this thesis, Section III), and it is unlikely that nitroxynil at the dose rate of 10 mg. per kg. will

kill all flukes down to 3 weeks of age other reasons must be sought. For instance, the drug may temporarily immobilise very young forms and so delay their development or an immune mechanism may be operating and so retard the growth of the parasite.

The findings described in this section of the thesis have important practical implications. Firstly, the drug is of value, at the dose rate of 10 mg. per kg. in arresting mortality during an outbreak of disease provided the treated animals are not so severely affected as to be almost on the point of death. Secondly, the drug at the same dose level, will eliminate faecal egg output for a sufficiently long period to allow it to be of some value in minimising pasture contamination.

Summary

This section describes the use of the fasciolicide 4-cyano-2-iodo-6-nitrophenol, M & B 10,755-nitroxynil ("Trodax") in the treatment of an outbreak of fasciolinosis in sheep infected under natural conditions. The drug was administered as the N-methylglucamine salt at a dose rate of 10 mg. per kg. and was given by intramuscular injection.

Following treatment there was a rapid improvement in the animals' general condition and the severe anaemia which had been present before treatment had disappeared 3 weeks after treatment. The serum protein fractions which had all been falling prior to treatment also showed a marked increase following treatment. This improvement in general condition and in the haematological and biochemical indices was maintained for at least 13 weeks after treatment.

Fluke eggs disappeared from the animal's faeces within 5 days of treatment and they were not observed again until 9 weeks post-treatment although it was 13 weeks after treatment before the majority of animals had positive faecal egg counts.

GENERAL SUMMARY

Section I

Field Studies on Clinical Parasitism in Young Dairy Cattle in the West of Scotland.

1. Investigation of 10 naturally-occurring outbreaks of parasitic gastro-enteritis in young cattle in South-west Scotland showed that Ostertagia ostertagi was the predominant parasite present. All the outbreaks were reported during the winter and early spring, the majority occurring in April and May. The main clinical signs were a rapid loss of bodyweight and a profuse diarrhoea. An anaemia developed in a proportion of cases and this was always mild to moderate in severity and frequently not suspected on clinical examination. The main changes in blood chemistry involved the plasma proteins and a hypoalbuminaemia was recorded with a marked increase in plasma pepsinogen levels.

2. Investigation of a further 10 outbreaks of clinical parasitism in the young b vine revealed, at necropsy, the presence of variable but usually significant numbers of O. ostertagi which were accompanied by relatively large numbers of mature Fasciola hepatica. This condition called the fascioliasis/ostertagiasis complex also occurred during the winter, the majority of cases being reported between January and March. The main features of clinical significance were gradual loss of bodyweight and the relative or complete absence of diarrhoea. A severe anaemia was present in all cases and readily appreciated on clinical examination. A hypoalbumin-aemia was noted but plasma pepsinogen levels were normal and only slightly elevated.

Section II

Experimental *Fasciola hepatica* Infections in Calves

3. Infection of two and a half month old calves with a single oral inoculation of 1,000 or 2,000 metacercariae of *F. hepatica* resulted in the establishment of an adult fluke burden capable of producing clinically recognisable signs. Although all the animals given 1,000 metacercariae died only two of the calves given 2,000 metacercariae died. The major clinical signs observed were loss of bodyweight and pallor of visible mucous membranes; diarrhoea was never present and submandibular oedema and ascites were not recorded. A moderate to severe anaemia developed in the calves and this was of the macrocytic, normochromic type; there was no reticulocytosis. Serum albumin levels were reduced in all infected animals. At post-mortem the percentage take was between 16 per cent and 37 per cent in all but two animals which were killed at the termination of the experiment. These latter calves had very low percentage takes and their livers showed bile duct thickening which was more severe than was the case in any of the other animals.

Section III

Experimental *Fasciola hepatica* Infections in Sheep

4. Infection of six-month old lambs with a single oral inoculation of 1,000 metacercariae of *F. hepatica* resulted in the development of clinical fascioliasis followed by death between 12 and 23 weeks after infection. A severe anaemia developed and was first apparent 5 to 6 weeks post-infection. Initially this anaemia was of normochromic and normocytic type but as the

disease progressed it became hypochromic and macrocytic in several cases; a reticulocytosis was present in every case. The degree of anaemia was approximately proportional to the number of flukes recovered at autopsy. A relative eosinophilia was noted first appearing during the second week of infection. At the same time as the anaemia appeared a hypoalbuminaemia developed and in those animals which survived the greatest length of time a frank hypoproteinaemia was present. Slight elevations in serum glutamic oxaloacetic transaminase and alkaline phosphatase were recorded commencing during the migratory phase of the parasite. The percentage take ranged from 10.8 per cent to 81.5 per cent with a mean of 48.5 per cent.

Section IV

Field Studies on Fascioliasis in Sheep

5. When a group of fifty lambs was allowed to graze permanent pasture known to have been responsible for outbreaks of fasciolíasis during the previous two years, clinical fasciolíasis was first apparent during the early part of October. Between October and the following March, forty of the lambs died, the remaining ten animals having been treated. None of the lambs died suddenly and all the animals dying between October and early January presented a subacute syndrome whilst two lambs dying at the end of March did so as a result of chronic fasciolíasis. The principal clinical signs recorded were weight loss, dullness, pallor of visible mucous membranes and, in many cases, resentment of palpation of the anterior abdomen. An

anaemia developed in almost all the lambs; this was of the macrocytic, hypochromic type and reticulocytes were observed in the peripheral circulation. The degree of anaemia at death was variable and appeared to be associated with the rate of development of the anaemia. Following an initial elevation in total serum protein levels, reduced levels were recorded; the elevation resulted from an increase in the globulins, particularly gamma-globulin, whilst the subsequent reduction was due to a fall in both serum albumin and globulin levels. At death a hypoalbuminaemia was always present and in many cases there was a frank hypoproteinaemia. The total number of flukes recovered from the lambs ranged from 110 to 1628.

6. Observations on the pasture populations of metacercariae of F. hepatica over one year from April to April were carried out using the fluke burden at necropsy of "tracer" lambs, which grazed for 4 or 5 weeks, to quantitate the level of herbage infections. Metacercariae were picked up throughout the period of observation but the maximum number was recovered from those "tracer" lambs grazing between mid-August and mid-September.

Section V

The Use of Nitroxynil in Ovine Fascioliasis

7. The efficiency of nitroxynil in the treatment of naturally acquired fascioliasis in sheep was studied. The drug was administered as the N-methylglucamine salt at a dose rate of 10 mg. per kg. and was given by intramuscular injection. Following treatment there was a rapid improvement in the animals' general condition and the severe anaemia which had been present before treatment had disappeared 3 weeks after treatment. The

serum protein fractions which had been falling prior to treatment also showed a marked increase after treatment. This improvement in general condition and in the haematological and biochemical indices was maintained for at least 13 weeks following treatment. Fluke eggs disappeared from the animals' faeces within 5 days of treatment and they were not observed again until 9 weeks post-treatment although it was 13 weeks after treatment before the majority of animals had positive faecal egg counts.

Alloroft, W.M. and Folley, S.J. (1941) Biochem. J., 35, 254.

Anderson, N. (1968) "Bovine Ostertagiasis", Ph.D. Thesis, University of Glasgow.

Anderson, N., Armour, J., Eadie, R.M., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1966) Am.J. vet. Res., 27, 1259.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D., and Urquhart, G.M. (1965) Vet. Rec., 77, 1196.

Armour, J. (1966) Personal communication.

Armour, J. (1967) "Ostertagia ostertagi Infections in the Bovine: Field and Experimental Studies." Ph.D. Thesis, University of Glasgow.

Balian, B. (1940a) Nuova Vet., 18, 134.

Balian, B. (1940b) Nuova Vet., 18, 154.

Barbieri (1935) Cited by Balian, 1940b.

Batte, E.G. and Swanson, L.E. (1952) Proc. Am. vet. med. Assoc., 88, 101.

Batte, E.G., Swanson, L.E. and Murphy, J.B. (1951a) J.Am.vet.med.Ass., 118, 139.

Batte, E.G., Swanson, L.E. and Murphy, J.B. (1951b) Am.J.vet.Res., 12, 158.

Belli, G. and Chini, P. (1954) Atti Soc. ital. Sci. Vet., 8, 671.

Bishop, O.N. (1966) in "Statistics for Biology", Longmans, Green & Co. Ltd., London.

Boray, J.C. and Happich, F.A. (1966) Vet. Rec., 79, 358.

Boray, J.C., Happich, F.A. and Andrews, J.C. (1965) Vet. Rec., 77, 175.

Boray, J.C., Happich, F.A. and Andrews, J.C. (1967) Vet. Rec., 80, 218.

Bremner, K.C. (1966) Aust.J.exp.Biol.med.Sci., 44, 251.

Bruford, J.W. and Fincham, I.H. (1945) Vet. Rec., 57, 421.

Bryant, A. (1935) Proc. Liverpool Biol. Soc., 49, 8.

Bugge, G. (1928) Berl. tierärztl. Wschr., 44, 189.

Bugge, G. (1935) Berl. tierärztl. Wschr., 101, 65.

Cameron, T.W.M. (1951) in "The Parasites of Domestic Animals", 2nd Edition, A & C Black, London.

Carrara, O. and Recalcati, M. (1961) Atti Soc.ital.Sci.Vet., 15, 542.

Chandler, A.C. (1920) J.agric.Res., U.S. Dept. Agric., 20, 193.

Clunies Ross, I. and Gordon, H.McL. (1936) in "The Internal Parasites and Parasitic Diseases of Sheep", Angus and Robertson, Ltd., Sydney.

Cohrs, P. (1967) in Nieberle and Cohrs "Textbook of the Special Pathological Anatomy of Domestic Animals, Pergamon Press, London.

Colegrave, A.J. (1968a) Vet.Rec. 82, 343.

Colegrave, A.J. (1968b) Vet.Rec. 82, 373.

Colombo de la Villa, G. (1955) Bull.Off.int.Epizoot., 43, 63.

Cornelius, C.E., Bishop, J.A., Switzer, J. and Rhode, E.A. (1959) Cornell Vet. 49(1), 116.

Cornelius, C.E. and Kaneko, J.J. (1963) in "Clinical Biochemistry of Domestic Animals", Academic Press, New York & London.

Coulson, C.B., Davie, P.J. and Evans, W.C. (1960) J.comp.Path., 70, 199.

Crighton, G.W. (1965) "Lymphosarcoma or Lymphatic Leukaemia of the Dog and Cat", Ph.D. Thesis, University of Glasgow.

Crofton, H.D. (1949) Parasitology, 39, 274.

Crofton, H.D. (1952) Parasitology, 42, 77.

Dacie, J.V. and Lewis, S.M. (1966) in "Practical Haematology", 3rd Edition, J & A Churchill Ltd., London.

Dargie, J.D., Holmes, P.H., MacLean, J.M. and Mulligan, W. (1967) Vet.Rec., 80, 390.

Dargie, J.D., Holmes, P.H., MacLean, J.M. and Mulligan, W. (1968) J.comp.Path., 78, 101.

Davis, M., Lucas, J.M.S., Rosenbaum, J. and Wright, D.E. (1966) Nature, Lond., 211, 882.

Dawes, B. (1961) J. Helminth., R.T. Leiper Suppl., p.41.

Dawes, B. (1962) J. Helminth., 36, 11.

Dawes, B. (1963a) Parasitology, 53, 123.

Dawes, B. (1963b) Nature, Lond., 198, 1011.

Dawes, B. and Hughes, D.L. (1964) Adv. in Parasitology, 2, 97.

Demidov, N.V. (1955) Veterinariya Moscow, 32, 29.

Dixon, K.E. (1964) J. Helminth., 38, 203.

Doby, J.M. and Chiche, G. (1965) Bull.Soc.Path.exot., 58, 209.

Docksen, J., Heringa, K.A. and Swiestra, D. (1949) Maandblad voor de Landbouwvoorlichtingsdienst, 6, 219.

Dorsman, W. (1959) Tijdschr. Diergeneesk, 84, 100.

Douvres, F.W. (1956) J. Parasit., 42, 626.

Douvres, F.W. (1957) Am.J.vet.Res., 18, 81.

Durie, P.H. (1962) Aust.J.Agric.Res., 13, 767.

Edwards, K., Jepson, R.P. and Wood, K.F. (1960) Br.med.J. (i), 30.

Enger, O. and Karbo, A. (1956) Norsk. Landbruk., 22, 60.

Enigk, K. (1958) Ceskoslov. Parasit., 5, 59.

Ershov, V.S. (1956) in "Parasitology and Parasitic Diseases of Livestock", State Publ. House for Agric. Literature, Moscow.

Euzeby, J. (1955) Bull.Off.int.Epizoot., 43, 79.

Facey, R.V. and Marsden, P.D. (1960) Br.med.J. (ii), 619.

Federman, M. (1959) Dtsch. tierärztl. Wschr., 66, 526.

Fisher, E.W. (1962) Br.vet.J. 118, 513.

Fluzy, F. and Leeb, F. (1926) Klin. Wschr., 5, 2054.

Ford, E.J.H. (1958) J.Anat., 92, 447.

Frederick, L.D. (1943) J.Am.vet.med.Ass., 102, 339.

Furmaga, S. and Gundlach, J.L. (1967a) Acta.Parasit.polon., 14, 313.

Furmaga, S. and Gundlach, J.L. (1967b) Acta.Parasit.polon., 15, 93.

Gardner, R.C.B. (1911) J.S.E.Agric.Coll.Wye, 20, 482.

Gebauer, O. (1939) Wien. tierärztl. Monats., 26, 33.

Gibson, T.E. (1959) Vet.Rec., 71, 362.

Gibson, T.E. (1963) Res.vet.Sci., 4, 258.

Gibson, T.E. (1965) "Veterinary Anthelmintic Medication", Commonwealth Agricultural Bureaux.

Gibson, T.E. (1966) In "Biology of Parasites", Academic Press, New York and London.

Goncharuk, I.S. (1959a) Sborn.nauch.Trud.Lovoski Zoovet.Inst., 2, 203.

Goncharuk, I.S. (1959b) Sborn.nauch.Trud.Lovoski Zoovet.Inst., 2, 213.

Gordon, H.McL. (1955a) Aust.vet.J., 31, 46.

Gordon, H.McL. (1955b) Aust.vet.J., 31, 102.

Gordon, H.McL. and Whitlock, H.V. (1939) J.Council Sci.Indust.Res., 12, 50.

Gracey, J.F. (1960) Survey of Livestock Diseases in Northern Ireland, H.M.S.O., London, England.

Gresham, G.A. and Jennings, A.R. (1962) in "An Introduction to Comparative Pathology", Academic Press, New York and London.

Groschaft, J. (1963) Ceskoslovenska Parasitologie, 10, 103.

Haiba, M.H. and Selim, M.K. (1960) Z. ParasitKde., 19, 535.

Hankiewicz, K. (1965) Medycyna wet., 21, 537.

Herlich, H. (1956) Proc.Helminth.Soc.Washington, 23, 102.

Hiepe, T. and Grünwoldt, J. (1965) Berl.Münch. tierärztl. Wschr., 78, 114.

Hindson, J.C. (1965) Vet.Rec., 77, 494.

Holman, H.H. (1945) J.comp.Path., 55, 229.

Holmes, P.H., Maclean, J.M., Dargie, J.D., Jennings, F.W. and Mulligan, W. (1967a) Vet.Rec., 80, 285.

Holmes, P.H., Maclean, J.M., Dargie, J.D., Jennings, F.W. and Mulligan, W. (1967b) Vet.Rec., 80, 420.

Hughes, D.L. (1963) "Some Studies on the Host-Parasite Relations of Fasciola hepatica". Ph.D. Thesis, University of London.

Hutyra, F., Marek, J., and Manninger, R. (1946) in "Special Pathology and Therapeutics of the Diseases of Domestic Animals". Balliere, Tindall and Cox, London.

Ibrovic, M. and Gall-Palla, V. (1959) Veterinaria, Sarej., 8, 531.

Jaggers, S. (1966) Personal Communication.

Jennings, F.W. (1962) "Biochemical and Immunological Studies on Some Helminth Diseases of Domestic Animals", Ph.D. Thesis, University of Glasgow.

Jennings, F.W., Armour, J., Lawson, D.D. and Roberts, R.J. (1966) Am.J.vet.Res., 27, 1249.

Jennings, F.W., Mulligan, W. and Urquhart, G.M. (1956) Expl.Parasit., 5, 458.

Jubb, K.V.F. and Kennedy, P.C. (1963) in "Pathology of Domestic Animals", Academic Press, New York and London.

Kendall, S.B. (1953) J. Helminth., 27, 17.

Kendall, S.B. and Parfitt, J.W. (1962) Br.vet.J., 118, 1.

Kind, P.R.N. and King, E.J. (1954) J.clin.Path., 7, 322.

Kirk, R.M. (1961) Br.med.J., (ii), 1333.

Knapp, S.E., Nyberg, P.A., Dutson, V.J. and Shaw, J.N. (1965) Am.J.vet.Res., 26, 1071.

Krull, W.H. (1934) Proc.Iowa Acad.Sci., 41, 309.

Krull, W.H. and Jackson, R.S. (1943) J.Wash.Acad.Sci., 33, 79.

Kuttler, K.L., Matthews, N.J. and Marble, D.W. (1963) Am.J.vet.Res., 24, 52.

Lagrange, E. and Gutmann, A. (1961) Riv.parassit., 22, 93.

Lämmler, G. (1960) Dt. tierärztl. Wschr., 67, 408.

Lang, B.Z. (1967) J.Parasit., 53, 21.

Lapage, G. (1956) in "Veterinary Parasitology", 1st Edition, Oliver and Boyd, Edinburgh, Scotland.

Lapage, G. (1962) in "Mönnig's Veterinary Helminthology and Entomology", 5th Edition, Balliere, Tindall and Cox, London.

Lee, R.P., O'Nuallian, T. and Power, J.H. (1966) Vet.Rec., 78, 196.

Leuckhart, R. (1881) Zool.Anz., 99, 641. (Cited by Pantelouris, 1965).

Lloyd, J.R. (1957) Vet.Rec., 69, 624.

Lucas, J.M.S. (1967) Br.vet.J., 123, 190.

Lutz, A (1892) Zentbl. Bakt. ParasitKde I, 11, 783 (Cited by Reinhard, 1957).

Martin, W.B., Thomas, B.A.C. and Urquhart, G.M. (1957) Vet.Rec., 69, 736.

Maximov, A.A. and Bloom, W. (1957) in "A Textbook of Histology", W.B. Saunders Company, Philadelphia and London.

Michel, J.F. (1966) Publication der IV Internationalen Tagung der Weltgesellschaft für Buiatrik, Zurich.

Mönnig, H.O. (1956) in "Veterinary Helminthology and Entomology", Balliere, Tindall and Cox, London.

Montgomerie, R.F. (1926) J.comp.Path., 39, 113.

Montgomerie, R.F. (1928) J. Helminth., 6, 167.

Montgomerie, R.F. (1931) J. Helminth., 9, 209.

Morgan, B.B. and Hawkins, P.A. (1953) in "Veterinary Helminthology", Burgess Publish. Co., Minneapolis, U.S.A.

Morozhkin, B.F., Kontina, A.A., Ivanskii, E.F. and Sutyagin, V.S. (1964) Veterinariya, Moscow, 41(10), 41.

Morrill, D.R. and Shaw, J.N. (1942) Bull.Oregon Agric.Exp.Stn., 408, 30.

Mozley, A.(1952) in "Molluscicides" Lewis & Co., London.

Mozley, A.(1957) in "Liver Fluke Snails in Britain", Lewis & Co., London.

Neill, D.W. (1963) in Joyce-Loebl Review, Team Valley, Gateshead, Spring Number.

Neuhaus, W. and Six, F. (1965) Berl.Münch.tierärztl.Wschr., 78, 67.

Nicolson, N. (1968) Personal communication.

Nikolic, B., Nikolic, V., Nevenic, V., Bugarski, M., Pavlovic, O., Ciric, V., Mladenovic, Z. and Polic, F. (1962) Acta.vet.Beogr., 12, (3/4), 55.

Nöller, W. and Schmid, F. (1927) Tierärztl., Rdsch., 33, 851.

Ollerenshaw, C.B. (1958) Agriculture, Lond., 65, 231.

Ollerenshaw, C.B. (1959) Vet.Rec., 71, 957.

Ollerenshaw, C.B. (1964) Proc. of the 1st Int. Cong. of Parasitology, Rome, 2, 1059.

Ollerenshaw, C.B. and Rowlands, W.T. (1959) Vet.Rec., 71, 591.

Olsen, O.W. (1945) J. Parasit. 31 (suppl.) 30.

Ono, Y. (1958) Bull.Off.int.Epizoot., 46, 555.

Ono, Y. and Isoda, M. (1951) Jap.J.vet.Sci., 13, 87.

Oshanova, N. (1959) Wiad. Parazyt., 5, 357.

Pantelouris, E.M. (1965) in "The Common Liver Fluke", Pergamon Press, Oxford.

Pearson, L.G. (1963) Expl. Parasit., 13, 186.

Peters, B.G. (1938) J. Helminth., 16, 213.

Peters, B.G. and Clapham, P.A. (1942) J. Helminth., 20, 115.

Popov, K.K. and Kalitina, Z.I. (1962) Zool. Zh., 41, 1793.

Pugh, D.M. (1965) Irish vet. J., 19, 223.

Ransom, B.H. (1911) U.S. Dept. of Agric. Bureau of Animal Industry, Bull.No.127.

Reid, J.F.S., Armour, J., Jennings, F.W., Kirkpatrick, K.S. and Urquhart, G.M. (1967) Vet.Rec., 80, 371.

Reinhard, E.G. (1957) Expl. Parasit., 6, 200.

Ritchie, J.S.D., Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W. and Urquhart, G.M. (1966) Am.J.vet.Res., 27, 659.

Roberts, E.W. (1950) Ann.trop.Med.Parasit., 44, 187.

Rose, J.H. (1959) Nature, Lond., 183, 1745.

Rose, J.H. (1963) Res.vet.Sci., 4, 450.

Ross, I.C. and McKay, A.C. (1929) Bull.Coun.sci.indust.Res.Aust., 45, 1.

Ross, J.G. (1966a) "Experimental Fascioliasis in Cattle", Ph.D. Thesis, Queen's University, Belfast.

Ross, J.G. (1966b) Irish vet.J., 20, 131.

Ross, J.G. (1966c) Br.vet.J., 122, 489.

Ross, J.G. (1967a) Vet.Rec., 80, 214.

Ross, J.G. (1967b) Vet.Rec., 80, 368.

Ross, J.G. and Dow, C. (1964) Br.vet.J., 120, 279.

Ross, J.G. and Dow, C. (1965a) Br.vet.J., 121, 18.

Ross, J.G. and Dow, C. (1965b) Br.vet.J., 121, 228.

Ross, J.G. and Dow, C. (1966) Vet.Rec., 68, 670.

Ross, J.G., Dow, C. and Todd, J.R. (1967) Vet.Rec., 80, 543.

Ross, J.G. and O'Hagan, J. (1966a) Vet.Rec., 78, 83.

Ross, J.G. and O'Hagan, J. (1966b) J. Helminth., 40, 375.

Ross, J.G. and Todd, J.R. (1965) Br.vet.J., 121, 55.

Ross, J.G., Todd, J.R. and Dow, C. (1966) J.comp.Path., 76, 67.

Rowcliffe, S.A. and Ollerenshaw, C.B. (1960) Ann.trop.Med.Parasit., 54, 172.

Sagar, J.L. (1962) Br.med.J., (i), 844.

Sazanov, A.M. (1961) Sbornik.Tekh.Inf.Inst.Gelm., 7/8, 53.

Schnappauf, H., Stein, H.B., Sipe, C.R. and Cronkite, E.P. (1967) Am.J.vet.Res., 28, 275.

Schumacher, W. (1938) Z. ParasitKde., 10, 608.

Schumacher, W. (1956) Z. ParasitKde., 17, 276.

Secretan, P. and Bickel, P. (1960) Arch.Mal.App.Digestif, 49, 642.

Sewell, M.M.H. (1966) Vet.Rec., 78, 98.

Sewell, M.M.H. (1967) Vet.Rec., 80, 579.

Shaw, J.N. (1932) J.Am.vet.med.Ass., 81, 76.

Shirai, M. (1927) Sci.Rep.Inst.infect.Dis. Tokyo Univ., 6, 511.

Sinclair, K.B. (1962) Br.vet.J., 118, 37.

Sinclair, K.B. (1964) Br.vet.J., 120, 212.

Smitsin, D.F. (1914) Zentble.Bakt.ParasitKde, 74, 280.

Sisson, S. (1958) in "Anatomy of the Domestic Animals", 4th Edition, W.B. Saunders Company, Philadelphia and London.

Smith, H.A. and Jones, T.C. (1957) in "Veterinary Pathology", Henry Kimpton, London.

Snedecor, G.W. (1956) "Statistical Methods", 5th Edition, Iowa State University Press (1965) printing.

Soulsby, E.J.L. (1965) in "Textbook of Veterinary Clinical Parasitology", Vol. 1, Helminths, Blackwell Scientific Publ., Oxford.

Soulsby, E.J.L. (1968) in "Helminths, Arthropods and Protozoa of Domesticated Animals", Balliere, Tindall and Cassell, London.

Stevenson, W. (1947) Parasitology, 38, 123.

Stewart, W.L. and Crofton, H.D. (1941) Vet.Rec., 53, 619.

Suppenar, R. (1964) Dtsch.tierärztl. Wschr., 71, 668.

Symons, L.E.A. and Boray, J.C. (1967) Vet.Rec., 81, 130.

Taglo, V.I. (1944) Rev.chil.Hist.nat., 46/47, 232.

Taylor, A.W. (1961) The Lancet, (ii), 1334.

Taylor, E.L. (1949) Proc.14th Int.Vet.Congr., London, 2, 81.

Taylor, E.L. (1964) in "Fascioliasis and the Liver Fluke", F.A.O. Agric.Studies, No. 64.

Taylor, E.L. and Mosley, A. (1943) Cited by Taylor, 1964.

Taylor, E.L. and Parfitt, J.W. (1957) Trans.Am.microsc.Soc., 76, 327.

Totley, J.H. (1959) J. Helminth., 33, 281.

Thienel, M. (1926) Münch. tierärztl. Wschr., 77, 771.

Thomas, A.P. (1881) J.Roy.Agric.Soc., England, 17, 1.

Thomas, A.P. (1882) Nature, Lond., 26, 606.

Thomas, A.P. (1883) Quart.J.micr.Sci., 23, 99.

Thomas, R.J. and Stevens, A.J. (1956) Vet. Rec., 68, 471.

Thorpe, E. (1963) "Experimental Fascioliasis in the Albino Rat", D.V.M. Thesis University of Glasgow.

Thorpe, E. (1965a) J.comp.Path., 75, 39.

Thorpe, E. (1965b) Res.vet.Sci., 6, 498.

Todd, J.R. and Ross, J.G. (1966) Expl. Parasit., 19, 151.

Tolgyesi, G. (1958) Acta.vet.hung., 8, 17.

Udall, D.H. (1943) in "The Practice of Veterinary Medicine", 4th Edition, Ithaca, New York.

Urquhart, G.M. (1954) Expl. Parasit., 3, 38.

Urquhart, G.M. (1955) "Experimental Fascioliasis in the Rabbit", Ph.D. Thesis, University of Glasgow.

Urquhart, G.M. (1956) J. Path. Bact., 71, 301.

Valcarenghi, G. and Molinari, P. (1959) Atti Soc.ital.Sci.vet., 13, 649.

Van Grembergen, G. (1950) Ann.Soc.Zool.Belg., 81, 15.

Walley, J.K. (1966) Vet.Rec., 78, 267.

Walker-Love, J. (1965) Personal Communication.

Watt, J.A., Nicolson, T.B. and Macleod, N.S.M. (1961) Vet.Rec., 73, 567.

Welchselbaum, T.E. (1946) Am.J.clin.Path., Tech.Sect., 10, 40.

Weinland, E. and von Brand, T.O. (1926) Z.vergl.Physiol., 4, 212.

Wirth, D. (1950) in "Grundlagen einer Klinischen Hämatologie der Haustiere" 2nd Edition, Urban and Schwarzenberg, Vienna & Innsbruck.

APPENDICES 1 to 5

Appendix 1

Calf Rearing on Dairy Farms in South-West Scotland

Calves aged four to eight months are usually put outside to graze for the first time in May or early June. Because of their age they are usually put on to a permanent paddock or paddocks, invariably adjoining the farm buildings, to allow of adequate supervision and to facilitate supplementary feeding. The time spent on these fields is variable and ranges from three weeks to six months. Some farmers use the small permanent calf paddock to acclimatise the young animals to the outside environment; others use it for a substantial part of the grazing season. However, essentially the time spent on this field depends on its size and the availability of grass. A common procedure is to move calves on to hay aftermath after August but in many cases the farmer moves the animals on to a larger area of permanent grass which, because of scarcity of pasture, is often wet. Where farmers graze hay aftermath they usually bring the calves back to the permanent paddock for a few weeks prior to housing in October or early November. If farmers are using a larger area of wet, permanent grass then they frequently allow stock to graze until later in the year and in some cases attempts are made to outwinter these animals.

