COMPARATIVE STUDY OF SUBCLINICAL FASCIOLIASIS

IN

SHEEP AND GOATS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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ABSTRACT

The literature on the identification of Fasciola spp. and their intermediate hosts, the general life cycle of Fasciola and aspects of the epidemiology, clinical signs, diagnosis and treatment of infections is reviewed.

Two experiments were carried out. The first involved 18 weeks observations on 5 adult male goats each infected with 150 metacercariae of F.hepatica and 5 uninfected controls. The second involved groups of 10 sheep and 10 goats each infected with 200 metacercariae with 5 uninfected controls of each species. In both experiments, faecal, haematological, biochemical and pathological examinations were conducted. The animals were also weighed regularly.

In the first experiment, although only 15-35 flukes were established, measurable and, in many cases, statistically significant changes in a variety of parameters were observed. A depression in packed cell volumes relative to controls of approximately 20% occurred. Though haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration levels remained within the normal ranges, erythrocyte levels in the infected group were significantly lower than in the controls and there was a tendency for the anaemia to become macrocytic. This suggests that goats may be particularly susceptible to the effects of blood loss associated with Fasciola infections though further work is needed to confirm this.

A marked peripheral eosinophilia and elevation in fibrinogen levels were observed in infected animals. Albumin levels decreased, globulin levels increased and the A/G ratio decreased significantly relative to the control group but all levels remained within the normal ranges.

In infected animals, gamma glutamyl transferase (GGT) and glutamyl dehydrogenase (GD) levels rose to beyond the limits of the normal ranges although aspartate aminotransferase (AST) levels, which were also significantly elevated, did not. The results indicate that serum GD and GGT are particularly sensitive indicators of damage to the liver parenchyma and bile ducts caused by F.hepatica in goats and that GD is more sensitive than AST. Serum bile acids were estimated but no significant change was detected.

The ratio between faecal egg counts and the numbers of adult flukes present at necropsy was consistently lower than described for sheep with a mean of approximately 13epg/fluke (range 9-23) at the final sampling and 18epg/fluke (range 11-29) in the previous week when the egg counts were highest. This is potentially of considerable diagnostic importance and needs further investigation.

In the second experiment, the number of flukes established was extremely low in both species (mean 0.85% & 2.95% in the sheep and goats respectively) although more goats than sheep became infected. Pre-existing liver pathology in the sheep was a further complication. Consequently, little information of value was generated by the infection of goats and no data that could be used for comparative purposes were obtained from the sheep infection.

However, combination of the data from the 13 infected goats from both experiments yielded some useful information in relation to serum enzyme levels. Correlations between the numbers of flukes recovered at necropsy and peak levels of serum enzymes and various haematological parameters in individual animals were examined though only those relating to enzyme levels were statistically significant. The correlation coefficients between peak enzyme levels and fluke numbers indicated that the relationship was strongest with GGT and weakest with AST. However, regression analysis showed that there was no predictive value in the relationship with any of the enzymes because of extremely wide confidence intervals for predicted fluke numbers.

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CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Fascioliasis is a term given to the disease caused by any member of the genus Fasciola (Trematoda:Fasciolidae). The major species involved in causing this disease in ruminants are Fasciola hepatica and F. gigantica. Other species of minor importance have been described by many researchers, but the damage they cause to domesticated animals as a whole is of little significance. It has, therefore, been decided to exclude them from this text, mainly because these species not only have similar life-cycles to that of Fasciola hepatica, but also their separation as distinct species is often doubtful and a matter of some dispute among researchers (Pantelouris, 1965). In general, F.gigantica is confined to the tropics and subtropics and F.hepatica to temperate zones. However, the latter also occurs in cooler regions of the tropics, eg. at higher altitudes.

The main morphological difference between the major two species is size; this and their specificity and preferences with respect to intermediate hosts for their parthenitae and their differing geographic locations are the major features differentiating them in various parts of the world.

The adult flukes may reach a size of 3.0 cm x 1.3cm (F.hepatica) and 7.5 cm x 1.2 cm (F.gigantica) in the mammalian host liver where they reside. They are leaf-shaped parasites with an anterior cone-like projection followed by "shoulders" which are prominent only in F.hepatica. Fasciola gigantica is bigger in size but more slender. Oral and ventral suckers are present and sharp spines cover the whole of the tegument.

Different biological and environmental factors play major roles in their life cycles. The only snail hosts for Fasciola species are in the genus Lymnaea (Galba). All Lymnaea species have the opening of the shell to the right when placed in the palm of the hand with apex of the shell pointing towards the fingertips. Characteristically they also have flattened triangular antennae. They are mostly relatively small snails, usually up to 1 cm in size. However, their size is variable depending on age and species. For example, mature L.truncatula are usually 4-5 mm long but some species may exceed 1 cm in length (eg. L.natalensis and L.columella) (Reinecke, 1983).

The snail L.truncatula, one of the major intermediate hosts of F. hepatica, is widely distributed through many regions of the world. It is an amphibious snail which is among the smallest in its group. It is dark-brown in colour and the shell has 4-5 whorls (Soulsby, 1965). Unlike most other lymnaeid snails, it can spend many hours out of water. It is commonly seen in poorly drained land, drainage ditches, broken drains and it can temporarily inhabit muddy gateways, and around drinking troughs etc. (Kendall, 1949). Other intermediate hosts and their geographical locations are listed in Table 1.1.

Lymnaea natalensis is one of the major intermediate hosts of F.gigantica and its distribution is over the tropical and sub-tropical areas of Africa, south of the Sahara. Other species are involved in other countries (Table 1.1). However, some of the species incriminated by workers as intermediate hosts of liver fluke may well be synonyms of L.truncatula or L.natalensis because most of them are not readily distinguishable from them on morphological or ecological grounds (Haroun et al. 1975; Peterson and Castelino, 1977; Reinecke, 1983; Urquhart et al. 1987). Lymnaea columella is considered to be an intermediate host for both F.hepatica and F.gigantica in southern Africa (Reinecke, 1983).

Mixed infections of domesticated animals with F.hepatica and F.gigantica are possible in the boundary regions of highland areas of some tropical and sub-tropical countries, eg. in Pakistan (Kendall, 1954), Ethiopia (Scott and Goll, 1977) and South Africa (Reinecke, 1983). However, with the exception of a few areas such as Papua-New Guinea and tropical Australia, fascioliasis in the tropics is mostly caused by F.gigantica (Fabiyi, 1986). Apart from their morphological and size differences, F.hepatica and F.gigantica are generally very similar parasites. Since they are both liver parasites, they tend to cause somewhat similar diseases in ruminants and other domesticated animals, but are by far most important in the former.

Table 1.1 Main snail hosts of Fasciola and their geographical distribution based on Soulsby (1982) and other references.

F.hepatica :		
	L.truncatula	Europe; Central Highlands of Ethiopia, Southern Africa; many other temperate areas of the world.
	L.tomentosa	Australia; New Zealand.
	L.columella	N.America; New.Zealand; Australia; S.Africa
	L.bulimoides	Caribbean; Southern USA.
	L.humilis	S.America.
	L.viator	S.America.
F.gigantica :		
	L.natalensis	Africa
	L.auricularia	Southern Europe; Southern USA; Middle East; N.Africa; Pacific Islands.
	L.acuminata	India; Pakistan
	L.rufescens	India; Pakistan
	L.rubiginosa	Malaysia
	L.columella	S.Africa

Liver fluke infection is of increasing importance in many parts of the world. Clinical studies on sheep and cattle with heavy infection have been described by many researchers and they have shown that there is a good correlation between the level of infection and bodyweight, erythrocyte number, packed cell volume, haemoglobin, plasma protein values, faecal egg counts and the extent of the liver damage.

Goats are susceptible to all species of Fasciola. However, the clinico-pathological picture of Fasciola infection in goats and the effect on the host have not been extensively studied and specific data on the disease in goats are scarce (Reddington et al., 1986).

A comparative study of sheep and goats under the same experimental conditions would provide direct comparative data and shed light on some specific aspects of the pathogenesis of sub-clinical infection of goats with F.hepatica. In this study, a preliminary experiment (Chapter 3) with goats examined biochemical and haematological changes of diagnostic and pathological significence at very low levels of infection. A further experiment was then carried out (Chapter 4) to determine whether the reaction to infection differs from that in similarly infected sheep.

The overall objective of this study was to develop better understanding of the pathological effects of fascioliasis in goats and to improve the diagnostic assessment of sub-clinical field cases.

1.2 Liver Fluke In New zealand

Fascioliasis in New Zealand was first observed by Park in 1896 around the Hawke's Bay region in the North Island (Brunsdon, 1966; Pullan and Whitten, 1972a). It gradually spread over a wider area in the east of the North Island but remained very restricted in distribution. Some foci of infection were also recorded in the South Island. Shortly after 1960 it was reported by several workers that fascioliasis had been rapidly spreading into larger areas of North Island and some northern parts of the South Island (Pullan & Whitten, 1972a).

In a discussion of a survey carried out in 1969, Pullan & McNab (1972a) indicated that, among other causes, the spread of liver fluke in the country had occurred as a result of constant movements of infected stock from the traditional sheep breeding area of Hawke's Bay, where Fasciola had been endemic for several years. Uncontrolled movement of animals from infected areas allowed continuous spread of the disease to other parts of the country (Faull, 1987). The other major factor in the spread was the colonisation of freshwater habitats by the exotic snail host, L.columella (see below) (Pullan & Mansfield, 1972). Fasciola hepatica is the only species of liver fluke found in New Zealand (Harris & Charleston, 1980). There are three snail hosts in New Zealand, namely : L.tomentosa, L.columella and L.truncatula of which the first two are more important in the spread of the disease.

1. L.tomentosa is an Australasian species which is indigenous to New Zealand. It occurs throughout the country (Pullan & Whitten, 1972a) and until comparatively recently was the major host of F.hepatica in both islands.

2. L.columella was first identified in New Zealand in 1969 (Pullan, 1969). A survey reported in 1972, indicated that it was already widespread in the North Island and present in the north of the South Island and near Timaru (Pullan et al., 1972). Since then it has been reported from several other locations in the South Island and is widespread along the West Coast (Charleston pers. comm.). It is found in seepages, swamps, farm dams, the margins of streams and drainage ditches. It has been reported as being three times as numerous as L.tomentosa in the west of the North Island (Faull, 1987) and appears to be replacing L.tomentosa in its habitats (Charleston pers. comm.).

3. L.truncatula is not important in the transmission of fascioliasis in New Zealand. It was apparently introduced in the 19th Century but is confined to a few small areas in the South Island (Pullan et al., 1972).

The economic significance of fascioliasis in New Zealand is indirectly indicated by liver condemnations. In a slaughterhouse survey carried out in 1972, it was estimated that 6% cattle livers and 3% sheep livers were affected by liver fluke infection (Pullan and McNab, 1972b). The survey indicated that the prevalence of disease and the overall economic loss from liver condemnation were much greater in the North Island than in the South Island.

In a similar survey conducted by Charleston et al. (1990) on cattle and sheep livers during the years 1984-1985, an overall prevalence in sheep of 7.5% and 1.1% respectively was recorded in North and South Islands and 4.4% nationally, with regional prevalences ranging up to 29%. The prevalence of infection in cattle was not recorded but infected animals were traced to every county in the North Island. In the South Island, most infected cattle originated from the north and west where a prevalence of over 30% had been recorded independently.

These two surveys clearly indicate that there is a substantial prevalence and widespread distribution of Fasciola in the North Island and parts of the South Island and that there has been a considerable increase in overall prevalence of liver fluke infection in the last 20-30 years.

Control of fascioliasis in New Zealand is mainly based on anthelmintic administration to animals both as therapeutic and prophylactic measures.

1.3 Life Cycle

The eggs of F.hepatica and F.gigantica measure on average about 90 x 150μm and 104 x 197μm respectively. They are ovoid or ellipsoid with a single operculum at one end. Their colour is dark yellow-brown and derives from the bile. The eggs of F.hepatica are passed in the faeces of the host animal and under suitable conditions of moisture and temperature above 10°C, a miracidium develops and hatches in water. At 26°C about 10-12 days is needed for hatching (Rowan, 1956; Soulsby, 1982). Under field conditions in Australia, the incubation period is 21 days in summer and may be up to 90 days in winter (Boray, 1963). F.gigantica eggs need 17 days to hatch at 26°C (Soulsby, 1982). However, the minimum temperature for hatching may be higher than for F.hepatica.

The miracidium is broad anteriorly with a small pointed protrusion commonly known as a rostellum. It also has two rounded dark spots situated at the anterior end on each side of the rostellum which are receptive to light. These spots resemble eyes, hence they are commonly referred to as "eye-spots". The tegument is covered with cilia.

The miracidium, liberated in water, actively swims by the action of the cilia in search of a suitable snail host. It is actually attracted to the specific snail by means of chemotaxis (Taylor, 1964). When the miracidium finds its suitable snail host (Lymnaea spp.), it quickly attaches to it and penetrates into it in a few minutes by producing enzymes which act on the external surface of the snail. The digestive enzymes are produced by penetration glands of the miracidium (Dawes, 1960). At this stage the miracidium penetrates into the snail leaving its ciliated tegument behind at the site of penetration. This new larval stage which is now in the tissue of the snail is known as the sporocyst.

The sporocyst is sac-like. After approximately one week, it releases 5-8 rediae which actively move to the hepatopancreas in the snail. The rediae may occasionally produce daughter-rediae but not regularly (Dawes, 1960).

The next stage produced in the snail are cercariae which develop inside the rediae and leave through a tiny opening commonly known as the "birth-pore". Under favourable conditions, each redia is capable of producing up to 40 cercariae and a total of 480 cercariae can be produced from only one miracidium (Kendall, 1965). The total development in the snail takes 4.5-7 weeks or more from the time of infection (Soulsby, 1982).

The cercaria is the last stage in the snail. When favourable conditions of temperature and moisture prevail, the cercariae leave the snail and attach to vegetation or any solid object available in the surroundings. The cercaria quickly encysts itself by secreting a covering fluid from its cystogenous glands to become an encysted metacercaria and it remains in this state until ingested by a suitable host animal while grazing (Kendall, 1949; Soulsby, 1982). The process of encystment is completed in about 15-60 min and a further 2-3 days is required for the metacercariae to be infective (Taylor, 1962).

The life-cycle continues when the metacercariae reach the duodenum of the definitive host and excystation takes place by the action of chemical stimuli of the host's intestinal content. Bile salts and a low redox potential are most important for excystation (Wikerhauser, 1960; Hanna & Jura, 1976). When the metacercariae are released in the intestine, they penetrate through the intestinal wall into the peritoneal cavity. Later, metacercariae migrate to the liver which after 2-3 days they enter through the capsule. After migrating in the liver parenchyma for 5-6 weeks, they enter the bile ducts to become adult liver fluke. The adult flukes contain both female and male reproductive organs, hence as hermaphrodites they produce eggs individually and release them into the faeces through the bile ducts of the host to start a new cycle. The minimum prepatent period for F.hepatica infection is 8 weeks. The complete life cycle of F.hepatica may take 15 weeks, but under normal field conditions development may take longer (Dawes, 1960; Pantelouris, 1965; Soulsby, 1982).

The epidemiology of fascioliasis is inseparably associated with the life cycle of the intermediate host. The ecology and habitat of the snails play a vital role in the normal development of the liver fluke. There are three important factors influencing the production of sufficient metacercariae to cause fascioliasis; availability of suitable snail habitats, temperature and moisture. At temperatures below $10^{o}C$, F.hepatica eggs do not develop (Rowan, 1956; Ollerenshaw, 1959). The eggs do not develop or hatch when they are still covered by faeces but may remain viable in them until favourable conditions prevail. At $26^{o}C$, eggs hatch in about 10-12 days if the eggs are in water (Rowan, 1956). They can also remain viable as developed or undeveloped eggs for several months at low temperatures and an accumulation of unhatched eggs may survive over the winter in temperate areas leading to mass hatching and liberation of miracidia in spring (Ollerenshaw, 1959).

The influence of temperature and moisture on the parthenitae in the snail has been studied by several workers who concluded that development of the larval stages in the snail host only occurs when the temperature is above $10^{o}C$ (Kendall and McCullough, 1951; Boray et al., 1969; Soulsby, 1982). Snails may continue to be active below 10^{o}C though the development of parthenitae is affected and the production of cercariae in the snail is markedly reduced. At very low temperatures, snails will cease activity and may enter a period of dormancy, sometimes referred to as hibernation. There can be high mortality of infected snails when the temperature increases above 20^{o}C (Taylor, 1949; Boray, 1963; Soulsby, 1982). However, snails are inclined to undergo aestivation, especially where the drying out of the snail habitat occurs and moisture becomes less available. Aestivation is a way of surviving hot, dry conditions.

The state of nutrition of the snail is one of the important factors for its development as well as the development of the parthenitae in it (Kendall, 1949; Ollerenshaw and Rowlands, 1959). The number of larval stages produced depends on the nutritional state of the snail. Given equal infections with miracidia, Kendall (1949) showed that larger snails may harbour about 10 times as many rediae as small snails. Similarly, the rediae inside larger and well-fed snails are larger and capable of producing many more cercariae. A single large snail can produce as many cercariae as 20 small snails (Ollerenshaw, 1959).

The density of snail populations is also an important epidemiological factor. Under natural conditions, a large number of snails in an area may hinder individual development and retard the growth of the snails because of scarcity of food in the crowded area. Thus the population density is liable to influence the size of individual snails and this is further reflected in the production of cercariae. More cercariae can be expected from fewer snails which are well nourished and more mature (Kendall, 1949). Boray (1963) observed that in two populations where snails measured 2-4mm in length and 8-11mm in another, the mean cercarial production per snail was 83 and 1201 respectively.

Studies made by Boray (1963) showed that 100% of metacercariae in water survived for 6 months and a few survived up to 10 months at 12-14^{o}C. But when stored at 2-5^{o}C, 10% survived for one year while only a few survived for 6 weeks at 25^{o}C. At average temperatures of just above freezing point, metacercariae may live as long as 11 months and at -2^{o}C remained viable for 8 weeks (Soulsby, 1965). Under pasture conditions, the metacercariae can survive a cold winter with very low temperatures. However, sufficient moisture and humidity is essential for the survival of metacercariae (Boray & Enigk, 1964)

Metacercariae may survive over one year on pasture but survival of numbers sufficient to cause a dangerous level of infection is always limited (Soulsby, 1965). They may be destroyed by direct sunlight within only a few days in tropical areas (Scott & Goll, 1977). Moist shade is the most favourable condition for maximum longevity (Taylor, 1949).

In tropical and subtropical areas where the temperatures are suitable for larval development and snail breeding all year round, cercarial release may occur throughout the year (Ford, 1959; Scott & Goll, 1977). In the central highland regions of Africa where F.hepatica is involved, it has been demonstrated that a marked seasonal increase of incidence of ovine fascioliasis occurs following the long rains. In this area of Africa, the long rains normally occur between July and September and are usually preceded by more variable short rains in February and March (Goll & Scott, 1978). The majority of parasites are acquired in October and November in these regions (Scott & Goll, 1977; Goll & Scott, 1978; Lemma et al., 1985). This may be because the cold and rainy season followed by warm and humid conditions provide optimum possibilities for the development of the parasites.

1.4 Pathogenesis and Pathology

The severity of fascioliasis depends upon the age and species of host involved, the phase of parasitic development in the host, its nutritional state and the number of metacercariae ingested. There are essentially two forms of fascioliasis, differing in pathogenesis. The first form occurs during migration of immature flukes in the liver parenchyma and is associated with liver parenchymal damage and haemorrhage. The second form of the disease occurs when the parasite has entered the bile ducts and results from the blood sucking activity of the fluke and the considerable damage inflicted on the epithelial lining of the bile ducts (Sinclair, 1966).

The main forms of fascioliasis are acute, subacute and chronic. Acute disease is usually rapidly fatal and mainly important in sheep and rarely, if ever, in cattle. The chronic disease is important in both sheep and cattle. Though it is of major economic importance, it is much less commonly fatal but can still be fatal in sheep (Sinclair, 1966; Urquhart et al., 1987). Fascioliasis in goats may resemble that of sheep, but little is known about it (Haroun, 1975).

1.4.1 Migration Phase : Acute - Subacute Disease

1.4.1.1 Clinical Consequences

In acute fascioliasis in sheep, death may occur 4-6 weeks after ingestion of a large number of metacercariae. It usually occurs 6-10 weeks after ingestion of about 500-2000 metacercariae by sheep and tends to occur earlier with larger infections (Urquhart et al., 1987). Acute disease occurs usually towards the end of summer in temperate regions or after the long rainy season in tropical areas (Scott and Goll, 1977).

In subacute disease fewer infected animals die and it is not as rapidly fatal as acute fascioliasis. Affected sheep may show clinical signs for 1-2 weeks prior to death. These include, anorexia, rapid loss of condition, pale mucous membranes, palpable enlarged liver, distension of the abdomen and abdominal pain, sub-mandibular oedema ("bottle-jaw") and ascites (Sinclair, 1966; Urquhart et al., 1987).

1.4.1.2 Tissue Damage and Histopathology

The acute form of fascioliasis is a traumatic hepatitis caused by the action of large numbers of migrating immature flukes resulting in considerable liver parenchymal damage. In severe cases, traumatic haemorrhage is an important consequence (Sinclair, 1966 and 1967; Ross et al., 1967). At necropsy, the liver is enlarged, and numerous haemorrhagic tracks of flukes can be seen on the surface. Fibrinous clots on the liver surface and peritoneum are common. The liver capsule is thickened and oedematous and in some cases may be covered by organised tags of fibrin. Blood-stained exudate is present in the peritoneal cavity. Small flukes approximately 1cm long can be squeezed from the cut surface of the liver (Sinclair, 1962).

In the subacute form, deaths result from traumatic damage to the liver and very large numbers of flukes entering the bile ducts. Similar lesions to those of acute form are seen but infiltration of the liver by leucocytes and fibrosis may be widespread. The migratory pathways are tortuous tunnels which appear on cross-section as haemorrhagic foci 2-3 mm in diameter. Older tunnels, which are in the process of healing, appear as pale yellow streaks due to the infiltration of eosinophils (Brunsdon, 1966).

1.4.1.3 Biochemical Changes

Increases in serum enzyme levels in infected animals are the major biochemical changes and they are of great significance in clinical pathology of the disease (Boyd, 1988).

1.4.1.4 Enzyme Activities Associated With Liver Damage

The enzymes listed in Table 1.2 are among the most detectable in the plasma when the liver is damaged. Only a few are specific to the liver although some are normally present in the liver in greater amounts than in other tissues. However, many are not good indicators of liver damage even though they are liver-specific, because the level of enzyme present is too small and the amount released is not high enough to be used for the detection of liver damage (Boyd, 1988).

The enzymes GD, ID and AST are sensitive indicators of parenchymal damage of the liver, while GGT is the most sensitive and reliable indicator of bile duct damage in ruminants infected with liver fluke (Boyd, 1988). Serum GD is a more sensitive indicator of liver necrosis than the serum AST during *F.hepatica* infection of sheep (Sewell, [illegible] 1967). The enzyme GD is largely associated with the parenchymal hepatic cell mitochondria. Repeated measurements of plasma GD will provide information on the progress of immature fluke through the parenchyma (Rowlands and Clampitt, 1979). Although both ID and GD are increased in the serum in sheep and calves with hepatic damage, the ID increase is transient due to its short plasma half-life and very rapid elimination and it is also unstable *in vitro*. GD is, therefore, recommended as the liver-specific cellular enzyme for diagnostic use in farm animals (Boyd, 1988). Serum GD and GGT were found to be the most sensitive of 10 enzymes examined for detecting liver parenchyma and bile duct damage in calves infected with liver fluke (Boyd, 1988).

The enzymes CPK, LAP, OCT and SAP do not provide sufficient diagnostic information on fascioliasis in ruminants to be useful. (Rowlands and Clampitt, 1979).

In an experimental infection of goats with *F.hepatica*, activities of both GD and ID were found to be higher than in cattle and sheep with significant increases in plasma levels (Hughes et al. 1973). Elevations in these enzyme levels occurred 2 and 3 weeks after infection respectively, with higher ID levels in more heavily infected goats (Hughes et al., 1974). Both enzymes are also sensitive indicators of *F.gigantica* infection in goats (Haroun et al., 1989).

Treacher et al., (1974) introduced 15-18 days old immature flukes directly into the biliary system of goats and increased plasma levels of GD and OCT were observed indicating that young flukes produce liver cell damage even when experimentally placed in the bile ducts.

Table 1.2 Major Serum Enzymes Involved In Liver Damage In Ruminants.

previous names	enzyme code	current abbreviation	liver specificity
GLDH ; GDH (Glutamyl Dehydrogenase)	1.4.1.3.	GD (Glutamic Dehydrogenase)	+
SD ; SDH (Sorbitol Dehydrogenase)	1.1.1.14.	ID (Iditol Dehydrogenase)	+
Arginase	3.5.3.1.	Arginase	+
OCT (Ornithine Carbamyl Transferase)	2.1.3.3.	OCT (Ornithine Carbamyl Transferase)	+

... cont

GGT (Gamma Glutamyl Transpeptidase)	2.3.2.2.	GGT (Gamma Glutamyl Transferase)	-
GOT ; SGOT (Glutamic oxalacetic transaminase)	2.6.1.1.	AST (Aspartate Amino Transferase)	-
TPN-SICD (Transphosphoridene Nucleotide-Isocitric Dehydrogenase)	1.1.1.42.	IC-DH (Isocitric Dehydrogenase)	-
LDH (Lactic Dehydrogenase)	1.1.1.27.	LDH (Lactic Dehydrogenase)	-
LAP (Leucine Aminopeptidase)	3.4.11.1.	LAP (Leucine Aminopeptidase)	-
AP (Alkaline Phosphatase)	-	SAP (Serum Alk.Phosphatase)	-
CPK (Creatine Phosphokinase)	-	CPK (Creatine Phosphokinase)	-

In cattle, AST plasma levels increased earlier and remained slightly elevated for a longer period after the flukes had entered the biliary system than GD and ID (Rowlands and Clampitt, 1979).

Haroun et al., (1975) examined the levels of the enzymes arginase and SAP in adult cattle naturally infected with F.gigantica and found that arginase was significantly increased while there were no significant changes in plasma levels of SAP.

1.4.1.5 Haematological And Serum Protein Changes

The extent of the haematological changes is determined mainly by the number of young migrating flukes in the liver. Progressive eosinophilia occurs during the migratory phase, accompanied later by a rise in serum globulins in sheep (Sinclair, 1962) and other ruminants (Rowlands and Clampitt, 1979). No significant changes in total protein, plasma albumin, erythrocytes, leucocytes, haemoglobin, packed cell volume or other parameters are detected in the early stage of the disease but most of these become significant at a later stage.

1.4.1.6 Black Disease

Sometimes the migratory phase is associated with "black disease" which is a concurrent bacterial infection occurring in temperate areas. It is more common in sheep than in cattle, and very little is known about it in goats. The causative organism in sheep is Cl.novyi type B (Pantelouris, 1965). Hepatic damage caused by migrating liver flukes provide the Clostridia, which are dormant in the tissue, with favourable conditions in which to multiply and cause hepatic necrosis and fatal toxaemia. If there is any sign of illness, it is brief and characterized by weak slow movement, drowsiness and rapid respiration. Affected animals are usually in good nutritional condition. In the liver, one or more necrotic lesions of about 2cm diameter are found and the pericardial sac is distended with straw coloured fluid. The skin appears black at postmortem. Sheep of all ages are susceptible but adult sheep are more often affected (Jubb and Kennedy, 1985). Affected animals are usually found dead without any previous clinical signs (Manktelow, 1984). Nowadays the use of a vaccine against this disease has made it less common (Soulsby, 1982).

1.4.2 Bile Duct Phase : Chronic Disease

Chronic fascioliasis is the most common form of the disease in both large and small ruminants occurring mainly in winter and spring in temperate zones. In sheep, clinical disease occurs 4-5 months after the ingestion of about 200-500 metacercariae (Urquhart et al. (1987). However, since sheep do not acquire resistance to infection they may accumulate flukes over long periods (Boyce et al., 1987).

1.4.2.1 Tissue Damage And Histopathology

As described by Jubb and Kennedy (1985), the pathological severity of chronic fascioliasis in sheep depends principally upon the number of adult fluke present in the biliary system. The flukes cause physical damage to and irritation of the bile duct epithelium by the action of their suckers and spines leading to cholangio-hepatitis of the large ducts. They eventually cause partial obstruction of the ducts resulting in progressive biliary retention. In severe infections, the bile ducts on the visceral surface become prominent.

According to Berry and Dargie (1978), the liver pathology in cattle is characterised by hepatic fibrosis and hyperplastic cholangitis of the large bile ducts arising from the severe erosion and necrosis of the epithelium caused by the feeding of flukes. Desquamative and ulcerative lesions in the bile ducts and the thickening of the bile ducts are more severe and prominent than in sheep. Thrombosis of the hepatic vessels associated with flukes damaging the small vessels is followed by obstruction of blood flow resulting in further necrosis and inflammatory reactions in the surrounding liver lobules (Sinclair, 1966; Sewell, 1966; Berry & Dargie, 1978). In addition, calcification of the bile ducts commonly develops. Enlargement of the gall bladder is also common and relatively more prominent in cattle than in small ruminants (Sinclair, 1967). Aberrant migration of flukes in cattle is common; as a result, parasites are often found in the lungs (Soulsby, 1982).

1.4.2.2 Biochemical Changes

In the bile duct phase of fascioliasis, the damage caused to the bile duct epithelium causes the release of the enzyme gamma glutamyl transferase (GGT) into the circulation. This enzyme increases its level in the serum mainly after the flukes have entered the bile ducts. In sheep, there is only a slight increase in serum GGT with toxic hepatocellular damage compared with a progressive, marked increase associated with cholestasis and bile duct damage (Ford, 1974).

Gamma glutamyl transferase (GGT) is also found in the kidney and in minor quantities in the intestines, but it is found in larger amounts in the liver than anywhere else in the body and it is almost entirely associated with the biliary epithelium. It is not released into the circulation until the bile duct damage occurs (Boyd, 1988). Elevations in plasma GGT are, therefore, indicative of the migration of flukes into the bile ducts, as well as continuing damage to the bile duct system (Simesen et al., 1973; Sykes et al., 1980; Boyd, 1988). However, it should be borne in mind that bile duct damage caused by other agencies (such as facial eczema) will also cause elevations in plasma GGT levels.

In an experiment with calves, Simesen et al., (1973) recorded that GGT activities increased as early as 4 weeks after infection and remained highly elevated for several months. This might be because during the migration of immature flukes in the liver parenchyma some damage to small bile ducts occurs before they actually enter them. The duodenal and peritoneal damaged cells released GGT in the blood before the immature flukes actually reached the liver which may have caused only a slight elevation.

However, contrary to the above experiment, Rowlands & Clampitt (1979) found that the serum GGT activity remained at a constant low level during the first 7 weeks of infection and then abruptly increased to a high peak, remaining high over several weeks after a single experimental infection of calves with 650 metacercariae indicating the migration of the young flukes into the bile ducts. The peak level was about 33 times the activity recorded during the first 7 weeks (Rowlands and Clampitt, 1979). However, Simesen et al., (1973) also considered that GGT is superior to AST in revealing the biliary phase of fascioliasis.

In sheep with chronic subclinical fascioliasis, GGT and GD are more sensitive indicators of disease than AST. In addition, GGT is more suitable as a diagnostic aid because of its tendency to remain at high levels as long as the biliary damage persists (Sykes et al., 1980; Boyd, 1988; Fuhui et al., 1989).

1.4.2.3 Haematological And Plasma Protein Changes

In chronic fascioliasis, the principal haematological consequences are anaemia, hypoalbuminaemia and hyperglobulinaemia. The total protein level, however, may remain within the normal range (Sinclair, 1966; Haroun et al., 1975; Urquhart et al., 1987). In sheep more than 0.5 ml blood loss per fluke occurs every day (Holmes et al., 1968). This leads to anaemia with severe changes in iron metabolism and, eventually, iron deficiency. In general the anaemia is slowly progressive. The rate at which it develops depends on the level of infection.

Sheep infected with 150 metacercariae every week for 20 weeks showed a marked and progressive drop in albumin, erythrocytes, haemoglobin, PCV and total WBC levels from the 7th week after the initial infection (Rowlands and Clampitt, 1979).

Serum protein fractionation indicates that gamma-globulin levels rise much more than the other globulins (Halliwell, 1985). At the same time, albumin levels fall progressively and to such a degree that the albumin/globulin ratio is significantly reduced during a prolonged period of infection. Albumin constantly flows through the damaged bile duct epithelium. On the other hand, the inflammatory reaction in the liver is associated with an immune response to the parasite as a result of which the gamma-globulin levels rise (Ross, 1967).

1.4.2.4 Clinical Consequences Of Chronic Infection

Chronic fascioliasis may continue for many years because the flukes can survive in sheep and possibly in goats for as long as the animals live. Infected sheep still contained flukes in the liver at slaughter 8 years after infection and some others died with live flukes in the liver 11 years after infection (Leiper, 1938; Egorov, 1954; cited by Pantelouris, 1965). It should also be noted that sheep may be infected year after year because of a lack of acquired resistance so that damage can be cumulative. Although significant breed differences were detected in faecal egg counts and fluke counts following experimental infection of 5 breeds of sheep with metacercariae of F.hepatica, there was no evidence of acquired protective immunity in any breed (Boyce, et al., 1987). However, there is some indication of protective effect of irradited metacercariae of F.gigantica against homologous challenge in sheep (Agadir et al., 1987) and cattle (Younis et al., 1986).

In cattle, the flukes tend to survive in the liver only for a few months (Pantelouris, 1965). Experimental studies have shown that cattle acquire a level of resistance to reinfection with F.hepatica (Ross, 1967; Doyle, 1971). However, it is not clear if this is immunologically based or due fibrosis of the liver and calcification of the bile ducts making conditions less suitable for the flukes (Soulsby, 1982; Urquhart et al.,1987)

Prolonged infection can have serious consequences for animal production. Hughes et al.(1974) reported that goats experimentally infected with a single infection of 200 metacercariae gradually lost weight. At 18 weeks post-infection, their group mean weight had been reduced by 3 kg and in others, each infected with 1000 metacercariae, was reduced by a mean of 9 kg during the same period.

In a similar experiment with sheep infected with a weekly dose of 150 metacercariae, Rowlands and Clampitt (1979) recorded a 30% loss in body weight at the end of the 20th week; however, they observed no important inter-group differences in body weight in calves infected with 650 and heifers with 1000 metacercariae each compared with their respective control groups during a relatively short period of time (17 weeks). This is probably because the number of innoculated metacercariae was too small and the post-infection period was not long enough to bring about significant changes. The effects on productivity are reviewed in a later section (1.6).

1.5. Diagnosis Of Fascioliasis

1.5.1 Clinical Signs

Clinical signs are important in the diagnosis of the disease but only in chronic and sub-acute cases are clinical signs regularly observed. In acute fascioliasis, death can occur with very little manifestation of clinical signs beforehand and diagnosis is made on the characteristic postmortem lesions (see section 1.4.1). The clinical signs of subacute fascioliasis are outlined in section 1.4.1.

The clinical signs of chronic fascioliasis characteristically include progressive loss of condition, severe emaciation, mild diarrhoea, anaemia, eosinophilia, hypoalbuminaemia, ascites and submandibular oedema and general loss of productivity (Soulsby, 1982 ; Reinecke, 1983; Urquhart et al., 1987). In sheep, wool production, quality and tensile strength deteriorate. It should be noted that significant effects on production can occur in the absence of obvious clinical signs.

1.5.2 Faecal Egg Counts

Faecal examination for the detection of liver fluke eggs establishes the diagnosis but the absence of eggs in the faeces does not altogether exclude the presence of the parasites as they may be at the migratory stage (Pantelouris, 1965; Boray, 1969).

Flotation techniques for the detection of eggs are not reliable because fluke eggs are large and dense and do not float readily (Happich and Boray, 1969a; Urquhart, 1987). They are also liable to rupture or become distorted due to the osmolarity of the solutions used for flotation (eg. silver nitrate solution, concentrated salt solution etc.) and, as a result, the identification of the eggs can be difficult (Happich and Boray, 1969a).

The Quantitative Sedimentation Technique developed by Boray and Pearson (1960), slightly modified by Happich and Boray (1969a), is relatively accurate, simple, quick and gives reproducible egg counts and no chemicals are necessary. This technique provides a reliable prediction of the number of fluke present. However, with heavy infections, there is reduction in the number of eggs produced by each fluke presumably as a result of overcrowding (Happich & Boray, 1969b).

1.5.3 Biochemical And Haematological Tests

Routine haematological tests and laboratory tests for estimation of plasma levels of enzymes have become most useful diagnostic aids. As discussed earlier, enzymes released by damaged parenchymal cells and cells of the bile duct epithelium can be measured to indicate the presence of the fluke in the liver, the phase of development and the severity of the disease (Haroun et al., 1975 & 1989; Boyd, 1988). GD and GGT levels are most often used, indicating parenchymal and bile duct damage respectively.

Though the detection of these enzymes is extremely useful in the diagnosis of fascioliasis, the methods used are relatively expensive as routine laboratory diagnostic procedures. Automatic analyzing machines are accurate and time-saving and also useful for wide range of biochemical analyses (Boyd, 1988). However, relative to cost of egg counts, they are still expensive and do not necessarily yield any more information on the status of the animal except in the migration phase of the infection.

Plasma protein and haematological parameters are useful in assessing the severity of the infection but are not, of themselves, diagnostic of fascioliasis. Their values must be interpreted in the light of the clinical signs and procedures more directly diagnostic of fascioliasis such as faecal examination. Normal values for sheep and goats are summarised in Table 1.3 which is derived from data used by the Animal Health Laboratories of the New Zealand Ministry of Agriculture & Fisheries (MAF).

Table 1.3 Normal ranges (& mean) values of haematological and biochemical parameters of sheep & goats as used by Animal Health Laboratories, New Zealand Ministry of Agriculture & Fisheries (MAF)

ITEM	UNIT	SHEEP	GOATS
Hb	g/dl	8-16 (12)	8-14 (11)
PCV	l/l	26-42 (33)	24-40 (30)
RBC	10^{12}/l	8-16 (12)	12-20 (15)
MCV	fl	28-40 (32)	17-24 (21)
MCHC	g/dl	31-38 (33)	30-35 (32)
WBC	10^{9}/l	4-12 (8)	6-21 (13.5)
Eosin.(Abs)	10^{9}/l	0-1.0 (0.4)	0-1.1 (4.5)
- (%)	%	0-10 (5)	1-8 (5)
T.Prot.	g/l	60-80	67-97
Alb.	g/l	24-30	22-32
Glob.	g/l	35-57	38-68
A/G	:1	0.42-0.76	0.34-0.77
Fib.	g/l	1-5	1-5
AST	U/l	49-141	63-187
GGT	U/l	0-52	29-64
GD	U/l	0-15	4-16
SBA	µM/l	0-60*	15-65**

*.. (Clinical Pathology Laboratory-Massey University)

**..(Established in this experiment)

1.5.4 Serum Bile Acids

Bile acids are synthesized in the liver from cholesterol in association with glycine and taurine conjugates. They are transported with the bile to the intestine where they are involved in digestion and absorption of lipids. Quantitation of total serum bile acids (SBA) has been found to be a sensitive and specific indicator of hepatobiliary disease in humans (Garry, 1989) and domestic animals (Kramer, 1988). Bile acids increase in the plasma of dogs, ponies, sheep and calves following liver damage caused by toxic substances such as CCl_4 (Anwer et al., 1976) and copper poisoning (West et al., 1987). Ligation of bile ducts or induced ketosis also produce a rise in total SBA concentration in sheep plasma (West et al., 1987) but no published material was found on the levels of SBA during Fasciola infection of domesticated animals. It would be interesting, therefore, to measure the SBA concentration in animals with fascioliasis to determine whether their elevation is a sensitive indicator of infection. This is investigated in Chapter 3.

1.5.5 Serological Antibody Tests

Serological tests such as Complement Fixation, Passive Haemagglutination and ELISA have been recommended by many reseachers for detecting the presence of Fasciola in ruminants. However, such serological tests are rarely used on a routine basis in ruminants because other diagnostic methods, such as faecal examination are simpler and more reliable (Pantelouris, 1965; Happich and Boray, 1969a; Soulsby, 1982; Urquhart, 1987). They may have some value in the detection of an immature infection in previously uninfected animals.

1.6 Economic Significance Of Effects On Health And Production

In many parts of the world, fascioliasis is one of the most important helminth infections affecting production in cattle and sheep (Fabiyi, 1986; Dargie, 1986). Though very little is known about the significance of fascioliasis for the productivity of goats, in many countries goats are becoming economically more and more important. In the tropics where the economic situation of the countries is very poor, regular control measures are often not practicable. Therefore, the scale of the economic losses can be much greater and relatively higher in tropical areas than in temperate regions.

High rates of infection in many African, Asian and South American countries which are economically dependant on livestock products can cause serious damage with significant economic losses in terms of wool, milk and meat production.

Some typical figures of rates of infection in cattle in tropical Africa and other similar areas have been cited by Fabiyi (1986) from previous records: Chad 6%; Central African Republic 45%; Ethiopia 30-90%; Malawi 50-70% and Uganda 53.7%.

