SOME ASPECTS OF TICK-BORNE FEVER IN SHEEP

by

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DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted solely by the author with the exception of the work presented in Chapter 5 and 6 which was carried out in collaboration with Dr. H.W. Reid and Dr. N.J.L. Gilmour respectively.

I also hereby certify that no part of this thesis has been submitted previously in any form to any university, but has been published in part as the following scientific papers.

Gilmour, N.G.L., Brodie, T.A. and Holmes, P.H. (1982). Tick-borne fever and pasteurellosis in sheep. Veterinary Record, 111, 512.

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SUMMARY

The condition known as tick-borne fever (TBF),which occurs in sheep and cattle, is due to a tick-transmitted rickettsia called Cytoecetes phagocytophila , and has frequently been suggested to have an immunosuppressive effect, predisposing animals to other disease conditions. The work in this thesis was directed towards examining C. phagocytophila as a pathogen per se and its role in other diseases of sheep such as tick pyaemia, louping-ill and pneumonic pasteurellosis. The mechanism whereby the immunosuppressive effect occurred was investigated and methods for the control or prevention of TBF and tick pyaemia explored.

Following experimental infection of lambs with TBF it was found that the only clinical signs were a transient pyrexia and an associated period of dullness and anorexia, most marked in very young lambs.

The haematological changes associated with infection were fairly severe and are the most likely explanation for the role the organism plays in predisposing animals to other diseases. Five to seven days after TBF inoculation was the period when the peak parasitaemia occurred, chiefly in the neutrophils and there was a modest neutrophilia at this time. At the same time the number of lymphocytes fell significantly, returning to pre-inoculation levels by 10 or 12 days after infection. Following the parasitaemic phase a severe neutropenia developed from the 10th day after infection and persisted for 5 days on average. Neutrophil numbers returned to normal by 24 days post-infection. Splenic enlargement was a consistent post-mortem feature in lambs suffering from tick-borne fever.

Assessment of the feasibility of inducing passive immunity to TBF in lambs via colostrum showed that, to be successful, a prolonged and continuous challenge of the dams during pregnancy was necessary, suggesting that such immunity is unlikely to occur in the field.

It was found that a long-acting tetracycline (LAT) drug could protect lambs from TBF for 5-10 days when it was given at the normal therapeutic dosage, but if the dosage was doubled the prophylactic effects were extended to at least 15 days. The use of this drug was subsequently studied in the field, in attempts to reduce lamb losses due to TBF, tick pyaemia and related conditions.

Experiments carried out to evaluate the effects of C. phagocytophila infection on the humoral immune system of lambs showed that the primary antibody response to two non-replicating antigens was not impaired by concurrent tick-borne fever. However from work on a live antigen (louping-ill virus) it was evident that the primary humoral response to the virus was impaired and the viraemia prolonged in sheep with concurrent TBF. Using a tuberculin test response as a measure of cell-mediated immunity it was found that the response was markedly reduced in sheep challenged with tuberculin during a TBF reaction. Thus C. phagocytophila appeared capable of impairing both the humoral and cell-mediated immune responses.

A concurrent tick-borne fever infection was found to exacerbate experimentally induced pneumonic pasteurellosis, louping-ill encephalitis and tick pyaemia.

Of particular interest was the finding that, in addition to increasing the severity of lesions and the likelihood of death from louping-ill, a number of sheep concurrently infected with C. phagocytophila and louping-ill virus succumbed to a systemic

mycosis.

Tick pyaemia was successfully reproduced experimentally following either intravenous or intradermal inoculation of Staphylococcus aureus to TBF-infected animals, although lesions occurred more readily after intravenous inoculation. When lambs were given S. aureus intravenously 5 days after TBF inoculation, 86% developed pyaemic lesions, but if bacteria were given in the absence of TBF only 20% became pyaemic. Intradermal inoculation of S. aureus resulted in pyaemic lesions only when inoculated during a TBF reaction. The timing of bacterial inoculation with relation to the stage of the tick-borne fever reaction was found to be important, lesions being most consistently produced when the staphylococci were given during the parasitaemic phase of TBF reactions (i.e. 5 days after TBF inoculation). The lungs were the most common site for abscess formation, followed by the joints in cases of experimentally induced tick pyaemia. A strain of S. aureus isolated from a field case of tick pyaemia was found to cause pyaemic lesions more consistently than a strain isolated from a case of ovine staphylococcal dermatitis. Necropsies carried out on a number of field cases of tick pyaemia showed that multiple abscess formation was common.

Field studies revealed that appreciable losses apparently associated with tick-borne diseases were being incurred on the hill sheep farms surveyed, although the incidence of tick pyaemia varied from year to year. By instituting control measures aimed at preventing the development of tick-borne fever and tick pyaemia (prophylaxis with a long-acting tetracycline drug) and reducing the tick challenge (dipping lambs) it was found that lamb losses could be

markedly reduced, especially in groups of lambs in which both dipping and prophylactic treatment were carried out. In addition lamb weight gains were improved in the treated groups.

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CHAPTER 1

LITERATURE REVIEW

Introduction

Tick-borne fever was first recognised as a specific disease entity in 1932 when Gordon and his co-workers were investigating louping-ill. Their experimental sheep, known to be immune to louping-ill, underwent a transient febrile reaction when placed on tick-infested pasture. This short-lived pyrexic condition was named tick-borne Fever (TBF) and was demonstrated to be immunologically distinct from louping-ill (Gordon, Brownlee, Wilson and MacLeod, 1932b). Later, Gordon, Brownlee and Wilson (1940) described the microscopic appearance of the agent of TBF in the cytoplasm of neutrophils and monocytes in blood smears stained by Giemsa.

Tick-borne fever has been identified in sheep and cattle in the United Kingdom (Gordon et al, 1932b), Eire (Collins, Hannon, Ferguson and Wilson, 1970), Norway (Overas, 1959), Finland (Tuomi, 1967a), India (Raghavachari and Reddy, 1959) and South Africa (Neitz, 1969).

Classification

The causative agent of tick-borne fever (TBF) in sheep is generally accepted as a member of the Order Ricketttsiales, although its final classification remains unresolved.

The organism was considered on morphological grounds to lie somewhere between Rickettsiae parasitic in animals i.e. R. canis, R. bovis and R. ovina (described by Donatien and Lestoquard in 1937 and later assigned to the genus Ehrlichia by Moshkovskii in 1945) and the Psittacosis virus group which was originally described by Bedson and Bland in 1932 (Foggie, 1951; Tuomi and von Bonsdorff, 1966). It was considered that the TBF agent was more closely related to the Rickettsial organisms and it was therefore classified as a member of the genus Rickettsia and given the specific name phagocytophila (Foggie, 1951). However, since the other members of the genus Rickettsia which were parasitic in animals were all found almost exclusively in circulating mononuclear cells while the agent of TBF parasitised neutrophil leucocytes, Foggie (1962) suggested that it should be placed in the genus Cytoecetes, whose type species was Cytoecetes microti, a parasite of the circulating neutrophils of the vole (Tyzzer, 1938) and whose morphology was very similar to that of TBF.

Nevertheless, in the 1974 edition of Bergey's Manual of Determinative Bacteriology, the assignment of the TBF organism to the genus Cytoecetes was rejected on the grounds that insufficient data was available on the type species Cytoecetes microti. It was placed instead in the genus Ehrlichia, of the tribe Ehrlichieae of the family Rickettsiaceae and remains there in the latest edition (Kreig, 1984).

Since then however, authors have used both generic names to describe the agent of TBF, for example Ehrlichia phagocytophila (Purnell et al, 1976; Ristic, 1978; Thrushfield et al, 1978) and Cytoecetes phagocytophila (Synge, 1976).

Morphology

The agent of TBF is visible under light microscopy following the staining of blood smears with the Romanowsky stains (Tuomi and von Bonsdorff, 1966). Among the more commonly used stains in examination of TBF are Leishman's (Foggie, 1951) and Giemsa (Gordon, Brownlee, Wilson and MacLeod, 1962). Under light microscopy with either of the above stains the organisms appear greyish-blue in colour (McEwen, 1947) although this colour can range from slate grey to dark purple (Gordon et al, 1962). They are found in the cytoplasm of circulating leucocytes, chiefly neutrophils although they have also been observed in eosinophils, monocytes and basophils (Foggie, 1951).

Several morphological forms of the agent of TBF have been described and although a reproductive cycle similar to that of the Chlamydia or the Rickettsias was suggested as an explanation for these morphological variations, the evidence for such a cycle remained inconclusive (Foggie, 1951; Gordon et al, 1962). The morphological forms described ranged from small rounded or rod-shaped bodies of 0.5 μ diameter to larger rounded bodies of up to 3 μ diameter and there were varying sizes of groups of bodies (called morulae) ranging from 2 to 4 μ in diameter. Some morulae consisted of solid, tightly packed masses of smaller bodies, while others were made up of a loose collection of bodies and in some cases gave the impression that they

were fragmenting or breaking up into irregular masses.

An electron microscopy study carried out by Tuomi and von Bonsdorff (1966) also showed that TBF appeared to have various morphological forms. These ranged from large oval particles (0.4 μ X 2 μ) down to small circular particles of 0.3 μ diameter. These particles were arranged in groups contained within cytoplasmic vacuoles separated from the cytoplasm by a cell membrane derived from the host cell cytoplasm. There was evidence that the large particles underwent binary fission. These findings led the authors to suggest that the TBF organism did in fact have a developmental cycle within the host cell.

Clinical Effects

Following a variable incubation period of 2-6 days, the most common and often the only clinical signs of TBF which appear are those of pyrexia and an associated dullness and anorexia (Gordon et al, 1932b; Foggie, 1951). The pyrexia is transient and although it has been reported to vary in duration from 2 to 19 days (Foggie, 1951), on average it lasts for 5-8 days (McEwen, 1947; Foggie, 1951).

However a number of authors have reported more severe clinical effects of TBF in sheep. A growth check on thriving sheep following TBF infection was noted by MacLeod and Gordon in 1933 and also by Stamp and Watt (1950), while Foggie (1951) described a severe weight loss in lambs (a mean of 7.5 lbs) during the week following TBF inoculation. Sheep have also been described as noticeably ill during the course of a TBF infection (Stamp and Watt, 1950; Overas, 1962a). In cattle a marked but temporary drop in milk yield has been observed

following TBF infection (Hudson, 1950; Tutt and Loving, 1955; Tuomi 1967a).

In experimentally infected cattle Tuomi (1967a) found that occasional dry coughing occurred in the majority of animals during the latter stages of the clinical reaction.

Jamieson (1947) reported that severe losses (23.75% mortality) had occurred among a group of ewe hoggs introduced on to tick infested hill pasture in April following a 6 month period during which they were wintered away on tick-free land. The author attributed the deaths to TBF since no evidence of any other infection was found among these animals. In later work the same author (Jamieson, 1950) reported two incidents involving abortions and deaths in gimmers originating from tick-free farms which were introduced on to farms known to be heavily tick infested. On one farm, of 68 gimmers (all vaccinated against louping-ill before arrival on the farm), 23 aborted and 11 died. On the second farm 41 gimmers aborted, of whom 16 died out of a group of 80 introduced onto the farm. In both cases TBF was found in blood smears from a number of the affected animals. Once again the author considered TBF to be the major factor in the events. In an experimental TBF infection of 10 ewes in late pregnancy, nine aborted and six ewes subsequently died (Jamieson, 1950). Other authors have suggested that the occasional animal had died as a direct result of TBF infection (MacLeod and Gordon, 1933; McEwen, 1947; Wilson, Foggie and Carmichael, 1964; Purnell and Brocklesby, 1978), although Jamieson's papers are the only ones describing such large numbers of deaths.

Abortion in sheep in tick areas was described by Harbour (1945) and since then the ability of the agent of tick-borne fever to cause

abortions in pregnant sheep has been demonstrated experimentally by several workers (Hudson, 1950; Jamieson, 1950; Stamp and Watt, 1950; Overas, 1959). In contrast, in studies carried out in Finland by Tuomi (1967a) pregnant cattle and sheep did not abort when infected with Finnish strains of TBF. Abortion due to TBF has also been reported in cattle by Wilson, Foggie and Carmichael (1964) who suspected that this was the agent responsible for abortions among heifers in late pregnancy which had recently been introduced onto tick-infested pasture.

Experimental infection of tups with TBF during the breeding season was found to result in a typical TBF reaction, following which the animals showed greatly reduced fertility for a period of months as assessed by semen quality (Watson, 1964b). Changes in sperm morphology were also reported in bulls infected with TBF and these persisted for about 15 weeks after infection (Retief, Neitz and McFarlane, 1971).

A haemorrhagic enteritis confined to the large intestine was reported to have occurred during three passages of a strain of TBF, although subsequent passages of the same strain failed to reproduce the condition (Foster, Foggie and Nisbet, 1968).

Post-mortem examination of uncomplicated cases of tick-borne fever have shown that the only abnormality present was an enlarged spleen (Gordon et al, 1932b; Jamieson, 1947; Gronstol and Ulvund, 1977).

Variations in the severity of the clinical (and haematological) effects of TBF have been noted and this may be related to differences between strains of TBF (Foggie, 1951) or may be due to different

susceptibilities among the experimental animals (Tuomi, 1967b).

Haematological Changes

The haematological changes that ensue as a result of TBF infection are fairly dramatic although of a transient nature and involve cells of the leucocyte series. An early report of these haematological events was given by Taylor and others (1941) who noted the occurrence of an initial modest neutrophilia which took place 2-4 days after TBF inoculation and was followed by a period of marked neutropenia lasting for 4-8 days which was in turn succeeded by a brief neutrophilia. Eosinophil numbers also decreased following infection, this depression lasting about the same length of time as the neutropenia. In addition they found that during a short period beginning 4 days after TBF inoculation there was a decrease in lymphocyte numbers lasting about 2 days. The effect of TBF on monocytes was irregular in Taylor *et al*'s study (1941) and no conclusions could be drawn.

Subsequent studies on tick-borne fever in which haematological studies were carried out have tended to agree with the findings of Taylor *et al*. Foggie (1956) in a study in one lamb found that the neutropenia began approximately 9 days after TBF infection and persisted for 7 days before the neutrophil count returned to a pre-infection level and later work by the same author using a much larger number of lambs confirmed that there was indeed a significant depression of the numbers of circulating neutrophils during a TBF reaction.

It has been shown that similar haematological changes occur in cattle infected with TBF, in which the development of a severe and prolonged neutropenia (Hudson, 1950; Tuomi, 1967a) was often preceded by a transient drop in the numbers of lymphocytes.

One other blood component has been reported to be affected during TBF reactions, namely the thrombocytes. Foster and Cameron (1968b) reported that a marked decline in platelet numbers occurred at the same time as the onset of the febrile reaction. However this was of short duration and the numbers had returned to normal within 7 to 10 days.

In addition to alterations in the circulating numbers of leucocytes caused by TBF infection, it has been suggested that the presence of rickettsial parasites in leucocytes may affect their functional integrity. Foster and Cameron (1970b) investigated this aspect of the condition and found that during the parasitaemic phase of a TBF reaction diapedesis was impaired in neutrophils. Later Woldehiwet (1979) reported that leucocyte migration was inhibited in animals during the early phase of parasitaemia.

Parasitaemic phase of tick-borne fever

In tick-borne fever in sheep and cattle the neutrophil is the main leucocyte parasitised and at the peak of a TBF reaction up to 90% of circulating neutrophils may contain rickettsial bodies (Raghavachari and Reddy, 1959; Foster and Cameron, 1968a). In addition to neutrophils, eosinophils, basophils and monocytes have been found parasitised (Foggie, 1951). Although the level of infection recorded in leucocytes other than neutrophils has been low

there is one report by Purnell and Brocklesby (1978) that a cattle strain of TBF could result in infection rates of up to 27% in monocytes.

The duration of parasitaemia appears to be variable both between individual animals and between strains of TBF (Foster and Cameron, 1970a) and has been reported to range between 2 and 11 days (Raghavachari and Reddy, 1959).

Transmission

In the series of experiments during which TBF was recognised as a separate disease entity from louping-ill, the tick Ixodes ricinus was found to be the natural vector of the disease in the United Kingdom (MacLeod and Gordon, 1932), while workers in India suspected that the TBF found there was transmitted by the tick Rhipicephalus haemophysaloides supino (Raghavachari and Reddy, 1959). In the U.K. transfer of TBF infection between stages of Ixodes ricinus has been shown to occur, although transfer of infection from the adult female tick through the egg has not been demonstrated and is not believed to occur (MacLeod and Gordon, 1933; Foggie, 1951).

Field studies have shown that lambs on tick infested pasture pick up a tick burden very soon after birth (Foster and Cameron, 1968a) and that within the first 2-3 weeks of life all lambs on such pastures where the ticks are carriers of TBF will have been infected with TBF (McEwen, 1947; Foster and Cameron, 1968). Apparently although not all of the ticks may be carrying TBF, only a very light tick infestation in a pasture is enough to transmit and maintain the disease (Foster, 1968) and the presence of only a small number of

ticks on an individual animal can result in infection (MacLeod and Gordon, 1933).

Experimental transmission of TBF has been successfully carrried out using infected ticks when the incubation period was found to vary from 3 to 13 days (MacLeod and Gordon, 1933). However, the majority of experimentally induced cases of TBF have been accomplished by the inoculation of whole blood collected from infected animals. Although the most commonly used route of administration has been intravenous inoculation, other parenteral routes have been used successfully. Subcutaneous inoculation was also satisfactory (Tuomi, 1967a; Scott and Koske, 1976) as were the intradermal and intramuscular routes although the latter two resulted in longer incubation periods than the others (Scott and Koske, 1976). Scott and Koske (1976) also tranferred infection via intraperitoneal inoculation although the success rate of infections was only 40 per cent. There is also one report by Hudson (1950) of successful infection of calves following intranasal inoculation, the resulting infection running a similar course to that found following intravenous or subcutaneous inoculation.

A TBF infection can be initiated following the inoculation of a very small quantity of infective blood, since Woldehiwet, Johnson and Scott (1980) produced infection in susceptible sheep by blood sampling, using a needle which had previously been used to collect blood from an infected animal.

In addition to infection with whole blood, serum (Foggie, 1951) and plasma (Foggie, 1951; Raghavachari and Reddy, 1959; Thrushfield, Synge and Scott, 1978) have been shown to be infective following intravenous inoculation into susceptible sheep although Foggie (1951)

pointed out that whole blood was more infective than plasma.

Tick Pyaemia

Foggie (1962) described tick pyaemia as 'an enzootic staphylococcal disease of lambs between 2 weeks and 2 months old', and there is no doubt that it is an important cause of economic loss through debilitated and dead lambs (Taylor, Holman and Gordon, 1941; Watson, 1964a). An early description of pyaemia in lambs associated with ticks was given by M'Fadyean in 1894 and this condition was later described as occurring in various tick-infested areas of Britain such as Scotland (Taylor et al, 1941), Northern England (Stewart and Ponsford, 1937) and Ireland (Foggie, 1943). The bacterial organism isolated from cases of pyaemia by M'Fadyean (1894) and Stewart and Ponsford (1937) was described by them as a Gram-positive micrococcus. Later Taylor and others (1941) considered that the organism they isolated from pyaemic abscesses in lambs was Staphylococcus aureus and this was confirmed by McDiarmid (1946b) who carried out extensive bacteriological tests on the organism.

Several groups of workers have shown that the incidence of tick pyaemia in the field situation varies from year to year although the cause of this unpredictability is unknown (Foster and Cameron, 1968a; Watt, Foster and Cameron, 1968). For example, high losses due to tick pyaemia were reported in a survey by Stewart and Ponsdorf in 1937 following necropsies performed on 209 dead lambs. They recorded that of these animals, a total of 100 were found to have pyaemic lesions and suggested that in 80 of these the pyaemic lesions alone were responsible for the lambs' deaths. In the same survey, on one farm

for which the numbers of lambs were supplied, the rates of lamb losses were 13% on one hirsel on which a louping-ill vaccine was used in some ewes and lambs and 16% on a second hirsel on which some lambs and ewes were dipped. Although these total losses appear relatively high, the losses due to tick pyaemia were found to be 4.7% on the first hirsel and 10.5% on the second. Taylor et al (1941) also reported a high rate of pyaemia cases (29%) among lambs on tick-infested farms in two successive years. In later work on the field incidence of pyaemia, Foster and Cameron (1968a) working with small numbers of animals reported that in two successive years 12% and 25% of the lambs at risk on tick-infested pasture became pyaemic. Nonetheless it was Foster's opinion (1968) that the level of field incidence of tick pyaemia very rarely exceeded 10% of the lambs which could be at risk.

Although Foggie (1956, 1959, 1962) remarked on several occasions that lightly tick infested farms had as many cases of tick pyaemia as those with heavy infestations, Watson (1964a) disagreed, stating that there was less pyaemia on farms with less ticks. A dipping experiment carried out by Watson, Brown and Wood (1966) tended to confirm Watson's view, in that there were fewer cases of pyaemia among dipped lambs with low tick burdens in comparison with the undipped control lambs which had much heavier tick burdens.

The most commonly recognised form of tick pyaemia is that in which joints are affected since crippled lambs are very obviously clinically affected and drawn to the shepherds' attention (Watson, 1964a). Various lesions may be found in affected lambs: staphylococci may have localised in joints and caused abscess formation and lameness; lambs may be presented as paraplegic or with other nervous

signs in which case the staphylococcal lesions involve the CNS; abscesses may be found in the internal organs resulting in a loss of condition in affected lambs; bacteria may be isolated from abscesses or from sites with no visible abscess formation; and combinations of the above lesions may be found in a single animal (McDiarmid, 1946a; Watson, 1964a). In addition to the chronic or pyaemic syndrome described above, an acute or septicaemic form of staphylococcal infection has been described (McDiarmid, 1946a, 1948; Foster and Cameron, 1968a).

Experimental production of tick pyaemia has been attempted on several occasions with varying degrees of success. In terms of pyaemia production, the intravenous inoculation of staphlylococci was the most successful route. McDiarmid (1948) inoculated nine normal lambs with a strain of Staph. aureus (originally isolated from a field case of tick pyaemia) and produced an acute septicaemic condition in two of them and pyaemic lesions in the remainder. Foggie (1948) also induced pyaemic lesions in normal lambs following intravenous inoculation with staphylococci and in a later series of experiments the same author found that lambs in the neutropenic phase of a TBF reaction were more likely to become pyaemic than normal lambs when given a similar dose of staphylococci intravenously (Foggie, 1956, 1957).

Other routes of staphylococcal inoculation have been attempted but they have failed to initiate pyaemic lesions although localised lesions of varying severity were produced. Taylor, Holman and Gordon (1941) inoculated Staph. aureus subcutaneously 4 days after TBF and failed to cause anything other than local lesions. Later Foggie (1957) also found that only local lesions were produced following

subcutaneous or intradermal staphylococcal inoculation, although the lesions were larger and more severe in lambs inoculated during a TBF-induced neutropenia compared with normal lambs.

In attempting to elucidate the role of the tick in the production of tick pyaemia, Foggie (1959) failed to induce pyaemic lesions in normal or neutropenic lambs following either intradermal inoculation with a staphylococcal suspension including tick salivary gland extract or the application of ticks to areas of skin contaminated with staphylococci.

The post-mortem findings in field cases of tick pyaemia were described by McDiarmid (1946a) who noted that the most common sites of abscess formation were the liver, joints of the limbs and the kidneys. Frequently abscesses were found in the meninges of the brain or spinal cord and in the pericardium and myocardium. Less commonly, abscesses were found in the diaphragm, thymus and adrenal glands and only rarely were the lungs affected. Watson (1964a) in a field survey of 45 dead and 63 living lambs collected from tick-infested farms listed the sites of abscess formation found and placed them in order of frequency as follows: carpal joints, liver, hock joints, kidneys, lungs, spleen, heart, subcutaneous tissues, elbow joints, hip joints, shoulder joints, stifle joints, skeletal muscle, spinal column and brain. However, these findings were biased by the fact that most of the lambs examined were selected on the basis that they were crippled lambs, i.e. lameness or paraplegia was a presenting sign, at least among the 63 live lambs collected. In a further examination of field cases of pyaemia, Foster and Cameron (1968a) found that of 10 lambs diagnosed as pyaemic, 6 had developed joint abscesses in addition to

internal abscesses while the remaining 4 only developed abscesses in internal organs, principally the liver and lungs. The distribution of lesions in experimentally produced pyaemia was described by McDiarmid (1948) who reported that the order of frequency of sites for abscess formation were the lungs, CNS, joints (including the costochondral junctions), kidneys, heart, skin and muscle. Although the spleen and liver of almost all these lambs were positive for staphylococci on culture, there was no abscess formation in these organs.

Therefore although there may be a tendency for staphylococci to localise in limb joints resulting in a predominance of abscesses in these sites, the liver, lungs and kidneys also appear to be principal sites of abscess formation.

TBF and other infections

It has often been suggested that in addition to tick pyaemia many other disease conditions have been predisposed to, or exacerbated by concurrent or recent infection of the animal with TBF. Among the more commonly mentioned conditions are louping-ill and pasteurellosis. MacLeod and Gordon (1932) in experimental studies found that infection with TBF just prior to, or concurrent with, louping-ill inoculation predisposed sheep to invasion of the central nervous system with the louping-ill virus and this finding was later confirmed by Gordon and others (1962). In addition, Taylor and his colleagues (1941) showed that exacerbation of the effects of louping-ill occurred when the virus was administered 2 days after TBF inoculation.

Pasteurellosis has been demonstrated in experimental sheep, giving rise to pneumonia and death shortly after infection with TBF

(Foggie, 1951, 1956; Foster and Cameron, 1970a) and it has been suggested that tick-borne fever may predispose sheep to pasteurellosis (Brandreth, 1978).

Experimental infection in sheep with parainfluenza-3 virus at the onset of the tick-borne fever parasitaemia resulted in a severe respiratory disease which led to deaths in some cases; in comparison, infection with the virus alone caused no clinical signs of disease (Batungbacal, 1979).

Tick-borne fever infection has been found in association with various other disease conditions, although its significance remains unknown. These conditions include listeric septicaemia (Gronstol and Ulvund, 1977), necrotic ileum in a lamb associated with a phycomycete (Angus, Renwick and Robinson, 1971), Johne's disease in a group of cattle (Hudson, 1950) and a complex of diseases involving mucosal disease and cobalt deficiency in calves (Grieg et al, 1977). In work carried out in India, Raghavachari and Reddy (1959) found that 61 of 127 sheep underwent a recrudescence of a latent Babesia infection following experimental inoculation with TBF. The same authors also reported that 90 per cent of their experimental sheep became severely diarrhoeic 7-8 days after infection with TBF and they suggested that this was due to the debilitating effect of TBF combined with the worm burden carried by the sheep.

There is evidence, however, that in some diseases concurrent TBF does not appear to exert an immunosuppressive effect. For example Purnell and others (1976) working on Babesia divergens in cattle found that there was no difference in the course of the babesiosis whether a concurrent TBF infection was present or not. Foggie and Nisbet (1964) found that not only did a concurrent TBF infection fail to exacerbate

an Eperythrozoon infection but the TBF pyrexia caused suppression of the Eperythrozoon parsitaemia and quashed the infection.

Immunity

The question of immunity to tick-borne fever remains controversial in the literature on the subject. Several factors serve to complicate the issue, among them the existence of immunologically variant strains of TBF; evidence of an age and breed-related immunity; the occurrence of premunity, relapsing infections and latency as features of the infection.

Homologous Immunity

In early work on TBF Gordon et al (1932b) found that sheep could become immune to homologous challenge, although in some cases it took two or more bouts of clinical TBF before this was achieved. This variability of response was also noted by Raghavachari and Reddy (1959) who found that some sheep became immune to challenge after only one TBF reaction while others would undergo a second TBF reaction following reinfection and become immune thereafter. More recently Woldehiwet, Johnson and Scott (1980) also noted some variability in response to a primary infection in that 80-100% of sheep became immune to homologous challenge, the remainder remaining susceptible. Other authors found that homologous immunity could be consistently induced following the primary reaction but have differed over how long it persisted. Stamp and Watt (1950) found that immunity lasted 12 months following experimental infection of sheep while in contrast most other reports suggest that immunity is short-lived. Scott (1975, 1977)

reported that 80% of sheep were immune to challenge for up to 6 months post-infection but that the percentage immune at 1 year post-infection had fallen to between 29% and 41% and by 2 years 19% were still immune. Experimental work by Foggie (1951) suggested that immunity was effective for up to 5-6 months and later work by Tuomi (1967a) on Finnish strains of TBF in cattle and sheep showed immunity to be effective for at least 4 months. Earlier experimental work on cattle TBF by Hudson (1950) revealed that in 11 out of 14 cattle immunity had waned by 6-12 months post-infection.

There is field evidence to indicate that immunity to TBF does occur, although there have been some reports that it is not persistent. Foster and Cameron (1970a) found that sheep on 2 farms were immune to challenge with TBF isolated on their respective farms of origin. Jamieson (1947), however, reported that sheep which were wintered away from home on a tick-free pasture for 6 months lost their immunity to TBF and were fully susceptible on their return. Similarly Overas (1962) found that immunity waned after sheep had spent only 14 weeks on tick-free pasture or 5 months housed. The same author also reported that lambs could undergo TBF reactions more than once during a single grazing season (Overas, 1962).

Foggie (1951) reported that immunity to TBF did not develop until 5 weeks after the primary infection and Raghavachari and Reddy (1959) presented similar findings in that a number of their experimental sheep reacted to a homologous challenge given 10 days after the end of the parasitaemia induced by the primary infection. However both these experiments involved very small numbers of sheep in each group and individual variations between sheep could not be discounted as contributary factors to the results. In addition, in these

experiments no account was taken of the fact that the secondary reactions noted may have been examples of spontaneous relapses (Foggie, 1951; Tuomi, 1967a). In contrast, Tuomi (1967a) in an experiment involving 30 sheep and cattle found that none were susceptible to homologous challenge when most of the animals were reinfected within 3 weeks of the cessation of the primary reaction. Similarly Scott and Koske (1976) reported that all experimental sheep were immune when challenged 4 weeks after recovery from TBF.

It has also been noted that where challenge was found to provoke a TBF reaction, the secondary reaction tends to be less severe than the primary (Gordon et al, 1932; Foggie, 1951; Overas, 1962; Scott and Koske, 1976).

In summary, it would seem that immunity to homologous challenge does exist, although it is temporary, and quite frequent re-challenge of the host is probably necessary to maintain resistance throughout life. Some residual immunity may exist even where animals succumb to re-infection since secondary reactions are reported to be milder than the initial TBF reaction.

Heterologous Immunity

Evidence that immunologically varying strains of TBF existed was presented by Foggie (1951) who found that TBF isolated from various sources produced different reactions in sheep and that there was also some degree of antigenic heterogeneity between the isolates when a cross-immunity experiment was carried out. Differences in the severity of the TBF reaction were also noted by Tuomi (1967b) and Foster and Cameron (1970a) when they examined isolates of TBF from different sources. Tuomi (1967b) on examining a number of isolates of

cattle TBF found that there was a range of incubation periods and variations in the duration and level of parasitaemia and pyrexia between isolates. When Foster and Cameron (1970a) examined two isolates of TBF from farms 12 miles apart they discovered that one provoked a more severe reaction than the other.

On the question of cross-immunity between strains of TBF, work from Finland on cattle strains of TBF showed that there was a wide range of heterologous strains of TBF producing little or no cross-immunity. However, some strains did possess a degree of cross-immunity in that a few of the more virulent strains would immunise animals against subsequent inoculation of a less virulent strain (Tuomi, 1967c). Foggie (1951) indicated that some form of cross-immunity may exist between strains when he pointed out that TBF reactions to challenge with heterologous strains were less severe than the primary reactions, while in their examination of two TBF isolates from farms in the South of Scotland, Foster and Cameron (1970a) found that a complete cross-immunity existed between the two strains. More recently Scott (1979) also claimed that reactions to heterologous challenge were milder than the primary reaction to TBF and he suggested that it was common for TBF isolates to display immunological variation, even when isolated from the same farm. This was in agreement with Tuomi's findings (1967c) regarding the existence of a large number of immunologically distinct strains of TBF, although in a later paper (Tuomi, 1967d) it was shown that even although strains were immunologically distinct they still showed common antigens since they all reacted with the same fluorescent immune serum. In contrast to Foggie's 1951 observations, Tuomi (1967b) reported that serial

inoculations of different strains did not appear to reduce the virulence of the subsequently inoculated strains.

In addition to studies of different strains of TBF affecting the same species, work has been carried out on inter-species infection with cattle and sheep strains of TBF. Early work by Hudson (1950) demonstrated that a sheep strain of TBF inoculated into cattle resulted in a more severe reaction in the new host than a cattle strain inoculated into sheep. The low infectivity of bovine strains for sheep was reported by Foggie and Allison (1960) and in further work Wilson, Foggie and Carmichael (1964) found that a cattle strain of TBF produced a mild disease in sheep when compared with naturally occurring ovine TBF. However some experimental work has indicated that cattle strains are not always of lower virulence than ovine strains, for instance , a bovine strain of TBF examined by Foggie (1951) appeared capable of inducing a reaction in sheep while an ovine strain did not produce any reaction in cattle. Similarly, in his studies on Finnish strains of TBF Tuomi (1967b) found that his cattle strains tended to have a higher virulence in the natural host, although a small number of strains were more virulent in sheep.

In summary it would seem that different strains of TBF exist. However, the capability of one strain to induce cross-immunity to other strains is variable.

Age and Breed-Related Immunity

A varying susceptibility of different age groups of sheep and cattle to TBF has been noted in some reports on the condition. In 1947, McEwen claimed that tick-borne fever was a relatively benign disease in lambs under 2 weeks of age when compared with Jamieson's

reports (1947) of death and abortions in older sheep reintroduced to tick-infested hill pasture after 6 months on tick-free ground. McEwen made the proviso that TBF may well have a more severe effect if affected lambs were exposed to bad weather conditions. Scott and Koske (1976) also reported that young animals appeared to have a higher resistance to TBF than hoggs and gimmers and added that in their experience aged adult sheep were similarly less susceptible to the effects of TBF infection.

Hudson (1950) working on TBF in cattle stated that calves below 6 months of age were less susceptible to TBF than older animals since a high proportion of the limited number of calves infected failed to respond to TBF inoculations known to be effective in older cattle. Later work on cattle TBF resulted in the finding that although both adult cattle and calves (up to 14 months of age) all reacted to TBF, the calves were less markedly affected (Tuomi, 1967a). Closer examination of Tuomi's experimental data indicated that the peak temperature reactions and the peak level of parasitised neutrophils were lower in calves than in adults while the duration of the temperature reaction and parasitaemia in the calves although slightly shorter, was similar to that of the adults.

A breed-related immunity to TBF may have been detected by Scott and Koske (1976) who reported that TBF reactions were milder in Scottish Blackface sheep than in other breeds.

Passive Immunity

Field observations (McEwen, 1947) showed that 39 out of 46 lambs born and reared on a tick-infested pasture had demonstrable TBF parasitaemias by the time they were 2 weeks old. This was in spite of

the fact that their dams had been present on the pasture during the previous year and in addition would have been exposed to tick bite prior to lambing. He therefore concluded that although the ewes were probably immune to TBF, either colostral transfer of immunity did not occur or the maternally derived antibody played a subordinate role and only served to reduce the severity of the condition rather than preventing it.

Premunity and the Carrier State

Premunity, defined as the maintenance of a state of protective immunity by the persistence of small numbers of the pathogenic organism in the body, has long been recognised as a feature of tick-borne fever. Premunity therefore by definition involves the maintenance of a carrier state. MacLeod and Gordon (1933) produced evidence of a carrier state when they reported that nymphal ticks could become infected by feeding on a sheep five weeks after it had recovered from TBF and these ticks could transmit TBF to a susceptible sheep after moulting to the adult stage. Since then other workers have observed that the carrier state persists for varying periods. For example, Raghavachari and Reddy (1959) found that their Indian strain of TBF resulted in a very short carrier state, their experimental animals' blood only being infective for up to 4 days after the parasitaemia subsided. They tested the blood by subinoculations until 11 days after the end of the parasitaemia, although they did not continue after that.

Similarly Tuomi (1967a) reported that one of his Finnish strains of TBF (J34-64) was poor at inducing a carrier state since only one

subinoculation of blood collected at various times between three days and eight and a half months after the cessation of the primary reaction resulted in TBF infection in susceptible subjects. The one successful subinoculation involved a very large inoculum (500 ml whole blood) collected nine days after the end of the donor's parasitaemia. Using the same strain of TBF Tuomi (1967a) found that subinoculated blood collected 7, 11 and 19 days after homologous challenge of the donors was not infective to susceptible animals.

In 1950, Hudson had found the carrier state to persist in some cattle for up to 14 days after the parasitaemia subsided while in sheep infected with an ovine strain blood was still infective 54 days post-parasitaemia. In contrast Foggie (1951) recorded very long carrier periods in two sheep; in one the carrier state persisted for 26 weeks while in the other blood was infective 25 months after the original infection. Foggie (1951) believed that under natural conditions the carrier state was relatively common since in his experience samples of blood collected from sheep on tick-infested farms always caused TBF reactions on subinoculation into susceptible sheep.

Some of the variable data from the carrier state studies described above may be accounted for by different strains of TBF possessing varying properties. Tuomi (1967a) compared his Finnish strains of TBF with the Indian strain of Raghavachari and Reddy (1959) and the British strains examined by Foggie (1951), concluding that his own strain appeared to be better at inducing a carrier state then the former and less capable than the latter. Further evidence of the role of strain differences in inducing the carrier state was provided by Woldehiwet, Johnson and Scott (1980) who described how when two

strains of TBF were inoculated into sheep and the carrier rates tested within 10 weeks of inoculation, one strain produced 26% carriers (10 of 39 sheep) while the other resulted in 80% carriers (8 of 10 sheep).

The immune status of carrier animals is not necessarily absolute. For instance Foggie (1951) found that when he reinfected 3 sheep with TBF about 6 months after they had undergone a primary TBF reaction, all 3 responded in spite of the fact that one of them was a carrier in that its blood transmitted infection immediately prior to challenge. In contrast, Scott (1977) found that when he classified carrier sheep as being those whose blood was infective, they were invariably immune. However, this immunity could be destroyed in a small proportion of carrier sheep following therapeutic immunosuppression. In addition more than 50% of non-carrier sheep proved to be immune (Scott, 1977). The latter finding, that animals could be immune without being carriers, i.e. the possible existence of a state of sterile immunity was in accordance with Hudson's suggestion (1950) that such a state could exist. He believed that it was a short-lived stage following premunity and that the animals quickly became susceptible again. From further observations Scott (1978) reported that sheep which had a sterile immunity could become carriers following a TBF challenge to which they did not respond.

