Toxoplasmosis : I A study of the fluctuation in antibody titre to *Toxoplasma gondii* in a flock of sheep. II A comparison between the three serological tests used in the study.

by

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SUMMARY

A review of toxoplasmosis is presented in the dissertation.

A flock of 40 sheep, were bled weekly for a period of 6 - 7 months (Nov. 1976 - May/June 1977). At least one of three serological tests were carried out on each serum sample.

The results showed a good degree of association between the three tests especially between the I.H.A.T. and the I.F.A.T. These two tests were considered to be easier to perform than the C.F.T., although the specificity of the I.H.A.T. was doubted. A titre of 1:8 or less was considered to be negative and the 1:16 as inconclusive for the I.H.A.T. and I.F.A.T.

Antibody titres showed little change over the period of study, a slight decrease perhaps. A similar state of decrease or of no change was noted when comparing titres before and after parturition.

In one ewe and lamb that showed serological evidence of infection around parturition it took four weeks for the antibody titres to rise four-fold.

Minor fluctuations in the results were put down to experimental variation, but larger fluctuations were considered to indicate re-infection or possible reactivation of latent infection. Periods of cold weather appeared to have no effect on titres, and vaccination had no apparent effect, except for one case of abortion which was not due to toxoplasma.

Ewes showed a higher reactor rate compared with gimmers but there was very little evidence of lateral transmission of infection within the flock although about five ewes showed a transient seroconversion shortly after tupping. The absence of disease transmission to sero-negative ewes at lambing time was considered to be possibly due to genetic factors within the host, or to low excretion rates of infection with a mild strain of the parasite.

Ewes with high antibody titres produced less lambs than the group with low or negative titres, and finally there was passive transfer of antibody between ewe and lamb.

INTRODUCTION

A flock of 40 sheep were bled at weekly intervals for a period of six to seven months (November, 1976 - May/June, 1977). Three serological tests were carried out on the serum samples. The purpose of the project was to study any fluctuations in the antibody levels to toxoplasma, and to compare the three tests used.

REVIEW OF LITERATURE.

Toxoplasmosis is a disease caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular protozoan parasite.

History:

T. gondii was first discovered in the North African rodent, the gondii, in 1908 by Nicolle & Manceaux. Independantly, Splendore in 1909 observed the parasite in rabbits: at that time it was thought to be host specific and this lead to the description of numerous species of *Toxoplasma*, one for each host species. It was not until 1952 that Weinman showed that all known species of Toxoplasma were experimentally capable of infecting most heterogenous homothermal animal species and so the parasite in all species of host were called *T. gondii*, the original name given by Nicolle and Manceaux.

Classification:

The classification according to Baker (1973) is as follows.

Kingdom	Animalia
Phylum	Protozoa
Sub-phylum	Sporozoa
Class	Telosporea
Sub-class	Coccidia
Order	Encoccidia
Sub-order	Eimeriina
Family	Toxoplasmatidae
Genus	*Toxoplasma*
Species	*T. gondii*

Aetiology:

The parasite is cresentic in form measuring 4-6 μ long by 2-3 μ wide. This trophozoite form of the parasite has a bluntly pointed anterior end and a rounded posterior end containing the nucleus. They die rapidly outside the host, in the carcase of dead animals and are also destroyed on drying, by changes in osmotic pressure and on exposure to low heat (Jacobs 1957).

Strains:

Strains of Toxoplasma isolated from various species of animals and birds are biologically and immunologically similar, (Sabin, 1939; Negbrega & Reis 1942). But strains of toxoplasma vary in their virulence, (Araujo, Williams, Grumet & Remington 1976). The main criteria for differentiating strains are virulence and the characteristics of the disease produced; the most virulent strains are those which are highly pathogenic for mice and also produce severe disease in other laboratory animals. With strains of low virulence there is usually a lower parasitaemia, less tissue invasion and shorter persistence of the parasite. Organisms isolated from animals which have been sick or dying of infection are usually more virulent than those that have been obtained from an animal showing no clinical evidence of disease

A sudden change in virulence was reported by Lainson (1955) who found that an avirulent strain isolated from rabbits as greatly enhanced in virulence by passage through multi-mammate rats. Beverley (1976) stated that virulence may be increased following infection in an unusually susceptible animal, or as a result of conversion into oocysts in cats and then back again into trophozoites in the original host species, or in the laboratory by rapidly repeated intraperitoneal passages in mice. Ito, Tsunoda, Nishikawa and Matsui (1975) showed that the same strain of Toxoplasma in different laboratory animal species showed differing pathogenicity.

Araujo et al (1976) as the result of their experiments using different breeds of mice, considered that the pathogenicity of a strain of T. gondii and the susceptibility of a given host may be linked to genetic factors within the host.

Distribution:

Toxoplasma is found in most parts of the world. However infection is more prevalent in countries with warm, moist climates than in those with cold, dry climates (Watson 1972); dry and high areas have lower rates than do coastline areas in the same county (Gibson & Coleman, 1958; Franti, Reimann, Behymer, Suther, Howarth & Ruppaner 1976); finally there is a higher prevalence of infection in irrigated areas (Franti et al, 1976).

Epidemiology & Pathogenesis.

Host susceptibility.

T. gondii has little host specificity and can infect any warm blooded animal but not cold blooded animals. (Baker, 1973).

Incidence.

While infection may be common in many species of mammals and birds, (Franti, Connolly, Reimann, Behymer, Ruppaner, Willadsen & Longhurst, 1975; Franti, Riemann, Behymer, Suther, Howarth & Ruppaner, 1976; Pake 1975) it does not often develop into clinically recognisable disease. It has been estimated that a quarter of a million human infections occur in Britain each year yet in only a thousand of these are there clinical manifestations, (Beverley, 1976). It was postulated by Beverley (1976) that similar disparity between infection and morbidity rates occur in most animals, and Watson (1972) stated that serological surveys indicate that between 30 and 60% of the adult populations in most countries have encountered infection with Toxoplasma.
Serological surveys have indicated that Toxoplasma infection is prevalent in livestock throughout the world. Approximate sero-positives for each species on a world wide basis are: (Turner, 1976)

Cattle	22%
Sheep	39%
Goats	22%
Horses	19%
Dogs	33%

Carnivores tend to show a higher percentage of infection (Franti et al, 1975 & 1976; Pake, 1975).

Numerous Toxoplasma antibody surveys in cats have been performed throughout the world; the prevalence of antibody has been found to range from 5 to 85% being higher in stray animals.

Pathogenesis.

The portal of entry is thought to be the nasopharynx or intestinal

mucosa. The former is suspected to be the more common as in a high proportion of human patients the upper deep cervical lymph nodes are the first to become enlarged (Beverley, 1976).
The trophozoite form of the parasite can only multiply in the cytoplasm of a nucleated host cell (Weinmann, 1952). In acute disease, each parasite actively penetrates a host cell membrane, takes up a position near the nucleus and becomes surrounded by a parasitophorous vacuole. In this vacuole, repeated mulitplication occurs every 6 hours, so distending the host cell; such a cell is known as a terminal colony or pseudocyst. This finally ruptures liberating up to 32 parasites each capable of invading another host cell. this rupturing of cells gives rise to focal areas of necrosis. In animals succumbing to infection, the parasitaemia can mount to high levels (Jacobs, 1957). Spread of these liberated parasites, within the host, may be local, haematogenous, via the lymphatics or across serous surfaces. Any type of cell can be invaded, but most commonly they are cells of the reticulo-endothelial system. Any organ can be involved, but most frequently affected are the brain, lung, heart muscle, liver, spleen and lymph nodes.

The sub-acute stage of the infection is characterised by the appearance of serum antibodies and cellular immunity to toxoplasma within the host; extracellular forms of the parasite are phagocytosed, so reducing the parasitaemia. Intracellular forms are not killed but their multiplication is slowed and another asexual stage develops - the tissue cyst. This marks the beginning of the chronic stage of the infection.
The tissue cyst originates as a slow growing collection of parasites inside a host cell and is surrounded by a thick parasitophorous vacuole. Gradually the whole of the contents of the host cell are used as nutrients for the developing tissue cyst; the cyst wall is elastic allowing for development and multiplication of the contained organisms. These are known as zoites; they continue to grow and multiply but at a very much reduced rate. They are a little smaller than trophozoites. Tissue cysts are most numerous in the central nervous system and are found more often in the grey matter

than white; they are also quite common in skeletal muscle, heart muscle and lungs, but are also occasionally found in the spleen, lymph nodes or other organs. They vary in size from about 20u, containing only a few zoites, up to 100u and containing thousands of zoites. Tissue cysts persist for many months or years, possibly for the life-time of the host.

The above cycle is known as the asexual cycle and occurs in mammals (including the felidae) and birds.

For years this cycle did not completely explain the clinical picture, especially the disease in herbivores, which was more difficult to explain than in carnivores.

After many years of work, mainly by Hutchinson & workers (Hutchinson, 1965 & 1967; Sheffield & Melton, 1969; Hutchinson, Dunachie, Siim & Work, 1970 & 1971 and Dubey and Frenkel, 1972) it transpired that T. gondii underwent a true coccidian life-cycle in the cat and that resistant oocysts of T. gondii were present in the faeces of infected cats. This is called the sexual cycle, with shizonts and gametes developing in the epithelial cells near the tips of the villi of the lower jejunum and upper ileum; after fertilisation the macrogamete is shed into the intestinal lumen as an unsporulated oocyst. Further development occurs in the voided faeces with the formation of two sporocysts, each containing 4 sporozoites, all within the oocyst wall. Sporulation occurs in 2 - 4 days depending on the temperature and availability of oxygen. The pre-patent period is 3-5 days and the patent period about 14 days during which time millions of oocysts may be shed.

Oocysts can survive for many months in moist conditions (Hutchinson, 1965) and are resistant to acids, alkalis and many disinfectants (Ito, Tsunoda, Shimada, Taki & Matsui, 1975). Although iodine is effective. They are susceptible to drying and are killed by heating to 90°C for 30 seconds or 50°C for 30 minutes (Ito, Tsunoda, Taki, Nishikawa, & Matsui, 1975. The domestic cat and some of the other cats

of the felidae family, i.e. Bengal cat, bobcats, ocelots, jaguars and European Lynx, but not panthers, leopards, or cheetahs, are the only animals known, at present, to produce the highly resistant oocysts.

Transmission.

Spread to other hosts can occur by any of the three known forms - trophozoites, zoites in tissue cysts, and sporozoites in oocysts. The chances of survival between hosts are least for trophozoites and much the greatest for sporozoites.

In animals with acute toxoplasmosis, parasites may be found in the urine, faeces, milk, conjunctival fluid and saliva, (Jacobs, 1957). The parasites in these secretions and excretions are the zoites, which are very delicate and do not survive long outside the host, and there has been shown to be little spread of toxoplasmosis among animals, even when closely confined in the laboratory (Hutchinson, 1965).

Basically the three main modes of transmission are carnivorism, faecal contamination and transplacental passage.

Carnivores (including the felidae), scavengers and omnivores can become infected by eating flesh containing tissue cysts including aborted foetuses and their placentae. Man can become infected by eating raw or undercooked meat containing viable tissue cysts whilst cats become infected by eating birds, small mammals, raw or undercooked meat or offal containing tissue cysts.

Studies performed on common species of domestic rats e.g. Rattus rattus and R. norvegicus indicates that they are chronic carriers of the tissue cysts, and probably serve as a reservoir of infection for the cat (Wallace, 1973). Other species, such as mice, hares, rabbits etc., may also act as reservoirs. Pasture contaminated by trophozoites and zoites from infected placentae, aborted foetuses or dead carcases partially eaten by scavengers, may be a means of infecting other herbivores. Oocysts are a vital link in the life cycle of toxoplasma infections in nature. As already discussed they are highly resistant and can survive for many months in a moist environment. Oocysts can infect any host, and it is likely

that most natural infections of herbivores are due to them. This method of transmission would explain an epidemic of toxoplasmosis amont hares in Denmark (Christiansen and Siim, 1951), and also human cases of toxoplasmosis in vegetarians. The contact between cats and animals such as hill sheep, is quite remote. In sheep, infection could be derived from transported feed-stuffs contaminated with cat faeces, or perhaps such reservoir hosts play an important role. The epidemiology of this aspect of toxoplasmosis has yet to be fully elucidated.

Senbusch & Senbusch (1976) state that direct or indirect personal hygiene, the duration of exposure and the infectivity of the cats should be considered in assessing the risk of infection to contact animals or man although Chave & Carballo, (1976) showed that people in contact with cats were more likely to have positive antibody titres compared with those people with no contact with cats

In a case of toxoplasmosis in a piggery toxoplasma oocysts were isolated from the soil which was thus considered to have been contaminated by cat faeces (Ito, Tsunoda, Tsutsumi, Matsui, Nishikawa, Iida & Sasaki, 1975). It has also been shown that oocysts can be transferred mechanically by earthworms, cockroaches, certain snails and slugs and dung flies without loss of infectivity (Frenkel, Ruiz, Chinchilla, 1975; Wallace, 1972).

Congenital infections are due to transplacental spread of trophozoites from mother to foetus during a maternal parasitaemia which occurs when a primary infection coincides with pregnancy; the foetal risk is generally greater in early pregnancy. Immunity develops after infection and so in sheep foetuses in subsequent pregnancies are safe from the more serious effects of infection; on the other hand in most small animals immunity is not so effective and congenital infections can occur in all pregnancies. Vertical transmission has been observed in rabbits, guinea-pigs, multi-mammate rats and mice - in the last species as far as the tenth generation; this may play a part in maintaining infection in nature by providing a continuing source of infection for cats and other predators. (Beverley, 1976).

Clinical Disease.

The incidence of clinical disease due to toxoplasmosis is low. Clinical manifestations develop when the pathological changes are sufficiently numerous and large enough to cause dysfunction; only in the rare overwhelming infections do the clinical manifestations indicate that several organs are affected simultaneously (Beverley, 1976). The clinical signs may vary from sudden death to very mild, with lymphadenopathy, as the only presenting manifestation or be completely assymptomatic. In an acute case the clinical signs include encephalitis, pneumonia, hepatitis, lymphadenopathy, myocardial irregularities and ulcerative gastro-enteritis.

Clinical cases of "acquired" toxoplasmosis have been recorded in dogs (Mello, 1910), man, cats, cattle, sheep, horses (Macrux, Lenci, Ishizuka, Miguelo & Cunha, 1975), hares (Christiansen, 1948; Christiansen & Siim, 1951) a variety of laboratory animals (Ito, Tsunoda, Nishikawa & Matsui, 1975) and domestic poultry (Ericksen & Harbor, 1953). In fact the disease has been described in most of domestic species and also in a wide range of zoo and free-living animals, (Munday, 1970). The incidence between species varies markedly; clinical cases in cattle are uncommon compared with sheep even when grazing the same pasture (Koestner & Cole, 1961; Hartley, 1964). Young animals appear to be more susceptible than adults (Siim, Biering-Sørensen & Møller, 1963; Drake & Hine, 1967).

Clinical cases may be precipitated by some "stress" factor (Beverley, 1957) e.g. a sudden change or in severe extremes of weather, or be complicated by other infections (Campbell, 1956), especially the virus of canine distemper (Hartley, 1956; Moller & Nielsen, 1964; Dobos-Kovacs & Kardevan, 1976; Gaal, 1976).

Congenital toxoplasmosis is known to occur in man (Hertzberg, 1952), sheep (Hartley & Marshall, 1957), cattle (Munday, Ryan, King & Corbould, 1966), swine (Pan, Young, Wang, Yeh, Pan & Chen, 1962; Morwaki, Hayashi, Minami & Ishitani, 1976), dogs (Koestner & Cole, 1960) and mink (Pridham, 1961) and is possibly responsible for a proportion of infections in many species. A recent study by Macrux, Lenci, Ishizuka, Miguelo & Cunha (1975) suggested that toxoplasma may be one of the causes of abortion and infertility in the mare.

Beverley (1976) set out four criteria to explain the presence or absence of congenital toxoplasmosis in different species.

i) The structure of the placenta varies.
A "thick" eg ovine placenta instead of acting as a barrier is more likely to slow down parasitic migration; multiplication then leads to placental lesions and a large population of parasites is available to infect the foetus. Placental damage may lead to insufficiency and foetal death.

ii) The length of gestation varies.
In those species with short pregnancies, there may be insufficient time for congenital lesions to develop. The foetus will be born infected but appear normal although lesions may develop in the first few weeks of life. In animals with longer gestations, infection in early pregnancy may kill the embryo whereas in mid-pregnancy lesions develop in the foetus which are similar to those in "acquired" infections. The foetus usually dies in such cases but can be born alive but with pathological changes. The clinical signs in such cases can include convulsions, fever, dyspnoea, blindness, and tremors. Dead embyros and small foetuses may be resorbed or they may be expelled from the uterus when they are rarely seen since they may be eaten by the mother or by scavengers. In such cases the dam may be classed as barren. Larger dead foetuses are retained 'in utero' for varying lengths of time before expulsion; they may be mummified macerated or in a reasonable state of preservation.

iii) The immuno-competence of the foetus varies.
Both human and ovine foetuses can produce antibody several weeks before the end of pregnancy and the foetal lamb can also exhibit functional cellular immunity. The presence of such paradoxically immune competence should lessen foetal parasitaemia but a cellular immunity, as well as eliminating infection, may contribute towards the pathological lesions themselves.

iv) Maternal immunity varies.
In large animals the immunity is solid and long lasting so

after an initial infection or abortion all subsequent foetuses are safe.