In Nigeria, the estimated annual loss for the Jos Plateau Region alone was over $40 million US dollars (Fabiyi, 1986). Though this figure includes weight losses, lower milk yields and deaths of infected animals, it may have been over estimated. An abattoir survey carried out by Preston & Castelino (1977) in Kenya confirmed that fascioliasis is an economically important important problem there. The annual loss resulting from liver damage was estimated as more than 160 thousand pounds Sterling in the slaughterhouse and its vicinity where the survey was conducted. The markedly reduced weight gains in cattle described by Preston & Castelino (1977) were associated with subclinical fascioliasis over a long period both under grazing conditions and with intensive high quality feeding.

In many developed countries, whole carcase condemnation and whole or partial liver condemnation at abattoirs are responsible for major economic losses. According to the American Association of Veterinary Parasitologists (Cited by Dargie, 1986), approximately 1.5 million cattle livers per year are condemned as fluke-infected in the USA.

The scale of the problem as estimated from liver condemnation rates has been quite high in United Kingdom in the past few decades. In recent years, the prevalence has declined to around 6% in the UK, probably as a result of the adoption of effective control methods. In Ireland, however, the prevalence is recorded as 38% (Fabiyi, 1986).

1.7 Treatment And Control

A great number of drugs have been used against fascioliasis in sheep and cattle but very few have been found to be effective against all stages of the infection. Many have toxic effects and the efficacy of others is only against either the adult fluke or flukes at the migration stage.

One of the most important drugs from many years back is carbon tetrachloride (CCl_4). It has been used against adult flukes and its effectiveness is well proven in sheep (Holmes, 1963). However, it exerts toxic effects on the liver of the host, especially in cattle when administered orally (Scott & Goll, 1977; Soulsby, 1982). The advantages of CCl_4 are its cheapness and its effectiveness against adult fluke in small dosage. Boray (1967) showed that it removed 31%, 39%, 75% and 99% of 4, 6, 8 and 12 week old flukes in sheep respectively. The oral administration to sheep of a solution of CCl_4 in liquid paraffin was also made easier with the advent of automatic drenchguns (Taylor, 1962; cited by Soulsby, 1982).

Subcutaneous injection of CCl_4 in cattle is also effective, but orally it is too toxic (Holmes, 1963). A mixture of 50% CCl_4 and 50% vegetable oil injected into cattle killed all mature flukes and caused no toxic effects (Holmes, 1963).

Other older drugs, not widely used nowadays, such as hexachlorethane, hexachlorophene, atabrin and some others have more or less similar efficacies to that of CCl_4. They are less toxic but effective only against mature flukes in cattle.

Tremendous advances have been made recently in both the efficacy and safety of anthelmintics. Some of the recent drugs are rafoxanide, nitroxynil, oxyclozanide, diamphenethide and triclabendazole. These drugs are highly effective in both sheep and cattle and several of them are also effective against immature flukes in sheep and other animals during acute fascioliasis (Armour & Urquhart, 1974; Boray et al., 1985; Urquhart et al., 1987).

Diamphenethide in cattle (Armour & Urquhart, 1974) and triclabendazole in sheep (Boray et al., 1985 have been proved effective against adults and migrating flukes at 2-3 weeks of infection. Diamphenethide (150mg/kg) and triclabendazole (10mg/kg) have an overall efficacy of 100% and 99.8% respectively in all susceptible farm animals (Hughes et al., 1974; Boray et al., 1985).

Rafoxanide and nitroxynil are highly effective in cattle and sheep against flukes of four and six weeks of age at a dose rate of 10mg/kg and 13.5mg/kg body weight respectively (Armour & Urquhart, 1974). In goats, nitroxynil at 15mg/kg and diamphenethide at 100mg/kg are effective against migrating flukes as young as one week old (Hughes et al., 1973; 1974).

The use of rafoxanide and nitroxynil is not recommended in lactating cows because of residue problems. The drug most frequently used in lactating cows, oxyclozanide, is free from residual or toxic effects, but it is mainly effective against the adult bile duct stage of liver fluke (Armour & Urquhart, 1974).

Effective control of fascioliasis may involve the use of anthelmintics, control of the intermediate host by the use of mulluscicides and reduction of exposure to infection by appropriate farm management (Boray et al., 1969; Armour, 1981; Soulsby, 1982; Urquhart et al., 1987).

The ideal approach to controlling of Fasciola infections in sheep and cattle is by integration of the available control methods (Urquhart et al., 1987) :

1. prophylactic use of anthelmintics to reduce pasture contamination;
2. use of molluscicides to limit snail population;
3. improved drainage to eliminate intermediate snail hosts &
4. fencing of habitats.

Following the use of rafoxanide and nitroxynil the faeces of treated animals should remain virtually free from eggs for at least 5-6 weeks (Armour & Corba, 1972; Edwards & Parry, 1972). However, this is a relatively short period and repeated treatments would be needed in many circumstances to effectively minimize egg shedding.

It is possible to eliminate pasture contamination with fluke eggs if regular treatments with triclabendazole are carried out every eight weeks which is within the prepatent period of the infection. Where the infection is restricted to localized regions, eradication of the parasite is a practical possibility if treatment is continued long enough for the infection in the snails and the metacrcariae on the pasture to die out (Boray et al., 1985). However, in many economically backward countries, where the use of older and cheap anthelmintics is a common practice, effective control of the parasite is not possible by this means (Armour, 1981).

Snails may be controlled by the use of molluscicides which are either sprayed in the water or over the area where the snail population occurs. Copper sulphate solution of 0.2 to 10 parts per million is effective in the destruction of snails but is not effective against their eggs. However, a simple mixture of one part $CuSO_4$ powder and two parts of sand applied at 25kg/hectare can be used to effectively reduce the population of L.truncatula snails (Armour, 1981). It can be toxic to grazing animals if consumed in large amounts and to fish if the water in which they live is contaminated.

One of the molluscicides more recently produced and recommended is N-tritylmorpholine. In an experimental trial with N-tritylmorpholine in Kenya, L.natalensis was successfully controlled for a period of 11 months before reinfestation occurred. The molluscicide is too expensive for repeated use on a large scale (Preston & Castelino, 1977). Some plants which are known to have molluscicidal properties (eg. Phytolaca dodecandra) are locally used as molluscicides in some areas in East Africa (Mkamwa, 1986; Kloos et al., 1987).

In tropical and subtropical areas, the humidity and the warmth create favourable conditions for the parasite and its intermediate host to remain active throughout the year. Therefore, control methods should be applied more frequently.

As for control in tropical countries, anthelmintic treatment seems to be the most reliable way to reduce fluke infections for the present. In most areas, since patterns of infection have not been adequately studied, there will be little on which to base a dosing programme. In such places treatment is commonly recommended at quarterly intervals relying upon the prepatent period of the parasite. However, rotational grazing combined with strategic treatment with anthelmintics is one of the most widely recommended methods in the developing world (Boray et al., 1969; Armour, 1981; Reinecke, 1983; Fabiyi, 1986; Urquhart et al., 1987).

CHAPTER TWO

MATERIALS AND METHODS

Two experiments were carried out. The first involved 18 weeks experimental observations on a group of 5 adult male goats each infected with 150 metacercariae of F.hepatica and 5 uninfected controls. The second experiment involved groups of 10 sheep and 10 goats each infected with 200 metacercariae with 5 uninfected controls of each species. This experiment lasted 16 weeks. In both experiments, faecal, haematological, biochemical and pathological examinations were conducted. Animals were also weighed regularly using an electronic scale (Micropower 2000. Donalds Electronics, New Zealand). The procedures common to both experiments are outlined in this chapter. Details of the experiments are presented in Chapters 3 & 4.

2.1. Metacercariae And Infection Procedures

The metacercariae used for the first experiment were obtained from The Regional Vet. Lab., New South Wales, Australia (Courtesy of Dr.J.C. Boray). Those for the second experiment were supplied by Ciba-Geigy (New Zealand) Ltd. from their laboratory at Kemp's Creek, N.S.W., Australia (Courtesy R.M.Nottingham).

2.1.1 Counting And Administration Procedure

The metacercariae were stored at 4°C for a few weeks until the animals were infected. The viability of the metacercariae was assessed one day before infection by both visual examination of individual metacercariae under the microscope and applying an in vitro hatch technique using pepsin and trypsin solutions consecutively (Wikerhauser, 1960). Twenty and 18 % viabilities were recorded for the two different batches of metacercariae respectively just before the respective experiments began.

To make up each infective dose, the metacercariae (mc) were individually counted in a petri dish under a dissection microscope and transferred with a Pasteur pipette onto a piece of paper tissue placed in a funnel on which the metacercariae were deposited.

The metacercariae on the tissue paper were carefully placed in 500 mg cellulose capsules and stored at 4°C until used the following day. The required number of capsules were prepared and later one fed to each of the animals in the infected groups. This was conducted by inserting the capsule into the back of the pharynx and stimulating swallowing by pouring water into the mouth of the infected animals.

2.2. Faecal Examination

Faecal samples were examined for fluke eggs using a modification of the Quantitative Sedimentation Technique described by Happich & Boray (1969); 4gm samples were used and the total number of eggs counted was taken as an estimate of eggs/gm since only approximately 25-32 % of eggs are recovered by this method (Happich & Boray, 1969). A Modified McMaster Technique in which 1 egg represented 50 eggs/gm was used for gastrointestinal nematode egg counts.

2.3. Haematology

Blood was collected weekly from each animal from the jugular vein into 10 ml (plain) and 5 ml (EDTA) vacutainer tubes.

2.3.1 EDTA Blood

Erythrocyte (RBC) counts were made by using the Unopette Method (Becton-Dickenson, New Jersey, USA) for dilution and a Neubauer Haemocytometer.

Packed Cell Volumes (PCV) were measured by a standard microhaematocrit method using micro capillary tubes (Terumo, Japan), a microcapillary centrifuge and reader (IEC Mass., USA). Duplicate microhaematocrit tubes were spun for 3 min for each sample on each occasion. After reading the PCV, the plasma in one tube was used to measure total plasma proteins concentration using a hand refractometer (ATAGO, Japan). The second tube was held in a waterbath at 56^{0}C for 3 minutes to precipitate the fibrinogen. The tube was then spun again to deposit the fibrinogen and the supernatant examined by refractometry, the difference between the two readings indicating the amount of fibrinogen present.

The haemoglobin (Hb) concentration and Total White Blood Cell (WBC) counts were estimated by means of a Cell-DYN 900 Haematology Analyzer (Sequoia-Turner Corp. USA).

The mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from the results of PCV, Hb and RBC values according to the following formula :

$$MCV = \frac{PCV \times 10}{RBC} \ fl \qquad MCHC = \frac{Hb}{PCV} \ g/dl$$

A differential WBC count was conducted according to Schalm (1985); blood smears were made and stained using a Modified Wright Stain (SIGMA. Thigmatic, Miss., USA). One hundred cells were differentiated in each smear.

2.4. Serum Biochemistry

Blood collected in plain vacutainers was kept at room temperature for 24 hr to allow clot retraction. The tubes were then centrifuged at 3000 x g for 10 minutes using a bench centrifuge (Sorvall GLC-1. Conn., USA) and the serum collected. Approximately equal amounts of serum were placed in each of two sterile bijou bottles. One aliquot was kept at 4^{0}C for biochemical analysis the following day. The other was frozen at -10^{0}C for future use.

2.4.1 Serum Enzymes

Serum Enzymes and Serum Bile Acids (SBA) were assayed with a random access automated chemistry analyzer (Cobas Mira - Roche, Switzerland) using standard kinetic methods according to instruction procedures recommended by the manufacturers:

AST..........Roche Diagnostics Uni-Kit.

GGT......... " " " "

GDH..........Boehringer Mannheim, Monotest.

SBA..........Nycomed Enzabile.

Concentrations were expressed as Units/liter (U/l).

2.4.2 Serum Proteins

Total Serum Protein (TP) (g/l) was measured by using both the automatic analyzer (Roche Standard Kinetic Method) and a hand refractometer (ATAGO, Japan).

Albumin (ALB),and globulin (GLOB) levels and albumin/globulin ratios (A/G) were measured using both the automatic analyzer and Cellulose Acetate Electophoresis (Standard Helena Laboratories Method) with automatic scanner (Quick Scan) and Automatic Integrator (Quick Quant III) (Helena Laboratories).

2.5 Necropsy Procedures

Animals were killed with an overdose of pentabarbitone administered intravenously. The abdominal and thoracic cavities were opened and all organs inspected for gross lesions. The livers were removed and examined; any lesions or abnormalities were recorded. The bile ducts and gall bladder were opened and any flukes present collected. The livers were then sliced into 1cm strips and soaked in warm saline for 30 minutes. The slices were washed and squeezed to remove any flukes or fragments of flukes remaining in them. The washing were poured through a 250 um sieve and all flukes & fragments of flukes collected. The parasites were preserved in 10% formol saline until counted.

2.6 Histopathology

Samples of liver and other tissues were fixed in 10% formol saline for histological preparation. Following standard paraffin embedding, sections were cut at 3 um and stained with Hematoxylin and Eosin.

2.7 Photography

Photographs of the histopathology of selected lesions were taken using an automatic photo-microscope (Olympus BH-2) and Ektacrome^{R} (Kodak) film. Photographs of gross pathological lesions were also taken.

2.8 Statistical Analyses

All statistical analyses were made by using Statistix Version 3.0 (NH Analytical Software, USA). Each group of animals was compared with its controls by using Two Sample T-tests to determine the **P** values between groups. Descriptive statistics were produced for each group and the tabular summaries of these are shown in Appendices 1 & 2. Correlation and regression analyses were also carried in some instances as described later.

All graphs were produced using SlideWrite Plus (Advanced Graphics Software INC., USA). In all graphs, group mean values are shown; the vertical bars indicate one standard error (**SEM**). Asterisks indicate significant differences between groups (*** = P<0.05**; **** = P<0.01**).

CHAPTER THREE

EXPERIMENT 1 : A PRELIMINARY EXPERIMENT ON GOATS

3.1 Introduction

The objective of this study was to provide preliminary information on parasitological, haematological, biochemical and pathological changes during subclinical caprine fascioliasis, the result of which could be of diagnostic value in the early stages of fascioliasis in goats.

3.2 Experimental Design And Procedures

Ten Angora goats used for this experiment were purchased in the Palmerston North region and believed to have been raised in a fluke-free area. They were housed in three separate groups and fed hay and water ad libitum throughout the experiment. At the start, faecal samples were examined for both gastrointestinal nematode and fluke infections. All proved to be free from Fasciola infection as there were no fluke eggs in their faeces. But since some goats were positive for gastrointestinal nematode parasites, all were drenched with Ivomec^{R} at the recommended dose rate (200 ug/kg). A second drenching was conducted one week before infection and further faecal examinations were made on the day of infection. No nematode or Fasciola eggs were found in these samples.

Fig.3.1 Mean Liveweight.

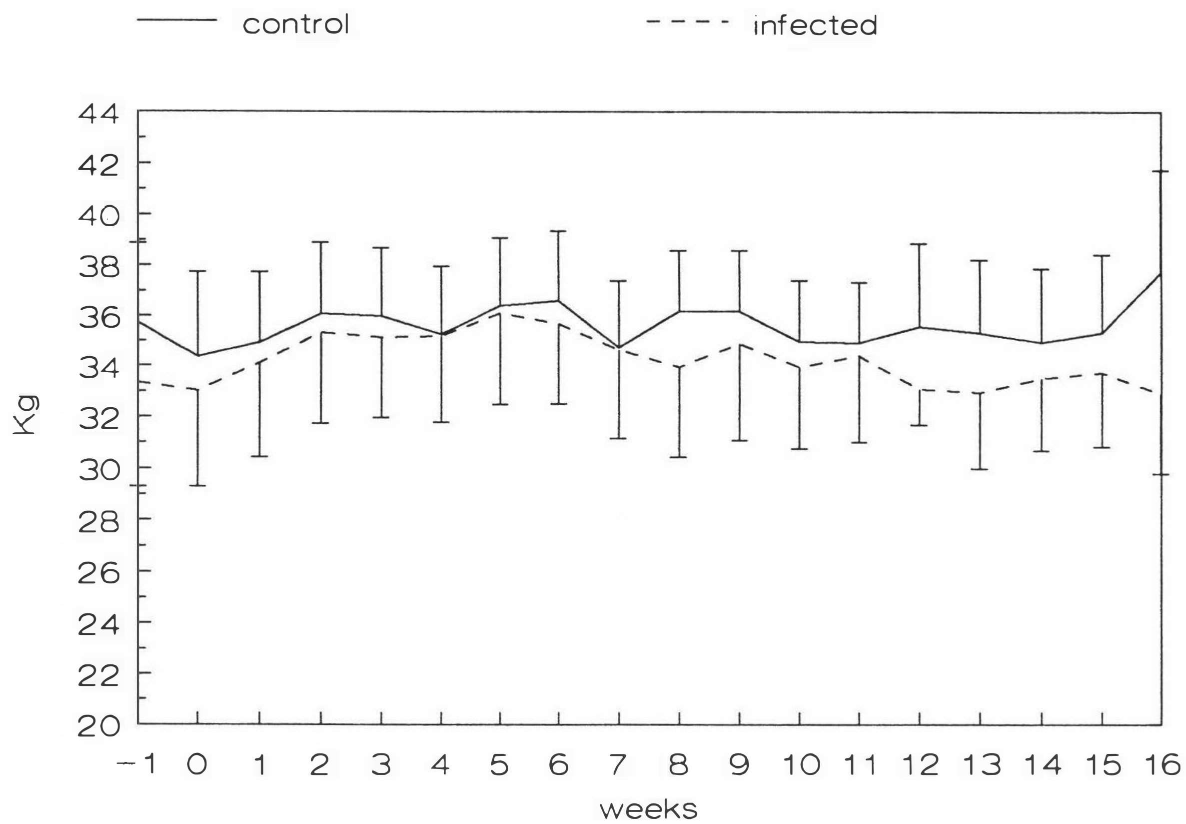
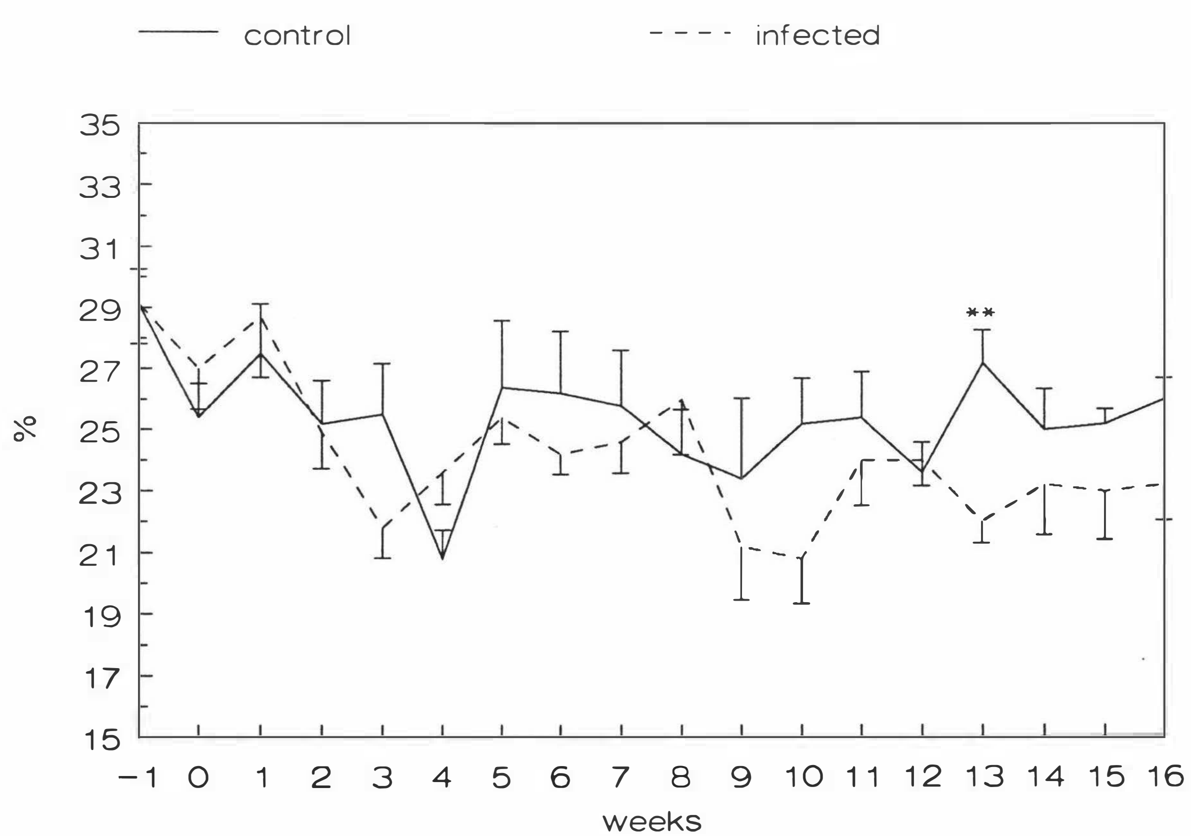


Fig.3.2 Packed Cell Volume.



The goats were randomly allocated to two equal groups and tagged with consecutive numbers 1-10. Group 1 (numbers 1-5) served as controls and Group 2 (numbers 6-10) was used for infection. Capsules each containing 150 metacercariae were prepared and administered to the goats in group 2 as described in Section 2.1.

The experiment was conducted for a total of 18 weeks starting one week before and ending 16 weeks after infection. All animals were weighed and bled at weekly intervals and clinical observations were recorded daily at feeding. Faecal sampling resumed at the 9th week after infection and continued at weekly intervals up to the end of the experiment.

Blood was taken into 5ml EDTA vacutainers for the assessment of haematological parameters as described in Chapter 2. Red blood cell (RBC) counts, PCV, Hb, MCV, MCHC, fibrinogen, total and differential WBC counts were estimated as described (Section 2.3.1).

Serum levels of AST, GGT and GD enzymes were measured together with serum bile acid (SBA), total protein, albumin and globulin levels and A/G ratios as described (Section 2.4.1).

At the end of the experiment all the animals were killed and necropsy carried out. The livers were examined grossly, weighed and flukes collected as described in section 2.5. Tissue samples were collected for histopathological examination and all interesting tissue changes were photographed.

3.3 RESULTS

3.3.1 Clinical Observations And Liveweights

Throughout the experiment the animals in both groups remained clinically normal. Liveweights are shown in Fig. 3.1. Weights at the end of the experiment are in Table 3.2. Summary statistics on liveweights are shown in Appendix 1.2a & b. It can be seen that at the beginning of the experiment the mean difference between Group 1 and Group 2 was 2.38kg. At the end of the experiment, at week 16 post-infection, there was a difference of 4.78kg. Over the experimental period there was a mean increase of 1.96kg in the control group while the mean weight of the infected group fell by almost 0.5kg. However, these differences were not statistically significant ($P>0.05$).

Fig.3.3 Haemoglobin Level.

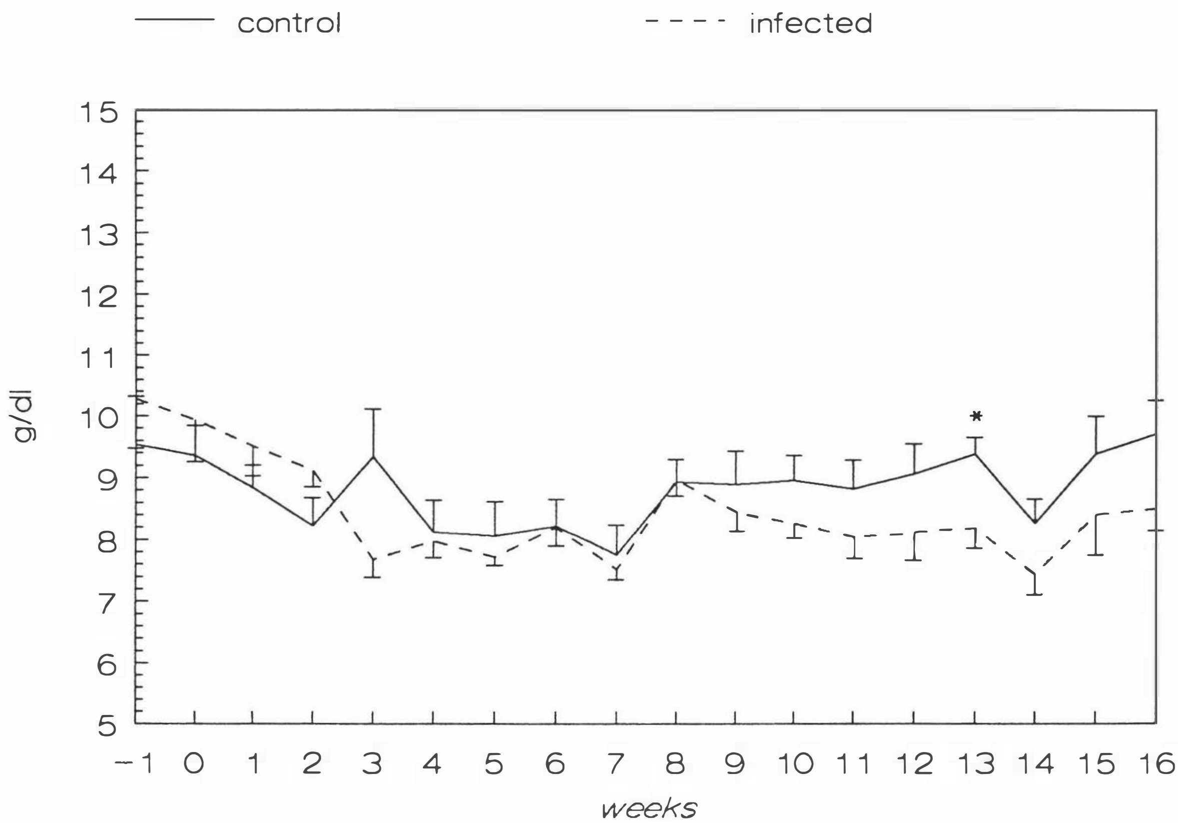


Fig.3.4 Erythrocyte Counts.

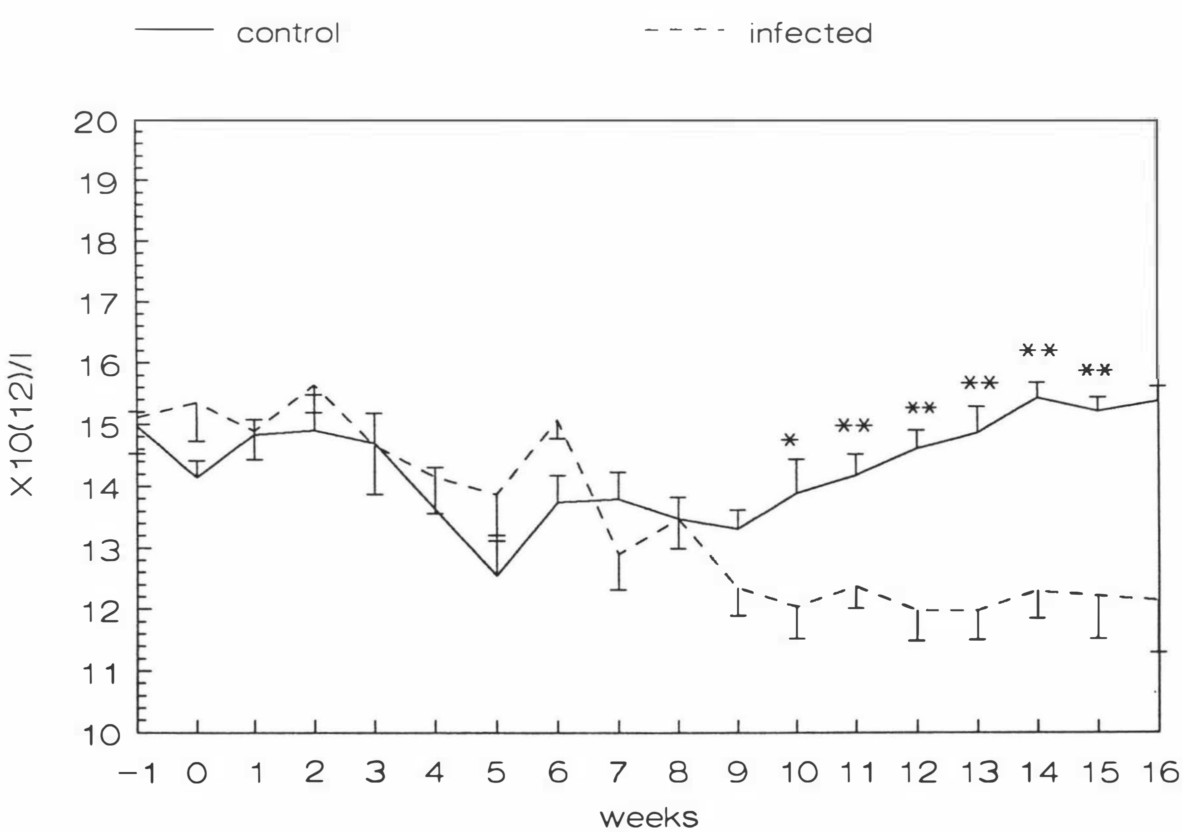


Fig.3.5 Mean Corpusc.Volume.

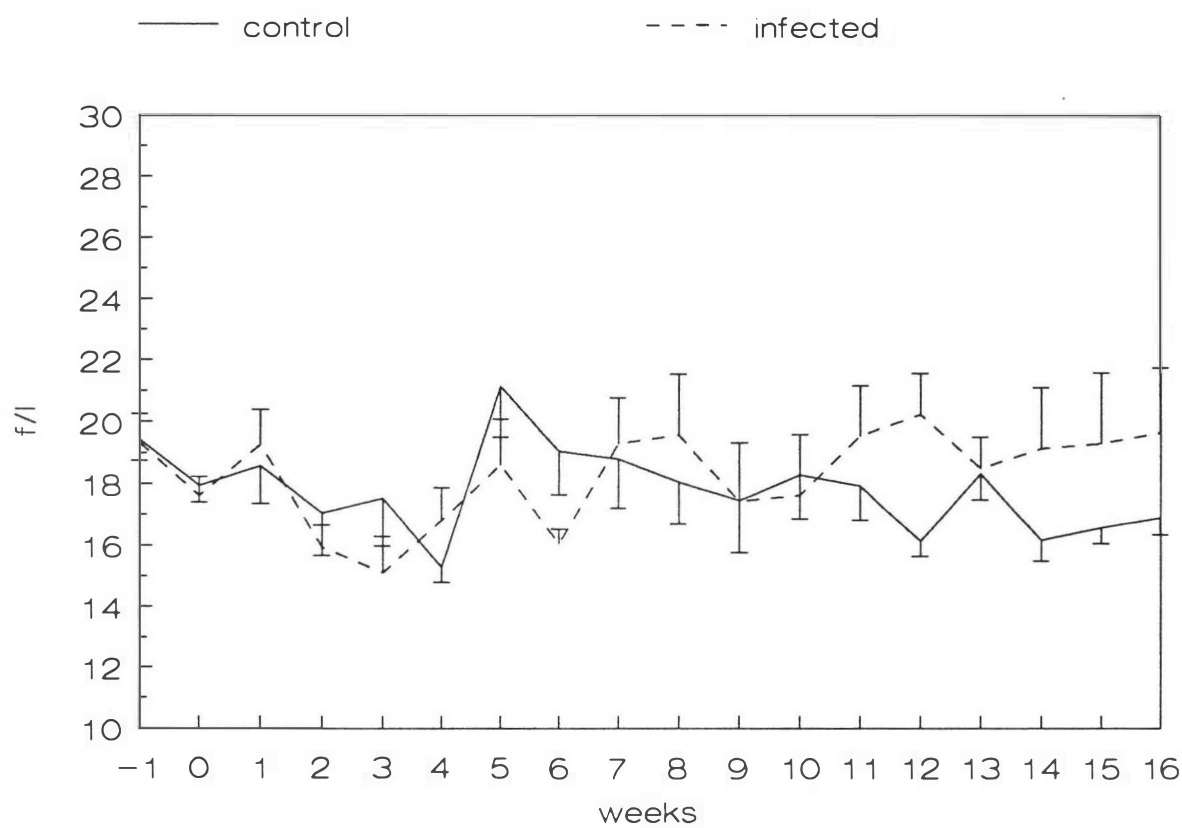
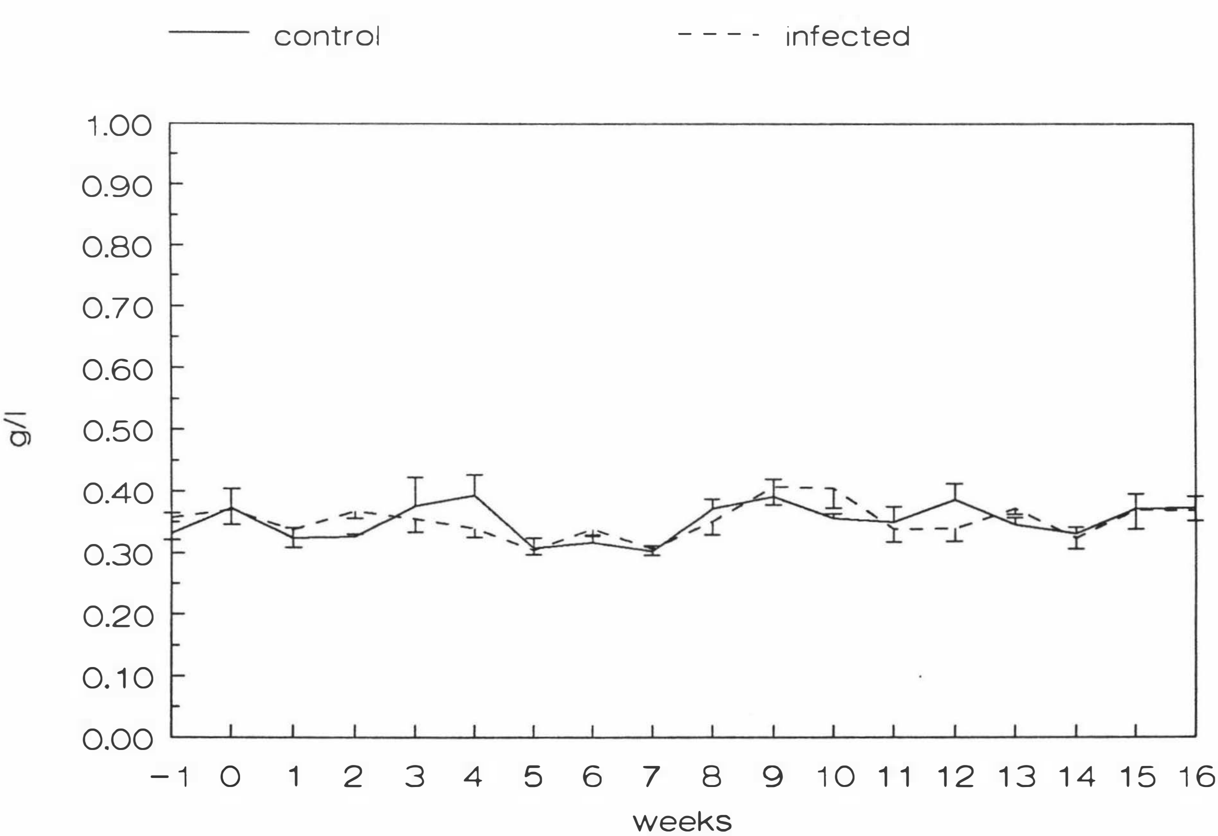


Fig.3.6 MCHC Level.



3.3.2 Faecal Egg Counts

Faecal egg counts in the infected group are shown in Table 3.1 and their descriptive statistics in Appendix 1.2. Infections in four of the 5 goats became patent 10 weeks post-infection and all were patent by week 11.

The egg counts progressively increased up to week 15 with a peak mean count of 389.4 epg. In the following week the counts from four goats had decreased. Goat 9 attained the highest count (680 epg) at week 15 and the highest fluke burden (35 adult flukes, ie. 23.5% of the infective dose). This same animal had the lowest body weight (27kg) and the lowest liver weight (490gm) in the infected group at necropsy (Table 3.2).

Table 3.1 Faecal Examination Results (Fasciola eggs/gm).

Weeks	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Group 1																		
1	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
2	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
3	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
4	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
5	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0
Group 2																		
6	-	-	-	-	-	-	-	-	-	-	0	0	120	140	434	450	570	450
7	-	-	-	-	-	-	-	-	-	-	0	4	57	98	112	150	202	250
8	-	-	-	-	-	-	-	-	-	-	0	15	97	145	150	250	175	130
9	-	-	-	-	-	-	-	-	-	-	0	34	150	220	250	535	680	405
10	-	-	-	-	-	-	-	-	-	-	0	9	45	201	310	280	320	189

Group Mean Epg	Weeks
12.4	10
93.8	11
160.2	12
271.2	13
333.0	14
389.4	15
285.4	16

Fig.3.7 Fibrinogen Level.

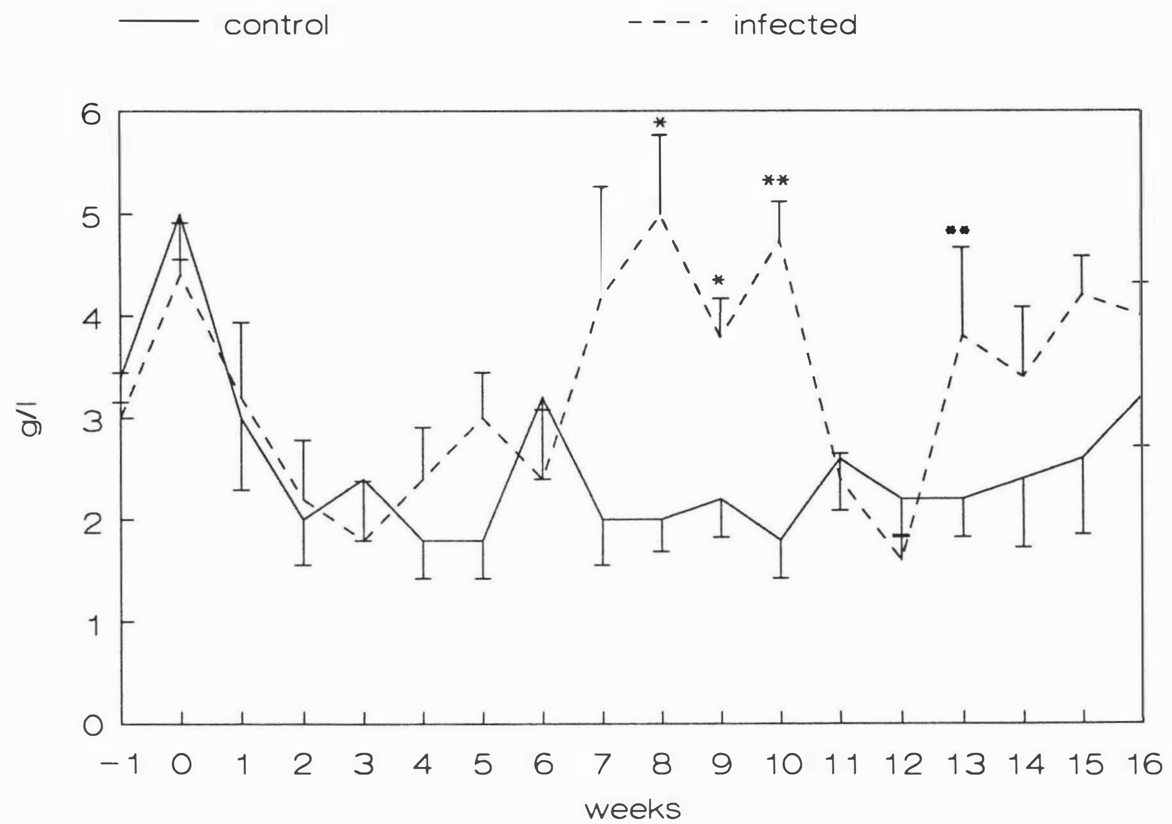


Fig.3.8 Total WBC Counts.