APPENDIX 2 - Table 1

Bodyweights (in lbs.) of Calves Following a Single Oral Inoculation with 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	107	121	120	115	116	3.2
1	116	122	130	127	124	3.1
2	125	132	137	132	132	2.5
4	122	134	140	132	132	3.7
6	125	135	142	138	135	4.1
8	130	141	148	144	141	3.9
10	132	140	143	148	141	3.3
11	129	140	148	148	141	4.5
12	136	145	150	145	144	2.9
13	128	134	150	143	139	4.9
14	127	128	148	141	136	5.1
15	133	125	141	141	135	3.8
16	131	D	140	137	136	-
17	123	-	129	D	126	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

Appendix 2 - Table 2

Bodyweights (in lbs.) of Calves Following a Single Oral Inoculation with 3,000 Metacercariae of Paramphistomum

Weeks After Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	129	135	109	118	116	2.7
1	133	135	110	122	124	3.0
2	136	137	129	131	134	2.2
4	134	145	125	130	134	4.3
6	136	132	116	128	128	4.4
8	136	140	129	140	136	4.0
10	139	146	134	141	138	4.7
11	139	150	128	141	138	5.4
12	136	150	120	142	137	6.4
13	133	154	117	143	134	8.6
14	D	158	D	145	150	"
15	"	149	"	146	148	"
16	"	151	"	150	151	"
17	"	153	"	144	150	"
18	"	156	"	147	152	"
19	"	160	"	144	152	"
20	"	157	"	140	149	"
21	"	174	"	155	178	"
22	"	167	"	166	177	"
23	"	167	"	172	180	"
24	"	169	"	173	181	"

D = Died

APPENDIX 2 - Table 3

Bodyweight (in lbs.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	120	120	111	117	3.0
1	124	126	125	125	0.3
2	135	131	133	133	1.2
4	143	132	129	135	4.3
6	149	130	132	137	6.0
8	155	140	140	145	5.0
10	163	150	144	153	6.2
11	168	155	144	156	6.9
12	168	161	148	159	5.9
13	173	161	147	160	7.5
14	181	169	150	167	9.0
15	177	168	152	166	7.3
16	181	174	153	169	8.4
17	189	170	155	171	9.8
18	185	180	157	174	8.6
19	190	186	157	178	10.4
20	208	195	175	193	9.6
21	217	207	186	203	9.1
22	217	211	194	207	6.9
23	228	215	200	214	8.1
24	234	221	206	220	8.1

APPENDIX 2 - Table 4

Total Red Cell Counts ($\times 10^6$/cu.mm.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	8.66	8.49	8.29	7.14	8.15	0.34
1	8.62	9.54	8.86	7.44	8.62	0.44
2	7.88	8.51	7.70	7.40	7.87	0.23
4	8.56	9.06	7.65	8.77	8.51	0.30
6	9.04	8.85	7.53	8.99	8.60	0.36
8	9.08	8.45	7.15	8.84	8.38	0.43
10	8.91	7.96	6.77	8.36	8.00	0.45
11	7.36	8.81	6.60	8.55	7.83	0.52
12	7.05	7.85	6.55	7.43	7.22	0.28
13	6.08	7.04	5.46	6.87	6.36	0.37
14	5.19	6.61	4.84	5.91	5.64	0.39
15	5.07	5.80	4.41	5.41	5.17	0.29
16	4.98	D	4.00	4.32	4.43	-
17	4.77	-	3.49	D	4.13	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

APPENDIX 2 - Table 5

Total Red Cell Counts ($x10^6$/cu.mm.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	8.46	8.49	6.92	7.67	7.89	0.37
1	8.20	9.51	7.22	8.65	8.40	0.48
2	9.90	8.67	6.23	7.77	7.39	0.77
4	9.96	8.20	6.57	8.88	8.40	0.70
6	10.80	9.33	6.42	9.77	9.08	0.94
8	9.71	9.10	6.53	8.38	8.43	0.69
10	8.53	8.29	5.96	7.96	7.69	0.56
11	5.81	8.00	5.71	8.08	6.90	0.66
12	4.29	7.88	4.99	7.68	6.21	0.92
13	3.52	7.31	D	7.08	5.97	1.23
14	D	6.41	"	6.42	6.42	"
15	"	6.41	"	6.35	6.38	"
16	"	6.34	"	6.18	6.26	"
17	"	6.17	"	5.65	5.91	"
18	"	6.32	"	5.89	6.11	"
19	"	6.26	"	5.85	6.06	"
20	"	6.08	"	5.21	5.65	"
21	"	5.91	"	5.35	5.63	"
22	"	6.99	"	5.53	6.26	"
23	"	6.76	"	5.50	6.13	"
24	"	7.18	"	5.72	6.45	"

D = Died

APPENDIX 2 - Table 6

Total Red Cell Counts ($x10^6$/cu.mm.) of Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	7.70	9.14	8.34	8.39	0.42
1	8.34	8.39	8.45	8.39	0.04
2	7.00	7.70	7.58	7.43	0.22
4	6.95	8.20	8.95	8.03	0.58
6	7.47	8.79	8.62	8.29	0.41
8	9.55	7.92	8.02	8.50	0.53
10	8.25	7.16	7.44	7.62	0.33
11	8.71	7.48	8.07	8.09	0.36
12	8.03	7.07	7.49	7.53	0.28
13	8.19	7.27	7.93	7.80	0.28
14	7.28	6.66	7.16	7.03	0.19
15	7.67	7.20	8.03	7.64	0.25
16	7.35	7.02	7.90	7.42	0.26
17	7.18	7.19	7.62	7.33	0.14
18	7.08	9.60	7.10	7.93	0.84
19	7.07	7.51	7.81	7.46	0.22
20	6.53	7.48	6.39	6.80	0.34
21	6.34	7.32	6.76	6.81	0.28
22	7.08	7.83	7.22	7.38	0.23
23	6.53	7.36	7.04	6.98	0.24
24	6.59	7.42	6.89	6.97	0.24

APPENDIX 2 - Table 7

Packed Cell Volume Percentages of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	35.0	41.0	37.0	30.0	35.8	2.29
1	33.0	39.0	34.0	29.0	33.8	1.03
2	32.0	36.5	32.0	30.0	32.6	1.38
4	35.0	36.5	32.5	39.5	35.9	1.46
6	33.0	36.5	30.0	37.0	34.6	1.60
8	36.5	42.5	27.5	37.5	36.0	3.12
10	38.5	39.0	29.0	40.0	36.6	2.55
11	31.0	42.0	29.5	39.5	35.5	3.10
12	29.5	36.5	25.5	34.5	31.5	2.48
13	26.5	33.5	24.0	32.0	29.0	2.25
14	23.5	30.5	22.5	30.0	26.6	2.11
15	24.5	27.5	20.5	27.5	25.0	1.66
16	23.5	D	19.5	21.5	21.5	"
17	24.0	"	16.0	D	20.0	"
18	D	"	D	"		
19	"	"	"	"		
20	"	"	"	"		
21	"	"	"	"		
22	"	"	"	"		
23	"	"	"	"		
24	"	"	"	"		

D = Died

APPENDIX 2 - Table 8

Packed Cell Volume Percentages of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	42.0	33.0	30.0	32.0	34.3	2.66
1	40.0	33.0	29.0	33.0	34.8	2.23
2	36.0	33.0	27.5	33.5	32.5	1.79
4	40.0	35.0	32.0	38.5	36.4	1.80
6	40.0	34.5	28.5	38.0	35.3	2.52
8	40.0	35.0	31.0	38.0	36.0	1.96
10	33.5	36.0	30.5	35.5	33.9	1.25
11	21.5	32.5	27.5	34.0	28.9	2.82
12	17.5	29.5	24.0	31.5	25.6	3.14
13	14.5	27.5	D	28.5	23.5	-
14	D	27.0	-	28.0	27.5	-
15	-	27.5	-	27.5	27.5	-
16	-	27.0	-	26.5	26.8	-
17	-	28.5	-	25.5	27.0	-
18	-	28.5	-	26.5	27.5	-
19	-	28.5	-	26.5	27.5	-
20	-	26.5	-	23.5	25.0	-
21	-	28.0	-	24.5	26.3	-
22	-	30.0	-	25.0	27.5	-
23	-	30.5	-	24.0	27.3	-
24	-	31.5	-	26.0	28.8	-

D = Died

APPENDIX 2 - Table 9

Packed Cell Volume Percentages of Calves Used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	32.0	32.0	39.0	34.3	2.34
1	33.0	31.0	31.0	31.7	0.67
2	31.5	30.5	28.5	30.2	0.88
4	29.5	34.0	35.0	32.8	1.69
6	34.5	36.0	33.5	34.7	0.73
8	37.0	31.5	32.0	33.5	1.76
10	34.5	30.5	30.5	31.8	1.33
11	34.5	30.0	33.5	32.7	1.37
12	31.5	29.5	31.5	30.8	0.67
13	32.0	29.0	34.0	31.7	1.46
14	31.0	28.5	30.0	29.8	0.73
15	30.0	28.5	38.0	32.2	2.95
16	30.0	28.5	32.5	33.7	1.17
17	29.5	30.0	31.5	30.3	0.61
18	28.5	31.0	30.5	30.0	0.76
19	29.0	32.5	32.0	31.2	1.09
20	26.5	30.0	27.0	27.8	1.09
21	26.5	30.5	29.0	28.7	1.17
22	27.5	31.5	29.0	29.3	1.17
23	29.0	30.0	29.0	29.3	0.34
24	31.5	31.5	29.5	30.8	0.67

APPENDIX 2 - Table 10

Haemoglobin Concentration (gm./100 ml.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	10.8	14.2	10.5	8.8	11.1	1.13
1	9.7	12.5	12.2	10.0	11.1	0.73
2	10.2	11.4	10.2	10.0	10.5	0.32
4	11.3	11.9	10.0	12.2	11.4	0.49
6	11.6	11.6	10.0	11.0	11.3	0.43
8	12.2	14.5	9.2	12.2	12.0	1.09
10	12.8	12.5	9.4	13.0	11.9	0.85
11	10.8	13.3	9.8	13.3	11.8	0.89
12	10.2	12.2	8.7	11.5	10.7	0.77
13	9.1	10.9	8.3	9.4	9.4	0.62
14	8.0	10.0	6.9	8.8	8.4	0.65
15	8.0	9.1	6.6	8.3	8.0	0.52
16	7.7	D	6.1	6.9	6.9	-
17	7.4	-	5.3	-	6.4	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Dead

APPENDIX 2 - Table 11

Haemoglobin Concentration (gm./100 ml.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	12.8	9.7	9.1	11.1	10.7	0.82
1	14.2	12.2	9.7	12.5	12.2	0.93
2	11.3	10.1	8.0	11.0	10.1	0.75
4	13.3	10.8	9.7	12.3	11.5	0.80
6	13.3	11.1	15.0	13.3	13.2	0.80
8	14.2	12.0	10.3	13.4	12.5	0.86
10	11.6	11.3	10.2	12.2	11.3	0.45
11	7.8	10.9	9.3	12.2	10.1	0.96
12	6.1	10.2	8.0	11.1	8.9	1.12
13	7.7	9.1	D	10.2	9.0	-
14	D	8.8	-	9.4	9.1	-
15	-	8.3	-	9.1	8.7	-
16	-	8.8	-	9.1	9.0	-
17	-	8.8	-	7.6	8.2	-
18	-	8.8	-	8.3	8.6	-
19	-	9.2	-	8.1	8.7	-
20	-	8.1	-	7.6	7.9	-
21	-	8.8	-	7.8	8.3	-
22	-	9.2	-	8.1	8.7	-
23	-	9.8	-	8.2	9.0	-
24	-	9.2	-	8.8	9.0	-

D = Died

APPENDIX 2 - Table 12

Haemoglobin Concentration (gm. per 100 ml.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	10.0	10.0	10.7	10.2	0.23
1	12.2	10.8	11.6	11.5	0.41
2	9.8	9.4	9.3	9.5	0.15
4	10.0	10.8	11.3	10.7	0.38
6	11.3	10.8	11.3	11.1	0.17
8	12.0	10.4	10.6	11.0	0.50
10	10.5	10.0	10.2	10.2	0.15
11	12.0	10.2	11.0	11.1	0.52
12	11.0	9.5	10.6	10.4	0.45
13	11.3	10.0	11.5	10.9	0.47
14	10.7	9.1	10.2	10.0	0.47
15	10.5	9.7	11.1	10.4	0.41
16	10.5	9.7	10.8	10.3	0.33
17	9.5	9.2	9.6	9.4	0.12
18	9.1	9.9	9.4	9.5	0.23
19	9.4	10.1	10.2	9.9	0.25
20	8.3	9.2	9.1	8.9	0.29
21	8.6	9.4	9.2	9.1	0.24
22	9.2	9.5	9.2	9.3	0.10
23	9.0	9.1	9.1	9.1	0.04
24	8.6	8.6	8.8	8.7	0.07

APPENDIX 2 - Table 13

Mean Corpuscular Volume (cu./μ) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	40	48	45	42	44	1.75
1	38	41	38	39	39	0.70
2	41	43	42	41	42	0.48
4	41	40	42	43	42	1.08
6	39	41	40	41	40	0.48
8	40	45	38	42	41	1.49
10	43	49	43	41	46	1.60
11	42	48	45	46	45	1.25
12	42	47	39	46	44	1.85
13	44	48	45	47	46	0.91
14	45	46	46	51	47	1.35
15	48	47	45	51	48	1.25
16	47	D	49	50	50	-
17	50	-	46	D	48	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

APPENDIX 2 - Table 14

Mean Corpuscular Volume (cu./u) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of Fasciola hepatica

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	50	39	43	42	44	2.33
1	49	37	40	40	42	2.60
2	36	38	44	43	40	1.93
4	40	43	49	43	44	1.89
6	37	37	44	40	40	1.66
8	41	39	47	45	43	1.83
10	39	43	51	45	45	2.25
11	D	41	48	42	42	2.27
12	"	37	48	41	42	2.29
13	"	38	D	40	40	"
14	"	42	"	44	43	"
15	"	43	"	43	43	"
16	"	43	"	43	43	"
17	"	46	"	45	46	"
18	"	45	"	45	45	"
19	"	46	"	45	46	"
20	"	44	"	45	45	"
21	"	47	"	46	47	"
22	"	43	"	45	44	"
23	"	45	"	44	45	"
24	"	44	"	47	46	"

D = Died

APPENDIX 2 - Table 15

Mean Corpuscular Volume (cu./μ) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of Fasciola hepatica

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	42	35	38	38	2.03
1	40	37	37	38	1.00
2	45	40	38	41	2.08
4	42	41	40	41	0.58
6	46	41	39	42	2.08
8	39	40	40	40	0.34
10	42	43	42	42	0.34
11	40	40	42	41	0.67
12	39	42	42	41	1.00
13	39	40	43	41	1.20
14	43	43	42	43	0.34
15	39	40	41	40	0.58
16	41	41	41	41	0.00
17	41	42	41	41	0.34
18	40	41	43	41	0.89
19	41	43	45	43	1.15
20	41	40	42	41	0.58
21	42	42	43	42	0.34
22	39	40	40	40	0.34
23	42	41	41	41	0.34
24	44	40	43	42	1.20

APPENDIX 2 - Table 16

Mean Corpusoular Haemoglobin Concentration (per cent) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	31	35	28	29	31	1.55
1	29	32	36	34	33	1.49
2	32	31	32	33	32	0.41
4	32	33	31	31	32	0.48
6	34	32	33	32	33	0.48
8	33	34	33	33	33	0.25
10	33	32	32	33	33	0.29
11	35	32	33	34	34	0.65
12	35	33	34	33	34	0.48
13	34	33	34	29	33	1.19
14	34	33	31	29	32	1.11
15	33	33	33	30	32	0.75
16	32	D	31	32	32	-
17	31	-	33	D	32	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

APPENDIX 2 - Table 17

Mean Corpuscular Haemoglobin Concentration (per cent) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	30	29	30	35	31	1.35
1	36	35	33	36	35	0.71
2	31	31	29	33	31	0.82
4	33	31	30	32	32	0.65
6	33	32	34	34	33	0.48
8	36	32	33	35	34	0.91
10	35	31	33	34	33	0.85
11	36	34	34	36	35	0.58
12	35	35	33	35	35	0.50
13	53	34	D	36	41	-
14	D	33	-	34	32	-
15	-	30	-	33	32	-
16	-	33	-	34	34	-
17	-	31	-	30	31	-
18	-	31	-	31	31	-
19	-	32	-	31	32	-
20	-	31	-	32	32	-
21	-	31	-	32	32	-
22	-	31	-	32	32	-
23	-	32	-	34	33	-
24	-	29	-	34	32	-

D = Died

APPENDIX 2 - Table 18

Mean Corpuscular Haemoglobin Concentration of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	31	31	27	30	1.34
1	37	35	37	36	0.67
2	31	31	33	32	0.67
4	34	32	32	33	0.67
6	33	30	34	32	1.20
8	32	33	33	33	0.34
10	30	33	33	32	1.00
11	35	34	33	34	0.58
12	35	32	34	34	0.89
13	33	34	32	34	0.89
14	34	32	34	34	0.67
15	35	34	33	34	0.58
16	35	34	33	34	0.58
17	32	31	30	31	0.58
18	32	32	31	32	0.34
19	32	31	32	32	0.34
20	31	31	34	32	1.00
21	32	31	32	32	0.34
22	33	30	32	32	0.89
23	33	30	31	32	0.89
24	30	29	30	30	0.34

APPENDIX 2 - Table 19

Total White Cell Counts ($x10^3$/cu.mm.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of Fasciola hepatica

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	14.6	9.2	13.8	8.6	11.6	1.54
1	12.7	12.1	17.9	10.4	13.3	1.62
2	11.3	11.2	19.1	9.5	12.8	2.15
4	14.2	11.2	17.0	9.3	12.9	1.69
6	17.8	15.3	18.3	11.3	15.7	1.60
8	16.1	16.5	20.4	11.8	16.2	1.76
10	17.5	14.3	19.2	13.2	16.1	1.39
11	15.0	17.0	18.7	13.2	16.0	1.20
12	20.6	13.4	18.4	14.2	16.7	1.71
13	13.7	19.3	14.7	15.9	15.9	1.22
14	14.0	19.8	13.9	14.1	15.5	1.45
15	18.1	16.2	14.4	18.8	16.9	1.00
16	16.5	D	13.0	14.6	14.7	-
17	14.4	-	12.7	D	13.6	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

APPENDIX 2 - Table 20

Total White Cell Counts ($x10^3$/cu.mm.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	8.7	8.1	8.9	10.9	9.2	0.61
1	11.1	13.3	9.3	14.3	12.0	1.12
2	10.6	11.7	9.8	11.7	11.0	0.46
4	10.3	16.4	7.4	11.4	11.4	1.88
6	13.3	13.7	15.1	13.6	14.0	0.40
8	12.3	11.2	12.7	11.1	11.8	0.40
10	12.7	11.8	10.7	12.3	11.9	0.43
11	14.4	12.8	12.9	12.4	13.2	0.44
12	12.2	9.6	13.0	11.6	11.6	0.73
13	15.7	8.9	D	9.1	11.2	2.23
14	D	13.6	-	11.0	12.3	-
15	-	10.3	-	11.4	10.9	-
16	-	8.8	-	9.5	9.2	-
17	-	13.1	-	11.5	12.3	-
18	-	12.7	-	12.6	12.7	-
19	-	13.3	-	14.6	14.0	-
20	-	11.3	-	9.7	10.5	-
21	-	12.4	-	14.7	13.6	-
22	-	9.3	-	11.6	10.5	-
23	-	12.1	-	11.8	12.0	-
24	-	12.6	-	12.1	12.4	-

D = Died

APPENDIX 2 - Table 21

Total White Cell Counts ($x10^3$/cu.mm.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	6.3	13.0	9.0	9.4	1.95
1	6.1	13.2	11.5	10.3	2.14
2	9.4	9.7	8.9	9.3	0.23
4	7.5	8.6	9.5	8.5	0.58
6	15.0	17.3	11.1	14.5	1.81
8	12.1	11.6	9.1	10.9	0.93
10	13.4	14.7	7.8	12.0	2.12
11	9.9	13.1	13.7	13.2	1.18
12	8.7	9.5	8.7	9.0	0.27
13	11.2	10.5	9.0	10.2	0.65
14	14.8	12.7	8.8	12.1	1.76
15	13.1	11.9	9.9	11.6	0.93
16	9.8	11.2	9.5	10.2	0.52
17	8.7	10.2	10.6	9.8	0.58
18	12.5	13.6	9.7	11.9	1.16
19	11.6	13.1	11.8	12.2	0.47
20	10.7	11.6	9.5	10.6	0.61
21	14.6	17.0	12.3	14.6	1.36
22	10.7	13.9	11.1	11.9	1.01
23	13.1	13.3	12.1	12.8	0.37
24	14.6	13.0	11.2	12.9	0.98

APPENDIX 2 - Table 22

Differential Cell Count (% Eosinophils) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	"	"	"	"	"	"
1	"	"	"	"	"	"
2	"	"	"	"	"	"
4	5.0	3.0	4.5	4.0	4.1	0.43
6	1.5	2.0	0	3.0	1.6	0.63
8	15.0	6.0	2.0	12.3	8.9	3.00
10	8.0	4.0	1.0	4.5	4.4	1.43
11	17.0	1.5	5.5	12.5	9.1	3.47
12	13.5	1.0	9.0	11.5	8.8	2.74
13	17.5	0	4.0	10.0	7.9	3.81
14	23.5	0	2.0	11.5	9.3	5.37
15	14.5	0	5.5	3.5	5.9	3.09
16	14.0	D	8.0	6.0	9.3	-
17	4.5	"	0	D	2.3	-
18	D	"	D	"		
19	"	"	"	"		
20	"	"	"	"		
21	"	"	"	"		
22	"	"	"	"		
23	"	"	"	"		
24	"	"	"	"		

D = Died

APPENDIX 2 - Table 23

Differential White Cell Count (% Eosinophils) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of Fasciola hepatica

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	"	"	"	"	"	"
1	"	"	"	"	"	"
2	"	"	"	"	"	"
4	2.0	0	0	0.5	0.6	0.47
6	11.5	1.0	20.5	2.5	8.9	4.52
8	5.0	5.0	5.0	0.5	3.9	1.01
10	0.5	10.5	2.0	1.0	3.5	2.35
11	1.5	13.0	0	3.5	4.5	2.92
12	0.5	10.0	D	2.5	4.3	"
13	D	4.5	"	6.5	5.5	"
14	"	4.5	"	8.5	6.5	"
15	"	4.5	"	5.5	5.0	"
16	"	4.0	"	5.0	4.5	"
17	"	2.0	"	9.0	5.5	"
18	"	6.0	"	5.5	5.8	"
19	"	18.0	"	5.5	11.8	"
20	"	12.0	"	6.5	9.3	"
21	"	14.0	"	8.5	11.3	"
22	"	5.0	"	5.0	5.0	"
23	"	4.0	"	5.5	4.8	"
24	"	3.0	"	5.0	4.0	"

D = Dead

APPENDIX 2 - Table 24

Differential White Cell Count (% Eosinophils) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Weeks	Calf Number			Mean	Standard Error
	1	2	3		
0	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	-
4	0	0.5	0.5	0.3	0.17
6	2.0	1.5	0.5	1.3	0.44
8	2.0	3.5	4.0	3.2	0.60
10	4.0	5.5	2.0	3.8	1.01
11	1.5	6.5	5.0	4.3	1.48
12	8.0	2.5	3.5	4.7	1.69
13	3.5	8.0	9.0	6.8	1.69
14	3.0	8.5	13.5	8.3	3.03
15	4.5	10.0	11.5	8.7	2.13
16	3.5	6.0	3.0	4.2	0.93
17	5.5	13.5	12.5	10.5	2.52
18	3.5	6.5	2.0	4.0	1.32
19	8.5	6.0	5.0	6.5	1.04
20	21.5	6.0	9.0	12.2	5.34
21	19.5	12.0	9.0	13.5	3.12
22	11.5	12.5	4.0	9.3	2.68
23	8.5	11.5	4.0	8.0	2.18
24	7.5	9.0	3.5	6.7	1.64

APPENDIX 2 - Table 25

Differential White Cell Count (% Neutrophils) of Calves Following a Single Oral Inoculation of 1,000 Metecercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	"	"	"	"	"	"
1	"	"	"	"	"	"
2	"	"	"	"	"	"
4	33.0	23.0	18.5	29.5	26.0	3.25
6	22.5	20.0	22.5	24.5	22.4	0.92
8	23.0	18.5	35.0	21.5	24.5	3.62
10	19.5	33.5	32.5	31.5	29.5	3.77
11	21.5	20.5	26.0	38.5	26.6	4.14
12	16.5	25.0	27.0	40.0	27.1	4.86
13	21.5	44.0	66.0	37.0	42.1	9.24
14	36.5	41.0	24.5	45.0	36.8	4.44
15	25.5	D	21.0	47.0	31.2	"
16	31.5	"	51.0	D	41.3	"
17	D	"	D	"		
18	"	"	"	"		
19	"	"	"	"		
20	"	"	"	"		
21	"	"	"	"		
22	"	"	"	"		
23	"	"	"	"		
24	"	"	"	"		

D = Dead

APPENDIX 2 - Table 26

Differential White Cell Count (% Neutrophils) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	-	-	-	-	-
4	47.5	33.0	45.5	35.0	40.3	3.65
6	32.5	23.0	18.5	18.0	23.3	3.60
8	44.5	26.0	39.0	28.5	34.5	4.36
10	59.0	21.0	42.5	32.0	38.6	10.11
11	58.0	12.5	48.0	27.0	36.4	10.25
12	66.5	19.0	D	20.0	35.2	-
13	D	35.5	-	27.0	31.3	-
14	-	53.5	-	31.5	43.5	-
15	-	13.0	-	26.5	19.8	-
16	-	31.0	-	46.0	38.5	-
17	-	31.0	-	31.0	31.0	-
18	-	35.0	-	34.0	34.5	-
19	-	19.5	-	29.0	24.3	-
20	-	21.5	-	29.5	25.5	-
21	-	8.5	-	17.5	13.0	-
22	-	34.5	-	31.0	32.8	-
23	-	24.5	-	25.5	25.0	-
24	-	31.5	-	30.0	30.8	-

D = Died

APPENDIX 2 - Table 27

Differential White Cell Count (% Neutrophils) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	-
4	24.0	38.0	30.0	30.7	4.06
6	28.0	19.5	29.5	25.7	3.11
8	30.0	13.0	15.5	19.5	5.30
10	16.0	23.0	33.0	24.0	4.93
11	21.5	16.0	38.5	25.3	6.77
12	27.5	11.5	13.5	17.5	5.03
13	40.0	31.5	25.0	32.2	4.34
14	37.0	25.5	22.5	28.3	4.42
15	33.5	16.5	22.5	24.2	4.98
16	29.5	21.5	29.5	26.8	2.67
17	37.0	24.0	27.0	29.3	3.93
18	35.0	24.0	45.5	34.8	6.21
19	31.0	23.0	31.0	28.3	2.67
20	22.0	26.0	24.0	24.0	1.15
21	18.5	6.5	22.0	15.7	4.69
22	41.0	34.0	35.0	36.7	2.19
23	30.0	30.0	27.5	29.2	0.83
24	23.0	31.5	25.5	26.7	2.52

APPENDIX 2 - Table 28

Differential White Cell Count (% Lymphocytes) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	"	"	"	"	"	"
1	"	"	"	"	"	"
2	"	"	"	"	"	"
4	65.5	75.0	81.5	67.5	72.4	3.67
6	62.5	74.0	75.5	63.5	68.9	3.41
8	69.0	77.5	64.0	74.0	71.1	2.95
10	63.5	65.0	61.0	56.0	61.4	1.97
11	65.0	78.5	65.0	50.0	64.6	5.82
12	66.0	75.0	68.0	50.0	64.8/11	5.28
13	55.0	56.0	32.0	51.5	48.6	5.63
14	49.0	59.0	70.0	51.5	57.4	4.71
15	60.5	D	71.0	47.0	59.5	"
16	64.0	"	49.0	D	56.5	"
17	D	"	D	"		
18	"	"	"	"		
19	"	"	"	"		
20	"	"	"	"		
21	"	"	"	"		
22	"	"	"	"		
23	"	"	"	"		
24	"	"	"	"		

D = Died

APPENDIX 2 - Table 29

Differential White Cell Count (% Lymphocytes) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	-	-	-	-	-
4	50.5	67.0	54.5	64.5	59.1	3.94
6	54.5	76.0	61.0	79.5	67.8	5.97
8	50.5	69.0	56.0	71.0	61.6	4.98
10	40.5	68.5	55.5	66.0	57.6	6.37
11	40.5	74.5	52.0	69.5	59.1	9.41
12	33.0	71.0	D	77.5	60.5	-
13	D	60.0	-	68.5	64.3	-
14	-	40.0	-	60.0	50.0	-
15	-	82.5	-	68.0	75.3	-
16	-	65.0	-	49.0	57.0	-
17	-	67.0	-	60.0	63.5	-
18	-	59.0	-	60.5	59.8	-
19	-	62.5	-	65.5	64.0	-
20	-	66.5	-	64.0	65.3	-
21	-	75.5	-	74.0	74.8	-
22	-	60.5	-	64.0	62.3	-
23	-	71.5	-	69.0	70.3	-
24	-	65.5	-	65.0	65.4	-

D = Died

APPENDIX 2 - Table 30

Differential White Cell Count (% Lymphocytes) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	-	-	-	-	-
1	-	-	-	-	-
2	-	-	-	-	-
4	76.0	61.5	69.5	69.0	4.19
6	70.0	79.0	70.0	73.0	3.00
8	68.0	83.5	80.5	77.3	4.75
10	80.0	71.5	65.0	72.2	4.34
11	77.0	77.5	76.5	77.0	0.29
12	64.5	86.0	83.0	77.8	6.72
13	56.5	60.5	66.0	61.0	2.75
14	60.0	66.0	64.0	63.3	1.76
15	62.0	73.5	66.0	67.2	3.37
16	67.0	72.5	67.5	69.0	1.76
17	57.5	62.5	60.5	60.2	1.45
18	61.5	69.5	52.5	61.2	4.91
19	60.5	71.0	64.0	65.2	3.09
20	56.5	68.0	67.0	63.8	3.68
21	62.0	81.5	69.0	70.8	5.70
22	47.5	53.5	61.0	54.0	3.91
23	61.5	58.5	68.5	62.8	2.96
24	69.5	59.5	71.0	66.7	3.61

APPENDIX 2 - Table 31

Total Serum Protein Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	5.6	5.0	4.9	NS	5.2	0.22
1	5.5	5.0	5.2	5.2	5.2	0.10
2	5.3	5.0	5.0	5.2	5.1	0.08
4	6.0	6.0	5.4	5.1	5.6	0.23
6	5.8	5.8	5.8	5.9	5.8	0.06
8	6.2	7.4	5.8	6.0	6.4	0.36
10	7.3	7.4	6.5	6.3	6.9	0.28
11	6.2	7.8	5.6	6.6	6.6	0.47
13	5.0	6.4	4.8	6.3	5.6	0.42
15	6.2	6.2	5.3	6.9	6.2	0.13
16	6.4	D	5.1	6.3	5.9	"
17	5.7	"	4.5	D	5.1	"
18	D	"	D	"		
19	"	"	"	"		
20	"	"	"	"		
21	"	"	"	"		
22	"	"	"	"		
23	"	"	"	"		
24	"	"	"	"		

D = Died

APPENDIX 2 - Table 32

Total Serum Protein Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	5.1	3.4	5.1	5.0	5.1	0.09
1	6.0	5.2	4.6	5.1	5.2	0.29
2	6.1	5.4	4.7	4.9	5.3	0.31
4	6.6	5.8	5.5	6.0	6.0	0.23
6	6.4	5.6	5.2	6.1	5.8	0.27
8	7.1	6.6	6.2	4.7	6.2	0.52
10	7.1	7.2	7.0	6.2	6.9	0.23
11	6.2	6.9	6.1	6.7	6.5	0.19
13	5.3	6.0	D	6.1	5.8	-
15	D	7.1	-	7.2	7.2	-
16	-	6.8	-	7.1	7.0	-
17	-	5.7	-	6.5	6.1	-
18	-	5.7	-	6.5	6.1	-
19	-	6.0	-	6.9	6.5	-
20	-	6.0	-	6.3	6.2	-
21	-	6.5	-	6.5	6.5	-
22	-	6.8	-	7.2	7.0	-
23	-	6.9	-	6.9	6.9	-
24	-	7.0	-	6.7	6.9	-

D = Died

APPENDIX 2 - Table 33

Total Serum Protein Levels (gms. per 100 ml.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	5.0	5.1	5.6	5.2	0.19
1	5.0	5.2	5.3	5.2	0.09
2	5.1	6.2	4.8	5.4	0.43
4	5.9	5.6	6.1	5.9	0.15
6	5.8	5.9	6.2	6.0	0.12
8	6.0	5.5	7.2	6.2	0.51
10	6.6	6.0	6.1	6.2	0.19
11	6.2	6.0	6.7	6.3	0.21
13	5.7	5.9	6.7	6.1	0.31
15	6.0	7.1	7.7	6.9	0.50
16	6.4	6.9	7.6	7.0	0.35
17	5.6	6.3	6.6	6.2	0.30
18	5.7	6.4	7.3	6.5	0.46
19	6.1	6.5	7.3	6.6	0.33
20	5.8	6.6	7.0	6.5	0.35
21	5.6	6.5	6.2	6.1	0.26
22	6.8	7.0	7.1	7.0	0.09
23	6.6	6.6	6.5	6.6	0.03
24	6.3	7.3	6.1	6.6	0.37

APPENDIX 2 - Table 34

Serum Alpha/Beta Globulin Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	1.27	1.13	0.90	NS	1.10	0.11
1	1.17	1.17	0.79	0.68	0.95	0.13
2	1.21	1.08	1.39	0.74	1.11	0.16
4	1.25	1.83	1.30	0.97	1.34	0.18
6	0.97	0.83	0.77	1.44	1.00	0.15
8	1.13	1.44	1.37	1.60	1.39	0.10
10	1.53	2.62	1.47	1.62	1.81	0.27
11	1.50	1.66	1.72	1.78	1.67	0.06
13	1.39	1.86	1.74	1.49	1.62	0.11
15	1.28	1.33	1.10	1.35	1.27	0.06
16	1.24	D	0.97	1.39	1.20	-
17	1.45	-	1.24	D	1.35	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Dead
NS = No Sample Available

APPENDIX 2 - Table 35

Serum Alpha/Beta Globulin Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	1.69	1.15	1.04	0.73	1.15	0.20
1	1.35	0.93	0.94	1.08	1.08	0.10
2	1.50	1.07	0.87	0.96	1.10	0.14
4	1.82	1.21	0.90	1.27	1.30	0.19
6	1.60	0.83	1.59	1.38	1.35	0.18
8	2.21	0.91	0.94	1.37	1.36	0.30
10	2.57	2.36	2.89	2.33	2.54	0.13
11	1.66	1.54	1.59	1.77	1.64	0.05
13	1.00	1.23	D	1.03	1.09	"
15	D	0.99	"	1.33	1.16	"
16	"	0.82	"	1.30	1.06	"
17	"	1.45	"	1.08	1.27	"
18	"	1.24	"	1.27	1.26	"
19	"	0.64	"	0.97	0.81	"
20	"	1.14	"	1.35	1.25	"
21	"	1.14	"	1.35	1.25	"
22	"	1.23	"	1.14	1.19	"
23	"	1.08	"	0.98	1.03	"
24	"	1.56	"	1.39	1.48	"

D = Died

APPENDIX 2 - Table 36

Serum Alpha/Beta Globulin Levels (gms. per 100 ml.) of Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	1.29	1.03	1.09	1.14	0.08
1	1.17	1.05	1.26	1.16	0.06
2	0.92	0.67	0.88	0.82	0.08
4	1.10	0.76	1.24	1.03	0.14
6	1.41	1.44	1.54	1.46	0.04
8	1.34	1.34	2.04	1.57	0.23
10	1.10	1.55	1.28	1.31	0.13
11	1.46	1.57	1.70	1.58	0.07
13	1.41	1.50	0.89	1.27	0.19
15	1.36	1.23	1.54	1.38	0.09
16	1.31	1.26	1.55	1.37	0.09
17	0.74	1.38	1.39	1.17	0.22
18	1.56	1.31	1.35	1.41	0.08
19	0.64	0.69	1.46	0.93	0.27
20	1.16	1.18	1.09	1.14	0.03
21	1.25	1.24	2.52	1.67	0.42
22	0.99	0.96	1.25	1.07	0.09
23	1.02	1.02	0.94	0.99	0.03
24	1.48	1.64	1.52	1.55	0.05

APPENDIX 2 - Table 27

Serum Gamma Globulin Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	2.63	2.17	2.14	NS	2.31	0.16
1	2.84	2.13	1.95	2.21	2.28	0.19
2	2.49	2.33	1.89	2.18	2.22	0.13
4	2.59	3.35	2.19	2.24	2.59	0.27
6	2.70	2.47	2.40	2.36	2.40	0.08
8	2.97	3.34	2.87	2.82	3.00	0.12
10	3.72	2.71	2.97	2.56	2.99	0.26
11	3.04	3.95	2.18	2.48	2.92	0.39
13	2.03	2.04	2.87	3.35	2.57	0.33
15	3.27	3.21	2.55	3.94	3.24	0.29
16	3.45	D	2.53	3.52	3.17	-
17	2.64	-	1.88	D	2.26	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died
NS = No Sample Available