The relationship between size of inoculum and rate of carrier detection was touched on briefly by Tuomi (1967a) who reported that a 500 ml whole blood sub-inoculum from an immune heifer resulted in the transfer of infection while an inoculum of 3 ml of the same blood failed to provoke a TBF reaction, leading him to suggest that the standard size of inocula used to detect carrier animals was too

small. However in this case the failed inoculum was given to a sheep while the recipient of the successful inoculation was a cow and a lower virulence of cattle derived strains for sheep had been noted before (Hudson, 1950; Overas, 1962) and may have had some bearing on the result. Recently Woldehiwet and others (1980) have suggested that the injection of large volumes of blood does not significantly increase the rate of carrier detection, although in some individual cases the larger inoculum may succeed in provoking a TBF reaction where the smaller volume fails.

A recognised feature of the carrier state is the occurrence of relapses of fever and parasitaemia which may be spontaneous or induced. Foggie (1951) noted that spontaneous relapses can occur occasionally in sheep 3 to 4 weeks after the primary TBF attack. Tuomi (1967a) described a similar phenomenon in both cattle and sheep: in 36 cattle experimentally infected with various strains of TBF secondary rises of temperature and parasitaemia occurred in 6 animals 3 to 8 days after the end of the primary TBF reactions. Furthermore, 4 animals had a parasitaemia without a fever and another 3 cattle had a secondary pyrexia but were not examined for a parasitaemia. In sheep 3 animals infected with one of the TBF strains had a relapse of infection 7, 12 or 13 days after the subsidence of the primary reaction.

Artificial induction of a TBF relapse can be achieved either by the administration of immunosuppressive drugs or following splenectomy. Foggie (1951) reported that a typical TBF reaction occurred when a sheep was splenectomised 48 weeks after it had undergone a primary TBF reaction. However, splenectomies carried out 2 weeks after the final TBF inoculation in 3 sheep immune to repeated

challenge failed to result in relapses of TBF although one died from babesiosis (Raghavachari and Reddy, 1959). The 2 survivors remained immune to TBF challenge after splenectomy. These three sheep, however, were never shown to be infected with TBF (i.e. by subinoculation) and they could in fact have possessed a sterile immunity. Tuomi (1967a) also failed to show recrudescence of TBF following the splenectomies of one sheep and one calf, 4 and 9 months respectively after recovery from a primary infection. However in the series of experiments from which these results were taken, Tuomi did not find that his strains of TBF produced a carrier state lasting for more than 5 weeks and so, as he suggested himself, the animals may have been clear of TBF by the time the splenectomies were carried out.

TBF in other species

Although tick-borne fever was first discovered in sheep in the U.K. in 1932 (Gordon and others, 1932) it was not proved to be a cause of disease in cattle in England until 1950 (Hudson, 1950) and was not recognised in Scottish cattle until 1960 (Foggie and Allison, 1960). As noted previously, experimental infection of sheep with cattle strains of TBF has been shown to produce a milder clinical response than when sheep strains were inoculated into cattle (Hudson, 1950; Overas, 1962; Wilson, Foggie and Carmichael, 1964). The infectivity of the cattle strain for sheep was shown to be low by Hudson (1950) and Foggie and Allison (1960).

Sheep strains of TBF have been experimentally inoculated into goats and the resulting clinical responses were found to be similar to those expected in sheep (MacLeod and Gordon, 1933; Raghavachari and

Reddy, 1959). Naturally occuring TBF infections in goats have also been reported from India (Raghavachari and Reddy, 1959) and amongst wild goats in Scotland (Foster and Greig, 1969).

Typical TBF bodies have been seen in the blood of red deer shot on the island of Rhum in Scotland and when sheep were inoculated with such blood a normal TBF reaction ensued (Foggie, 1962). It has also been reported that TBF was isolated from roe and fallow deer in the New Forest (McDiarmid, 1965).

Although attempts were made to reproduce TBF in laboratory animals such as guinea-pigs, mice and rabbits (Hudson, 1950; Foggie, 1951) and ferrets (Hudson, 1950), it was not until 1961 that successful infection was achieved by using splenectomised mice and guinea-pigs (Foggie and Hood, 1961). However this work was a limited success in mice in that only splenectomised mice were capable of maintaining the infection. Better results were obtained with guinea-pigs since, following adaptation to splenectomised guinea-pigs, the TBF organism was capable of being maintained in normal guinea-pigs. The strains of TBF that were maintained in these laboratory animals remained infective for sheep (Foggie and Hood, 1961).

Other species in which TBF infection failed to become established following experimental inoculation were pigs, a horse and an elk calf (Tuomi, 1967a).

Serology

The diagnosis of an acute TBF infection is relatively simply carried out by examining a giemsa-stained blood smear for intracellular parasites. It has long been recognised that the

development of a serological test would allow for more accurate retrospective diagnosis of TBF than that achieved by demonstration of the carrier state and greatly aid investigation of the condition.

Tuomi (1967d) carried out some studies using immunofluorescent staining techniques and developed a direct immunofluorescent method whereby he demonstrated that labelled immunoglobulin from cattle with experience of TBF selectively stained TBF bodies present in leucocytes on a smear. In addition he found that serum antibody could be detected in cattle by this method 2 weeks after the TBF reaction had ended.

Snodgrass and Ramachandran (1971) went some way towards developing a complement fixation (C.F.) test for TBF in sheep. They reported that specific complement fixing antibodies developed within 20 days of TBF inoculation and continued to rise thereafter. From the results of an experiment involving two sheep they suggested that there was a connection between the presence of complement fixing antibodies and immunity. Woldehiwet and others (1980) also reported that C.F. titres developed 2-3 weeks after TBF infection, rapidly reached a peak and persisted at that level for 2-4 weeks before gradually declining.

Serological assessment of the cross-reactivity between strains of TBF, using both the immunofluorescent staining technique (Tuomi, 1967d) and the C.F. test (Woldehiwet et al, 1980), has shown that all the strains examined cross-react, suggesting that they share one or more common antigens (Tuomi, 1967d).

Preservation of infective TBF blood stabilates

The storage of infective TBF blood has been necessary in order to preserve stabilates of different strains and to allow the use of the same strain and passage of a TBF stabilate on several occasions. Hudson (1950) reported that he had successfully stored citrated infective cattle blood for 48 hours at room temperature or for up to 14 days at 4-8°C. Foggie (1951) working with ovine TBF stated that he had successfully stored citrated blood for up to 10 days at room temperature without loss of infectivity and for 13 days at 4°C. In later studies Tuomi (1967a) recorded that infective blood could confidently be stored at 4°C for up to 7 days but that longer periods of storage resulted in less regular preservation of infectivity, although on one occasion blood was infective after 30 days storage. However Tuomi (1967a) also noted that preserved blood tended to result in an extended incubation period in comparison with fresh blood.

Successful preservation of infective blood for long periods by storage at very low temperatures (-74°C to -79°C) was reported by Foggie, Lumsden and McNeillage (1966) and Tuomi (1967a). The former authors added 7.5% glycerol or dimethylsulphoxide (DMSO) as cryopreservative agents and found that blood retained its infectivity for at least 18 months while Tuomi (1967a), who did not add a cryopreservative, reported infectivity for at least 8 months after storage. Similarly Pierce et al (1974), investigating various cryopreservation regimes found that no cryopreservative was necessary.

In vitro culture of the agent of TBF

The development of an in vitro culture system for TBF would be a useful tool for further investigation of the organism. Embryonated hen eggs have been employed on several occasions, in attempts to culture the agent in the yolk sacs; however, all attempts were unsuccessful (Hudson, 1950; Tuomi, 1967a; Thrushfield, Synge and Scott, 1978). Other culture methods attempted have included conventional bacteriological media and tissue culture systems (Tuomi, 1967a) involving various techniques proved to be successful in the culture of human rickettsias such as pulmonary macrophage cultures (Thrushfield et al, 1978). Again, none of these techniques resulted in the successful cultivation of the TBF organism.

Chemotherapy of tick-borne fever

A fairly wide variety of drugs have been used experimentally in attempts to suppress or prevent tick-borne fever. Three of these had limited success, while the remainder had little or no effect.

There have been two reports of oxytetracycline therapy being instituted in outbreaks of TBF in herds of dairy cattle, and in both cases the fever fell rapidly and the markedly depressed milk yield quickly returned to normal (Venn and Woodford, 1956; Foggie and Allison, 1960).

Experimental investigations of the action of oxytetracycline against TBF have given slightly varying results, although the dosing regimes may have been the cause of discrepancies. Tuomi (1967e) found that when he gave cattle five daily injections beginning 3 days after TBF inoculation, the TBF reaction was suppressed and no relapse

occurred. The same author also reported that he could prevent a TBF reaction from taking place by starting oxytetracylcine therapy 3 hours before TBF inoculation and continuing it for 2 days. In contrast, Evans (1972) gave his sheep treatment with oxytetracycline at the onset of parasitaemia which resulted in the rapid suppression of the reaction, although relapses occurred frequently. When he delayed treatment the result was the same and the relapses just as common.

A more detailed series of experiments by Synge (1976) showed that oxytetracycline treatment on the first day of parasitaemia rapidly abated fever and parasitaemia but relapses occurred 6 to 12 days after therapy. Following the relapse, the animals were solidly immune. When Synge (1976) treated for 3 successive days instead of giving a single treatment the same series of events was observed.

Sulphamethazine has also proved useful against TBF and its effects are similar to those of oxytetracycline. Foggie (1951) recorded that 3 days of sulphamethazine therapy instituted during a TBF parasitaemia suppressed the reaction but that a relapse occurred about a week later, while a 5 day course of therapy would prevent relapses. However, the animal given the 5 days therapy was not immune to challenge 3 weeks later. Similarly Evans (1972) using sulphamethazine and Synge (1976) using sulphadimidine both found that a single treatment at the onset of parasitaemia only temporarily suppressed the reaction; Synge noted that his sheep were immune following the relapse. Earlier, Tuomi (1976e) had found that relapses did not occur when he treated cattle with sulphamethazine, although his dosage regime was different from the others in that he treated 3 hours before TBF inoculation, 4 hours after the inoculation and

subsequently once daily for the following 2 days.

The third drug reported to be effective against TBF was alpha-ethoxyethylglyoxal dithiosemicarbazone. Both Evans (1972) and Synge (1976) observed that a single dose given at the onset of parasitaemia suppressed the reaction. However they differed on the question of relapses, Evans claiming that they did not occur while Synge reported that the animals invariably relapsed, although he found that 3 daily doses of the drug would prevent relapses.

Treatment with a penicillen and dihydrostreptomycin mixture was reported to have abated the pyrexia but had no effect on the TBF parasitaemia (Evans, 1972).

Other therapeutic agents tested against TBF include penicillen (Foggie and Allison, 1960; Tuomi, 1967e), streptomycin (Tuomi, 1967e; Synge, 1976) and chloramphenicol (Tuomi, 1967e; Evans, 1972; Synge, 1976), but none was successful in treating or preventing the condition.

CHAPTER 2

GENERAL MATERIALS AND METHODS

In this chapter only materials and methods used throughout the thesis are described. Special techniques unique to each chapter are described in that chapter.

(a) Tick-borne Fever

Isolates of tick-borne fever

Four isolates of tick-borne fever were used in the course of the experimental work presented in the thesis. The origin of each isolate is given below. Strain A (Argyll strain) was used as the infective dose in all the experimental TBF infections. Strains C, H, and M were only used on a small number of occasions in the experiments described.

Strain A (Argyll Strain) - Isolated from sheep on a hill farm at Glendaruel, Argyll in 1977. It was passaged 4 times in sheep and the blood collected on the 4th passage was used as the reserve stabilate.

Strain C (Compton Strain) - This strain of TBF was kindly supplied by Dr David Lewis (Pfizer Ltd, Sandwich, Kent) when he was working at the ARC Institute, Compton. It was originally from hill sheep in the Nidderdale area of Yorkshire.

Strains H and M - These two strains were isolated from hill sheep on Arran during the field survey. Strain H originated from sheep on Farm A2 and Strain M from Farm A1.

The isolates of Strain H and Strain M were made as follows. A random selection of 5 adult ewes were selected on each farm during the period of tick activity from April to June and a 10 ml heparinised blood sample collected from each ewe. The 5 samples from each farm were pooled and a 5 ml sample of each farm's pooled blood was inoculated intravenously into separate susceptible experimental sheep. At the peak of the TBF reaction (usually 5 days after inoculation) heparinised blood was collected from the culture animals and this blood was stored as the stabilate.

Preparation and Storage of Stabilates

Heparinised blood was collected from culture sheep 5 days after infective blood inoculation. The amount of heparinised blood collected varied but on average 150 ml of blood was taken from each animal from the jugular vein into three 50 ml sterile syringes each containing 700 units of Heparin (Sigma Chemical Co., St. Louis, U.S.A.). The blood from the syringes was pooled and glycerol was added in a proportion of 12.5% and the mixture stirred gently on a magnetic mixer for 1 hour. The blood with the glycerol as cryopreservative agent was then divided into 4.5 ml aliquots and placed first in the vapour phase of a liquid nitrogen container and allowed to freeze slowly before being finally stored in liquid nitrogen at -196^{o}C.

Experimental Infection of Sheep with TBF Stabilates

The number of 4.5 ml aliquots of stabilate required for infection were removed from the liquid nitrogen, thawed rapidly under warm water and inoculated into the experimental animals as soon as possible after thawing. Each experimental sheep (regardless of age) was inoculated intravenously in the jugular vein with 1.5 ml of stabilate.

Monitoring of TBF Reactions

Infected sheep were examined clinically on a daily basis, rectal temperatures were recorded and 5 ml blood samples collected into EDTA tubes (William Sarstedt (U.K.) Ltd, Leicester, England).

Haematological Procedures

Total leucocyte counts were carried out in an electronic particle counter (Coulter Electronics Ltd, England). Thin blood smears were prepared, air dried, fixed in methanol for 5 minutes and stained with a buffered 10% Giemsa solution (BDH Chemicals Ltd, Poole, England) for 30 minutes. The stained smears were examined under an oil immersion lens (X1000) and the percentage of neutrophils, lymphocytes, eosinophils and monocytes ascertained by counting 200 cells. The percentage parasitised neutrophils was also assessed at the same time by examining 100 neutrophils although in some smears due to the low neutrophil counts found during the latter stages of TBF reactions only 50 neutrophils were examined. The percentage count for each leucocyte type was then multiplied by the total leucocyte count to give numbers of each cell type per ml of whole blood.

Experimental Animals

All sheep used in the experiments described were from tick-free areas and thus were known not to have had previous exposure to tick-borne fever infection. They were housed indoors on straw bedding.

Statistical Methods

The statistical procedures applied to the data in this thesis were the Chi-squared Test which was used on non-parametric data and the Students t-Test applied to parametric data (Snedecor, 1956).

CHAPTER 3

STUDIES ON TICK-BORNE FEVER

Introduction

This chapter describes a series of experiments undertaken to investigate various aspects of the agent of TBF, *Cytoecetes phagocytophila*. The course of an uncomplicated tick-borne fever reaction with the strain of *C. phagocytophila* used in subsequent experiments throughout this thesis was examined both clinically and haematologically. Subsequently the effects, both therapeutic and prophylactic, of a long-acting tetracycline (LAT) drug on TBF and the possibility of inducing passive immunity in young lambs by immunising their dams to TBF was studied. Finally, various reports that TBF infection resulted in splenomegaly (Gordon *et al*,1932b; Jamieson, 1947; Gronnstol and Ulvund, 1977) were confirmed in this study.

SECTION I. UNCOMPLICATED TICK-BORNE FEVER INFECTION

Experiment 3a: Uncomplicated C. phagocytophila infection in lambs

Materials and Methods

Two crossbred lambs aged approximately 4 months (Nos. 42 and 49) and three crossbred lambs aged 4 weeks (Nos. 73, 75 and 76) were inoculated intravenously with C. phagocytophila Strain A on Day 0. Daily rectal temperatures were taken and 5 ml blood samples were collected for haematological examination.

Results

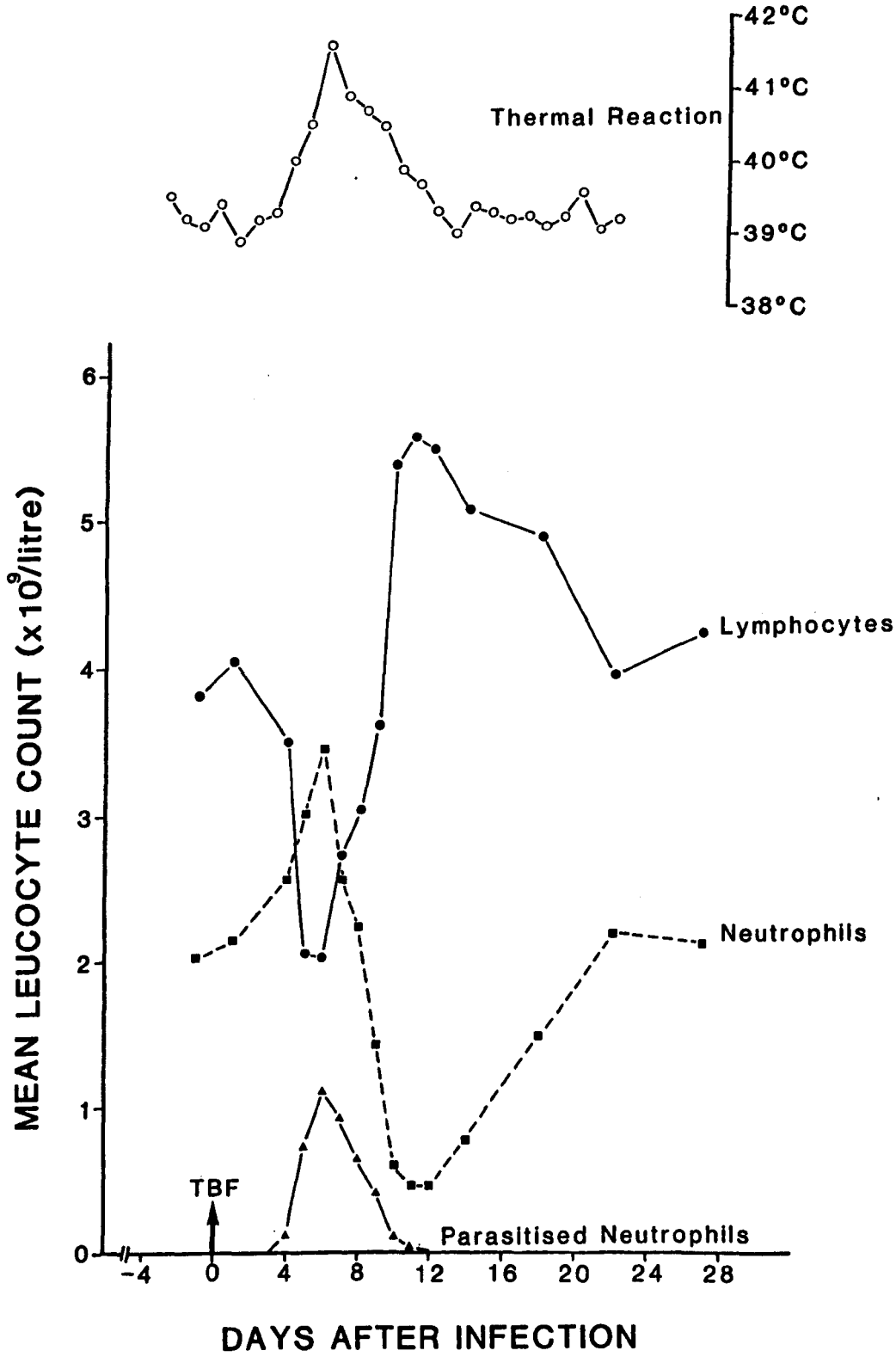
Clinical Signs: The lambs appeared unaffected by TBF inoculation apart from a transient period of dullness and anorexia during the febrile phase of the TBF reaction. This period of dullness was, however more obvious and marked in younger animals than in older ones and since the former were being fed ewe milk replacer on a lamb-bar system, their daily consumption of milk was noticeably reduced during the febrile period.

Pyrexia: The mean rectal temperatures are shown in Figure 3 (i) and daily temperatures are presented in Table 3a. The mean incubation period before the lambs became febrile (> 40.5°C) was 5.2 (± 1.1) days and the mean duration of the febrile response was 4.2 (± 0.8) days. The mean peak febrile reponse was 41.6 (± 0.2)°C and occurred on Day 6. When the pre-inoculation mean temperature (Day -1) was compared with post-inoculation mean temperatures it was found that the mean rectal temperatures were significantly raised on Days 6, 7, 8 and 9.

Parasitaemia: The mean numbers of parasitised neutrophils are illustrated in Figure 3 (i) and the daily figures are presented in Table 3b. The mean incubation period before intracellular parasites were visible by light microscopy was 4.8 (± 0.8) days, and the mean duration of parasitaemia was 6.8 (± 0.8) days. Lambs 42, 49, and 73 developed both parasitaemia and fever on the same day while lambs 75 and 76 did not become parasitaemic until one day after the fever commenced. The mean peak number of parasitised neutrophils occurred on Day 6 and on that day the mean percentage of neutrophils containing parasites was 36.8% (± 7.0%). The peak percentage of parasitised neutrophils ranged from 41% to 56%. On occasions parasitised eosinophils and monocytes were also noted. Plates 1 and 2 show the typical appearance of parasitised cells under light microscopy (x1000, oil immersion). The parasitic cellular inclusions stained a grey-blue colour in the cytoplasm of neutrophils, eosinophils and monocytes and were either composed of amorphous clusters, formed a ring-like structure or appeared as small discrete bodies. When present in eosinophils (Plate 1) they were best visualised using a green filter (Filter No. Grun VG9, Leitz, Wetzlar, Germany) while in examining neutrophils a filter was unnecessary (Plate 2).

Neutrophils: The mean neutrophil counts are illustrated in Figure 3(i) and those of individual animals in Figure 3 (ii). The daily figures are presented in Table 3c. The graphs for the individual lambs show a similar pattern to that of the mean figures, namely that 6 days after TBF inoculation the neutrophil count rose to a transient peak of 3.48 (± 0.64) x 10^9/litre and thereafter fell

FIGURE 3(i). EVENTS DURING A TICK-BORNE FEVER REACTION.

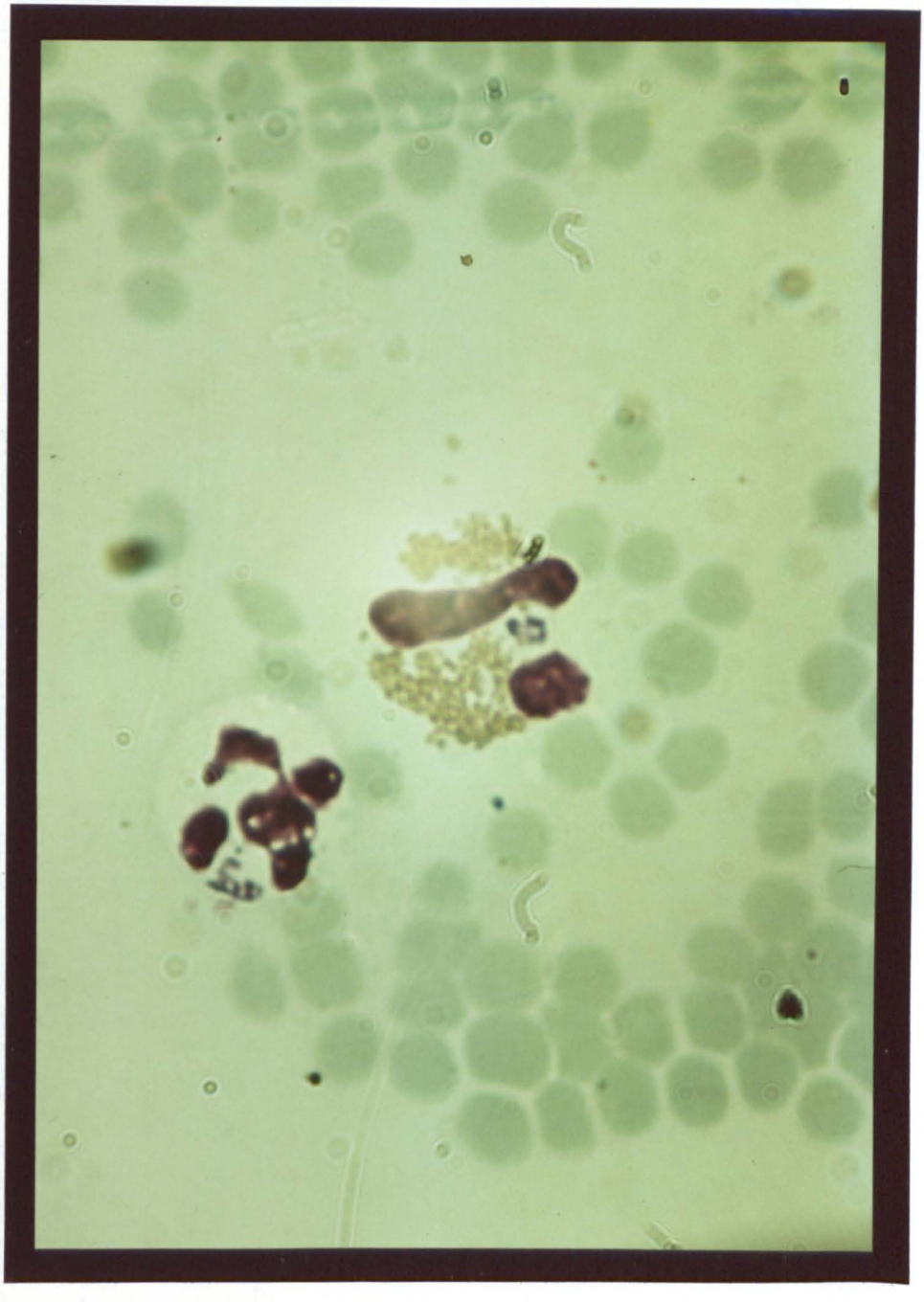


to reach a nadir on Day 12 of 0.46 (± 0.23) x 10^9/litre, following which the neutrophils rose slowly again to return to pre-inoculation levels by Day 22. There was no evidence of a reactive neutrophilia. The mean neutrophil numbers were significantly higher than pre-inoculation levels on Day 6 ($P < 0.05$) and from Day 10 to Day 14 inclusive they were significantly depressed ($P < 0.05$). One lamb became neutropenic (i.e. the neutrophil count was below 0.7 x 10^9/litre (Schalm et al, 1975)) on Day 9, three on Day 10 and one on Day 11.

Lymphocytes: The mean lymphocyte counts are illustrated in Figure 3(i) and those of individual animals in Figure 3 (iii). The daily figures are presented in Table 3d. The graphs for individual animals show a similar pattern to that of the mean figures, namely that there was a fall in lymphocyte numbers between Day 4 and Day 5, reaching a nadir of 2.02 (± 0.63) x 10^9/litre on Day 6, followed by a return to levels greater that those of the pre-inoculation sample reaching a peak of 5.56 (± 1.53) x 10^9/litre on Day 11 and from this peak they returned to pre-inoculation levels by Day 22. The post-inoculation mean lymphocyte counts were significantly lower than the pre-inoculation levels on Days 5 and 6 ($P < 0.05$, $P < 0.01$ respectively).

Monocytes: The mean monocyte counts are illustrated in Figure 3 (iv). The daily figures are presented in Table 3e. There was a fall in mean monocyte numbers beginning on Day 6, which reached a nadir on Day 11, following which they recovered to pre-inoculation levels. A small number of monocytes were noted to be parasitised.

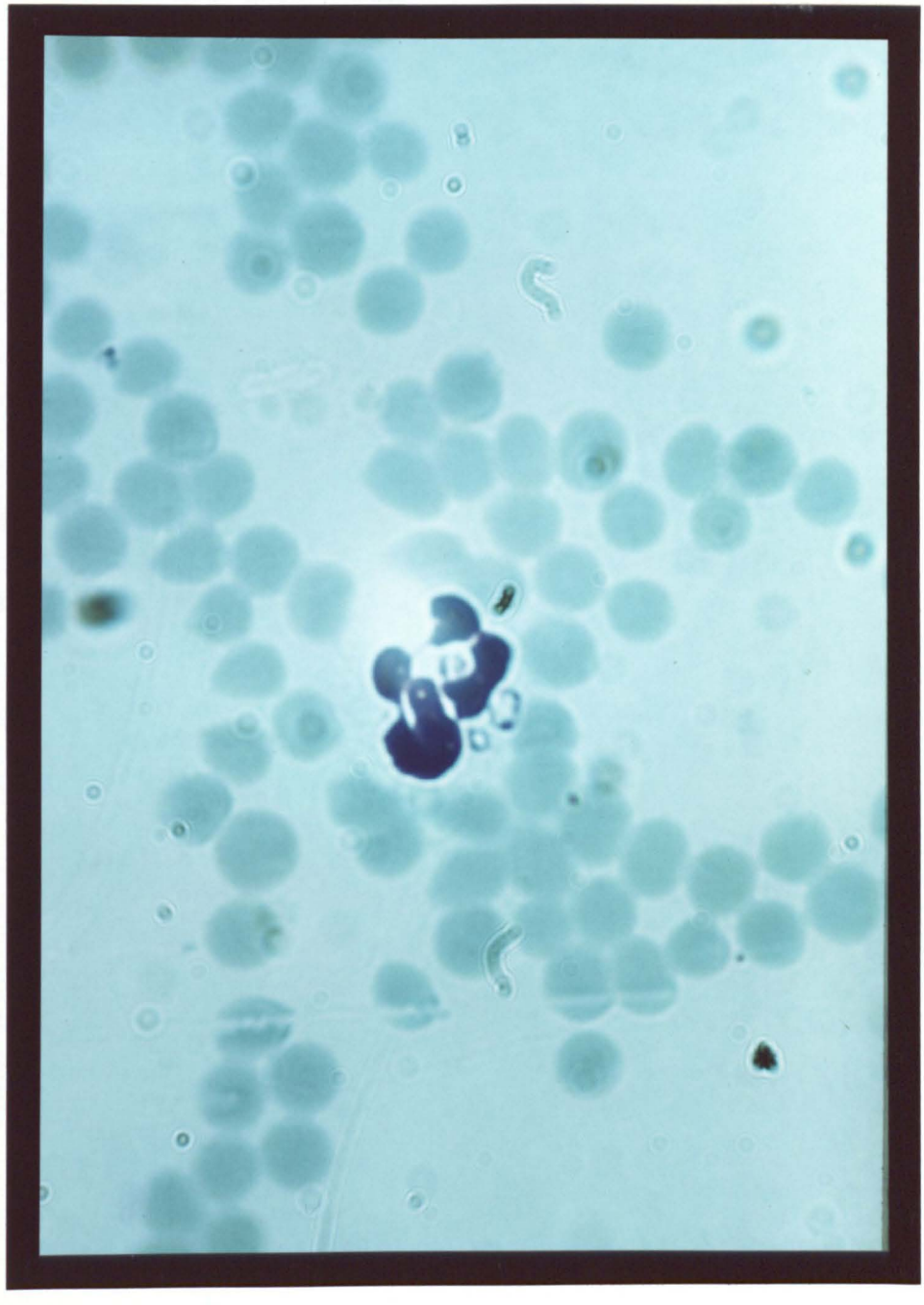
PLATE 1



Blood smear stained with Geimsa, showing parasites in an eosinophil and a neutrophil

(x 1000 oil immersion, using a green filter)

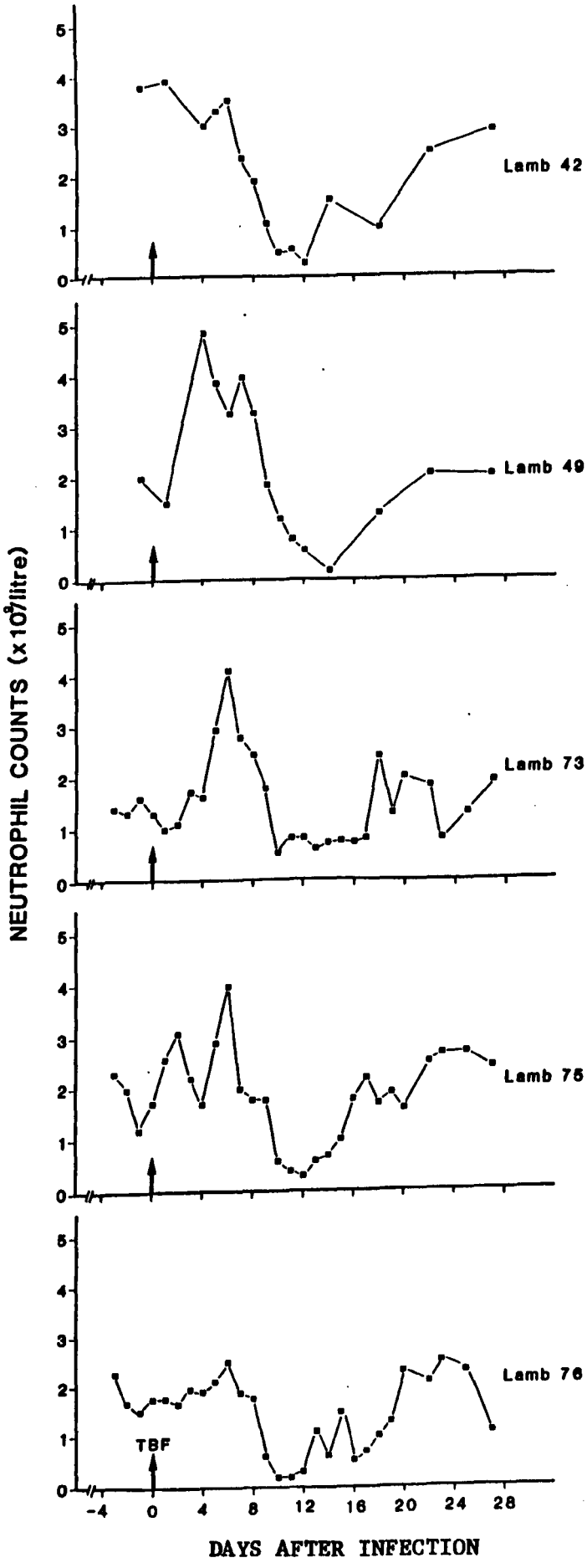
PLATE 2



Blood smear stained with Geimsa, showing parasites in a neutrophil

(x 1000 oil immersion, no filter)

FIGURE 3(ii). NEUTROPHIL COUNTS OF FIVE LAMBS DURING A TICK-BORNE FEVER REACTION.



Eosinophils: The mean eosinophil counts are illustrated in Figure 3(iv). The daily figures are presented in Table 3f. There was a fall in mean eosinophil numbers that began shortly after TBF inoculation and resulted in virtually no eosinophils being present in the peripheral blood from Day 5 onwards until they began to reappear by Day 22. Parasites were occasionally seen in the cytoplasm of eosinophils as noted earlier.

ALTERNATIVE METHOD OF ANALYSIS

The five lambs in this study could be regarded as two groups divided on the basis of age - 4 week old lambs (nos 73, 75, 76) and 4 month old lambs (nos 42 and 49). When the results were examined on this basis, using analysis of variance according to a two - factor design with repeated time measurements, some differences were found between the responses of the older and younger lambs. These are summarised below.

Rectal temperature (Table 3a) : there is a significant group and time interaction ($P < 0.01$) between the groups, i.e. they have a different response pattern.

Parasitised neutrophils (Table 3b) : as above there is a significant group and time interaction ($P < 0.01$).

Neutrophil counts (Table 3c) : no difference between groups but a significant difference between times ($P<0.01$).

Lymphocyte counts (Table 3d) : as above for Table 3c.

Monocyte counts (Table 3e) : significant difference between groups only ($P < 0.05$).

Eosinophil counts (Table 3f) : no differences between groups or times.

FIGURE 3(iii). LYMPHOCYTE COUNTS OF FIVE LAMBS DURING A TICK-BORNE FEVER REACTION.