In small animals the reverse is true. The immunity is not so effective and so foetuses in all pregnancies are at risk. Afrer an initial infection, there can be recrudescences with each pregnancy. This can lead to vertical transmission through many generations.

Ovine Toxoplasmosis.

An early report of "acquired" toxoplasmosis in sheep was made by Olafson and Monlux (1942). Wickham and Carne (1950) recorded a similar case. The association of toxoplasma with ovine perinatal mortality was first recorded by Hartley, Jebson & MacFarlane. (1954) and then by Hartley and Marshall (1957) in New Zealand.

In 1961, Beverley and Watson showed that toxoplasma infection was associated with ovine abortion in Yorkshire; they demonstrated that antibodies to toxoplasma were much higher in aborting ewes. In this and subsequent surveys (Beverley and Mackay, 1962; Beverley and Watson, 1962; Watson and Beverley, 1971a) they showed that the incidence in a flock may vary from one or two ewes up to 40% (Hartley, Jebson & McFarlane, 1954). Some ewes which were classed as barren were found to be strongly positive on serological testing for toxoplasma and suggested that such infertility would add to the number of lambs lost in actual abortions.

In 1962, Beverley and Watson showed that cases of ovine abortion could be due to dual infections and that primary infection with toxoplasma during pregnancy may not be of primary diagnostic significance.

Ewes that aborted one year were found to be immune and to lamb normally in subsequent years. (Watson & Beverley, 1971a). On a farm where abortions were occurring each year, they demonstrated that it was the replacement sheep that were affected and suggested that these bought-in replacements were non-immune. The conclusion was that infection was acquired during the months after mating whilst purchased sheep were running with the indigenous stock;

to eliminate this the replacements were bought in three months early, so that they became infected and immune before pregnancy, (Watson & Beverley, 1971b). In summarising their conclusions (Watson & Beverley, 1971a) stated that infection in the first third of pregnancy leads to foetal death and resorption whilst infection in mid-pregnancy may cause foetal death and abortion in up to 50% of ewes; infection in late pregnancy is unlikely to cause foetal death although some lambs may be weak at birth, with fever and dyspnoea, and die within 3 - 4 days.

Acute toxoplasmosis, with fever, dyspnoea, generalized tremor, abortions and stillbirths can rarely occur in adult ewes but the majority present as an abortion syndrome , during the last 3 - 4 weeks of pregnancy, or with neonatal deaths. After the initial abortion, further cases usually continue sporadically for 3 - 4 weeks and retained foetal membranes may also present a problem (Hartley, Jebson & McFarlane, 1954).

In general, the rate of seroconversion seems to be higher in sheep on lowland pastures compared with those on the hill. In some endemic areas, all sheep in a flock can be seroconverted (Miller, 1976). Beverley and Watson (1961) suggested that contact between sheep on lowland pasture was greater than between sheep on hill grazing; on the hill, contact with cats would be less and the environment would be more hostile to oocysts.

Munday (1970) showed that wethers possessed a lower reactor rate (12%) compared with that of ewes and rams (16%). He suggested that the majority of infection is acquired postnatally and that congenital transmission only provides a small proportion of the total sheep infection.

Munday also found that infections occurred throughout all seasons of the year and that the number of positive reactors was directly proportion to rainfall.

Finally he demonstrated the difference in reactor rates for different breeds. i.e.

Merinos - 10%
Merino X's - 18%
British breeds - 31%

Pathology.

The pathological lesions in acute cases include lymphadenopathy, splenomegaly, hepatomegaly and sero-sanguinous exudate in the body cavities. The lungs are also affected and this varies from small nodules in the dog to frank pneumonia in the calf or pig. In the dog and other carnivores there are often ulcers in the small intestine (Soulsby, 1969).

In lambs, the aborted conceptus may be mummified with sub-cutaneous oedema and an excess of fluid in the body cavities (Hartley & Marshall, 1957; Beverley, Watson & Spence, 1971) but such findings are not pathonomic.

The appearance of the affected ovine placenta is characteristic; there are multiple white or yellow focal lesions at various depths in the cotyledonary tissues, which often progress to necrosis. They are macroscopic, vary in number and may become confluent, when they would inhibit the separation of the placenta at parturition. The inter-cotyledonary areas are not affected (Hartley, Jebson & McFarlane, 1954; Beverley, Watson & Payne, 1971).

Pathological changes are more obvious in the placenta than in the foetus, and placental insufficiency is probably the primary cause of foetal death (Beverley, Watson & Payne, 1971).

In the lamb the microscopic changes are inflammatory and reactive. The former consist of focal lymphocytic infiltrations in such organs as the brain, lung, liver and placenta. In cardiac and striated muscle the infiltrations tend to be interstitial, and is associated with fibre necrosis. Inflammatory changes are usually greatest about 4 - 6 weeks after infection; resolution occurs thereafter, or in the case of necrosed tissue replacement occurs by fibrosis or calcification as in muscle, brain and placenta.

Reactive changes occur in lymph nodes, spleen and thymus. The main finding is of folliculor hyperplasia which usually takes 8 - 18 weeks to diminish to normal. The frequency and intensity of the histological changes vary from species to species, and are usually more pronounced in young animals than in adults (Beverley, 1976).

Pseudocysts can be found in the vicinity of lesions and also in areas free of inflammation. (Frenkel, 1956; Dubey, 1966) but they do not usually provoke an inflammatory response. Occasionally, when a pseudocyst ruptures, the contents will present a small focus for a delayed-type hypersensitivity reaction.

Pseudocysts can be isolated from a variety of organs including the brain, lungs, myocardium, liver, kidney, spleen, muscle, lymph nodes and placenta (Beverley, 1976; Moriwaki, Hawashi, Minami & Ishitani, 1976); such isolation can occur in both acquired and congenital cases of toxoplasmosis (Hartley & Marshall, 1957). Trophozoites can be demonstrated in the tissues of early clinical cases.

Immunity.

The sub-acute form of toxoplasmosis is characterized by the appearance of humoral and cellular immunity within the host, which rapidly clear the blood and tissues of the proliferative form of the parasite (Soulsby, 1969; Turner, 1976; Beverley, 1976).

Shirahata and Shimizu (1974) showed that in pigs, IgM antibody (by the I.F.A.T.) appeared at the end of the first week after infection. This was followed a few days later by IgG antibody. The IgM titre rose sharply and then fell and disappeared by 7 weeks post-infection (p.i.). The IgG rose gradually with a peak at 4 - 5 weeks p.i., and persisted until a secondary challenge at 12 weeks. p.i., which gave a further increase in IgG but no reappearance of IgM.

Butavaeva and Levit (1975) showed that complement-fixing antibodies in the coypu appeared about 17 days p.i.,. Costa, Araujo, Costa, Lima and Nascimento (1977) showed that antibodies (Dye test and I.F.A.T.) to Toxoplasma were detected on the 7th. day p.i., with peak titres occurring between days 9 - 21. They showed a difference in antibody response using tissue cysts or oocysts and different routes of infection. Calves were shown to have parasitaemias some time after the initial infection when they appeared healthy and their titres had declined. This was also demonstrated by Remington, Melton and Jacobs (1961). Later Dubey (1976) and Turner (1976) showed that an initial

infection in the cat could be reactivated with renewed oocyst production. Immunity in the cat appears to be variable and not absolute to the intestinal stages.

Cellular immunity also occurs within the host infected with Toxoplasmosis (Lai, 1975; Pelster, 1975). Lymphokimes also enhance resistance to the parasite (Lai, 1975). Depression of the cellular immune response by "stress" or other infectious agents e.g. canine distemper, can activate latent toxoplasmosis (Hartley, 1956; Moller & Nielson, 1964; Gaal, 1976; Dobos-Kovace & Kardevan, 1976). The use of immunosuppressants has a similar effect (Turner, 1976; Watson, 1972).

Immunity appears to be closely related to the strain of the parasite (Ito, Tsunoda, Nishikawa & Matsui, 1975) and to genetic factors working within the host (Araujo, Williams, Grumet & Remington, 1976). Considering immunity in sheep, it appears that under certain circumstances such as inter current disease toxoplasma re-infection can be super-imposed on the immune state, leading to abortions in immune stock. Generally once a ewe has become infected she is immune at least to homologous strains of the parasite.

Hartley and Moyle (1968) showed that the majority of ewes producing congenitally affected lambs had high antibody (D.T.) titres, but there were considerable fluctuations and they proposed that re-infection or reactivation of infection was occurring. There was a general decline in antibodies over the following 10 months. Hartley and Marshall (1957) and Beverley and Watson (1961) showed that aborting ewes had increasing D.T. titres within 3 weeks p.i. Munday (1970) using the I.F.A.T. showed that in aborting ewes the titres rose 14 days after abortion and were still rising by day 49.

The relationships between maternal - foetal immunity and abortions, stillbirths and congenital infections have already been discussed under the section "Clinical Disease - Ovine Toxoplasmosis".

Young lambs may possess high levels of antibodies to Toxoplasma at birth (Remington, Miller & Brownlee, 1968). These may be due to passive

transfer of antibody (IgG) from the dam to the progency, and if so decline to zero over the first 3 - 6 months of life (Hartley, 1966). Conversely the lamb may be congenitally infected with or without clinical coincidence. In such cases antibodies show a rise to significant levels over the first few weeks of life and remain high for many months; these antibodies are of the IgM fraction and can thus be differentiated from the transplacental IgG.

Hartley (1966) found a definite seasonal effect on titres, he showed that titres tended to rise in the Autumn and Winter months and this was noted in ewes, wethers and lambs irrespective of the husbandry system.

Diagnosis.

Several methods are available for the diagnosis of toxoplasmosis. These are outlined below.

i) Biopsies or post-mortem material for:-
   i) isolation of the parasite.
   ii) Histology.
   iii) Impression smears.

ii) Serology.

In the case of abortions and stillbirths in ovine toxoplasmosis, the appearance of the placenta, with white milary lesions on the cotyledons, is pathonomic (Beverley, Watson & Payne, 1971).

Isolation of the parasite.

Mice are inoculated intraperitoneally with an emulsion of the suspect tissue (Munday, 1970; Archer, Watson & Hunter, 1971; Watson, 1972). The mice may die in a few days with a fulmenating parasitaemia if it is a virulent strain, or may survive when they are euthanized 8 - 10 weeks after inoculation; in this case the brain emulsions are examined microscopically for the presence of cysts. According to Munday (1970) the above method is preferable to the rise of embryonated eggs, tissue culture or intra-cerebral inoculation of mice (Abbas, 1967). The recovery rate of the

organism was found to be greater from the diaphragm, using a pepsin digest technique, compared with that of the brain where the organism was only isolated from the brain of ewes with high antibody titres (Munday, 1970; Jacobs, 1963).

Histology

In abortion or stillbirth cases the best organ for histology is the placenta (Munday, 1970; Beverley, Watson & Payne, 1971) but the tissues must be in a reasonable state of preservation (Watson and Beverley, 1971b). However, histology can be carried out on biopsies or post-mortem material, from both acquired and congenital cases of toxoplasmosis and from a wide range of tissues (Munday, 1970; Beverley, Watson & Spence, 1971) to check for pathological changes and the presence of the parasite.

Impression smears.

Impression smears can be taken from biopsy, post-mortem or placental tissues and then a fluorescent antibody test carried out on the smear (Archer, Beverley, Fry & Watson, 1966). In experienced hands this method has been found to be specific and sensitive when compared with mouse inoculation (Archer & Watson, 1971) and it is less laborious than histology. (Archer, Watson & Hunter, 1971).

Serology.

Testing for toxoplasma antibodies in acquired cases in aborting dams or in a congenitally affected animals is helpful to confirm a diagnosis of toxoplasmosis.

There are many novel serological tests including a skin protection test (Sabin & Olitsky, 1937), precipitation test (O'Connor, 1957), flocculation test (Siim & Lind, 1960; Bozdeck & Jira, 1961), completment-fixation inhibition test (Harboe & Reenaas, 1957), plate haemolysin test (Jackson, O'Connor & Hall, 1974) indirect immuno-peroxidase test (Ourth, Matre, Helgeland & Tonder, 1974), indirect complement-fixation test (Roudna, 1975), radio-immunoassay (Gehle, Smith & Fuccillo, 1976) and enzyme-immunoassay (Voller, Bidwell, Bartlett, Fleck, Perkins and Oladehin, 1976).

Five tests are more commonly employed in routine toxoplasma diagnosis.

i) Dye test (D.T.) (Sabin & Feldman, 1948)

There are several modifications of the D.T. e.g. Beverley and Beattie (1952), Frenkel and Jacobs (1958), Moyle (1965).

Although the dye test is specific, sensitive and reproducible, it is technically cumbersome, slow and requires the use of live pathogenic Toxoplasma parasites, with the risk of laboratory infection, and an accessory factor from human serum which is not always readily available. It demands the maintenance of standard strains of Toxoplasma in the laboratory by frequent passage through mice and the preparation of a suitable freshly prepared peritoneal exudate from mice (Ludlam, 1959).

Beverley (1976) and Holmes, Illman and Beverley (1977) state that a high D.T. titre indicates a recent infection, but that "high" varied between the species e.g. in the thousands for sheep coypu, rabbit, guinea-pig and man and in the hundreds for cats, dogs, pigs, rats and birds.

The titres tend to remain high for periods of months up to years, the time being related to the height of the initial titre.

Beverley and Watson (1959) devised three levels of positivity for ovine toxoplasmosis.

1:16 or less	represents	a negative.
1:32 - 1:64	"	a moderate positive.
1:128 or more	"	a definite positive.

Hartley and Moyle (1968) agree with this interpretation and consider that a titre of 1:16 is positive in laboratory mice. They also stated that 95% of ewes producing congenitally affected lambs had a D.T. titre of 1:256 or more.

Hartley and Marshall (1957) and Beverley and Watson (1961) showed

that aborting ewes had increasing D.T. titres up to 1:3,000 within 3 weeks of infection.

ii) Complement-fixation test (C.F.T.) The C.F.T. is exacting and time consuming to perform but is highly specific (Huldt, 1958; Fulton and Fulton, (1965) used an antigen prepared from washed suspensions of Toxoplasma organisms derived from the peritoneal exudate of infected cotton rats. Cook and Pope (1959) and Fulton and Fulton (1965), using sheep sera, considered that a titre of 1:8 or more was positive, whereas Kean (1972) considered a titre of 1:4 or more to be positive.

iii) Indirect-Haemagglutination Test (I.H.A.T.)
Jacobs and Lunde (1957) used tannic acid treated cells while Jennis (1966) devised a technique using pyruvic aldehyde treated cells which remained stable for longer; the test is attractive because of its simplicity (Thornburn and Williams, 1972). The specificity of the IHAT was doubted by Lunde & Jacobs (1965) when they found that Toxoplasma had common antigens with Besnoitia, however, Suggs, Walls and Kagan (1968) showed that the R.H. strain of Toxoplasma had no such common antigens and recommended its use for routine diagnostic purposes and the toxoplasma I.H.A.T. was concluded to be adequate for survey purposed to show the prevalence of the disease (Lunde & Jacobs, 1958; Walls, Kagan & Turner, 1967).

iv) Direct Agglutination Test. (D.A.T.)
Fulton and Turk (1959) used whole formalinized Toxoplasma organisms as antigen. The standardization and reproducibility of the antigen preparation is much more difficult to achieve than in the other tests, and the reliability of the test was doubted by several workers including Sharman, Williams, Thorburn and Williams (1972).

v) Immuno-fluorescent Antibody Test. (I.F.A.T.)
The IFAT was first described by Goldman (1957); various modifications have been devised, including Kelen, Ayllon - Leindl & Labzoffsky (1962), Munday & Corbould (1971) and McKinney (1973), but its specificity was questioned by Araujo, Barnett, Gentry and Remington

(1971). They showed that some patients suffering from systemic lupus erythmatosis gave a false positive reaction in the IFAT for toxoplasmosis.

The IFAT in sheep is, however, regarded as being specific for Toxoplasma (Munday and Corbould, 1971; McKinney, 1973) and because of its ease of operation, economy and safety, they recommended that it should replace the other serological tests in toxoplasmosis. There are some limitations since a U.V. microscope is required and one technician can only examine small numbers of slides because of fatigue (Walton, Benchoff & Brooks, 1966); they regarded a titre of 1:8 or more as positive. Conversely Munday (1970) and McKinney (1973) considered that a titre of 1:16 or more was positive, whereas Costa, Araujo, Costa, Lima & Nascimento (1977) found titres up to 1:16 in their negative control calves.

Comparison of the tests.

A satisfactory serological confirmation of diagnosis of toxoplasmosis requires the demonstration of a four-fold, or greater, rise in antibody titre, on paired serum samples but frequently the first sample is taken rather late when the titre is at or past the peak.