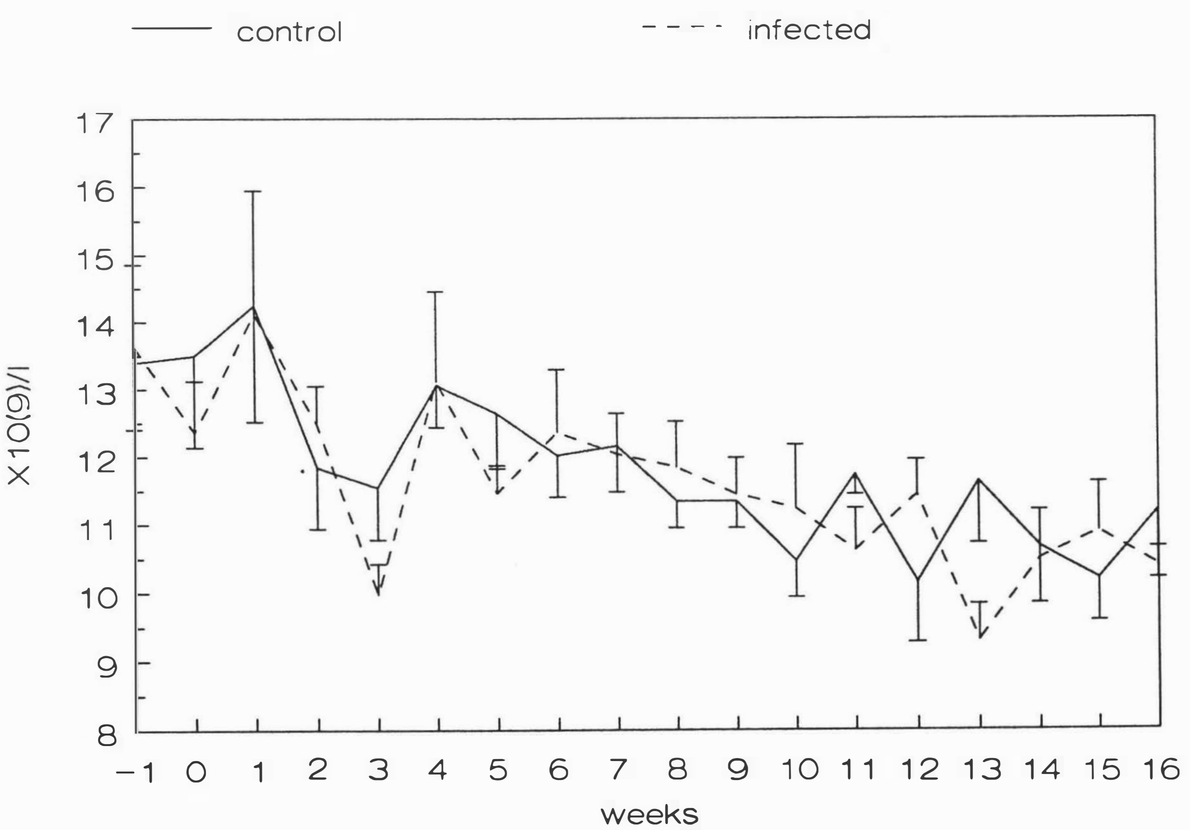


Fig.3.9 Abs. Eosinophil Count.

—— control - - - - infected

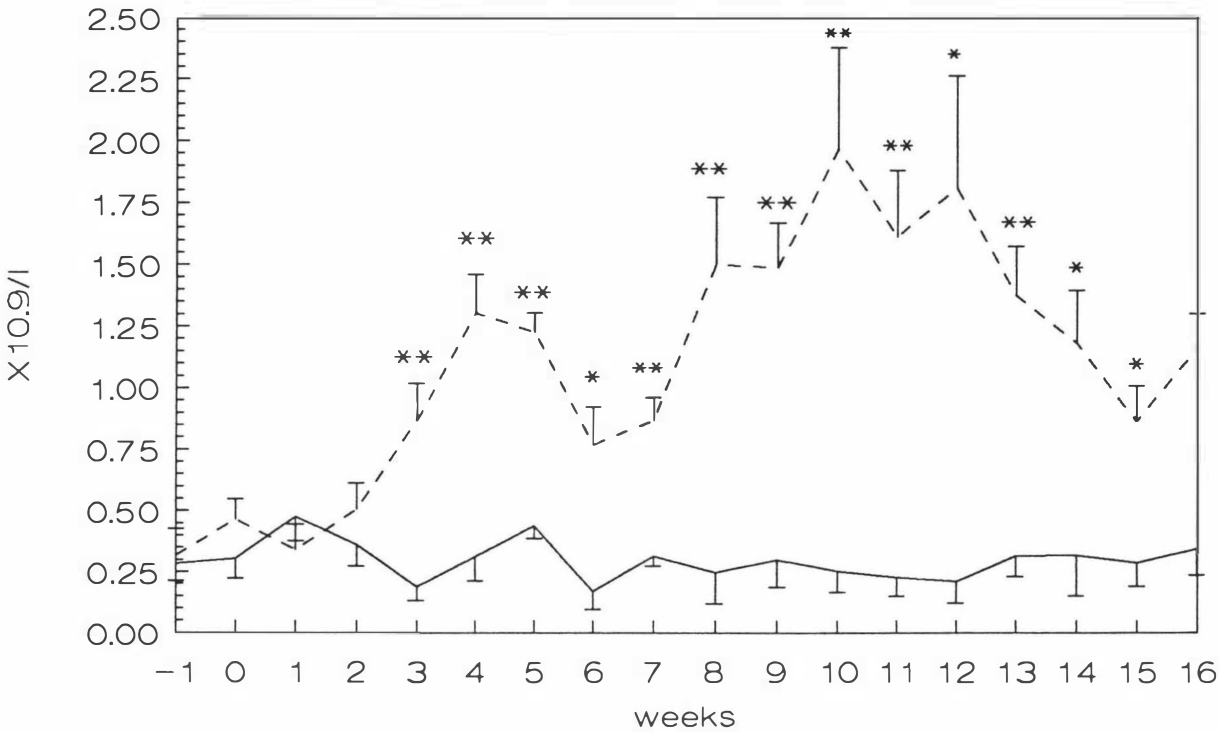
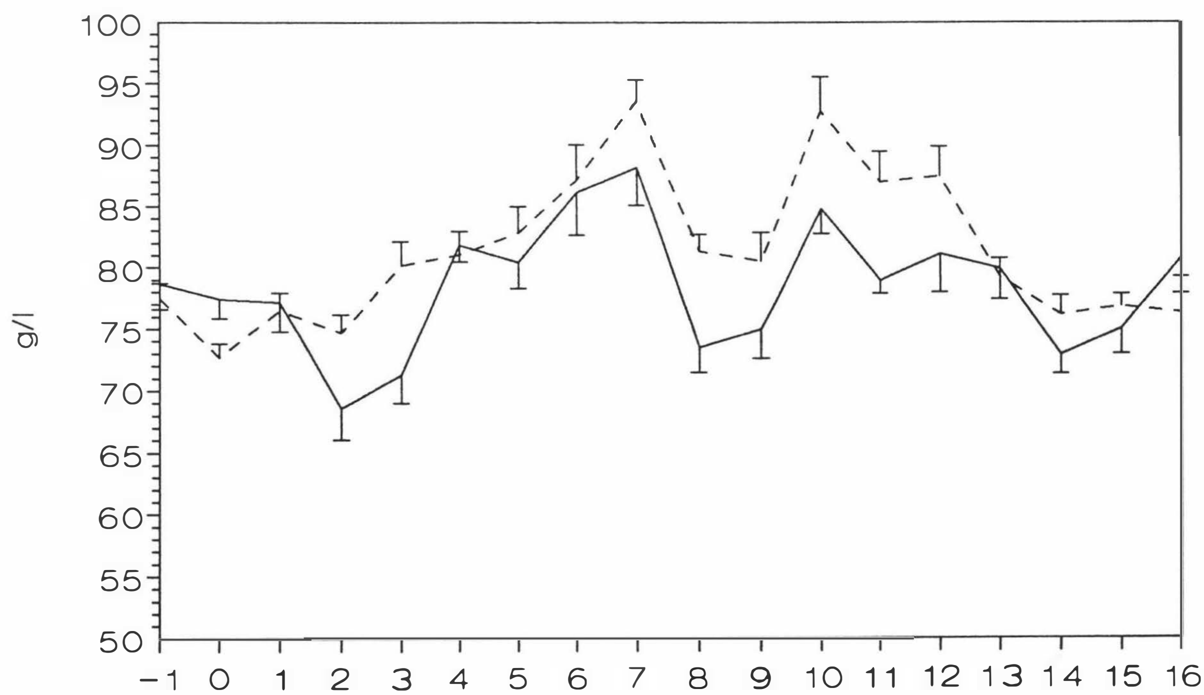


Fig.3.10 Total Protein (Cobas Mira)

—— control - - - - infected



3.3.3 Haematological Parameters

The PCV, Hb and RBC data are shown in Figs. 3.2, 3.3 and 3.4 respectively. Descriptive statistics for haematological parameters (including MCV, MCHC & fibrinogen levels) are shown in Appendices 1.3a/b-1.8a/b. It can be seen that there was a slight decrease in the levels of PCV and Hb in the infected group which became more obvious after week 9. However, only at week 13 were the two groups significantly different ($P<0.05$).

The RBC levels became significantly different between groups from week 10 onwards ($P<0.01$). At week 16, the mean value in the infected group had decreased to 12.15 X 10^{12}/l (lower limit of normal range: see Table 1.3) whereas the control group remained close to the mean of the normal range.

The changes in RBC counts, Hb and PCV were not parallel, suggesting a change in erythrocyte size and/or haemoglobin concentration, so MCV and MCHC values were estimated. These are shown in Figs. 3.5 and 3.6 respectively. There were no significant differences ($P>0.05$) between the values obtained in the groups although there was a tendency towards an increased mean cell volume in the infected group.

As shown in Fig.3.7, fibrinogen levels were significantly higher in the infected group from week 8 ($P<0.05$) and highly significant differences from controls were recorded at weeks 10 and 13 ($P<0.01$). Starting from week 7, the levels were around the upper limit of the normal ranges. In the last 3 weeks of the experiment, the difference between groups was not significant. Values of normal ranges are shown in Table 1.3.

Though there was a gradual decrease in total WBC counts in both the infected and control groups (Fig. 3.8), the levels at the end of the experiment reaching the lower limits of the normal range, no significant difference was recorded between the two groups.

Absolute eosinophil counts are shown in Fig. 3.9. While the control group recorded consistently low levels and remained within the normal range, the infected group showed highly significantly raised levels from week 3 and these remained high for the remainder of the experiment exceeding the normal range for much of the period. Normal ranges for leucocyte counts are given in Table 1.3 and Appendices 1.9a/b and 1.10a/b show the descriptive statistics for total WBC and eosinophil counts respectively.

Fig.3.11 Total Protein (Refractometry).

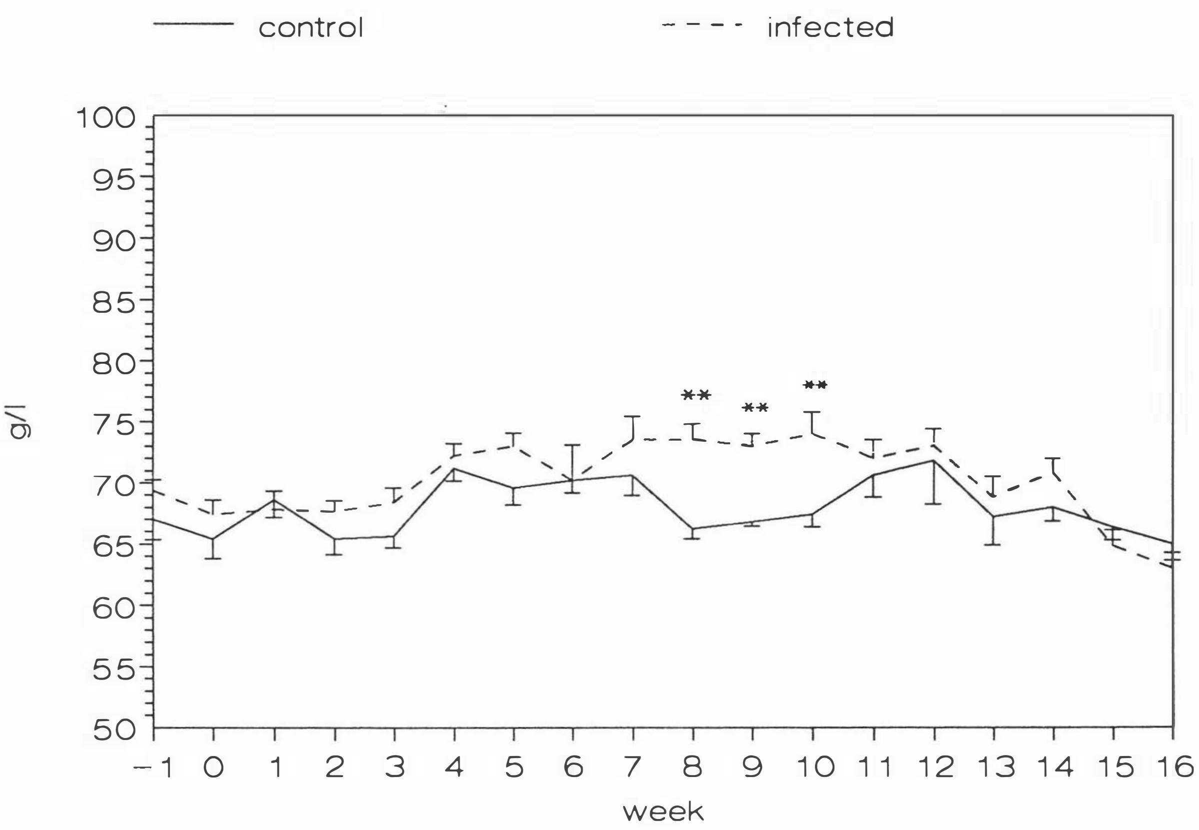


Fig.3.12 Serum Albumin Level.

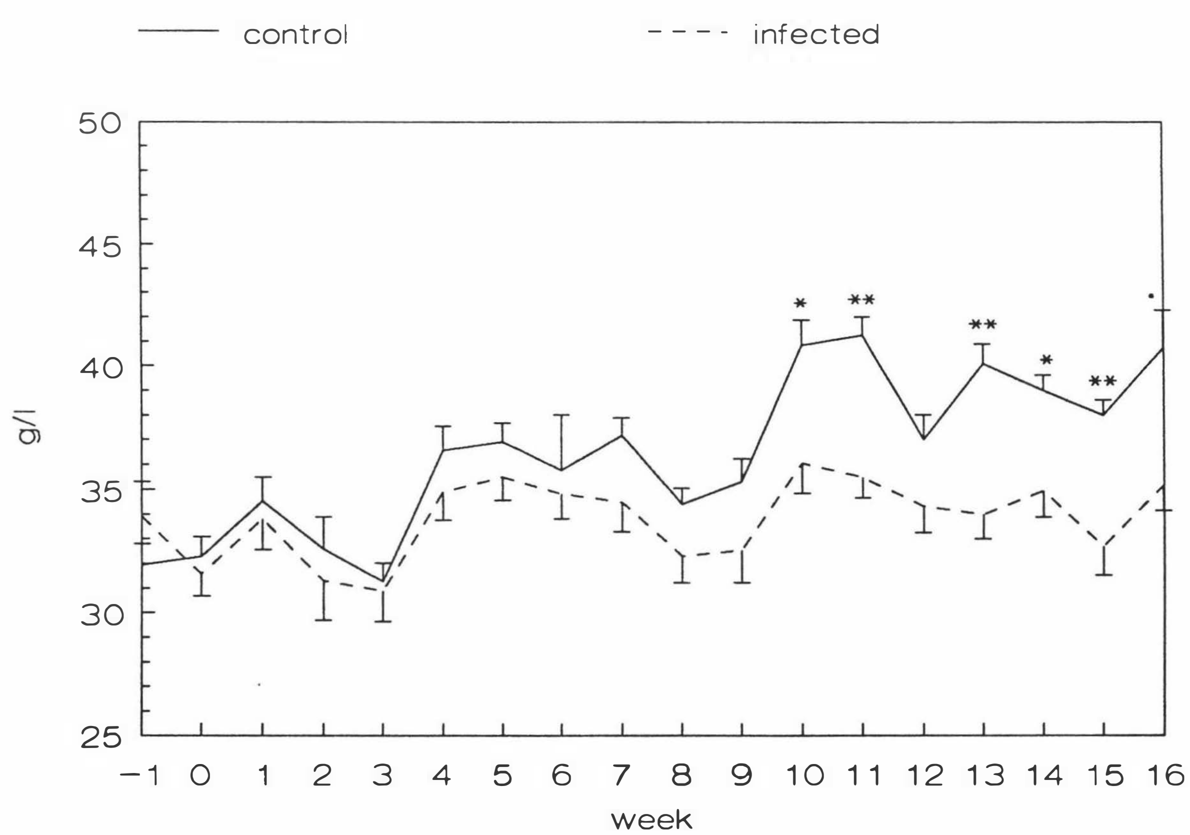


Fig.3.13 Serum Globulin Level.

—— control - - - - infected

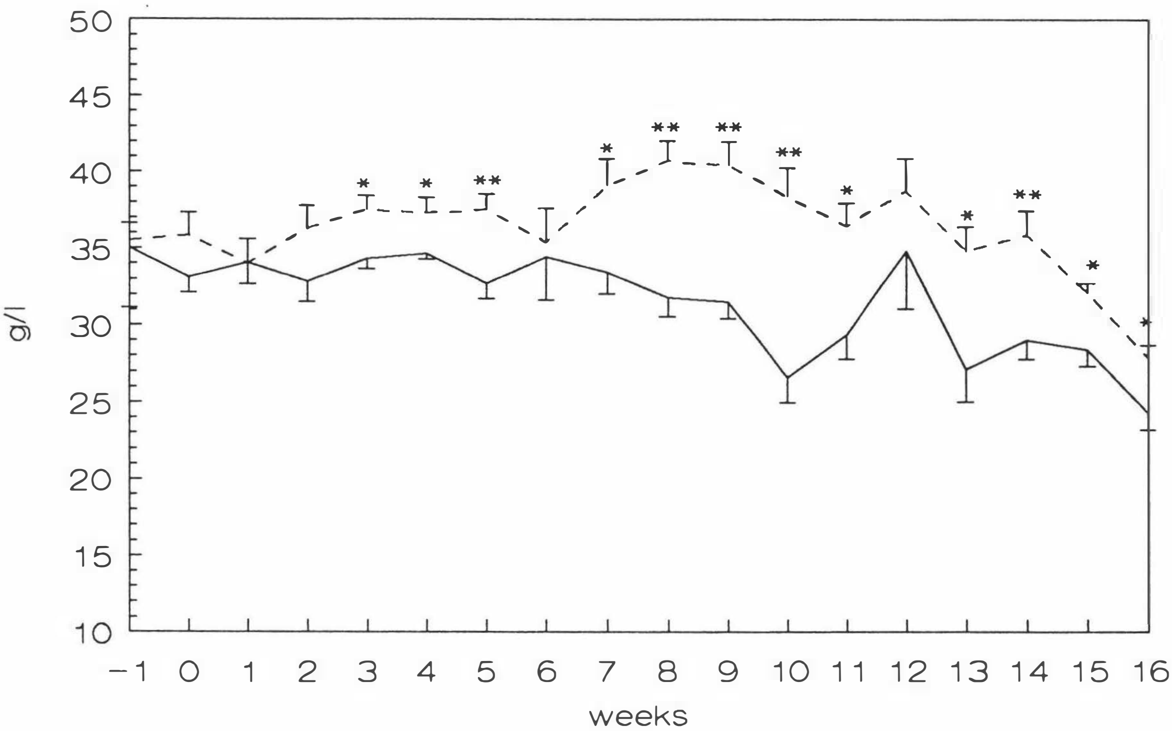
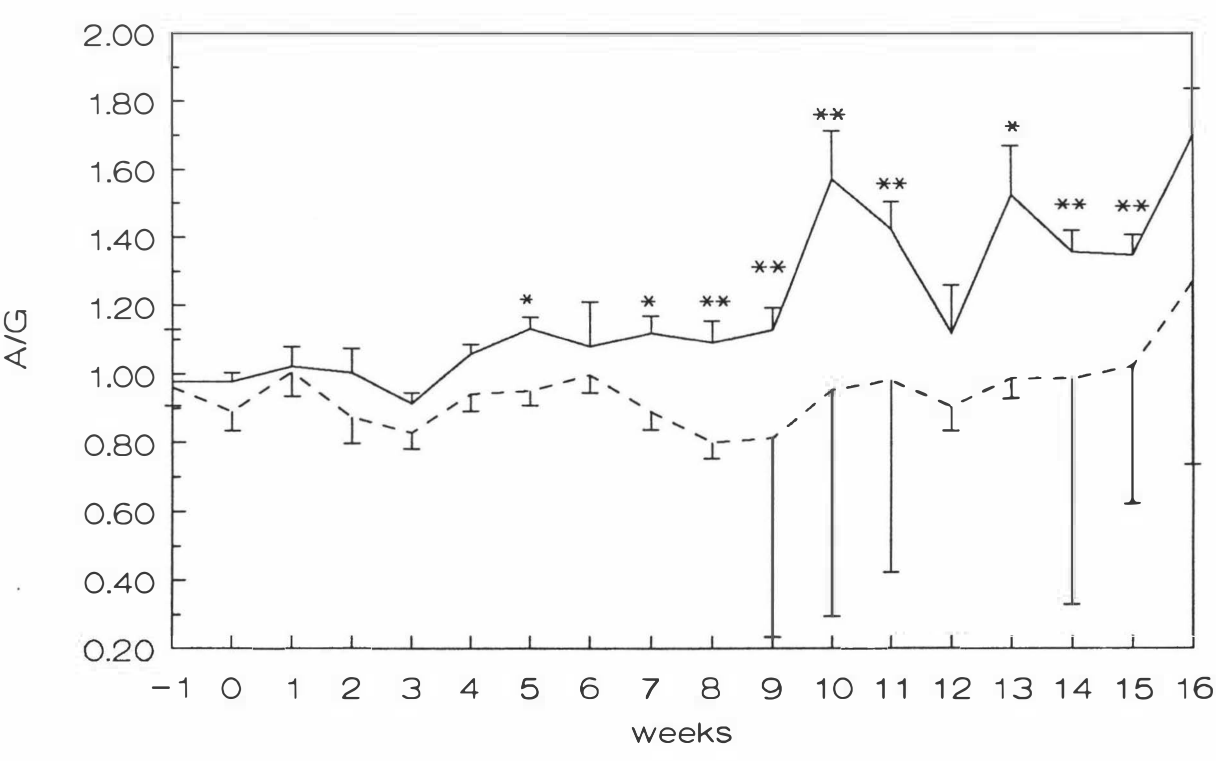


Fig.3.14 Alb/Glob Ratio.

—— control - - - - infected



3.3.4 Biochemical Parameters

The total proteins, albumin and globulin levels and the A/G ratio data are shown in Figs. 3.10, 3.11, 3.12, 3.13 and 3.14 respectively. Appendices 1.11a/b-1.15a/b show the descriptive statistics. Normal ranges are given in Table 1.3.

Total proteins as measured by automatic analyzer (Cobas Mira) and hand refractometer are shown in Figs. 3.10 and 3.11. The results show that there were substantial differences between the two measurements. Data obtained from the analyzer were much higher and tended to fluctuate more than the refractometer readings. With both readings, the levels in the two groups gradually increased up to week 7 and suddenly dropped to a lower level and then returned to their original levels at week 10. This was more prominent in the analyzer readings. There were highly significant differences between the two groups as judged by refractometry at weeks 8, 9 and 10 ($P<0.01$), but at other times differences between the groups were not significant. Moreover, levels of total serum protein recorded by both methods remained within the normal range.

The mean values of serum albumin in the infected group remained constant around the upper limit of the normal range. However, the level in the control group gradually increased to around 40 g/l and was significantly higher than in the infected group from week 10 as shown in Fig. 3.12.

Figure 3.13 shows that there were significantly higher serum globulin levels in the infected group from week 3 on, except for weeks 6 and 12. There was a tendency for levels in both groups to fall towards the end of the experiment.

As shown in Fig. 3.14, the mean A/G ratio in the infected group remained constantly below 1.0 throughout the experiment except for the last week. In the period between week 6 and week 15 a significantly lower level was recorded for the infected group, although in some weeks (9, 10 and 11) the variation in levels in the infected group, reflected in the large standard errors, was very high. On the other hand, unusually high levels occurred irregularly in the control group in the period between weeks 10 and 14 and this increased the significance of the difference from controls during this period. While group mean levels remained within normal ranges, those in some infected animals fell below the lower limit.

Fig.3.15 Serum AST Level.

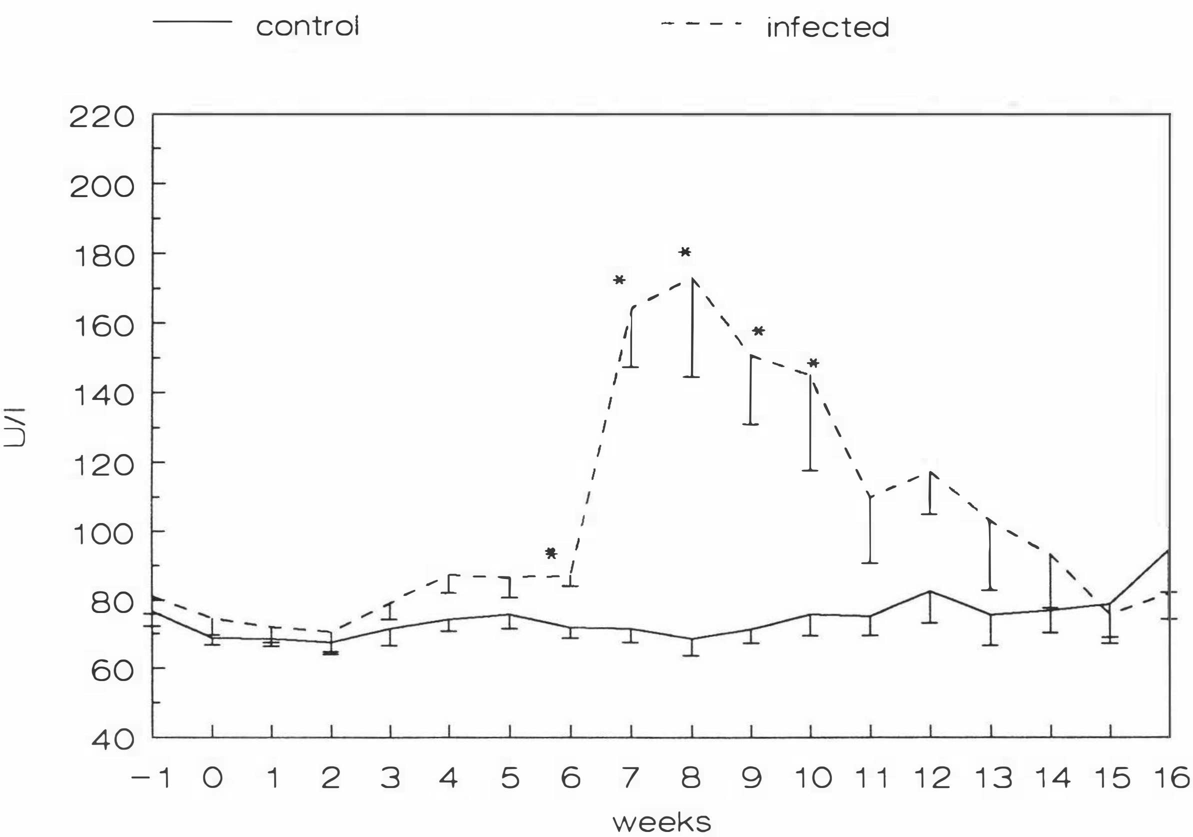


Fig.3.16 Serum GGT Level.

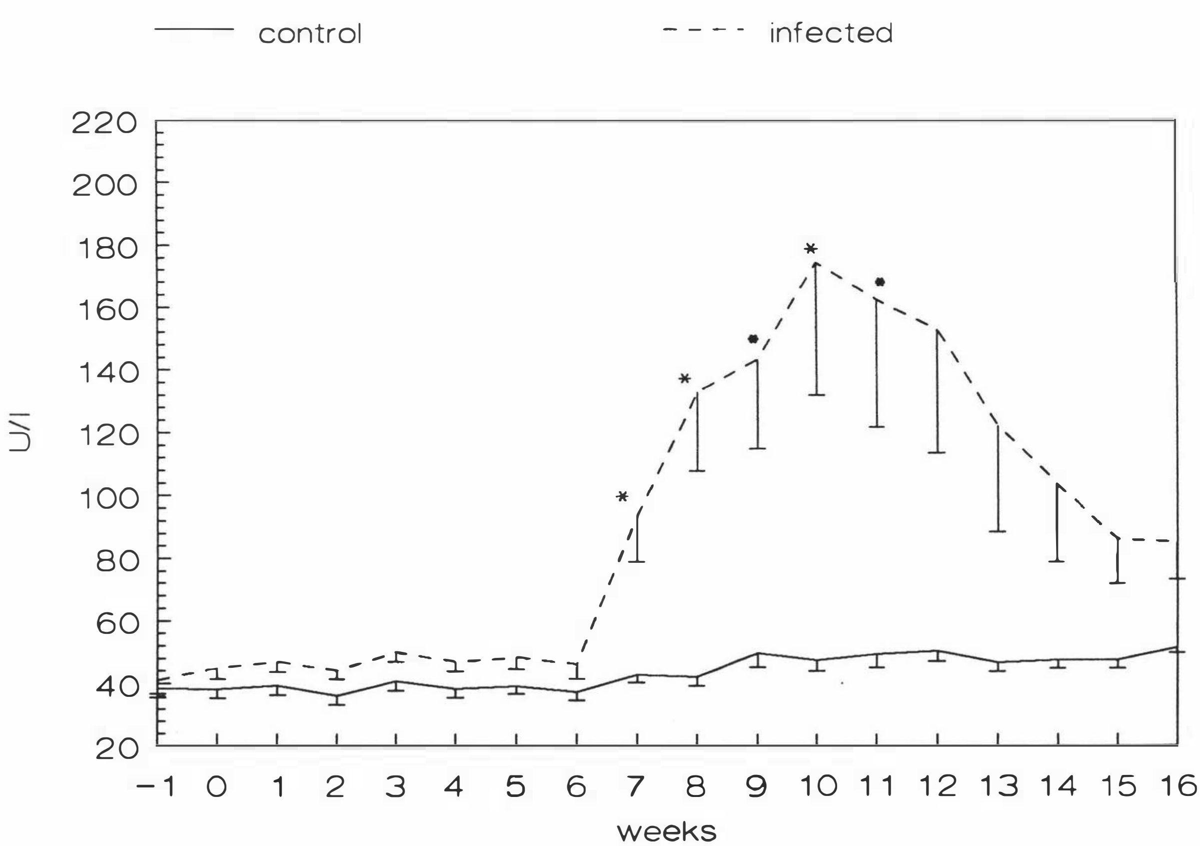


Fig.3.17 Serum GD Level.

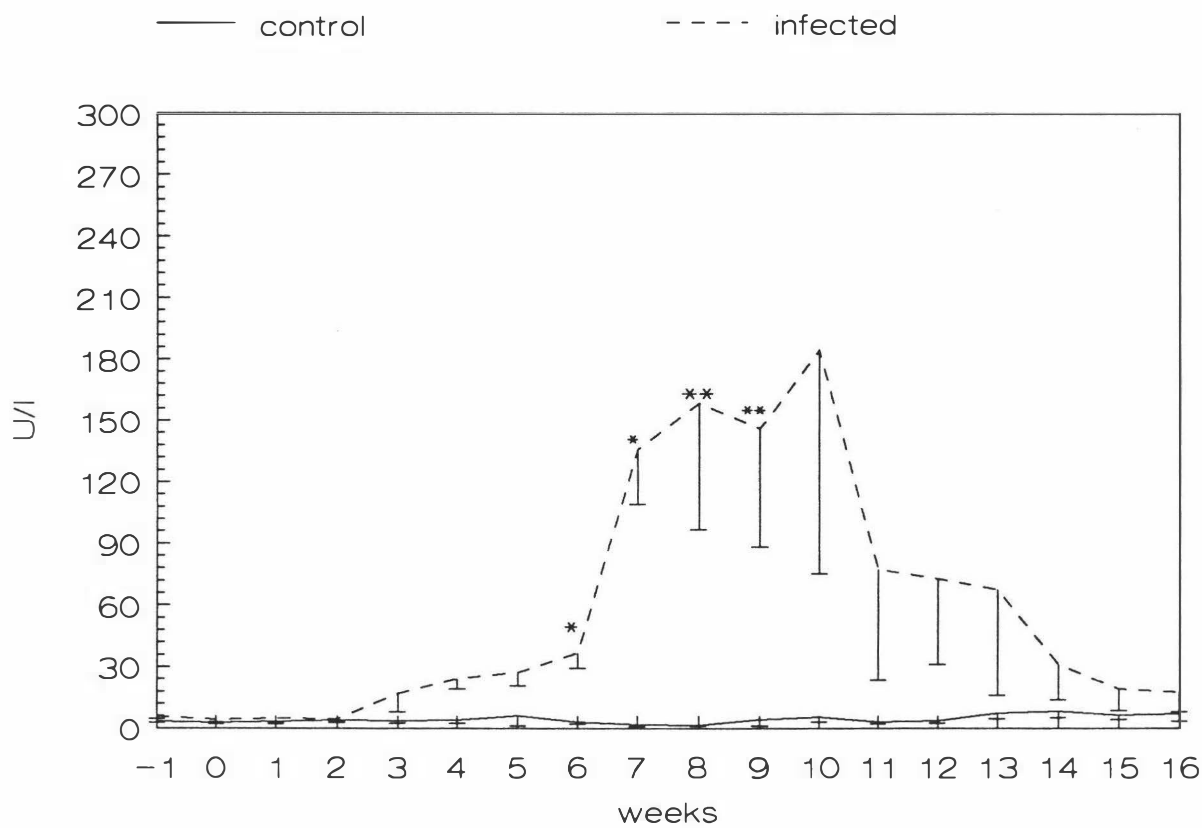
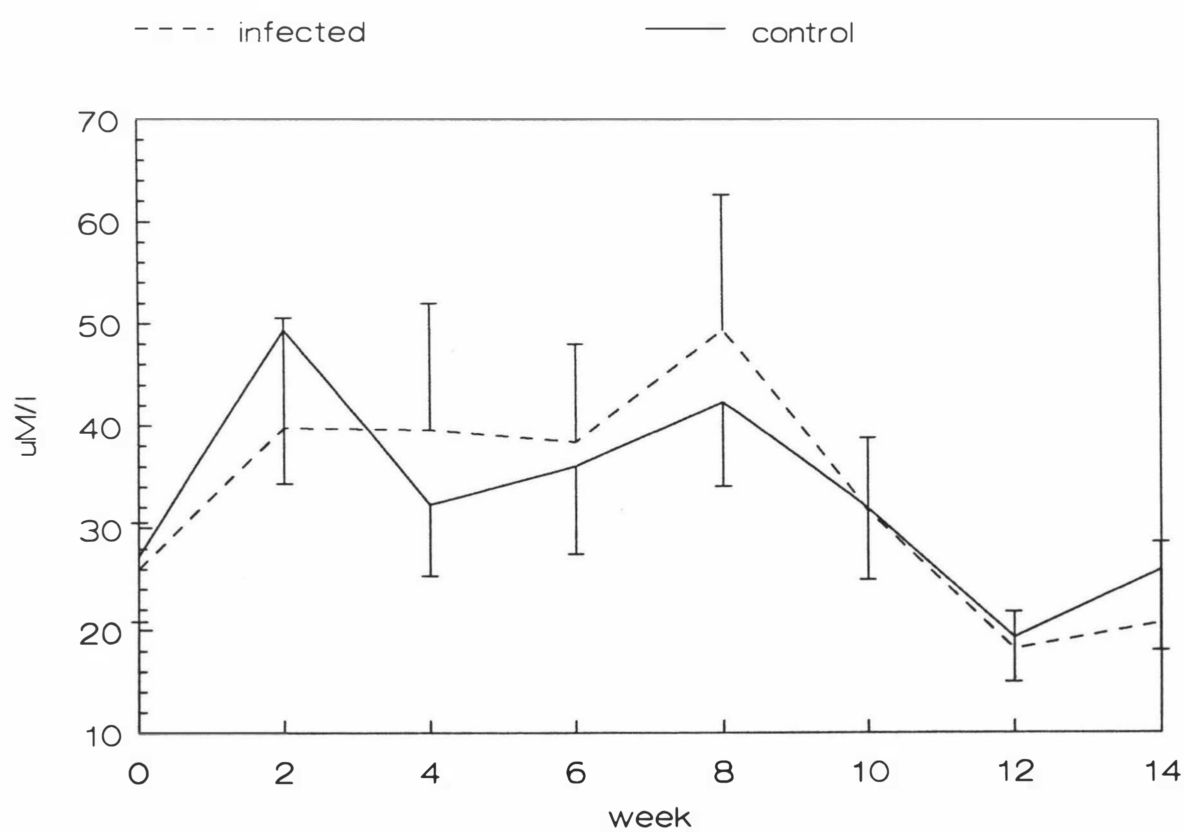


Fig.3.18 Serum Bile Acid.



3.3.5 Serum Enzymes

The levels of the enzymes AST, GGT and GD examined in this experiment are shown in Figs. 3.15, 3.16 and 3.17, and Appendices 1.16a/b-18a/b show the corresponding descriptive statistics.

As shown in Fig. 3.15, the level of AST remained low and approximately constant in the control animals throughout the experiment. In the infected group, it remained unchanged up to week 3 post-infection. The level in the infected group started to increase gradually up to week 6 but was not significantly greater than the control animals up to this time. At week 6 post-infection, the difference became significant. A more than 2-fold increase was recorded in the infected group at this time with a peak mean value of 173 U/l, which is just below the upper limit of the normal range at week 8. Some animals, however, scored well above the normal levels at weeks 7, 8, 9 and 10 post-infection. Although the enzyme levels gradually decreased, significantly higher levels were recorded at weeks 9 and 10 ($P<0.05$). At week 15, the level in the infected group returned to the starting point.

The level of GGT remained low up to week 7 when a sharp increase was recorded in the infected group as shown in Fig. 3.16. Further increases were recorded at weeks 8 and 9 with a peak reached at week 10 at a mean level of 174 U/l. The levels then gradually decreased but at the end of the experiment were still above the upper limit of the normal level (85 U/l).

Serum GD levels are shown in Fig. 3.17. In infected goats, increased levels above the normal limits were recorded as early as week 3 post-infection. Levels significantly higher than in control goats were recorded at weeks 6 and 7 ($P<0.05$) and 8 and 9 ($P<0.01$). The peak level was recorded at week 10 although it was not statistically significantly different from controls because of the extreme variability in levels in the infected animals. From then on, the level gradually decreased, reaching the initial normal level by the end of the experiment.

Serum bile acid (SBA) levels were estimated at 2-weekly intervals but, as shown in Fig. 3.18 and Appendix 1.19a & b, no statistically significant differences between the two groups were seen. Though there was large variability between individual animals in both groups, the mean levels in the two groups were almost equal on the day of infection and remained very similar throughout. The levels in both groups fluctuated irregularly and from week 8 gradually declined and at week 12 were lower than the initial level.

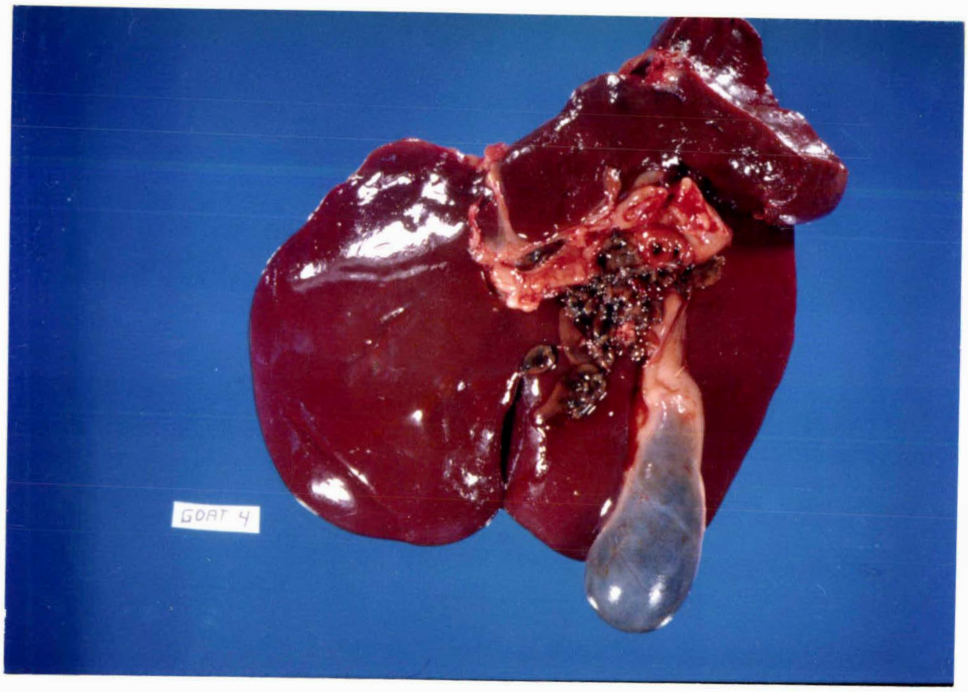


Fig. 3.19 Adult flukes concentrated in the lumen of the large bile ducts at the entrance of the gall bladder recovered at necropsy 16 weeks post infection.