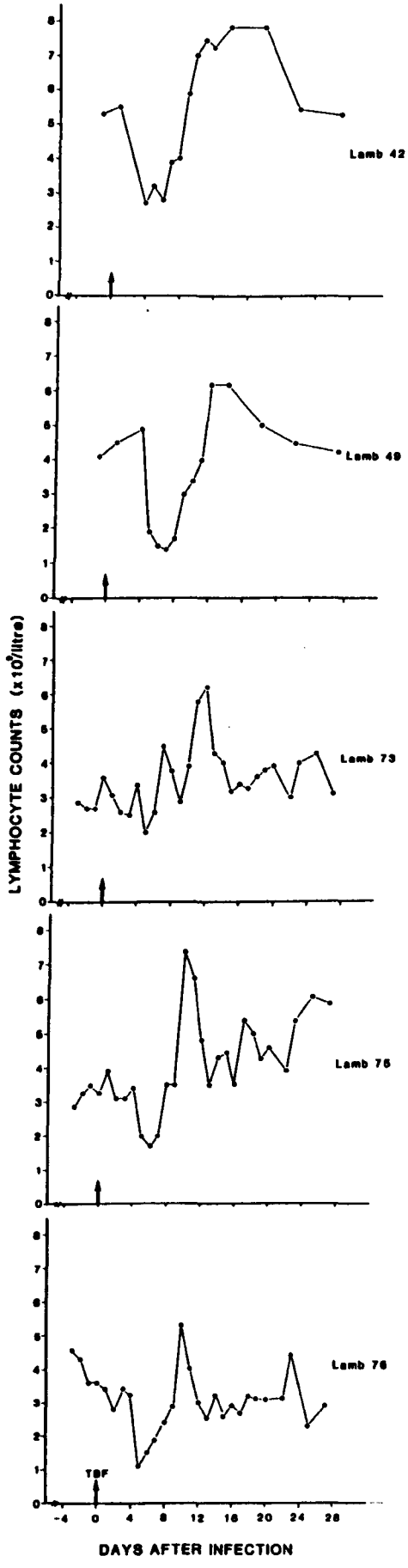
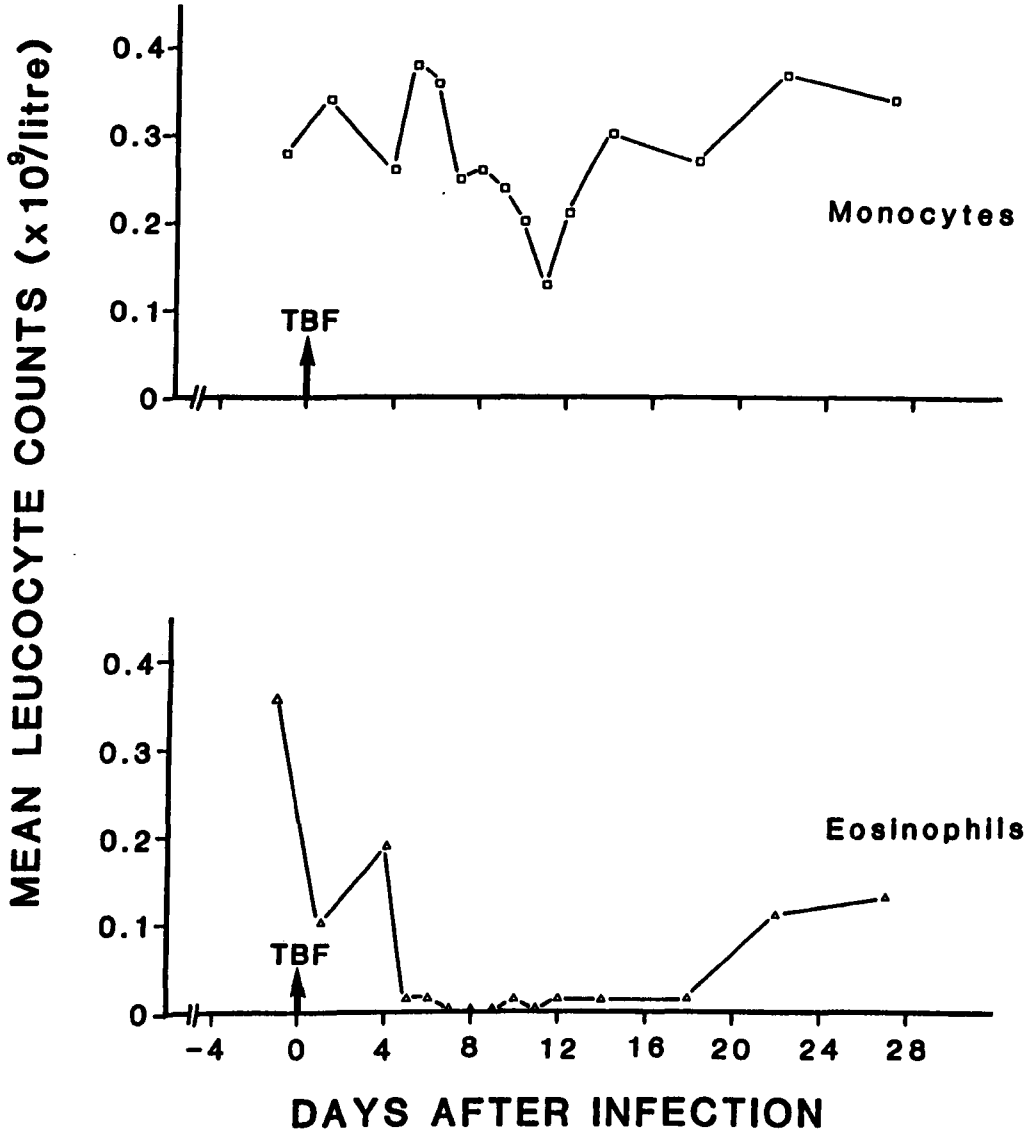


FIGURE 3(iv). MEAN MONOCYTE AND EOSINOPHIL COUNTS DURING A TICK-BORNE FEVER REACTION.



Discussion

The results of the uncomplicated Cytoecetes phagocytophila infection (Expt. 3a) were in general agreement with the findings of other workers on TBF reactions. The clinical signs consisted only of a period of anorexia and depression during the febrile period which lasted approximately 4 days; in the present study this was most marked in the younger lambs. It suggests that although tick-borne fever may in itself be a fairly innocuous clinical entity it could cause deaths in young, depressed, anorexic lambs in the field when they are unable to keep up with their mothers at a time when frequent feeding and maternal care is most important to their survival, particularly in inclement weather conditions. Further evidence for this point of view was furnished by MacLeod and Gordon (1933) who pointed out that lambs on hill pastures went through an unthrifty phase at about 3 weeks of age and there were unexplained deaths in lambs at this time, coincident with the spring rise of the tick population. Later Stamp and Watt (1950) and Foggie (1951) reported a check in growth of sheep and lambs infected with TBF and in the latter paper, Foggie (1951) found that weight gains were not only reduced but the lambs actually lost weight following TBF infection.

The incubation period in TBF infections is usually measured by the number of days up to the onset of fever. However in different authors' works, different points on the temperature scale have been taken above which a sheep or lamb was regarded as febrile. In the present experimental study, in common with Scott (1983), the febrile point was taken to be 40.5°C or greater. Thus the mean incubation period was 5.2 (± 1.1) days and the animals were febrile for a mean of

4.2 (± 0.8) days. This appears to be a somewhat milder response than that reported by Taylor et al (1941) and Scott (1983) although similar to that reported by Foster and Cameron (1970a). The degree and duration of parasitaemia found in the present study also tended to be of a lesser magnitude than reported by other workers (Raghavachari and Reddy, 1959; Gordon et al, 1962) in which the percentage of parasitised neutrophils rose to 90% or more.

The transient rise in neutrophils found to occur on the 6th day after inoculation in the present study was also a feature noted by Taylor et al (1941), although they found it to occur on either the 2nd or the 4th day post-inoculation, and by Foggie (1951) who noted that in his sheep it coincided with the onset of pyrexia. Thus in both these reports and in the present study the transient rise in neutrophils appeared to coincide with the onset of pyrexia and also with the onset of parasitaemia.

The subsequent neutropenia which developed in the lambs in the present work was found to begin to develop from Day 9 or 10 post-inoculation and the mean numbers of circulating neutrophils were significantly depressed from Days 10 to 14. This characteristic period of neutropenia has been noted by others working on TBF infections although the exact time of onset, severity and time taken for recovery to pre-inoculation levels has been found to vary slightly (Taylor et al, 1941; Foggie, 1957; Overas, 1962; Foster and Cameron, 1970a; Munro et al, 1982). The time taken for recovery to pre-inoculation levels in the present study was about 20 days which is similar to that found by other authors (Raghavachari and Reddy, 1959) although Munro et al (1982) reported the neutrophil numbers were still depressed 21 days post-inoculation.

In addition to changes in the numbers of neutrophils, the functional capabilities of neutrophils during the parasitaemic phase of TBF was investigated by Foster and Cameron (1970b) and Woldehiwet (1979); the former suggested that diapedesis was impaired in parasitised neutrophils and the latter that leucocyte migration was reduced during the early phase of the parasitaemia.

The initial rise in the neutrophil count, coinciding with the peak of parasitaemia (around Day 6) is most probably due to a non-specific release of mature neutrophils from an existing pool such as the bone marrow or spleen; the abscence of immature neutrophils suggests that increased granulocytosis is not occurring at this stage. These cells were already infected or became so soon after release and a study of TBF in goats by Van Miert (1984) reports a similar finding. This was supported by the findings of Woldehiwet and Scott (1982a) that a neutrophilia induced by corticosteroid therapy in TBF infected animals was associated with increased granulocytosis and an influx of immature uninfected neutrophils from the bone marrow, thus indicating that the increased granulocytosis induced by steroid therapy was different from that induced by TBF infection.

The fall in neutrophil numbers to very low levels by about Day 10 in the present study could have been due to destruction of the parasitised neutrophils either by the disruption caused by C. phagocytophila or by an immune response in the reticulo-endothelial system or possibly a combination of both. Work on electron microscopy of the TBF organism carried out by Tuomi and von Bonsdorff (1966) and Woldehiwet and Scott (1982c) would suggest that it is unnecessary for the agent of TBF to cause lysis of cells since there is evidence for

the extrusion of vacuoles containing TBF particles from the granulocytes. Tuomi and von Bonsdorff (1966) also noted that, contrary to their expectations, they found that infected granulocytes did not show obvious signs of degeneration. It is therefore possible that the removal of infected neutrophils from the circulation by an immune response in the reticulo-endothelial system may explain the neutropenia that occurs in tick-borne fever reactions.

Most workers believe that the neutropenia which follows TBF infection is responsible for immunosuppressive effects (Foggie, 1956; 1957; Raghavachari and Reddy, 1959); however Foster and Cameron (1968a) noted that clinical signs of tick pyaemia could occur before the onset of the TBF-induced neutropenia.

However, the effects of Cytoecetes phagocytophila on the leucocytes are not confined to the neutrophils since significant changes among the lymphocytes have also been noted. Taylor et al (1941) first remarked on a transient fall in the lymphocyte numbers which began 4 days post-inoculation in TBF-infected lambs. Later work by Batungbacal and others (1982) and Munro et al (1982) confirmed this although the fall in lymphocytes occurred slightly later in the former experiment. The present study also revealed a significant fall in lymphocytes on Days 5 and 6 post-inoculation which was succeeded by a rise to above pre-inoculation levels reaching a peak on Day 11 and returning to pre-inoculation levels by Day 22. The cause of this fall in lymphocyte numbers remains unknown, although Batungbacal and others (1982) closely examined the fall in lymphocyte numbers and found that is was due to a selective and significant fall in the number of B-lymphocytes, while the T-lymphocyte numbers fell by an insignificant amount. Also, in work on TBF in cattle (Hudson,1950), it was noted

that lymphoid tissue in the spleen and lymph nodes was depleted of lymphocytes in TBF-infected animals. However the stage of the TBF reaction at which the necropsies were undertaken was not reported.

The numbers of eosinophils and monocytes were also found to alter following TBF inoculation in the present study. The eosinophils virtually disappeared from the circulation from Day 5 to Day 22 while the monocytes showed a more transient depression which began on Day 6 and reached a nadir by Day 11, following which the numbers returned to pre-inoculation levels. These findings, coupled with the fact that both parasitised eosinophils and monocytes were seen would suggest that the reduction in numbers could be a direct result of parasitism. Other authors have noted both eosinophils and monocytes containing TBF inclusions (Foggie, 1951; Raghavachari and Reddy, 1959; Tuomi, 1967a) although the percentage of such cells infected was generally low; however Scott (1983) states that a prolonged eosinopenia may be a feature of TBF infections.

When the results of TBF inoculation were analysed by the alternative method described on Page 46 there were some differences between the responses of the older and younger lambs, with the older lambs tending to respond slightly more severely.

SECTION II. ATTEMPTS TO INDUCE PASSIVE IMMUNITY TO TBF INFECTION IN LAMBS

Introduction

Immunity to tick-borne fever has frequently been recorded, although it appears to be neither longlasting nor complete (Hudson, 1950; Foggie, 1951; Overas, 1962). The role of passive immunity to TBF acquired via colostrum was considered by McEwen (1947) who suggested that from his observations it did not occur in the field situation. The following experiments were carried out in order to find out whether passive immunity could be induced and if so, whether it might occur under field conditions.

Experiment 3b: An attempt to induce passive immunity in lambs by frequent TBF challenge of their dams during pregnancy

Materials and Methods

Animals

A group of nine crossbred ewes aged 4-5 years were involved in this experiment. They were all bred on lowland areas and were known to have had no contact with ticks. Five of these ewes were inoculated on 3 occasions at monthly intervals with TBF before mating and were subsequently challenged at monthly intervals until they lambed. The remaining four ewes acted as uninfected controls. The final date of TBF challenge was 4/3/82, approximately 3 weeks before the first batch of ewes lambed and 5 weeks before the second group lambed.

On all occasions vials from the same batch of stabilate of TBF were used.

Reproductive procedures

All nine ewes were reproductively synchronised in an attempt to achieve a close lambing pattern. Progesterone-impregnated sponges (Veramix Sheep Sponge, Upjohn Ltd) were inserted intravaginally in all ewes on 13/10/81 and removed 14 days later on 26/10/81 and on that day all ewes were injected with a PMSG preparation (Folligon, Intervet Ltd). Forty-eight hours later a tup wearing a sire-sign harness and marking crayon was introduced to the ewes and within 24 hours all nine had been served. The tup was left to run with the ewes and four of them were later served again between the 13th and 16th of November.

Five of the ewes lambed naturally between 20/3/82 and 24/3/82 and the remaining four which had returned to service were each induced to lamb by administering 4 ml of a short-acting steroid intramuscularly on 6/4/82 (Dexamethasone Aqueous Suspension, Berk Pharmaceuticals Ltd). These ewes subsequently lambed between 7/4/82 and 10/4/82. Five ewes produced live twins, two had a single lamb and the other two each produced one healthy twin and one weakly twin which died soon after birth. The reproductive data for the nine ewes is given below.

Ewe No.	TBF challenged or control	Service date	Lambing date	Lambs born
72	TBF	16/11/81	10/4/82	Single lamb
73	TBF	29/10/81	20/3/82	Twins
74	TBF	16/11/81	8/4/82	Twins, 1 weakly, died
75	TBF	13/11/81	7/4/82	Twins, 1 weakly, died
76	TBF	29/10/81	24/3/82	Twins
77	Control	16/11/81	8/4/82	Single lamb
78	Control	29/10/81	22/3/82	Twins
79	Control	29/10/81	24/3/82	Twins
80	Control	29/10/81	24/3/82	Twins

Lambs

All lambs were infected between 8 and 14 days of age with the same TBF stabilate used on the ewes. Just before infection with TBF, the lambs from the TBF infected dams 72, 74 and 75 were bled and a pooled sample of heparinised blood from these three lambs was inoculated into a susceptible lamb. Three weeks after TBF inoculation the lambs of TBF infected ewes 73 and 76 were bled and a pooled sample of heparinised blood from these four lambs was inoculated into a susceptible lamb.

Results

Maternal infection

All ewes responded to the first infection with TBF and underwent a transient pyrexia and parasitaemia. None of the subsequent monthly challenges caused any clinical or parasitaemic reaction and the ewes appeared to be solidly immune.

Lamb infection

All lambs from the control ewes were susceptible to TBF infection becoming pyrexic and developing a parasitaemia either 5 or 6 days after infection. In contrast, the lambs of the hyperimmunised ewes which lambed naturally (ewes 73 and 76) were immune to TBF challenge, neither developing a fever nor having visible parasites in their neutrophils. However the lambs from the hyperimmunised ewes which were induced to lamb (ewes 72, 74, 75) were not completely immune since one became fevered and parasitaemic 7 days after infection while the other two reacted 9 days post-inoculation. These three lambs had

lower percentages of parasited neutrophils than the lambs from control ewes.

Lamb subinoculation

The pooled blood samples from the lambs of ewes 72, 74 and 75 collected before they were infected with TBF, did not cause a TBF reaction when inoculated into a susceptible lamb.

However the pooled blood sample from the lambs of ewes 73 and 76, collected 21 days after they had been inoculated with TBF and failed to react, resulted in a TBF reaction when inoculated into a susceptible lamb.

Experiment 3c: An attempt to induce passive immunity in lambs by a single TBF challenge of their dams during pregnancy

Introduction

Following the results of the first experiment in trying to induce passive immunity in lambs to TBF by hyperimmunising ewes it was decided to repeat the work using a more realistic TBF challenge regime. To approximate the field situation in which there are two periods of peak tick activity, corresponding to the activation of two separate tick populations (spring and autumn feeders) it was decided to inoculate the ewes with TBF before mating and rechallenge them approximately 3 weeks before they were due to lamb.

Materials and Methods

Animals

Seven crossbred ewes aged 4-5 years were used in this experiment,

four of whom were immunised against TBF, the remaining three acted as controls. For all TBF inoculations vials from the same batch of TBF stabilate were used.

Reproductive procedures

As in the previous experiment the oestrus cycle of the ewes was synchronised using vaginal sponges impregnated with progesterone (Veramix Sheep Sponges, Upjohn Ltd) which were inserted on 2/12/82 and removed on 16/12/82 at which time all the ewes were inoculated with 500 i.u. of PMSG (Folligon, Intervet Ltd). The sheep were then artificially inseminated 55 hours later. Only two animals held to the artificial insemination, the remaining five being served by a tup between 2/1/83 and 4/1/83. One of the TBF immunised ewes was later found to be barren and was removed from the experiment. The remaining six ewes lambed successfully and the reproductive data is presented below along with the dates of infection with TBF. One of the twin lambs born to ewe 96 died at 10 days of age due to a selenium/Vitamin E deficiency (White Muscle Disease) and was not involved in the TBF inoculation experiment.

Ewe No	Date of service	Date of 1st TBF inoculation	Date of booster TBF inoculation	Lambing date	Lambs born
90	2/1/83	17/11/82	6/5/83	---	Barren
91	3/1/83	17/11/82	6/5/83	26/5/83	Twins
92	16/12/82	17/11/82	20/4/83	11/5/83	Single lamb
93	16/12/82	17/11/82	20/4/83	11/5/83	Single lamb
96	4/1/83	Control	Control	26/5/83	Twins, 1 died
97	2/1/83	Control	Control	28/5/83	Single lamb
98	2/1/83	Control	Control	26/5/83	Twins

Lambs

All lambs were infected with the same stabilate used on the immunised ewes. Inoculation took place when the lambs were 10-12 days old.

Results

Maternal infection

All four ewes responded to the first TBF inoculation and experienced a TBF reaction, with a pyrexia and parasitaemia (ranging from 20-60% parasitised neutrophils) 5 days after inoculation. When these ewes, now three in number, were given the booster dose of TBF stabilate 22 or 24 weeks later they reacted with a mild bout of tick-borne fever characterised by incubation periods of between 7 and 9 days, low levels of parasitaemia (2-18% of neutrophils parasitised) for 1-3 days and a pyrexia lasting 1-2 days only.

Lamb infection

All lambs from the control ewes were susceptible to TBF inoculation and became pyrexic and parasitaemic from 5 days post-infection. All four lambs from the immunised ewes (ewes 91, 92, 93) were also susceptible to TBF and became pyrexic and parasitaemic between 6 and 8 days after inoculation, although the parasitaemic and pyrexic reactions appeared to be marginally more severe in the lambs from control ewes (Table 3g).

Discussion

The results of the experiment in which ewes were hyperimmunised against Cytoecetes phagocytophila were interesting in that clear indications of passive immunity were found in all 4 of the offspring of the hyperimmunised ewes which lambed naturally. In the 3 offspring of the hyperimmunised ewes which were induced to lamb by cortisone therapy TBF reactions did occur but were of a less severe nature than those of lambs from control ewes.

One possible explanation for this differenc is that as these ewes lambed 1 month later than the naturally lambing ewes, and were all given their final challenge inoculation of TBF on 6/3/82, there was almost a 2-month gap between the final infection of TBF and the day of lambing, perhaps allowing for a waning of immunity. However against this theory is the fact that a primary infection of TBF has been shown to produce immunity lasting for 5 months or more (Stamp and Watt, 1950; Foggie, 1951; Scott, 1975; 1977). A second explanation could be that, by using a relatively large dose of a corticosteroid drug to induce parturition, ewes so treated may suffer a reduction in both humoral and cell-mediated immune responses (Webb and Winkelstein, 1980) which could adversely affect the transfer of passive immunity to their offspring. Thirdly both the amount of colostral immunoglobulins available, and the efficiency of their transfer to lambs, can vary for several reasons (Halliday, 1970).

There was apparently no transfer of infection from the ewe to the lambs via colostrum within the first 7-13 days life as evidenced by the failure of pooled blood from the lambs to induce a TBF reaction when inoculated into a susceptible lamb. However following

experimental infection of the lambs of the repeatedly challenged ewes which lambed naturally, there was evidence that they became symptomless carriers of Cytoecetes phagocytophila. A simliar case was reported by Foster (1968) in which a lamb on tick-infected pasture showed neither pyrexia nor parasitised neutrophils; however its blood contained Cytoecetes phagocytophila since its inoculation into a susceptible lamb produced a TBF reaction.

It is possible that the explanation of this "subclinical" infection with TBF is that the presence of maternally derived antibody suppressed the normal replication of C. phagocytophila but failed to destroy it. Presumably it subsequently persisted in whatever form it assumes in sheep which have recovered from TBF and remain as carriers; in these, parasites are not apparently visible on blood smears and yet the blood remains infective for susceptible animals.

The carrier state in TBF has often been reported to occur following infection although its duration has been found to vary (Hudson, 1950; Foggie, 1951). As yet the location of the organism during this period of premunity is unknown. Woldehiwet and Scott (1982b) believed that carrier animals had high levels of complement fixing antibodies, especially IgM and suggested that antigenic challenge must be continuous. The most likely explanation for the whereabouts of the carrier organism is that they are present in long-lived phagocytic cells such as tissue macrophages and may periodically be released into the circulation in small numbers. Support for this comes from work carried out by Munro et al (1982) in which they report the presence of TBF organisms inside macrophages in the lungs; also in recent work, Scott and Horsburgh (1983) record that the use of very large inocula of whole blood does not increase the likelihood of

carrier detection, suggesting that the carrier state does not persist solely by the survival of a small number of parasitised circulating leucocytes. The latters' findings agree with the generally held opinion that animals may be carriers and yet have no visible infection in the neutrophils.

The second experiment was concerned with an attempt to induce passive immunity in a manner more closely related to what might happen in the field. This showed that immunity to reinfection in the ewes had waned by 5 months after the first inoculation in that the challenge TBF infections largely resulted in reactions, although these were less severe than primary ones. The lambs also were not immune to TBF infection, although again there was some evidence that the onset of the TBF reactions were slightly delayed.

Thus, with the strain of TBF used, it would appear that colostrally acquired immunity to TBF may occur in lambs, but only under conditions of severe and continuous challenge of the ewes. Where such a challenge does not exist immunity can wane over a 5-month period so that ewes are generally susceptible to reinfection and little, if any, maternal immunity is transferred to the lamb via the colostrum. These findings would bear out the field observations of MacEwen (1947) and Foster and Cameron (1968a) among others, who found that even in situations where TBF was endemic and ewes may have been expected to be immune to TBF challenge, the high rate of clinical TBF infection among very young lambs would suggest that there was little or no transmission of passive immunity.

SECTION III: STUDIES ON THE USE OF A LONG-ACTING TETRACYCLINE DRUG AS A PROPHYLACTIC MEASURE AGAINST C. PHAGOCYTOPHILA INFECTION

Introduction

The use of various chemotherapeutic agents in the treatment of tick-borne fever has been reported in the past and short-acting oxytetracycline and sulphamethazine were proved to be the best among the few drugs effective (Foggie, 1951; Tuomi, 1967e; Evans, 1972; Synge,1976), although relapses were likely to occur after treatment (Evans, 1972; Synge, 1976). Tuomi (1976e) suggested that TBF could be prevented by treating animals with a short-acting oxytetracycline 3 hours before infection and continuing the therapy for the following two days. The following experiments were designed to test the prophylactic and therapeutic effects on TBF of a long-acting oxytetracycline (Terramycin L/A, Pfizer Ltd, Sandwich, Kent).

Experiment 3d: The effects of LAT at the therapeutic dose (20 mg/kg) on TBF infection

Materials and Methods

In this preliminary experiment, five 10-month old cross-bred sheep were treated on one occasion with long-acting oxytetracycline at various times in relation to tick-borne fever inoculation and one animal acted as an untreated control. The dose rate of the drug given was the recommended therapeutic dose of 20 mg/kg; each animal weighed approximately 40 kg and was given a dose of 4 ml intramuscularly.

The drug was administered either 15, 10 or 5 days prior to TBF inoculation, or at the same time as TBF or 5 days after TBF when the reaction was already in progress. All the animals were challenged with the same stabilate of TBF 28 days after the initial infection.

Results

The results are presented below.

Lamb No	Day of Treatment	Response to 1st TBF infection (Day 0)	Response to TBF challenge infection on Day 28
46	-15	Normal TBF reaction	Immune to challenge
48	-10	Normal TBF reaction	Immune to challenge
53	-5	No reaction	Normal TBF reaction
54	0	No reaction	Normal TBF reaction
55	+5	Suppressed reaction	Normal TBF reaction
56	Control	Normal TBF reaction	Immune to challenge

The drug was effective in preventing the development of a TBF reaction when given at the same time as TBF or up to 5 days prior to infection, its prophylactic effect ceasing between 5 and 10 days prior to infection. When given during a TBF reaction it rapidly suppressed both the pyrexia and the parasitaemia (within 24 hours the parasitaemia fell from 26% to 4%, the rectal temperature fell from 41.6^{o}C to 40.1^{o}C and within 48 hours no parasites were visible in peripheral blood smears) and no relapse occurred during the following 23 days. The control animal underwent a normal TBF reaction which started on Day 5. When the animals were challenged with TBF 28 days after the initial inoculation only those which had previously experienced a full TBF reaction were immune. Therefore the total prevention of a TBF reaction by long-acting oxytetracycline or the suppression of a TBF reaction resulted in a continued susceptibility

to challenge. The control animal was immune to challenge.

Experiment 3e: The effect of a larger dose (40 mg/kg) of LAT on TBF infections

Introduction

In this experiment, which used lambs of less than 10 kg body weight, the dose of LAT was increased to approximately double the therapeutic dose rate.

Materials and Methods

Nine cross-bred lambs aged three weeks were divided into three groups of 3 lambs. Two ml of long-acting oxytetracycline was administered intramuscularly to each of the first group on Day -15, to each of the second group on Day -10 and to each of the third group on Day -5. On Day 0 all nine lambs were intravenously inoculated with a TBF stabilate along with three control lambs. The lambs were challenged with the same TBF stabilate 28 days after the primary TBF inoculation. The dose of long-acting oxytetracycline used was at least double the therapeutic dose since none of the lambs weighed more than 10 kg.

Results

The three control lambs all responded normally to the TBF inoculation and underwent normal febrile reactions and parasitaemias. In contrast, none of the lambs given the prophylactic treatment showed any signs of infection. However when challenged 28 days later the control lambs were immune but all nine of the treated lambs were

susceptible and developed normal TBF reactions.

These results would suggest that the higher dose rate resulted in prophylaxis against TBF for between 15 and 33 days.

Experiment 3f: An attempt to quantify the period of time over which LAT has a prophylactic effect on TBF infection

Introduction

Following the results of the previous two experiments the following experiment was designed to ascertain more exactly the duration of the prophylactic period against TBF when double the therapeutic dose of long-acting oxytetracycline was administered.

Materials and Methods

Fifteen blackface lambs aged 4 months were divided into 5 groups of three lambs. Each lamb was weighed and given a prophylactic treatment of long-acting oxytetracycline at double the therapeutic dose (i.e 40 mg/kg) at various times in relation to inoculation with a tick-borne fever stabilate. The first group of lambs were each treated 38 days prior to TBF inoculation (i.e. Day -38), the second group on Day -33, the third group on Day -28, the fourth group on Day -23 and the fifth group on Day -18. All lambs were inoculated on Day 0 with the TBF stabilate. One lamb (No.5) died of urolithiasis on Day -19 and thus there were only two lambs remaining in that group.

Results

The results are presented in Table 3h. With two exceptions the lambs all responded to infection with both pyrexia and parasitaemia.

However, those animals which were infected with TBF between 18 and 23 days after prophylactic treatment showed a delay in the onset of the reaction i.e their incubation periods were extended. The mean incubation period for those animals treated 28 days or more before infection was 5.86 ($\pm$ 0.69) days while animals treated 18 or 23 days before infection had a mean incubation period of 9.2 ($\pm$ 1.48) days and the incubation period of the latter group was significantly longer than that of the former ($P < 0.001$).

Discussion

The experimental work in this section shows that at approximately double the recommended therapeutic dose, LAT will prevent the development of TBF in lambs for at least 15 days in the face of experimental intravenous inoculation of infected blood. There was little evidence that the prophylactic effects of LAT extended much past 15 days although the result of Experiment 3f suggested that when given 18-23 days prior to TBF challenge, it ameliorated the TBF reaction, resulting in significantly longer incubation periods. These results suggest that the dose of LAT administered to lambs in the field work (Chapter 8) is likely to have prevented the development of TBF for at least 15 days post administration.

The findings of the experiment concerned with the administration of LAT at the manufacturers recommended therapeutic dosage on Day 5 of a TBF reaction was similar to that reported by Scott and Horsburgh in 1982, in that fevers and parasitaemia were rapidly suppressed with no relapse, although the animals were often not immune to subsequent challenge. These findings are in contrast with those obtained

following the use of a short-acting oxytetracycline (SAT) drug reported by Synge and Scott (1976). This also suppressed TBF reactions when given at the clinical stage, although relapses occurred some days later; while less severe than a primary reaction, these seemed to be a prerequisite for the acquisition of immunity.

All of these results indicate that when TBF is inoculated into a susceptible sheep but fails to produce a TBF reaction due to either the prophylactic or curative effects of a tetracycline drug, immunity to TBF is impaired or absent.

The existence of TBF organisms in the extracellular portions of the blood and their ability to infect new cells have been demonstrated in both in vivo and in vitro experiments. Thus Foggie (1951), Thrushfield et al (1978) and Van Miert et al (1984) showed that serum or plasma from infected sheep was infective for susceptible animals; and Woldehiwet and Scott (1982e) showed that the proportion of infected leucocytes increased following blood culture.

Therefore while it is perhaps unlikely that tetracycline is effective against intracellular parasites, the relatively short 6-7 hour half-life of the circulating neutrophil (Allison, 1982), combined with the persistence of blood levels of LAT in sheep for up to 5 days (Pfizer Technical Information Update, Ref. No. 8201) would allow for the destruction of all the parasites during their extracellular phase prior to invasion of new leucocytes. Presumably the susceptibility of LAT treated sheep to reinfection is due to insufficient antigen being available to stimulate full immunity.

In contrast, the short-acting tetracycline drug while temporarily suppressing the extracellular parasitaemia, allows the survival of a

number of intracellular parasites which permits the reaction to recrudesce 6-12 days later, following which the animal becomes immune.

SECTION IV: ASSESSMENT OF SPLENIC ENLARGEMENT AS A SEQUEL TO TBF INFECTION

Introduction

In uncomplicated cases of tick-borne fever the only abnormality reported at post-mortem examination has been an enlarged spleen. Gordon and others (1932b) were the first workers to remark on this and although they noted a macroscopic enlargement of the spleen, the few cases on which they carried out histological examinations failed to show definite pathological changes. Similarly both Jamieson (1947) and Gronstol and Ulvund (1977) reported splenic enlargement in sheep which had died following a recent attack of TBF. Hudson (1950) noted a similar change in the spleen of cattle killed during a TBF reaction; however histopathology revealed only a slight swelling of the Malpighian Corpuscles although the lymphoid tissue was said to appear as if 'drained of lymphocytes'. Following the slaughter of the lambs used in one of the experiments (Exp. 6b) carried out at the Moredun Institute on the role of TBF in pneumonic pasteurellosis, the spleens were weighed and compared with the animals' body weights.

Experiment 3q: Assessment of splenomegaly as a sequel to TBF infection

Materials and Methods

Fourteen hysterectomy-derived cross-bred lambs were used and they were 4 weeks of age at the start of the experiment. They were divided randomly into two groups of seven and housed in separate rooms of an

SPF building.

Seven lambs were inoculated with a TBF stabilate on Day 0 and seven days later (Day 7) all fourteen lambs were infected with an aerosol of P. haemolytica serotype A1 as described in Chapter 6. All the lambs were killed on Day 14 and necropsied.

Results

The bodies of the lambs were weighed at necropsy and then the spleen of each animal was removed and weighed separately. The figures are presented in Table 3i.

Both the mean spleen weight and the mean spleen weight as a percentage of total body weight were significantly greater in the TBF-infected group compared with the animals which had not been given TBF ($p < 0.05$), although there was no significant difference between the mean body weights of each group.

The mean spleen weight of the TBF infected group was 1.5 times greater than that of the animals which had not experienced TBF.

Discussion

These results clearly agree with the observations of earlier workers and give them a quantitative basis. Gronstol and Ulvund (1977) observed that the spleens of TBF affected sheep were approximately 1.5 times normal size and the findings here support this.

Summary

The sequence of clinical and haematological effects of a TBF infection was found to occur as follows. Following an average incubation period of about 5 days there was a pyrexia lasting for 4 days, during which the animals were dull and anorexic, most noticeably very young lambs. From Days 5-7 post-inoculation there was a slight neutrophilia and the numbers of parasitised neutrophils were at a peak, lymphocyte numbers were significantly depressed and there was also a fall in eosinophil numbers. By Days 10-12 post-inoculation a severe neutropenia had developed, lymphocyte numbers had returned to pre-inoculation levels, numbers of circulating monocytes had fallen and there were virtually no circulating eosinophils. Neutrophil numbers retured to pre-inoculation levels by Day 24 post-inoculation.

Following attempts to induce passive immunity to TBF in lambs via colostrum, it was found that a degree of passive immunity could be induced, although this was dependent on frequent challenge of the dam prior to and during pregnancy. When pregnant ewes, first infected with TBF one month before mating, were challenged once during pregnancy there was little evidence of any transfer of passive immunity to their offspring and indeed the ewes' own immunity to TBF appeared to have waned during the 5 months between infection and challenge.

Studies on the effects of a long-acting tetracycline (LAT) on C. phagocytophila infection revealed that it could rapidly suppress active TBF reactions without relapses occurring, following which however, animals remained susceptible to reinfection. At the normal therapeutic dose (20 mg/kg) it also had a prophylactic effect,

preventing the development of TBF reactions for between 5 and 10 days. At double the therapeutic dose rate the period of effective prophylaxis was extended to between 15 and 18 days.

It was found that lambs slaughtered 14 days after TBF inoculation had significantly enlarged spleens, which were on average one and a half times the weight of those of lambs not given TBF.

TABLE 3a: Daily rectal temperatures of 5 lambs during a TBF reaction

Day	Rectal temperature of each lamb (°C)					Mean (Std Dev)
	42	49	73	75	76	
-1	39.4	39.9	38.8	39.4	39.0	39.1 (0.42)
0	39.4	40.0	39.0	39.4	39.0	39.4 (0.41)
1	39.8	39.5	38.9	38.5	38.0	38.9 (0.73)
2	39.9	39.5	39.1	39.0	38.9	39.2 (0.28)
3	40.1	39.4	39.0	39.6	38.5	39.3 (0.61)
4	41.0	41.1	39.6	39.5	39.0	40.0 (0.95)
5	41.7	42.0	40.3	40.0	38.7	40.5 (1.34)
6	41.8	41.7	41.7	41.4	41.3	41.6 (0.21)***
7	40.2	41.1	41.3	41.1	40.8	40.9 (0.43)***
8	40.5	40.8	41.0	40.8	40.6	40.7 (0.19)***
9	40.0	41.4	40.3	40.7	40.3	40.5 (0.54)**
10	40.0	40.3	40.6	39.9	38.5	39.9 (0.81)
11	40.0	40.0	40.5	39.4	38.8	39.7 (0.65)
12	39.6	39.4	39.9	39.0	38.7	39.3 (0.48)
13	39.0	39.5	39.0	39.2	38.3	39.0 (0.44)
14	39.9	39.0	39.6	39.7	38.6	39.4 (0 54)
15	39.7	39.3	39.3	39.4	38.9	39.3 (0.29)
16	39.8	39.2	39.2	38.8	38.8	39.2 (0.41)
17	40.2	39.1	39.0	39.6	38.8	39.3 (0.56)
18	40.2	39.0	39.3	39.0	38.0	39.1 (0.79)
19	40.0	39.2	39.5	38.9	38.2	39.2 (0.67)
22	39.7	39.0	39.1	39.3	38.9	39.2 (0.32)

Significant differences from mean pre-inoculation temperature (Day -1)

** $P < 0.01$

*** $P < 0.001$

TABLE 3b: Numbers of parasitised neutrophils in 5 lambs during a TBF reaction.