Karim and Ludlam (1975) put forward the theory of two types of antigen

i) Cell wall or cuticular antigen
ii) Soluble or somatic antigen

In normal antigen preparations the D.T., D.A.T., C.F.T., and IgG/IgM I.F.A.T. measure the reaction between the cell wall antigen and specific antibody. Whereas the IHAT measures the reaction between the soluble, somatic antigen and the H.A. antibody. The titres in the cuticular antigen tests rise quickly after infection, the titres of the specific IgM- IFAT being proportionately lower than the other tests.

Conversely the titres in the I.H.A.T. rise more slowly but persist for longer and so a low IHAT titre combined with a high titre in one of the other four tests, especially the IgM-IFAT suggests a recent infection.

In general a high IHAT titre may be considered to represent a past infection, or in the case of progeny to represent passive transfer of antibody from the dam. If the titres to the other tests are concurrently high this may indicate a re-infection or reactivation of infection within the host animal.

In considering the various tests for routine diagnostic purposes, those using cell-wall derived antigens will give the highest titres in the early stages of the disease (Karim and Ludlam, 1975); the relative merits of each test type has already been reviewed earlier in this section but it is important to note that the selection of the strain and the method of preparation of the antigen will affect results in the test. Thorburn and Williams (1972) consider that the IHAT measured IgM and the D.T. measured IgG in their test procedures and was in agreement with Munday (1970). Remington and Miller (1966) described a technique of identifying 19S (IgM) and 7S (IgG) globulin antibodies. They inactivated IgM in the serum by using dimercaptoethanol, but heat treatment may also be used for a crude differentation e.g. 65°C for 30 minutes (Osiyemi, 1976).

Good correlation between the D.T. and C.F.T. has been shown by Bleir, Kabelitz and Siegert (1953) and Fleck (1961); between the D.T. and I.H.A.T. by Jacobs (1967), Sharman, Williams, Thorburn and Williams (1972), Jacobs and Lunde (1957), Fleck (1961) and Fairchild, Greenwald and Decker (1967); between the D.T. and D.A.T. by Sharman, Williams, Thorburn and Williams (1972) and Fulton and Turk (1959); between the D.T. and I.F.A.T. by Fairchild, Greenwald, and Decker (1967) and Munday and Corbould (1971); between the I.H.A.T. and D.A.T. by Sharman, Williams, Thorburn and Williams (1972); and finally between the I.H.A.T. and C.F.T. by Fleck (1961).

Jacobs (1967) considered the I.H.A.T. to be less sensitive than the D.T. although the titres may be higher (Fairchild, Greenwald & Decker, 1967). Huldt (1958) showed that the sensitivity of the C.F.T. was less than the D.T. and Fulton and Fulton (1965) found the titres determined by the C.F.T. to be lower than those determined by D.T. Jacobs (1973) found

the sensitivity between the D.T. and I.F.A.T. to be similar, but all these differences probably merely reflect the nature of the antigen-antibody reaction involved in each test. Discrepancies have arisen and Beverley, Watson and Payne (1971) describe serum antibody tests to toxoplasma as being "unreliable".

Beverley and Mackay (1962) and Osiyemi (1976) found some aborting ewes to have high IHAT titres but this finding was inconsistent since others had low or negative titres. Kelen, Ayllon-Leindl & Labzoffsky (1962) and Osiyemi (1976) found instances where the D.T. titres indicated infection and yet the I.F.A.T. titres were negative.

These differences may be attributed to different routes of infection in combination with different forms of the parasite (Costa et al., 1977).

Jacobs and Lunde (1957b) state that a four-fold difference in titre could be found in different D.T.'s performed on the same serum. Osiyemi (1976) descirbes descrepancies on the same serum to be due to improper shaking of thawed serum samples and "performer effect" and concluded that two laboratories may consistently produce different resilts on the same specimens, although using the same basic technique. He also pointed out the value of using more than one test on a serum sample to clarify the interpretation and stressed the need to consider results on a flock basis rather than in individual animals.

MATERIALS AND METHODS

A flock of 40 sheep were bled at weekly intervals from 10th November, 1976 to 4th May, 1977 and 15 ewes were bled until 8th June, 1977. The flock consisted of:

1 Tup (North Country Cheviot);
16 Half-bred ewes;
8 Blackface ewes;
15 Blackface gimmers.

History

The flock was managed on traditional lines. The gimmers were mixed with the older ewes in July and grazed together. A teaser ram was put in with the flock from 18/10/76 - 15/11/76 and then the tup (N. Country Cheviot) was run with the flock from 15/11/76 until 6/12/76. A raddle was used to show the ewes that had been tupped. A Suffolk ram was put in after the Cheviot ram to tup any ewes that the cheviot may have missed.

The ewes were fed increasing amounts of concentrates from the beginning of March until four weeks after lambing.

Two weeks before the commencement of lambing the ewes were dosed with "Covexin 8" (Burroughs Wellcome) to boost the ewes' immunity to the clostridial diseases and also to transfer immunity to their progeny. One ewe, a gimmer, (No. 93) aborted the day after dosing.

Lambing commenced on 9/4/77 and finished on 1/6/77, although the majority were within the first five weeks from the start of lambing. Three ewes were barren. After lambing each lamb(s) was ear tagged and rubber rings used for castration and tail docking.

After lambing, the ewe plus her lamb(s) were removed from the flock and put into pens for a few days and then the ewe was dosed in "Nilzan" and the ewe and lamb(s) turned onto new pastures.

The individual tupping and lambing dates are given on the results sheets for each ewe and her lamb.

Tests

Each serum sample was examined for toxoplasma antibodies by the indirect haemagglutination test (IHAT) and complement fixation test (CFT). Due to the limited time the indirect fluorescent antibody test (IFAT) was only carried out on a limited number of ewes (and their lambs).

The IHAT and CFT were commenced before the bleeding period had ended and so the sera were tested in batches of 5 bleeds (ie. 5 bleeds at 40 sheep per bleed = 200 sera). Where this was too large a number to process it was divided into three groups of 13, 13 and 14 sheep each sheep having 5 bleeds giving 65, 65 and 70 sera/group.

The IFAT was carried out after the completion of bleeding so the whole series of bleeds for one sheep (plus her selected lamb) were carried out together.

Every time a group of sera where tested, controls were also used and underwent the same process as the test sera. Results for the test sera were only taken when the control sera titres were correct or within one dilution, either way, of the normal. Several positive controls and a negative were included for each batch of sera tested.

(i) The indirect haemagglutination test (IHAT)

Def: In the IHAT the reaction between a particulate antigen and its specific antibody is made visible by linking the antigen onto the surface of red blood cells.

The IHAT was first performed by Jacobs and Lunde (1957) using tannic acid treated red cells coated with antigen. These cells were not very stable and so Jennis (1966) devised an IHAT using pyruvic aldehyde treated red cells. The latter cells being stable up to six months at 4°C.

The method and reagents used were as follows (H. Thorburn and H. Williams, 1972).

Reagents:

(i) H.A. antigen

Pyruvic aldehyde treated sheep erythrocytes (Jennis, 1966) were sensitized

with toxoplasma soluble antigen (cotton-rat peritoneal exudate) by the method described by Thorburn and Williams (1972).

The above was supplied by Dr. H. Williams of Raigmore Hospital, Inverness.

(ii) Diluent

This was sterile normal saline containing 2% foetal calf serum.

Equipment

Reusable "Microtiter" pipette dropper and capillary transfer pipettes (henceforth called a "loop") each of 0.05 ml capacity were used in conjunction with reusable "Microtiter" Lucite acrylic microtitration plates (12.7 x 9.5 cm, 96 conical "V" wells). All the above were produced by "Cooke Laboratory Products".

Method:

One (0.05 ml) drop of diluent was added to all wells of each row being used in the test. One row being used for each serum sample.

A "loop" containing (0.05 ml) serum was placed in the first well of a row, mixed and transferred to the second well of that row and the process repeated to the end of that row. The loop was removed, washed and dried. This was repeated for each serum sample, including the control sera. This gave doubling dilutions of the serum from 1/2 up to 1/256 (any sera positive at 1/256 were then titrated out to 1/4096).

One (0.05 ml) drop of sensitized red cells (pyruvic aldehyde treated) was added to each well. The plates were shaken, placed on damp blotting paper (to reduce any static electricity effects), covered and left to incubate at room temperature for 2 hours before reading.

The last dilution showing 100% agglutination was used as the end-point.

Several control sera were included with each batch tested and test results only taken when the control sera gave the correct titre, or within 1 dilution either way.

After use, the plates were rinsed in water and soaked in "Haemasol" overnight, and then washed (several times in tap and then diluted water) and dried before re-use .

(ii) The Complement Fixation Test. (CFT)

The method used was that described by Bradstreet & Taylor (1962). This is a complement fixation test applicable to the diagnosis of virus diseases and was used for the diagnosis of Q-fever at Edinburgh V.I. Centre. It was also used as the CFT for toxoplasma diagnosis by Dr. Williams at Raigmore Hospital, Inverness, (the reference laboratory of Scotland for toxoplasmosis).

Definition :

The principle of the CF test is that the heated inactivated serum (antibody) sample is mixed with complement and antigen. If an antigen-antibody reaction takes place, complement is fixed. This is demonstrated by testing for residual complement. Sheep cells, sensitized with anti-sheep cell haemolytic serum, are added. If lysis does not occur, complement has been fixed in the first reaction, and the test is positive. If there is lysis, free complement has been available, indicating that it was not fixed in the first reaction, and that the test is negative.

Equipment :

1. Disposable "U" well microtiter plates were used.
2. 0.025ml pipette dropper.
3. 0.025ml capillary transfer pipettes (henceforth called a "loop").
4. Clean 10ml and 1ml pipettes.

0.025ml constitutes "one volume".

Reagents :

1. Veronyl buffered Saline (V.B.S.)

V.B.S. tablets (containing calcium and magnesium) form "Oxoid" were used. Each tablet was dissolved in 100ml distilled water by immersing the bottle in a water bath at 56°C and gave a pH of 7.2.

2. Sheep Red Cells

The sheep cells were obtained from "Gibco Biocult Diagnostics". They were purchased in Alsever's solution and remain suitable for use for three months when stored at 4°C.

For use on the day of the test the cells were washed at least three times in V.B.S. and the supernatant removed.

A 4% suspension of cells was made up in V.B.S. For a batch of 65 sera, 0.8ml of packed cells was added to 19.2ml V.B.S.

3. Complement.

This was ob tained from "Burroughs Wellcome & Co". The source of complement is guinea-pig serum preserved by Richardson's method (Richardson, 1941).

The lyphophilized complement should be reconstituted by the addition of the appropriate volume of distilled water (this volume is stated on the bottle label). This preserved complement was hypertonic and was reconstituted by diluting 1/8 in distilled water. This results in a 1/10 dilution of the original serum.

Further dilution of complement is made with dilutent (V.B.S.).

The freeze-dried material was stored at 4°C and remains potent for 1 - 2 years.

After reconstitution the undiluted stock solution was stored at 4°C and remains potent for 7 days.

Dilutions from the stock solution were prepared freshly and discarded if used within the working day. If it was not used immediately then it was placed in the refrigerator at 4°C until use that day.

4. CFT antigen.

This was supplied by Dr. Williams of Raigmore Hospital, Inverness.

Peritoneal exudate was removed from 12-15 week old cotton rats 66hrs. after inoculation with the RH strain of toxoplasma. Each exudate was harvested individually and not pooled together.

The exudate was spun and washed twice in saline and then resuspended in distilled water. This underwent freezing and thawing 3 times and

and was then spun lightly to remove the gross particles and then the supernatant removed.

Foetal bovine serum (approximately 2%) was added to the supernatant to act as a stabilizer. This was then inactivated at 56°C for 1 hour, it was then stored at -20°C.

5. Positive Control Antiserum.

This was supplied by Dr. Williams of Raigmore Hospital, Inverness.

The antiserum was obtained from humans with high CFT titres.

6. Negative Control Antiserum.

Foetal bovine serum was used.

Titration of haemolytic serum and Complement.

This was done to determine the optimal concentration of the haemolytic serum and the titre of the complement, and was carried out as a "chessboard" titration in a microtiter plate.

Using a pippette dropper, two drops (0.025ml/drop) of V.B.S. were added to each well of the plate. This represents one volume of serum and one volume of antigen in the CFT. A third drop of V.B.S. was added to the control column in place of complement.

Dilutions of complement with a 20% difference in concentration between each were prepared in tubes. This was done in the following way. Into the second and each subsequent tube 1ml of V.B.S. was pipetted. In the first tube 6ml of 1/30 dilution of complement was made as follows:

	0.25ml preserved complement 1.75ml distilled water	=	1/10 dilution of complement.
Add	4ml V.B.S.	=	1/30 " " "

From the first tube 4ml of the diluted complement was transferred to the second tube. The contents in the second tube were mixed using the pipette and 4ml transferred to the third tube, and so on to the end of the row. This gave 9 complement dilutions i.e. 1/30, 1/38, 1/47, 1/59, 1/73, 1/92, 1/114, 1/143, 1/179.

One drop (0.025ml) of each complement dilution was added into the appropriate column of the plate.

The plate was covered to prevent evaporation and incubated at 37°C for 1½ hours.

A series of doubling dilutions of haemolytic serum from 1/25 to 1/800 were prepared in 1 ml amounts. A tube containing 1 ml of V.B.S. was prepared as a control.

A 4% suspension of sheep cells was prepared, as described earlier under reagents. 1 ml of this suspension was added to each 1 ml dilution of haemolytic serum, mixed and left at room temperature for at least 30 minutes before use.

From the appropriate tube, 1 (0.025ml) drop of the sensitized cells was added to each well of the appropriate row, as below:

Reciprocal Dilutions of Haemolytic serum.	Recipricol dilutions of Complement									Control
	30	38	47	59	73	92	114	143	179	
25	0	0	0	0	2	4	4	4	4	4
50	0	0	0	0	tr	3	4	4	4	4
100	0	0	0	0	tr	2	4	4	4	4
200	0	0	0	0	tr	2	4	4	4	4
400	0	0	2	4	4	4	4	4	4	4
800	4	4	4	4	4	4	4	4	4	4
Control	4	4	4	4	4	4	4	4	4	4

The plate was shaken, covered and incubated at 37°C for 30 minutes, being shaken again after 15 minutes. The titration was then read. An example is given above in the diagram.

Readings

0	=	No cells remaining
Tr	=	Approximately 10% cells remaining
1	=	" 25% " "
2	=	" 50% " "
3	=	" 75% " "
4	=	" 100% " "

The optimal sensitizing concentration (O.S.C.) of haemolytic serum is in that dilution which gives most lysis with the highest dilution of complement. In the titration the optimal sensitizing dilution was found to be 1/200 and the haemolytic serum was used at this dilution, for the test proper.

One unit of complement (HC_{50}) is in the dilution which gives 50% lysis (reading 2) at the optimal sensitizing concentration of the haemolytic serum. The complement was titrated daily.

In the test proper, three units of complement were used (i.e. three times the HC_{50}). In the figure above the HC_{50} was in the 1/92 dilution and therefore on that day the complement was used at 1/30 (3 x 1/92).

Titration of Antigen and Positive Control Standard Antiserum.

This was done to determine the optimal concentration of the antigen and the titre of the positive control serum. It was carried out as a "chessboard" titration as below.

Reciprocal Dilutions of Antiserum	Reciprocal Dilutions of Antigen					
	10	25	50	75	100	Control
32	4	4	4	4	4	0
69	4	4	4	4	4	0
128	4	4	4	4	4	0
256	4	4	4	4	4	0
512	4	4	4	4	tr	0
1024	4	4	4	3	0	0
2048	0	tr	2	1	0	0
4096	0	0	0	0	0	0
8192	0	0	0	0	0	0
16384	0	0	0	0	0	0
32768	0	0	0	0	0	0
Control	0	0	0	0	0	0

Doubling dilutions of the antiserum were made and 1/10, 1/25, 1/50, 1/75 and 1/100 dilutions of the antigen were also made.

The pipette was washed out once between each dilution.

The test was set up as follows in a microtiter plate.

One volume of diluent was added to the control row and the control column.

One volume of each of the serum dilutions was added to each well of the appropriate row.

One volume of each of the antigen dilutions was added to each well of the appropriate column.

One volume of complement ($3HC_{50}$) was added to each well.

Complement Control.

Dilutions of complement were made in the plate, so that readings were obtained for values of $3HC_{50}$ units of complement, one unit, and a half a unit. A control for the sensitized cells was also included.

The plate was covered and incubated at 37°C for 1½ hours.

The sensitized red cells were made up and allowed to stand for at least ½ hour at room temperature before one volume of the sensitized red cells were added to each of the wells in the plate.

The plate was incubated for a further 30 minutes at 37°C, shaking at the beginning and after 15 minutes. The plate was then read.

In the diagram on previous page:-

a) The optimal dilution of the antigen was 1/50 i.e. that dilution which gave the most fixation with the highest dilution of serum. This was the dilution, used in the tests with the unknown sera, for that batch of antigen. Each new batch of antigen was retitrated.

b) The titre of the serum was 1/2048 i.e. that dilution which gave a reading of 2 with the optimal dilution of antigen.

Each new batch of serum was titrated.

A reading of 2 is expected at the titre of the standard serum, but

because here the cells are most sensitive to differences in complement concentration, a reading from trace to 3 is acceptable. At twice the titre (i.e. in the proceeding well) there should be a reading of 4, and at half the titre (i.e. in the well following) there should be a reading of 0 - 1.