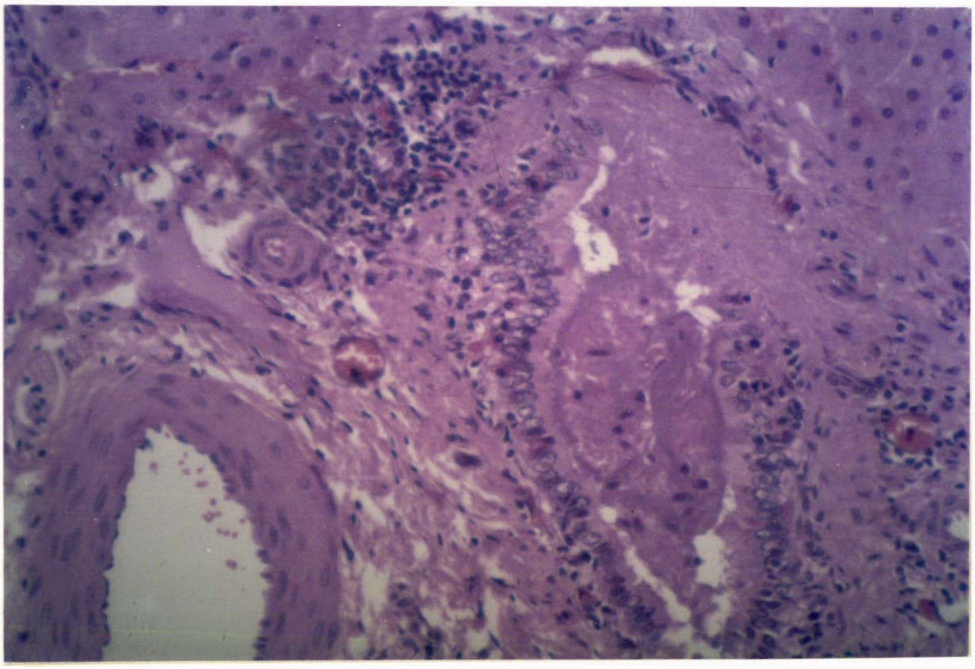


Fig. 3.20 Degenerating remains of a young fluke in amedium sized bile duct surrounded by inflammatory cells and globule leucocytes infiltrating the basal layer of the epithelium.

3.3.6 Pathological Investigation

At the end of the experiment, all the animals were necropsied. Table 3.2 shows that on the day of slaughter, the mean weight of the control group was higher than that of the infected group while the mean liver weight of infected goats was higher than in the controls (Table 3.2). However, these differences were not statistically significant.

The mean number of flukes recovered was 21.8 giving a mean establishment rate of 14.7% (Table 3.2). At the start of the experiment, 20% viability was estimated by in vitro excystation which makes the fluke recovery slightly less than expected. The majority of flukes recovered were found in the large bile ducts near the entrance to the gall bladder. These were fully adult flukes (Fig. 3.19).

Table 3.2 Necropsy Results.

Group 1.	Liver Wt. gm	Live Wt.Kg Necropsy	Fluke Recovery	% Recovery
Group 1				
1	520	34.4	-	-
2	510	29.5	-	-
3	570	48.2	-	-
4	610	46.5	-	-
5	480	29.8	-	-
Mean	538.0	37.68	-	-
Group 2				
6	750	44.9	20	13.5
7	600	32.1	18	12.5
8	590	30.2	15	10.0
9	490	27.0	35	23.5
10	530	30.3	21	14.0
Mean	592.0	32.9	21.8	14.7

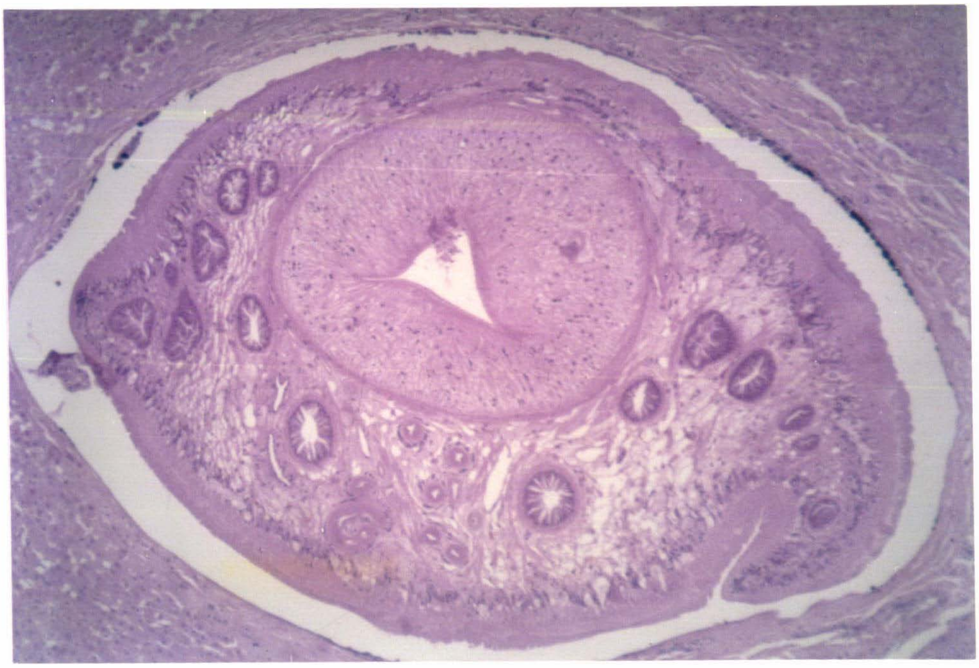


Fig. 3.21 Cross section of a migrating fluke in the parenchyma surrounded by fibrous tissue.

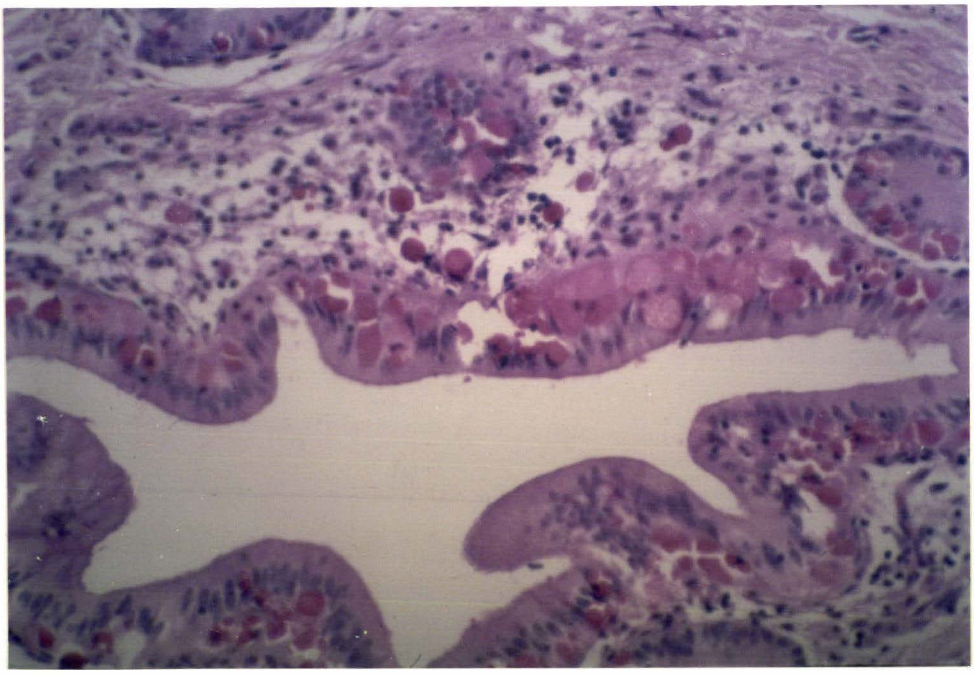


Fig. 3.22 Large number of globule leucocytes are shown in and around the basal layer of the epithelium of a large bile duct.

Gross pathological lesions were confined to the liver. A few greyish white, rounded, clearly demarcated, fibrous nodules (mostly 1-3mm in diameter but varying up to about 1 cm) were found on both diaphragmatic and visceral surfaces of all livers. These were not characteristic of fascioliasis and possibly caused by migratory taeniid larvae. Other small, whitish migratory lesions were visible in the infected group on the visceral surfaces of the accessory and left lobes which were indicative of fluke penetration of the liver.

Histopathological examination revealed that all the samples of liver from infected goats showed varying levels of tissue damage and tissue reaction. Migratory lesions of varying sizes were seen. Some focal areas of tissue reaction infiltrated with inflammatory cells and larger areas of eosinophil infitration were observed around migratory lesions, together with numerous giant cells and inflammatory pigment cells surrounding the invaded area. The degenerating remains of some flukes were seen in the liver parenchyma (Fig. 3.20) and also one live fluke (Fig. 3.21).

Some larger bile ducts showed various degrees of epithelial damage. The connective tissue wall of some bile ducts was thickened. Large numbers of globule leucocytes were also observed in several areas, infiltrating in and around the basal layer of the epithelium of larger bile ducts (Fig. 3.22). No major haemorrhage or tissue damage was observed in any of the samples. The livers of the control animals showed no migratory lesions or inflammatory reactions attributable to liver fluke.

CHAPTER FOUR

EXPERIMENT 2 : EXPERIMENTAL INFECTION IN SHEEP AND GOATS

4.1 Introduction

The objective of this study was to provide information on the similarities or differences between sheep and goats during experimental subclinical fascioliasis.

4.2 Experimental Design

Fifteen locally purchased Romney adult female sheep and 15 Angora goats were used for this experiment. They were all believed to be free of fluke infection when purchased.

The experiment was conducted for a total of 16 weeks starting one week before and ending 14 weeks after infection. The animals were housed in several small groups and fed with hay and water *ad libitum*. Faecal samples were examined two weeks before infection. All proved to be free from *Fasciola* infection but since some animals were positive for gastrointestinal nematode parasites, all were drenched with Ivomec^{R} at the recommended dose rate (200 ug/kg). Faecal examinations were also made on the day of infection and found to be negative for both *Fasciola* and nematode eggs.

The sheep and goats were randomly allocated to two groups of each species with 10 infected and 5 control animals and tagged with consecutive numbers 1-30.

The metacercariae used in this experiment were assessed for viability by in vitro excystation (Wikerhauser, 1960) and 18% viability was recorded one day before the animals were infected. Twenty 500mg cellulose capsules were loaded with 200 metacercariae each as described in section 2.3.1 and the following day one administered to each of the 10 sheep and 10 goats in the infection groups.

All animals were weighed on the day of infection and 8 and 14 weeks after infection. Clinical observations were recorded daily at feeding. Faecal samples were examined for Fasciola eggs each week from 9 weeks post-infection.

Packed cell volumes (PCV), Hb, total and differential WBC counts, total protein, albumin, globulin and A/G ratios and AST, GGT and GD levels were measured at weekly intervals. In this experiment, total serum protein levels were estimated only by refractometry and differential protein analysis made by electophoresis.

At the end of the experiment the animals were killed and necropsy carried out as described in Chapter 2.

The laboratory procedures used in this experiment were conducted as described in various sections of Chapter 2.

4.3 RESULTS

4.3.1 Clinical Observations And Liveweights.

No clinical signs of parasitism were observed in the infected sheep and goats and no deviation from the normal state of health was detected in the control groups throughout the experiment. But since eight of the sheep proved to be pregnant, which was not suspected when they were purchased, several of the infected and the control sheep lambed at different stages of the experimental period and one control animal was pregnant at necropsy. The lambs were allowed to stay with their mothers for only three days when they were permanently removed. Two goats in the infected group were also pregnant and kidded during the experiment.

Fig.4.1a Liveweight : Sheep

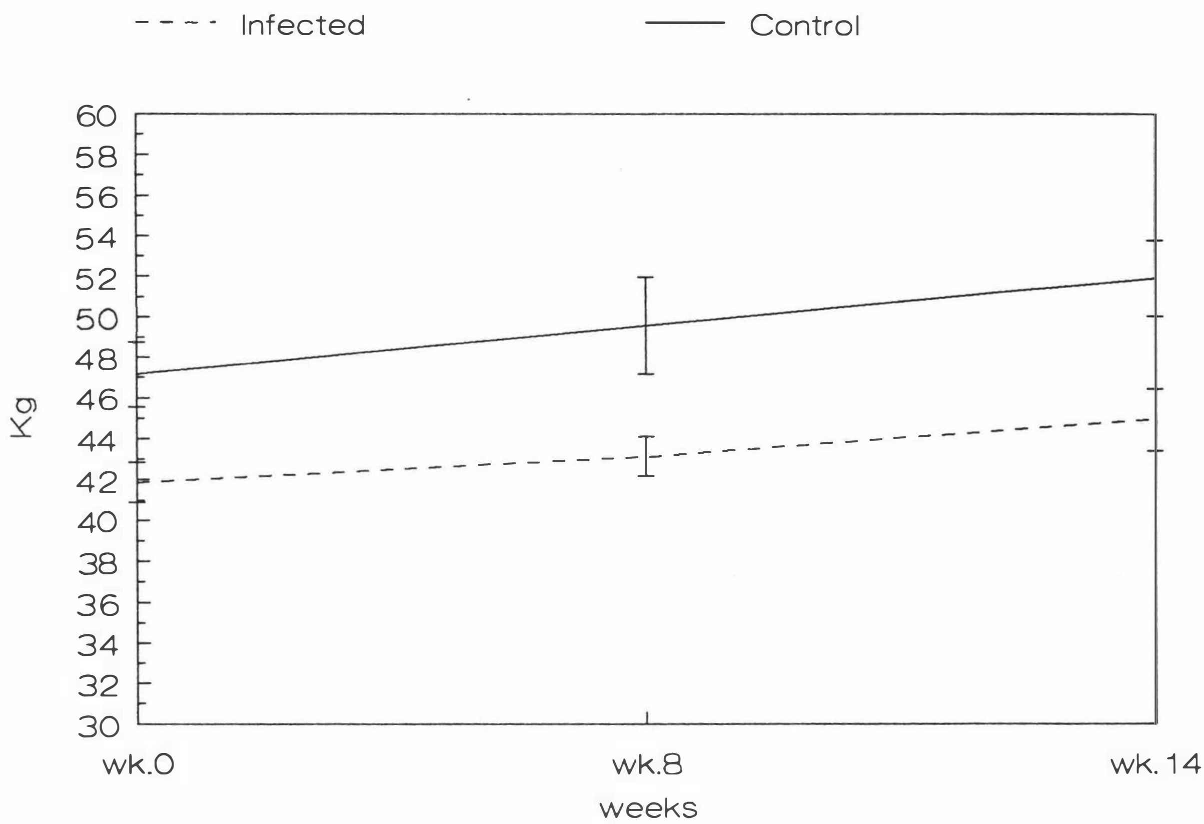
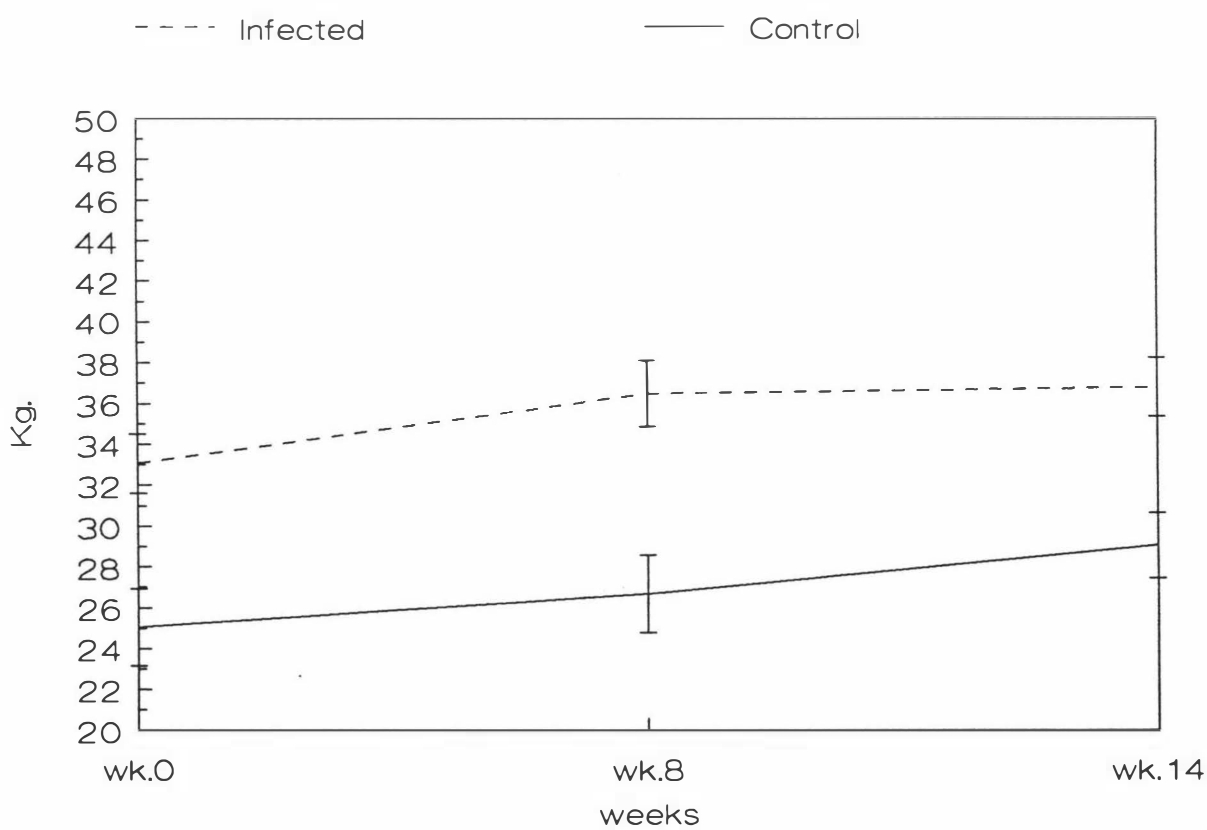


Fig.4.1b Liveweight : Goats



Liveweights are shown in Figs. 4.1a and 4.1b for sheep and goats respectively. The weight data are summarised in Appendices 2.1a, b, c and d. By chance, the random allocation to groups led to significant differences in group mean weights at the start. For this reason, it is more appropriate to consider weight gains. As indicated in the Figure, over the 14 week period after infection, the control sheep gained, on average, more (4.76kg) than the infected sheep (2.13kg). However, this difference is confounded by the effects of lambing on bodyweight. To some degree this is also true for the goats although in this case the overall mean gains in the infected and control groups were almost identical (3.72kg and 3.80kg respectively). As the weight changes in sheep are confused by the effects of parturition, the effect of infection cannot be distinguished. Unlike the sheep, the mean weight difference between the groups of goats had increased by week 8 but once again declined to the initial level at the end of the experiment as shown in Fig. 4.1b.

4.3.2 Faecal Egg Counts

Faecal egg counts of both infected sheep and goats are shown in Table 4.1. Infections became patent in only 4 and 8 of the 10 sheep and goats respectively. The egg counts were extremely low throughout the patent period and lower in sheep than in goats.

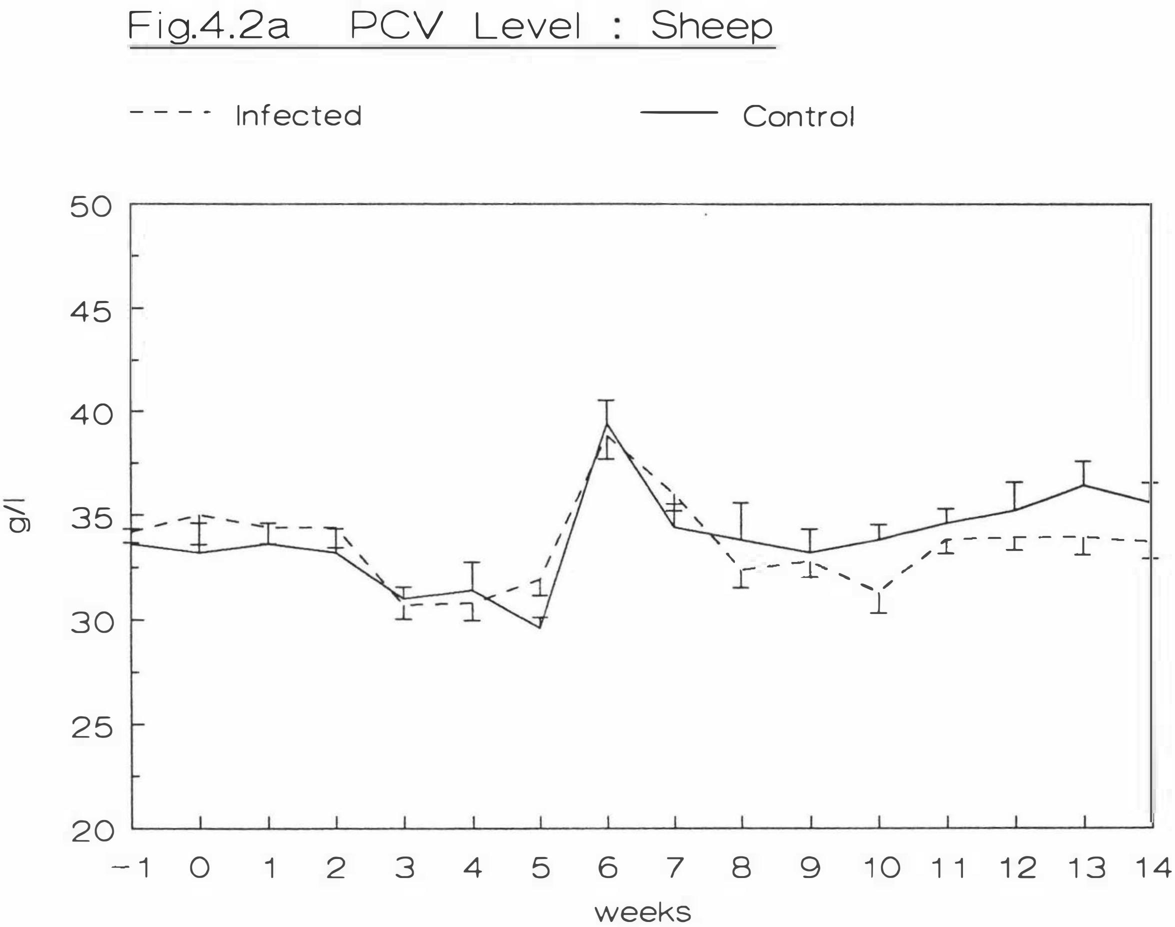


Fig.4.2a PCV Level : Sheep
- - - - Infected
Control
50
45
40
35
30
25
20
g/l
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
weeks

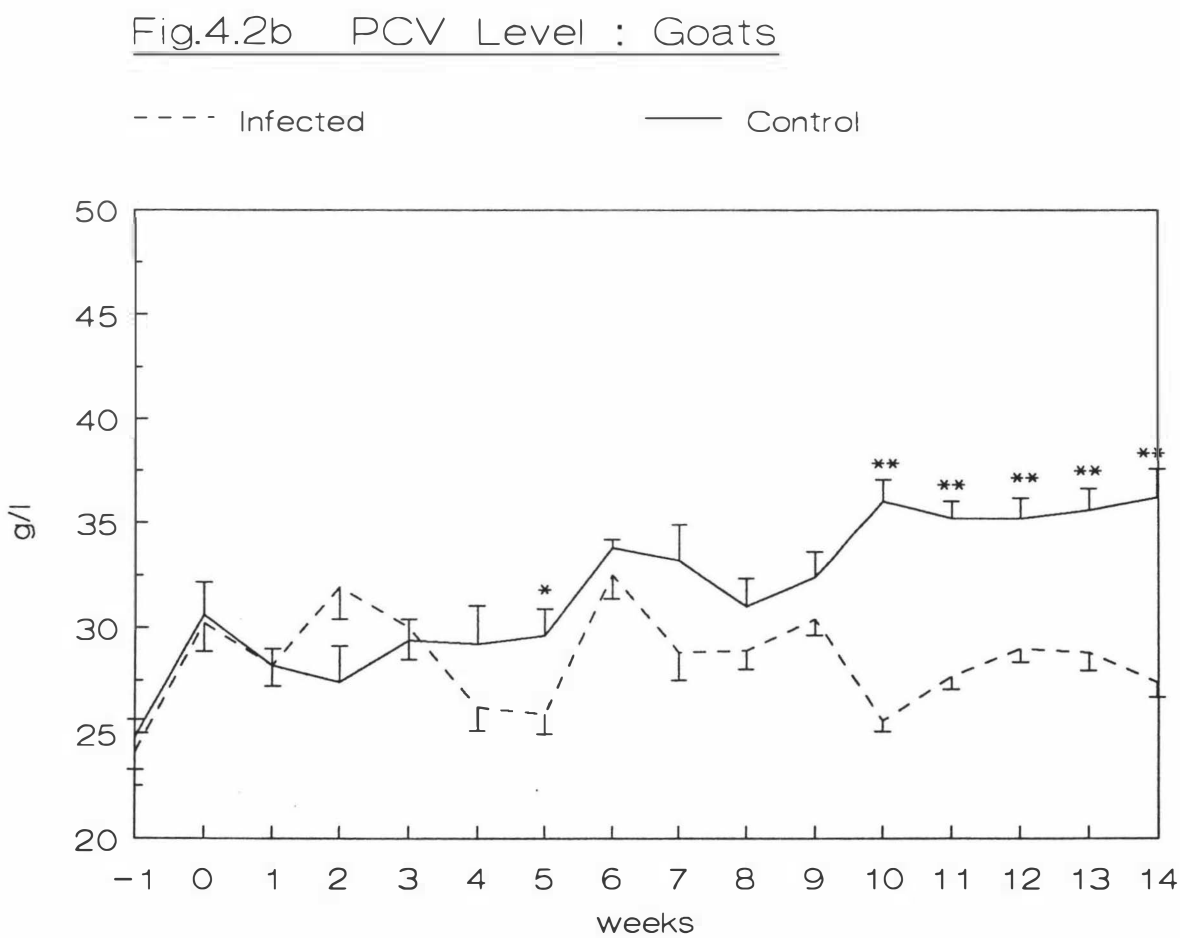


Fig.4.2b PCV Level : Goats
- - - - Infected
Control
50
45
40
35
30
25
20
g/l
*
**
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
weeks

Fig.4.3a Haemoglobin Level : Sheep

---- Infected —— Control

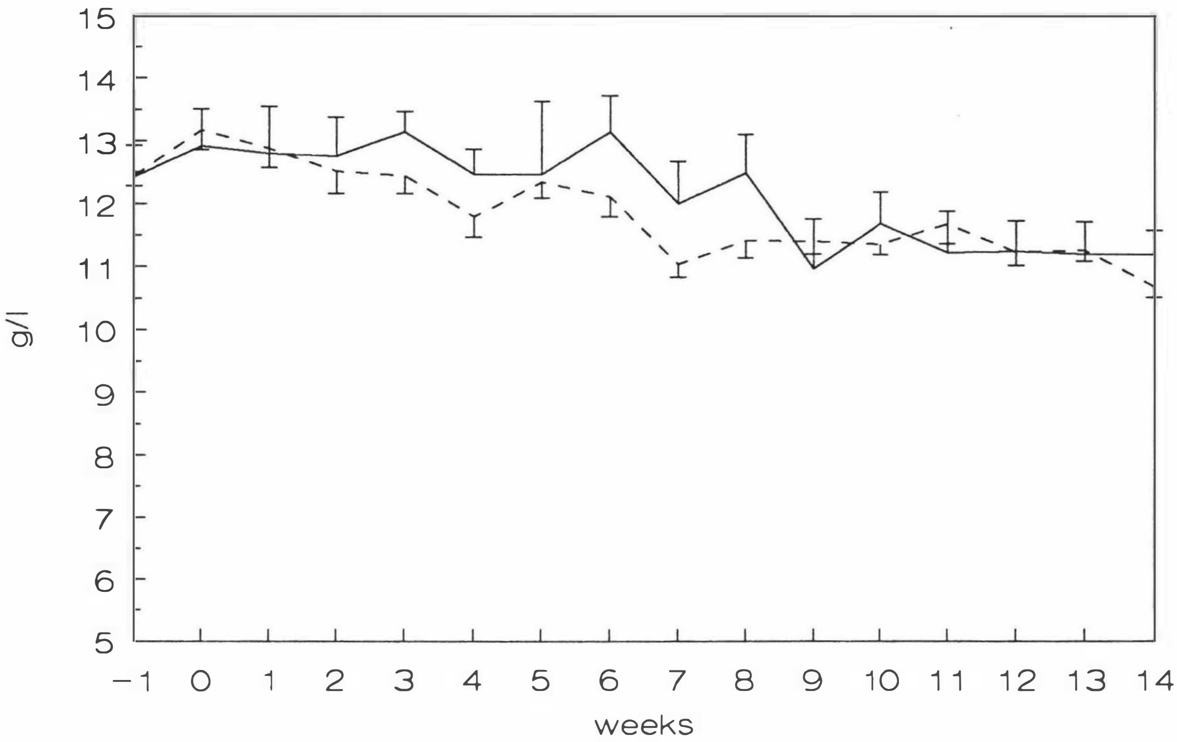


Fig.4.3b Haemoglobin Level : Goats

---- Infected —— Control

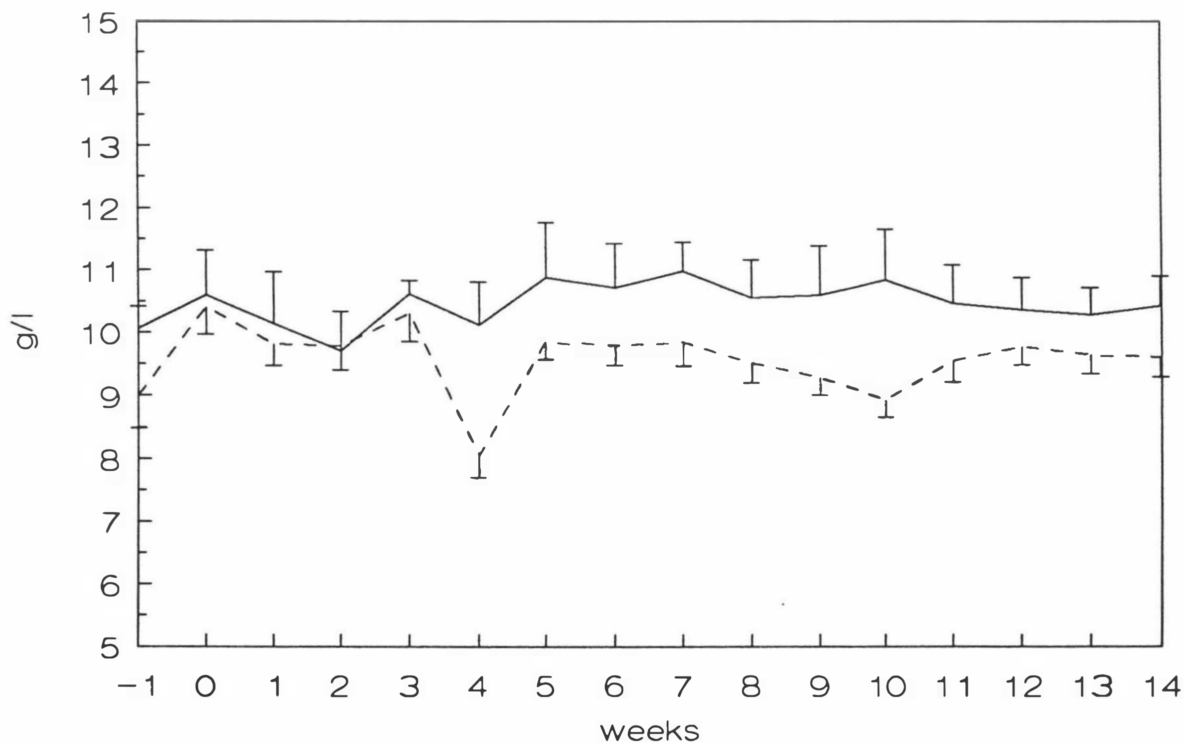


Table 4.1 Faecal Examination Results (Fasciola eggs/gm).

Weeks :	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sheep :																
1	-	-	-	-	-	-	-	-	-	-	0	0	15	10	17	20
2	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
3	-	-	-	-	-	-	-	-	-	-	0	0	0	0	15	10
4	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
5	-	-	-	-	-	-	-	-	-	-	0	5	0	10	15	5
6	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
7	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
8	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
9	-	-	-	-	-	-	-	-	-	-	0	0	0	0	30	50
10	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Goats :																
1	-	-	-	-	-	-	-	-	-	-	10	15	13	20	53	15
2	-	-	-	-	-	-	-	-	-	-	0	0	5	0	5	10
3	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
4	-	-	-	-	-	-	-	-	-	-	10	15	10	9	25	20
5	-	-	-	-	-	-	-	-	-	-	0	13	2	5	20	20
6	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
7	-	-	-	-	-	-	-	-	-	-	0	5	13	24	30	19
8	-	-	-	-	-	-	-	-	-	-	0	0	4	15	20	50
9	-	-	-	-	-	-	-	-	-	-	0	5	3	0	10	30
10	-	-	-	-	-	-	-	-	-	-	0	0	10	20	3	5

4.3.3 Haematological Parameters

The mean values ($\pm$ SE) of PCV, Hb, WBC and eosinophil counts in sheep and goats are shown in Figs. 4.2a & b, 4.3a & b, 4.4a & b, 4.5a & b and 4.6a & b respectively. Summarised and descriptive statistics are given in Appendices 2.2-2.5. Normal levels for all parameters are given in Table 1.3. Though significant differences in PCV, Hb & WBC occurred between groups as described below, levels remained within normal limits.

In the sheep (Fig. 4.2a), apart from a temporary elevation in both groups at weeks 6 & 7, the PCV level remained unchanged with no significant differences between infected and control groups. However, in the goats a significant difference between infected and control animals was recorded at week 5 and this difference increased particularly from week 9. From week 10 on, the reduction in PCV in infected animals was highly significant ($P<0.01$). In both sheep and goats levels remained within normal ranges.

In both species, haemoglobin levels showed no statistically significant differences between groups ($P>0.05$) (Fig. 4.3a, b) although in the goats, the infected group mean values were lower than in the control group throughout most of the experiment.

The total WBC counts in sheep showed no statistically significant differences between the two groups and were in the normal range. However, as shown in Fig. 4.4b, the levels recorded for infected goats were generally lower than in the controls. In the infected group at week 5 there was a peak of 11×10^9/l; thereafter, counts gradually declined to 7.5×10^9/l at the end of the experiment whereas, at this stage, the level for the control group was 9×10^9/l. Significantly different levels were recorded only at weeks 13 and 14 ($P<0.05$). Nevertheless, the counts in both groups remained in the normal range.

Absolute eosinophil levels in both sheep and goats showed significant increases in infected animals and at times exceeded the normal range. In sheep (Fig. 4.5a), the counts increased sharply at week 7 reaching a peak level of 0.765×10^9/l with significantly raised levels at weeks 8, 9, 11 and 13 ($P<0.05$). In goats (Fig. 4.5b), the levels in the infected animals increased more than 2.5 fold at week 3 and reached a peak of 1.07×10^9/l at week 5. The levels remained significantly higher than in controls until the end of the experiment ($P<0.01$).