Day	No. Infected neutrophils ($x10^9/l$) (% infected) in each lamb					Mean (Std Dev)
	42	49	73	75	76	
2	--- *	---	---	---	---	
3	---	---	---	---	---	
4	0.12 (4%)	0.39 (8%)	---	---	---	0.10 (0.17)
5	1.29 (39%)	1.95 (50%)	---	0.15 (5%)	0.19 (9%)	0.72 (0.86)
6	1.61 (46%)	1.12 (34%)	1.64 (40%)	1.00 (27%)	0.93 (37%)	1.11 (0.62)
7	0.29 (12%)	0.68 (17%)	1.57 (56%)	0.52 (26%)	1.56 (38%)	0.92 (0.60)
8	0.49 (26%)	0.30 (9%)	0.84 (35%)	0.83 (46%)	0.74 (41%)	0.64 (0.24)
9	0.20 (18%)	0.59 (31%)	0.52 (9%)	0.58 (32%)	0.12 (20%)	0.40 (0.22)
10	0.02 (4%)	0.25 (21%)	0.07 (14%)	0.10 (16%)	0.008 (4%)	0.09 (0.09)
11	---	0.08 (10%)	0.02 (2%)	0.008 (2%)	---	0.02 (0.03)
12	---	---	---	---	---	

* no parasitised neutrophils found

TABLE 3c: Neutrophil counts in 5 lambs during a TBF reaction

Day	Neutrophil counts of lambs ($x10^9$/litre)					Mean (Std Dev)
	42	49	73	75	76	
-1	3.8	2.0	1.6	1.2	1.5	2.02 (1.04)
1	3.9	1.5	1.0	2.6	1.8	2.16 (1.13)
4	3.0	4.9	1.6	1.7	1.9	2.62 (1.39)
5	3.3	3.9	2.9	2.9	2.1	3.02 (0.66)
6	3.5	3.3	4.1	4.0	2.5	3.48 (0.64)*
7	2.4	4.0	2.8	2.0	1.9	2.62 (0.85)
8	1.9	3.3	2.4	1.8	1.8	2.24 (0.64)
9	1.1	1.9	1.8	1.8	0.6	1.44 (0.57)
10	0.5	1.2	0.5	0.6	0.2	0.60 (0.37)*
11	0.6	0.8	0.8	0.4	0.2	0.56 (0.26)*
12	0.3	0.6	0.8	0.3	0.3	0.46 (0.23)*
14	1.6	0.2	0.7	0.7	0.6	0.76 (0.51)*
18	1.0	1.3	2.4	1.7	1.0	1.48 (0.59)
22	2.5	2.1	1.8	2.5	2.1	2.20 (0.30)
27	3.0	2.1	1.9	2.4	1.1	2.10 (0.70)

Signficant differences from pre-inoculation count (Day -1)

* $P < 0.05$

TABLE 3d: Lymphocyte counts in 5 lambs during a TBF reaction

Day	Lymphocyte counts of lambs ($x10^9/l$)					Mean (Std Dev)
	42	49	73	75	76	
-1	5.3	4.1	2.7	3.5	3.6	3.84 (0.96)
1	5.5	4.5	3.1	3.9	3.4	4.08 (0.95)
4	2.7	4.9	3.4	3.4	3.2	3.52 (0.82)
5	3.2	1.9	2.0	2.0	1.1	2.04 (0.75)*
6	2.8	1.5	2.6	1.7	1.5	2.02 (0.63)**
7	3.9	1.4	4.5	2.0	1.9	2.74 (1.37)
8	4.0	1.7	3.8	3.5	2.4	3.08 (0.99)
9	5.9	3.0	2.9	3.5	2.9	3.64 (1.29)
10	7.0	3.4	3.9	7.4	5.3	5.40 (1.79)
11	7.4	4.0	5.8	6.6	4.0	5.56 (1.53)
12	7.2	6.2	6.2	4.8	3.0	5.48 (1.63)
14	7.8	6.2	4.0	4.3	3.2	5.10 (1.87)
18	7.8	5.0	3.6	5.0	3.2	4.92 (1.80)
22	5.4	4.5	3.0	3.9	3.1	3.98 (1.00)
27	5.2	4.2	3.1	5.9	2.9	4.26 (1.30)

Significant differences from pre-inoculation count (Day -1)

* $P < 0.05$

** $P < 0.01$

TABLE 3e: Monocyte counts in 5 lambs during a TBF reaction

Day	Monocyte counts of lambs ($x10^9/l$)					Mean (Std Dev)
	42	49	73	75	76	
-1	0.60	0.35	0.18	0.25	0	0.28 (0.22)
1	0.31	0.79	0.04	0.21	0.33	0.34 (0.28)
4	0.50	0.62	0	0	0.16	0.26 (0.29)
5	0.57	0.44	0.15	0.55	0.21	0.38 (0.19)
6	0.51	0.30	0.36	0.37	0.26	0.36 (0.10)
7	0.48	0.29	0	0.17	0.29	0.25 (0.18)
8	0.66	0.21	0.19	0.22	0	0.26 (0.24)
9	0.37	0.54	0.05	0	0.22	0.24 (0.22)
10	0.31	0.47	0.05	0.08	0.11	0.20 (0.18)
11	0.16	0.36	0.14	0	0	0.13 (0.15)
12	0.66	0.28	0.07	0.05	0	0.21 (0.27)
14	1.04	0.27	0	0.16	0.04	0.30 (0.43)
18	0.47	0.40	0.12	0.14	0.22	0.27 (0.16)
22	1.02	0.43	0.20	0.07	0.11	0.37 (0.39)
27	1.21	0.20	0.10	0.09	0.12	0.34 (0.49)

TABLE 3f: Eosinophil counts in 5 lambs during a TBF reaction.

Day	Eosinophil counts of lambs ($x10^9/l$)					Mean (Std Dev)
	42	49	73	75	76	
-1	0	0	0	1.24	0.57	0.36 (0.55)
1	0.16	0	0.04	0.28	0	0.10 (0.12)
4	0.06	0	0.05	0.83	0	0.19 (0.36)
5	0	0	0	0.06	0	0.01 (0.03)
6	0	0	0	0	0.04	0.01 (0.02)
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0.05	0	0	0	0.01 (0.02)
11	0	0	0	0	0	0
12	0	0	0	0.05	0	0.01 (0.02)
14	0	0	0	0.05	0	0.01 (0.02)
18	0	0	0	0.07	0	0.01 (0.03)
22	0	0.07	0	0.41	0.05	0.11 (0.17)
27	0	0.07	0.05	0.53	0	0.13 (0.23)

TABLE 3g: Results of Experiment 3c: An attempt to induce passive immunity in lambs by a single TBF challenge of their dams during pregnancy.

	Day 4	5	6	7	8	9	10	11	12
Percentage parasitised neutrophils									
Lambs from imm. ewes									
71L	0	0	0	0	42	34	22	ND*	ND
72L	0	0	0	10	34	31	26	ND	ND
92L	0	0	12	37	32	21	0	0	0
93L	0	0	0	0	3	41	45	33	0
Mean (S.D.)	0	0	3 (6)	11.8 (17.5)	27.8 (17.1)	31.8 (8.3)	23.3 (18.5)	-	-
Lambs from con. ewes									
73L	0	0	40	56	35	29	14	2	0
75L	0	5	27	26	46	32	16	2	0
76L	0	9	37	38	41	20	4	0	0
77L	0	32	24	17	28	11	0	0	0
Mean (S.D.)	0	11.5 (14.2)	32.0 (7.7)	34.3 (16.9)	37.5 (7.8)	23.0 (9.5)	8.5 (7.7)	1 (1.2)	0
Rectal temperature (^{o}C)									
Lambs from imm. ewes									
71L	ND	ND	40.4	40.4	41.0	41.4	40.3	ND	ND
72L	ND	ND	40.7	40.8	41.0	40.0	40.3	ND	ND
92L	39.7	40.0	40.6	41.9	41.4	41.0	40.5	39.0	ND
93L	39.4	39.5	39.7	40.0	40.4	41.7	41.8	41.8	ND
Mean (S.D.)	-	-	40.4 (0.5)	40.4 (0.3)	41.0 (0.4)	41.0 (0.7)	40.7 (0.7)	-	-
Lambs from con. ewes									
73L	39.6	40.3	41.7	41.3	41.0	40.3	40.6	40.5	39.9
75L	39.5	40.0	41.4	41.1	40.8	40.7	39.9	39.4	39.0
76L	39.0	38.7	41.3	40.8	40.6	40.3	38.5	38.8	38.7
77L	41.2	41.2	42.3	41.3	41.0	39.9	39.3	39.5	39.2
Mean (S.D.)	39.8 (1.0)	40.1 (1.0)	41.7 (0.5)	41.1 (0.2)	40.9 (0.2)	40.3 (0.3)	39.6 (0.9)	39.6 (0.7)	39.2 (0.5)

* Not done.

TABLE 3h: Results of Experiment 3f: An attempt to quantify the period of time over which LAT has a prophylactic effect on TBF infection.

Lamb	Day of treatment	Day TBF inoculated	1st day on which each lamb was both pyrexic (>40.3°C) and parasitised	% of neutrophils infected on the on the first day of parasitaemia
1	-38	0	6	55%
2	-38	0	6	24%
3	-38	0	7	57%
4	-33	0	6	70%
5 *	-	-	-	-
6	-33	0	5	65%
7	-28	0	6	54%
8	-28	0	5	21%
9	-28	0	Never	Nil
10	-23	0	11	14%
11	-23	0	9	47%
12	-23	0	9	50%
13	-18	0	10	38%
14	-18	0	Never	Nil
15	-18	0	7	15%

* Lamb No. 5 died of urolithiasis on Day -19

TABLE 3i: Results of Experiment 3g: Assessment of splenomegaly as a sequel to TBF infection

Lamb No	Body Weight (kg)	Spleen Weight (g)	Spleen weight as a percentage of body weight
TBF and *P. haemolytica*			
701	9.6	43.15	0.45%
703	10.2	72.8	0.71%
706	7.0	41.9	0.60%
707	11.6	56.6	0.49%
710	10.6	49.8	0.47%
712	8.6	53.6	0.62%
716	11.0	50.4	0.46%
Mean values	9.8 kg	52.61 g	0.543%
P. haemolytica alone			
704	10.4	28.3	0.27%
708	8.4	63.7	0.75%
709	10.2	29.8	0.29%
711	11.0	30.7	0.28%
713	10.4	27.4	0.26%
714	11.4	28.7	0.25%
717	11.2	33.8	0.30%
Mean values	10.4 kg	34.63 g	0.343%

CHAPTER 4

STUDIES ON THE EFFECTS OF TICK-BORNE FEVER ON THE IMMUNE SYSTEM

SECTION I. HUMORAL IMMUNITY

Experiment 4a.

An assessment of the effects of TBF infection on a primary humoral immune response to unrelated antigens in 7 month old lambs

Introduction

This experiment was designed to test whether the primary humoral response of lambs to two antigens was altered if the antigens were presented while the lambs were undergoing a TBF reaction, either coincidentally with TBF, 5 days after TBF inoculation (i.e. during the parasitaemic phase of the reaction) or 9 days after TBF inoculation (i.e. during the neutropenic phase of the reaction).

Materials and Methods

Animals

Twelve 7-month old crossbred lambs were divided into four groups of three. Three of these groups of lambs were each inoculated with 1.5 ml of a TBF stabilate on Day 0. The remaining three lambs acted as controls and did not receive TBF. The first group of three TBF infected lambs along with one of the control lambs were each inoculated subcutaneously in the left axilla with 1 ml of louping-ill vaccine (LIV) and in the right axilla with 2 ml of horse red blood cells (HRBC) on Day 0. The second group of three TBF infected lambs, along with a second control lamb, were inoculated with the two antigens on Day 5, and a third group of three TBF infected lambs, along with the final control lamb inoculated on Day 9.

Antigens

Louping-ill vaccine: this was obtained from Dr Hugh Reid of the Moredun Institute, Edinburgh. The vaccine was inactivated antigen from tissue cultures infected with louping-ill virus, in oily adjuvant.

Horse red cells: The red cell suspension inoculated into the sheep was prepared as follows. A total of 60 ml of whole blood was collected from an adult horse, defibrinated and centrifuged. After centrifugation 30 ml of red cells were obtained and washed 3 times in phosphate buffered saline; each sheep was inoculated with 2 ml of this suspension of packed red cells.

Serological Procedures - serum collection and storage

a. LIV Test method - The method described by Reid and Doherty (1971a) was used to assess the level of haemagglutination inhibiting antibodies.

b. HRBC Test method - the method according to Herbert (1978) was used to assess the level of haemagglutinating antibodies by the direct method. The sera were heat-inactivated by being placed in a water-bath at 37°C for 30 minutes prior to the test.

Serum samples were collected at intervals of several days from all lambs simultaneously. Because of the staggered nature of the antigen inoculation, the serum samples from each group were on differing numbers of days post-inoculation. Geometric mean titres were calculated according to the method of Paul and White (1973) in which the initial serum dilution for each test (1/10 for louping-ill vaccine and 1/6 for horse red blood cells) was expressed as a reciprocal and

accorded a $\log_2$ value of 1 and the test results were given as the corresponding $\log_2$ titres for the end points of each serum. Titres of less than the initial serum dilution were given a $\log_2$ of 0.

Results

The antibody titres found in each animal to the two antigens are detailed in Tables A1 and A2 in the appendix. The geometric mean titres of each group of three lambs are presented in Tables 4a and 4b and illustrated in Figures 4 (i) and 4 (ii).

Dealing first with the LIV antigen in which pre-inoculation titres were absent, when the mean titres of each group were compared with those of the controls, there was a significant difference on only once occasion in one group, i.e. on Day 17, when the lambs inoculated with louping-ill vaccine on Day 5 of a TBF reaction had a significantly higher titre than the control lambs.

A comparison of the results to the HRBC antigen indicated that in 3 of the 4 groups the mean titres after inoculation were significantly higher than the pre-inoculation titres. In the fourth group, in which the antigen was given on the same day as TBF, the titres, although raised, just failed to achieve significance. However this was probably due to the very small numbers of animals involved, since a statistical comparison of the titres of the various groups over each sampling period (as carried out for the LIV antigen results above) showed no significant differences.

In conclusion there was little difference in the primary antibody response between the groups of lambs infected with TBF and the control animals, when challenged with either of the antigens.

FIGURE 4(i). MEAN RECIPROCAL HAEMAGGLUTINATION INHIBITING (HAI) ANTIBODY TITRES AGAINST HORSE RED BLOOD CELLS (HRBC) IN LAMBS, SOME OF WHICH WERE INFECTED WITH TICK-BORNE FEVER.

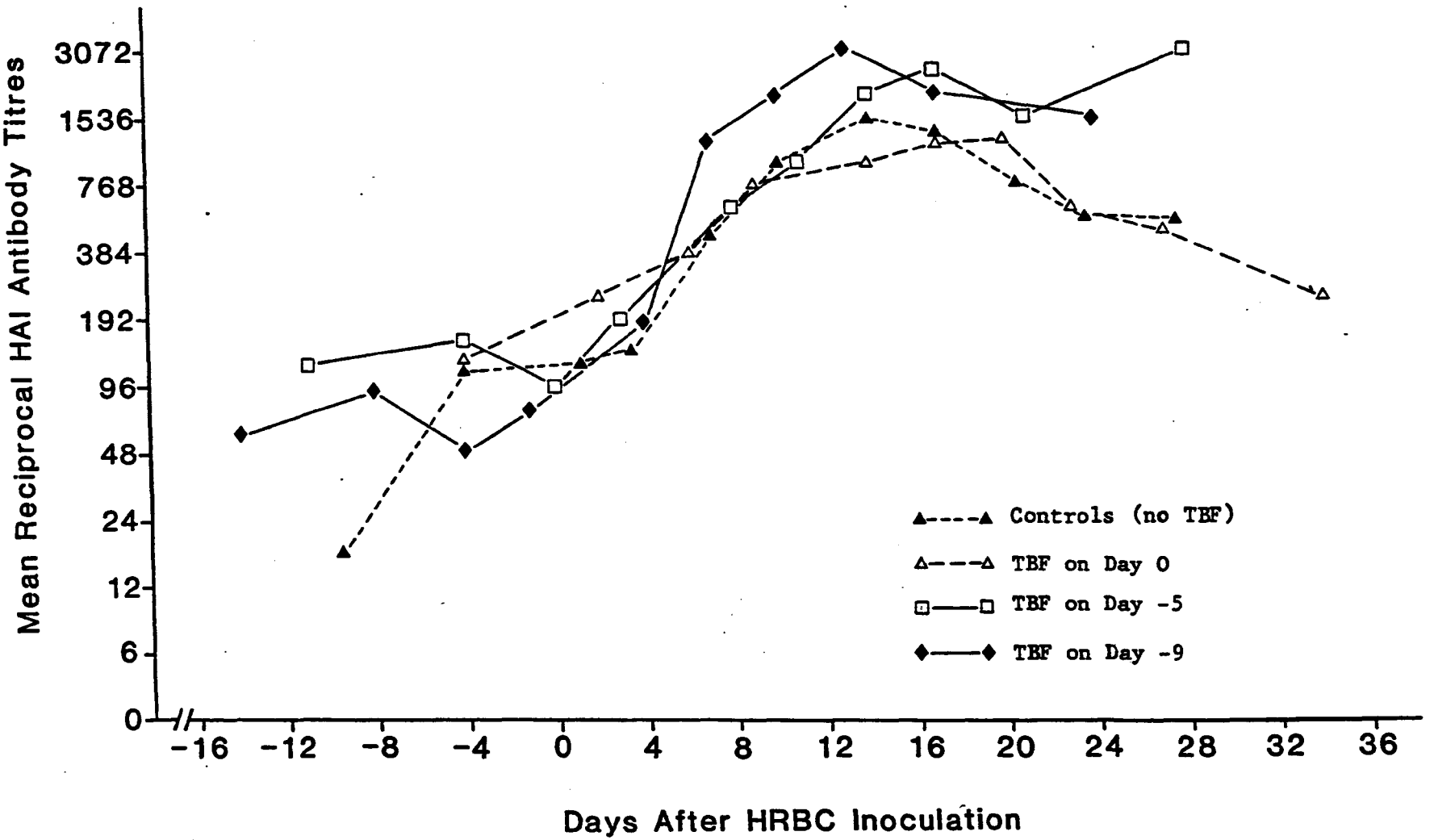
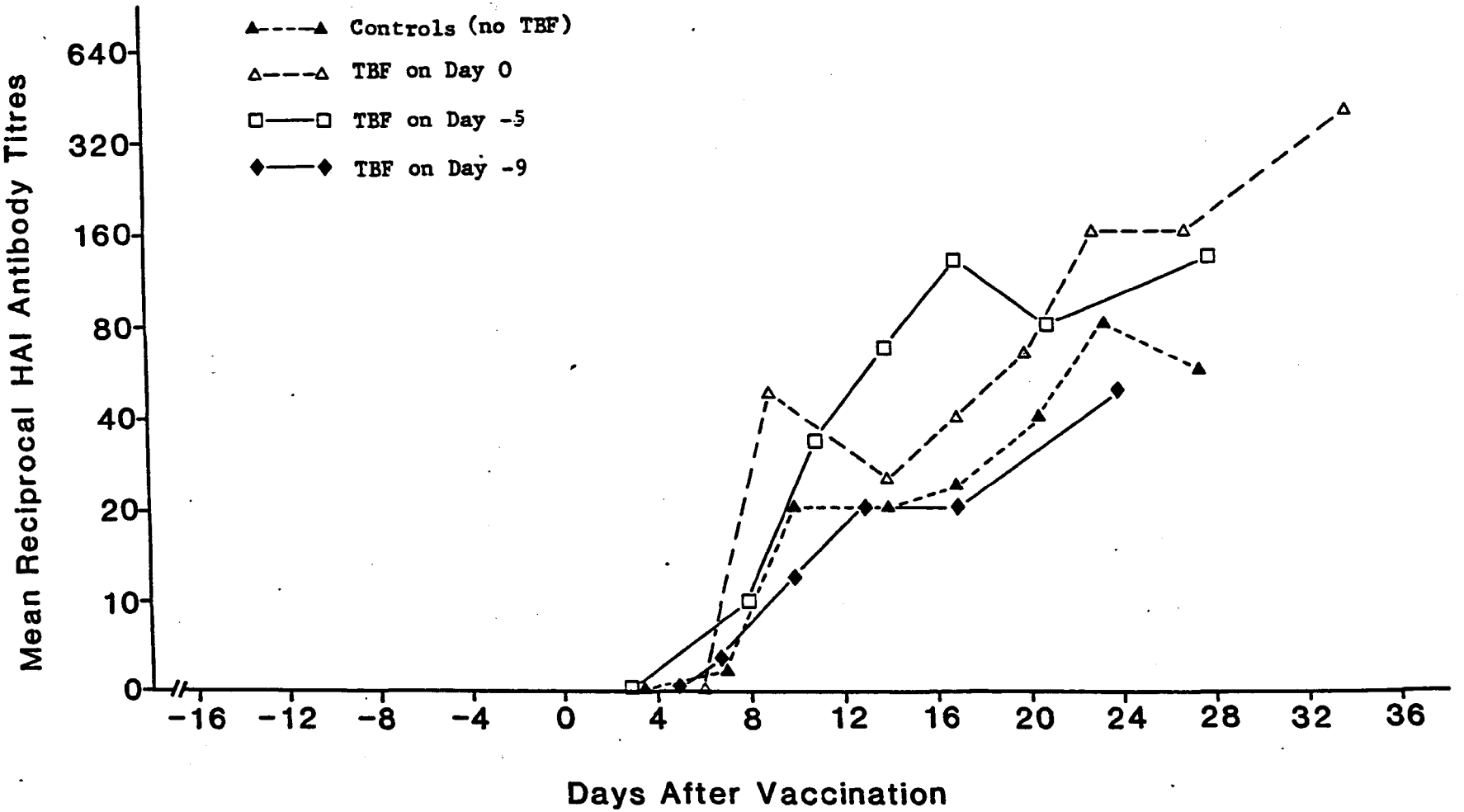


FIGURE 4(ii). MEAN RECIPROCAL HAEMAGGLUTINATION INHIBITING (HAI) ANTIBODIES TO LOUPING-ILL VIRUS (LIV) IN LAMBS, SOME OF WHICH WERE INFECTED WITH TICK-BORNE FEVER (TBF).



Discussion

Batungbacal and Scott (1982a) in a study of the effects of TBF infection on the humoral response to a commercial bacterial vaccine (Clostridium chauvoei) found that both the primary and secondary responses were depressed in animals inoculated during the febrile phase of a TBF reaction. In the present study, carried out in a small number of lambs, the primary humoral response to a standard antigen (horse red cells) and an inactivated viral antigen (louping-ill vaccine) were found to be apparently unaffected when the antigens were presented at various stages during a TBF reaction. However in Batungbacal and Scott's work, the humoral response, although depressed, was still present.

It is difficult to make a direct comparison between these experiments since the antigens used, and also the strain of C. phagocytophila inoculated, varied; however one might conclude, in the present state of knowledge, that the humoral response to some antigens may be depressed by a concurrent acute TBF reaction. Thus Batungbacal and Scott (1982b) also reported that the HI antibody response to parainfluenza-3 (PI-3) virus was depressed in animals inoculated with virus during a TBF reaction and their susceptibility to PI-3 increased in terms of both viral persistence and clinical signs. Also in the present study (Chapter 5), when sheep were infected with louping-ill virus (LIV) during a TBF reaction their haemagglutination inhibiting antibody response was suppressed and their susceptibility to virus increased. However this is not always the case and indeed the reverse, i.e. an elevation of titre in a TBF-infected sheep, may also be recorded.

Other authors have found that animals undergoing acute infections may demonstrate a reduced capability to respond to vaccination. Examples of these include acute toxoplasmosis causing reduced responses to Clostridium welchii type D vaccine in louping-ill virus vaccine in mice (Buxton et al, 1979); trypanosomiasis in cattle reducing the response to a clostridial vaccine (Holmes et al, 1974); and T. brucei infection in mice lowering the protection afforded to mice by vaccination against louping-ill virus prior to virus challenge (Reid et al, 1979) as well as suppressing the antibody response to vaccination (Whitelaw et al, 1979). Presumably the mechanism of immunosuppression in each of these infections is different. For example in murine Trypanosomiasis it has been suggested that a B-cell mitogen, secreted by the trypanosomes, is the cause of the plasma cell hyperplasia in the lymphoid tissue; this non-specific stimulation appears to pre-empt specific antibody production to antigen inoculated during this reaction.

The situation in TBF infections is described by Batungbacal et al (1982) as that of a fall in lymphocyte numbers due to a significant fall in the B-lymphocyte component and a slight reduction in the numbers of T-lymphocytes. However the underlying mechanism is still unknown.

SECTION II. CELL-MEDIATED IMMUNITY

Experiment 4b.

An assessment of the effects of TBF infection on cell-mediated immunity in 7 month old lambs using a tuberculin test.

Introduction

A preliminary experiment was carried out to assess the effects of tick-borne fever infection on the cell-mediated response of sheep. The tuberculin test was chosen as it is both simple to perform and is based on an *in vivo* expression of a delayed-type hypersensitivity (DTH) response (Stites, 1976). The method followed in designing the experiment was that of Cole and Morris (1971).

Materials and Methods

Twelve 7-month old Scottish Blackface lambs were divided into four groups of three animals. Each group of three animals was treated as follows: each of a pair of lambs were sensitised to BCG by the subcutaneous inoculation on the neck of 0.1 ml of BCG vaccine (Glaxo Laboratories Ltd, Middlesex, England) containing 1.5×10^6 viable BCG organisms. Sensitivity was tested 2, 4, 6 or 8 weeks later (depending on which of the four groups the lambs were in) by the intradermal inoculation of 0.1 ml of Bovine Tuberculin PPD (Ministry of Agriculture, Central Veterinary Laboratory, Weybridge, England) into the wool-free skin on the medial aspect of the thigh. One of each of the pairs of lambs was inoculated with TBF 5 days before tuberculin challenge. The third lamb in each group acted as a control and was

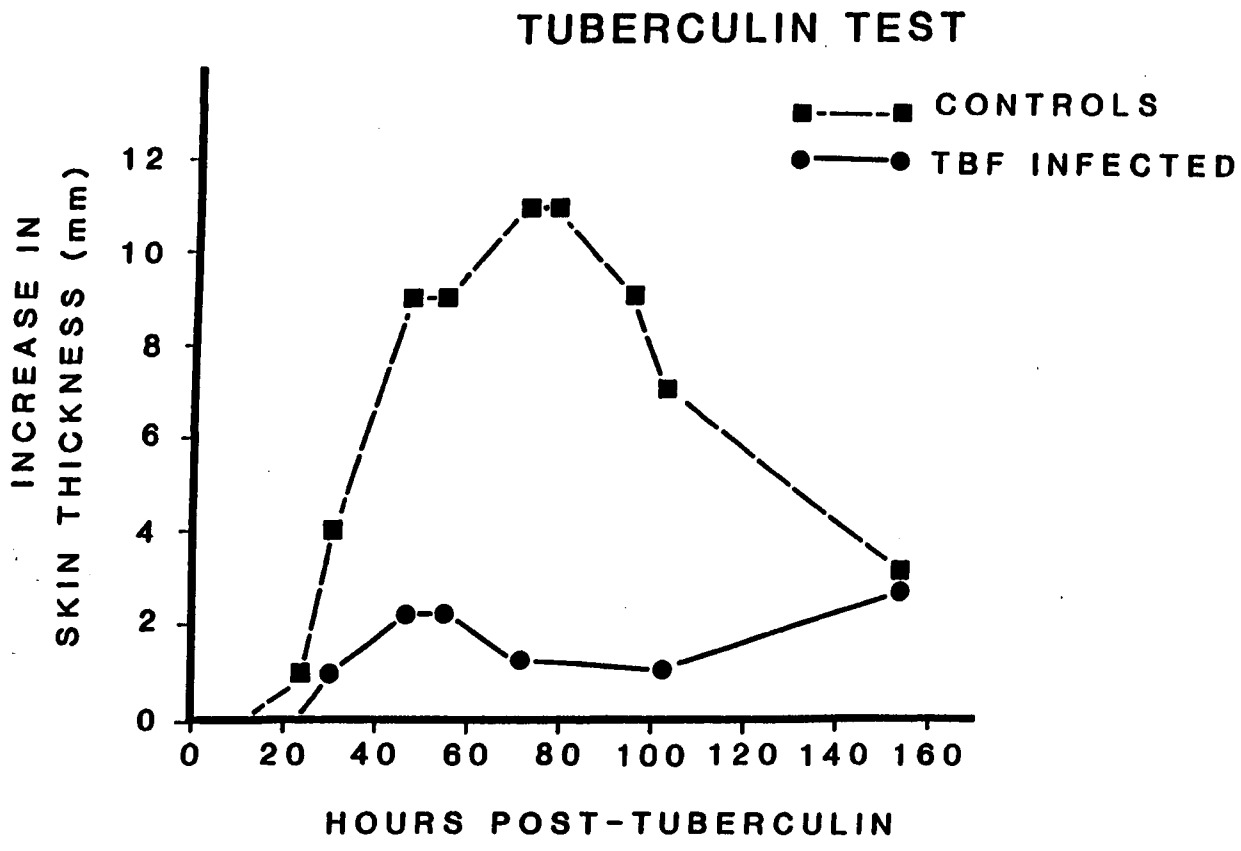
only inoculated with tuberculin at the same time as the other two lambs. The experimental design is illustrated below. Following the intradermal inoculation of tuberculin the skin thickness at the site was measured twice daily for the following 7 days using a pair of tuberculin test calipers (Willington Medicals Ltd, Reading, England).

Sheep Number	Day of inoculation			Interval between BCG and tuberculin
	BCG	TBF	Tuberculin	
45	-9	0	5	2 weeks
46	-9	-	5	2 weeks
53	-	-	5	---
47	-23	0	5	4 weeks
48	-23	-	5	4 weeks
54	-	-	5	---
49	-37	0	5	6 weeks
50	-37	-	5	6 weeks
55	-	-	5	---
51	-51	0	5	8 weeks
52	-51	-	5	8 weeks
56	-	-	5	---

Results

The increases in skin thickness at the site of tuberculin inoculation are presented in detail for each lamb in Table 4c. The longer the time interval between BCG sensitisation and tuberculin stimulation, the greater the response in both the TBF infected animals and the controls. The animals infected with TBF just prior to tuberculin stimulation consistently demonstrated a reduced response

FIGURE 4(iii). INCREASE IN SKIN THICKNESS IN A BCG-SENSITISED LAMB INOCULATED WITH TUBERCULIN ON DAY 5 OF A TICK-BORNE FEVER REACTION.



8 WEEKS BETWEEN BCG SENSITISATION AND TUBERCULIN CHALLENGE

which was most marked in the animal which had an 8-week gap between the BCG and tuberculin inoculations (see Figure 4 (iii)). The lambs which were inoculated with tuberculin without prior sensitisation with BCG did not show any skin reactions other than slight reddening and an increase in skin thickness by 1 mm during the first 24 hours post-inoculation and these lambs have been omitted from Table 4c.

Experiment 4c.

An assessment of the effects of TBF infection on cell-mediated immunity in young lambs aged 2 weeks using a tuberculin test.

Introduction

Following the results of the previous experiment which suggested that TBF could significantly affect tuberculin test reactions in 7-month old sheep, it was decided to investigate its effect on a similar test in much younger lambs. The interval between BCG sensitisation and tuberculin challenge was, however, restricted to two weeks in order to simulate field conditions in which young lambs aged 4-8 weeks are most likely to be infected with TBF.

Materials and Methods

Sixteen two-week old crossbred lambs were divided into one group of ten animals and two groups of three. The group of ten and one of the groups of three lambs were each inoculated subcutaneously with 0.1 ml of BCG vaccine containing 1.5×10^6 viable organisms (Glaxo). Nine days later the group of ten lambs were each inoculated with a TBF stabilate (Day 0). Five days after that (Day 5), all sixteen lambs

were inoculated intradermally into the wool-free skin on the medial aspect of the thigh with 0.1 ml of Bovine Tuberculin PPD (Min. of Agriculture). The skin thickness at the site of inoculation was measured once or twice daily for the following 4 days using a pair of tuberculin test calipers (Willington Medicals Ltd).

The experiment structure is detailed in Table 4d which also gives the results.

Results

The changes in skin thickness following the tuberculin test are detailed in Table 4d. The response to tuberculin inoculation in the BCG sensitised control lambs (Nos. 82, 86 and 93) was very low in comparison to the reactions found in the sensitised control lambs of the previous experiment. The sensitised TBF infected lambs showed a virtually identical low response. No reaction was shown to tuberculin inoculation in the unsensitised control lambs.

Discussion

One of the simplest and most useful methods of assessing the cell mediated immunity of an animal is the intradermal skin test for delayed hypersensitivity. It is however often of doubtful value in the very young as a means of diagnosing defects in cell-mediated immunity since they may often fail to react (Stites, 1976). This was borne out in the present work by the failure of lambs sensitised with BCG organisms at 2 weeks of age to respond to a tuberculin challenge two weeks later. In older lambs, however, there was a clear

difference in the response to the tuberculin test between lambs undergoing TBF reactions at the time of tuberculin challenge and those free from TBF infection. The reduced responsiveness of the TBF-infected lambs was apparent no matter whether there was a 2, 4, 6 or 8 week interval between sensitisation and challenge, although the longer the interval, the greater the response of the uninfected control lamb, resulting in a greater difference between the infected and control lambs' responses.

These results, with the reservation that only small numbers of animals were used, indicate that concurrent TBF infection has a damaging effect on the ability of previously sensitised sheep to mount a cell-mediated immune response to challenge with tuberculin. This result differs in degree from that of Batungbacal and Scott (1982a), who in a similar type of study in adult sheep but with a different antigen (DNCB) and a different experimental protocol, found that there was no significant impairment of the delayed-type hypersensitivity response in sheep infected with TBF, although responses as measured by increases in skin thickness were consistently but not significantly less in TBF-infected groups than in normal TBF-free sheep.

Perhaps this difference merely reflects the small numbers of sheep used in these experiments and the fact that different experimental protocols were used.

Finally, if one therefore assumes that TBF depresses the cell-mediated immune responses to a variable degree, it is somewhat difficult to assess the significance of this in young lambs since in the experiment involving 2-week old lambs, cell-mediated responses failed to occur even in the control lambs.

Summary

There was no evidence that the primary humoral response of 7-month old lambs to two antigens (horse red blood cells and louping-ill vaccine) was depressed by tick-borne fever infection. However in 7-month old lambs it was found that their ability to mount a cell-mediated response to tuberculin was impaired when they were undergoing a TBF reaction. Young lambs aged 4 weeks at the time of tuberculin challenge were unable to mount a cell-mediated response regardless of whether or not they were infected with C. phagocytophila.

Table 4a. Geometric mean titres to horse red blood cells in sheep inoculated at various times during a TBF reaction.

Lamb Groups	Geometric Mean Titres (log_2) (S.D.)						
		Days after Horse red cell inoculation					
	Pre-inoculation (Day -4)	6 - 8	9 - 11	13 - 14	17	20 - 21	27 - 28
Controls (HRBC on Day 0)	5.33 (1.15)	7.33 (1.53)	8.33 (1.15)*	9.00 (1.00)*	8.67 (1.15)*	8.00 (1.41)[1]	7.50 (0.71)[1]
TBF on Day 0 HRBC on Day 0	5.33 (1.15)	7.00 (2.65)	8.00 (1.73)	8.33 (2.08)	8.33 (2.31)	7.67 (1.53)	7.33 (1.53)
TBF on Day -5 HRBC on Day 0	5.67 (0.58)	7.67 (1.53)	8.33 (0.58)**	9.33 (0.58)**	9.67 (0.58)**	9.00 (1.00)**	9.67 (1.53)*
TBF on Day -9	4.00 (3.00)	8.33 (2.52)	9.00 (1.73)	9.67 (1.15)*	9.00 (1.73)	- [2]	- [2]

1: only two animals

2: not done

Significantly higher than pre-inoculation titres (t-test)

* P 0.05
** P 0.01

Table 4b. Geometric mean titres to louping-ill vaccine in lambs vaccinated at various times during a TBF reaction

Lamb Groups	Geometric Mean Titres (log_2) (S.D.)						
		Days after louping-ill vaccination					
	Pre-vaccination (Day -4)	6 - 8	9 - 11	13 - 14	17	20 - 21	27 - 28
Controls (Vaccine Day 0)	0	0.33 (0.58)	2.00 (2.00)	2.00 (2.00)	2.33 (1.15)	3.00 (0.00)[1]	3.50 (0.71)[1]
TBF on Day 0 Vaccine on Day 0	0	0	3.33 (1.53)	2.33 (2.08)	3.00 (2.65)	3.67 (2.31)	5.00 (2.65)
TBF on Day -5 Vaccine on Day 0	0	1.00 (1.73)	2.66 (2.31)	3.67 (0.58)	4.67 (0.58)	4.00 (0.00)	4.67 (0.58)
TBF on Day -9 Vaccine on Day 0	0	0	1.33 (1.53)	2.00 (2.65)	2.00 (1.73)	- [2]	- [2]

1: only two animals

2: not done

TABLE 4c. Tuberculin Test reactions in normal 7 month-old lambs compared with lambs infected with tick-borne fever.

Lamb No.	TBF infection	Interval between BCG and Tuberculin inoculations	Increase in skin thickness (mm)											
			Hours post-Tuberculin											
			7	25	31	47	55	71	79	96	103	125	149	167
45	TBF	2 weeks	0	0	0	1.5	0	0	0	0	ND*	0	0	0
46	-		0	4	7	5	5	4	4	3	ND	1	1	1
47	TBF	4 weeks	1	1	1	2	2	1	1	1	0	ND	ND	0
48	-		1	1.5	3	5	6	5	5	3	3	ND	ND	1
49	TBF	6 weeks	0	0	1	2	1	1	1	1	1	ND	ND	1
50	-		1	3	4	7	7	6	8	5	5	ND	ND	3
51	TBF	8 weeks	0	0	1	2	2	1	1	1	1	ND	3	ND
52	-		0	1	4	9	9	11	11	9	7	ND	3	ND

* Not Done

TABLE 4d. Tuberculin Test results in normal 2-week old lambs compared with lambs infected with tick-borne fever

Lamb No.	Day of inoculation			Increase in skin thickness (mm) Hours post-tuberculin					
	BCG	TBF	Tuberculin	6	27	47	55	76	99
Sensitised control lambs									
82	-9	- *	5	0	1	1	1	0	0
86	-9	-	5	0	0	0	1	0	0
93	-9	-	5	0	3	2	1	0	0
Sensitised TBF infected lambs									
81	-9	0	5	0	0	0	0	0	0
84	-9	0	5	0	0	0	0	0	0
87	-9	0	5	0	0	0	0	0	0
89	-9	0	5	0	0	0	0	0	0
91	-9	0	5	0	1	2	2	1	0
95	-9	0	5	0	1	1	0	0	0
96	-9	0	5	0	1	0	0	0	0
99	-9	0	5	0	0	0	0	0	0
103	-9	0	5	0	0	0	0	0	0
105	-9	0	5	0	0	0	0	0	0
Unsensitised control lambs									
90	-	-	5	0	0	0	0	0	0
94	-	-	5	0	0	0	0	0	0
102	-	-	5	0	0	0	0	0	0

* Not given

CHAPTER 5

STUDIES ON THE EFFECTS OF A CONCURRENT TICK-BORNE FEVER INFECTION ON EXPERIMENTALLY INDUCED LOUPING-ILL VIRUS ENCEPHALITIS

Introduction

Workers responsible for the initial isolation of louping-ill virus (LIV) experienced difficulty in reproducing the disease in sheep by parenteral inoculation of virus. Limited evidence suggested that sheep infected with Cytoecetes phagocytophila, the cause of tick-borne fever (TBF), regularly developed typical signs of louping-ill and it was proposed that TBF facilitated invasion of the central nervous system (CNS) by louping-ill virus (Macleod and Gordon, 1932). Subsequent studies which indicated that virus invasion of the CNS following peripheral inoculation with louping-ill virus invariably occurred (Zlotnik et al, 1970; Doherty and Reid, 1971a, b) did not support this hypothesis. However, increased susceptibility to louping-ill virus has been demonstrated in mice concurrently infected with Trypanosoma brucei (Reid et al, 1979) and in mice and lambs infected with Toxoplasma gondii (Buxton et al, 1980; Reid et al, 1982) and attributed to the immunosuppressive effects of these protozoa. Since TBF infection of sheep has been shown to depress immune responses (Batungbacal and Scott, 1982a, b; Gilmour, Brodie and Holmes, 1982), the increase in susceptibility to louping-ill virus of sheep with concurrent TBF could arise from a suboptimal immune response. The following experiments were designed to examine the interaction between TBF and louping-ill virus following experimental infection of sheep.

Materials and Methods

In the two experiments reported in this chapter the materials and methods were by and large identical. Where variations occurred these

are recorded in the relevant section for each experiment.

TBF inoculation was as described in Chapter 2. For louping-ill infection sheep were inoculated subcutaneously with a known quantity of plaque-forming units (pfu) of the SB526 isolate of louping-ill virus prepared from suckling mouse brains, as described by Reid and Doherty (1971a).

In both experiments animals were observed at approximately 12-hour intervals and rectal temperatures were recorded daily. Animals which died or became severely affected were necropsied and survivors were killed on Day 26 (21 days after virus inoculation). At post-mortem examination, portions of approximately 1 cm^3 of cerebrum, cerebellum, brain-stem, representative lymph nodes and spleen were collected and stored at -80°C until homogenised and tested for virus as previously described (Reid et al, 1982). When it became apparent following histological examination that systemic mycosis had affected a number of animals, samples of selected organs were cultured on Sabauraud's 1% Ionager (Oxoid). Small fragments of tissue were aspirated into a pasteur pipette and embedded in the agar plates which were incubated aerobically at 37°C and examined daily for evidence of fungal growth. The remainder of the brain together with other organ samples were placed in 10% Baker's formal saline for histological examination.

To assess the severity of histopathological changes in the brain, coronal slices through the anterior pole of cerebrum, corpus striatum, thalamus, hippocampus, midbrain (anterior end of the red nucleus and another at the posterior end of the red nucleus), cerebellar peduncles and three levels of medulla were taken together with a sagittal

section through the cerebellar vermis. These were then processed in paraffin wax and sections 6-um thick were cut and stained with haematoxylin and eosin and selected sections were stained by the Grocott-Gromori methenamine silver method or by the Periodic acid Schiff technique (Bancroft and Stevens, 1977).

Experiment 5 a.

Assessment of the effects of a TBF infection on the course of an experimentally induced louping-ill virus infection in adult rams

Materials and Methods

Eighteen 4 to 7 year old Cheviot or Cheviot-cross rams which had been reared in an environment free of the sheep tick Ixodes ricinus were divided into two groups of 8 and one of 2. The group of two rams and one of the groups of 8 were all inoculated i/v with the TBF inoculum on Day 0 and five days later (Day 5) both groups of 8 sheep were inoculated subcutaneously with $10^{6.1}$ plaque-forming units of the SB526 louping-ill virus isolate. The remaining two rams were injected with a clarified suspension of normal brain on the same day.

Blood was collected daily for virus and antibody assays (Reid and Doherty, 1971a, b) and for the preparation of blood films. Virus titres were determined using a plaque method and antibody was assayed by a haemagglutination inhibition (HI) test. Blood films were stained with Giemsa to assess the intensity of the TBF parasitaemia.

Results

Clinical Observations

The 2 rams infected with TBF alone showed no obvious clinical signs apart from a raised rectal temperature while 3 of the 8 rams infected with louping-ill virus alone showed only transient clinical signs and made uneventful recoveries. In contrast, all 8 dually infected animals developed clinical signs and 3 died and 5 were killed *in extremis*. First signs were observed on Day 11 when two of these animals appeared depressed and by Day 12 six were depressed and four ataxic; two of the latter became comatose and were killed. Over the next two days, the remaining six animals having developed severe dysentery, trembling and ataxia, died or became recumbent and were killed.

Pathology

Gross

No gross lesions were observed in any of the animals given either TBF or louping-ill virus alone. In contrast, all dually infected tups showed marked congestion of the meningeal vessels. Haemorrhage into the intestinal lumen was apparent in six cases and varied from mild leakage, with associated petechiation of the mucous membranes, to very severe with blood clots 10 mm in diameter in the ileum and up to 50 mm in diameter in the large bowel. In half the necropsies, haemorrhagic necrosis was present in the retropharyngeal, perscapular, popliteal and mediastinal lymph nodes. Infarcts were found in the livers or kidneys of three animals and cardiac petechiation was also present in three cases. In the abomasal mucosa of one there were striking rings

of haemorrhage up to 15 mm in diameter with pale centres.

Microscopic

In the sheep given TBF only, a few large lymphoid cells were present in the cerebral and cerebellar meninges with mild scattered accumulations around blood vessels. No other abnormalities were found.

In the sheep which received louping-ill virus alone, mild to moderate non-suppurative meningo-encephalitis was present in seven brains with mild accumulations of lymphoid cells in the cerebral and cerebellar meninges. Lymphoid vascular cuffing was present in the cerebrum, cerebellum and brain-stem of all these animals. Focal gliosis, although mild, was present in the cerebral white matter, thalamus, midbrain, cerebellar white matter, pons and medulla. Neurone necrosis was observed in only 4 animals and was confined to the pons and medulla and neuronophagia was seen in only one brain. The remaining tup in the group had only very mild encephalitis composed of a few perivascular cuffs in the medulla. Pathological changes in the group injected with TBF and louping-ill virus were more complex with lesions also being present in non-neural tissues. Five showed neuropathological changes characteristic of louping-ill virus infection to varying degrees, i.e. a non-suppurative meningoencephalitis with lymphoid meningitis and perivascular cuffing, within the brain-stem focal gliosis, neurone necrosis and neuronophagia. The other three brains had, in addition to changes characteristic of louping-ill virus infection, inflammatory exudate composed primarily of polymorphonuclear cells and, in one case, infarction of meningeal blood vessels in the forebrain and malacia in

the adjacent cortex and white matter. Special staining revealed in this area heavy infiltrations of non-septate branching fungal hyphae within infarcted blood vessels, vessel walls and meninges.

In 7 dually infected tups there was a mild, focal, interstitial lymphoid nephritis. In 2 of these, infarction had occurred and the resulting 'cone' of dead tissue was heavily infiltrated by non-septate branched fungal hyphae. There was a clear line of demarcation between the infarct and normal tissue, with the latter not infiltrated by fungal hyphae. Fungi were not found in the liver but in 6 animals there were mild periportal accumulations of lymphoid cells and in one, thrombosis and focal necrosis. Morphologically similar hyphae associated with severe necrosis were present in various lymph nodes of 4 of the rams, although haemorrhages were present in seven.

Pulmonary oedema and congestion were present in 7 cases with purulent exudative foci of pneumonia in 2. Histological changes in the gastrointestinal tract were not very marked. The abomasal lesions noted macroscopically in one sheep also showed non-septate branching fungal hyphae extending into the lamina propria and adjacent blood vessels.

In all, non-septate branching fungi were demonstrated histologically in 5 animals of this group although changes characteristic of fungal infection were found in 7.

Microbiology

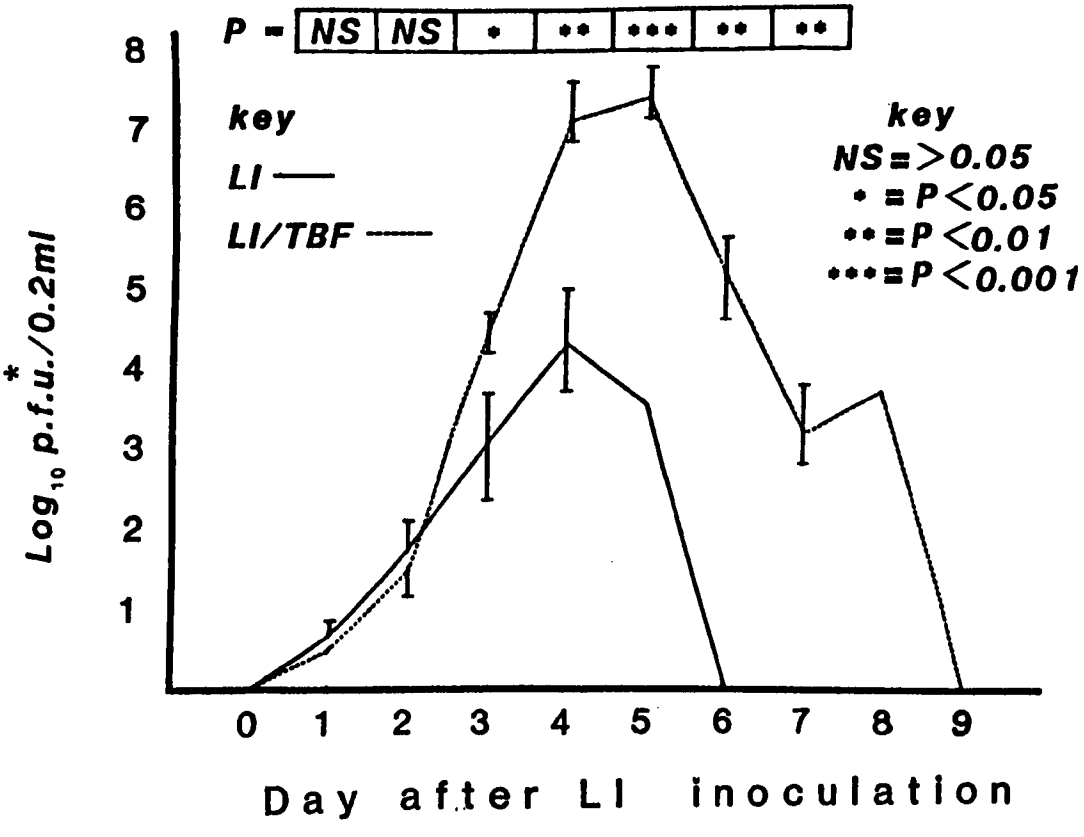
Louping-ill virus was isolated from the plasma of all but one (No. 13) of the tups given the virus. Evidence of infection in this animal relied on the detection of serum HI antibody from 6 days after

LIV inoculation (Day 11) and the presence of mild neuropathological changes at necropsy. In the remainder, viraemia was detected from the day following virus inoculation and rose rapidly in titre through the ensuing three days (Fig. 5(i)). Compared to animals infected with louping-ill virus alone, the viraemia in dually infected animals was greater and more persistent, maximum titres in this group ranging from $10^{6.19}$ to $10^{8.53}$ pfu per 0.2 ml of plasma compared to $10^{0.7}$ to $10^{6.19}$ in the group infected with louping-ill virus alone. Viraemia in the dually infected group persisted until 7 days after virus infection in seven of the animals while in the group infected with virus alone, virus was present in only five rams 4 days after virus infection and persisted until the fifth day in only one.

Virus was recovered from the brains of all the dually infected animals (Table 5 a) but from none of the animals infected with the virus alone that were killed on Day 26. Virus was also present in most of the lymph node and spleen specimens taken from the dually infected animals. From tissues of 7 of the eight dually infected animals cultured on Sabauraud's agar numerous white colonies with extensive aerial mycelial growth were recovered. These moulds were subcultured and identified as Rhizomucor pucillus.

Haemagglutination inhibiting antibodies were not detected in any of these sheep before Day 11 when all 8 of those infected with virus alone were positive (Table 5b). Thereafter titres in this group rose rapidly with the exception of two animals in which viraemia was low or not detected. In contrast, in the dually infected group, antibody was detected in only 4 of the 8 on Day 12 and, even by Day 13, one of the surviving animals had failed to seroconvert.

FIGURE 5(i). THE MEAN (± S.E.) TITRE OF LOUPING-ILL VIRUS IN SHEEP GIVEN LOUPING-ILL VIRUS (LI) ALONE OR VIRUS FIVE DAYS AFTER INOCULATION WITH TICK-BORNE FEVER (LI/TBF).



* Plaque-forming Units

On Day 5, 7 of the 8 dually infected rams and both TBF control animals had patent TBF infections and by the following day the eighth ram was also parasitaemic. The percentage of parasite-infected neutrophils in both of the above groups ranged from 20% to 60% (mean 39%) on Day 5 and rickettsial bodies were visible in neutrophils for 4 to 7 days (mean 4.8 days) after TBF infection.

Experiment 5b.

Confirmation of the effects of a dual infection with TBF and Louping-ill virus in adult and immature sheep

Introduction

Following the results of Experiment 5a in which an unexpectedly high mortality was encountered associated with a systemic mycosis it was decided to repeat the experiment to ascertain whether this was a chance happening or if the syndrome was readily reproducible. In addition to mature sheep, younger rams were also infected to check their response and confirm that this condition was not confined to aged animals.

Materials and Methods

Five adult rams aged 2-3 years and five 7 month old Cheviot lambs, all of which had been reared in a tick-free environment were used. All 10 were inoculated with TBF on Day 0 and 5 days later (Day 5) all were infected subcutaneously with $10^{6.4}$ plaque forming units of the SB526 isolate of louping-ill virus.

Blood was collected for evaluation of the TBF parasitaemia. Pathological examinations were carried out as described above, but virological and serological studies were not conducted in this experiment nor was fungal isolation undertaken.

Results

Clinical Observations

Nine of the 10 dually infected animals died or were killed *in extremis* on Days 10 to 16. Three had developed dystentery or profuse diarrhoea prior to death and 6 showed signs of ataxia or tremor while 1 died suddenly having shown no obvious clinical signs. The surviving ram had a mucoid diarrhoea on Day 13 and thereafter made an uneventful recovery and was killed on Day 26.

Pathology

Gross

Marked lesions were present in 7 of the 9 animals that died or were killed *in extremis*. Haemorrhage of the small and large intestine were present in 2 and enlarged lymph nodes, frequently oedematous and haemorrhagic, were seen in 6; the retropharyngeal node was most frequently involved. Infarcts of the liver and kidney were seen in 3 and 4 of the sheep respectively. In most sheep, the meningeal blood vessels appeared congested.

Microscopic

The neuropathological changes observed were not typical of louping-ill meningo-encephalitis. In the first animals to succumb

inflammatory changes were not present but there was widespread vascular haemorrhage and extensive areas of necrosis of lymph nodes, lung and liver in which many bacteria were observed. Of the remaining animals all had a severe non-suppurative meningo-encephalitis with vascular cuffing by large lymphoblast-like cells which was particularly severe in the cerebral cortex. In addition, macrophages containing necrotic debris were present in the cuffs. Five of the sheep to succumb had necrotic lesions in organs other than the brain and these were seen to be associated with fungal invasion. No lesions could be detected in the surviving ram that was killed on Day 26.

On Day 5, all 10 animals had patent TBF infections, the percentage of infected neutrophils ranging from 37% to 68% (mean 58%). Virological studies were not conducted in this experiment.

Discussion

The response of the sheep to infection with louping-ill virus alone is variable (Pool *et al*, 1930; Reid and Doherty, 1971b), thus the mild reaction of the group of 8 rams given virus alone is not exceptional; the absence of clinical signs in the rams infected only with TBF is also in accordance with the benign nature of this disease (Woldehiwet, 1983). In contrast, in two separate experiments, sheep infected with TBF prior to exposure to louping-ill virus experienced a very much more severe reaction from which 17 out of 18 died or were killed *in extremis*. In the first experiment (Expt. 5a) old male sheep were employed and the second experiment (Expt. 5b) was therefore performed to compare the response of mature animals and 7 month old lambs. Both categories of animal proved equally susceptible,

indicating that the advanced age of the animals employed in the first experiment did not influence the result. The mortality that occurred in the dually infected group was, however, not entirely due to an increased susceptibility to louping-ill virus but was also apparently associated with a profound depression of the animals' normal defence mechanisms.

In a previous study of the effect of toxoplasmosis on louping-ill virus infection in mice and sheep (Buxton et al, 1980; Reid et al, 1980; Reid et al, 1982) the mortality in dually infected animals compared to those infected with louping-ill virus alone was considerably greater. However, the clinical course was extended and the deaths occurred later. This pattern of delayed but increased mortality has been observed in numerous studies of virus encephalitis in immunosuppressed hosts (Bhatt and Jacoby, 1976; Camenga et al, 1974) and it was concluded that the Toxoplasma infection exerted an immunosuppressive effect which enhanced the pathogenicity of louping-ill virus. In contrast, in the present study deaths in dually infected animals commenced on the 6th day after louping-ill virus inoculation and continued to the 11th day with most dying earlier than would be expected for animals given virus alone (Reid and Doherty, 1971b). However, the viraemias in the dually infected lambs were markedly greater and more prolonged while the HI antibody response either did not develop or was delayed, both features suggesting an immunosuppressed response to louping-ill virus.

These results were similar to those reported by Batungbacal and Scott (1982b), following dual infection of sheep with TBF and parainfluenza-3 (PI-3) virus. In their experiment, Batungbacal and Scott found that the production of antiviral antibodies was depressed

and PI-3 virus was excreted for a longer period in the dually infected group compared with lambs given PI-3 virus alone, suggesting a reduced immune response in the TBF infected group.

In addition, in the present work high titres of louping-ill virus were found in non-neuronal tissues, whereas virus is normally restricted to the CNS in sheep that succumb to infection with virus alone but has been found in extraneural tissue in lambs with concurrent acute toxoplasmosis (Reid et al, 1982). Elimination of virus from plasma and tissues is mediated through antibody to the virus (Reid and Doherty, 1971b; Reid et al, 1971) so that the persistence of virus in extraneural tissue further supports the concept that the immune response of the TBF-infected animals to louping-ill virus was severely compromised.

In accordance with previous studies of sheep infected with louping-ill virus alone (Doherty and Reid, 1971a, b) evidence that virus entered the CNS of all surviving animals was confirmed by the consistent presence of mild to moderate encephalitis detected in brains collected on Day 26. The wider distribution of the lesions in the sheep infected with both TBF and louping-ill virus suggests that viral invasion of the CNS was augmented. It is probable that this reflected a greater intensity of viral replication in these animals, as indicated by the magnitude of the viraemia.

However, the cause of the increased mortality in the dually infected sheep was not solely attributable to increased susceptibility to louping-ill virus, the lesions of which are restricted to the CNS.

In both experiments the dually infected sheep developed dysentery and the majority had extensive haemorrhagic lesions in the intestine

and/or elsewhere. Histopathological examination revealed widespread necrotic lesions and in some of these the presence of fungal hyphae was demonstrated. The isolation of Rhizomucor pucillus from the tissues of 7 of the 8 dually infected rams in Experiment 5a suggests that this fungus was specifically involved.

It is interesting to note that the syndrome described here bears some similarity to bovine petechial fever as described by Danskin and Burdin (1963). They did not however find evidence of a secondary infectious agent and attributed the condition to the rickettsial agent alone.

Although neutropenia and immunosuppression are known to be associated with TBF (reviewed by Scott, 1984), the fact that this fatal syndrome occurred only in the dually infected sheep indicates that its aetiology is dependent on concurrent infection with TBF and louping-ill. That there was no evidence of overwhelming bacterial infection implies either that the perturbation of the host's defences operates very locally at sites where R. pucillis is present or that it selectively abrogates homeostatic mechanisms responsible for maintaining equilibrium with mycotic symbiots.

An apparently similar syndrome has been described in British sheep on two previous occasions. Haemorrhagic enteritis was reported in sheep following experimental inoculation of TBF (Foster et al, 1968) and intestinal mycosis was reported in a lamb (Angus et al, 1971) also given TBF. In the light of the present findings, it is possible that on these two occasions blood used for the TBF infections also, incidentally, contained louping-ill virus.

The probability of the syndrome described in this paper occurring naturally will depend on the duration of the enhanced susceptibility

to louping-ill virus infection following exposure to TBF. In the current experiments louping-ill virus was given only 5 days after injection of TBF and thus, if this interval is critical, the syndrome may occur only infrequently. A further feature militating against the natural occurrence of the syndrome is that colostral antibody is very efficient at protecting lambs from louping-ill virus (Reid and Boyce, 1976) but does not normally provide any protection from TBF. Thus the interval between lambs becoming infected with TBF and louping-ill is likely to be considerable. However, sheep introduced to tick-infested pasture for the first time are most likely to become infected with both agents together and the consequences of dual infection reported here could explain the very high mortality attributed to louping-ill virus in this category of animal.

Summary

Sixteen out of eighteen sheep infected with louping-ill virus 5 days after TBF was given died, while none of the eight given louping-ill virus alone died. The dead sheep showed not only pathological and clinical evidence of increased susceptibility to louping-ill virus infection but in many cases there was also evidence of a systemic mycotic infection with the fungus Rhizomucor pucillus. The mechanism underlying this syndrome remains unclear.

Table 5a. Isolation of louping-ill virus and Rhizomucor pucillus from tissues of sheep infected with tick-borne fever and louping-ill virus

Sheep No.	Day of Death	Cerebrum	Cerebellum	Brain Stem	Spleen	Prescapular lymph node	Mediastinal lymph node
1	13	5.7[1]	6.5+	6.8	5.2	6.3+	6.2+
2	14	-*	2.3	4.1	-*	3.2*	-
6	12	4.6*	5.2	5.7	6.8	6.9*	3.6+
9	12	3.9	4.7	4.4	-	-	-
11	13	5.5	6.4	6.7	6.7+	6.7*	5.8*
15	14	4.8	6.5	6.2	3.5	4.8*	4.0
16	13	3.3+	5.2	4.8	-	-	4.4+
17	14	6.9	6.7	7.0	5.0	6.7+	6.6*

[1] = $\log_{10}$ plaque forming units per 0.2 gm of tissue

* = tissues from which Rhizomucor pucillus was recovered

+ = tissues from which Rhizomucor pucillus could not be recovered

Table 5b. Haemagglutination inhibiting antibody response to louping-ill virus (LIV) in sheep infected with virus alone and with tick-borne fever (TBF) and louping-ill virus.

Sheep No.	Treatment	Day 10	11	12	13	14	15
1	LIV + TBF	<	<	<	D		
2	"	<	<	160	2560	5120	D
6	"	<	<	10	D		
9	"	<	<	10	D		
11	"	<	<	<	320	D	
15	"	<	<	<	320	1280	D
16	"	<	<	320	1280	D	
17	"	<	<	<	<	D	
5	LIV	<	160*	2560	10240	5120	2560
7	"	<	160	1280	5120	10240	2560
8	"	<	320	1280	2560	2560	1280
10	"	<	20	640	640	320	640
12	"	<	80	80	80	80	80
13	"	<	40	80	80	80	40
14	"	<	40	640	5120	10240	5120
18	"	<	80	640	1280	640	160

< = titre of less than 1/10 recorded

D = animal dead

* = reciprocal of titre

CHAPTER 6

STUDIES ON THE EFFECTS OF A CONCURRENT TICK-BORNE FEVER INFECTION ON EXPERIMENTALLY INDUCED P. HAEMOLYTICA PNEUMONIA

Introduction

Suggestions of a connection between C. phagocytophila infection and subsequent pasteurellosis have been made on more than one occasion. In the course of experiments involving tick-borne fever infection it was reported that pneumonic pasteurellosis developed shortly after TBF infection (Foggie, 1951; Foster and Cameron, 1970a) and it was suggested that TBF may have been the predisposing factor. Brandreth (1978) also suggested that TBF may predispose sheep to pasteurellosis and later work reported by Batungbacal and Scott (1980), in which Pasteurella haemolytica was isolated from nasal swabs in a higher proportion of sheep clinically affected with TBF than from control animals, also indicated a connection. In view of these findings, and bearing in mind the fact that the majority of outbreaks of pneumonic pasteurellosis occur between April and July (Gilmour, 1978), which in hill flocks is also the time of peak tick activity, it was decided to investigate experimentally the role of TBF in pneumonic pasteurellosis.

Experiment 6a.

Infection of lambs with P. haemolytica serotype A2 during a TBF reaction

Materials and Methods

Experimental Animals

Eighteen six-week old caesarian-derived, colostrum-deprived specific pathogen free (SPF) lambs were used. They were kept in

isolation in SPF accommodation at the Moredun Institute and fed on a diet of cow's milk, hay and commercial lamb rearing pellets.

The eighteen lambs were divided into two groups of eight and one group of two and each group was kept in a separate room.

Infection procedure

A stabilate of tick-borne fever was administered I/V to each of 8 lambs in one group on Day 0 and seven days later (Day 7) both groups of eight lambs were exposed to an aerosol of P. haemolytica serotype A2 according to the method of Gilmour and others (1975). In this, the lambs' heads were placed in a specially constructed box into which the aerosol was delivered. The lambs remained in this position for a period of 15 minutes, the air within containing an aerosol concentration of $\log_{10}$ 6.8 colony forming units per litre (CFU/L) calculated from an impinger count. The remaining two lambs were inoculated with the TBF stabilate on Day 0 and were kept as TBF controls. This serotype was chosen since it was the most commonly isolated from cases of ovine pneumonia (Fraser et al, 1982).

Clinical observations

The lambs were examined clinically for a period of 5 days after P. haemolytica infection (i.e. from Day 8 to Day 12) and were necropsied on the seventh day (Day 14). In order to differentiate the responses of the lambs a scoring method was used for both clinical observations and pneumonic lesions found at necropsy (Gilmour, Sharp and Gilmour, 1982). This system involved scoring for four main features of illness: a pyrexia of greater than 40.6^{o}C; dullness or lethargy; abnormal respiration (i.e. abdominal respiration or dyspnoea); death. A score of one point was given for each sign the

lambs showed on each day. If a lamb was dead it was given 4 points. Thus on each day a lamb could score between 0 and 4 points.

Necropsy procedure

Lambs were necropsied on the day they died and surviving lambs were killed and examined 7 days after aerosol infection with P. haemolytica. The extent of any lesions of pneumonic pasteurellosis were drawn on lung outline charts and a score was calculated based on the percentage of the lung chart with lesions. Lungs with no lesions scored 0; if 5-10% of the lung was affected the lamb was given a score of 5; for 11-25% affected the score was 10 and if more than 25% of the lung was affected the score was 20. In addition 2 g samples were cut from areas of lung showing lesions and cultured for estimation of the concentration of P. haemolytica per gram of lung.

Results

All ten lambs inoculated with TBF had developed pyrexias of greater than 40.6°C by Day 5 and nine of them were parasitaemic on that day. All ten lambs were parasitaemic by Day 7 when exposed to the P. haemolytica aerosol.

The two TBF control animals remained clinically unaffected other than a transient period of dullness associated with the pyrexic phase of the reaction. At necropsy there were no pneumonic lesions.

One of the animals given only P. haemolytica died on Day 10, but death was not due to pasteurellosis and it was therefore omitted from the experiment. Of the animals infected with pasteurellosis alone, three died on Day 10, one on Day 12 and two on Day 13, only one animal

surviving to be killed on Day 14. Clinical evidence of infection was noted in all except the animal surviving to Day 14 and at necropsy all seven animals showed lesions typical of pneumonic pasteurellosis and P. haemolytica was isolated in culture from the lungs of six of them.

Of the animals infected with P. haemolytica and TBF, one died on Day 9, two died on Day 10, one on Day 11 and two on Day 13, only two animals surviving to be killed on Day 14. Clinical evidence of infection was noted in all except one of the two animals surviving till Day 14 and typical lesions of pasteurella pneumonia were seen in all except that animal at necropsy. P. haemolytica was isolated in culture from the lungs of 6 of the 7 lambs with lesions.

The clinical and lesion scores for each animal are summarised in Table 6a along with the mean scores for each group. There was no significant difference between the mean clinical, lesion or total scores in the groups given P. haemolytica alone or in combination with TBF, although the scores were slightly higher in the latter group. (The Mann-Whitney test was used to analyse the results).

Experiment 6b.

Infection of SPF lambs with P. haemolytica serotype A1 during a TBF reaction

Introduction

The results of Expt. 6a had been unexpectedly severe in the group given P. haemolytica alone since the majority had died. It was therefore decided to repeat the experiment but to use P. haemolytica serotype A1 instead of serotype A2 although the other details of the

experiment were virtually unchanged. A1 was selected because, apart from being another serotype involved in pneumonic pasteurellosis there was more laboratory information on the experimental production of pasteurellosis with this serotype (Gilmour, personal communication).

Materials and Methods

Experimental animals

Fourteen four-week old hysterectomy-derived colostrum-deprived SPF lambs were used. They were divided into two groups of seven and each group was kept in a separate room of the isolation unit.

Infection procedure

Each lamb in one of the groups of seven was inoculated intravenously with 1.5 ml of TBF blood stabilate on Day 0 and seven days later (Day 7), both groups of lambs were exposed to an aerosol of *P. haemolytica* serotype A1 as described in Expt. 6a. The aerosol concentration in the infection chamber was $\log_{10}$ 6.05 colony forming units per litre and the lambs were exposed to it for 15 minutes.

Clinical and necropsy procedures

The lambs were observed clinically for a period of 5 days following *P. haemolytica* infection (from Day 8 to Day 12) and were killed and examined at necropsy on Day 14. The same scoring system as before was used both for assessing the clinical effects of infection and the pathological lesions.

Results

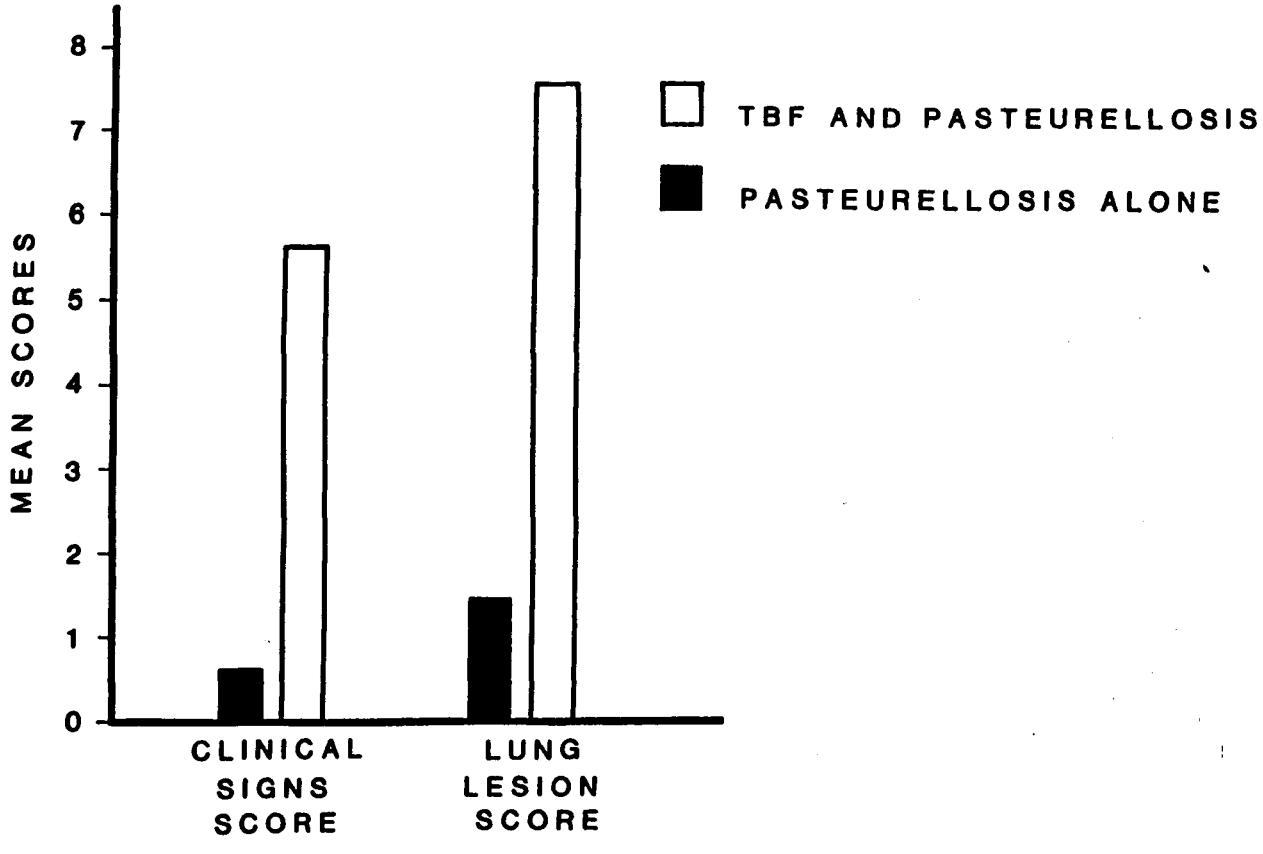
By Day 5 six of the seven lambs infected with TBF were parasitaemic and by Day 7 all were parasitaemic.

Only 3 of the lambs infected with *P. haemolytica* alone showed clinical signs of infection although none of the three had pneumonic lesions at necropsy. In this group of seven lambs only two animals had lung lesions, neither of whom had shown clinical signs of pasteurellosis. *P. haemolytica* was only isolated from the lungs of one animal in this group.

By contrast all seven lambs with the dual infection showed clinical signs of pasteurellosis, and all but one had pneumonic lesions at necropsy. The clinical and lesion scores for this group of lambs were very much higher than those found in the lambs infected with *P. haemolytica* alone. *P. haemolytica* was isolated in culture from the lungs of 4 animals in this group.

The results of the experiment are presented in Table 6b and illustrated graphically in Fig 6 (i). There were significant differences between the mean clinical scores ($P < 0.05$) and mean total scores ($P < 0.05$) while the mean lung lesion scores just failed to achieve significance ($P > 0.05$) in the two groups of lambs with the dual infected group having significantly higher scores. (The Mann-Whitney test was used to analyse the results).

FIGURE 6(i). A COMPARISON OF THE CLINICAL SIGNS AND LUNG LESIONS FOUND IN CONTROL LAMBS INFECTED WITH PASTEURELLA HAEMOLYTICA ONLY, AND IN LAMBS INFECTED WITH PASTEURELLA HAEMOLYTICA 7 DAYS AFTER TBF INOCULATION.



Discussion

Pasteurella haemolytica is an opportunist pathogen and as such speculation about the precipitating factors in an outbreak of pneumonic pasteurellosis is justified. Although various predisposing causes have been implicated such as virus infection (principally Para-influenza-3 (PI-3) virus) or stress of various types, e.g. handling, transport or environmental stress, the true cause or causes remain unknown due to the sporadic nature of the condition (Gilmour, 1978, 1980).

Suggestions that TBF may play a predisposing role in outbreaks of pneumonic pasteurellosis in sheep have, up till the present time, largely been based on circumstantial evidence such as reports of pasteurellosis developing shortly after TBF infection (Foggie, 1951; Foster and Cameron, 1970a). The experiments in this chapter have demonstrated that lambs undergoing a TBF reaction are more likely to become pneumonic and show more severe clinical and pathological signs than normal lambs following exposure to an aerosol of *P. haemolytica*. This was most evident when the A1 serotype was involved. The results of the experiment in which the A2 serotype was employed, although inconclusive due to the unexpectedly severe condition provoked (Gilmour *et al*, 1975), did show a trend towards increased pathogenicity amongst the group infected with both TBF and *P. haemolytica*. A variability of response to experimental inoculation of *P. haemolytica* has been noted occasionally (Gilmour, personal communication).

In two other recent studies on the effects of concurrent TBF infection on respiratory pathogens similar findings have been

reported. Batungbacal and Scott (1982b) investigated the results of a combined TBF and PI-3 virus infection in sheep, while Munro et al (1982) examined sheep experimentally infected with TBF and Chlamydia psittaci. In both investigations the pathological findings in animals which were dually infected were more severe than in those affected with either TBF or the respiratory pathogen alone. Both groups of workers also noted that if the respiratory pathogen was administered a few days after TBF inoculation then the pathogenic effects were greater than in animals which had received both agents simultaneously.

Although the exact mechanism of the increased susceptibility to these respiratory pathogens induced by TBF remains unknown, it is probably due to a combination of factors. Munro et al (1982), from histopathological studies, suggested that C. phagocytophila exerts its effect by interfering with local immune defence mechanisms in the lung, notably the mononuclear cells and macrophages. Batungbacal and Scott (1982b) reported that in TBF-infected sheep there were reduced titres of both specific haemagglutination inhibiting (HI) and virus neutralising (VN) antibodies to PI-3 virus compared with the response in TBF-free sheep following viral infection. Moreover they suggest that as a direct result of this, virus infection was prolonged in such animals.

Sequential infection with PI-3 virus and P. haemolytica has been shown to result in a large proportion of the animals developing pneumonia, while either agent on its own had little effect (Davies et al, 1977; Sharp et al, 1978). In a later study on P. haemolytica infection in sheep, Davies and Penwarden (1981) stated that the relative importance of resident alveolar macrophages and infiltrating neutrophils in the clearance of pathogenic bacteria from the lungs

remained unknown. There was some evidence however that in the clearance of P. haemolytica from the lungs of sheep, the heavy neutrophil infiltration observed was important (Davies and Penwarden, 1981).

The timing of the respiratory pathogen's inoculation in the present study varied a little from that used by the above workers, and also from that used elsewhere in this thesis. The reason for this was that, at the time when these two experiments were designed, it was not clear whether the high parasitaemia and reduced lymphocyte count present at Day 5 of a TBF reaction or the developing neutropenia at Day 9 might be more important. Consequently a mid-point (Day 7) was selected for the day of infection with P. haemolytica. Taking into consideration the findings reported elsewhere in this thesis and those of Davies and Penwarden (1981), Batungbacal and Scott (1982b) and Munro et al (1982), it would be interesting to repeat this experiment, introducing P. haemolytica infection on Day 5 of a TBF reaction in an attempt to ascertain whether timing is important to the opportunist pathogen.

Summary

Specific pathogen free lambs infected with an aerosol of P. haemolytica serotype A2 became clinically ill and the majority died of pneumonic pasteurellosis, regardless of whether or not a concurrent TBF infection was present. Examination of the scores for lung lesions and clinical signs however revealed that the scores were slightly higher in lambs with concurrent tick-borne fever. In a second experiment designed to confirm this effect, specific pathogen free

lambs were infected with an aerosol of Pasteurella haemolytica serotype A1 on Day 7 of a TBF reaction. These lambs had combined higher clinical signs and lung lesion scores than control lambs infected with either agent alone, indicating that concurrent tick-borne fever had depressed the animals' immune response to P. haemolytica.

TABLE 6a

Results of infection with *P. haemolytica* serotype A2 and TBF

The degree of experimental pasteurellosis produced by aerosol inhalation of serotype A2 in normal lambs compared with lambs undergoing a TBF reaction.