The complement Fixation Test.

0.1 ml amounts of the test sera were added to 0.7 ml of V.B.S. in bijou bottles giving a dilution of 1/8. The complement in the sera was inactivated by heating at 56°C in a water-bath for 30 minutes.

One volume of V.B.S. was added to wells 2 - 8 of each row being used.

One volume of the 1/8 diluted serum was added to wells 1, 2 and 8 of each row.

A "loop" was then placed in well 2, mixed and transferred to well 3 and the cycle repeated to well 7. After this the loop was washed and dried.

This gave doubling dilutions of each serum being tested from 1/8 to 1/512 with a serum control in well 8.

The positive control standard antiserum and any test sera positive at 1/512 were titrated out to 1/8192.

One volume of antigen was added to all wells except well 8 of each row.

One volume of $3HC_{50}$ Complement (the complement was titrated daily to ascertain the HC_{50} and then the $3H_{50}$) was added to all wells of each row.

The plate was shaken, covered and incubated at 37°C for 1½ hours.

One volume of sensitized red cells was added to all wells of each row, shaken, covered and incubated at 37°C for 30 minutes, the plate being shaken after 15 and 30 minutes.

The plates were then allowed to stand at 4°C overnight to allow the

cells to settle before reading. The end-point was taken as the highest dilution giving 50% lysis.

Several positive sera and a negative serum were included with each batch of sera tested, as well as a complement control both with and without antigen. Results were only taken if the complement control was correct and the control sera were correct or within one dilution of the normal.

Any serum sample showing anti-complementary activity in the serum control (well 8) was noted in the results.

(iii) The Indirect Fluorescent Antibody Test (IFAT)

Def : The test is based on two successive reactions. The first between the fixed toxoplasma antigens and specific antibody globulin. The second between the antibody - globulin - coated toxoplasma and a fluorescent labelled anti-globulin antibody.

Since Weller and Coons (1954) first described the successful IFAT in microbiology, the test has been used and adapted as a useful technique for research and various diagnostic procedures including toxoplasmosis.

The technique used was that described in the "Wellcome" pamphlet "Parasitic Antigens for Fluorescent Antibody Tests", as enclosed with their toxoplasma antigen for the IFAT. This is based upon the technique described by Goldman (1957).

Reagents.

(i) The Antigen:

This was prepared from Toxoplasma gondii R.H. strain passaged in rodents. It was freeze-dried from a small volume of 0.85% saline.

Each vial of antigen was reconstituted with 0.5ml distilled water, shaken on a mixer and then passed through a fine hypodermic needle several times in order to break up any clumps of the parasite to give a suspension of individual parasites.

The antigen was obtained from "Wellcome Reagents Ltd".

(ii) Fluorescein Conjugated IgG Fraction. Rabbit anti-Sheep gammaglobulin (Lot 8319) was obtained from Cappel Laboratories Inc., Downington P.A. 19335 U.S.A. It is conjugated with Fluorescein Isothiocyanate. After conjugation it was chromographed with DEAE - Sephadex A50 to remove the overcharged molecules. The 5ml vial of freeze-dried conjugated antiserum was restored by adding 5ml of sterile distilled water. The vial was rotated gently to reconstitute.

0.1ml amounts were then pipetted into 3ml, stoppered, plastic tubes and stored at -20°C until use.

The conjugate was titrated with a range of sera to determine the working dilution. The working dilution was found to be 1/20.

(iii) Phosphate buffered Saline. (P.B.S) pH 7.6.

This was prepared as follows:-

Na Cl	8.5gm.
$Na_2 HPO_4$	1.28gm.
$Na H_2PO_4$	0.156gm.

Distilled water to 1 litre.

(iv) Absolute methyl Alcohol.

(v) Chromic acid.

This was prepared as follows:-

Potassium dichromate	10gm.
Distilled water	75ml.
Concentrated Sulphuric acid	25ml.

This was used for cleaning the microscope slides.

Equipment.

(i) Clean microscope slides.

New microscopic slides were soaked for 2-3 hours (minimum) in chromic acid. Then washed for 2-3 hours (minimum) in running tap water and then rinsed twice in distilled water and finally dried at 160°F.

(ii) Microscope slide rack.

(iii) Two square staining jars - one for the alochol and the other for PBS.

(iv) A 3mm. loop.

(v) Glycerine

(vi) Humid chamber

A one foot square sandwich box with a piece of damp blotting paper in the bottom and a lid was used.

(vii) One - ml syringes with 23G 5/8 hypodermic needles.

(viii) A can of "Teflon" spray.

(ix) A 0.05ml pipette dropper.

(x) 0.05ml capillary transfer pipettes (henceforth called a "loop")

(xi) "Microtiter" disposal "U" well plates

(xii) Leitz Orthoplan Fluorescent microscope with a HPO/200 mercury vapour lamp, KP500 excitation filter, BG12 and BG38 FITC filter, phloem illumination, K510 suppression filter, a X54 oil immersion objective lens and X6.3 binocular eyepieces.

Method.

Using the 3mm. wire loop, twenty-four drops of glycering in 3 rows of 8 drops per row were placed on each chromic acid cleaned microscope slide. The slides was sprayed with "Teflon". The glycering spots were washed off in hot water and the slides dried.

Using the 3mm loop, a drop of the antigen suspension was placed in each "well" on the teflon-coated slides. The antigen was allowed to dry on the slides, and was fixed for 10 minutes in absolute methyl alcohol. The slide was washed in P.B.S. for 5 minutes.

To dilute the sera, one 0.05ml drop of PBS was placed in each well, from 1 - 10, in each row being used of the "Microtiter" plate. One 0.05ml "loop" of serum was placed in the first well, mixed and transferred to the second well and the process continued to the tenth well. Then the loop was washed and dried, and the process repeated for each serum

sample. This gave doubling dilutions of each serum from 1/2 to 1/1024.

Using a 1ml syringe and hypodermic needle a drop of serum was placed on the coated slides, starting with the highest dilution, as follows.

Sheep No.	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
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

Three sera were placed on each slide. The slides were then placed in the humid chamber at room temperature for 30 minutes.

The sera were rinsed off the slides with P.B.S. and the slides washed in P.B.S. for 30 minutes with two changes of P.B.S. and occasional agitation.

The slides were dried and one drop of conjugate, using a syringe and needle, added to each "well" on the slides.

The slides were placed in the humid chamber at room termperature for 30 minutes.

The conjugate was rinsed off with PBS, and the slides washed for one hour in 3 changes of P.B.S. and with occasional agitation.

The slides were dried, and then wrapped in tissue paper and then foil and stored in a box in a cool place until they were read on the microscope over the next few days. Non-fluorescing oil was used.

Two Sheep's sera (30 bleeds each, plus their lambs) per day were stained and examined the following day, where possible.

Control sera (a positive and negative) plus a PBS control were included in each batch examined. Results of the test sera were only taken when the P.B.S. and negative controls were negative and the positive was correct, or within one dilution.

The end-point was taken as the highest dilution giving bright fluorescence i.e. a bright, regular, continuous fluorescence over the whole and periphery of the majority of the parasites at that dilution.

RESULTS.

The results of each individual ewe and her respective lamb, and the results for the tup are shown on separate sheets i.e. tables 1 - 40. Each table gives the sheep no., breed, tupping and lambing dates, the number of lambs produced (those dead being noted), the lamb no. and the results of the three tests for the ewe and her lamb, or in the case of barren ewes, just the ewe's results and the same for the tup.

Table 41 shows the total fluctuations of titre from the beginning to the end of bleeding for each sheep and each test. Changes in titre between consecutive readings were translated into the number of dilutions to rise or fall and summated to indicate the overall change.

Table 42 shows the Geometric Mean Titres (G.M.T.) for the I.H.A.T. and the I.F.A.T. for 15 ewes, weeks 1 - 10 and 21 - 30.

Table 43 shows the G.M.T. for the I.H.A.T. for 24 ewes and the tup, weeks 1 - 10 and 21 - 25.

Table 44 shows the G.M.T. for an equal number of bleeds before and after parturition (this varied between 3 and 5 bleeds before and after). Sheep with only 1 or 2 bleeds after parturition were omitted. In the case of barren ewes and the tup a point around which the weekly bleeds were taken was made to coincide with the time of parturition.

Table 45 shows the reciprocal titres of the control sera for the three tests.

Table 46 shows the relationship between titre level and breed.

Table 47 shows the relationship between titre level and the number of lambs born per ewe and also the number born alive and dead.

Table 48 shows the relationship between positive or negative antibody status in the dam and her lamb at the time of parturition or just after.

Figure 1 shows the corresponding titres for the I.H.A.T. and C.F.T. and

the results of the X^2 test.

Figure 2 shows the corresponding titres for the I.H.A.T. and I.F.A.T. and the results of the X^2 test.

Figure 3 shows the corresponding titres for the C.F.T. and I.F.A.T. and the results of the X^2 test.

Figure 4 Natural logarithm (ln.) of G.M.T. of the C.F.T. against ln I.H.A.T. titre. Data taken from figure 1.

Figure 5 - Ln of G.M.T. of I.F.A.T. against ln I.H.A.T. titre. Date taken from figure 2.

Figure 6 - Ln of G.M.T. of C.F.T..against ln I.F.A.T. titre. Data taken from figure 3.

TOXOPLASMA PROJECT SHEET

SHEEP NO: 302

BREED: HALFBRED

TUPPED* 27/11/76

LAMBED** 25/4/77

NO. OF LAMBS: TRIPLETS (2 dead)

LAMB NO: 61G

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	128	32	32				
2	17/11/76	128	64	64				
3 *	24/11/76	128	64	32				
4	1/12/76	128	64	32				
5	10/12/76	128	64	32				
6	15/12/76	64	64	32				
7	23/12/76	256	64	64				
8	29/12/76	32	64	32				
9	6/1/77	128	64	64				
10	12/1/77	128	128	32				
11	21/1/77	64	128	16				
12	26/1/77	64	64	32				
13	2/2/77	64	32	16				
14	9/2/77	64	32	8				
15	23/2/77	64	64	32				
16	2/3/77	32		8				
17	9/3/77	32		16				
18	16/3/77	32		64				
19	23/3/77	32		16				
† 20	29/3/77	16		8				
21	6/4/77	32		16				
22	13/4/77	32		8				
23	20/4/77	16		8				
24 **	27/4/77	32		8	128		16	
25	4/5/77	32		8	64		16	
26	11/5/77	32		8	64		Neg	
27	18/5/77	64		16	32		Neg	
28	25/5/77	16		16	32		Neg	
29	1/6/77	32		32	32		Neg	
30	8/6/77	64		16	16	32	8	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 313

BREED: Halfbred

TUPPED* 16.11.76, 29.11.76

LAMBED** 13.4.77

NO. OF LAMBS: TWO

LAMB NO: 93

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	*	10/11/76	16	16					
2		17/11/76	16	16					
3	*	24/11/76	8	16					
4		1/12/76	8	32					
5		10/12/76	16	16					
6		15/12/76	16	16					
7		23/12/76	16	16					
8		29/12/76	16	16					
9		6/1/77	8	16					
10		12/1/77	8	16					
11		21/1/77	Neg	16					
12		26/1/77	Neg	16					
13		2/2/77	Neg	16					
14		9/2/77	Neg	16					
15		23/2/77	Neg	16					
16		2/3/77	Neg						
17		9/3/77	Neg						
18		16/3/77	Neg						
19		23/3/77	8						
† 20		29/3/77	Neg						
21		6/4/77	8						
22	**	13/4/77	Neg			Neg			
23		20/4/77	8			Neg			
24		27/4/77	Neg			Neg			
25		4/5/77	Neg			Neg			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 322

BREED: Halfbred

TUPPED* 27.11.76

LAMBED** 26.4.77

NO. OF LAMBS: ONE

LAMB NO: 132

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	32	Neg				
2	17/11/76	8	32	8				
3 *	24/11/76	8	32	Neg				
4	1/12/76	16	32	Neg				
5	10/12/76	Neg	32	Neg				
6	15/12/76	32	16	Neg				
7	23/12/76	64	16	8				
8	29/12/76	128	256	Neg				
9	6/1/77	128	128	Neg				
10	12/1/77	64	64	Neg				
11	21/1/77	32	32	Neg				
12	26/1/77	16	32	8				
13	2/2/77	8	32	Neg				
14	9/2/77	8	16	Neg				
15	23/2/77	8	16	Neg				
16	2/3/77	8		Neg				
17	9/3/77	8		Neg				
18	16/3/77	Neg		Neg				
19	23/3/77	8		8				
† 20	29/3/77	Neg		Neg				
21	6/4/77	8		Neg				
22	13/4/77	8		Neg				
23 **	20/4/77	16		Neg				
24	27/4/77	16		Neg	Neg		Neg	
25	4/5/77	8		Neg	Neg		Neg	
26	11/5/77	Neg		Neg	Neg		Neg	
27	18/5/77	Neg		Neg	Neg		Neg	
28	25/5/77	Neg		Neg	Neg		Neg	
29	1/6/77	8		Neg	32		Neg	
30	8/6/77	Neg		Neg	16		Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 325

BREED: HALFBRED

TUPPED* 28.11.76

LAMBED** 26.4.77

NO. OF LAMBS: ONE

LAMB NO: 62

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	128	16					
2	17/11/76	128	16					
3 *	24/11/76	128	16					
4	1/12/76	128	16					
5	10/12/76	128	16					
6	15/12/76	128	16					
7	23/12/76	128	32					
8	29/12/76	256	32					
9	6/1/77	256	16					
10	12/1/77	128	Neg					
11	21/1/77	64	Neg					
12	26/1/77	128	Neg					
13	2/2/77	128	8					
14	9/2/77	128	8					
15	23/2/77	256	8					
16	2/3/77	64						
17	9/3/77	256						
18	16/3/77	256						
19	23/3/77	64						
† 20	29/3/77	64						
21	6/4/77	128						
22	13/4/77	64						
23 **	20/4/77	64						
24	27/4/77	64			64			
25	4/5/77	64			64			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

†. ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 412

BREED: HALFBRED

TUPPED* 1.12.76

LAMBED** 26.4.77

NO. OF LAMBS: TWO

LAMB NO: 63

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	256	32					
2		17/11/76	256	32					
3		24/11/76	512	16					
4	*	1/12/76	256	32					
5		10/12/76	256	32					
6		15/12/76	128	32					
7		23/12/76	256	32					
8		29/12/76	128	32					
9		6/1/77	64	32					
10		12/1/77	256	32					
11		21/1/77	128	64					
12		26/1/77	128	64					
13		2/2/77	128	64					
14		9/2/77	128	64					
15		23/2/77	64	32					
16		2/3/77	128						
17		9/3/77	128						
18		16/3/77	128						
19		23/3/77	128						
† 20		29/3/77	128						
21		6/4/77	64						
22		13/4/77	64						
23		20/4/77	32						
24	**	27/4/77	32			128			
25		4/5/77	32			64			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 527

BREED: HALFBRED

TUPPED* 15.11.76

LAMBED** 13.4.77

NO. OF LAMBS: TWO

LAMB NO: 91

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	*	10/11/76	16	32					
2		17/11/76	16	64					
3		24/11/76	8	32					
4		1/12/76	8	32					
5		10/12/76	8	32					
6		15/12/76	Neg	32					
7		23/12/76	Neg	32					
8		29/12/76	Neg	32					
9		6/1/77	Neg	32					
10		12/1/77	8	32					
11		21/1/77	8	16					
12		26/1/77	8	32					
13		2/2/77	8	32					
14		9/2/77	8	16					
15		23/2/77	8	32					
16		2/3/77	16						
17		9/3/77	8						
18		16/3/77	16						
19		23/3/77	16						
† 20		29/3/77	16						
21		6/4/77	8						
22	**	13/4/77	Neg			Neg			
23		20/4/77	Neg			Neg			
24		27/4/77	Neg			Neg			
25		4/5/77	Neg			Neg			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 535

BREED: HALFBRED

TUPPED* 24.11.76

LAMBED** BARREN

NO. OF LAMBS: NO LAMB

LAMB NO:

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	512	256	64				
2	17/11/76	1024	256	32				
3 *	24/11/76	2048	256	64				
4	1/12/76	1024	128	128				
5	10/12/76	512	128	128				
6	15/12/76	256	64	256				
7	23/12/76	256	128	256				
8	29/12/76	512	64	512				
9	6/1/77	512	128	1024				
10	12/1/77	512	128	1024				
11	21/1/77	128	64	128				
12	26/1/77	64	128	128				
13	2/2/77	512	128	128				
14	9/2/77	512	128	64				
15	23/2/77	512	128	64				
16	2/3/77	512		128				
17	9/3/77	128		64				
18	16/3/77	128		32				
19	23/3/77	128		32				
† 20	29/3/77	128		64				
21	6/4/77	256		64				
22	13/4/77	128		32				
23	20/4/77	64		32				
24	27/4/77	128		32				
25	4/5/77	128		32				
26	11/5/77	32		32				
27	18/5/77	64		32				
28	25/5/77	64		32				
29	1/6/77	64		16				
30	8/6/77	32		32				

†. ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 536

BREED: HALFBRED

TUPPED* 16.11. 76

LAMBED** 9. 4. 77

NO. OF LAMBS: THREE

LAMB NO: 95

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	*	10/11/76	8	16	Neg				
2		17/11/76	Neg	32	Neg				
3		24/11/76	Neg	32	Neg				
4		1/12/76	8	32	Neg				
5		10/12/76	64	16	Neg				
6		15/12/76	32	16	Neg				
7		23/12/76	256	16	Neg				
8		29/12/76	16	16	Neg				
9		6/1/77	16	8	Neg				
10		12/1/77	8	8	Neg				
11		21/1/77	16	8	Neg				
12		26/1/77	32	16	Neg				
13		2/2/77	16	16	Neg				
14		9/2/77	Neg	16	Neg				
15		23/2/77	Neg	16	Neg				
16		2/3/77	8		Neg				
17		9/3/77	Neg		Neg				
18		16/3/77	Neg		Neg				
19		23/3/77	Neg		Neg				
† 20		29/3/77	Neg		Neg				
21		6/4/77	8		Neg				
22	**	13/4/77	8		Neg	Neg		Neg	
23		20/4/77	Neg		Neg	Neg		Neg	
24		27/4/77	Neg		Neg	Neg		Neg	
25		4/5/77	Neg		Neg	Neg		Neg	
26		11/5/77	8		Neg	Neg		Neg	
27		18/5/77	Neg		Neg	Neg		Neg	
28		25/5/77	Neg		8	Neg		Neg	
29		1/6/77	Neg		Neg	Neg		Neg	
30		8/6/77	Neg		Neg	Neg	8	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 539

BREED: HALFBRED

TUPPED* 29.11.76

LAMBED** 10.5.77

NO. OF LAMBS: ONE

LAMB NO: 69

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	Neg	32	32				
2	17/11/76	8	32	32				
3 *	24/11/76	8	32	32				
4	1/12/76	Neg	32	32				
5	10/12/76	8	16	64				
6	15/12/76	32	8	64				
7	23/12/76	32	8	32				
8	29/12/76	32	8	32				
9	6/1/77	32	Neg	32				
10	12/1/77	32	Neg	8				
11	21/1/77	16	16	8				
12	26/1/77	16	16	8				
13	2/2/77	8	16	8				
14	9/2/77	8	16	Neg				
15	23/2/77	16	16	8				
16	2/3/77	8		Neg				
17	9/3/77	Neg		16				
18	16/3/77	Neg		8				
19	23/3/77	Neg		Neg				
† 20	29/3/77	Neg		Neg				
21	6/4/77	Neg		Neg				
22	13/4/77	Neg		32				
23	20/4/77	Neg		32				
24	27/4/77	Neg		16				
25	4/5/77	Neg		32				
26 **	11/5/77	Neg		16	Neg		16	
27	18/5/77	Neg		16	Neg		8	
28	25/5/77	Neg		32	Neg		8	
29	1/6/77	8		32	Neg		Neg	
30	8/6/77	Neg		8	Neg	16	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 542

BREED: HALFBRED

TUPPED* 18.11.76 6.12.76

LAMBED** 3.5.77

NO. OF LAMBS: ONE

LAMB NO: 65

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	32	64	16				
2	*	17/11/76	16	32	8				
3		24/11/76	8	32	16				
4	*	1/12/76	8	16	8				
5		10/12/76	8	16	16				
6		15/12/76	16	16	8				
7		23/12/76	128	64	8				
8		29/12/76	32	16	16				
9		6/1/77	32	32	8				
10		12/1/77	16	32	Neg				
11		21/1/77	8	32	8				
12		26/1/77	Neg	32	Neg				
13		2/2/77	8	32	Neg				
14		9/2/77	Neg	32	Neg				
15		23/2/77	Neg	32	Neg				
16		2/3/77	Neg		Neg				
17		9/3/77	8		Neg				
18		16/3/77	8		Neg				
19		23/3/77	8		Neg				
† 20		29/3/77	8		Neg				
21		6/4/77	Neg		Neg				
22		13/4/77	Neg		Neg				
23		20/4/77	Neg		Neg				
24	**	27/4/77	8		Neg				
25		4/5/77	Neg		Neg	Neg		Neg	
26		11/5/77	Neg		Neg	Neg		Neg	
27		18/5/77	Neg		Neg	Neg		8	
28		25/5/77	Neg		Neg	Neg		Neg	
29		1/6/77	Neg		Neg	Neg		Neg	
30		8/6/77	Neg		Neg	Neg	Neg	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 572

BREED: HALFBRED

TUPPED* 24.11.76

LAMBED** 18.4.77

NO. OF LAMBS: THREE

LAMB NO: 98 (Lamb 99 fostered on to ewe No. 42)

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	Neg	64					
2	17/11/76	Neg	64					
3 *	24/11/76	8	64					
4	1/12/76	8	64					
5	10/12/76	8	64					
6	15/12/76	8	16					
7	23/12/76	16	16					
8	29/12/76	Neg	16					
9	6/1/77	8	16					
10	12/1/77	16	16					
11	21/1/77	8	32					
12	26/1/77	16	32					
13	2/2/77	8	32					
14	9/2/77	8	16					
15	23/2/77	8	16					
16	2/3/77	8						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	16						
21	6/4/77	Neg						
22 **	13/4/77	Neg						
23	20/4/77	Neg			Neg			
24	27/4/77	Neg			Neg			
25	4/5/77	Neg			N.S.			Not Sampled
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 575

BREED: HALFBRED

TUPPED* 27.11.76

LAMBED** 10.5.77

NO. OF LAMBS: ONE

LAMB NO: 70

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	16	Neg				
2	17/11/76	Neg	16	Neg				
3 *	24/11/76	Neg	16	Neg				
4	1/12/76	8	16	Neg				
5	10/12/76	N.S.	N.S.	N.S.				Not Sampled
6	15/12/76	32	16	Neg				
7	23/12/76	32	8	Neg				
8	29/12/76	16	Neg	Neg				
9	6/1/77	32	Neg	Neg				
10	12/1/77	32	Neg	Neg				
11	21/1/77	16	16	Neg				
12	26/1/77	32	16	Neg				
13	2/2/77	16	8	Neg				
14	9/2/77	16	8	Neg				
15	23/2/77	Neg	16	Neg				
16	2/3/77	Neg		Neg				
17	9/3/77	Neg		Neg				
18	16/3/77	8		Neg				
19	23/3/77	Neg		Neg				
† 20	29/3/77	Neg		Neg				
21	6/4/77	Neg		Neg				
22	13/4/77	Neg		Neg				
23	20/4/77	Neg		Neg				
24	27/4/77	Neg		Neg				
25	4/5/77	Neg		Neg				
26 **	11/5/77	Neg		Neg	Neg		Neg	
27	18/5/77	Neg		Neg	Neg		Neg	
28	25/5/77	Neg		Neg	Neg		Neg	
29	1/6/77	Neg		Neg	Neg		Neg	
30	8/6/77	Neg		Neg	Neg	8	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 589

BREED: HALFBRED

TUPPED* 15.11.76 29.11.76

LAMBED** 22.5.77

NO. OF LAMBS: TWO

LAMB NO: 71

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	16	16	16				
2	17/11/76	16	16	8				
3 *	24/11/76	16	16	Neg				
4	1/12/76	16	16	8				
5	10/12/76	8	32	8				
6	15/12/76	16	16	8				
7	23/12/76	16	16	Neg				
8	29/12/76	Neg	16	Neg				
9	6/1/77	Neg	16	Neg				
10	12/1/77	8	16	Neg				
11	21/1/77	Neg	16	Neg				
12	26/1/77	Neg	16	Neg				
13	2/2/77	Neg	16	Neg				
14	9/2/77	Neg	16	Neg				
15	23/2/77	Neg	16	Neg				
16	2/3/77	Neg		Neg				
17	9/3/77	Neg		Neg				
18	16/3/77	Neg		Neg				
19	23/3/77	Neg		8				
† 20	29/3/77	8		Neg				
21	6/4/77	Neg		Neg				
22	13/4/77	Neg		Neg				
23	20/4/77	Neg		Neg				
24	27/4/77	Neg		Neg				
25	4/5/77	Neg		16				
26	11/5/77	Neg		16				
27 **	18/5/77	Neg		16				
28	25/5/77	Neg		Neg	Neg		Neg	
29	1/6/77	Neg		Neg	Neg		Neg	
30	8/6/77	Neg		Neg	Neg	16	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 594

BREED: HALFBRED

TUPPED* NOT TUPPED

LAMBED** BARREN

NO. OF LAMBS: NO LAMBS

LAMB NO:

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	128	Neg	Neg				
2	17/11/76	128	16	Neg				
3	24/11/76	64	16	Neg				
4	1/12/76	64	16	Neg				
5	10/12/76	32	16	Neg				
6	15/12/76	8	32	Neg				
7	23/12/76	8	32	Neg				
8	29/12/76	32	32	Neg				
9	6/1/77	Neg	64	Neg				
10	12/1/77	8	64	Neg				
11	21/1/77	8		Neg				
12	26/1/77	16		Neg				
13	2/2/77	16		Neg				
14	9/2/77	16		Neg				
15	23/2/77	16		Neg				
16	2/3/77	32		Neg				
17	9/3/77	8		Neg				
18	16/3/77	16		Neg				
19	23/3/77	32		Neg				
† 20	29/3/77	32		Neg				
21	6/4/77	16		Neg				
22	13/4/77	8		Neg				
23	20/4/77	8		Neg				
24	27/4/77	8		Neg				
25	4/5/77	Neg		Neg				
26	11/5/77	8		Neg				
27	18/5/77	32		Neg				
28	25/5/77	16		Neg				
29	1/6/77	32		Neg				
30	8/6/77	32		Neg				

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 2027

BREED: HALFBRED

TUPPED* 20.11.76 6.12.76

LAMBED** 4.5.77

NO. OF LAMBS: ONE

LAMB NO: 68

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	32	32					
2	*	17/11/76	16	32					
3		24/11/76	8	32					
4	*	1/12/76	Neg	64					
5		10/12/76	Neg	32					
6		15/12/76	16	16					
7		23/12/76	16	16					
8		29/12/76	8	16					
9		6/1/77	8	16					
10		12/1/77	8	16					
11		21/1/77	8						
12		26/1/77	Neg						
13		2/2/77	Neg						
14		9/2/77	Neg						
15		23/2/77	Neg						
16		2/3/77	Neg						
17		9/3/77	Neg						
18		16/3/77	Neg						
19		23/3/77	8						
† 20		29/3/77	Neg						
21		6/4/77	Neg						
22		13/4/77	Neg						
23		20/4/77	Neg						
24		27/4/77	Neg						
25	**	4/5/77	Neg			Neg			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 2040

BREED: HALFBRED

TUPPED* 28.11.76

LAMBED** 27.4.77

NO. OF LAMBS: ONE

LAMB NO: 67

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	128	32					
2	17/11/76	128	32					
3 *	24/11/76	64	32					
4	1/12/76	128	32					
5	10/12/76	64	32					
6	15/12/76	8	32					
7	23/12/76	Neg	32					
8	29/12/76	32	16					
9	6/1/77	16	16					
10	12/1/77	8	16					
11	21/1/77	16						
12	26/1/77	32						
13	2/2/77	16						
14	9/2/77	32						
15	23/2/77	32						
16	2/3/77	32						
17	9/3/77	32						
18	16/3/77	64						
19	23/3/77	64						
† 20	29/3/77	64						
21	6/4/77	32						
22	13/4/77	16						
23	20/4/77	16						
24 **	27/4/77	16						
25	4/5/77	16			64			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 41

BREED: BLACKFACE

TUPPED* 16.11.76

LAMBED** 11.4.77

NO. OF LAMBS: ONE

LAMB NO: 101

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	2048	32					
2	17/11/76	1024	32					
3	24/11/76	4096	32					
4	1/12/76	4096	32					
5	10/12/76	2048	32					
6	15/12/76	256	64					
7	23/12/76	N.S.	N.S.					Not Sampled
8	29/12/76	128	64					
9	6/1/77	256	64					
10	12/1/77	256	64					
11	21/1/77	128						
12	26/1/77	512						
13	2/2/77	256						
14	9/2/77	256						
15	23/2/77	256						
16	2/3/77	256						
17	9/3/77	128						
18	16/3/77	256						
19	23/3/77	256						
† 20	29/3/77	256						
21	6/4/77	128						
** 22	13/4/77	256			256			
23	20/4/77	128			256			
24	27/4/77	128			128			
25	4/5/77	256			128			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 42

BREED: BLACKFACE

TUPPED* 17.11.76

LAMBED** 11.4.77

NO. OF LAMBS: ONE

LAMB NO: 103 (died) Lamb 99 fostered on from ewe 572.

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	1024	16	32				
2	*	17/11/76	1024	16	32				
3		24/11/76	1024	16	16				
4		1/12/76	512	16	16				
5		10/12/76	1024	16	8				
6		15/12/76	512	32	Neg				
7		23/12/76	256	32	Neg				
8		29/12/76	128	32	Neg				
9		6/1/77	64	32	Neg				
10		12/1/77	256	32	8				
11		21/1/77	512		8				
12		26/1/77	512		16				
13		2/2/77	256		Neg				
14		9/2/77	256		Neg				
15		23/2/77	128		Neg				
16		2/3/77	128		16				
17		9/3/77	256		16				
18		16/3/77	256		8				
19		23/3/77	128		Neg				
† 20		29/3/77	256		Neg				
21		6/4/77	128		Neg				
22	**	13/4/77	256		Neg	128		16	
23		20/4/77	128		8	128		16	Lamb 103
24		27/4/77	128		8	64		16	
25		4/5/77	128		8	Neg		Neg	
26		11/5/77	128		16	Neg		Neg	
27		18/5/77	256		8	Neg		Neg	Lamb 99
28		25/5/77	32		8	Neg		Neg	
29		1/6/77	256		8	Neg		Neg	
30		8/6/77	128		8	Neg	Neg	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 43

BREED: BLACKFACE

TUPPED* 25.11.76

LAMBED** 22.4.77

NO. OF LAMBS: TWO

LAMB NO: 128

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	512	32					
2	17/11/76	512	32					
3 *	24/11/76	512	16					
4	1/12/76	512	32					
5	10/12/76	512	16					
6	15/12/76	32	64					
7	23/12/76	256	32					
8	29/12/76	64	64					
9	6/1/77	128	32					
10	12/1/77	256	64					
11	21/1/77	128						
12	26/1/77	128						
13	2/2/77	128						
14	9/2/77	128						
15	23/2/77	64						
16	2/3/77	256						
17	9/3/77	128						
18	16/3/77	256						
19	23/3/77	256						
† 20	29/3/77	256						
21	6/4/77	128						
22	13/4/77	64						
23 **	20/4/77	64						
24	27/4/77	128			> 256			
25	4/5/77	128			> 256			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

†. ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 44

BREED: BLACKFACE

TUPPED* 28.11.76

LAMBED** 23.4.77

NO. OF LAMBS: TWO

LAMB NO: 118

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	256	32					
2	17/11/76	512	32					
3 *	24/11/76	512	32					
4	1/12/76	512	16					
5	10/12/76	512	16					
6	15/12/76	128	64					
7	23/12/76	16	8					
8	29/12/76	256	64					
9	6/1/77	256	64					
10	12/1/77	256	64					
11	21/1/77	64						
12	26/1/77	64						
13	2/2/77	64						
14	9/2/77	128						
15	23/2/77	128						
16	2/3/77	128						
17	9/3/77	64						
18	16/3/77	128						
19	23/3/77	128						
† 20	29/3/77	128						
21	6/4/77	64						
22	13/4/77	64						
23 **	20/4/77	256						
24	27/4/77	64			>256			
25	4/5/77	64			128			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 47

BREED: BLACKFACE

TUPPED* 16.11.76

LAMBED** BARREN

NO. OF LAMBS: NO LAMBS

LAMB NO:

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	512	8	16				
2	17/11/76	512	16	16				
3	24/11/76	512	16	16				
4	1/12/76	512	16	16				
5	10/12/76	256	32	16				
6	15/12/76	256	64	16				
7	23/12/76	256	64	Neg				
8	29/12/76	256	128	8				
9	6/1/77	128	64	Neg				
10	12/1/77	64	32	64				
11	21/1/77	128		16				
12	26/1/77	128		8				
13	2/2/77	128		32				
14	9/2/77	128		16				
15	23/2/77	256		16				
16	2/3/77	256		Neg				
17	9/3/77	256		16				
18	16/3/77	256		Neg				
19	23/3/77	256		Neg				
† 20	29/3/77	64		Neg				
21	6/4/77	64		Neg				
22	13/4/77	256		16				
23	20/4/77	512		Neg				
24	27/4/77	64		8				
25	4/5/77	128		Neg				
26	11/5/77	128		Neg				
27	18/5/77	128		16				
28	25/5/77	32		16				
29	1/6/77	256		16				
30	8/6/77	32		16				

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 421

BREED: BLACKFACE

TUPPED* 28.11.76

LAMBED** 24.4.77

NO. OF LAMBS: TWO

LAMB NO: 124

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	16					
2	17/11/76	8	16					
3	24/11/76	16	16					
* 4	1/12/76	16	16					
5	10/12/76	8	16					
6	15/12/76	16	16					
7	23/12/76	16	64					
8	29/12/76	8	16					
9	6/1/77	Neg	8					
10	12/1/77	Neg	8					
11	21/1/77	8						
12	26/1/77	Neg						
13	2/2/77	Neg						
14	9/2/77	Neg						
15	23/2/77	Neg						
16	2/3/77	8						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21	6/4/77	Neg						
22	13/4/77	Neg						
23	20/4/77	Neg						
** 24	27/4/77	Neg			Neg			
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 586