Fig.4.4a WBC Counts : Sheep

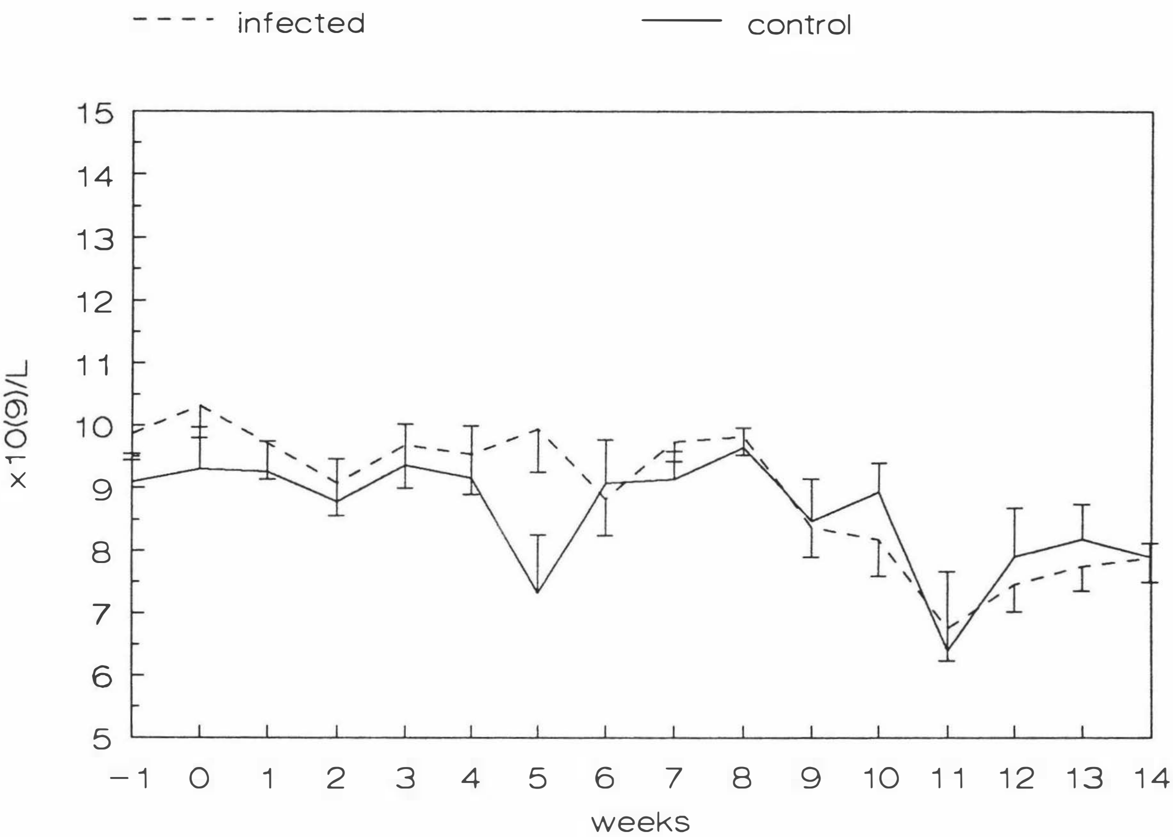


Fig.4.4b WBC Counts : Goats

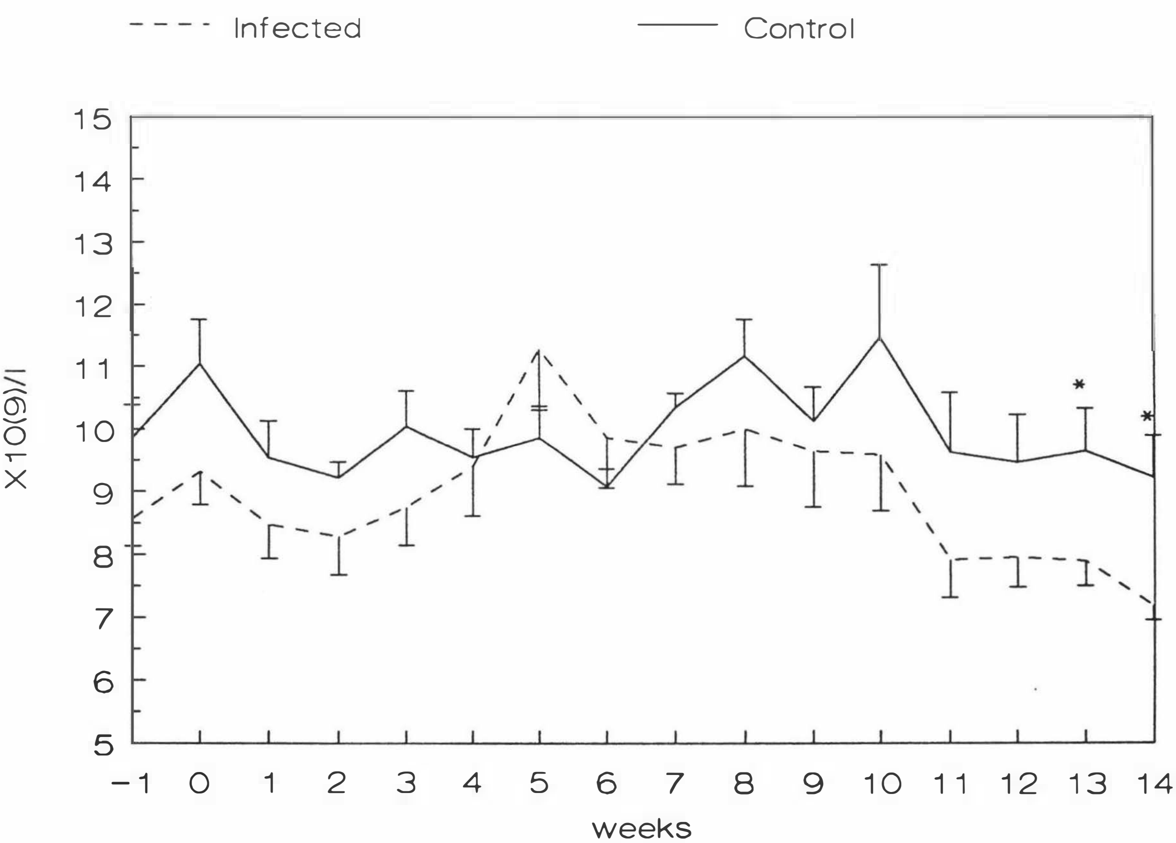


Fig.4.5a Eosinophil Level : Sheep

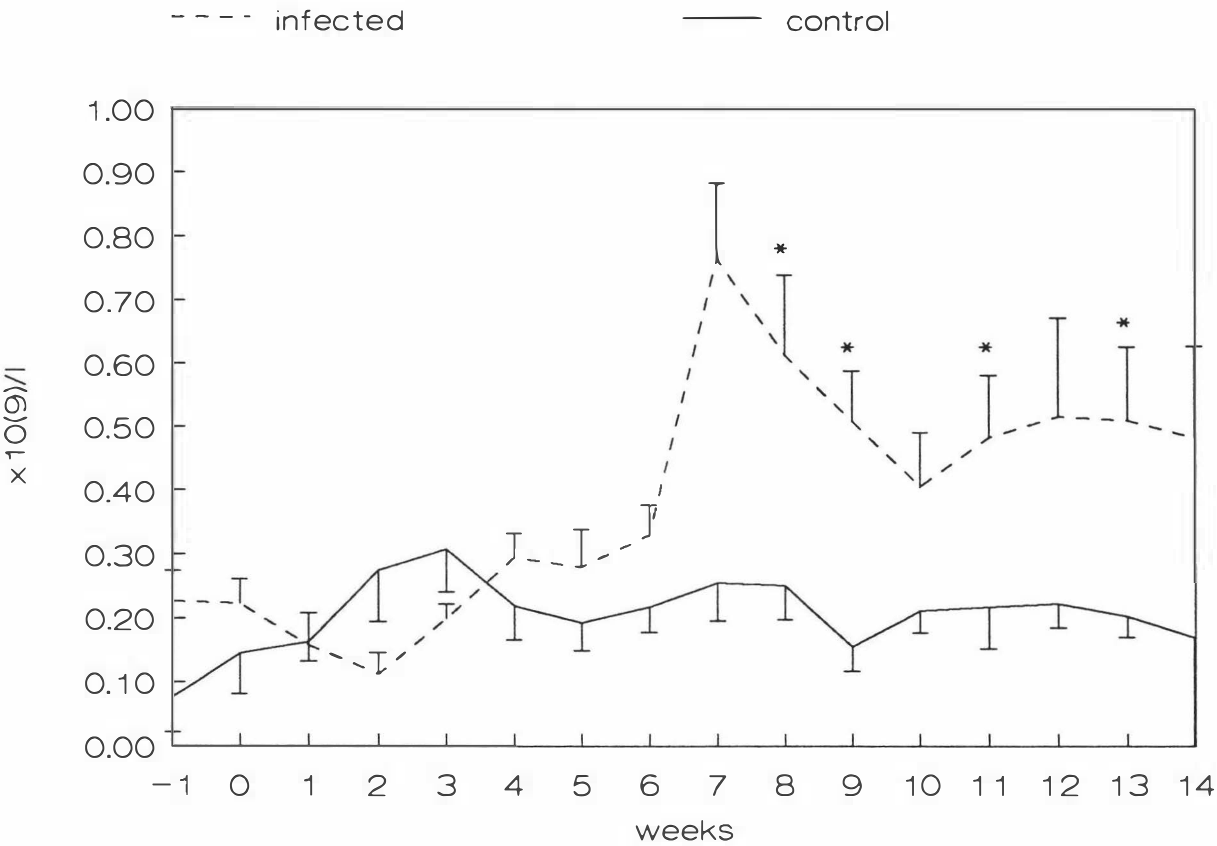
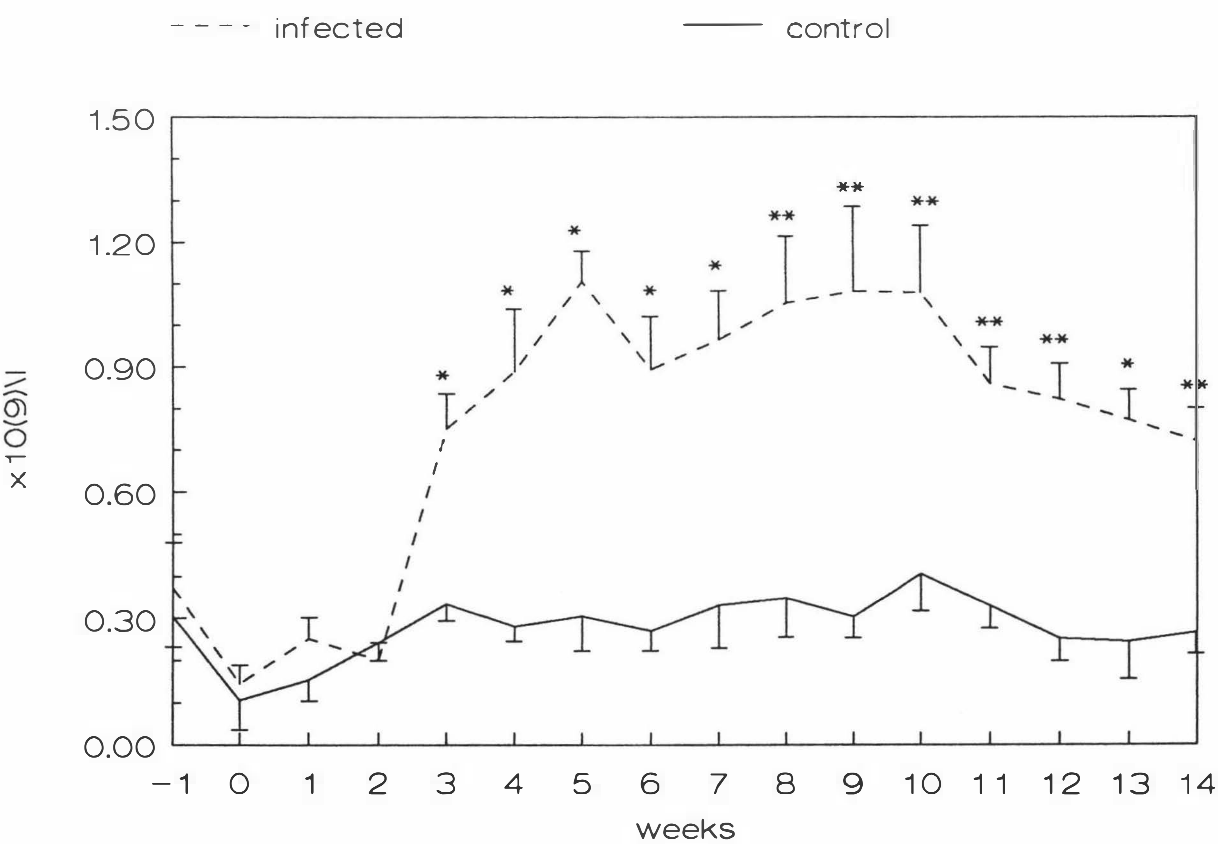


Fig.4.5b Eosinophil Level : Goats



4.3.4 Biochemical Parameters

4.3.4.1 Serum Proteins

The total serum protein, albumin, globulin and A/G ratio data are shown in Figs. 4.6a, 4.6b, 4.7a, 4.7b, 4.8a, 4.8b, 4.9a and 4.9b respectively. The data are summarised in Appendices. 2.6-2.9 respectively; (See also Appendix 3)

Total serum protein levels tended to decline in the control group of sheep throughout the experiment and levels in the infected sheep were significantly higher than the controls at the later stage of the experiment (ie. weeks 11, 12 and 13). In the goats, significantly higher levels than in the controls were recorded at weeks 5, 7 & 8 with a peak level at week 7 of 81.10 g/l. (Fig. 4.6b). At the end of the experiment, mean levels in the two groups were almost identical. In both sheep and goats total serum protein levels remained within the normal range.

The serum albumin levels in the infected sheep, as shown in Fig.4.7a, were significantly lower than controls in weeks 6, 7, 9 and 14 ($P<0.05$-0.01). As shown in Fig. 4.7b, the serum albumin in goats remained unchanged up to week 9. Thereafter, the mean level in infected animals was slightly lower than in controls and significantly different at weeks 10, 13 and 14 ($P<0.05$). In both sheep and goats levels were generally within the normal range (Table 1.3).

In sheep, serum globulin levels gradually decreased in both groups. However, they tended to be higher in the infected group and were significantly higher at weeks 2, 7, 9 and 10 ($P<0.05$). As shown in Fig. 4.8a, the globulin level in the control group dropped considerably between weeks 5 and 10 with a dramatical fall at week 9 to 20g/l. By week 11 the level had recovered and was similar to that of the infected group for the remainder of the experiment. From week 1, levels in control sheep were below the normal range. This was also the case in the infected group from week 8 onwards. In goats, there was no significant difference between the two group means throughout the experiment (Fig. 4.8b) and levels remained stable in the normal range.

In both groups of sheep the albumin/globulin ratio increased over the first five weeks of the experiment. The mean ratio in the control sheep was significantly higher than in infected animals as early as week 2 post-infection, as shown in Fig. 4.9a, and it remained slightly higher up to week 8 when the level suddenly rose to a peak of 1.80. It then gradually declined down to the initial level at week 11, but this level was still significantly different from controls ($P<0.01$) and remained higher for the rest of the experiment.

Fig.4.6a Serum Protein : Sheep

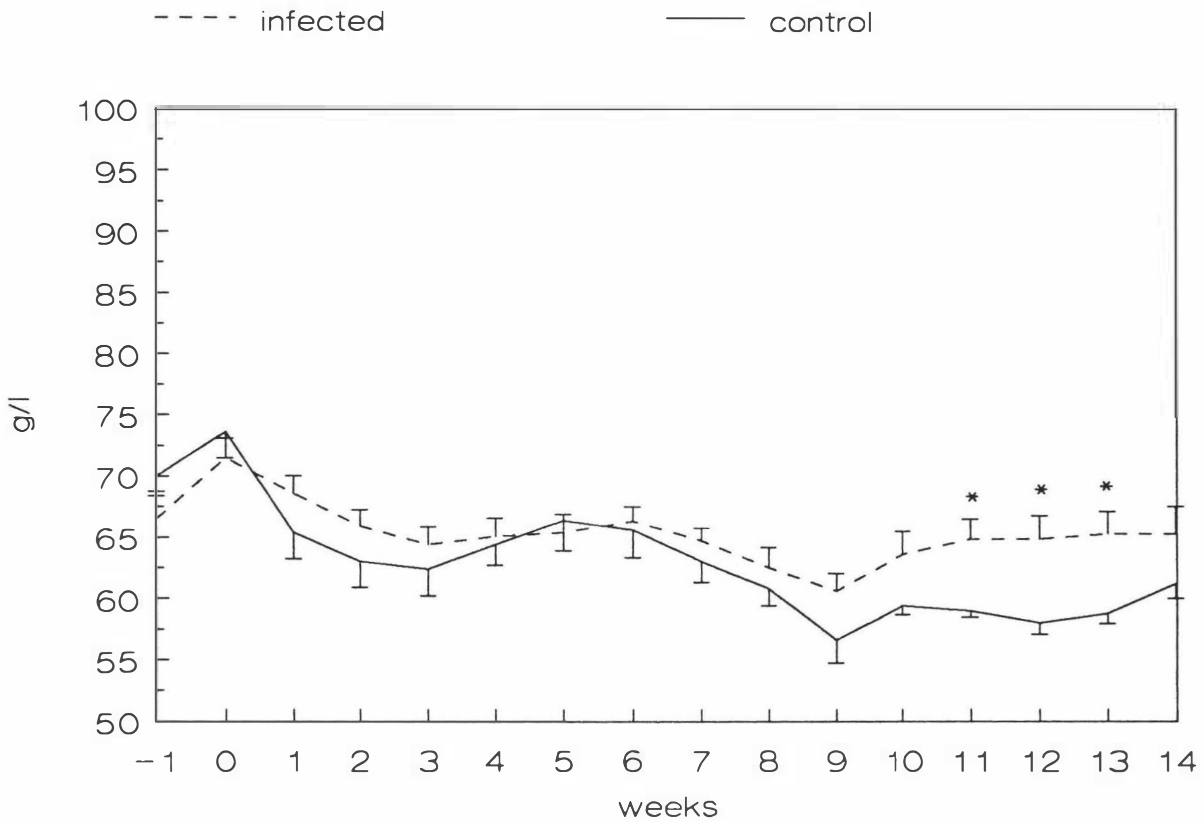


Fig.4.6b Serum Protein : Goats

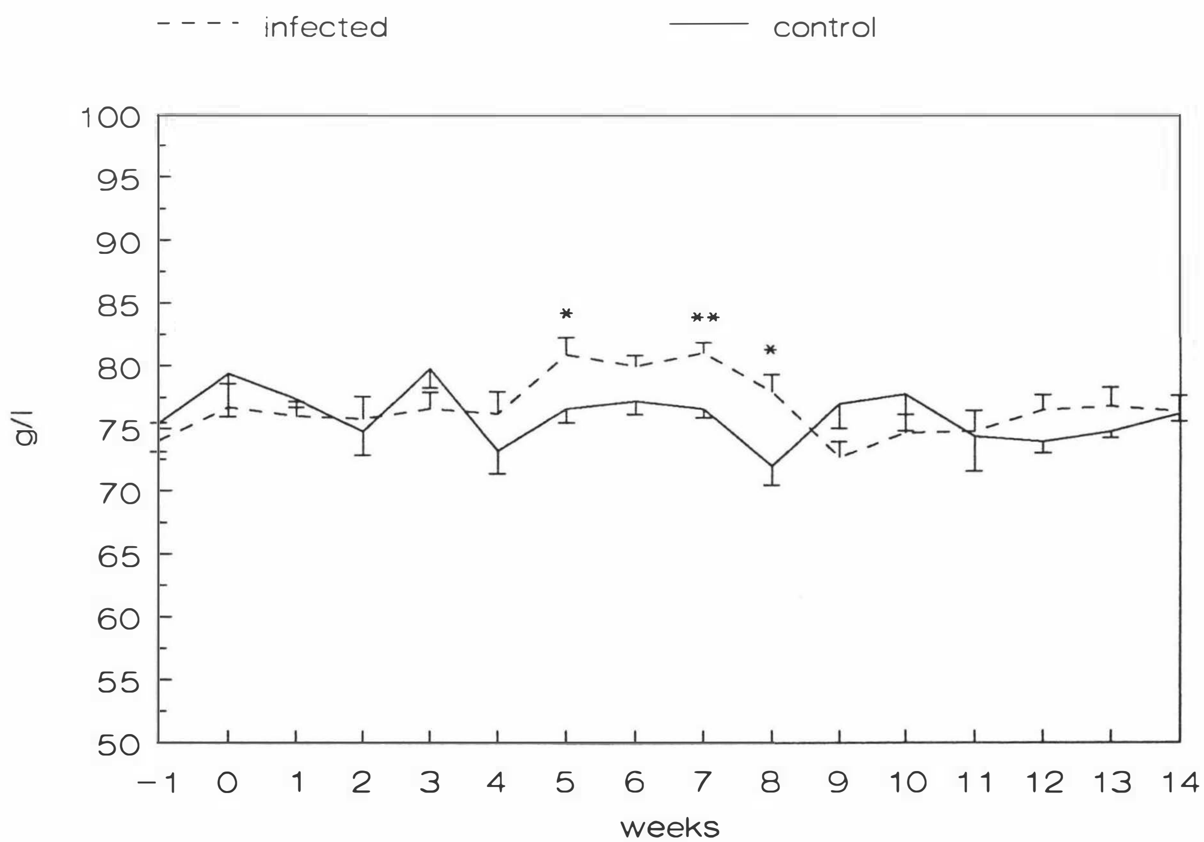


Fig.4.7a Serum Albumin : Sheep

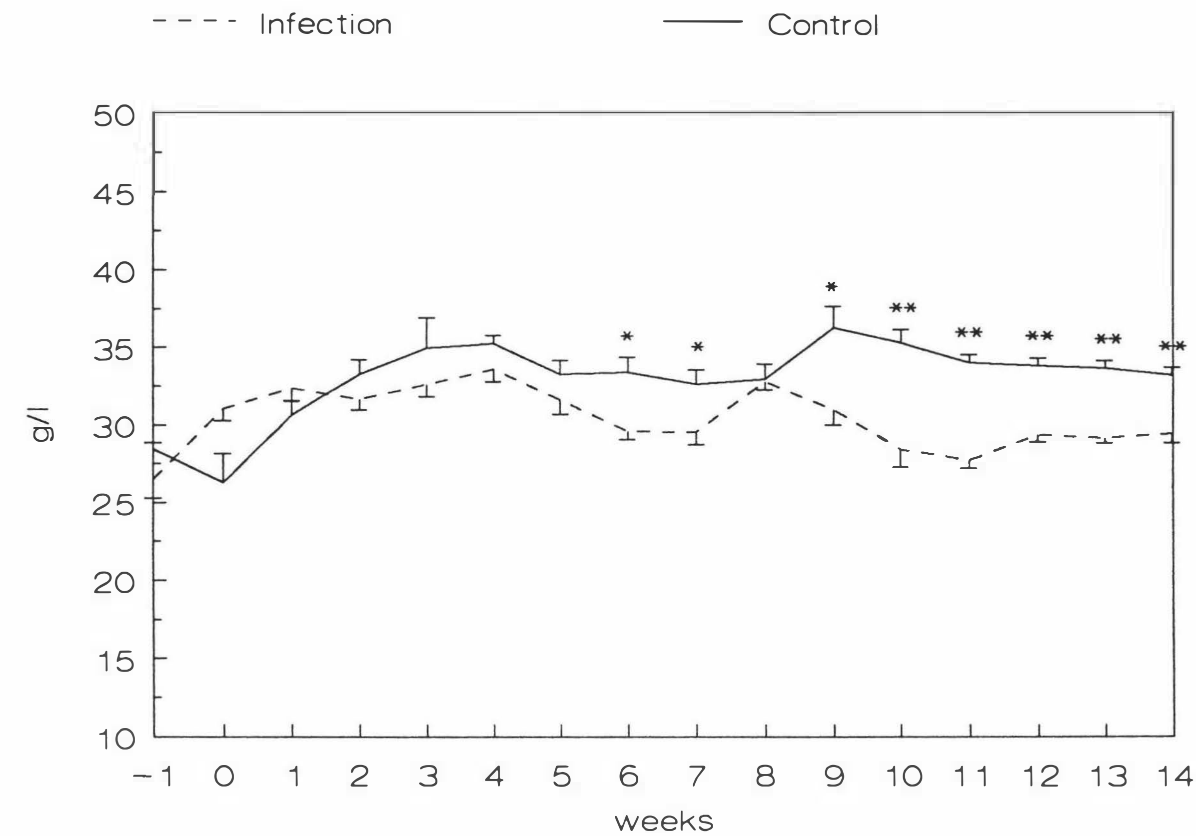
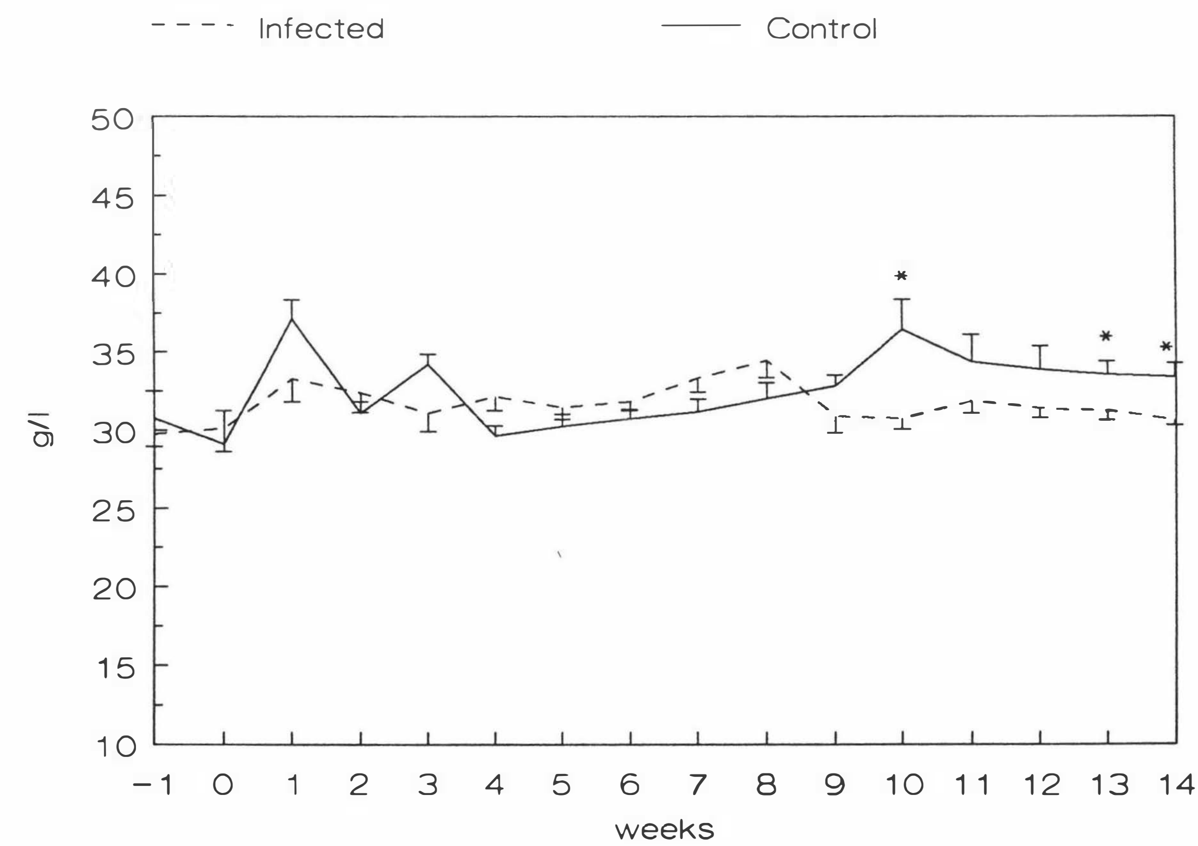


Fig.4.7b Serum Albumin : Goats



In goats, the A/G ratio remained almost constant and was not significantly different between groups throughout the experiment except in weeks 10 and 14 as shown in Fig. 4.9b. Levels were within the normal range throughout.

4.3.4.2 Enzymes

The serum AST, GGT and GD levels in sheep and goats are shown in Figs. 4.10a, 4.10b, 4.11a, 4.11b, 4.12a and 4.12b respectively.
The data are summarised in Appendices 2.10-2.12 respectively. Normal levels are given in Table 1.3.

The AST levels in sheep showed no significant differences between the two groups throughout the experiment as shown in Fig. 4.10a. In the first three weeks, the AST level in the control animals rose slightly and then declined while in the infected group it declined to a similar level at week 2 from a slightly higher starting point. Over the next four weeks levels remained steady in both groups and then rose to week 9. In the infected group, levels then fell to rise again from week 11 to the end of the experiment whereas in the controls, a peak level was reached at week 11 and this was followed by a decline. Throughout, levels remained within normal ranges (Table 13) although there were considerable variations in level within both groups.

In the goats, however, the level of AST in the infected group started to rise at week 5 and was significantly higher than the controls at week 7 ($P<0.05$). At week 8 the level reached a peak of 218 U/l (Fig. 4.10b) (exceeding the normal range). However, for the remainder of the experiment the difference from the controls was not statistically significant ($P>0.05$) as, even though the levels were still moderately elevated, they were very variable within the infected group. The level of AST gradually decreased reaching the initial level by the end of the experiment. Levels in some control animals increased towards the end of the experiment.

Fig.4.8a Serum Globulin : Sheep

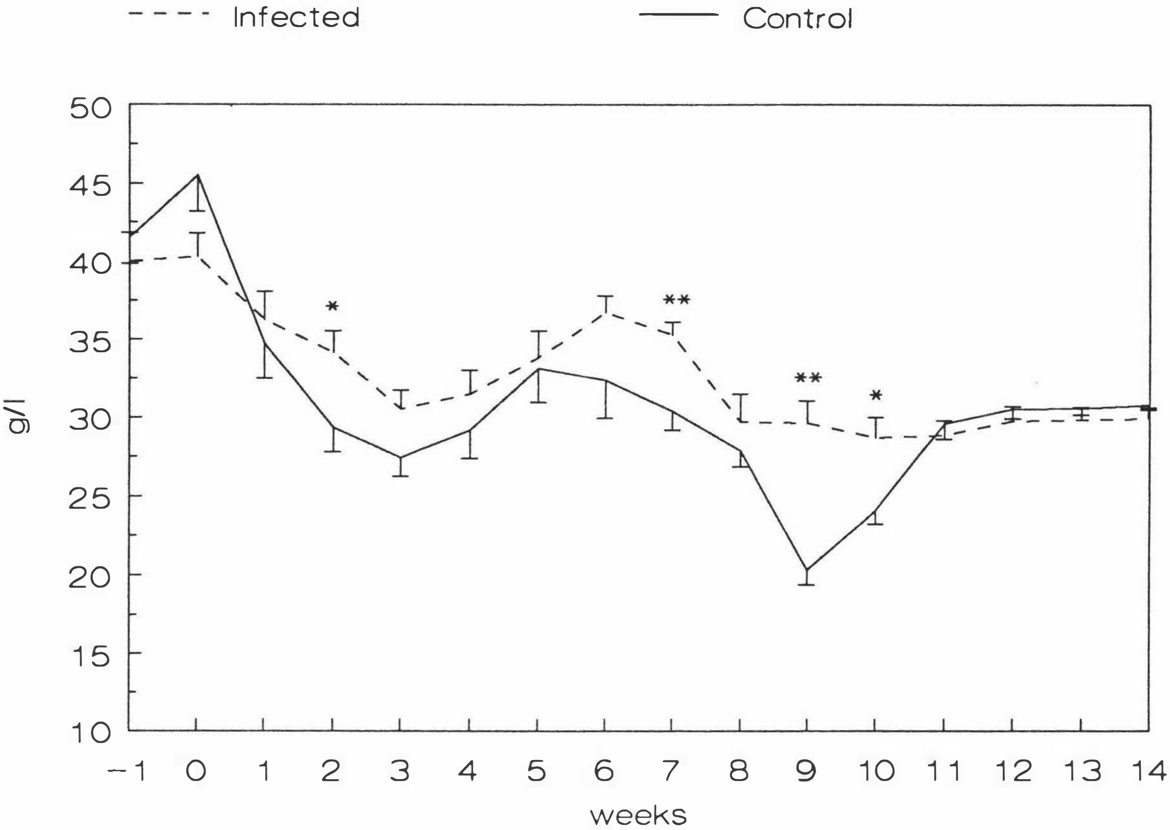


Fig.4.8b Serum Globulin : Goats

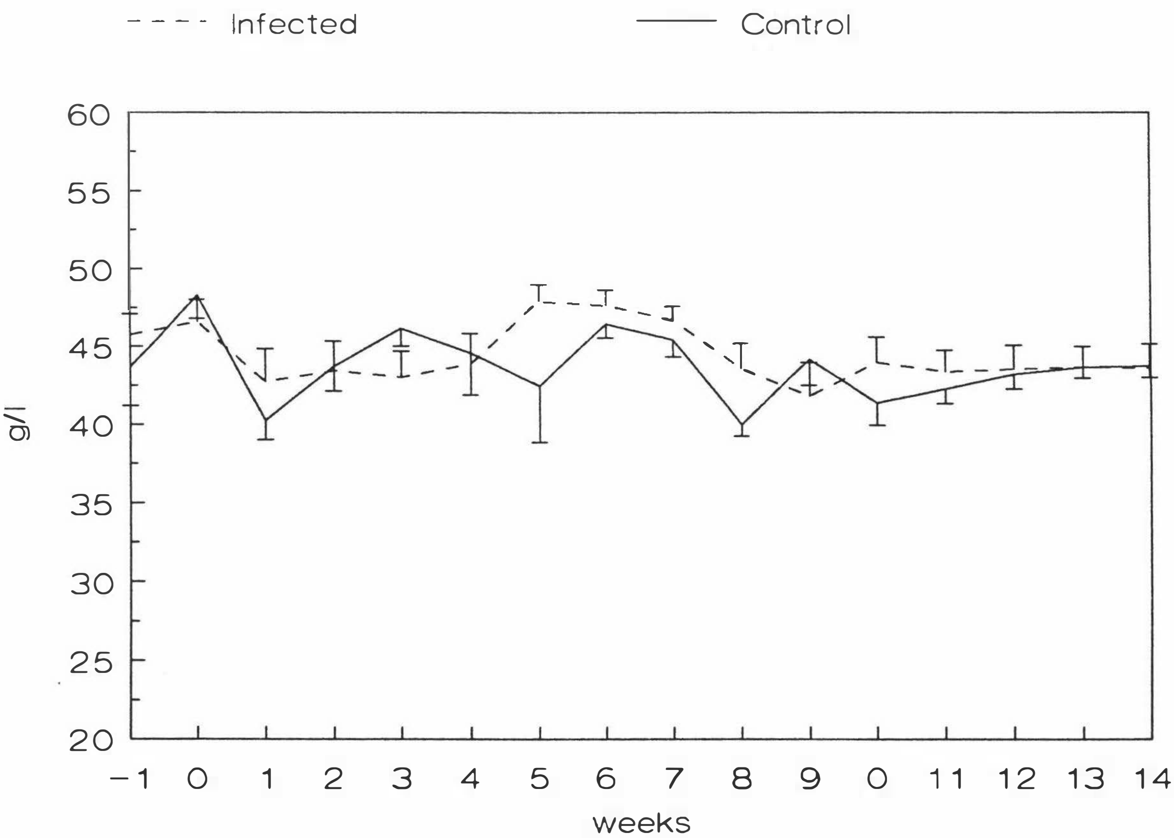


Fig.4.9a A/G Ratio : Sheep

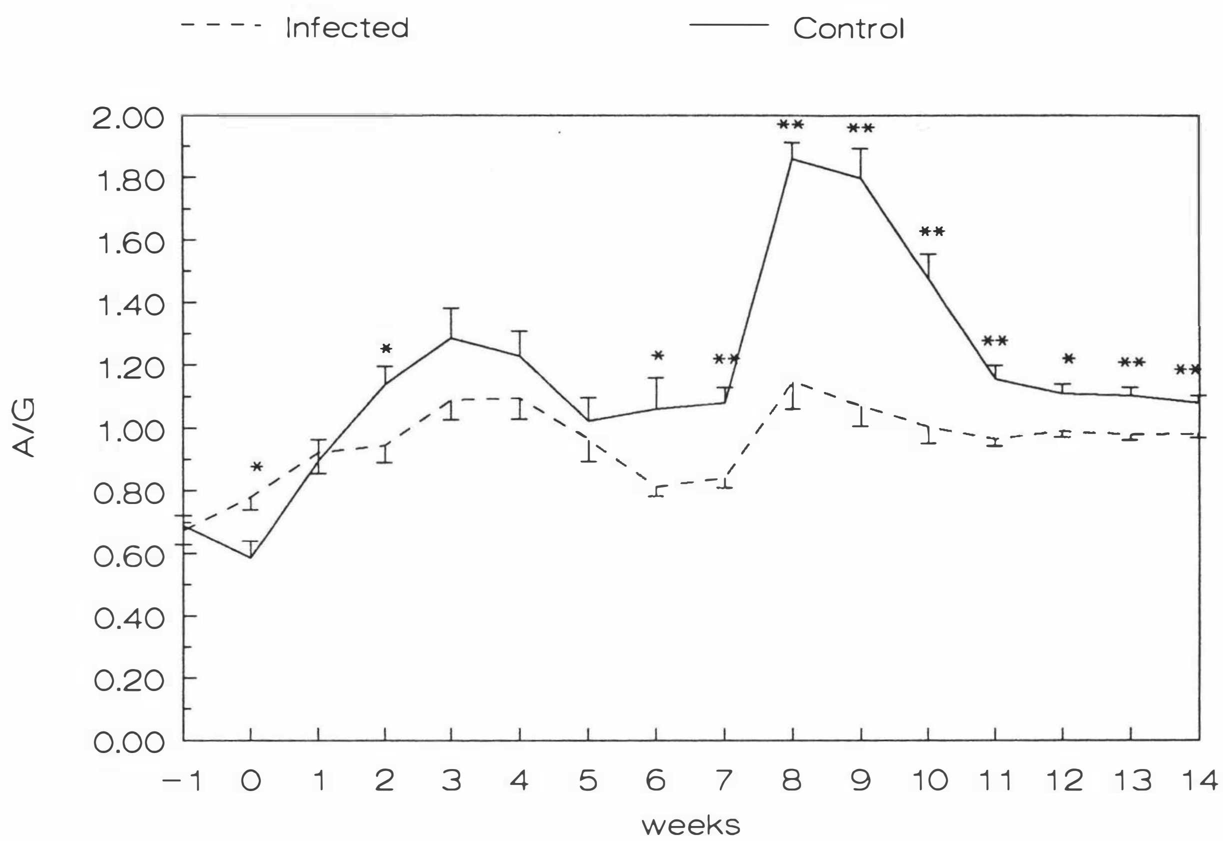
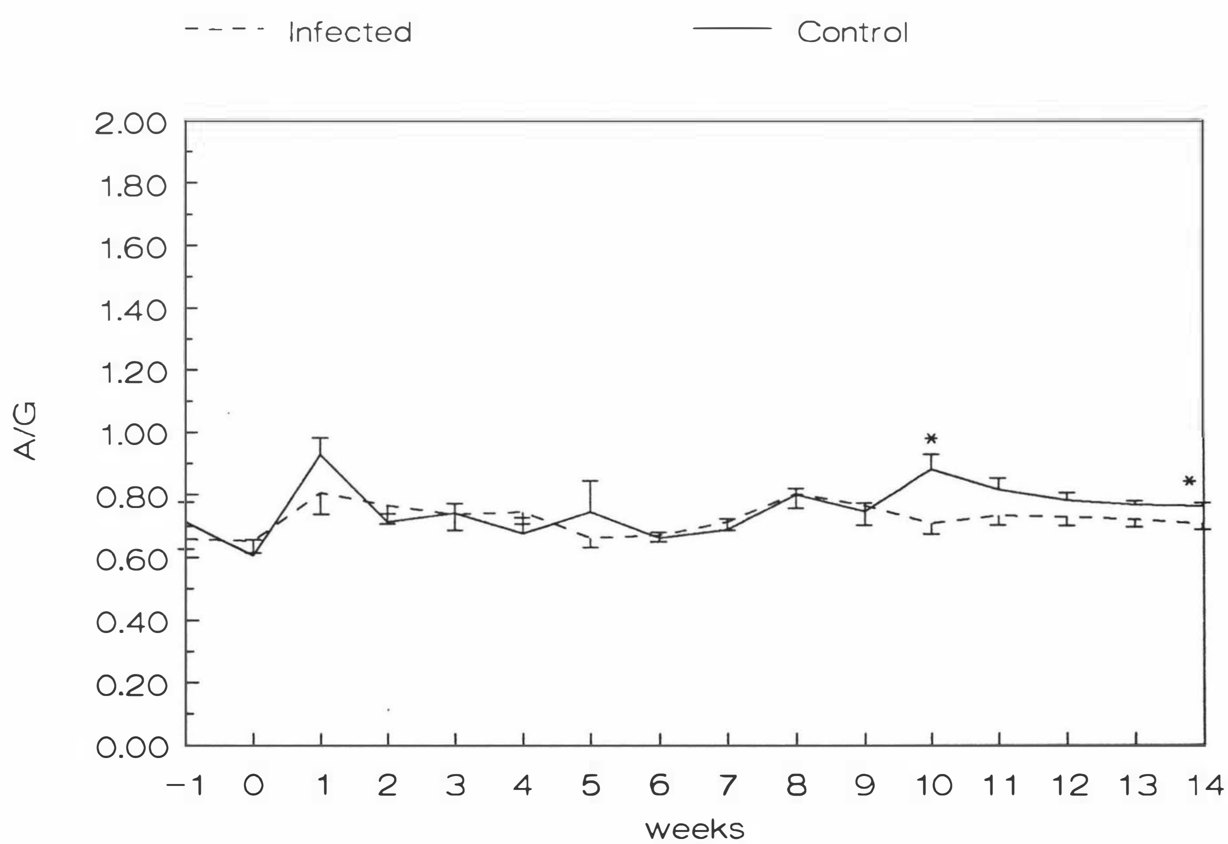


Fig.4.9b A/G Ratio : Goats



The GGT levels in both groups of sheep were in the upper part of normal range at the start of the experiment and later exceeded normal levels in both groups. The level in the infected sheep (Fig. 4.11a) increased slowly from the start of the experiment and more rapidly from week 7. Levels were significantly different from controls at week 9 and remained higher for the remainder of the experiment ($P<0.05$). Levels also increased slowly in control animals throughout the experiment. In goats, (Fig. 4.11b) the mean serum GGT level in the infected group increased sharply between weeks 7 & 8 to a level of 70 U/l but significant differences from controls were recorded only at weeks 8 and 12 because of the wide range of levels in individual animals. Mean levels exceeded the normal range in weeks 8-10. The level gradually decreased from week 10 and returned to the initial level by week 14. Levels in control goats showed no trends during the experiment and remained within the normal range.

The serum GD level (Fig. 4.12a) in infected sheep remained at normal levels up to week 7 but from then on increased steadily to 230 U/l at week 14. In the control group, the level remained within the normal range up to week 10 but, at week 11, the level increased three-fold from the previous week and then returned almost to the normal range in the following few weeks. Since there were large variations of levels in individual animals, no significant differences between groups were recorded.

The serum GD levels in infected goats (Fig. 4.12b) started to rise at week 4 and significantly elevated levels were recorded at weeks 4, 5, 6 and 7. Although many of the remaining weeks also showed high mean levels of GD, differences from controls were not statistically significant because of the extremely wide range of levels in the infected group. The mean level declined from week 8 to the end of the experiment though remaining above normal levels until the last week. The control group level remained low up to week 10 and gradually increased for the following few weeks and then returned almost to its initial level by the end of the experiment.