Infection Group	Lamb No.	Clinical Score (Day 8-12)	Lung Lesion Score	Total Score
P. haemolytica on Day 7 (serotype A2)	8	13	20	33
	11	1	10	11
	19	14	5	19
	27	14	20	34
	39	0	5	5
	47	7	20	27
	60	15	10	25
Group mean scores (+ S.D.)		9.14 (+6.47)	12.86 (+6.99)	22.0 (+10.94)
TBF on Day 0 *P. haemolytica* on Day 7 (serotype A2)	25	10	20	30
	36	12	20	32
	46	5	20	25
	55	15	10	25
	64	18	20	38
	71	14	20	34
	81	0	0	0
	84	17	10	27
Group mean scores (+ S.D.)		11.38 (+6.19)	15.0 (+7.56)	26.38 (+11.58)
TBF on Day 0	7	0	0	0
	18	1	0	1

TABLE 6b

Results of Infection with *P. haemolytica* serotype A1 and TBF

The degree of experimental pasteurellosis produced by aerosol inhalation of serotype A1 in normal lambs compared with lambs undergoing a TBF reaction.

Infection Group	Lamb No.	Clinical Score (Day 8-12)	Lung Lesion Score	Total Score
P. haemolytica on Day 7 (serotype A1)	704	1	0	1
	708	2	0	2
	709	0	0	0
	711	0	0	0
	713	0	5	5
	714	0	5	5
	717	0	0	1
Group mean scores (+ S.D.)		0.57 (+0.79)	1.43 (+2.44)	2.0 (+2.16)
TBF on Day 0 *P. haemolytica* on day 7 (serotype A1)	701	2	0	2
	703	2	5	7
	706	8	5	13
	707	6	5	11
	710	1	20	21
	712	13	11	23
	716	8	5	13
Group mean scores (+ S.D.)		5.71 (+4.35)	7.14 (+6.36)	12.86 (+7.36)

CHAPTER 7

STUDIES ON THE ROLE OF TICK-BORNE FEVER IN EXPERIMENTALLY INDUCED TICK PYAEMIA

Introduction

One of the most widely recognised secondary infections linked with tick-borne fever is tick pyaemia (or Enzootic Staphylococcosis). Consequently it was felt that some experimental work should be carried out in an attempt to define more clearly the role of TBF in the condition and to provide some basic information related to the field work described later.

Although intravenous inoculation of normal lambs with large doses of S.aureus had been shown to result in pyaemic lesions fairly regularly (Foggie, 1948; McDiarmid, 1948), if inoculation was carried out in animals during the neutropenic phase of a TBF reaction, the dose of staphylococci required was found to be much lower (Foggie, 1956). Other routes of staphylococcal inoculation such as intradermal or subcutaneous injection had failed to result in pyaemic lesions in lambs at various stages during TBF reactions (Taylor et al, 1941; Foggie, 1957).

The experimental work described below was undertaken to attempt to reproduce tick pyaemia and to assess the role of TBF in its aetiology.

Bacteriological materials and methods

(a) Strains of Staphylococcus aureus

The strains of S. aureus used in the following experimental infections were all isolated from naturally occurring cases of tick pyaemia from hill sheep farms on the Island of Arran, except for one strain kindly provided by Dr. B.N.J. Parker (Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratories, Weybridge) which

was isolated from skin lesions in lambs with Pustular Dermatitis. The strains were given laboratory strain numbers and these are listed below along with the origin of the isolate.

Origin of ovine strains of *S. aureus*

S. aureus Strain No.	Source of original sample	Farm or area of origin
3	Pus from joint lesion	Farm A2, Arran
9	Pus from joint lesion	Farm A1, Arran
10	Pus from fetlock joint lesion	Farm A3, Arran
12	Pus from joint lesion	Farm A5, Arran
C149	Purulent skin lesions of suckling lambs with Pustular Dermatitis	Central Veterinary Laboratories, Weybridge

(b) Preparation and storage of staphylococcal stabilates for challenge inoculation of sheep

Stage 1. Isolation of *S. aureus*

The original samples of purulent material containing *S. aureus* had been stored in liquid nitrogen at -196°C. These were thawed at 37°C and 10 ml amounts of Nutrient Broth (Oxoid Ltd, Basingstoke, England) were inoculated with a loopful of the pus. The infected nutrient broths were incubated for 18-24 hours at 37°C, after which each culture was used to inoculate a series of three media - Nutrient Agar (Oxoid Ltd), Mannitol Salt Agar (Gibco Europe Ltd, Paisley, Scotland) and 10% Sheep Blood Agar (see Appendix). After 24-48 hours

incubation at 37°C the cultures were checked for the presence of colonies of Gram-positive cocci by Gram's staining method. Confirmed colonies of Gram-positive cocci were then subcultured onto the same three media as above and incubated for 24 hours at 37°C. The resulting cultures were checked for purity by examining several colonies by Gram's staining method and performing the Coagulase Test (Coagulase Test method in Appendix).

Stage 2. Preparation of pure inocula of S. aureus

Three or four morphologically similar colonies from the pure cultures of S. aureus prepared on sheep-blood agar in Stage 1 above were inoculated into 15 ml amounts of sterile nutrient broth and incubated at 37°C for 18 hours. 2.5 ml of these starter cultures were seeded into 50 ml amounts of pre-warmed Bernheimer and Schwartz medium (see Appendix) contained in 250 ml flanged Erlenmeyer flasks and incubated at 37°C in an orbital shaking incubator operating at 150 rpm. After 24 hours incubation the cultures were centrifuged in sterile bottles at 17000 g for 10 minutes at 4°C. The bacterial pellets were resuspended in 50 ml of sterile diluent and the centrifugation and washing procedure was repeated once. The washed pellets of cells were resuspended in a volume of diluent to a concentration of approximately 10^{10} organisms/ml which was measured spectrophotometrically. The viable count of the inocula were estimated in terms of colony forming units per ml (cfu/ml) using the method of Postgate (1969).

The diluent used in Stage 2 depended on whether the inoculum was to be administered by the intravenous or intradermal route. Sterile normal saline, 0.85% (Oxoid Ltd) was the diluent for intravenous

inocula while sterile peptone water prepared on a 10% w/v basis (Oxoid Ltd) was the diluent for intradermal inocula.

Stage 3 Storage of S. aureus inocula

The suspensions of S. aureus prepared in Stage 2 were dispensed asceptically in sterile 2 ml ampoules (Flow Laboratories Ltd, Irvine, Scotland) which were sealed and stored immersed in liquid nitrogen at -196°C. One ampoule was used for each experimental infection, thus ensuring that a standard challenge dose was used in each experiment. The ampoule was removed from the liquid nitrogen, thawed at 37°C and diluted to the appropriate cell concentration using the diluent relevant to the proposed route of inoculation.

(c) Isolation and identification of S. aureus from post-mortem samples

Collection of samples

Two methods of collection were used and their use depended on the volume of pus present in a lesion. Where there was a large volume of pus a sample of pus was aspirated into a sterile syringe and stored in the syringe at 4°C until cultured. Where there was little or no pus or it was desired to sample the cut surface of an organ, the area was swabbed using a commercial transport swab system (Exogen Ltd, Clydebank, Scotland) in which the swab was stored in sterile Amies Transport Medium. The swabs were stored in their medium at 4°C until cultured.

Culture of samples

Material from post-mortems was cultured using the method described in section (b) Stage 1 for the isolation and identification of S. aureus. Pus samples went through the whole procedure of Stage 1 while swab samples were inoculated directly onto the three culture media and the method continued from that point.

Experimental work

Preliminary experiment to assess the virulence of S. aureus strains and the dosage required for pathogenic effects

Introduction

A preliminary experiment was set up to examine two criteria on which future work would be based. Firstly, which strain of staphylococci would be the most likely to result in pyaemic lesions and secondly, at which stage of the TBF reaction was inoculation of staphylococci most likely to produce pyaemic lesions.

Materials and Methods

Strains 3, 9, 10 and 12 of Staphylococcus aureus, whose origins are described above, were inoculated intradermally at 5 different dose rates into each of 4 lambs approximately 7 months old. The inoculations were carried out on the clipped belly of the lambs in a single longitudinal row for each strain, starting with a dose of 10^4 organisms and going in steps of 10^1 up to 10^8. All lambs also received a control intradermal inoculation of peptone water, the diluent used. Two of the lambs received S. aureus alone while the

other two were inoculated with TBF intravenously on the same day.

Results

On the day following inoculation all strains at concentrations of 10^6, 10^7 and 10^8 had produced a blue-coloured bruise-type lesion at the site of inoculation while at the lower doses either a small pustule had formed or there was no reaction. Two days later all the animals had developed a severe cellulitis extending over the whole abdomen, which subsequently developed into large subcutaneous abscessess.

Conclusion

There was unfortunately, a confluence of lesions from which it was impossible to determine differences between strains or dosages. It was therefore decided to repeat the experiment using only the lower concentrations of S. aureus.

Experiment 7a:

Assessment of the virulence of S. aureus strains and the degree required for pathogenic effects following intradermal inoculation

Materials and Methods

Lambs were again given 4 strains of S. aureus as above but at concentrations of 10^4, 10^5 and 10^6 organisms. Eight 7 month old lambs were inoculated in 4 groups of 2 animals as described below.

Group 1 - Intradermal S. aureus only

Group2 - Intradermal S. aureus and intravenous TBF coincidentally

Group 3 - Intradermal S. aureus 5 days after intravenous TBF

Group 4 - Intradermal S. aureus 9 days after intravenous TBF

Results

Of the four staphylococcal isolates tested, one strain of staphylococci (strain 9) appeared to be more virulent than the others, strains 3 and 12 were similar in severity while strain 10 rarely resulted in any lesions.

The intradermal lesions in each group of lambs are summarised below:

Group 1: in both lambs all except strain 10 resulted in intradermal abscess formation ranging in diameter from 0.2 cm at the lower dose up to 2.5 cm at the highest dose. Strain 9 produced the largest reaction - 2.5 cm at dose 10^6.

Group 2: similar reactions to those of Group 1, once again strain 10 showing little effect, only producing a 0.3 cm diameter abscess at the 10^6 dose. Strain 9 caused abscessess ranging in size from 1 cm up to 2.5 cm at the high dose. Strains 3 and 12 caused abscesses from 0.5 cm up to 1 cm at the high dose.

Group 3: both lambs developed a diffuse, purple coloured swelling within 24 hours which progressively thickened, spread across the whole abdomen and became firm and cellular in nature. It was not possible to differentiate between doses or strains because of the cellulitis. At post-mortem examination the skin was underrun with pus and areas of skin were beginning to slough.

Group 4: one lamb developed a similar cellulitis-type lesion to those in Group 3, although it was of a less severe nature. The other lamb showed no reaction to strain 10, strain 9 caused abscesses ranging from 1 cm to 2 cm while strains 3 and 12 only resulted in abscess formation of 1.5 and 1.0 cm respectively at the highest dose.

All the animals were post-mortemed 26 days after S. aureus inoculation by which time the intradermal lesions were healed in all except those animals which had developed cellulitis. The latter still had pus under-running the abdominal skin and skin was starting to slough in some areas. There was no evidence of pyaemic (or systemic) spread of staphylococci.

Conclusions

Infection, therefore, appeared to be most severe in animals inoculated with staphylococci 5 days after TBF inoculation, while intradermal inoculation 9 days into a TBF reaction resulted in less severe lesions. Inoculation of staphylococci at the same time as TBF produced the mildest lesions which were of a similar order to those caused in lambs given staphylococci only.

This experiment therefore suggested that staphylococcal strain 9 was the most virulent and it was this one that was used in all subsequent experimental work in attempts to reproduce tick pyaemia. It also suggested that the lesions might be more severe in natural infections when inoculation took place during a TBF reaction, especially when the staphylococci were introduced 5 days after TBF infection. It was therefore decided to repeat the experiment using younger lambs around the age when field cases of pyaemia occurred and to use only Strain 9 as the inoculum.

Experiment 7b.

Intradermal inoculation of S. aureus at various times during the course of a TBF reaction

Materials and Methods

A total of 16 lambs aged 2-3 weeks were divided into 4 groups of 4. Only one strain of staphylococci was used (strain 9) and it was inoculated at a dose of 10^5, 10^4 and 10^3 organisms in 0.1 ml of peptone water in duplicate rows on each lamb's clipped belly. A

peptone water inoculation was also made separately on each lamb as a control. At the time of intradermal inoculation some groups of lambs were at varying stages of TBF infections as detailed below.

Group 1 intradermal S. aureus only

Group 2 intradermal S. aureus and TBF coincidentally

Group 3 intradermal S. aureus 5 days after TBF

Group 4 intradermal S. aureus 9 days after TBF

Results

The results are summarised in Table 7a. Post-mortem examinations were carried out when the lambs were slaughtered 16 days after staphylococcal inoculation except in the case of lambs 36 and 23 which died 2 and 9 days respectively after S. aureus inoculation.

Intradermal reactions were evident within 24 hours and by 48 hours after inoculation lesions were present. The intradermal abscesses produced were in some cases topped by pustules and in others appeared to lie more deeply in the dermis. The majority of the abscesses came to a head and burst, releasing thick creamy pus and subsequently healed by granulation. By the time the lambs were killed the intradermal lesions were almost healed.

At post-mortem 3 lambs were found to have pneumonic lesions and another 3 had evidence of systemic spread of staphylococci. The pneumonic cases all had collapse and fibrosis of the apical and intermediate lobes of the lungs with in addition some consolidation in ventral areas of the diaphragmatic lobes. There were fibrinous adhesions to the chest wall and between areas of lung. No abscesses were present in the lungs.

Lamb 33 had only one internal abscess which was situated at the diaphragmatic border of the liver and adhered to the diaphragm. The abscess was thick-walled, filled with thick caseous pus and 2 cm in diameter. Lamb 23 died 9 days after staphylococcal inoculation following a 5 day period during which it was noted to be dull, anorexic and losing condition. At post-mortem there were multiple small (0.2 cm diameter) abscesses scattered over the superficial muscles of the lamb in the connective tissue and there were also three larger (1 cm diameter) abscesses in the connective tissue on the brisket, left thoracic wall and dorsal to the left shoulder joint. In addition both kidneys were swollen and almost liquified internally and the right kidney had pus lying between the capsule and the kidney tissue. There was pleurisy in the left side of the chest, both lungs were peppered with small 1-2 mm abscesses and there was a very severe pericarditis. Lamb 24 had bilateral pleurisy and both pairs of apical and intermediate lobes were collapsed and fibrous and contained numerous small abscesses 1-3 mm in diameter. In addition the left diaphragmatic lobe had two abscesses of 2 cm diameter at its caudal border.

Intradermal inoculation of S. aureus coincidentally with TBF or on its own appeared to provoke no response either at the site of inoculation or systemically. In contrast, marked lesions were produced at the inoculation site when given on Day 5 or Day 9 of a TBF reaction with the results being slightly more severe in the Day 9 group. In addition to marked local lesions the latter two groups also showed evidence of systemic spread with abscess formation in other areas unrelated to the site of inoculation. The animals in the Day 9

group were more severely affected in this respect, 2 out of 3 showing pyaemic spread while only 1 in 4 of the Day 5 group became pyaemic.

Conclusion

It was possible to cause pyaemic lesions following intradermal inoculation with S. aureus, although these only occurred when inoculation took place while the animal was undergoing a TBF reaction.

Experiment 7c.

Intravenous inoculation of S. aureus in normal 6 month old lambs and 6 month old lambs on Day 5 of a TBF reaction

Introduction

The preliminary work (Expt. 7a) on intradermal S. aureus inoculation had shown that Day 5 of a TBF reaction appeared to result in the most severe local lesions. It had also shown that S. aureus strain 9 was the most virulent and this strain was therefore used in all subsequent staphylococcal inoculations. Consequently in a preliminary experiment to assess the feasibility of producing pyaemia following intravenous inoculation of S. aureus it was decided to give the staphylococci on Day 5 of a TBF reaction.

Materials and Methods

Six 6-month old lambs were split into two groups of 3, the first group being inoculated with TBF on Day 0 and 5 days later both groups were intravenously inoculated with a dose of 2×10^5 S. aureus.

Sixteen days later (on Day 21) all animals were necropsied.

Results

The results are presented in Table 7b. Multiple pyaemic abscesses occurred in two out of three of the TBF-inoculated lambs but only a single abscess was found in one animal in the group which did not get TBF.

Conclusions

The results of this experiment, tempered by the small numbers of lambs used, indicated that pyaemia was exacerbated in the presence of a concurrent TBF reaction since not only did more lambs develop pyaemic lesions but those lesions were much more widespread than in the animal given S. aureus only.

Experiment 7d.

Intravenous inoculation of S. aureus in normal 2-3 month-old lambs and 2-3 month-old lambs on Day 5 of a TBF reaction

Introduction

Although pyaemia had been successfully induced in Experiment 7c, it was decided to repeat the experiment using younger lambs to confirm the result and to check that young lambs were equally susceptible to infection under the same conditions.

Materials and Methods

Eight lambs aged 10-12 weeks were divided into two groups of 4, the first group being inoculated with TBF on Day 0 and 5 days later all 8 lambs were intravenously inoculated with a dose of 2 X 10^5 S. aureus. All lambs were necropsied 16 days later (on Day 21).

Results

The results are presented in Table 7c. All four lambs inoculated with S. aureus on Day 5 of a TBF reaction developed pyaemic abscesses while only two of four lambs inoculated with the bacteria alone did so.

Conclusions

As in Experiment 7c the inoculation of S. aureus during a TBF reaction resulted in more cases of pyaemia than when the bacteria were given alone.

Experiment 7e.

Intravenous inoculation of S. aureus in 2-3 week-old lambs at various times during the course of a TBF reaction

Introduction

Following the outcome of Experiment 7b in which the intradermal inoculation of S. aureus into lambs in the neutropenic phase of a TBF reaction (i.e. on Day 9) resulted in a greater production of pyaemic

lesions than inoculation on Day 5, it was decided to vary the timing of intravenous inoculation of S. aureus in relation to TBF.

Materials and Methods

Sixteen lambs aged 2-3 weeks were split into four groups of 4 and treated as below. The TBF inoculations were staggered and the S. aureus inoculations all took place on the same day. However for ease of comparison with other experiments it would be preferable to consider the day of TBF inoculation as Day 0 in all groups and to alter the day of staphylococcal inoculation accordingly.

Group 1 S. aureus 2×10^5

Group 2 S. aureus 2×10^5 and TBF intravenously on Day 0 (coincident infection)

Group 3 S. aureus 2×10^5 intravenously on Day 5 (5 days after TBF infection)

Group 4 S. aureus 2×10^5 intravenously on Day 9 (9 days after TBF infection)

All lambs were necropsied 16 days after staphylococcal inoculation except for those which died earlier and were examined on the day of death.

Results

The results of the experiment are presented in Table 7d.

Conclusions

The results, tempered by the small numbers of animals in the groups, indicate that the production of pyaemic lesions in the experimental lambs occurred most readily when the staphylococci were inoculated on Day 5 of the TBF reaction rather than in the neutropenic phase (Day 9). The lesions produced in animals inoculated on Day 5 were also more systemically widespread than those in the other groups of lambs.

Experiment 7f.

Intravenous inoculation of S. aureus in 2-3 week-old lambs prior to inoculation with tick-borne fever

Introduction

The exact time relationship between staphylococcal infection and TBF inoculation in the field situation has never been established. In the previous experiments the bacterial infection had been introduced either coincidentally with TBF or following it. This protocol was based on the generally accepted theory that the staphylococci were introduced into the tissues via the tick bite.

In this experiment it was decided to examine a different theory which presupposed that S. aureus may be present from around birth as a latent infection and tick pyaemia may in fact be a result of these latent foci flaring up following tick-borne fever infection. According to Anderson (personal communication) many staphylococcal infections arise when latency is disturbed by some immunosuppressive

factor.

In order to achieve a close similarity to field conditions it was decided to give multiple infections with different strains of TBF to simulate what might occur in lambs on tick-infested pastures.

Materials and Methods

Twelve lambs aged 2-3 weeks were split into two groups of 6, all of whom were intravenously inoculated with S. aureus (2 X 10^5 organisms) on Day -7. The first group received no further infection, the second group were inoculated with the usual strain of TBF (Strain A) on Day 0, followed by inoculation with TBF strains C, H and M on Days 5, 10 and 15. Apart from two lambs killed early due to severe lameness the remainder were necropsied 35 days after staphylococcal infection.

Results

The results are presented in Table 7e. The multiple infections with four different isolates of TBF resulted in what appeared to be a normal TBF reaction to the first strain inoculated, with a normal length and level of parasitaemia and pyrexia, with no further bouts of fever or parasitaemia in response to subsequent inoculations of the other strains.

Both the lambs which developed joint lesions in the TBF group became lame before tick-borne fever was inoculated, although the lameness progressively worsened after TBF. The day of onset of development of the brisket abscess and the small subcutaneous abcess

was unknown.

Conclusions

The results of this small experiment did not lend unequivocal support to the theory that a latent staphylococcal infection could be induced to become active following TBF infection. However before it could be dismissed as a viable theory more extensive experimentation would be necessary.

Experiment 7g.

Intravenous inoculation of a strain of S. aureus from a case of staphylococcal dermatitis at various times during the course of a TBF reaction

Introduction

Up until this experiment all the strains of S. aureus used in infections originated from samples of pus from naturally pyaemic lambs. In view of the fact that sheep can suffer from ovine staphylococcal dermatitis characterised by deep necrotic skin ulceration with no systemic involvement, it was thought interesting to attempt to reproduce pyaemia in young lambs with a strain of S. aureus from a natural outbreak of staphylococcal dermatitis. This isolate was kindly provided by Dr B.N.J. Parker, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratories, Weybridge, Surrey.

Materials and Methods

The experiment was designed similarly to Expt. 7e with S. aureus being inoculated at varying times after TBF. The TBF inoculations were staggered while all the groups of lambs were inoculated intravenously with S. aureus on the same day. However for ease of comparison with other experiments the terminology used in naming the days of infection with either agent indicates that the TBF infections all took place on the same day (Day 0) while the staphylococcal inoculations were staggered. The sheep used were 2-month old lambs divided into three groups of 3 and one group of 2 and inoculated as below.

Group 1 - S. aureus 2 X 10^5 intravenously on Day 0

Group2- S. aureus 2 X 10^5 intravenously and TBF on Day 0

Group 3 - S. aureus 2 X 10^5 intravenously on Day 5 (5 days after TBF infection)

Group 4 - S. aureus 2 X 10^5 intravenously on Day 9 (9 days after TBF infection)

The lambs were all necropsied 16 days after staphylococcal inoculation.

Results

The results are presented in Table 7f. Fewer pyaemic lesions resulted from inoculation of the same number of organisms of the dermatitis strain of S. aureus compared with the pyaemic strain used previously. The lesions produced were of a fairly mild nature, especially those in the lungs which were small (0.3 and 0.5 cm in

diameter) and heavily encapsulated.

Conclusions

This would indicate that strains of S. aureus associated with staphylococcal dermatitis are probably unlikely to be responsible for tick pyaemia since under the most favourable experimental conditions, evidence of systemic spread was negligible. The lesions produced occurred only in the group infected at Day 5 of the TBF reaction, concurring with the results of the previous experiments that intravenous inoculation of staphylococci on the fifth day of a TBF reaction was most likely to result in pyaemic lesions.

Summary of the experiments carried out with reference to the reproducibility of the pyaemic abscesses and the sites in which these occurred

In order to summarise the experimental reproduction of tick pyaemia following intravenous inoculations of S. aureus at varying times in relationship to tick-borne fever infection, Table 7g was prepared. It was made up from the results of Expts. 7c, 7d, 7e, 7f and 7g.

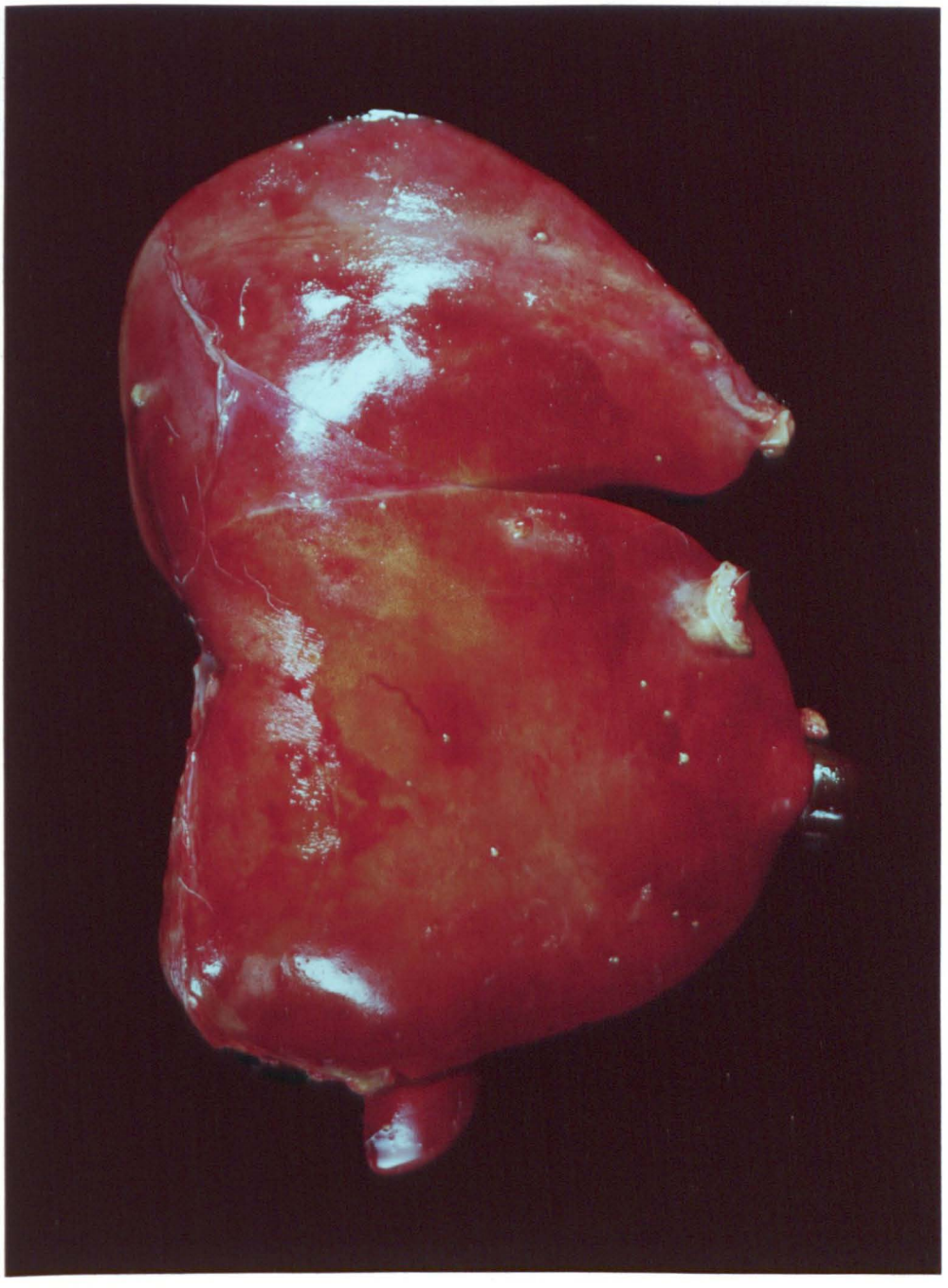
The proportion of animals which became pyaemic following S. aureus infection on Day 5 of a TBF reaction was much greater than at other times in the TBF reaction or when bacterial infection took place alone. Taking all the animals in which TBF was involved along with S. aureus the proportion which became pyaemic was 16/28 (57%) compared with only 4/20 (20%) pyaemic when S. aureus was given on its

own, and this difference was highly significant ($P < 0.001$). There were no significant differences between the numbers of lambs which developed pyaemia when inoculated with S. aureus alone, at the same time as TBF or 9 days after TBF inoculation ($P > 0.05$). However there was a highly significant difference between the numbers that developed pyaemia following S. aureus alone and those given S. aureus on Day 5 of a TBF reaction ($P < 0.001$). There was a less significant difference between the numbers that developed pyaemia following the inoculation of S. aureus on day 5 of a TBF reaction and those inoculated with S. aureus on either Day 0 or Day 9.

To summarise the sites at which lesions occurred following intravenous S. aureus infection, with a strain originally isolated from a natural case of tick pyaemia, the data from Expts. 7c, 7d, 7e, and 7f were amalgamated and are presented in Table 7h. The most common sites for lesions to occur in these experiments were the lungs, followed by the joints or bones. Lesions in the nervous system or associated with the clinical manifestation of nervous signs were rare, as were lesions in the kidneys or liver.

Characteristic lesions are shown in Plates 3, 4 and 5. Plate 3 shows an affected liver with abcesses dotted over its surface, some of which have formed adhesions to the diaphragm. A number of the smaller lesions were firm and calcified in nature. Plate 4 shows an affected spleen with pyaemic lesions both on the surface and in the substance of the organ. The largest surface abcess present adhered to the peritoneum. Plate 5 shows an affected carpal joint with a markedly thickened hypertrophic joint capsule containing areas of organised pus. These examples of lesions were all from lambs killed 16 days after S. aureus inoculation in the above series of experiments.

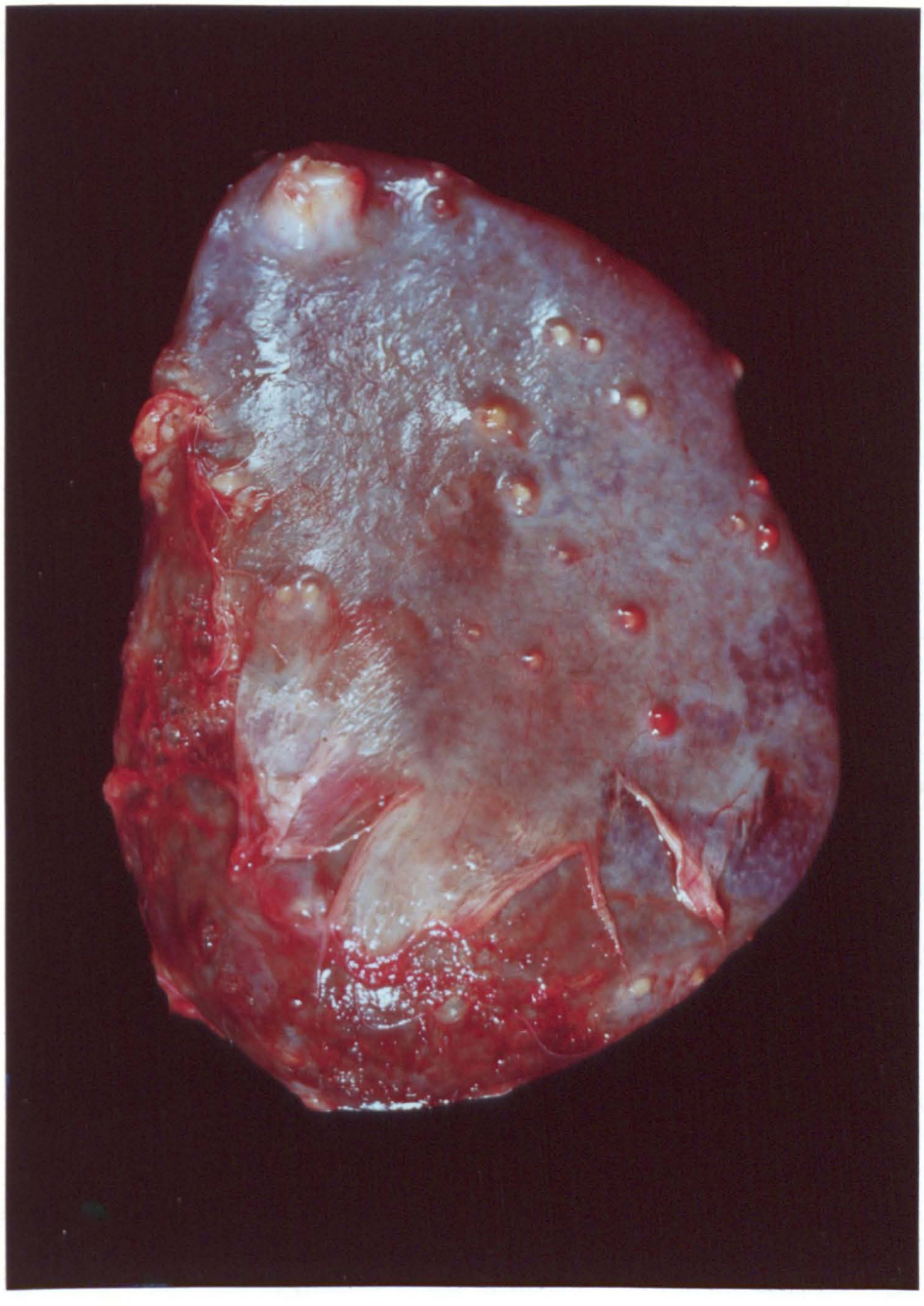
Plate 3



Experimentally induced pyaemic lesions in lambs

- an affected liver

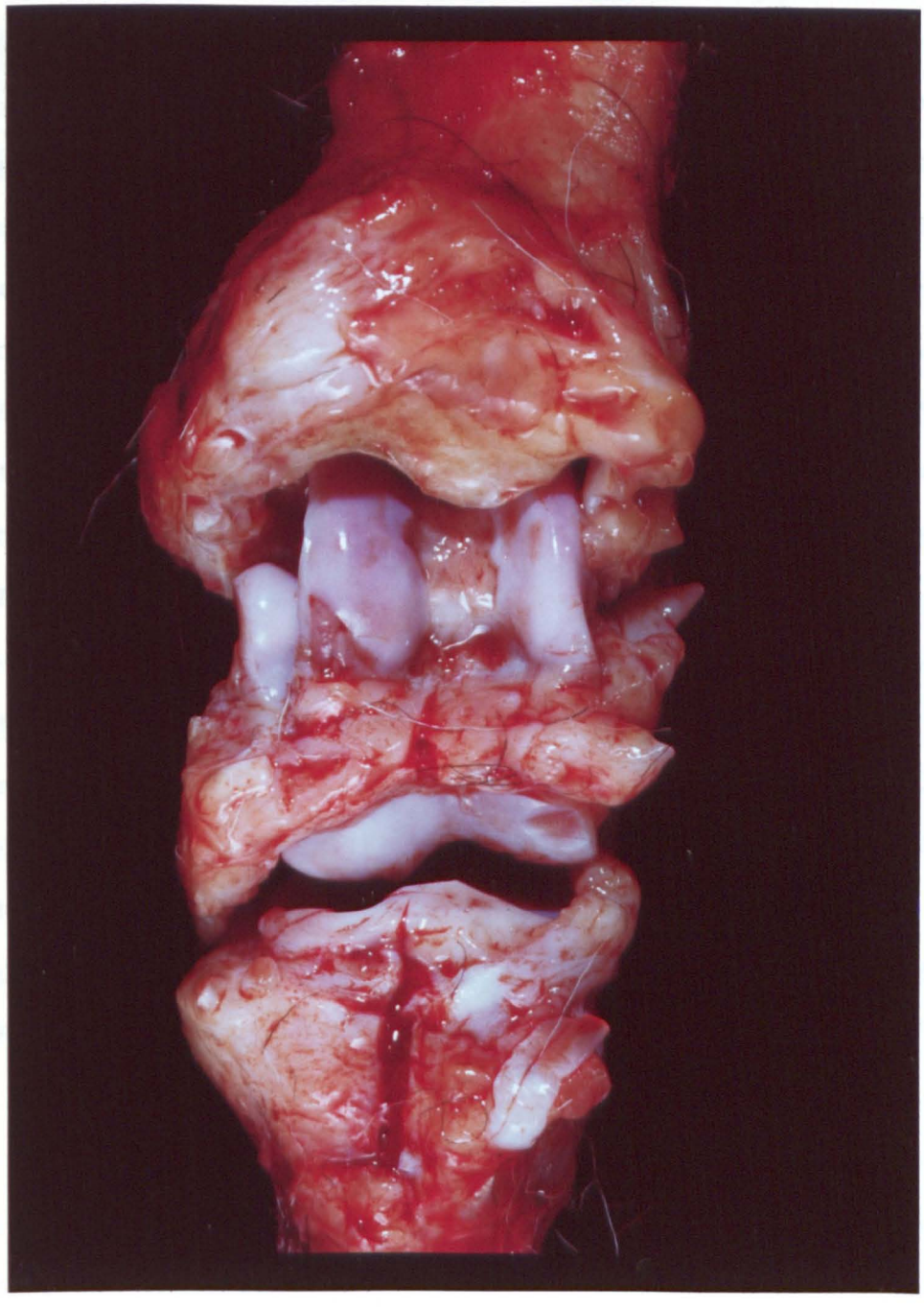
Plate 4



Experimentally induced pyaemic lesions in lambs

- an affected spleen.

Plate 5



Experimentally induced pyaemic lesions in lambs

- an affected carpal joint.

Discussion

For many years in the study of the aetiology of tick pyaemia, investigators have tried by various methods to reproduce the disease under experimental conditions. Most recognised an association between tick-borne fever and tick pyaemia and introduced it into the experimental design. The work carried out in the present study has shown that animals undergoing a TBF reaction were indeed more susceptible to staphylococcal infection than those free from TBF. Furthermore the degree of susceptibility appeared to be related to the point during a TBF reaction at which the staphylococcal infection was introduced. From Table 7g showing the results of intravenous (i/v) inoculation of *S. aureus* at various times in relation to the course of TBF reactions, it is apparent that by far the highest proportion of pyaemia cases resulted when bacteria were inoculated 5 days after *Cytoecetes phagocytophila* inoculation. In fact 12 of 14 lambs inoculated at this time developed pyaemia, compared with 4 of 20 lambs inoculated with the same dose in the absence of TBF infection. Otherwise there was little difference between the incidence of pyaemia amongst lambs inoculated with staphylococci, either concomitantly with TBF or 9 days after TBF, and those inoculated with *S. aureus* only.

In common with other workers, the development of pyaemia was by no means certain, even under the optimal conditions described above. In only two experiments (Expts. 7d and 7e) did 100% of the lambs in any group become pyaemic while in the remainder, despite all conditions within each group being equal, the results were variable. Similarly Foggie, in his extensive studies on the experimental production of pyaemia, with and without concurrent TBF infection,

found that animals were variable in their susceptibility to S. aureus infection (Foggie, 1948; 1956).

Foggie (1956) believed that the role of TBF in increasing the susceptibility of lambs to S. aureus resulted from the neutropenic state induced by the former. The results of the present study are somewhat at variance from Foggie's findings, in that lambs inoculated with S. aureus during the neutropenic phase of a TBF reaction (i.e. those inoculated 9 days into a TBF reaction) were found to be little more susceptible than lambs inoculated with S. aureus either simulataneously with, or in the absence of TBF. However the dosage and strain of S. aureus was different from that employed by Foggie so a straight comparison is difficult. In addition, Foggie did not examine the effects of staphylococcal inoculation around the peak parasitaemic phase of a TBF reaction (i.e. at Day 5 as in the present study).

Almost certainly the quantity of S. aureus inoculated will influence the incidence of pyaemia and in an attempt to quantify this, Foggie carried out experiments in 1956 and 1957 inoculating varying doses of staphylococci intravenously following which the control animals (those not undergoing TBF reactions) were unaffected at all doses while almost all the animals inoculated during the neutropenic phase of TBF developed pyaemic lesions; however he did not comment on whether the pyaemic lesions were more severe at the higher dosages or not. In contrast, in the present study it was found that although a higher proportion of animals became pyaemic if inoculated during a TBF infetion than animals uninfected with TBF, a number of such control lambs also developed pyaemia. Foggie used lower doses of S. aureus than were used here and it is possible that if a lower infective

dosage, which would not cause pyaemia in the control lambs, had been used in the present study a more clear-cut picture of the influence of TBF on staphylococcal infection could have been obtained. However it was felt that it was better to continue to use the same dose of S. aureus that had been used in the first i/v experiment (Expt. 7c) in order to keep some factors constant throughout the study.

In earlier work in 1948, Foggie had tried to quantify doses of S. aureus for pyaemia production in lambs of varying ages where TBF was not involved, and had concluded that young lambs required a lower dose of staphylococci to cause pyaemic lesions than older lambs; he also found that if very large doses of bacteria were adminstered intravenously, pyaemia would be the inevitable result, even in older animals. The results were judged on lambs showing clinical signs of pyaemia within 5 days of inoculation which persisted for at least 7 days. Since the most obvious sign of pyaemic infection is often taken to be lameness due to joint invasion it is probable that this was the criterion which Foggie used. However it has been shown, both in the present study and in the literature (McDiarmid, 1946a; Watson, 1964a; Foster and Cameron, 1968a), that almost symptomless cases of pyaemia may occur (at least in the early stages) due to internal abscess formation without joint involvement. Thus it is possible that in this study by Foggie, some pyaemic animals went unnoticed. Further evidence that increasing the infective dose of bacteria will increase the severity of the lesions was furnished by the results of the intradermal inoculation of S. aureus in the present study, which clearly showed the gradually increasing severity of lesions as the dose increased.

A further factor found to influence the incidence of pyaemia in the present study was the route of inoculation. In addition to the i/v route, the intradermal inoculation of S. aureus was attempted in an effort to simulate a possible tick bite inoculation. The results of this showed that the pyaemic spread of staphylococci could be the sequel of intradermal inoculation. However this only occurred when the bacteria were inoculated into animals during the course of a TBF reaction, particularly during the neutropenic phase (Day 9 inoculation). This production of pyaemic lesions following intradermal inoculation of S. aureus in lambs has not been recorded previously. Foggie (1956) inoculated both normal and neutropenic lambs with similar doses of S. aureus intradermally but in his experiment none developed pyaemic lesions. He later attempted to further simulate the field situation of tick inoculation by injecting a combination of staphylococci and tick salivary gland extract intradermally, but that too failed to result in pyaemia (Foggie, 1959).

The timing of staphylococcal invasion in relation to TBF infection in field cases remains obscure. Although lambs may become infected with ticks within the first 2 days of life (Foster and Cameron, 1968a), and have almost all become infected with TBF within the first 2 weeks of life (McEwen, 1947; Foster and Cameron, 1968a), the peak incidence of tick pyaemia is generally agreed to occur later, at about 3-8 weeks of age (Foggie, 1948). Thus it would appear that in the field the staphylococcal infection occurs somewhat later than the tick-borne fever reaction and this is discussed further in Chapter 9.

According to Anderson (personal communication) latent focci of staphylococci can exist and may cause infection when the animals'

defence mechanisms are compromised, for example, by tick-borne fever. The results of the experiment undertaken to examine this theory (Expt. 7f) were inconclusive and require further investigation.

Further aspects of the relative timing of TBF and pyaemia exist. As noted previously the most obvious sign of the clinical onset of pyaemic lesions is lameness, however not all animals affected with pyaemia suffer from joint lesions and thus in many cases the incubation period is not known. In the present study in cases where lameness became apparent, it was possible to relate this to the time after staphylococcal and TBF inoculation. Two animals given TBF and S. aureus simultaneously on Day 0 became lame 4 days later; five lambs inoculated with S. aureus on the fifth day of the TBF reaction (Day 5) became lame 5-7 days later; three animals infected with S. aureus in the absence of TBF infection became lame 6-8 days later. These results indicate that there may be a relatively short interval of 4-8 days between the intravenous inoculation of staphylococci and the onset of clinical signs in cases where bacteria localise in joints or muscles. This is similar to the 7-day interval recorded by McEwen (1947) in a field case and the 3-10 day intervals reported by Foster and Cameron (1968a) in a series of field cases. Foggie, in his experimental work on pyaemia in 1956 and 1957 does not mention when his experimental animals developed clinical signs of pyaemia prior to necropsy.

In an earlier study by McDiarmid (1948) seven lambs inoculated with a dose of approximately $3 \times 10^6 - 3 \times 10^7$ S. aureus organisms in the absence of TBF became listless and depressed within a few hours after inoculation and within 24 hours these signs became more marked,

impairment of joint movement and incoordination was noticed and all the lambs died within 3-7 days post-inoculation. This was a much more severe reaction than any in the present study and may have been due to the high dose of S. aureus - 15 to 150 times higher than that used in the present study. In the same experiment, two lambs given even higher doses of staphylococci succumbed to acute septicaemia and died within 31 hours of inoculation.

In the literature regarding field cases of tick pyaemia, in all instances the affected lambs are young, up to about 3 months of age. There are no reports of older animals suffering from tick pyaemia and Foggie in 1956 suggested that Taylor and his colleagues' (1941) failure to produce pyaemia in lambs following subcutaneous inoculation of staphylococci could have been due to his experimental lambs being 3-4 months old, i.e. over the age at which the disease normally occurs in the field. Nonetheless, in the present study pyaemia was produced in lambs of 6 months of age (Expt. 7c), using the same dose of staphylococci as in younger animals. This finding is in contrast to Foggie's suggestion (1948) that younger animals are susceptible to lower doses of staphylococci than older animals. However it is possible that the doses of staphylococci administered in the present study were larger than those inoculated by Foggie, overcoming the resistance of the older sheep. In the field, the fact that the disease is confined to young animals may be due to a combination of factors such as their susceptibility to quite small inocula of S. aureus, the absence of antibodies to staphylococci (Foggie, 1948) and the prevalence of active TBF infection at this age. It is perhaps significant that older sheep introduced to tick areas for the first time, such as bought-in replacement hoggs or bought-in tups, may be

susceptible to tick-borne fever (Jamieson, 1950; Watson, 1964b) although there are no reports of the occurrence of pyaemia.

It is interesting to note that the occurrence of various lesion sites in the present study was slightly different from that found in the present field survey (Chapter 8) and field surveys carried out by others (McDiarmid, 1946a; Watson, 1964a) and experimental infections (McDiarmid, 1948). McDiarmid and Watson in their field surveys both found that the liver was the most consistently affected internal organ showing abscess formation, while in his laboratory studies McDiarmid (1948) reported that the lung was the most common site of internal abscess formation. In the present study, in agreement with McDiarmid (1948), the lungs were the most common site of internal abscess formation; however, in contrast with his findings the nervous system and spinal cord were rarely affected in experimental infection in this study. McDiarmid (1948) also noted that the liver was rarely a site of abscess formation in his experimental infection despite the fact that in 5/7 cases he isolated S. aureus from the liver. Staphylococcal isolation from apparently grossly uninfected organs was not attempted in the present study.

The differences in lesion distribution between the present experimental study and that of McDiarmid (1948) must be tempered by the facts that the latter study employed only 9 animals (of which two died of an acute septicaemic form of S. aureus infection) and the methodology of the experiments differed in several facets. Firstly, the dose of S. aureus used by McDiarmid was higher than that of the present study; secondly, the strains and sources of S. aureus differed although both were originally isolated from field cases of

pyaemia (McDiarmid's strain came from a liver lesion while that of the present study was cultured from a joint lesion); thirdly, in the present survey most of the lambs which developed pyaemia were also infected with TBF whereas TBF was not a factor in McDiarmid's experiment. Any or all of these factors may have influenced the site and severity of lesion formation.

Differences in lesion distribution between natural (field) and laboratory infection of S. aureus may, to a large extent depend on the basis for selection of the cases examined by necropsy. In laboratory experiments all subjects are generally necropsied while in the field they are generally selected on the basis of prior joint lesions and thus inapparent (or subclinical) field cases with internal abscess formation but no joint lesions would not be necropsied. It was shown in the present work that, among four strains of S. aureus isolated from joint lesions of field cases of pyaemia on Arran, the capacity to cause local lesions following intradermal inoculation was variable; thus it would not be surprising to find that their virulence following intravenous inoculation could also vary and, similarly, that isolates from other sources would vary.

Recently there has been some investigative work carried out on staphylococcal dermatitis in sheep (Fraser et al, 1982) and it was felt that it would be interesting to see whether such strains of staphylococci could be involved in pyaemia production. The results of the experiment carried out (Expt. 7g) again demonstrated that the i/v inoculation of one such strain of S. aureus during the febrile phase of a tick-borne fever reaction (i.e. on Day 5) was more likely to result in pyaemic lesions than bacterial inoculation alone or at other stages in a TBF reaction. However the pyaemic lesions were in general

less severe with this strain of staphylococci compared with those produced by the true "tick pyaemia" strain used in the other experiments. Nevertheless, the numbers of lambs involved were small and further studies would be necessary to confirm this.

Although an acute or septicaemic form of staphylococcal infection has been recorded both in experimental work (McDiarmid, 1948) and in field investigations (McDiarmid, 1946a; Foster and Cameron, 1968a) no such cases occurred in the present study, perhaps because the dose of S aureus used was low or it was of a less virulent strain than those involved in the acute cases reported.

Summary

In a series of experiments in lambs it was found that although both intradermal and intravenous inoculation of Staphlococcus aureus could cause pyaemic lesions in animals concurrently infected with tick-borne fever, the latter route more consistently resulted in lesions. A second factor influencing the occurrence of lesions was the relative timing of S. aureus inoculation during a TBF reaction. In the case of intravenous inoculation, the peak of parasitaemia (Day 5) was the optimal time for lesion production (86% of lambs affected), followed by Day 9 or Day 0 inoculation (29% of lambs affected) while S. aureus inoculation in the abscence of TBF infection resulted in lesions in only 20% of the lambs. This suggested that concurrent TBF infection had reduced the lambs' abilities to overcome a systemic bacterial infection.

The sites at which lesions occurred following experimental induction of tick pyaemia were examined and it was found that the most

commonly affected organs were the lungs (35% of animals had pyaemic lung lesions), followed by the joints (24%). It was noted that animals could have pyaemic lesions without joint involvement following experimental inoculation of S. aureus and presumably this could also occur in the field situation. This would suggest that the true field incidence of tick pyaemia may be higher than that reported, since such reports generally take into account only lame lambs.

The incidence of experimentally induced pyaemia was compared following the intravenous inoculation of two different strains of S. aureus. It was found that a strain of S. aureus isolated from a field case of tick pyaemia caused lesions more consistently than a strain from a case of staphylococcal dermatitis.

The incubation period between the inoculation of S. aureus and the onset of lameness indicating joint localisation was found to be relatively short, ranging from 4 to 8 days.

TABLE 7a

Results of Expt. 7b: Intradermal inoculation of S. aureus at various times during the course of a TBF reaction

Group	Sheep No.	Intradermal dose of S. aureus 10^3	10^4	10^5	Post-mortem findings
S. aureus on Day 0	22	NR*	NR	NR	NAD**
	32	NR	NR	NR	NAD
	37	NR	NR	NR	Pneumonia
	38	NR	NR	NR	Pneumonia
S. aureus and TBF on Day 0	26	NR	NR	NR	NAD
	29	NR	NR	NR	NAD
	30	NR	NR	NR	NAD
	31	NR	NR	NR	NAD
S. aureus on Day 5 of a TBF reaction	27	NR	Pustule	1 cm Abcess	NAD
	28	NR	NR	1 cm Abcess	NAD
	33	NR	NR	0.5 cm Abcess	2 cm liver abcess
	34	NR	NR	NR	NAD
S. aureus on Day 9 of a TBF reaction	23	NR	NR	1 cm Abcess	Multiple abcesses
	24	NR	NR	0.7 cm Abcess	Lung abcesses
	35	NR	1 cm abcess	3 cm Abcess	NAD
	36	Died 2 days post-inoculation of S. aureus			Pneumonia

* NR - no reaction

** NAD - No Abnormality detected

TABLE 7b

Results of Expt. 7c: Intravenous inoculation of *S. aureus* in normal 6-month old lambs on Day 5 of a TBF reaction

Group	Sheep No.	Clinical Signs	Site (and No.) of pyaemic lesions or abscesses	Presence (+) or absence(-) of pyaemia
S. aureus on Day 5	59	-	None	-
	67	-	None	-
	68	-	Pericardium (1)	+
TBF on Day 0 *S. aureus* on Day 5	51	-	None	-
	55	-	Diaphragm (1); spleen (1); lungs (multiple)	+
	56	Lame from Day 10	Hock joint (1) Lungs (multiple)	+

TABLE 7c

Results of Expt. 7d: Intravenous inoculation of *S. aureus* in normal 2-3 month old lambs and 2-3 month old lambs on Day 5 of a TBF reaction

Group	Sheep No.	Clinical Signs	Site (and No.) of pyaemic lesions or abscesses	Presence (+) or absence (-) of pyaemia
S. aureus on Day 5	20		None	-
	21		Lungs (1); Staph. pericarditis	+
	34	Lame from Day 8	Elbow joint (1); external thorax (1)	+
	37		None	-
TBF on Day 0, *S. aureus* on Day 5	19		Lungs (multiple)	+
	25	Lame from Day 12	Stifle joint (1)	+
	26		Lungs (multiple)	+
	33		Lungs (multiple)	+

TABLE 7d

Results of Expt 7e: Intravenous inoculation of *S. aureus* in 2-3 week old lambs at various times during the course of a TBF reaction

Group	Sheep	Clinical Effects	Site (and No.) of pyaemic lesions or abcesses	Presence (+) or absence (-) of pyaemia
S. aureus on Day 0	1	-	None	-
	4	-	None	-
	11	-	None	-
	20	-	Lung (1)	+
TBF on Day 0 *S. aureus* on Day 0	10	Lame from Day 4 Died on Day 9	Inner thoracic wall (1) and purulent pleural effusion	+
	13	Lame from Day 4	Hip joint (1) spleen (2)	+
	17	-	None	-
	18	-	None	-
TBF on Day 0 *S. aureus* on Day 5	6	Lame from Day 10	Biceps muscle (1) kidney cortex (1) Lungs (multiple)	+
	15	Lame from Day 10 Coughing from Day 19	Biceps muscle (1) Lungs (multiple)	+
	16	Lame from Day 11	Below wing of ilium (1); spleen (1); Quadriceps femoralis muscle (1) Lungs (multiple)	+
	19	Coughing from Day 19	Lungs (multiple)	+
TBF on Day 0 *S. aureus* on Day 9	2	Coughing from Day 19	Lungs (3)	+
	7	Coughing from Day 19	Liver (1); Lungs (multiple)	+
	9	-	None	-
	14	-	None	-

TABLE 7e

Results of Expt. 7f: Intravenous inoculation of *S. aureus* in 2-3 week old lambs prior to the inoculation of tick-borne fever.

Group	Sheep No.	Clinical Signs	Site (and No.) of Pyaemic lesions or abscesses	Presence (+) or absence(-) of pyaemia
S. aureus on Day 7	83	-	None	-
	90	-	None	-
	94	-	None	-
	102	-	None	-
	104	-	None	-
	107	-	None	-
S. aureus on Day -7, followed by TBF strains A,C,H and M on Days 0, 5, 10 and 15 respectively	85	-	Brisket (1) with sternal erosion	+
	92	-	None	-
	97	Lame from Day-1 killed D 8	Hip joint (1) with bone erosion	+
	100	-	Subcutaneous abscess (1)	+
	101	-	None	
	106	Lame from Day-1 killed D 8	Carpal joint (1); below wing of ileum (1) - bone erosion at both sites	+

TABLE 7f

Results of Expt. 7g: Intravenous inoculation of a strain of *S. aureus* from a case of staphylococcal dermatitis at various times during the course of a TBF reaction

Group	Sheep No.	Clinical Effects	Site (and no.) of pyaemic lesions or abscesses	Presence (+) or absence (-) of pyaemia
S. aureus on Day 0	71	-	None	-
	72	-	None	-
TBF on Day 0 *S. aureus* on Day 0	47	-	None	-
	51	-	None	-
	52	-	None	-
TBF on Day 0 *S. aureus* on Day 5	46	-	Subcutaneous on chest wall (1)	+
	49	-	Lungs (2)	+
	50	-	None	-
TBF on Day 0 *S. aureus* on Day 9	45	-	None	-
	48	-	None	-
	53	-	None	-

TABLE 7g

Summary of the incidence of pyaemic lesions following intravenous inoculation of *S. aureus* at various times during the course of a TBF reaction

Group	Number (%) of animals		Total
	Pyaemic	Unaffected	
S. aureus alone	4 (20%)	16 (80%)	20
S. aureus same day as TBF (Day 0)	2 (29%)	5 (71%)	7
S. aureus 5 days after TBF (Day 5)	12 (86%)	2 (14%)	14
S. aureus 9 days after TBF (Day 9)	2 (29%)	5 (71%)	7

TABLE 7h

The sites of pyaemic lesions following intravenous inoculation of *S. aureus* at varying times during the course of a TBF reaction

Site	No. of lambs (%)
Lungs	13 (35.1%)
Joints (or bone involvement)	9 (24.3%)
Muscles	3 (8.1%)
Spleen	3 (8.1%)
Subcutaneous tissue	3 (8.1%)
Pericardial sac	2 (5.4%)
Diaphragm	1 (2.7%)
Kidney	1 (2.7%)
Liver	1 (2.7%)
Pleural cavity	1 (2.7%)

CHAPTER 8

FIELD STUDIES CARRIED OUT TO ASSESS THE IMPORTANCE OF TICK PYAEMIA, TICK-BORNE FEVER AND RELATED CONDITIONS IN LAMB LOSSES IN S.W. SCOTLAND, INCLUDING AN INVESTIGATION OF SUITABLE PROPHYLACTIC MEASURES

Introduction

For many years the incidence and significance of tick-borne disease on hill sheep farms has remained a matter of some debate. In an attempt to assess the importance of tick pyaemia, tick-borne fever and related conditions in one area of Scotland the following field survey was undertaken over the three years from 1981 to 1983. All the farms in the survey were hill sheep farms whose ewes lambed on in-bye ground beginning in the middle of April, the ewes and lambs being held there for approximately the first 3-4 weeks of life before going out onto hill pastures. The farmers stated that they were losing a large number of lambs between the time the lambs are put out on to the hill and when they re-gather them 4-8 weeks later. They attributed most of these losses to tick-borne disease, in particular tick pyaemia.

A. PRELIMINARY FIELD SURVEY 1981

Introduction

During April to June 1981 preliminary studies were carried out on four hill sheep farms on the island of Arran off the west coast of Scotland. The farms selected were chosen on the advice of the local veterinary surgeon who considered that they were among the farms worst affected by tick pyaemia on the island.

The objectives of the initial study were firstly to assess the extent of the disease problem, both in terms of tick pyaemia and tick-borne fever and secondly to make some attempt at finding a satisfactory method of prophylaxis.

Materials and Methods

On the first visit to the farms on the 28th April, 1981, between 70 and 100 lambs on each farm were eartagged (Dalton's Rototags, Henley-on-Thames, England). One third of the tagged lambs on each farm were given blue tags and these were given a prophylactic treatment of 2 ml of a long-acting penicillin (Penidural L-A; Wyeth Laboratories, Maidenhead, England), the remaining two-thirds being untreated controls given yellow tags. In addition, heparinised blood samples were collected from a number of the tagged lambs on that day and also on further visits on 12th May, 27th May and 9th June. These samples were used to make thin blood smears which were later stained and examined for the presence of TBF organisms in the leucocytes. On the final visit to the farms for that year on 9th June an attempt was made to assess the number of lamb losses over the survey period by noting the numbers of tagged lambs which failed to return.

Results

Over the four blood samplings a total of 820 smears were collected. The percentage of smears found to be positive (i.e. TBF inclusions were seen in the leucocytes) was variable: on 28th April 11.4% were positive, on 12th May 19.3%, on 27th May 2.7% and on 9th June 2.4%). There was little difference in the level of TBF infection between lambs treated with Penidural L-A and the controls. The results of the numbers of lambs which returned in June are shown in Table 8a. The figures show very high levels of missing lambs on some farms - up to 37% in one case.

Discussion

If it was to be assumed that the failure of these lambs to return in June meant that they were dead then this would be an alarmingly high level of mortality. However no such conclusion may be drawn since it was certain that some ewes and lambs were not gathered by the farmers and, in addition to that, the figures for missing lambs includes those which had lost their eartags and were therefore unidentifiable. Nevertheless, in spite of these inaccuracies, the figures indicate that lamb losses from unknown causes were not inconsiderable. In view of the fact that these figures of non-returning lambs could not be assumed to represent lamb losses it was not possible to assess the effects of Penidural L-A prophylaxis on the incidence of disease. However, since the lambs were tagged with different coloured ear tags for treated and untreated groups, the farmers could differentiate between them and all the farmers remarked that the treated lambs appeared to have thrived better than the controls during the survey period from April to June.

The results of the 1981 survey suggested that by 4-6 weeks of age most lambs had been been infected with the agent of tick-borne fever regardless of whether Penidural L-A prophylaxis had been given. It should be noted however that a small percentage of older lambs on the hill were still susceptible to TBF infection until at least the 9th June. It also appeared that the method of assessing the benefits of prophylactic antibiotic cover based on the return of lambs in June was inadequate and so it was decided that in subsequent years the weight gains of lambs would be taken into account, since it might indicate the level of subclinical disease and provide a further index of the

benefits of treatment. In addition it was decided to stress to the farmers the importance of gathering as many of the sheep as possible for the final weighing and they were also asked to keep records of tagged lambs found dead or crippled over the survey period. The antibiotic prophylaxis was changed to a long-acting oxytetracycline (LAT) drug (Terramycin L/A: Pfizer Ltd, Kent, England) since experimental work had shown that it was effective against both the TBF organism (Scott and Horsburgh, 1982; experiments in Chapter 3) and strains of S. aureus isolated from pyaemic lesions from affected lambs on Arran (E. Leggett, personal communication).

B. 1982 FIELD SURVEY

Materials and Methods

The same four farms on Arran were involved in the field survey in 1982. Two visits were made in 1982, the first one on 26th April and the second one 52 days later on the 17th June. At the first visit in April a number of lambs were ear-tagged on each farm, half of which were given a prophylactic treatment of Terramycin L/A, the remainder being left as untreated controls. The lambs on each farm were all given the same colour of ear tag and those lambs with an even-numbered tag were treated.

In addition all lambs were weighed using a spring balance (Salter Ltd, England) and their weights recorded in pounds (lbs). The prophylactic dose of Terramycin L/A given was 2 ml per lamb which was approximately double the therapeutic dose rate recommended by the manufacturer (Terramycin L/A Data Sheet; Pfizer Ltd., Kent, England).

In the 1982 survey, the figures for losses were based on lambs whose fate was known, i.e. found dead or with clinical evidence of pyaemia. Crippled lambs were classified as lamb losses along with dead lambs because, in the experience of the farmers, such animals often failed to thrive regardless of treatment and in many cases died. Lambs noted to be thriving poorly and which may have had visceral abscesses were not classified as cases of pyaemia as necropsy was not carried out.

Similarly, the mean weight gain data were based on only those lambs for which both first and second weighing figures were available. Thus those lambs which did not return at the time of the second weighing due possibly to death or to being missed during the gathering and those known to have died due to events unrelated to disease such as road accidents, were excluded from the results; also excluded were lambs which had lost their ear tags.

On all farms the lambs were a mixture of singles and twins although the majority of them were single lambs.

Results

Lamb Losses

The lamb losses, in terms of morbidity and mortality, are presented in Table 8b from which it is apparent that in contrast to the untreated lambs, there were no deaths amongst the treated groups, all affected lambs in the latter being presented as lame lambs with one or more joint abscesses. However statistical comparison of lamb losses between treated and untreated lambs on individual farms showed no significant difference, although on 3 of the 4 farms the losses

incurred by the latter groups were higher than those in the former. Nevertheless, when the four farms were considered as a block, there were significantly fewer losses in the treated groups ($p < 0.05$) and the losses were reduced from 7.9% to 2.6% following treatment.

Weight Gains

The data showing weight gains over the survey period are presented in Table 8c and they were examined statistically using the t-Test. Although there were no significant differences between treated and untreated groups on individual farms, there was, nevertheless, a strong indication that the former performed better. On three of the four farms the extra mean weight gained by the treated lambs over the controls was between + 1.49 lbs and + 2.27 lbs (i.e. between 6.7% and 11.1% extra over the weight gained by the controls). On the fourth farm (A4), the treated lambs performed slightly less well. However, when the four farms were considered as a block, the increased weight gain by the treated lambs was significant ($p < 0.05$).

Lambs of Unknown Fate

From Table 8b it was apparent that of the total numbers of lambs originally ear-tagged in the survey, the proportions of those which did not return for the second weighing or had lost their ear tags were 13.6% and 16.2% respectively in the treated and untreated groups. There was no statistical difference between these figures.

C. 1983 FIELD SURVEY

Materials and Methods

During the final year of the field survey the same four farms on Arran were again studied but in addition five mainland farms were added to the survey. These five farms were all known to their local veterinary surgeons as units on which tick pyaemia had been a problem in recent years. Three of the farms were in Argyllshire (M1, M2 and M3), one was on the banks of Loch Lomond (M5) and one was near Moffat in Dumfries and Galloway region. (M4). All five were hill sheep farms carrying mainly Scottish Blackface ewes although farms M1 and M3 were using a cross-Suffolk tup.

The same routine as in 1982 was used on all 9 farms, namely a first visit on which lambs were tagged, weighed and a proportion of them treated and a second visit on which lambs were reweighed and data on deaths and disease collected. Due to the large number of farms in the trial it was not possible to have the same time interval between the visits on all farms and the intervals between the first and second weighings are given in the Table 8d.

Farm M4 had a much shorter interval between 1st and 2nd weighings in order to fit in with the management on the farm. The mean interval between weighings on all 9 farms was 41.4 ($\pm$6.9) days. The lambs were thus weighed for the first time at approximately 3 weeks of age and for the second time at about 9 weeks old.

Terramycin L/A was again given at the same dose rate as the previous year namely 2 ml per lamb treated at the first visit to the farm. On two of the Arran farms (A1 and A2), a number of twins were tagged at birth and one of each pair treated then and again at 3 weeks

of age, the other remaining untreated, to compare the effects of LAT in siblings. The dose of LAT was 1ml/lamb at birth and 2ml/lamb at 3 weeks old. On one of the mainland farms (M5) an additional small trial to compare the effects of a long-acting penicillin (Lentrax, May & Baker, Ltd., Dagenham, England) with those of LAT was undertaken at the request of the owner. No untoward side-effects were noted following the injection of either prophylactic antibiotic.

On four of the nine farms investigated in 1983, the farmers decided to dip all the lambs at about 3 weeks of age, prior to putting them out onto the hill ground, in an attempt to further reduce their losses from tick-borne diseases.

In the survey, the figures for lamb losses were based on lambs whose fate was known. Losses was the term applied to lambs known to have died or shown clinical evidence of pyaemia. Similarly, the mean weight gain data were based on only those lambs for which both first and second weighing figures were available. Thus those lambs which did not return at the time of the second weighing and those known to have died due to events unrelated to disease such as road accidents, were excluded from the results; also excluded were those lambs which had lost their ear tags.

On all farms the lambs were a mixture of singles and twins although the majority of them were single lambs.

Results

The results of the 1983 field survey are presented in Tables 8e - 8m. For ease of comparison with the previous year's results, the Arran farms were considered separately from those on the mainland.

Lamb losses

The lamb losses over the survey period are detailed in Tables 8e and 8f. On all four Arran farms and on three of the five mainland farms the lamb losses incurred in the untreated group were greater than those in the treated groups. On the remaining two farms, the disease incidence appeared to be very low and there were no losses in either group. However, although there were no significant differences between the treated and untreated group losses on individual farms, when the farms were considered collectively the losses were significantly reduced from 10.7% to 2.8% ($p < 0.01$) on the Arran farms and from 3.0% to 0.3% ($p < 0.05$) on the mainland farms following treatment. When the nine farms in the survey for 1983 were considered as a whole, the difference between the losses in treated and untreated groups became even more marked: 1.4% in the former and 7.0% in the latter ($p < 0.001$).

Weight gains

The mean weight gains in treated and untreated lambs were compared on individual farms and on the Arran and mainland farms as blocks; the results are presented in Tables 8g and 8h. On an individual farm basis there were greater weight gains in the treated groups on all nine farms; however, these increased weight gains were statistically significant only on the four farms with the largest numbers of lambs in the survey - i.e farms A1, A2, M1 and M4 ($p < 0.001$, $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively). When the Arran farms were considered as a block, the mean gain in the treated group was significantly higher than in the controls ($p < 0.001$) and similarly in the block of mainland farms (excluding farm M4) ($p <$

0.001). The reason for the exclusion of farm M4 was that the interval between weighings was much shorter than on the other farms (see Table 8b) and consequently the lower weight gains of the lambs on this farm severely biased the results when the farms were considered as a block.

Comparison of sibling twins

From farm A1, 30 pairs and from farm A2, 8 pairs of sibling twins were tagged and one of each pair were treated at birth and again 3 weeks later. All of the twins from farm A2, both treated and untreated, survived till at least 9 weeks of age and were present and weighed at birth, 3 weeks and 9 weeks. However on Farm A1, of the original 30 pairs only 19 pairs were present and weighed on all three occasions. Two of the untreated twins had very low birthweights compared to their siblings and both died within 4 weeks of birth; a further five untreated twins (of which one was known to have died) and four treated lambs were not present at the 9 weeks of age weighing. Thus in terms of lamb losses there was no difference between the treated and untreated twins.

When the weight gains of treated and untreated sibling twins on both farms were compared (Table 8i), there were no differences in weight gains between the two groups over the periods birth-3 weeks or 3-9 weeks of age, however the treated twins had better weight gains over the period birth-9 weeks of age ($p < 0.05$).

Comparison of long-acting penicillin with long-acting tetracycline

On farm M5 when the performance of a group of lambs treated with long-acting penicillin was compared with that of lambs treated with LAT and living on the same hill ground there was no difference in lamb losses between the groups on each type of prophylaxis; one lamb in

either group died of pneumonia. However, the perfomance of the lambs in the LAT treated group was better than those in the group given long-acting penicillin - their weight gains were significantly higher ($p < 0.001$) and are illustrated in Table 8j.

Comparison of treatment regimens

On four of the farms (A2, M1, M2, M4) all of the lambs were dipped at about 3 weeks of age, regardless of whether LAT had been administered or not. This resulted in a total of four possible regimens to which lambs in the survey could belong: i.e. (A). Both Dipped and LAT treated; (B). Treated LAT only; (C). Dipped only; (D). Neither Dipped nor LAT treated. Thus on farms A2, M1, M2 and M4 lambs could only be in Regimens A or C while on the remaining five farms only Regimens B and D existed. Although these regimens existed on differenct farms, it was considered valuable to compare the lamb losses in each to assess the possible benefits of dipping lambs (Table 8k). The lambs which were both dipped and LAT treated had very low lamb losses of 0.6% compared to 10.3% losses in lambs which were neither dipped nor LAT treated ($p < 0.001$) while either dipping or LAT treatment alone also significantly reduced losses (3.7% and 2.5% respectively) when compared with those of the group which was neither dipped nor LAT treated ($p < 0.05$). There was no significant difference in losses between the groups LAT treated only or dipped only.

D. NECROPSY FINDINGS IN LAME LAMBS

Materials and methods

During the three years of the field survey, a number of lame lambs, both from within and outside the survey group with joint abscesses were presented by the farmers. A total of 23 such animals, the most severely affected, were necropsied and the remainder were treated with antibiotics.

Results

Detailed findings of the necropsy examinations of 23 lame lambs investigated during the three years of the field survey are given in Table 81. Of the 23 lambs, a total of 16 (69.6%) had multiple abscesses in areas other than joints, 3 (13.0%) had abscesses in multiple joints but no internal abscesses, 2 (8.7%) had a single joint abscess and 2 (8.7%) were paraplegic, possibly due to a spinal abscess. The abscess sites were also tabulated according to frequency of observation and this data is presented in Table 8m. The most common site among the joint lesions was the hock joint, followed by the carpal, pastern, stifle, fetlock, elbow and shoulder joints in descending order of frequency. Amongst the internal organs and tissues involved as abscess sites, the most commonly affected was the liver, followed by the lungs, spleen, kidneys, subcutaneous tissues, peritoneum and suspected spinal abscess in descending order of frequency.

Plate 6 shows the necropsy of a field case of tick pyaemia which had multiple abscess formation. There were abscesses scattered over

the peritoneum and the serosal surfaces of abdomenal viscera, in the substance of the liver, adhesion of the diaphragm to the liver, abscesses in the lungs and adhesion of the lungs to the diaphragm and to an abscess on the inner aspect of the thoracic wall.

Plate 6

Necropsy of a field case of tick pyaemia showing multiple abscess formation.

Discussion

Most hill sheep farmers in the West of Scotland will assert that tick-borne disease is the major cause of losses amongst young lambs on their farms. In fact, however, reliable data on the causes of lamb mortality and morbidity during the first nine weeks or so of life are scarce. It has proven extremely difficult in the past to assess the true level of losses due to tick-borne diseases on hill or upland farms due to the system of management imposed on the farmers by the rough terrain and the large areas of land involved. For example, lambs are only gathered infrequently, during which a fair proportion may evade the net and those which die are rapidly scavenged or may lie unnoticed in a ditch or bog. The available information is reviewed in Chapter 1 and from this it would appear that lamb losses due to tick pyaemia may range from 5% up to 29%.

Against this background, the field survey presented here was undertaken with several aims in mind. The aims were to assess the level of infection with Cytoecetes phagocytophila, the number of lamb losses and the possible benefits of antibiotic prophylaxis and dipping of lambs.

Throughout the three years of this field survey, a problem arose when trying to assess the numbers of lamb losses (i.e. dead or pyaemic lambs), in that, of the numbers of lambs originally tagged, a fairly large proportion failed to appear in June for the second weighing. In 1981, this proportion amounted to 20%, in 1982 to 15% and in 1983 to 10%. Although some were known to have died either on the hill or from road accidents, the majority were apparently not gathered in from the hill. There were two reasons to support this. First that the farmers

were unconcerned and, in fact, believed themselves to have missed them on the hill, and secondly the missing lambs were almost equally divided between the treated and untreated groups. Therefore since it was felt that these lambs of 'fate unknown' were in all probability not 'losses', they were in effect removed from the numbers of lambs during calculations on the lamb losses and weight gain data. It is interesting to note that the percentage of such missing lambs steadily declined over the three years of the survey. It is possible that this may have been due to improved shepherding and an awareness of the situation occasioned by the survey itself. In future surveys of this nature, it would be useful to ear-tag the ewes also, since if both the ewe and its offspring are absent in June the chances are that they are still alive and on the hill, having been missed during the gathering. Another improvement would be to double tag the lambs since a number of lambs in the present survey lost their ear tags.

In the first survey in 1981 (following in the pioneering steps of Watt, Foster and Cameron, 1968) the prophylactic antibiotic used was long-acting benzathine penicillin, known to be effective against S. aureus. However, studies on the effects of long-acting tetracycline (LAT) on the agent of TBF (see Chapter 3) showed that at double the therapeutic dose rate this drug could protect lambs against TBF infection for at least 15 days, in addition to its being effective against S. aureus. Therefore in subsequent years it was decided to change to prophylaxis using LAT rather than long-acting penicillin.

The dosage of long-acting penicillin used in lambs by Watt et al (1968) was, on a weight basis, several times the dose recommended by the manufacturer for use in man for prophylaxis of rheumatic fever (Penidural L-A Data Sheet, Wyeth Laboratories, Berks, England). In

contrast, the dose of LAT used in the present survey was approximately double the therapeutic dose recommended by the manufacturer. It is not known at present for how long a single injection of LAT at this dosage will prevent the development of pyaemia; however in work on cattle, Breeze and Gay (1981) have shown that detectable levels of antibiotic were present in the blood for up to nine days following a single intramuscular injection at the manufacturer's recommended dosage, i.e. half the dosage used in lambs in this survey.

In assessing the possible benefits to be gained from antibiotic prophylaxis and dipping, two aspects were considered. The first of these was a comparison of the level of lambs losses incurred in treated and untreated lambs on each farm or group of farms. However, for the reasons described previously, this alone was felt to be inadequate and a comparison of live-weight gains in treated and untreated lambs was introduced as a second measure of morbidity. The improvements to be gained from prophylaxis were thus examined separately from these two aspects and lamb losses will be dealt with first.

When the figures for lamb losses were examined and treated and untreated groups compared regardless of whether or not the lambs were dipped, there was a significant reduction in the level of losses amongst treated lambs in both 1982 and 1983. In 1982, losses amongst treated lambs on the Arran farms were only 2.6% compared with 7.9% losses in the untreated group. In 1983, the losses amongst treated lambs on Arran were only 2.8% compared with 10.7% in the untreated group and similarly for treated lambs on the mainland farms the losses were low (0.3%) compared with those of the untreated controls (3.0%).

However, the greatest reduction in lamb losses was found when the 1983 lamb loss figures were considered and the role of dipping lambs taken into account. As described previously, lambs could be divided into four groups, each of which were given a different regimen of preventive measure.

The greatest reduction in lamb losses was achieved in farms on which a combination of dipping lambs and LAT prophylaxis was applied. The regimen of dipping plus LAT treatment reduced lamb losses to 0.6%, compared with losses of 10.3% where no preventive measures were implemented. Comparison of lamb losses incurred by groups in the LAT treatment only regimen (2.5%) with those incurred by groups in the dipping only regimen (3.7%) showed no difference in losses, although both were significantly lower than in the group which was neither dipped nor treated (10.3%).

An earlier assessment of dipping lambs as a prophylactic measure against tick pyaemia was carried out by Watson and others in 1966. They found that dipping lambs twice at an interval of 3 weeks markedly reduced both lamb losses and the number of ticks on the lambs; in addition it resulted in improved weight gains by the dipped animals. Consequently they suggested that, although the practical application of such a dipping programme could present managemental problems on the type of farms suffering from a tick-borne disease problem, it was a worthwhile method of reducing lamb losses. The findings of the present survey lend support to this advice but would further suggest that on farms where there is a problem of tick-related lamb losses the administration of a long-acting tetracycline drug as a further prophylactic measure would be a useful adjunct to single dipping.

There has been little previous work done to ascertain the value

of antibiotic prophylaxis on farms with a tick pyaemia problem. Foggie (1962) made a brief mention of an attempt using a long-acting penicillin, but, apart from stating that it failed to prevent pyaemia, he gave no further details. The work carried out by Watt and others (1968) has been the most extensive to date and showed more promising results with the same drug that Foggie had used; the incidence of clinical cases of pyaemia was reduced from a mean of 10.46% in untreated controls to a mean of 0.46% in treated lambs. In this work however they excluded those farms on which the disease incidence was low, so overall these percentages may have been lower had they taken into consideration all the lambs originally in their trial. The 1983 data for the farms on Arran and the mainland shows lower percentages of clinical cases of pyaemia than those in Watt et al's trial (Arran farms - treated lambs, 0.4%, untreated lambs 4.9%; Mainland farms - treated lambs 0.3%, untreated lambs 1.7%); nevertheless significant reductions of lamb losses were found in the present survey. Watt and his co-workers however have made no mention of whether any lambs died during their trial and so it remains unclear as to whether the cases of clinical pyaemia quoted included only the classic type presented as lame lambs with joint abscesses, or whether they included dead lambs subsequently necropsied and found to be suffering from pyaemic lesions.

In considering the benefits of antibiotic prophylaxis on the farms in the survey in terms of increased live-weight gains, comparisons between treated and untreated groups were made regardless of whether or not dipping of lambs was practiced on the farms.

There was clear evidence of enhanced live-weight gains following

LAT prophylaxis. In both the 1982 and 1983 surveys, there was a significantly greater weight gain in the treated lambs in the group of four farms on Arran and the group of five farms on the mainland in 1983. In addition, on individual farms in both 1982 and 1983 (with one exception in 1982), the LAT treated lambs consistently gained more weight than the untreated controls.

Further evidence of the benefits to performance following prophylactic administration of LAT was furnished by the comparison of weight gains in sibling twins, only one of which was treated. The treated twins gained significantly more weight than their untreated siblings over the period from birth to 9 weeks of age; in addition the treated twins performed better over both the birth to 3 weeks and 3-9 weeks of age periods, although the increased benefit during these periods was not statistically significant. Since these animals were sibling twins it is probable that the extra weight gain was a result of the LAT prophylaxis.

In the small-scale trial undertaken to compare the beneficial effects of prophylactic injection of a long-acting penicillin (Lentrax; May & Baker Ltd., Dagenham, England) with those of LAT, the disease incidence was very low. Nonetheless the LAT treated lambs gained significantly more weight than those treated with Lentrax. Since both are broad spectrum antibiotics and S. aureus and P. haemolytica amongst others are sensitive to both antibiotics, this difference tends to suggest that the benefits gained from the LAT prophylaxis may indeed be due to its effect on the agent of tick-borne fever, Ctyoecetes phagocytophila. However, further investigations on this should be carried out since the level of disease challenge in this trial was apparently low and neither the level of tick challenge

nor the incidence of tick-borne fever was known.

As mentioned above, LAT is effective against, amongst other things, Pasteurella haemolytica (Gilmour, Sharp and Gilmour, 1982) in addition to the agents of TBF and tick pyaemia. Thus its use may have prevented tick-borne fever infection for a period of 2-4 weeks (see Chapter 3) with its associated pyrexia and immunosuppression, thereby reducing the incidence of conditions such as tick pyaemia and pasteurellosis both by direct antibacterial action and also by its effects in suppressing the agent of TBF. Furthermore, inapparent or subclinical forms of tick pyaemia exist in which only internal organs are affected and the only effect may be weight loss or a check in growth (Foster and Cameron, 1968a). Thus the increased weight gains following LAT treatment may also be due, to some extent, to the prevention of such subclinical forms of pyaemic infection.

In addition, the possible role of TBF per se in weight loss should be noted. Foggie, (1951) stated that he found a significant mean weight loss of between 1.6 lbs and 7.5 lbs in two groups of lambs in the week following an experimental infection with one of two strains of tick-borne fever. At the same time he also pointed out in his studies on TBF in more than 440 sheep, that many animals lost condition following infection, although he made no comment on how much this was due to the effects of TBF and how much was due to the pneumonic infections that often occurred subsequent to TBF infections. In contrast, although Foster and Cameron (1968a) found that while pyaemia caused a significant drop in daily liveweight gain in affected lambs, TBF infection alone had little effect on liveweight gain.

In their trial using long-acting penicillin, Watt and his

colleagues (1968) point out that the timing of drug prophylaxis was an important factor in the success of their trial. Foggie (1948) had noted the peak incidence of pyaemia to occur at about 3-5 weeks of age and Watt et al (1968) agreed with this, estimating that most cases occurred from 2-6 weeks of age. Therefore they administered their prophylactic dose of antibiotic immediately prior to the expected appearance of the condition and found a marked reduction in its incidence. Similarly, in the present study, the timing of the prophylaxis was just before the expected occurrence of pyaemia and the incidence of pyaemia and the overall lamb losses were reduced.

Tick infestation and subsequent tick-borne fever has been found to occur in lambs from an early age and the majority of lambs on tick-infested pasture are said to be infected within the first two weeks of life (McEwen, 1947; Foster and Cameron, 1968a). The age range of lambs most commonly found suffering from tick pyaemia has also been studied. In 1948, in a survey of 54 pyaemic lambs, Foggie found that the age range over which pyaemia occurred was from 1-8 weeks of age, with a mean of 3.8 weeks of age. Of these 54 cases, a total of 12 (22%) were presented by the 3rd week of life.

These findings with regard to the relative timing of TBF infection and tick pyaemia in the field fit well with the experimental conclusions in the present study (Chapter 7) and those of others that TBF acts as the predisposing factor for staphylococcal infection. However, results of the present field survey are apparently at some variance with the above findings in that cases of tick pyaemia were generally not noted by the farmers until the lambs were 5-7 weeks of age, although results of the 1981 survey on the field incidence of TBF indicated that at least 30% of the lambs had been infected with TBF by

3 weeks of age, before going on to the hill. Thus there is apparently a gap of some 2-4 weeks between TBF and the onset of clinical pyaemia although the results from the experimental induction of tick pyaemia (Chapter 7) suggest that it is likely to develop shortly after TBF inoculation. This topic will be more fully discussed in Chapter 9.

As noted earlier, the true incidence of tick pyaemia in the field remains unknown because of the fact that in almost all surveys carried out to date, only lame lambs or lambs found dead, whose clinical signs were unknown, were necropsied. For example in a 1964 study by Watson, of 63 lame or non-thriving lambs submitted for necropsy, only 9.5% were presented as suffering from loss of condition, the remainder being either lame, paraplegic or showing nervous signs. In common with the present survey, Watson found that the lungs, liver, spleen and kidneys were the most commonly affected visceral organs and the hock and carpal joints were the most commonly affected joints. Earlier, McDiarmid (1946a) had suggested a slightly different distribution of lesions following his investigations in which he found that the most common internal sites for abscess formation were the liver and kidneys with the spleen sometimes involved, but rarely the lungs. However such differences may well depend on the method of selection of the pyaemic cases examined.

Summary

A preliminary field survey was carried out in 1981 to assess the incidence of TBF infection and estimate the extent of morbidity and mortality in lambs due to tick pyaemia. It was found that by 3 weeks

of age, just prior to going on to hill ground, at least 30% of the lambs had been infected with TBF. Considerable numbers of lambs (up to 37% on one farm) apparently went missing between turnout on to the hill at about 3 weeks of age and marking of lambs 6-7 weeks later. In surveys conducted over the two subsequent years it was concluded that most of these lambs which did not return from the hill at marking time had been missed during the gathering.

Control programs involving prophylactic injection of a long-acting tetracycline drug and dipping lambs at 3 weeks of age were assessed during the second and third year of the survey. The results showed that such preventive measures markedly reduced the numbers of dead or pyaemic lambs. Lamb groups which were both treated and dipped incurred lamb losses of only 0.6% compared with losses of 10.3% in control groups in which neither preventative measures was carried out. Groups which were either dipped or treated also suffered fewer losses (3.7% and 2.5% respectively) than control groups. In addition to reducing the levels of morbidity and mortality, the prophylactic antibiotic treatment was found to have significantly improved lamb growth. This was considered to be due both to the direct antibacterial action of the long-acting tetracycline and its suppression of TBF reactions.

A number of necropsies performed on lame lambs demonstrated that multiple abscess formation was common. The most frequently observed sites of abscess formation were the hock and carpal joints and the liver and lungs.

Table 8a

Numbers of lambs failing to return in June in the 1981 preliminary survey

Farm	Lambs not returning/Total Tagged Lambs (%)	
	Penidural L/A Treated	Untreated Controls
A1	7/19 (37%)	18/50 (36%)
A2	3/25 (12%)	4/56 (7%)
A3	5/26 (19%)	18/52 (35%)
A4	2/31 (17%)	10/75 (13%)
Totals	17/101 (17%)	50/233 (21%)

TABLE 8b: 1982 Arran field survey: Lamb losses incurred on four hill sheep farms from the 3rd to the 10th week of life

Farm	Lamb Group (No. originally tagged/No. of known* fate)		No. of lambs dead or pyaemic** (i.e. known lamb losses)		% Lamb losses (based on No. of known fate)	
A1	Treated lambs	81/69	1	(lame)	1.4%	
	Untreated lambs	81/70	3	(all dead)	4.3%	
A2	Treated lambs	82/71	3	(all lame)	4.2%	
	Untreated lambs	82/69	11	(6 dead, 5 lame)	16.2%	
A3***	Treated lambs	52/43	2	(both lame)	4.7%	
	Untreated lambs	52/40	1	(lame)	2.5%	
A4	Treated lambs	57/52	Nil		Nil	
	Untreated lambs	57/50	3	(2 dead, 1 lame)	6.0%	
Totals	Treated lambs	272/235	6	(all lame)	2.6%	$P<0.05$
	Untreated lambs	272/228	18	(11 dead, 7 lame)	7.9%	

* Lambs which were known to have died due to road accidents etc, those not gathered in off the hill and those which although gathered were unidentifiable due to having lost their eartags have been excluded.

** The pyaemic lambs were all cases with joint abcesses. Poorly thriving lambs which may have had internal pyaemic lesions were not included.

*** On this farm the lambs were dipped at 3 weeks of age prior to going to the hill ground

TABLE 8c 1982 Arran field survey: mean weight gains by lambs on four hill sheep farms from the 3rd to the 10th week of life

Farm	Lamb Group (No. of Lambs)*	Mean Weight Gains (lbs) (± S.D.)	Significance (t-Test)
A1	Treated (67)	23.59 (± 5.01)	NS
	Untreated (63)	22.10 (± 5.57)	
A2	Treated (71)	22.75 (± 5.85)	NS
	Untreated (59)	20.48 (± 7.14)	
A3	Treated (42)	26.32 (± 7.07)	NS
	Untreated (40)	24.55 (± 7.22)	
A4	Treated (52)	28.07 (± 5.47)	NS
	Untreated (48)	28.53 (± 5.27)	
All Farms	Treated (232)	24.83 (± 7.56)	$p < 0.05$
	Untreated (210)	23.58 (± 8.22)	

* The number of lambs includes only those which were present and weighed on both occasions.

Table 8d

Intervals betweeen first and second weighings in the 1983 survey

Farm	Date of first weighing and treatment	Date of second weighing	No. of days between weighings
A1	12/5/83	21/6/83	40
A2	12/5/83	21/6/83	40
A3	12/5/83	21/6/83	40
A4	12/5/83	21/6/83	40
M1	11/5/83	30/6/83	50
M2	3/5/83	23/6/83	51
M3	9/5/83	24/6/83	46
M4	5/5/83	2/6/83	28
M5	9/5/83	16/6/83	38

TABLE 8e: 1983 Arran field survey: Lamb losses incurred on four hill sheep farms from the 3rd to the 9th week of life

Farm	No. of lambs (No. originally tagged/No. of known* fate)		Whether lambs dipped at 3 weeks old	Lamb Losses No. Dead	No. Pyaemic	Total Losses (%)	
A1	Treated lambs	125/108	No	4	1	5 (4.6%)	
	Untreated lambs	121/106		7	6	13 (12.3%)	
A2	Treated lambs	81/80	Yes	2	-	2 (2.5%)	
	Untreated lambs	81/78		5	3	8 (10.3%)	
A3	Treated lambs	26/18	No	-	-	nil	
	Untreated lambs	27/17		-	1	1 (5.9%)	
A4	Treated lambs	50/41	No	-	-	nil	
	Untreated lambs	50/43		2	2	4 (9.3%)	
All Four Farms	Treated lambs	282/247		6 (2.4%)	1 (0.4%)	7 (2.8%)	$P < 0.01$
	Untreated lambs	279/244		14 (5.7%)	12 (4.9%)	26 (10.7%)	

* This figure excludes lambs which failed to return for the second weighing in June, returned but were unidentifiable due to lost eartags or which were known to have died due to accidents unrelated to disease (e.g. road accidents etc).

TABLE 8f: 1983 Mainland field survey: Lamb losses incurred on five hill sheep farms from the 3rd to the 9th week of life.

Farm	No. of Lambs (No. originally tagged/No. of known* fate)		Whether lambs dipped at 3 weeks old	Lamb Losses No. Dead	No. Pyaemic	Total Losses (%)	
M1	Treated lambs	74/65	Yes	-	-	Nil	
	Untreated lambs	74/59		-	-	Nil	
M2	Treated lambs	50/47	Yes	-	-	Nil	
	Untreated lambs	50/48		-	-	Nil	
M3	Treated lambs	27/22	No	-	1	1 (4.5%)	
	Untreated lambs	28/24		-	3	3 (12.5%)	
M4	Treated lambs	129/126	Yes	-	-	Nil	
	Untreated lambs	60/56		-	1	1 (1.8%)	
M5	Treated lambs	50/50	No	-	-	Nil	
	Untreated lambs	44/43		3	-	3 (7.0%)	
All Five Farms	Treated lambs	330/310		-	1	1 (0.3%)	P< 0.05
	Untreated lambs	256/230		3	4	7 (3.0%)	

* This figure includes lambs which failed to return for the second weighing in June, returned but were unidentifiable due to lost eartags and those which were known to have died due to accidents unrelated to disease (e.g. road accidents etc)

TABLE 8g 1983 Arran field survey: mean weight gains by lambs on four hill sheep farms from the 3rd to the 9th week of life

Farm	Lamb Group (No. of Lambs)*	Mean Weight Gain (lbs) (± S.D.)	Signficance (t-Test)
A1	Treated (79)	20.81 (± 4.14)	p < 0.001
	Untreated (78)	17.22 (± 4.58)	
A2	Treated (76)	20.62 (± 4.32)	p < 0.05
	Untreated (68)	18.80 (± 5.66)	
A3	Treated (18)	19.78 (± 3.65)	NS
	Untreated (17)	16.70 (± 5.94)	
A4	Treated (45)	23.78 (± 4.86)	NS
	Untreated (44)	22.37 (± 5.18)	
All Arran Farms	Treated (218)	21.27 (± 4.32)	p < 0.001
	Untreated (207)	18.79 (± 5.20)	

* The number of lambs includes only those which were present and weighed at both weighings.

TABLE 8h 1983 Mainland field survey: mean weight gains by lambs on five hill sheep farms from the 3rd to the 9th week of life

Farm	Lamb Group (No. of Lambs)*	Mean Weight Gain (lbs) (± S.D.)	Significance (t-test)
M1	Treated (66)	28.72 (± 5.17)	$p < 0.01$
	Untreated (59)	25.68 (± 5.52)	
M2	Treated (47)	26.18 (± 4.08)	NS
	Untreated (48)	25.29 (± 4.52)	
M3	Treated (24)	24.61 (± 5.21)	NS
	Untreated (22)	21.50 (± 5.60)	
M4	Treated (121)	13.42 (± 2.79)	$p < 0.001$
	Untreated (55)	11.73 (± 2.98)	
M5	Treated (46)	25.19 (± 3.53)	NS
	Untreated (40)	24.62 (± 3.67)	
Four ** Mainland Farms	Treated (183)	26.64 (± 4.54)	$p < 0.001$
	Untreated (169)	24.77 (± 4.87)	

* The number of lambs includes only those which were present and weighed at both weighings.

** Farm M4 excluded because the length of time between the first and second weighings was very short (4 weeks), resulting in much lower weight gains over the survey period which biased the data.

TABLE 8i: 1983 Arran field survey: A comparison of mean weight gains from the 3rd to the 9th week of life in sibling twin lambs from two hill sheep farms which were either LAT treated or untreated.

Time Period	Lamb Group	(n)*	Mean Wt. Gain (lbs) (± S.D)	Mean Difference (± S.D.)	Significance of Difference (Paired t-Test)
Birth-3 weeks	Treated	27	9.02 (± 1.33)	+ 0.76 (± 0.382)	NS
	Untreated	27	8.26 (± 2.32)		
3-9 weeks	Treated	27	21.70 (± 2.66)	+ 2.24 (± 1.107)	NS
	Untreated	27	19.46 (± 5.57)		
Birth-9 weeks	Treated	27	30.74 (± 3.36)	+ 3.04 (± 1.318)	$P < 0.05$
	Untreated	27	27.70 (± 6.48)		

* There were 19 pairs of twins from farm A1 and 8 pairs of twins from farm A2

TABLE 8j 1983 Mainland field survey: a comparison of mean weight gain from the 3rd to the 9th week of life in lambs on a hill sheep farm either treated with LAT or Lentrax.

Farm	Lamb Group (No. of Lambs)*	Mean Weight Gain (lbs) (± S.D.)	Significance (t-Test)
M5	LAT Treated (75)	20.39 (± 2.97)	$p < 0.001$
	Lentrax Treated (71)	18.56 (± 2.66)	

* The number of lambs includes only those which were present and weighed at both weighings.

TABLE 8k 1983 field survey: a comparison of the lamb losses incurred from the 3rd to the 9th week of life by four different regimens on nine hill sheep farms.

Regimen (No. of Lambs)	No. Dead	No. Pyaemic	No. Losses (%)
A. Dipped and LAT treated (318)	2	0	2 (0.6%)
B. LAT treated only (239)	4	2	6 (2.5%)
C. Dipped only (241)	5	4	9 (3.7%)
D. Neither dipped nor LAT treated (233)	12	12	24 (10.3%)

A vs D - $p < 0.001$

B vs D - $p < 0.01$

C vs D - $p < 0.01$

TABLE 81 Necropsy results of 23 field cases of tick pyaemia presented as lame lambs during the field surveys carried out on hill sheep farms during 1981-83.

FARM AND	YEAR	LAMB NO.*	TREATED	SITE (AND NUMBER) OF ABCESSES
A1	1981	Untagged	--	Carpus (1); Stifle (1); Kidneys (both); multiple abcesses in Lungs, Spleen and Peritoneum
		Untagged	--	Carpus (both); Hock (both); Kidneys (both); multiple abcesses in Spleen, Lungs and Liver
		Untagged	--	Hock (1); Liver (3); Kidneys (both)
		Untagged	--	Hock (1); Subcutaneous tissues (2).
A2	1981	Y17	--	Hock (1); Pastern (1); Kidneys (both); Lungs (multiple)
		Y23	--	Hock (1); Liver (2); Spleen (multiple)
		Untagged	--	Hock (both); Pastern (1)
		Untagged	--	Pastern (1); Lungs (multiple)
		Untagged	--	Elbow (1); Liver (2)
A1	1982	G5	LAT	Carpus (both); Shoulder (1); Hock (1)
		Untagged	--	Paraplegic - possible spinal abcess
		Untagged	--	Carpus (1); Liver (2); Lungs (1)
		Untagged	--	Carpus (1); Fetlock (1); Spleen (1); Diaphragm (1)
A2	1982	Y305	--	Hock (1); Pastern (1); Liver (multiple)
		Y344	LAT	Carpus (1); multiple abcesses in Liver and Spleen
		Y314	LAT	Carpus (both); Fetlock (1 fore, 1 hind); Pastern (1); Hock (1); Subcutaneous tissue (2); multiple abcesses in Liver, Lungs, Spleen, Kidneys and Peritoneum

TABLE 81 (cont). Necropsy results of 23 field cases of tick pyaemia as lame lambs during the field surveys carried out on hill sheep farms during 1981-83.

FARM AND YEAR		LAMB NO.*	TREATED	SITE (AND NUMBER) OF ABCESSES
A3	1982	Y134	LAT	Pastern (both hind); Carpus (1); Liver (multiple)
		Y143	--	Hock (1); Stifle (1); Carpus (1); multiple abcesses in Liver, Lungs, Spleen and Skeletal muscles.
A4	1982	Y207	--	Stifle (1).
A1	1983	Y21	--	Carpus (1); Lungs (2); Liver (multiple)
		B75	-	Hock (1)
A3	1983	Untagged	--	Carpus (1); Hock (1)
M4	1983	Y338	--	Paraplegic - possible spinal abcess.

* Lambs with tag numbers were those which were part of the group of lambs in the field survey on each farm. Those lambs which were untagged were from among the lambs on each farm not in the survey.

TABLE 8m Sites of the pyaemic lesions found in 23 crippled lambs from hill sheep farms necropsied during the field survey.

SITE OF ABCESS FORMATION	NUMBER OF LAMBS AFFECTED (%)	
Hock joints (one or both)	12	(52.2%)
Carpal joints (one or both)	11	(47.8%)
Liver	11	(47.8%)
Lungs	8	(34.8%)
Spleen	7	(30.4%)
Pastern Joints (one or more)	6	(26.1%)
Kidneys (one or both)	5	(21.7%)
Stifle joints (one or both)	3	(13.0%)
Subcutaneous tissues	3	(13.0%)
Fetlock joints (one or more)	2	(8.7%)
Peritoneum	2	(8.7%)
Suspected spinal abcess	2	(8.7%)
Elbow joints (one or both)	1	(4.3%)
Shoulder joints (one or both)	1	(4.3%)
Diaphragm	1	(4.3%)
Skeletal muscle	1	(4.3%)

CHAPTER 9

GENERAL CONCLUSIONS AND DISCUSSION

It is generally recognised than an uncomplicated infection with the agent of tick-borne fever (Cytoecetes phagocytophila) results in a mild disease characterised by a brief pyrexia associated with a short period of anorexia and dullness in most sheep. In addition, temporary changes in the haematological parameters of infected animals have been observed. Such uncomplicated infections are generally relatively innocuous although on occasions the pyrexia itself has been reported to result in more severe effects such as reduced spermatogenesis in rams (Watson, 1964) and abortion in ewes (Jamieson, 1950).

Perhaps the most important facet of the disease, however, lies in its putative role as an immunosuppressive or predisposing factor in other diseases.

In the present study the role of TBF in sheep disease was investigated from several aspects. The first of these was an examination of uncomplicated C. phagocytophila infection to determine the course of the clinical disease and to investigate both the haematological and immunological changes in sheep following TBF infection. Following on from these investigations, three of the diseases most commonly linked with TBF (namely tick pyaemia, louping-ill and pasteurellosis) were experimentally reproduced in association with TBF infection to determine whether TBF could indeed act as a predisposing factor. Finally, field studies were carried out for 3 years on hill sheep farms with a tick-borne disease problem in attempts to elucidate the role of TBF in the field and to assess control measures instituted to reduce lamb losses due to tick-borne disease on these farms.

The clinical signs noted in the experimentally infected animals in this study were very mild except in very young lambs being fed on a

milk bar system. In these animals the notable depression and anorexia would suggest that tick-borne fever infection per se may give rise to lamb losses by sheer debilitation, thus preventing them from being able to accompany their dams, particularly in inclement weather or on rough terrain. Thus TBF may be a contributory factor in cases of lambs which are deemed to have died of starvation or exposure.

In attempts to determine the means by which C. phagocytophila could cause depressed immune responses and consequent predisposition to other diseases, two aspects were studied.

The first of these aspects was the haematological changes during and after an episode of clinical TBF. The sequence of changes found in the present study was very similar to that described by other authors and variations from their findings were possibly due to differences in the strains of TBF. The most important haematological changes found were probably the drop in lymphocyte numbers coinciding with the peak numbers of parasitised neutrophils at around Days 5-7 post inoculation and the development of a neutropenia begining around Day 10 which was not fully resolved until 2 weeks later. In addition, eosinophils were almost absent from the peripheral blood and monocyte numbers were depressed from around Day 5 post inoculation until Day 24. The significance of these changes from the point of view of disease must depend not only on the fact that at various times during a TBF reaction one or more cellular components in the blood are significantly reduced but that, at least in the case of parasitised neutrophils, they may also be functionally impaired.

Functional impairment of parasitised neutrophils has been demonstrated by Foster and Cameron (1970b) and Woldehiwet (1979) in

terms of reduced migratory capabilities. However, up to the present time, little work has been done to examine any effects which TBF may have on other neutrohil functions such as phagocytosis and intracellular killing.

The second aspect studied was the effects of C. phagocytophila infection on the humoral and cell-mediated immune responses. From experiments reported in this thesis there is evidence that both of these types of immune response were impaired following TBF infection.

Although the primary humoral response to dead antigens was not found to be affected in TBF-infected lambs in the present study, the antibody response to live antigen (louping-ill virus) was depressed in lambs with concurrent TBF infection. However, work reported by Batungbacal and Scott (1982a) demonstrated that TBF infection can depress both primary and secondary humoral responses to non-replicating antigens. In addition, the cell-mediated immune response to a mycobacterial antigen (bovine tuberculin test) was depressed in sheep old enough to mount such a response. These findings indicate that TBF infection may have wide-ranging and variable effects on the immune response.

Both antibody and cell-mediated responses are complex processes dependent on a variety of components for their final effects. The fact that during a TBF reaction there appears to be a constant and chronological sequence to the haematological changes would suggest that antibody and cell-mediated immune responses could well be affected at different stages during the reaction and that timing is likely to be an important factor in the successful establishment of secondary infections. Thus presentation of antigen at different times

during TBF reactions could account for variations in the degree of immune response impairment reported by different groups of workers.

The role of C. phagocytophila as a predisposing factor in other diseases was studied experimentally in three different diseases: tick pyaemia, louping-ill and pneumonic pasteurellosis. In each case, concurrent TBF was found to increase the severity of the disease compared with that in control animals not infected with TBF.

In experiments with tick pyaemia it was found that 9/10 (90%) lambs inoculated with S. aureus 5 days after TBF inoculation developed pyaemic lesions compared with only 4/20 (20%) control lambs inoculated with S. aureus alone. Lambs infected with staphylococci either simultaneously with a TBF inoculum or 9 days after TBF also developed pyaemia more consistently than control lambs (29% in both cases).

These findings raise several points. Firstly, it is apparent that a concurrent TBF infection can increase the chances of pyaemic lesions developing although it would appear that the timing of bacterial inoculation relative to the stage of TBF infection is important in this respect.

Secondly, the fact that a proportion of the control animals developed pyaemic lesions, suggests that the numbers of staphylococci inoculated may have been a little high. In further experiments on experimental induction of tick pyaemia, it would be useful to titrate an inoculum of S. aureus which would produce pyaemic lesions in TBF-infected sheep but not in controls.

Thirdly, a high proportion (15/25) of the sheep which developed pyaemic lesions following i/v inoculation of staphylococci did not become lame and appeared either unthrifty or even normal. This suggests that in the field such cases may occur in significant

numbers but are not readily diagnosed as being due to tick pyaemia.

Experimental work on the other two diseases studied i.e. louping-ill and pasteurellosis, did not investigate the effects of altering the timing of bacterial or virus inoculation with respect to the stage of the TBF reaction. In the light of the results in Chapter 7 discussed above, an investigation of this could be worthwhile.

The difference between the clinical and pathological findings in TBF and non-TBF infected animals after infection with louping-ill virus was an intriguing and unexpected finding, since most of the dually infected sheep also developed a severe systemic mycosis. Indeed this was probably the major contributory factor in the deaths of 17 of these 18 sheep. It is possible that the combined effect of louping-ill virus and TBF infection resulted in such an immunologically depressed animal that the opportunist mycotic pathogen Rhizomucor pucillis was free to invade. This finding does, however, suggest that louping-ill virus may have had a role in previous reports of a haemorrhagic syndrome associated with simple TBF infection (Foster, Foggie and Nisbet, 1968) and a systemic mycosis also associated with TBF infection (Angus et al, 1971). Similarities also arise between the clinical picture in the present study in dually-infected animals and that recorded by Danskin and Burdin (1963) in cattle affected by bovine petechial fever (Ondiri disease), presently attributed solely to a rickettsial organism Cytoecetes ondiri.

When experimental work was done to compare the clinical signs and lung lesion scores in TBF and P. haemolytica infected lambs with those of control lambs given P. haemolytica alone, the difference between the two groups was highly significant, the clinical signs and lung

lesion scores being most severe in the dually infected group. Again, because of the possible importance of temporal relationships it is possible that had P. haemolytica been inoculated on Day 5, rather than Day 7, of a TBF reaction, a more severe disease may have been induced.

If, indeed, Day 5 of a TBF reaction is the optimum day for the establishment of a secondary bacterial or viral infection, this might suggest that, in addition to lymphocyte depletion, impairment of neutrophil function is important since neutropenia occurs at a later stage.

As reviewed in the literature (Chapter 1) there is a clear connection between the peak age incidence of TBF in young lambs and the age of onset of tick pyaemia in that the latter usually closely follows the former. However in the present work, there appears to be a slight anomaly. From the experimental work on tick pyaemia, most cases occurred when staphylococci were inoculated 5 days after TBF and the lambs became lame about 4-7 days later. Thus in the experimental situation it seems that there are 9-12 days between TBF infection and the onset of clinical pyaemia. However from the field work, at least 30% of lambs were infected with TBF by 3 weeks of age and yet none were noted pyaemic until they were 5-7 weeks old, i.e. an apparent gap of between 2-4 weeks between TBF infection and the onset of pyaemia.

There are several possible explanations for this situation.

a. It may be that not all of the lambs were infected with TBF before going to the hill and those lambs which became pyaemic did so following a primary infection with TBF.

b. If one assumes that in a field situation where TBF is endemic all lambs have been infected with TBF by 2 weeks of age (Foster and Cameron, 1968a), then perhaps those which succumb to pyaemia

later on do so following a subsequent infection with a different strain of TBF to which they are not immune.

c. It is possible that although lambs are infected with TBF some time before staphylococcal invasion, the immunosuppressive effect of TBF may be longer-lasting than currently envisaged, giving rise to pyaemia at a later age.

d. A final possibility is that the tick rise is variable according to the weather and thus the age incidence of tick-borne fever and consequently tick pyaemia may vary from year to year.

One of the most important findings from the field survey was the significantly reduced morbidity and mortality amongst lamb groups that were either dipped or prophylactically treated with LAT and the even more marked reduction found in lamb groups in which both preventive measures were applied. The reduction in morbidity and mortality from 10.3% in undipped and untreated groups to 0.6% in groups both dipped and treated was an economically viable proposition for the farmers and the results would justify advising farmers to carry out both preventive measures on farms with a tick-borne disease problem.

An additional benefit from LAT treatment was the improved productivity of treated lambs (measured by improved weight gains), although the cost effectiveness requires further assessment. The farmers themselves thought that their treated lambs looked better and it may be that the small extra weight gains improved the appearance of the treated groups. It is possible that the improved weight gains amongst treated lambs resulted from the suppression of subclinical infections such as tick pyaemia and bacterial pneumonia and also possibly by reducing the weight loss that has been reported following

a simple TBF infection (Foggie, 1951).

It is worth noting that of Britain's total sheep population of around 35 million, 16 million are ewes, of which about 7 million live in hill areas. The vast majority of these and their lambs are exposed to the sheep tick Ixodes ricinus. On the modest assumption that the incidence of tick pyaemia is around 3%, possibly around 200,000 lambs develop tick pyaemia annually, most of which die or fail to produce any profit. This also takes no account of the possible role of TBF in predisposing to other diseases. Such a level of losses certainly justify continued investigations into the control of tick-borne diseases of sheep.

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APPENDIX

APPENDIX - BACTERIOLOGICAL METHODS

Sheep Blood Agar

Take 900 ml of Nutrient Agar (Oxoid Ltd) and autoclave for 15 minutes at 15 lbs/in^2. Cool agar to 45^{o}C and asceptically add 100 ml of heparinised sterile sheep blood.

Coagulase Test Method

Five ml volumes of Nutrient Broth (Oxoid Ltd) were inoculated with isolated colonies of Gram-positive cocci and incubated overnight at 37^{o}C. Five drops of broth culture were added to both 0.5 ml of a 1:5 dilution of sheep plasma in saline and to 0.5 ml of a 1:5 dilution of human plasma in saline. The two tests were incubated at 37^{o}C in a water bath and the tubes were examined for clotting, indicating a coagulase positive strain of S. aureus.

Bernheimer and Schwartz Medium (Bernheimer and Schwartz, 1963).

Prepare Yeast Extract Diffusate as follows: dissolve 200 g of Yeast Extract (Difco Laboratories, Detroit, U.S.A.) in 500 ml distilled water by steaming and after cooling pour into a 50 cm length of 2 " Visking Dialysis Tubing (Scientific Instrument Centre, London, England) which had been previously soaked in 70% ethanol. Immerse the dialysis sac in 1500 ml distilled water in a 5 litre beaker and stir for 72 hours at 4^{o}C. Discard the dialysis sac and its contents and make the volume of the diffusate up to 1600 ml with distilled water. Normally duplicate batches were prepared to yield 3200 ml of diffusate.

Prepare medium as follows:

Yeast extract diffusate	3200 ml
Bacto Casamino Acids (Difco Labs)	64.0 g
Glucose	8.0 g
Nicotinic Acid	3.7 mg
Aneurine Hydrochloride	0.4 mg

Adjust the pH to 7.1 with 1 N NaOH and autoclave the medium for 30 minutes at 15 lbs/in^2.

TABLE A1. Reciprocal Haemagglutination-inhibiting Titres to Horse Red Blood Cells (HRBC)

Sheep Number	Reciprocal Titres at each sampling time Days after HRBC administration									
	-4	2	6	9	14	17	20	23	27	34
TBF on Day 0 HRBC on Day 0										
47	192	384	3072	3072	3072	6144	6144	1536	1536	384
53	192	192	192	384	1536	384	768	768	384	384
65	48	192	96	384	192	384	384	192	192	96
45-Control (No TBF)	192	96	192	384	768	768	384	384	384	384

TABLE A1 (cont). Reciprocal haemagglutination-inhibiting Titres to Horse Red Blood Cells (HRBC)

Sheep Number	Reciprocal Titres at each sampling time: Days after HRBC administration									
	-11	-4	0	3	8	11	14	17	21	28
TBF on Day -5 HRBC on Day 0										
46	96	96	96	96	1536	768	1536	3072	3072	6144
48	192	192	96	192	192	768	1536	1536	768	768
64	96	192	96	384	768	1536	3072	3072	1536	3072
59-Control	6	48	96	96	384	1536	1536	3072	1536	768

TABLE A1 (cont). Reciprocal Haemagglutination-inhibiting Titres to Horse Red Blood Cells (HRBC)

Sheep number	Reciprocal Titres at each sampling time Days after HRBC administration									
	-14	-8	-4	-1	4	7	10	13	17	24
TBF on Day -9 HRBC on Day 0										
44	192	384	384	384	384	6144	6144	6144	6144	6144
62	24	48	6	48	192	192	768	1536	768	384
66	48	48	48	24	96	768	768	1536	768	768
60-Control (No TBF)	192	96	192	192	192	1536	1536	3072	768	768

TABLE A2. Reciprocal Haemagglutination-inhibiting Titres to Louping-ill Vaccine

Reciprocal Titres at each sampling time

Sheep Number	Days after vaccination									
	-4	2	6	9	14	17	20	23	27	34
TBF on Day 0 Vaccine on Day 0										
47	10	10	10	40	40	80	160	640	320	1280
53	10	10	10	20	10	10	10	20	20	80
65	10	10	10	160	80	160	160	320	640	640
45-Control (No TBF)	10	10	10	80	20	40	40	80	80	80

TABLE A2 (cont). Reciprocal Haemagglutination-inhibiting Titres to Louping-ill vaccine

Reciprocal Titres at each sampling time

Sheep Number	Days after vaccination									
	-11	-4	0	3	8	11	14	17	21	28
TBF on Day -5 Vaccine on Day 0										
46	10	10	10	10	40	80	80	160	80	160
48	10	10	10	10	10	80	40	80	80	160
64	10	10	10	10	10	10	80	160	80	80
59-Control (No TBF)	10	10	10	10	10	10	10	10	40	40

TABLE A2 (cont). Reciprocal Haemagglutination-inhibiting Titres to Louping-ill vaccine

Sheep Number	Reciprocal Titres at each sampling: Days after vaccination									
	-14	-8	-4	-1	4	7	10	13	17	24
TBF on Day -9 Vaccine on Day 0										
44	10	10	10	10	10	10	10	10	10	40
62	10	10	10	10	10	10	10	10	10	20
66	10	10	10	10	10	10	40	160	80	160
60-Control (no TBF)	10	10	10	10	10	10	20	80	40	80