APPENDIX 2 - Table 38

Serum Gamma Globulin Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	3.42	2.31	2.09	2.02	2.46	0.23
1	2.88	1.94	1.68	2.06	2.14	0.26
2	2.90	2.22	1.87	2.43	2.34	0.23
4	2.76	2.44	2.19	2.82	2.55	0.15
6	2.94	2.52	1.94	2.88	2.57	0.23
8	3.00	3.12	2.87	1.34	2.57	0.41
10	2.59	2.81	2.20	1.78	2.35	0.23
11	3.24	3.55	3.36	3.19	3.34	0.08
13	3.19	3.30	D	3.36	3.28	0.05
15	D	3.73	-	3.89	3.81	-
16	-	3.79	-	3.77	3.78	-
17	-	2.14	-	3.60	2.87	-
18	-	2.94	-	3.55	3.25	-
19	-	3.58	-	4.14	3.86	-
20	-	2.95	-	2.48	2.72	-
21	-	2.89	-	3.69	3.29	-
22	-	3.75	-	4.21	3.98	-
23	-	3.95	-	3.95	3.95	-
24	-	3.82	-	3.87	3.85	-

D = Died

APPENDIX 2 - Table 39

Serum Gamma-Globulin Levels (gms. per 100 ml.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	1.97	2.29	2.21	2.16	0.10
1	2.01	2.30	2.21	2.17	0.09
2	2.16	2.03	2.77	2.32	0.23
4	2.24	2.14	2.65	2.34	0.16
6	2.21	2.26	3.02	2.50	0.26
8	2.68	2.39	2.46	2.51	0.09
10	3.03	1.84	2.50	2.46	0.34
11	2.62	2.52	2.99	2.71	0.14
13	1.91	3.08	2.25	2.41	0.35
15	2.40	3.18	3.68	3.09	0.37
16	2.94	3.17	3.84	3.32	0.27
17	2.72	2.23	2.65	2.53	0.15
18	2.72	3.12	3.61	3.15	0.26
19	2.88	3.52	3.69	3.36	0.25
20	2.57	3.18	3.60	3.12	0.30
21	2.26	3.16	3.57	3.00	0.39
22	3.59	3.53	3.50	3.54	0.03
23	3.46	3.36	3.22	3.35	0.07
24	3.00	3.56	2.80	3.12	0.23

APPENDIX 2 - Table 40

Serum Albumin Levels (gms. per 100 ml.) of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	1.70	1.70	1.86	NS	1.75	0.06
1	1.89	1.69	2.47	2.31	2.09	0.18
2	1.69	1.59	1.66	2.18	1.78	0.14
4	2.15	1.81	1.92	1.93	1.95	0.07
6	2.13	2.50	2.63	1.69	2.24	0.21
8	2.09	2.62	1.93	2.10	2.19	0.15
10	2.05	2.06	2.16	2.12	2.10	0.02
11	1.65	2.18	1.70	1.64	1.79	0.13
13	1.57	1.89	1.79	1.46	1.68	0.10
15	1.65	1.64	1.65	1.62	1.64	0.01
16	1.71	D	1.60	1.39	1.57	-
17	1.60	-	1.38	-	1.49	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died

APPENDIX 2 - Table 41

Serum Albmin Levels (gms. per 100 ml.) in Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	1.98	1.94	1.97	2.00	1.97	0.01
1	1.77	2.33	1.98	1.96	2.01	0.12
2	1.69	2.10	2.02	1.50	1.83	0.14
4	2.02	2.15	2.46	1.90	2.13	0.12
6	1.85	2.23	1.67	1.84	1.90	0.12
8	1.89	2.56	2.45	2.00	2.23	0.17
10	1.95	2.04	1.91	2.09	2.00	0.04
11	1.32	1.81	1.15	1.74	1.51	0.16
13	1.10	1.47	D	1.70	1.42	0.17
15	D	2.38	-	1.98	2.18	-
16	-	2.19	-	2.03	2.11	-
17	-	2.12	-	1.82	1.97	-
18	-	1.52	-	1.68	1.60	-
19	-	1.78	-	1.80	1.79	-
20	-	1.90	-	2.47	2.19	-
21	-	1.90	-	2.47	2.19	-
22	-	1.82	-	1.85	1.84	-
23	-	1.87	-	1.97	1.92	-
24	-	1.75	-	1.50	1.63	-

D = Died

APPENDIX 2 - Table 42

Serum Albumin Levels (gms. per 100 ml.) of the Calves used as Uninfected Controls for those Calves given a Single Oral Inoculation of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	1.74	1.78	2.31	1.94	0.18
1	1.82	1.85	1.84	1.84	0.01
2	2.02	3.50	1.15	2.22	0.69
4	2.56	2.68	2.20	2.48	0.14
6	2.18	2.20	1.64	2.01	0.18
8	1.98	1.47	2.70	2.05	0.36
10	2.48	2.61	2.32	2.47	0.08
11	2.12	1.91	2.00	2.01	0.06
13	2.38	1.32	3.56	2.42	0.65
15	2.24	2.68	2.48	2.47	0.13
16	2.16	2.47	2.21	2.28	0.10
17	2.14	2.70	2.56	2.47	0.17
18	1.55	1.97	2.35	1.96	0.23
19	2.58	2.29	2.14	2.34	0.16
20	2.07	2.24	2.31	2.21	0.07
21	2.07	2.10	2.05	2.07	0.02
22	2.22	2.61	2.25	2.36	0.13
23	2.13	2.22	2.33	2.23	0.06
24	1.82	2.10	1.78	1.90	0.10

APPENDIX 2 - Table 43

Albumin:Globulin Ratios of Calves Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number 1	2	3	4	Mean	Standard Error
0	0.43	0.51	0.62	NS	0.52	0.06
1	0.52	0.50	0.90	0.80	0.68	0.12
2	0.47	0.47	0.50	0.72	0.54	0.08
4	0.56	0.43	0.55	0.60	0.54	0.04
6	0.58	0.76	0.83	0.40	0.64	0.12
8	0.51	0.55	0.50	0.45	0.50	0.03
10	0.39	0.39	0.50	0.51	0.45	0.04
11	0.37	0.39	0.44	0.33	0.38	0.03
13	0.46	0.45	0.39	0.30	0.40	0.04
15	0.36	0.37	0.45	0.31	0.38	0.03
16	0.37	D	0.46	0.28	0.38	-
17	0.39	-	0.44	D	0.42	-
18	D	-	D	-		
19	-	-	-	-		
20	-	-	-	-		
21	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
24	-	-	-	-		

D = Died
NS = No Sample Available

APPENDIX 2 - Table 44

Albumin:Globulin Ratios of Calves Following a Single Oral Inoculation of 2,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Calf Number				Mean	Standard Error
	1	2	3	4		
0	0.39	0.56	0.63	0.45	0.51	0.05
1	0.42	0.51	0.76	0.62	0.58	0.07
2	0.38	0.64	0.75	0.44	0.55	0.09
4	0.44	0.74	0.75	0.46	0.60	0.09
6	0.41	0.67	0.47	0.43	0.50	0.06
8	0.36	0.64	0.66	0.74	0.60	0.08
10	0.38	0.39	0.38	0.51	0.42	0.03
11	0.27	0.25	0.23	0.35	0.30	0.03
13	0.26	0.32	D	0.39	0.32	-
15	D	0.50	-	0.37	0.44	-
16	-	0.48	-	0.40	0.44	-
17	-	0.59	-	0.39	0.49	-
18	-	0.36	-	0.35	0.36	-
19	-	0.42	-	0.35	0.39	-
20	-	0.46	-	0.64	0.55	-
21	-	0.57	-	0.44	0.51	-
22	-	0.37	-	0.34	0.36	-
23	-	0.37	-	0.40	0.39	-
24	-	0.33	-	0.31	0.32	-

D = Died

APPENDIX 2 - Table 45

Albumin:Globulin Ratios of the Calves used as Uninfected Controls for those Calves given Single Oral Inoculations of 1,000 or 2,000 Metacercariae of *Fasciola hepatica*

Week	Calf Number			Mean	Standard Error
	1	2	3		
0	0.53	0.54	0.70	0.59	0.06
1	0.57	0.55	0.53	0.55	0.01
2	0.63	1.30	0.32	0.76	0.29
4	0.78	0.92	0.56	0.75	0.10
6	0.60	0.60	0.36	0.52	0.08
8	0.49	0.86	0.60	0.65	0.11
10	0.60	0.77	0.61	0.66	0.05
11	0.52	0.47	0.43	0.47	0.03
13	0.72	0.29	1.14	0.72	0.25
15	0.60	0.52	0.47	0.53	0.04
16	0.51	0.56	0.41	0.49	0.04
17	0.62	0.73	0.63	0.67	0.04
18	0.37	0.44	0.47	0.43	0.03
19	0.73	0.55	0.42	0.57	0.09
20	0.55	0.51	0.49	0.52	0.02
21	0.59	0.48	0.47	0.51	0.04
22	0.48	0.58	0.47	0.51	0.04
23	0.48	0.51	0.56	0.52	0.02
24	0.41	0.40	0.41	0.41	0.01

APPENDIX 2 - Table 46

Faecal Egg Counts of Calves Following A Single Oral Inoculation of 1,000 (Group 1) and 2,000 (Group 2) Metacercariae of *Fasciola hepatica*

	Week	0-9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	Calf No.															
Group 1	1	-ve	-ve	-ve	-ve	200	200	100	100	525	D					
	2	-ve	100	50	50	-ve	25	175	D							
	3	-ve	-ve	-ve	-ve	-ve	100	100	50	100	D					
	4	-ve	-ve	-ve	100	50	100	-ve	75	D						
	Mean	-	25	13	38	63	106	94	75	313						
	S.E.	-	25	13	24	47	36	36	-	-						
Group 2	1	-ve	150	-ve	500	150	D									
	2	-ve	-ve	-ve	150	-ve	-ve	100	75	50	-ve	-ve	25	-ve	25	
	3	-ve	-ve	50	150	100	D									
	4	-ve	-ve	50	-ve	-ve	75	75	75	250	-ve	-ve	50	150	50	
	Mean	-	38	25	200	63	38	88	75	150	-	-	38	75	38	
	S.E.	-	38	14	106	38	-	-	-	-	-	-	-	-	-	

APPENDIX 3 - Table 1

Total Red Cell Counts (millions per cu. mm.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	11.80	12.01	13.21	11.11	12.20	12.13	11.43	11.91	11.98	0.22
1	11.30	11.52	12.08	10.86	11.44	12.06	11.66	11.88	11.60	0.15
2	10.28	10.04	10.74	9.52	11.64	11.86	12.00	11.04	10.89	0.32
3	10.88	11.06	11.38	10.06	11.60	11.14	11.78	11.16	11.13	0.19
4	11.22	10.38	10.84	9.98	10.76	11.10	11.22	11.10	10.83	0.16
5	9.32	8.58	8.54	10.60	9.76	10.02	8.82	10.32	9.50	0.28
6	9.56	8.48	9.92	10.58	8.68	10.04	7.86	9.66	9.35	0.32
7	8.44	7.04	7.60	9.62	8.68	8.60	6.02	8.02	8.00	0.39
8	7.74	5.86	7.06	7.64	4.84	8.68	6.48	9.72	7.25	0.55
9	7.72	4.84	6.30	6.40	5.08	7.86	5.86	7.34	6.43	0.41
10	6.74	4.50	5.00	6.18	5.38	7.96	4.22	7.20	5.90	0.47
11	7.04	4.34	5.16	6.22	4.78	8.10	4.22	5.62	5.69	0.48
12	6.60	3.54	4.48	5.92	Cl.	7.78	3.08	4.88	5.18	0.64
13	7.26	2.78	4.16	5.40	3.90	7.34	3.04	D.	5.18	0.74
14	6.52	D.	3.64	4.40	D.	7.38	D.		5.49	1.02
15	5.18		3.20	4.60		7.06			5.01	0.80
16	4.65		D.	3.29		6.41			4.78	--
17	4.12			2.40		6.40			4.31	--
18	4.10			1.82		6.25			4.06	--
19	3.62			D.		5.66			4.64	--
20	2.59					5.77			4.18	--
21	2.37					4.24			3.31	--
22	D.					4.44				
23						4.07				
24						D.				

D = Died

Cl = Clotted Sample

APPENDIX 3 - Table 2

Packed Cell Volume Percentage of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	24.5	36.5	38.0	34.0	38.0	38.5	35.5	35.5	36.3	0.54
1	32.0	32.0	36.0	31.0	36.5	37.0	36.5	35.0	34.5	0.86
2	30.5	30.0	33.0	29.0	35.0	32.5	35.0	31.0	32.0	0.80
3	30.5	31.5	33.0	30.5	32.0	32.0	31.0	31.0	31.4	0.31
4	31.5	31.5	33.0	31.5	31.5	32.0	31.5	32.0	31.8	0.19
5	28.5	28.0	30.0	34.5	31.0	29.5	24.5	28.5	29.3	1.01
6	27.0	25.5	29.5	32.5	25.0	28.5	21.5	27.5	27.1	1.16
7	21.0	22.0	24.5	30.0	25.0	28.5	20.0	25.5	24.6	1.24
8	24.5	18.0	21.0	23.5	14.0	25.5	19.0	26.0	21.4	1.49
9	23.5	16.5	21.0	21.5	19.0	24.5	18.5	23.5	21.0	1.00
10	20.0	15.5	17.0	23.0	18.5	25.5	14.0	20.0	19.2	1.35
11	22.5	15.0	17.5	24.0	16.0	25.5	13.5	17.0	18.9	1.59
12	24.5	14.5	17.5	22.0	C1.	25.5	9.5	16.5	18.6	2.18
13	27.0	10.5	17.0	23.5	14.5	25.0	10.0	D	18.2	2.64
14	27.0	D	16.0	22.0	D	26.0	D	-	22.8	2.50
15	22.5	-	14.0	20.0	-	24.0	-	-	20.1	2.20
16	20.5	-	D	17.5	-	24.5	-	-	20.8	-
17	17.5	-	-	12.0	-	25.0	-	-	18.2	-
18	17.5	-	-	8.0	-	24.0	-	-	16.5	-
19	15.5	-	-	D	-	22.0	-	-	18.8	-
20	8.5	-	-	-	-	20.0	-	-	14.3	-
21	8.5	-	-	-	-	14.5	-	-	11.5	-
22	D	-	-	-	-	16.5	-	-		
23	-	-	-	-	-	15.0	-	-		
24	-	-	-	-	-	D	-	-		

D = Died C = Clotted Sample

APPENDIX 3 - Table 3

Haemoglobin Concentration (gms. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	11.6	11.8	12.9	11.3	12.8	13.0	12.4	12.3	12.3	0.32
1	11.4	11.5	13.0	11.4	12.4	13.2	12.5	12.6	12.3	0.26
2	10.9	10.5	11.3	9.7	12.7	11.6	12.2	11.4	11.3	0.33
3	11.1	10.8	11.5	10.5	10.8	11.4	10.4	11.1	11.0	0.14
4	11.2	10.6	10.6	10.2	10.6	11.1	10.2	10.8	10.7	0.12
5	10.2	9.4	11.1	11.6	9.7	10.0	8.8	10.1	10.1	0.32
6	8.8	8.2	8.4	10.8	8.3	9.7	7.2	9.2	8.8	0.39
7	8.6	7.3	7.9	9.7	8.0	9.3	6.6	8.4	8.2	0.36
8	8.0	5.8	7.1	7.2	4.5	8.4	6.8	9.1	7.1	0.52
9	7.7	5.5	6.7	6.5	6.5	8.5	5.5	8.0	7.0	0.35
10	6.5	5.0	5.4	7.2	6.2	8.8	4.5	6.4	6.3	0.48
11	6.5	4.7	5.4	6.9	4.8	8.3	4.5	5.7	5.9	0.46
12	7.8	4.5	5.0	7.2	Cl.	8.8	3.4	5.4	6.0	0.74
13	6.8	3.1	5.2	6.9	3.9	8.0	2.8	D	5.5	0.98
14	8.3	D	4.5	6.5	D	8.5	D	-	7.0	0.95
15	7.2	-	4.0	6.2	-	7.8	-	-	6.3	0.83
16	6.7	-	D	5.3	-	7.9	-	-	6.6	-
17	4.7	-	-	3.1	-	7.2	-	-	5.0	-
18	5.3	-	-	1.8	-	7.3	-	-	4.8	-
19	4.2	-	-	D	-	7.0	-	-	5.6	-
20	2.6	-	-	-	-	6.5	-	-	4.6	-
21	2.0	-	-	-	-	4.1	-	-	3.2	-
22	D	-	-	-	-	5.3	-	-		
23	-	-	-	-	-	4.7	-	-		
24	-	-	-	-	-	D	-	-		

D = Died C = Clotted Sample

APPENDIX 3 - Table 4

Mean Corpuscular Volume (cu./u) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	29	30	29	31	31	32	31	30	30.4	0.37
1	28	28	30	28	32	31	31	29	29.6	0.57
2	30	30	31	30	30	27	29	28	29.4	0.46
3	28	28	29	30	26	29	26	28	28.0	0.52
4	28	30	32	32	29	29	28	29	29.4	0.46
5	31	33	33	33	32	29	28	28	31.1	0.92
6	28	30	30	30	29	28	27	28	28.8	0.41
7	25	31	32	31	29	33	33	32	30.8	0.94
8	32	31	30	31	29	29	29	27	29.8	0.56
9	30	34	33	34	37	31	32	32	32.9	0.76
10	30	34	34	37	34	32	33	28	32.8	0.98
11	32	35	34	39	33	31	32	30	33.3	1.00
12	37	41	39	37	Cl.	33	31	34	36.0	1.33
13	37	D	41	44	37	34	33	D	37.7	1.44
14	41	-	44	50	D	35	D	-	42.5	1.32
15	43	-	44	43	-	34	-	-	41.0	2.55
16	44	-	D	53	-	38	-	-	45.0	-
17	42	-	-	50	-	39	-	-	43.7	-
18	43	-	-	44	-	38	-	-	41.7	-
19	43	-	-	D	-	40	-	-	41.5	-
20	33	-	-	-	-	35	-	-	34.0	-
21	36	-	-	-	-	34	-	-	35.0	-
22	D	-	-	-	-	37	-	-		
23	-	-	-	-	-	37	-	-		
24	-	-	-	-	-	D	-	-		

D = Died　　　　Cl = Clotted Sample

APPENDIX 3 - Table 5

Mean Corpuscular Haemoglobin Concentration (per cent) of Lambs Following Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	34	32	34	35	34	34	35	35	33.9	0.32
1	36	36	36	37	34	36	34	36	35.6	0.35
2	36	35	34	33	36	36	35	37	35.3	0.45
3	36	34	35	34	34	36	34	36	34.9	0.32
4	36	34	32	32	34	35	32	34	33.6	0.53
5	36	36	37	34	31	34	36	35	34.9	0.67
6	33	32	28	34	33	34	33	33	32.5	0.64
7	41	33	32	32	32	33	33	33	33.6	1.07
8	33	32	34	31	32	33	36	35	33.3	0.60
9	33	33	32	30	34	35	33	34	33.3	0.59
10	33	32	32	31	34	35	32	32	32.6	0.46
11	29	31	31	29	30	33	33	34	31.3	0.67
12	31	31	29	33	Cl.	35	36	33	32.6	0.95
13	33	30	31	29	27	32	28	D	30.0	0.81
14	31	D	28	30	D	33	D	-	30.5	1.04
15	32	-	29	31	-	33	-	-	31.3	0.86
16	33	-	D	30	-	32	-	-	31.7	-
17	27	-	-	26	-	29	-	-	27.3	-
18	30	-	-	23	-	30	-	-	27.7	-
19	27	-	-	D	-	31	-	-	29.0	-
20	31	-	-	-	-	33	-	-	32.0	-
21	24	-	-	-	-	28	-	-	26.0	-
22	D	-	-	-	-	32	-	-		
23	-	-	-	-	-	31	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

Cl = Clotted Sample

APPENDIX 3 - Table 6

Reticulocyte Counts (per cent) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	0	0	0	0	0	0	0	0	0	-
1	0	0.2	0	0	0	0	0	0	<0.1	-
2	0	0.2	0	0.4	0	0	0.1	0.1	0.1	0.05
3	0	0.1	0	0.3	0.6	0	0.1	0.1	0.2	0.07
4	0	0	0	0.2	0.5	0	0	0	0.1	0.06
5	0	0	0	0.2	0.3	0	0.2	0	0.1	0.04
6	0	0	0	0.2	0.1	0.4	0.7	0.3	0.2	0.09
7	0	0	0	1.4	0	0	1.5	0.2	0.4	0.23
8	0.2	0.5	0	4.0	2.5	0.3	0.8	0.8	1.1	0.49
9	0.2	0.4	0.3	4.1	1.8	0	0.4	0.6	1.0	0.49
10	0.2	2.0	1.8	1.8	1.1	0	5.4	1.5	1.7	0.59
11	1.8	2.2	2.1	4.4	1.9	0	2.6	1.0	1.9	0.44
12	5.5	13.9	6.6	10.8	Cl	0.2	6.1	4.4	6.8	1.68
13	6.5	14.6	9.0	18.0	2.7	0.7	9.0	D	8.6	2.32
14	6.7	D	3.7	19.2	D	1.5	D	-	7.8	3.95
15	7.2	-	3.6	4.3	-	2.0	-	-	4.3	1.09
16	13.0	-	D	18.4	-	1.4	-	-	10.9	-
17	11.0	-	-	23.2	-	2.1	-	-	12.1	-
18	10.7	-	-	11.4	-	1.7	-	-	11.3	-
19	9.8	-	-	D	-	4.5	-	-	7.2	-
20	12.5	-	-	-	-	2.8	-	-	7.7	-
21	17.6	-	-	-	-	1.8	-	-	9.7	-
22	D	-	-	-	-	5.2	-	-		
23	-	-	-	-	-	4.9	-	-		
24	-	-	-	-	-	D	-	-		

Cl = Clotted Sample D = Died

APPENDIX 3 - Table 7

Total White Cell Counts (thousands per cu. mm.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	11.6	15.4	15.2	13.8	14.8	13.9	11.8	16.4	14.1	0.64
1	12.9	18.1	16.5	14.7	13.9	14.2	11.5	16.0	14.7	0.74
2	14.7	14.6	17.0	19.0	16.6	13.4	11.9	18.2	15.7	0.86
3	14.8	13.7	15.4	18.1	15.8	15.1	9.8	16.5	14.9	0.86
4	11.5	13.1	15.1	15.6	12.9	13.8	14.1	9.8	13.2	0.66
5	12.8	14.3	14.2	14.1	13.3	13.2	10.3	15.4	13.5	0.53
6	9.5	16.2	15.3	14.7	12.2	14.7	15.6	15.7	14.2	0.80
7	10.8	17.2	16.5	11.3	14.2	13.9	16.4	12.1	14.1	0.88
8	13.0	20.1	16.2	12.5	19.3	13.7	11.1	16.9	15.4	**1.16**
9	12.2	16.8	15.1	12.8	12.2	12.6	12.3	15.6	13.7	**0.65**
10	13.1	17.1	13.2	12.5	14.9	15.2	13.1	14.6	14.2	0.54
11	16.5	24.3	15.2	11.1	13.4	14.2	9.4	13.8	14.7	1.58
12	11.6	14.2	14.0	8.6	Cl	15.5	9.0	15.0	12.6	1.08
13	10.3	15.0	13.4	10.3	12.9	13.0	9.0	D	11.5	0.75
14	10.7	D	14.3	7.9	D	11.3	D	-	11.1	1.31
15	13.8	-	8.2	7.1	-	13.7	-	-	10.7	1.78
16	8.2	-	D	6.8	-	13.0	-	-	9.3	-
17	9.1	-	-	6.8	-	14.1	-	-	10.7	-
18	6.8	-	-	7.7	-	13.3	-	-	9.3	-
19	7.9	-	-	D	-	10.7	-	-	9.3	-
20	8.5	-	-	-	-	11.4	-	-	10.0	-
21	7.4	-	-	-	-	12.4	-	-	9.9	-
22	D	-	-	-	-	11.3	-	-		
23	-	-	-	-	-	9.2	-	-		
24	-	-	-	-	-	D	-	-		

Cl = Clotted Sample

D = Died

APPENDIX 3 - Table 8

Differential Leucocyte Count (% Eosinophils) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	1.5	1.0	1.5	1.5	2.5	1.0	4.0	2.0	1.9	0.36
1	3.0	1.5	2.0	1.5	6.5	1.0	5.0	2.5	2.9	0.68
2	20.0	6.0	17.5	14.0	14.5	17.5	15.0	14.5	14.9	0.46
3	20.5	11.5	16.5	26.0	16.0	14.0	14.5	20.5	17.4	1.64
4	18.5	15.0	27.5	13.0	8.0	7.0	10.5	14.5	14.3	2.32
5	19.0	12.5	15.0	16.0	9.0	20.0	19.5	19.5	16.3	1.41
6	23.5	20.5	27.0	15.0	19.0	19.0	24.5	18.0	21.4	1.72
7	20.5	28.5	25.0	13.0	26.5	25.5	27.5	23.0	21.7	1.77
8	22.5	32.0	32.5	25.0	23.5	26.0	22.5	18.0	25.3	1.74
9	29.5	26.5	27.0	31.0	9.0	29.0	19.0	19.5	23.8	2.64
10	31.5	25.0	24.0	24.5	16.0	25.0	31.5	26.5	25.5	1.73
11	34.5	25.5	28.0	15.0	13.5	24.5	30.0	29.0	25.0	2.58
12	17.0	8.0	34.0	6.5	Cl	19.5	18.0	32.0	19.3	4.01
13	16.0	0.5	39.5	7.5	15.0	19.0	4.5	D	14.6	4.86
14	21.5	D	31.0	3.5	D	8.5	D	-	16.1	6.25
15	21.5	-	7.5	4.5	-	8.5	-	-	10.5	3.77
16	18.5	-	D	8.0	-	7.5	-	-	11.3	-
17	19.0	-	-	7.0	-	5.0	-	-	10.3	-
18	16.5	-	-	1.5	-	6.0	-	-	8.0	-
19	4.0	-	-	D	-	7.5	-	-	5.8	-
20	3.0	-	-	-	-	5.0	-	-	4.0	-
21	2.5	-	-	-	-	6.0	-	-	4.3	-
22	D	-	-	-	-	2.5	-	-		
23	-	-	-	-	-	6.0	-	-		
24	-	-	-	-	-	D	-	-		

Cl = Clotted Sample

D = Died

APPENDIX 3 - Table 9

Differential Leucocyte Count (% Neutrophils) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	26.0	28.5	26.5	38.5	30.0	24.5	26.5	20.5	27.6	1.83
1	25.5	31.5	26.5	40.0	25.0	28.5	22.5	19.0	27.3	2.24
2	18.0	26.5	15.0	31.0	17.5	18.0	24.5	15.0	20.7	2.09
3	15.5	15.0	17.5	28.0	22.0	23.0	16.0	14.0	18.9	1.74
4	12.5	11.5	9.5	34.5	23.5	15.0	20.5	14.5	17.7	2.85
5	18.5	17.0	15.0	17.5	19.5	15.0	10.0	11.5	15.4	1.15
6	12.5	15.5	11.0	41.0	19.5	16.0	12.5	13.0	17.6	3.47
7	13.5	14.0	12.0	33.5	24.5	15.0	21.5	14.5	18.3	2.86
8	22.0	20.0	9.0	19.5	44.5	17.0	18.0	16.5	20.8	2.82
9	21.0	22.0	16.0	20.5	43.0	15.5	16.0	16.5	21.3	3.23
10	22.0	17.0	15.0	29.0	43.5	15.0	22.0	14.0	22.2	3.30
11	15.0	17.0	14.3	24.5	47.5	18.5	20.5	18.5	22.0	3.81
12	20.5	28.5	15.5	20.0	C1	19.5	23.0	13.5	20.1	3.44
13	20.0	30.0	13.5	26.5	38.5	16.0	45.0	D	27.1	4.41
14	16.5	D	13.0	33.5	D	23.0	D	-	21.5	4.51
15	17.0	-	20.5	30.5	-	19.0	-	-	21.8	3.00
16	16.5	-	D	23.5	-	28.0	-	-	22.7	-
17	25.0	-	-	32.0	-	29.0	-	-	28.7	-
18	13.5	-	-	45.0	-	21.5	-	-	26.7	-
19	16.0	-	-	D	-	16.0	-	-	16.0	-
20	24.5	-	-	-	-	16.5	-	-	20.5	-
21	34.5	-	-	-	-	26.5	-	-	30.5	-
22	D	-	-	-	-	28.0	-	-		
23	-	-	-	-	-	24.0	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

C = Clotted Sample

APPENDIX 3 - Table 10

Differential Leucocyte Count (% Lymphocytes) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of Fasciola hepatica

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	67.5	70.5	72.0	60.0	67.5	76.5	69.5	77.5	69.9	1.98
1	71.5	67.0	71.5	58.0	68.5	70.5	72.5	73.5	69.1	1.75
2	62.0	67.5	67.5	55.0	68.0	66.5	60.5	67.5	64.1	1.64
3	64.0	73.5	66.0	46.0	62.0	68.0	69.5	65.5	63.7	2.84
4	69.0	73.5	62.0	52.5	68.5	78.0	69.0	71.5	68.0	2.74
5	62.5	70.5	70.0	67.5	71.5	65.0	70.5	69.5	68.4	1.11
6	64.0	66.0	62.0	44.0	61.5	65.0	58.0	69.0	60.9	2.66
7	66.0	57.5	63.0	53.5	49.0	59.5	51.0	62.5	57.8	2.16
8	55.5	48.0	58.5	55.5	32.0	57.0	60.0	65.5	54.0	3.89
9	49.5	51.5	57.0	48.5	48.0	55.5	65.0	64.0	54.9	2.39
10	46.5	58.0	61.0	46.5	40.5	60.0	66.5	59.5	52.3	2.86
11	51.0	57.5	57.7	60.5	39.0	57.0	49.5	52.5	53.1	2.42
12	62.5	63.5	50.5	73.5	C1	61.0	59.0	54.5	60.6	2.76
13	64.0	69.5	47.0	66.0	46.5	65.0	50.5	D	58.2	3.25
14	62.0	D	56.0	63.0	D	68.5	D	-	62.4	2.56
15	61.5	-	7.20	65.0	-	72.5	-	-	67.8	2.70
16	65.0	-	D	68.5	-	64.5	-	-	66.0	-
17	56.0	-	-	61.0	-	66.0	-	-	61.0	-
18	70.0	-	-	53.5	-	72.5	-	-	65.3	-
19	80.0	-	-	D	-	76.5	-	-	78.3	-
20	72.5	-	-	-	-	78.5	-	-	75.5	-
21	63.0	-	-	-	-	67.5	-	-	65.3	-
22	D	-	-	-	-	69.5	-	-		
23	-	-	-	-	-	70.0	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

C = Clotted Sample

APPENDIX 3 - Table 11

Total Serum Proteins (gms. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	7.0	7.4	8.1	7.0	7.2	7.3	8.0	7.0	7.4	0.14
1	6.5	6.1	7.1	6.2	6.6	6.7	7.7	6.6	6.7	0.18
2	7.2	6.9	7.8	6.8	7.4	7.4	7.8	6.9	7.3	0.14
3	7.7	7.4	8.0	6.9	7.5	8.0	7.7	7.3	7.6	0.13
4	7.0	6.8	8.0	.5	6.7	6.8	7.8	6.9	7.1	0.19
5	7.0	7.8	8.1	7.1	8.0	7.8	7.3	7.3	7.6	0.15
6	7.8	8.5	6.7	7.2	8.4	8.0	8.9	6.4	7.7	0.32
7	7.2	8.7	7.9	6.5	8.8	7.8	8.2	5.5	7.6	0.40
8	9.2	9.8	9.0	7.7	8.4	8.9	8.6	5.6	8.4	0.45
9	8.8	9.2	7.4	8.0	8.0	8.9	7.6	6.9	8.1	0.28
10	9.6	9.0	8.5	7.1	5.5	8.6	8.2	8.7	8.2	0.45
11	9.7	7.6	9.4	6.7	9.0	8.6	7.9	9.9	8.6	0.40
12	7.5	5.5	8.8	5.5	9.5	8.6	6.7	D	7.4	0.65
13	6.9	5.2	8.3	5.1	7.6	7.6	5.3	-	6.6	0.55
14	6.1	D	6.9	4.8	D	7.4	D	-	6.3	0.55
15	6.3	-	5.5	4.6	-	7.8	-	-	6.1	0.68
16	5.3	-	D	3.6	-	6.8	-	-	5.2	-
17	4.8	-	-	3.2	-	6.4	-	-	4.8	-
18	4.0	-	-	3.1	-	5.7	-	-	4.3	-
19	3.7	-	-	D	-	5.6	-	-	4.7	-
20	3.3	-	-	-	-	5.0	-	-	4.2	-
21	3.3	-	-	-	-	4.2	-	-	3.8	-
22	D	-	-	-	-	4.4	-	-		
23	-	-	-	-	-	3.9	-	-		
24	-	-	-	-	-	D	-	-		