Fig.4.10a AST : Sheep

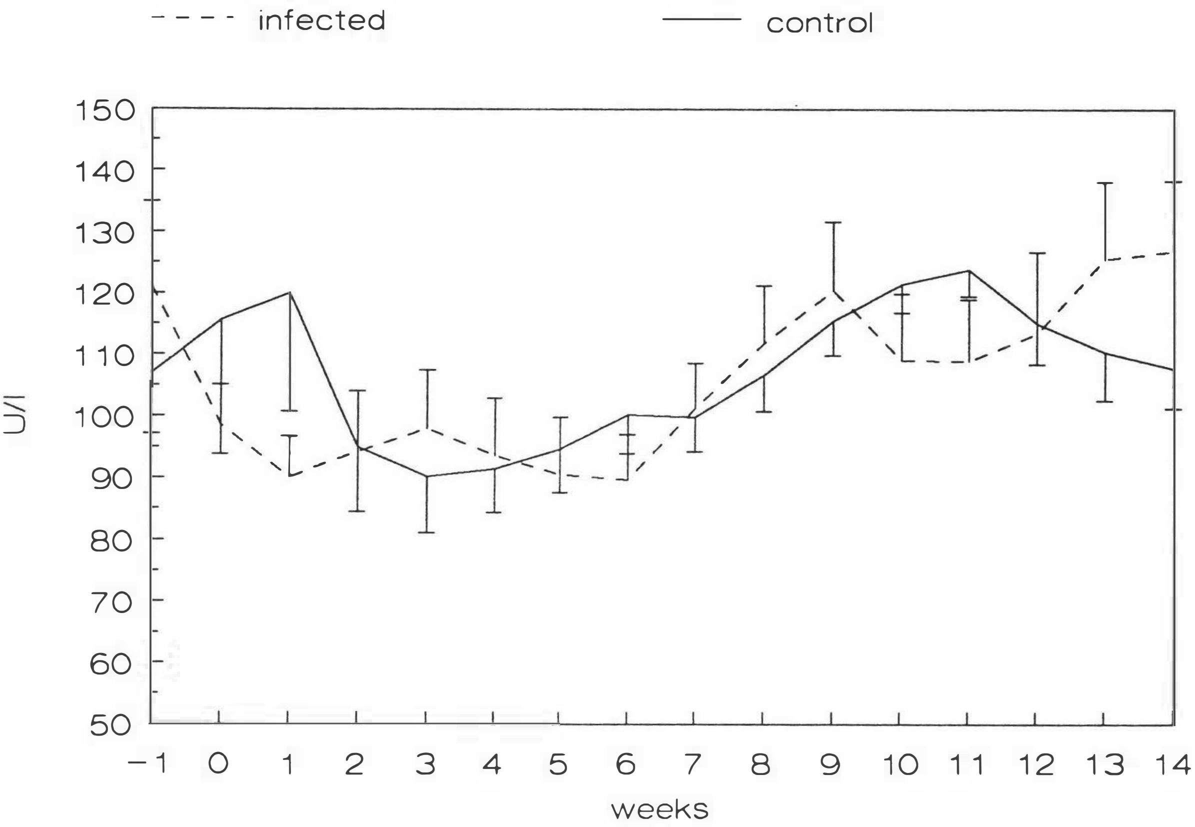


Fig.4.10b AST : Goats

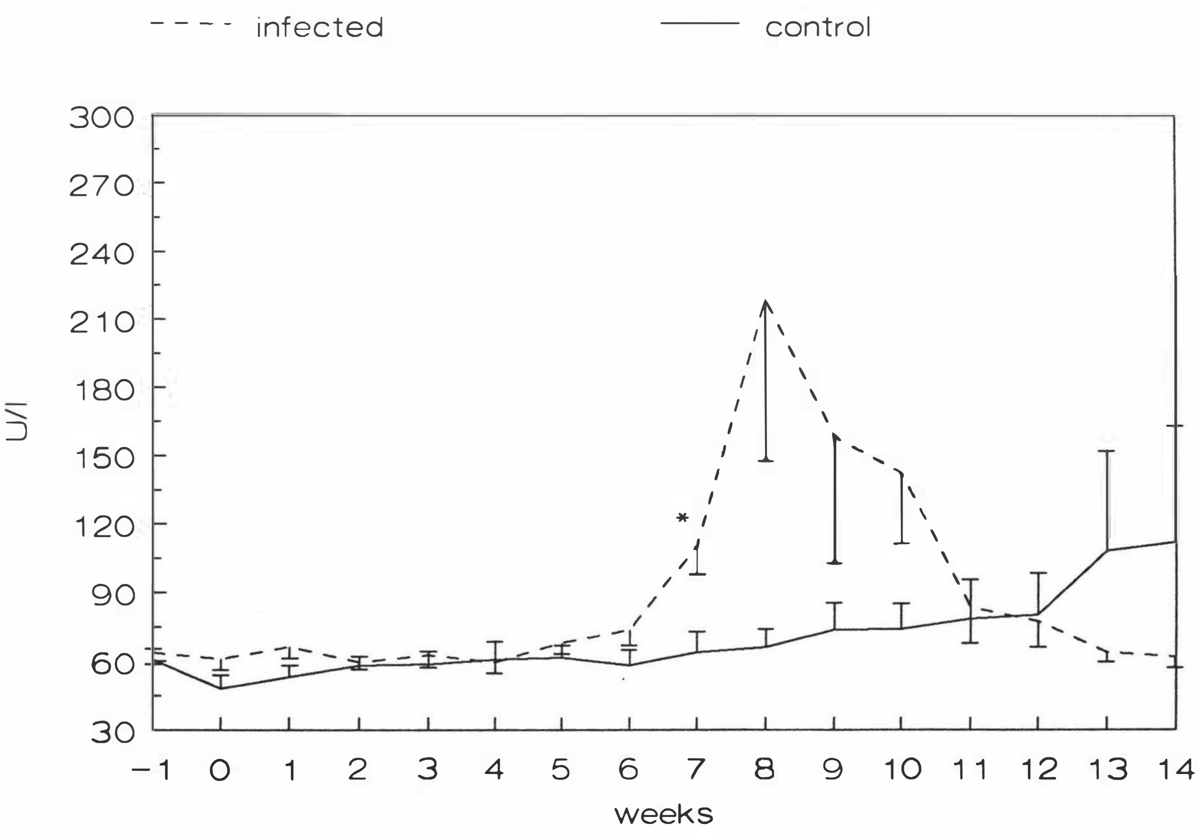


Fig.4.11a GGT : Sheep

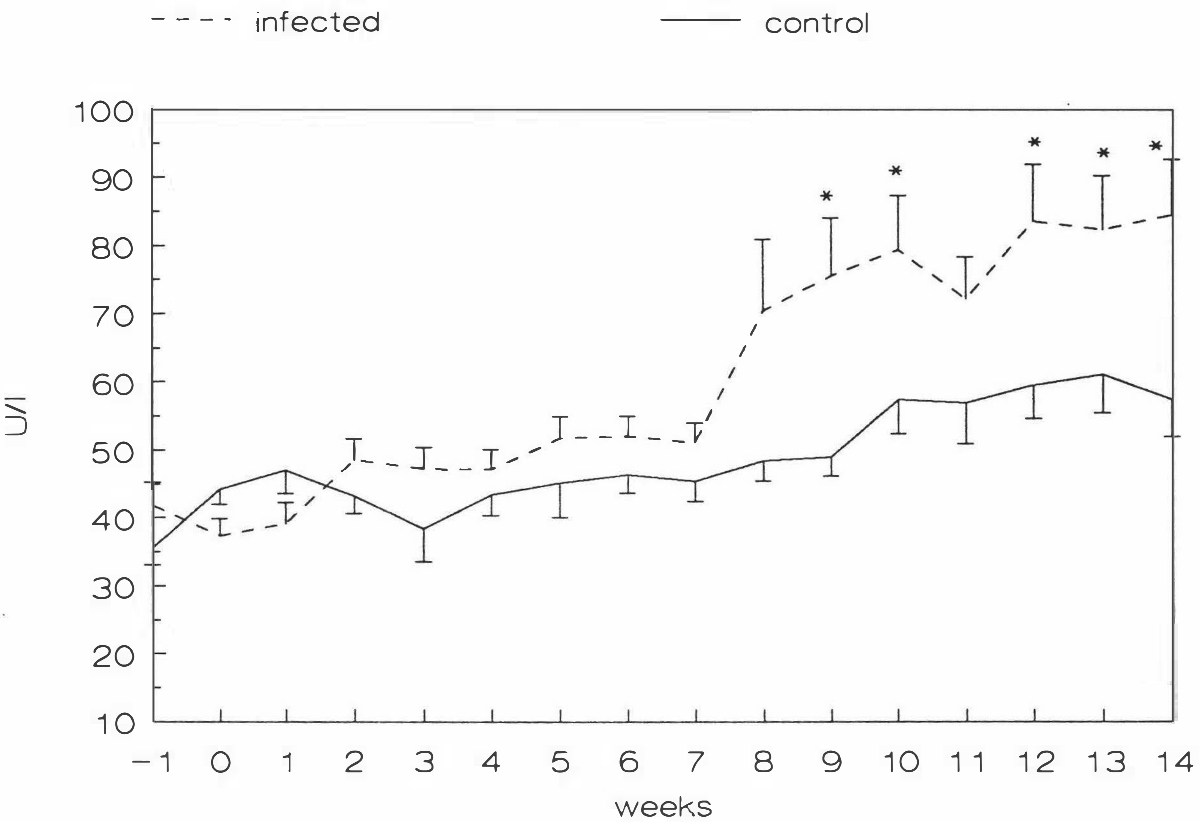
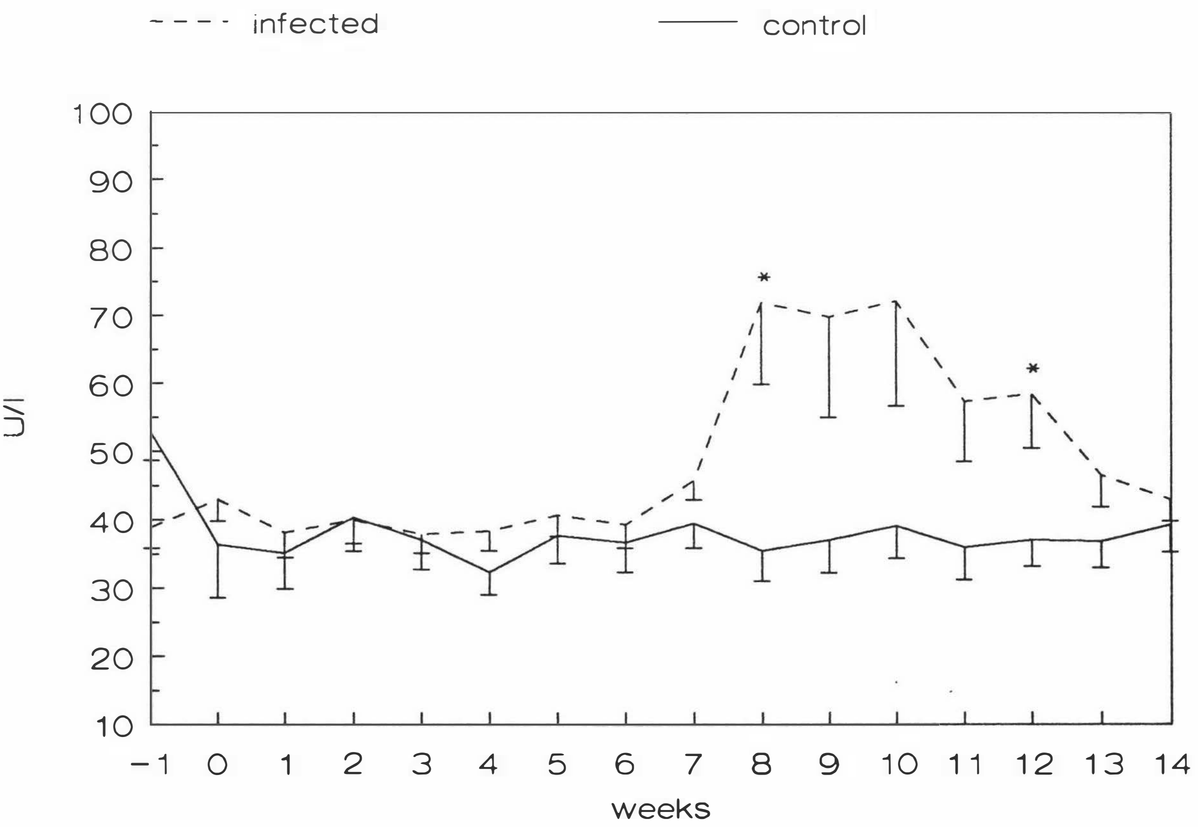


Fig.4.11b GGT : Goats



4.3.5 Necropsy Results

As shown in Table 4.2, flukes were recovered from only 4 and 8 out of 10 infected sheep and goats respectively and the number of flukes recovered in each case was very small. The in vitro viability test carried out one day before the animals were infected, indicated an 18% viability of the metacercariae. However, the mean establishment rate in the sheep was only 0.85% and in goats was 2.95%

Prominent gross pathological lesions were observed in the majority of the sheep livers. In most cases, the left lobes of sheep livers were shrunken to a variable degree. Some lobes were irregular in shape with larger or smaller areas of congestion on their surface. Incision of the livers also showed varying degrees of fibrosis in some from both control and infected animals. The left lobe of Sheep 12 from the control group was totally atrophied and smaller than the accessory lobe (Fig. 4.13). The lesions indicated that many of the sheep could have been previously affected by mild chronic facial eczema.

Table 4.2 Adult Flukes Recovered At Necropsy.

Animal No.	Fluke Recovered	
	Sheep	Goats
1	3	4
2	0	5
3	4	0
4	0	5
5	5	7
6	0	0
7	0	7
8	0	12
9	6	11
10	0	8
Mean recovery rate :	1.7	5.9
Establishment rate :	0.85%	2.95%

Fig.4.12a GD : Sheep

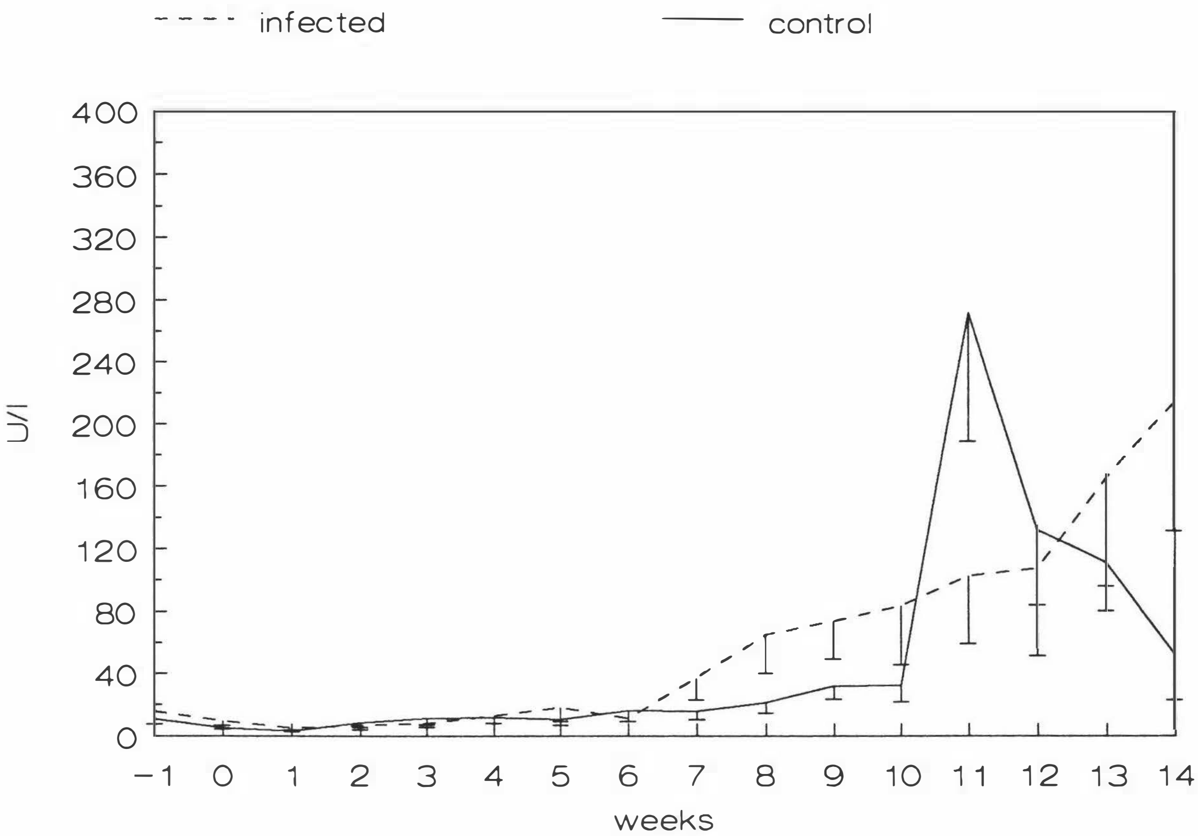
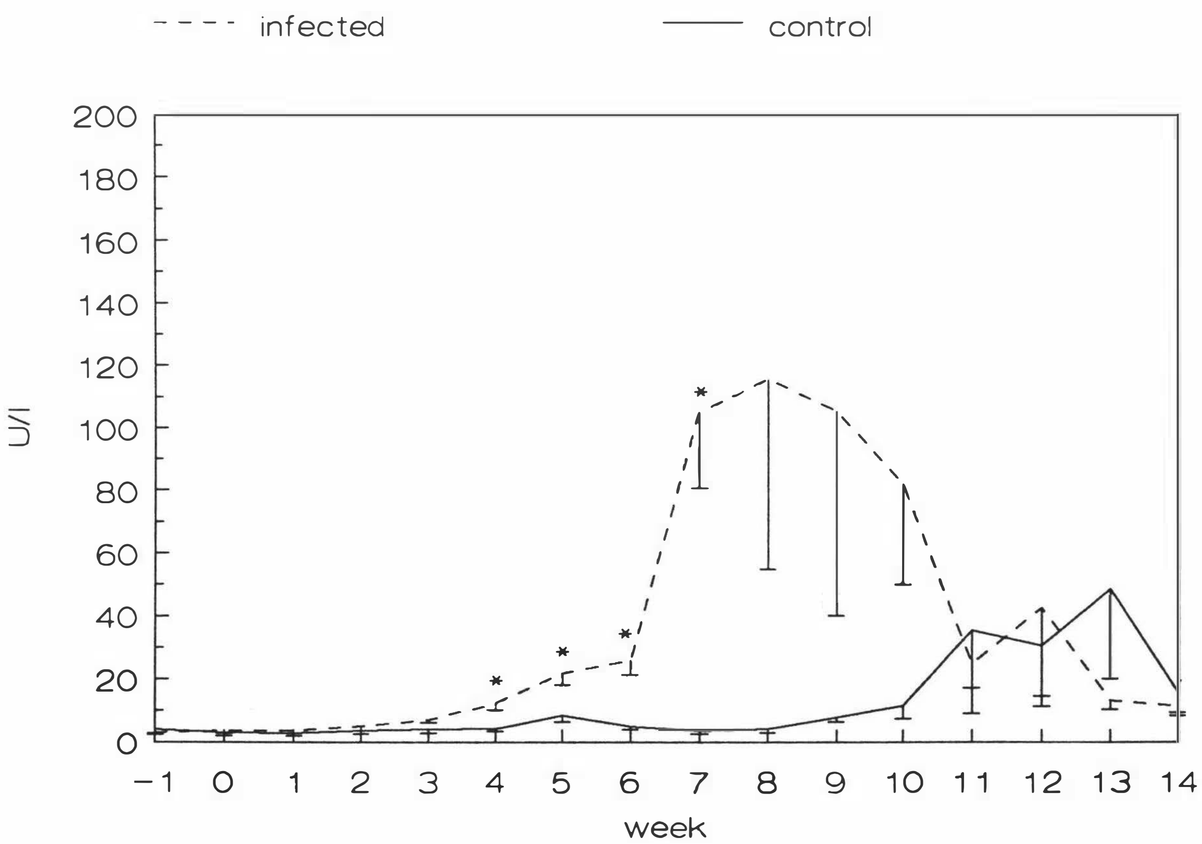


Fig.4.12b GD : Goats



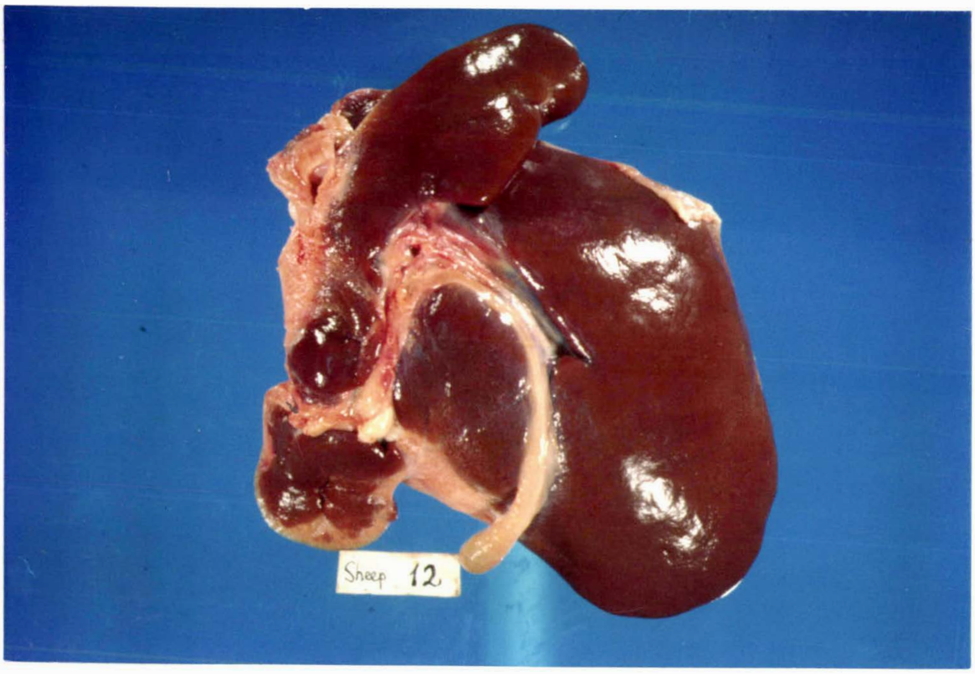


Fig.4.13 Liver of Sheep 12 showing the left lobe totally atrophied reduced to a size smaller than the accessory lobe.

Fig 4.14 The left lobe of the liver of Goat 21 showing a large area of mild fibrosis.

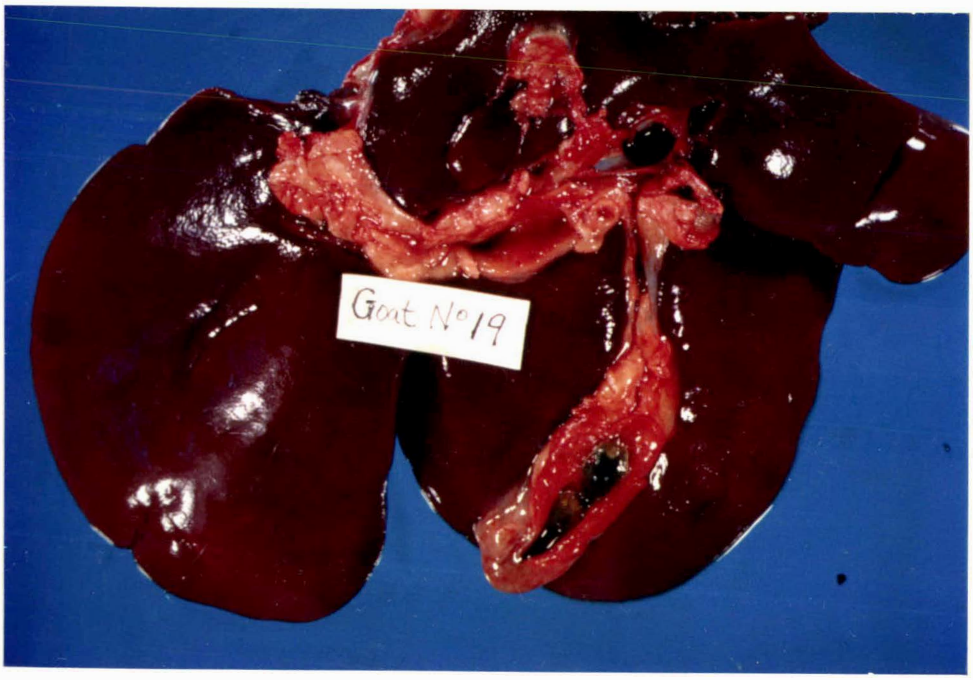


Fig. 4.15 Liver of goat 19 showing large flukes in the gall bladder and no fluke present in the bile ducts.

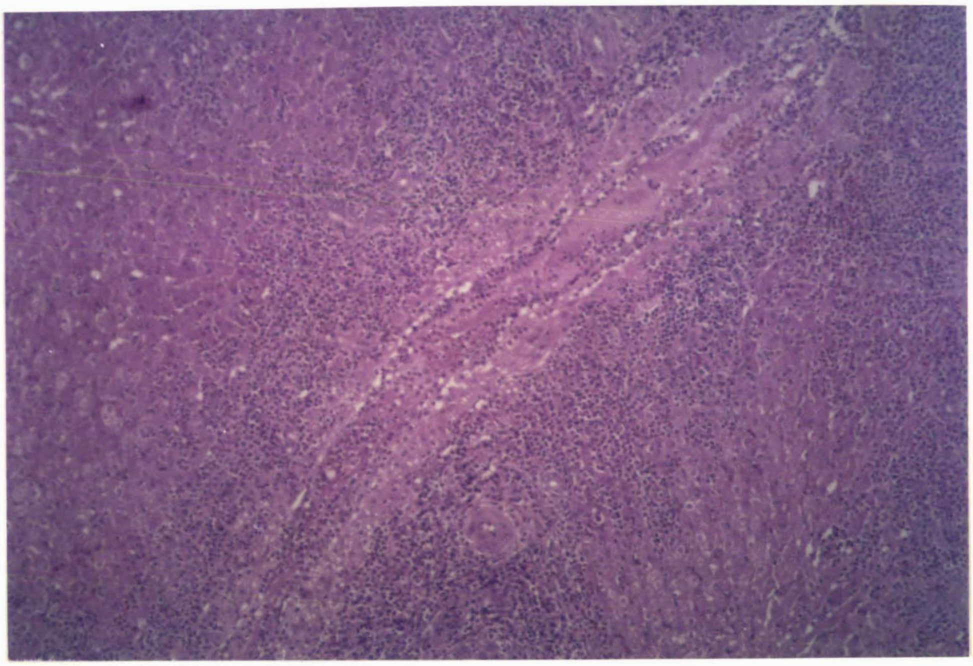


Fig. 4.15 Migratory tracks in the parenchyma with a large number of giant cells and other inflammatory cells infiltrating the tissue along the sides of the track.

Other lesions also present in the livers of some sheep and goats were similar to those explained in Experiment 1 but relatively much smaller in number and size. However, the liver of goat 21 showed a large area of mild fibrosis on the surface of the left lobe. No fluke was recovered from this goat at necropsy (Fig. 4.14).

Apart from occasional lesions possibly attributable to migrating Fasciola and a few isolated small rounded lesions observed on the surface, the livers of the infected goats looked relatively normal at necropsy.

In the infected group of sheep, the adult flukes were recovered from the large bile ducts near the entrance to the gall bladder. However, in the goats, the majority of the flukes were found in the gall bladder (Fig. 4.15).

Histological examination of the sheep livers from both infected & control groups showed variable tissue reactions with mononuclear leucocytes and other inflammatory cells infiltrating mainly in and around the connective tissue surrounding the bile ducts in some portal areas. The lesions were consistent with chronic facial eczema. Microscopic examination of the liver sections from infected goats showed changes similar to those described in the previous chapter but less marked. Migratory tracks were observed in some areas of the parenchyma. The lesions were consistent with the low level fluke infection (Fig. 4.16).

The livers of control groups of both sheep and goats showed no evidence of Fasciola infection.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

Fasciola is prevalent in most areas of the world and results in economic losses in many countries involved in livestock production, primarily through the loss of animal production and the condemnation of infected livers. A large amount of research on subclinical chronic fascioliasis has been done on sheep & cattle. Studies on sheep infected with F.hepatica have shown that there is a good correlation between the level of infection and bodyweight, as well as with a variety of haematological parameters and serum enzymes at a relatively low level of infection (Rowlands & Clampitt, 1979). However, fascioliasis in goats, either in its own right or in comparison with other ruminants, has not received adequate attention. The lack of information in goats prompted this study into subclinical infection and a comparison with similarly infected sheep.

5.1 Experiment 1

In the first experiment, although there were only five infected animals and only 15-35 flukes established, measurable and, in many cases, statistically significant changes in a variety of parameters were observed. Particularly noteworthy are the changes in PCV and serum enzyme levels. It is remarkable that a depression in PCV relative to controls of approximately 20% occurred with so few flukes established in the bile ducts for a comparatively short period of time. Though Hb, MCV and MCHC levels remained well within the normal ranges, the RBC levels in the infected group were significantly lower than in the controls and reached the lower limit of the normal range by the end of the experiment. This suggests that goats may be particularly susceptible to the effects of blood loss associated with Fasciola infections though further work is needed to confirm this. The moderate anaemia which developed showed a tendency to become macrocytic and normochromic.

The marked peripheral eosinophilia beginning 3 weeks after infection and continuing until the end of the experiment was similar in pattern to that described from sheep (Butterworth, 1984) and it is highly consistent with what occurrs in cattle infected with F.gigantica (Haroun & Housein, 1975). The increase of eosinophils in the peripheral circulation during fascioliasis is believed to be caused by excretory/secretory (E/S) antigenic substances released by the migrating fluke and is consistent with the observations in mice described by Milboume & Howell (1990). It is also interesting to note here that no major changes were recorded in the other leucocytes except that the initial preponderance of lymphocytes over neutrophils was reversed from week 3 on.

Similarly, changes in the serum proteins were particularly interesting. Even at the low level of infection established, early changes in plasma protein levels were evident with a tendency for significantly decreased albumin and increased globulin levels relative to controls. This is also consistent with changes reported by other investigators in both sheep and cattle (Rowlands & Clampitt, 1979). Hypoalbuminaemia and hyperglobulinaemia and a decrease in the A/G ratio are attributable to albumin losses on the one hand and increased globulin production on the other. Though these were significantly different from the control group, all levels remained within the normal ranges.

One difficulty encountered in assessing plasma protein changes was the inconsistency in total protein levels indicated by Cobas Mira automatic analyzer and refractometry. The fact that the two procedures gave differing results is, perhaps, not surprising since they are based on totally different methods of measuring protein concentrations. Of concern was the degree of fluctuation in levels as estimated by the Cobas Mira procedure which was not reflected in the refractometer results. The reasons for the variation in the Cobas Mira estimation are unknown and in the second experiment only refractometry was used to measure total protein.

There was an elevation of fibrinogen level in the infected group with significant differences from the control. However, it did not exceed the normal range which indicates that there was only a moderate inflammatory reaction in progress. This is consistent with subclinical mild infection in sheep as described by Sinclair (1966).

Increases in enzyme levels followed the general pattern described in goats (Hughes et al., 1973 & 1974) and sheep (Ford, 1974; Sykes et al., 1980) and were again remarkable given the low level of infection. Gamma glutamate transferase and GD levels rose to beyond the limits of the normal range although AST levels, which were also signifcantly elevated, did not. The results indicate that GD and GGT enzymes are particularly sensitive indicators of damage to the liver parenchyma and bile ducts caused by F.hepatica in goats. This is consistent with the findings in sheep described by Sykes et al., (1980).

The relationship between faecal egg counts and the numbers of Fasciola present at necropsy is of interest as Happich & Boray (1969b) have shown that with infections of up to 100 flukes in sheep, each fluke is represented by approximately 33 epg. In the first experiment with 5 goats, the rates was consistently lower with a mean of approximately 13 epg/fluke (range 9-23) at the last sampling and 18 epg/fluke (range 11-29) in the previous week when the egg counts were at their highest. Whether this reflects the relatively young age of the infection (15-16 weeks after infection) or a difference between goats and sheep or a chance occurrence would be well worth investigating as it is of considerable diagnostic interest.

Total serum bile acid (SBA) has been reported as a sensitive and specific indicator of liver disease and it increases in plasma of sheep, cattle and other domestic animals following liver damage (Anwer et al., 1976; West et al., 1987; Garry, , 1989). However, no data are available on the effect of liver damage caused by liver fluke on SBA activity in goats. In this experiment, an attempt was made to investigate the activities of SBA in infected goats compared with controls. The failure to detect an effect of infection on SBA is interesting but not surprising given the level of infection established. Serum bile acid can be used to assess alterations in liver function in sheep (West et al., 1987), test liver function in domestic animals (Anwer et al.,1976) and to detect hepatobiliary diseases in human (Garry. , 1989). Moreover, SBA is elevated mainly during cholestasis (West et al., 1987). Whether or not it is elevated with higher levels of Fasciola infection has not been investigated. The normal level of SBA as estimated from the control group in the first experiment is comparable with the normal range recently established for sheep (Thompson, K. pers. comm.).

5.2 Experiment 2

The second experiment, intended to provide comparative data from goats and sheep with an infection level higher than in the first experiment, was unsuccessful. Several factors contributed to this and, regrettably, time did not allow it to be repeated. The principal problem was the low level of infectivity of the metacercariae for both species. It is difficult to understand why such a low establishment rate was obtained. The metacercariae were kept in water at approximately 4°C after they were received about 7 weeks before they were used. Normally, under such conditions, metacercariae retain their infectivity for up to 365 days (Boray & Enigk, 1964).

After the infective doses had been prepared, the capsules containing the metacercariae on moist tissue were also kept at 4°C. Both visual examination of the metacercariae and *in vitro* excystation the day before they were used to infect the animals, indicated that a substantial proportion of them were viable (18% of them excysted *in vitro*). In the first experiment, there was good agreement between the *in vitro* excystment rate and establishment. However, the second experiment suggests that an ability to excyst *in vitro* may not necessarily indicate an ability to infect the host.

The number of flukes established in the two species did not differ significantly, although more goats than sheep became infected and there was a tendency for a better establishment in goats. Whether or not the difference was a chance occurrence, or attributable to an inherently greater susceptibility of goats or a decreased susceptibility of the sheep because of their existing liver pathology, could not be determined. However, Boyce et al.(1987) have shown that there are differences in resistance to experimental infection with *F.hepatica* in exotic and domestic breeds of sheep resulting in significant breed differences in mean faecal egg counts and fluke counts. So there could well be differences between sheep and goats. The results of an investigation into the susceptibility of goats described by Reddington et al. (1986), indicate that the goat is extremely susceptible to both primary and secondary infections with *F.hepatica*.

Although it has been suggested that T.hydatigena infection may protect sheep against Fasciola infection (Campbell et al., 1977) the fact that focal lesions possibly caused by this parasite were present is probably of no relevance to the low establishment rate. Other work has failed to show any protective effect of the taeniid infection (Rickard et al., 1982). In addition, similar focal lesions were present in some goat livers in the first experiment in which relatively satisfactory establishment was obtained. It should be borne in mind, of course, that the observed lesions, although probably parasitic in origin judging from their histopathology, may not have been due to T.hydatigena which is now an uncommon parasite in New Zealand (Charleston pers.comm).

The liver abnormalities already present in the sheep, but only apparent at necropsy, were the second major complication of the experiment as they were associated with significant changes in serum enzymes and plasma proteins in the uninfected control animals. The fact that several of the animals were pregnant and gave birth during the experiment was a further complication although it was probably of comparatively little significance.

As a consequence of these problems, little information of value was generated by the infection of the goats and no data that could be used for comparative purposes were obtained from the sheep infections. The very small numbers of flukes in the few infected animals did not produce sufficient changes to even allow comparisons with the uninfected sheep in the same group. The questions as to the relative susceptibilities of sheep and goats to infection and the comparability of their respective responses to Fasciola remain.

5.3 Combined Goat Data

Despite the very low establishment rate in the goats in the second experiment, there was still a measurable and significant decrease in PCV and marked increases of serum enzyme levels. Combination of the data from the 13 infected goats from both experiments yielded some useful information in relation to serum enzyme levels. The correlations between the numbers of flukes recovered at necropsy and peak levels of serum enzyme levels and various haematological parameters in individual animals were examined though only those relating to enzyme levels were significant (Table 5.1).

Table 5.1 Correlation coefficients of peak levels of AST, GGT and GD versus number of flukes recovered in 13 infected goats.

A) Untransformed Data (Enzyme v Fluke)

	AST	GGT	GD
AST	1.0000		
GGT	0.7932	1.0000	
GD	0.7689	0.8504	1.0000
FLUKES	0.6039	0.9249	0.7820

B) Log-Transformed Data (log-Enzyme v Fluke)

	LOGAST	LOGGGT	LOGGD
LOGAST	1.0000		
LOGGGT	0.9937	1.0000	
LOGGD	0.9878	0.9866	1.0000
FLUKES	0.8274	0.8692	0.8551

CASES INCLUDED 13 MISSING CASES 0

Fig.5.1 AST VERSUS FLUKES

```
         AST
  900.0 +
        I
        I
        I
        I+
  600.0 +    +
        I         +
        I
        I
        I
  300.0 +
        I                    +                  +
        I     +        +      +
        I +  +     +       +
        I +
    0.0 +
        -+---------+---------+---------+---------+-
        4.0      12.0      20.0      28.0      36.0
                FLUKES 13 CASES PLOTTED
```

The correlation coefficients between peak enzyme levels and fluke numbers are shown in Table 5.1 and the relationships are shown graphically in Figs. 5.1 (AST), 5.2 (GD) and 5.3 (GGT). It is interesting to note that the relationship is strongest with GGT levels and weakest with AST (Table 5.1a). This is consistent with published data for sheep (Ford, 1974; Sykes et al., 1980) and cattle (Anderson et al., 1977; Rowlands & Clampitt, 1979). The relationships are strengthened by log transformation of the enzyme levels as this reduces the variances of the data as shown in Table 5.1b.

Fig.5.2 GD VERSUS FLUKES

```
      GD
900.0 +
      I
      I
      I
      I    +
600.0 +                                      +
      I
      I
      I
      I    +
300.0 +     +              +
      I+         +
      I                  +
      I +       +    +      +
      I
  0.0 + +
      -+---------+---------+---------+---------+-
      4.0       12.0      20.0      28.0      36.0
              FLUKES 13 CASES PLOTTED
```

The question was then considered as to whether or not the relationships between enzyme levels and fluke numbers were sufficiently close for the former to be useful as predictors of the latter. Regression analysis of both untransformed and transformed data showed, however, that there was no predictive value in the relationship with any of the enzymes because of the extremely wide confidence intervals for predicted values. These reflect both the wide variation in individual enzyme level responses and the small number of animals studied.

Fig.5.3 GGT VERSUS FLUKES

```
    GGT
320.0 +                                     +
      I
      I
      I
      I
230.0 +                   +
      I
      I
      I+
      I
140.0 +    +        +
      I                    +
      I        +
      I +       +       +
      I +
 50.0 +    ++
     -+---------+---------+---------+---------+-
     4.0       12.0      20.0      28.0      36.0
             FLUKES   13 CASES PLOTTED
```

Although larger amounts of data would, no doubt, improve the predictive value of the relationships, the wide individual variation in responses may mean that they remain of little practical use in this respect. It is interesting to note that Hawkins (1984) observed that peak sorbitol dehydrogenase levels in infected sheep were also not related to the number of flukes present and concluded that this enzyme is an unreliable indicator of the size or the duration of fluke burdens. However, serum enzymes clearly can provide strong indirect evidence for the presence of liver fluke in goats during the prepatent period of infection and combined with other parameters, such as PCV, Hb and faecal egg counts, assist in the assessment of the severity of the infection both before and after patency.

5.4 Conclusion

In conclusion, this study has shown that very low levels of Fasciola infection in goats result in significant elevations of serum enzymes and that GGT and GD, particularly, are of diagnostic value with low-level subclinical infections. It has also shown that significant changes in some haematological and biochemical parameters can occur with infections of fewer than 35 flukes in goats. Further investigations are needed to fully describe the changes caused by Fasciola infection in goats.