BREED: BLACKFACE

TUPPED* 27.11.76

LAMBED** 25.4.77

NO. OF LAMBS: ONE

LAMB NO: 127

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	256	16					
2	17/11/76	128	16					
3 *	24/11/76	256	16					
4	1/12/76	256	16					
5	10/12/76	512	16					
6	15/12/76	64	32					
7	23/12/76	Neg	8					
8	29/12/76	64	32					
9	6/1/77	8	32					
10	12/1/77	64	32					
11	21/1/77	128						
12	26/1/77	64						
13	2/2/77	128						
14	9/2/77	128						
15	23/2/77	128						
16	2/3/77	256						
17	9/3/77	256						
18	16/3/77	128						
19	23/3/77	64						
† 20	29/3/77	64						
21	6/4/77	64						
22	13/4/77	64						
23 **	20/4/77	64						
24	27/4/77	64			32			
25	4/5/77	64			32			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 970

BREED: BLACKFACE

TUPPED* 23.11.76

LAMBED** 15.4.77

NO. OF LAMBS: THREE

LAMB NO: 106 (LAMB NO.107 fostered on to ewe 394)

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	1024	16					
2	17/11/76	1024	32					
* 3	24/11/76	256	32					
4	1/12/76	256	32					
5	10/12/76	512	32					
6	15/12/76	64	128					
7	23/12/76	128	128					
8	29/12/76	16	128					
9	6/1/77	64	128					
10	12/1/77	32	64					
11	21/1/77	128						
12	26/1/77	256						
13	2/2/77	256						
14	9/2/77	256						
15	23/2/77	8						
16	2/3/77	128						
17	9/3/77	128						
18	16/3/77	256						
19	23/3/77	128						
† 20	29/3/77	128						
21	6/4/77	128						
22	13/4/77	64						
** 23	20/4/77	128			256			
24	27/4/77	256			>256			
25	4/5/77	128			>256			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 54

BREED: BLACKFACE GIMMER

TUPPED* 27.11.77

LAMBED** 10.5.77

NO. OF LAMBS: TWO

LAMB NO: 135

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	16	Neg				
2	17/11/76	32	16	Neg				
3 *	24/11/76	64	Neg	Neg				
4	1/12/76	32	Neg	Neg				
5	10/12/76	32	Neg	Neg				
6	15/12/76	8	16	16				
7	23/12/76	16	16	Neg				
8	29/12/76	32	16	Neg				
9	6/1/77	Neg	16	Neg				
10	12/1/77	16	16	8				
11	21/1/77	8		Neg				
12	26/1/77	8		Neg				
13	2/2/77	Neg		Neg				
14	9/2/77	Neg		Neg				
15	23/2/77	Neg		Neg				
16	2/3/77	Neg		8				
17	9/3/77	Neg		8				
18	16/3/77	Neg		Neg				
19	23/3/77	Neg		Neg				
† 20	29/3/77	8		Neg				
21	6/4/77	Neg		Neg				
22	13/4/77	Neg		8				
23	20/4/77	Neg		Neg				
24	27/4/77	Neg		Neg				
25	4/5/77	Neg		Neg				
26 **	11/5/77	8		Neg	Neg		Neg	
27	18/5/77	Neg		Neg	Neg		Neg	
28	25/5/77	Neg		Neg	Neg		Neg	
29	1/6/77	8		Neg	Neg		Neg	
30	8/6/77	Neg		Neg	Neg	Neg	Neg	

†. ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 91

BREED: BLACKFACE GIMMER

TUPPED* ?

LAMBED** 20.4.77

NO. OF LAMBS: THREE

LAMB NO: 112

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	32					
2	17/11/76	8	32					
3	24/11/76	16	32					
4	1/12/76	32	32					
5	10/12/76	64	32					
6	15/12/76	32	32					
7	23/12/76	16	8					
8	29/12/76	16	32					
9	6/1/77	16	32					
10	12/1/77	8	32					
11	21/1/77	8						
12	26/1/77	8						
13	2/2/77	Neg						
14	9/2/77	Neg						
15	23/2/77	8						
16	2/3/77	32						
17	9/3/77	32						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21	6/4/77	8						
22	13/4/77	8						
23 **	20/4/77	Neg			Neg			
24	27/4/77	Neg			Neg			
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 92

BREED: BLACKFACE GIMMER

TUPPED* 24.11.76

LAMBED** 19.4.77

NO. OF LAMBS: TWO

LAMB NO: 108

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	64	16					
2		17/11/76	64	16					
3	*	24/11/76	64	32					
4		1/12/76	32	32					
5		10/12/76	64	16					
6		15/12/76	64						
7		23/12/76	8						
8		29/12/76	16						
9		6/1/77	16						
10		12/1/77	16						
11		21/1/77	8						
12		26/1/77	8						
13		2/2/77	8						
14		9/2/77	16						
15		23/2/77	16						
16		2/3/77	8						
17		9/3/77	Neg						
18		16/3/77	Neg						
19		23/3/77	Neg						
† 20		29/3/77	Neg						
21		6/4/77	8						
22		13/4/77	Neg						
23	**	20/4/77	8			8			
24		27/4/77	8			Neg			
25		4/5/77	8			Neg			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 93

BREED: BLACKFACE GIMMER

TUPPED* 16.11.76

LAMBED** ABORTED 30.3.77

NO. OF LAMBS: TWO

LAMB NO: (LAMB 140 FOSTERED ON TO EWE 93)

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	512	32	16				
2	17/11/76	512	32	16				
3	24/11/76	256	32	32				
4	1/12/76	256	Neg	8				
5	10/12/76	512	Neg	8				
6	15/12/76	512		16				
7	23/12/76	16		Neg				
8	29/12/76	256		32				
9	6/1/77	256		8				
10	12/1/77	128		16				
11	21/1/77	128		8				
12	26/1/77	128		16				
13	2/2/77	128		Neg				
14	9/2/77	64		16				
15	23/2/77	64		16				
16	2/3/77	32		16				
17	9/3/77	64		16				
18	16/3/77	64		16				
19	23/3/77	64		16				
† 20	29/3/77	32		16				30.3.77 aborted IFAT 16 IHAT 64
21	6/4/77	128		16				
22	13/4/77	128		16				
23	20/4/77	128		16	Neg		Neg	
24	27/4/77	64		16	Neg		Neg	
25	4/5/77	64		16	Neg		Neg	
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 94

BREED: BLACKFACE GIMMER

TUPPED* 27.11.76

LAMBED** 23.4.77

NO. OF LAMBS: TWO

LAMB NO: 119

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	Neg					
2	17/11/76	8	Neg					
3 *	24/11/76	16	Neg					
4	1/12/76	16	Neg					
5	10/12/76	32	Neg					
6	15/12/76	32						
7	23/12/76	256						
8	29/12/76	16						
9	6/1/77	16						
10	12/1/77	Neg						
11	21/1/77	16						
12	26/1/77	16						
13	2/2/77	16						
14	9/2/77	32						
15	23/2/77	128						
16	2/3/77	64						
17	9/3/77	16						
18	16/3/77	16						
19	23/3/77	8						
† 20	29/3/77	8						
21	6/4/77	8						
22	13/4/77	Neg						
23	20/4/77	64						
** 24	27/4/77	16			Neg			
25	4/5/77	8			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 95

BREED: BLACKFACE GIMMER

TUPPED* 16.11.76 3.12.76

LAMBED** 26.5.77

NO. OF LAMBS: TWO

LAMB NO: 138

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	Neg	8	Neg				
2	17/11/76	8	8	Neg				
3	24/11/76	8	8	Neg				
4 *	1/12/76	Neg	8	Neg				
5	10/12/76	Neg	Neg	Neg				
6	15/12/76	Neg		Neg				
7	23/12/76	Neg		Neg				
8	29/12/76	Neg		Neg				
9	6/1/77	Neg		Neg				
10	12/1/77	Neg		Neg				
11	21/1/77	8		Neg				
12	26/1/77	8		Neg				
13	2/2/77	Neg		Neg				
14	9/2/77	Neg		Neg				
15	23/2/77	Neg		8				
16	2/3/77	8		Neg				
17	9/3/77	8		8				
18	16/3/77	Neg		8				
19	23/3/77	Neg		Neg				
† 20	29/3/77	Neg		8				
21	6/4/77	Neg		8				
22	13/4/77	Neg		Neg				
23	20/4/77	Neg		Neg				
24	27/4/77	Neg		Neg				
25	4/5/77	Neg		Neg				
26	11/5/77	8		Neg				
27	18/5/77	Neg		8				
28 **	25/5/77	Neg		Neg	Neg		N.S.	No Sample
29	1/6/77	Neg		Neg	Neg		Neg	
30	8/6/77	Neg		Neg	Neg	Neg	Neg	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 96

BREED: BLACKFACE GIMMER

TUPPED* 16.11.76

LAMBED** 9.4.77

NO. OF LAMBS: TWO

LAMB NO: 104

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	16	16					
2	17/11/76	16	16					
3	24/11/76	8	16					
4	1/12/76	8	16					
5	10/12/76	8	16					
6	15/12/76	8						
7	23/12/76	Neg						
8	29/12/76	8						
9	6/1/77	8						
10	12/1/77	8						
11	21/1/77	Neg						
12	26/1/77	16						
13	2/2/77	8						
14	9/2/77	Neg						
15	23/2/77	8						
16	2/3/77	8						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	8						
21	6/4/77	Neg						
22 **	13/4/77	Neg			Neg			
23	20/4/77	Neg			Neg			
24	27/4/77	Neg			Neg			
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 97

BREED: BLACKFACE GIMMER

TUPPED* 28.11.76

LAMBED** 18.4.77

NO. OF LAMBS: TWO

LAMB NO: 110

BLEED NO		DATE	EWE			LAMB			COMMENTS
			IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1		10/11/76	8	16					
2		17/11/76	8	16					
3	*	24/11/76	32	16					
4		1/12/76	64	Neg					
5		10/12/76	64	8					
6		15/12/76	16						
7		23/12/76	16						
8		29/12/76	16						
9		6/1/77	16						
10		12/1/77	8						
11		21/1/77	8						
12		26/1/77	8						
13		2/2/77	8						
14		9/2/77	8						
15		23/2/77	256						
16		2/3/77	Neg						
17		9/3/77	Neg						
18		16/3/77	Neg						
19		23/3/77	Neg						
† 20		29/3/77	Neg						
21		6/4/77	Neg						
22		13/4/77	Neg						
23	**	20/4/77	Neg			Neg			
24		27/4/77	Neg			Neg			
25		4/5/77	Neg			Neg			
26		11/5/77							
27		18/5/77							
28		25/5/77							
29		1/6/77							
30		8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 98

BREED: BLACKFACE GIMMER

TUPPED* 30.11.76

LAMBED** 27.4.77

NO. OF LAMBS: TWO

LAMB NO: 133

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	16	16					
2	17/11/76	8	Neg					
3 *	24/11/76	8	Neg					
4	1/12/76	Neg	16					
5	10/12/76	Neg	Neg					
6	15/12/76	Neg						
7	23/12/76	8						
8	29/12/76	Neg						
9	6/1/77	Neg						
10	12/1/77	Neg						
11	21/1/77	8						
12	26/1/77	Neg						
13	2/2/77	Neg						
14	9/2/77	Neg						
15	23/2/77	Neg						
16	2/3/77	Neg						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21	6/4/77	Neg						
22	13/4/77	Neg						
23	20/4/77	Neg						
24 **	27/4/77	Neg						
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Coverin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 99

BREED: BLACKFACE GIMMER

TUPPED* 28.11.76

LAMBED** 25.4.77

NO. OF LAMBS: TWO

LAMB NO: 130

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	16	Neg					
2	17/11/76	16	Neg					
3 *	24/11/76	8	Neg					
4	1/12/76	8	Neg					
5	10/12/76	Neg	Neg					
6	15/12/76	Neg						
7	23/12/76	8						
8	29/12/76	Neg						
9	6/1/77	8						
10	12/1/77	Neg						
11	21/1/77	8						
12	26/1/77	Neg						
13	2/2/77	Neg						
14	9/2/77	Neg						
15	23/2/77	Neg						
16	2/3/77	Neg						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21	6/4/77	Neg						
22	13/4/77	Neg						
23 **	20/4/77	Neg						
24	27/4/77	Neg			64			
25	4/5/77	Neg			32			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 212

BREED: BLACKFACE GIMMER

TUPPED* 27.11.76

LAMBED** 22. 4.77

NO. OF LAMBS: ONE

LAMB NO: 116

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	Neg	Neg					
2	17/11/76	16	Neg					
3 *	24/11/76	64	Neg					
4	1/12/76	32	Neg					
5	10/12/76	16	Neg					
6	15/12/76	16						
7	23/12/76	8						
8	29/12/76	8						
9	6/1/77	8						
10	12/1/77	Neg						
11	21/1/77	Neg						
12	26/1/77	Neg						
13	2/2/77	Neg						
14	9/2/77	N.S.						Not Sampled
15	23/2/77	Neg						
16	2/3/77	8						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	8						
21	6/4/77	Neg						
22	13/4/77	Neg						
23 **	20/4/77	Neg						
24	27/4/77	Neg			Neg			
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 348

BREED: BLACKFACE GIMMER

TUPPED* 28.11.76

LAMBED**24.4.77

NO. OF LAMBS: TWO

LAMB NO: 122

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	8	Neg	16				
2	17/11/76	8	Neg	16				
3 *	24/11/76	8	Neg	8				
4	1/12/76	8	Neg	16				
5	10/12/76	Neg	Neg	16				
6	15/12/76	Neg		16				
7	23/12/76	Neg		Neg				
8	29/12/76	Neg		Neg				
9	6/1/77	Neg		Neg				
10	12/1/77	8		Neg				
11	21/1/77	8		Neg				
12	26/1/77	8		8				
13	2/2/77	Neg		Neg				
14	9/2/77	Neg		Neg				
15	23/2/77	Neg		8				
16	2/3/77	8		8				
17	9/3/77	Neg		8				
18	16/3/77	Neg		16				
19	23/3/77	Neg		Neg				
† 20	29/3/77	Neg		Neg				
21	6/4/77	Neg		Neg				
22	13/4/77	8		8				
23 **	20/4/77	Neg		16				
24	27/4/77	32		32	Neg		[illegible]	
25	4/5/77	32		64	Neg		Neg	
26	11/5/77	16		64	Neg		8	
27	18/5/77	64		64	Neg		8	
28	25/5/77	32		16	Neg		16	
29	1/6/77	32		16	8		32	
30	8/6/77	16		16	8	64	32	

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 394

BREED: BLACKFACE GIMMER

TUPPED* 23.11.76

LAMBED** 17.4.77

NO. OF LAMBS: ONE (Dead)

LAMB NO: (Foster 107 from ewe 970)

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	Neg	16					
2 *	17/11/76	Neg	8					
3	24/11/76	Neg	16					
4	1/12/76	Neg	16					
5	10/12/76	Neg	Neg					
6	15/12/76	16						
7	23/12/76	512						
8	29/12/76	16						
9	6/1/77	16						
10	12/1/77	Neg						
11	21/1/77	8						
12	26/1/77	8						
13	2/2/77	Neg						
14	9/2/77	8						
15	23/2/77	8						
16	2/3/77	8						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21	6/4/77	8						
22 **	13/4/77	Neg						
23	20/4/77	Neg			8			
24	27/4/77	Neg			32			
25	4/5/77	Neg			8			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: 414

BREED: BLACKFACE GIMMER

TUPPED* 16.11.76

LAMBED**12. 4.77

NO. OF LAMBS: ONE

LAMB NO: 102W

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1 *	10/11/76	Neg	16					
2	17/11/76	Neg	16					
3	24/11/76	8	16					
4	1/12/76	8	16					
5	10/12/76	8	16					
6	15/12/76	Neg						
7	23/12/76	Neg						
8	29/12/76	Neg						
9	6/1/77	Neg						
10	12/1/77	Neg						
11	21/1/77	Neg						
12	26/1/77	8						
13	2/2/77	Neg						
14	9/2/77	Neg						
15	23/2/77	16						
16	2/3/77	Neg						
17	9/3/77	Neg						
18	16/3/77	Neg						
19	23/3/77	Neg						
† 20	29/3/77	Neg						
21 **	6/4/77	8						
22	13/4/77	8			Neg			
23	20/4/77	8			Neg			
24	27/4/77	Neg			Neg			
25	4/5/77	Neg			Neg			
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TOXOPLASMA PROJECT SHEET

SHEEP NO: TUP

BREED: NORTH COUNTRY CHEVIOT

TUPPED*

LAMBED**

NO. OF LAMBS:

LAMB NO:

BLEED NO	DATE	EWE			LAMB			COMMENTS
		IHAT	CFT	IFAT	IHAT	CFT	IFAT	
1	10/11/76	64	32					
2	17/11/76	64	32					
3	24/11/76	64	32					
4	1/12/76	64	32					
5	10/12/76	64	32					
6	15/12/76	32						
7	23/12/76	32						
8	29/12/76	N.S.						Not Sampled
9	6/1/77	128						
10	12/1/77	32						
11	21/1/77	128						
12	26/1/77	64						
13	2/2/77	64						
14	9/2/77	64						
15	23/2/77	128						
16	2/3/77	32						
17	9/3/77	32						
18	16/3/77	32						
19	23/3/77	128						
† 20	29/3/77	128						
21	6/4/77	32						
22	13/4/77	64						
23	20/4/77	64						
24	27/4/77	64						
25	4/5/77							
26	11/5/77							
27	18/5/77							
28	25/5/77							
29	1/6/77							
30	8/6/77							

† ewe dosed with Covexin 8

TABLE 41. Total fluctuations of titre from beginning to end of bleeding for each sheep and each test.

SHEEP	IHAT	CFT	IFAT		LAMB	IHAT	IFAT
302	-2	+1	+4		61	-3	0
313	-2	0	-		93	0	-
322	0	-1	0		132	+1	0
325	-1	-1	-		62	0	-
412	-4	0	-		63	-1	-
527	-2	0	-		91	0	-
535	+1	-1	-1			Barren	-
536	+1	0	+1		95	0	0
539	0	-1	-2		69	0	-2
542	-3	-1	-2		65	0	0
572	0	-2	-		98	0	-
575	-1	0	0		70	0	0
589	-2	+1	-2		71	0	0
594	-2	+4	0			Barren	-
2027	-3	0	-		68	-	-
2040	-4	-1	0		67	-	-
41	-3	+1	-		101	-1	-
42	-3	+1	-1		)103	-1	0)
					) 99	0	0)
43	-2	-1	-		128	0	-
44	-2	+1	-		118	-1	-
47	-4	+2	-1			Barren	
421	0	-1	-		124	0	-
586	-2	+1	-		127	0	-
970	-3	+2	-		106	0	-
54	-1	0	0		135	0	0
91	-1	0	-		112	0	-
92	-3	0	-		108	-1	-
93	-3	-3	-1		140	0	0
94	0	0	-		119	0	-
95	0	-1	0		138	0	0
96	-1	0	-		104	0	-
97	-1	-1	-		110	0	-
98	-2	-2	-		133	-	-
99	-2	0	-		130	-1	-
212	0	0	-		116	0	-
348	+1	0	0		122	+1	+1
382	-1	-2	-1		125	0	0
394	0	-2	-		107	0	-
414	0	0	-		102	0	-
TUP	0	0	-		-	-	-
TOTALS	-57	-7	-6			-7	-1

Footnote : Changes in titre between consecutive readings were translated into no. of dilutions rise or fall (+ or -) and summated to indicate overall change.

TABLE 42 : Geometric mean titres (G.M.T.) for I.H.A.T. & I.F.A.T. for 15 ewes, weeks 1 - 10 and 21 - 30.

SHEEP NO.	I.H.A.T. - BLEEDS.		I.F.A.T. - BLEEDS	
	1 - 10	21 - 30	1 - 10	21 - 30
302	111.4	32.0	39.4	12.1
322	21.1	4.0	1.5	0
535	588.16	78.8	194.0	21.1
536	8.0	1.9	0	1.2
539	10.6	1.2	32.0	16.0
542	19.7	1.2	8.6	0
575	10.1	0	0	0
589	8.0	0	3.0	2.3
594	22.6	11.3	0	0
42	415.9	137.2	5.3	5.7
47	274.4	111.4	9.8	4.9
54	11.3	1.5	1.6	1.2
95	1.5	1.23	0	1.5
348	2.8	13.0	4.9	18.4
382	27.4	5.3	2.5	1.2
G.M.T. OF TOTAL SAMPLE	24.35	6.10	4.63	2.97

* N.B. I.F.A.T. G.M.T. e.g. ewe 95, G.M.T. for weeks 1 - 10 = 0, and for weeks 21 - 30 = 1.5. This is due to one titre of 1:8 in the latter group and is regarded as non-specific. Similar low G.M.T. should also be ignored.

TABLE 43 : Geometric mean titres (G.M.T.) for I.H.A.T. for 24 ewes and tup, weeks 1 - 10 and 21 - 25.

	I.H.A.T.	- BLEEDS
SHEEP NO.	1 - 10	21 - 25
313	12.1	2.3
325	147.1	73.5
412	207.9	42.2
527	4.0	1.5
572	4.9	0
2027	7.5	0
2040	27.9	18.4
41	812.7	168.9
43	238.9	97.0
44	238.9	84.4
421	7.0	0
586	68.6	64.0
970	157.6	128.0
91	17.1	2.3
92	32.0	5.3
93	238.0	97.0
94	15.9	9.2
96	7.5	0
97	18.4	0
98	2.5	0
99	4.0	0
212	9.2	0
394	4.3	1.5
414	1.9	1.5
TUP	54.9	53.8
G.M.T. TOTAL SAMPLE	24.66	7.22

TABLE 44 : Geometric mean titres (G.M.T.) for an equal number of bleeds before and after parturition (this varies between 3 and 5 bleeds before and after). N.B. Some sheep, with only 1 or 2 bleeds after parturition, were omitted.

SHEEP NO.	I.H.A.T.		I.F.A.T.		NO OF BLEEDS	COMMENTS
	BEFORE	AFTER	BEFORE	AFTER		
302	24.3	32.0	10.6	10.6	5	
313	2.9	1.7	-	-	4	
322	6.1	2.6	1.5	0	5	
527	13.5	0	-	-	4	
535	147.0	73.5	42.2	32	5	Control-Barren(P=22/23)
536	1.5	2.3	0	0	5	
539	0	1.5	13.9	18.4	5	
542	2.3	0	0	0	5	
575	0	0	0	0	5	
589	0	0	16.0	0	3	
594	18.4	7.0	0	0	5	Control-Barren(P=22/23)
41	215.3	181.0	-	-	4	
42	194.0	147.0	2.6	6.1	5	
47	147.0	147.0	1.7	2.6	5	Control-Barren(P=22/23)
970	101.6	161.3	-	-	3	
54	0	2.3	1.5	0	5	
91	2.3	0	-	-	3	
92	1.5	8	-	-	3	
93	48.5	97.0	16	16	5	Aborted.
95	2.0	0	2.0	0	3	
96	1.7	0	-	-	4	
97	0	0	-	-	3	
348	1.5	32.0	2.6	42.2	5	
382	10.6	3.5	1.5	0	5	
394	2.0	0	-	-	3	
414	1.7	2.8	-	-	4	
TUP	64.0	53.8	-	-	4	Control. (P=20/21)
G.M.T. TOTAL SAMPLE	6.05	7.03	3.10	3.25		

Footnote : P = point around which week bleeds were taken

TABLE 45 : Reciprocal titres of the controls for I.H.A.T., C.F.T. and I.F.A.T.

I.H.A.T.

CONTROL	H	M	N	A	B	C	D	E	F
	1024	32	Neg						
	512	64	16						
	1024	32	Neg						
	512	32	Neg						
	512	32	Neg						
	128	16	Neg	256	16	Neg			
	128	8	Neg	512	8	Neg			
	512	8	Neg	1024	16	Neg			
	128	16	Neg	256	16	8			
	256	16	Neg	512	16	Neg			
	512	16	-	512	32	Neg			
	128	8	-	512	16	Neg			
	128	8	-	128	16	Neg			
				256	16	Neg			
				256	64	Neg			
				1024	32	-	256	16	Neg
							256	16	Neg
							512	16	Neg
TOTAL FLUCTUATION	-2	-2	0	+2	+1	0	+1	0	0

CFT

CONTROL	H	M	N	POS.	FBS
	64	16	16	2048+	Neg
	32	32	64	8192	Neg
	16	16	16	1024	Neg
	16	8	32	8192	Neg
	16	8	16	8192+	Neg
	-2	-1	0	-	0

I.F.A.T.

D	FBS	PBS
32	Neg	Neg
16	Neg	Neg
16	Neg	Neg
32	Neg	Neg
32	Neg	Neg
32	Neg	Neg
32	Neg	Neg
32	Neg	Neg
32	Neg	Neg
32	Neg	Neg
0	0	0

TABLE 46 : The relationship between titre level and breed are shown below.

		Breed			
		Half-breds	Blackface		TUP
			Ewes	Gimmers	
Titre-Group	High (≥1:128)	6	7	1 (aborted)	1
	Low (≤1: 64)	10	1	13	

Table 47 : The relationship between titre level and number of lambs born per ewe and also number born alive (L) and dead (D).

		No. of lambs per ewe						Barren
		Singles.		Twins		Triplets		Ewes
Total No.		L	D	L	D	L	D	
Titre-Group	High (≥1:128)	5	-	6	2	4	2	3
	Low (≤1: 64)	7	1	28	-	9		-

Total - 59 live lambs per 39 ewes = 1.51 lambs per ewe.

High titre group - 15 live lambs per 14 ewes = 1.07 lambs per ewe.

Low titre group - 44 live lambs per 25 ewes = 1.76 lambs per ewe.

Table 48 : The relationship between positive (+) or negative (-) antibody status in the dam and her lamb at the time of parturition or just after.

Dam / Lamb	+	-
+	12	1
-	1	23

Fig. 1 : Corresponding titres for I.H.A.T. and C.F.T. plotted in table below. X^2 carried out.

I.H.A.T.	C.F.T. 0	8	16	32	64	128	256
4096				2			
2048				2			1
1024			5	2		1	1
512	1	1	10	9	1	5	1
256	1	1	5	15	11	2	
128	3	2	8	9	14	3	
64	4	1	7	18	5	4	
32	9	4	10	7	4		
16	7	7	34	11	2	1	
8	11	3	39	29	5		
0	10	8	27	14	4		

$X^2_{60} = 202.01$

significant > 0.1%

FIG. 2: Corresponding titres for I.H.A.T. and I.F.A.T. plotted in table below. X^2 carried out.

I.H.A.T.	0	8	16	32	64	128	256	512	1024
1024		1	1	3		1			
512	2	1	8		3	4		1	2
256	8	7	6	2	2		2		
128	10	5	8	10	4	1			
64	10	4	12	6	2	1			
32	29	8	11	8	3				
16	22	17	3		1				
8	55	12	6	5	1				
0	161	17	16	6					

I.F.A.T.

$X^2_{64} = 351.43$

Significant $> 0.1\%$

FIG. 3 : Corresponding titres for C.F.T. and I.F.A.T. plotted in table below. X^2 carried out.

C.F.T.	0	8	16	32	64	128	256	512
256	1			1	2			
128	1	1	1	1	2	4	1	
64	5	1	2	8	3	1	1	1
32	21	9	4	6	1			
16	41	15	15	2	1			
8	14		4	3	1			
0	13	4	9	1				

I.F.A.T.

$X^2_{42} = 147.82$

Significant $>$ 0.1%

Fig. 4 : Natural logarithm (ln) of G.M.T. of C.F.T. against ln I.H.A.T. titre. Data taken from Fig. 1.

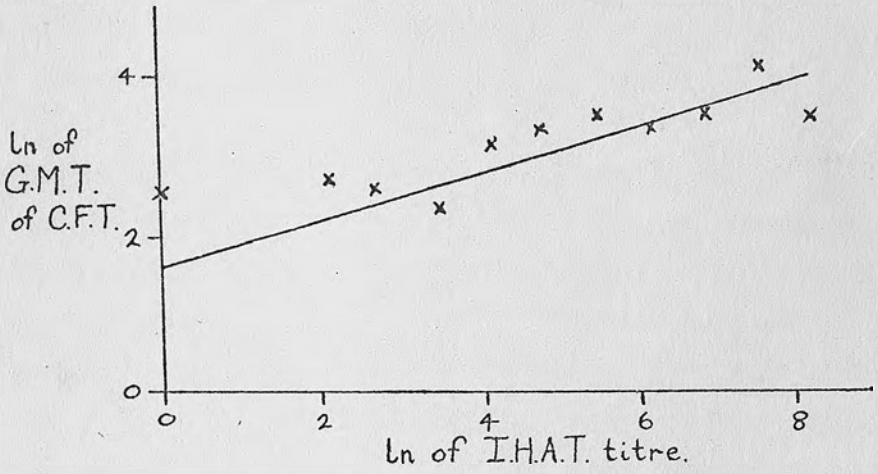


Fig. 5 : Ln of G.M.T. of I.F.A.T. against ln I.H.A.T. titre Data taken from Fig. 2.

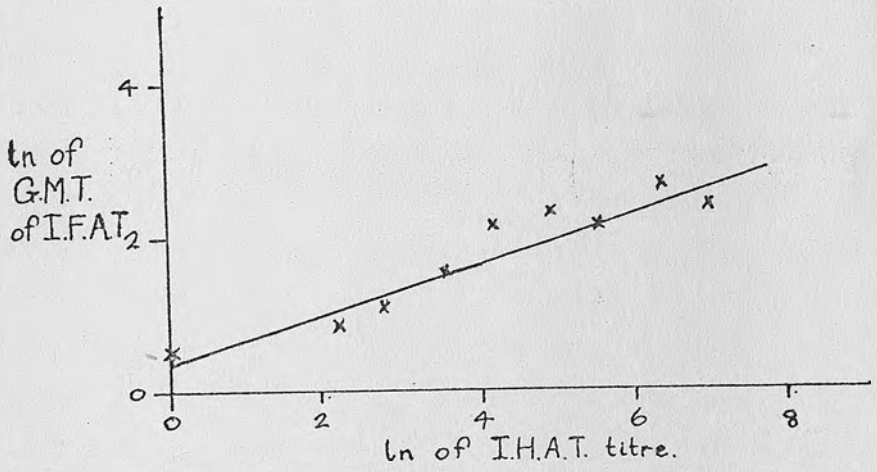
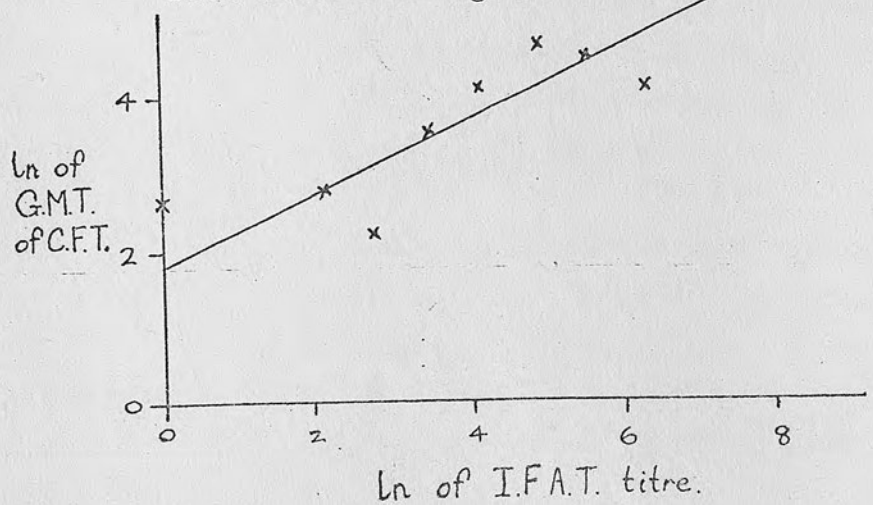


Fig. 6 : Ln of G.M.T. of C.F.T. against ln I.F.A.T. titre Data taken from Fig. 3.



DISCUSSION.

The I.H.A.T. using antigen kindly supplied by Dr Williams, was the easiest test to perform in agreement with the findings of Thorburn and Williams (1972); The test was reliable and of 18 batches that were tested only one batch had to be repeated.

Table 45 shows the results of the control sera; due to inexperience the author considers that in the first batches of sera tested the end titres were taken one dilution further than they should have been. Therefore in control "H" if the two readings of 1024 are taken as being too high then the rest fall into a pattern of reciprocal titres of 512, 256 and 128; Similarly with "M" and "N", if 64 and 16 are considered to be incorrectly interpreted; the same situation applies to "A" & "B" where the odd titre is particularly high. Controls "C", "D", "E" and "F" are within the acceptable limits.

Although it is realised that fluctuating control titres invalidate experimental results it is considered that slight titre differences are unavoidable in inexperienced hands. It must also be pointed out that the antigen was prepared for the human I.H.A.T., the sheep specimens were not preabsorbed and therefore non-specific reactions may account for some of the titre variations; variability of the control results may also be attributible to variations of antigen between batches. Although several different batches of antigen were used over the period of testing, no record was kept of the dates on which changes were made and so no batch effects could be determined in retrospect.

From the results in Tables 1 - 40 the author considers that a titre of 1:8 or less should be interpretated as negative and a titre of 1:16 as inconclusive; the occasional titre of 1:16 may be due for example to transient contact with toxoplasma antigen.

The C.F.T. was found to be the most difficult test technically to perform and was the most unreliable. Tables 1 - 40 show the results of 5 batches of tests, but an equal number of batches were carried out which had to be rejected because of unacceptable control results. The C.F.T. is a complex test especially in a micro-system but in spite

of this, correctly controlled results are very consistent and reliable.

The I.F.A.T. was less demanding and more satisfactory especially as Corbould (1971) and McKinney (1973) regard the test to be specific. In these studies adjustment to the use of the microscope may have contributed to the higher readings in the first few ewes and lambs tested e.g. ewe 535; later technical expertise was improved, although the microscope time was limited by fatigue as also noted by Walton, Benchoff & Brooks (1966). The author considered that "end-points" may have varied from time to time even within a single batch; therefore, to become consistent, the operator must be reading sufficient numbers of slides regularly with a familiar microscope and illumination system. The use of I.F.A.T's specifically to measure different immunoglobulin classes may be useful to assess the stage of infection. In this context Remington & Miller (1966) described a technique using dimercaptoethanol to inactivate IgM antibody while Osiyemi, (1976) used the cruder method of heat inactivation of IgM by heating the test serum to 65°C for 30 minutes. Shirahata and Shimizu (1974) showed that in IgM rises first in an primary infection and that IgG appears a few days later; in secondary infections only IgG antibody is apparent.

In this study the I.F.A.T. used measured IgG. Table 45 shows the consistent reciprocal titres of the control sera, and under the test conditions used a titre of 1:8 was considered to be negative, in agreement with the interpretations made by Munday (1970) and McKinney (1973). As in the I.H.A.T. a titre of 1:16 was considered to be inconclusive although no association was noted between 1:16 titres of the two tests. Costa, Araujo, Costa, Lima & Nascimento (1977) found titres of up to 1:16 in their negative control calves and in view of this it is possible that 1:16 titres in the current studies may relate to non-specific reactions or may be due to recrudescence of latent infections or to transient contact with the parasite. The interpretation of 1:16 titres must be related to serial results in individual animals.

Figures 1,2,3,4,5 & 6 show the degree of association between the three tests; figures 1,2 and 3 acted as contingency tables and a X^2 test

was carried out on each table, the value being shown under each figure. The X^2 shows there is a high degree of association between the three tests. The best degree of association was between the I.H.A.T. and I.F.A.T. as shown by Figure 5, but the association between the C.F.T. and I.H.A.T. or I.F.A.T. is not so convincing with C.F.T. titres commonly recorded in the absence of I.H.A.T. or I.F.A.T. response. This may be explained by the anti-complementary titres of some sera which were included in the results without comment. At higher titres the C.F.T. shows better correlation with the I.H.A.T. and I.F.A.T. As an alternative explanation, it is suggested that the C.F.T. may be measuring different classes of antibody when compared with other tests and the diagrams (Figs, 1 - 6) show that the I.H.A.T. attains higher titres compared with the C.F.T. and I.F.A.T. In agreement with Kelen, Allyou-Leindl & Labzoffsky (1962) and Osiyemi (1976) it was found that negative I.F.A.T. titres could be found in conjunction with positives I.H.A.T. titres (especially in the range 1:8 - 1:32) and occasionally the reverse was true, in support of the findings of Beverley and Mackay (1962) and Osiyemi (1976).

Table 41 shows the total fluctuations of titre for each ewe and lamb over the bleeding period. There is a general trend downwards and although the I.H.A.T. appears to show the greatest fall it must be remembered that I.H.A.T. results are also the largest group over the longest period in time since the I.H.A.T. was the only test to be carried out on all samples. The net result is that most of the test titres show a general decrease in titre from Autumn 1976 to Summer 1977 in support of the observations made by Hartley (1966). Tables 42 and 43, showing the Geometric mean titres (G.M.T.) for the first and last ten bleeds (the last five bleeds in the case of table 43) also show this general decline in titre and in fact all the I.H.A.T. titres decreased except in the case of ewe 348 who showed a significant increase, the I.F.A.T. G.M.T. for this ewe also rose. the other ewes showed a decrease in I.F.A.T. G.M.T.; the largest and not surprisingly decrease in G.M.T. occurred where the titres were highest originally.

Table 44 shows the G.M.T. for an equal number of bleeds before and after

parturition. The majority of ewes show either a decrease or no change in G.M.T. on comparison of G.M.T. results before and after parturition; again a rise in I.F.A.T. G.M.T. from 2.6 - 6.1 in ewe 42 is due to presumably non-specific 1:8 titres within the groups. Ewe 348 shows a marked rise in G.M.T. over the period of parturition. Table 36 shows that in this ewe the I.H.A.T. titre started to rise after parturition, rose three-fold by the week after parturition and to four-fold by the fourth week: Conversely the I.F.A.T. started to rise before parturition and showed a four-fold rise by the second week after parturition. The corresponding lamb (No 122) was negative by I.H.A.T. until it was 6 weeks old when it showed a titre of 1:8. The week before parturition the ewe had a negative I.H.A.T. so 'infection' appears to have occurred at or about the time of parturition. This infection and rise in I.H.A.T. antibodies may have occurred too late for the I.H.A.T. antibodies to be transferred to the colostrum which would explain the negative I.H.A.T. in her lamb at the bleeding after parturition; the C.F.T. titre for the final week was 1:64 and thus indicative of infection. Conversely, in this lamb, the I.F.A.T. showed a titre of 1:16 in the first week of life and became negative by the second week and this may have been due to a low level of passive maternal antibody since at the time of parturition the ewe had an I.F.A.T. titre of 1:16. In the lamb the I.F.A.T. titre was beginning to rise and reached a titre of 1:32 by the sixth week of life.

In the results as a whole antibody fluctuations from week to week tended to be of low magnitude irrespective of the original titres; such fluctuations may be associated with experimental error, as the control sera showed similar fluctuations. It was concluded that these variable results could be due to experimental error or possibly to re-infection or reactivation of infection at that time as proposed by Hartley and Moyle (1968). Costa et al (1977) found that calves showed differing antibody titres depending on the route of administration and the form of the parasite used for experimental infection. In these studies vaccination (with the clostridial vaccine) had no effect on antibody titres except in the case of ewe 93, who aborted the day after vaccination and it was of interest to note that this was the only Blackface gimmer to show any significant titres, especially to the I.H.A.T., throughout the whole period of bleeding. However, titres

were not impressive since the G.M.T. after the abortion only showed a one-fold rise in titre by the I.H.A.T. and no change by the I.F.A.T; it was concluded that the abortion in this ewe was probably related to the "stress" imposed by the vaccination. From the results in table 28 it appears that this ewe had been seroconverted at some time prior to bleeding and it is possible that vaccination may have reactivated this infection. However, the aborted foetuses underwent a routine screening for toxoplasma as well as for other causes of abortion and the results were all negative.

The stressful effect of harsh weather, suggested by Beverley (1957) as a factor involved in precipitating clinical toxoplasmosis, was studied. According to the Meteorological data from Turnhouse Airport, Edinburgh, the coldest days and nights were on the 3rd December, 1976 and 12th January, 1977, but there were no associated rises in titre in the flock.

If the ewes are grouped under two headings i.e. high I.H.A.T. titres ($\geqslant$ 1:128) and low I.H.A.T. titres ($\leqslant$1:64) and related to breed and the number of live and stillborn lambs produced then the results are as follows. Six halfbred ewes, seven Blackface ewes and one Blackface gimmer (No 93) and the tup were all in the high titre group. Ten halfbred ewes, one Blackface ewe and thirteen Blackface gimmers were in the low or negative titre group. It is interesting to note that some gimmers e.g. 95 were negative at the beginning of bleeding and despite being mixed with sero-converted ewes, tupped, undergoing a harsh winter prior to pregnancy and lambing they remained sero-negative. In fact in this flock the information shows that infection does not necessarily occur when sero-converted and sero-negative ewes are together even through an "infected" lambing. There was only one such case in the flock. She was ewe 348 (a Blackface gimmer) who has already been discussed earlier in this section. This information disputes the findings of Beverley and Watson (1971) and Watson & Beverley (1971b) who suggested that lateral transmission was common and that mixing uninfected gimmers with an indigenous "infected" flock 3 months before tupping was a method of natural immunization. However, they were dealing with flocks where abortions had occurred and in this flock there was no abortions

due to toxoplasma. This may be explained in two ways. Firstly, the failure of infection between sheep may be due to the genetic constitution of affected individuals. Araujo, Willaims, Grumet and Remington (1976) showed that in mice there were striking differences in susceptibility to toxoplasma in different mouse strains and that susceptibility altered with dosages, and a similar principle may apply to ovine toxoplasmosis. Secondly strain differences in the parasite itself may explain the different disease status and clinical history of this flock in comparison with those flocks studied by Beverley & Watson (1971) and Watson & Beverley (1971b).

Turner (1976) suggests that ewes may be infected by concentrates contaminating by faeces of cats shedding toxoplasma oocysts. These experimental ewes were fed concentrates from March to June, 1977 and the farm did possess cats, but only ewe 348 showed serological evidence of infection around parturition, but as no other ewes were infected, this explanation seems unlikely.

Munday (1970) using the I.F.A.T. found that wethers showed a lower reactor rate (12%) than ewes and rams (16%). Using tables 1 - 40 the reactor rate for the gimmers is 40% and for the ewes and tup is 45.5% as any sheep showing a titre of 1:32 or more was classed as a positive reactor. Using a titre of 1:32 or more as positive for the I.H.A.T. and C.F.T. the reactor rates are as follows.

Reactor rate.	Ewes & Tup.	Gimmers.	Whole flock.
I.H.A.T.	76%	66.6%	72.5%
C.F.T.	96%	20 %	65 %
I.F.A.T.	45.5%	40 %	43.8%

These results agree with Munday (1970) in that the gimmers show a lower reactor rate when compared with that for ewes and tups; this suggests that infection does occur other than congenitally, and suggests that some ewes encounter the parasite and become infected after the first year of life.

The I.H.A.T. shows the high reactor rates and this may be due to the

fact that it is less specific than the Dye test (Jacobs, 1967) and this specificity was also doubted by Lunde & Jacobs (1964) when they found that Toxoplasma had common antigens with Besnoitia, although Suggs, Walls and Kagan (1968) showed that the R.H. strain had no such common antigens with Besnoitia. Proportionately in these results the I.F.A.T. and I.H.A.T. show similar reactor rates suggesting again that they are measuring similar antibodies. Since an IgG conjugate was used the evidence suggests that the I.H.A.T. is also measuring IgG; this is in conflict with Thorburn and Williams (1972) who considered that their modification of the I.H.A.T. showed a greater affinity for IgM in their human serology work.

In this flock the CFT results show a great difference in reactor rate between the older ewes and the young gimmers, which suggest that it is measuring a different type of antibody from that of the I.H.A.T. and I.F.A.T. Karim & Ludlam (1975) considered that their modification of the C.F.T. measured the cuticular antigen antibody reaction, and their results correlated with parallel reactions demonstrated by the IgM-I.F.A.T. using whole paraiste antigens. Unfortunately the current C.F.T. results were not comparable with those of other workers because of the different methods of antigen production employed and the different host sera under test.

Table 47 shows the number of live and stillborn lambs in the high and low titre groups and also the proportion of singles, twins and triplets produced as well as barren ewes. In the "high" group 1.07 live lambs were produced per ewe; in the "low" group this rose to 1.76 live lambs per ewe whilst average for the flock was 1.51 live lambs per ewe. The "high titre" group thus show a marked decrease in live lambs produced per ewe over the "low titre" group, although the interpretation is confused by possible age and breed factors within such a small group. In studies carried out last year, 1975-1976 (Osiyemi, 1976) it was found that ewes 325, 535, 542 and 572 were barren in conjunction with high titres ($\geq$1:64) to the C.F.T., D.T. and/or I.F.A.T.; Using heat inactivation of IgM Osiyemi showed that these ewes had significant IgM levels to toxoplasma. During the current study (1976-1977) ewe 535 was still barren, two ewes (325 & 542) produced single lambs and ewe

572 produced triplets. Reproductive failure in 1975-76 may have been due to toxoplasma infection early in pregnancy with resulting embryonic loss and absorption and subsequent "recovery" would be in agreement with Watson & Beverley (1971a) who showed that ewes becoming infected and aborting one year were immune and produced lambs normally in subsequent years.

Here, it is interesting to note that the titres of ewes between 1975/1976 have remained stable when comparing them with the 1976/1977 titres; in fact all the ewes that are present in both sets of results show virtually no change in titres. Ewe 313 has remained sero-negative for 2 years and conversely ewe 535 has retained high titres for about 2 years. A group of five halfbred ewes (including ewe 313) have been through two complete cycles of tupping, by a sero-converted tup, and lambing without becoming infected.

In these studies several ewes i.e. 322, 536, 539, 542 and 575 and gimmers i.e. 54, 91, 92, 94, 97, 212, 382 and 394 showed a rise and fall in I.H.A.T. over a period varying from 4 - 10 weeks; ewe 322 also showed a corresponding rise in the C.F.T. and ewe 539 showed a corresponding rise in the I.F.A.T. The gimmers tended to have the higher titres from the beginning of bleeding which could possibly be related to the stresses they incurred after being brought down from the hill, coupled with the beginnings of a harsh winter (Beverley, 1957, suggested that harsh weather was the precipitating factor for some clinical cases of toxoplasmosis in dogs), may well have resulted in challenge with toxoplasma (from sero-converted ewes?), but the infection being quickly overcome. All had twins or triplets except ewes 212 & 394 who produced singles (394's lamb was stillborn). The rise in titre in the Halfbred ewes tended to occur 2 - 3 weeks after tupping which suggests the possibility of venereal transmission by the tup; there were five Halfbred ewes which showed this rise in titre 2 - 3 weeks after tupping and all had single lambs, except 536 which had triplets. These titre responses may have been due to the "stress" of mixing, tupping, hormonal and climate disturbances or in fact this whole series of high titre results may have been due to some batch effect in the testing procedures, yet another explanation, as described earlier, is that of the effect of

genetic variations in serological response within individual groups of ewes.

Table 48 shows the relationship between the antibody status of the dam and lamb at around the time of parturition and shows that where the ewe is sero-converted there is passive transfer of antibody to the lamb. This was shown to occur in 12 lambs and agrees with Hartley (1966) who also found that there was passive transfer of antibody from dam to progeny and that the titres faded over the following 3 months. Twenty-three lambs born to sero-negative ewes were also negative and in one case did the lamb possess antibodies where the dam was sero-negative; Similar cases were noted by Hartley (personal communication to Munday, 1970). The lamb may well have sucked at a sero-converted ewe for its first feed since Hartley (1966) suggests "mismothering" as an explanation for these anomalies between mother and lamb. In one case i.e. ewe 348, the dam was showing a rising I.H.A.T. titre and the lamb at birth was negative by this test; it is suggested that the ewe may have been too late for antibodies to the I.H.A.T. to enter the colostrum, hence the lamb was negative until the sixth week when it was itself to show the beginnings of a rising titre. In this same lamb the I.F.A.T. showed a titre of 1:16 in the first week of life and the mother had a corresponding titre of 1:16 four days before parturition. In the lamb the titre waned by the second week but by the 3rd week had started rising again to show four-fold rise in titre by the fifth week; The initial fall was probably due to waning maternal antibody but the rising titre tends to suggest that the lamb was also infected, as was its mother, and that infection occurred at or around parturition in the ewe and that infection was also acquired by the lamb. In all the sero-converted lambs the I.H.A.T. and I.F.A.T. show similar titres although the I.H.A.T. tends to be slightly higher. However, in lamb to ewe 348 the I.H.A.T. took 5 - 6 weeks to show signs of a rise which would possibly be more indicative of an affinity for later developing antibody. The IgG I.F.A.T. started to rise by week 3 - 4 but as whole parasites were used in the I.F.A.T. - it could be expected to have a broader antigenic display, whereas the I.H.A.T. was more specifically measuring antibodies to somatic antigen which takes longer to appear (Karim and Ludlam, 1975).

CONCLUSIONS.

The I.H.A.T. was easy to perform although its specificity was doubted. Due to the method of production of the antigen the results obtained and their clinical interpretation suggested that the I.H.A.T. was measuring the antibody to somatic antigen of T. gondii. A titre of 1:8 or less was considered to be negative and 1:16 was classed as inconclusive.

The C.F.T. was found to be difficult to perform and the results were inconsistent possibly because of the exacting demand of the microtitre system. In this study no guideline was established for C.F.T. interpretation.

The I.F.A.T. was easy to perform despite the limitations imposed by operator fatigue.

In this test a titre of 1:8 or less was considered to be negative and 1:16 as inconclusive. In the interests of accuracy the operator should examine slides regularly using the same microscope and illumination system. The I.F.A.T. System used measured antibody of the IgG class specifically.

There was a good degree of association between the results of the three tests, although the association was strongest between the I.H.A.T. and I.F.A.T.

In the flock, over the period of bleeding, the titres to all tests for each sheep diminished or remained stable. Titres were unaffected over the period of parturition except in one ewe in which the I.H.A.T. and I.F.A.T. titres showed a fourfold rise over a period of one month. The lamb of this ewe was also seroconverted.

Minor fluctuations in titre were attributed to experimental variation but larger fluctuations were considered as possibly due to infection or reactivation of infection; Vaccination had no effect on antibody titre except in one case and no rise in titre could be associated with particularly cold periods of weather.

Ewes showed a higher reactor rate than gimmers but in the flock under study there was no evidence of transmission between sero-converted and sero-negative ewes although some ewes showed a transient rise in titre.after tupping.

In the small group under study less live lambs were produced per ewe in the high titre group compared with those ewes in the low titre or negative antibody group. In all cases there was some evidence of passive transfer of antibody from sero-converted ewes to their lambs.

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