D= Died

APPENDIX 5 - Table 12

Serum Albumin Levels (gms. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	2.79	2.76	3.08	3.29	3.20	3.47	3.77	3.36	3.22	0.44
1	2.81	2.92	2.89	2.53	3.16	2.81	3.27	3.18	2.95	0.09
2	2.72	2.63	2.81	2.75	2.91	2.92	2.77	2.93	2.81	0.04
3	2.81	3.11	2.82	2.98	2.07	3.00	2.58	2.84	2.78	0.36
4	2.78	2.47	2.77	2.72	2.27	2.32	2.69	2.55	2.57	0.08
5	2.54	2.40	2.31	2.60	2.35	2.10	1.97	2.15	2.30	0.08
6	2.88	2.29	2.14	3.28	2.18	2.49	1.89	2.27	2.43	0.16
7	2.84	1.95	2.09	2.82	2.07	2.06	2.02	2.02	2.23	0.13
8	2.79	2.24	2.46	2.40	1.97	2.34	2.16	1.51	2.23	0.13
9	2.27	1.91	1.79	2.09	1.74	2.00	1.65	1.30	1.84	0.10
10	2.57	1.75	2.01	2.15	1.32	1.92	1.73	1.56	1.87	0.13
11	2.35	1.65	2.16	2.10	1.77	2.28	1.74	2.19	2.03	0.10
12	1.93	1.21	1.89	1.67	1.91	1.93	1.27	2.10	1.74	0.11
13	1.86	1.14	1.68	1.67	1.39	1.80	0.85	D	1.48	0.16
14	1.81	D	1.40	1.44	D	1.98	D	-	1.66	0.15
15	1.79	-	1.12	1.28	-	1.84	-	-	1.51	0.20
16	1.70	-	D	1.16	-	1.47	-	-	1.44	-
17	1.48	-	-	0.81	-	1.42	-	-	1.24	-
18	1.13	-	-	0.70	-	1.32	-	-	1.05	-
19	0.87	-	-	D	-	1.18	-	-	1.03	-
20	1.00	-	-	-	-	1.19	-	-	1.10	-
21	0.83	-	-	-	-	0.96	-	-	0.90	-
22	D	-	-	-	-	1.01	-	-		
23	-	-	-	-	-	0.86	-	-		
24	-	-	-	-	-	D	-	-		

APPENDIX 13 - Table 13

Serum -Globulin Levels (gms. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of Fasciola hepatica

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	1.32	1.31	1.34	1.17	1.20	1.02	1.05	1.03	1.18	0.20
1	0.51	0.64	1.03	0.84	1.08	0.99	1.10	0.89	0.89	0.08
2	1.49	1.21	1.42	1.18	1.47	1.13	1.61	1.25	1.35	0.06
3	1.64	1.26	1.40	1.06	1.88	1.07	1.26	1.50	1.38	0.10
4	1.36	1.27	1.37	1.55	1.78	1.63	1.65	1.35	1.47	0.06
5	1.36	1.52	1.64	1.44	1.55	1.64	1.53	1.51	1.52	0.03
6	1.13	1.85	0.84	1.08	1.57	1.69	0.45	1.20	1.23	0.16
7	0.74	1.54	1.41	0.83	1.49	1.52	0.57	0.67	1.10	0.15
8	1.79	2.11	1.76	1.54	1.76	1.83	1.65	1.05	1.69	0.12
9	1.99	1.77	1.56	1.85	1.85	1.94	1.55	1.33	1.73	0.08
10	1.67	1.75	1.65	1.65	1.19	1.45	1.40	1.60	1.56	0.06
11	1.99	1.77	1.78	1.62	1.78	2.07	1.57	2.05	1.83	0.07
12	1.79	1.14	1.56	1.19	1.95	1.63	0.84	1.59	1.46	0.13
13	1.25	0.80	1.42	1.18	0.86	1.29	0.83	D	1.09	0.10
14	1.40	D	1.31	1.33	D	0.94	D	-	1.25	0.10
15	1.75	-	0.62	1.34	-	1.48	-	-	1.30	0.25
16	1.02	-	D	0.98	-	1.58	-	-	1.19	-
17	1.54	-	-	1.24	-	1.46	-	-	1.41	-
18	0.90	-	-	0.98	-	1.20	-	-	1.03	-
19	1.08	-	-	D	-	1.44	-	-	1.26	-
20	0.89	-	-	-	-	1.05	-	-	0.97	-
21	0.91	-	-	-	-	0.96	-	-	0.94	-
22	D	-	-	-	-	0.90	-	-		
23	-	-	-	-	-	0.80	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

APPENDIX 3 - Table 14

Serum -Globulin Levels (gms. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	3.09	3.34	3.67	2.54	2.01	2.81	3.19	2.61	2.91	0.14
1	3.18	2.54	3.18	2.83	2.36	2.80	3.34	2.53	2.84	0.13
2	2.99	3.06	3.57	2.86	3.03	3.35	3.41	2.71	3.12	0.10
3	3.25	3.03	3.79	2.86	3.55	3.93	3.86	2.96	3.40	0.15
4	2.86	3.06	3.87	2.23	2.66	3.05	3.47	2.99	3.02	0.17
5	3.10	3.88	4.15	3.06	4.11	4.05	3.80	3.24	3.67	0.16
6	3.80	4.36	3.72	2.84	4.65	3.82	4.06	3.83	3.11	0.19
7	3.62	5.21	4.40	2.85	5.24	4.23	5.61	3.71	4.36	0.33
8	4.61	5.45	4.78	3.76	4.67	4.73	4.80	2.94	4.47	0.27
9	4.54	5.52	4.05	4.06	4.42	4.96	4.40	2.96	4.36	0.26
10	5.37	5.52	4.84	3.30	4.98	5.23	4.98	3.74	4.75	0.35
11	5.36	4.17	5.45	2.99	5.45	4.25	4.59	4.46	4.59	0.30
12	3.78	3.98	5.35	2.62	5.63	5.03	3.68	6.21	4.53	0.43
13	3.79	3.26	5.20	2.25	5.35	4.51	2.94	D	3.9	0.65
14	2.90	D	4.18	2.03	D	3.98	D	-	3.27	0.50
15	2.76	-	3.76	1.98	-	4.48	-	-	3.24	0.55
16	2.48	-	D	1.46	-	3.75	-	-	2.56	-
17	1.97	-	-	1.14	-	3.52	-	-	2.21	-
18	1.75	-	-	1.45	-	3.18	-	-	2.12	-
19	1.41	-	-	D	-	2.98	-	-	2.20	-
20	1.56	-	-	-	-	2.76	-	-	2.16	-
21	1.53	-	-	-	-	2.28	-	-	1.90	-
22	D	-	-	-	-	2.49	-	-		
23	-	-	-	-	-	2.24	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

APPENDIX 3 - Table 15

Albumin:Globulin Ratios of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	0.63	0.59	0.61	0.89	0.80	0.84	0.89	0.92	0.77	0.05
1	0.76	0.92	0.69	0.69	0.92	0.74	0.74	0.93	0.80	0.04
2	0.61	0.62	0.56	0.68	0.65	0.66	0.55	0.74	0.63	0.12
3	0.57	0.73	0.54	0.76	0.38	0.60	0.50	0.64	0.59	0.04
4	0.66	0.53	0.53	0.72	0.51	0.52	0.53	0.59	0.57	0.03
5	0.57	0.44	0.40	0.32	0.41	0.37	0.37	0.45	0.45	0.03
6	0.58	0.37	0.47	0.83	0.35	0.45	0.27	0.45	0.47	0.06
7	0.65	0.29	0.36	0.77	0.21	0.36	0.33	0.46	0.44	0.06
8	0.44	0.30	0.38	0.45	0.31	0.36	0.33	0.38	0.37	0.02
9	0.35	0.27	0.32	0.35	0.28	0.29	0.28	0.30	0.31	0.01
10	0.37	0.24	0.31	0.43	0.31	0.29	0.26	0.26	0.31	0.02
11	0.32	0.28	0.30	0.46	0.24	0.36	0.28	0.34	0.32	0.02
12	0.33	0.28	0.27	0.44	0.25	0.29	0.23	0.27	0.30	0.02
13	0.37	0.28	0.25	0.49	0.22	0.31	0.18	D	0.30	0.04
14	0.42	D	0.25	0.43	D	0.37	D	-	0.37	0.04
15	0.44	-	0.26	0.45	-	0.32	-	-	0.34	0.12
16	0.48	-	D	0.47	-	0.28	-	-	0.41	-
17	0.44	-	-	0.34	-	0.28	-	-	0.34	-
18	0.39	-	-	0.29	-	0.30	-	-	0.30	-
19	0.31	-	-	D	-	0.27	-	-	0.25	-
20	0.43	-	-	-	-	0.31	-	-	0.33	-
21	0.34	-	-	-	-	0.30	-	-	0.32	-
22	D	-	-	-	-	0.30	-	-		
23	-	-	-	-	-	0.28	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

APPENDIX 3 - Table 16

Serum Glutamic Oxaloacetic Transaminase Levels (S-F Units) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	78	88	88	167	167	105	105	88	111	12.7
1	125	92	98	146	190	105	288	127	146	23.1
2	200	86	158	146	150	75	168	120	138	25.0
3	200	240	420	290	234	200	262	270	265	25.0
4	380	334	392	244	274	244	334	360	320	21.0
5	238	356	411	238	274	312	400	380	326	25.0
6	360	380	420	190	330	240	555	400	359	39.6
7	444	672	672	360	360	398	838	444	524	63.4
8	290	314	400	196	314	420	274	314	315	24.9
9	544	210	292	476	358	184	222	222	314	47.5
10	600	180	360	408	206	360	265	222	325	49.0
11	216	105	210	140	170	200	282	170	187	56.3
12	146	85	170	132	292	200	200	127	169	22.3
13	103	108	142	90	393	175	158	D	167	39.4
14	101	D	78	108	D	167	D	-	114	19.0
15	120	-	107	103	-	N.S.	-	-	110	5.1
16	120	-	D	85	-	190	-	-	132	-
17	120	-	-	85	-	190	-	-	132	-

D = Died N.S. = No Sample Available

APPENDIX 3 - Table 17

Serum Glutamic Pyruvic Transaminase Levels (S-F Units) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	14	10	36	0	21	21	21	14	17.1	3.90
1	12	14	14	20	30	26	30	10	19.5	2.90
2	25	25	17	24	17	25	32	18	22.9	1.85
3	26	23	21	15	21	5	20	15	18.5	2.51
4	21	0	5	15	5	20	17	17	12.5	2.82
5	13	6	17	17	12	20	20	20	15.6	1.76
6	8	6	9	5	8	10	5	6	7.1	0.67
7	20	22	22	25	25	20	20	14	21.0	1.24
8	15	13	13	17	12	17	13	20	15.0	0.98
9	21	15	20	17	13	24	15	15	17.5	1.34
10	24	18	10	20	18	22	24	15	18.9	1.69
11	8	8	6	10	18	6	16	5	9.6	1.71
12	6	13	13	5	41	6	10	0	11.8	4.46
13	14	16	15	14	40	14	19	D	18.9	3.59
14	15	D	15	5	D	8	D	-	10.8	2.53
15	20	-	20	28	-	20	-	-	22.0	2.00
16	11	-	D	17	-	22	-	-	17.0	-
17	11	-	-	14	-	22	-	-	16.0	-

D = Died

APPENDIX 3 - Table 18

Serum Alkaline Phosphatase Levels (K.A. Units) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	16	10	8	10	9	16	13	10	12	1.10
1	14	14	7	6	10	12	10	6	10	1.20
2	14	17	7	9	11	17	21	8	13	1.80
3	17	15	9	15	10	17	6	8	12	1.50
4	17	20	12	17	19	24	16	10	17	1.56
5	18	25	12	14	16	23	12	9	16	1.98
6	20	14	12	16	16	23	16	10	16	1.37
7	17	15	9	14	15	22	11	6	14	1.75
8	19	13	8	12	11	22	9	8	13	1.83
9	14	10	7	11	6	24	9	5	11	2.15
10	13	7	6	11	5	22	8	4	10	2.08
11	10	5	6	11	4	24	4	5	9	2.39
12	8	7	7	13	3	20	3	7	9	1.98
13	14	9	8	10	4	21	4	D	10	2.25
14	12	D	6	9	D	24	D	-	13	3.94
15	16	-	6	9	-	25	-	-	14	4.20
16	15	-	D	6	-	26	-	-	15	-
17	15	-	-	6	-	25	-	-	15	-

D = Died

APPENDIX 3 - Table 19

Serum Bilirubin Levels (mgm. per 100 ml.) of Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
0	0.4	<0.1	0.2	0.4	0.3	0.2	0.2	<0.1	0.2	0.04
1	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.2	0.02
2	0.3	0.4	0.3	0.1	0.2	0.2	0.2	0.3	0.3	0.03
3	0.1	0.1	0.3	0.2	0.4	0.2	0.2	0.2	0.2	0.03
4	0.4	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.2	0.04
5	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.02
6	0.2	0.4	0.1	0.1	0.1	0.4	0.1	0.3	0.2	0.04
7	0.2	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.02
8	0.2	0.5	<0.1	0.1	0.2	0.3	0.1	0.2	0.2	0.05
9	0.2	0.4	0.4	0.3	0.4	0.2	0.3	0.2	0.3	0.03
10	0.3	0.1	0.1	<0.1	0.4	0.2	0.4	0.3	0.2	0.05
11	0.2	0.2	0.3	0.2	0.5	0.2	0.6	0.3	0.3	0.07
12	0.2	0.2	0.4	<0.1	0.6	0.2	0.8	0.5	0.4	0.09
13	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.1	0.2	0.02
14	0.2	D	0.3	0.3	D	0.3	0.5	D	0.3	0.05
15	0.2	-	0.2	0.2	-	0.2	D	-	0.2	0.00
16	0.2	-	D	0.2	-	0.3	-	-	0.2	-
17	0.4	-	-	0.2	-	0.3	-	-	0.3	-

D = Died

APPENDIX 3 - Table 20

Faecal Egg Counts (e.p.g.) in Lambs Following a Single Oral Inoculation of 1,000 Metacercariae of *Fasciola hepatica*

Week after Infection	Lamb Number								Mean	Standard Error
	Y 81	Y 82	Y 83	Y 84	Y 85	Y 86	Y 87	Y 88		
1 - 9	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	--	---
10	-ve	75	-ve	-ve	-ve	-ve	-ve	-ve	9	9
11	NS	200	25	-ve	-ve	-ve	-ve	-ve	32	28
12	-ve	825	-ve	50	50	-ve	-ve	-ve	116	110
13	220	NS	50	325	75	50	50	D	128	48
14	125	D	225	575	D	510	D	-	309	97
15	525	-	300	300	-	125	-	-	313	82
16	325	-	D	475	-	25	-	-	275	132
17	75	-	-	150	-	100	-	-	108	22
18	225	-	-	125	-	75	-	-	142	44
19	125	-	-	D	-	75	-	-	100	---
20	125	-	-	-	-	500	-	-	313	---
21	100	-	-	-	-	275	-	-	188	---
22	D	-	-	-	-	250	-	-		
23	-	-	-	-	-	175	-	-		
24	-	-	-	-	-	D	-	-		

D = Died

Appendix 4 -

Bodyweights (lbs) of Lambs Grazing at

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	72	58	68	60	78	56	53	79	52	72
1/8	69	65	67	63	80	61	60	81	52	76
16/8	79	69	75	65	90	70	70	84	60	86
29/8	80	71	79	70	94	77	70	81	63	90
12/9	86	73	90	79	100	82	78	90	60	88
26/9	77	73	85	78	99	87	83	92	70	101
11/10	71	78	81	78	98	90	86	96	65	105
25/10	D	78	80	78	86	88	88	97	68	109
9/11	-	77	70	D	D	84	88	98	62	110
16/11	-	78	D	-	-	89	90	94	65	112
29/11	-	70	-	-	-	76	90	90	58	109
6/12	-	65	-	-	-	72	84	83	D	106
13/12	-	D	-	-	-	67	82	82	-	106
20/12	-	-	-	-	-	D	74	75	-	103
27/12	-	-	-	-	-	-	74	73	-	100
3/1	-	-	-	-	-	-	74	69	-	95
10/1	-	-	-	-	-	-	D	D	-	94
24/1	-	-	-	-	-	-	-	-	-	93
7/2	-	-	-	-	-	-	-	-	-	96
21/2	-	-	-	-	-	-	-	-	-	92
6/3	-	-	-	-	-	-	-	-	-	89
28/3	-	-	-	-	-	-	-	-	-	90
										D

D = Died

Table 1

Brookleas Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
62	58	55	63	47	56	50	49	50	45	59.2	2.31
65	59	57	64	48	58	53	53	52	48	61.6	2.22
80	71	72	78	55	54	66	65	65	60	70.7	2.29
76	75	76	74	61	66	64	67	67	60	73.1	2.08
88	75	80	76	64	72	69	67	65	64	77.3	2.47
90	83	85	75	70	70	70	74	69	70	80.1	2.30
96	90	88	68	72	75	75	74	70	58	80.7	2.86
95	88	82	D	73	71	72	D	D	D	83.5	2.88
99	94	92	-	72	D	77	-	-	-	85.3	4.06
102	95	95	-	71	-	77	-	-	-	88.0	4.21
99	88	87	-	60	-	69	-	-	-	81.5	4.87
98	79	83	-	58	-	62	-	-	-	79.0	4.86
99	72	80	-	58	-	57	-	-	-	78.1	5.60
91	D	72	-	51	-	D	-	-	-	78.0	7.17
95	-	74	-	D	-	-	-	-	-	82.4	6.79
94	-	71	-	-	-	-	-	-	-	80.6	5.73
89	-	D	-	-	-	-	-	-	-	91.5	-
87	-	-	-	-	-	-	-	-	-	90.0	-
83	-	-	-	-	-	-	-	-	-	89.5	-
86	-	-	-	-	-	-	-	-	-	89.0	-
82	-	-	-	-	-	-	-	-	-	85.5	-
76	-	-	-	-	-	-	-	-	-	83.0	-
D											

APPENDIX 4 -

Packed Cell Volume Percentage of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	40.0	37.5	01.	38.0	42.0	39.5	41.0	42.0	38.5	41.0
1/8	42.5	33.5	43.0	37.0	37.0	35.0	37.5	40.5	37.5	38.5
16/8	35.5	33.0	42.0	33.5	33.5	35.0	38.0	34.0	38.0	36.5
29/8	37.5	35.5	39.5	33.0	32.5	32.5	35.5	36.0	34.5	34.5
12/9	31.0	32.5	36.0	34.0	30.5	33.5	36.0	36.5	34.5	34.5
26/9	30.5	26.0	30.0	28.5	26.0	28.5	32.0	33.0	24.5	34.5
11/10	27.0	24.5	25.5	24.0	26.0	25.5	28.5	32.5	23.0	32.0
25/10	D	20.0	20.0	22.0	16.0	23.5	25.0	26.0	22.0	33.0
9/11	-	17.5	15.5	D	D	20.0	25.0	26.0	20.0	33.0
16/11	-	01.	D	-	-	18.0	21.0	25.0	17.0	32.5
29/11	-	11.0	-	-	-	11.5	18.0	23.0	13.5	29.0
6/12	-	10.0	-	-	-	12.0	16.5	20.0	D	29.0
13/12	-	D	-	-	-	10.0	15.0	21.0	-	30.0
20/12	-	-	-	-	-	D	16.0	17.5	-	28.5
27/12	-	-	-	-	-	-	14.0	16.0	-	26.5
3/1	-	-	-	-	-	-	10.0	13.0	-	29.0
10/1	-	-	-	-	-	-	D	D	-	29.5
24/1	-	-	-	-	-	-	-	-	-	24.5
7/2	-	-	-	-	-	-	-	-	-	23.0
21/2	-	-	-	-	-	-	-	-	-	19.0
6/3	-	-	-	-	-	-	-	-	-	20.0
28/3	-	-	-	-	-	-	-	-	-	19.0
										D

D = Died

Table 2

at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
37.5	41.0	40.0	41.5	40.0	39.5	40.5	36.0	40.5	40.0	39.8	0.38
39.0	39.0	36.5	39.5	38.0	40.5	38.5	33.5	Cl.	40.5	38.3	0.60
34.0	37.5	36.5	39.0	30.5	34.5	37.0	32.5	33.0	37.5	35.6	0.61
36.0	34.5	37.5	35.5	34.5	32.0	40.5	33.0	33.0	37.5	35.1	0.52
34.0	34.0	34.5	34.0	Cl.	31.0	36.5	30.5	31.5	36.5	33.8	0.48
Cl.	28.5	34.0	31.0	31.0	32.0	37.0	28.5	32.0	37.0	30.8	0.80
32.0	32.0	29.5	28.5	31.0	21.5	28.5	23.5	25.0	35.0	27.8	0.84
32.0	27.0	26.5	D	22.5	21.5	26.0	D	D	D	24.2	1.16
33.0	25.0	25.0	-	22.5	D	25.0	-	-	-	23.9	1.55
29.5	17.5	21.0	-	20.0	-	19.0	-	-	-	22.1	1.60
27.5	14.0	16.5	-	18.0	-	15.5	-	-	-	18.0	1.84
22.5	13.0	16.0	-	15.0	-	14.0	-	-	-	16.8	1.80
23.5	10.0	15.0	-	16.0	-	15.0	-	-	-	17.3	2.17
20.0	D	14.0	-	13.5	-	D	-	-	-	18.3	2.27
23.0	-	12.5	-	10.0	-	-	-	-	-	16.9	2.56
18.5	-	5.0	-	D	-	-	-	-	-	15.1	4.12
21.0	-	D	-	-	-	-	-	-	-	25.3	-
21.0	-	-	-	-	-	-	-	-	-	22.8	-
22.0	-	-	-	-	-	-	-	-	-	22.5	-
19.0	-	-	-	-	-	-	-	-	-	19.0	-
17.0	-	-	-	-	-	-	-	-	-	18.5	-
11.0	-	-	-	-	-	-	-	-	-	15.0	-
D											

Cl. = Clotted Sample

Appendix 4 -

Haemoglobin Concentrations (gms. per 100 ml) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	13.9	13.3	Cl.	13.0	14.7	13.6	14.0	14.1	12.7	14.7
1/8	13.9	12.8	15.0	11.9	13.9	12.5	13.3	15.0	14.0	15.0
16/8	11.9	11.8	14.9	12.3	13.0	12.2	13.6	12.2	12.5	14.2
29/8	10.9	12.2	13.9	11.1	11.9	11.4	12.0	11.5	11.9	12.8
12/9	10.3	10.8	12.5	11.9	10.9	11.9	11.4	13.7	12.0	11.4
26/9	10.1	8.7	10.2	9.0	9.0	9.5	12.0	12.0	5.7	10.7
11/10	8.4	7.2	7.2	7.9	7.9	7.5	9.1	12.0	6.8	10.4
25/10	D	5.8	6.0	6.5	4.4	6.7	7.4	8.5	6.5	9.6
9/11	-	4.3	3.7	D	D	6.3	7.7	7.9	5.5	10.9
16/11	-	Cl.	D	-	-	4.4	6.0	7.2	3.9	9.9
29/11	-	2.1	-	-	-	3.1	4.9	6.3	3.2	8.9
6/12	-	2.0	-	-	-	2.7	4.3	5.1	D	8.2
13/12	-	D	-	-	-	2.2	3.6	5.3	-	8.6
20/12	-	-	-	-	-	D	4.1	4.8	-	7.9
27/12	-	-	-	-	-	-	3.3	4.1	-	7.7
3/1	-	-	-	-	-	-	1.9	2.7	-	8.2
10/1	-	-	-	-	-	-	D	D	-	8.2
24/1	-	-	-	-	-	-	-	-	-	7.2
7/2	-	-	-	-	-	-	-	-	-	6.3
21/2	-	-	-	-	-	-	-	-	-	5.4
6/3	-	-	-	-	-	-	-	-	-	4.8
28/3	-	-	-	-	-	-	-	-	-	4.8
										D

D = Died

Table 3

Grazing at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
12.2	13.9	14.2	15.6	14.0	14.8	14.3	12.2	12.8	14.2	13.8	0.21
12.6	13.9	14.4	13.9	13.2	14.1	14.2	11.6	Cl.	14.4	13.7	0.23
12.2	13.2	10.8	13.5	11.3	9.2	12.8	11.1	12.0	12.2	12.5	0.23
12.3	12.8	12.2	12.8	11.5	10.8	13.3	10.8	10.7	12.5	12.0	0.20
10.7	11.6	12.3	11.4	Cl.	10.8	12.2	10.0	9.6	12.9	11.5	0.23
Cl.	11.0	10.8	10.5	10.8	11.5	12.9	9.8	10.0	12.8	10.4	0.38
10.8	10.5	10.4	6.6	10.8	7.2	10.0	8.0	8.3	12.8	9.0	0.43
10.1	8.5	8.4	D	7.5	6.5	7.2	D	D	D	7.3	0.39
9.9	6.3	7.2	-	6.1	D	6.8	-	-	-	6.9	0.60
8.7	4.3	5.4	-	5.9	-	4.9	-	-	-	6.1	0.63
8.0	3.4	4.4	-	5.1	-	4.1	-	-	-	4.9	0.64
6.5	2.7	4.1	-	4.1	-	3.4	-	-	-	4.3	0.60
5.9	1.7	3.3	-	3.7	-	3.4	-	-	-	4.2	0.71
5.3	D	3.1	-	3.4	-	D	-	-	-	4.8	0.66
6.0	-	2.7	-	2.4	-	-	-	-	-	4.4	0.85
5.3	-	0.8	-	D	-	-	-	-	-	3.8	1.33
5.9	-	D	-	-	-	-	-	-	-	7.1	-
5.6	-	-	-	-	-	-	-	-	-	6.4	-
6.1	-	-	-	-	-	-	-	-	-	6.2	-
4.9	-	-	-	-	-	-	-	-	-	5.2	-
3.7	-	-	-	-	-	-	-	-	-	4.3	-
3.1	-	-	-	-	-	-	-	-	-	4.0	-
D											

Cl. = Clotted Sample

Appendix 4 -

Total Red Cell Counts (millions per cu. mm.) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	13.78	11.78	Cl.	12.46	12.02	12.56	12.76	13.58	13.04	13.70
1/8	16.10	12.00	15.90	15.10	12.90	11.56	14.00	15.30	13.90	14.70
16/8	12.92	11.64	14.08	11.82	11.20	12.64	13.48	12.56	13.78	14.16
29/8	11.68	11.60	13.30	13.68	10.88	10.40	11.98	12.42	12.02	12.80
12/9	11.06	10.60	12.64	12.00	9.62	11.68	11.62	13.06	11.76	12.56
26/9	10.82	8.82	10.54	10.46	7.78	9.74	10.62	11.06	7.22	11.38
11/10	9.76	6.86	7.68	8.90	7.80	7.24	9.12	10.54	7.20	11.26
25/10	D	5.54	5.36	7.92	4.66	7.32	7.68	9.40	6.76	11.00
9/11	-	4.04	3.62	D	D	6.68	7.16	8.66	5.32	11.22
16/11	-	Cl.	D	-	-	5.46	5.96	8.70	4.44	10.72
29/11	-	3.36	-	-	-	4.38	5.34	7.26	3.40	9.32
6/12	-	2.42	-	-	-	3.10	4.70	7.52	D	9.74
13/12	-	D	-	-	-	2.54	4.28	6.92	-	9.56
20/12	-	-	-	-	-	D	4.48	5.84	-	8.66
27/12	-	-	-	-	-	-	3.64	5.04	-	8.56
3/1	-	-	-	-	-	-	2.64	3.92	-	8.96
10/1	-	-	-	-	-	-	D	D	-	9.00
24/1	-	-	-	-	-	-	-	-	-	6.96
7/2	-	-	-	-	-	-	-	-	-	6.62
21/2	-	-	-	-	-	-	-	-	-	5.74
6/3	-	-	-	-	-	-	-	-	-	5.34
28/3	-	-	-	-	-	-	-	-	-	5.46
										D

D = Died

Table 4

Grazing at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
12.82	12.38	13.22	13.06	13.16	12.26	12.52	11.70	11.28	12.30	12.65	0.18
14.36	15.54	11.32	15.70	12.80	14.24	15.96	14.06	Cl.	12.30	14.09	0.36
9.90	11.10	10.20	13.90	10.40	7.40	12.60	13.60	10.40	12.30	11.95	0.51
11.94	12.02	11.70	11.94	11.32	9.78	12.82	11.52	9.32	12.52	11.78	0.22
14.66	11.10	10.50	11.94	Cl.	9.34	11.88	11.20	8.60	11.62	11.44	0.44
Cl.	9.18	10.60	10.40	10.16	10.32	11.90	10.82	8.96	11.82	10.14	0.29
10.66	9.93	9.29	5.68	10.32	6.78	9.33	9.22	7.30	10.62	8.77	0.36
13.21	8.76	7.78	D	7.88	7.18	8.12	D	D	D	7.90	0.56
11.64	7.48	6.46	-	7.42	D	6.38	-	-	-	7.17	0.71
10.88	5.86	5.58	-	7.00	-	5.32	-	-	-	6.99	0.73
9.72	4.52	4.60	-	5.68	-	3.88	-	-	-	5.59	0.65
8.58	4.10	4.08	-	4.98	-	3.28	-	-	-	5.25	0.79
7.44	3.02	3.42	-	4.70	-	3.10	-	-	-	5.00	0.81
7.10	D	3.16	-	4.10	-	D	-	-	-	5.56	0.84
6.90	-	2.54	-	3.58	-	-	-	-	-	5.04	0.94
6.52	-	1.24	-	D	-	-	-	-	-	4.66	1.38
6.24	-	D	-	-	-	-	-	-	-	7.62	-
5.64	-	-	-	-	-	-	-	-	-	6.30	-
5.64	-	-	-	-	-	-	-	-	-	6.13	-
4.96	-	-	-	-	-	-	-	-	-	5.35	-
4.26	-	-	-	-	-	-	-	-	-	4.80	-
4.36	-	-	-	-	-	-	-	-	-	4.91	-
D											

Cl. = Clotted Sample

Appendix 4 -

Mean Corpuscular Volume (cu. μ) of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	29.0	31.8	Cl.	30.5	34.9	31.4	32.1	30.9	29.5	29.9
1/8	26.4	27.9	27.0	24.5	28.7	30.4	26.8	27.5	27.0	26.2
16/8	27.5	28.4	29.8	28.3	29.9	27.7	28.2	27.1	27.6	26.0
29/8	28.7	30.6	29.7	24.1	29.9	31.3	29.6	29.0	28.7	27.0
12/9	28.0	30.7	28.5	28.3	31.7	28.7	31.0	27.9	29.3	27.5
26/9	28.2	29.5	28.5	27.2	33.4	29.3	30.1	29.8	33.9	30.3
11/10	27.7	35.7	32.2	27.0	33.3	35.2	31.3	30.8	31.9	28.4
25/10	D	36.1	37.3	27.8	34.3	32.1	32.6	27.7	32.4	30.0
9/11	-	43.3	42.8	D	D	29.9	34.9	30.0	37.6	29.4
16/11	-	Cl.	D	-	-	33.0	35.2	28.7	38.3	30.3
29/11	-	32.7	-	-	-	26.3	33.7	31.7	30.9	31.1
6/12	-	41.3	-	-	-	38.7	35.4	26.6	D	29.7
13/12	-	D	-	-	-	36.7	33.5	30.3	-	31.4
20/12	-	-	-	-	-	D	35.7	30.0	-	32.9
27/12	-	-	-	-	-	-	38.0	34.0	-	30.4
3/1	-	-	-	-	-	-	37.9	33.2	-	32.4
10/1	-	-	-	-	-	-	D	D	-	32.8
24/1	-	-	-	-	-	-	-	-	-	35.7
7/2	-	-	-	-	-	-	-	-	-	34.7
21/2	-	-	-	-	-	-	-	-	-	33.1
6/3	-	-	-	-	-	-	-	-	-	37.5
28/3	-	-	-	-	-	-	-	-	-	34.6
										D

D = Died

Table 5

at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
29.3	33.1	30.3	31.8	30.4	31.3	32.3	30.8	35.9	32.5	31.5	0.41
27.1	25.1	32.2	25.2	29.7	28.4	24.1	23.8	Cl.	33.0	27.2	0.64
34.3	33.8	35.8	28.1	29.3	36.7	29.4	23.9	31.7	30.5	29.7	0.73
30.2	28.7	32.1	29.7	30.5	32.7	31.6	28.6	35.4	30.0	29.9	0.51
23.2	30.6	32.9	28.5	Cl.	33.2	30.7	27.2	36.6	31.4	29.8	0.66
Cl.	31.0	32.1	29.2	30.5	31.0	31.1	26.3	35.7	31.3	30.4	0.52
30.0	32.2	31.8	50.2	30.0	31.7	30.5	25.5	34.2	33.0	32.1	0.80
24.2	30.8	34.1	D	28.6	29.9	32.0	D	D	D	31.3	0.89
28.4	33.4	38.7	"	30.3	D	39.2	"	"	"	34.8	1.55
27.1	29.9	37.6	"	28.6	"	35.7	"	"	"	32.4	1.28
28.3	30.9	35.9	"	31.8	"	40.0	"	"	"	32.1	1.10
26.2	31.7	39.0	"	36.7	"	42.7	"	"	"	34.8	1.88
31.6	33.3	45.3	"	34.0	"	48.4	"	"	"	36.1	2.15
28.2	D	44.3	"	32.9	"	D	"	"	"	34.0	2.32
33.3	"	49.2	"	41.0	"	"	"	"	"	37.7	2.77
28.4	"	40.3	"	D	"	"	"	"	"	34.4	2.10
33.7	"	D	"	"	"	"	"	"	"	33.3	"
37.1	"	"	"	"	"	"	"	"	"	36.4	"
39.0	"	"	"	"	"	"	"	"	"	36.9	"
38.3	"	"	"	"	"	"	"	"	"	35.7	"
39.9	"	"	"	"	"	"	"	"	"	38.7	"
25.0	"	"	"	"	"	"	"	"	"	29.8	"
D											

Cl. = Clotted Sample

Appendix 4 -

Mean Corpuscular Haemoglobin Concentration (per cent) of

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	34.8	35.5	Cl.	34.2	35.0	34.4	34.1	33.6	33.0	35.9
1/8	32.7	38.2	34.9	32.2	37.6	33.8	35.5	37.0	37.3	39.0
16/8	33.5	35.8	35.5	36.7	38.8	34.9	35.8	35.9	32.9	36.8
29/8	32.5	34.4	35.2	33.6	36.6	35.1	33.8	31.9	34.5	37.1
12/9	33.2	33.2	34.7	35.0	35.7	35.5	31.7	37.5	34.8	33.0
26/9	33.1	32.7	34.0	31.6	34.6	33.3	37.5	36.4	23.3	31.0
11/10	31.1	29.4	28.2	32.9	30.4	29.4	31.9	36.9	29.6	32.5
25/10	D	29.0	30.0	29.5	27.5	28.5	29.6	32.7	29.5	29.1
9/11	-	24.6	23.9	D	D	31.5	30.8	30.4	27.5	33.0
16/11	-	Cl.	D	-	-	24.4	28.6	28.8	22.9	30.5
29/11	-	19.1	-	-	-	26.9	27.2	27.4	23.7	30.7
6/12	-	20.0	-	-	-	22.5	26.1	25.5	D	28.3
13/12	-	D	-	-	-	22.0	24.0	25.2	-	28.3
20/12	-	-	-	-	-	D	25.6	27.4	-	27.7
27/12	-	-	-	-	-	-	23.6	25.6	-	29.6
3/1	-	-	-	-	-	-	19.0	20.8	-	28.3
10/1	-	-	-	-	-	-	D	D	-	27.8
24/1	-	-	-	-	-	-	-	-	-	29.4
7/2	-	-	-	-	-	-	-	-	-	27.3
21/2	-	-	-	-	-	-	-	-	-	28.4
6/3	-	-	-	-	-	-	-	-	-	24.0
28/3	-	-	-	-	-	-	-	-	-	25.3
										D

D = Died

Table 6

Lambs Grazing at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
32.5	33.9	35.5	37.6	35.0	37.5	35.3	33.9	31.6	35.5	34.7	0.33
32.3	35.6	39.5	35.2	34.7	34.8	36.9	34.6	Cl.	35.6	35.7	0.49
35.9	35.2	29.6	34.6	37.0	37.6	34.6	34.2	36.4	32.5	35.3	0.47
34.2	37.1	32.5	36.1	33.3	33.8	32.8	32.7	32.4	33.3	34.2	0.36
31.5	34.1	35.7	33.5	Cl.	34.8	33.4	32.8	30.5	35.3	34.0	0.42
Cl.	40.7	31.8	33.9	34.8	36.0	34.9	34.4	31.3	34.6	33.7	0.79
33.8	32.8	35.3	23.2	34.8	33.5	35.1	34.0	33.2	36.6	32.2	0.73
31.6	31.5	31.7	D	33.3	30.2	27.7	D	D	D	30.1	0.46
30.0	25.2	28.8	-	27.1	D	27.2	-	-	-	28.3	0.84
29.5	24.6	25.7	-	29.5	-	25.8	-	-	-	27.0	0.84
29.1	24.3	26.6	-	34.0	-	29.3	-	-	-	27.1	1.18
28.8	20.6	25.6	-	27.3	-	24.3	-	-	-	24.9	0.94
25.6	17.0	21.3	-	24.1	-	22.7	-	-	-	23.4	1.04
26.5	D	22.1	-	25.2	-	D	-	-	-	25.8	0.83
26.1	-	21.6	-	19.0	-	-	-	-	-	24.2	1.52
28.6	-	16.0	-	D	-	-	-	-	-	22.5	2.53
28.1	-	D	-	-	-	-	-	-	-	28.0	-
26.7	-	-	-	-	-	-	-	-	-	28.1	-
27.7	-	-	-	-	-	-	-	-	-	27.5	-
25.8	-	-	-	-	-	-	-	-	-	27.1	-
21.8	-	-	-	-	-	-	-	-	-	22.9	-
19.1	-	-	-	-	-	-	-	-	-	22.2	-
D											

Cl. = Clotted Sample

Appendix 4 -

Reticulocyte Counts (per cent) of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	0	0	Cl.	0	0	0	0	0	0	0
1/8	0	0	0	0	0	0	0	0	0	0
16/8	0	0	0	0	0	0	0	0	0	0
29/8	0	0	0	0	0	0	0	0	0	0
12/9	0	0	0	0	0	0	0	0	0	0
26/9	0	0	0	0	0	0	0	0	0	0
11/10	0	0	0	0	0	0	0	0	0	0
25/10	D	0	8	6	0	0	0	0	1	0
9/11	-	15	0	D	D	0	0	0	0	0
16/11	-	Cl.	D	-	-	8	0	0	2	0
29/11	-	24	-	-	-	8	4	0	18	0
6/12	-	16	-	-	-	12	6	0	D	0
13/12	-	D	-	-	-	9	14	0	-	0
20/12	-	-	-	-	-	D	15	5	-	0
27/12	-	-	-	-	-	-	10	7	-	0
3/1	-	-	-	-	-	-	6	15	-	0
10/1	-	-	-	-	-	-	D	D	-	0
24/1	-	-	-	-	-	-	-	-	-	4
7/2	-	-	-	-	-	-	-	-	-	0
21/2	-	-	-	-	-	-	-	-	-	4
6/3	-	-	-	-	-	-	-	-	-	6
28/3	-	-	-	-	-	-	-	-	-	3
										D

D = Died

Table 7

at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
0	0	0	0	0	0	0	0	0	0	0	-
0	0	0	0	0	0	0	0	Cl.	0	0	-
0	0	0	0	0	0	0	0	0	0	0	-
0	0	0	0	0	0	0	0	0	0	0	-
0	0	0	0	Cl.	0	0	0	0	0	0	-
Cl.	0	0	0	0	0	0	0	0	0	0	-
0	0	0	0	0	0	0	0	0	0	0	-
0	0	0	D	0	0	0	D	D	D	1.0	0.64
1	14	7	-	5	D	8	-	-	-	4.2	1.63
0	0	8	-	0	-	2	-	-	-	2.0	1.03
0	12	4	-	0	-	2	-	-	-	6.5	2.47
0	10	4	-	0	-	6	-	-	-	5.4	1.81
1	12	9	-	2	-	10	-	-	-	6.3	1.85
8	D	14	-	8	-	D	-	-	-	8.3	2.29
4	-	11	-	10	-	-	-	-	-	7.0	1.75
5	-	17	-	D	-	-	-	-	-	8.6	3.20
6	-	D	-	-	-	-	-	-	-	3.0	-
5	-	-	-	-	-	-	-	-	-	4.5	-
10	-	-	-	-	-	-	-	-	-	5.0	-
12	-	-	-	-	-	-	-	-	-	8.0	-
9	-	-	-	-	-	-	-	-	-	7.5	-
26	-	-	-	-	-	-	-	-	-	14.5	-
D											

Cl. = Clotted Sample

Appendix 4 - Table 8

The Terminal Haematological Values of Lambs Dying between October 1967 and March 1968 as a Result of Naturally Acquired Fascioliasis

October 1967:

Lamb No.	P.C.V.(%)	Hb (gms/100 ml.)	R.B.Cs. ($\times 10^6$/cu.mm.)	M.C.V. (cu.μ)	M.C.H.C. (%)	Reticulocytes (%)
P42	13.0	3.8	3.42	38.0	29.2	1
R94	28.5	6.6	5.65	50.2	23.2	0
P74	28.5	8.2	8.06	35.5	28.7	0
R81	27.5	7.8	10.02	27.7	31.1	0
R85	16.0	4.4	4.66	34.3	27.5	0
R96	21.5	6.5	7.18	29.9	30.2	0
Mean	22.5	6.2	6.50	35.9	28.3	0.2
S.E.	2.77	0.73	0.98	3.24	1.14	-

November 1967:

R84	22.5	5.8	7.26	31.0	25.9	1
P69	12.5	2.4	3.50	35.7	19.2	22
P71	13.5	2.9	3.84	35.2	21.5	20
R83	15.5	3.7	3.62	42.8	23.9	8
P81	13.5	3.7	4.40	30.7	27.4	0
P40	10.5	2.2	2.64	39.9	20.9	14
P66	9.5	1.9	2.72	31.2	20.0	16
Mean	13.9	3.2	4.00	35.2	22.7	11.6
S.E.	1.62	0.50	0.59	1.79	1.17	3.32

Appendix 4 - Table 8 (continued)

Lamb No.	P.C.V.(%)	Hb (gms/100 ml.)	R.B.Cs. (x10⁶/cu.mm.)	M.C.V. (cu. μ)	M.C.H.C. (%)	Reticulocytes (%)
December 1967:						
P44	12.0	2.3	3.40	35.3	19.2	0
P65	8.5	1.7	1.54	55.2	20.0	24
R89	13.5	3.2	3.40	40.0	23.7	18
R82	10.0	2.0	2.00	50.0	20.0	16
P34	10.0	2.2	2.98	33.6	22.0	14
P75	13.5	3.2	3.62	37.3	23.5	8
R86	11.0	2.0	2.52	43.7	18.2	30
R92	11.5	1.7	2.94	39.1	14.8	14
R97	15.5	3.2	2.98	52.0	20.6	14
P78	10.0	1.7	2.85	35.1	17.0	30
R95	10.0	1.9	2.64	37.9	19.0	10
Mean	11.4	2.3	2.81	41.7	19.8	16.2
S.E.	0.62	0.19	0.19	2.24	0.80	2.74
January 1968:						
R93	5.0	0.8	1.24	40.3	16.0	17
R87	9.0	1.5	2.22	40.5	16.7	13
R88	9.0	1.2	1.98	45.5	13.3	15
Mean	7.7	1.2	1.81	42.1	15.3	15.0
S.E.	1.33	0.20	0.30	1.70	1.04	1.15
March 1968:						
R90	19.0	5.3	5.52	34.4	27.9	4
R91	8.0	1.7	2.44	32.8	21.3	20
Mean	13.5	3.5	3.98	33.6	24.6	12.0
S.E.	5.50	1.80	1.54	0.80	3.30	8.00

Total Serum Protein Levels (gms per 100 ml) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	6.1	6.6	6.7	7.0	7.0	6.6	6.6	5.8	6.5	6.6
1/8	6.5	6.3	6.6	6.5	6.3	6.7	6.3	5.0	6.1	6.9
16/8	6.3	6.5	6.9	5.9	6.5	6.9	6.5	5.9	7.0	6.6
29/8	6.5	6.9	8.0	6.9	7.2	7.2	7.0	6.1	7.4	7.4
12/9	6.7	6.9	8.7	7.3	7.3	7.5	7.5	6.8	7.4	7.6
26/9	8.9	7.0	9.3	7.7	8.1	7.5	7.8	7.3	8.0	8.0
11/10	9.9	7.0	8.2	8.5	7.0	7.7	7.2	7.7	7.0	8.0
25/10	D	5.4	6.9	6.9	6.9	4.9	5.9	7.6	7.7	6.8
9/11	-	5.0	6.8	D	D	6.7	7.4	7.6	7.2	5.9
16/11	-	4.5	D	-	-	6.0	5.1	7.3	6.7	6.7
29/11	-	3.6	-	-	-	4.9	5.0	7.6	4.4	6.0
6/12	-	2.8	-	-	-	4.1	5.0	7.1	D	6.1
13/12	-	D	-	-	-	5.2	5.5	5.7	-	3.2
20/12	-	-	-	-	-	D	3.8	5.8	-	5.9
27/12	-	-	-	-	-	-	3.0	4.8	-	6.1
3/1	-	-	-	-	-	-	3.6	4.2	-	6.0
10/1	-	-	-	-	-	-	D	D	-	5.6
24/1	-	-	-	-	-	-	-	-	-	5.9
7/2	-	-	-	-	-	-	-	-	-	5.1
21/2	-	-	-	-	-	-	-	-	-	5.5
6/3	-	-	-	-	-	-	-	-	-	5.4
28/3	-	-	-	-	-	-	-	-	-	6.3
										D

D = Died

Table 9

Grazing at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
6.2	6.3	6.7	6.3	6.5	6.2	6.2	6.3	6.3	6.5	6.5	0.07
5.7	5.9	6.1	5.7	6.1	6.1	5.5	5.9	5.6	6.0	6.1	0.10
5.7	6.3	6.3	5.8	6.1	5.9	6.0	5.8	6.3	5.8	6.3	0.09
6.0	6.7	7.0	6.4	6.5	6.3	6.9	6.6	6.8	6.8	6.8	0.10
6.8	7.1	7.4	6.8	7.1	7.8	N.S.	7.3	7.2	6.8	7.3	0.11
7.2	7.0	8.1	7.8	7.4	8.6	8.1	8.1	7.2	7.0	7.8	0.14
7.6	6.3	7.6	6.2	5.9	7.5	5.8	6.9	6.4	5.7	7.2	0.23
6.9	6.5	7.9	D	8.0	8.7	7.9	D	D	D	7.0	0.27
6.7	6.1	6.4	"	7.8	D	7.0	"	"	"	6.7	0.23
6.2	6.1	5.5	"	7.5	"	5.9	"	"	"	6.2	0.30
5.3	4.9	4.1	"	7.3	"	4.4	"	"	"	5.2	0.38
5.1	4.0	4.3	"	7.2	"	4.7	"	"	"	5.0	0.84
3.3	3.6	3.1	"	4.8	"	5.3	"	"	"	4.4	0.39
4.9	D	3.7	"	5.8	"	D	"	"	"	5.0	0.42
5.2	"	3.0	"	4.6	"	"	"	"	"	4.5	0.51
4.7	"	3.1	"	D	"	"	"	"	"	4.3	0.50
5.0	"	D	"	"	"	"	"	"	"	5.3	"
5.8	"	"	"	"	"	"	"	"	"	5.6	"
4.6	"	"	"	"	"	"	"	"	"	4.9	"
4.8	"	"	"	"	"	"	"	"	"	5.2	"
4.0	"	"	"	"	"	"	"	"	"	4.7	"
3.7	"	"	"	"	"	"	"	"	"	5.0	"
D											

N.S. = No Sample Available

Appendix 4 -

Serum Albumin Levels (gms per 100 ml) of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	3.35	2.46	3.63	2.91	3.07	2.23	2.84	2.20	N.S.	N.S.
1/8	1.80	2.21	2.11	2.02	2.26	1.99	2.03	1.81	1.94	2.36
16/8	2.22	2.07	2.02	1.75	2.11	2.03	1.74	2.04	2.24	2.35
29/8	2.08	2.82	2.76	2.07	2.95	2.38	1.91	3.28	2.38	2.72
12/9	2.68	2.17	2.72	2.87	2.48	2.50	2.18	2.28	2.41	2.49
26/9	1.65	1.92	1.71	2.68	1.72	2.86	1.63	1.68	1.42	1.74
11/10	1.53	1.21	1.49	1.73	1.50	1.75	1.68	1.83	1.45	2.02
25/10	D	1.41	1.23	1.44	1.59	1.24	1.47	1.39	1.66	2.40
9/11	"	1.17	0.98	D	D	1.64	1.96	1.70	1.50	2.00
16/11	"	1.28	D	"	"	1.47	1.45	1.64	1.46	2.10
29/11	"	1.04	"	"	"	N.S.	1.37	1.73	0.87	2.20
6/12	"	0.79	"	"	"	0.86	1.54	1.47	D	1.92
13/12	"	D	"	"	"	0.87	1.29	1.37	"	0.95
20/12	"	"	"	"	"	D	1.18	1.26	"	2.29
27/12	"	"	"	"	"	"	0.91	1.46	"	2.68
3/1	"	"	"	"	"	"	0.50	0.86	"	1.98
10/1	"	"	"	"	"	"	D	D	"	1.88
24/1	"	"	"	"	"	"	"	"	"	1.48
7/2	"	"	"	"	"	"	"	"	"	1.41
21/2	"	"	"	"	"	"	"	"	"	1.40
6/3	"	"	"	"	"	"	"	"	"	1.34
28/3	"	"	"	"	"	"	"	"	"	1.65
										D

D=Died

Table 10

at Brookleen Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
2.54	3.00	2.32	2.74	2.52	2.86	2.78	2.94	2.54	2.10	2.72	0.10
2.42	2.48	2.29	2.23	2.50	2.71	2.35	2.52	2.31	2.13	2.22	0.06
N.S.	2.15	2.13	1.99	1.85	1.95	2.18	2.03	N.S.	1.75	2.03	0.04
1.76	2.40	2.31	1.99	2.19	1.74	1.94	3.46	2.11	2.09	2.37	0.11
2.07	2.51	2.39	2.12	2.50	2.46	N.S.	2.23	2.34	2.43	2.41	0.05
2.85	1.91	3.03	2.53	2.16	2.30	2.15	2.38	2.15	2.10	2.13	0.11
3.28	3.00	2.28	1.66	1.30	1.66	1.25	1.46	1.78	2.72	1.83	0.13
2.52	2.19	2.02	D	1.86	2.53	2.14	D	D	D	1.94	0.19
2.15	1.79	1.95	"	1.84	D	1.86	"	"	"	1.71	0.09
2.09	1.80	2.13	"	1.80	"	1.42	"	"	"	1.69	0.09
1.83	1.23	1.08	"	1.52	"	1.02	"	"	"	1.39	0.13
1.84	0.88	0.93	"	1.20	"	0.96	"	"	"	1.24	0.13
1.08	0.55	0.54	"	0.94	"	1.19	"	"	"	0.98	0.10
1.57	D	1.01	"	1.36	"	D	"	"	"	1.45	0.19
2.14	"	0.64	"	0.70	"	"	"	"	"	1.42	0.34
1.62	"	0.57	"	D	"	"	"	"	"	1.11	0.30
1.56	"	D	"	"	"	"	"	"	"	1.72	"
1.08	"	"	"	"	"	"	"	"	"	1.28	"
0.96	"	"	"	"	"	"	"	"	"	1.19	"
1.14	"	"	"	"	"	"	"	"	"	1.27	"
0.97	"	"	"	"	"	"	"	"	"	1.16	"
0.63	"	"	"	"	"	"	"	"	"	1.14	"
D											

N.S. = No Sample Available

Serum Alpha/beta Globulin Levels(gms per 100ml) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	1.58	1.84	1.16	1.75	1.56	1.61	1.58	2.46	N.S.	N.S.
1/8	1.80	1.78	1.80	2.02	1.90	1.70	1.91	2.10	2.12	2.03
16/8	2.25	2.36	2.14	2.28	2.15	2.23	2.07	2.59	2.36	2.18
29/8	1.63	1.88	1.78	1.96	1.56	1.46	2.27	1.21	2.19	2.03
12/9	1.79	2.16	2.31	1.77	1.63	1.88	2.01	2.11	2.04	1.76
26/9	2.25	1.91	2.47	1.87	2.77	1.24	2.18	2.24	2.38	2.16
11/10	2.37	1.88	1.87	2.37	2.01	2.24	1.92	2.56	1.79	2.08
25/10	D	1.32	1.47	1.48	1.64	1.20	1.42	1.35	1.62	1.53
9/11	"	1.37	1.60	D	D	1.25	1.99	1.95	1.44	1.39
16/11	"	1.32	D	"	"	1.29	1.08	1.96	1.99	1.70
29/11	"	1.10	"	"	"	N.S.	1.14	2.01	1.17	1.66
6/12	"	0.78	"	"	"	0.88	0.96	1.44	D	1.52
13/12	"	D	"	"	"	1.45	1.33	1.62	"	0.64
20/12	"	"	"	"	"	D	0.93	1.47	"	1.31
27/12	"	"	"	"	"	"	0.79	1.34	"	1.48
3/1	"	"	"	"	"	"	1.11	0.19	"	1.25
10/1	"	"	"	"	"	"	D	D	"	1.11
24/1	"	"	"	"	"	"	"	"	"	1.48
7/2	"	"	"	"	"	"	"	"	"	1.16
21/2	"	"	"	"	"	"	"	"	"	1.39
6/3	"	"	"	"	"	"	"	"	"	1.46
28/3	"	"	"	"	"	"	"	"	"	1.55
										D

D = Died

Table 9

Grazing at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
6.2	6.3	6.7	6.3	6.5	6.2	6.2	6.3	6.3	6.5	6.5	0.07
5.7	5.9	6.1	5.7	6.1	6.1	5.5	5.9	5.6	6.0	6.1	0.10
5.7	6.3	6.3	5.8	6.1	5.9	6.0	5.8	6.3	5.8	6.3	0.09
6.0	6.7	7.0	6.4	6.5	6.3	6.9	6.6	6.8	6.8	6.8	0.10
6.8	7.1	7.4	6.8	7.1	7.8	N.S.	7.3	7.2	6.8	7.3	0.11
7.2	7.0	8.1	7.8	7.4	8.6	8.1	8.1	7.2	7.0	7.8	0.14
7.6	6.3	7.6	6.2	5.9	7.5	5.8	6.9	6.4	5.7	7.2	0.23
6.9	6.5	7.9	D	8.0	8.7	7.9	D	D	D	7.0	0.27
6.7	6.1	6.4	"	7.8	D	7.0	"	"	"	6.7	0.23
6.2	6.1	5.5	"	7.5	"	5.9	"	"	"	6.2	0.30
5.3	4.9	4.1	"	7.3	"	4.4	"	"	"	5.2	0.38
5.1	4.0	4.3	"	7.2	"	4.7	"	"	"	5.0	0.84
3.3	3.6	3.1	"	4.8	"	5.3	"	"	"	4.4	0.39
4.9	D	3.7	"	5.8	"	D	"	"	"	5.0	0.42
5.2	"	3.0	"	4.6	"	"	"	"	"	4.5	0.51
4.7	"	3.1	"	D	"	"	"	"	"	4.3	0.50
5.0	"	D	"	"	"	"	"	"	"	5.3	"
5.8	"	"	"	"	"	"	"	"	"	5.6	"
4.6	"	"	"	"	"	"	"	"	"	4.9	"
4.8	"	"	"	"	"	"	"	"	"	5.2	"
4.0	"	"	"	"	"	"	"	"	"	4.7	"
3.7	"	"	"	"	"	"	"	"	"	5.0	"
D											

N.S. = No Sample Available

Appendix 4 -

Serum Albumin Levels (gms per 100 ml) of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	3.35	2.46	3.63	2.91	3.07	2.25	2.84	2.20	N.S.	N.S.
1/8	1.80	2.21	2.11	2.02	2.26	1.99	2.03	1.81	1.94	2.36
16/8	2.22	2.07	2.02	1.75	2.11	2.03	1.74	2.04	2.24	2.35
29/8	2.08	2.82	2.76	2.07	2.95	2.38	1.91	3.28	2.38	2.72
12/9	2.68	2.17	2.72	2.87	2.48	2.50	2.18	2.28	2.41	2.49
26/9	1.65	1.92	1.71	2.68	1.72	2.86	1.63	1.68	1.42	1.74
11/10	1.53	1.21	1.49	1.73	1.50	1.75	1.68	1.83	1.45	2.02
25/10	D	1.41	1.23	1.44	1.59	1.24	1.47	1.39	1.66	2.40
9/11	-	1.17	0.98	D	D	1.64	1.96	1.70	1.50	2.00
16/11	-	1.28	D	-	-	1.47	1.45	1.64	1.46	2.10
29/11	-	1.04	-	-	-	N.S.	1.37	1.73	0.87	2.20
6/12	-	0.79	-	-	-	0.86	1.54	1.47	D	1.92
13/12	-	D	-	-	-	0.87	1.29	1.37	-	0.95
20/12	-	-	-	-	-	D	1.18	1.26	-	2.29
27/12	-	-	-	-	-	-	0.91	1.46	-	2.68
3/1	-	-	-	-	-	-	0.50	0.86	-	1.98
10/1	-	-	-	-	-	-	D	D	-	1.88
24/1	-	-	-	-	-	-	-	-	-	1.48
7/2	-	-	-	-	-	-	-	-	-	1.41
21/2	-	-	-	-	-	-	-	-	-	1.40
6/3	-	-	-	-	-	-	-	-	-	1.34
28/3	-	-	-	-	-	-	-	-	-	1.65
										D

D=Died

Table 10

at Brooklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
2.54	3.00	2.32	2.74	2.52	2.86	2.78	2.94	2.54	2.10	2.72	0.10
2.42	2.48	2.29	2.23	2.50	2.71	2.35	2.52	2.31	2.13	2.22	0.06
N.S.	2.15	2.13	1.99	1.85	1.95	2.18	2.03	N.S.	1.75	2.03	0.04
1.76	2.40	2.31	1.99	2.19	1.74	1.94	3.46	2.11	2.09	2.37	0.11
2.07	2.51	2.39	2.12	2.50	2.46	N.S.	2.25	2.34	2.43	2.41	0.05
2.85	1.91	3.03	2.53	2.16	2.30	2.15	2.38	2.15	2.10	2.13	0.11
3.28	3.00	2.28	1.66	1.30	1.66	1.25	1.46	1.78	2.72	1.83	0.13
2.52	2.19	2.02	D	1.86	2.53	2.14	D	D	D	1.94	0.19
2.15	1.79	1.95	-	1.84	D	1.86	-	-	-	1.71	0.09
2.09	1.80	2.13	-	1.80	-	1.42	-	-	-	1.69	0.09
1.83	1.23	1.08	-	1.52	-	1.02	-	-	-	1.39	0.13
1.84	0.88	0.93	-	1.20	-	0.96	-	-	-	1.24	0.13
1.08	0.55	0.54	-	0.94	-	1.19	-	-	-	0.98	0.10
1.57	D	1.01	-	1.36	-	D	-	-	-	1.45	0.19
2.14	-	0.64	-	0.70	-	-	-	-	-	1.42	0.34
1.62	-	0.57	-	D	-	-	-	-	-	1.11	0.30
1.56	-	D	-	-	-	-	-	-	-	1.72	-
1.08	-	-	-	-	-	-	-	-	-	1.28	-
0.96	-	-	-	-	-	-	-	-	-	1.19	-
1.14	-	-	-	-	-	-	-	-	-	1.27	-
0.97	-	-	-	-	-	-	-	-	-	1.16	-
0.63	-	-	-	-	-	-	-	-	-	1.14	-
D											

N.S. = No Sample Available

Serum Alpha/beta Globulin Levels(gms per 100ml) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	1.58	1.84	1.16	1.75	1.56	1.61	1.58	2.46	N.S.	N.S.
1/8	1.80	1.78	1.80	2.02	1.90	1.70	1.91	2.10	2.12	2.03
16/8	2.25	2.56	2.14	2.28	2.15	2.23	2.07	2.59	2.36	2.18
29/8	1.63	1.88	1.78	1.96	1.56	1.46	2.27	1.21	2.19	2.03
12/9	1.79	2.16	2.31	1.77	1.63	1.88	2.01	2.11	2.04	1.76
26/9	2.25	1.91	2.47	1.87	2.77	1.24	2.18	2.24	2.38	2.16
11/10	2.37	1.88	1.87	2.37	2.01	2.24	1.92	2.56	1.79	2.08
25/10	D	1.32	1.47	1.48	1.64	1.20	1.42	1.35	1.62	1.53
9/11	-	1.37	1.60	D	D	1.25	1.99	1.95	1.44	1.39
16/11	-	1.32	D	-	-	1.29	1.08	1.96	1.99	1.70
29/11	-	1.10	-	-	-	N.S.	1.14	2.01	1.17	1.66
6/12	-	0.78	-	-	-	0.88	0.96	1.44	D	1.52
13/12	-	D	-	-	-	1.45	1.33	1.62	-	0.64
20/12	-	-	-	-	-	D	0.93	1.47	-	1.31
27/12	-	-	-	-	-	-	0.79	1.34	-	1.48
3/1	-	-	-	-	-	-	1.11	0.19	-	1.25
10/1	-	-	-	-	-	-	D	D	-	1.11
24/1	-	-	-	-	-	-	-	-	-	1.48
7/2	-	-	-	-	-	-	-	-	-	1.16
21/2	-	-	-	-	-	-	-	-	-	1.39
6/3	-	-	-	-	-	-	-	-	-	1.46
28/3	-	-	-	-	-	-	-	-	-	1.55
										D

D = Died

Table 11

Grazing at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
1.50	1.54	1.60	2.06	1.57	1.47	1.36	1.18	1.83	1.97	1.65	0.07
1.30	1.57	1.53	1.74	1.31	1.59	1.32	1.26	1.52	1.32	1.68	0.06
N.S.	2.20	1.66	1.82	1.89	2.05	1.58	1.69	N.S.	1.64	2.06	0.07
2.64	2.33	2.03	2.35	2.04	2.19	2.24	1.59	2.29	2.09	1.99	0.08
2.08	2.51	2.12	2.28	2.15	2.48	N.S.	2.27	2.68	2.06	2.11	0.06
1.91	2.26	1.63	1.86	1.60	1.88	2.21	1.62	2.33	1.94	2.04	0.08
1.55	1.43	1.93	1.82	1.60	1.97	1.60	1.31	1.89	1.36	1.88	0.08
1.68	2.02	1.57	D	1.86	1.80	1.49	D	D	D	1.56	0.06
1.44	1.87	1.69	-	2.01	D	1.10	-	-	-	1.59	0.09
1.40	1.72	0.87	-	1.61	-	1.17	-	-	-	1.46	0.11
1.32	1.64	1.11	-	1.38	-	1.22	-	-	-	1.38	0.10
1.37	1.16	0.97	-	1.47	-	1.08	-	-	-	1.16	0.09
0.84	1.11	0.79	-	1.03	-	1.35	-	-	-	1.13	0.11
1.09	D	1.07	-	1.35	-	D	-	-	-	1.20	0.08
1.33	-	0.91	-	1.37	-	-	-	-	-	1.20	0.11
1.16	-	0.69	-	D	-	-	-	-	-	0.88	0.20
1.18	-	D	-	-	-	-	-	-	-	1.15	-
1.39	-	-	-	-	-	-	-	-	-	1.44	-
1.19	-	-	-	-	-	-	-	-	-	1.18	-
1.27	-	-	-	-	-	-	-	-	-	1.33	-
1.13	-	-	-	-	-	-	-	-	-	1.30	-
1.21	-	-	-	-	-	-	-	-	-	1.38	-
D											

N.S. = No Sample Available

Appendix 4 -

Serum Gamma-Globulin Levels (gms per 100 ml) of Lambs

Date	Lamb Numbers									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	1.16	2.30	1.91	2.35	2.36	2.75	2.18	1.14	N.S.	N.S.
1/8	2.90	2.13	2.69	2.45	2.15	3.01	2.37	1.09	2.05	2.51
16/8	1.83	2.06	2.74	1.87	2.24	2.63	2.69	1.27	2.40	2.08
29/8	2.78	2.19	3.46	2.87	2.69	3.36	2.82	1.61	2.84	2.65
12/9	2.23	2.57	3.67	2.65	3.18	3.11	3.31	2.40	2.95	3.35
26/9	4.99	3.17	5.11	3.14	3.61	3.30	3.99	3.37	4.19	4.09
11/10	6.00	3.92	4.85	4.40	3.49	3.71	3.60	3.33	3.76	3.90
25/10	D	2.67	4.19	3.98	3.67	2.46	3.00	4.86	4.42	2.87
9/11	-	2.46	4.22	D	D	3.81	3.45	3.95	4.26	2.51
16/11	-	1.90	D	-	-	3.25	2.55	3.70	3.25	2.90
29/11	-	1.46	-	-	-	N.S.	2.49	3.86	2.36	2.13
6/12	-	1.24	-	-	-	2.36	2.70	4.19	D	2.66
13/12	-	D	-	-	-	2.89	2.88	2.81	-	1.61
20/12	-	-	-	-	-	D	1.70	3.06	-	2.29
27/12	-	-	-	-	-	-	1.30	2.00	-	1.99
3/1	-	-	-	-	-	-	1.99	3.21	-	2.77
10/1	-	-	-	-	-	-	D	D	-	2.61
24/1	-	-	-	-	-	-	-	-	-	2.93
7/2	-	-	-	-	-	-	-	-	-	2.54
21/2	-	-	-	-	-	-	-	-	-	2.72
6/3	-	-	-	-	-	-	-	-	-	2.59
28/3	-	-	-	-	-	-	-	-	-	3.10
										D

D = Died

Table 12

Grazing at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
2.16	1.76	2.78	1.50	2.41	1.87	2.06	2.18	1.93	4.22	2.17	0.16
1.99	1.85	2.29	1.73	2.29	1.80	1.82	2.12	1.77	2.55	2.18	0.10
N.S.	1.96	2.51	1.98	2.36	1.90	2.24	2.08	N.S.	2.41	2.18	0.09
1.60	1.96	2.65	2.06	2.27	2.37	2.71	1.55	2.40	2.63	2.47	0.14
2.64	2.09	2.89	2.40	2.45	2.86	N.S.	2.78	2.18	2.31	2.74	0.10
2.44	2.83	3.44	3.41	3.65	4.42	3.75	4.11	2.72	2.96	3.63	0.16
2.77	1.87	3.39	2.72	2.99	3.87	2.95	4.13	2.73	1.62	3.50	0.22
2.70	2.28	2.31	D	4.29	3.48	4.28	D	D	D	3.43	0.22
3.11	2.44	2.76	"	3.95	D	4.04	"	"	"	3.41	0.21
2.71	2.57	2.48	"	4.06	"	3.31	"	"	"	2.97	0.19
2.15	2.03	1.91	"	4.40	"	2.16	"	"	"	2.50	0.29
1.88	1.95	2.40	"	4.53	"	2.66	"	"	"	2.66	0.32
1.37	1.94	1.76	"	2.83	"	2.76	"	"	"	2.32	0.21
2.24	D	1.62	"	3.09	"	D	"	"	"	2.33	0.26
1.72	"	1.45	"	2.53	"	"	"	"	"	1.83	0.18
1.92	"	1.01	"	D	"	"	"	"	"	2.18	0.38
2.25	"	D	"	"	"	"	"	"	"	2.43	"
2.73	"	"	"	"	"	"	"	"	"	2.83	"
2.45	"	"	"	"	"	"	"	"	"	2.50	"
2.39	"	"	"	"	"	"	"	"	"	2.56	"
1.90	"	"	"	"	"	"	"	"	"	2.25	"
1.86	"	"	"	"	"	"	"	"	"	2.48	"
D											

N.S. = No Sample Available

Appendix 4 -

Serum Total Globulin Levels (gms per 100 ml) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	2.75	4.14	3.07	4.09	2.93	4.37	3.76	3.60	N.S.	N.S.
1/8	4.70	3.91	4.49	4.47	4.05	4.71	4.28	3.19	4.17	4.54
16/8	4.08	4.42	4.88	4.15	4.39	4.86	4.76	3.86	4.76	4.26
29/8	4.41	4.07	5.24	4.83	4.25	4.82	5.09	2.82	5.03	4.68
12/9	4.02	4.73	5.98	4.42	4.81	4.99	5.32	4.51	4.99	5.11
26/9	7.24	5.08	7.58	5.01	6.38	4.64	6.17	5.61	6.57	6.25
11/10	8.37	5.80	6.72	6.77	5.50	5.95	5.52	5.89	5.55	5.98
25/10	D	3.99	5.66	5.46	5.31	3.66	4.42	6.21	6.04	4.40
9/11	-	3.83	5.82	D	D	5.06	5.44	5.10	5.70	3.90
16/11	-	3.22	D	-	-	4.54	3.63	5.64	5.24	4.60
29/11	-	2.56	-	-	-	N.S.	3.63	5.87	3.53	3.79
6/12	-	2.02	-	-	-	3.24	3.46	5.63	D	4.18
13/12	-	D	-	-	-	4.34	4.21	4.43	-	2.25
20/12	-	-	-	-	-	D	2.63	4.53	-	3.60
27/12	-	-	-	-	-	-	2.09	3.34	-	3.47
3/1	-	-	-	-	-	-	3.10	3.40	-	4.02
10/1	-	-	-	-	-	-	D	D	-	3.72
24/1	-	-	-	-	-	-	-	-	-	4.56
7/2	-	-	-	-	-	-	-	-	-	3.60
21/2	-	-	-	-	-	-	-	-	-	3.66
6/3	-	-	-	-	-	-	-	-	-	4.05
28/3	-	-	-	-	-	-	-	-	-	4.65
										D

D = Died

Table 13

Grazing at Brookleas Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
3.66	3.30	4.38	3.56	3.98	3.34	3.42	3.36	3.76	6.19	3.82	0.17
3.28	3.42	3.81	3.47	3.60	3.39	3.15	3.38	3.29	3.87	3.86	0.12
N.S.	4.16	4.17	3.80	4.25	3.95	3.82	3.77	N.S.	4.05	4.24	0.09
4.24	4.29	4.68	4.41	4.31	4.56	4.95	3.14	4.69	4.72	4.46	0.13
4.72	4.60	5.01	4.68	4.60	5.34	N.S.	5.05	4.86	4.37	4.85	0.10
4.35	5.09	5.07	5.27	5.25	6.30	5.96	5.73	5.05	4.90	5.68	0.19
4.32	3.30	5.32	4.54	4.58	5.84	4.55	5.44	4.62	2.98	5.38	0.27
4.38	4.30	3.88	D	6.15	5.28	5.77	D	D	D	5.01	0.21
4.55	4.31	4.45	"	5.96	D	5.14	"	"	"	4.94	0.21
4.11	4.29	3.35	"	5.69	"	4.48	"	"	"	4.44	0.26
3.47	3.67	3.02	"	5.78	"	3.38	"	"	"	3.67	0.54
3.25	3.11	3.37	"	6.00	"	3.74	"	"	"	3.80	0.38
2.21	3.05	2.55	"	3.86	"	4.11	"	"	"	3.45	0.31
3.33	D	2.69	"	4.44	"	D	"	"	"	3.54	0.34
3.05	"	2.36	"	3.90	"	"	"	"	"	3.04	0.52
3.08	"	1.70	"	D	"	"	"	"	"	3.06	0.71
3.43	"	D	"	"	"	"	"	"	"	3.58	"
4.12	"	"	"	"	"	"	"	"	"	4.34	"
3.64	"	"	"	"	"	"	"	"	"	3.62	"
4.11	"	"	"	"	"	"	"	"	"	3.89	"
3.03	"	"	"	"	"	"	"	"	"	3.54	"
3.07	"	"	"	"	"	"	"	"	"	3.86	"
D											

N.S. = No Sample Available

Appendix 4 -

Albumin:Globulin Ratios of Lambs Grazing

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
19/7	1.22	0.60	1.18	0.71	0.78	0.51	0.75	0.61	N.S.	N.S.
1/8	0.38	0.56	0.47	0.45	0.56	0.42	0.47	0.57	0.47	0.52
16/8	0.54	0.47	0.41	0.42	0.48	0.42	0.36	0.53	0.47	0.55
29/8	0.47	0.69	0.53	0.43	0.69	0.49	0.38	1.16	0.47	0.58
12/9	0.67	0.46	0.46	0.65	0.52	0.50	0.40	0.50	0.48	0.49
26/9	0.23	0.38	0.23	0.53	0.27	0.62	0.26	0.30	0.22	0.28
11/10	0.18	0.21	0.22	0.26	0.27	0.29	0.30	0.31	0.26	0.34
25/10	D	0.35	0.22	0.26	0.30	0.34	0.33	0.22	0.27	0.55
9/11	-	0.32	0.17	D	D	0.32	0.36	0.29	0.26	0.51
16/11	-	0.40	D	-	-	0.32	0.40	0.29	0.28	0.46
29/11	-	0.41	-	-	-	N.S.	0.27	0.29	0.25	0.58
6/12	-	0.39	-	-	-	0.27	0.45	0.26	D	0.46
13/12	-	D	-	-	-	0.26	0.31	0.31	-	0.42
20/12	-	-	-	-	-	D	0.45	0.28	-	0.64
27/12	-	-	-	-	-	-	0.43	0.44	-	0.79
3/1	-	-	-	-	-	-	0.16	0.17	-	0.49
10/1	-	-	-	-	-	-	D	D	-	0.50
24/1	-	-	-	-	-	-	-	-	-	0.34
7/2	-	-	-	-	-	-	-	-	-	0.38
21/2	-	-	-	-	-	-	-	-	-	0.34
6/3	-	-	-	-	-	-	-	-	-	0.36
28/3	-	-	-	-	-	-	-	-	-	0.35
										D

D = Died

Table 14

at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
0.69	0.91	0.54	0.77	0.63	0.86	0.81	0.87	0.67	0.34	0.75	0.05
0.74	0.72	0.60	0.64	0.70	0.80	0.75	0.75	0.70	0.55	0.59	0.03
N.S.	0.52	0.51	0.52	0.44	0.49	0.57	0.54	N.S.	0.43	0.48	0.01
0.41	0.56	0.49	0.45	0.51	0.38	0.39	1.10	0.45	0.44	0.55	0.05
0.44	0.55	0.48	0.45	0.54	0.46	N.S.	0.45	0.48	0.55	0.50	0.02
0.66	0.37	0.60	0.48	0.41	0.36	0.36	0.41	0.43	0.43	0.39	0.03
0.76	0.41	0.43	0.36	0.28	0.28	0.28	0.27	0.39	0.91	0.35	0.04
0.57	0.51	1.03	D	0.33	0.48	0.37	D	D	D	0.41	0.05
0.47	0.41	0.44	"	0.31	D	0.36	"	"	"	0.35	0.03
0.51	0.42	0.64	"	0.32	"	0.32	"	"	"	0.40	0.03
0.53	0.34	0.36	"	0.26	"	0.30	"	"	"	0.40	0.04
0.18	0.35	0.28	"	0.20	"	0.26	"	"	"	0.31	0.03
0.40	0.18	0.21	"	0.24	"	0.29	"	"	"	0.10	0.03
0.47	D	0.39	"	0.31	"	D	"	"	"	0.42	0.05
0.70	"	0.27	"	0.18	"	"	"	"	"	0.47	0.10
0.53	"	0.34	"	D	"	"	"	"	"	0.34	0.08
0.45	"	D	"	"	"	"	"	"	"	0.48	"
0.26	"	"	"	"	"	"	"	"	"	0.30	"
0.26	"	"	"	"	"	"	"	"	"	0.32	"
0.31	"	"	"	"	"	"	"	"	"	0.33	"
0.21	"	"	"	"	"	"	"	"	"	0.29	"
0.21	"	"	"	"	"	"	"	"	"	0.28	"
D											

N.S. = No Sample Available

Appendix 4 - Table 15

The Terminal Blood Biochemistry Values of Lambs Dying between October 1967 and March 1968 as a Result of Naturally Acquired Fascioliasis

Lamb No.	Total Protein (gms/100 ml.)	A/G Ratio (gms/100 ml.)	Albumin (gms/100 ml.)	Globulin (gms/100 ml.)	g-Globulin (gms/100 ml.)	Tot.Globulin (gms/100 ml.)
October 1967:						
P42	7.7	0.20	1.27	1.31	5.12	6.43
R94	7.1	0.23	1.34	2.19	3.55	5.74
P74	6.7	0.27	1.82	1.92	2.96	4.88
R81	11.6	0.19	1.83	1.61	8.16	9.77
R85	6.9	0.30	1.59	1.64	3.67	5.31
R96	8.7	0.48	2.53	1.80	3.48	5.28
Mean	8.1	0.30	1.73	1.75	4.49	6.24
S.E.	0.75	0.05	0.19	0.12	0.79	2.79
November 1967:						
R84	8.2	0.24	1.60	1.89	4.71	6.60
P69	5.3	0.28	1.15	1.27	2.88	4.15
P71	5.0	0.32	1.22	1.38	2.39	3.77
R83	6.0	0.17	0.98	1.60	4.22	5.82
P81	6.6	0.23	1.22	0.68	4.70	5.38
P40	3.8	0.14	0.46	1.25	2.09	3.34
P66	4.0	0.23	0.74	1.22	2.04	3.26
Mean	5.7	0.23	1.05	1.33	3.29	4.62
S.E.	0.61	0.02	0.14	0.14	0.46	0.50

Appendix 4 - Table 15 (continued)

Lamb No.	Total Protein (gms/100 ml.)	A/G Ratio (gms/100 ml.)	Albumin (gms/100 ml.)	Globulin (gms/100 ml.)	g-Globulin (gms/100 ml.)	Tot.Globulin (gms/100 ml.)
December 1967:						
P44	6.9	0.34	1.73	0.66	4.51	5.17
P65	4.4	0.32	1.06	1.01	2.33	3.34
R89	4.4	0.25	0.87	1.17	2.36	3.53
R82	2.8	0.39	0.79	0.78	1.24	2.02
P34	3.2	0.26	0.66	0.98	1.55	2.53
P75	5.5	0.43	1.04	0.93	3.53	4.46
R86	5.2	0.26	0.87	1.45	2.89	4.34
R92	3.6	0.18	0.55	1.11	1.94	3.05
R97	5.3	0.29	1.19	1.35	2.76	4.11
P76	3.2	0.40	0.91	0.99	1.30	2.29
R95	4.6	0.18	0.70	1.37	2.53	3.90
Mean	4.5	0.30	0.94	1.07	2.45	3.52
S.E.	0.37	0.03	0.10	0.07	0.30	0.30
January: 1968:						
R93	3.1	0.34	0.57	0.69	1.01	1.70
R87	4.3	0.15	0.55	1.10	2.65	3.75
R88	4.6	0.19	0.74	1.24	2.61	3.85
Mean	4.0	0.23	0.62	1.01	2.09	3.10
S.E.	0.46	0.06	0.06	0.17	0.54	0.70
March 1968:						
R90	6.0	0.32	1.46	1.46	3.08	4.54
R91	3.2	0.19	0.52	1.00	1.68	2.68
Mean	4.6	0.26	0.99	1.23	2.38	3.61
S.E.	1.40	0.07	0.47	0.23	0.70	0.93

Appendix 4 -

Faecal Egg Counts (fluke eggs per gram) of Lambs

Date	Lamb Number									
	R 81	R 82	R 83	R 84	R 85	R 86	R 87	R 88	R 89	R 90
1/8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
16/8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
30/8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12/9	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
26/9	-ve	100	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11/10	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
25/10	D	400	550	150	100	300	400	200	400	150
9/11	-	1200	550	D	D	600	450	250	750	250
16/11	-	800	D	-	-	700	450	500	1500	650
29/11	-	450	-	-	-	350	450	600	1500	300
6/12	-	450	-	-	-	500	700	N.S.	D	250
13/12	-	D	-	-	-	600	650	650	-	450
20/12	-	-	-	-	-	D	650	1500	-	600
27/12	-	-	-	-	-	-	300	600	-	750
3/1	-	-	-	-	-	-	50	1800	-	200
10/1	-	-	-	-	-	-	D	D	-	600
24/1	-	-	-	-	-	-	-	-	-	500
7/2	-	-	-	-	-	-	-	-	-	1590
21/2	-	-	-	-	-	-	-	-	-	400
6/3	-	-	-	-	-	-	-	-	-	500
28/3	-	-	-	-	-	-	-	-	-	700
										D

D = Died

Table 16

Grazing at Brocklees Farm, July 1967 to March 1968

Lamb Number											
R 91	R 92	R 93	R 94	R 95	R 96	R 97	R 98	R 99	R100	Mean	S.E.
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	5	5
-ve	50	100	-ve	-ve	50	50	-ve	-ve	-ve	13	6
150	50	200	D	N.S.	-ve	50	D	D	D	211	48
100	450	800	-	50	D	350	-	-	-	483	94
300	400	400	-	100	-	450	-	-	-	568	110
200	600	700	-	250	-	500	-	-	-	536	107
100	150	800	-	150	-	550	-	-	-	406	81
200	250	500	-	500	-	900	-	-	-	522	71
300	D	750	-	100	-	D	-	-	-	650	197
100	-	200	-	1000	-	-	-	-	-	492	143
150	-	5900	-	D	-	-	-	-	-	1620	1118
450	-	D	-	-	-	-	-	-	-	525	-
900	-	-	-	-	-	-	-	-	-	700	-
850	-	-	-	-	-	-	-	-	-	1220	-
450	-	-	-	-	-	-	-	-	-	425	-
350	-	-	-	-	-	-	-	-	-	425	-
2150	-	-	-	-	-	-	-	-	-	1425	-
D											

N.S. = No Sample Available

Appendix 4 - Table 17

The Liver Weights at Post-Mortem of, Terminal Fluke Faecal Egg Counts of, and the Numbers and Size Distribution of *F. hepatica* Recovered at Autopsy from, Lambs Dying of Naturally Acquired Fascioliasis

Lamb No.	Date of P.M.	e.p.g. at P.M.	Liver weight (gms)	Number of *F. hepatica*				
				Total	< 6mm.	6-12 mm.	> 12mm.	% > 12mm.
October 1967:								
P70	12/10	N.S.	-	714	698	162	54	7.6
R94	12/10	N.S.	-	287	100	171	16	5.5
R98	12/10	-ve	-	251	197	45	9	3.6
R99	12/10	250	-	346	153	171	22	6.3
R100	13/10	50	-	110	37	47	26	23.6
P74	13/10	-ve	-	252	109	99	44	17.5
P42	14/10	-ve	-	759	595	117	47	6.2
R81	20/10	-ve	-	174	130	42	2	1.1
P43	30/10	550	-	1065	372	454	239	22.4
Mean		121	-	440	266	145	51	10.4
S.E.		93	-	109	79	43	24	2.8
November 1967:								
R84	1/11	-ve	1315	400	264	120	16	4.0
P69	1/11	400	1154	677	317	316	44	6.5
P71	1/11	400	1074	795	105	497	193	24.3
R83	15/11	775	1750	1628	619	813	196	12.0
P31	16/11	1750	-	519	302	196	21	4.0
P36	16/11	600	985	766	136	494	136	17.5
P81	23/11	-ve	1024	361	183	140	38	10.5
P40	29/11	600	1312	761	422	251	88	20.9
P66	29/11	700	1210	1002	428	509	65	6.5
Mean		581	1229	768	308	371	89	11.8
S.E.		173	85	127	54	75	23	2.5

N.S. = No Sample Available

Appendix 4 - Table 17 (continued)

Lamb No.	Date of P.M.	e.p.g. at P.M.	Liver weight (gms)	Number of F. hepatica				
				Total	< 6mm.	6-12 mm.	> 12mm.	% > 12mm.
December 1967:								
P44	1/12	400	1230	703	182	291	230	32.7
P65	4/12	400	965	1011	438	480	93	9.2
R89	4/12	3850	1100	1142	453	451	238	20.8
R82	6/12	550	1360	797	172	427	198	24.8
P34	8/12	400	1200	584	231	240	113	19.3
P75	8/12	300	1350	885	454	278	154	17.4
R86	15/12	600	1276	909	238	406	265	29.2
R92	15/12	200	1362	1041	549	303	189	18.2
R97	15/12	900	1279	1222	258	416	548	44.8
P78	21/12	500	1364	1268	604	331	333	26.3
R95	28/12	1250	1228	479	86	235	158	33.0
Mean		850	1247	913	333	351	229	25.1
S.E.		313	37	77	52	26	38	2.9
January 1968:								
R93	3/1	5900	1165	434	276	158	90	20.7
R87	5/1	-ve	1100	652	441	128	68	10.4
R88	5/1	1300	1400	817	305	294	218	26.7
Mean		2400	1222	634	341	193	125	19.3
S.E.		418	91	111	51	51	47	4.8
March 1968:								
R90	28/3	700	910	449	0	53	396	88.2
R91	28/3	2150	840	378	0	54	324	85.7
Mean		1425	875	414	0	54	360	87.0
S.E.		725	35	36	-	0.5	36	1.3

APPENDIX 4 - Table 18

Bodyweights (lbs.) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	40	39	37	*	38.6	0.9
Grazed 19/7/67 to 16/8/67	1/8	41	39	40		40.0	0.6
	16/8	42	40	44		42.0	1.2
	31/8	50	50	48		49.3	0.7
Housed thereafter	14/9	59	60	55		58.0	1.5
	27/9	63	64	60		62.3	1.2
GROUP B	16/8	58	53	53	54	54.5	1.2
Grazed 16/8/67 to 20/9/67	29/8	59	49	49	47	51.0	2.7
	12/9	56	53	49	47	51.3	2.0
	28/9	62	55	47	49	53.3	3.4
Housed thereafter	12/10	60	56	46	51	53.3	3.0
	23/10	58	52	42	47	49.8	3.4
	30/10	56	50	40	46	48.0	3.4
GROUP C	20/9	70	67	65	69	67.8	1.1
Grazed 20/9/67 to 16/11/67	4/10	70	70	66	65	67.8	1.3
	18/10	70	68	63	70	67.8	1.7
	30/10	72	70	66	71	69.8	1.3
Housed thereafter	13/11	73	72	68	74	71.8	1.3
	27/11	74	71	69	76	72.5	1.6
GROUP D	18/10	79	68	73	69	72.3	2.5
Grazed 18/10/67 to 16/11/67	1/11	80	68	72	68	72.0	2.8
	15/11	85	70	74	68	74.3	3.8
	27/11	82	67	74	67	73.5	3.6
Housed thereafter	11/12	80	61	68	64	68.3	4.2
	28/12	83	60	66	63	68.0	5.1

* Died shortly after going out to grass.

APPENDIX 4 - Table 18 (continued)

	Date	Lamb Numbers 1	2	3	4	Mean	Standard Error
GROUP E	16/11	48	68	62	53	57.8	4.5
	29/11	49	70	64	55	59.5	4.7
Grazed 16/11/67 to 13/12/67	13/12	44	70	69	57	60.0	6.1
	29/12	46	65	59	56	56.5	4.0
Housed	12/1	52	68	63	63	61.5	3.4
thereafter	26/1	56	73	69	68	66.5	3.7
GROUP F	14/12	70	76	70	65	70.3	2.3
	27/12	74	78	71	67	72.5	2.3
Grazed 13/12/67 to 18/1/68	10/1	69	66	68	65	67.0	0.9
	19/1	70	69	70	68	69.3	0.4
Housed	2/2	71	68	69	69	69.3	0.6
thereafter	16/2	69	69	67	70	68.8	0.6
	1/3	67	65	65	72	67.3	1.7
GROUP G	18/1	67	80	62	65	68.5	4.0
	2/2	66	75	60	67	67.0	3.1
Grazed 18/1/68 to 13/3/68 *	21/2	69	75	58	68	67.5	3.5
	13/3	70	68	60	66	66.0	2.2
Housed	28/3	68	60	61	69	64.5	2.3
thereafter	10/4	67	65	60	69	65.3	1.9
	25/4	68	68	60	66	65.5	1.89
GROUP H	11/3	69	69	68	66	68.0	0.7
	25/3	70	68	66	62	66.5	1.7
Grazed 13/3/68 to 8/4/68	8/4	72	67	66	63	67.0	1.9
	22/4	81	72	69	71	73.3	2.7
Housed	10/5	86	76	78	77	79.3	2.3
thereafter	21/5	91	80	90	80	85.3	3.0

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 19

Packed Cell Volume Percentages of Tracer Lambs at Brooklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	31.5	34.0	35.0	*	33.5	1.04
	1/8	33.0	35.5	35.5		34.7	0.83
Grazed 19/7/67 to 16/8/67	16/8	26.0	30.5	32.0		29.5	1.80
	31/8	29.5	32.0	29.0		30.2	0.93
Housed	14/9	33.5	29.5	33.0		32.0	1.26
thereafter	27/9	39.5	30.5	38.5		36.2	2.85
GROUP B	16/8	35.5	36.5	40.0	33.5	36.4	1.36
	29/8	Cl.	35.0	37.5	34.0	35.5	1.04
Grazed 16/8/67 to 20/9/67	12/9	32.5	31.5	36.5	34.0	33.6	1.09
	28/9	29.0	32.5	34.0	31.5	31.8	1.05
Housed	12/10	32.0	28.0	33.5	29.0	30.6	1.28
thereafter	23/10	28.5	28.5	33.0	24.0	28.5	1.84
	30/10	26.0	22.0	25.0	19.0	23.0	1.58
GROUP C	20/9	37.0	40.5	37.5	43.5	39.6	1.51
	4/10	Cl.	40.0	36.5	40.0	38.8	1.17
Grazed 20/9/67 to 18/10/67	18/10	38.5	39.5	34.0	38.0	37.5	1.21
	30/10	38.0	42.0	33.5	40.0	38.4	1.82
Housed	13/11	33.5	37.0	33.0	37.0	35.1	1.09
thereafter	27/11	35.0	32.0	30.0	33.0	32.5	1.04
GROUP D	18/10	43.5	40.0	47.0	47.0	44.4	1.68
	1/11	41.0	37.5	48.0	46.0	43.1	2.38
Grazed 18/10/67 to 16/11/67	15/11	39.0	37.0	47.5	44.0	41.0	2.38
	27/11	43.0	35.0	45.0	44.0	41.8	2.29
Housed	11/12	38.0	34.5	44.0	40.0	39.1	1.98
thereafter	28/12	38.0	28.0	37.0	35.0	34.5	2.25

* Died shortly after going out to grass.

APPENDIX 4 - Table 19 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	35.0	45.0	34.0	30.0	36.0	3.19
	29/11	41.0	43.0	39.0	30.0	38.3	2.87
Grazed 16/11/67	13/12	34.5	40.0	36.0	30.0	35.1	2.07
to 13/12/67	29/12	35.0	35.0	31.0	33.0	33.5	0.96
Housed	12/1	33.0	38.0	35.0	36.0	35.5	1.04
thereafter	26/1	37.0	37.5	41.0	34.5	37.5	1.34
GROUP F	13/12	38.0	34.0	38.0	37.0	36.8	0.95
	27/12	40.0	38.0	39.0	36.0	38.3	0.85
Grazed 13/12/67	10/1	35.5	34.5	35.0	35.0	35.0	0.20
to 18/1/68	19/1	35.0	35.0	35.0	37.0	35.5	0.50
Housed	2/2	35.0	33.0	43.0	35.0	36.5	2.22
thereafter	16/2	39.0	35.0	37.0	26.0	34.3	2.87
	1/3	40.5	35.0	34.0	32.0	35.4	1.82
GROUP G	18/1	38.0	38.0	37.5	42.0	38.9	1.05
	2/2	36.0	43.0	38.0	45.0	40.5	2.10
Grazed 18/1/68	21/2	34.0	37.5	34.0	38.5	36.0	1.17
to 13/3/68 *	13/3	38.0	42.5	35.0	37.5	38.3	1.56
Housed	28/3	28.0	34.0	31.0	33.0	31.5	1.32
thereafter	10/4	29.0	31.0	35.0	33.0	32.0	1.29
	25/4	36.0	36.0	30.0	38.0	35.0	1.73
GROUP H	11/3	37.0	34.0	39.0	33.5	35.9	1.30
	28/3	38.0	37.5	40.0	36.0	37.9	0.83
Grazed 13/3/68	8/4	36.0	35.0	40.0	34.0	36.3	1.31
to 8/4/68	22/4	30.0	28.5	34.0	31.0	30.9	1.16
Housed	10/5	32.0	29.0	34.0	31.0	31.5	1.04
thereafter	21/5	31.0	30.0	36.5	31.5	32.3	1.45

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 20

Haemoglobin Concentrations (gms. per 100 ml.) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	11.4	12.2	12.4	*	12.0	0.31
Grazed 19/7/67 to 16/8/67	1/8	11.8	11.3	12.2		11.8	0.26
	16/8	9.3	11.1	10.4		10.3	0.52
	31/8	9.4	10.2	9.2		9.6	0.31
Housed thereafter	14/9	11.7	10.2	11.7		11.2	0.50
	27/9	13.0	10.1	13.9		12.3	1.15
GROUP B	16/8	11.1	13.0	13.6	11.9	12.4	0.56
Grazed 16/8/67 to 20/9/67	29/8	Cl.	11.6	13.4	12.0	12.3	0.55
	12/9	11.2	10.2	12.6	11.9	11.5	0.51
	28/9	10.9	10.4	13.9	10.9	11.5	0.80
Housed thereafter	12/10	12.0	8.9	12.1	9.9	10.7	0.79
	23/10	9.6	9.4	10.4	7.9	9.3	0.52
	30/10	7.9	7.2	7.9	6.0	7.3	0.45
GROUP C	20/9	12.5	14.9	14.3	16.7	14.6	0.87
Grazed 20/9/67 to 18/10/67	4/10	Cl.	15.4	13.6	15.5	14.8	0.62
	18/10	13.3	13.7	12.2	12.0	12.8	0.41
	30/10	12.0	13.0	11.3	13.0	12.3	0.42
Housed thereafter	13/11	11.8	12.3	10.3	12.3	11.7	0.47
	27/11	11.8	9.9	10.3	11.1	10.8	0.42
GROUP D	18/10	15.4	15.4	16.4	16.6	16.0	0.32
Grazed 18/10/67 to 16/11/67	1/11	12.7	11.6	15.8	16.1	14.1	1.12
	15/11	12.7	11.6	15.4	13.7	13.4	0.81
	27/11	14.4	11.6	15.4	14.2	13.9	0.81
Housed thereafter	11/12	11.6	11.1	14.0	12.7	12.4	0.64
	28/12	11.6	7.5	10.6	11.5	10.3	0.96

* Died shortly after going out to grass.

APPENDIX 4 - Table 20 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	12.3	14.7	10.4	9.6	11.8	1.13
	29/11	12.5	14.1	12.0	9.2	12.0	1.02
Grazed 16/11/67 to 13/12/67	13/12	10.8	11.6	10.3	8.5	10.3	0.66
	29/12	10.6	10.3	9.6	8.7	9.8	0.42
Housed	12/1	10.7	9.5	10.6	9.6	10.1	0.32
thereafter	26/1	11.3	11.3	12.3	8.9	11.0	0.72
GROUP F	14/12	12.0	11.0	12.0	12.0	11.8	0.25
	17/12	12.3	12.0	12.1	11.3	11.9	0.22
Grazed 13/12/67 to 18/1/68	10/1	11.1	11.5	11.6	11.0	11.3	0.15
	19/1	10.6	11.0	11.3	11.3	11.1	0.17
Housed	2/2	10.6	9.9	13.4	10.6	11.1	0.78
thereafter	16/2	11.6	11.0	11.0	7.4	10.3	0.96
	1/3	12.0	10.6	9.0	8.2	10.0	0.85
GROUP G	18/1	11.0	11.3	12.0	13.0	11.8	0.44
	2/2	10.8	13.0	12.0	13.7	12.4	0.63
Grazed 18/1/68 to 13/3/68 *	21/2	10.3	11.1	10.3	12.3	11.0	0.47
	13/3	11.8	13.4	11.5	11.3	12.0	0.48
Housed	28/3	8.7	10.3	9.1	9.1	9.3	0.35
thereafter	10/4	7.7	8.7	9.7	9.2	8.8	0.43
	25/4	9.7	9.6	8.6	10.3	9.6	0.35
GROUP H	11/3	10.1	9.2	9.9	8.9	9.5	0.28
	25/3	9.9	10.6	12.0	9.9	10.6	0.49
Grazed 13/3/68 to 8/4/68	8/4	9.2	9.7	11.0	10.6	10.1	0.41
	22/4	8.2	7.5	9.6	8.4	8.4	0.44
Housed	10/5	7.9	7.5	9.4	8.2	8.3	0.41
thereafter	21/5	7.7	7.3	9.4	7.7	8.0	0.47

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 21

Total Red Cell Counts (millions per cu. mm.) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	10.92	10.40	10.30	*	10.54	0.19
Grazed 19/7/67 to 16/8/67	1/8	11.00	12.20	11.90		11.70	0.36
	16/8	9.40	10.80	10.10		10.10	0.40
	31/8	11.12	12.12	10.28		11.17	0.53
Housed thereafter	14/9	10.30	11.22	10.88		10.80	0.27
	27/9	13.88	10.72	11.10		11.90	1.00
GROUP B	16/8	11.00	14.20	12.80	12.70	12.68	0.66
Grazed 16/8/67 to 20/9/67	29/8	Cl.	14.38	12.84	12.40	13.21	0.60
	12/9	10.02	10.48	12.46	15.68	12.16	1.29
	28/9	8.73	10.18	12.28	12.16	10.84	0.85
Housed thereafter	12/10	10.54	8.84	11.40	10.92	10.43	0.56
	23/10	8.06	8.20	10.50	8.42	8.80	0.57
	30/10	8.42	7.36	8.72	7.76	8.07	0.31
GROUP C	20/9	11.90	13.48	11.36	14.10	12.71	0.65
Grazed 20/9/67 to 18/10/67	4/10	Cl.	13.66	12.11	13.06	12.94	0.45
	18/10	13.76	13.88	11.64	13.28	13.14	0.52
	30/10	13.46	14.72	11.56	13.66	13.35	0.68
Housed thereafter	13/11	12.40	12.00	10.72	12.90	12.01	0.47
	27/11	12.16	10.98	9.86	11.60	11.15	0.49
GROUP D	18/10	15.82	13.36	13.66	18.60	16.61	1.27
Grazed 18/10/67 to 16/11/67	1/11	15.26	13.90	17.44	19.46	16.52	1.22
	15/11	14.32	13.68	16.42	17.76	15.55	0.94
	27/11	14.20	12.02	15.24	15.40	14.22	0.78
Housed thereafter	11/12	13.14	12.26	15.90	15.66	14.24	0.91
	28/12	13.34	11.88	12.78	12.36	12.59	0.31

* Died shortly after going out to grass.

APPENDIX 4 - Table 21 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	13.56	15.84	12.96	10.98	13.34	1.00
	29/11	14.38	15.30	14.54	10.72	13.74	1.02
Grazed 16/11/67 to 13/12/67	13/12	12.12	13.14	12.74	12.26	12.57	0.23
	29/12	12.46	11.88	12.08	10.68	11.78	0.38
Housed	12/1	10.40	11.54	10.16	10.10	10.55	0.34
thereafter	26/1	11.98	11.0	13.68	9.10	11.44	0.96
GROUP F	14/12	12.20	13.10	11.60	11.90	12.20	0.32
	27/12	13.28	12.46	13.48	11.50	12.68	0.45
Grazed 13/12/67 to 18/1/68	10/1	11.78	10.95	12.34	11.04	11.53	0.33
	19/1	11.10	11.38	11.30	11.30	11.27	0.06
Housed	2/2	9.64	8.92	11.35	10.60	10.13	0.53
thereafter	16/2	11.86	10.76	11.54	6.86	10.26	1.16
	1/3	14.24	11.64	9.44	7.84	10.79	1.39
GROUP G	18/1	10.24	11.88	11.36	12.92	11.60	0.56
	2/2	10.60	12.80	10.46	12.92	11.70	0.67
Grazed 18/1/68 to 13/3/68 *	21/2	10.04	11.48	9.92	12.12	10.89	0.54
	13/3	11.40	12.84	10.38	11.82	11.61	0.51
Housed	28/3	9.78	10.30	8.90	8.76	9.44	0.37
thereafter	10/4	8.02	8.16	9.20	8.32	8.43	0.27
	25/4	13.70	11.38	9.34	11.36	11.45	0.87
GROUP H	11/3	9.86	9.41	9.87	9.15	9.57	0.18
	25/3	11.20	11.44	12.12	10.80	11.39	0.28
Grazed 13/3/68 to 8/4/68	8/4	10.16	10.72	12.02	10.68	10.90	0.40
	22/4	9.40	8.42	10.38	9.48	9.42	0.40
Housed	10/5	8.68	8.36	10.28	9.24	9.14	0.42
thereafter	21/5	9.14	8.10	10.48	8.04	8.94	0.57

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 22

Mean Corpuscular Volume (cu./μ) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	29	35	34	*	32	1.53
	1/8	30	29	30		29.7	0.34
Grazed 19/7/67	16/8	28	28	32		29.3	1.34
to 16/8/67	31/8	27	26	28		27	0.58
Housed	14/9	33	27	30		30	1.73
thereafter	27/9	29	28	35		30.7	2.19
GROUP B	16/8	32	26	31	26	28.8	1.60
	29/8	Cl.	24	29	27	26.7	1.45
Grazed 16/8/67	12/9	32	30	29	32	30.8	0.75
to 20/9/67	28/9	35	32	28	26	29.8	1.65
Housed	12/10	30	32	29	27	29.5	1.04
thereafter	25/10	35	35	31	29	32.5	1.50
	30/10	31	30	29	25	28.8	1.31
GROUP C	20/9	31	30	33	31	31.3	0.63
	4/10	Cl.	29	30	31	30	0.58
Grazed 20/9/67	18/10	28	29	29	29	28.8	0.24
to 18/10/67	30/10	28	29	29	29	28.8	0.24
Housed	13/11	27	31	31	29	29.5	0.96
thereafter	27/11	29	29	30	28	29	0.41
GROUP D	18/10	28	30	25	25	27	1.22
	1/11	27	27	28	24	26.5	0.87
Grazed 18/10/67	15/11	27	27	29	25	27	0.82
to 16/11/67	27/11	30	29	30	29	29.5	0.29
Housed	11/12	29	28	28	26	27.8	0.63
thereafter	28/12	29	28	29	28	28.5	0.29

* Died shortly after going out to grass.

APPENDIX 4 - Table 22 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	26	28	26	27	26.8	0.47
	29/11	29	28	27	28	28	0.41
Grazed 16/11/67	13/12	29	30	28	25	28	1.08
to 13/12/67	29/12	28	30	26	31	28.8	1.11
Housed	12/1	32	34	34	36	34	0.82
thereafter	26/1	31	34	30	38	33.3	1.80
GROUP F	14/12	31	26	33	31	30.3	1.49
	27/12	30	31	29	31	30.3	0.47
Grazed 13/12/67	10/1	30	32	28	32	30.5	0.96
to 18/1/68	19/1	32	31	31	33	31.8	0.47
Housed	2/2	36	37	38	33	36	1.08
thereafter	16/2	33	33	32	38	34	1.35
	1/3	28	30	36	41	33.8	2.95
GROUP G	18/1	37	32	33	33	33.8	1.11
	2/2	34	34	36	35	34.8	0.48
Grazed 18/1/68	21/2	34	33	34	32	33.3	0.48
to 13/3/68 *	13/3	33	33	34	32	33.0	0.41
Housed	28/3	29	33	35	38	33.8	1.89
thereafter	10/4	36	38	38	40	38.0	0.82
	25/4	26	32	32	33	30.8	1.60
GROUP H	11/3	38	36	40	37	37.8	0.85
	25/3	34	33	33	33	33.3	0.25
Grazed 13/3/68	8/4	35	33	33	32	33.3	0.63
to 8/4/68	22/4	32	34	33	33	33.0	0.41
Housed	10/5	37	35	33	34	34.8	0.85
thereafter	21/5	34	37	35	39	36.3	1.11

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 23

Mean Corpuscular Haemoglobin Concentration (per cent) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	36	36	35	*	35.7	0.34
Grazed 19/7/67 to 16/8/67	1/8	36	32	34		34	1.15
	16/8	36	36	33		35	1.00
	31/8	32	32	32		32	0.00
Housed thereafter	14/9	35	35	36		35.3	0.34
	27/9	33	33	36		34	1.00
GROUP B	16/8	31	36	34	36	34.3	1.18
Grazed 16/8/67 to 20/9/67	29/8	Cl.	33	36	35	34.7	0.89
	12/9	35	32	35	35	34.3	0.75
	28/9	38	32	41	35	36.5	1.94
Housed thereafter	12/10	38	32	36	34	35	1.29
	23/10	34	33	32	33	33	0.41
	30/10	30	33	32	32	32.8	0.63
GROUP C	20/9	34	37	38	38	36.8	0.94
Grazed 20/9/67 to 18/10/67	4/10	Cl.	38	37	39	38	0.58
	18/10	35	35	36	32	34.5	0.89
	30/10	32	31	34	33	32.5	0.65
Housed thereafter	13/11	35	33	31	33	33	0.82
	27/11	34	31	34	34	33.3	0.75
GROUP D	18/10	35	39	35	35	36	1.0
Grazed 18/10/67 to 16/11/67	1/11	31	31	33	35	32.5	0.98
	15/11	33	31	32	31	32.8	0.47
	27/11	34	33	34	32	33.3	0.47
Housed thereafter	11/12	31	32	32	32	31.8	0.24
	28/12	31	27	29	33	30	1.29

* Died shortly after going out to grass.

APPENDIX 4 - Table 23 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	35	33	31	32	32.8	0.85
	29/11	31	33	31	31	31.5	0.50
Grazed 16/11/67	13/12	31	29	29	28	29.3	0.63
to 13/12/67	29/12	30	29	31	26	29	1.08
Housed	12/1	32	25	30	27	28.5	1.55
thereafter	26/1	31	30	30	26	29.3	1.11
GROUP F	14/12	32	32	32	32	32	0.00
	27/12	31	32	31	31	31.3	0.24
Grazed 13/12/67	10/1	31	33	33	31	32	0.58
to 18/1/68	19/1	30	31	32	31	31	0.41
Housed	2/2	30	30	31	30	30.3	0.24
thereafter	16/2	30	31	30	28	29.8	0.63
	1/3	30	30	26	26	28	1.15
GROUP G	18/1	29	30	32	31	30.5	0.65
	2/2	30	30	32	30	30.5	0.50
Grazed 18/1/68	21/2	30	30	30	32	30.5	0.50
to 13/3/68 *	13/3	31	32	33	30	31.5	0.65
Housed	28/3	31	30	29	28	29.5	0.65
thereafter	10/4	27	28	28	28	27.8	0.25
	25/4	27	27	29	27	27.5	0.50
GROUP H	11/3	27	27	25	27	26.5	0.50
	25/3	26	28	30	28	28.0	0.82
Grazed 13/3/68	8/4	26	28	28	31	28.3	1.03
to 8/4/68	22/4	27	26	28	27	27.0	0.41
Housed	10/5	25	26	28	26	26.3	0.63
thereafter	21/5	25	24	26	24	24.8	0.48

* Snow on ground for 4 weeks of this time.

APPENDIX 4 - Table 24

Total Serum Protein Levels (gms. per 100 ml.) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968.

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	7.2	6.0	5.3	*	6.17	0.56
Grazed 19/7/67	1/8	7.6	6.2	3.9		5.90	1.08
to 16/8/67	16/8	7.6	6.9	6.2		6.90	0.40
	31/8	7.1	6.8	5.7		6.53	0.43
Housed	14/9	7.2	7.0	6.2		6.80	0.31
thereafter	27/9	7.8	8.0	7.1		7.63	0.27
GROUP B	16/8	6.0	5.9	6.2	5.7	5.95	0.10
Grazed 16/8/67	29/8	6.8	6.3	7.6	6.1	6.70	0.33
to 20/9/67	12/9	7.0	6.7	7.8	6.4	6.98	0.30
	28/9	7.0	7.1	7.7	6.6	7.10	0.23
Housed	12/10	7.1	6.9	8.2	6.8	7.25	0.32
thereafter	23/10	6.7	6.9	8.8	6.9	7.33	0.49
	30/10	6.4	6.3	7.6	6.2	6.63	0.33
GROUP C	20/9	6.1	6.0	5.5	6.4	6.00	0.19
Grazed 20/9/67	4/10	6.2	5.7	5.9	6.8	6.15	0.24
to 18/10/67	18/10	6.5	6.3	6.7	7.8	6.83	0.34
	30/10	6.4	6.4	6.5	7.8	6.78	0.34
Housed	13/11	7.1	6.8	7.2	7.9	7.25	0.23
thereafter	27/11	6.8	6.0	6.2	7.3	6.58	0.30
GROUP D	18/10	6.9	6.5	7.3	6.5	6.80	0.19
Grazed 18/10/67	1/11	6.9	7.0	7.4	7.3	7.15	0.12
to 16/11/67	15/11	6.3	6.5	7.1	7.1	6.75	0.21
	27/11	7.1	7.0	6.8	7.3	7.05	0.10
Housed	11/12	7.1	7.2	6.7	6.9	6.98	0.11
thereafter	28/12	6.3	6.0	6.1	7.1	6.38	0.25

* Died shortly after going out to grass.

APPENDIX 4 - Table 24 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	6.4	6.5	6.4	7.0	6.58	0.14
	29/11	5.4	6.3	7.0	6.6	6.33	0.34
Grazed 16/11/67 to 13/12/67	13/12	5.4	5.7	6.5	6.1	5.93	0.24
	29/12	5.6	6.2	5.7	6.4	5.98	0.19
Housed	12/1	6.3	6.6	6.9	6.9	6.68	0.14
thereafter	26/1	6.2	6.3	7.1	7.1	6.68	0.25
GROUP F	14/12	5.2	6.8	7.1	5.3	6.10	0.49
	27/12	4.9	7.1	7.5	5.6	6.28	0.61
Grazed 13/12/67 to 18/1/68	10/1	6.6	8.9	8.0	6.3	7.45	0.61
	19/1	7.0	8.7	8.9	4.3	7.23	1.06
Housed	2/2	6.5	7.1	6.9	6.6	6.78	0.14
thereafter	16/2	6.3	7.0	7.4	6.8	6.88	0.23
	1/3	7.9	8.1	8.2	7.1	7.83	0.25
GROUP G	18/1	6.2	6.6	6.1	6.1	6.3	0.12
	2/2	6.6	6.6	6.1	6.3	6.4	0.12
Grazed 18/1/68 to 13/3/68 *	21/2	7.0	6.4	6.3	6.9	6.7	0.18
	13/3	7.8	7.3	7.0	7.3	7.4	0.17
Housed	28/3	6.4	6.4	6.9	6.8	6.6	0.13
thereafter	10/4	6.3	5.6	6.6	6.8	6.3	0.26
	25/4	6.9	6.8	7.0	7.8	7.1	0.23
GROUP H	11/3	5.9	5.8	6.5	6.0	6.1	0.16
	25/3	5.6	5.8	5.9	6.0	5.8	0.09
Grazed 13/3/68 to 8/4/68	8/4	6.7	6.6	6.7	8.0	7.0	0.33
	22/4	7.0	7.5	7.2	8.0	7.4	0.22
Housed	10/5	7.3	6.5	6.9	7.7	7.1	0.26
thereafter	21/5	7.1	6.5	7.1	7.7	7.1	0.24

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 25

Serum Albumin Levels (gms. per 100 ml.) of Tracer Lambs at Brocklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	1.86	2.02	2.28	*	2.05	0.12
Grazed 19/7/67 to 16/8/67	1/8	1.80	1.89	1.63		1.77	0.08
	16/8	1.34	2.39	1.85		1.86	0.30
	31/8	1.95	2.14	1.72		1.94	0.12
Housed thereafter	14/9	2.45	2.46	2.09		2.33	0.12
	27/9	2.24	2.94	2.04		2.41	0.27
GROUP B	16/8	2.15	2.07	1.92	1.93	2.02	0.06
Grazed 16/8/67 to 20/9/67	29/8	2.24	2.27	2.38	2.25	2.29	0.04
	12/9	2.16	2.63	2.20	1.57	2.14	0.22
	28/9	1.97	1.88	1.68	1.85	1.85	0.06
Housed thereafter	12/10	2.36	2.60	2.31	2.43	2.43	0.06
	23/10	2.21	2.27	2.23	2.35	2.27	0.03
	30/10	1.77	1.89	1.75	1.65	1.77	0.05
GROUP C	20/9	2.35	2.73	2.15	2.96	2.55	0.18
Grazed 20/9/67 to 18/10/67	4/10	2.01	1.88	2.00	2.66	2.14	0.18
	18/10	2.26	2.87	2.52	2.18	2.46	0.15
	30/10	1.95	2.15	2.09	3.07	2.32	0.25
Housed thereafter	13/4	1.25	2.26	2.14	2.36	2.00	0.25
	27/11	2.11	2.14	1.95	2.86	2.27	0.20
GROUP D	18/10	2.64	3.29	2.64	2.41	2.75	0.19
Grazed 18/10/67 to 16/11/67	1/11	2.70	2.93	2.41	3.04	2.77	0.14
	15/11	2.61	2.26	2.50	2.82	2.55	0.12
	27/11	2.68	2.54	2.30	3.60	2.78	0.28
Housed thereafter	11/12	2.46	2.14	2.05	2.07	2.18	0.10
	28/12	1.95	1.72	2.23	2.27	2.04	0.13

* Died shortly after going out to grass.

APPENDIX 4 - Table 25 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	2.79	3.09	2.83	2.53	2.81	0.12
	29/11	2.32	2.27	2.47	2.27	2.33	0.05
Grazed 16/11/67	13/12	1.73	1.93	1.86	1.61	1.78	0.07
to 13/12/67	29/12	2.01	2.29	1.85	2.04	2.05	0.09
Housed	12/1	2.51	2.33	2.81	2.60	2.56	0.10
thereafter	26/1	2.80	2.36	2.54	2.27	2.49	0.12
GROUP F	14/12	2.16	2.42	3.11	2.16	2.46	0.22
	27/12	2.28	2.65	3.26	2.23	2.61	0.24
Grazed 13/12/67	10/1	2.00	2.07	2.45	2.80	2.33	0.18
to 18/1/68	19/1	1.83	2.15	2.37	1.25	1.90	0.24
Housed	2/2	2.14	2.26	2.20	1.67	2.07	0.13
thereafter	16/2	2.13	1.83	2.13	2.08	2.04	0.07
	1/3	1.99	1.96	2.19	1.86	2.00	0.07
GROUP G	18/1	1.78	2.67	2.50	2.47	2.36	0.20
	2/2	2.55	2.41	2.57	2.84	2.59	0.09
Grazed 18/1/68	21/2	2.06	2.19	2.14	1.97	2.09	0.05
to 13/3/68 *	13/3	2.18	2.30	2.16	2.08	2.18	0.05
Housed	28/3	1.69	1.44	2.17	2.02	1.83	0.16
thereafter	10/4	1.60	1.53	2.06	1.98	1.80	0.13
	25/4	1.86	1.93	2.07	2.16	2.01	0.07
GROUP H	11/3	1.97	1.95	2.18	2.17	2.07	0.06
	25/3	2.21	2.15	2.24	2.48	2.27	0.07
Grazed 13/3/68	8/4	2.22	2.28	2.16	2.28	2.24	0.03
to 8/4/68	22/4	2.41	2.47	2.02	2.22	2.28	0.10
Housed	10/5	2.41	2.71	1.92	2.41	2.36	0.16
thereafter	21/5	2.09	1.93	1.91	2.39	2.08	0.11

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 26

Albumin:Globulin Ratios of Tracer Lambs at Brooklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	0.35	0.56	0.76	*	0.56	0.12
Grazed 19/7/67	1/8	0.31	0.44	0.71		0.49	0.12
to 16/8/67	16/8	0.30	0.48	0.42		0.40	0.05
	31/8	0.38	0.46	0.43		0.42	0.02
Housed	14/9	0.52	0.54	0.51		0.52	0.01
thereafter	27/9	0.40	0.58	0.62		0.53	0.07
GROUP B	16/8	0.56	0.54	0.45	0.51	0.52	0.02
Grazed 16/8/67	29/8	0.49	0.56	0.45	0.59	0.52	0.03
to 20/9/67	12/9	0.45	0.65	0.39	0.33	0.46	0.07
	28/9	0.39	0.36	0.28	0.38	0.35	0.03
Housed	12/10	0.50	0.63	0.39	0.56	0.52	0.05
thereafter	23/10	0.49	0.49	0.34	0.52	0.46	0.04
	30/10	0.38	0.43	0.30	0.36	0.37	0.03
GROUP C	20/9	0.63	0.83	0.64	0.86	0.74	0.06
Grazed 20/9/67	4/10	0.48	0.49	0.51	0.64	0.53	0.04
to 18/10/67	18/10	0.53	0.84	0.60	0.39	0.59	0.09
	30/10	0.44	0.51	0.47	0.62	0.51	0.04
Housed	13/11	0.21	0.50	0.42	0.43	0.39	0.06
thereafter	27/11	0.45	0.56	0.45	0.64	0.53	0.05
GROUP D	18/10	0.62	1.02	0.56	0.59	0.70	0.11
Grazed 18/10/67	1/11	0.64	0.72	0.53	0.72	0.65	0.05
to 16/11/67	15/11	0.71	0.53	0.54	0.66	0.61	0.04
	27/11	0.51	0.64	0.52	0.61	0.60	0.03
Housed	11/12	0.53	0.42	0.44	0.43	0.46	0.03
thereafter	28/12	0.44	0.40	0.57	0.47	0.47	0.04

* Died shortly after going out to grass.

APPENDIX 4 - Table 26 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	0.77	0.91	0.79	0.57	0.76	0.07
Grazed 16/11/67 to 13/12/67	29/11	0.75	0.56	0.55	0.53	0.60	0.05
	13/12	0.47	0.51	0.40	0.36	0.44	0.03
	29/12	0.57	0.59	0.49	0.47	0.53	0.03
Housed thereafter	12/1	0.66	0.55	0.69	0.61	0.63	0.03
	26/1	0.82	0.60	0.56	0.47	0.61	0.08
GROUP F	13/12	0.70	0.55	0.78	0.69	0.68	0.05
Grazed 13/12/67 to 18/1/68	27/12	0.87	0.60	0.77	0.66	0.73	0.06
	10/1	0.43	0.43	0.44	0.80	0.53	0.09
	19/1	0.36	0.33	0.36	0.41	0.37	0.02
Housed thereafter	2/2	0.49	0.47	0.38	0.34	0.42	0.04
	16/2	0.51	0.35	0.40	0.44	0.43	0.03
	1/3	0.34	0.32	0.36	0.36	0.35	0.01
GROUP G	18/1	0.40	0.68	0.69	0.68	0.61	0.07
Grazed 18/1/68 to 13/3/68 *	2/2	0.63	0.58	0.73	0.82	0.69	0.23
	21/2	0.42	0.52	0.51	0.40	0.46	0.03
	13/3	0.39	0.46	0.45	0.40	0.43	0.02
Housed thereafter	28/3	0.36	0.29	0.46	0.42	0.38	0.04
	10/4	0.35	0.38	0.45	0.41	0.40	0.02
	25/4	0.37	0.40	0.42	0.38	0.39	0.01
GROUP H	11/3	0.50	0.51	0.50	0.57	0.52	0.02
Grazed 13/3/68 to 8/4/68	25/3	0.65	0.59	0.61	0.71	0.64	0.03
	8/4	0.50	0.53	0.47	0.40	0.48	0.03
	22/4	0.53	0.49	0.39	0.38	0.45	0.04
Housed thereafter	10/5	0.49	0.72	0.39	0.45	0.51	0.07
	21/5	0.42	0.42	0.37	0.45	0.42	0.02

* Snow on Ground for 4 weeks of this period.

APPENDIX 4 - Table 27

Serum Gamma Globulin Levels (gms. per 100 ml.) of Tracer Lambs at Brookleas Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	4.07	2.67	1.78	*	2.84	0.67
Grazed 19/7/67 to 16/8/67	1/8	4.10	2.30	1.36		2.59	0.80
	16/8	3.00	3.25	2.42		2.89	0.24
	31/8	3.03	2.77	2.14		2.65	0.27
Housed thereafter	14/9	2.77	2.49	2.07		2.44	0.20
	27/9	3.49	2.89	2.68		3.02	0.24
GROUP B	16/8	1.83	1.91	2.30	1.80	1.96	0.11
Grazed 16/8/67 to 20/9/67	29/8	2.50	2.11	3.17	1.72	2.38	0.31
	12/9	2.68	2.20	3.66	2.67	2.80	0.31
	28/9	2.60	2.99	3.89	2.62	3.03	0.30
Housed thereafter	12/10	2.83	2.64	4.16	2.60	3.06	0.37
	23/10	2.99	2.92	5.03	2.84	3.45	0.53
	30/10	3.13	2.75	4.36	2.85	3.27	0.37
GROUP C	20/9	2.10	1.60	1.78	1.68	1.79	0.11
Grazed 20/9/67 to 18/10/67	4/10	2.47	2.00	1.95	2.45	2.22	0.14
	18/10	2.56	1.80	2.85	3.56	2.69	0.36
	30/10	3.03	3.03	3.13	3.42	3.15	0.09
Housed thereafter	13/4	3.70	2.68	3.11	3.52	3.25	0.23
	27/11	3.76	2.89	3.26	3.40	3.33	0.18
GROUP D	18/10	2.45	1.72	2.81	2.34	2.33	0.23
Grazed 18/10/67 to 16/11/67	1/11	2.45	2.20	3.20	2.40	2.56	0.22
	15/11	2.71	2.75	2.97	2.89	2.83	0.06
	27/11	3.16	3.33	3.20	4.70	3.60	0.37
Housed thereafter	11/12	3.17	3.45	3.33	3.24	3.30	0.06
	28/12	3.17	3.26	2.91	3.99	3.33	0.23

* Died shortly after going out to grass.

APPENDIX 4 - Table 27 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	1.87	2.05	1.70	2.46	2.02	0.16
	29/11	1.59	2.03	2.54	2.45	2.15	0.22
Grazed 16/11/67	13/12	2.36	2.19	3.03	3.25	2.71	0.26
to 13/12/67	29/12	2.18	2.20	2.34	2.86	2.40	0.16
Housed	12/1	1.96	2.09	2.77	2.43	2.31	0.18
thereafter	26/1	1.74	2.30	2.61	2.98	2.41	0.26
GROUP F	13/12	1.50	3.07	2.78	1.93	2.32	0.36
	27/12	1.38	3.17	3.19	2.07	2.45	0.44
Grazed 13/12/67	10/1	2.92	4.52	3.61	2.39	3.36	0.46
to 18/1/68	19/1	3.23	4.44	4.36	1.78	3.45	0.62
Housed	2/2	2.80	3.45	3.33	3.17	3.19	0.14
thereafter	16/2	2.58	3.51	3.72	2.98	3.20	0.26
	1/3	3.62	4.18	3.58	3.35	3.68	0.18
GROUP G	18/1	2.70	2.02	2.25	2.04	2.25	0.16
	2/2	2.77	2.53	2.39	1.88	2.39	0.19
Grazed 18/1/68	21/2	3.38	2.51	2.65	2.83	2.84	0.19
to 13/3/68 *	13/3	3.62	3.23	3.14	3.33	3.33	0.10
Housed	28/3	3.23	3.38	3.36	3.06	3.26	0.07
thereafter	10/4	3.16	2.64	3.30	3.12	3.06	0.14
	25/4	3.36	2.99	2.96	3.70	3.25	0.17
GROUP H	11/3	2.25	2.23	2.90	2.08	2.37	0.18
	25/3	1.86	2.05	1.29	2.25	1.86	0.21
Grazed 13/3/68	8/4	2.60	2.51	2.72	3.82	2.91	0.31
to 8/4/68	22/4	2.42	3.06	3.50	3.76	3.19	0.29
Housed	10/5	3.17	2.49	3.32	3.60	3.15	0.26
thereafter	21/5	3.13	2.78	3.38	3.27	3.14	0.13

* Snow on ground for 4 weeks of this period.

APPENDIX 4 - Table 28

Serum Alpha/Beta Globulin Levels (gms. per 100 ml.) of Tracer Lambs at Brecklees Farm, July 1967 to March 1968

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP A	18/7	1.27	1.31	1.24	*	1.27	0.03
Grazed 19/7/67	1/8	1.69	1.81	0.91		1.47	0.28
to 16/8/67	16/8	1.27	1.80	1.94		1.67	0.20
	31/8	2.12	1.88	1.84		1.95	0.09
Housed	14/9	1.98	2.04	2.03		2.02	0.02
thereafter	27/9	2.03	2.16	2.37		2.19	0.10
GROUP B	16/8	2.02	1.92	1.98	1.97	1.98	0.03
Grazed 16/8/67	29/8	2.06	1.92	2.13	2.12	2.06	0.05
to 20/9/67	12/9	2.16	1.86	1.95	2.16	2.03	0.08
	28/9	2.13	2.13	2.05	2.12	2.11	0.01
Housed	12/10	1.91	1.59	1.73	1.76	1.75	0.06
thereafter	23/10	1.50	1.71	1.52	1.70	1.61	0.05
	30/10	1.49	1.66	1.49	1.69	1.58	0.05
GROUP C	20/9	1.65	1.67	1.57	1.76	1.66	0.03
Grazed 20/9/67	4/10	1.72	1.81	1.95	1.68	1.79	0.06
to 18/10/67	18/10	1.68	1.63	1.32	2.06	1.67	0.15
	30/10	1.42	1.21	1.28	1.54	1.36	0.07
Housed	13/11	2.15	1.85	1.95	2.01	1.99	0.06
thereafter	27/11	0.93	0.97	1.01	1.04	0.99	0.03
GROUP D	18/10	1.80	1.49	1.86	1.75	1.73	0.08
Grazed 18/10/67	1/11	1.74	1.86	1.79	1.86	1.81	0.03
to 16/11/67	15/11	0.98	1.49	1.63	1.40	1.38	0.14
	27/11	1.26	1.04	1.10	1.20	1.15	0.05
Housed	11/12	1.46	1.61	1.32	1.59	1.50	0.07
thereafter	28/12	1.18	1.02	0.96	0.84	1.00	0.07

* Died shortly after going out to grass.

APPENDIX 4 - Table 28 (continued)

	Date	Lamb Number 1	2	3	4	Mean	Standard Error
GROUP E	16/11	1.75	1.36	1.87	2.01	1.75	0.14
	29/11	1.49	2.00	1.99	1.88	1.84	0.12
Grazed 16/11/67 to 13/12/67	13/12	1.32	1.58	1.61	1.24	1.44	0.09
	29/12	1.41	1.70	1.50	1.49	1.53	0.06
Housed	12/1	1.84	2.19	1.32	1.87	1.81	0.18
thereafter	26/1	1.66	1.64	1.95	1.89	1.79	0.08
GROUP F	13/12	1.54	1.31	1.21	1.21	1.32	0.08
	27/12	1.23	1.27	1.06	1.30	1.22	0.06
Grazed 13/12/67 to 18/1/68	10/1	1.68	1.71	1.94	1.11	1.61	0.18
	19/1	1.93	2.09	2.17	1.26	1.86	0.21
Housed	2/2	1.56	1.39	1.37	1.76	1.52	0.09
thereafter	16/2	1.59	1.65	1.56	1.74	1.64	0.04
	1/3	2.30	1.95	2.44	1.89	2.15	0.13
GROUP G	18/1	1.72	1.91	1.36	1.59	1.65	0.12
	2/2	1.28	1.66	1.14	1.58	1.42	0.12
Grazed 18/1/68 to 13/3/68 *	21/2	1.56	1.71	1.56	2.09	1.73	0.12
	13/3	2.01	1.77	1.69	1.90	1.84	0.07
Housed	28/3	1.48	1.58	1.37	1.72	1.54	0.07
thereafter	10/4	1.52	1.43	1.24	1.70	1.47	0.10
	25/4	1.67	1.87	1.97	1.93	1.86	0.07
GROUP H	11/3	1.68	1.61	1.43	1.75	1.62	0.07
	25/3	1.53	1.59	1.24	1.26	1.41	0.09
Grazed 13/3/68 to 8/4/68	8/4	1.88	1.81	1.83	1.91	1.86	0.02
	22/4	2.16	1.97	1.68	2.02	1.96	0.10
Housed	10/5	1.72	1.29	1.66	1.70	1.59	0.10
thereafter	21/5	1.88	1.79	1.81	2.04	1.88	0.06

* Snow on ground for 4 weeks of this period.

Appendix 4 - Table 29

The Numbers and Size Distribution of *F. hepatica* Recovered at Autopsy of Tracer Lambs Grazing at Brocklees Farm, April 1967 to April 1968

Period Grazed	Number of *F. hepatica*				Numbers of *F. hepatica* ingested per week of period grazed	
	Total	< 6 mm.	6-12 mm.	> 12 mm.	Individual	Mean
15/4	16	0	8	8	2	
-19/7*	22	4	16	2	3	2
	14	2	11	1	2	
	2	1	1	0	0.3	
19/7	49	2	35	12	12	
-16/8	75	34	41	0	19	11
	8	0	8	0	2	
16/8	212	122	85	5	43	
-20/9	91	50	41	0	18	41
	229	128	94	7	46	
	379	215	150	14	76	
20/9	25	3	17	5	6	
-18/10	46	13	26	7	12	10
	22	3	19	0	6	
	69	18	40	7	17	
18/10	28	6	19	3	7	
-16/11	62	59	3	0	16	18
	65	31	32	2	16	
	130	48	69	13	33	

* "Permanent" lambs removed at weaning. Assumed ingesting significant quantities of grass only over the eight weeks prior to removal from grazing.

Appendix 4 - Table 29 (continued)

Period Grazed	Number of *F. hepatica*				Numbers of *F. hepatica* ingested per week of period grazed	
	Total	< 6 mm.	6-12 mm.	> 12 mm.	Individual	Mean
16/11	28	7	17	4	7	
- 13/12	116	59	41	16	29	13
	32	19	13	0	8	
	32	18	13	1	8	
13/12	51	11	16	4	6	
-18/1	62	31	25	6	12	10
	80	22	43	15	16	
	78	49	18	11	16	
18/1	21	8	7	6	5	
-13/3**	25	4	11	10	6	7
	21	7	10	4	5	
	49	5	20	24	12	
13/3	11	1	8	2	3	
- 8/4	12	10	2	0	3	7
	51	28	18	5	13	
	34	15	15	4	9	

** Snow on ground for four weeks of period grazed.

APPENDIX 5 - Table 1

Bodyweight (lbs.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P34	P35	P41	P47	P51	P52	P56	P61	P72	P75		
	9/11	87	78	85	80	99	77	80	70	74	80	81.0	2.5
	23/11	87	78	84	79	95	79	80	70	73	73	79.8	2.3
Treated →	6/12	80	73	80	80	94	75	75	69	60	65	75.1	3.0
(8/12)	13/12	D	71	79	77	94	75	73	65	59	D	74.1	3.7
	20/12	-	66	78	79	91	73	72	65	55	-	72.4	3.8
	27/12	-	73	80	81	95	75	78	66	61	-	76.1	3.6
	3/1	-	74	79	84	94	78	78	67	63	-	77.1	3.4
	10/1	-	70	75	78	90	71	71	64	60	-	72.4	3.2
	24/1	-	69	73	73	89	72	74	64	63	-	72.1	2.8
	7/2	-	75	75	74	85	64	73	62	65	-	71.4	2.5
	21/2	-	74	73	74	82	67	71	61	64	-	70.8	2.3
	6/3	-	71	70	69	85	68	69	62	60	-	69.3	2.6
Group B		R82	R86	R87	R88	R90	R91	R92	R93	R95	R97		
	9/11	77	84	88	98	110	99	94	92	72	77	89.1	3.7
	16/11	78	89	90	94	112	102	95	95	71	77	90.3	3.9
	29/11	70	76	90	90	109	99	88	87	60	69	83.8	4.7
	6/12	65	72	84	83	106	98	79	83	58	62	79.0	4.9
	13/12	D	67	82	82	106	99	72	80	58	57	78.1	5.6
	20/12	-	D	74	75	103	91	D	72	51	D	77.7	7.3
	27/12	-	-	74	73	100	95	-	74	D	-	83.2	5.9
	3/1	-	-	74	69	95	94	-	71	-	-	80.6	5.7
	10/1	-	-	D	D	94	89	-	D	-	-	91.5	-
	24/1	-	-	-	-	93	87	-	-	-	-	90.0	-
	7/2	-	-	-	-	96	83	-	-	-	-	89.5	-
	21/2	-	-	-	-	92	86	-	-	-	-	89.0	-
	6/3	-	-	-	-	89	82	-	-	-	-	85.5	-

D = Died

APPENDIX 5 - Table 2

Total Red Cell Counts (millions per cu. mm.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	5.40	7.00	7.58	9.78	9.82	6.04	6.38	5.50	6.02	5.84	6.94	0.52
	23/11	4.36	5.72	6.96	6.52	6.56	4.98	5.22	4.88	4.00	4.50	5.37	0.32
	6/12	2.78	5.36	6.02	5.70	7.40	4.76	4.32	3.46	3.16	3.62	4.66	0.46
Treated → (8/12)	13/12	D	6.32	6.21	7.68	7.88	6.96	6.26	5.98	4.92	D	6.53	0.34
	20/12	-	7.88	8.16	9.72	9.24	9.30	8.72	9.58	5.46	-	8.51	0.49
	27/12	-	8.68	9.82	10.38	10.36	9.64	9.60	9.54	6.42	-	9.31	0.45
	3/1	-	8.64	10.86	10.74	11.04	9.30	10.14	9.76	7.48	-	9.75	0.44
	10/1	-	9.74	10.38	11.54	11.26	9.92	11.16	10.68	8.68	-	10.42	0.34
	24/1	-	8.66	9.58	10.92	8.54	8.84	9.84	8.90	8.50	-	9.19	0.30
	7/2	-	10.96	9.02	11.44	7.74	8.96	9.48	9.38	9.38	-	9.54	0.41
	21/2	-	10.26	9.82	12.32	9.38	10.06	9.30	9.08	9.42	-	9.96	0.37
	6/3	-	9.56	8.24	10.02	8.70	8.20	6.72	8.00	7.04	-	8.31	0.40
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	4.04	6.68	7.16	8.66	11.22	11.64	7.48	6.46	7.42	6.38	7.71	0.72
	16/11	Cl.	5.46	5.96	8.70	10.72	10.88	5.86	5.58	7.00	5.32	7.28	0.75
	29/11	3.36	4.38	5.34	7.26	9.32	9.72	4.52	4.60	5.68	3.88	5.81	0.71
	6/12	2.42	3.10	4.70	7.52	9.72	7.68	4.10	4.08	4.98	3.28	5.25	0.80
	13/12	D	3.54	4.28	6.92	9.56	7.44	3.02	3.42	4.70	3.10	5.00	0.81
	20/12	-	D	4.48	5.84	8.66	7.10	D	3.16	4.10	D	5.56	0.84
	27/12	-	-	3.64	5.04	8.56	6.90	-	2.54	3.58	-	5.04	1.00
	3/1	-	-	2.64	3.92	8.96	6.52	-	1.24	D	-	4.66	1.38
	10/1	-	-	D	D	9.00	6.24	-	D	-	-	7.68	-
	24/1	-	-	-	-	6.96	5.64	-	-	-	-	6.30	-
	7/2	-	-	-	-	6.62	5.64	-	-	-	-	6.13	-
	21/2	-	-	-	-	5.74	4.96	-	-	-	-	5.35	-
	6/3	-	-	-	-	5.34	4.26	-	-	-	-	4.80	-

D = Died Cl. = Clotted Sample

APPENDIX 5 - Table 3

Packed Cell Volume Percentages of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	18.5	22.0	22.5	27.5	31.0	23.0	23.5	20.0	19.5	18.5	22.6	1.27
	23/11	18.5	19.0	19.0	22.0	24.0	17.0	19.5	17.0	13.0	15.0	18.4	1.01
Treated ⟹	6/12	12.0	17.5	17.0	19.0	27.0	17.0	16.0	13.0	12.0	13.5	16.4	1.41
(8/12)	13/12	D	19.0	19.5	25.0	27.0	20.0	20.0	20.0	17.5	D	21.0	1.15
	20/12	-	23.0	21.0	26.0	30.0	25.0	25.0	26.0	18.0	-	24.3	1.28
	27/12	-	25.0	29.0	31.0	33.5	25.0	28.0	28.0	20.0	-	27.4	1.46
	3/1	-	25.0	30.0	29.5	33.5	28.5	30.5	29.5	25.0	-	28.7	1.16
	10/1	-	28.5	27.0	31.5	33.5	26.0	31.0	29.0	25.0	-	28.9	1.03
	24/1	-	29.0	29.0	32.0	30.0	27.0	27.5	28.0	26.0	-	28.6	0.56
	7/2	-	31.0	27.0	33.5	29.5	28.0	28.0	28.0	27.5	-	29.1	0.78
	21/2	-	29.0	27.0	33.5	33.0	28.0	27.0	27.0	28.5	-	29.1	0.94
	6/3	-	31.0	28.0	30.0	31.0	26.5	21.0	25.5	24.0	-	27.1	1.26
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	17.5	20.0	25.0	26.0	33.0	33.0	25.0	25.0	22.5	26.0	25.3	1.55
	16/11	13.0	18.0	21.0	25.0	32.5	29.5	17.5	21.0	20.0	19.0	21.7	1.85
	29/11	11.0	11.5	18.0	23.0	29.0	27.5	14.0	16.5	18.0	15.5	18.4	1.97
	6/12	10.0	12.0	16.5	20.0	29.0	22.5	13.0	16.0	15.0	14.0	16.8	1.79
	13/12	D	10.0	15.0	21.0	30.0	23.5	10.0	15.0	16.0	15.0	17.3	2.17
	20/12	-	D	16.0	17.5	28.5	20.0	D	14.0	13.5	D	18.3	2.27
	27/12	-	-	14.0	16.0	26.0	23.0	-	12.5	10.0	-	16.9	2.56
	3/1	-	-	10.0	13.0	29.0	18.5	-	5.0	D	-	15.1	4.11
	10/1	-	-	D	D	29.5	21.0	-	D	-	-	25.3	-
	24/1	-	-	-	-	24.5	21.0	-	-	-	-	22.8	-
	7/2	-	-	-	-	23.0	22.0	-	-	-	-	22.5	-
	21/2	-	-	-	-	19.0	19.0	-	-	-	-	19.0	-
	6/3	-	-	-	-	20.0	17.0	-	-	-	-	18.5	-

D = Died

APPENDIX 5 - Table 4

Haemoglobin Concentration (gms. per 100 ml.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P34	P35	P41	P47	P51	P52	P56	P61	P72	P75		
	9/11	5.5	6.5	6.5	8.2	9.9	6.8	7.5	5.5	6.5	6.8	7.0	0.41
	23/11	4.9	5.4	5.9	6.3	7.5	4.4	5.1	4.4	3.7	4.4	5.2	0.35
	6/12	2.7	4.6	5.1	4.6	7.5	3.4	3.6	2.7	2.9	3.2	4.0	0.47
Treated → (8/12)	13/12	D	4.8	4.8	6.1	7.2	4.8	4.9	4.8	3.9	D	5.2	0.36
	20/12	-	5.7	5.9	7.9	8.2	6.5	7.5	6.5	4.4	-	6.6	0.45
	27/12	-	6.8	7.9	8.4	9.4	7.0	7.7	7.9	5.4	-	7.6	0.42
	3/1	-	7.0	8.7	8.6	9.9	7.5	8.6	8.0	6.5	-	8.1	0.38
	10/1	-	7.9	7.4	9.1	10.3	7.4	8.6	8.2	7.0	-	8.2	0.38
	24/1	-	7.5	8.4	9.6	8.2	7.5	7.9	7.9	7.5	-	8.1	0.25
	7/2	-	8.9	8.0	10.3	8.4	7.9	8.7	7.5	8.4	-	8.5	0.30
	21/2	-	8.7	8.2	9.9	9.9	7.5	7.7	7.0	8.0	-	8.4	0.38
	6/3	-	8.4	8.0	8.6	9.2	7.0	5.6	6.8	7.0	-	7.6	0.42
Group B		R82	R86	R87	R88	R90	R91	R92	R93	R95	R97		
	9/11	4.3	6.3	7.7	7.9	10.9	9.9	6.3	7.2	6.1	6.8	7.3	0.60
	16/11	Cl.	4.4	6.0	7.2	9.9	8.7	4.3	5.4	5.9	4.9	6.3	0.65
	29/11	2.1	3.1	4.9	6.3	8.9	8.0	3.4	4.4	5.1	4.1	5.0	0.58
	6/12	2.0	2.7	4.3	5.1	8.2	6.5	2.7	4.1	4.1	3.4	4.3	0.60
	13/12	D	2.2	3.6	5.3	8.6	5.9	1.7	3.3	3.7	3.4	4.2	0.71
	20/12	-	D	4.1	4.8	7.9	5.3	D	3.1	3.4	D	4.8	0.66
	27/12	-	-	3.3	4.1	7.7	6.0	-	2.7	2.4	-	4.4	0.85
	3/1	-	-	1.9	2.7	8.2	5.3	-	0.8	D	-	3.8	1.33
	10/1	-	-	D	D	8.2	5.9	-	D	-	-	7.1	-
	24/1	-	-	-	-	7.2	5.6	-	-	-	-	6.4	-
	7/2	-	-	-	-	6.3	6.1	-	-	-	-	6.2	-
	21/2	-	-	-	-	5.4	4.9	-	-	-	-	5.2	-
	6/3	-	-	-	-	4.8	3.7	-	-	-	-	4.3	-

D = Died Cl. = Clotted Sample

APPENDIX 5 - Table 5

Mean Corpuscular Volumes (cu./μ) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	34.3	31.4	29.7	28.1	31.6	38.1	36.8	36.4	32.4	31.7	33.1	1.03
	23/11	42.4	33.2	27.3	33.7	36.6	34.1	37.3	35.0	32.5	33.3	34.5	1.22
Treated →	6/12	43.2	32.6	28.2	33.3	36.5	35.7	37.0	37.5	38.0	37.3	35.0	1.25
(8/12)	13/12	D	30.0	31.4	32.5	34.3	28.8	31.9	33.4	35.6	D	32.2	0.79
	20/12	-	29.2	25.8	26.7	32.5	26.8	28.7	27.2	33.0	-	28.7	0.96
	27/12	-	28.8	29.6	30.0	32.3	25.9	29.2	29.4	31.2	-	29.6	0.66
	3/1	-	28.9	27.6	27.5	30.3	30.6	30.0	30.2	30.7	-	29.5	0.46
	10/1	-	29.2	26.0	27.3	30.0	26.1	27.9	27.1	28.8	-	27.8	0.51
	24/1	-	33.5	29.4	29.3	35.0	30.5	29.8	31.4	30.6	-	31.2	0.73
	7/2	-	28.3	30.0	29.3	38.1	31.3	28.5	30.0	29.3	-	30.6	1.12
	21/2	-	28.3	27.5	27.2	35.2	27.8	29.9	29.7	30.2	-	29.5	0.91
	6/3	-	32.4	33.9	30.0	35.6	32.3	31.2	32.0	34.1	-	32.7	0.63
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	43.3	29.9	34.9	30.0	29.4	28.4	33.4	38.7	30.3	39.2	33.8	1.62
	16/11	Cl.	33.0	35.2	28.7	30.3	27.1	29.9	37.6	28.6	35.7	31.8	1.25
	29/11	32.7	26.3	33.7	31.7	31.1	28.3	30.9	35.9	31.8	40.0	32.2	1.20
	6/12	41.3	38.7	35.4	26.6	29.7	26.2	31.7	39.0	36.7	42.7	34.8	1.88
	13/12	D	36.7	33.5	30.3	31.4	31.6	33.3	45.3	34.0	48.4	36.1	2.15
	20/12	-	D	35.7	30.0	32.9	28.2	D	44.3	32.9	D	34.0	2.32
	27/12	-	-	38.0	34.0	30.4	33.3	-	49.2	41.0	-	37.7	2.77
	3/1	-	-	37.9	33.2	32.4	28.4	-	40.3	D	-	34.4	2.10
	10/1	-	-	D	D	32.8	33.7	-	D	-	-	33.3	-
	24/1	-	-	-	-	35.7	37.1	-	-	-	-	36.4	-
	7/2	-	-	-	-	34.7	39.0	-	-	-	-	36.9	-
	21/2	-	-	-	-	33.1	38.3	-	-	-	-	35.7	-
	6/3	-	-	-	-	37.5	39.9	-	-	-	-	38.7	-

D = Died Cl. = Clotted Sample

APPENDIX 5 - Table 6

Mean Corpuscular Haemoglobin Concentrations (per cent) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	29.7	29.6	28.9	29.8	32.0	29.6	31.9	27.5	33.3	36.8	30.9	0.85
	23/11	26.5	28.4	31.0	28.6	31.3	25.9	26.2	25.9	28.4	29.3	28.2	0.64
	6/12	22.5	26.3	30.0	24.2	27.8	20.0	22.5	20.8	24.2	23.7	24.2	0.98
Treated → (8/12)	13/12	D	25.3	24.5	24.4	25.9	24.0	24.5	24.0	22.2	D	24.4	0.38
	20/12	-	24.8	28.5	30.4	27.3	26.0	30.0	25.0	24.4	-	27.1	0.84
	27/12	-	35.2	27.2	27.1	28.1	28.0	27.5	28.2	27.0	-	28.5	0.97
	3/1	-	28.0	29.0	29.2	29.6	26.3	28.2	27.1	28.3	-	28.2	0.39
	10/1	-	27.7	27.4	28.9	30.7	28.5	27.8	28.3	28.0	-	28.4	0.37
	24/1	-	26.0	29.0	30.0	27.3	27.8	28.8	28.2	28.8	-	28.2	0.43
	7/2	-	28.7	30.0	30.6	28.5	28.2	31.0	26.8	30.5	-	29.3	0.52
	21/2	-	30.0	30.4	29.5	30.0	26.8	28.5	25.9	28.1	-	28.7	0.58
	6/3	-	27.0	28.6	28.5	30.0	26.4	26.7	27.7	29.2	-	27.9	0.48
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97	Mean	S.E.
	9/11	24.6	31.5	30.8	30.4	33.0	30.0	25.2	28.8	27.1	27.2	29.0	0.90
	16/11	Cl.	24.4	28.6	28.8	30.5	29.5	24.6	25.7	29.5	25.8	28.4	1.03
	29/11	19.1	26.9	27.2	27.4	30.7	29.1	24.3	26.6	34.0	29.3	26.3	1.00
	6/12	20.0	22.5	26.1	25.5	28.3	28.8	20.6	25.6	27.3	24.3	24.9	0.94
	13/12	D	22.0	24.0	25.2	28.3	25.6	17.0	21.3	24.1	22.7	23.4	1.04
	20/12	-	D	25.6	27.4	27.7	26.5	D	22.1	25.2	D	25.8	0.83
	27/12	-	-	23.6	25.6	29.6	26.1	-	21.6	19.0	-	24.2	1.52
	3/1	-	-	19.0	20.8	28.3	28.6	-	16.0	D	-	22.5	2.53
	10/1	-	-	D	D	27.8	28.1	-	D	-	-	28.0	-
	24/1	-	-	-	-	29.4	26.7	-	-	-	-	28.1	-
	7/2	-	-	-	-	27.3	27.7	-	-	-	-	27.5	-
	21/2	-	-	-	-	28.4	25.8	-	-	-	-	27.1	-
	6/3	-	-	-	-	24.0	21.8	-	-	-	-	22.9	-

D = Died Cl. = Clotted Sample

APPENDIX 5 - Table 7

Reticulocyte Counts (per cent) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P34	P35	P41	P47	P51	P52	P56	P61	P72	P75		
	9/11	0	0	0	0	0	0	0	2	0	1	0.3	0.21
	23/11	0	0	0	1	1	2	2	1	1	2	1.0	0.26
	6/12	4	0	3	5	0	16	8	8	4	8	5.6	1.49
Treated → (6/12)	13/12	D	0	2	1	0	4	8	0	4	D	2.4	1.00
	20/12	-	0	0	0	0	0	0	0	0	-	0	-
	27/12	-	0	0	0	0	0	0	0	0	-	0	-
	3/1	-	0	0	0	0	0	0	0	0	-	0	-
	10/1	-	0	0	0	0	0	0	0	0	-	0	-
	24/1	-	0	0	0	0	0	0	0	0	-	0	-
	7/2	-	0	0	0	0	0	0	0	0	-	0	-
	21/2	-	0	0	0	0	0	0	0	0	-	0	-
	6/3	-	0	0	0	0	0	5	0	0	-	0.4	-

	Date	R82	R86	R87	R88	R90	R91	R92	R93	R95	R97	Mean	S.E.
Group B	9/11	15	4	1	1	0	1	14	7	5	8	5.6	1.71
	16/11	Cl.	8	0	0	0	0	0	8	0	2	2.0	1.15
	29/11	24	8	4	0	0	0	12	4	0	2	5.4	2.42
	6/12	16	12	6	0	0	0	10	4	0	6	5.4	1.81
	13/12	D	9	14	0	0	1	12	9	2	10	6.3	1.85
	20/12	-	D	15	5	0	8	D	14	8	D	8.3	2.29
	27/12	-	-	10	7	0	4	-	11	10	-	7.0	1.75
	3/1	-	-	6	15	0	5	-	17	D	-	8.6	3.20
	10/1	-	-	D	D	0	6	-	D	-	-	3.0	-
	24/1	-	-	-	-	4	5	-	-	-	-	4.5	-
	7/2	-	-	-	-	0	0	-	-	-	-	0	-
	21/2	-	-	-	-	4	12	-	-	-	-	8.0	-
	6/3	-	-	-	-	6	9	-	-	-	-	75.	-

D = Died Cl. = Clotted Sample

APPENDIX 5 - Table 8

Total Serum Protein Levels (gms. per 100 ml.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P34	P35	P41	P47	P51	P52	P56	P61	P72	P75		
	9/11	4.9	6.6	6.3	6.9	6.5	7.3	6.3	7.3	8.0	6.8	6.7	0.26
	23/11	4.3	5.3	6.7	5.7	7.4	5.0	6.1	5.1	6.2	7.1	5.9	0.31
	6/12	3.2	4.6	5.8	5.0	6.6	4.4	5.2	4.0	5.2	5.5	5.0	0.30
Treated → (8/12)	13/12	D	7.1	3.9	3.5	6.4	5.7	6.2	6.7	5.9	D	5.7	0.46
	20/12	-	5.5	6.5	7.0	7.6	6.7	7.2	6.8	7.1	-	6.8	0.22
	27/12	-	5.7	6.8	6.5	7.6	6.8	7.2	7.6	7.8	-	7.0	0.25
	3/1	-	5.7	6.8	6.5	6.8	7.0	7.6	7.3	7.8	-	6.9	0.24
	10/1	-	6.1	6.5	6.4	6.9	6.9	6.5	7.3	8.3	-	6.9	0.25
	24/1	-	6.9	7.8	7.1	7.4	7.0	8.1	8.2	8.8	-	7.7	0.24
	7/2	-	6.3	7.0	7.0	7.0	7.2	7.9	7.7	8.5	-	7.3	0.24
	21/2	-	6.4	7.1	7.2	7.7	7.4	8.4	8.1	9.2	-	7.7	0.31
	6/3	-	6.5	7.2	6.8	7.2	7.0	7.2	7.7	8.3	-	7.2	0.20
Group B		R82	R86	R87	R88	R90	R91	R92	R93	R95	R97		
	9/11	5.0	6.7	7.4	7.6	5.9	6.7	6.1	6.4	7.8	7.0	6.7	0.27
	16/11	4.5	6.0	5.1	7.3	6.7	6.2	6.1	5.5	7.5	5.9	6.1	0.29
	29/11	3.6	4.9	5.0	7.6	6.0	5.3	4.9	4.1	7.3	4.4	5.3	0.41
	6/12	2.8	4.1	5.0	7.1	6.1	5.1	4.0	4.3	7.2	4.7	5.0	0.84
	13/12	D	5.2	5.5	5.7	3.2	3.3	3.6	3.1	4.8	5.3	4.4	0.39
	20/12	-	D	3.8	5.8	5.9	4.9	D	3.7	5.8	D	5.0	0.42
	27/12	-	-	3.0	4.8	6.1	5.2	-	3.0	4.6	-	4.5	0.51
	3/1	-	-	3.6	4.2	6.0	4.7	-	3.1	D	-	4.3	0.50
	10/1	-	-	D	D	5.6	5.0	-	D	-	-	5.3	-
	24/1	-	-	-	-	5.9	5.2	-	-	-	-	5.6	-
	7/2	-	-	-	-	5.1	4.6	-	-	-	-	4.9	-
	21/2	-	-	-	-	5.5	4.8	-	-	-	-	5.2	-
	6/3	-	-	-	-	5.4	4.0	-	-	-	-	4.7	-

D = Died

APPENDIX 5 - Table 9

Serum Albumin Levels (gms. per 100 ml.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	1.47	1.57	1.67	2.51	2.00	2.32	1.48	1.65	1.89	1.33	1.79	0.12
	23/11	1.15	1.10	1.61	2.03	1.30	1.60	1.72	1.58	1.12	1.52	1.47	0.10
	6/12	0.66	1.06	1.47	1.38	1.96	1.23	1.33	0.93	0.91	1.04	1.20	0.12
Treated → (8/12)	13/12	D	2.15	1.00	1.01	1.46	1.49	1.65	1.70	1.06	D	1.44	0.14
	20/12	-	1.52	2.05	2.18	2.13	2.42	2.28	1.74	1.58	-	1.99	0.12
	27/12	-	1.92	2.51	2.65	2.86	2.76	2.67	2.85	1.79	-	2.50	0.15
	3/1	-	1.86	2.06	2.42	2.41	2.71	2.53	2.21	1.88	-	2.26	0.11
	10/1	-	2.08	2.24	3.52	2.98	2.39	2.30	2.23	2.33	-	2.51	0.17
	24/1	-	1.90	2.05	2.00	1.98	1.79	1.77	2.09	2.05	-	1.95	0.04
	7/2	-	2.14	1.66	1.91	1.49	1.48	1.60	2.05	2.02	-	1.79	0.09
	21/2	-	1.87	1.96	2.03	2.13	1.82	1.95	2.14	2.46	-	2.05	0.07
	6/3	-	1.96	2.04	2.19	2.00	1.83	1.66	2.00	2.13	-	1.98	0.06
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	1.17	1.64	1.96	1.70	2.00	2.15	1.79	1.95	1.84	1.86	1.81	0.08
	16/11	1.28	1.47	1.45	1.64	2.10	2.09	1.80	2.13	1.80	1.42	1.72	0.10
	29/11	1.04	N.S.	1.37	1.73	2.20	1.83	1.23	1.08	1.52	1.02	1.45	0.14
	6/12	0.79	0.86	1.54	1.47	1.92	1.84	0.88	0.93	1.20	0.96	1.24	0.13
	13/12	D	0.87	1.29	1.37	0.95	1.08	0.55	0.54	0.94	1.19	0.98	0.10
	20/12	-	D	1.18	1.26	2.29	1.57	D	1.01	1.36	D	1.45	0.19
	27/12	-	-	0.91	1.46	2.68	2.14	-	0.64	0.70	-	1.42	0.34
	3/1	-	-	0.50	0.86	1.98	1.62	-	0.57	D	-	1.11	0.30
	10/1	-	-	D	D	1.88	1.56	-	D	-	-	1.72	-
	24/1	-	-	-	-	1.48	1.08	-	-	-	-	1.28	-
	7/2	-	-	-	-	1.41	0.96	-	-	-	-	1.19	-
	21/2	-	-	-	-	1.40	1.14	-	-	-	-	1.27	-
	6/3	-	-	-	-	1.34	0.97	-	-	-	-	1.16	-

D = Died

APPENDIX 5 - Table 10

Serum Alpha/Beta Globulin Levels (gms. per 100 ml.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	1.30	1.83	1.48	1.88	1.52	1.91	1.68	1.80	1.83	1.37	1.66	0.07
	23/11	0.56	0.88	0.79	0.96	0.91	1.05	1.07	0.87	0.82	1.09	0.90	0.05
	6/12	0.98	1.17	1.16	1.24	1.07	1.09	1.02	0.95	1.58	0.93	1.12	0.06
Treated → (8/12)	13/12	D	1.72	1.05	0.79	1.52	1.80	1.53	1.63	1.37	D	1.39	0.54
	20/12	-	1.59	1.40	1.78	1.57	1.68	1.66	1.53	1.52	-	1.59	0.04
	27/12	-	1.41	1.55	1.67	1.76	1.76	1.68	1.41	1.16	-	1.55	0.07
	3/1	-	1.48	1.54	1.46	1.35	1.41	1.33	1.44	1.23	-	1.41	0.04
	10/1	-	1.33	1.21	1.28	1.06	1.16	1.07	1.06	1.12	-	1.16	0.04
	24/1	-	1.78	1.80	1.84	1.82	1.90	2.04	1.69	1.71	-	1.82	0.04
	7/2	-	1.71	1.56	1.58	1.77	1.82	1.76	1.51	1.66	-	1.67	0.04
	21/2	-	1.78	1.50	1.69	1.72	1.78	1.81	1.71	1.75	-	1.72	0.03
	6/3	-	1.68	1.58	1.63	1.76	1.79	1.86	1.82	1.88	-	1.75	0.04
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	1.37	1.25	1.99	1.95	1.39	1.44	1.87	1.69	2.01	1.10	1.61	0.11
	16/11	1.32	1.29	1.08	1.96	1.70	1.40	1.72	0.87	1.61	1.17	1.41	0.11
	29/11	1.10	N.S.	1.14	2.01	1.66	1.32	1.64	1.11	1.38	1.22	1.40	0.10
	6/12	0.78	0.88	0.96	1.44	1.52	1.37	1.16	0.97	1.47	1.08	1.16	0.09
	13/12	D	1.45	1.33	1.62	0.64	0.84	1.11	0.79	1.03	1.35	1.13	0.11
	20/12	-	D	0.93	1.47	1.31	1.09	D	1.17	1.35	D	1.20	0.08
	27/12	-	-	0.79	1.34	1.48	1.33	-	0.91	1.37	-	1.20	0.11
	3/1	-	-	1.11	0.19	1.25	1.16	-	0.69	D	-	0.89	0.20
	10/1	-	-	D	D	1.11	1.18	-	D	-	-	1.15	-
	24/1	-	-	-	-	1.48	1.39	-	-	-	-	1.44	-
	7/2	-	-	-	-	1.16	1.19	-	-	-	-	1.18	-
	21/2	-	-	-	-	1.39	1.27	-	-	-	-	1.33	-
	6/3	-	-	-	-	1.46	1.13	-	-	-	-	1.30	-

D = Died

APPENDIX 5 - Table 11

Serum Gamma-Globulin Levels (gms. per 100 ml.) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	2.12	3.20	3.14	2.50	2.97	3.07	3.14	3.85	4.27	4.10	3.24	0.21
	23/11	2.58	3.31	4.30	2.71	5.20	2.35	3.31	2.65	4.70	4.48	3.56	0.32
	6/12	1.55	2.37	3.17	2.40	3.56	2.07	3.12	2.11	2.71	3.53	2.66	0.21
Treated → (8/12)	13/12	D	3.23	1.85	1.70	3.41	2.42	3.02	3.37	3.47	D	2.81	0.25
	20/12	-	2.39	3.04	3.03	3.90	2.60	3.26	3.53	3.99	-	3.22	0.20
	27/12	-	2.37	2.74	2.18	2.98	2.26	2.85	3.34	4.84	-	2.95	0.30
	3/1	-	2.48	3.19	2.62	3.04	2.88	3.74	3.64	4.69	-	3.29	0.25
	10/1	-	2.69	2.95	2.96	2.85	3.35	3.11	4.01	4.84	-	3.35	0.26
	24/1	-	3.22	3.95	3.26	3.60	3.31	4.29	4.42	5.04	-	3.89	0.23
	7/2	-	2.45	3.78	3.51	3.74	3.90	4.52	4.14	4.82	-	3.86	0.25
	21/2	-	2.75	3.64	3.48	3.84	3.80	4.53	4.26	4.99	-	3.91	0.24
	6/3	-	2.86	3.59	2.99	3.44	3.38	3.68	3.88	4.29	-	3.51	0.16
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	2.46	3.81	3.45	3.95	2.51	3.11	2.44	2.76	3.95	4.04	3.25	0.21
	16/11	1.90	3.25	2.55	3.70	2.90	2.71	2.57	2.48	4.08	3.31	2.95	0.20
	29/11	1.46	N.S.	2.49	3.86	2.13	2.15	2.03	1.91	4.40	2.16	2.26	0.38
	6/12	1.24	2.36	2.70	4.19	2.66	1.88	1.95	2.40	4.53	2.66	2.66	0.32
	13/12	D	2.89	2.88	2.81	1.61	1.37	1.94	1.76	2.83	2.76	2.32	0.21
	20/12	-	D	1.70	3.06	2.29	2.24	D	1.62	3.09	D	2.33	0.26
	27/12	-	-	1.30	2.00	1.99	1.72	-	1.45	2.53	-	1.83	0.18
	3/1	-	-	1.99	3.21	2.77	1.92	-	1.01	D	-	2.18	0.38
	10/1	-	-	D	D	2.61	2.25	-	D	-	-	2.43	-
	24/1	-	-	-	-	2.93	2.73	-	-	-	-	2.83	-
	7/2	-	-	-	-	2.54	2.45	-	-	-	-	2.50	-
	21/2	-	-	-	-	2.72	2.39	-	-	-	-	2.56	-
	6/3	-	-	-	-	2.59	1.90	-	-	-	-	2.25	-

D = Died N.S. = Not Sample Available

APPENDIX 5 - Table 12

Albumin:Globulin Ratios of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P 34	P 35	P 41	P 47	P 51	P 52	P 56	P 61	P 72	P 75		
	9/11	0.43	0.31	0.36	0.57	0.45	0.46	0.31	0.29	0.31	0.24	0.37	0.03
	23/11	0.37	0.26	0.32	0.55	0.20	0.47	0.39	0.45	0.20	0.27	0.35	0.04
	6/12	0.26	0.30	0.34	0.37	0.42	0.39	0.32	0.31	0.21	0.23	0.32	0.02
Treated → (8/12)	13/12	D	0.43	0.35	0.41	0.30	0.35	0.36	0.34	0.22	D	0.35	0.02
	20/12	-	0.38	0.46	0.45	0.39	0.57	0.46	0.34	0.29	-	0.42	0.03
	27/12	-	0.49	0.59	0.69	0.60	0.68	0.59	0.60	0.30	-	0.57	0.04
	3/1	-	0.47	0.44	0.59	0.55	0.63	0.50	0.44	0.32	-	0.49	0.04
	10/1	-	0.52	0.54	0.83	0.76	0.53	0.55	0.44	0.39	-	0.57	0.05
	24/1	-	0.38	0.36	0.39	0.36	0.34	0.28	0.34	0.30	-	0.34	0.01
	7/2	-	0.51	0.31	0.37	0.27	0.26	0.25	0.36	0.31	-	0.33	0.03
	21/2	-	0.41	0.38	0.39	0.38	0.33	0.31	0.36	0.37	-	0.37	0.01
	6/3	-	0.43	0.39	0.47	0.39	0.35	0.30	0.35	0.35	-	0.38	0.02
Group B		R 82	R 86	R 87	R 88	R 90	R 91	R 92	R 93	R 95	R 97		
	9/11	0.32	0.32	0.36	0.29	0.51	0.47	0.41	0.44	0.31	0.36	0.38	0.02
	16/11	0.40	0.32	0.40	0.29	0.46	0.51	0.42	0.64	0.32	0.32	0.41	0.03
	29/11	0.41	N.S.	0.27	0.29	0.58	0.53	0.34	0.36	0.26	0.30	0.37	0.04
	6/12	0.39	0.27	0.45	0.26	0.46	0.18	0.35	0.28	0.20	0.26	0.31	0.03
	13/12	D	0.26	0.31	0.31	0.42	0.49	0.18	0.21	0.24	0.29	0.30	0.03
	20/12	-	D	0.45	0.28	0.64	0.47	D	0.39	0.31	D	0.42	0.05
	27/12	-	-	0.43	0.44	0.79	0.70	-	0.27	0.18	-	0.47	0.10
	3/1	-	-	0.16	0.17	0.49	0.53	-	0.34	D	-	0.34	0.08
	10/1	-	-	D	D	0.50	0.45	-	D	-	-	0.48	-
	24/1	-	-	-	-	0.34	0.26	-	-	-	-	0.30	-
	7/2	-	-	-	-	0.38	0.26	-	-	-	-	0.32	-
	21/2	-	-	-	-	0.34	0.31	-	-	-	-	0.33	-
	6/3	-	-	-	-	0.36	0.21	-	-	-	-	0.29	-

D = Died N.S. = No Sample Available

APPENDIX 5 - Table 13

Faecal Egg Counts (fluke eggs per gram) of Lambs with Naturally Acquired Fascioliasis Following Treatment with Nitroxynil (Group A) and of Infected but Untreated Control Lambs (Group B)

	Date	Lamb Number										Mean	S.E.
Group A		P34	P35	P41	P47	P51	P52	P56	P61	P72	P75		
	9/11	700	300	200	350	450	1000	700	300	-ve	400	440	91.2
	23/11	950	450	400	1100	50	750	500	1450	150	-ve	580	15.1
Treated ⟶	6/12	600	850	400	500	N.S.	250	650	1000	400	300	550	83.7
(8/12)	13/12	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	D	-ve	-
	20/12	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-ve	-
	27/12	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-ve	-
	3/1	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-ve	-
	10/1	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-ve	-
	24/1	-	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-ve	-
	7/2	-	50	-ve	-ve	N.S.	-ve	-ve	-ve	-ve	-	7	7
	21/2	-	50	-ve	-ve	-ve	50	250	100	-ve	-	56	30.5
	6/3	-	100	50	50	-ve	50	150	150	50	-	75	18.9
Group B		R82	R86	R87	R88	R90	R91	R92	R93	R95	R97		
	9/11	1200	600	450	250	250	100	450	800	50	350	450	
	16/11	800	700	450	500	650	300	400	400	100	450	475	
	29/11	450	350	450	600	300	200	600	700	250	500	440	
	6/12	450	500	700	N.S.	250	100	150	800	150	550	400	80.8
	13/12	D	600	650	650	450	200	250	500	500	900	522	71.3
	20/12	-	D	650	1500	600	300	D	750	100	D	650	196.7
	27/12	-	-	300	600	750	100	-	200	1000	-	492	142.9
	3/1	-	-	50	1800	200	150	-	5900	D	-	1620	1117.9
	10/1	-	-	D	D	600	450	-	D	-	-	525	-
	24/1	-	-	-	-	500	900	-	-	-	-	700	-
	7/2	-	-	-	-	1590	850	-	-	-	-	1220	-
	21/2	-	-	-	-	400	450	-	-	-	-	425	-
	6/3	-	-	-	-	500	350	-	-	-	-	425	-

D = Died N.S. = No Sample Available