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APPENDIX 1

DESCRIPTIVE STATISTICS : EXPERIMENT 1

Appendix 1.1 EPG

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
9	0	0	0	0	0
10	12.40	13.32	5.955	0.000	34.00
11	93.80	43.56	19.48	45.00	150.0
12	160.8	49.36	22.08	98.00	220.0
13	271.2	166.6	74.52	112.0	534.0
14	333.0	156.3	69.89	150.0	535.0
15	389.4	225.3	100.7	175.0	680.0
16	285.4	138.8	62.08	130.0	453.0

Appendix 1.2a Liveweight (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	33.34	9.057	4.050	24.30	48.40
0	33.02	8.329	3.725	24.50	46.40
1	34.10	8.202	3.668	25.70	47.60
2	35.32	8.026	3.589	28.10	49.00
3	35.12	7.094	3.172	28.40	47.10
4	35.18	7.599	3.398	28.80	48.30
5	36.10	8.111	3.627	29.90	50.20
6	35.68	7.132	3.189	30.50	48.10
7	34.62	7.747	3.465	28.30	47.90
8	33.94	7.809	3.492	27.90	47.40
9	34.88	8.509	3.805	27.90	49.40
10	33.94	7.117	3.183	28.20	46.00
11	34.38	7.543	3.373	29.60	47.60
12	33.08	7.811	3.493	25.20	45.90
13	32.94	6.587	2.946	27.90	44.10
14	33.50	6.276	2.807	28.80	44.10
15	33.72	6.425	2.874	29.00	44.50
16	32.90	6.955	3.110	27.00	44.90

Appendix 1.2b Liveweight (Cnotrol)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	35.72	7.072	3.163	26.80	45.10
0	34.36	7.526	3.366	26.00	44.90
1	34.92	6.301	2.818	27.80	42.80
2	36.06	6.373	2.850	28.90	44.40
3	35.96	6.058	2.709	29.90	44.50
4	35.26	6.026	2.695	29.10	43.20
5	36.40	5.988	2.678	29.80	44.00
6	36.60	6.118	2.736	29.60	44.90
7	34.72	5.955	2.663	28.00	42.70
8	34.74	5.428	2.427	29.20	42.50
9	36.16	5.404	2.417	30.50	43.20
10	34.96	5.426	2.426	28.50	41.60
11	34.90	5.424	2.426	28.70	41.40
12	35.54	6.266	2.802	29.20	44.40
13	35.30	6.469	2.893	29.20	44.90
14	34.92	6.595	2.949	28.10	44.70
15	35.30	6.950	3.108	28.90	45.70
16	37.68	9.059	4.051	29.50	48.20

Appendix 1.3a PCV (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	29.12	2.911	1.302	25.00	32.60
0	27.00	3.000	1.342	22.00	30.00
1	28.70	4.438	1.985	24.00	34.00
2	24.90	2.655	1.187	22.50	29.00
3	21.80	2.168	0.970	20.00	25.00
4	23.60	2.302	1.030	20.00	26.00
5	25.40	1.949	8.718E-01	22.00	27.00
6	24.20	1.483	6.633E-01	22.00	26.00
7	24.60	2.302	1.030	21.00	27.00
8	26.00	4.062	1.817	20.00	30.00
9	21.20	3.899	1.744	15.00	25.00
10	20.80	3.271	1.463	16.00	25.00
11	24.00	3.317	1.483	20.00	27.00
12	24.00	1.871	8.367E-01	22.00	26.00
13	22.00	1.581	7.071E-01	20.00	24.00
14	23.20	3.633	1.625	18.00	27.00
15	23.00	3.536	1.581	19.00	27.00
16	23.20	2.588	1.158	20.00	26.00

Appendix 1.3b PCV (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	29.10	2.608	1.166	26.00	32.50
0	25.40	2.485	1.111	23.00	29.50
1	27.50	3.606	1.612	22.00	31.00
2	25.20	3.174	1.420	21.00	29.50
3	25.50	3.742	1.673	20.00	30.00
4	20.80	2.080	9.301E-01	18.00	23.50
5	26.40	4.879	2.182	21.00	32.00
6	26.20	4.550	2.035	19.00	30.00
7	25.80	4.025	1.800	20.00	30.00
8	24.20	3.271	1.463	20.00	28.00
9	23.40	5.899	2.638	16.00	30.00
10	25.20	3.347	1.497	20.00	29.00
11	25.40	3.362	1.503	23.00	30.00
12	23.60	2.191	0.980	21.00	27.00
13	27.20	2.387	1.068	24.00	30.00
14	25.00	3.000	1.342	22.00	30.00
15	25.20	1.095	4.899E-01	24.00	27.00
16	26.00	1.581	7.071E-01	24.00	28.00

Appendix 1.4a Haemoglobin (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	10.28	1.801	8.052E-01	8.100	12.30
0	9.940	1.514	6.772E-01	8.300	12.30
1	9.520	1.085	4.852E-01	8.000	10.90
2	9.120	5.848E-01	2.615E-01	8.400	9.900
3	7.680	6.496E-01	2.905E-01	6.900	8.700
4	7.980	6.140E-01	2.746E-01	7.500	9.000
5	7.720	3.114E-01	1.393E-01	7.200	8.000
6	8.200	6.671E-01	2.983E-01	7.300	9.000
7	7.520	3.701E-01	1.655E-01	7.200	8.100
8	8.980	6.099E-01	2.728E-01	8.400	10.00
9	8.440	6.656E-01	2.977E-01	7.700	9.400
10	8.260	5.320E-01	2.379E-01	7.700	8.900
11	8.040	7.635E-01	3.415E-01	7.200	8.900
12	8.120	0.996	4.454E-01	7.100	9.500
13	8.180	7.225E-01	3.231E-01	7.400	9.200
14	7.440	7.369E-01	3.295E-01	6.600	8.300
15	8.400	1.451	6.488E-01	6.600	10.40
16	8.500	7.714E-01	3.450E-01	7.500	9.300

Appendix 1.4b Haemoglobin (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	9.540	1.742	7.788E-01	7.400	11.10
0	9.360	1.090	4.874E-01	7.800	10.50
1	8.840	8.264E-01	3.696E-01	7.600	9.500
2	8.220	1.035	4.630E-01	6.700	9.300
3	9.340	1.731	7.743E-01	6.600	11.40
4	8.120	1.150	5.142E-01	6.500	9.300
5	8.060	1.242	5.555E-01	6.300	9.400
6	8.220	0.971	4.341E-01	6.600	9.100
7	7.760	1.078	4.823E-01	6.500	9.200
8	8.940	8.081E-01	3.614E-01	7.900	9.800
9	8.900	1.190	5.320E-01	7.400	10.10
10	8.960	8.905E-01	3.982E-01	7.600	9.900
11	8.820	1.047	4.684E-01	7.700	10.20
12	9.060	1.092	4.885E-01	7.900	10.80
13	9.380	5.975E-01	2.672E-01	8.500	10.10
14	8.260	8.961E-01	4.007E-01	7.000	9.100
15	9.380	1.361	6.086E-01	7.300	10.50
16	9.700	1.235	5.523E-01	8.100	11.20

Appendix 1.5a RBC (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	15.12	1.329	5.945E-01	14.23	17.30
0	15.35	1.370	6.127E-01	14.14	17.65
1	14.90	1.051	4.699E-01	13.12	15.92
2	15.64	0.975	4.362E-01	14.11	16.50
3	14.66	1.765	7.893E-01	13.20	17.50
4	14.16	1.321	5.907E-01	11.95	15.21
5	13.87	1.675	7.492E-01	12.48	16.53
6	15.09	6.834E-01	3.056E-01	14.20	16.10
7	12.90	1.281	5.730E-01	11.31	14.23
8	13.48	1.092	4.882E-01	11.91	14.81
9	12.34	1.011	4.520E-01	11.13	13.50
10	12.03	1.156	5.171E-01	10.45	13.50
11	12.38	8.255E-01	3.692E-01	11.28	13.15
12	11.98	1.104	4.939E-01	10.17	13.11
13	11.98	1.064	4.757E-01	10.72	13.57
14	12.31	1.004	4.490E-01	11.21	13.55
15	12.23	1.565	6.998E-01	10.85	14.50
16	12.15	1.891	8.459E-01	10.00	13.90

Appendix 1.5b RBC (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	14.97	5.260E-01	2.352E-01	14.50	15.75
0	14.14	6.156E-01	2.753E-01	13.50	15.15
1	14.84	5.437E-01	2.432E-01	13.91	15.31
2	14.91	1.297	5.799E-01	13.13	16.25
3	14.70	1.093	4.887E-01	13.20	16.00
4	13.64	1.496	6.691E-01	12.05	15.65
5	12.55	1.475	6.598E-01	11.04	14.32
6	13.75	0.985	4.405E-01	12.91	15.35
7	13.80	0.970	4.337E-01	12.60	14.97
8	13.48	7.883E-01	3.525E-01	12.61	14.50
9	13.32	6.925E-01	3.097E-01	12.51	14.12
10	13.90	1.218	5.449E-01	12.40	15.24
11	14.19	7.437E-01	3.326E-01	13.25	15.20
12	14.62	6.775E-01	3.030E-01	13.78	15.32
13	14.88	9.410E-01	4.208E-01	13.76	16.05
14	15.45	5.502E-01	2.460E-01	14.53	15.90
15	15.23	5.100E-01	2.281E-01	14.60	15.91
16	15.40	5.214E-01	2.332E-01	14.60	15.92

Appendix 1.6a MCV (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	19.32	2.103	9.404E-01	17.52	22.78
0	17.60	1.420	6.349E-01	15.56	19.31
1	19.25	2.547	1.139	15.87	22.35
2	15.94	1.574	7.038E-01	14.24	17.90
3	15.10	2.653	1.187	11.43	17.48
4	16.81	2.404	1.075	13.15	19.25
5	18.62	3.261	1.458	13.31	21.58
6	16.06	1.055	4.718E-01	14.66	17.61
7	19.31	3.277	1.466	14.79	22.10
8	19.56	4.408	1.972	13.50	24.35
9	17.44	4.208	1.882	11.11	20.66
10	17.63	4.396	1.966	11.85	23.92
11	19.55	3.623	1.620	15.61	23.94
12	20.23	2.968	1.327	16.78	24.58
13	18.50	2.255	1.009	15.48	20.52
14	19.13	4.392	1.964	13.28	24.09
15	19.31	5.058	2.262	13.10	24.24
16	19.65	4.694	2.099	14.39	25.00

Appendix 1.6b MCV (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	19.43	1.547	6.919E-01	17.11	20.69
0	17.94	1.220	5.458E-01	16.61	19.47
1	18.57	2.724	1.218	14.77	21.57
2	17.05	3.117	1.394	14.43	22.47
3	17.53	3.498	1.564	12.50	20.68
4	15.30	1.154	5.159E-01	13.69	16.60
5	21.14	3.645	1.630	16.06	25.39
6	19.05	3.131	1.400	14.72	22.62
7	18.80	3.568	1.596	15.24	23.81
8	18.05	3.032	1.356	14.34	21.88
9	17.47	3.818	1.707	12.48	21.25
10	18.28	3.192	1.428	14.13	22.50
11	17.94	2.495	1.116	15.13	20.68
12	16.13	1.093	4.888E-01	15.24	17.92
13	18.34	1.908	8.533E-01	14.95	19.44
14	16.16	1.536	6.867E-01	15.14	18.87
15	16.58	1.193	5.334E-01	15.47	18.49
16	16.90	1.234	5.520E-01	15.08	18.42

Appendix 1.7a MCHC (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.576E-01	8.009E-02	3.582E-02	2.485E-01	4.560E-01
0	3.696E-01	5.246E-02	2.346E-02	3.214E-01	4.556E-01
1	3.378E-01	6.549E-02	2.929E-02	2.813E-01	4.449E-01
2	3.681E-01	2.697E-02	1.206E-02	3.414E-01	3.958E-01
3	3.551E-01	4.826E-02	2.158E-02	3.080E-01	4.350E-01
4	3.401E-01	3.293E-02	1.473E-02	2.885E-01	3.750E-01
5	3.048E-01	1.518E-02	6.790E-03	2.852E-01	3.273E-01
6	3.389E-01	2.037E-02	9.111E-03	3.250E-01	3.750E-01
7	3.073E-01	2.440E-02	1.091E-02	2.769E-01	3.429E-01
8	3.510E-01	4.764E-02	2.130E-02	2.900E-01	4.200E-01
9	4.068E-01	6.300E-02	2.818E-02	3.522E-01	5.133E-01
10	4.053E-01	7.145E-02	3.195E-02	3.480E-01	5.125E-01
11	3.392E-01	4.656E-02	2.082E-02	2.667E-01	3.905E-01
12	3.398E-01	4.712E-02	2.107E-02	2.808E-01	3.955E-01
13	3.719E-01	1.969E-02	8.807E-03	3.524E-01	4.000E-01
14	3.248E-01	4.016E-02	1.796E-02	2.720E-01	3.667E-01
15	3.697E-01	6.958E-02	3.111E-02	2.778E-01	4.522E-01
16	3.686E-01	3.680E-02	1.646E-02	3.077E-01	4.048E-01

Appendix 1.7b MCHC (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.312E-01	7.486E-02	3.348E-02	2.467E-01	4.269E-01
0	3.734E-01	6.852E-02	3.064E-02	2.644E-01	4.304E-01
1	3.244E-01	3.665E-02	1.639E-02	2.710E-01	3.654E-01
2	3.262E-01	1.041E-02	4.653E-03	3.153E-01	3.423E-01
3	3.758E-01	1.039E-01	4.648E-02	2.491E-01	4.900E-01
4	3.941E-01	7.212E-02	3.225E-02	2.955E-01	4.944E-01
5	3.081E-01	3.747E-02	1.676E-02	2.806E-01	3.739E-01
6	3.167E-01	2.349E-02	1.050E-02	2.933E-01	3.474E-01
7	3.022E-01	2.136E-02	9.554E-03	2.733E-01	3.250E-01
8	3.721E-01	3.343E-02	1.495E-02	3.435E-01	4.250E-01
9	3.919E-01	6.302E-02	2.818E-02	3.367E-01	4.625E-01
10	3.570E-01	1.415E-02	6.329E-03	3.414E-01	3.800E-01
11	3.508E-01	5.483E-02	2.452E-02	3.036E-01	4.435E-01
12	3.865E-01	5.791E-02	2.590E-02	3.222E-01	4.696E-01
13	3.461E-01	2.597E-02	1.161E-02	3.269E-01	3.917E-01
14	3.313E-01	2.385E-02	1.066E-02	3.033E-01	3.640E-01
15	3.723E-01	5.302E-02	2.371E-02	2.920E-01	4.208E-01
16	3.730E-01	4.136E-02	1.850E-02	3.296E-01	4.308E-01

Appendix 1.8a Fibrinogen (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.000	1.000	4.472E-01	2.000	4.000
0	4.400	1.140	5.099E-01	3.000	6.000
1	3.200	1.643	7.348E-01	1.000	5.000
2	2.200	1.304	5.831E-01	1.000	4.000
3	1.800	1.304	5.831E-01	1.000	4.000
4	2.400	1.140	5.099E-01	1.000	4.000
5	3.000	1.000	4.472E-01	2.000	4.000
6	2.400	1.517	6.782E-01	1.000	4.000
7	4.200	2.387	1.068	1.000	7.000
8	5.000	1.732	7.746E-01	4.000	8.000
9	3.800	8.367E-01	3.742E-01	3.000	5.000
10	3.800	8.367E-01	3.742E-01	3.000	5.000
11	2.400	5.477E-01	2.449E-01	2.000	3.000
12	1.600	5.477E-01	2.449E-01	1.000	2.000
13	4.400	8.944E-01	4.000E-01	3.000	5.000
14	3.400	1.517	6.782E-01	2.000	5.000
15	4.200	8.367E-01	3.742E-01	3.000	5.000
16	4.000	7.071E-01	3.162E-01	3.000	5.000

Appendix 1.8b Fibrinogen (control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.400	5.477E-01	2.449E-01	3.000	4.000
0	5.000	1.000	4.472E-01	4.000	6.000
1	3.000	1.581	7.071E-01	1.000	5.000
2	2.000	1.000	4.472E-01	1.000	3.000
3	2.400	1.342	6.000E-01	1.000	4.000
4	1.800	8.367E-01	3.742E-01	1.000	3.000
5	1.800	8.367E-01	3.742E-01	1.000	3.000
6	3.200	1.789	8.000E-01	1.000	5.000
7	2.000	1.000	4.472E-01	1.000	3.000
8	2.000	7.071E-01	3.162E-01	1.000	3.000
9	2.200	8.367E-01	3.742E-01	1.000	3.000
10	1.800	8.367E-01	3.742E-01	1.000	3.000
11	2.600	1.140	5.099E-01	1.000	4.000
12	2.200	8.367E-01	3.742E-01	1.000	3.000
13	2.200	8.367E-01	3.742E-01	1.000	3.000
14	2.400	1.517	6.782E-01	1.000	4.000
15	2.600	1.673	7.483E-01	0.000	4.000
16	3.200	1.095	4.899E-01	2.000	5.000

Appendix 1.9a WBC (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	13.64	2.726	1.219	11.10	17.40
0	12.36	1.705	7.626E-01	10.80	14.80
1	14.12	4.086	1.827	9.600	18.00
2	12.48	1.287	5.757E-01	10.70	14.00
3	9.980	0.996	4.454E-01	8.500	10.90
4	13.12	2.994	1.339	9.300	16.80
5	11.46	9.127E-01	4.082E-01	10.00	12.50
6	12.36	2.088	9.336E-01	9.400	14.30
7	12.04	1.346	6.022E-01	10.90	14.30
8	11.84	1.527	6.831E-01	10.40	14.30
9	11.44	1.212	5.418E-01	10.20	13.30
10	11.24	2.098	9.384E-01	9.700	14.80
11	10.62	1.375	6.151E-01	8.600	12.20
12	11.44	1.148	5.134E-01	9.900	12.80
13	9.300	1.196	5.348E-01	8.000	11.00
14	10.50	1.584	7.085E-01	8.400	12.60
15	10.90	1.600	7.155E-01	9.200	12.80
16	10.40	5.831E-01	2.608E-01	9.900	11.30

Appendix 1.9b WBC (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	13.40	2.215	0.990	10.40	16.00
0	13.50	3.034	1.357	10.40	17.50
1	14.24	3.844	1.719	8.500	18.40
2	11.84	2.011	8.992E-01	9.200	14.10
3	11.54	1.701	7.607E-01	9.600	13.40
4	13.06	1.397	6.250E-01	11.70	15.10
5	12.64	1.828	8.177E-01	10.80	14.80
6	12.02	1.385	6.192E-01	10.50	14.10
7	12.16	1.524	6.816E-01	10.70	14.60
8	11.34	8.735E-01	3.906E-01	10.20	12.20
9	11.34	8.735E-01	3.906E-01	10.20	12.20
10	10.46	1.180	5.278E-01	9.400	12.40
11	11.74	6.693E-01	2.993E-01	10.80	12.40
12	10.16	1.983	8.869E-01	7.200	12.00
13	11.64	2.053	9.179E-01	10.30	15.20
14	10.68	1.875	8.387E-01	8.300	13.00
15	10.20	1.396	6.245E-01	8.500	11.70
16	11.20	2.221	0.993	8.800	13.70

Appendix 1.10a Eosinophil (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.168E-01	2.410E-01	1.078E-01	0.000	6.660E-01
0	4.560E-01	1.858E-01	8.307E-02	2.160E-01	6.700E-01
1	3.416E-01	2.300E-01	1.028E-01	1.620E-01	7.200E-01
2	5.058E-01	2.361E-01	1.056E-01	2.600E-01	8.400E-01
3	8.690E-01	3.347E-01	1.497E-01	5.100E-01	1.417
4	1.306	3.494E-01	1.562E-01	9.300E-01	1.680
5	1.227	1.763E-01	7.885E-02	1.100	1.512
6	7.674E-01	3.438E-01	1.538E-01	3.000E-01	1.140
7	8.690E-01	2.066E-01	9.238E-02	5.640E-01	1.144
8	1.501	6.076E-01	2.717E-01	8.720E-01	2.280
9	1.488	4.065E-01	1.818E-01	1.064	2.023
10	1.966	3.154E-01	1.411E-01	1.660	2.460
11	1.612	5.994E-01	2.681E-01	9.000E-01	2.400
12	1.808	1.021	4.567E-01	4.300E-01	3.200
13	1.374	4.478E-01	2.003E-01	8.900E-01	2.000
14	1.180	4.805E-01	2.149E-01	7.600E-01	1.760
15	8.620E-01	3.229E-01	1.444E-01	4.900E-01	1.280
16	1.156	3.225E-01	1.442E-01	7.900E-01	1.590

Appendix 1.10b Eosinophil (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	2.844E-01	1.769E-01	7.913E-02	1.040E-01	4.800E-01
0	3.054E-01	1.865E-01	8.341E-02	1.460E-01	5.800E-01
1	4.758E-01	2.227E-01	9.960E-02	1.630E-01	7.500E-01
2	3.618E-01	1.954E-01	8.737E-02	2.260E-01	7.050E-01
3	1.880E-01	1.250E-01	5.589E-02	0.000	2.970E-01
4	3.126E-01	2.278E-01	1.019E-01	0.000	6.000E-01
5	4.382E-01	1.145E-01	5.119E-02	2.860E-01	5.550E-01
6	1.680E-01	1.636E-01	7.318E-02	0.000	3.660E-01
7	3.152E-01	9.375E-02	4.193E-02	2.100E-01	4.230E-01
8	2.474E-01	2.906E-01	1.300E-01	0.000	7.440E-01
9	3.006E-01	2.553E-01	1.142E-01	1.150E-01	7.320E-01
10	2.532E-01	1.984E-01	8.871E-02	0.000	5.300E-01
11	2.264E-01	1.743E-01	7.794E-02	0.000	4.300E-01
12	2.082E-01	1.974E-01	8.826E-02	0.000	5.300E-01
13	3.158E-01	1.938E-01	8.668E-02	1.060E-01	6.300E-01
14	3.164E-01	3.751E-01	1.678E-01	0.000	0.960
15	2.870E-01	2.238E-01	1.001E-01	0.000	5.850E-01
16	3.440E-01	2.454E-01	1.098E-01	8.000E-02	7.300E-01

Appendix 1.11a Serum Protein (Cobas Mira) (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	77.48	2.773	1.240	73.80	80.40
0	72.66	2.630	1.176	69.00	76.40
1	76.52	3.256	1.456	73.10	80.30
2	74.72	3.345	1.496	68.90	77.10
3	80.16	4.397	1.967	74.30	86.40
4	81.06	4.258	1.904	77.10	85.80
5	82.84	4.845	2.167	76.40	89.30
6	87.14	6.553	2.931	77.10	95.00
7	93.64	3.738	1.672	89.60	98.30
8	81.30	3.215	1.438	78.20	85.50
9	80.54	5.178	2.316	76.40	88.30
10	92.76	6.222	2.783	84.20	98.60
11	87.02	5.576	2.493	79.80	93.10
12	87.48	5.431	2.429	78.80	92.60
13	79.26	3.288	1.471	75.80	84.30
14	76.22	3.406	1.523	71.40	80.60
15	76.88	2.229	0.997	74.30	80.20
16	76.36	6.462	2.890	70.10	86.80

Appendix 1.11b Serum Protein (Cobas Mira) (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	78.66	4.586	2.051	74.00	86.30
0	77.42	3.426	1.532	72.90	81.10
1	77.16	5.286	2.364	71.90	85.00
2	68.60	5.696	2.547	60.20	74.90
3	71.30	5.071	2.268	65.60	77.90
4	81.82	2.951	1.320	79.40	86.90
5	80.46	4.722	2.112	75.20	85.90
6	86.12	7.739	3.461	75.20	95.50
7	88.18	6.873	3.074	78.10	93.80
8	73.52	4.527	2.024	66.60	76.80
9	75.00	5.269	2.356	69.80	83.60
10	84.76	4.476	2.002	78.10	89.10
11	78.96	2.337	1.045	77.10	82.80
12	81.10	6.849	3.063	74.80	90.50
13	79.90	5.488	2.454	73.10	85.80
14	72.92	3.399	1.520	69.30	77.20
15	75.08	4.658	2.083	70.80	82.90
16	80.76	6.354	2.842	74.50	90.10

Appendix 1.12a Serum Protein (Refractometry) (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	68.80	1.789	8.000E-01	66.00	70.00
0	64.60	3.362	1.503	61.00	69.00
1	66.80	2.387	1.068	64.00	70.00
2	64.60	2.074	9.274E-01	62.00	67.00
3	68.40	2.608	1.166	65.00	72.00
4	65.60	5.477E-01	2.449E-01	65.00	66.00
5	78.00	6.964	3.114	72.00	86.00
6	72.40	3.209	1.435	70.00	78.00
7	90.20	13.54	6.053	74.00	110.0
8	73.00	2.828	1.265	70.00	76.00
9	78.60	3.975	1.778	72.00	82.00
10	73.20	3.271	1.463	70.00	78.00
11	76.60	4.722	2.112	74.00	85.00
12	70.40	3.286	1.470	68.00	76.00
13	69.40	4.506	2.015	63.00	75.00
14	64.80	4.604	2.059	60.00	70.00
15	62.80	3.564	1.594	60.00	68.00
16	62.40	2.302	1.030	60.00	65.00

Appendix 1.12b Serum Protein (Refractometry) (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	67.40	3.782	1.691	61.00	70.00
0	64.60	3.647	1.631	60.00	70.00
1	67.00	3.162	1.414	62.00	70.00
2	63.80	2.168	0.970	61.00	66.00
3	65.60	2.074	9.274E-01	63.00	68.00
4	63.60	3.050	1.364	60.00	67.00
5	67.60	2.608	1.166	64.00	70.00
6	69.80	1.789	8.000E-01	68.00	72.00
7	66.60	3.286	1.470	62.00	71.00
8	66.20	1.789	8.000E-01	64.00	68.00
9	73.00	4.123	1.844	70.00	80.00
10	67.20	1.643	7.348E-01	65.00	69.00
11	66.20	3.899	1.744	60.00	70.00
12	66.80	1.304	5.831E-01	65.00	68.00
13	66.80	2.950	1.319	64.00	70.00
14	188.8	271.8	121.6	65.00	675.0
15	66.40	2.510	1.122	63.00	70.00
16	66.60	1.517	6.782E-01	65.00	68.00

Appendix 1.13a Serum Albumin (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	33.90	2.443	1.093	31.50	37.80
0	31.58	2.028	9.069E-01	29.20	34.00
1	33.80	2.767	1.237	30.20	37.30
2	31.30	3.610	1.614	26.40	36.50
3	30.88	2.773	1.240	26.50	33.90
4	34.90	2.542	1.137	30.90	37.70
5	35.48	2.043	9.135E-01	32.10	37.30
6	34.82	2.277	1.019	31.30	37.60
7	34.48	2.640	1.180	30.00	36.80
8	32.30	2.434	1.089	28.80	34.70
9	32.54	2.961	1.324	28.00	35.70
10	36.06	2.718	1.216	33.20	40.40
11	35.50	1.875	8.385E-01	32.80	37.70
12	34.32	2.385	1.066	30.60	37.20
13	33.98	2.172	0.971	31.60	37.40
14	34.94	2.354	1.053	32.80	38.30
15	32.70	2.576	1.152	29.50	35.70
16	35.16	2.296	1.027	33.20	38.00

Appendix 1.13b Serum Albumin (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	31.96	7.497	3.353	19.50	39.00
0	32.28	1.821	8.145E-01	29.20	33.70
1	34.54	2.136	0.955	32.80	38.10
2	32.60	2.882	1.289	29.40	36.40
3	31.28	1.663	7.439E-01	29.80	33.40
4	36.58	2.174	0.972	34.70	40.20
5	36.92	1.699	7.599E-01	35.30	39.00
6	35.78	5.020	2.245	27.20	39.50
7	37.18	1.582	7.074E-01	35.20	39.40
8	34.40	1.470	6.573E-01	32.90	36.20
9	35.30	2.084	9.322E-01	31.90	37.10
10	40.84	2.342	1.047	38.10	44.00
11	41.26	1.688	7.547E-01	38.80	43.40
12	37.00	2.268	1.014	33.30	39.10
13	40.08	1.821	8.145E-01	38.60	43.20
14	39.00	1.396	6.245E-01	37.50	40.70
15	38.00	1.386	6.197E-01	36.30	39.90
16	40.70	3.512	1.571	37.00	44.90

Appendix 1.14a Serum Globulin (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	35.50	2.474	1.106	33.00	38.50
0	35.82	3.390	1.516	33.00	41.20
1	34.00	3.571	1.597	30.70	39.40
2	36.30	3.314	1.482	32.50	40.60
3	37.52	2.055	9.189E-01	35.10	40.50
4	37.30	2.182	0.976	34.30	39.70
5	37.52	2.350	1.051	34.70	40.80
6	35.38	4.980	2.227	28.70	42.40
7	39.12	3.793	1.696	35.20	44.20
8	40.70	2.971	1.329	35.90	44.10
9	40.46	3.481	1.557	36.20	45.00
10	38.34	4.219	1.887	34.60	45.40
11	36.50	3.349	1.498	31.30	40.20
12	38.68	4.810	2.151	33.90	46.40
13	34.82	3.595	1.608	29.90	38.70
14	35.86	3.532	1.579	32.70	41.40
15	32.10	1.444	6.458E-01	30.50	34.40
16	27.84	1.917	8.571E-01	26.20	30.80

Appendix 1.14b Serum Globulin (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	35.04	8.727	3.903	29.60	50.50
0	33.12	2.269	1.015	30.80	36.50
1	34.06	3.134	1.402	30.90	39.10
2	32.80	2.908	1.300	28.50	36.60
3	34.32	1.545	6.909E-01	32.60	36.40
4	34.62	7.855E-01	3.513E-01	33.30	35.30
5	32.68	2.172	0.971	30.00	35.50
6	34.42	6.228	2.785	28.50	44.80
7	33.42	3.158	1.412	29.70	37.80
8	31.80	2.848	1.274	28.30	35.10
9	31.50	2.418	1.081	28.90	35.10
10	26.56	3.581	1.601	21.00	31.00
11	29.34	3.513	1.571	24.70	33.60
12	34.80	8.369	3.743	25.90	43.40
13	27.12	4.749	2.124	19.00	30.30
14	29.00	2.766	1.237	26.10	33.50
15	28.40	2.433	1.088	26.10	31.90
16	24.30	2.395	1.071	20.10	26.10

Appendix 1.15a A/G Ratio (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	0.961	1.219E-01	5.452E-02	8.182E-01	1.105
0	8.901E-01	1.225E-01	5.480E-02	7.233E-01	1.030
1	1.006	1.589E-01	7.108E-02	8.274E-01	1.215
2	8.742E-01	1.728E-01	7.726E-02	6.502E-01	1.123
3	8.265E-01	1.040E-01	4.649E-02	6.883E-01	0.966
4	9.401E-01	1.103E-01	4.932E-02	7.903E-01	1.099
5	9.497E-01	9.617E-02	4.301E-02	8.470E-01	1.075
6	0.996	1.148E-01	5.133E-02	8.396E-01	1.126
7	8.889E-01	1.198E-01	5.356E-02	7.500E-01	1.045
8	7.989E-01	1.051E-01	4.698E-02	6.990E-01	9.499E-01
9	8.127E-01	1.296E-01	5.796E-02	6.222E-01	9.337E-01
10	0.952	1.468E-01	6.567E-02	7.841E-01	1.168
11	0.981	1.249E-01	5.585E-02	8.657E-01	1.173
12	9.025E-01	1.554E-01	6.949E-02	6.595E-01	1.039
13	0.985	1.318E-01	5.894E-02	8.605E-01	1.147
14	0.985	1.471E-01	6.579E-02	8.116E-01	1.171
15	1.020	8.951E-02	4.003E-02	8.895E-01	1.105
16	1.268	1.194E-01	5.342E-02	1.078	1.407

Appendix 1.15b A/G Ratio (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	0.978	3.426E-01	1.532E-01	3.861E-01	1.258
0	0.977	6.121E-02	2.738E-02	9.178E-01	1.077
1	1.022	1.290E-01	5.768E-02	8.926E-01	1.233
2	1.004	1.564E-01	6.996E-02	8.306E-01	1.211
3	9.132E-01	6.964E-02	3.114E-02	8.407E-01	1.025
4	1.057	6.427E-02	2.874E-02	0.983	1.155
5	1.133	7.417E-02	3.317E-02	1.047	1.219
6	1.081	2.906E-01	1.300E-01	6.071E-01	1.386
7	1.120	1.110E-01	4.966E-02	0.984	1.247
8	1.092	1.406E-01	6.286E-02	9.373E-01	1.261
9	1.129	1.448E-01	6.475E-02	9.088E-01	1.284
10	1.571	3.157E-01	1.412E-01	1.258	2.095
11	1.423	1.832E-01	8.192E-02	1.244	1.672
12	1.120	3.097E-01	1.385E-01	8.433E-01	1.510
13	1.523	3.228E-01	1.444E-01	1.310	2.053
14	1.355	1.432E-01	6.403E-02	1.119	1.490
15	1.346	1.338E-01	5.982E-02	1.194	1.529
16	1.697	3.110E-01	1.391E-01	1.480	2.234

Appendix 1.16a Serum AST (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	81.00	11.53	5.158	65.00	96.00
0	74.60	11.15	4.986	65.00	93.00
1	72.00	10.12	4.528	64.00	88.00
2	70.60	13.05	5.836	58.00	90.00
3	79.00	10.72	4.796	72.00	98.00
4	87.20	11.63	5.200	74.00	100.0
5	86.60	13.24	5.921	76.00	103.0
6	87.40	7.503	3.356	81.00	99.00
7	164.2	37.65	16.84	118.0	218.0
8	173.0	63.49	28.40	115.0	255.0
9	150.8	44.27	19.80	100.0	213.0
10	145.2	61.30	27.41	83.00	232.0
11	109.8	43.11	19.28	72.00	179.0
12	117.4	27.99	12.52	88.00	159.0
13	103.2	45.57	20.38	70.00	177.0
14	93.00	34.39	15.38	64.00	133.0
15	75.80	15.25	6.822	62.00	95.00
16	81.80	16.65	7.446	66.00	107.0

Appendix 1.16b Serum AST (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	76.60	9.685	4.331	64.00	88.00
0	68.80	4.438	1.985	62.00	74.00
1	68.60	4.879	2.182	63.00	74.00
2	67.60	7.925	3.544	59.00	77.00
3	71.60	11.33	5.066	57.00	86.00
4	74.20	7.430	3.323	65.00	85.00
5	75.80	9.602	4.294	63.00	84.00
6	71.80	6.907	3.089	63.00	79.00
7	71.60	8.933	3.995	61.00	82.00
8	68.60	10.78	4.823	55.00	78.00
9	71.40	9.290	4.155	57.00	80.00
10	75.80	13.94	6.232	64.00	92.00
11	75.20	12.85	5.748	63.00	95.00
12	82.40	20.71	9.261	62.00	114.0
13	75.60	20.18	9.026	58.00	110.0
14	76.80	14.75	6.598	64.00	101.0
15	78.60	25.56	11.43	59.00	119.0
16	94.40	27.41	12.26	68.00	127.0

Appendix 1.17a Serum GGT (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	41.00	9.513	4.254	29.00	55.00
0	45.00	7.842	3.507	33.00	55.00
1	47.00	7.583	3.391	37.00	58.00
2	44.80	5.630	2.518	37.00	52.00
3	50.60	6.656	2.977	40.00	57.00
4	47.40	7.057	3.156	40.00	57.00
5	48.40	8.649	3.868	38.00	60.00
6	46.20	10.83	4.841	30.00	60.00
7	93.60	32.67	14.61	59.00	142.0
8	133.2	56.22	25.14	76.00	203.0
9	143.4	63.22	28.27	89.00	245.0
10	174.6	95.10	42.53	95.00	318.0
11	162.6	90.91	40.65	82.00	301.0
12	153.0	87.82	39.27	79.00	291.0
13	122.4	75.62	33.82	68.00	248.0
14	104.0	56.07	25.07	69.00	201.0
15	86.20	32.14	14.37	64.00	141.0
16	85.00	26.08	11.66	66.00	127.0

Appendix 1.17b Serum GGT (control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	38.40	6.107	2.731	30.00	46.00
0	38.20	6.301	2.818	29.00	46.00
1	39.40	6.580	2.943	29.00	45.00
2	36.20	6.380	2.853	26.00	43.00
3	40.80	6.686	2.990	31.00	48.00
4	38.40	6.465	2.891	29.00	45.00
5	39.20	5.495	2.458	31.00	45.00
6	37.40	6.066	2.713	28.00	44.00
7	42.80	5.404	2.417	34.00	48.00
8	42.20	6.221	2.782	33.00	50.00
9	49.80	9.834	4.398	39.00	65.00
10	47.60	7.668	3.429	38.00	56.00
11	49.40	9.555	4.273	37.00	60.00
12	50.40	7.369	3.295	40.00	58.00
13	46.80	6.611	2.956	38.00	54.00
14	47.60	5.899	2.638	39.00	53.00
15	47.60	5.941	2.657	38.00	53.00
16	51.40	3.782	1.691	46.00	55.00

Appendix 1.18a Serum GD (infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	5.484	2.575	1.151	2.550	7.950
0	4.352	2.770	1.239	3.900E-01	7.990
1	4.930	4.530	2.026	1.170	12.82
2	4.428	1.428	6.387E-01	3.150	6.430
3	16.85	19.85	8.878	7.800E-01	50.50
4	24.17	11.49	5.139	11.36	40.33
5	27.29	14.99	6.706	12.74	52.03
6	36.74	16.55	7.399	20.40	62.70
7	135.9	59.84	26.76	46.96	197.8
8	158.5	138.0	61.71	30.31	329.9
9	145.9	128.8	57.60	24.18	365.0
10	184.6	244.3	109.2	18.78	610.0
11	77.31	120.0	53.66	3.580	289.9
12	72.68	92.72	41.47	3.670	235.5
13	67.56	114.8	51.36	9.410	272.5
14	31.30	38.65	17.28	10.62	100.2
15	19.26	23.58	10.54	2.710	60.50
16	17.68	21.45	9.594	3.370	55.60

Appendix 1.18b Serum GD (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.330	1.301	5.816E-01	1.210	4.360
0	2.764	1.684	7.532E-01	1.700E-01	4.450
1	3.454	3.183	1.423	7.300E-01	8.810
2	3.898	2.659	1.189	1.550	8.480
3	3.740	2.693	1.204	7.800E-01	7.900
4	3.828	3.460	1.547	3.900E-01	9.670
5	6.034	11.16	4.991	6.900E-01	25.99
6	3.144	2.282	1.021	1.300E-01	6.520
7	1.702	2.188	0.979	1.300E-01	5.180
8	1.616	8.663E-01	3.874E-01	3.500E-01	2.420
9	4.230	7.161	3.203	4.700E-01	17.01
10	5.588	5.559	2.486	1.380	14.68
11	3.100	2.155	0.964	1.080	6.780
12	3.712	3.244	1.451	6.500E-01	8.680
13	7.350	6.522	2.917	3.110	18.87
14	8.274	6.894	3.083	1.300	17.44
15	6.530	5.326	2.382	2.810	15.85
16	6.928	7.778	3.478	1.170	19.95

Appendix 1.19a Serum Bile Acid (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
2	26.02	10.04	4.488	13.80	37.10
4	39.76	24.09	10.77	16.80	80.40
6	39.60	27.64	12.36	13.20	77.40
8	38.36	21.58	9.653	22.70	75.60
10	49.39	29.67	13.27	23.80	92.80
12	31.62	15.95	7.131	16.10	56.70
14	18.20	8.021	3.587	9.900	30.20
16	20.62	17.82	7.971	2.110	49.10

Appendix 1.19b Serum Bile Acid (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
2	27.20	14.27	6.380	10.60	49.20
4	49.40	33.81	15.12	16.00	97.50
6	32.24	15.47	6.919	15.30	57.00
8	36.06	19.24	8.606	6.300	56.10
10	42.30	18.39	8.222	20.50	58.50
12	31.90	15.63	6.990	13.20	51.30
14	19.30	9.704	4.340	6.300	29.20
16	25.86	17.56	7.852	8.800	53.90

APPENDIX 2

DESCRIPTIVE STATISTICS : EXPERIMENT 2.

Appendix 2.1 Liveweights of Sheep & Goats.

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
1a Infected (Sheep)					
0	41.87	3.122	0.987	38.20	47.70
8	43.09	3.045	0.963	38.20	47.30
14	44.00	4.874	1.541	36.40	50.90
1b Control (Sheep)					
0	47.16	3.580	1.601	42.10	52.00
8	49.56	5.360	2.397	40.70	55.30
14	51.92	4.168	1.864	46.60	57.20
1c Infected (Goats)					
0	33.06	4.551	1.439	26.80	38.50
8	36.49	5.107	1.615	29.70	43.50
14	36.78	4.601	1.455	30.30	42.30
1d Control (Goats)					
0	25.04	4.173	1.866	21.20	31.90
8	26.68	4.246	1.899	22.20	33.00
14	29.02	3.555	1.590	25.50	34.20

Appendix 2.2a PCV : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	34.20	1.687	5.333E-01	32.00	37.00
0	35.00	4.522	1.430	24.00	39.00
1	34.40	2.459	7.775E-01	30.00	38.00
2	34.40	3.098	0.980	29.00	38.00
3	30.70	2.111	6.675E-01	28.00	34.00
4	30.80	2.616	8.273E-01	28.00	35.00
5	31.90	2.378	7.520E-01	29.00	35.00
6	38.80	3.553	1.123	33.00	44.00
7	36.00	2.539	8.028E-01	32.00	40.00
8	32.40	2.797	8.844E-01	29.00	36.00
9	32.80	2.440	7.717E-01	29.00	37.00
10	31.30	3.129	0.989	26.00	35.00
11	33.80	2.098	6.633E-01	30.00	36.00
12	33.90	1.853	5.859E-01	31.00	37.00
13	33.90	2.685	8.492E-01	30.00	38.00
14	33.70	2.584	8.172E-01	30.00	38.00

Appendix 2.2b PCV : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	33.60	1.673	7.483E-01	32.00	36.00
0	33.20	3.194	1.428	29.00	37.00
1	33.60	2.302	1.030	30.00	36.00
2	33.20	2.588	1.158	30.00	37.00
3	31.00	1.225	5.477E-01	30.00	33.00
4	31.40	2.966	1.327	28.00	36.00
5	29.60	1.140	5.099E-01	28.00	31.00
6	39.40	2.510	1.122	37.00	42.00
7	34.40	2.510	1.122	30.00	36.00
8	33.80	4.025	1.800	29.00	38.00
9	33.20	2.490	1.114	30.00	35.00
10	33.80	1.643	7.348E-01	31.00	35.00
11	34.60	1.517	6.782E-01	32.00	36.00
12	35.20	3.033	1.356	32.00	40.00
13	36.40	2.608	1.166	35.00	41.00
14	35.60	2.074	9.274E-01	33.00	38.00

Appendix 2.2c PCV : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	24.10	2.601	8.226E-01	20.00	28.00
0	30.20	4.211	1.332	23.00	35.00
1	28.20	3.048	0.964	24.00	32.00
2	31.90	4.748	1.501	24.00	40.00
3	30.00	4.853	1.535	22.00	36.00
4	26.20	3.736	1.181	21.00	32.00
5	25.90	3.035	0.960	21.00	31.00
6	32.50	3.567	1.128	25.00	36.00
7	28.80	4.158	1.315	20.00	34.00
8	28.90	2.807	8.876E-01	24.00	33.00
9	30.40	2.413	7.630E-01	26.00	33.00
10	25.60	1.713	5.416E-01	24.00	29.00
11	27.70	2.003	6.333E-01	25.00	31.00
12	29.00	2.055	6.498E-01	26.00	33.00
13	28.80	2.616	8.273E-01	25.00	34.00
14	27.40	2.221	7.024E-01	24.00	31.00

Appendix 2.2d PCV : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	24.80	1.924	8.602E-01	22.00	27.00
0	30.60	3.507	1.568	25.00	34.00
1	28.20	1.789	8.000E-01	26.00	30.00
2	27.40	3.847	1.720	23.00	32.00
3	29.40	2.191	0.980	26.00	32.00
4	29.20	4.087	1.828	25.00	34.00
5	29.60	2.881	1.288	26.00	32.00
6	33.80	8.367E-01	3.742E-01	33.00	35.00
7	33.20	3.768	1.685	29.00	39.00
8	31.00	3.000	1.342	28.00	35.00
9	32.40	2.702	1.208	29.00	36.00
10	36.00	2.345	1.049	34.00	40.00
11	35.20	1.789	8.000E-01	34.00	38.00
12	35.20	2.168	0.970	32.00	38.00
13	35.60	2.302	1.030	32.00	38.00
14	36.20	3.033	1.356	32.00	40.00

Appendix 2.3a Haemoglobin Level : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	12.44	5.103E-01	1.614E-01	11.50	13.20
0	13.17	0.998	3.155E-01	11.10	14.30
1	12.88	0.951	3.007E-01	10.80	14.10
2	12.51	1.122	3.548E-01	10.50	14.50
3	12.44	8.834E-01	2.794E-01	11.50	13.80
4	11.79	1.017	3.216E-01	10.20	13.60
5	12.34	7.919E-01	2.504E-01	11.60	13.80
6	12.11	0.989	3.129E-01	10.60	13.90
7	11.03	6.430E-01	2.033E-01	10.30	11.80
8	11.41	8.582E-01	2.714E-01	9.800	12.70
9	11.40	6.616E-01	2.092E-01	10.20	12.60
10	11.35	5.233E-01	1.655E-01	10.70	12.30
11	11.67	0.993	3.141E-01	10.70	13.60
12	11.23	6.848E-01	2.166E-01	10.50	12.20
13	11.26	5.621E-01	1.778E-01	10.60	12.00
14	10.69	5.607E-01	1.773E-01	10.00	11.50

Appendix 2.3b Haemoglobin Level : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	12.42	1.152	5.152E-01	10.80	14.00
0	12.92	1.318	5.894E-01	11.50	14.50
1	12.80	1.676	7.497E-01	11.20	15.30
2	12.76	1.381	6.177E-01	11.20	14.20
3	13.14	7.301E-01	3.265E-01	12.30	13.90
4	12.46	9.127E-01	4.082E-01	11.30	13.60
5	12.46	2.609	1.167	8.200	14.40
6	13.14	1.309	5.853E-01	11.70	14.70
7	12.00	1.522	6.804E-01	10.20	13.70
8	12.48	1.397	6.248E-01	10.70	13.70
9	10.96	1.770	7.916E-01	8.900	12.70
10	11.68	1.112	4.974E-01	10.50	13.10
11	11.22	1.453	6.499E-01	9.000	12.80
12	11.24	1.078	4.823E-01	9.800	12.50
13	11.20	1.151	5.148E-01	10.00	12.50
14	11.20	8.456E-01	3.782E-01	10.10	12.00

Appendix 2.3c Haemoglobin Level : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	8.990	1.595	5.045E-01	6.500	11.00
0	10.40	1.378	4.356E-01	7.700	12.00
1	9.820	1.116	3.530E-01	7.700	11.10
2	9.790	1.230	3.889E-01	7.900	12.00
3	10.30	1.415	4.475E-01	8.300	12.90
4	9.050	1.106	3.497E-01	7.600	10.70
5	9.860	8.796E-01	2.782E-01	8.400	11.30
6	9.800	0.981	3.102E-01	8.600	11.50
7	9.850	1.199	3.793E-01	8.500	11.60
8	9.510	0.972	3.075E-01	8.100	11.10
9	9.280	8.690E-01	2.748E-01	7.500	10.30
10	8.930	8.680E-01	2.745E-01	7.900	10.30
11	9.550	1.036	3.277E-01	7.900	11.20
12	9.780	9.016E-01	2.851E-01	8.500	11.50
13	9.850	9.372E-01	2.964E-01	8.400	11.30
14	9.610	0.972	3.075E-01	8.000	11.40

Appendix 2.3d Haemoglobin : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	10.06	7.829E-01	3.501E-01	9.100	11.10
0	10.60	1.609	7.197E-01	8.100	12.50
1	10.14	1.851	8.280E-01	7.600	12.50
2	9.700	1.405	6.285E-01	7.400	11.10
3	10.62	2.073	9.270E-01	8.300	13.90
4	10.12	1.545	6.909E-01	8.200	12.20
5	10.88	1.972	8.817E-01	8.100	13.40
6	10.72	1.587	7.095E-01	9.000	12.90
7	10.98	1.055	4.716E-01	9.800	12.70
8	10.56	1.361	6.088E-01	8.800	12.40
9	10.60	1.756	7.855E-01	8.000	12.90
10	10.84	1.815	8.115E-01	8.700	13.70
11	10.46	1.399	6.258E-01	8.800	12.20
12	10.36	1.163	5.202E-01	8.900	11.60
13	10.28	0.983	4.398E-01	9.000	11.50
14	10.42	1.076	4.810E-01	8.900	11.60

Appendix 2.4a Total WBC Count : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	9.870	1.024	3.239E-01	8.800	12.10
0	10.31	1.619	5.120E-01	8.600	13.20
1	9.710	1.814	5.736E-01	7.200	12.30
2	9.080	1.645	5.202E-01	6.900	12.00
3	9.680	2.171	6.865E-01	7.900	14.60
4	9.530	2.022	6.393E-01	8.000	14.80
5	9.940	2.177	6.885E-01	7.300	14.70
6	8.820	1.838	5.811E-01	6.900	11.80
7	9.730	0.991	3.134E-01	8.200	11.50
8	9.820	9.461E-01	2.992E-01	8.600	11.30
9	8.380	1.524	4.818E-01	6.100	10.80
10	8.180	1.861	5.884E-01	6.000	11.30
11	6.750	1.638	5.180E-01	5.000	9.700
12	7.450	1.365	4.316E-01	6.000	10.30
13	7.750	1.257	3.976E-01	6.600	10.90
14	7.880	1.236	3.910E-01	6.300	10.50

Appendix 2.4b Total WBC Count : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	9.100	7.517E-01	3.362E-01	8.100	10.10
0	9.300	1.505	6.731E-01	7.100	10.80
1	9.260	1.083	4.844E-01	8.000	10.60
2	8.780	1.525	6.822E-01	6.700	10.70
3	9.360	1.479	6.615E-01	7.300	11.10
4	9.160	1.853	8.286E-01	6.100	11.10
5	7.320	2.078	9.292E-01	5.100	10.00
6	9.080	1.534	6.859E-01	6.700	10.70
7	9.140	0.999	4.468E-01	8.200	10.80
8	9.640	7.092E-01	3.172E-01	8.900	10.60
9	8.480	1.497	6.696E-01	6.900	10.30
10	8.940	1.038	4.643E-01	7.200	9.800
11	8.400	2.822	1.262	6.100	13.00
12	7.900	1.746	7.810E-01	7.000	11.00
13	8.180	1.260	5.634E-01	7.500	10.40
14	7.900	4.950E-01	2.214E-01	7.300	8.500

Appendix 2.4c Total WBC Count : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	8.570	1.398	4.420E-01	6.100	10.70
0	9.320	1.678	5.308E-01	7.400	12.00
1	8.480	1.722	5.444E-01	6.400	11.80
2	8.280	1.918	6.064E-01	5.700	11.40
3	8.730	1.936	6.121E-01	6.100	11.40
4	9.400	2.490	7.875E-01	5.600	12.60
5	11.28	3.087	0.976	8.200	18.10
6	9.860	2.537	8.022E-01	6.000	13.30
7	9.700	1.835	5.802E-01	6.900	13.20
8	10.00	2.877	9.098E-01	5.900	14.20
9	9.640	2.825	8.932E-01	6.700	14.50
10	9.580	2.816	8.905E-01	5.500	13.90
11	7.910	1.905	6.025E-01	5.500	11.70
12	7.950	1.498	4.738E-01	6.500	10.70
13	7.890	1.245	3.937E-01	6.000	10.50
14	7.780	7.510E-01	2.375E-01	6.200	8.500

Appendix 2.4d Total WBC Count : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	9.860	1.172	5.240E-01	8.600	11.70
0	11.04	1.595	7.132E-01	9.100	13.40
1	9.540	1.326	5.930E-01	7.600	10.90
2	9.220	5.718E-01	2.557E-01	8.600	10.10
3	10.04	1.270	5.680E-01	8.300	11.80
4	9.540	1.036	4.632E-01	8.100	10.60
5	9.860	1.122	5.016E-01	8.800	11.40
6	9.080	6.140E-01	2.746E-01	8.300	10.00
7	10.34	4.980E-01	2.227E-01	9.600	10.80
8	11.16	1.337	5.980E-01	10.00	13.20
9	10.12	1.215	5.435E-01	8.000	11.00
10	11.46	2.624	1.173	8.800	15.70
11	9.620	2.136	0.955	6.600	12.20
12	9.460	1.695	7.580E-01	7.100	11.50
13	9.640	1.524	6.816E-01	7.500	11.20
14	9.220	1.482	6.629E-01	6.800	10.50

Appendix 2.5a Eosinophil Count : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	2.266E-01	1.502E-01	4.749E-02	0.000	5.050E-01
0	2.239E-01	1.183E-01	3.741E-02	8.600E-02	4.560E-01
1	1.579E-01	1.594E-01	5.040E-02	0.000	3.950E-01
2	1.119E-01	1.080E-01	3.416E-02	0.000	2.760E-01
3	2.000E-01	7.069E-02	2.236E-02	8.500E-02	2.920E-01
4	2.947E-01	1.210E-01	3.825E-02	9.000E-02	4.440E-01
5	2.798E-01	1.868E-01	5.906E-02	0.000	7.350E-01
6	3.296E-01	1.491E-01	4.715E-02	1.000E-01	5.920E-01
7	7.651E-01	3.740E-01	1.183E-01	1.020E-01	1.305
8	6.135E-01	3.975E-01	1.257E-01	1.740E-01	1.380
9	5.085E-01	2.515E-01	7.953E-02	2.670E-01	1.050
10	4.059E-01	2.676E-01	8.463E-02	1.400E-01	9.300E-01
11	4.828E-01	3.102E-01	9.811E-02	1.650E-01	1.120
12	5.154E-01	4.951E-01	1.566E-01	8.900E-02	1.700
13	5.099E-01	3.690E-01	1.167E-01	1.340E-01	1.040
14	4.827E-01	4.562E-01	1.443E-01	6.900E-02	1.260

Appendix 2.5b Eosinophil Count : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	7.660E-02	1.229E-01	5.495E-02	0.000	2.820E-01
0	1.448E-01	1.417E-01	6.338E-02	0.000	3.240E-01
1	1.630E-01	6.804E-02	3.043E-02	9.700E-02	2.490E-01
2	2.756E-01	1.819E-01	8.136E-02	8.000E-02	5.350E-01
3	3.080E-01	1.483E-01	6.632E-02	1.720E-01	5.550E-01
4	2.194E-01	1.194E-01	5.338E-02	1.110E-01	3.920E-01
5	1.926E-01	9.849E-02	4.405E-02	1.220E-01	3.600E-01
6	2.170E-01	8.814E-02	3.942E-02	8.800E-02	3.210E-01
7	2.554E-01	1.326E-01	5.932E-02	9.200E-02	4.300E-01
8	2.510E-01	1.189E-01	5.320E-02	1.000E-01	4.240E-01
9	1.546E-01	8.491E-02	3.797E-02	6.900E-02	2.910E-01
10	2.114E-01	7.699E-02	3.443E-02	9.500E-02	2.940E-01
11	2.178E-01	1.477E-01	6.604E-02	6.100E-02	3.900E-01
12	2.220E-01	8.228E-02	3.680E-02	1.400E-01	3.300E-01
13	2.028E-01	7.421E-02	3.319E-02	1.040E-01	3.000E-01
14	1.698E-01	1.166E-01	5.216E-02	7.500E-02	3.650E-01

Appendix 2.5c Eosinophil Counts : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.724E-01	3.395E-01	1.073E-01	0.000	9.400E-01
0	1.453E-01	1.388E-01	4.389E-02	0.000	4.080E-01
1	2.516E-01	1.616E-01	5.111E-02	7.100E-02	5.900E-01
2	2.019E-01	1.296E-01	4.100E-02	0.000	4.000E-01
3	7.521E-01	2.640E-01	8.350E-02	3.720E-01	1.080
4	8.888E-01	4.767E-01	1.507E-01	1.680E-01	1.710
5	1.107	2.292E-01	7.249E-02	6.450E-01	1.335
6	8.932E-01	4.071E-01	1.287E-01	3.000E-01	1.729
7	0.965	3.759E-01	1.189E-01	3.120E-01	1.584
8	1.053	5.106E-01	1.615E-01	3.120E-01	1.846
9	1.083	6.411E-01	2.027E-01	4.020E-01	2.755
10	1.080	5.065E-01	1.602E-01	3.850E-01	2.085
11	8.590E-01	2.796E-01	8.843E-02	4.400E-01	1.349
12	8.230E-01	2.666E-01	8.430E-02	3.250E-01	1.125
13	7.716E-01	2.335E-01	7.383E-02	3.600E-01	1.050
14	7.224E-01	2.476E-01	7.830E-02	3.100E-01	1.120

Appendix 2.5d Eosinophil Counts : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.044E-01	1.613E-01	7.216E-02	1.170E-01	5.050E-01
0	1.060E-01	1.566E-01	7.002E-02	0.000	3.480E-01
1	1.552E-01	1.126E-01	5.035E-02	0.000	2.700E-01
2	2.422E-01	9.256E-02	4.140E-02	1.720E-01	3.760E-01
3	3.338E-01	8.639E-02	3.863E-02	2.080E-01	4.150E-01
4	2.816E-01	8.054E-02	3.602E-02	1.840E-01	3.720E-01
5	3.048E-01	1.841E-01	8.234E-02	8.800E-02	5.700E-01
6	2.700E-01	1.062E-01	4.751E-02	1.840E-01	4.500E-01
7	3.304E-01	2.258E-01	1.010E-01	1.020E-01	6.480E-01
8	3.472E-01	2.078E-01	9.293E-02	1.000E-01	5.900E-01
9	3.036E-01	1.127E-01	5.042E-02	2.100E-01	4.400E-01
10	4.052E-01	1.970E-01	8.810E-02	1.760E-01	6.280E-01
11	3.298E-01	1.182E-01	5.284E-02	1.320E-01	4.450E-01
12	2.524E-01	1.205E-01	5.389E-02	7.100E-02	4.000E-01
13	2.442E-01	1.980E-01	8.856E-02	9.000E-02	5.500E-01
14	2.660E-01	1.121E-01	5.011E-02	1.360E-01	4.200E-01

Appendix 2.6a Total Proteins : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	66.50	7.091	2.242	60.00	75.00
0	71.30	5.056	1.599	65.00	80.00
1	68.60	4.551	1.439	60.00	75.00
2	65.90	4.358	1.378	60.00	70.00
3	64.40	4.551	1.439	58.00	70.00
4	65.10	4.630	1.464	55.00	74.00
5	65.40	4.671	1.477	55.00	71.00
6	66.30	3.831	1.212	58.00	72.00
7	64.70	3.302	1.044	57.00	70.00
8	62.50	5.191	1.641	55.00	75.00
9	60.60	4.502	1.424	50.00	66.00
10	63.60	5.929	1.875	50.00	70.00
11	64.80	5.329	1.685	52.00	70.00
12	64.80	6.197	1.960	50.00	71.00
13	65.30	5.697	1.802	52.00	71.00
14	65.30	7.056	2.231	51.00	75.00

Appendix 2.6b Total Proteins : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	70.00	3.536	1.581	65.00	75.00
0	73.60	4.722	2.112	68.00	80.00
1	65.40	4.827	2.159	61.00	71.00
2	63.00	4.690	2.098	58.00	70.00
3	62.40	4.827	2.159	55.00	68.00
4	64.40	3.782	1.691	60.00	70.00
5	66.40	5.595	2.502	60.00	75.00
6	65.80	5.119	2.289	61.00	74.00
7	63.00	3.742	1.673	60.00	69.00
8	60.80	3.194	1.428	57.00	65.00
9	56.60	4.099	1.833	50.00	60.00
10	59.40	1.673	7.483E-01	58.00	62.00
11	59.00	1.225	5.477E-01	57.00	60.00
12	58.00	2.121	9.487E-01	55.00	60.00
13	58.80	1.924	8.602E-01	56.00	61.00
14	61.20	2.683	1.200	59.00	65.00

Appendix 2.6c Total Proteins : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	74.00	4.595	1.453	65.00	81.00
0	76.70	5.982	1.892	68.00	85.00
1	76.00	3.682	1.164	71.00	81.00
2	75.80	5.692	1.800	69.00	85.00
3	76.60	4.274	1.352	70.00	85.00
4	76.20	5.712	1.806	71.00	85.00
5	80.90	4.458	1.410	77.00	92.00
6	80.00	2.708	8.563E-01	75.00	85.00
7	81.10	2.470	7.810E-01	78.00	86.00
8	78.00	4.295	1.358	72.00	85.00
9	72.70	3.974	1.257	70.00	80.00
10	74.70	4.620	1.461	70.00	82.00
11	74.80	5.329	1.685	70.00	84.00
12	76.50	3.866	1.222	72.00	83.00
13	76.80	4.826	1.526	70.00	88.00
14	76.40	4.033	1.275	70.00	84.00

Appendix 2.6d Total Proteins : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	75.40	5.128	2.293	70.00	81.00
0	79.40	7.701	3.444	72.00	90.00
1	77.40	1.517	6.782E-01	75.00	79.00
2	74.80	4.324	1.934	70.00	80.00
3	79.80	3.347	1.497	76.00	85.00
4	73.20	4.087	1.828	70.00	80.00
5	76.60	2.408	1.077	74.00	80.00
6	77.20	2.280	1.020	74.00	80.00
7	76.60	1.517	6.782E-01	75.00	78.00
8	72.00	3.391	1.517	68.00	76.00
9	77.00	4.359	1.949	70.00	81.00
10	77.80	6.573	2.939	72.00	88.00
11	74.40	6.269	2.804	70.00	85.00
12	74.00	2.121	9.487E-01	71.00	76.00
13	74.80	1.095	4.899E-01	73.00	76.00
14	76.20	1.304	5.831E-01	75.00	78.00

Appendix 2.7a Serum Albumin : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	26.50	3.864	1.222	21.30	33.70
0	31.02	2.425	7.669E-01	27.10	34.90
1	32.36	2.709	8.568E-01	28.60	38.50
2	31.66	2.192	6.932E-01	26.80	34.50
3	32.64	2.634	8.331E-01	26.80	36.20
4	33.60	2.630	8.317E-01	28.70	36.90
5	31.60	2.939	9.294E-01	26.60	36.50
6	29.60	1.778	5.623E-01	26.50	33.20
7	29.53	2.487	7.864E-01	25.50	33.10
8	32.80	1.744	5.514E-01	30.30	35.30
9	30.97	3.077	0.973	26.20	36.00
10	28.41	3.565	1.127	24.30	35.20
11	27.72	1.740	5.501E-01	25.10	30.20
12	29.35	1.636	5.173E-01	26.20	31.20
13	29.15	1.138	3.600E-01	27.00	31.00
14	29.40	1.837	5.808E-01	26.10	32.00

Appendix 2.7b Serum Albumin : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	28.46	8.735E-01	3.906E-01	27.60	29.90
0	26.30	4.117	1.841	20.40	32.00
1	30.62	2.142	0.958	28.40	33.90
2	33.26	2.092	9.357E-01	30.70	36.40
3	34.98	4.333	1.938	30.10	39.70
4	35.24	1.189	5.316E-01	34.10	37.10
5	33.26	1.993	8.914E-01	31.10	36.30
6	33.40	2.185	0.977	30.20	35.30
7	32.62	2.077	9.287E-01	30.10	34.80
8	32.94	2.197	0.983	29.90	35.70
9	36.26	3.135	1.402	33.00	40.40
10	35.28	1.983	8.868E-01	32.50	37.90
11	34.00	1.196	5.348E-01	33.10	35.50
12	33.80	1.042	4.658E-01	32.50	35.20
13	33.64	1.115	4.986E-01	31.80	34.70
14	33.20	1.093	4.889E-01	32.00	34.50

Appendix 2.7c Serum Albumin : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	29.76	2.631	8.319E-01	26.50	33.80
0	30.11	4.732	1.496	22.30	38.50
1	33.27	4.674	1.478	26.20	41.00
2	32.38	4.024	1.273	25.10	37.10
3	31.06	3.602	1.139	25.00	35.50
4	32.12	2.788	8.818E-01	29.00	38.10
5	31.46	2.533	8.011E-01	29.00	37.50
6	31.80	1.719	5.437E-01	29.20	35.20
7	33.31	2.844	8.994E-01	27.20	36.70
8	34.44	3.448	1.090	28.30	40.00
9	30.89	3.428	1.084	26.30	37.40
10	30.77	2.197	6.948E-01	27.90	34.90
11	31.86	2.437	7.706E-01	27.60	35.20
12	31.40	1.867	5.905E-01	28.10	33.50
13	31.23	1.917	6.063E-01	27.20	33.60
14	30.63	1.052	3.327E-01	28.20	32.10

Appendix 2.7d Serum Albumin : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	30.74	3.946	1.765	24.70	33.90
0	29.10	4.797	2.145	25.20	36.90
1	37.14	2.725	1.218	33.40	40.10
2	31.08	1.632	7.297E-01	29.30	33.40
3	34.20	1.930	8.631E-01	32.20	36.80
4	29.64	1.376	6.153E-01	27.70	31.20
5	30.24	1.704	7.620E-01	28.20	32.10
6	30.72	1.365	6.102E-01	29.10	32.50
7	31.16	1.832	8.195E-01	29.10	33.10
8	32.00	2.261	1.011	30.00	35.10
9	32.84	1.539	6.882E-01	31.20	35.20
10	36.42	4.348	1.944	30.20	42.10
11	34.56	3.935	1.760	30.00	40.10
12	33.86	3.402	1.521	30.50	39.00
13	33.56	1.889	8.447E-01	31.00	35.80
14	33.40	1.944	8.695E-01	30.90	36.00

Appendix 2.8a Serum Globulin : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	41.54	3.777	1.689	37.00	47.40
0	45.50	5.099	2.280	40.20	53.30
1	34.78	5.075	2.270	29.50	42.60
2	29.34	3.503	1.566	25.60	33.60
3	27.42	2.609	1.167	24.60	30.70
4	29.16	3.994	1.786	24.40	35.00
5	33.14	4.923	2.201	26.40	38.70
6	32.36	5.362	2.398	26.00	38.50
7	30.38	2.676	1.197	26.80	34.20
8	27.86	2.215	0.991	25.10	30.90
9	20.34	2.102	9.400E-01	17.00	22.20
10	24.12	1.965	8.789E-01	22.10	26.50
11	29.56	2.117	9.469E-01	27.50	32.40
12	30.50	1.336	5.975E-01	28.50	32.10
13	30.54	9.209E-01	4.118E-01	29.00	31.40
14	30.76	6.348E-01	2.839E-01	30.00	31.50

Appendix 2.8b Serum Globulin : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	41.54	3.777	1.689	37.00	47.40
0	45.50	5.099	2.280	40.20	53.30
1	34.78	5.075	2.270	29.50	42.60
2	29.34	3.503	1.566	25.60	33.60
3	27.42	2.609	1.167	24.60	30.70
4	29.16	3.994	1.786	24.40	35.00
5	33.14	4.923	2.201	26.40	38.70
6	32.36	5.362	2.398	26.00	38.50
7	30.38	2.676	1.197	26.80	34.20
8	27.86	2.215	0.991	25.10	30.90
9	20.34	2.102	9.400E-01	17.00	22.20
10	24.12	1.965	8.789E-01	22.10	26.50
11	29.56	2.117	9.469E-01	27.50	32.40
12	30.50	1.336	5.975E-01	28.50	32.10
13	30.54	9.209E-01	4.118E-01	29.00	31.40
14	30.76	6.348E-01	2.839E-01	30.00	31.50

Appendix 2.8c Serum Globulin : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	45.74	4.289	1.356	39.50	52.90
0	46.60	4.505	1.424	39.30	55.00
1	42.73	6.673	2.110	34.00	54.80
2	43.42	6.076	1.921	32.90	51.80
3	43.01	5.320	1.682	35.30	50.30
4	43.86	6.169	1.951	36.60	56.00
5	47.89	3.300	1.044	42.50	54.60
6	47.61	3.121	0.987	44.50	54.00
7	46.89	2.709	8.567E-01	42.90	51.90
8	43.56	5.110	1.616	37.00	51.90
9	41.82	6.757	2.137	32.60	53.70
10	43.96	5.089	1.609	38.10	52.50
11	43.84	4.416	1.397	38.30	52.50
12	43.53	4.800	1.518	35.40	51.60
13	43.62	4.301	1.360	37.20	51.30
14	43.59	3.652	1.155	38.50	50.20

Appendix 2.8d Serum Globulin : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	43.66	5.541	2.478	36.10	50.10
0	48.30	3.311	1.481	44.50	53.50
1	40.26	2.768	1.238	36.90	44.60
2	43.72	3.574	1.598	40.70	48.10
3	46.16	2.526	1.130	44.00	50.50
4	44.56	6.005	2.686	38.80	52.30
5	42.44	8.048	3.599	28.10	47.00
6	46.44	1.977	8.841E-01	43.50	48.30
7	45.44	2.539	1.136	41.90	47.80
8	40.00	1.661	7.430E-01	37.30	41.30
9	44.16	3.683	1.647	38.80	47.60
10	41.38	3.239	1.449	36.80	45.90
11	42.24	2.027	9.064E-01	39.50	45.00
12	43.20	2.152	0.962	40.10	45.20
13	43.62	1.486	6.644E-01	42.20	45.50
14	43.72	1.612	7.207E-01	41.70	45.50

Appendix 2.9a A/G Ratio : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	6.758E-01	1.506E-01	4.762E-02	5.504E-01	1.027
0	7.813E-01	1.283E-01	4.056E-02	6.393E-01	1.036
1	9.216E-01	2.118E-01	6.699E-02	6.745E-01	1.262
2	9.446E-01	1.706E-01	5.396E-02	7.500E-01	1.353
3	1.089	1.962E-01	6.206E-02	8.590E-01	1.403
4	1.094	2.070E-01	6.544E-02	7.906E-01	1.353
5	0.963	2.167E-01	6.854E-02	6.751E-01	1.327
6	8.131E-01	9.618E-02	3.042E-02	7.250E-01	1.044
7	8.415E-01	1.017E-01	3.216E-02	7.105E-01	1.000
8	1.147	2.716E-01	8.588E-02	7.943E-01	1.792
9	1.071	2.085E-01	6.594E-02	7.318E-01	1.365
10	1.004	1.695E-01	5.360E-02	8.182E-01	1.313
11	0.966	7.307E-02	2.311E-02	8.531E-01	1.093
12	0.992	6.578E-02	2.080E-02	8.908E-01	1.117
13	0.981	5.729E-02	1.812E-02	8.853E-01	1.054
14	0.984	4.492E-02	1.421E-02	9.010E-01	1.042

Appendix 2.9b A/G Ratio : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	6.900E-01	6.945E-02	3.106E-02	5.823E-01	7.568E-01
0	5.857E-01	1.234E-01	5.517E-02	4.286E-01	7.442E-01
1	8.966E-01	1.483E-01	6.634E-02	6.667E-01	1.068
2	1.144	1.243E-01	5.558E-02	1.031	1.290
3	1.286	2.140E-01	9.571E-02	1.085	1.602
4	1.228	1.813E-01	8.110E-02	1.000	1.459
5	1.022	1.660E-01	7.425E-02	8.428E-01	1.273
6	1.060	2.218E-01	9.918E-02	8.436E-01	1.346
7	1.080	1.092E-01	4.883E-02	0.987	1.239
8	1.188	1.182E-01	5.287E-02	1.027	1.308
9	1.796	2.153E-01	9.628E-02	1.568	2.061
10	1.474	1.819E-01	8.135E-02	1.226	1.715
11	1.155	9.575E-02	4.282E-02	1.025	1.291
12	1.110	6.843E-02	3.060E-02	1.012	1.193
13	1.103	5.971E-02	2.670E-02	1.013	1.179
14	1.080	5.108E-02	2.284E-02	1.016	1.150

Appendix 2.9c A/G Ratio : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	6.582E-01	1.029E-01	3.255E-02	5.312E-01	8.228E-01
0	6.525E-01	1.214E-01	3.840E-02	4.231E-01	8.280E-01
1	8.074E-01	2.188E-01	6.920E-02	4.781E-01	1.206
2	7.655E-01	1.811E-01	5.728E-02	5.030E-01	1.128
3	7.386E-01	1.605E-01	5.075E-02	5.000E-01	0.969
4	7.461E-01	1.246E-01	3.940E-02	5.179E-01	9.269E-01
5	6.621E-01	9.462E-02	2.992E-02	5.568E-01	8.824E-01
6	6.712E-01	6.509E-02	2.058E-02	5.626E-01	7.910E-01
7	7.131E-01	7.987E-02	2.526E-02	5.939E-01	8.182E-01
8	8.049E-01	1.507E-01	4.765E-02	5.582E-01	1.081
9	7.659E-01	1.983E-01	6.269E-02	4.898E-01	1.147
10	7.101E-01	1.091E-01	3.449E-02	5.486E-01	8.703E-01
11	7.342E-01	9.907E-02	3.133E-02	5.676E-01	8.538E-01
12	7.290E-01	8.869E-02	2.805E-02	5.833E-01	8.701E-01
13	7.214E-01	7.552E-02	2.388E-02	6.212E-01	8.038E-01
14	7.066E-01	5.695E-02	1.801E-02	6.076E-01	7.844E-01

Appendix 2.9d A/G Ratio : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	7.142E-01	1.412E-01	6.316E-02	5.768E-01	9.391E-01
0	6.059E-01	1.151E-01	5.149E-02	4.953E-01	7.672E-01
1	9.290E-01	1.233E-01	5.515E-02	7.489E-01	1.087
2	7.139E-01	5.928E-02	2.651E-02	6.561E-01	8.029E-01
3	7.430E-01	6.412E-02	2.868E-02	6.832E-01	8.364E-01
4	6.774E-01	1.147E-01	5.130E-02	5.296E-01	8.041E-01
5	7.462E-01	2.237E-01	1.000E-01	6.000E-01	1.142
6	6.625E-01	4.199E-02	1.878E-02	6.205E-01	7.143E-01
7	6.890E-01	7.683E-02	3.436E-02	6.318E-01	7.900E-01
8	8.002E-01	4.902E-02	2.192E-02	7.317E-01	8.582E-01
9	7.472E-01	6.284E-02	2.810E-02	6.807E-01	8.224E-01
10	8.815E-01	9.385E-02	4.197E-02	7.225E-01	0.957
11	8.179E-01	7.871E-02	3.520E-02	7.229E-01	8.911E-01
12	7.830E-01	5.472E-02	2.447E-02	7.245E-01	8.647E-01
13	7.691E-01	2.520E-02	1.127E-02	7.294E-01	7.973E-01
14	7.636E-01	2.198E-02	9.828E-03	7.410E-01	7.965E-01

Appendix 2.10a Serum AST Levels : Sheep (infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	121.2	43.50	13.76	76.00	203.0
0	98.60	20.51	6.486	70.00	136.0
1	90.10	20.93	6.617	62.00	131.0
2	94.20	31.27	9.889	64.00	166.0
3	97.90	30.27	9.572	66.00	162.0
4	93.60	29.53	9.337	58.00	163.0
5	90.50	29.51	9.331	59.00	151.0
6	89.70	23.04	7.286	62.00	142.0
7	101.3	23.17	7.326	70.00	153.0
8	111.8	29.74	9.403	71.00	164.0
9	120.4	35.63	11.27	78.00	193.0
10	109.0	34.37	10.87	79.00	177.0
11	108.9	31.71	10.03	73.00	165.0
12	113.4	41.93	13.26	79.00	211.0
13	125.6	39.68	12.55	87.00	191.0
14	126.9	35.87	11.34	77.00	195.0

Appendix 2.10b Serum AST Levels : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	107.0	22.03	9.854	84.00	143.0
0	115.6	48.77	21.81	75.00	195.0
1	120.8	43.01	19.24	88.00	191.0
2	95.00	23.40	10.46	70.00	133.0
3	90.20	20.30	9.080	68.00	122.0
4	91.40	15.57	6.961	73.00	112.0
5	94.60	15.61	6.983	76.00	112.0
6	100.2	14.02	6.272	82.00	114.0
7	99.80	12.54	5.607	88.00	118.0
8	106.6	13.16	5.887	89.00	123.0
9	115.4	12.36	5.528	100.0	132.0
10	121.4	10.26	4.589	105.0	131.0
11	123.8	9.628	4.306	110.0	133.0
12	115.0	14.73	6.588	103.0	140.0
13	110.4	17.63	7.884	93.00	137.0
14	107.8	14.69	6.568	96.00	133.0

Appendix 2.10c Serum AST Levels : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	63.80	16.53	5.228	37.00	88.00
0	60.90	14.87	4.701	39.00	91.00
1	66.30	16.43	5.194	45.00	98.00
2	59.40	9.698	3.067	43.00	72.00
3	62.70	16.90	5.344	44.00	103.0
4	59.50	14.67	4.639	41.00	90.00
5	67.80	14.83	4.690	46.00	97.00
6	73.50	20.77	6.568	46.00	110.0
7	110.3	39.91	12.62	43.00	182.0
8	218.9	225.8	71.41	49.00	652.0
9	157.8	174.8	55.28	47.00	595.0
10	142.3	98.21	31.06	56.00	293.0
11	85.30	49.43	15.63	54.00	222.0
12	77.00	34.52	10.91	42.00	162.0
13	63.60	12.89	4.078	48.00	95.00
14	61.80	15.40	4.869	43.00	90.00

Appendix 2.10d Serum AST Levels : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	60.60	10.90	4.874	46.00	70.00
0	48.00	13.56	6.066	31.00	65.00
1	53.20	10.71	4.790	39.00	68.00
2	58.00	8.944	4.000	47.00	69.00
3	58.60	12.46	5.573	48.00	79.00
4	60.80	16.93	7.572	44.00	88.00
5	61.60	11.80	5.278	48.00	79.00
6	58.00	15.03	6.723	43.00	83.00
7	63.80	19.83	8.868	48.00	98.00
8	66.00	17.36	7.765	46.00	93.00
9	73.40	26.36	11.79	47.00	117.0
10	73.80	24.86	11.12	60.00	118.0
11	78.20	38.71	17.31	54.00	147.0
12	79.80	41.19	18.42	57.00	153.0
13	108.2	97.81	43.74	57.00	283.0
14	111.8	114.7	51.31	58.00	317.0

Appendix 2.11a Serum GGT Levels : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	41.80	10.98	3.473	23.00	63.00
0	37.30	8.138	2.574	24.00	51.00
1	39.10	10.16	3.213	24.00	58.00
2	48.50	9.902	3.131	32.00	61.00
3	47.20	9.998	3.162	29.00	63.00
4	47.00	9.428	2.981	30.00	59.00
5	51.80	10.24	3.238	34.00	69.00
6	52.00	9.452	2.989	35.00	67.00
7	51.00	9.286	2.936	41.00	68.00
8	70.60	32.99	10.43	34.00	153.0
9	75.70	26.73	8.451	36.00	116.0
10	79.50	25.19	7.966	45.00	122.0
11	72.30	19.47	6.157	43.00	97.00
12	83.70	26.28	8.309	49.00	137.0
13	82.50	24.55	7.764	52.00	119.0
14	84.60	25.70	8.128	57.00	130.0

Appendix 2.11b Serum GGT Levels : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	35.60	5.727	2.561	27.00	43.00
0	44.20	4.970	2.223	37.00	50.00
1	47.00	7.583	3.391	39.00	58.00
2	43.20	5.848	2.615	35.00	51.00
3	38.40	10.81	4.833	20.00	48.00
4	43.40	6.914	3.092	36.00	52.00
5	45.20	11.56	5.171	27.00	56.00
6	46.40	6.107	2.731	39.00	53.00
7	45.40	6.542	2.926	36.00	50.00
8	48.40	6.656	2.977	39.00	55.00
9	49.00	6.205	2.775	41.00	57.00
10	57.40	11.15	4.986	44.00	69.00
11	57.00	13.55	6.058	42.00	71.00
12	59.60	11.10	4.966	47.00	69.00
13	61.20	12.79	5.722	47.00	73.00
14	57.40	12.14	5.428	42.00	73.00

Appendix 2.11c Serum GGT Levels : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	38.90	9.678	3.060	29.00	55.00
0	43.00	10.07	3.183	30.00	59.00
1	38.20	11.40	3.605	22.00	57.00
2	40.00	10.79	3.412	22.00	60.00
3	38.00	8.969	2.836	24.00	57.00
4	38.50	9.396	2.971	25.00	60.00
5	40.80	9.897	3.130	26.00	61.00
6	39.40	10.86	3.436	24.00	63.00
7	45.80	8.917	2.820	31.00	60.00
8	72.10	38.28	12.10	32.00	150.0
9	69.90	46.86	14.82	23.00	165.0
10	72.20	48.59	15.36	27.00	176.0
11	57.40	27.70	8.760	23.00	109.0
12	58.60	25.50	8.064	26.00	109.0
13	46.60	14.70	4.648	27.00	76.00
14	43.10	10.09	3.192	26.00	59.00

Appendix 2.11d Serum GGT Levels : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	52.80	9.257	4.140	40.00	61.00
0	36.40	17.37	7.769	18.00	55.00
1	35.20	11.88	5.314	15.00	45.00
2	40.40	10.90	4.874	28.00	53.00
3	37.20	9.910	4.432	25.00	47.00
4	32.40	7.570	3.385	24.00	43.00
5	37.80	9.176	4.104	28.00	49.00
6	36.80	9.783	4.375	26.00	49.00
7	39.60	8.173	3.655	29.00	51.00
8	35.60	9.965	4.456	26.00	48.00
9	37.20	11.01	4.923	27.00	51.00
10	39.20	10.62	4.748	28.00	51.00
11	36.00	10.49	4.690	26.00	49.00
12	37.20	8.758	3.917	29.00	48.00
13	37.00	8.803	3.937	28.00	47.00
14	39.40	8.905	3.982	29.00	49.00

Appendix 2.12a Serum GD Levels : Sheep (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	15.51	24.54	7.760	5.400	85.10
0	9.500	8.774	2.775	1.600	25.00
1	5.299	4.909	1.552	6.900E-01	16.70
2	6.680	8.653	2.736	1.300	30.50
3	7.955	7.910	2.501	9.500E-01	25.00
4	13.05	15.82	5.002	2.800	55.20
5	17.98	27.40	8.665	1.500	93.30
6	11.23	6.425	2.032	3.500	21.80
7	37.01	44.77	14.16	6.800	160.1
8	64.87	78.46	24.81	7.900	252.0
9	73.96	78.24	24.74	19.60	230.0
10	83.42	119.2	37.69	13.70	310.0
11	102.4	137.3	43.42	10.80	378.2
12	107.3	176.9	55.95	8.200	540.6
13	165.6	220.2	69.65	8.640	625.6
14	213.3	258.3	81.69	16.40	824.0

Appendix 2.12b Serum GD Levels : Sheep (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	10.64	6.708	3.000	4.800	20.40
0	5.280	2.316	1.036	3.100	8.300
1	3.080	0.9230	0.4128	2.200	4.600
2	8.360	6.007	2.686	1.200	14.90
3	11.30	8.828	3.948	1.600	22.50
4	11.82	8.611	3.851	1.500	22.00
5	10.44	8.331	3.726	1.300	21.20
6	16.26	15.52	6.941	1.500	40.70
7	15.67	12.10	5.412	1.100	25.70
8	21.42	15.12	6.760	8.680	45.90
9	31.94	19.19	8.581	2.800	52.10
10	32.48	24.27	10.85	10.00	65.50
11	271.6	185.7	83.04	13.10	477.9
12	132.4	108.1	48.35	21.20	285.4
13	110.9	68.88	30.80	11.30	195.2
14	53.12	67.69	30.27	5.700	172.7

Appendix 2.12c Serum GD Levels : Goats (Infected)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	2.940	1.615	5.106E-01	7.000E-01	5.100
0	3.620	2.761	8.730E-01	4.000E-01	9.400
1	3.550	2.554	8.075E-01	9.000E-01	9.900
2	5.010	4.282	1.354	1.900	16.70
3	7.020	2.398	7.582E-01	2.900	9.600
4	12.39	7.090	2.242	4.400	23.80
5	21.84	11.42	3.610	4.400	35.30
6	25.98	15.30	4.838	7.500	44.20
7	105.3	77.99	24.66	15.40	232.3
8	115.6	191.9	60.69	4.700	622.0
9	105.4	206.6	65.34	11.70	664.0
10	82.14	101.3	32.05	11.10	342.4
11	24.97	24.91	7.879	6.400	91.50
12	42.60	88.74	28.06	3.070	294.1
13	13.23	9.282	2.935	3.670	35.88
14	11.28	9.308	2.943	4.200	36.40

Appendix 2.12d Serum GD Levels : Goats (Control)

WEEKS	MEAN	S.D.	SE(MEAN)	MINIMUM	MAXIMUM
-1	3.960	2.663	1.191	7.000E-01	7.000
0	2.960	2.141	0.957	1.600	6.700
1	2.800	1.756	7.855E-01	6.000E-01	5.300
2	3.520	2.262	1.012	1.400	6.500
3	3.980	2.839	1.270	1.400	8.700
4	4.140	1.730	7.737E-01	1.400	5.900
5	8.480	4.498	2.012	1.900	13.90
6	5.020	2.254	1.008	2.800	8.000
7	3.540	2.323	1.039	9.000E-01	7.000
8	4.160	2.834	1.268	4.000E-01	7.600
9	7.822	3.320	1.485	2.600	10.60
10	11.44	8.970	4.011	2.900	25.50
11	35.38	58.85	26.32	2.700	140.3
12	30.66	43.28	19.35	2.810	107.1
13	48.72	64.11	28.67	2.920	153.7
14	15.78	14.29	6.389	1.800	36.40

APPENDIX 3

Electrophoresis Serum Protein Fractionation Results Of Sheep & Goats On Cellulose Acetate Strips

Sheep:

Goats: