THE EFFECT OF THEILERIOSIS ON THE REPRODUCTIVE FUNCTION OF BORAN/FRIESIAN CROSS HEIFERS

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DECLARATION

This thesis has been composed by me and describes my own work except where collaboration is gladly acknowledged.

DEDICATION

To my wife Muthoni and children Mukami, Kubai, Njeri and Karimi for their love, patience and encouragement.

ABSTRACT

This thesis describes studies that were undertaken to evaluate the effect of *T. parva* infection on the reproductive function of Boran/Friesian cross heifers. The study was conducted in four separate experiments. In Experiments 1 and 2, the animals were monitored for cyclical status post-infection. In Experiments 3 and 4, the infected animals were divided into two groups: one group was monitored for cyclical status and the other for both cyclical and pregnancy status after exposure to a bull. The reproductive function was assessed by routine monitoring of oestrus behaviour, palpation of the genital reproductive tract *per rectum* and determination of serum progesterone (P4) concentrations. Further, in view of observed acyclicity in the early work, attempts were made to assess the pituitary response to gonadotrophin releasing hormone (GnRH) in a group of animals (Ch. 5) in an attempt to explain partly the mechanisms involved in the impairment. Animals were monitored for the progression of disease by clinical and parasitological responses. Further, subject to death or sacrifice, a macroscopic and histological examination of target and various other organs and tissues was undertaken in an effort to establish the part played by observable pathological changes in the development of impaired reproductive function.

In Experiment 1, ten heifers were infected with 1:20 *T. parva* stabilate while four others remained as uninfected controls. Two of the infected animals died, one each on days 17 and 29. Clinical reactions in the recovered animals ranged from mild to moderate. P4 profiles and ovarian structures revealed that three of eight recovered animals had luteal dysfunction post-infection although two of the three showed clinical oestrus during this particular period. The other five recovered animals cycled regularly. The results from clinical and pathological studies did not indicate any direct adverse effect of infection on reproductive function. All the four controls cycled throughout the study period.

In Experiment 2, ten animals were infected with an undiluted stabilate while

four others acted as uninfected controls. Eight of ten infected animals died of severe disease. Of the eight fatal cases, six were cyclic pre- and post-infection based on P4 profiles, ovarian structures and/or behavioural data. P4 profiles and ovarian structures revealed that the other two animals were acyclic before and after infection although clinical oestrus was detected in both pre-infection. Both of the recovered animals underwent extended periods of acyclicity post-infection. There was no evidence linking the acyclicity to the active parasitosis or pathological changes observed in the reproductive organs. All control animals cycled throughout the study period.

In Experiment 3, 14 heifers were infected with undiluted stabilate and treated at the height of clinical reaction. One of eight animals subjected to serial kill, was acyclic before and after infection based on P4 profile and ovarian changes although it was seen in heat pre-infection. Of the remaining seven, one animal became acyclic while all the other six revealed clinical oestrus or/and cyclical ovarian changes after infection. Two of six animals exposed to a bull became pregnant while the others were cycling regularly at seven months post-infection. All controls cycled throughout the study period.

In Experiment 4, eight animals were immunized by infection and treatment method while four others acted as uninfected controls. Three of four animals monitored for cyclicity cycled regularly post-immunization. The other had a persistent *corpus luteum* which regressed spontaneously after an extended luteal period of 42 days and cycled regularly subsequently. One of four animals exposed to a bull became pregnant while the others cycled regularly to week 20 post-immunization. All the controls cycled throughout the study period.

The evidence from the four experiments indicated that *T. parva* did not directly affect reproductive function. Pathological lesions were observed in the reproductive organs but these were mild and did not affect normal reproductive physiological activity. It is postulated that the impaired reproductive function was due to an indirect effect linked to loss in condition following infection.

INTRODUCTION

Theileriosis is the most important disease of livestock in East and Central Africa and constitutes a major constraint to the development and expansion of the cattle industry. Of the five *Theileria* species recognized in Africa south of the Sahara (Uilenberg, 1981; Young, 1981), *T. parva* is of the greatest economic importance. *T. parva* causes three distinguishable syndromes based on clinical and parasitological parameters.

Of the three syndromes, East Coast fever (ECF) is the most important mainly due to its wider distribution. ECF is often fatal. Experimentally, it was reported to have a morbidity rate of 87.5% and a mortality rate of about 95% (Brocklesby *et al.*, 1961). The main economic losses due to ECF are based on direct mortality. However, despite its importance as a disease of livestock, precise figures on animal losses are difficult to obtain. In Kenya, it was estimated that 50000-70000 mature cattle and 12000 of 115000 calves born to artificial insemination die of ECF yearly (Duffus, 1977). It has also been estimated that between 53% (Duffus, 1977) and 80% (Dolan and Young, 1981) of the dairy herds in Kenya are exposed to the tick *Rhipicephalus appendiculatus* which is the main vector of ECF and that at least 1% of the animals at risk die of the disease each year.

Bovine malignant theileriosis has only been described in Zimbabwe (Lawrence, 1981). Most outbreaks occur in the high veld and coincide with the high tick build-up during the high rainfall season from January to March. Clinically, it is less severe than ECF and on tick passage, the causative agent did not transform to the type causing ECF (Lawrence, 1981).

Corridor disease is a cattle disease derived from the African buffalo (*Syncerus caffer*) and is probably the origin of the cattle-derived forms (Young *et al.*, 1988). The parasite is widespread in the buffalo population and virtually every buffalo within the distribution of *R. appendiculatus* is infected (Young *et al.*, 1978). Although there are

reports of the disease being fatal in buffalo (Neitz, 1957), these are rare. However, in cattle the disease is highly fatal.

The primary vector of *T. parva* is *R. appendiculatus* although other *Rhipicephalus* species are capable of transmitting disease (Neitz, 1957; Brocklesby *et al.*, 1966). Transmission is trans-stadial. In the vertebrate host, two stages of the parasite are recognized: the intralymphocytic macroschizont, and the intraerythrocytic form, the piroplasm. Within the tick, the infective particles can be maintained for periods of almost two years before the tick feeds on a susceptible host (Young *et al.*, 1983a), and this feature therefore introduces great difficulties in the control of theileriosis. On the other hand, it is now widely recognized that a *T. parva* carrier state usually develops following natural recovery from *T. parva* infection, immunization or chemotherapy (Neitz, 1957; Barnett and Brocklesby, 1966a; Dolan, 1981, 1986a,b; Moll *et al.*, 1981; Young *et al.*, 1981; Mutugi *et al.*, 1988a; Kariuki, 1991). Ticks feeding on carrier animals can become infected and transmit the infection to susceptible hosts. It is thought that *T. parva* carrier state is probably more important in the epidemiology of theileriosis than ticks.

The response to infection with *T. parva* depends on the virulence of the particular subspecies and strain of the parasite involved, the quantum of the infective dose and the resistance of the host. *Theileria* can cause either asymptomatic infection or syndromes that may be severe, moderate or mild. Occasionally, the disease is presented as a chronic wasting syndrome (Dolan, 1981). Reactions vary widely but are more severe in susceptible exotic breeds. In endemic areas in which the majority of the cattle population occurs, a high proportion of the animals develop resistance from sustained low dose infection.

In *T. parva* infections, the pathogenesis is either directly or indirectly dependent on the destructive effect of schizonts on the lymphoblastoid cells. The pathological picture is dominated by changes which occur in the major lymphoid organs, in the peripheral lymphoid tissues and various other organs and tissues as well

as in the lymphocytes in circulation. Initially, the response to the parasite is lymphoproliferative followed by widespread dissemination of infected lymphoblastoid cells while later stages are associated with lymphocytolysis.

Most of the economic losses due to theileriosis are as a result of direct losses from death. However, equally important are the high cost of tick control, loss in productivity in recovered animals, high cost of research inputs directed at the development of a safe and effective vaccine, high cost of treatment as well as the constraint it introduces to the introduction of high-yielding cattle into endemic areas. Any strategies aimed at the eradication of *T. parva* infections have to take consideration of its unique host-parasite relationship; in the tick vector, in the bovine host and in the buffalo population. In Africa, *Rhipicephalus* species infest cattle in large numbers (Walker, 1974) and they also have a wide mammalian host range within both the domestic and wild animals. To date, the main thrust at the control of theileriosis is directed at the reduction of the tick vector population since its total eradication is not practical. Chemical control is the most widely practised method but this has its many challenges which include the cost of acaricides and tick resistance to acaricides. The problem is further complicated since *R. appendiculatus* is a three-host tick and between hosts when it is inaccessible to chemical control it can maintain the parasite for long periods (Young *et al.*, 1983a). Further, *T. parva* can be maintained in a carrier state within the cattle and buffalo populations.

Since it was realized that animals that recover from theileriosis develop a solid immunity (Neitz, 1953), several immunization methods have been tried to control the disease (Purnell, 1977). Of the methods that have been tried so far, the most successful to date is the infection and treatment method or chemoprophylaxis using tetracyclines (Radley *et al.*, 1975a,b). Animals treated in this way may develop inapparent to mild reactions but subsequently become immune. However, one of the drawbacks to this method is that a carrier status usually ensues (Mutugi *et al.*, 1988a; Kariuki, 1991). The effect of infection and treatment on productivity is equivocal with

some reports indicating no loss (Morzaria *et al.*, 1988) and others indicating loss in weight (Mutugi *et al.*, 1988a). Possibilities exist for the development of a safe and effective vaccine using *T. parva* antigens targeted against the sporozoite or macroschizont-infected cell. However the research is only in the developmental stage.

Until recently, there was no known cure for *T. parva* infections. Two specially developed compounds have undergone extensive investigation and are now available for clinical use; these are parvaquone (Clexon, Wellcome) and halofuginone (Terit, Hoechst). Both compounds are schizonticidal. However, they do not completely eliminate the parasite and treated animals suffer from persistent parasitosis (Dolan, 1981,1986a,b) and thus act as reservoirs of infection. Evidence has also been presented indicating that treated animals suffer some loss of productivity (Dolan, 1986a,b).

From a clinical perspective, *T. parva* infection causes a debilitating syndrome (Henning, 1956). As more epidemiological studies are done, it is being recognized that the poor productivity of animals in endemic areas may be associated with persistent parasitosis (Young *et al.*, 1981). In addition, abortions in pregnant animals and long anoestrous periods were linked to infection with *T. parva* infections (Oteng, 1977). The linkage of low productivity to persistent parasitosis to date is basically one of association since no work has been done to elucidate this relationship.

Evidence linking *T. parva* infection to impairment of reproductive function so far is only anecdotal. This linkage has not been verified and if this allusion happens to be true, it is not known whether the effect is due to a direct effect of the parasite on the reproductive organs or to an indirect effect of the disease.

This thesis reports on:

1. Studies aimed at investigating whether animals recovering from a low or high dose *T. parva* infection suffer impaired reproductive function.
2. Studies aimed at investigating whether animals treated with parvaquone at the height of clinical reaction suffer an impairment of reproductive function.

3. Studies aimed at investigating whether immunization by infection and treatment method has an adverse effect on the reproductive function.

To achieve this, all animals in this study were monitored for cyclical status and in addition, a number of animals were also assesssed for fertility in the last two experiments. Further, the reproductive organs were examined for pathology in an attempt to explain whether the impaired function was due to damage of target organs.

It was hoped that the knowledge gained in this study would establish whether *T. parva* affects reproductive function in the female bovid and what mechanisms may be involved. The results would contribute partly towards our understanding of the long-term effects of theileriosis on productivity.

CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

This literature review will concentrate on two major aspects that were interrelated in this study. The main focus of the study was to investigate the effect of theileriosis on the reproductive function with particular emphasis on the cyclical status. Initially, a review of the disease with particular emphasis on the areas investigated will be presented. In the second part, the review will concentrate on reproductive physiology in the female bovid. Reference will be made to other species when no available literature could be obtained on cattle.

1.2 East Coast fever

Our knowledge of theilerioses has been summarized in a number of good reviews (Neitz, 1957; Wilde, 1967; Purnell, 1977) and symposia (Henson and Campbell, 1977; Irvin *et al.*, 1981; Dolan, 1989) in which an exhaustive and comprehensive body of information available is presented.

1.2.1 Aetiology

East Coast fever is caused by a *sporozoa* in the genus *Theileria*. The principal characteristics of the *Theileriidae* are the presence of schizont stages in lymphoid cells, the presence of erythrocytic forms and transmission by ticks. The generally-accepted bovine species recognized in Africa are *T. parva, T. annulata, T. mutans, T. orientalis, T. taurotragi and T. velifera* (Uilenberg *et al.*, 1985).

The taxonomic sub-speciation of *T. parva* has always been subject to discussion and revision. Three subspecies based on trinomial nomenclature, namely,

T. p. parva, T. p. lawrencei and *T. p. bovis* had been adopted for *T. parva* (Uilenberg, 1981). This distinction was based on clinical and epidemiological parameters. However, it has been demonstrated by use of monoclonal antibodies and DNA probes (Conrad *et al.*, 1989) that genetically the three subspecies are not distinguishable and descriptive terms, such as cattle-derived for *T. p. parva* and *T. p. bovis* and buffalo-derived for *T. p. lawrencei* have been recommended for adoption (Anon, 1989a).

East Coast fever (ECF) is classically caused by a cattle-derived form of *T. parva*. *T. parva* (buffalo-derived) is the causative agent of Corridor disease in cattle. The parasite was originally described from buffalo (*Syncerus caffer*) by Neitz (1955). On tick passage through cattle, the buffalo-derived *T. parva* becomes indistinguishable from the cattle-derived *T. parva* (Barnett and Brocklesby, 1966c; Young and Purnell, 1973; Young *et al.*, 1973), suggesting that the two parasites are of common origin. Bovine malignant theileriosis is caused by a bovine-derived *T. parva* and has only been described in Zimbabwe (Lawrence, 1981). It is distinguishable from ECF by its less severe clinical reaction and lower mortality.

In the present study, a buffalo-derived form of *T. parva* was used. The term ECF or theileriosis will be used to encompass disease syndromes caused by the species and distinction between cattle- and buffalo-derived form of disease made only where marked variations exist.

1.2.2 Life cycle of *T. parva*

A summary of the life cycle of *T. parva* is presented in Fig. I-1.

1.2.2.1 In the tick vector

All theilerial parasites are tick-transmitted. The life cycle of the parasite is linked very closely with that of the tick and the two are best considered together. Transmission occurs trans-stadially. *R. appendiculatus* is a three-host tick; this means that the larval and nymph instars can become infected with *T. parva* when they feed on

cattle or buffalo and transmission is only possible through nymphs or adults which moult from the previous instar.

Piroplasms in the erythrocytes of the infected host are the parasite stages infective for the tick. The infected red blood cells are lysed in the tick gut releasing piroplasms which differentiate into micro-gametes and macro-gametes. These gametogenic stages are recognizable in the gut of ticks 1 to 4 days after repletion on the bovine host. Pairs of gametes undergo anisogamy to produce zygotes which enter cells of the gut wall (Schein *et al.*, 1977). During and following the period of moulting, the zygotes differentiate into motile kinetes which are released into the haemolymph (Fujisaki *et al.*, 1988), migrate to the salivary glands and invade the E cells of type III acini (Fawcett *et al.*, 1982a,b, 1985; Binnington *et al.*, 1983; Young *et al.*, 1983b).

After initial division to form sporoblasts, parasites remain quiescent until the now-moulted tick (now nymph or adult) begins feeding on a new host. The sporoblasts then undergo rapid division and maturation to form sporozoites (Fawcett *et al.*, 1985). The normal feeding stimulus results in the parasite maturation in the salivary glands after about 3 days, with the peak production of mature infective sporozoites on days 4 and 5 (Purnell *et al.*, 1973). Sporogony can also be induced by artificial incubation of the ticks at 28-37^{o}C (Young *et al.*, 1979, 1984) and may occasionally occur before feeding under natural conditions in which there is a high ambient temperature (Ochanda *et al.*, 1988). Maturation of sporozoites occurs more rapidly in nymphs than in adults. However the infection rates are reported to be higher in adult ticks (Purnell *et al.*, 1973). Ticks fed over piroplasm parasitaemias of 41-50% were found to have a higher infection rate than those in the lower ranges although there was a marked variation in the infection levels between individual ticks (Purnell *et al.*, 1974).

Although under natural conditions, moulting of the tick usually occurs between 30 and 80 days after completion of feeding, it is reported the tick may survive for over two years before feeding again on a new host (Young *et al.*, 1983a, 1987). Studies on

the longevity of *T. parva* in its tick vector so far undertaken have shown that the parasite does not remain infective as long as the tick lives. Studies using ticks experimentally infected with *T. parva* have shown that they remain infective for 643 days, although the level of infection declines progressively from about 250 days onwards (Young *et al.*, 1983a).

1.2.2.2 Life cycle in the mammalian host

Following tick or artificial inoculation of sporozoites into a susceptible bovine host, the first evidence of infection is the appearance several days later of macroschizonts within lymphoblastoid cells in the regional lymph node. The earliest that parasites have been detected is 4 days after infection (Wilde, 1967). The immediate fate of sporozoites after inoculation is still not known, mainly because of the difficulty of detecting the parasite during this period. The information on the cell type first entered by the infective particles in the bovine host is also not clear although several theories have been forwarded.

In the view of Wilde (1967), two main possibilities exist: firstly that the parasite on injection enters a cell type of the skin or subcutaneous tissue, possibly a histiocyte, develops there for some time and subsequently migrates to a lymphoid cell or secondly that the parasite is carried to the local drainage lymph node and there enters a cell of the lymphatic system or possibly of the reticuloendothelial system. Other investigators suggested that *T. parva* came into contact with bovine leucocytes and became closely associated with these cells either at the site of injection by the tick or in the local drainage lymph node (Brown *et al.*, 1969; 1978).

Initial electron microscopic studies however, did not reveal the presence of infective particles in association with all cells undergoing hyperplasia (Jarrett and Brocklesby, 1966). *In vitro* studies indicated that sporozoites can enter lymphocytes within a matter of minutes (Fawcett *et al.*, 1982c). Consequently the data are equivocal but entry into lymphocytes is favoured.

Penetration of the lymphocytes by the parasite was reported to result in changes in cellular morphology characterised by increased amounts of cytoplasm and enlargement of the Golgi apparatus. During the 24 h following invasion, the parasite becomes located in the Golgi region of the cell, and after another 1-2 days, nuclear division occurs resulting in multinucleate macroschizonts (Stagg *et al.,* 1981). Division of the macroschizont occurs synchronously with the host cell division so that there is a clonal expansion of the cell population initially infected (Irvin *et al.,* 1982).

The next parasite stage detected is the microschizont. This is distinguished from the macroschizont by having a large number of small regular shaped nuclei. The mechanism by which microschizonts derive from the macroschizont is not clearly understood, but it was thought that this occurs by a process of nuclear budding (Jarrett and Brocklesby, 1966). Microschizonts give rise to merozoites which enter erythrocytes by penetration of the cell membrane and develop to piroplasms. Jarrett *et al.* (1969) and Radley *et al.* (1974) found that the production of piroplasms is time dependent, occurring around day 12-13 after parasite inoculation irrespective of the size of the infective dose. However, Dolan *et al.* (1984b) found the time to piroplasmaemia to be dose dependent. Piroplasms of *T. parva* do not appear to divide readily, nor have dimorphic gametocyte forms been recognised. However, more than one piroplasm have been detected in erythrocytes and this is attributed to multiple invasion rather than multiplication of the parasite within erythrocytes (Jarrett *et al.,* 1969). Piroplasm-infected blood can be ingested by ticks feeding on the host and the disease cycle is thus perpetuated.

1.2.3 Epidemiology

East Coast fever is endemic in a large area of East and Central Africa (Neitz, 1957). The presence of the disease closely follows the distribution of the tick vector, *R. appendiculatus* (Young, 1981; Norval *et al.*, 1991). Other ticks were reported to transmit ECF experimentally (Neitz, 1957; Brocklesby, 1965; Brocklesby *et al.*, 1966),

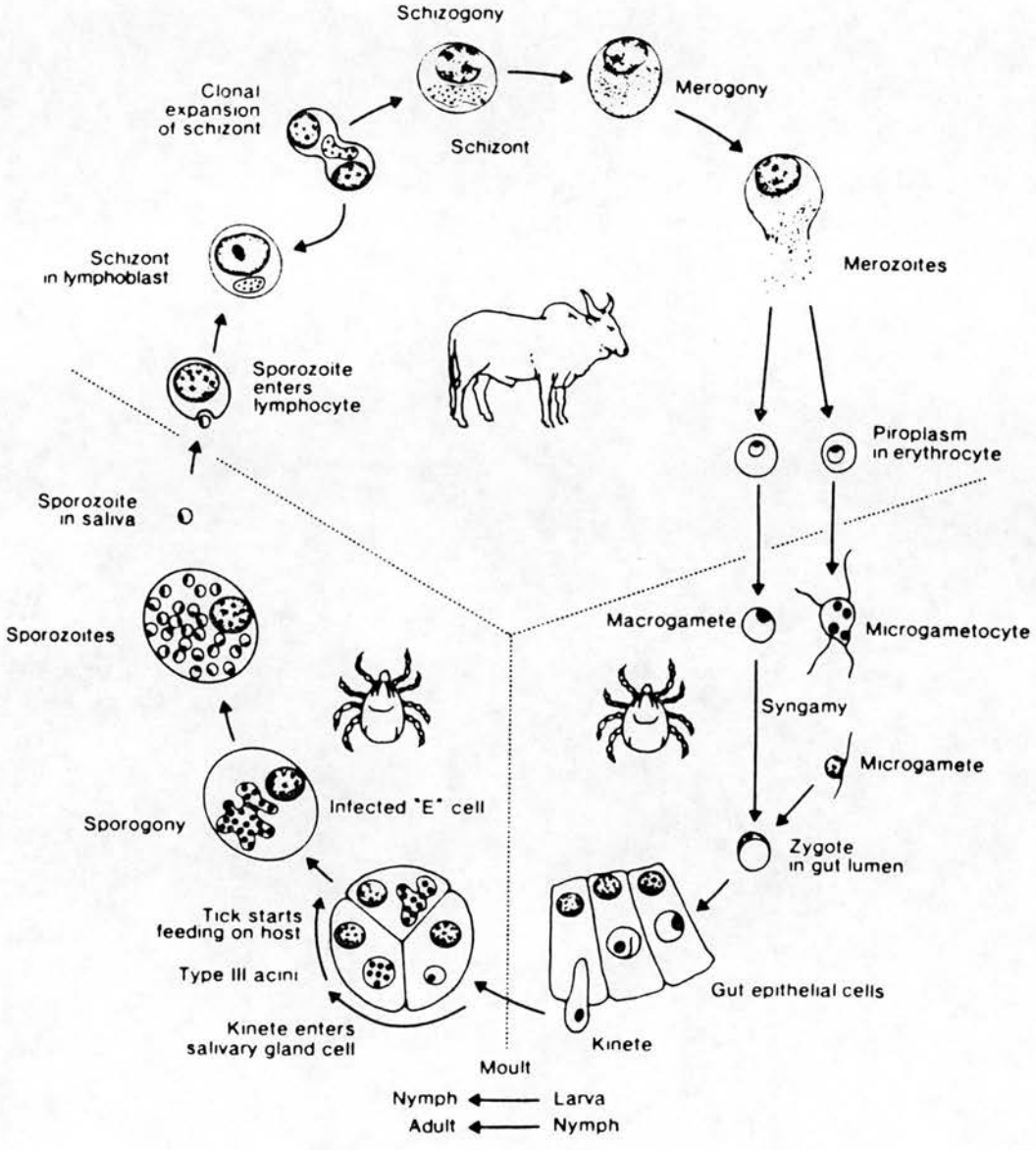


Fig. I-1.

Life cycle of *Theileria parva* in cattle and in the ixodid tick *Rhipicephalus appendiculatus*. Figure prepared by A.S. Young.

but were not considered to play any significant role in the field (Barnett, 1968). *R. appendiculatus* thrives at altitudes from sea-level to over 2100 m above sea-level providing there is adequate vegetation and rainfall above 20 inches a year (Walker, 1974). In some areas, conditions favourable for the tick development prevail throughout the year, so that there is a relatively constant challenge. In other areas, there is a marked seasonal variation in the number of ticks, following temperature and rainfall patterns.

The role of the African buffalo (*Syncerus caffer*) in the epidemiology of ECF syndrome is well recognized (Brocklesby and Barnett, 1966a). Buffalo act as a constant reservoir from which infection can be maintained in the resident tick population (Young *et al.*, 1978). Although there are reports of deaths due to the buffalo-derived parasite in buffaloes, these are rare (Brocklesby and Barnett, 1966b), but in cattle, this parasite is usually pathogenic and fatal. The disease syndromes of ECF and Corridor disease have many features in common though they are usually distinguishable. Lymphocyte parasitosis and piroplasmaemia is lower in Corridor disease compared to ECF (Neitz, 1957; Young *et al.*, 1973).

An important feature of *T. parva* infection in cattle and in buffalo is that a carrier state can develop so that ticks feeding on them can become infected over a period of several years. Although the carrier state of the buffalo-derived *T. parva* had been known to exist for many years in naturally recovered buffalo and cattle (Barnett and Brocklesby, 1966c) a similar state for the cattle-derived parasite was only demonstrated more recently (Young *et al.*, 1981). This oversight could have arisen due to the emphasis that has been placed on studies using a laboratory stock, *T. parva* (Muguga), one of the few parasite stocks not shown to cause a carrier state. As more epidemiological studies are done, it is becoming apparent that in endemic areas, most cattle are carriers of *T. parva* (Young *et al.*, 1986; Kariuki, 1991). Young *et al.* (1981, 1986) found that the carrier state of *T. parva* approached 100% in adult cattle raised in endemic area and Kariuki (1991) has confirmed this in two other areas in

Kenya. It is likely that the carrier state plays a greater role than ticks in the maintenance of infection in endemic areas. A carrier state may also develop following immunization by infection and treatment (Mutugi *et al.*, 1988a) or chemotherapy (Dolan, 1981).

Theileria parva carrier state can be demonstrated by feeding clean *R. appendiculatus* ticks to suspect cattle and subsequently examining the salivary glands of the dropped ticks for infection and/or using such ticks for transmission studies in susceptible hosts (Young *et al.*, 1981). Culture of peripheral blood lymphocytes has been used to demonstrate the carrier state in immunized and naturally recovered cattle (Mutugi *et al.*, 1988a; Kariuki, 1991).

1.2.4 Pathogenesis

The infectivity and virulence of various species of *Theileria* in domestic ruminants has been discussed by Barnett (1977). *Theileria parva* is almost exclusively a pathogen of cattle; the only other species of proven susceptibility are the closely-related buffaloes (Barnett and Brocklesby, 1966b). Stagg (1981) confirmed this observation by showing that *T. parva* only infected lymphocytes of cattle and African buffalo.

With regard to cellular changes which occur in lymphoid organs, Morrison *et al.* (1981) considered the infection to progress through three phases, namely, an initial period when infection is not detectable, a second period when infection becomes patent throughout the lymphoid system and is associated with marked hyperplasia, and a final period when the level of parasitism is high and there is disorganization and cellular depletion of the lymphoid organs.

During the prepatent period when parasites cannot be detected, the only apparent evidence of infection is an increase in activity characterized by a slight increase in cellularity of the node and a marked increase in cellular output in efferent lymph. Radley *et al.* (1974) suggested that this unknown stage in the life cycle of the

parasite may be disguised either by being associated with the host cell nucleus, by having a different staining reaction to the conventional macroschizont, or by being present in a different site or in a limited focus within the lymph node.

Following the initial detection of the parasite, there is a dramatic increase in cellularity of the regional drainage lymph node. Parasitised and blast lymphoid cells increase during this phase. Coincident with their appearance, there is an increase in cell output and markedly elevated levels of parasitised and blast lymphoid cells in efferent lymph (DeMartini and Moulton, 1973b). The pattern of cellular changes in lymph nodes distant from the site of inoculation and in the spleen is similar to that in the regional node, except that the parasites are first detected about two days later and the increases in cellularity are less pronounced and occur more gradually (DeMartini and Moulton, 1973a).

In the later stages of the disease, there is a wide lymphocytolysis with depletion of lymphocytes both in the solid organs and in the recirculating pool (Steck, 1928; Barnett, 1960). The lymphocytolysis affects both parasitised and non-parasitised cells. The mechanism by which lymphocytolysis occurs is not known. While infection with the parasite itself may result in the destruction of some cells, it is thought that immune-mediated cytotoxicity plays a role in the widespread cell death that occurs (Morrison *et al.*, 1986).

The infected cells spread not only to secondary lymphoid tissues but also to the thymus and bone marrow and to a variety of non-lymphoid tissues (De Kock, 1957), being particularly numerous in the *lamina propria* of the gastro-intestinal tract and the interstitial tissue of the lungs (Bwangamoi *et al.*, 1971). The presence of large numbers of parasitised cells in the gastrointestinal tract and lungs probably reflects the accumulation of infected cells at sites in which there is normally a high rate of lymphocyte traffic.

1.2.5 Clinical signs

Henning (1956) summarized the clinical signs that may be encountered in ECF. The first indication of disease is a rise in rectal temperature above 39.5°C. The incubation period varies widely depending on the size of the infective dose and the strain of parasite but is usually between 8-25 days (Henning, 1956; Neitz, 1957; Brocklesby, 1962; Jura and Losos, 1980). The mean incubation period also varies but is usually close to 14 days (Brocklesby, 1962). Other clinical symptoms appear only after the elevation of body temperature. These include a gradual loss in appetite, cessation of rumination, excessive salivation and lachrymation, staring hair coat, decreased lactation, drying of the muzzle and drooped ears. Swelling of the superficial lymph nodes which may be progressive as the disease advances is a characteristic symptom. Difficult and distressed breathing precedes a short cough. Just before death, dyspnoea may become so severe that the animal becomes recumbent and usually dies of respiratory failure. Constipation is sometimes observed, although Henning (1956) considered diarrhoea a more common symptom, being observed from around day 8-10 after developing fever. The evacuations are tar-like, blood-tinged and slimy. The animals become progressively weaker and become recumbent as a result of posterior paresis. Death usually occurs around 25-30 days after infection. Loss of condition is considered to be common, but sometimes an animal keeps on feeding for some time so that loss in condition is not so evident (Henning, 1956).

Depending on the virulence of the parasite, the host's resistance and the mode of transmission, Neitz (1957) classified clinical manifestations due to *Theileria parva* infection into acute, subacute, mild and inapparent forms. In an attempt to standardize the description of disease reactions caused by *Theileria parva* in cattle, a broad classification of reactions using clinical and parasitological parameters were recommended recently (Anon, 1989b). These classifications comprise of no reaction or no apparent reaction; mild reaction; moderate reaction and severe reaction followed by recovery or death.

The clinical signs described for the cattle-derived and buffalo-derived *T. parva* syndromes are basically the same with minor variations. The characteristics that may differentiate the buffalo-derived from the cattle-derived infection include the higher severity (Neitz, 1957) and manifestation of nervous signs (Mettam, 1934; Mettam and Carmichael, 1936; Jura and Losos, 1980) in the former.

Under experimental conditions, ECF has been reported to have a high morbidity (87.6%) and mortality (95%) rate (Brocklesby *et al.*, 1961). However, although death is the invariable outcome, mild forms have been described in the laboratory and in the field (Barnett and Brocklesby, 1966a; Shannon, 1973).

Apart from the high losses incurred by animal deaths, other losses include reduced productivity in animals recovering from ECF. Oteng (1977) estimated that between 5-10% of calves recovering from ECF remain unthrifty, are often stunted and may not gain their pre-infection weights for at least six months. He also reported that adult animals recovering from the disease take up to 18 months before starting to gain weight even under good management conditions. Young *et al.* (1981) suggested that *T. parva* carrier status may have a detrimental effect on the productivity of affected cattle and this could explain for the slow growth and small size of cattle in ECF endemic areas.

Recovery after treatment has also been found to be associated with lowered weight gains (Dolan, 1986a,b). Dolan (1986a) examined the long term weight changes, carrier status and the nature of chronic ECF in experimentally infected animals treated with parvaquone. Four months after inoculation, the treated recovered cattle had regained their starting weights. However, the treated recovered animals which developed a carrier status regained their starting weights more slowly and were between 50-100 kg lighter than the controls at 10 months post-inoculation. The group that did not develop a carrier status regained weight similarly to the uninfected controls.

1.2.6 Pathology

1.2.6.1 Gross changes

The pathological changes in ECF have been comprehensively described by various investigators (Steck, 1928; Henning, 1956; Neitz, 1957; De Kock, 1957; Barnett, 1960; Munyua *et al.,* 1973). According to Neitz (1957), the lesions of cattle-derived and buffalo-derived infections show little variation.

Henning (1956) summarized the pathological lesions that may be encountered in East Coast fever. He observed that in rapidly fatal cases, the general condition of the carcase is good, but in protracted cases there is evidence of loss of condition. The gross lesions include a gelatinous or haemorrhagic subcutaneous oedema, enlarged lymphatic glands which are moist and hyperaemic on section.

In the abdominal cavity, there is a fair amount of straw coloured fluid and scattered petechial haemorrhages of the peritoneum. The abomasum is generally hyperaemic, swollen due to submucosal oedema and frequently contains multiple mucosal erosions and ulcers especially in the pyloric region. The intestines are haemorrhagic and the Peyer's patches are hypertrophied and stand out prominently. The liver is generally enlarged, yellowish in colour and frequently mottled with greyish-white foci. The kidneys have a variegated appearance from greyish-white nodules, the "pseudoinfarcts", which are believed to be aggregates of lymphoid cells.

In the thoracic cavity, there is usually a hydrothorax. The lungs are usually enlarged and oedematous on section. The bronchioles, bronchi and trachea contain variable amounts of froth which frequently oozes through the nostrils.

These are the gross lesions that are likely to be encountered and have been reported by others. However, rare gross lesions reported include brain and meningeal haemorrhages in cerebral theileriosis (Henning, 1956), anaemia (De Kock, 1957), degeneration of certain muscle groups (Neitz, 1957) and macroscopic lymphoid aggregates in the gall bladder and intestines (Bwangamoi *et al.*, 1971).

1.2.6.2 Histological changes

The microscopic pathological picture in theileriosis is dominated by the changes that occur in the major lymphoid organs and the peripheral lymphoid tissues in various other organs and tissues.

The first lesion which develops at the site of inoculation of the parasite consists of raised nodules characterized by epidermal ulcers, haemorrhage, oedema and infiltration by inflamatory cells predominantly of the mononuclear type (Kimeto, 1978).

Microscopically, the lesions in the major lymphoid organs comprise of proliferating lymphoblastoid cells and varying amounts of necrosis. The sequential changes in the local lymph nodes reported by Barnett (1960) consisted initially of multiplication of lymphocytes and reticular cells followed later by degeneration and necrosis of these lymphoblastoid cells as well as the small lymphocytes. Other changes included an eosinophilic exudation into the germinal centres, small haemorrhages and increased numbers of neutrophils, eosinophils, macrophages and plasma cells especially in the medullary sinusoids. Similar early (DeMartini and Moulton, 1973a) and late changes (De Kock, 1957; Munyua *et al.*, 1973) have been described. Changes in the spleen and other distant lymph nodes are comparable to but of less intensity than those observed in the regional node (De Kock, 1957; Munyua *et al.*, 1973)

In the myeloid tissue there is a depletion of immature erythroid and granulocytic cells but an increase in cells of the lymphocytic series (De Kock, 1957). Barnett (1960) studied serial changes in myeloid tissue of cattle infected with *T. parva* and concluded that the changes occurred in three phases: a transient stage of stimulation, a prolonged destruction and an increase in the immature cells of all series. He attributed these changes to a diffusible factor or toxin released by the parasites. The changes observed on the second day of fever included dilated sinusoids with red blood cells, a reduction in mature neutrophils and eosinophils and an increase in the immature myeloid elements. Arrested myelopoiesis was observed on the third day of fever and from the fourth day, increasing degenerative and necrotic changes were

observed. Barnett (1960) observed that extensive changes occurred during the late stages of the disease. With the destruction of the myeloid cells, numerous macrophages with phagocytosed cell fragments were seen. Animals surviving beyond the ninth day of fever showed a recovery response with a densely cellular myeloid tissue.

The lesions which have provoked most interest in the past are the macroscopic lymphoid aggregates, the so-called "pseudoinfarcts" in the kidneys. They have been described in detail by Steck (1928), who studied their development, and by other investigators (De Kock 1957; Barnett, 1960). Except on rare occasions, the proliferations of lymphocytic cells only occur in the cortex and extend from the region of the arcuate vessels to the capsule. These aggregates vary in shape and size. These proliferations are usually found around the walls of the interstitial and arcuate blood vessels or in the immediate neighbourhood of a glomerulus. When large, these aggregates cause a certain amount of pressure atrophy of the tubules and degeneration of the tubular epithelium.

In the liver the lesions usually consist of a mononuclear cell infiltration of periportal zone (Steck, 1928; De Kock, 1957). The infiltrating cells are usually associated with the walls of blood vessels and bile ducts. Distended sinusoids containing a high number of medium and large lymphocytes and later granulocytes and macrophages were observed in most animals by Barnett (1960). Degeneration of the liver parenchymal cells although observed, was not predominant (Steck, 1928; De Kock, 1957; Barnett, 1960; Munyua *et al.,* 1973).

Lesions in the lungs resembled those seen in the liver but were less developed (Steck, 1928). De Kock (1957) described aggregates of lymphocytes in the walls of bronchi, bronchioles and blood vessels, congested alveolar capillaries, oedema and hyperaemia.

In the gastrointestinal tract, lesions include erosions and ulceration of the mucous membrane (De Kock, 1957; Munyua *et al.,* 1973). The erosions result from

localized superficial necrosis associated with extensive proliferation of lymphoblastoid cells in the *lamina propria*. The proliferating lymphocytes were also reported to cause some pressure atrophy of the glandular tissue.

Description of lesions in other organs and tissues are scanty. In the adrenals, a mononuclear cell infiltration accompanied by a certain amount of atrophy (De Kock, 1957) or occlusion of trabecular vessels by lymphoid cells (Dolan, 1986a) are reported. The predominant lesion in the brain and *pia-mater* consisted of partial or complete blockage of the smaller arteries and arterioles by dense masses of lymphoblastoid cells and perivascular cuffing (Losos, 1986).

A search of the literature revealed no descriptions of histologic changes in the gonads or tubular genital tract suggesting either that they were not affected or that they were totally overlooked.

In the pituitary gland, De Kock (1957) described aggregates of lymphocytes in the pituitary stalk. These aggregates were usually found around small blood vessels and acini. Dolan (1986a) also found congestion and vascular occlusion by lymphocytes in small scattered foci in the adenohypophysis.

1.2.6.3 Haematology

It has been recognised that a profound leucopaenia occurs in fatal *T. parva* infection and that the different white blood cells are equally affected (Steck, 1928; Barnett, 1960; Wilde, 1966; Muhammed *et al.*, 1975; Maxie *et al.*, 1982).

Leucopaenia may occur at (Steck, 1928) or after (Barnett, 1960) the onset of fever. Leucopaenia may develop progressively until death (Steck, 1928; Barnett, 1960) or be arrested followed by a leucocytosis (Barnett, 1960; Hill and Matson, 1970). In prolonged or recovering cases, leucopaenia is gradual and less intense (Barnett, 1960). Mutugi *et al.*, (1988b) observed the degree of leucocyte depression to increase with lowering dilution of parasite stabilate and concluded that the degree of leucopaenia depended on the severity of the theilerial reaction.

The packed cell volume is reported to decrease in ECF (Muhammed *et al.*, 1975; Maxie *et al.*, 1982). In contrast, no significant change in the packed cell volume, haemoglobin concentration and red blood cell concentration were reported in cattle infected with buffalo-derived *T. parva* (Maxie *et al.*, 1982; Mutugi *et al.*, 1988b). Haemoglobin and red blood cell concentrations were found to parallel changes in packed cell volume in ECF (Maxie *et al.*, 1982).

In addition to the above changes a thrombocytopaenia (Maxie *et al.*, 1982) and hypoproteinaemia (Muhammed *et al.*, 1975; Maxie *et al.*, 1982) have been reported in *T. parva* infections.

Because there is a decrease in all the formed elements of the blood, *T. parva* infection may be termed as a pancytopaenia. However, the pathogenesis of this condition is poorly understood although a number of theories have been advanced. Steck (1928) and Barnett (1960) were of the opinion that this was partly due to cellular destruction while Wilde (1966) attributed it to an arrest in bone marrow maturation. Morrison *et al.* (1986) on the other hand suggested it was as a result of cell-mediated immune lymphocytolysis.

1.2.7 Immunization

Many methods of immunization have been attempted over the years to protect cattle against theileriosis. Because of the extensive literature involved, the reader is referred to a number of excellent reviews considered to be of particular relevance (Purnell, 1977; Brown, 1981; Radley, 1978, 1981; Morrison *et al.*, 1986; Young *et al.*, 1988). Of the many approaches attempted to-date, only immunization by infection and treatment has proven to be suitable and the following brief review will be restricted to this method.

Immunization by infection and treatment involves two simultaneous processes; one of actively infecting cattle with the parasite and the second of treating the recipient cattle with a chemoprophylactic agent which acts in the early stages of the disease and

results in mild or inapparent reactions and production of immune status.

Neitz (1953) reported that chlortetracycline hydrochloride (Aureomycin) was protective when administered intravenously in repeated (8-12 treatments) doses (10mg/kg) at arbitrary irregular intervals commencing 24 h after tick infestation. He concluded that the drug was schizonticidal. Ten to seventeen intravenous injections of oxytetetracycline hydrochloride (1mg/day) were also protective when given on the first but not on the third day of fever (Neitz, 1957). Brocklesby and Bailey (1962) also reported success in immunizing cattle by using oral tetracyclines (15mg/kg) for 28 days commencing from day of tick infestation. However these techniques were never adopted probably because they were cumbersome and expensive and also at that time, infection of cattle depended on infection with ticks of variable infectivity. The latter was solved by the development of methods to cryopreserve infected tick stabilates capable of inducing reproducible infections (Cunningham *et al.*, 1973).

A number of experiments were done to reduce the drug regimen required for immunization. Radley *et al.* (1975a) were able to immunize against homologous challenge by inoculation with *T. parva* (Muguga) stabilate followed by four daily injections of tetracycline (Terramycin Q; 5mg/kg). Reactions to challenge with homologous strains were mild but more severe with heterologous strains. In their second experiment, animals were immunized against five strains of *T. parva* following the same procedure as in the first experiment. The animals underwent mild reactions and were subsequently immune to challenge with the homologous strain and to *T. parva* (Muguga). Their results indicated that animals could be immunized by stabilate infection and four daily treatments of oxytetracycline starting on day of infection. With the development of long acting tetracycline, Radley *et al.* (1975b) were able to immunize animals by administration of 10mg/kg on the day of infection. The method was subsequently refined to involve two doses of 10mg/kg (day 0 and 4) using short-acting oxytetracycline, or one dose of 20mg/kg using long-acting oxytetracycline (day 0 only).

Although the prophylaxis regimen given above were adequate, a further problem arose from the heterogeneity of parasite stocks. Thus immunization against one stock of *T. parva* resulted in protection against some, but not all stocks of the parasite (Radley *et al.* 1975a). The possibility of using a combination of three theilerial strains for infection and chemoprophylaxis immunization was explored by Radley *et al.* (1975b). In the first experiment, animals were immunized against a combination of three strains or against one or two elements of the combination separately and challenged with either of two strains known to break through *T. parva* (Muguga) immunity. The results showed that animals immunized against the combination had mild or inapparent reactions to challenge while many of those immunized against one or two strains either had severe reactions or died. It was also demonstrated that animals immunized with the combination of theilerial strains withstood challenge from parasites isolated from areas far removed from the source of immunizing parasites. Field trials were conducted using the same parasite combination used by Radley *et al.* (1975b) and by Uilenberg *et al.* (1976, 1977). While all the controls died of ECF during undipped exposure, none of the immunized animals died of ECF, although some animals died from babesiosis, heartwater and other causes.

Subsequently, studies have been conducted to explore the possibility of using the newly developed theilericidal compounds in immunization procedures. Dolan *et al.* (1984a) showed that parvaquone given at 20mg/kg could control *T. parva* infection without development of clinical disease if given between 8 and 12 days after parasite inoculation, and this was followed by development of immunity. Buparvaquone has also been shown to be an effective drug for chemoprophylaxis when administered at 2.5mg/kg on the day of infection (Mutugi *et al.*, 1988b). However the use of parvaquone has not been followed up partly because it is expensive while more work needs to be done using buparvaquone before it is adopted for routine use.

Cattle that recover from infection or are immunized by infection and treatment produce antibodies to the parasite. The indirect fluorescent antibody (IFA) test has

been the most useful and extensively used serological procedure for detection of prior exposure of animals to ECF because it offers standardized antigens (Burridge and Kimber, 1972). Standardisation was made possible after the development of techniques for propagating *T. parva* macroschizonts in cell culture systems by Malmquist *et al.* (1970). Other serological methods attempted include immunodiffusion, capillary tube agglutination test, complement fixation, indirect haemagglutination and enzyme linked immunosorbent assay (ELISA) and have been reviewed by Cowan (1981).

To date, there is no conclusive evidence that antibodies to *T. parva* play a significant role in immunity. The evidence was supported by the failure to induce immunity either by use of colostral antibodies (Barnett *et al.*, 1957), passive immunization (Muhammed *et al.*, 1975) or by active immunization using macroschizont infected cell lines (Wagner *et al.*, 1974). However despite the general consensus that antibody does not play a role in protection in ECF, a few studies have shown that they probably play a partial role. Gray and Brown (1981) showed that sera collected after 23 days post-infection has a strong neutralization effect on the infectivity of sporozoites co-incubated with bovine lymphocytes while Dolan *et al.* (1985) reported that sera collected 21-28 days post-infection had schizonticidal properties *in vitro*.

Because immunity could not be related directly to the action of antibodies, it was suggested that resistance in ECF might be associated with cell-mediated mechanisms. Initial evidence of cell-mediated immunity was suggested by the observation that the migration of cultured lymphoblastoid cells was inhibited by peripheral leucocytes obtained from cattle vaccinated against *T. parva* (Muhammed, 1975).

The first strong evidence that cell-mediated immune mechanisms operate in ECF was presented by Pearson *et al.* (1979). These workers reported that irradiated *T. parva* transformed cells when cultured with autologous or allogeneic peripheral

blood leucocytes stimulated the latter to proliferate. When the transformed cells were cultured with autologous leucocytes from an immune autologous donor, cytotoxic cells were generated that killed the autologous infected cell line and to a lesser extent, allogeneic infected cell lines.

After this initial observation, a number of studies examined the induction of cytotoxic cells *in vivo* in infected and immune challenged animals. The results are reviewed by Eugui *et al.* (1981) and Morrison *et al.* (1986). Two types of cytotoxic responses were recognised, namely, a non-specific cytotoxicity and a specific cytotoxicity. The non-specific cytotoxicity was found in lethal and sublethal primary infections and was manifested against allogeneic infected cells and mouse tumour cells but not against autologous infected cells. This cytotoxic response appeared terminally and was suggested to be one of the important factors contributing to the lymphocytolysis and panleucopenia characteristic of ECF.

In contrast to the non-specific cytotoxicity, immune animals challenged with sporozoites or autologous macroschizont infected cells manifested powerful cytotoxic activity on autologous cells only. This response lasted only a few days and was observed 10-12 days after the first sporozoite challenge. After infection and treatment with tetracycline, specific cytotoxicity against autologous infected cells was observed 14 to 21 days later (Eugui *et al.,* 1981). Since these cytotoxic cells could lyse only autologous but not allogeneic *Theileria* transformed cells nor mouse tumor cells, it was concluded that they were genetically restricted and therefore behaved like cytotoxic T-lymphocytes (Eugui *et al.*, 1981; Gooderis *et al.*, 1986; Morrison *et al.*, 1986).

1.2.8 Chemotherapy

There has been a concentrated search for an effective curative agent for ECF and a wide range of drugs have been tested in attempts to cure the disease. The advances made in this field have been reviewed by Dolan (1981) and McHardy (1989).

Menoctone was the first compound demonstrated to have antitheilerial effects both *in vitro* and *in vivo* (McHardy *et al.*, 1976). In a preliminary study, McHardy *et al.* (1976) screened twenty compounds for their antitheilerial effect *in vitro* and found that only menoctone had an appreciable effect on the parasite. Menoctone was selected for further testing in cattle. Stabilate-infected animals were first treated on the first day of fever and schizont parasitosis with 5mg/kg followed by five daily treatments at 1mg/kg of menoctone. All the seven treated animals recovered; temperatures returned to normal levels and schizonts could not be detected in lymph-node smears. Degeneration of schizonts was observed within two days of the first injection of menoctone. Six of the seven untreated animals died of ECF. McHardy (1978) later screened more compounds and observed that only two compounds; menoctone and methotrexate showed significant antitheilerial activity. Menoctone was about 100 times more active than methotrexate. Menoctone however, was expensive and difficult to synthesise and alternative analogues were developed for testing against ECF.

One analogue, 993C (parvaquone, Clexon-Wellcome), was reported to have antitheilerial action and was chosen for further evaluation. *In vitro* and *in vivo* studies revealed that parvaquone was highly efficacious against the schizont stage of the parasite (Morgan and McHardy, 1982; McHardy *et al.*, 1983). These authors reported that treatment with a single dose of 20mg/kg or double dose of 10mg/kg at 48 h interval from day 5-8 of fever and parasitosis resulted in elimination of parasites, reduction of fever, arrest of leucopaenia and eventual recovery.

Dolan *et al.* (1984a) extended these studies by infecting cattle with *T. parva* either by the application of ticks to modulate a field situation or by tick stabilate followed by parvaquone treatment at 20mg/kg on the second day of fever and presence of schizonts. In both groups, there was a marked response to treatment with a reduction in fever, degeneration of macroschizonts within 24 to 48 h and recovery of white blood cell count. Fever was reduced in 1 to 3 days but secondary febrile periods

were detected in some animals. There were recrudescences of parasitosis in both groups and in one animal, parasites were detected until death, extending over more than 50 days of infection. Parvaquone treatment arrested the fall in white blood cell count and animals showed a reactive lymphocytosis which developed in 4-5 days. Two of seven treated animals died in each group while all the seven controls died. In a second experiment, five stabilate isolates from different locations within East Africa, were used. Treatment was by 10mg/kg of parvaquone 48 hours apart starting on the third day of fever. Variable mortality was reported with all isolates while all control animals died. Further work revealed that parvaquone was most efficacious if administered before the development of severe clinical disease (Dolan *et al.*, 1988).

All the above studies on the effects on parvaquone on treatment of ECF were carried out under laboratory conditions. A field trial was undertaken in which parvaquone was used to treat ECF cases reported by farmers in various locations in Kenya (Chema *et al.,* 1986). Of 187 cases treated with two doses of 10mg/kg at 48 hour intervals, 94.1% (170/187) survived. Of the 17 animals that died, 6 died before the second treatment and many of these had been treated in the terminal stage of the disease. The results of this trial indicated that parvaquone provides an effective therapy for clinical ECF in field situations.

A new naphthoquinone analogue, buparvaquone (Butalex, Coopers) has been developed and its efficacy against theilerial infection is reviewed by McHardy (1989). Although it was reported to have cured 91% (62/68) ECF field cases at a single dose of 2.5mg/kg intramuscularly (McHardy, 1989). Mutugi *et al.* (1988b) however, reported 2 out of 3 deaths in cattle treated at the above dose rate on day 8 post-infection. Compared to parvaquone, buparvaquone has a higher *in vitro* activity against *T. parva* and a longer plasma half-life (McHardy, 1989). More studies need to be done to evaluate its potential use as a chemotherapeutic compound.

Halofuginone, the active principle of the coccidiostat Stenorol was first used as the hydrobromide but later as a soluble lactate (Schein and Voigt, 1979, 1981). Their

studies indicated that halofuginone was effective against *T. parva* in artificially infected cattle at 1.2mg/kg of halofuginone *per os*. All infected cattle treated on the first or sixth day of fever and presence of macroschizonts recovered. Treatment reduced the febrile response and eliminated schizonts in 2-4 days after treatment. At 0.6mg/kg of halofuginone, fever and schizont parasitosis were transiently reduced. Doses above 3mg/kg of halofuginone caused signs of acute toxicity, such as diarrhoea, conjunctivitis, subnormal temperature and cachexia.

1.3 Reproductive physiology in cattle

1.3.1 Introduction

This study was initiated because although little is known, it is generally accepted that ECF affects the normal reproductive function of cattle. The main objective of this study was to verify this anecdotal evidence partly by examining the effect of infection with specific regard to the cyclical status and fertility in the female bovid. The literature reviewed in the following sections will therefore, be limited mainly to the relevant areas of this study.

1.3.2 The bovine oestrous cycle

The average length of the oestrous cycle in the cow is 21 days but with considerable variability (range, 17-24 days). However, cycles of less than 17 days or more than 24 days are reported (Chapman and Casida, 1937; Moeller and Van deMark, 1951). Heifers tend to have cycle lengths of one day shorter than cows (Chapman and Casida, 1937). Studies on zebu cattle kept in the tropics showed that the oestrous cycle lengths tend to be longer in *Bos indicus* than in *Bos taurus*. Cycle lengths of 22 days for Angoni, 23 days for Barotzi and 24 days for Boran breeds in Central Africa are reported (Rakha *et al.*, 1970). Llewellyn *et al.* (1987) reported an average cycle length of 23 days, with a range of 19-26 days in Borans in East Africa.

Oestrus or standing heat, usually designated as day 0, lasts 15-18 h, during which time the cow is receptive to a bull and will allow mating. The duration of oestrus tends to be shorter in *Bos indicus* than in *Bos taurus* (Rakha *et. al.*, 1970). In addition, behavioural signs associated with oestrus appear to be less marked in *Bos indicus* (Adeyemo *et al.*, 1979). Short oestrous periods have been reported in many tropical countries. Cuevas and Hagen (1966) reported mean periods of 13.2 h for Holsteins in a hot humid climate in Mexico. In Nigeria, the duration of oestrus was found to range between 9-14 h in about 48% of Fulani heifers (Adeyemo *et al.*, 1979). These short periods of oestrus have been attributed to several factors. Heat stress has been shown to shorten the duration and reduce the intensity of oestrus (Gangwar *et al.*, 1965). Ovulation occurs about 25-30 h after the onset of oestrus (Swanson and Hafs, 1971).

The oestrous cycle can be divided into four periods; proestrus, oestrus, metoestrus and dioestrus. Proestrus or the period of preparation lasts 2-3 days (day 18-20 of the previous cycle). Oestrus, usually designated as the period of desire or sexual receptivity occurs on day 0. During metoestrus, there is a sudden cessation of heat signs. Metoestrus lasts from day 1-4 of the cycle. The dioestrus period is dominated by the *corpus luteum* (CL) which has formed from the *corpus haemorrhagicum* and lasts from day 4-17.

In the bovid, the oestrous cycle can more appropriately be described to comprise of two phases; a luteal phase which extends from the formation of the CL after ovulation until its regression towards the end of the cycle, and a follicular phase which begins when the CL regresses and ends at ovulation. A brief review of the features characteristic of each phase are presented in the following sections.

1.3.2.1 Luteal phase

The luteal phase starts at ovulation. Based on rectal palpation, the luteal phase is characterized by the presence of a *corpus luteum* in the ovaries and flaccid uterine

horns. The first palpable evidence of the initiation of the luteal phase is the presence of an ovulation depression (OVD) which is detectable 12-24 h after the rupture of the ovulatory follicle. The OVD is felt as a soft circumscribed area approximately 1 cm in diameter and is either slightly raised or level with the ovarian surface. The OVD has a fluid filled cavity that may persist throughout a large part of the funtional life span of the CL. A blood clot formed by extravasation of blood into the cavity is present due to which the young CL is referred to as a *corpus haemorrhagicum*.

The CL grows rapidly within the first week after oestrus and reaches its mature size by day 7 (Donaldson and Hansel, 1965). The changes of the CL are quite dramatic and permit recognition of several stages based on its size and consistency (Zemjanis, 1970; Arthur *et al.*, 1989). On rectal palpation, the developing CL is less than 1 cm in diameter and soft in consistency during day 2-3, 1-2 cm and soft in consistency between day 3-5 and over 2 cm and soft in consistency between day 5-7 of the cycle (Zemjanis, 1970). The mature CL is palpable between day 8-17 of the cycle and is firm in consistency, measuring between 2.0-2.5 cm in diameter but with marked variability. The palpable features that are characteristic of the mature CL include an increase in the size of the ovary so that the ovary bearing the CL is sometimes double its initial size, distortion of the form of the ovary, presence of a surface crown which is an extension of luteal tissue in the form of a prominence of varying size and width, a demarcation line between the CL and the rest of the ovarian tissue and its homogenous consistency as compared to the granular nature of the rest of the ovary. The shape of the mature CL varies from oval, irregularly square or rectangular (Arthur *et al.*, 1989). From about day 18 the CL starts regressing. On palpation, the regressing CL is 1-2 cm in diameter and firm in consistency between day 18-20 and less than 1 cm and hard in consistency from oestrus to the middle of the next cycle.

Palpable uterine changes characteristic of the luteal phase include a mild tonus between day 1-4 due to postoestrous endometrial oedema, flaccid uterine horns between day 4-15 and a slight increase in tonus from day 16-18 of the cycle.

During the luteal phase, progesterone (P4) and 20β-hydroxyprogesterone are produced by the CL. P4 is the major hormone produced by the CL. It is produced from cholesterol through a process of steroidogenesis. Luteinizing hormone (LH) acts as the luteotrophin in cows through its steroidogenic effects. LH is released in a pulsatile fashion (Rahe *et al.*, 1980). LH levels during the luteal phase range between 0.2-2ng/ml. During the early luteal phase, LH release is characterised by high frequency and low amplitude while, during the mid-cycle they are characterised by low frequency and high amplitude (Hansel and Convey, 1983). LH exerts its steroidogenic effects by increasing adenylate cyclase and cyclic AMP (cAMP) (Niswender *et al.*, 1981). The luteotrophic properties of LH were demonstrated both *in vitro* and *in vivo* (Hansel, 1967). LH was shown to increase luteal tissue weights, P4 concentrations and when given at mid-cycle, prolonged the lifespan of the CL and the oestrous cycle. Gonadotrophin releasing hormone (GnRH) by its virtue to cause release of LH from the anterior pituitary can also act as a luteotrophic hormone.

P4 concentration in the blood rises and falls as the CL grows and regresses respectively. It rises from basal levels (<1ng/ml) from day 0-4 post-oestrus and reaches a peak (>4ng/ml) around day 7 and remains elevated until it falls steeply to basal levels 3 to 2 days before ovulation (Hansel and Echternkamp, 1972; Peters and Ball, 1987). P4 concentrations reflect the activity of the CL and are, therefore, of potential value in mapping out oestrous cycles as well as identifying the time of ovulation based on P4 profiles (Pope *et al.*, 1976; Bulman and Lamming, 1978; Heap and Holdsworth, 1981; Bloomfield *et al.*, 1986).

Essentially, P4 is connected with activities associated with the preparation for pregnancy and its maintenance. P4 is therefore, elevated during pregnancy and the luteal phase. The luteal phase is therefore, characterised by a period of sexual quiescence, growth of and secretion from the endometrial glands.

Although the luteal period is dominated by the CL and sexual quiescence, numerous studies have shown that there are waves of follicular development during

this period. Oestrogens (oestradiol 17β and oestrone) are reported to increase during the early luteal period (Glencross *et al.*, 1973). The large oestrogen active follicles that appear at this time are likely sources of this oestrogen (Ireland and Roche, 1983). Luteal phase oestrogen concentrations in the blood are about 200-300pg/ml (Hansel and Echternkamp, 1972). Follicle stimulating hormone (FSH) levels are relatively low during the luteal period (Bolt and Rollins, 1983) although a small peak has been reported between days 2-5 of the cycle in cows (Dobson, 1978; Ireland and Roche, 1983).

1.3.2.2 Follicular phase

Dramatic changes take place during the follicular phase. Based on rectal palpation of the genital tract, the features that are characteristic of the follicular phase consist of the rapid development of an ovulatory follicle, increased uterine tonus and CL regression. During proestrus and oestrus the follicle is recognized as a slightly bulging smooth and round surface area on the ovary measuring between 2-2.5 cm in diameter. The actual diameter may be equal to or larger than that determined at examination depending on how deep the follicle is embedded in the ovarian stroma. The follicular surface is fluctuating but the tension increases as more fluid is secreted into the cavity as oestrus approaches. During the rest of the oestrous period and even in pregnancy the follicles are less than 1 cm in diameter.

In cattle relatively little is known of the precise mechanisms that control folliculogenesis. Investigations on the follicular dynamics have used a number of approaches: counts of follicles in ovaries obtained at slaughter (Rajakoski, 1960; Marion *et al.*, 1968; Ireland and Roche, 1983), counts of follicles in ovaries obtained at slaughter coupled with measurement of steroids in the follicular fluid (Fortune *et al.*, 1988), following individual follicles by marking them with dye (Matton *et al.*, 1981), observation of the regrowth of large follicles following destruction of large follicles on the ovaries (Matton *et al.*, 1981) and ultrasonic imaging of the ovaries (Pierson and

Ginther, 1984,1987; Savio *et al.*, 1988; Sirois and Fortune, 1988). Conflicting ideas about the pattern of development have been advanced. In the view of some (Matton *et al.*, 1981), development is continuous with rapid growth occurring during the last 18-24 h prior to ovulation. Rajakoski (1960) on the other hand postulated that there are two waves of growth of follicles $\geq$ 5mm in diameter during the bovine oestrous cycle; one occurring between days 3 and 4 of the cycle resulting in a large atretic follicle and the second beginning around days 12 to 14 and culminating in ovulation. Most of the studies favour the view that follicles develop in 2-3 waves with the ovulating follicle being recruited from the last wave of follicles. Real time, B-mode ultrasonography which offers the advantage that the same follicles can be examined repeatedly has confirmed that follicles develop in waves (2-4) and while other follicles regress spontaneously, there is a selective accelerated growth of the preovulatory follicle in the 3 days preceding ovulation.

Follicular growth to the antral stage may occur independent of gonadotrophin action though the rate of pre-antral follicle growth is accelerated by gonadotrophins. Analysis of gonadotrophin secretions during the cycle reveal no obvious changes that might explain control of growth and subsequent regression of follicles. It is not clear whether episodic fluctuations in gonadotrophin secretion may be related to follicular dynamics. Matton *et al.* (1981) demonstrated that large follicles are quickly replaced following cautery of all follicles $\geq$ 5 mm diameter suggesting possibly that intra-ovarian factors play a role in the development of follicles. Inhibin, a glycoprotein present in follicular fluid, was shown to suppress the secretion of FSH (Ireland *et al.*, 1983; Quirk and Fortune, 1986) and is therefore considered to play a role in follicular dynamics. In cattle, this was revealed by studies in which immunization against a partly purified sheep follicular fluid was reported to significantly increase FSH concentrations and the number of large follicles (Price *et al.*, 1987). The above studies suggest that inhibin negatively controls FSH secretion but whether it plays this role at the hypothalamic or the pituitary level is not known. It was also found that the number

of dominant follicles that developed in the ovary ipsilateral to the *corpus luteum* were greater than in the contralateral ovary (Savio *et al.* 1988) suggesting that progesterone influences follicular development .

The mechanism by which pre-ovulatory follicles are selected is not known. Of the numerous follicles that are recruited for development, only one usually matures and ovulates in the cow while the others degenerate. The basic causes of follicular atresia are not adequately understood. Morphological features of atretic follicles have been described in cattle (Rajakoski, 1960; Erickson, 1966; Marion *et al.*, 1968). It was suggested that follicular atresia may be brought about either by inadequate supply of FSH, LH or oestrogen, or by excess LH, oestrogen or progesterone (Marion *et al.*, 1968). Atresia may begin at any stage of follicular development and may follow one or any of several processes which can roughly be classified as early, definite or late atresia (Marion *et al.*, 1968).

Palpation of the uterus reveals a moderate tonus and marked irritability to manipulation during proestrus (day 19-20). On the day of oestrus, there is marked tonus as a result of endometrial oedema and myometrial contractility.

The series of events which culminate in ovulation are triggered off by the involution of the CL (luteolysis). The mechanism of CL involution has not been clearly elucidated. It is generally considered, however, that prostaglandin F2 alpha ($PGF_2\alpha$) produced in the uterus may be the natural luteolysin in the cow (Hansel *et al.*, 1973). Evidence supporting this idea includes the fact that hysterectomy prolongs the life of the CL (Ginther *et al.*, 1967; Brunner *et al.*, 1969). Evidence of the luteolytic role of $PGF_2\alpha$ came from the exogenous administration of this compound during the luteal phase resulting in luteolysis (Ireland and Roche, 1983).

As more follicles develop following luteolysis, they acquire the capacity to secrete more oestrogens under the influence of LH and FSH. Theca cells secrete androgens in response to LH and these in turn are aromatised to oestrogens by the granulosa cells. The highest level (20 pg/ml) of oestrogen secretion occurs during the

late follicular phase of the cycle (Peters and Ball, 1987). During the pre-ovulatory gonadotrophin surge, there is a marked decrease of oestradiol to baseline levels.

Oestrogens are responsible for the behavioural and genital tract changes observed during the follicular (proestrus and oestrus) phase. Changes in the genital tract include a swollen and congested vulva, turgid uterus, secretion of clear viscid mucus, increased vascularity of the endometrium and growth of the endometrial glands. Behavioural manifestations include restlessness, mounting or being mounted by other animals, sniffing and being sniffed, bellowing, head-butting and reduced appetite (Peters and Ball, 1987). Standing firmly to be mounted is reported to be the only true sign of oestrus (Whitmore, 1980), while the other traits are secondary since they are non-specific and extend into the proestrous or metoestrous periods.

Levels of FSH and LH are high during the follicular phase. Surge release of FSH and LH occur coincidentally at or near oestrus (Akbar *et al.*, 1974). The increase in oestradiol that occurs during this period is the stimulus that triggers the gonadotrophin surge. Removal of the negative feedback due to progesterone also appears to be prerequisite at this time. LH starts to rise 5 days before oestrus and culminates in a surge release of 10-50 ng/ml during oestrus (Hansel and Echternkamp, 1972). Frequent sampling shows that LH is released in a pulsatile manner (Rahe *et al.*, 1980) and is characterised by pulses of high frequency and low amplitude during the pre-surge period. FSH levels tend to decline during the follicular phase but peak at oestrus (Akbar *et al.*, 1974). LH and FSH levels decline to baseline concentrations following their surge release (Hansel and Convey, 1983).

1.3.2.3 Endocrine control of the oestrous cycle

Most of the recent advances in reproductive endocrinology have been made possible by the development of techniques for accurate, sensitive and specific measurement of concentrations of various reproductive hormones during the last thirty years. The data available shows that concentrations of reproductive hormones change

throughout the oestrous cycle. The whole mechanism is not fully understood but the available information allows at least a possible series of events to be put together to explain the morphological and behavioural changes that take place during, and the mechanisms regulating, the oestrous cycle.

At the start of the CL involution but before the gonadotrophin surge, low levels of GnRH are being released from the hypothalamus and are passed through the hypophyseal-portal vessels, to the anterior pituitary gland. The GnRH brings about the low release of gonadotrophins which act to induce follicular growth in the ovaries and these follicles start to secrete oestrogens. Oestrogens bring about the changes in the tubular genital tract and stimulate hypothalamic and reticular nuclei to bring about the behavioural pattern observed in proestrus. At this time, oestrogens act on the hypothalamus via a negative feedback mechanism to block the release of large amounts of GnRH. The negative feedback mechanism of oestrogens on the hypothalamus/pituitary is not known, but is suggested by experimental evidence where exogenous administration of oestradiol in ovariectomised animals is reported to decrease FSH concentrations and LH pulse amplitude (Kesner and Convey, 1982; Price and Webb, 1988).

With the continued growth of follicles, the circulating blood concentration of oestrogens rises and through a positive feedback mechanism, stimulates the release of large amounts of GnRH to the pituitary. Following the involution of the CL, the negative feedback effect of P4 on the anterior pituitary is removed and the oestrogens are able to exert their positive feedback effect on the anterior pituitary. The negative feedback effect of P4 is suggested by experimental evidence in which exogenous P4 administration results in decreased LH concentrations (Beck *et al.*, 1976; Kesner *et al.*, 1981) and LH pulse amplitude (Price and Webb, 1988). The mechanism by which oestrogens induce gonadotrophin surge is not completely understood. However, it is reported that the capacity of the pituitary to release gonadotrophins is greatest during oestrus (Convey, 1973) or after exogenous administration of oestradiol (Kesner *et al.*,

1981) suggesting that oestrogens sensitize the pituitary to GnRH. Combining the positive feedback effect of oestrogens and the removal of the negative feedback effect of P4, this period is therefore, characterized by rising levels of FSH and LH which culminate in a surge release. During this period LH pulses are of high frequency and low amplitude.

Following the surge release of gonadotrophins, ovulation occurs. Due to the change induced in the pre-ovulatory follicle, oestrogen secretion is diminished and oestrous behaviour is terminated. At the same time, either because of depletion of gonadotrophins or to the refractoriness of the pituitary gland to GnRH, concentrations of LH and FSH decline to baseline levels. Low levels of LH which are being secreted at this time initiate luteinization of *theca interna* and granulosa cells of the ovulated follicle. The luteinized cells start secreting P4. Progesterone produced during the luteal phase acts negatively on the pituitary to block release of large amounts of gonanotrophins. However, small amounts of gonadotrophins are released which are able to bring about follicular development and production of oestrogens during the luteal period. Due to the negative effect of both oestrogens and P4, FSH and LH concentrations are low, with LH pulses characterized by pulses of low frequency and high amplitude. P4 brings about the changes observed in the tubular genital tract and probably by acting negatively on the hypothalamus, blocks initiation of behavioural oestrus by oestrogens produced during the luteal phase.

Since the cow is polyoestrus, a mechanism of removing P4 dominance to allow for further cyclicity is necessary. The mechanism of CL involution has not been clearly elucidated. However, available evidence indicates that $PGF_2\alpha$ is partly responsible. The signal that triggers off the secretion and release of $PGF_2\alpha$ is not known. McCracken (1984) suggested a working hypothesis however, in which it is suggested that due to the declining action of P4 in the uterus towards the end of the luteal phase, oestradiol is able to stimulate the synthesis of oxytocin receptors and that $PGF_2\alpha$ is produced after oxytocin, originating from the brain and CL, couples with its receptors.

That this may be a possible mechanism is supported from evidence of the luteolytic effect of oestradiol (Hansel *et al.*, 1973; Kindhal *et al.*, 1981; McCracken *et al.*, 1981) and oestrone sulphate (Eley *et al.*, 1979).

The mechanism of $PGF_2\alpha$ as a luteolysin is still not well established. However it is thought that it causes reduced blood flow to the ovary containing the CL through endothelial cell swelling, resulting in reduced perfusion rate (Niswender *et al.*, 1976). $PGF_2\alpha$ appears to exert its effect locally on the CL, and hence its utero-ovarian transfer (Ginther and del Campo, 1974). This is partly supported by the observation of Furr *et al.* (1981) that exogenous administration of $PGF_2\alpha$ had no effect on FSH and LH secretion in heifers suggesting that it plays no role in CL involution through the hypothalamo-pituitary axis.

1.3.3 Assessment of reproductive status

In the previous sections, behavioural, morphologic and endocrine changes that are associated with the oestrous cycle have been discussed. These changes may be used singly or in combination to assess the cyclical status in cattle. Behavioural, ovarian and P4 concentration changes were used in this study and the brief review presented here will be relevant to them and, where necessary, evaluated against other applied techniques.

Oestrous behaviour is displayed through the periods of proceptivity and receptivity. Although the various traits are used, these have their limitations in that they may not be specific for the oestrous period (Williamson *et al.*, 1972; Whitmore, 1980); their expression varies within and between animals and their intensity of response may be influenced by environmental factors such that oestrous signs are subdued (Hafez, 1974). Standing to be mounted is reported to be the only true sign of oestrus and lasts 8-18 h (Whitmore, 1980) although with wide variations. However, the success rate of oestrus detection based on standing to be mounted is subject to wide variations (Williamson *et al.*, 1972; Llewelyn *et al.*, 1987) and is influenced by

the duration spent, and time of observing for oestrus as well as the number and experience of the observers. Since about 70% of mountings occur between 18.00-6.00 h (Whitmore, 1980), increasing the observation time improves the detection rate although, even with a continuous (24 h) monitoring by a team of observers for 21 days, the detection rate was only 89% (Williamson *et al.*, 1972). False positives arise when non-oestrous or pregnant animals allow mounting (Williamson *et al.*, 1972; Appleyard and Cook, 1976) thus complicating the accuracy of detection.

Corpus luteum diagnosis by palpation *per rectum* is frequently used to assess the cyclical status in cattle. The criteria used include ovarian size, evidence of ovulation, demarcation of the CL from the rest of the ovarian stroma and surface crowns (Zemjanis, 1970). Changes in size and consistency are used to assess the luteal stages and relate them to the stage of the oestrous cycle (Zemjanis, 1970). However, palpation *per se* may not be a good predictor of luteal status. Cows diagnosed by palpation *per rectum* to have a functional CL were in agreement with P4 concentrations in only 85% of the palpations and at the same time, the sizes and consistencies of the CL were neither related to each other nor to the milk P4 concentrations (Watson and Munro, 1980). These authors concluded that the assessment of the CL although reasonably accurate, was too subjective in assessment of functional status of the CL. Elsewhere, agreement between palpation of a CL and P4 concentration in Zebus was 77% (Pathiraja *et al.*, 1986) and 61% (Llewelyn *et al.*, 1987). In another study, rectal palpation and P4 concentration were compatible in 77% of 142 examinations (Boyd and Munro, 1979). These authors found that most of the incompatibilities occurred early or late in the cycle suggesting that the function and the recognition by rectal palpation of the CL becomes dissociated at either end of the oestrous cycle. Absolute errors in CL detection may also be made depending on the skill of the person palpating.

Real-time B mode transrectal ultrasonic imaging of the ovaries has provided a more superior method of assessing luteal tissue. A comprehensive review is presented

by Griffin and Ginther (1992). Pierson and Ginther (1987) reported a 100% agreement between ultrasonic scanning and post-mortem diagnosis of CL bearing ovary in heifers when imaged between days 12-14 post-ovulation. In another study, milk P4 concentrations were highly correlated to CL size as determined by ultrasound diagnosis (Sprecher *et al.*, 1989). The same authors found a higher compatibility to P4 concentrations when using ultrasound (90%) than with rectal palpation (70%).

Progesterone levels display a cyclical pattern during repeated cycles and the P4 profile is now widely used to assess cyclicity in cattle (Bulman and Lamming, 1978). P4 increases steadily from basal levels during the follicular phase to plateau levels during the luteal phase. The CL is the major source of P4 in the cow and declining P4 levels are seen coincidentally wih luteolysis.

If pregnancy ensues, then the CL is rescued from involution (referred to as *corpus verum*) and P4 levels maintained. In practice, a high P4 concentration 24 days after mating is indicative of conception. The establishment of pregnancy requires that the luteal phase of the oestrous cycle should be prolonged and ovulation suppressed. The prolongation of the lifespan of the CL in response to an embryo is referred to as the maternal recognition of pregnancy. The CL is required for maintenance of pregnancy in the cow. Removal of the CL prior to day 117 of gestation resulted in abortion (McDonald *et al.*, 1953). During early gestation, plasma progesterone levels rise above those found in cycling cows (Lukaszewska and Hansel, 1980). These high levels of progesterone were reported to decline 1-2 days prior to parturition reflecting the lysis of the CL (Edqvist *et al.*, 1981).

The maintenance of the CL during pregnancy is thought to be due to the presence of the conceptus in the uterus. The day of maternal recognition of pregnancy in the cow is around days 16 and 17 (Betteridge *et al.*, 1980). Two concepts have been proposed for the maintenance of the CL during gestation; an antiluteolytic concept related to the conceptus and a luteotrophin mechanism related to the development of the allantochorion placenta. The role of the conceptus playing an

antiluteolytic role is suggested by the observation that $PGF_2\alpha$ concentrations were reduced in pregnant sheep (Thorburn *et al.*, 1973) and by the prolongation of the CL lifespan when 14-16 day sheep embryo suspensions were infused into the uterine lumen (Rowson and Moor, 1967). Further, conceptus secretory proteins (CSP) or bovine trophoblast protein-1 (bTP-1) synthesized by and secreted by the bovine conceptus administered between day 15-21 of the oestrous cycle extended the lifespan of the CL suggesting that they accommodate luteal maintenance during early gestation via attenuation of endometrial $PGF_2\alpha$ (Knickerbroker *et al.*, 1986). These conceptual proteins have a high homology with alpha interferons (IFN-α) (Poyser, 1984). IFN-α has been shown to increase the life span of the CL and the inter-oestrus interval (Plante *et al.*, 1989) suggesting that it plays a role in the maternal recognition of pregnancy. The increase in plasma progesterone concentration during early pregnancy was also thought to be due to a luteotrophic signal produced by the conceptus. Evidence to support this concept was demonstrated by incubating dispersed luteal cells with blastocyst homogenates which resulted in progesterone synthesis (Beal *et al.*, 1981). The available evidence therefore suggests that the conceptus exerts a direct action on the maintenance of the CL during pregnancy although it is not clear whether this is due to the antiluteolytic or luteotrophic signals working separately or in concert.

Although P4 concentrations may be used to assess successful conception and pregnancy, they need to be complemented by other diagnostic aids like rectal palpation of the uterus (Arthur *et al.*, 1989) or ultrasound scanning (Griffin and Ginther, 1992) since some conditions of the uterus may be associated with a persistent CL. Palpable uterine changes characteristic of pregnancy consist of: 1) an increase in size of the uterine horn bearing the conceptus as early as day 35 after conception, 2) fluctuation of the uterine horn on ballotment, 3) position of the uterus in the pelvic or abdominal cavity, 4) presence of foetal membranes (membrane slip of the allantochorion from day 30; cotyledons from about day 60), 5) presence of a foetus from about day 120 as well as 6) hypertrophy and presence of fremitus in the middle uterine artery from about 75

days of conception (Zemjanis, 1970; Arthur *et al.*, 1989).

1.3.4 Effect of nutrition on reproductive function

An insufficient supply of energy, or undernutrition, is probably one of the most common causes of nutritional reproductive disorders in cattle. On the farm, a case of malnutrition can seldom be attributed to a single factor; it could be as a result of underfeeding or disease.

The relationship between fertility and body weight or body condition is basically one of correlation not primarily of causation because both parameters are functions of nutritional status. The evaluation models that have been developed have therefore been based on liveweight or body condition scores, reflecting nutritional reserves demanded for maintenance and breeding.

Prolonged restriction in dietary intake was reported to induce anoestrus in sexually mature cattle (Bond *et al.*, 1958; Beal *et al.*, 1978; Richards *et al.*, 1989). This effect was partially attributed to a decrease in LH secretion. Beal *et al.* (1978) observed that cows on restricted diets released more LH in response to GnRH suggesting that energy restriction impairs LH secretion by decreasing hypothalamic release of GnRH. In similar studies, Richards *et al.* (1989) noted that luteal activity ceased (progesterone <1ng/ml) after 26 $\pm$ 1 weeks of reduced nutrient in 91% (10/11) of the dietary restricted group and that this was associated with a reduction of the initial body weight, body condition scores and absence of behavioural oestrus. Apparently, more energy was needed for the resumption of oestrus since animals were reported to cease cycling at a higher weight loss than at the initiation of oestrus (Bond *et al.*, 1958; Richards *et al.*, 1989). Richards *et al.* (1989) reported lower LH concentrations and LH frequency at the initiation of oestrus in the reduced nutrient group but no changes in plasma oestradiol concentrations and concluded that nutrient restriction compromises the hypothalamic-pituitary function.

In contrast, other studies suggested that anoestrus under energy restriction may be due to a reduction of the ability of ovarian tissue to respond to LH (Gombe and Hansel, 1973).

These authors observed lower plasma P4 concentrations but higher LH concentrations in energy restricted heifers compared to their energy maintained counterparts.

In summary, most of the work done so far supports the theory that nutritional anoestrus in cattle involves disruption of the LH release although direct effects on the ovary are also possible.

1.4 Conclusions

This literature review has revealed that reactions to *T. parva* infection are extremely variable. However, the literature dealing with the effect of theileriosis on reproduction in cattle is very scanty, possibly because it is overlooked due to the high mortality rates caused by the disease. However it is realized that animals recovering from primary infection suffer a reduction in productivity which may be long term (Dolan, 1981; Moll *et al*., 1981; Young *et al*., 1981). Oteng (1977) reported that heifers recovered from ECF took up to two years before breeding while mature female cattle cease breeding for at least 12 months with abortions occurring in pregnant animals. These reports were based on field observations and there appears to be no controlled studies to confirm these observations.

The only available reports on the effect of theileriosis on reproductive function were on *Theileria annulata* infections. Khvan *et al.* (1981) reported two abortions out of 22 cases of acute disease, abortions and disturbances of the oestrous cycle in 15 animals suffering from moderately severe *T. annulata* infection while no reproductive disorders were observed in mild cases. In another study, anoestrus was linked with latent *T. annulata* infection (Bhaskaran and Patil, 1982). On treatment, these animals resumed cycling and became pregnant on breeding. Lower haemoglobin concentrations in the infected animals was linked to the observed acyclicity (Bhaskaran and Patil, 1982). Khvan *et al.* (1981) reported atrophy of the ovaries and endometritis in animals suffering from acute *T. annulata* infections. However, in *T. annulata* the pathogenesis is mainly due to the destruction of red blood cells by the parasite. Therefore, there may be no justification in comparing the effect of the two parasites on reproductive function.

Reports describing lesions caused by *Theileria parva* on reproductive endocrine organs are equally scanty. Of the various reproductive organs, lesions have only been reported in the pituitary gland (De Kock, 1957; Dolan, 1986a). No abnormalities of the hypothalamus, uterus or ovaries have been reported in *T. parva* infections. No studies on reproductive hormones have been done to enable a clearer picture of the role of theileriosis on reported reproductive disorders and a sequential pathophysiology of endocrine mechanisms in theileriosis is yet to be adequately studied.

In an experiment designed to investigate the effect of immunization on fertility, Dolan and Mutugi (1989) immunized heifers with *T. parva* by infection and treatment on a large ranch in Kenya and exposed them to bulls at various periods. They reported a pregnancy rate of 63% at 5 months post-immunization compared to the yearly pregnancy rate of 70% of the other herds. There were 24 abortions which could not be associated with infectious abortion. They attributed the low in-calf rate at 5 months and abortions to nutritional influences since the plane of nutrition was poor from a prolonged drought. Elsewhere, Young *et al.* (1990) reported no adverse effect due to immunization in two pregnant cows. In a subsequent study, 50 heifers were immunized with the same stabilate of *T. parva* and maintained on a good plane of nutrition (Dolan and Mutugi, 1989). The calving rate in the immunized and control groups was 86% and 80% respectively. It was concluded that on a good plane of nutrition and in the absence of management interventions and disease challenge, immunization had no adverse effect on the fertility of Boran heifers.

The work reported in this thesis investigated the effect of infection without treatment or infection coupled with either chemotherapy or chemoprophylaxis on the reproductive function of Boran/Friesian cross heifers. Three parameters: palpable changes in the reproductive organs, measurement of P4 concentrations and behavioural changes were routinely used to monitor reproductive status.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Animals

2.1.1 Cattle

Boran/Friesian cross heifers, 18 to 20 months of age were purchased from a farm in Kenya which practised good tick control and which had no recent history of East Coast fever. As a pre-requisite for selection, the animals were tested for negative titres to theileriosis in the indirect fluorescent antibody test (IFAT) using cultured *T. parva* schizont antigen (Burridge and Kimber, 1972) and also assessed for cyclical status by rectal palpation of the reproductive organs and measurement of serum progesterone levels at a 10 days interval.

The experimental heifers were kept in a one hectare fenced paddock at the National Veterinary Research Centre, Muguga, Kenya Agricultural Research Institute. They were maintained on hay and ranch cubes supplemented with mineral licks (Maclik, Cooper Kenya Ltd.). Water was provided *ad libitum*. The animals were dewormed every three months with Levafas, a broad spectrum anthelmintic, (1.5% levamisole-hydrochloride, 7.5mg/kg; 3% W/V oxycyclozamide, 15mg/kg; Norbrook Laboratories Ltd., UK). They were dipped twice weekly in Asuntol (coumaphos, 700 ppm - Bayer, E.A. Ltd). The animals were also tagged with plastic ear tags impregnated with acaricide (Anchor Ear Force tags; Permethrin (3-phenoxyphenyl) methyl (±) -cis/trans -3 (2-2) dichloroethenyl) 2, 2 - dimethylcyclopropane - carboxylate; Boehringer Ingelheim Animal Health Inc., Missouri, USA). The ear tags reinforced protection against infestation with the brown ear tick, *R. appendiculatus* (Young *et al.*, 1985). The only exception to the above management practice was when

selected heifers were transferred for a period of 9-12 days to tick-proof barns for tick feeding to isolate *Theileria* parasites (2.8) when acaricidal ear tags were removed and tick control temporarily stopped.

Holstein/Friesian steers, over 12 months of age, with negative titres (<1:40) to *T. parva* schizont antigen were purchased from the same farm for transmission studies. These were kept in tick-proof barns and maintained on hay, ranch cubes and mineral supplements. Water was provided *ad libitum*. A Holstein/Friesian bull from the Centre's dairy herd was put together with selected animals from the late treatment and immunization experimental groups in a separate paddock but under similar management practices as above.

The distribution of the animals used in this study is presented in Table II-1 and an overview of the monitoring protocol in Table II-2. For fuller details, the reader is referred to the specific experimental designs in later chapters.

Table II-1 **Distribution of animals in the study**

Experiment	1	2	3	4
Infected	10	10	14	8
Controls	4	4	4	4
Total	14	14	18	12

2.1.2 Ticks

Rhipicephalus appendiculatus nymphs from the Muguga colony which has been maintained on rabbits since 1952 were used in parasite isolation attempts. The ticks were maintained as described by Bailey (1960).

2.1.3 Rabbits

Rabbits were conventionally kept New Zealand White type from the small

animal colony at the National Veterinary Research Centre, Muguga.

2.2 *Theileria* parasite

The *Theileria* parasite used was *T. parva* stabilate IL3081 kindly donated by the International Laboratory for Research in Animal Diseases (ILRAD). The parasite isolate was derived from buffalo 7014 caught at Ol-Pejeta ranch in Laikipia District, Kenya (Mutugi *et al.*, 1988a).

The stabilate was prepared as described by Cunningham *et al.* (1973) and contained five tick equivalents per ml. Initial studies by Dolan (unpublished results) using 1 ml of the stabilate in Boran cattle showed the time in days to macroschizonts, fever, piroplasms and death was 6.5, 6.5, 11.5 and 11.5, respectively. At a dilution of 1:10, this stabilate was reported to cause death in all five Friesian/Hereford crosses in 16.4 (mean) days and in three out of five cattle in 20.5 (mean) days at a dilution of 1:100 (Ngumi, Personal communication). Another isolate of *T. parva*, designated as stabilate 199, derived from buffalo 7014 was also reported to be infective to all Friesian steers and to cause deaths in 3/3, 2/3 and 2/3 at dilutions of 1:1, 1:10 and 1:100 respectively (Mutugi *et al.*, 1988a,b). At 1:1000 dilution, stabilate 199 was infective to two out of three cattle and caused death in one (Mutugi *et al.*, 1988b).

2.3 Method of Infection

The *T. parva* stabilate (IL3081) was stored in 0.5 ml aliquots in plastic straws in a liquid nitrogen container. Before inoculation, the plastic straws containing the stabilate were removed from the container, rapidly thawed by rubbing them between the palms of the hands and the contents dispensed into a sterile universal bottle. Appropriate dilutions were made using Eagle's Minimum Essential Medium with 3.5% bovine plasma albumin and 7.5% glycerol. The contents were mixed together and allowed to equilibrate for thirty minutes. The animals received 1 ml of various stabilate dilutions inoculated subcutaneously below and behind the left ear gland.

2.4 Clinical examination

Rectal temperature was taken daily between 08.00 - 09.00 h, 3-14 days before infection and after infection. Temperature monitoring was extended to more than seven days in all animals after the last febrile reaction in a group of experimental animals. Rectal temperature of 39.5^{o}C or more was considered to indicate fever. Animals were also observed for changes in appetite, nasal and ocular discharge, eye lesions, nervous signs and diarrhoea.

The prepatent period was defined as the length of time from infection to the first appearance of macroschizonts in the local drainage lymph node (left parotid lymph node, see below). Incubation period was defined as the length of time from infection to the first day of fever.

Animals were weighed once weekly on a weighbridge from about two months before experimental infection and continued after infection until the experiment was terminated.

2.5 Haematology

Animals were bled twice or three times weekly between 08.00 and 09.00 h. Sixteen gauge, 4 cm needles were used for jugular venepuncture. About 7 ml of blood was collected in 7 ml bijou bottles containing 0.05 ml of 20% dipotassium ethylene diamine tetra-acetate (EDTA) and thoroughly mixed.

Total white blood cell counts (WBC) and red blood cell concentration (RBC) were estimated with an electronic particle counter (Coulter Counter Model ZM). Haemoglobin (Hb) concentrations were estimated using a Coulter Haemoglobinometer. Packed cell volume (PCV) was estimated by using the microhaematocrit method. Differential leucocyte counts were performed on Giemsa stained blood smears; 200 cells were counted.

2.6 Monitoring of cattle for theilerial parasites

Needle biopsies were taken from the left parotid lymph node (referred to as left ear gland - LEG) from day 5 after inoculation of the parasite. Smears were made on glass slides, air dried, fixed in methanol and stained in Giemsa. They were examined for the presence of schizonts and lymphoid cell hyperplasia under oil immersion lens (x100). When the local drainage lymph nodes became positive or from day 10 post-inoculation, needle biopsies were taken from the right parotid and right prescapular lymph nodes and prepared smears examined for the presence of schizonts. Lymph node biopsies from the three glands were continued at daily intervals until animals died or no schizonts or clinical signs were seen for more than seven days. Peripheral blood smears were made from the tail-tip at daily intervals after schizonts were detected in the regional lymph node or from day 10 after inoculation and examined for the presence of piroplasms until the animals died or no clinical signs were seen for more than seven days.

2.7 Serology

About 10 ml of blood was collected from the jugular vein into a sterile vacutainer tube before experimental infection and at various intervals after infection and in uninfected controls as indicated in the specific protocols. The blood was allowed to clot and serum collected into 7 ml sterile bijou bottles after centrifugation at 2700xg for 15 minutes. The indirect fluorescent antibody test (IFAT) was carried out as described by Burridge and Kimber (1972). In this study a bovine lymphoid cell line (C_2P38) infected with *T. parva* schizont was used as a source of antigen. The sera were tested using 4-fold dilutions from 1:10 in phosphate buffered saline (PBS), pH 7.0. A negative control at 1:20 and a positive control at 1:160 dilution were included as a check in the test. Titres of $\leq$ 1:40 were considered negative while titres of $\geq$ 1:160 were considered positive. Those between were designated indeterminate.

2.8 Tick-infection and transmissions

Infected animals selected for isolation attempts of *Theileria* parasites using ticks were thoroughly cleaned to remove traces of acaricide on the body and to remove wax from the ears. The hair was clipped off the ears and the animals washed using teepol and water with particular attention being paid to the ears. This was followed the following day by cleaning the ears with dry cotton wool followed by rinsing with water. The washing was repeated on the third day.

One thousand *R. appendiculatus* nymphs were applied to each of the ears in ear bags and allowed to engorge and drop. The time interval from tick attachment to engorgement was 5-6 days. The ticks were allowed to moult and harden and then stored in flat-bottomed tubes in an incubator maintained at 28°C and 80% relative humidity. Prior to dissection or transmission attempts, the adult ticks were fed on rabbits for three days.

2.8.1 Demonstration of tick salivary gland infection

To demonstrate the presence of *T. parva* in the salivary glands of ticks from the parasite isolation attempts, 50 female and 50 male ticks were used from different batch collections. The ticks' salivary glands were dissected and examined for infection under a microscope after staining in Feulgen's stain as described by Blewett and Branagan (1973) and Young and Leitch (1982).

2.8.2 Transmission

For transmission studies, susceptible steers with no antibodies to *T. parva* using the IFAT (Burridge and Kimber, 1972) were used. They were prepared as described above for the tick feeding. Equal numbers of both sexes of adult ticks (2.8) were applied to the ears in ear bags and the animals monitored clinically as described in 2.4, for parasitosis as described in 2.6 and for antibody response as described in 2.7.

2.9 Culture of peripheral blood lymphocytes (PBL)

Parasite isolation from peripheral blood lymphocytes (PBL) was attempted as described by Brown (1979). Culture of PBL was done from selected infected animals in experiments 3 and 4. Blood was collected once at various stages of disease as shown in the experimental designs.

2.9.1 Separation of PBL

The area above the jugular vein was thoroughly swabbed with cotton wool wetted with 70% ethanol. Blood was collected via jugular venepuncture using 7 ml vacutainer tubes containing sodium EDTA (Vacutainer Systems - New Jersey). The blood was mixed with the anticoagulant by gentle agitation of the tube at the collection point.

The blood was centrifuged at 1060xg for 20 minutes at 4^{o}C (2300 rpm) to obtain the buffy coat. The supernatant plasma was removed with a 5 ml pipette and discarded. About 1 ml buffy coat was recovered and diluted with 2 ml phosphate buffered saline (PBS) pH 6.8. The buffy coat was carefully layered to an equal volume of Ficoll-Na Metrozoate (Ficol 400; Pharmacia Fine Chemicals, Uppsala, Sweden; Sodium metrozoate - 32.8% w/v; - Sigma Chemical Co., St Louis, Missouri, USA) and centrifuged at 600xg for 25 minutes at 4^{o}C (1700 rpm). The supernatant on the top of the interface was removed and discarded and mononuclear cells at the interface recovered using a 5 ml pipette. These were resuspended in 9 ml PBS, pH 6.8 and washed twice at 200xg for 10 minutes at 4^{o}C (1000 rpm). The supernatant was discarded in each case and the final pellet suspended in 10 ml MEM. The final concentration was adjusted to 1x10^{7} cells/ml. Cytocentrifuge smears were made on a cytospin, stained with Giemsa and examined for the presence of schizonts.

2.9.2 Establishment and maintenance of cultures

The PBL (1x10^{7}/ml) were seeded on to a monolayer of bovine embryonic

spleen cells (BESP) containing 10ml of fresh growth medium and incubated at 37°C in 5% $C0_2$ air.

The bovine embryonic spleen primary cells used as feeder layer was established as described by Malmquist *et al.*(1970). The medium for the cell culture was Eagle's Minimum Essential Medium (MEM) with Earle's salts supplemented with L-asparagine (0.1gm/litre). Penicillin, streptomycin and kanamycin were added at 100 IU/ml of medium each. Just before use, the medium was supplemented with 20% heat inactivated foetal calf serum, 2mM L-glutamine and 0.5ug/ml gentamycin and the pH adjusted to 6.8 using HEPES.

Two days after seeding the feeder layers with PBL, 3 ml of fresh growth medium was added. Two days later, 8 ml of the culture was removed, centrifuged at 200xg (1000 rpm) and the pellet resuspended in 5 ml fresh growth medium, returned to the culture flask and incubated as before. This procedure was repeated at regular intervals of 1-3 times per week until a cell line was established. Normally, a cell line would establish in 3 weeks. A poor PBL source of poor or no activity, would be maintained up to 12 weeks.

Assessment of cultures was initially done by regular examination on an inverted microscope for signs of transformation. When activity was seen, a cytospin smear was prepared, air dried, fixed in methanol and stained in 5% Giemsa. Cells were examined for the presence of schizonts and/or mitosis.

2.10 Behavioural observations for oestrus

Observations for behavioural signs of heat were carried out three times a day for 20 minutes between 7.00-8.00, 12.00-13.00 and 16.00-17.00 h. The following indices were recorded for any animal exhibiting heat and recorded as single scores for each sign: restlessness, loss of appetite, back-mounting, front-mounting, allowing mounting, sniffing or being sniffed by other animals, head-butting, bellowing, raised tail, swelling of the vulva and vulval mucal discharges. These indices are recorded as

heat scores in figures presented.

2.11 Rectal palpation

The reproductive tracts of the animals were examined by palpation *per rectum* once per week. The ovaries were palpated for the presence of ovarian structures. The presence of a *corpus luteum* (CL) was recorded and classified into one of six categories according to its consistency and estimated size. The developing CL fell into three classifications: less than 1 cm in diameter and soft in consistency, 1-2 cm in diameter and soft in consistency, more than 2 cm in diameter and soft in consistency (A1, A2 and A3 respectively). A mature CL (B) was over 2 cm in diameter and firm in consistency. Regressing CL were classified into two categories: more than 1 cm in diameter and hard in consistency, and less than 1 cm in diameter and hard in consistency (C2 and C1 respectively). The presence of follicles were categorised either as large, medium or small. Absence of detectable structures was also recorded. The uterus was palpated for tonus which was classified from 1-3: flaccid (1), turgid (3) and intermediate tonus (2). Animals which were exposed to a bull were palpated for absence or presence of pregnancy.

2.12 Serum progesterone determination

2.12.1 Sample collection and storage

Animals were bled twice a week at intervals of 3 and 4 days for serum samples used to determine levels of P4 concentrations. The animals were always bled between 8.00 to 9.00 h. About 10 ml of blood was collected by jugular venepuncture into sterile universal bottles, allowed to clot and serum separated by centrifugation at 1150xg (2500 rpm) for 15 minutes. Serum was separated and stored at -20^{o}C until used.

2.12.2.1 IAEA Supplied Assay

Serum progesterone concentrations were determined using a specific radioimmunoassay (RIA) kit supplied by the International Atomic Energy Agency (IAEA) (Animal Production Unit, IAEA Agricultural Laboratory, Siebersdorf, Vienna, Austria). The kit was developed for the measurement of progesterone in plasma/serum in domestic animals, including cattle. The kit consisted of polypropylene tubes coated with antibodies to progesterone, buffered progesterone label (^{125}I-progesterone; <5uCi/105 ml of solution), seven vials of progesterone standards (0, 0.3, 1.6, 6.4, 15.9, 31.8 and 63.6 nmol/litre) processed in human serum and containing sodium azide and gentamycin as preservatives, and two quality control (QC) samples containing low, and high concentrations of progesterone. This is a direct assay needing no extraction of progesterone and analysis was done following the protocol supplied with the kits.

Briefly, antibody coated tubes were set up in duplicate per standard, QC and unknown serum samples. Quality control sample tubes were placed at the beginning, middle and end of each assay. Polystyrene tubes (LP3-Luckham, England) were used for total counts (TC). Samples were allowed to thaw and then together with standards and QC's, allowed to stabilize to room temperature. After vortexing, 100 µl each of standards, QC's and samples were pipetted into the bottom of appropriately labelled tubes. This was followed by dispensing of 1 ml of ^{125}I-progesterone label into each tube including total count tubes using a multi-dispenser pipette (Eppendorf repeater). The mixture in the antibody coated tubes was vigorously mixed on a vortex mixer and incubated at 4^{o}C overnight. After the incubation, all tubes except total count tubes were decanted into a disposal container. The mouth of decanted tubes were struck sharply on a wad of absorbent paper and allowed to drain on more absorbent paper on a tube rack in an inverted position for 5-10 minutes. Radioactivity was counted in all tubes in a manual well, single tube gamma counter for 20 seconds. The mean number of counts in the total count tubes (TC) and in tubes containing no progesterone (B0) were recorded and the maximum binding (MB) of radio-labelled progesterone

determined by dividing B0 mean counts by TC expressed as percentage. The MB was a measure of efficiency of progesterone to bind to antibody in the coated tubes. The binding of all standards, quality controls and unknown samples was calculated by dividing their mean counts by the mean counts of B0 tubes and expressed as a percentage. A calibration (Standard) curve was drawn on logit-log graph paper with the average percent bound (B/B0) of standards on the vertical (y) axis and the progesterone standard concentrations on the horizontal (x) axis. A straight line was drawn through the points on the graph. Progesterone concentrations of the unknown serum samples and QC's were read by extrapolation of their percentage binding against standard progesterone concentrations on the standard curve which were expressed in nmol/litre.

Intra-assay and inter-assay coefficient of variation were calculated using readings of pooled quality control samples used in the assays.

2.12.2.2 WHO Matched Reagent Programme

Serum progesterone concentrations in the immunized group of experimental animals were determined according to the WHO Matched Reagent Programme protocols (Method Manual, 1986). Progesterone antiserum, standards, dextran and charcoal were supplied by the WHO Matched Reagent Programme, (London) and tritiated progesterone was obtained from the Radiochemical Centre, Amersham, England.

2.12.2.2.1 Materials

Progesterone antiserum: Raised in rabbits against progesterone -3 (0- carboxymethyl) oximino-BSA. The specificity to progesterone, cortisol, testosterone, 17β hydroxyprogesterone and 20α dihydroprogesterone was 100%, 0.005%, 0.1%, 1% and 2.7% respectively. Antiserum was supplied in lyophilized form and a working

solution was made by reconstitution with 10 ml assay buffer.

Progesterone standards: Standards were provided in ethanolic solution at a concentration of 250nmol/L. 100 µl of the solution was mixed with 10 ml assay buffer, heated at 40°C for 30 minutes and kept at 4°C as a stock solution containing 2.5 nmol/L. Six assay standards (2500, 1250, 625, 313, 156 and 78 pmol/L) were set up by doing a serial double dilution of the stock solution in assay buffer.

Progesterone tracer: This was obtained from Amersham International, England as 1, 2, 6, 7 - ^{3}H progesterone in amounts of 3.7 MBq (100µCi) with a specific activity of 3.15 TBq (85Ci) per nmol (TRK 413, Batch No. 71). A stock solution containing 370KBq was made up by adding 10 ml toluene. A working solution was made by evaporating off the toluene from 150 µl of stock solution and redissolving the extract in 15 ml assay buffer. The working solution contained 3.7 KBq/ml and gave counts of approximately 10,000 counts per minute. Recovery tracer was made by taking 100 µl stock solution and reconstituting with 1 ml assay buffer after evaporating off the solvent.

Dextran-charcoal reagent: This was made up as follows:

Activated charcoal	0.625g
Dextran	0.0625g

Made to 100 ml in assay buffer.

Assay buffer: This was made up as follows:

Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$)	-	3.05g
Disodium hydrogen phosphate (Na_2HPO_4)	-	11.6g
Sodium chloride	-	8.8g
Sodium azide	-	0.1g
Gelatin	-	1.0g

Made to 1 litre in distilled water. The pH was adjusted to 7.3 and the buffer stored at 4°C.

Scintillation cocktail: This was made by dissolving 7.5g of 2,5-diphenyloxazole (PPO;

Sigma Chemical Co., Missouri, USA) in 1 litre of sulphur-free analytical grade toluene (BDH, England).

2.12.2.2.3 Assay procedure: Serum samples and quality control pools (low and high progesterone) were vortexed and 200 µl pipetted into extraction tubes. 4 ml of analytical grade diethyl ether (BDH, England) was added to each tube and into two empty tubes (ether blanks-EB), the tubes were stoppered and the contents vortexed. These were further mixed on a shaker for 5 minutes and transferred to a deep-freezer for 45 minutes. The solvent ether layer was decanted into a second set of tubes and the ether evaporated off on a warm water-bath. 2 ml of assay buffer was added to the dry extracts and vortexed thoroughly. The assay was set up in duplicate using polystyrene LP3 tubes (Luckham, England) as summarized below:

	Buffer (µl)	Std,QC, Sample, Ether blank (µl)	P4 antiserum (µl)	^{3}H-P4 (µl)
TC	800	-	-	100
NSB	600	-	-	100
Bo	500	-	100	100
Std,QC,Sample, & Ether blank	-	500	100	100

These were vortexed and incubated overnight at 4°C. 200 µl of chilled dextran-charcoal reagent was added to all tubes (except TC), vortexed and incubated at 4°C for 45 minutes. These were then centrifuged at 4°C in a refrigerated centrifuge for 15 minutes. The supernatant was decanted with minimum delay into 6 ml scintillation vials. 4 ml of scintillation cocktail was added to all tubes including TC and the contents allowed to equilibriate in the dark for a minimum of 5 hours. Counting

was performed in a liquid scintillation counter for 2 minutes. Progesterone concentrations were determined using a RIA computer package (WHO Immunoassay D.P. Programme (A5.4) - Edwards, P.R.).

An estimate of extraction efficiency was monitored in every assay by including two recovery samples into every assay batch. In brief, 10 µl of recovery tracer was added to 200 µl of serum sample, vortexed and left to equilibrate for 30 minutes. The samples were extracted as described above but the extract was directly transferred into scintillation vials and the ether evaporated off. The dry extract was dissolved in 500 µl of assay buffer. Recovery total counts were prepared by adding 10 µl of recovery tracer directly into scintillation vials. 4 ml scintillation cocktail was added to both recovery total and recovery vials and the contents allowed to equilibrate and counted as described earlier. Recovery was determined by dividing recovery counts by recovery total counts and expressed as a percentage. The percentage recovery was 74.4 ± 4.4% (mean ± SD; n=24). The intra-assay CV was 6%. Results were not corrected for recovery and the results presented in Figs VI-1 to VI-5 should therefore be considered to represent about 74% of real values.

Thirty two samples assayed using the antibody coated tube method were also analysed using the WHO Matched Reagent Protocols for comparison. The means in the two methods were 6.2 ± 1.1 and 6.8 ± 1.3 (mean ± SEM) nmol/L for the WHO Matched Reagent Programme protocols and the IAEA method, respectively. There was no significant difference between the two values ($p > 0.05$). The compared values are presented in Table II-3. The P4 values presented for the WHO Matched Reagent Protocol were not corrected for recovery.

2.13 Plasma luteinising hormone assays

Radioimmunoassays for plasma luteinising hormone (LH) to monitor hypophyseal response to gonadotrophin releasing hormone were planned for animals in the late treatment group. The animals sampled are given in the experimental design.

2.13.1 Collection and storage of samples

Preparation of animals for sample collection was started on the same day between 7.00-8.00 h.

Materials

Catheter storage bags: Prepared by using small (7.5x12mm) plastic bags which were covered externally with adhesive tape leaving an open end with an overlying flap cover. A hole was made on one side of the bag for passing the catheter through .

Catheters: Polyvinyl tube, internal diameter 1.0mm, external diameter 1.5mm (Dural Plastics and Engineering, NSW, Australia). Catheter stoppers were made by cutting off tips of 1ml syringes with a hot blade.

Heparin: Heparin solution for flushing the interiorised catheters was prepared by diluting heparin (Radiufarma Int., Italy - 5000 I.U/ml) to 1:50 with sterile distilled water to give a final concentration of 100 I.U./ml. A stock solution containing 500 I.U./ml was similarly prepared for the collected blood. 50 µl of the stock solution was transferred into individual sterile universal bottles.

GnRH: A synthetic formulation of GnRH (Fertagyl, Intervet International B.V -Boxmeer, Holland) containing 0.1mg GnRH/ml was used.

Needles: 14G, 4cm long.

Disinfectants: Savlon (Chlorhexidine glycolate 1.5% w/v, cetrimide 15% w/v - ICI, UK) made up to 1:30 in water.

Methods: An area over the jugular vein slightly larger than that of the catheter storage bag was shaved and cleaned with spirit. After a jugular venepuncture using 14G sterile needle, a piece of polyvinyl tube which had been sterilized in savlon solution overnight was introduced through the needle and fed in to reach to the base of the heart leaving about two feet exteriorised. The needle was removed, the exteriorised catheter passed into the plastic storage bag and 1-2 ml of heparin (100 IU/ml) injected into the catheter

to prevent clotting. The catheter was stoppered, folded into the bag and the latter attached to the skin using adhesive tape. The animals were left for two hours only interrupted by hourly flushing of the catheter before sampling started.

Sampling was done at -60, -40, -20, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min relative to GnRH injection (100μg IM). Before samples were collected, an equal amount of heparin/blood equal to the heparin used for flushing the catheter was removed and using a separate sterile syringe, 5 ml of blood was withdrawn and collected into the heparinized bijou bottles to give a final concentration of 50 IU heparin per ml of blood. The blood was mixed, stored over ice and centrifuged at 1150xg(2500 rpm) for 15 minutes within three hours of collection. Plasma was separated and stored at -20^{o}C. For animals in which the entire procedure was repeated the alternate jugular veins were used.

2.13.2 Radioimmunoassay: The WHO Matched Reagent Programme (Method Manual, 1986) protocol for LH assay was adopted with modification for the separation of bound from free radioligand. Initially, an antiserum to bovine LH (bLH-F88) supplied by Professor W. Butt (Birmingham, Midland Hospital for Women) was validated for use. This was later disregarded in favour of human anti-LH serum (WHO Matched Reagent Programme). Similar materials and methods were used during the validation of both the bLH-F88 and human LH antisera.

2.13.3 Validation of LH antisera

Materials

Anti-bovine LH serum (bLH-F88 antiserum): Stock solutions of bLH-F88 antiserum were prepared by diluting the original sample to 1:4 in assay buffer and stored in aliquots of 100 μl at -20^{o}C.

Anti-human LH serum: Provided by the WHO Matched Reagent Programme in

lyophilized form. It was raised in rabbits and had a specificity of 100, 0.5, 0.2, 50 and 0.6% against hLH68/40, hFSH AFP/57AC, LHαNM/14, LHβNM/14 and hTSH AFP/1001C, respectively. The working solution was prepared by reconstitution of vial contents with 10 ml assay buffer.

LH standards: Supplied by the WHO Matched Reagent Programme and prepared from human pituitary. It was provided in lyophilised form with each bottle containing 50 milli units (mU) of the WHO International Reference preparation (IRP) of pituitary LH. Working standards were prepared by reconstituting the contents of one vial with 1 ml of assay buffer and allowed to dissolve in 10 minutes before being vortexed thoroughly. The solution now containing 50 I.U/L WHO IRP was further diluted to prepare solutions containing 25.0, 12.5, 6.25, 3.125 and 1.56 I.U/L WHO IRP.

Tracer for LH: Provided in lyophilised iodinated form (^{125}I-LH) form by the WHO Matched Reagent Programme. A stock solution was prepared by reconstituting the vial contents with 1 ml of assay buffer and thoroughly vortexed after allowing to dissolve for 5-10 minutes. This was stored at 4^{o}C until used. Working solutions were made using 200 µl of stock solution diluted to 10.5ml in assay buffer. Second antibody used was donkey anti-rabbit serum (DARS) diluted to 1:15 in assay buffer.

Normal rabbit serum (NRS): 1:400 dilution prepared in 0.1 M-EDTA.

Polyethylene glycol (PEG): Used at a concentration of 4.8% (w/v) made with physiological saline.

2.13.4 Development of assay methods

A. Determination of optimal concentration of bLH-F88 As.

A 1:50 dilution of stock solution (1:4) was prepared by reconstituting 100 µl of the antiserum to 1.25 ml with assay buffer. This was diluted further to 1:25600 through serial double dilution in assay buffer. The optimum concentration of this antiserum was determined following methodology given below.

*	Buffer µl	bLH As µl	^{125}I-LH µl	DARS µl	NRS µl	PEG µl
TC	-	-	100	-	-	-
NSB	400	-	100	100	100	500
bLH As	300	100	100	100	100	500
		**				

* TC, NSB, bLH-F88 As done in triplicate

** Overnight incubation (Room temperature)

After overnight incubation at room temperature, NRS, DARS and 4.8% PEG, were added, the contents vortexed, incubated for 30 minutes at room temperature and centrifuged at 1600xg (3000 rpm) at 4°C. The supernate was aspirated using a vacuum pump and the precipitate counted on a gamma counter. Percentage binding for each dilution was determined. The greatest dilution (1:3800) of LH antiserum which fell within the linear region (optimum area) of the titration assay was selected and used later to establish a standard curve Fig II-1. At this dilution, the antiserum was too avid for the tracer. Repeated assays using a dilution of 1:38,000 bound 37.04 ± 1.16% (mean ± SEM) of tracer and this concentration was adopted for subsequent work.

B: Standard curve

The standard curve assay was set up as summarized below: The contents were vortexed and incubated at room temperature overnight. They were then separated as described above (2.13.4 A). The precipitate was counted on a gamma counter and the percentage binding plotted against the specific standard LH dilutions. A typical standard curve is presented in Fig. II-2.

Representative mean percentage bindings over the LH standard concentration range are presented in Table II -4.

	Assay buffer	Standard	bLH As	^{125}I-LH
	µl	µl	µl	µl
TC	-	-	-	100
NSB	400	-	-	100
Bo	300	-	100	100
Std 1: 1.56 I.U/L	200	100	100	100
Std 2: 3.125 I.U/L	200	100	100	100
Std 3: 6.25 I.U/L	200	100	100	100
Std 4: 12.5 I.U/L	200	100	100	100
Std 5: 25.0 I.U/L	200	100	100	100
Std 6: 50.0 I.U/L	200	100	100	100

2.13.5 Specificity

Specificity of the bLH-F88 As was tested for parallelism using bovine plasma samples from the experimental animals. Two bovine plasma samples were selected from a 40 (A) and 240 (B) minute post-GnRH injection pool and four preparations made from each as follows:

1. 200 µ l sample + 100 µl assay buffer
2. 100 µl " + 200 µl "
3. 50 µl " + 250 µl "
4. 25 µl " + 275 µl "

Assays were set up following the same protocol as used in the standard curve assay but with the substitution of the bovine sample preparations for standards. Fig.II-3 is representative of the results obtained from a number of assays and shows lack of parallelism with the standard curve.

2.13.6 Validation human LH antiserum for use on bovine plasma samples.

The assay was tested for parallelism of bovine samples to the standard curve using the same protocol as in 2.13.5. Anti-hLH serum was substituted for anti-bLH-F88 serum. The results are shown in Fig II-4 and demonstrated a high degree of parallelism.

2.13.7 Radioimmunoassay of LH in bovine plasma samples.

The assay was set up as in the summarised format below. The same procedures adopted in the validation trials were followed.

LH assay protocol on bovine samples (summarized)

	Buffer µl	Std,QC, Sample µl	LH As µl	^{125}I-LH µl	NRS µl	DARS µl	4.8% PEG µl
TC	-	-	-	100	-	-	-
NSB	400	-	-	100	-	-	-
Bo	300	-	100	100	100	100	100
Stds.	200	100	100	100	100	100	100
QCs	200	100	100	100	100	100	100
Samples	100	200	100	100	100	100	100
			*				

* - Overnight incubation at room temperature

To assess the suitability of the assay, plasma samples collected on day 35 and 56 post-infection were assayed. Two quality controls comprising of human plasma supplied with the assay kit were included.

2.14 Pathology

Materials for pathological studies were from animals in experiments 1-3. The particulars are presented in the various experimental designs. The four control animals in the first experiment provided control materials.

Following death or sacrifice, the animals underwent a full post-mortem examination. Gross findings were recorded and tissues for light microscopy collected and fixed in 10% neutral buffered formalin. Tissues for light microscopy in experiments 1 and 2 (Chapter 3 and 4) comprised of the pituitary gland, ovaries, uterus and adrenal gland. In addition to these, a range of other tissues were collected from animals in experiment 3 (Chapter 5).

After fixation, the tissues were dehydrated in graded alcohols and embedded in paraffin wax. Sections were cut at 3-4 μm and stained routinely with haematoxylin and eosin (H & E). Stained sections were examined microscopically at both low and high magnification and representative lesions photographed.

2.15 Statistics

Data were analysed by two-sample analysis of variance using the statigraphics (Statg) computer programme (Statistical Graphics Corp. 1985-89). Comparisons were made between groups or within a group where values were compared to the initial pre-infection group means. A value of $p < 0.05$ was considered significant.

Table II-2 **Monitoring protocol: General overview**

Oestrus detection	Thrice daily
Body weight	Once weekly
Rectal palpations	Once weekly
Serum P4	Twice weekly
Clinical examination	
Symptoms	Daily
Temperature	Daily
Haematology	x 3 (Expts. 1&2) or x2 (Expts. 3&4) weekly
Parasitology*	
LEG (a)	Daily after day 5 post-infection
RPG	Daily after (a) positive or day 10 post-infection
Blood smears	Daily after (a) positive or day 10 post-infection
Serology	Pre- and post-infection
Parasite isolation	Post-infection
Necropsy	After death or sacrifice

* - Infected animals
LEG - Left ear gland
RPG - Right prescapular gland

Table II-3 **Comparison of serum P4 concentrations as measured by the IAEA or WHO Matched Reagent Programme assay methods**

Anim. #	Date of sampling	IAEA method		WHO method*	
		P4 conc.**	CV	P4 conc.	CV
602B	23.7.90	0	0.9	0	0.8
020B	"	20	3.3	13.9	0.4
587B	"	2.1	3.7	1.9	0.3
025B	"	2.4	3.1	2.6	1.3
586B	"	0.3	0.4	0.7	0.3
022B	"	13.0	4.1	10.8	0.8
026B	"	17.5	2.5	16.0	6.8
029B	"	1.4	0.6	1.4	0.5
036B	"	2.0	0.9	0.8	0.9
594B	"	1.9	0.5	1.8	4.5
024B	"	10.0	0.7	7.4	4.6
037B	"	9.0	1.6	5.1	5.7
583B	"	27.0	1.1	21.6	9.9
579B	"	7.2	1.7	6.7	1.1
590B	20.9.90	10.5	1.7	9.9	0.1
591B	"	1.0	2.8	0.6	4.2
597B	"	3.4	0.3	5.2	5.1
803B	"	1.4	1.5	2.9	7.6
580B	24.9.90	2.6	4.9	1.0	9.0
584B	"	13.5	0.3	18.2	2.3
588B	"	0	2.8	0	11.2
590B	"	1.0	2.2	0.3	0.4
597B	"	0	3.0	0	2.0
598B	"	8.0	0.9	11.6	5.7
803B	"	6.8	2.3	5.3	3.7
804B	"	5.4	1.7	6.0	6.6
805B	"	24.0	3.2	18.2	6.3
806B	"	16.0	0.1	17.2	1.6
580B	"	6.4	4.5	8.1	11.8
584B	"	1.2	3.8	0.7	4.7
590B	"	0.4	0.2	0.6	4.7
597B	"	1.6	1.9	0.4	4.3

* - Not corrected for recovery

** - nmol/L

Table II-4 **Percentage binding over the standard curve using bovine Lh antiserum (anti bLH F88), human LH (hLH) standards and ^{125}I-hLH**

LH standard I.U/L	Standard curves n	%B/B0 Mean ± SEM	CV
0	5	37.0 ± 1.16	7.0
1.56	5	98.5 ± 0.36	0.8
3.125	5	93.0 ± 0.49	1.2
6.25	5	88.0 ± 0.94	2.4
12.5	5	76.6 ± 0.67	2.0
25.0	5	61.0 ± 0.94	3.4
50.0	5	47.4 ± 0.67	3.2

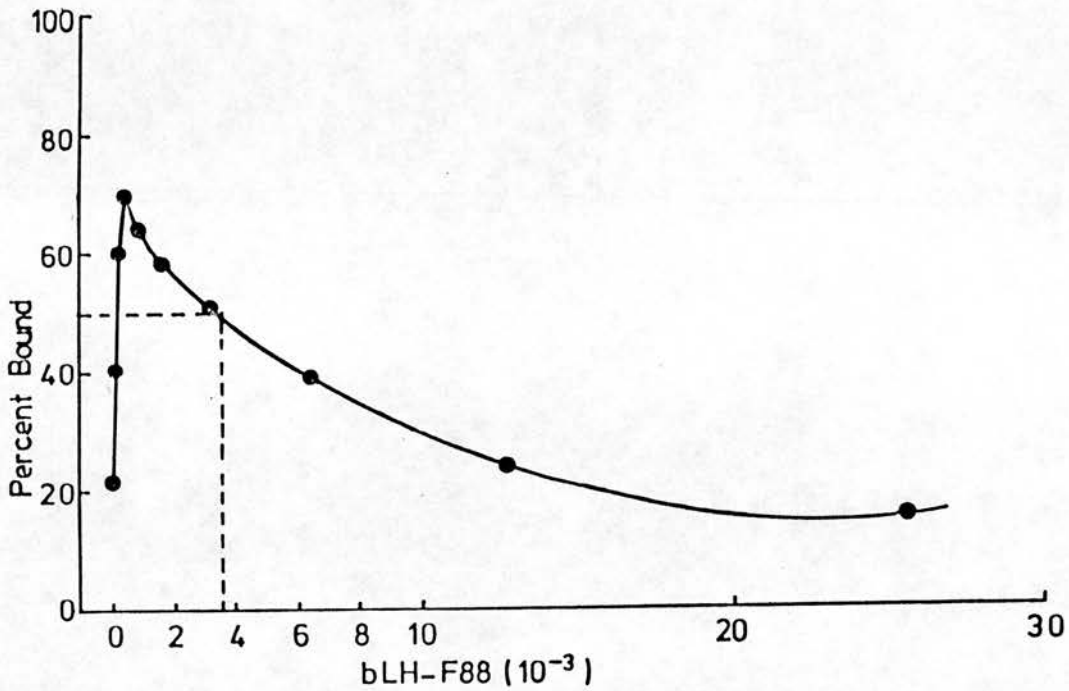


Fig.II-1.

Titration assay curve to determine the optimal working concentration of bovine LH antiserum (bLH-F88) with ^{125}I-LH tracer. A 50% binding occurred at 1:3800 antiserum dilution (dotted line) as the binding declined with increasing dilution of antiserum.

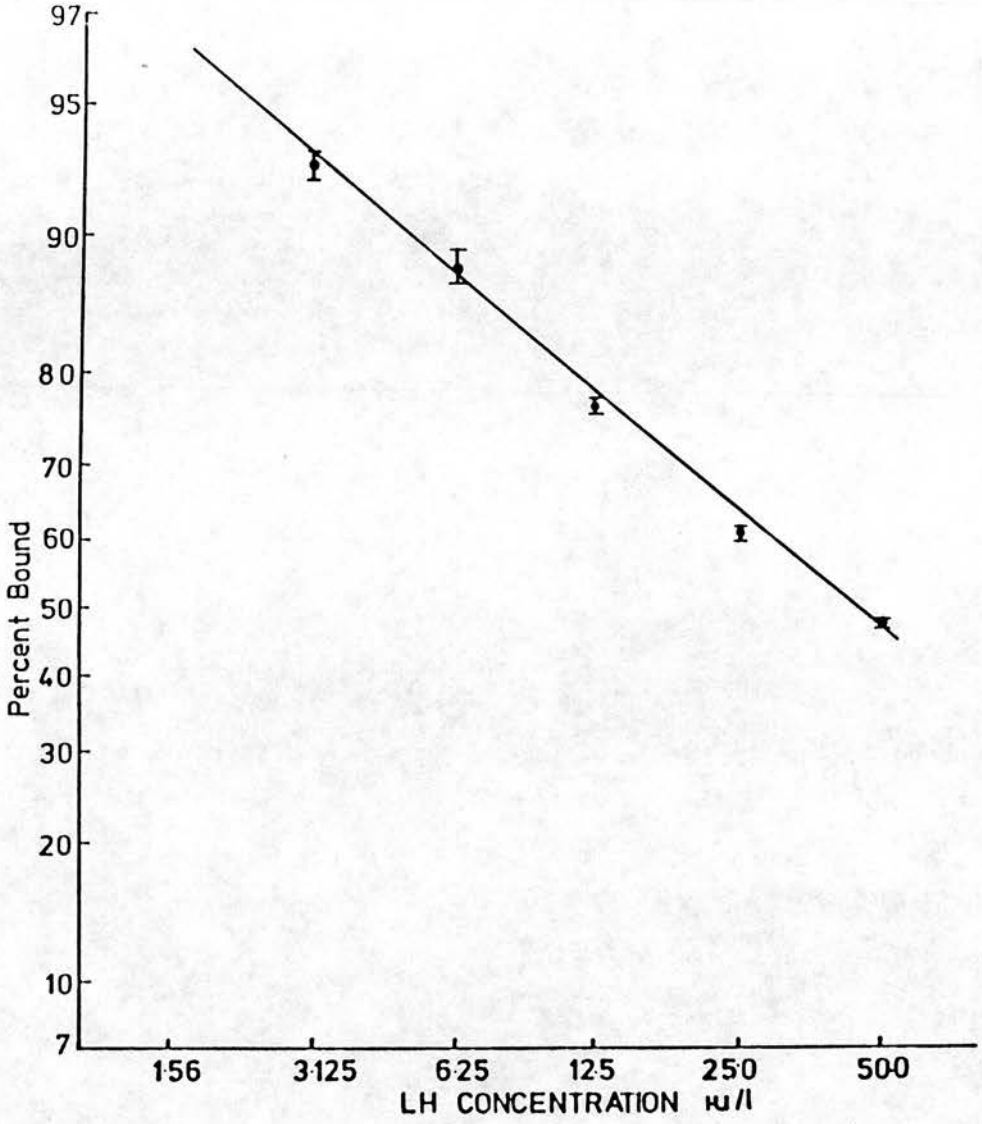


Fig.II-2.

Standard assay curve. The assay was set with bovine LH antiserum (bLH-F88), ^{125}I-hLH and hLH standards. Percent binding (Mean ± SEM).

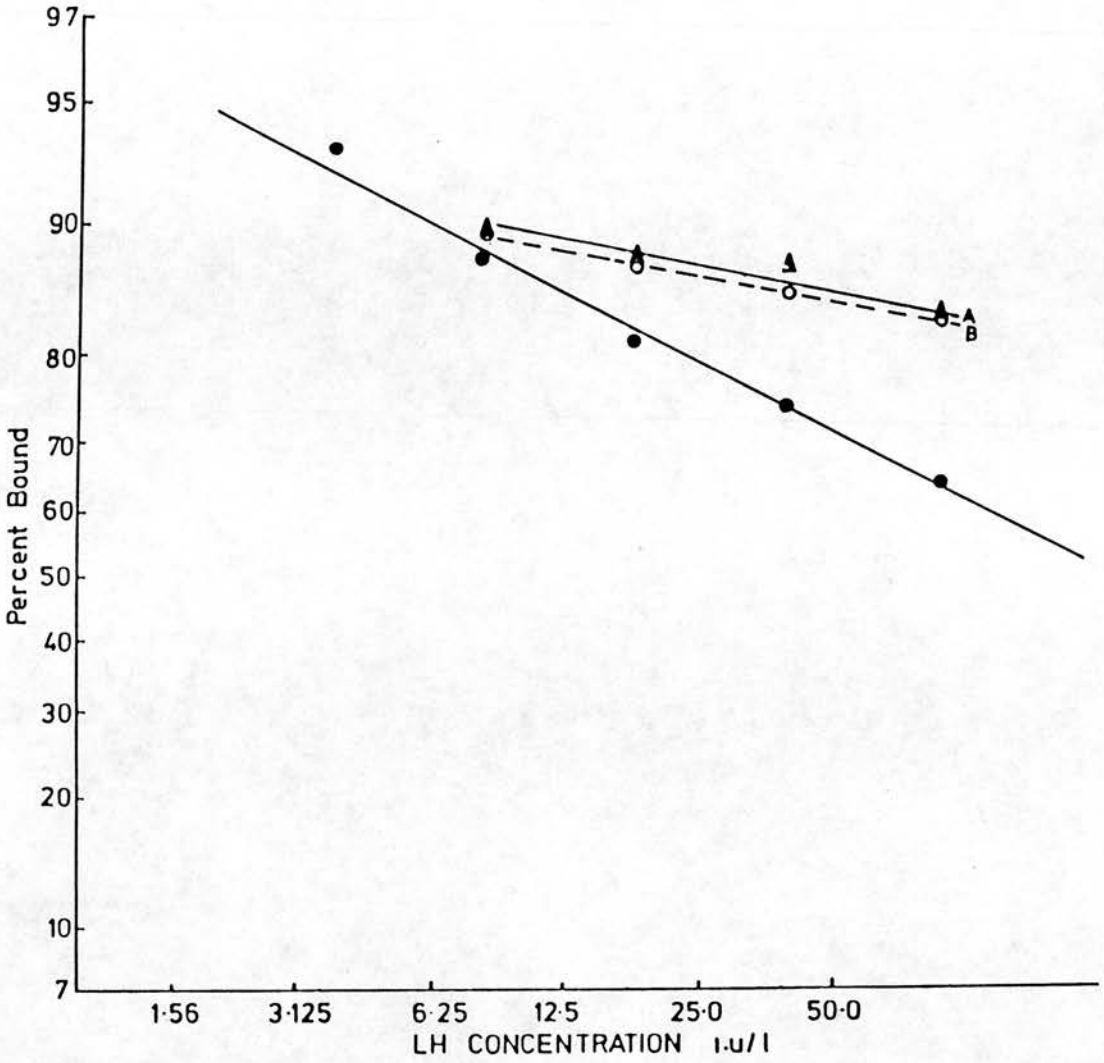


Fig.II-3.

Determination of specificity of the standard assay (•) set using bovine LH antiserum (bLH-F88), ^{125}I-hLH and hLH standards to measure LH in bovine plasma samples (A and B).

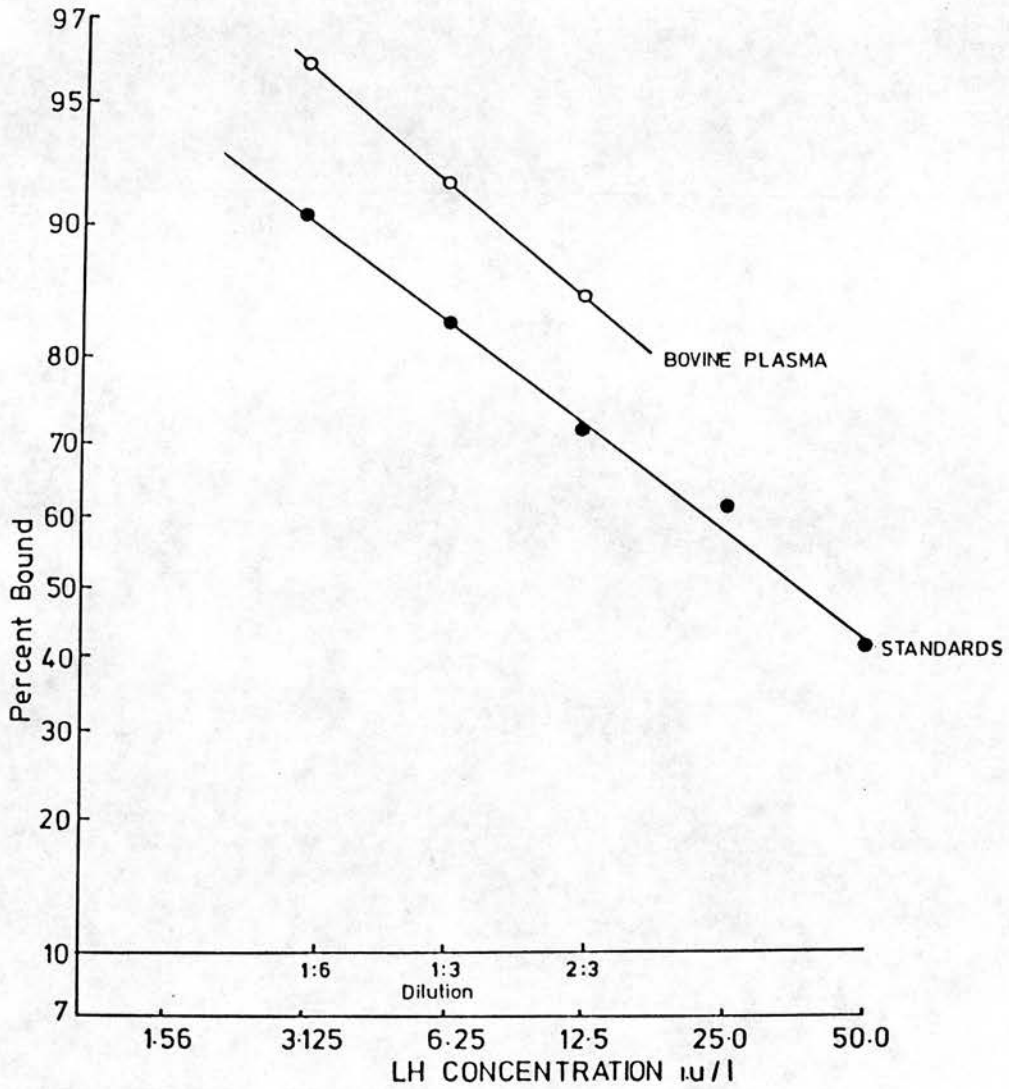


Fig.II-4.

Determination of specificty of the standard assay (•) set using human LH antiserum (hLH), ^{125}I-hLH and hLH standards to measure LH in bovine plasma (o).

CHAPTER THREE

THE EFFECT OF USING 1:20 *T. PARVA* STABILATE IL3081 ON THE REPRODUCTIVE FUNCTION OF BORAN/FRIESIAN CROSS HEIFERS

3.1 Introduction

The initial objective of this study was to investigate the effect of acute theileriosis on reproductive function in cattle with particular reference to the pathology of participatory organs like the pituitary gland, ovaries and uterus. Dolan (unpublished observations) had found the parasite intended for use in this work caused mortality in 14.5 and 16.5 days at dilutions of 1:1 and 1:10, respectively and no mortalities in two animals at 1:50 dilution. Further work in our laboratory showed that the parasite, *T. parva* stabilate IL3081 caused 100% (5/5) mortality in yearling bovine steers in 14-23 days when used at 1:10 dilution. A 1:20 dilution of *T. parva* stabilate was chosen with the hope that it would introduce severe reactions but that the period from infection to death would be long enough to accomodate at least one cycle length.

The result of using 1 ml 1:20 dilution of *T. parva* stabilate IL3081 to inoculate our 10 Boran/Friesian cross heifers were unexpected; 2 animals died and of the 8 survivors, 5 could be classified to have suffered from mild and the other 3 from moderate forms of theileriosis. As the overall objective of the research was to investigate the effect of theileriosis on reproductive function in cattle, the outcome of this experiment gave an opportunity to look at the effects of mild and moderate disease forms on bovine reproduction.

3.2 Materials and methods

3.2.1 Materials

3.2.1.1 Animals

These are described in General Materials and Methods (2.1).

3.2.1.2 Parasite

Details of the *T. parva* stabilate are described in General Materials and Methods (2.2). A 1:20 dilution of stabilate was made by reconstituting 1 ml of undiluted stabilate in 19 ml of Eagle's Minimum Essential Medium with 3.5% bovine plasma albumin and 7.5% glycerol as described in General Materials and Methods (2.3).

3.2.2 Experimental design

Fourteen Boran/Friesian cross heifers were allocated randomly into two groups of ten and four animals each. Each of the ten animals was inoculated with 1 ml of 1:20 *T. parva* stabilate as described in General Materials and Methods (2.3). The day of stabilate inoculation was referred to as day 0. Two of these animals died and were designated as infected-died group; five survived and cycled subsequently and were designated as infected-cyclic group and three survived and failed to cycle post-infection and were designated as infected-acyclic group. The four animals that were not infected acted as controls.

The monitoring regimen is presented in a summarized form in Table III-1. The methodologies were as described in detail in General Materials and Methods.

Serum P4 concentrations were analysed by the IAEA supplied assay. The inter-assay coefficients of variation were 12.5% (n=5) at 16.4 $\pm$ 2.1 nmol/L (mean $\pm$ SD) and 12.8% (n=5) at 4.1 $\pm$ 0.5 nmol/L for the high and low quality controls (QCs), respectively. The intra-assay coefficients of variation were 4.5% and 3.3% for the high and low QCs, respectively.

Table III-1 **Summarized experimental protocol (Experiment 1)**

Parameter monitored	Frequency and duration
Oestrus behaviour	Daily (x3):Week-16 to 28
Body weight	Weekly: Week-16 to 28
Serum progesterone*	Weekly (x2): Week-12 to 14 or 23**
Rectal palpations	Weekly: Week-16 to 14 or 23**
Haematology	Weekly (x3): Week-3 to 14
Rectal temperature	Daily: Day-3 to 42
Lymph node biopsies***	
LEG	Daily: Day 5 to 30 latest
RPG	Daily: Day 8 to 30 latest
Blood smears***	Daily: Day 8 to 35 latest
Parasite isolation studies**	Day 103 to 113
Serology	Days 0, 18, 29, 35 and 180
Necropsies	At death or Days 180 to 200

* IAEA Supplied assay (2.12.2.1)

** Animals 325A, 328A and 341A

*** Infected animals only

3.3 Results

3.3.1 Clinical signs

Nine out of the ten infected animals developed fever. The mean time to the onset of fever was 13.0 ± 2.7 (range 10-18) days (Table III-2). The febrile reaction period ranged from 2-8 (mean 4.9 ± 1.9) days. Three of nine animals (Nos. 320A, 324A and 341A) suffered intermittent febrile reactions. One infected animal (No. 327A) maintained normal temperature throughout. Representative temperature changes are shown in Figs III-1 to III-5. The major clinical signs were observed during the period of elevated temperature. During this period, all infected animals except Nos. 327A and 317A were anorectic, dull, showed signs of respiratory distress, developed seromucinous ocular discharge and enlargement of local lymph-nodes. Diarrhoea was observed in No. 317A from day 25 to 27 and in No. 322A from day 26 to its death on day 29. Nervous signs were seen in one animal (No. 323A) and consisted of ataxia, muscle tremors and apparent blindness in the terminal stages of the disease. Rectal temperatures in control animals remained below 39.5°C (Fig. III-6) and the animals remained healthy over the study period.

3.3.2 Parasitology

The presence of macroschizonts in lymph-node biopsies in representative animals is shown in Figs. III-1 to III-5. The time to appearance and disappearance of macroschizonts in the left ear gland (LEG) and right prescapular (RPG) lymph node biopsies is presented in Table III-2. All the infected heifers developed a macroschizont parasitosis with a mean ± SD prepatent period of 10.5 ± 2.0 (range 8-13) days. Macroschizonts appeared in the contra-lateral prescapular gland (RPG) a little later (12.9 ± 1.6, range 9 to 15 days). Macroschizonts were seen either continuously or intermittently until they disappeared. The mean time to disappearance of macroschizont parasitosis in the LEG and RPG was 20.5 ± 2.6 (range 17-26) and 23.2

± 2.7 (range 17-27) days, respectively. One animal (323A) had a persistent macroschizont parasitosis until the time of death while in animal No. 322A, which died on day 29 post-infection, macroschizonts were not seen beyond day 23 of infection (Fig. III-1). The time to appearance and disappearance of macroschizonts in the LEG and RPG among the infected-died, infected-cyclic and infected-acyclic groups was not significant.

3.3.3 Haematology

3.3.3.1 White blood cell count (WBC)

White blood cell concentration change presented from 17 days pre-infection to day 99 of infection are presented in Figs. III-1 to III-5 for representative animals. White blood cell concentration showed a generalised leucopaenia in all the infected animals. However, the degree of depression in the leucocyte counts varied from animal to animal and the degree, time to maximum depression and time to recovery of pre-infection levels are presented in Table III-3. The most marked depression was to 31.6% of pre-infection level (3000 μl^{-1} from 9500 μl^{-1}) while the least was to 82.2% (8100 μl^{-1} from 9500 μl^{-1}). The depression was highest in two animals in the acyclical status (Nos. 325A and 341A) which after regaining their pre-infection levels, underwent further prolonged periods of leucopaenia. Heifer No. 328A which also underwent an acyclic period suffered the least degree of leucopaenia and had no exacerbations after recovery of pre-infection levels. A marked leucocyte depression also occurred in the two fatal cases. Animal No. 323A died before, while No. 322A died after recovery of pre-infection levels (Fig. III-1). As a group, animals that survived infection and remained cyclical underwent the least leucopaenia and although exacerbations occurred, these were of lesser duration compared to those of the two acyclic cases. Leucocyte concentration did not vary markedly in control animals. Data presented in Fig. III-6 for 318A is typical of the group..

The mean leucocyte concentrations for the various groups are presented in Table III-4. Mean leucocyte levels appeared to start declining in the infected groups from day 8 and 11. However, significant declines from pre-infection levels were seen on days 6, 8, 13 and 15 in the infected-fatal group; days 13 and 15 in the infected-cyclic group and days 15 and 25 in the infected-acyclic group. Compared to controls, all infected groups had significant reduced mean leucocyte concentrations on various days from the second to third week post-infection (Table III-4). There were no significant differences among the infected groups. Mean leucocyte concentrations did not differ significantly in the control group over the study period.

3.3.3.2 Differential leucocyte count

Peripheral lymphocyte concentration declined in all animals following infection, and initial declines were observed as early as day 4 post-infection. The degree of lymphocyte depression varied. The time to and degree of depression at nadirs are presented in Table III-5. The degree of depression varied and ranged from 25.3% of pre-infection level (6200 down from 8300 μl^{-1}) to 71.6% (1900 down from 6700 μl^{-1}). Recovery to pre-infection levels occurred in all recovered animals although intermittent falls in concentration were observed in 5/8 animals after initial recovery.

Neutropaenia developed in all the infected animals with nadirs reached between days 11-25 post-infection. Neutrophil concentrations declined by as much as 78.3% (500 down from 2300 μl^{-1} in 320A) of pre-infection levels (Table III-5).

Lymphocyte and neutrophil concentrations fluctuated in the controls. The lowest levels observed compared to respective concentrations on day 0 are presented in Table III-5.

3.3.3.3 Red blood cell concentration (RBC)

The RBC concentration in all heifers was between 7.0-8.5 μl^{-6} pre-infection. A decline in RBC concentration (7.2 ± 0.94 μl^{-6} down to 6.17 ± 0.62 μl^{-6}) was

observed in the infected-acyclic animals between the third and fifth weeks of infection followed by recovery to pre-infection levels in the sixth week. No marked change was seen in the infected-cyclic, infected-died and control groups. RBC concentration showed no significant difference between control, infected-died, infected-cyclic and infected-acyclic groups before and to day 22. The RBC concentrations in the infected-acyclic group however, were signifificantly lower than those in the infected-cyclic and controls from day 22-36. There was no significant difference between control and infected-cyclic groups.

3.3.3.4 Haemoglobin concentration

Haemoglobin concentrations were in the range of 9.37 ± 0.95 to 13.35 ± 1.08g dl^{-1} in control animals and infected animals pre-infection. Significantly ($p<0.05$) lower levels were seen between infected-acyclic and infected-cyclic animals between days 29 and 48 and between infected-acyclic and controls between days 29 and 50. There were no significant differences between control and infected-cyclic or within the control groups throughout the study period.

3.3.3.5 Packed cell volume

The packed cell volume (PCV) was within the range of 29-40% (mean 32.7 ± 1.91 %) pre-infection. The PCV did not change significantly ($p>0.05$) within the infected-cyclic, infected-acyclic or in the control group over the study period. However, there were significantly lower levels in the infected-acyclic group from days 29 to 48 and days 29 to 60 compared to the infected-cyclic and control groups, respectively. There was no significant difference in PCV values between the infected-cyclic and control groups over the study period.

3.3.4 Body weight change

Representative body weight (bw) changes are presented in Figs. III-1 to III-6. All cattle except Nos. 318A and 323A lost weight during the early post-infection period when they were handled and sampled more frequently than they were before infection. Infected animals started to lose weight around 1-5 weeks after infection (Table III-6 and Fig. III-7). Infected animals lost between 2.6 to 19.6% of their initial body weight, maximum weight loss occurring between weeks 3 and 6 (Table III-6). Of the infected recovering animals, the acyclic group lost more weight than the cyclic group (17.5 $\pm$ 1.4% vs 9.3 $\pm$ 5.7%). Control animals had the smallest weight loss (0.5-1.1%).

Animals began to gain weight after their nadirs. Animals that suffered the smallest weight loss regained their pre-infection bw more rapidly (Table III-6). Both the infected-cyclic and infected-acyclic groups gained weight in parallel to the controls (Fig. III-7). However the infected-cyclic group reached its pre-infection group mean bw earlier than the infected-acyclic group.

3.3.5 Serology

Positive titres in IFAT to *T. parva* schizont antigen were detected in five (317A, 324A, 327A, 328A and 341A) out of nine animals as early as 18 days post-infection and in all animals that survived infection on days 29, 35 and 180. Serum collected on day 15 from heifer No. 323A which died on day 17 had negative serological titres (Table III-2). None of the control animals showed positive serological titres during the experimental period.

3.3.6 Parasite isolation studies

3.3.6.1 Tick salivary gland infection

No infected salivary gland acini were detected in ticks fed on the infected-

acyclic heifers No. 325A, 328A and 341A.

3.3.6.2 Transmission studies

Attempts at transmission using pooled ticks which had been fed on heifers No. 325A, 328A and 341A were negative. The steer used for transmission attempts neither developed clinical disease signs nor showed positive serological titres in IFAT to *T. parva* schizont antigen.

3.3.7 Cyclical status

3.3.7.1 Behavioural

Before the time of infection, between two and four heat periods were observed in each of the animals. Table III-7 gives the total number of observed heat signs (scores) in individual animals before and after infection and in uninfected controls. Before infection, oestrous signs for individual animals ranged from 1-10 out of a possible score of 11 (mean 5.7 ± 3.0, n=49). The frequency of detected heat signs during the pre-infection and post-infection periods were summed for all the animals and are presented in Table III-8. The major presented oestrous manifestations before the start of the experiment were vulval mucous discharge, mounting other animals, restlessness and a swollen vulva which were detected on 49, 38, 34 and 28 occasions, respectively out of a total of 49 heats. Standing to be mounted as a sign of receptivity was detected on 22/49 occasions (Table III-8).

During the post-infection period, vulval mucous discharge was again the major presented sign being detected in 51/51 heats. A swollen vulva, head-butting, mounting others, being sniffed and restlessness were observed on 43, 43, 41, 40 and 30 occasions, respectively out of the 51 heats. Allowing mounting was seen in more than half (32/51) of observed heats.

The number of oestrous scores per oestrus varied widely in individual animals

and between animals for satisfactory statistical comparisons to be made.

3.3.7.2 Serum progesterone concentration

Serum P4 concentrations were used as a tool in the confirmation of oestrus. P4 concentration profiles for representative animals are presented in Figs. III-1 to III-6. All animals recorded P4 concentrations below the detection limit (0.3 nmol/L) of the assay while the peak values observed in individual animals ranged from 13.0 nmol/L (328A) to 35.0 nmol/L (341A). In a cycle serum P4 concentrations rose from basal levels to peak levels where they remained elevated over one to three consecutive samples before declining to basal levels. The peak cycle periods exhibited different range values within individual animals and among animals. Two animals (322A and 328A) had their plateau P4 values limited to between 7.5 to 12.5 nmol/L while the others had most of the plateau P4 values above 10.0 nmol/L.

To present a profile of serum P4 concentrations throughout the cycle, values were pooled from 55 cycles completed by seven animals and related to the day of oestrus. The mean values are presented in Table III-9 and in Fig. III-8. Fig III-8 demonstrates a basal period of P4 concentration when levels were below 1nmol/L from day 0 to 3, an elevation phase to day 7 and a plateau phase with P4 values ranging from 10.65 $\pm$ 1.28 (mean $\pm$ SEM) to 16.0 $\pm$ 1.42 nmol/L between days 7 and 16. P4 concentrations then declined to basal levels reaching a mean value of 1.63 $\pm$ 0.61 nmol/L on day 20.

3.3.7.3 Ovarian structures

Pooled data of ovarian structures palpated during the cyclic periods were summarized and are presented in Table III-12 where they are related to the phase of the oestrous cycle based on P4 profiles. The luteal phase consisted of rising and plateau while the follicular phase consisted of falling and basal P4 concentrations, respectively.

A total of 88 periods of plateau P4 concentrations were presented during which a mature CL was palpated at least once in 87/88 occasions. However, of all palpated mature CL, 90% (153/170) and 10% (17/170) were palpated during the luteal and follicular phases, respectively (Table III-12). Of the 44 developing CL, 97.7% (43/44) and 2.3% (1/44) were palpated during the luteal and follicular phases, respectively. Comparative figures for regressing CL were 65.1% (28/43) and 34.9% (15/43) for the luteal and follicular phases, respectively. Follicles were palpated in both follicular (34/55) and luteal (21/55) phases. No structures were palpable on 24 occasions; 13/24 and 11/24 during the follicular and luteal periods, respectively.

Corpora lutea (developing and mature) and absence of CL (regressing CL, follicles and no palpable structures) were pooled and correlated to the luteal and follicular phases (Table III-13). Agreement between the palpable structures and P4 concentrations were 87.1% and 75.7% during the luteal and follicular phases, respectively. The overall agreement between rectal palpation results and P4 concentrations was 84.3%. Overall, a CL could not be detected in 86.2% (25/29) examinations when P4 concentrations were rising (early luteal phase) while all (17/17) of the mature CL palpated during the follicular phase occurred during falling P4 concentrations.

3.3.7.4 Cyclical statuses in various groups

In the preceding sections (3.3.5.1-3), an overview of behavioural, P4 concentration and rectal palpation data was presented as standards of assessing reproductive function. In this section, the cyclical status of various groups will be assessed on these criteria.

3.3.7.4.1 Infected-died

From behavioural data, P4 profiles and palpable ovarian structures, each of the two (322A and 323A) infected animals that died had three cycles pre-infection. The

cycle lengths ranged from 18-24 days (Tables III-10 and III-11). Oestrus was not detected in either animal post-infection. However P4 profiles and palpable ovarian structures revealed cyclic changes before they died. Fig. III-1, which is representative for this group revealed a complete cycle post-infection in 322A.

3.3.7.4.2 Infected-cyclic

From behavioural data, P4 profiles and palpable ovarian structures, animals in this group each had two to three cycles pre-infection. Cycle lengths ranged from 19-23 days with individual mean cycle lengths of 19.5 $\pm$ 0.5 (mean $\pm$ SD) to 22.3 $\pm$ 0.5 days and an overall group mean of 21.0 $\pm$ 1.3 days (Table III-10).

Based on behavioural data, three animals had long inter-oestrus intervals (44-56 days) in the early infection period while one animal (326A) had a short inter-oestrus period of 5 days (Table III-10). However, P4 profiles and palpable ovarian structures revealed 4-5 normal cycles in each animal. Mean post-infection cycle lengths in individual animals varied from 20.6 $\pm$ 1.4 to 23.0 $\pm$ 1.2 with a group mean of 21.7 $\pm$ 1.6 (range 19-24) days (Table III-11). P4 profiles revealed that oestrus was not detected once in two animals (317A and 320A), twice in 324A and was associated with the luteal phase resulting in a 5 days inter-oestrus interval in 326A. The P4 profile, rectal palpation and behavioural data presented for No. 317A (Fig. III-2) was typical of this group.

3.3.7.4.3 Infected-acyclic

Based on behavioural data pre-infection, each of the animals in this group had three to four cycles with individual mean lengths of 20.0 $\pm$ 1.6 to 24.0 $\pm$ 0.5 and a group mean of 21.9 $\pm$ 2.5 (range, 18-28) days (Table III-10). P4 profiles however, revealed the cycle lengths to range from 18-23 (mean $\pm$ SD, 21.4 $\pm$ 1.6) days (Table III-11).

After infection, all the three animals had long (43-97 days) inter-oestrus

intervals based on behavioural data. Each of the animals had three to five cycles to week 23 post-infection (Table III-10). However, P4 profiles revealed two cycles instead of five behavioural based cycles in 325A (Table III-11, Fig. III-3), two instead of three in 328A (Table III-11, Fig. III-4) and and five in 341A (Table III-11, Fig. III-5). The group mean inter-oestrus interval was 32.5 $\pm$ 22.6 days as compared to P4 cycle lengths of 20.8 $\pm$ 3.3 days post-infection.

Extended (>30 days) periods of basal P4 concentrations and absence of ovarian structures were noted in all three animals post-infection. This was more pronounced in 325A (72 days initially and $\geq$31 days later) and in 328A (77 days initially and $\geq$34 days later). In 341A, basal P4 concentrations were maintained for 42 days followed by regular cycles. Oestrus was detected in each of 325A and 341A but not in 328A during the initial extended period of basal P4 concentration. Based on P4 profile and ovarian structures, these three animals were regarded to have undergone a period of acyclicity post-infection. On the other hand, if behavioural detection was added to the above two tools, a critical evaluation would only classify 328A to have undergone an acyclic period of about 77 days.

3.3.7.4.4 Controls

From behavioural data, each of the four controls had 6-8 cycles which ranged from 18-41 days (Table III-10). However P4 profiles and ovarian structures revealed one missed oestrus associated with an inter-oestrus interval of 41 days in 318A. The group mean P4 cycles before and after the start of the experiment were 20.6 $\pm$ 1.3 and 20.6 $\pm$ 1.9 days, respectively (Table III-11). The P4 profile, behavioural data and palpable ovarian structures are presented for 318A which was typical of the group.

3.3.8 Pathology

3.3.8.1 Gross findings

All the eight recovered and control animals were in good body condition at the time of sacrifice. On post-mortem examination none of the above animals had any gross pathological lesions (Table III-14).

Animal No. 323A which died on day 17 post-infection was in good body condition while No. 322A which died on day 29 post-infection was in a poorer condition compared to its pre-infection status. Froth was seen exuding through the nostrils in 323A.

The lymph nodes were markedly enlarged, hyperaemic and petechiated on cut surface in 323A. Its spleen was enlarged and the malpighian corpuscles prominent on section. In 322A, the lymph nodes and spleen were of normal size and consistency.

In both 322A and 323A the abomasal folds were oedematous. In addition, there were petechial haemorrhages on the serosal and mucosal surfaces of the abomasum in 323A. Petechial haemorrhages were present in the small intestine, caecum and rectum in 323A and only in the rectum in 322A. The small intestine and colon were hyperaemic in 323A. Although no macroscopic lesions were detectable in the small intestines, caecum and colon of 322A, they contained very fluid and foul smelling contents. An enlarged liver and serosal petechial haemorrhages of the gall bladder were detected in 323A.

There were no gross changes involving the kidneys of 322A. In 323A, there were petechial haemorrhages on the peri-renal fat and on the surface. The kidneys were slightly enlarged. On section, the cortex was hyperaemic. The adrenal glands were also hyperaemic on cut section in 323A.

Gross changes in the respiratory tract were only observed in 323A. The lungs were markedly enlarged and oedematous. Scattered petechial haemorrhages were present on the visceral pleura. On section, straw coloured fluid oozed out. There

were also scattered hyperaemic foci on the visceral pleura. The trachea, bronchi and bronchioles contained a frothy exudate. There were epicardial petechial haemorrhages in 323A.

The meningeal blood vessels were congested in 322A. There were no detectable gross pathological changes in the pituitary glands in either of the infected or uninfected control animals.

The gross findings in the reproductive tracts of both infected and control animals are presented in Table III-15. Small, medium and tertiary follicles were observed in either or both ovaries. They also revealed the presence of a mature or regressing CL. A central cavity was seen in the CL of five infected animals. These cavities contained no fluid and had diameters ranging from 7 to 12mm. No other detectable gross lesions were found in the uteri apart from serosal petechial haemorrhages in 323A and 328A.

3.3.8.2 Histological findings

3.3.8.2.1 Uterus

The histological findings in the uterus in both infected and control animals are presented in Table III-16.

Three out of the ten infected animals showed uterine changes which included cellular infiltration, hyperaemia and oedema. Of these, heifer 322A which died of infection on day 29 post-infection, the only change was a mild mononuclear cell infiltration in the superficial endometrium. Heifer 320A had a mild mononuclear cell infiltration of the superficial layer of the endometrial propria-submucosa in addition to oedema of the perimetrium. Changes were seen in all the uterine layers of 323A which died on day 17 post-infection. These included a mild cellular infiltration of the endometrium and perimetrium, moderate cellular infiltration of the myometrium, endometrial hyperaemia and oedema of the endometrium, myometrium and

perimetrium. The cells observed in the endometrium and perimetrium comprised of mononuclear leucocytes, neutrophils and eosinophils which were diffusely scattered in the stroma. In the myometrium, the infiltrating cells were chiefly mononuclear leucocytes scattered between bundles of inner circular smooth muscle cells. Occasional mitotic figures were observed among the mononuclear cells.

There were no abnormal findings in the uterine tissues of control animals. Two animals (315A and 316A) which were in the follicular phase had mild endometrial hyperaemia and oedema.

3.3.8.2.2 Ovaries

Histologically, the control animals had normal ovaries containing variable numbers of primordial to tertiary follicles and CL. Various degrees of follicular atresia were observed especially involving the majority of tertiary follicles.

Variable numbers of primordial to tertiary follicles were present in ovaries of all infected animals. Heifer No. 341A however, had a decreased number of all types of follicles compared to the controls and other infected animals. A mild focal mononuclear cell infiltration of the cortical stroma, medulla and CL was observed in 320A (Table III-16). In the medulla, the infiltrating cells were centred in the adventitia of small to medium sized blood vessels. Heifer No. 323A had a mild mononuclear cell infiltration of the medulla and a severe mononuclear cell infiltration of the CL. Degeneration of luteal cells as indicated by nuclear pyknosis was noted.

3.3.8.2.3 Pituitary gland

The pituitary glands of control animals were normal. In the adenohypophysis, acidophils were the predominant cell type. Histologic findings in eight of the ten infected animals were similar to the controls. Two of the infected animals showed a patchy mononuclear cell infiltration in the adenohypophysis (Table III-16). Heifer 322A had focal mononuclear cell infiltration around glandular acini as well as in

perivascular sites in the adenohypophysis and in addition, it had a mild degree of adenohypophyseal hyperaemia. Heifer 341A had a focal mononuclear cell infiltration in the adenohypophysis and perivascular cellular infiltration in the posterior lobe.

3.3.8.2.4 Adrenal gland

The histological findings in the adrenal glands of the infected and control animals are presented in Table III-16. Normal histological architecture was maintained in all the control animals.

Histologic changes were found in the adrenal glands of 6 out 10 infected heifers. These changes included cellular infiltration and hyperaemia. The infiltrating cells were predominantly mononuclear and were found in the *zona glomerulosa* in four animals, *zona fasciculata* in one animal, *zona reticularis* in one animal and in the medullary zone in two animals. The infiltrating cells tended to have a focal distribution in the *zona glomerulosa* and a focal or diffuse distribution in the sinusoidal spaces in the *zona fasciculata, reticularis* and medulla. Mild hyperaemia of the *zona fasciculata* and *reticularis* was present in three animals (320A, 325A and 341A). Comparatively, animal 322A which died of infection on day 29 had more defined changes than the others (Table (III-16).

3.4 Discussion

In this study, the disease syndrome caused by a 1:20 dilution of *T. parva* (buffalo-derived) stabilate IL3081 was similar to that described elsewhere. Of the ten infected heifers, two reacted severely and died, seven reacted clinically and recovered and one exhibited no clinical disease although parasites were detected in lymph-node biopsy material. The uninfected controls which were kept together with the infected group remained healthy throughout the experimental period.

Fever was the first clinical manifestation of disease. The incubation period was found to vary from 10-18 (mean 13.0 ± 2.7) days and compared favourably with

similar reports by Neitz (1957) and Brocklesby (1962). Using an undiluted stabilate of this isolate, Dolan (unpublished results) reported an incubation period of 6.5 days while Mutugi *et al.* (1988b) reported incubation periods of 7.4, 11.3, 14.7 and 16.0 with similar isolate at 1:1, 1:10, 1:100 and 1:1000 dilutions, respectively. The febrile reaction period ranged from 2-8 (mean 4.9 ± 1.9) days and fever was either continuous or intermittent as reported by others (Henning, 1956; Neitz, 1957; Brocklesby, 1962). The prepatent period was shorter than the incubation period in all but one of the nine animals (320A) where the presence of parasites coincided with the day of fever. The time to detection of macroschizonts in the regional lymph node varied from 7-13 (mean 10.5 $\pm$ 2.0) days. Using undiluted buffalo-derived *T. parva* stabilates, Jura and Losos (1980) and Dolan (unpublished results) reported a prepatent period of 5.75 and 6.5 days, respectively. At various dilutions of a related isolate to that used in this work, Mutugi *et al.* (1988b) reported prepatent periods of 6.0, 7.0, 10.0 and 11.5 at 1:1, 1:10, 1:100 and 1:1000 dilutions, respectively.

Neitz (1957) classified disease reactions resulting from *T. parva* (buffalo-derived) to range from peracute to mild forms. However, based on the most recent mode of classification of theilerial reactions in cattle (Anon, 1989b), the reactions in the ten infected heifers could be classified to be severe in two animals that died, moderate in two and mild in six others. This classification however has its inherent limitations when applied to buffalo-derived theileriosis due to its relatively low degree of parasitosis. In this regard, one animal (327A) which only exhibited a parasitosis but no clinical symptoms was put together in the mild reactor group with others that reacted clinically.

Apart from fever, other clinical signs were typical of theileriosis as described in the literature (Henning, 1956; Neitz, 1957; Jura and Losos, 1980). These included swollen lymph nodes, dullness, anorexia, respiratory distress and ocular discharge. Although Henning (1956) considered diarrhoea to be a common symptom, only two animals manifested this sign in this study. Henning (1956) and Neitz (1957) reported

diarrhoea to occur from about one week following the initial temperature rise. In this study, one mild (317A) and one severe (322A) reactor developed diarrhoea one and two weeks following temperature rise, respectively. Nervous signs similar to those described by Mettam and Carmichael (1936) were observed terminally in one animal (323A). Of the eight surviving animals, five animals which cycled through the post-infection period based on P4 profile were classified as moderate (324A) and mild (317A, 320A, 326A, 327A) reactions, while of the three considered to be acyclic, one (325A) was a moderate and the others (328A, 341A) mild reactors.

Panleucopaenia is the most commonly reported haematologic abnormality in *T. parva* infection of cattle (Steck, 1928; Barnett, 1960; Maxie *et al.*, 1982). Before infection, the total leucocyte count in all the 14 heifers in this study ranged from 7500-17100 (mean 11300 ± 2400) μl^{-1}. These values compared favourably to those reported for Friesian-Holstein steers used at the Centre (Maxie *et al.*, 1982) but higher than those reported for exotic animals (4000 - 10000 μl^{-1}) and could be due to breed and/or environmental influences (Doxey, 1977). All the ten infected animals suffered a leucopaenia of varying magnitude. Whereas Barnett (1960) reported that leucopaenia preceded fever by 2-7 days, the reverse was true in this study. There did not appear to be a correlation between the onset and degree of leucopaenia with the clinical nor cyclical status. For example, 325A which had a moderate reaction suffered more severe leucopaenia than acute cases. Similarly, although two acyclic animals (325A and 341A) suffered more marked leucopaenia, another acyclic animal (328A) suffered least of the surviving animals. The leucopaenia was as a result of neutropaenia and lymphopaenia. Hill and Matson (1970) reported a terminal leucocytosis. This was also observed in all our infected animals except one which died early (323A). Intermittent exacerbations following recovery to pre-infection levels was recorded in 7/8 animals.

The erythrocyte values were maintained around the pre-infection values in most infected and control animals post-infection. The red blood cell concentration of

the 14 heifers ranged between 7.0 to 8.5 μl^{-6} pre-infection. This compared favourably with a range of 5-9 μl^{-6} reported in *Bos taurus* (Doxey, 1977). Red blood cell concentration was mostly maintained above 6.5 μl^{-6} except in one acyclic animal (325A) where levels intermittently fell to 5.5 from 7.5 μl^{-6} from the third to fifth week post-infection. This is the period when the infected-acyclic group exhibited lower values compared to control and infected-cyclic groups. Similar changes were seen for the haemoglobin concentration and packed cell volume although the onset of the decline occurred later (day 28-50). A variable degree of anaemia was reported in ECF (Maxie *et al.*, 1982) but was not associated with piroplasmaemia. The cause of anaemia in ECF is unknown but could be due to a depression of the bone marrow or lowered nutritional status. In our study, piroplasms were also not seen and therefore, the cause of low erythrocyte values could not be explained. However, this could have been of nutritional origin since animals in this study lost condition after infection.

Loss of condition especially in protracted ECF cases is a common finding (Henning, 1956). All but one infected animal which died on day 17 post-infection suffered variable degrees of weight loss. Body weight recovery to pre-infection levels took longer in the acyclic than in cyclic infected animals. Dolan (1986a) reported slower recovery in ECF animals habouring a carrier status compared to those without. However, we were unable to demonstrate a carrier status in the three acyclic animals about three months post-infection. Each of the two animals that underwent either a moderate or mild reaction lost more weight and took longer to recover the lost weight. These four animals had longer febrile reaction periods than the other recovered animals suggesting that the magnitude of weight loss may be related to the course of disease.

Gross lesions characteristic of ECF (Henning, 1956; De Kock, 1957; Neitz, 1957) were observed in only two animals (322A and 323A) that died of severe reaction. Macroscopic lesions were more profound in 323A that died on day 17 post-infection. No typical ECF lesions were found in the other animals that were sacrificed after about six months of recovery suggesting either that lesions did not develop or

that the lymphoblastoid cells associated with the pathogenesis were removed through cell-mediated mechanisms (Morrison *et al.*, 1986). Based on size and presence of follicles and a CL, the ovaries were judged to be functional in all the infected heifers and controls following death or sacrifice. No significant gross lesions were observed in the uterus apart from serosal haemorrhages in the two fatal cases. Steck (1928) reported a few red pinpoint spots on the uterine serosa of a heifer infected with theilerial parasites. However there may be no significance to the serosal haemorrhages in the uterus since they are reported to occur frequently in virgin heifers (McEntee, 1990).

Histological studies revealed a mononuclear cell infiltration as the predominant lesion. In the endometrium, a mild mononuclear infiltration occurred in three animals. Presence of mononuclear cells in the endometrium is expected throughout the cycle and their presence is only considered abnormal when they have a dense and multifocal distribution (McEntee, 1990). Although the presence of mononuclear cells in the endometrium could have been a normal finding, a myometrial infiltration in one of the animals may suggest the infiltrations in the uterus to be ECF associated. A mononuclear cell infiltration was found in the ovaries of three animals. This was of mild severity except in the CL of 323A where it was marked. Despite this latter observation, the P4 levels in this animal prior to death were high (>10 nmol/L) suggesting that the infiltration did not interfere with luteal function in this particular animal. Dolan (1986a) reported congestion and vascular occlusion by lymphocytes in the adenohypophysis. In our study, the main finding was a focal perivascular infiltration in one of the fatal reactors and in one animal that registered a period of acyclicity. A mononuclear cell infiltration was observed in the adrenals. Similar changes have been reported (De Kock, 1957; Dolan, 1986a).

Williamson *et al.* (1972) obtained a high (89%) rate of oestrus detection by observing a group of animals in a dairy herd continuously. However, poorer detection rates (56%) were obtained by herdsmen based on twice daily observations in the same

study. Llewelyn *et al.* (1987) also obtained unsatisfactory results (27%) by observing Boran cows for 20 min, four times daily. In our study, observation of the heifers for 20 min, three times daily resulted in an oestrus detection rate of 54% based on allowing mounting to be the criterion for true oestrus (Whitmore, 1980). Although it was reported that increasing the number of observations may improve the oestrus detection rate, there may be considerable variability in the results possibly attributed to the fact that about 70% of mounting interactions occur at night (Whitmore, 1980). Oestrus was not observed for at night in this study and this could have affected our detection rate. However when other parameters considered to be non-specific for true oestrus detection were considered, this study obtained an oestrus detection rate of 90.9% when assessed against P4 profiles. Of the range of parameters considered, apart from standing to be mounted, vulval mucous discharge was observed in all the detected heats and therefore, gave a reliable indication that the animal was around oestrus.

Agreement of rectal palpation results with serum P4 concentrations was 87.1% in the luteal phase and 75.7% in the follicular phase. The overall agreement of 84.3% compares favourably with results reported elsewhere (Boyd and Munro, 1979; Watson and Munro, 1980; Pathiraja *et al.*, 1986) where detection rates ranged between 77-85%. Incompatibilities between rectal palpation results and P4 concentrations in this study occurred when a CL could not be detected when P4 concentrations were high or when a CL was detected in the follicular phase. The latter was mainly observed during the period of falling P4 concentrations. Reports from other studies revealed no correlation between CL size and consistency and luteal function. Most of the subjective errors were made early or late in the cycle (Boyd and Munro, 1979; Watson and Munro, 1980). Our results agree with this observation thus limiting the usefulness of rectal palpation results *per se* in assessing cyclical status. Of the three methods, P4 concentrations therefore, offered the most reliable method of assessing the cyclical status.

Based on behavioural oestrus detection, P4 profiles and palpable ovarian structures, it was revealed in this study that *T. parva* can cause a disruption of the oestrous cycle in some animals. Several animals failed to exhibit behavioural oestrus and three heifers underwent variable extended periods of over thirty days when serum P4 was maintained at basal levels.

In this experiment, no ovarian structures were detected in one of the acyclic heifers (328A) throughout the period of basal P4 concentrations. In the other two infected-acyclic heifers, an absence of palpable ovarian structures was noted in the majority of palpations. However, a follicle or a regressing, developing (325A) or a mature (341A) CL was palpated at least on one occasion during this period. Oestrus was expressed at least once in all three acyclic animals during the period of extended basal P4 concentration. If oestrus detection was the only tool for diagnosing the cyclical status, then only 328A would be judged to have been acyclic. P4 profiles however suggested an interruption of cyclical ovarian activity in these three animals.

One of the mechanisms that could lead to an impairment of the ovarian cyclicity could be an interference with the pituitary secretion and/or release of gonadotrophic hormones (FSH and LH). Sub-optimal levels of FSH and LH would result in failure to recruit ovulatory follicles or induce ovulation or luteinization. The three acyclic animals were sacrificed when all had resumed cycling as judged from behavioural data and only one (341A) had mild mononuclear cell infiltration in the pituitary. Similarly, adenohypophyseal lesions were found in animals that died of disease but cycled suggesting that the lesions were not severe enough to impair gonadotrophin secretion. Another possible cause of the ovarian dysfunction could be due to secretion and release of sub-optimal levels of GnRH which would have resulted in failure of gonadotrophin release. Alternatively, the acyclicity could have been due to lack of responsiveness of the ovary to pituitary gonadotrophins.

Oestrus was demonstrated on several occasions in two acyclic animals but this was not followed by cyclical ovarian changes. Oestrogens are required among other

things for behavioural oestrus manifestations. Follicles were not detected prior to these observed heats and therefore, the source of oestrogen responsible for the behavioural oestrus at these times is not known. However, only the largest follicles are usually palpable (Arthur *et al.*, 1989) and their detection is subject to much experimental error. *In vivo*, ultrasonography offers the most accurate technique of evaluating follicular dynamics (Fortune *et al.*, 1988; Griffin and Ginther, 1992). It is therefore very difficult to comment on the follicular activity in this study when no ultrasonography was done. However behavioural oestrus unaccompanied by luteal function would suggest ovulatory failure or inadequate LH levels for luteinization.

Behavioural oestrous manifestations were subdued in eight of ten infected animals during the disease reaction period which was characterised by fever, panleucopaenia and parasitosis. Oestrus was not manifested during cyclic basal P4 periods in the three acyclic and three of the five infected cycling animals. P4 profiles however indicated maintained cyclicity during this early phase suggesting that the infection did not interfere with cyclical status but the general malaise may have subdued oestrus manifestation. That this was a possible cause was demonstrated by one of the two infected-cyclic animals (327A) which showed oestrous signs during the early period of infection. This animal had no febrile reaction but developed a patent infection.

The observed acyclicity occurred after completion of one or two cycles in the three heifers post-infection. During this period, body temperatures were normal, a partial recovery of circulating leucocytes had been restored and packed cell volume, red blood cell and haemoglobin concentrations were within the pre-infection levels. However, significantly lower values for packed cell volume, red blood cell and haemoglobin concentration were noted in the infected-acyclic group compared to either the infected-cyclic or control groups. This was also the period when all infected animals were suffering from reduction in body weight. Although all the infected animals lost weight during the post-infection period, the highest loss was noted in the

infected-acyclic group. The one heifer (327A) which continued to show both oestrus and cyclical ovarian changes throughout the study period lost only 2.6% of her initial weight. A partial to complete resumption of cyclical status occurred in the acyclic animals after they had regained their pre-infection weights. Reduced body weight could be due to low levels of nutrient intake. In this study, infected animals were anorectic following infection and this could possibly account for the weight loss.

Our study did not go far enough to examine the actual cause of luteal dysfunction. Palpation *per rectum* was either not specific or sensitive enough to accurately detect follicular dynamics and in the absence of ultrasound scanning of the ovaries during the acyclic period, it is difficult at this stage to link the impaired gonadal function to its lack of responsiveness. Gonadotrophins (FSH and LH) were not assessed to find out whether adequate amounts were being produced for folliculogenesis, ovulation, CL growth and maintenance.

However, although this study did not address the mechanisms involved in impairment of ovarian function, several reports have linked body weight loss with anoestrus in cattle (Bond *et al.*, 1958; Richards *et al.*, 1989). A reduction in nutrient intake was reported also to result in anoestrus (Beal *et al.*, 1978). The relationship between body weight and fertility is one of correlation, not primarily of causation because body weight is basically a function of nutritional status. Gombe and Hansel (1973) suggested that the gonadal hypofunction observed following decreased nutrient intake was due to a reduction of ovarian response to gonadotrophins since P4 concentrations in their study were low in spite of high LH levels suggesting that gonadal dysfunction was not at the pituitary level. In contrast Richards *et al.* (1989) reported low LH levels in low nutrient group heifers suggesting that gonadal dysfunction was at the pituitary level. Consequently, the data is equivocal and although decreased LH secretion is favoured (Griffin and Ginther, 1992), the hypofunction could have been at either level.

In conclusion, this study demonstrated that infection with *T. parva* may

interfere with the reproductive function of Boran/Friesian heifers. There was little evidence to suggest that the acyclicity was due to a direct effect of infection and it is suggested that it was due to an indirect effect of weight loss associated with infection. Further studies on the hypothalamo-pituitary-ovarian axis may give more information on the mechanisms leading to the ovarian dysfunction.

Table III-2 **Reactions of Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 or in controls**

Anim. #	Day to								
	Schi-zonts: LEG +ve	Schi-zonts: LEG -ve	Schi-zonts: RPG +ve	Schi-zonts: RPG -ve	Fever	Reco-very	Death	Ab. resp-onse δ	Reac-tion
317A	12	18	14	23	18	24	-	+	Mild (2)*
320A	11	18	13	23	11	24	-	+	Mild (3)
322A	12	21	13	23	11	-	29	+	Acute (8)
323A	7	17	9	17	10	-	17	-	Acute (6)
324A	8	21	12	21	11	22	-	+	Mode-rate (6)
325A	10	23	13	27	11	28	-	+	Mode-rate (6)
326A	13	26	14	27	16	28	-	+	Mild (3)
327A	13	22	15	24	-	25	-	+	Mild (.)
328A	9	20	12	23	16	24	-	+	Mild (4)
341A	10	19	14	24	13	25	-	+	Mild (4)
Mean **	10.5 ± 2.0	20.5 ± 2.6	12.9 ± 1.6	23.2 ± 2.7	13.0 ± 2.7	25.0 ± 1.9	23.0 ± 6.0		
315A	.	.	.	.	.	.	.	.	-
316A	.	.	.	.	.	.	.	.	-
318A	.	.	.	.	.	.	.	.	-
329A	.	.	.	.	.	.	.	.	-

δ (+) –Titre of > 1:40 to *T. parva* schizont antigen

* - Days of febrile reaction period

** - Mean ± SD

LEG = Left ear gland; RPG = Right prescapular gland

Table III-3 **Peripheral blood leucocyte concentration in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081**

Animal #	Status	Max. WBC decline (%)*	Day to		Exacerbation δ
			Max. WBC decline	Recovery	
322A	Died	41.0	18	27	.
323A	Died	35.9	15	-	.
Mean ± SD		38.4 ± 2.6**	17.5 ± 1.5	27	
317A	Cyclic	51.9	15	29	+
320A	Cyclic	45.3	15	22	+
324A	Cyclic	54.3	20	27	+
326A	Cyclic	68.3	20	27	+
327A	Cyclic	80.7	18	20	+
Mean ± SD		60.1 ± 12.7	17.6 ± 2.2	25.0 ± 3.4	
325A	Acyclic	31.6	20	29	+
328A	Acyclic	82.2	15	18	.
341A	Acyclic	34.5	13	34	+
Mean ± SD		49.4 ± 23.2	16 ± 2.9	27 ± 8.2	

* - Percentage of pre-infection levels

** - Standard deviation

δ - Refers to further decline after initial recovery

Table III-4 **Leucocyte concentration in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

	Leucocyte concentration 10^3 μl^{-1}			
Day	Died	Cyclic	Acyclic	Controls
0	11.5 ± 0.3	10.3 ± 2.1	10.3 ± 1.1	13.2 ± 2.9
4	10.3 ± 0.6	11.6 ± 0.8	10.0 ± 0.7	13.2 + 3.7
6	10.1 ± 0.3*	12.0 ± 1.5	11.3 ± 0.8	13.1 ± 0.8
8	8.3 ± 0.6*	10.6 ± 2.0	10.8 ± 2.9	13.9 + 4.2
11	7.1 ± 1.6	7.8 + 3.3	8.2 + 1.6	13.9 + 4.2
13	4.8 + 0.4*	6.8 + 2.0*	5.8 + 2.7	13.5 ± 2.5
15	5.4 + 1.2*	6.0 + 2.1*	5.5 + 1.9*	13.4 + 2.1
18	4.6	7.7 + 1.9	5.8 + 2.6	13.0 ± 3.0
20	6.9	7.9 + 2.1	7.0 ± 3.0	12.5 ± 3.1
22	9.5	9.9 ± 1.7	9.9 ± 4.9	12.1 ± 2.8
25	9.6	8.5 + 1.1	6.5 + 1.9*	13.3 ± 3.8
27	11.3	11.4 ± 1.8	9.8 ± 2.4	14.1 ± 3.0
29	15.9	11.9 ± 1.0	11.5 ± 2.6	12.7 ± 2.9
32		11.9 ± 1.0	14.9 ± 7.2	13.8 ± 2.4
34		11.5 ± 1.1	14.7 ± 4.9	13.3 ± 2.2
36		11.1 ± 1.2	12.6 ± 3.3	13.6 ± 2.1
39		12.0 ± 1.9	13.1 ± 4.2	13.9 ± 3.4
41		10.2 ± 0.8	11.0 ± 2.9	13.7 ± 2.9

* :Significant ($p<0.05$) difference to pre-infection value

Underlined :Significant ($p<0.05$) difference with controls

Table III-5 **Peripheral blood lymphocyte and neutrophil concentration in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 or in controls**

		LYMPHOCYTES			NEUTROPHILS		
		Day to			Day to		
Animal #	Status	Max. decline	Recovery	Exacer-bation	Max. decline	Recovery	Exacer-bation
322A	Died	13 (69.4)*	-	.	11 (53.6)	20	-
323A	Died	27 (67.5)	-	.	15 (46.7)	.	.
317A	Cyclic	15 (48.9)	48	.	15 (48.7)	27	+
320A	Cyclic	15 (43.1)	22	-	15 (78.3)	20	-
324A	Cyclic	13 (47.6)	29	+	15 (56.7)	22	+
326A	Cyclic	13 (59.4)	25	+	15 (27.8)	27	-
327A	Cyclic	27 (25.3)	32	+	22 (46.7)	32	+
325A	Acyclic	20 (71.6)	29	-	25 (77.8)	29	+
328A	Acyclic	13 (26.9)	25	+	25 (37.5)	27	-
341A	Acyclic	13 (64.0)	34	+	13 (74.4)	34	+
315A	Control	34 (43.3)	39	.	22 (21.1)	25	.
316A	Control	4 (17.3)	6	.	4 (27.3)	6	.
318A	Control	39 (16.0)	41	.	22 (35.7)	25	.
329A	Control	29 (13.0)	32	.	8 (21.9)	11	.

* - Percentage decline at nadirs of pre-infection levels

Table III-6 **Changes in cyclical status and body weights in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

		Time to (Weeks)			Percent*	
Animal #	Status	Initial bw loss	Max. bw loss	Regain	Max. bw loss	bw at wk 14
322A	Died	1	4	.	17.4	.
323A	Died	.	.	.	.	.
Mean		1	4	.	17.4	.
317A	Cyclic	1	3	9	7.1	5.4
320A	Cyclic	1	6	14	9.9	1.2
324A	Cyclic	2	6	20	19.6	-3.8
326A	Cyclic	2	4	11	7.4	8.1
327A	Cyclic	5	5	7	2.6	7.5
Mean ± SD		2.2 ± 1.5*	4.8 ± 1.2	12.2 ± 4.5	9.3 ± 5.7	3.7 ± 2.5
325A	Acyclic	2	6	28	19.3	-11.7
328A	Acyclic	1	6	14	15.9	0.8
341A	Acyclic	2	5	13	17.2	1.9
Mean ± SD		1.7 ± 0.5	5.7 ± 0.5	18.3 ± 6.8	17.5 ± 1.4	-3.0
315A	Control	1	1	2	0.6	9.4
316A	Control	3	3	4	0.5	12.7
318A	Control	.	.	.	.	8.7
329A	Control	1	1	2	1.1	6.7
Mean ± SD		1.7 ± 0.9	1.7 ± 0.9	2.7 ± 0.9	0.7 ± 0.3	9.4 ± 2.2

* - Expressed relative to bw at Week 0

** - SD

Table III-7 **Number of oestrus signs (scores) recorded in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

		Number of heats relative to infection								
Animal #	Status	-4	-3	-2	-1	1	2	3	4	5
322A	Died	4	6	2	6	7	.	.	.	.
323A	Died	2	9	1	8	.	.	.	.	.
Mean ± SD		3.0 ± 1.0	7.5 ± 1.5	1.5 ± 0.5	7.0 ± 1.0	7.0				
317A	Cyclic	5	8	4	6	ND	9	3	8	8
320A	Cyclic		7	4	10	ND	5	9	6	8
324A	Cyclic	7	4	4	ND	ND	6	ND	6	9
326A	Cyclic		2	4	10	4	1	8	5	6
327A	Cyclic		8	7	10	7	7	8	8	8
Mean ± SD		6.0 ± 1.0	5.8 ± 2.4	4.6 ± 1.2	9.0 ± 1.7	5.5 ± 1.5	5.6 ± 2.7	7.0 ± 2.3	6.6 ± 1.2	7.8 ± 1.0
325A	Acyclic		1	1	10	8	2*	2*	6*	8
328A	Acyclic	2	2	6	10	8	ND	4	1*	4*
341A	Acyclic	2	6	2	10	ND	ND	5*	4	7
Mean ± SD		2.0	4.3 ± 2.4	3.0 ± 2.2	10	8	2.0	3.7 ± 1.2	3.7 ± 2.1	6.3 ± 1.7
315A	Control			7	1	7	9	10	7	8
316A	Control	5	8	6	7	7	9	10	7	8
318A	Control	9	10	1	9	ND	8	3	8	9
329A	Control	1	7	9	3	6	9	9	4	10
Mean ± SD		5.0 ± 3.3	8.3 ± 1.2	5.8 ± 2.9	5.0 ± 3.2	6.7 ± 0.5	8.8 ± 0.4	8.0 ± 2.9	6.5 ± 1.5	8.8 ± 0.8

ND = Not detected; * Observed heat not accompanied by rise in P4 concentration

Table III-8 **Summary of oestrus signs (scores) recorded in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

Oestrus sign	Pre-infection	Post-infection				
	Entire group (n=49)	Died (n=1)	Cyclic (n=22)	Acyclic (n=9)	Controls (n=19)	Entire group (n=51)
Mucous discharge	49	1	22	9	19	51
Restlessness	34	1	9	4	16	30
Mounting others	38	1	20	4	16	41
Swollen vulva	28	1	19	7	16	43
Allowing mounting	22	1	12	5	14	32
Being sniffed	20	1	17	5	17	40
Bellowing	18	.	6	1	7	14
Inappetence	9	.	9	4	14	27
Front mounting	21	.	7	1	5	13
Head butting	22	1	22	5	15	43
Raised tail	1	.	1	.	2	3

n= Number of heats

Table III-9 **Serum P4 concentrations related to day of oestrus (day 0) derived from 55 cycles compeleted by seven Boran/Friesian cross heifers**

Day of cycle	P4 concentration (mean ± SEM)	Number of observations
0	0.23 ± 0.07	15
1	0.30 ± 0.09	19
2	0.40 ± 0.09	16
3	0.75 ± 0.69	15
4	2.96 ± 0.61	16
5	3.82 ± 0.77	19
6	4.90 ± 0.97	13
7	10.65 ± 1.28	15
8	10.34 ± 0.79	18
9	11.2 ± 1.19	19
10	12.54 ± 1.19	14
11	12.65 ± 0.68	23
12	14.58 ± 1.02	21
13	16.0 ± 1.42	9
14	12.95 ± 0.72	9
15	15.72 ± 1.12	19
16	15.37 ± 1.51	15
17	11.8 ± 1.2	19
18	8.56 ± 1.66	16
19	6.21 ± 1.61	16
20	1.63 ± 0.61	13

Table III-10 **Oestrus cycle lengths in Boran/Friesian cross heifers infected with *T. parva* stabilate IL3081 and in controls**

Animal #	Status	Cycle length in days			
		Pre-infection		Post-infection	
			Mean*		Mean
322A	Died	20,18,19	19.0 ± 0.8	20	
323A	Died	24,21,19	21.0 ± 2.1	.	
Mean			20.2 ± 2.0		
317A	Cyclic	19,23,21	21.0 ± 1.6	44,22,20,23	27.3 ± 9.7
320A	Cyclic	20,21	20.5 ± 0.5	44,20,20,19	25.8 ± 10.5
324A	Cyclic	19,20	19.5 ± 0.5	56,30,20 19	31.3 ± 14.5
326A	Cyclic	21,20,22	21.0 ± 0.8	19,5,24,23,21	18.4 ± 6.9
327A	Cyclic	22,22,23	22.3 ± 0.5	25,22,22,22	22.8 ± 1.3
Mean			21.0 ± 1.3		24.8 ± 10.6
325A	Acyclic	28,22,22	24.0 ± 2.8	43,19,16,21, 37	27.2 ± 10.7
328A	Acyclic	19,21,18,22	20.0 ± 1.6	97,20,20	45.7 ± 36.3
341A	Acyclic	23,22,22	22.3 ± 0.5	63,26,20,22, 19	30.0 ± 16.7
Mean			21.9 ± 2.5		32.5 ± 22.6
315A	Control	22,21	21.5 ± 0.5	20,22,22,27	22.8 ± 2.6
316A	Control	20,19,22,23	21.0 ± 1.6	22,21,19,20	20.5 ± 1.3
318A	Control	21,18,20	19.7 ± 1.2	41,23,18,21	25.8 ± 9.0
329A	Control	20,20,21	20.3 ± 0.6	20,20,20,20, 20	20
Mean			20.6 ± 1.3		22.1 ± 5.1

*= Mean ± SD

Table III-11 **Oestrus cycle lengths in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

Animal #	Status	Cycle length (days)			
		Pre-infection period		Post-infection period	
			Mean**		Mean
322A	Died	20, 18, 19	19.0 ± 0.8	20	20
323A	Died	24, 21, 19	21.3 ± 2.1	.	.
Mean			20.2 ± 2.0		20.0
317A	Cyclic	19, 23, 21	21.0 ± 1.6	22, 21*, 22, 20, 23	21.6 ± 1.0
320A	Cyclic	20,21	20.5 ± 0.5	23, 21, 20, 20, 19	20.6 ± 1.4
324A	Cyclic	19, 20, 20	19.7 ± 0.4	22, 23, 21, 20, 19	21.0 ± 1.4
326A	Cyclic	21, 20, 22	21.0 ± 0.8	24, 24, 23, 21	23.0 ± 1.2
327A	Cyclic	22, 22, 23	22.3 + 0.5	25, 22, 22, 22	22.8 +1.3
Mean			20.9 ± 1.3		21.7 ± 1.6
325A	Acyclic	23, 22, 22	22.3 ± 0.5	29, 18	23.5 ± 5.5
328A	Acyclic	19, 21 18, 22	20.0 ± 1.6	20, 17	18.5 ± 1.5
341A	Acyclic	23, 22, 22	22.3 ± 0.5	22, 19, 20, 21, 21	20.6 ± 1.0
Mean			21.4 ± 1.6		20.8 ± 3.3
315A	Control	22, 21	21.5 ± 0.5	20, 22, 22, 27	22.8 ± 2.6
316A	Control	20, 19, 22, 23	21.0 ± 1.6	22, 21, 19, 20	20.5 ± 1.3
318A	Control	21, 18, 20	19.7 ± 1.2	19, 22, 23, 18, 21	20.6 ± 1.9
329A	Control	20, 20, 21	20.3 ± 0.6	20, 20, 20, 20, 20	20.0
Mean			20.6 ± 1.3		20.9 ± 1.9

* Underlined = Cycles adjusted using P4 profiles

** mean ± SD

Table III-12 **Distribution of palpable ovarian structures in relation to the oestrous cycle in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls**

Status and palpable structure	Number of observations	Structure present in	
		Luteal phase	Follicular phase
Infected, died			
Developing CL (A1-3)	1	1	.
Mature CL (B)	21	19	2
Regressing CL (C1-2)	4	3	1
Follicles (D)*	2	0	2
NDS (E)	10	6	4
Infected, cyclic			
Developing CL (A1-3)	19	18	1
Mature CL (B)	71	67	4
Regressing CL (C1-2)	23	8	15
Follicles (D)	4	.	4
NDS (E)	10	6	4
Infected,acyclic**			
Developing CL (A1-3)	3	3	.
Mature CL (B)	24	20	4
Regressing CL (C1-2)	4	2	2
Follicles (D)	5	2	3
NDS (E)	3	1	2
Controls			
Developing CL (A1-3)	21	21	.
Mature CL (B)	54	47	7
Regressing CL (C1-2)	12	2	10
Follicles (D)	7	1	6
NDS (E)	10	3	7

NDS - No detectable structures

* - Follicles palpated in presence of CL omitted

** - Examinations restricted to period before extended P4 concentrations

Table III-13 **Summary of palpable ovarian structures in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and controls related to the oestrous cycle**

# of examinations	Luteal phase		Follicular phase	
	CL*	No CL**	CL	No CL
# of exams : 299	196	29	18	56
% of total 100	65.6	9.7	6.0	18.7
% to phase	87.1	12.9	24.3	75.7
Overall agreement	84.3% (252/299)			

* - Developing and mature CL

** - Regressing CL, Follicles and no detectable structures

Table III-14 **Gross findings in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 or in controls**

Animal #	322A	323A	317A	320A	324A	326A	327A	325A	328A	341A	315A	316A	318A	329A
Days to death	29	17	RR	RR	RR	RR	RR	RR	RR	RR	Cont-rol	Cont-rol	Cont-rol	Cont-rol
Condition at death	Poor	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good
Abomasum	oe	oe,h	.	.	.	.	.	.	.	.	.	.	.	.
Small intestine	.	h,hr	.	.	.	.	.	.	.	.	.	.	.	.
Large intestine	h	h,hr	.	.	.	.	.	.	.	.	.	.	.	.
Liver	.	e	.	.	.	.	.	.	.	.	.	.	.	.
Gall bladder	.	h	.	.	.	.	.	.	.	.	.	.	.	.
Lymph nodes	.	e,h, hr	.	.	.	.	.	.	.	.	.	.	.	.
Spleen	.	e,wp	.	.	.	.	.	.	.	.	.	.	.	.
Kidneys	.	h,hr	.	.	.	.	.	.	.	.	.	.	.	.
Urinary bladder	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trachea/ bonchi	.	f	.	.	.	.	.	.	.	.	.	.	.	.
Lungs	.	oe,h, hr	.	.	.	.	.	.	.	.	.	.	.	.
Heart	.	h	.	.	.	.	.	.	.	.	.	.	.	.
Brain	c	.	.	.	.	.	.	.	.	.	.	.	.	.

RR = Reacted and recovered; c = congestion; e = enlarged; f = froth; h = haemorrhage; hr = hyperaemia; oe = oedema; wp = prominent white pulp

Table III-15 **Gross findings in the reproductive tract of Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 or in controls**

Animal #	Stage in cycle at death	Right ovary			Left ovary			Uterus
		Size*	Follicles	CL**	Size	Follicles	CL	
Infected								
322A	Luteal	32x22x20	.	20; cavity	30x15x10	Medium	.	.
323A	Luteal	22x15x16	Small	.	34x24x24	.	22; cavity	h (serosal)
317A	Luteal	30x16x14	.	18	24x13x11	Small	.	.
320A	Luteal	30x20x25	Small	22	25x15x15	Medium	.	.
324A	Luteal	40x20x20	.	18; cavity	30x18x18	Medium	.	.
326A	Luteal	32x12x17	Medium	10	30x20x10	Medium	18	.
327A	Follicular	26x15x15	Tertiary	10	22x12x10	Small	.	.
325A	Luteal	30x20x20	.	17; cavity	25x15x15	Small	.	.
328A	Luteal	30x12x12	Tertiary	8; Regressing	26x18x15	Tertiary	18; cavity	h(serosal)
341A	Luteal	29x19x20	.	12, Regressing	25x20x20	Tertiary	.	.
Controls								
315A	Follicular	30x18x18	Tertiary	8, Regressing	28x15x15	Medium	.	.
316A	Follicular	24x15x12	Tertiary	.	22x14x12	Small	10, Regressing	.
318A	Luteal	34x20x20	Small	15	28x16x15	Tertiary	.	.

* - Size of ovary in mm
** - Diameter of CL in mm
h - Haemorrhages

Table III-16 **Histological findings in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 or in controls**

Animal #	322A	323A	317A	320A	324A	326A	327A	325A	328A	341A	315A	316A	318A	329A
Cycle stage	L	L	L	L	L	L	F	L	L	L	F	F	L	L
Status	Died	Died	Cy	Cy	Cy	Cy	Cy	Acy	Acy	Acy	Cont.	Cont.	Cont	Cont.
Endometrium														
C. infilt.	1	1	.	1	.	.	.	.	.	.	.	.	.	.
Oedema	.	1,hr	.	.	.	.	.	.	.	.	.	1,hr	1,hr	.
Myometrium														
C. infilt.	.	2	.	.	.	.	.	.	.	.	.	.	.	.
Oedema	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Perimetrium														
C. infilt.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
Oedema	.	1	.	2	.	.	.	.	.	.	.	.	.	.
Ovary :														
C. infilt.														
O. cortex	.	.	.	1	.	.	.	.	.	.	.	.	.	.
O. medulla	.	1	.	1	.	.	.	.	.	.	.	.	.	.
CL	1	3	.	1	.	.	.	.	.	.	.	.	.	.
Pituitary														
C. infilt.														
Ant. lobe	1	.	.	.	.	.	.	.	.	1	.	.	.	.
Post. lobe	.	.	.	.	.	.	.	.	.	1	.	.	.	.
Adrenal														
C. infilt.														
Z.g	2	1	.	1	.	.	.	.	.	.	.	.	.	.
Z.f	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Z.r	1	.	.	.,hr	.	.	.	.	.	.	.	.	.	.
medulla	1	.	.	1	.	.	.	.	.	.	.	.	.	.

L = luteal; F = follicular phase; Cy = cyclic; Acy = acyclic; Cont. = control; C. infilt. = cell infiltration; Z.g. = *zona glomerulosa*; Z.f.= *zona fasciculata*; Z.r. = *zona reticularis*; . = No change, 1 = Mild change,
2 = Moderate change, 3 = Severe change; hr = hyperaemia

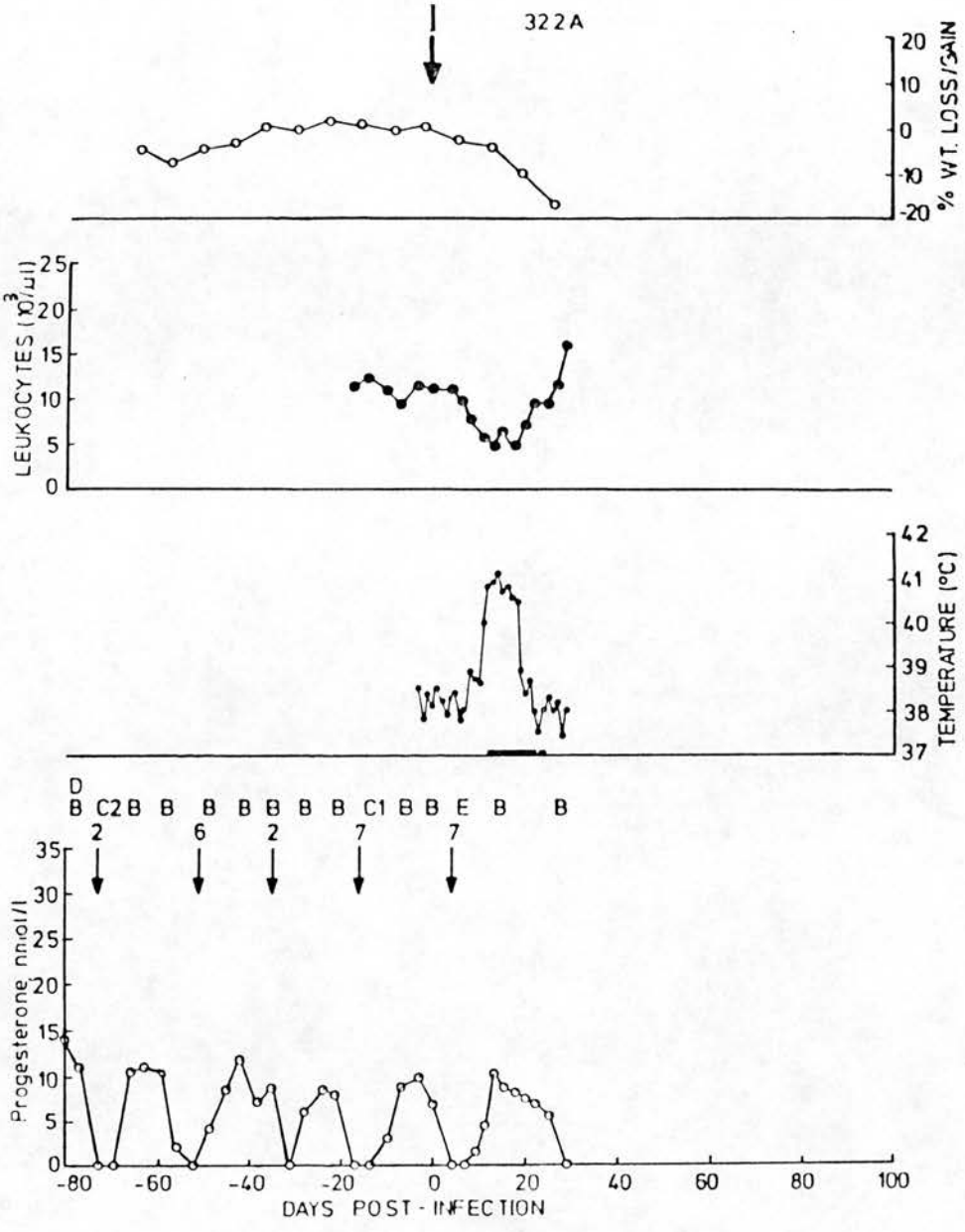


Fig.III-1.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 322A infected with 1:20 *T. parva* stabilate IL3081 (Infected, died). Weight is expresed as a percentage of pre-infection body weight. Ovarian structures are represented by B = Mature CL; C1-2 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

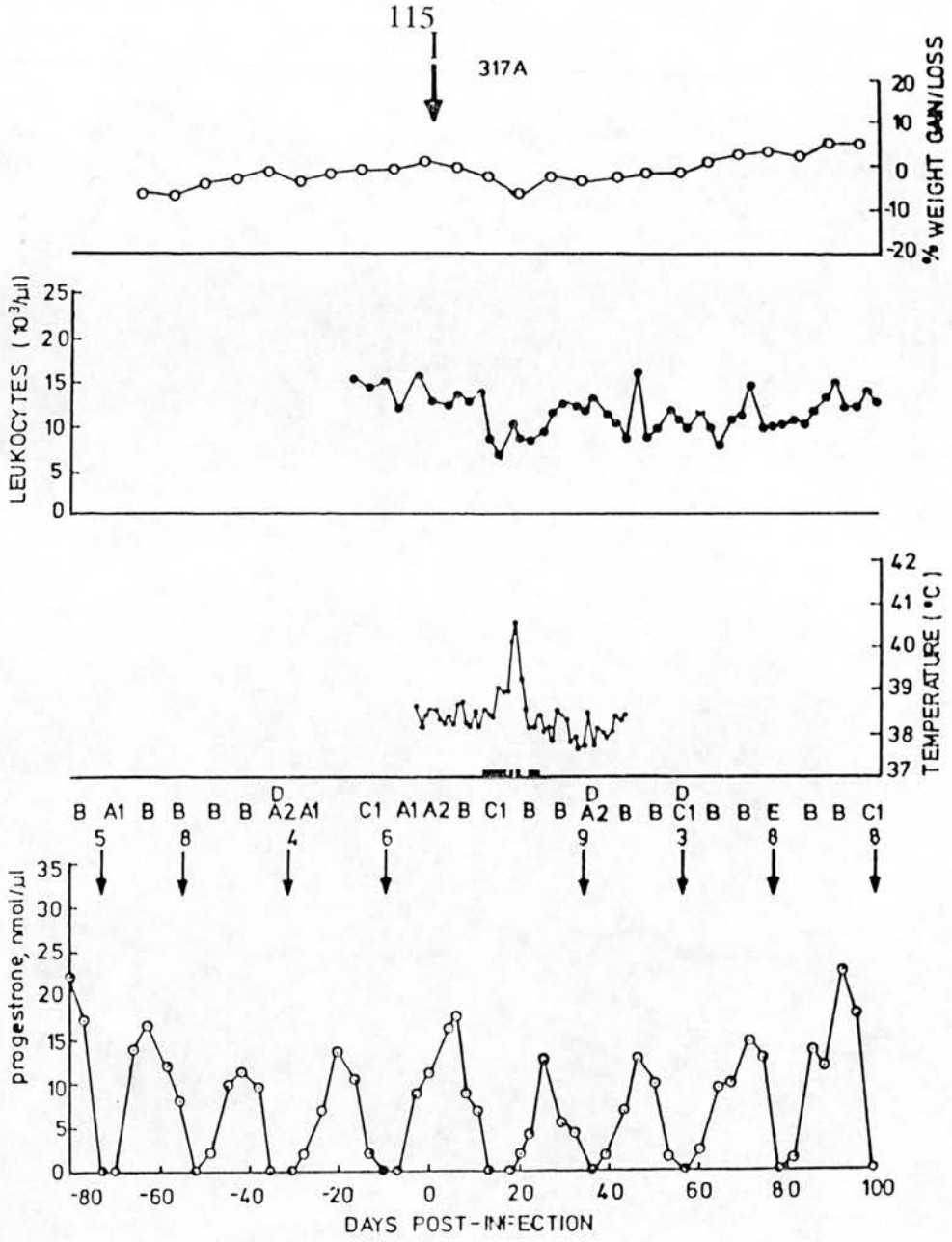


Fig.III-2.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 317A infected with 1:20 *T. parva* stabilate IL3081 (Infected, cyclic). Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A1-2 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

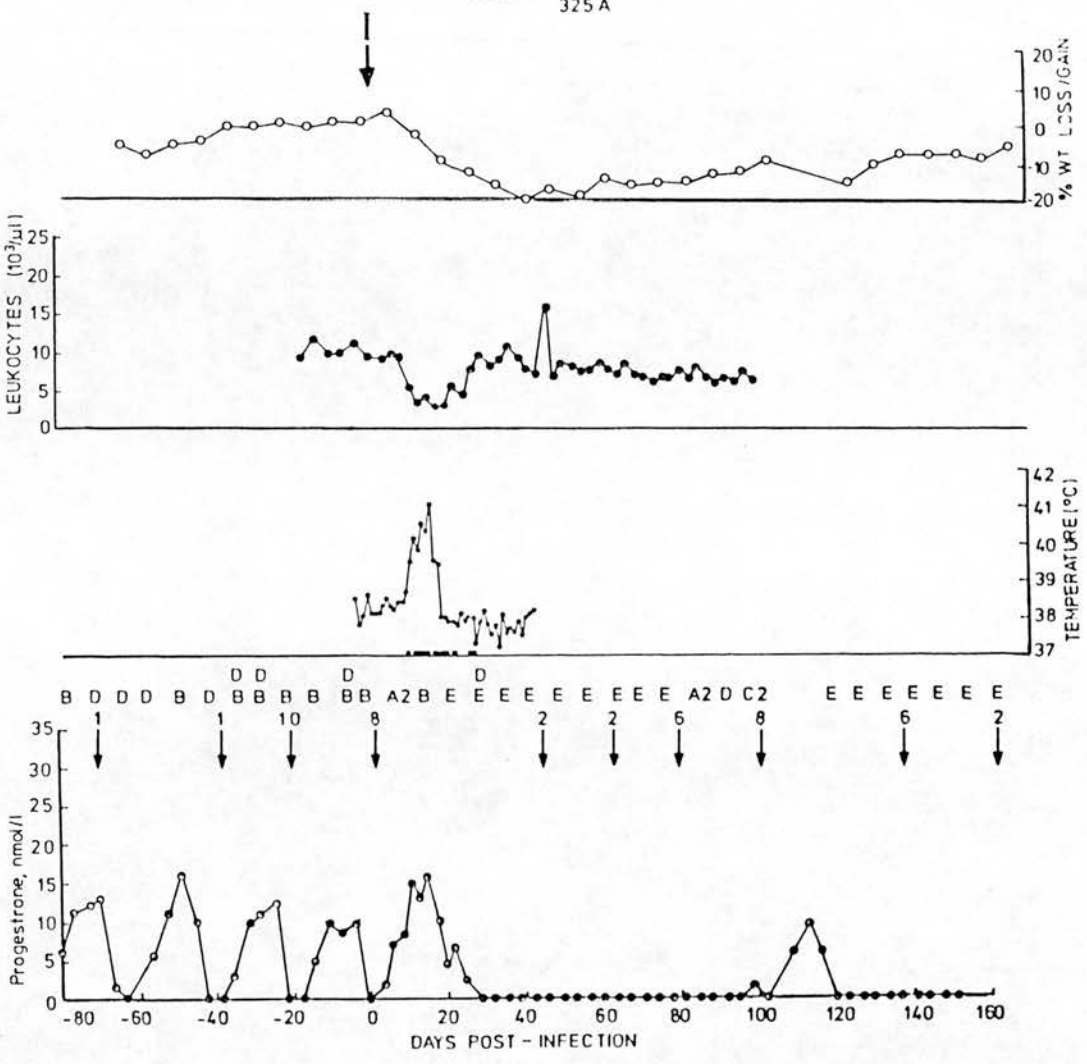


Fig.III-3.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 325A infected with 1:20 *T. parva* stabilate IL3081 (Infected, acyclic). Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A2 = Developing CL; B = Mature CL; C2 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

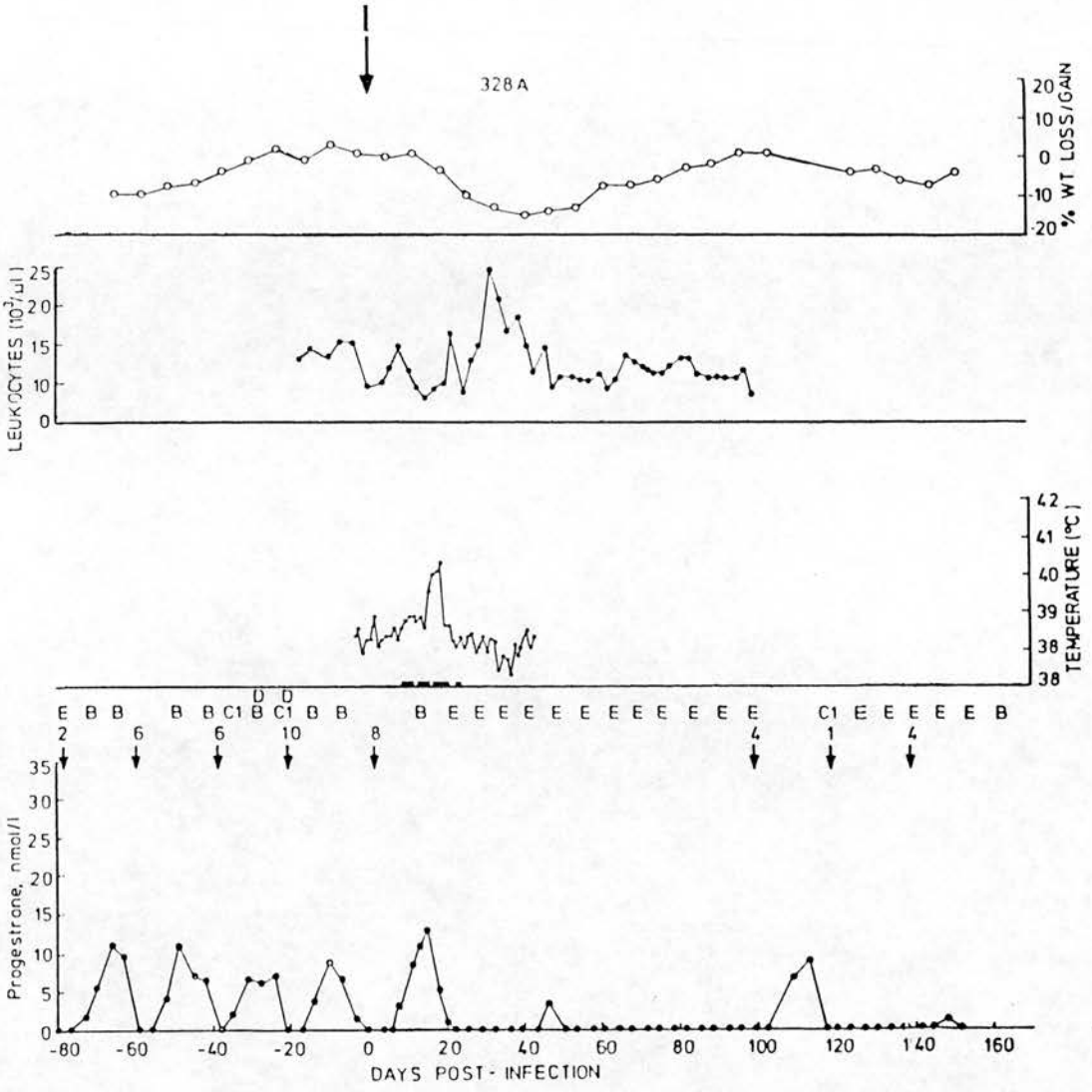


Fig.III-4.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 328A infected with 1:20 *T. parva* stabilate IL3081 (Infected, acyclic). Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

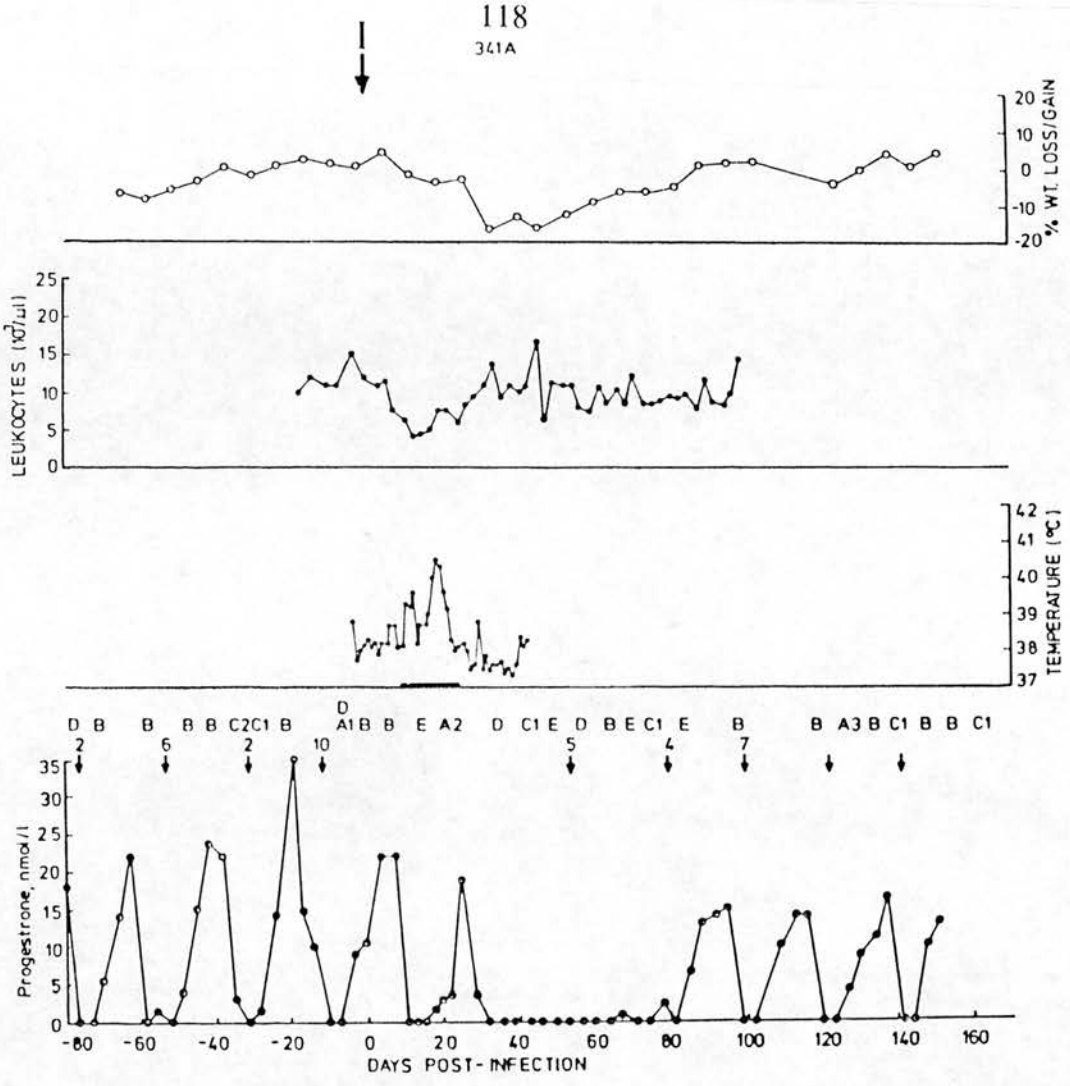


Fig.III-5.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 341A infected with 1:20 *T. parva* stabilate IL3081 (Infected, acyclic). Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A1-3 = Developing CL; B = Mature CL; C1-2 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

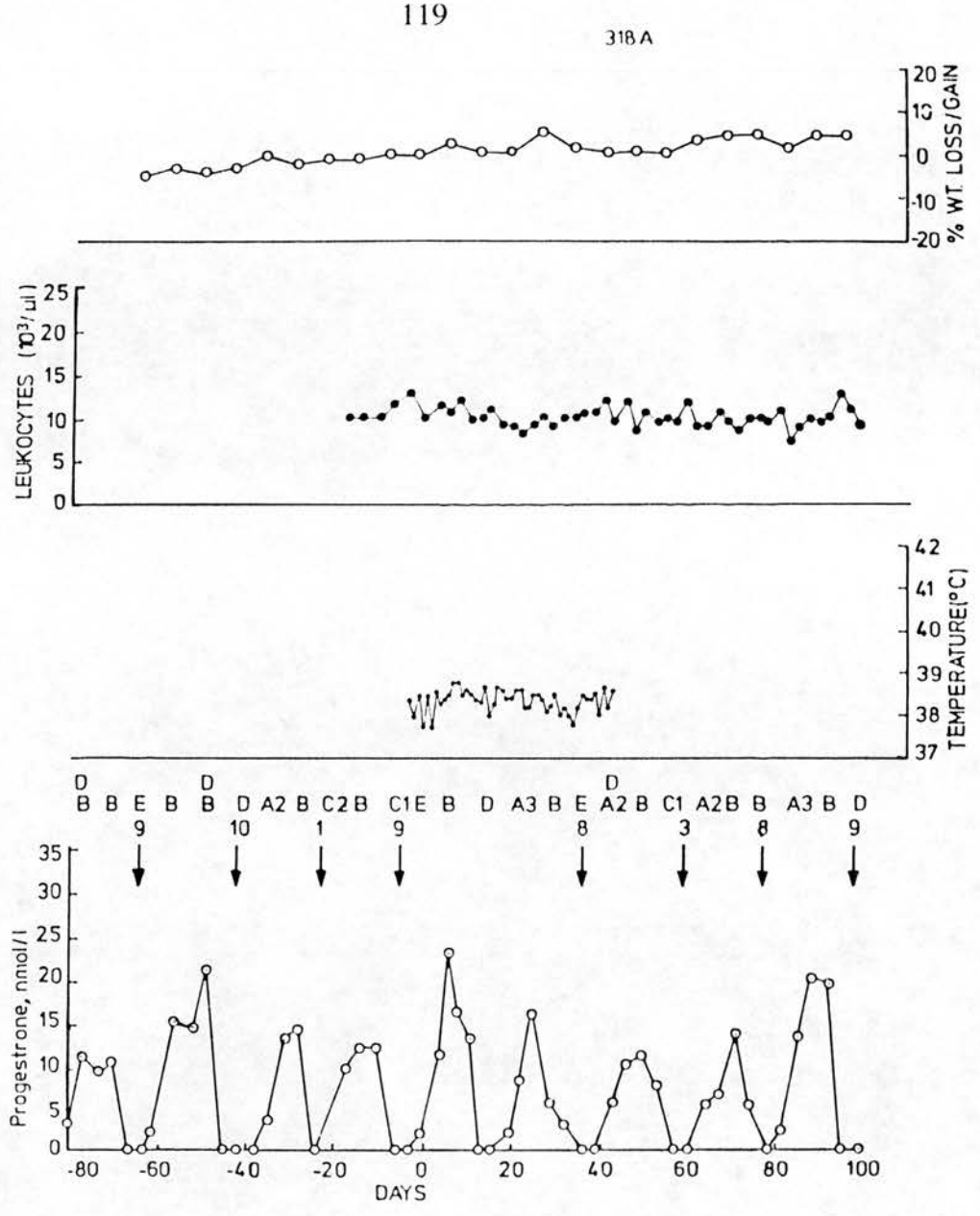


Fig.III-6.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and body weight change in control heifer No 318A. Weight is expressed as a percentage of initial weight. Ovarian structures are represented by A2-3 = Developing CL; B = Mature CL; C1-2 = Regressing CL; D = Follicles; E = No detectable structures. Arrows = Occurrence of oestrus and oestrous scores in superscript.

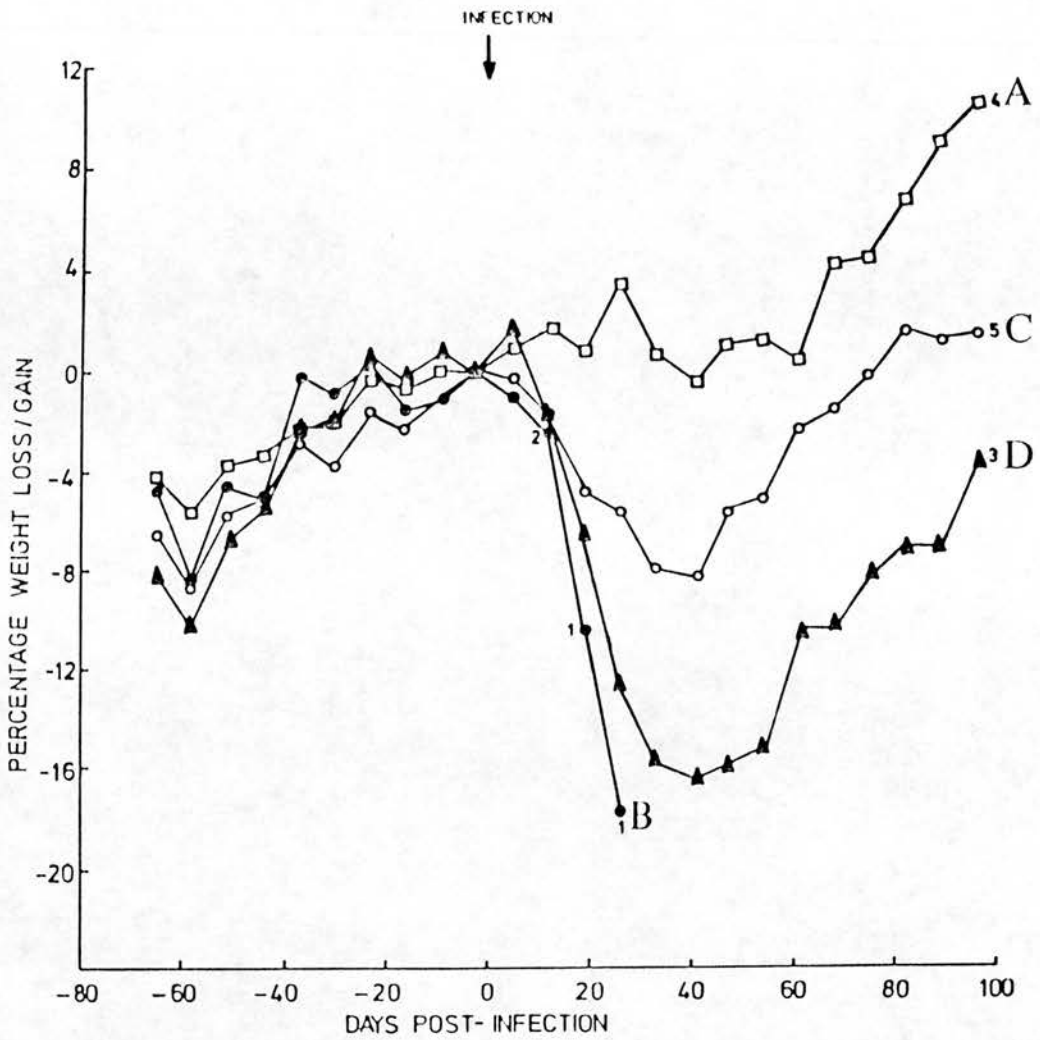


Fig.III-7.

Mean percentage weight changes in Boran/Friesian cross heifers infected with 1:20 *T. parva* stabilate IL3081 and in controls. A = Controls; B = Infected, died; C = Infected, cyclic; D = Infected, acyclic. Numbers against the group mean weights represent number of animals.

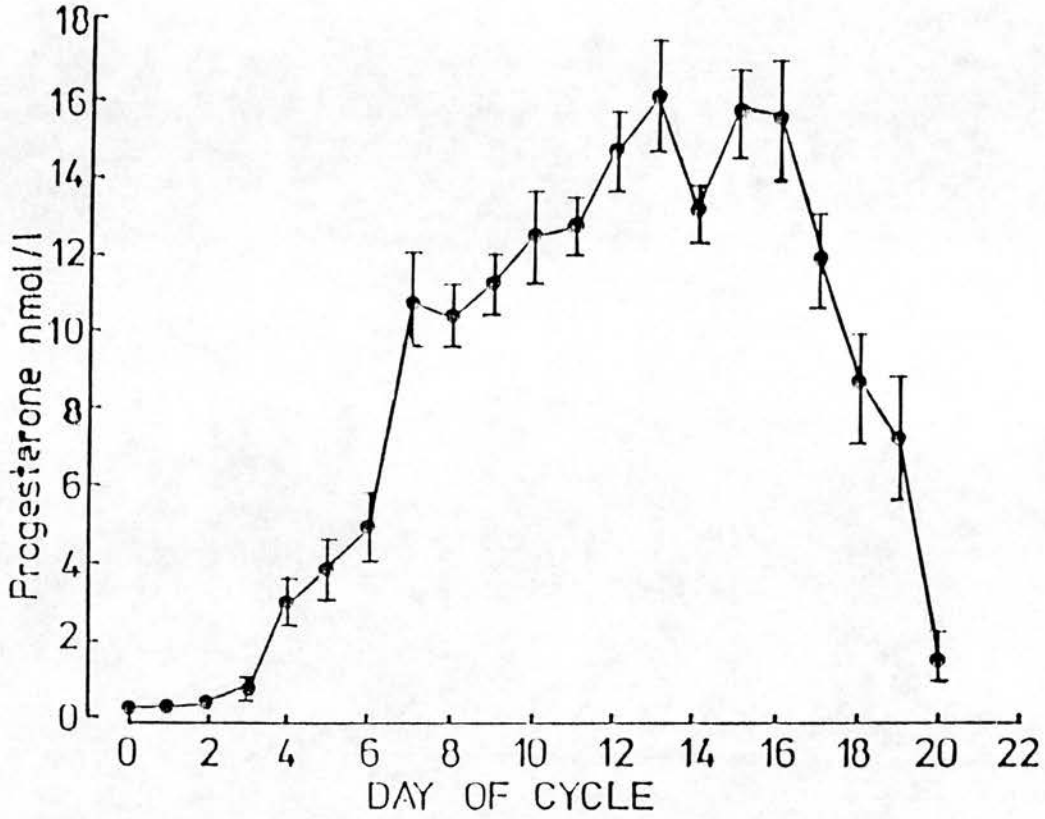


Fig.III-8.

Serum progesterone concentration change over an oestrous cycle in Boran/Friesian cross heifers. Values (Mean ± SEM) were from 55 cycles completed by 7 heifers. Day 0 is the day of observed oestrus.

CHAPTER FOUR

THE EFFECT OF ACUTE THEILERIOSIS ON THE REPRODUCTIVE FUNCTION OF BORAN/FRIESIAN CROSS HEIFERS

4.1 Introduction

It is known that reproductive function in the cow is controlled by a complex mechanism involving primarily four endocrine organs: the hypothalamus, pituitary, ovaries and uterus. Although anecdotal evidence has been presented to implicate *T. parva* infection with disturbances in the reproductive function of cattle, little information exists to explain the mechanisms involved. This experiment was designed therefore with the objective of assessing possible pathological damage with particular emphasis on three key endocrine organs (pituitary, ovaries and uterus) whose dysfunction could directly or indirectly adversely affect reproductive efficiency.

It was considered that animals suffering from inapparent or mild infections may not have pronounced pathological changes and hence the need for an acute syndrome for these studies. In an earlier experiment (Chapter 3), it was found that a 1:20 dilution of *T. parva* stabilate IL3081 caused death in 2/10 Boran/Friesian cross heifers. This experiment was therefore designed to use a more concentrated preparation of same infective material. Ten normally cycling Boran/Friesian cross heifers were given 1 ml of undiluted *T. parva* stabilate IL3081 while four others remained as uninfected controls. All the animals were monitored for cyclicity through behavioural oestrus detection, determination of serum progesterone concentration and rectal palpation of the reproductive tract. After death or sacrifice, a complete post mortem examination was performed and selected tissues taken and processed for light microscopy.

4.2. Materials and methods

4.2.1 Materials

4.2.1.1 Animals

These are described in General Materials and Methods (2.1).

4.2.1.2 Parasite

Details of the *T. parva* stabilate are described in General Materials and Methods (2.2)

4.2.2 Experimental design

Fourteen Boran/Friesian cross heifers were randomly allocated into two groups of ten and four animals each. The ten animals were inoculated with 1 ml of undiluted *T. parva* stabilate as described in General Materials and Methods (2.3). The day of stabilate inoculation was referred to as day 0. The four heifers were not infected and acted as controls.

The monitoring regimen is presented in a summarized format in Table IV-1. The methodologies were adopted as described in detail in General Materials and Methods.

Serum P4 concentrations were analysed using the IAEA supplied assay (2.12.2.1). The inter-assay coefficients of variation were 10.6% (n=16) at 28.9 $\pm$ 3.1 (mean $\pm$ SD) nmol/L and 8.8% (n=16) at 6.8 $\pm$ 0.6 nmol/L for the high and low QCs, respectively. The mean intra-assay CVs were 5.2% and 4.6% for the high and low QCs, respectively.

Table IV-1 **Summarized experimental protocol (Experiment 2)**

Parameter monitored	Frequency and distribution	
	Infected group	Control group
Oestrus behaviour	Daily(x3): d-94 to death or week 19 (021B) week 28 (035B)	Daily(x3): d-94 to week 3 (026B; 036B) week 19(023B;037B)
Body weight	Weekly: Week-12 to termination of experiment	
Serum progesterone	Twice weekly: d-62 to	
	death or week 19 (021B) week 28 (035B)	week 3 (026B; 036B) week 19 (023B; 037B)
Rectal palpations	Weekly: Week-12 to	
	death or week 12 (021B) week 12, 20-28 (035B)	week 3 (026B; 036B) week 12 (023B; 037B)
Haematology	weekly (x3): Week-12 to	
	death or sacrifice	week 3 (026B; 036B) week 19 (023B; 037B)
Rectal temperature	Daily: d-17 to	
	death or d 56 (021B; 035B)	d 35
Stabilate inoculation	d 0	ND
Lymph node biopsies		
LEG	d 5 to d 40	ND
RPG	Earliest d 7 to d 40	ND
Blood smears	Earliest d 7 to d 40	ND
Serology	Fatal cases: last P4 sample d 18, 28, 35 (021B;035B)	d 18, 28, 35
Parasite isolation studies	d 88 to 100 (021B;035B)	ND
Necropsy	On death or d 134 (021B) d 210 (035B)	ND

LEG - Left ear gland; RPG - Right prescapular gland

d - Day post-infection

ND - Not done

4.3 Results

4.3.1 Clinical signs

The time to a febrile response in individual animals is presented in Table IV-2 and representative temperature changes in Figs. IV-1 to IV-4. The first indication of disease in the infected animals was a rise in rectal temperature. All animals in the infected group developed a febrile reaction within 6 to 9 days (mean incubation period, 7.3 days). The febrile reaction period lasted for 3-9 days (Table IV-2) and was continuous in 70% (7/10) and intermittent in three other animals. Eight of the ten animals died 13-21 days post-infection. In 6/8 animals that died of infection, the temperature dropped to subnormal or normal levels before. The other two animals died while their temperature was elevated over 39.5°C. The two recovered cattle maintained normal temperature after a febrile period of 5 and 7 days.

One to two days after temperature elevation to or above 39.5°C, the animals became dull and listless, lost appetite and had a staring hair coat. Other signs included swollen superficial lymph nodes, serous nasal and ocular secretions and as the disease progressed, difficult breathing and an occasional cough. A foetid watery diarrhoea was observed terminally in heifer No. 018B and for about two weeks in the recovered animals from the third week of infection. Rapid losses in condition were observed terminally in 7/8 animals that died. Death was usually sudden and copious amounts of froth was always found exuding from the nostrils. Animals that recovered lost more condition which was not completely regained before sacrifice after several months. Control animals maintained normal temperatures (Fig. IV-4) and remained healthy throughout the course of the experiment.

4.3.2 Parasitology

All infected animals developed a patent macroschizont parasitosis. The time to the appearance and disappearance of macroschizonts in the left ear lymph node (LEG)

which was the drainage gland of the site of inoculation and the right prescapular lymph node (RPG) are presented in Table IV-2. The prepatent period was 6.8 ± 1.0 (mean ± SD) days. Macroschizonts were persistently seen in lymph node biopsies up to the time of death except in one animal (018B) where they were not observed in the LEG one day before death. In the two animals that survived infection, the disappearance of macroschizonts in the LEG was on days 13 and 28 and in the RPG on days 20 and 17 (Nos. 021B and 035B, respectively). All animals had continuous macroschizont parasitosis except animal No. 035B which showed periods of parasite recrudescences (Fig. IV-3).

Three animals developed a piroplasm parasitaemia between 9-11 (mean, 10.0 ± 0.8) days after infection (Table IV-2). In all the three animals, the level of parasitaemia was less than 1% and was observed on only one or two occasions (Table IV-2).

4.3.3 Haematology

4.3.3.1 White blood cell (WBC) concentration

The white blood cell concentration at the start of the experiment was in the range of 7200-21600 μl^{-1}. Cell concentrations fluctuated slightly in all animals during the pre-infection period. Leucocyte values varied in individual animals and ranged between 7500 ± 900 to 19900 ± 800 μl^{-1}.

Changes in leucocyte concentration are presented through Figs. IV-1 to IV-3 for representative infected animals and in Fig. IV-4 for a representative control. In the infected animals, a decline in leucocyte concentration was recorded from day 6 to 13 post-infection. The highest decline was by 73% (from 14800 down to 4000 μl^{-1}) in 019B while the least was by 40.9% (from 9300 down to 5500 μl^{-1}) in 031B (Table IV-3). As a group, the infected animals had significantly ($p<0.05$) lower leucocyte concentrations to the pre-infection value on days 6, 15, 29, 32 and 36 (Table IV-4).

When compared to the control group, significantly ($p<0.05$) lower leucocyte concentrations were recorded on days 6-8 and thereafter on day 15, 32 and 36 in the infected group (Table IV-4 and Fig. IV-5). All infected animals showed a leucocytosis after registering maximum decline. However, five animals (019B, 027B, 028B 032B and 034B) never regained while three animals (018B, 031B and 340A) regained their pre-infection values before death. The two recovered animals (021B and 035B) recorded fluctuating leucocyte concentrations during the infection period (Figs. IV-2 and IV-3). The leucocyte concentration did not vary markedly in the controls.

4.3.3.2 Differential leucocyte concentration

Changes in the differential leucocyte count for the infected and control animals are presented in Table IV-5 and Fig. IV-5. Leucopaenia was as a result of concomitant lymphopaenia, neutropaenia and eosinopaenia. The pre-infection lymphocyte concentration in the infected group was about 9000 μl^{-1}. After infection, lymphocyte concentration declined with significantly ($p<0.05$) lower levels compared to controls occurring on days 6, 11 and 15, 27 to 32 and 36 to 41 (Table IV-5). The lowest lymphocyte concentration for the infected group was observed on day 11 (from about 9000 down to about 5400 μl^{-1}). A significant lymphopaenia to the pre-infection level occurred on days 6, 11, 27 to 32 and 36-41 (Table IV-5). In individual animals, nadir lymphocyte concentrations were reached after 6 to 13 days post-infection. The highest lymphocyte decline of 73.3% (from 10500 down to 2800 μl^{-1}) was seen in 019B (Table IV-3). Three animals (018B, 031B and 340A) had recovered their pre-infection values while the rest had not before death. In the recovered animals, there were either short periods of lymphocytosis followed by a prolonged period of lymphopaenia or intermittent lymphocytosis and lymphopaenia. Lymphocyte concentrations did not vary markedly in the control group (Table IV-5 and Fig. IV-5).

Changes in neutrophil concentration were almost similar to those observed for lymphocytes (Fig. IV-5). However, there was a non-significant rise of neutrophil

concentration one day after infection (from about 3500 up to about 4500 μl^{-1}). The decline started on day 6 post infection but only reached significant ($p<0.05$) levels on day 15 (Table IV-5). A significant ($p<0.05$) neutrophilia occurred on days 25 and 27 in the infected group. Compared to the control group, the infected animals had a significant ($p<0.05$) neutropaenia on days 8 and 15 and a significant ($p<0.05$) neutrophilia on days 25 and 27 post-infection (Table IV-5). In individual infected animals, the degree of neutropaenia ranged from 74.5% (from 4000 down to 1020 μl^{-1}) in 019B to 29.2% (from 2600 down to 1840 μl^{-1}) in 031B at nadir concentrations which were reached between day 6 to 18 post-infection (Table IV-3). Neutrophil concentration in the two recovered animals continued to fluctuate between 3000-6000 μl^{-1}. Neutrophil concentrations did not vary significantly in the control group (Table IV-5).

Eosinophil concentration before infection was about 450 μl^{-1} (range 150-700 μl^{-1}). There was a non-significant eosinophilia on day 4 post-infection. A significant ($p<0.05$) eosinopaenia developed on day 11 and thereafter except on day 34 in the infected group (Table IV-5). Eosinophil concentrations were significantly ($p<0.05$) lower than in the control group on day 15 post-infection. In the control group, eosinophil concentrations fluctuated during the experimental period with significantly lower levels occurring on days 22, 27 and 39.

4.3.3.3 Red blood cell (RBC) concentration

The mean red blood cell concentration for the 14 heifers was 8.5 ± 0.7 μl^{-6} before infection. The infected and control group values did not differ significantly ($p<0.05$) at this time. A significant ($p<0.05$) reduction from pre-infection levels was observed on days 8, 13, 20, 25 and 29 and thereafter in the infected group (Table IV-6). Significantly ($p<0.05$), lower concentrations were recorded on days 8, 13, 25, 29, 34 and thereafter in the infected group compared to the controls (Table IV-6). Red blood cell concentration did not vary significantly in the control group except on day

29 of the experiment when a significant ($p<0.05$) reduction was observed (Table IV-6).

4.3.3.4 Haemoglobin concentration

The fourteen heifers had a mean haemoglobin concentration of about 13g dl^{-1} before infection during which there was no significant difference between the infected and control groups (Table IV-6). Haemoglobin concentration started declining on day 8 post-infection and reached a nadir 29 days post-infection (down to 6.25 from 12.69g dl^{-1}) before starting a slow recovery in the infected group (Table IV-6; Fig. IV-6). A significant ($p<0.05$) reduction from the pre-infection concentration occurred on days 8 to 18, 25, 29 and thereafter in subsequent samples in the infected group (Table IV-6). Haemoglobin concentration was significantly ($p<0.05$) lower than in the control group on days 8 to 13, 25, 32 and thereafter (Table IV-6). Haemoglobin concentration fluctuated within the control group with significantly ($p<0.05$) lower levels recorded on days 18 to 22, 27 to 29 and 36 of the experiment (Table IV-6).

4.3.3.5 Packed cell volume

Changes in packed cell volume are presented in Table IV-6 and Fig. IV-6. The packed cell volume decreased significantly ($p<0.05$) in the infected group on days 8, 11and 13 and then rose to pre-infection levels on day 22 post-infection. A significant ($p<0.05$) decrease ensued in the two recovered animals on all sampled days from day 25. Comparison with the uninfected controls showed significantly ($p<0.05$) lower levels on days 8 to 15, 25, 27 and thereafter on all sampled days from day 32 post-infection (Table IV-6). The packed cell volume fluctuated in the control group with significantly ($p<0.05$) lower levels occurring on days 18 and 29 of the experiment (Table IV-6).

4.3.4 Body weight change

Before the start of the experiment, animals in both groups weighed between 240 and 377kg. All the animals recorded relatively constant body weights during this period (Figs. IV-1 to IV-4). All infected animals started to lose weight during the first week of infection (Table IV-7). All the eight animals dying of infection had not regained the initial body weight at the time of death. The two recovered animals lost between 28-29% of their pre-infection weight before starting a slow recovery 4 to 5 weeks after infection (Figs. IV-2 and IV-3). However, 021B had not regained her pre-infection weight at the time of sacrifice nor had 035B at 134 days and about six months post-infection, respectively. Three control animals lost about 1 to 3% of the initial body weight during the first week of the experiment and went on to record constant or increasing body weights during the study period.

4.3.5 Serology

None of the eight animals that died had developed positive titres to *T. parva* schizont antigen before death. The two surviving heifers (Nos. 021B and 035B) had positive titres on days 28 and 35 post-infection. All control animals had negative titres to *T. parva* schizont antigen on days 28 and 35 of the experiment (Table IV-2).

4.3.6 Parasite isolation studies

4.3.6.1 Tick salivary gland infection

Examination of salivary glands of 100 *R. appendiculatus* ticks which had been fed on each of the two recovered animals three months after infection failed to show any infection.

4.3.6.2 Transmission studies

Transmission attempts using 600 pooled *R. appendiculatus* adult ticks (300

from each recovered animal) on one steer were not successful. The steer did not react clinically, no parasites were detected in lymph gland biopsies and it did not sero-convert for *T. parva* antibodies.

4.3.7 Cyclical status

Three parameters, viz: behavioural oestrus detection, analysis of serum progesterone concentration and palpation of the ovaries *per rectum* were used to monitor the cyclical status. The data collected are presented through Figures IV-1 to IV-4 for representative animals and discussed in subsequent sections below. As monitoring tools, the three methods are discussed briefly to highlight their accuracy and usefulness with data collected from the entire group.

4.3.7.1 Behavioural

A total of 62 basal P4 concentration periods were revealed by distinct P4 profiles during the study period. Behavioural oestrus was observed in 58/62 (93.5%) of the basal P4 periods. From the P4 profiles, oestrus was missed twice in 035B before infection and once in each of 018B and 021B post-infection. Oestrus was also observed during basal P4 concentrations without distinct P4 cycles in three animals (027B, 032B and 035B). The number of behavioural signs per oestrus ranged from 2-8. The most commonly observed oestrous signs were restlessness, vulval mucous discharge, head-butting, mounting other animals, allowing mounting and a swollen vulva.

4.3.7.2 Serum progesterone concentration

Serum progesterone concentrations varied from undetectable (<0.3 nmol/L) to 21.0 nmol/L. Distinct luteal P4 plateau concentrations ranged from about 5 nmol/L to between 15-20 nmol/L.

4.3.7.3 Ovarian structures

A mature CL was palpated in 43/44 (97.7%) of the cycles completed by the infected and control animals. Developing and regressing CL were palpated during the luteal phase while regressing CL were palpated during both the follicular and luteal phases. Follicles were detected during both the follicular and luteal phase as well as the period of basal P4 concentrations in infected acyclic animals.

4.3.7.4 Cyclical statuses in various groups

4.3.7.4.1 Infected group

Cycle lengths based on behavioural data are presented in Table IV-8. Before infection, animals in this group had each, one to three cycles ranging from 15-26 days. The mean cycle lengths within animals ranged from 18.0 $\pm$ 0.8 to 25.5 $\pm$ 0.5 (mean $\pm$ SD) days and with a group mean of 21.8 $\pm$ 6.2 days. However based on P4 profiles and palpated ovarian structures, the results were incompatible in three animals. Cycle lengths based on P4 profiles are presented in Table IV-9. Based on these data, animals had each, one to three cycles ranging from 16-26 days. The mean length of P4 cycles within animals ranged from 18.0 $\pm$ 0.8 to 25.5 $\pm$ 0.5 days while the entire group mean was 20.5 $\pm$ 2.8 days.

Heifers Nos. 027B and 032B had basal P4 concentrations for 24 and 17 days, respectively prior to infection. The only palpable ovarian structures during this low P4 concentration period in these two animals were regressing CL in 027B and follicles in 032B. Oestrus had been detected six and three days before infection in 027B and 032B, respectively.

Following infection, of the eight animals that were cycling normally pre-infection, five animals died before showing oestrus. However, there was evidence indicative of maintained cyclicity post-infection based on P4 profiles and/or palpated ovarian structures. Heifer No. 028B was typical of these cases and its data are

presented in Fig. IV-1. One animal (018B) completed a P4 cycle post-infection but maintained basal P4 concentrations for 11 days before it died.

Of the two recovered animals, 021B completed one P4 cycle post-infection and then maintained basal P4 concentrations for 105 days (Fig. IV- 2). Neither oestrus nor ovarian structures indicative of regular cyclicity were detected in this animal during the extended period of basal P4 concentrations. Oestrus was manifested on two occasions during the extended period of basal P4 concentrations in 035B which also recovered. However, this animal maintained basal P4 concentrations and had no evidence of cyclical ovarian changes to day 200 post-infection (Fig.IV-3).

The two animals (027B and 032B) which had revealed luteal dysfunction pre-infection maintained basal P4 concentrations post-infection. Follicles were detected in 032B while 027B had no palpable ovarian structures post-infection. Neither of these two animals manifested heat post-infection.

4.3.7.4.2 Controls

Based on behavioural data, P4 profiles and palpable ovarian structures, all controls cycled regularly throughout the study period. Each animal had three cycles and three to four cycles before and after the start of the experiment, respectively. The cycle lengths ranged from 19-26 days. Within animal and group mean cycle lengths are presented in Table IV-8. Heifer No. 023B was typical of the control group and its P4 profile, palpated ovarian structures and behavioural data is presented in Fig. IV-4.

4.3.8 Pathology

4.3.8.1 Gross findings

The major gross lesions in the animals dying of ECF or sacrificed following recovery are presented in Table IV-10.

The general condition of the carcase was good to fair in seven of eight fatal

cases and poor in one (018B). However, although the latter appeared to have lost condition on visual inspection, it had a fair distribution of subcutaneous fat. Heifer 021B which was sacrificed on day 134 post-infection was in poor condition and had gelatinous subcutaneous fat while 035B sacrificed on day 210 post-infection was in fair condition. A yellowish discolouration of subcutaneous fat was observed in 028B.

Of the two animals which recovered, 035B had no gross lesions at necropsy while 021B only revealed a shrunken spleen and a few scattered pseudoinfarcts in both kidneys. Various lesions characteristic of ECF were found in all the fatal cases.

There was oedema of the abomasal folds in six of eight fatal cases. The abomasal oedema was marked in five and mild in one (031B) of these animals. Mucosal haemorrhages were present in the abomasum in six of the fatal cases. These were petechial in four cases, ecchymotic in one (027B) and both petechial and ecchymotic in another (034B). Hyperaemia of the abomasal mucosa was seen only in 018B. Two animals (028B and 031B) had multiple ulcers of the abomasal mucous membrane. In 028B, caseous necrotic material was found in the depressions.

The wall of the small intestine was thickened in two cases (019B and 034B). Petechial haemorrhages in the intestine were found in four animals. These were either serosal (034B and 340A), mucosal (028B) or in both the serosa and mucosa (019B). Hyperaemia of the intestinal mucosa was observed in one case (031B) and a yellowish mucosal discolouration in another (018B). Mucosal haemorrhages were present in the large intestine of five fatal cases. These were distributed in the colon, caecum and rectum of four animals and only in the colon of the other (018B). In 034B and 340A the haemorrhage was very severe and was accompanied by frank blood in the rectal lumen. Serosal haemorrhages of the large intestine were observed in two (019B and 034B) of these animals. In addition to the haemorrhages, heifer 031B had a hyperaemic colon and caecum.

The liver was enlarged in five of the fatal cases. Congestion was observed in three of the livers which were enlarged. The livers of two animals were icteric on cut

surface. In seven of eight fatal cases, the gall-bladder was distended with turbid bile while the other had petechial mucosal haemorrhages.

The lymph nodes were markedly enlarged in all but one (031B) of the fatal cases. Haemorrhages were present in lymph nodes of two animals. Five animals had hyperaemic lymph nodes. Oedema of the lymph nodes was observed in only one animal.

An enlarged spleen was seen in three of the fatal cases while in one (031B) it appeared slightly shrunken in size. On the cut surface, the malpighian corpuscles were observed to be prominent in three animals. The cut surface had a meaty appearance in one animal (027B). Of the animals sacrificed, 021B had a small shrunken spleen which displayed prominent connective tissue on cut surface.

Renal pseudoinfarcts were seen in five of the infected animals. On cut surface, these were reddish in three animals (018B, 019B and 027B), white in 021B and of both colours in 031B. The renal cortex was congested in five animals. In one of the fatal cases (340A), the kidneys had a soft consistency. Petechial mucosal haemorrhages were observed in the urinary bladder of six animals.

The most significant lesions were observed in the respiratory tract in animals dying of the infection. Following death, copious amounts of froth were observed oozing through the nose in six of the fatal cases. A variable amount of yellow fluid was found in the pleural cavity in three of these (027B, 028B and 034B). In 034B the pleural fluid was about two litres. Petechial haemorrhages were observed in the visceral pleura of two animals (034B and 340A). The lungs were grossly enlarged and heavy in all fatal cases except in 018B. On section, straw coloured oedematous fluid oozed out. The same animals exhibited a fibrinous interstitial pneumonia. The only gross change in 018B was an intense hyperaemia in the lungs. Mild hyperaemia was also found in four other fatal cases. Variable amounts of froth were present in the trachea and bronchi of all fatal cases. In addition, petechial haemorrhages and a tenacious mucoid exudate were found in the trachea and bronchi of 019B and 031B,

respectively.

A slight increase in pericardial fluid, yellowish in colour, was found in 019B and 032B. Petechial haemorrhages were seen in the pericardium of 028B and in the pericardium, epicardium and endocardium of 034B.

The gross anatomic findings in the reproductive tracts of the infected animals are presented in Table IV-11. The ovaries were generally ovoid except in 034B where the right ovary was elongated and had a CL at both ends. The type of follicles found ranged from small sized to tertiary. CL were absent in four animals. The other animals had either a regressing or mature CL on either of the ovaries. Serosal petechial haemorrhages of the uterus were observed in three animals (027B, 028B and 340A). No other abnormalities were found in the opened uteri apart from a scanty quantity of brownish exudate in 032B.

4.3.8.2 Histologic findings

4.3.8.2.1 Uterus

The histologic findings in the uteri of infected animals are presented in Table IV-12.

A mild endometrial cellular infiltration was found in five of the ten infected animals. The infiltrating cells were predominantly mononuclear. Scattered polymorphonuclear leucocytes and macrophages were observed in the superficial endometrial submucosa in 019B and 027B. An increased number of medium and large-sized lymphocytes were observed in the lumina of capillary and medium-sized blood vessels especially in the superficial endometrium in 340A. A mild hyperaemia was observed in 032B and 034B and a mild endometrial oedema in 018B, 032B and 340A.

In the myometrium a mild to moderate mononuclear cell infiltration was found in four of the infected animals (Table IV-12). The infiltrating cells were located

between bundles of smooth muscle cells or in the adventitia of blood vessels located between the inner and outer circular muscles.

Changes in the perimetrium were found in four of the ten infected animals (Table IV-12). The changes comprised of hyperaemia alone (018B and 034B) or moderate to severe cellular infiltration accompanied by oedema (032B and 340A). In 340A, the perimetrium was widely distended by oedema fluid. Mononuclear cells predominated over the sparsely distributed stromal connective tissue elements. In 340A, the perimetrial blood vessel lumina were plugged with lymphocytes.

4.3.8.2.2 Ovaries

Primordial to tertiary follicles were seen in the ovaries of all the infected animals. Primordial, primary and tertiary follicles were abundant while secondary follicles were generally scanty. Histological changes in the ovaries comprised mainly of cellular infiltrations (Table IV-12).

A mononuclear cellular infiltration of the ovarian cortex was observed in four of the infected animals. The infiltration was mild in three out of the four and moderate in the other (Table IV-12). The infiltrating cells were diffusely distributed in the stromal tissue. A mild to moderate mononuclear cellular infiltration was also observed in the medulla of four animals (Table IV-12). In addition, the medullary blood vessels contained large numbers of mononuclear cells (Fig. IV-7). A mild hyperaemia was also observed in 018B. The CL was included in ovarian sections of two animals. In these, a moderate to severe mononuclear cell infiltration of the CL was found (Fig. IV-8). Several blood vessels in the CL had lumina plugged by medium and large sized lymphocytes.

4.3.8.2.3 Pituitary gland

Histological findings in the pituitary glands of the infected animals are presented in Table IV-12.

In the pituitary gland, a mild mononuclear cell infiltration of the adenohypophysis was found in 018B, 028B and 032B. Large numbers of lymphocytes were observed in the lumina of a few blood vessels in the adenohypophysis of 028B. No changes were observed in the intermediate and posterior lobes of the pituitary gland.

4.3.2.4 Adrenal gland

The histological findings in the adrenal glands of the infected animals are presented in Table IV-12.

Of the ten infected animals, five animals had no lesions in the adrenal glands. A mild to moderate mononuclear cell infiltration of the *zona glomerulosa* was observed in five of the infected animals. A mild to moderate hyperaemia was also observed in the *zona glomerulosa* of three of the five animals. Four of the five animals had a mild mononuclear cell infiltration in the *zona fasciculata*. In the *zona reticularis*, there was a mild to moderate mononuclear cell infiltration in four of the five animals and mild to moderate hyperaemia in all of them. Changes in the medulla comprised of a mild to moderate mononuclear cell infiltration accompanied by a mild to moderate hyperaemia except in 028B where only cellular infiltration was present.

4.4 Discussion

In this experiment, it was demonstrated that an undiluted *T. parva* (buffalo-derived) stabilate IL3081 was highly pathogenic and caused death in 8/10 (80%) Boran/Friesian cross heifers. All the infected animals reacted to the parasite and fever was the first clinical manifestation of disease. According to Neitz (1957), the reactions could be classified into three categories: peracute in six animals where fever persisted for 5-8 days and subacute in two animals where the symptoms were severe but the animals survived. However, according to Anon (1989b), the eight animals that died of disease would be categorised as severe reactors while the two recovered animals

would be categorised as mild reactors.

The uninfected controls which were kept together with the infected group remained healthy throughout the study period. In the infected group, the clinical signs were similar to those described for *T. parva* infection (Henning, 1956; Neitz, 1957). The incubation period in this study ranged from 6-9 (mean 7.3 $\pm$ 1.2) days. In an earlier experiment, the incubation period was found to range from 10-18 (mean 13.0 $\pm$ 2.7) days when the same stabilate was used at 1:20 dilution. The incubation period noted here was close to that reported by Dolan (unpublished results) of 6.5 days or Mutugi *et al.* (1988a,b) of 7.4 and 9.5 days, respectively using a similar undiluted stabilate. The mean febrile reaction period was longer with the undiluted stabilate compared to that noted for the 1:20 stabilate dilution (6.2 $\pm$ 1.8 vs 4.9 $\pm$ 1.9 days). As reported by others (Henning, 1956; Neitz, 1957; Brocklesby, 1962) fever was either continuous or intermittent with some animals dying during elevated temperature phase (>39.5^{o}C) or after temperatures had dropped to subnormal levels.

Other clinical signs were preceded by fever by 1-2 days. These resembled those described in the literature (Henning, 1956; Neitz, 1957; Jura and Losos, 1980). These included swollen lymph nodes, dullness, anorexia, staring hair coat, serous nasal and ocular discharge, difficult breathing and an occasional cough. Dolan (unpublished results) and Mutugi *et al.* (1988a,b) reported the mean time to death to be between 11.5 and 14.7 days respectively. In this study, the mean time to death was 14.5 $\pm$ 2.5 (range 13-21) days and therefore compares favourably with their findings.

The prepatent period ranged from 6-9 (mean 6.8 $\pm$ 1.0) days. This compared favourably with that of Dolan (unpublished results) and Mutugi *et al.* (1988a,b) of between 6-6.5 days. Unlike as in the 1:20 *T. parva* infection, a transient piroplasmaemia occurred in three animals. One of these was in 035B which survived and the others in fatal cases. The mean time to detection of piroplasms of 10.0 $\pm$ 0.8 days was close to that reported elsewhwere (Dolan, unpublished results). Only two surviving animals developed serological response while those dying of disease did not.

These findings are similar to those of Mutugi *et al.* (1988a,b).

No evidence of a carrier state of *T. parva* was obtained by parasite isolation attempts in the two surviving animals. It is possible for cattle to develop a carrier state after natural recovery from buffalo-derived *T. parva* (Barnett and Brocklesby, 1966a). It was not clear whether our failure to demonstrate carrier state could be related to the low or lack of detectable parasitaemia in the survivors.

The white blood cell concentrations in the uninfected animals compared favourably to those observed in earlier studies (Chapter 3) and to those reported by others (Maxie *et al.*, 1982). All the infected animals suffered variable degrees of leucopaenia. The leucopaenia was a result of concomitant lymphopaenia, neutropaenia and eosinopaenia. Maxie *et al.* (1982) reported similar findings in *T. parva* (buffalo-derived) infection. Hill and Matson (1970) reported a terminal leucocytosis/lymphocytosis. All our infected animals also had a leucocytosis following their nadirs. The leucocytosis in our animals was the result of a concomitant lymphocytosis and neutrophilia. White blood cell concentration did not vary markedly in the controls.

Doxey (1977) indicated the normal red blood cell concentration ranges between 5.0-9.0 μl^{-6}. Our pre-infection mean red blood cell concentration of 8.40 $\pm$ 0.57 μl^{-6} and 8.88 $\pm$ 0.85 μl^{-6} in the infected and control groups respectively, therefore, compare favourably. Red blood concentration was significantly reduced following infection. In contrast, a significant reduction in red blood cell concentration was reported only in cattle-derived but not in buffalo-derived *T. parva* infections (Maxie *et al.*, 1982). Changes in haemoglobin concentration and PCV paralleled changes in RBC concentration in this study. The decrease in the erythrocyte value was more marked as the disease progressed during which values were limited to the two surviving animals. The cause of the reduced erythrocyte values is not known. Maxie *et al.* (1982) found no evidence of haemolysis but noted a hypoproteinaemia in *T. parva* infections. A hypoproteinaemia possibly could explain our findings since

animals in this experiment lost condition after infection.

Loss of condition has been reported in *T. parva* infection (Henning, 1956; Neitz, 1957). In this experiment, all the infected animals suffered variable degrees of weight loss. The eight heifers that died of the infection lost between 5-18% of their initial body-weight while the two recovered animals lost 28% and 29% of their initial body weight. The body weight loss in each of the two recovered animals was more than in any of the recovered animals in an earlier experiment (Chapter 3). Recovery was slow in the two recovered animals and both animals had not regained their pre-infection weights up to four and six months post-infection unlike in our earlier experiment (Chapter 3).

At necropsy, gross lesions which have been described for theileriosis (Steck, 1928; Henning, 1956; De Kock, 1957; Neitz, 1957; Barnett, 1960; Munyua *et al.*, 1973) were observed in major organs and viscera in all animals that died of acute disease. However, of the recovered animals, only the animal sacrificed on day 134 post-infection had a shrunken spleen and renal pseudoinfarcts suggesting that the lesion development in *T. parva* infections is not progressive. Apart from serosal haemorrhages in the uterus which were also reported in our earlier work (Chapter 3) no other gross changes except for a scanty exudate in one animal were observed in the reproductive organs examined. However, histological findings in this animal did not indicate an endometritis. In the ovaries, follicles at various stages of development and in some animals a CL were observed suggesting functional ovarian activity.

Histological changes in the ovaries, uterus and the pituitary gland were very similar to those reported in our earlier work (Chapter 3), consisting mainly of a mononuclear cell infiltration. Of the ten infected animals there were no histological lesions in the ovaries, uterus and pituitary of the two recovered animals and one animal that died of acute disease suggesting that there may be no simple relationship between the severity of infection and development of lesions. Alternatively, it may be postulated that in severe reactions, some animals die before allowing enough time for

the development of lesions in these organs while in recovered cattle, there are mechanisms responsible for the arrest/reversal of the progression of lesion development. Generally, lesions in the reproductive tract were mild and observed in a few animals suggesting that these are unfavourable sites.

In the endometrium, the mild and diffuse mononuclear cell infiltration suggested no abnormality (McEntee, 1990). However, the mild to moderate mononuclear cell infiltration of the myometrium and perimetrium in some animals suggested some abnormal cellular presence that could be linked to theileriosis. In the ovaries only the CL, when it was involved, appeared to be severely affected. However, in spite of the moderate to severe cellular infiltration, P4 levels revealed maintained luteal activity indicating no adverse effect of the infiltration. In the pituitary, focal mononuclear cell infiltrations and increased numbers of lymphocytes in vascular lumina were observed. Dolan (1986a) on the other hand reported vascular occlusion by lymphoid cells in the adenohypophysis. The changes observed in the adrenal glands were similar to those reported by Dolan (1986a) and in our earlier work (Chapter 3).

All the ten infected animals were cycling regularly before infection based on behavioural oestrus detection. However, ovarian structures and P4 profiles revealed that two of these animals (027B and 032B) had a period of about three weeks of luteal dysfunction preceding infection. The cause of the luteal dysfunction was not known but since these two animals had exhibited oestrus, this would suggest a disturbance of gonadotrophin (FSH and LH) secretion or non-responsiveness of the ovary to gonadotrophins. No oestrus was exhibited post-infection in 8/10 animals that died of severe disease. However, P4 profiles and ovarian structures revealed functional ovarian changes in six of the eight and none in the two animals (027B and 032B) which had exhibited basal P4 pre-infection. One animal (018B) however, failed to exhibit oestrus during basal P4 concentrations following a cycle completed after infection. These results suggest that the infection did not interfere with ovarian

function in the six animals but interfered with oestrus manifestation in one.

The two animals that recovered from infection had long periods post-infection when oestrus was not detected, P4 concentrations remained at basal levels and rectal palpations only revealed occasional small ovarian follicles suggesting that these animals were acyclic. Although oestrus was detected after an inter-oestrus interval of 162 days in 035B, there was no evidence of subsequent luteal function suggesting either lack of pituitary gonadotrophins or impaired ovarian responsiveness to gonadotrophins.

Although various changes were found in reproductive endocrine organs i.e the pituitary, uterus and ovaries in various animals at necropsy, cyclical status and by inference, endocrine mechanisms were not compromised in any of the six animals that died of severe disease. On the other hand, one of the recovered animals (021B) completed a cycle during the macroschizont parasitosis and clinical reaction period but became acyclic subsequently in the absence of clinical disease and parasitosis suggesting that the impairment of the cyclical status in this particular animal was not as a direct effect of the parasite. A further animal (035B) ceased cycling during the period of active parasitosis and remained acyclic during the recovery period and it is therefore not clear whether the parasitosis and/or clinical reaction contributed to the acyclicity during the early post-infection period in this particular animal.

A striking feature in the two acyclic animals was the observation that cessation of oestrus manifestation and cyclical ovarian changes occurred concomitantly with severe loss in body weight. Both of these animals lost over 28% of their pre-infection bw which was not regained at the termination of the experiment. 021B was 16% below her initial bw at the time of sacrifice and had not resumed cycling. On the other hand, 035B exhibited oestrus when she achieved a liveweight of about 4% below her pre-infection bw. At the time cyclicity was arrested, 021B and 035B had lost about 19.4% and 10% of their pre-infection weights, respectively. Results in this experiment are, therefore, similar to our earlier findings (Chapter 3) where loss in condition appeared linked with acyclicity.

Our findings imply that weight loss may be involved in disruption of cyclicity following infection with *T. parva*. Studies in which body weights were decreased by gradual reduction in nutrient intake resulted in cessation of oestrous cycles which was restored when at about 88% of the initial body weight (Richards *et al.*, 1989). Other reports have also associated cessation of oestrus to low levels of nutrient intake in previously normally cycling animals (Bond *et al.*, 1958; Beal *et al.*, 1978). The physiological mechanisms whereby undernutrition, and by implication, loss in body weight causes anoestrus in cattle is understood only partially. Lack of ovarian activity has been attributed to reduced gonadotrophin secretion with decreased follicular development, absence of oestrus and lack of ovulation. Our samples were not evaluated for gonadotrophins (FSH and LH) or oestradiol. In those studies where some of these were measured, it was reported that LH was reduced while oestradiol was within normal concentrations in energy restricted heifers (Richards *et al.*, 1989). This may explain the presence of occasional follicles in our two animals and the absence of CL following behavioural oestrus during extended basal P4 concentration periods. LH concentrations in nutritionally restricted cattle however remain controversial with some reports indicating low concentrations (Beal *et al.* 1978; Richards *et al.*, 1989), high concentrations (Gombe and Hansel, 1973) or no change (Harrison and Randel, 1986).

Lack of luteal activity would suggest a disturbance of LH secretion in our study. Whether the disturbance originated at the pituitary or hypothalamic level was not evaluated. At necropsy, no pituitary lesions were observed in these two recovered animals which failed to cycle while lesions observed in the fatal cases were not linked to impairment of cyclical status suggesting that other mechanisms were involved. Beal *et al.* (1978) reported lower P4 concentrations in energy restricted compared to energy adequate groups following GnRH challenge which suggested that nutrient restriction compromises pituitary function.

Behavioural data, P4 concentrations and palpable ovarian structures were

adequate to determine that the uninfected controls had normal oestrous cycles throughout the experiment. Two animals in this group had a transient loss of body weight of 1-3% but cycled throughout.

In conclusion, this experiment demonstrated that:

a) *T. parva* parasitosis did not adversely affect the reproductive function during the early phase of infection.

b) Pathological lesions observed in the reproductive endocrine organs did not reflect any adverse effect on cyclical status.

c) Acyclicity was associated with loss in body weight and by inference lowered nutritional status as reflected in reduced erythrocyte values in acyclic animals.

It is postulated that loss in body weight influenced cyclic ovarian activity and oestrus in the heifers infected with *T. parva*. Further work is required to elucidate whether this adverse effect was at the hypothamic, pituitary or ovarian level.

Table IV-2 **Reactions of Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Anim. #	Days to								
	Schi-zonts LEG +ve	Schi-zonts LEG -ve	Schi-zonts RPG +ve	Schi-zonts RPG -ve	Piropl-asms	Fever	Reco-very	Death	Ab. resp-onse*
018B	7	20	10	21	9 (2)	9 (5)	.	21	.
019B	6	15	10	15	.	7 (7)	.	15	.
021B	9	14	12	20	.	9 (5)	20	.	+
027B	7	13	9	13	.	6 (7)	.	13	.
028B	6	13	9	13	.	8 (3)	.	13	.
031B	6	13	9	13	.	8 (4)	.	13	.
032B	6	13	9	13	.	6 (8)	.	13	.
034B	7	14	9	14	.	6 (9)	.	14	.
035B	8	28	10	17	11 (2)	8 (7)	28	.	+
340A	6	14	9	14	10 (1)	6 (7)	.	14	.
Mean ± SD	6.8 ± 1.0	15.7 ± 4.6	9.6 ± 0.9	15.3 ± 2.9	10.0 ± 0.8	7.3 ± 1.2	24.0 ± 4.0	14.5 ± 2.5	
023B	.	.	.	.	.	.	.	.	.
026B	.	.	.	.	.	.	.	.	.
036B	.	.	.	.	.	.	.	.	.
037B	.	.	.	.	.	.	.	.	.

* (+) -Titre of > 1:40 to *T. parva* schizont antigen

( ) -Number in parenthesis indicates days of piroplasmaemia or fever

LEG = Left ear gland; RPG = Right prescapular gland

Table IV-3 **Leucocyte concentration changes in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Animal #	Day to (*) and percentage (**) maximum cell concentration decline					
	Total leucocytes		Lymphocytes		Neutrophils	
	*	**	*	**	*	**
Infected						
018B	8	51.8	8	53.9	13	52.7
019B	13	73.0	13	73.3	13	74.5
021B	13	55.5	11	59.5	15	68.6
027B	6	65.1	6	66.7	6	60.1
028B	8	44.2	11	36.5	8	56.6
031B	6	40.9	6	46.3	6	29.2
032B	11	64.7	11	64.9	11	71.6
034B	11	71.6	11	72.8	11	63.3
035B	11	45.8	11	40.9	18	60.4
340A	11	59.6	11	58.7	11	52.3
Controls						
023B	29	18.1	29	32.2	.	.
026B	29	21.2	32	6.4	22	49.8
036B	18	28.8	11	25.3	20	53.8
037B	18	37.4	18	31.0	20	70.8

Table IV-4 **Leucocyte concentration in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Day post-infection	Leucocyte concentration 10^3 μl^{-1}	
	Infected	Controls
-1	13.02 ± 3.99	14.98 ± 2.32
2	13.28 ± 3.99	14.08 ± 1.71
4	12.54 ± 3.85	14.98 ± 3.14
6	9.11 ± 3.77*	15.30 ± 2.31
8	11.10 ± 3.08	18.0 ± 3.72
11	9.19 ± 5.60	13.75 ± 2.89
13	10.05 ± 4.12	14.23 ± 3.31
15	8.36 ± 3.07*	13.03 ± 2.59
18	10.40 ± 6.15	12.25 ± 3.90
20	16.67 ± 8.28	12.23 ± 2.40
22	11.20 ± 4.10	13.35 ± 2.53
25	15.30 ± 8.0	13.0 ± 3.03
27	13.10 ± 0.28	13.10 ± 3.01
29	8.05 ± 0.35*	11.78 ± 2.05
32	7.70 ± 0.71*	14.50 ± 1.89
34	12.20 ± 7.21	14.18 ± 3.33
36	7.35 ± 0.64*	14.58 ± 2.65
39	8.70 ± 1.84	14.40 ± 2.74
41	8.60 ± 3.11	14,.53 ± 2.43

* - Significantly ($p<0.05$) lower than pre-infection levels

Underlined - Significant difference ($p<0.05$) between groups

Table IV-5 **Lymphocyte, neutrophil and eosinophil concentrations in Boran/Friesian cross heifers infected with undiluted *T.parva* stabilate IL3081 or in controls**

Day p-i	Lymphocytes 10^3 μl^{-1}		Neutrophils 10^3 μl^{-1}		Eosinophils	
	Infected	Controls	Infected	Controls	Infected	Controls
-1	9.02 ± 2.87	9.88 ± 1.03	3.46 ± 0.96	4.37 ± 1.96	424 ± 236	512 ± 152
1	8.27 ± 2.83	10.10 ± 0.97	4.57 ± 1.87	3.55 ± 0.82	333 ± 169	335 ± 137
4	8.44 ± 2.84	9.70 ± 1.76	3.39 ± 0.75	5.02 ± 1.79	609 ± 359	317 ± 99
6	5.91 ± 2.19*	7.60 ± 3.86	2.89 ± 1.43	4.66 ± 1.30	214 ± 142	445 ± 376
8	7.32 ± 2.12	10.50 ± 1.36	3.33 ± 1.20	6.22 ± 2.10	339 ± 230	593 ± 140
11	5.37 ± 3.13*	8.50 ± 1.86	2.66 ± 1.72	4.46 ± 1.04	191 ± 115*	592 ± 454
13	7.13 ± 2.84	9.70 ± 1.76	2.73 ± 1.22	3.84 ± 1.46	138 ± 124*	399 ± 366
15	6.15 ± 2.61	9.30 ± 1.34	1.67 ± 0.62*	3.29 ± 0.67	23 ± 26*	318 ± 187
18	7.93 ± 3.84	8.30 ± 1.64	2.18 ± 0.93	3.57 ± 1.36	117 ± 102*	302 ± 327
20	9.40 ± 3.96	8.20 ± 0.55	5.93 ± 1.57	2.81 ± 1.62	0*	850 ± 695
22	7.55 ± 2.65	9.70 ± 1.67	3.58 ± 0.38	3.46 ± 1.01	62 ± 62*	152 ± 185*
25	6.95 ± 1.55	9.30 ± 1.39	8.14 ± 1.87*	3.36 ± 1.22	76 ± 1*	285 ± 143
27	5.50 ± 0.65*	9.40 ± 1.30	7.70 ± 0.80*	3.56 ± 1.49	163 ± 30*	175 ± 73*
29	4.35 ± 1.15*	4.35 ± 1.15	3.46 ± 1.43	3.24 ± 0.86	80 ± 37*	251 ± 169
32	3.40 ± 0.9*	9.60 ± 1.44	4.26 ± 1.40	4.31 ± 1.43	20 ± 20*	367 ± 274
34	5.85 ± 1.85	9.50 ± 1.97	6.01 ± 2.89	4.35 ± 1.67	173 ± 173	256 ± 240
36	3.60 ± 0.4*	9.50 ± 1.18	3.54 ± 0.04	4.71 ± 1.44	110 ± 7*	278 ± 330
39	3.75 ± 1.35*	9.70 ± 1.83	4.63 ± 0.03	4.61 ± 1.19	161 ± 61*	126 ± 94*
41	3.80 ± 0.1*	8.90 ± 1.39	5.15 ± 1.6	5.31 ± 1.05	140 ± 76*	245 ± 148

p-i - post-infection
* - Significantly ($p<0.05$) different to pre-infection levels
Underlined - Significant difference ($p<0.05$) between groups

Table IV-6 **Red blood cell concentration, haemoglobin concentration and packed cell volume in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Day p-i	RBC concentration 10^6 μl^{-1}		Hb. concentration gm dl^{-1}		PCV %	
	Infected	Controls	Infected	Controls	Infected	Controls
-1	8.40 ± 0.57	8.88 ± 0.85	12.69 ± 1.15	13.75 ± 0.99	32.0 ± 2.9	35.3 ± 2.2
1	8.4 ± 0.52	8.38 ± 0.63	11.96 ± 0.74	12.50 ± 0.88	33.0 ± 1.9	34.5 ± 1.9
4	8.05 ± 0.90	8.63 ± 0.50	12.40 ± 1.31	13.18 ± 1.07	34.2 ± 3.2	35.5 ± 2.9
6	8.20 ± 0.89	8.75 ± 1.19	12.02 ± 0.99	12.95 ± 1.17	32.8 ± 2.6	34.8 ± 3.8
8	7.05 ± 0.69*	8.13 ± 0.25	10.73 ± 0.79*	13.43 ± 1.07	29.7 ± 1.3*	36.5 ± 3.1
11	8.35 ± 1.0	8.63 ± 0.48	11.59 ± 0.94*	12.88 ± 0.45	28.6 ± 2.3*	33.5 ± 2.1
13	6.55 ± 0.83*	8.0 ± 0.41	10.21 ± 1.35*	12.75 ± 0.71	28.3 ± 2.8*	34.0 ± 2.8
15	7.50 ± 1.50	8.88 ± 0.85	10.46 ± 1.81*	12.60 ± 0.61	28.5 ± 4.4	34.0 ± 0.8
18	8.17 ± 0.29	8.13 ± 0.85	10.40 ± 0.89*	10.95 ± 0.75*	30.0 ± 1.7	30.3 ± 1.5*
20	7.0 ± 0.5*	7.75 ± 0.65	10.73 ± 1.31	11.43 ± 0.86*	31.0 ± 2.6	31.5 ± 3.0
22	6.75 ± 1.77	7.75 + 0.29	9.15 ± 2.33	11.43 ± 1.27*	32.5 ± 0.7	33.5 ± 2.9
25	5.75 ± 0.35*	7.88 ± 0.48	8.45 ± 1.48*	12.30 ± 0.54	24.5 ± 2.1*	33.5 ± 2.4
27	7.0 ± 1.41	7.63 ± 0.75	9.15 ± 3.04	11.30 ± 0.45*	22.5 ± 0.7*	32.3 ± 2.9
29	4.5 ± 0.71*	6.75 ± 0.87*	6.25 ± 1.77*	9.70 ± 1.34*	23.5 ± 2.1*	29.0 ± 3.4*
32	5.0 ± 0.71*	8.0 ± 1.83	7.40 ± 1.27*	11.75 ± 1.86	22.5 ± 2.1*	35.5 ± 3.1
34	5.25 ± 1.06*	8.5 ± 0.41	7.85 ± 0.64*	12.43 ± 0.85	24.5 ± 0.7*	36.0 ± 1.4
36	6.75 ± 0.35*	9.25 ± 0.5	7.75 ± 0.78*	11.13 ± 0.66*	22.0 ± 1.4*	31.3 ± 2.5
39	5.5 ± 0.71*	7.88 ± 0.25	8.65 ± 1.34*	12.13 ± 0.62	26.0 ± 2.1*	34.0 ± 2.1
41	5.5 ± 0.07*	8.63 ± 0.48	8.55 ± 0.07*	12.38 ± 0.49	26.0 ± 0.7*	35.0 ± 1.6

* - Significantly ($p<0.05$) lower than pre-infection levels
Underlined - Significant difference ($p<0.05$) between groups
p.i - Day post-infection

Table IV-7 **Changes in cyclical status and body weights in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Animal #	Status	Weeks to			Max.bw loss (%)	Days to resumed oestrus
		Initial bw loss	Max. bw loss	bw regain		
018B	Infected	1	3	.	18	.
019b	Infected	1	2	.	14	.
021B	Infected	1	4	.	29	.
027B	Infected	1	2	.	5	.
028B	Infected	1	2	.	9	.
031B	Infected	1	2	.	12	.
032B	Infected	1	2	.	8	.
034B	Infected	1	2	.	17	.
035B	Infected	1	3	.	28	165*
340A	Infected	1	2	.	8	.
023B	Control	1	1	2	2	Cyclic**
026B	Control	1	1	2	3	Cyclic
036B	Control	1	1	2	1	Cyclic
037B	Control	.	.	.	.	Cyclic

* - Day to observed oestrus

** - Cycled throughout

Table IV-8 **Cycle lengths in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Animal #	Status	Cycle length (days)			
		Pre-infection		Post-infection	
			Mean ± SD		Mean ± SD
018B	Infected	18, 19, 17	18.0 ± 0.8	.	.
019B	Infected	21, 22, 20	21.0 ± 0.8	.	.
021B	Infected	18, 25	21.5 ± 3.5	.	.
027B	Infected	21, 21	21	.	.
028B	Infected	26, 25	25.5 ± 0.5	.	.
031B	Infected	21, 20	20.5 ± 0.5	.	.
032B	Infected	20, 23, 15	19.3 ± 3.3	.	.
034B	Infected	19, 18, 21	19.3 ± 1.2	.	.
035B	Infected	24	24	162, 24	
340A	Infected	26, 18, 16	20.0 ± 4.3	.	.
Mean			21.8 ± 6.2		
023B	Control	23, 19, 22	21.3 ± 1.7	21, 23, 20, 22, 22	21.6 ± 1.0
026B	Control	20, 23, 25	22.7 ± 2.1	20, 22, 23, 21	21.5 ± 1.1
036B	Control	19, 26, 21	22.0 ± 2.9	25, 22, 22, 21	22.5 ± 1.5
037B	Control	20, 20, 20	20	22, 20, 21, 23, 19	21.0 ± 1.4
Mean			21.5 ± 2.2		21.3 ± 1.3

Table IV-9 **Adjusted cycle lengths* in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 or in controls**

Animal #	Status	Cycle length (days)			
		Pre-infection		Post-infection	
			Mean ± SD		Mean ± SD
018B	Infected	18, 19, 17	18.0 ± 0.8	18	18
019B	Infected	21, 22, 20	21.0 ± 0.8	.	.
021B	Infected	18, 25	21.5 ± 3.5	28	28
027B	Infected	21	21	.	.
028B	Infected	26, 25	25.5 ± 0.5	.	.
031B	Infected	21, 20	20.5 ± 0.5	.	.
032B	Infected	20, 23	21.5 ± 1.5	.	.
034B	Infected	19, 18, 16	19.3 ± 1.2	.	.
035B	Infected	21, 21, 21	21	.	.
340A	Infected	26, 18, 16	20.0 ± 4.3	.	.
Mean			20.5 ± 2.8		22.5 ± 5.5
023B	Control	23, 19, 22	21.3 ± 1.7	21, 23, 20,22,22	21.6 ± 1.0
026B	Control	20, 23, 25	22.7 ± 2.9	20, 22, 23, 21	21.5 ± 1.1
036B	Control	19, 26, 21	22.0 + 2.9	25, 22, 22, 21	22.5 ± 1.5
037B	Control	20, 20, 20	20	22, 20, 21, 23, 19	21.0 ± 1.3
Mean			21.5 ± 2.2		21.3 ± 1.3

* - Cycle lengths adjusted using P4 profiles

Table IV-10 **Gross findings in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081**

Animal #	018B	019B	021B	027B	028B	031B	032B	034B	035B	340A
Days to death	21	15	134	13	13	13	13	14	210	14
Condition at death	Poor	Fair	Poor	Good	Good	Fair	Good	Fair	Fair	Fair
Abomasum	oe,hr	oe,h	.	h	h,u	oe,u	oe,h	oe,h	.	oe,h
Small intestine	.	h	.	.	h	hr	.	h	.	h
Large intestine	h	h	.	.	.	h,hr	.	h	.	h
Liver	.	e	.	i	e,i	e,c	.	e,c	.	e,c
Gall bladder	tb	tb	.	h	tb	tb	tb	tb	.	tb
Lymph nodes	e,hr	e,h	.	e,hr	e,hr	oe,hr	e	e,h	.	e,hr
Spleen	wp	e	s	e	e,wp	s	wp	.	.	.
Kidneys	c,pi	c,pi	pi	pi	.	c,pi	.	c	.	c
Urinary bladder	.	h	.	.	h	h	h	h	.	h
Trachea/ bronchi	f	f,h	.	f	f	f,m	f	f	.	f
Lungs	hr	oe,pn	.	oe,pn	oe,pn,hr	oe,pn,hr	oe,pn	oe,pn,h	.	oe,pn,h
Heart	.	hp	.	.	h	.	hp	h	.	.
Brain	.	.	.	.	.	.	.	.	.	.

c = congestion; e = enlarged; f = froth; h = haemorrhages; hp = hydropericardium; hr = hyperaemia; i = icterus; m = mucoid exudate; oe = oedema; pi = pseudoinfarcts; pn = pneumonia; s = shrunken; tb = turbid bile; u = ulcers; wp = prominent white pulp

Table IV-11 **Gross findings in the reproductive tract of Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081**

Animal #	Stage in cycle at death	Right ovary			Left ovary			Uterus
		Size*	Follicles	CL**	Size	Follicles	CL	
018B	Follicular	30x20x12	Tertiary	.	22x17x12	Tertiary	.	.
019B	Luteal	37x18x13	Tertiary	.	37x18x13	Small	Regressing	.
021B	Acyclic	25x15x13	Tertiary	.	25x15x18	Small	.	.
027B	Acyclic	20x10x10	Tertiary	.	20x10x10	Tertiary	.	h (serosal)
028B	Luteal	35x20x15	.	.	30x15x20	Small	25; cavity	h (serosal)
031B	Luteal	28x18x12	Tertiary	.	25x18x18	.	25	.
032B	Acyclic	30x20x17	Tertiary	.	30x17x15	.	.	Exudate
034B	Luteal	50x13x13	.	18	20x18x18	Small	.	.
035B	Luteal	26x17x12	Tertiary	.	25x15x12	Small	Regressing	.
340A	Luteal	50x37x25	Small	28	37x25x25	Small	.	h (serosal)

* - Size of ovary in mm
** - Diameter of CL in mm
h - Haemorrhages

Table IV-12 **Histological findings in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081**

Animal #	018B	019B	021B	027B	028B	031B	032B	034B	035B	340A
Cycle stage	F	L	Acy	Acy	L	L	Acy	L	L	L
Status	Died	Died	RR	Died	Died	Died	Died	Died	RR	Died
Endometrium										
Cell infiltration	.	1	.	1	.	.	1	1	.	1
Oedema	1	.	.	.	.	.	1,hr	.,hr	.	1
Myometrium										
Cell infiltration	.	.	.	1	.	.	2	2	.	2
Perimetrium										
Cell infiltration	.	.	.	.	.	.	2	.	.	3
Oedema	.,hr	.	.	.	.	.	1	.,hr	.	2
Ovary										
Cell infiltration										
Ov. cortex	.	.	.	2	1	.	1	.	.	1
Ov. medulla	1	.	.	2	2	.	1	.	.	.
CL	.	.	.	.	.	.	.	3	.	2
Pituitary gland										
Cell infiltration										
Anterior lobe	1	.	.	.	1	.	1	.	.	.
Posterior lobe	.	.	.	.	.	.	.	.	.	.
Adrenal gland										
Cell infiltration										
Z.g.	1	.	.	1	2	.	.	2	.	1
Z.f.	.	.	.	1	1	.	.	1	.	1
Z.r.	.	.	.	1	2	.	.	2	.	2
medulla	2	.	.	1	2	.	.	1	.	2

L = luteal; F = follicular phase; Acy = acyclic; RR = reacted and recovered; Z.g = *zona glomerulosa*; Z.f. = *zona fasciculata*; Z.r. = *zona reticularis*, . = No change; 1 = Mild change; 2 = Moderate change; 3 = Severe change; hr = hyperaemia

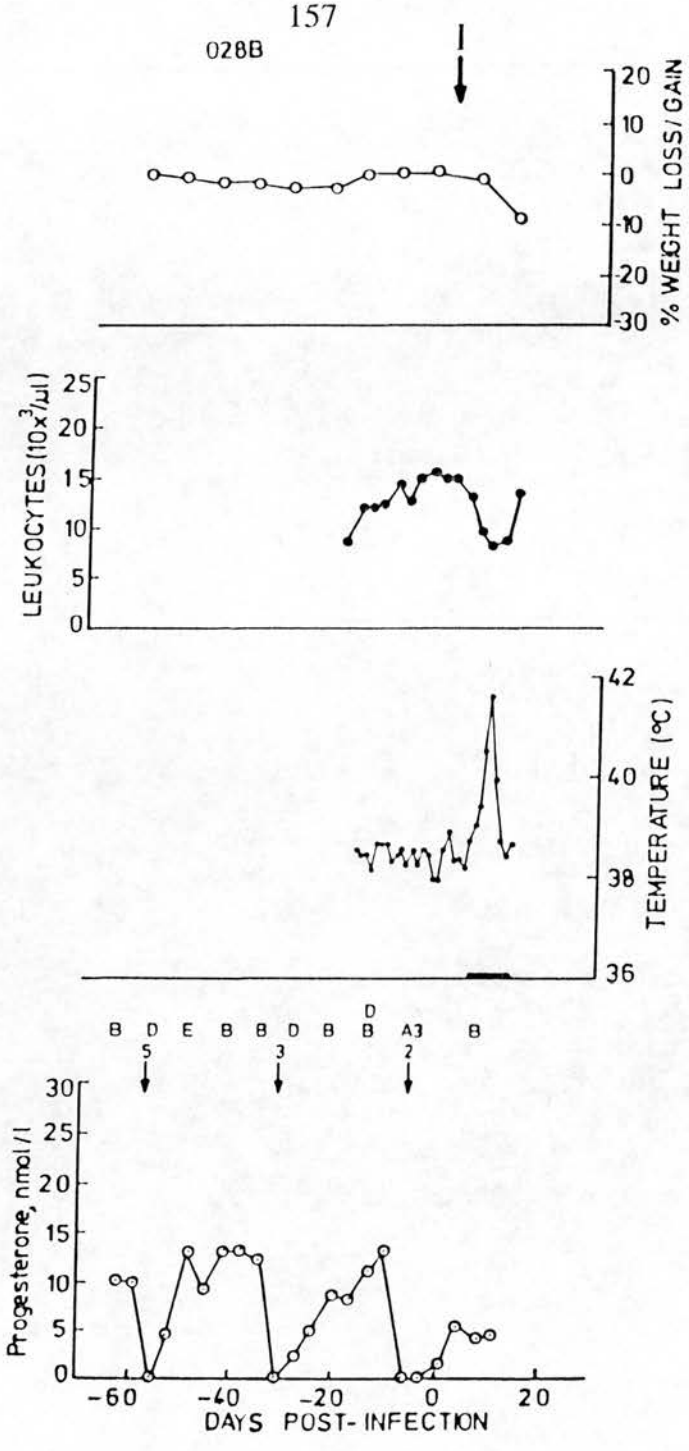


Fig. IV-1.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 028B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; D = Follicles; E = No detectable structures. Arrows indicate observed oestrus and oestrous scores in superscript. Arrow [I] = Infection.

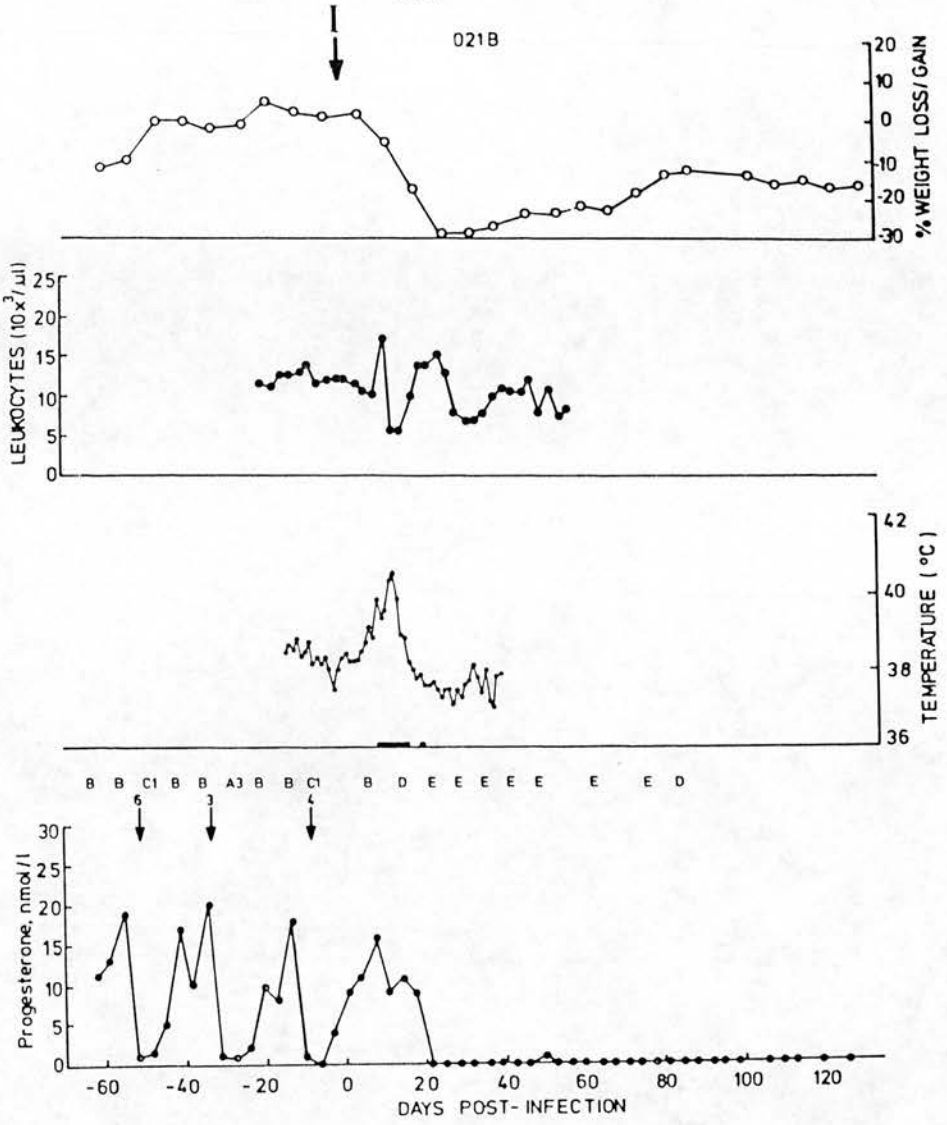


Fig.IV-2.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 021B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate observed oestrus and oestrous scores in superscript. Arrow [I] = Infection.

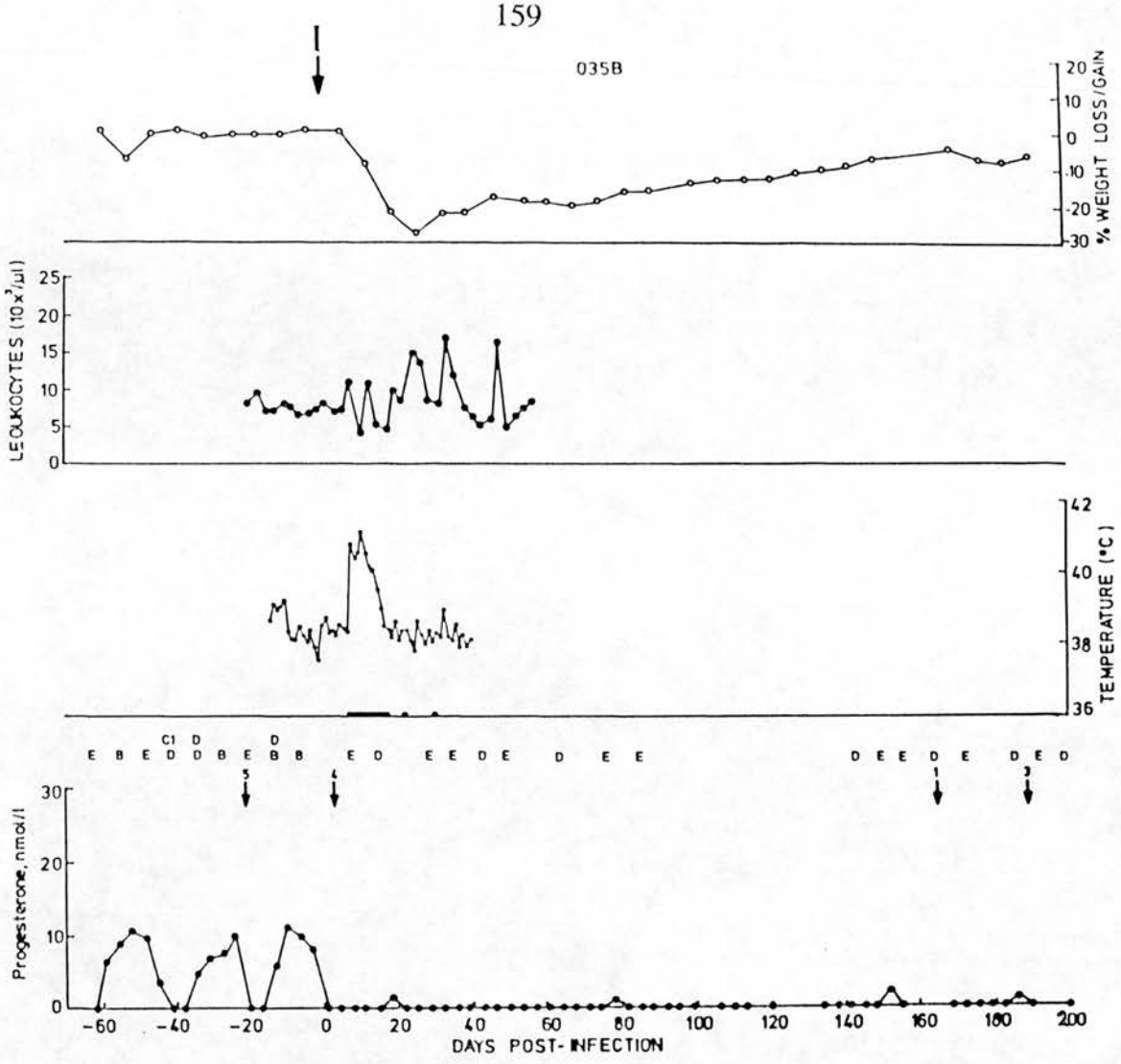


Fig.IV-3.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and body weight change in heifer 035B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate occurrence of oestrus and oestrous scores in superscript. Arrow [I] = Infection.

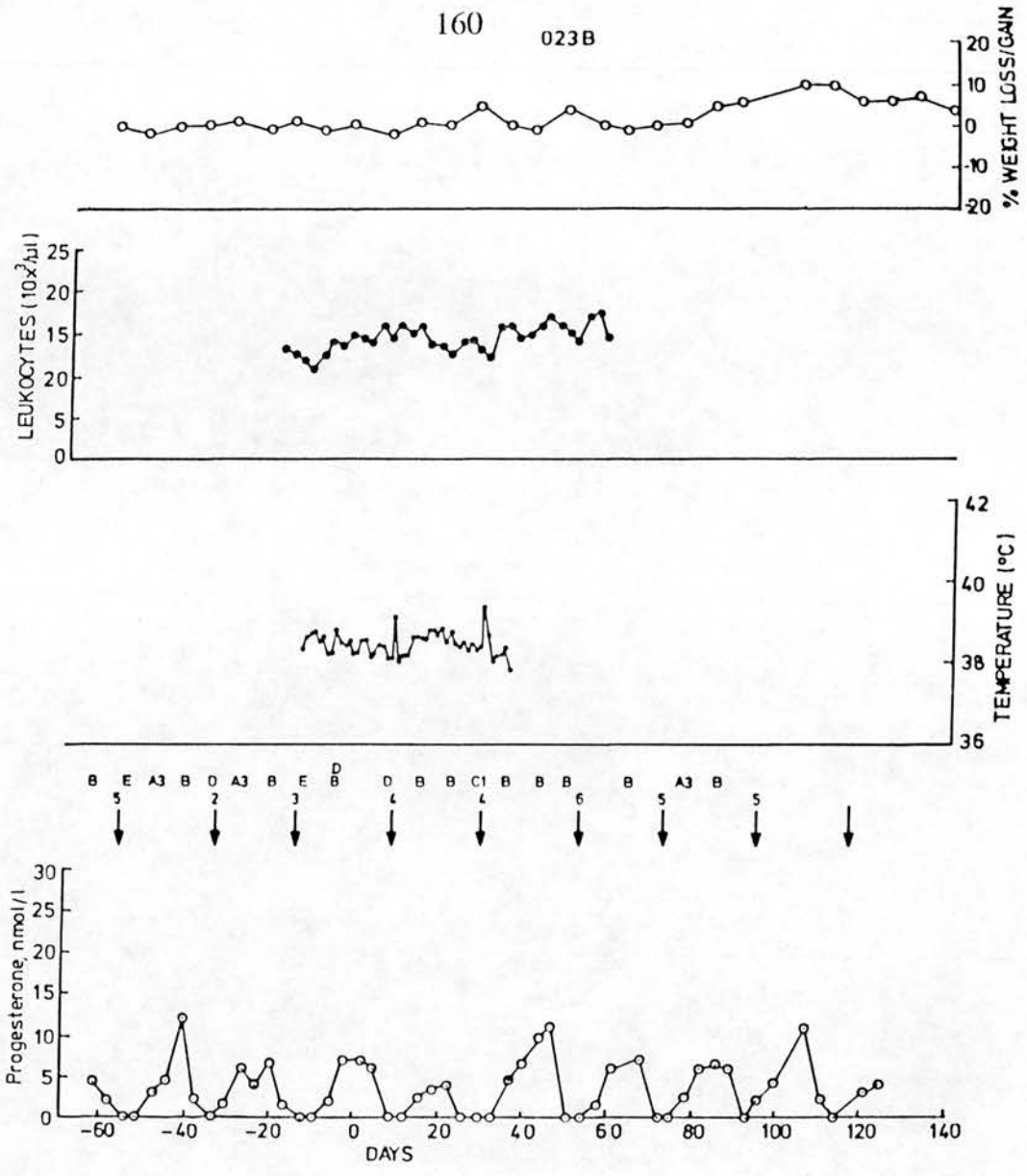


Fig.IV-4.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and body weight change in control heifer No. 023B. Weight is expressed as a percentage of initial weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows indicate observed oestrus and oestrous scores in superscript.

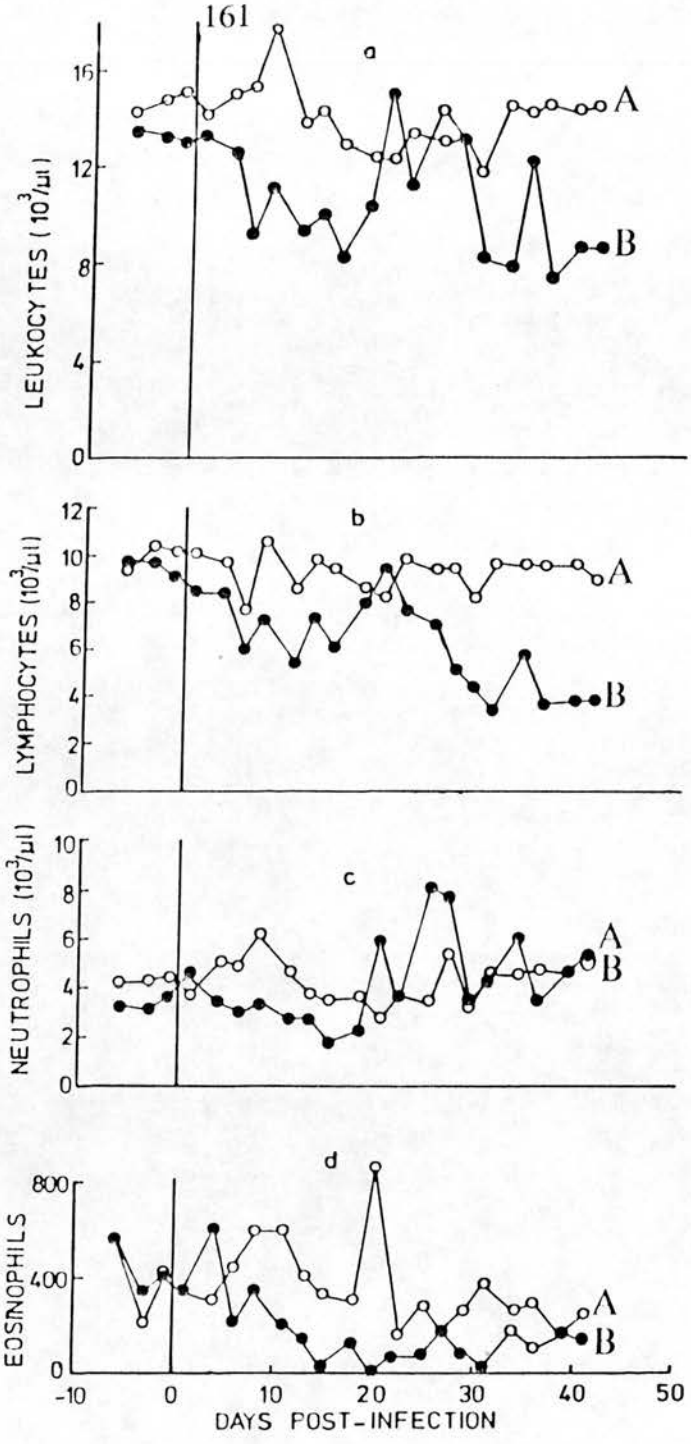


Fig.IV-5.

Changes in leucocyte (a), lymphocyte (b), neutrophil (c) and eosinophil (d) concentrations in control (A) and Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 (B).

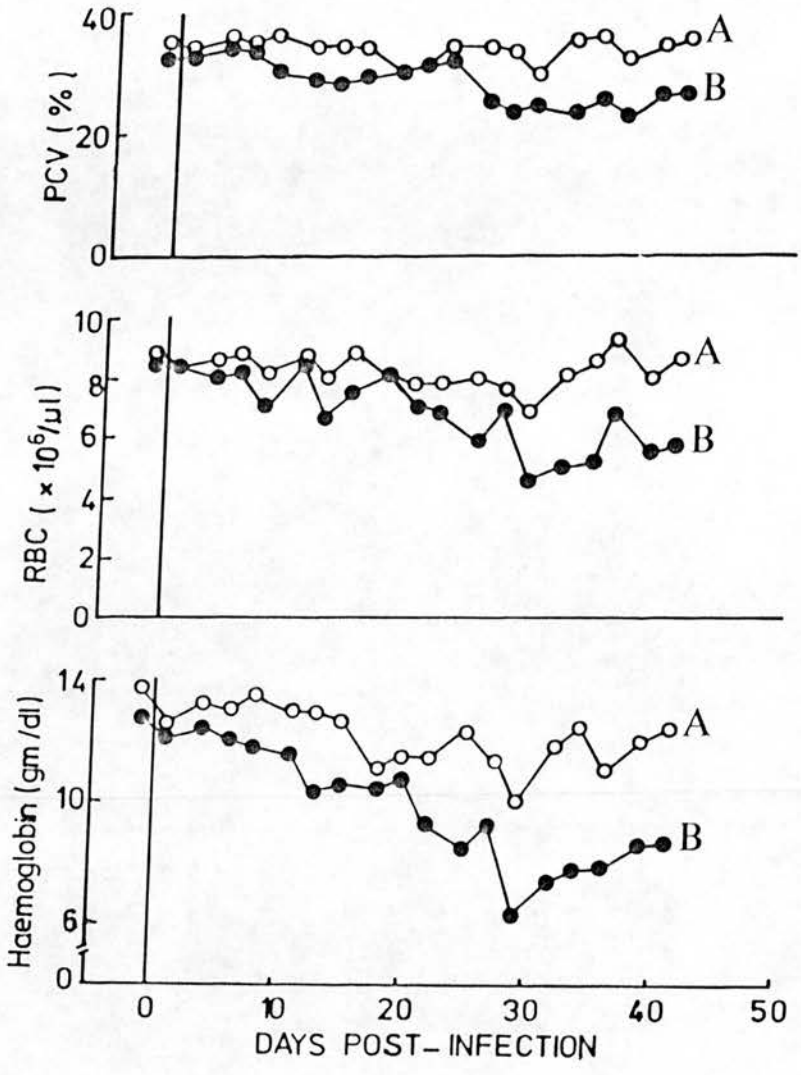


Fig.IV-6.

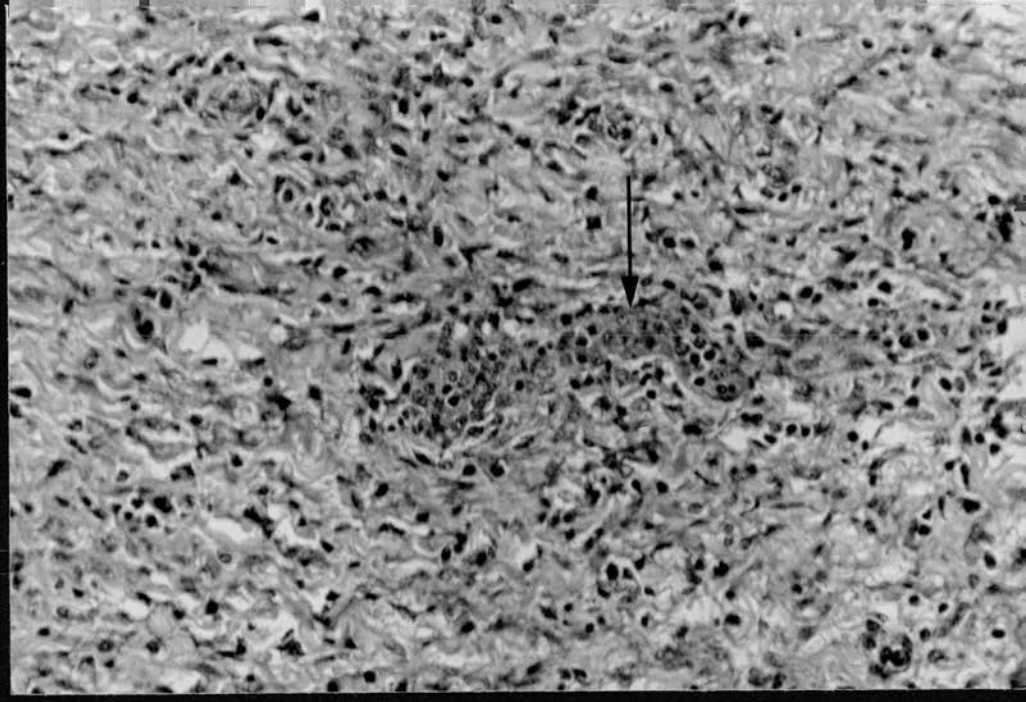
Changes in packed cell volume (PCV), red blood cell concentration (RBC) and haemoglobin concentration in control (A) and in Boran/Friesian cross heifers infected with undiluted *T. parva* stabilate IL3081 (B).

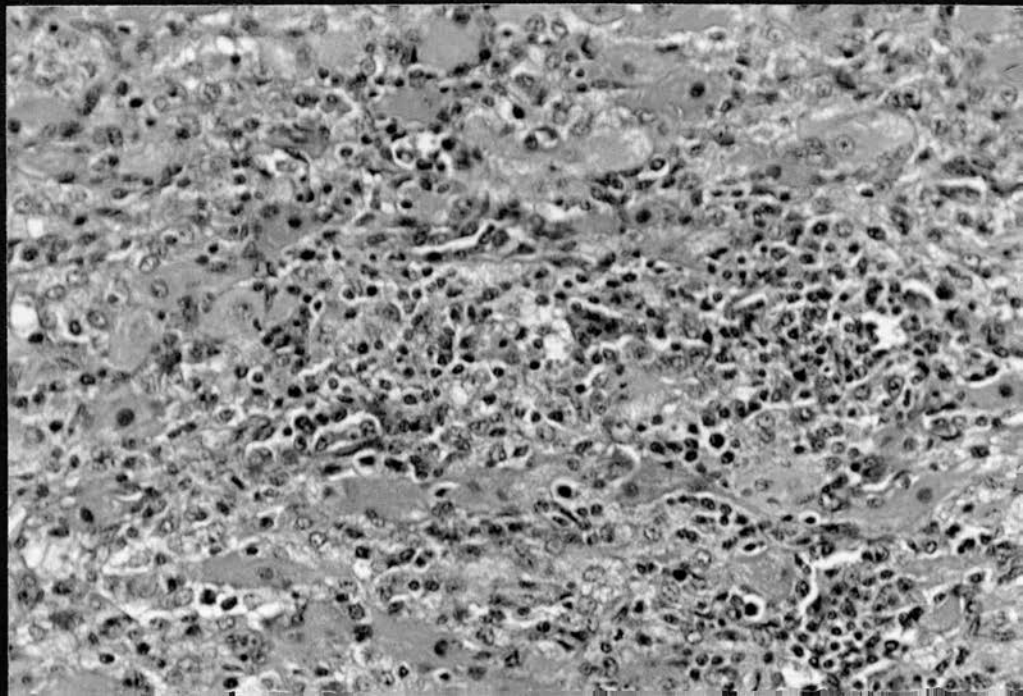
Fig. IV-7.

Section of ovary of heifer No.340A which died on day 14 post-infection showing a mononuclear cell infiltration of the ovarian medulla. Increased number of mononuclear cells in vascular lumen [Arrow]. H&E, (x350).

Fig. IV-8.

Section of ovary of heifer No. 340A which died on day 14 post-infection showing a moderate infiltration of the *corpus luteum* by mononuclear cells. H&E, (x350)





CHAPTER FIVE

THE EFFECT OF INFECTION AND TREATMENT AGAINST THEILERIOSIS ON THE REPRODUCTIVE FUNCTION OF BORAN/FRIESIAN CROSS HEIFERS

5.1 Introduction

The initial demonstration by McHardy *et al.* (1976) that a quinone, menoctone, had anti-theilerial activity against *T. parva* parasites both *in vitro* and *in vivo* was a major breakthrough which led to later developments of related compounds that are now available for chemotherapeutic control of bovine theileriosis. While mortality rates are known to be extremely high in the absence of treatment, it is however widely known that recovery from theileriosis does not confer a sterile immunity and may be followed by parasite recrudescence as well as clinical exacerbations. Anecdotal evidence suggests that recovered cattle suffer some varying degree in loss of productivity. However, no investigations have been carried out to find out the long term effects of chemotherapy in *T. parva* infection on reproductive function.

The objective of the study was therefore to investigate, under controlled laboratory conditions, whether after infection and treatment the female bovine suffered any loss in reproductive function. To achieve this objective, reproductively sound heifers were infected and treated at the height of clinical disease. Subsequently they were monitored, with particular reference to cyclical behaviour, by use of behaviourial detection of oestrus, palpation of the reproductive tract and analysis of serum progesterone concentrations.

In addition, in light of earlier evidence of impaired cyclical status, a decision was taken to examine the pituitary responsiveness to exogenous gonadotrophins and fertility status by exposing some animals to a fertile bull. Further, the decision was taken to, in light of the experimental protocol of serial kill, that a range of major organs and viscera would also be examined histologically with the intention of providing some idea of disease progression after treatment.

5.2 Materials and methods

5.2.1 Materials

5.2.1.1 Animals

These are described in General Materials and Methods (2.1).

5.2.1.2 Parasite

Details of the *T. parva* stabilate are described in General Materials and Methods (2.2)

5.2.1.3 Drugs used

1) A synthetic prostaglandin $F_2\alpha$ analogue, Luprostiol, formulated at 7.5mg/ml (Prosolvin, Intervet International BV, Holland).
2) A synthetic gonadotrophin releasing hormone (GnRH analogue, Fertagyl, Intervet International BV, Holland). The active ingredient was formulated at 100μg/ml.
3) Parvaquone (2-hydroxy-3-cyclohexyl-1, 4-naphthoquinone, Clexon, Coopers Animal Health Ltd., UK). The active ingredient was formulated at 150mg/ml in dimethyl sulphoxide and corn oil.
4) A short-acting formulation of oxytetracycline hydrochloride with magnesium chloride and propylene glycol formulated at 100mg/ml active base (Medamycin 100, Techamerica Group Inc., U.S.A.).

5.2.2 Experimental design

A summary of the experimental design is presented in Table V-1.

Thirteen days and again three days to the start of the experiment, all the 18 heifers were given 2 ml of Prosolvin intramuscularly to synchronize them into oestrus.

From behavioural data and P4 profiles, 16 of the eighteen heifers were successfully synchronized to be around oestrus at the time of infection. Of the other two animals, one animal (602B) was observed in oestrus on the day of infection but its P4 profile pre-infection and post-infection revealed basal P4 concentrations indicative of luteal dysfunction. The other animal (586B) responded to the first $PGF_2\alpha$ but not the second and was in the early luteal phase at the time of infection.

The animals were allocated at random into two groups of 14 and 4 animals each. Each of the fourteen animals was inoculated with 1 ml of undiluted stabilate as described in General Materials and Methods (2.3). The day of stabilate inoculation is referred to as day 0. Once a febrile reaction was observed in any infected animal, it was given a single dose of Medamycin at 10mg/kg bw with the objective of alleviating clinical reactions. Heifer No 036B was however from the onset treated with Clexon/Medamycin due to sudden development of severe clinical reaction. Further, following severe clinical disease reactions, animals were treated with Clexon/Medamycin at a dose rate of 10mg/kg bw of each compound. Twelve animals received a double dose of Clexon/Medamycin 48 h apart. Of the other two animals, 025B responded very favourably to the first treatment and was not retreated while 023B was given a repeat double treatment eight weeks post-infection following a clinical exacerbation.

The 14 infected animals were randomly allocated into two groups of eight and six animals. The eight animals (designated as group A) were further allocated into four groups of two animals each and subjected to a serial kill on days 18, 38 and 83. Of the two animals expected to be killed lastly, one died on day 101 and the other was killed on day 105 post-infection. Further, animals in group A were administered with 100μg GnRH (Fertagyl) intramuscularly and sampled for LH as described in General Materials and Methods (2.13). Each of the eight animals was challenged twice with GnRH except the two animals (592B and 602B) which were killed on day 18 post-infection. The last four animals in the serial kill (025B, 030B, 023B and 586B) were

also studied for carrier status as described in General Materials and Methods (2.8 and 2.9). The other six infected animals (designated as group B) were exposed to a bull from day 23 post-infection. Since animals in this group were seen to be cycling at the expected time of terminating the experiment, a decision was made to monitor them to day 163 for P4 concentrations and to day 200 for behavioural oestrus.

The four uninfected animals were each administered with Clexon/Medamycin at the same dose rate as the infected animals on day 10 and acted as controls. Further, they were sampled for LH on each occasion alongside the group A animals.

All cattle except those killed on day 18 post-infection were bled on day 34 and their sera tested on IFAT for *T. parva* antibodies. Blood for serology for animals killed on day 18 were collected immediately before the animals were killed.

The monitoring protocol is presented in Table V-1. The methodologies are described in detail in General Materials and Methods.

Serum P4 concentrations were analysed by the IAEA supplied assay kit. The inter-assay coefficients of variation were 11.2% (n=9) at 9.0 $\pm$ 0.7 (mean $\pm$ SD) nmol/L, 14.8% (n=9) at 24.7 $\pm$ 2.5 nmol/L and 9.4% (n=9) at 48.1 $\pm$ 3.1 nmolL for the low, medium and high quality controls, respectively. The intra-assay CVs were 4.7%, 5% and 4.1% for the low, medium and high QCs, respectively.

Table V-1 **Summarized experimental protocol (Experiment 3)**

Parameter monitored	Frequency and duration		
	Group A	Group B	Controls
Oestrus behaviour	Daily (x3) : d -90 to d 100	Daily (x3) : d -90 to d 200	Daily (x3) : d -90 to d 100
Body weight	Weekly : Week -12 to 14		
Serum P4*	Twice weekly : d -57 to d 100	Twice weekly : d -57 to d 163	Twice weekly : d -57 to d 100
Rectal palpations	Weekly : Week -12 to 14		
Rectal temperature	Daily : d -6 to d 34 or death		
Haematology	Twice weekly : Week -12 to 14		
$PGF_2\alpha$ 1st	d -13		
$PGF_2\alpha$ 2nd	d -3		
Infection	d 0		ND
L/N biopsies			
LEG	Daily: d 5 to recovery		ND
RPG	Daily : Earliest d 9 to recovery		ND
Blood smears	Daily : Earliest d 9 to recovery		
Treatment			
Medamycin	1st day of fever		
Clexon/ Medamycin	x1 to x4 (see Table V-2)		d 10
Serology	d 18 :592B;602B d 34 : others	d 34	
GnRH Challenge & LH sampling	592B,602B:d 14 587B,020B:d 14,35 025B,030B:d 35,56 023B,586B:d 56,91	ND	024B,583B: d 14,35,56,91 037B,582B: d 14,35,56
Parasite isolation studies	025B,030B:d 64 to 71 023B,586B:d 85 to 101	ND	ND
Cell culture	025B,030B:d 58 023B,586B :d 97	d97	ND
Exposure to bull	ND	d 23-200	ND
Necropsy	592B,602B:d 18 587B,020B:d 38 025B,030B:d 83 023B :d 101 586B :d 105	d 210-220	ND

* - IAEA supplied assay (2.12.2.1)

d - Days relative to infection

5.3 Results

5.3.1 Clinical signs

The animal responses to infection and chemotherapy are summarized and presented in Table V-2. Temperature changes for representative animals are presented in Figs. V-1 to V-6.

All the infected animals developed fever within 6-9 (mean $\pm$ SD, 6.9 $\pm$ 0.9) days after infection. Temperatures dropped to normal within a day in two animals (025B and 594B) following treatment with Medamycin. However, the effect was transitory and both animals developed a febrile relapse within a day of the temperature decline.

Following the first Clexon/Medamycin treatment, temperatures dropped to normal levels in two animals (025B and 036B) within a day. Both animals had recrudescences of fever but 025B (Fig. V-3) recovered without, while 036B (Fig. V-6) recovered after a repeat treatment with Clexon/Medamycin. All the other 12 infected animals responded to the second Clexon/Medamycin treatment with temperatures dropping to normal within 1-2 days. Of the twelve animals, two (020B and 030B) recovered without a febrile relapse while the other ten developed intermittent febrile reactions. Of the latter 10 animals, two were killed on day 18 before temperatures had dropped to normal (Fig. V-1) while in the other eight, temperatures eventually dropped to and were maintained at below 39.5^{o}C. However heifer No.023B had a clinical exacerbation on day 66 post-infection with elevated body temperatures above 39.5^{o}C for four days. Temperature dropped to and was maintained at normal levels following a double treatment with Clexon/Medamycin. The febrile reaction period ranged from 6-14 (mean, 8.7 $\pm$ 2.8) days for the twelve recovered animals (Table V-2).

The major clinical signs were observed once fever had set in. All the infected animals developed a staring hair coat, became dull and anorectic, had laboured respiration and developed serous nasal and ocular discharge. A cough which persisted for two to seven days developed in three animals (025B, 036B and 592B), a stiff gait

in five animals (022B, 026B, 030B, 036B and 602B) and submandibular oedema in five (022B, 026B, 030B, 036B and 602B). Submandibular oedema developed suddenly during the initial temperature rise in three animals (022B, 030B and 036B) and during the second febrile reaction period in 026B and 602B. Peripheral lymph node enlargement occurred in all infected animals within 5 to 9 days of infection. There was an alleviation of most of the clinical signs after Clexon/Medamycin treatment but intermittent periods of dullness and anorexia were observed in all animals up to the third week of infection.

Three animals (023B, 026B and 594B) developed a bilateral corneal opacity eight weeks post-infection. This cleared in two weeks except in 023B which eventually became blind.

Heifer No. 023B developed typical signs indicative of theileriosis which included fever, swollen peripheral lymph nodes and a serous nasal and ocular discharge eight weeks post-infection. No parasites were however detected in lymph node biopsy and blood smears although it responded to Clexon/Medamycin treatment. Two weeks later, it became anorectic and recumbent over a period of two weeks. It became progressively weaker and emaciated until death 101 days post-infection.

The four control animals (024B, 037B, 582B and 583B) remained healthy throughout the study period. Rectal temperatures monitored at the same time as in the infected animals remained at normal levels (Table V-2). Temperature changes presented for 024B (Fig. V-7) were characteristic of the controls.

5.3.2 Parasitology

The mean pre-patent period was 6.3 ± 0.9 (range 5-8) days. Macroschizonts were detected on the first day (day 5) of sampling of the regional drainage lymph node (LEG) in three animals (022B, 029B and 592B). Macroschizonts were detected earlier in the regional lymph node than in the contra-lateral prescapular gland (RPG) in all animals except in No. 587B where they appeared on the same day. The mean time to

the appearance of macroschizonts in the RPG was 9.4 ± 1.3 (range 8-12) days. The difference in the time to detection of parasites in both glands ranged from 0 to 6 days and there was no clear-cut evidence to correlate this difference to the early or late prepatency in individual animals.

Medamycin which was administered at 10mg/kg bw on the first day of fever did not seem to affect the detection of macroschizonts in the sampled lymph glands. After the first treatment of Clexon/Medamycin, macroschizonts disappeared in 025B within two days but were detected for a single day three days later. This animal was not retreated. Parasites disappeared briefly after the first treatment with Clexon/Medamycin in two (036B and 602B) of the thirteen animals which received a repeat treatment. All these thirteen animals which were retreated responded to the second treatment. In these, parasites took up to four days to disappear from lymph node biopsy material. However, eleven of the thirteen animals had a macroschizont recrudescence (Figs. V-1, V-4 and V-6 are representative). Persistence of macroschizont parasitosis was longest in heifer No. 023B (Fig. V-4). The animals recovered after 14-26 (mean, 20.3 ± 3.7) days of infection (Table V-2).

Piroplasm parasitaemia was detected in 12/14 infected animals between 12 to 20 (mean, 15.3 ± 3.4) days of infection (Table V-2). Piroplasm parasitaemia was below 1%, intermittent and was not detected beyond day 24 of infection. No macroschizonts or piroplasms were detected in lymph node biopsies or blood smears, respectively in heifer No. 023B sixty six days after infection when it presented a clinical syndrome characteristic of theileriosis.

5.3.3 Haematology

5.3.3.1 White blood cell concentration (WBC)

Representative white blood cell concentration changes are presented in Figs. V-1 to V-7 and mean group values in Table V-3. The mean leucocyte concentration for

the eighteen animals one day pre-infection was 12700 $\pm$ 2100 μl^{-1}. There was no significant ($p>0.05$) difference between the potential infected and control groups before infection. After infection, a leucopaenia developed in all infected animals reaching a nadir between days 5-27 post-infection. As a group, infected animals suffered a significant ($p<0.05$) leucopaenia from the pre-infection level on days 5 and 9 (Table V-3) before Clexon/Medamycin treatment administered between days 9 to 12 post-infection.

Comparison with the controls showed a significant ($p<0.05$) leucopaenia on day 9 in the infected group. White blood cell concentration did not vary significantly ($p>0.05$) from the pre-infection levels and with the controls between days 13-20 post-infection.

From day 23 to 27, the infected group recorded another period of low leucocyte concentrations which significantly differed from their pre-infection levels and that of the controls (Table V-3).

In individual infected animals, it was observed that treatment with Clexon/Medamycin resulted in the arrest of leucopaenia followed by a leucocytosis within one or two days of treatment (Figs. V-1 to V-6). However, throughout the remaining experimental period there were intermittent elevations and depressions in leucocyte concentrations except in the two animals (592B and 602B) which had high leucocyte concentrations at the time they were sacrificed on day 18 post-infection. After 30 days of infection, leucocyte concentration were higher than the pre-infection level in 4/12 animals, about the same in 5/12 and lower in 3/12.

Leucocyte concentrations in the control group did not change significantly ($p>0.05$) from the pre-infection level during the period studied.

5.3.3.2 Differential leucocyte concentration

The mean peripheral blood lymphocyte concentration pre-infection was 8800 $\pm$ 1700 μl^{-1} for the 18 heifers. Lymphocyte concentration for potential infected and

control groups did not vary significantly ($p>0.05$) during the pre-infection period (Table V-3). In the infected group, a lymphopaenia soon developed with lymphocyte concentrations reaching lowest levels concomitantly with the WBC nadirs in individual animals. The highest decline was down to 30 percent of pre-infection levels (from 7300 down to 2200 μl^{-1}) in heifer No. 030B, while the least decline was down to 75.5% of pre-infection levels (from 5300 down to 4000 μl^{-1}) in heifer No. 597B.

A lymphocytosis was observed in all animals after treatment. An absolute lymphocytosis developed in the two animals sacrificed on day 18 post-infection (592B and 602B), while in the remaining twelve animals, lymphocyte concentrations rose and declined intermittently. By day 37 of infection, 6/12 infected animals had lymphocyte concentrations equal to or above the pre-infection levels while the other six had lower concentrations. As a group, the infected animals had significantly ($p<0.05$) lower lymphocyte concentrations on days 5, 9, 23 and 27 post-infection when compared to their pre-infection levels. They also showed significantly ($p<0.05$) lower values compared to the controls on day 9 of infection. Although lymphocyte concentrations fluctuated constantly in the control group, no significant ($p>0.05$) difference was registered from the pre-infection concentration (Table V-3).

Neutropaenia developed in 12/14 infected animals with nadirs reached from days 5 to 23 of infection. Neutrophil recovery was, as in the case of WBC and lymphocyte concentrations, characterised by intermittent elevations and depressions. Six of twelve infected animals taken beyond day 18 of infection recorded elevations in neutrophil concentrations on days 30, 34 and 37 when values were sometimes two to three times those pre-infection. As a group, the infected group showed significantly ($p<0.05$) lower neutrophil concentrations to both their pre-infection concentrations and to the controls on days 9, and 20 to 23 post-infection. A significant ($p<0.05$) neutrophilia to pre-infection concentrations occurred on day 30 post-infection (Table V-3). Neutrophil concentrations in the control group did not differ significantly during the study period.

Eosinophil concentrations were very variable in individual animals throughout the study period. The within animals pre-infection values of the 18 heifers ranged from 0-440 (mean 183 $\pm$ 128) μl^{-1}. The mean values for the infected and control groups are presented in Table V-3. A significant ($p<0.05$) eosinopaenia as compared to pre-infection concentration was observed on days 9, 16 and 23 post-infection. Eosinophil concentrations in the infected animals were significantly ($p<0.05$) lower compared to controls on day 16 post-infection. Although eosinophil concentrations fluctuated widely in the control group, they did not differ significantly through the study period.

5.3.3.3 Red blood cell concentration (RBC)

The change in RBC concentration before and after infection and in controls is presented in Table V-4. The mean concentrations pre-infection did not differ significantly between the potential control and infection groups (Table V-4). Red blood cell concentrations declined after infection and were significantly ($p<0.05$) lower on days 9, 13, 20 and 23 within that group than they were pre-infection. Significantly ($p<0.05$) higher concentrations were observed 30 days after infection. Comparison with the treated uninfected controls showed significantly ($p<0.05$) lower concentrations on days 9, 13, 16, 23, 27 and 30 in the infected group (Table V-4).

Red blood cell concentrations within the uninfected control group did not differ significantly ($p>0.05$).

5.3.3.4 Packed cell volume (PCV)

The change in PCV before and after infection and in the control groups is presented in Table V-4. Before infection, the eighteen heifers had a mean PCV of 33.7 $\pm$ 2.0%. The values did not differ significantly ($p>0.05$) between controls and the 14 animals which were later infected (Table V-4).

There was a significantly ($p<0.05$) higher PCV on day 2 post-infection followed by a decline which reached significant ($p<0.05$) levels from the pre-infection

value on day 9 and on each sampling day thereafter to and including day 23 post-infection (Table V-4).

A comparison with the control group values showed that the infected group had significantly ($p<0.05$) lower PCV on day 9 and on subsequent sampling days to day 30 except on day 20. PCV values fluctuated constantly in the control group but did not differ significantly ($p>0.05$) through the experimental period.

5.3.3.5 Haemoglobin concentration

The mean haemoglobin concentration for the eighteen heifers was 12.7 ± 1.2g dl^{-1} pre-infection. The concentrations for both control and infected groups did not differ significantly ($p>0.05$) pre-infection (Table V-4). The change in concentration in both groups to day 37 post-infection are presented in Table V-4. In the infected group, the concentrations were significantly ($p<0.05$) lower on days 9, 13, 16, 20 and 23 to the pre-infection value. Comparison with the control group showed significantly ($p<0.05$) lower concentrations in the infected group on days 9, 16, 23, 27, and 30.

Haemoglobin concentrations did not vary significantly ($p>0.05$) in the control group over the study period.

5.3.4 Body weight

Representative bw changes within animals are presented in Figs. V-1 to V-7. The presented change represents body weight relative to the immediate value pre-infection.

For the two months pre-infection, all the eighteen animals had maintained steady body weight with minor fluctuations of less than 10kg between weekly measurements. Initial body weights ranged from 256kg to 453kg pre-infection.

All but two (025B and 029B) of the fourteen infected animals suffered weight loss which ranged from 2.3 to 27.2% (Table V-5). The time to an initial weight loss in the infected animals ranged from week 1 to 3 post-infection (Table V-5). The time to

maximum weight loss relative to the initial decline showed it to be sudden in five infected animals (0592B, 602B, 020B, 030B and 036B) and protracted in others.

Eight of the infected heifers which suffered weight loss regained their pre-infection weights within 3 to 10 weeks of their initial loss. By week 14 post-infection, ten of these including the two which suffered no weight loss had gained weights ranging from 1.6% to 12.1% above their pre-infection weights (Table V-5). Of the animals that did not regain their pre-infection body weight, two (592B and 602B) were sacrificed early (day 18), one (587B) was sacrificed on day 38 while 023B died 101 days post-infection.

Two controls lost weight (024B and 582B) but in both cases, the loss was small and only occurred for one week (Table V-5).

5.3.5 Serology

Results of serological responses are presented in Table V-2. Serum samples collected from two infected and treated heifers (592B and 602B) immediately before sacrifice 18 days after infection were negative in IFAT to *T. parva* schizont antigen. Positive titres were detected in sera collected on day 34 post-infection from all the other twelve infected animals. All the four uninfected controls were negative on day 34 of the experiment.

5.3.6 Parasite isolation studies

5.3.6.1 Tick salivary gland infection

Tick infections were attempted only in four animals from group A which were assumed to be representative for the infected and treated animals. Animals in group B were not used because their isolation in individual barns during tick feeding would have removed them from the bull thus interfering with fertility studies. No infected salivary gland acini were detected in ticks which had fed and dropped off on day 70 in

025B and 030B or on 023B and 586B on day 101 post-infection.

5.3.6.2 Transmission studies

These were not done because although susceptible animals had been selected and paid for, they could not be received due to quarantine restrictions placed on the originating farm due to an outbreak of lumpy skin disease.

5.3.6.3 Culture of peripheral blood lymphocytes (PBL)

Culture of PBL at various periods after the infection and treatment was done in ten out of the fourteen heifers and the results are presented in Table V-6. Macroschizonts were demonstrated in PBL cultures from two animals (Nos. 586B and 594B) bled 97 days after infection. Mitosis was observed in PBL culture in two other heifers (Nos. 022B and 036B) also bled 97 days after infection but no parasites were demonstrable on stained smear preparations. Two animals bled on day 58 and another four bled on day 97 of infection neither showed cellular transformation nor presence of schizonts in smear preparations.

5.3.7 Reproductive status

5.3.7.1 Behaviourial

A summary of the oestrous signs (scores) is presented in Table V-7. Within animals, the number of manifested signs ranged from 1-11 per observed oestrus. The major presented signs were vulval mucous discharge, restlessness, mounting other animals, swollen vulva and standing to be mounted which were observed on 95.2% (99/104), 95.2% (99/104), 81.7% (85/104), 68.3% (71/104) and 62.5% (65/104) of the manifested heats, respectively.

Oestrus was detected in 82.6% (95/115) periods of basal P4 concentrations in all animals up to day 100 post-infection. Three heats were observed during the luteal

phase thus giving false positives of 3.1% (3/98).

5.3.7.2 Serum progesterone concentrations

Serum samples coinciding with the day of oestrus gave values ranging from undetectable (0.3 nmol/L) to 1.0 nmol/L. Values of <1.0 nmol/L were therefore considered to be basal. Luteal phase progesterone concentrations varied considerably within and between animals. Plateau progesterone concentrations ranged from 5-30nmol/L.

5.3.7.3 Ovarian structures

Pooled data of ovarian stuctures palpated are summarized and presented in Table V-10.

A developing or mature CL was palpated at least once in 75/79 (94.9%) of luteal phases. During the luteal phase, developing and mature CL were palpated in 15 and 159 occasions, respectively in 222 palpations. Agreement between a palpable CL and P4 concentrations was therefore, 78.4% (174/222). Of the other 48 palpations, a regressing CL, follicles or no palpable structures were palpated on 8, 25 and 15 occasions, respectively. A mature CL was palpated in 1/90 (1.1%) of the palpations during the follicular phase. A regressing CL, follicles and no detectable structures were palpated on 20, 24 and 45 occasions during the follicular phase. Agreement of palpable structures with P4 concentrations during the follicular phase was therefore 98.9% (89/90).

The overall accuracy of palpated structures with P4 concentrations was therefore, 84.3% (263/312) for both phases.

5.3.7.4 Reproductive statuses in various groups

5.3.7.4.1 Group A

From behavioural data, animals in this group each had two to three cycles ranging from 15-27 (mean $\pm$ SD, 21.3 $\pm$ 2.7) days pre-infection (Table IV-8). P4 profiles from day 57 pre-infection however revealed that one heifer in this group (602B) had luteal dysfunction while the others cycled pre-infection (Table IV-9). Following the second $PGF_2\alpha$ injection, seven of eight animals in this group including 602B were observed in oestrus on or one day after infection while P4 profile revealed that 586B was in the luteal phase.

From behavioural data post-infection, no oestrus was detected in the two animals (592B and 602B) killed on day 18. However, P4 profiles and ovarian structures revealed an incomplete cycle in 592B (Fig. V-1) and basal P4 concentrations in 602B indicative of continued luteal dysfunction in this animal.

Of the animals killed on day 38 post-nfection, heifer No. 587B manifested oestrus on day 4, seven days after the second $PGF_2\alpha$ injection. Subsequently, this animal and 020B were not observed on heat but P4 profiles and ovarian structures revealed a cycle of 25 days in each animal post-infection (Table V-9). Heifer No. 020B was killed during rising P4 concentrations (Fig. V-2) while heifer No. 587B was killed during basal P4 concentrations.

Based on behavioural data, both animals (025B and 030B) which were killed on day 83 post-infection had each two cycles ranging from 15-26 days post-infection (Table V-8). However, P4 profiles and ovarian structures revealed three cycles in each animal ranging from 15-34 days (Table V-9) post-infection. Heifer No. 025B was typical of the two and its data are presented in Fig. V-3.

After infection, heifer No. 586B which was killed on day 105 had three cycles of 41, 22 and 25 days post-infection based on behavioural data (Table V-8). However, P4 profile and ovarian structures revealed three cycles of 29, 22 and 25 days (Table

V-9) post-infection. This animal had 14 days of basal P4 concentrations after its first cycle. Heifer No. 023B was not seen in oestrus after infection. However, P4 profile and ovarian structures revealed a cycle of 28 days immediately post-infection (Table V-9). This was followed by basal P4 concentrations and no palpable ovarian structures until it died on day 101 post-infection (Fig. V-4).

In summary, of the eight animals in group A, one of the eight had luteal dysfunction throughout the study period although behavioural oestrus was seen pre-infection. Of the other seven animals, behavioural oestrus was observed in three post-infection. Five of the seven animals revealed either an initiation of/or maintained P4 cycles post-infection. Two of the seven animals however revealed variable periods of luteal dysfunction ranging from 14-$\geq$72 days.

5.3.7.5 Group B

From behavioural data, each of the six group B heifers that were exposed to the bull were cycling before infection. At least three cycles were recorded in each animal before the administration of $PGF_2\alpha$ for oestrus synchronization. The within animal mean cycle length ranged from 19.3 $\pm$ 2.1 to 22.0 $\pm$ 4.1 days (Table V-8). P4 profiles from about two months pre-infection were indicative of cyclicity pre-infection in each animal (Table V-9). Basal P4 concentrations and oestrus were observed either one day before or on the day of infection in each animal indicating successful oestrus synchronization.

From behavioural data post-infection, each of the six animals had at least one cycle which was longer than the within-animal pre-infection value. The long cycle lengths ranged from 37 to 83 days. P4 profiles and palpated ovarian changes to day 100 post-infection however revealed that 4/6 (022B, 029, 036B and 579B) animals were cycling throughout this period (representative data for 022B and 036B in Figs. V-5 and V-6, respectively are characteristic of the four animals). Each of the four animals had 3-5 cycles ranging from 17 to 29 days in length to day 100 post-infection.

Heifers No. 026B and 594B each had only one cycle length (83 and 37 days, respectively) from behavioural data for the first 100 days post-infection. P4 profiles in these two animals revealed cyclic changes post-infection which however were interrupted by extended periods of basal P4 concentrations (11 and 38 days in 026B and 594B, respectively) after the first cycle in both animals.

Behavioural data indicated that all the six group B heifers were cycling at four months post-infection. P4 profiles revealed that 4/6 (029B, 036B, 579B and 594B) were still cycling to day 163 post-infection (Fig. V-8). From behavioural data, three of the above (029B, 579B and 594B) were still cycling six months post-infection. Heifer No. 022B and 026B which were last seen in oestrus on days 126 and 123 respectively maintained elevated P4 concentrations to day 163 post-infection (Fig.V-8). Rectal palpation of the uterus and ovaries 184 days post-infection revealed changes indicative of pregnancy in 022B and 026B only.

Of twelve observed post-infection heats to day 100 post-infection, only four were observed to be mated (once in each of 026B and 029B and twice in 036B). More mated heats were observed after day 100 post-infection to the termination of the experiment. The number of observed mated heats in individual animals varied from 33.3% (1/3) to 100% (3/3). Of the animals that were considered pregnant by rectal palpation, 022B had one and 026B had two observed matings, respectively (Table V-8).

5.3.7.6 Controls

From behavioural data, all the control animals were cycling before the start of the experiment. Each animal had three cycles with within animal mean cycle lengths of 20.0 ± 0.8 to 21.0 ± 0.8 days (Table V-8) before $PGF_2\alpha$ administration. Oestrus was synchronized to within a day or day 0 of the experiment. P4 profiles and ovarian structures from day 57 before the start of the experiment to day 100 confirmed all the controls to be cycling regularly with cycle lengths ranging from 19-24 days (Table V-

9). The data presented in Fig. V-7 for 024B was characteristic of this group.

5.3.7.7 LH assays

Luteinizing hormone (LH) assays were perfomed on 151 samples collected from both infected and control animals on days 35 and 56 post-infection in four separate assays. Two quality controls (QCs) were included in the assay. The inter-assay coefficients of variation (CVs) were 9.8% (n=4) at 12.2 ± 1.2 I.U/L (mean ± SD) and 8.6% (n=4) at 15.2 ± 1.3 I.U/L for the low and high QCs respectively. The intra-assay CVs were 2.8% and 2.5% for the low and high QCs respectively.

The average batch coefficient of variation of the 151 samples was 12.8%. Of the 151 samples, 47% (71/151) had undetectable LH concentrations. The highest LH concentration determined was 16.5 I.U/L. However, about 80% of the samples became gelatinous during the overnight incubation before separation of the bound from unbound fraction. Due to this, assayable LH concentrations varied widely in duplicate samples. Further, assayable LH concentrations did not increase and decline following GnRH challenge in individual animals but fluctuated from high to low over the sampled period. To resolve this problem, two different approaches were tried:

1.Samples were centrifuged at 1650xg (3000 rpm) for 30 min and the supernatant separated. The supernatant was then assayed as described in General Materials and Methods (2.13.7). Out of the 17 samples which were centrifuged and assayed, four had no gelling while both, and one of each duplicate samples had gelled in eleven and two samples, respectively. The degree of gelling varied from mild (15 tubes), moderate (5 tubes) and severe (4 tubes).

2.By modifying the buffer by addition of phenyl sulfonyl fluoride (PSF) to make a final concentration of buffer-0.1%PSF. To test its suitability, the modified buffer and the original assay buffer were compared. 100µl of each buffer was added to 200µl of each of six bovine plasma samples and left overnight at room temperature. Aliquots of undiluted samples were also included. There was no gelling of any samples after being

left at room temperature overnight. However, substitution of the assay buffer with the modified buffer in the assay did not resolve the gelling of the incubates in the assay and the LH assays were therefore discontinued.

5.3.8 Pathology

5.3.8.1 Gross findings

The gross post-mortem findings in the infected animals sacrificed serially are presented in Table V-11.

The animals sacrificed on days 18 and 38 post-infection were in fair body condition. The two animals (025B and 030B) sacrificed on day 83 and 586B sacrificed on day 105 post-infection were in good body condition. Heifer 023B which died on day 101 post-infection was emaciated and had gelatinous subcutaneous fat.

The gastro-intestinal tract, liver and gall bladder appeared normal in all the animals. The lymph nodes were normal in 7 of the 8 animals but enlarged and congested in 023B. The spleen was of normal size in all the animals. Petechial serosal haemorrhages were observed on the spleen of 023B while the cut surface exhibited a moderate prominence of the red and white corpuscles in four animals. Pseudoinfarcts were found in the kidneys of four animals. These were few in number except in 587B where they were numerous. The kidneys of 023B were shrunken and pitted on the surface. Froth was present in the trachea and bronchi of 602B and 023B. In addition, the latter had tracheal and bronchial petechiation of the mucosa. Gross changes were observed in the lungs of five animals. These changes comprised of emphysema (592B), red hepatization of scattered lobules (602B, 020B), oedema and grey hepatization (023B) and hyperaemia (587B). Hydropericardium was seen in 587B and 023B. The fluid was slight in quantity and straw coloured. Epicardial haemorrhages were also present in 023B. All other organs and tissues appeared normal on macroscopic examination.

The gross findings in the reproductive tract are presented in Table V-12. Animals in the luteal or follicular phase of the cycle generally had large ovaries with one of the ovaries being larger than the other. Of the two acyclic animals (based on P4 concentrations), 602B had similar sized ovaries, while 023B had small ovaries compared to the rest of the animals. Small, medium and large follicles were observed except in 023B where follicles were not observed. A CL was observed in five of the eight animals. There were no visible gross lesions in the uterus in any of the group A heifers.

The animals that were exposed to a bull were killed on day 210 post-infection. No gross pathological lesions were seen in any of the six heifers. Each of two heifers (022B and 026B) had a foetus in the uterus. The crown-rump length of the foetus in both animals was about 14 cm.

5.3.8.2 Histological findings

5.3.8.2.1 Uterus

The surface epithelium of the endometrium was intact and was either simple columnar or pseudostratified in all the group A animals except in 023B in which it was denuded. Cellular infiltration was found in the endometrium of six animals (Table V-13). Except in 023B, the infiltrated cells were predominantly mononuclear and were distributed in the superficial endometrial submucosa (030B) or throughout the submucosa (592B, 602B, 020B and 587B). The cellular infiltration was either focal (Figs. V-9 and V-10) or diffuse (Fig. V-11). In addition to the stromal mononuclear cell infiltration, large sized lymphocyles were frequently seen in the lumina of small blood vessels in the superficial endometrium and in medium sized blood vessels in the deeper layer of the endometrium (Fig. V-12 and V-13). In 023B, the endometrium contained very few glands and the infiltrating cells consisted mainly of macrophages and neutrophils (Fig. V-14). A mild endometrial hyperaemia was observed in 592B.

A mild to moderate mononuclear cell infiltration was observed in the myometrium of three animals (Table V-13). The infiltrating cells were located between bundles of smooth muscle cells or perivascularly in the *tunica adventitia* (Fig. V-15). A number of small and medium sized blood vessels in the myometrium contained large numbers of mononuclear cells.

Changes in the perimetrium were observed in only one animal (592B). These consisted of a distended perimetrium with clear empty spaces, a moderate mononuclear cell infiltration and moderate hyperaemia. The mononuclear cell infiltration involved mainly the perivascular tissue and to a lesser degree, the distended perimetrial stroma.

5.3.8.2.2 Ovaries

Primordial to tertiary follicles were found in variable numbers in individual animals. Ovaries of 602B, 587B and 586B contained numerous follicles of all types while the other animals had few follicles. In 023B, teriary follicles were absent in the sections examined. Some of the follicles observed were either healthy while others showed degeneration of granulosal cells.

The ovarian cortical and medullary stroma had a mild infiltration by mononuclear cells in three animals (Table V-13). The infiltrating cells were diffusely distributed within the connective tissue stroma. In one animal (602B), blood vessels in an atretic follicle had lumina containing large numbers of mononuclear cells. A mild hyperaemia was observed in the cortex of 025B.

The CL was examined in three animals. A moderate mononuclear cell infiltration was observed in 587B while the CL of the other two appeared normal (Table V-13).

5.3.8.2.3 Pituitary gland

Pathological changes in the pituitary gland were observed in two animals (Table V-13). A few scattered foci of mononuclear cell infiltration were observed in

the adenohypophysis of 587B (Fig. V-16 and V-17). In addition, a mild hyperaemia confined to the peripheral zones of the adenohypophysis was observed in the same animal. In the neurohypophysis, a perivascular mononuclear cell infiltration was observed in 592B and 587B (Fig. V-18). Focal degeneration and necrosis of epithelial cells lining the cleft between the adenohypophysis and intermediate lobe was observed in 587B. The underlying tissue was infiltrated by lymphocytes and a few macrophages and plasma cells (Fig. V-19).

5.3.8.2.4 Adrenal gland

The histological findings in the adrenal gland are presented in Table V-13. Of the eight animals, pathological lesions were absent in three animals (020B, 025B and 586B), two animals had hyperaemia (030B and 023B) and three animals had a cellular infiltration accompanied or not accompanied by hyperaemia (592B,602B and 587B).

In the *zona glomerulosa*, a mild focal mononuclear cell infiltration (Fig. V-20) was observed in 592B and 602B. A focal mononuclear cell infiltration was also observed in the *zona fasciculata* (Fig. V-21) and *zona reticularis* (Fig. V-22) of 592B and 602B. In addition, a number of animals had a mild hyperaemia in the *zona glomerulosa*, *fasciculata* and *reticularis*. (Table V-13). The mononuclear cell infiltration was more marked in the medulla (Fig. V-23). All the three animals (592B, 602B and 587B) had a moderate cellular infiltration into the medulla. The infiltrating cells were located perivascularly. Large numbers of mononuclear cells were also seen in the lumina of medullary blood vessels. A mild hyperaemia was observed in the medulla of 030B and 023B.

5.3.8.2.5 Small intestine

Lesions in the small intestine comprised mainly of mononuclear cell infiltration of the *lamina propria* (Fig. V-24). The degree of infiltration varied from mild to severe (Table V-13). Localized areas of epithelial necrosis were observed where there

was a severe cellular infiltration in the superficial *lamina propria*. Although the infiltrating cells were predominantly mononuclear, moderate numbers of eosinophils were also observed in 592B. The submucosa and muscular tunic had no cellular infiltration.

5.3.8.2.6 Liver

The predominant change in the liver was a patchy distribution of mononuclear cells in the periportal zone (Fig. V-25). The infiltrating cells were usually located in the perivascular *tunica adventitia*. The severity of infiltration varied from mild to moderate (Table V-13). A number of sinusoids also contained low numbers of mononuclear cells. There were no histological changes in the livers of three animals (Table V-13).

5.3.8.2.7 Kidney

A mild to marked cellular infiltration of the renal parenchyma was observed in all animals. Except in two animals (020B and 587B), the cellular infiltrate was confined to the cortex (Table V-13). The cellular aggregates were focal but varied substantially in size (Fig. V-26). The infiltrating cells were predominantly mononuclear. A significant proportion of these cells were seen around the inter-lobular and arcuate blood vessels or at the vascular pole of the glomerulus.

In mild and moderate interstitial cell infiltration, the renal tubular epithelium appeared histologically normal. Where the infiltrations were marked, degeneration of the renal tubular epithelium and an apparent displacement of tubules by cellular infiltrates was observed (Fig. V-26). In 023B, glomeruli enclosed within marked cellular aggregates were shrunken. Fibrosis was also observed in the renal cortex of 023B.

The mononuclear cell infiltration in the medulla was mild in 587B and moderate in 020B and had a focal distribution. Medullary congestion was observed in the two

animals as well as in three other animals (Table V-13)

5.3.8.2.8 Lungs

Histological changes were observed in the lungs of four of the eight animals (Table V-13). The predominant change was a mononuclear cell infiltration whose severity varied from mild to moderate (Table V-13). Cell aggregates were observed around bronchi, bronchioles and blood vessels accompanying them. In the affected lobules, the septae were distended by eosinophilic amorphous material and mononuclear cell infiltrates. The alveolar walls were thickened by mononuclear cell infiltrations (Fig. V-27). The subpleura was distended in 592B and lymphatic and blood vessels in the region had a moderate monuclear cell infiltrations around them and moderate to marked mononuclear cell aggregates in their lumina.

5.3.8.2.9 Heart

A mild mononuclear cell infiltration was observed in the heart of only one animal (592B). The infiltrating cells were located between muscle bundles (Fig. V-28) which were otherwise normal.

5.3.8.2.10 Brain

A mild to moderate perivascular mononuclear cell infiltration of blood vessels in the mid-brain (Fig. V-29) was observed in six of the eight animals. Increased numbers of mononuclear cells were also seen in the lumina of blood vessels of 592B and 602B. Meningeal blood vessels were moderately congested in 025B and 587B. In addition, 025B had some subdural brain haemorrhages.

5.4 Discussion

The objective of this experiment was to investigate whether Boran/Friesian cross heifers suffered any loss in reproductive function following infection with *T. parva* and treated after clinical manifestation of disease. Animals were infected with 1

ml undiluted *T. parva* stabilate IL3081 and treated with a short-acting formulation of oxytetracycline or in combination with parvaquone between day 1 and 5 of fever. The aim of the initial oxytetracycline treatment was to slow down the clinical reaction before parvaquone treatment.

All the fourteen infected heifers developed clinical signs similar to those described in earlier experiments (Chapters 3 and 4). One peculiar presentation was a submandibular oedema not observed in earlier work but described in the literature (Neitz, 1957). Fever developed in 6-9 (mean 6.9 $\pm$ 0.9) days post-infection. Treatment with Medamycin at 10mg/kg bw on the initial day of fever only resulted in a transient drop of temperature in two animals. Treatment was not continued but there are reports of successful treatment of ECF using oxytetracycline at 15mg/kg bw for 5 days (Brown *et al.* 1977) or at 30mg/kg bw for 5 days (Dolan, 1981). Treatment with Clexon produced a rapid and marked effect on the disease. Fever was reduced within one day in two animals with the first injection and in all others after the second. Two animals maintained normal temperatures after parvaquone treatment while the others developed remissions. Rapid reductions of fever followed by remissions in a few animals following parvaquone treatment are also reported (Morgan and McHardy, 1982; McHardy *et al.*, 1983; Dolan *et al.*, 1984a). Two animals were sacrificed on day 18 before complete recovery. Of the other twelve animals, all recovered except one animal which developed typical clinical signs of ECF 66 days post-infection. No parasites were however, demonstrated in lymph node biopsies or blood smears. This animal became progressively weaker, emaciated and recumbent before death. Dolan *et al.* (1984a) described a similar case, but in contrast, the treated animal exhibited parasitosis for over 70 days up to the time of death.

The changes in the parasitological parameters following treatment resembled those described earlier (Morgan and McHardy, 1982; McHardy *et al.,* 1983; Dolan *et al.*, 1984a). The mean prepatent period of 6.3 $\pm$ 0.9 days was close to that noted (6.8 $\pm$ 1.0 days) in an earlier experiment (Chapter 4) where a similar quantity of undiluted

stabilate was used as in this experiment. Treatment with Medamycin had no effect on the parasitosis. However, a transient disappearance of parasites was observed after the first treatment with Clexon/Medamycin in three animals and in all thirteen animals following the second treatment. Parasite recrudescence was observed in the majority of animals. Similar observations have been made following parvaquone treatment (Morgan and McHardy, 1982; McHardy *et al.*, 1983; Dolan *et al.*, 1984a, 1988).

The total white cell count before infection was within normal levels before infection (Doxey, 1977). Following infection, the white blood cell count fell rapidly in all animals. The infected animals recorded a significant leucopaenia initially on day 9 post-infection compared to the controls. The fall was rapidly arrested in all animals after treatment with Clexon/Medamycin followed by a leucocytosis. The initial fall in total white cell count followed by recovery after Clexon treatment were similar to those reported elsewhere (Morgan and McHardy 1982, Dolan *et al.* 1984a, 1988). Animals in this experiment were treated during severe clinical reaction and either had higher (4/12), similar (5/12) or lower (3/12) leucocyte counts after treatment compared to pre-infection levels. Changes in lymphocyte, neutrophil concentrations showed parallel changes to total white cell count.

Red blood cell concentration, packed cell volume and haemoglobin concentration fell below pre-infection levels on most sampling days starting from day 9 to the third week post-infection. Treatment with Clexon/Medamycin apparently did not arrest this decline. The erythrocyte values were within the pre-infection levels from about four weeks in the infected group. These low values could have been due to a depression of bone marrow erythropoesis. Hill and Matson (1970) noted a lymphocytosis and a depression of bone marrow erythropoesis although other workers report no changes in erythrocyte values during *T. parva* (buffalo-devived) infection (Maxie *et al.*, 1982).

Animals suffering from ECF, and especially protracted cases lose condition (Henning, 1956). In this experiment, 12/14 infected animals underwent variable

degrees of weight loss. After treatment , most of the animals that had lost between 4.6-12.5% of their initial body weight, recovered and had higher weights than their pre-infection body weight by 14 weeks post infection. Of the animals that never recovered their pre-infection weights, two were sacrificed early before clinical recovery while the other two had lost more weight than the others. One of these had developed a protracted illness. Loss in body weight in treated ECF cases is similarly reported (McHardy *et al.*, 1983; Dolan, 1986a). Low weight gains were associated with a carrier status (Dolan, 1986a) or protracted recovery (McHardy *et al.*, 1983). In this experiment there was no correlation between bw loss/gain with the carrier status since animals that had poor weight gain revealed no carrier status. In this experiment, animals that lost more weight took longer to regain their initial bw suggesting that there was a relationship between regain and the magnitude of weight loss.

At post-mortem examination, animals sacrificed between day 18-83 post-infection were in fair to good condition. The gross findings in these treated animals were characteristic of ECF but fewer as compared to those noted in animals previously which died of severe disease in the absence of treatment (Chapter 4). The paucity of observed gross lesions in individual animals especially those killed before recovery (day 18) suggests that the teatment slowed down the development of disease.

Histological findings in the ovaries, uterus and the pituitary gland were similar to those reported in earlier work (Chapters 3 and 4), consisting predominantly of mononuclear cell infiltration. In one animal that died on day 101 post-infection, the denudation of the endometrial epithelium was a finding which could be due to post-mortem change. Histological changes in the major organs and viscera comprised mainly of mononuclear cell infiltrations and were similar to those reported elsewhere (Steck, 1928; De Kock, 1957; Barnett, 1960; Munyua *et al.*, 1973; Dolan, 1986a).

Of the three methods used to assess the reproductive status, serum P4 dertermination was the most reliable. In the assessment of cyclical status, agreement of observed oestrus to basal P4 concentrations was 82.6% while there were 3.1% false

positives. However, standing to be mounted as a true sign of oestrus (Whitmore 1980) accounted for only 62.5% of the observed heats. This figure was close to the 56% detection rate based on twice daily observation, but lower than 89% reported for continuous observation for oestrus (Williamson *et al.*, 1972). Of the non-specific oestrous signs, vulval mucous discharge and restlessness gave the best indication that the animal was around oestrus.

Agreement of rectal palpation results with serum P4 concentrations was 78.4% in the luteal phase and 98.9% in the follicular phase. The overall agreement of 84.3% compares favourably with results reported elsewhere (Boyd and Munro, 1979; Watson and Munro, 1980) and to our earlier results (Chapter 3). In this experiment, the ovarian structures palpated during the luteal phase were the ones mainly inconsistent with the P4 profiles. During this period, regressing CL, follicles and no structures were palpable suggesting that subjective errors were made in assessing the palpable structures based on size and consistency. Similar problems in relating CL size and consistency to functional CL status are reported (Boyd and Munro,1979; Watson and Munro, 1980).

All infected animals except 602B had cyclical ovarian changes during the early infection phase when clinical disease manifestations like fever and panleucopaenia were present and when parasites were demonstrable in the peripheral lymph nodes. This suggests that the parasite or clinical disease did not adversely affect cyclical status. In the two animals that were exposed to a bull and suffered acyclicity post-infection, this period was associated with loss in body weight. Of these, 586B which lost about 7.1% of her initial weight, P4 concentrations were basal for only about two weeks. However, 023B which remained acyclic for about nine weeks without resumption of cyclical status had an intermittent clinical syndrome, lost about 27.2.% of her initial weight and at death was about 20% below her pre-infection weight. Evidence of pathological lesions in the pituitary or ovaries of these animals that could have compromised cyclical status were absent. Animals which had histological changes in

these organs actually cycled normally suggesting no hormone-based disturbance in these animals. The relationship that weight-loss and by implication loss in condition adversely affects cyclicity has been discussed earlier. It is suggested that weight loss played an important role in causing acyclicity in these animals.

It was intended in this experiment to examine whether the disturbances of the cyclical status observed in our earlier work might have been due to a functional disturbance in the pituitary. However, determination of plasma LH levels after exogenous administration of GnRH were unsuccessful mainly due to problems associated with plasma samples. There was no gelling of undiluted plasma samples or in those where the original or modified buffer had been added suggesting that the problem was due to the interaction of the materials in the incubate. Since no problems were encountered during the assay with the standards or quality control plasma samples both of which were of human origin, it is thought that the antiserum and/or labelled LH could have acted with certain components in the bovine samples to cause the gelling. Whether this was as a result of physical or chemical interactions is not known. Contamination of the samples either with microorganisms or chemicals arising from glassware, during blood collection and separation or during the assay could also have been contributory. No macroscopic growths were seen in the samples before or after incubation to suggest microbial contamination. However, no culturing for microorganisms was done.

Attempts at examining the pituitary gland responsiveness to GnRH were therefore disappointing despite considerable effort and a variety of attempts. However, examination of the cyclical patterns of animals that were sampled for LH revealed that all but two of the infected animals were cyclic on P4 profile at the time of GnRH challenge. Of these two infected acyclic animals, one (602B) revealed an acyclic pattern on P4 profile and palpable ovarian changes before and after infection although behavioural data had indicated it was cycling pre-infection. The other animal (023B) was cycling normaly based on P4 profile, palpable ovarian changes and

behavioural data pre-infection and acyclic post-infection. Therefore, in retrospect, if the LH assay had been successful, only data from one animal would have been useful to explain whether the pituitary secretion/release of LH was partly responsible for the acyclic status observed following infection with *T. parva*. It must be considered that the data from one animal could not have provided a basis for conclusive evidence of the direct effect of the disease on LH secretion although it may have provided a pointer to the possible mechanisms involved.

In group B heifers which were exposed to a bull, two of the six animals experienced a period when P4 concentrations remained at basal levels and in addition there were no palpable ovarian structures. This period was short (11 days) in 026B and longer (38 days) in 594B. These findings suggest that the two animals suffered from ovarian dysfunction during this period. Prior to this, parasites had ceased to be observed in lymph-node biopsies and temperature and haematological values had returned to normal. However, the initial body weight had not been regained in 026B while in 594B, the body weight was around and later above the pre-infection weight. In 026B, the transient period of ovarian dysfunction may have been associated primarily with loss in condition. This animal had lost 12.5% of her initial weight which took about seven weeks for the regain. The cause of ovarian dysfunction in 594B is not known. Rectal palpation did not reveal any ovarian or uterine abnormalities in this animal.

After exposing the six group B heifers to a bull for about six months, two animals were considered pregnant from P4 profiles and confirmed by rectal palpation and at slaughter. Mating was observed from the third week post-infection when the bull was introduced and continued to be observed throughout the experimental period. The pregnant animals were observed to be mated on either one or two occasions out of three detected heats in each. Non-pregnant animals had 2-4 matings each. Oestrus was not observed on 1-3 periods of basal P4 concentrations and it is not known whether matings occurred during these periods. Failure to have a conceptus at the end

of the experiment could be due to a number of factors.

In the female, conceptual failures could be due to anatomical aberrations; functional, for example in delayed ovulation or anovulation; failure of fertilization or due to early embryonic death. At slaughter, there was no evidence of anatomical anomalies in the four non-pregnant heifers. Delayed ovulation after the animal is mated would result in fertilization failure. Normally, ovulation occurs in from 25-30 hours after the onset of oestrus (Swanson and Hafs, 1971). Delayed ovulation would require several rectal examinations to be confirmed. In this study, rectal examinations were done once a week and therefore would not have been adequate to reveal this abnormality if it was present. Similarly, ultrasound scanning of the ovaries which is reported to be more accurate (Griffin and Ginther, 1992) was not done. Anovulation could result from insufficient amounts of LH or lack of LH receptors in the follicle. However, the presence of a CL and P4 profile following oestrus to these mated heats suggests that ovulation had occurred.

Cycles in individual animals that were not pregnant at the termination of the experiment ranged from 19-25 to over 30 days. Cycles of less than 25 days following mating may suggest either failure of fertilization or successful fertilization followed by early loss of conceptus. The extended luteal phase in the long cycles could be as a result of embryonic death or endometritis. However, there was no clinical evidence of endometritis nor vaginitis.

It was not known in this study whether the bull's breeding perfomance affected fertility studies in this experiment. Plasse *et al.* (1970) reported psychological interactions which may inhibit libido in some males and suggested use of several bulls. Daly (1971) suggested a mating intensity (bull/cow ratio) of 2.5-3%. In our study, one bull was used for ten heifers and was considered adequate. Mating was not observed during all heat periods. However, a more accurate assessment, for example, by use of heat-mount detectors would have been necessary to confirm whether animals were served on all observed and unobserved heats. Other areas which were not assessed are

the bull's breeding efficiency and freedom from genital infections as well as comparative fertility studies in uninfected controls.

In conclusion, this study demonstrated that some animals that recovered after treatment from severe theileriosis suffered some impairment of reproductive function. This impairment was not due to a direct effect of the parasite but to an indirect effect associated with loss in condition following infection.

Table V-2 **Reactions of Boran/Friesian cross heifers to infection with *T. parva* stabilate IL3081 with treatment or in treated controls**

Animal #	Days to						Ab. response δ
	Schizonts	Fever	Treatment		Piroplasms	Recovery	
			M	M/C			
592B	5	7	7	10,12	12	.*	.
602B	6	6	6	10,12	12	.*	.
020B	6	7(6)	7	9,11	.	15	+
587B	8	6(9)	6	9,11	20	20	+
025B	7	7(6)	7	11	12	19	+
030B	6	7(6)	7	9,11	12	23	+
023B	6	6(14)	6	8,10, 66,68	19	26	+
586B	7	7(7)	7	10,12	19	20	+
022B	5	6(8)	6	8,10	.	14	+
026B	7	8(9)	8	10,12	12	23	+
029B	5	8(11)	8	9,11	19	20	+
036B	7	9(7)	.	9,11	19	23	+
579B	6	6(7)	6	8,10	15	16	+
594B	7	6(14)	6	10,12	12	25	+
Mean ± SD	6.3 ± 0.9	6.9 ±0.9			15.3 ± 3.4	20.3 ± 3.7	
024B**	.	.	.	10	.	.	.
037B**	.	.	.	10	.	.	.
582B**	.	.	.	10	.	.	.
583B**	.	.	.	10	.	.	.

δ (+) - Antibody titre of >1:40 to *T. parva* schizont antigen
M - Medamycin
M/C - Medamycin/Clexon
* - Schizonts seen up to day of sacrifice
** - Controls
Numbers in parentheses represent days of febrile reaction

Table V-3 **Leucocyte and differential leucocyte concentration in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 and in controls**

Day p-i	LEUCOCYTES		LYMPHOCYTES		NEUTROPHILS		EOSINOPHILS	
	(10^3 μl^{-1})							
	A&B	C	A&B	C	A&B	C	A&B	C
-1	13.0 ± 1.7	12.0 ± 3.3	8.9 ± 1.6	8.4 ± 1.9	3.6 ± 1.0	3.4 ± 1.5	201 ± 130	118 ± 92
2	13.0 ± 4.1	14.0 ± 3.3	8.0 ± 2.4	8.7 ± 1.0	3.6 ± 1.2	4.9 ± 2.4	387 ± 296	351 ± 407
5	10.0* ± 2.5	11.9 ± 2.1	6.7* ± 1.6	7.3 ± 1.4	3.0 ± 1.5	4.1 ± 0.8	224 ± 204	444 ± 368
9	7.2* ± 1.8	12.9 ± 3.0	4.4* ± 1.0	8.0 ± 2.1	2.6* ± 1.3	4.5 ± 1.3	95* ± 119	372 ± 315
13	12.6 ± 4.6	14.9 ± 3.6	10.0 ± 4.1	10.4 ± 1.8	2.8 ± 1.6	4.3 ± 2.0	116 ± 130	135 ± 189
16	13.9 ± 6.1	13.2 ± 2.6	10.8 ± 4.8	9.5 ± 3.2	2.9 ± 1.1	3.6 ± 0.6	74* ± 97	360 ± 187
20	11.2 ± 3.0	13.5 ± 3.1	8.5 ± 2.5	7.8 ± 2.4	2.5* ± 0.6	5.4 ± 1.9	125 ± 128	349 ± 307
23	9.5* ± 2.2	13.0 ± 4.2	6.8* ± 1.8	8.2 ± 2.1	2.6* ± 0.8	4.3 ± 2.0	35* ± 66	467 ± 462
27	10.1* ± 3.6	15.1 ± 4.5	6.8* ± 2.7	9.5 ± 1.6	3.1 ± 1.5	4.7 ± 2.6	166 ± 189	484 ± 427
30	14.4 ± 7.0	18.1 ± 4.7	9.4 ± 3.8	11.2 ± 3.0	6.1* ± 2.9	6.1 ± 2.1	312 ±288	345 ± 468
34	16.0 ± 5.5	15.1 ± 4.6	10.2 ± 3.3	10.0 ± 2.7	5.4 ± 2.9	4.7 ± 2.0	269 ± 254	153 ± 264
37	13.4 ± 4.8	13.4 ± 4.8	8.4 ± 3.1	8.2 ± 2.9	4.9 ± 2.0	6.1 ± 2.4	179 ± 264	736 ± 601

A&B - Groups A and B

C - Controls

* - Significantly different from pre-infection (p-i) levels ($p<0.05$)

Underlined - Signicantly different from controls ($p<0.05$)

Table V-4 **RBC, PCV and haemoglobin concentration in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 and in controls**

Day p-i	RBC 10^6 µl^{-1}		PCV %		Hb gm dl^{-1}	
	A&B	C	A&B	C	A&B	C
-1	8.25 ± 0.78	8.63 ± 1.25	33.6 ± 2.5	33.8 ± 4.4	12.75 ± 1.07	12.6 ± 1.47
2	8.21 ± 0.85	8.38 ± 0.75	36.1* ± 3.1	38.8 ± 0.5	12.29 ± 1.02	13.0 ± 1.83
5	8.21 ± 1.01	8.63 ± 0.95	32.9 ± 2.8	36.3 ± 5.9	11.84* ± 1.0	12.7 ± 1.95
9	7.29* ± 0.73	8.5 ± 0.7	30.9* ± 2.5	34.0 ± 3.8	10.96* ± 0.91	12.43 ± 1.16
13	7.18* ± 0.82	8.25 ± 0.96	30.0* ± 2.8	35.0 ± 2.6	10.95* ± 1.04	12.2 ± 1.75
16	7.79 ± 1.03	9.38 ± 0.75	30.6* ± 3.2	33.8 ± 4.4	10.91* ± 1.2	14.07 ± 1.0
20	7.63 ± 0.68*	8.13 ± 1.03	30.7* ± 3.0	34.0 ± 0.5	11.26* ± 1.05	12.15 ± 0.78
23	7.38* ± 0.91	9.13 ± 0.63	29.8* ± 2.9	36.3 ± 3.2	11.12* ± 1.04	13.73 ± 0.85
27	8.04 ± 1.03	9.5 ± 0.91	32.7 ± 4.2	39.3 ± 2.1	12.08 ± 1.64	14.68 ± 0.75
30	9.13* ± 0.91	10.5 ± 1.08	33.3 ± 3.6	39.5 ± 3.1	12.13 ± 1.3	14.5 ± 0.78
34	8.17 ± 0.94	9.0 ± 1.41	32.8 ± 4.1	37.5 ± 3.1	12.35 ± 1.4	13.95 ± 0.75
37	8.67 ± 1.05	9.13 ± 1.31	34.5 ± 3.6	38.3 ± 4.5	12.47 ± 1.37	13.9 ± 1.61

* - Significantly diffferent from pre-infection (p-i) levels ($p<0.05$)
Underlined - Significantly different from controls ($p<0.05$)

Table V-5 **Changes in cyclical status and body weights in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 and in controls**

Animal #	Day killed	Weeks to			Max. bw*		Acyclic**
		Initial bw loss	Max. bw loss	bw regain	Loss	Gain	
Group A							
592B	18	2	2	.	4.3	.	.
602B	18	2	2	.	2.3	.	.
020B	38	2	2	5	5.6	1.6	.
587B	38	1	4	.	15.1	.	.
025B	83	.	.	.	.	8.9	.
030B	83	2	2	6	4.6	7.3	.
023B	101	1	9	.	27.2	.	>72
586B	105	2	3	6	7.1	3.2	14
Group B							
022B		2	4	7	9.0	9.0	.
026B		1	4	11	12.5	12.1	11
029B		.	.	.	.	11.0	.
036B		3	3	8	5.9	4.1	.
579B		2	6	11	9.1	9.3	.
594B		2	4	5	6.7	8.5	38
Controls							
024B		3	3	4	0.8	10.5	.
037B		.	.	.	.	8.8	.
582B		2	2	3	2.5	8.3	.
583B		.	.	.	.	13.4	.

* - Maximum bw loss/gain expressed as a % of initial weight

** - Acyclic period in days based on serum P4 profile

Table V-6 **Parasite isolation studies in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081**

Animal #	Salivary gland infection		Cell culture	
	Day p.i*	Result	Day p.i**	Result
592B	ND		ND	
602B	ND		ND	
020B	ND		ND	
587B	ND		ND	
025B	70	-	58	-
030B	70	-	58	-
023B	101	-	97	-
586B	101	-	97	+(schizonts)
022B	ND		97	+(mitosis)
026B	ND		97	-
029B	ND		97	-
036B	ND		97	+(mitosis)
579B	ND		97	-
594B	ND		97	+(schizonts)

p-i - Post-infection

* - Day of tick feeding

** - Day of peripheral blood leucocyte isolation

ND - Not done

Table V-7 **Frequency of oestrus signs in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 or in controls**

	Frequency of oestrus signs							
	Pre-infection				Post-infection			
	A	B	C	Total	A	B	C	Total
n	25	29	15	69	12	8	15	35
Oestrus sign								
Mucous discharge	24	28	14	66	10	8	15	33
Restlessness	25	29	14	68	11	7	13	31
Mounting others	22	24	13	22	9	5	12	26
Swollen vulva	10	16	8	34	11	6	10	37
Allowing mounting	19	13	8	40	7	5	13	25
Being sniffed	4	4	2	10	6	1	3	10
Head butting	5	3	2	10	3	2	3	8
Bellowing	1	5	2	8	1	2	6	9
Inappetence	.	1	.	1	7	5	3	15
Front mounting	2	2	1	5	1	1	1	3
Raised tail	1	2	.	3	1	1	.	2

A = Group A; B = Group B; C = Control group
n - Number of heats

Table V-8 **Oestrous cycle lengths in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 or in controls**

Animal #	Cycle length in days					
	Pre-infection		Post-infection			
		Mean		Mean		
Group A						
592B	21,22, 20,20	20.8 ± 0.8	ND (18)*	.		
602B	21,20,25	22.0 ± 2.2	ND (18)	.		
020B	26,17,21	21.3 ± 1.7	ND (38)	.		
587B	20,24, 15,21	20.0 ± 3.2	ND (38)	.		
025B	22,23,19	21.3 ± 1.7	26,21 (83)	23.5 ± 2.5		
030B	16,20,27	21.0 ± 4.5	23,15 (83)	19.0 ± 4.0		
023B	22,24,23	23.0 ± 0.8	ND (101)	.		
586B	23,22,20	21.7 ± 1.2	41,22,25 (105)	29.3 ± 8.3		
Mean	n=26	21.3 ± 2.7	n=7	24.7 ± 7.4		
Group B					Pregnancy status	Service ratio**
022B	19,21,22	20.7 ± 1.2	61,34	47.5 ± 13.5	126*** (P)	1/3
026B	27,17,22	22.0 ± 4.1	83	83	123 (P)	2/3
029B	17,19,22	19.3 ± 2.1	24,22,50	32.0 ± 12.8	199 (NP)	4/6
036B	19,23,23	21.7 ± 1.9	30,44	37.0 ± 7.0	109 (NP)	3/3
579B	20,21, 21,21	20.8 ± 0.4	61,19,19	33.0 ± 19.8	189 (NP)	3/6
594B	18,22,20	20.0 ± 1.6	37	37	194 (NP)	2/5
Mean	n=19	20.7 ± 2.3	n=12	40.3 ± 19.3		
Controls						
024B	22,21,20	21.0 ± 0.8	23,23,24	23.3 ± 0.5		
037B	20,20,22	20.7 ± 0.9	24,22, 22,21	22.3 ± 1.1		
582B	19,20,21	20.0 ± 0.8	23,19,44	28.7 ± 11.0		
583B	20,23,19	20.7 ± 1.7	22,21, 21,22	21.5 ± 0.5		
Mean	n=12	20.6 ± 1.2	n=14	23.6 ± 5.8		

ND - Not detected on heat
* - Numbers in parenthesis indicate day of sacrifice or death
** - Number of observed services/observed heats
*** - Day of last observed oestrus and mating
P = Pregnant; NP = Not pregnant

Table V-9 **Oestrous cycle lengths in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 or in controls**

Animal #	Cycle length in days*		Acyclic period
	Pre-infection	Post-infection	
Group A			
592B	22	.	.
602B	.	.	
020B	23	25	.
587B	23	25	.
025B	23	26,21,26	.
030B	21	34,15,19	.
023B	24	28	>72
586B	22	29,22,25	14
Mean	22.6 ± 0.9	24.7 ± 4.7	
Group B			
022B	22	23,20,19,23	.
026B	22	31,21,21	11
029B	21	24,22,21,20	.
036B	24	30,23,23	.
579B	21,21	21,20,21,19,19	.
594B	23	27,21	38
Mean	22.0 ± 1.1	22.3 ± 3.2	
Controls			
024B	21	23,23,24,23	.
037B	22	24,22,22,21	.
582B	21	23,19,22,21	.
583B	19	22,21,21,22	.
Mean	20.8 ± 1.1	22.1 ± 1.2	

* - Cycle lengths adjusted using P4 profiles

Table V-10 **Distribution of palpable ovarian structures in relation to the oestrous cycle in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081 or in controls**

Palpable structure*	Number of observations	Structure present in	
		Luteal phase	Follicular phase
Group A			
Developing CL (A1-3)	7	7	.
Mature CL (B)	50	49	1
Regressing CL (C1-2)	6	2	4
Follicles (D)	15	8	7
NDS (E)	21	4	17
Group B			
Developing CL (A1-3)	3	3	.
Mature CL (B)	63	63	.
Regressing CL (C1-2)	14	5	9
Follicles (D)	22	13	9
NDS (E)	26	6	20
Controls			
Developing CL (A1-3)	5	5	.
Mature CL (B)	47	47	.
Regressing CL (C1-2)	8	1	7
Follicles (D)	12	3	9
NDS (E)	13	5	8

* - Heifer No. 602B and all acyclic periods were omitted in this data

NDS - No detectable structures

Table V-11 **Gross findings in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081**

Animal #	592B	602B	020B	587B	025B	030B	023B	586B
Days to death	18	18	38	38	83	83	101	105
Condition at death	Fair	Fair	Fair	Fair	Good	Good	Poor	Good
GIT and Liver	.	.	.	.	.	.	.	.
Lymph nodes	.	.	.	.	.	.	e,c	.
Spleen	wp, rp	wp, rp	.	wp, rp	.	wp	h	.
Kidneys	.	pi	.	pi	pi	pi	s	.
Trachea/bronchi	.	f	.	.	.	.	f, h	.
Lungs	em	pn	pn	hr	.	.	oe, pn	.
Heart	.	.	.	hp	.	.	hp, h	.

c = congestion; e = enlarged; em = emphysema; f = froth; h = haemorrhage; hp = hydropericardium; hr = hyperaemia; oe = oedema; pi = pseudoinfarcts; pn = pneumonia; rp = prominent red pulp; wp = prominent white pulp;

Table V-12 **Gross findings in the reproductive tract of Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL3081**

Animal #	Stage in cycle at death	Right ovary			Left ovary			Uterus
		Size*	Follicles	CL**	Size	Follicles	CL	
592B	Luteal	25x10x8	Small	.	35x20x10	.	25	.
602B	Acyclic	30x15x10	Small	.	30x20x10	Tertiary	.	.
020B	Luteal	30x20x20	Small	10	15x15x10	Medium	.	.
587B	Follicular	25x15x15	Medium	.	28x18x15	Medium	.	.
025B	Luteal	20x15x15	Small	.	25x20x15	Medium	22	.
030B	Luteal	30x20x15	Small	.	35x17x17	Small	20	.
023B	Acyclic	20x10x12	.	.	18x10x10	.	.	.
586B	Luteal	26x15x15	Medium	Regressing	25x15x12	Medium	.	.

* - Size of ovary in mm
** - Diameter of CL in mm

Table V-13 **Histological findings in Boran/Friesian cross heifers infected and treated against *T. parva* stabilate IL 3081**

Animal #	592B	602B	020B	587B	025B	030B	023B	586B
Cycle stage	L	Acy	L	F	L	L	Acy	L
Day of death	18	18	38	38	83	83	101	105
Uterus								
Endometrium	2;hr	2	1	1	.	1	1	.
Myometrium	1	2	.	1	.	.	.	.
Perimetrium	2;hr	.	.	.	.	.	.	.
Ovary								
O. cortex	1	1	0	1	.;h	.	.	.
O. medulla	1	.	.	2	.	.	.	.
CL	.	.	.	.	.	.	.	.
Pituitary								
Ant. lobe	.	.	.	1;hr	.	.	.	.
Post. lobe	1	.	.	1	.	.	.	.
Adrenal gland								
Z.g.	1	1	.	.	.	.	.	.
Z.f.	1;hr	1	.	.;hr	.	.	.	.
Z.r.	1;hr	2	.	2	.	.;hr	.;hr	.
Ad. medulla	2	2	.	2	.	.;hr	.;hr	.
GIT								
S. intestine	2;hr	3	2	1	1	3	.	.
Liver	2	2	1	1	.	.	1	.
Kidney								
Cortex	2	2	1	1	1	2	3	.
Medulla	.;hr	.	2;hr	1;hr	.;hr	.,hr	.	.
Lungs	2	1	.	2;hr	.	.	2	.
Heart	1	.	.	.	.	.	.	.
Skeletal muscle	.	.	.	.	.	.	.	.
Brain (mid)	2	.	2	2;c	2;c	1	1	.

Acy = acyclic; F = follicular; L = Luteal; GIT = gastrointestinal tract; Z.g. = *zona glomerulosa*; Z.f. = *zona fasciculata*; Z.r. = *zona reticularis*; c = congestion; h = haemorrhage; hr = hyperaemia;
Cell infiltraion: . = no change, 1 = mild, 2 = moderate, 3 = severe

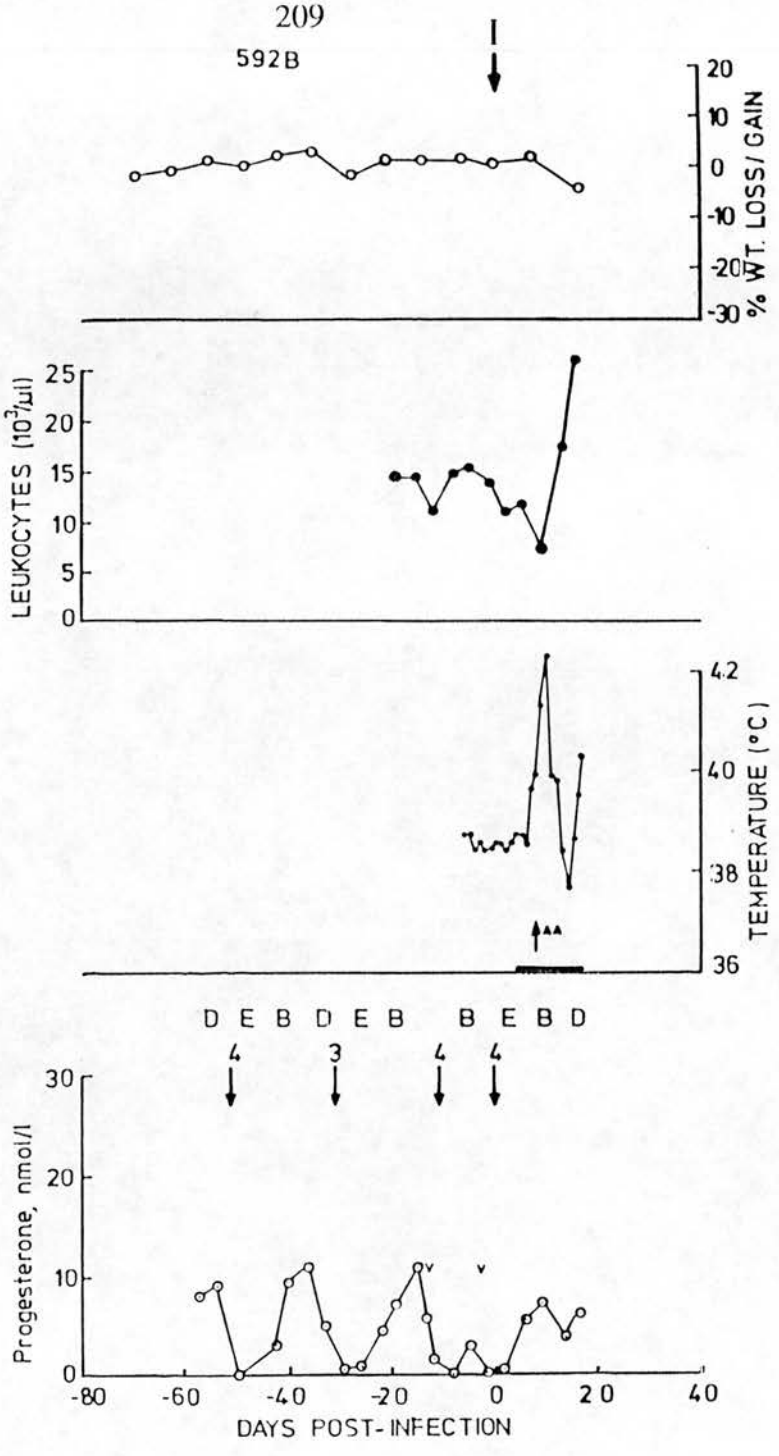


Fig.V-1.

Serum progesterone concentration, ovarian structures, rectal temperature, shizont parasitosis (bar), leucocyte concentration and weight change in heifer 592B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by B = Mature CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous cores in superscript. V = $PGF_2\alpha$; ↑ = Medamycin; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

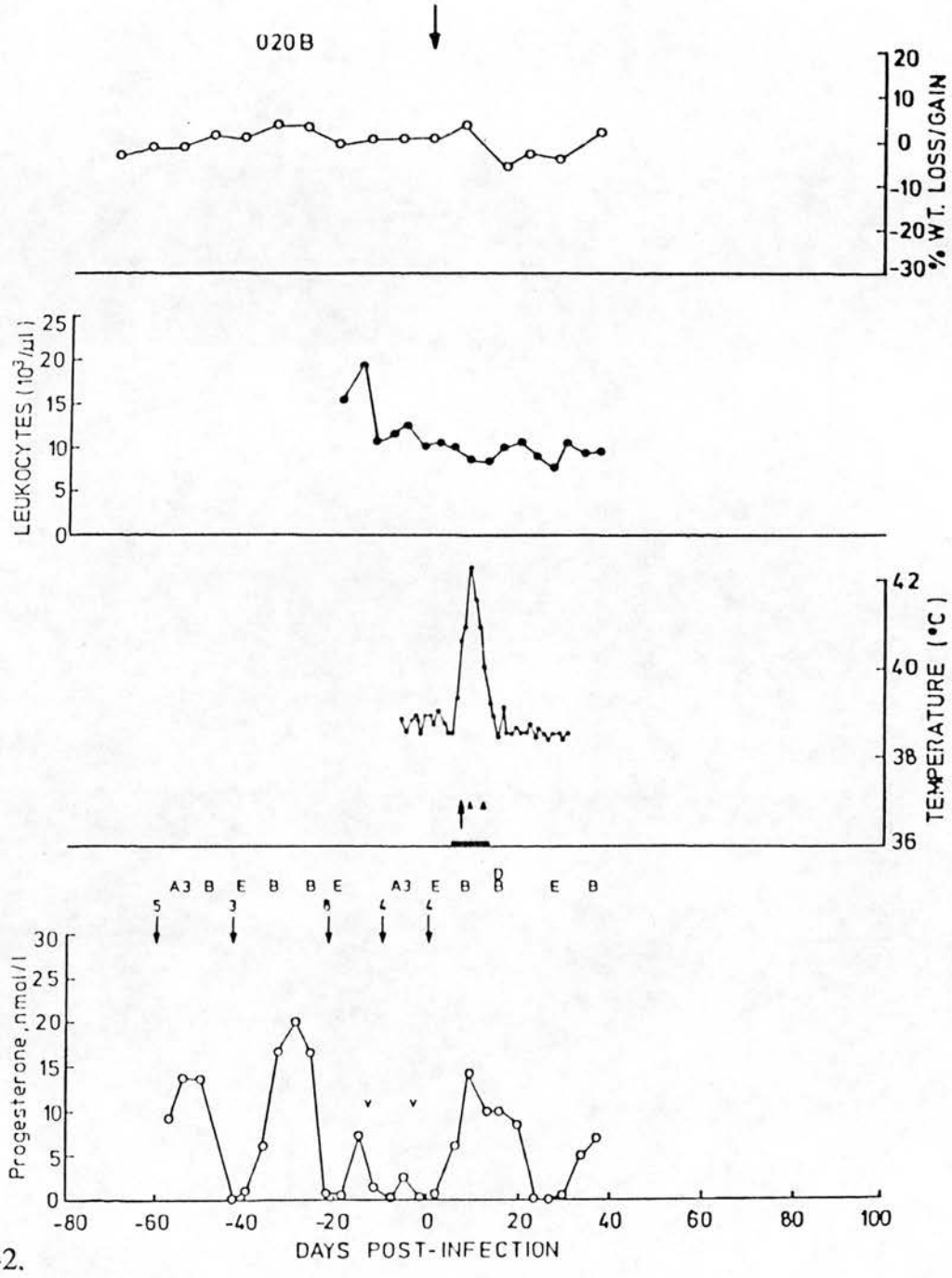


Fig.V-2.

Serum progesterone concentration, ovarian structures, rectal temperature, shizont parasitosis (bar), leucocyte concentration and weight change in heifer 020B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. v = $PGF_2\alpha$; ↑ = Medamycin; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

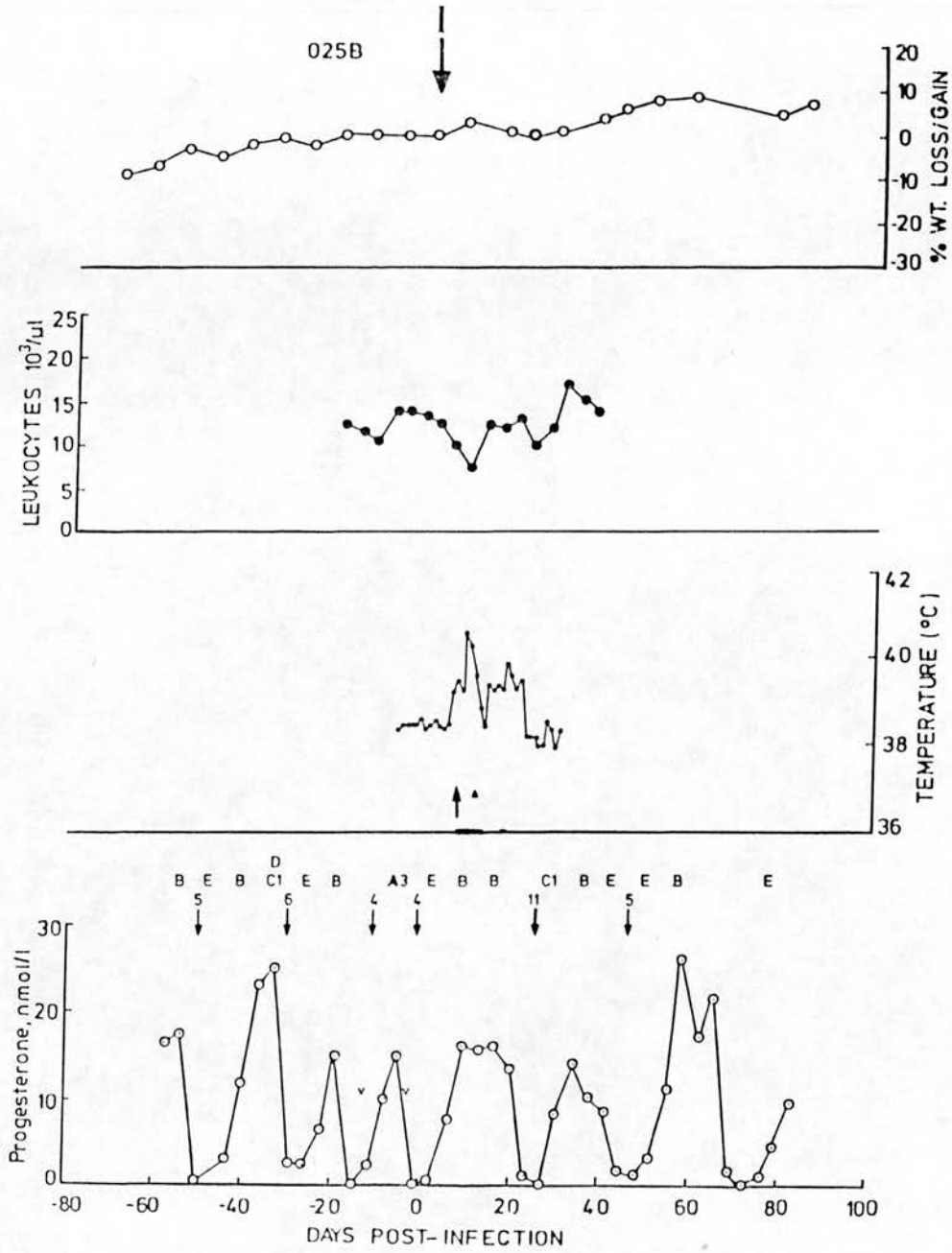


Fig.V-3.

Serum progesterone concentration, ovarian structures, rectal temperature, shizont parasitosis (bar), leucocyte concentration and weight change in heifer 025B infected with undiluted *T. parva* stabilate I13081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$; ↑ = Medamycin; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

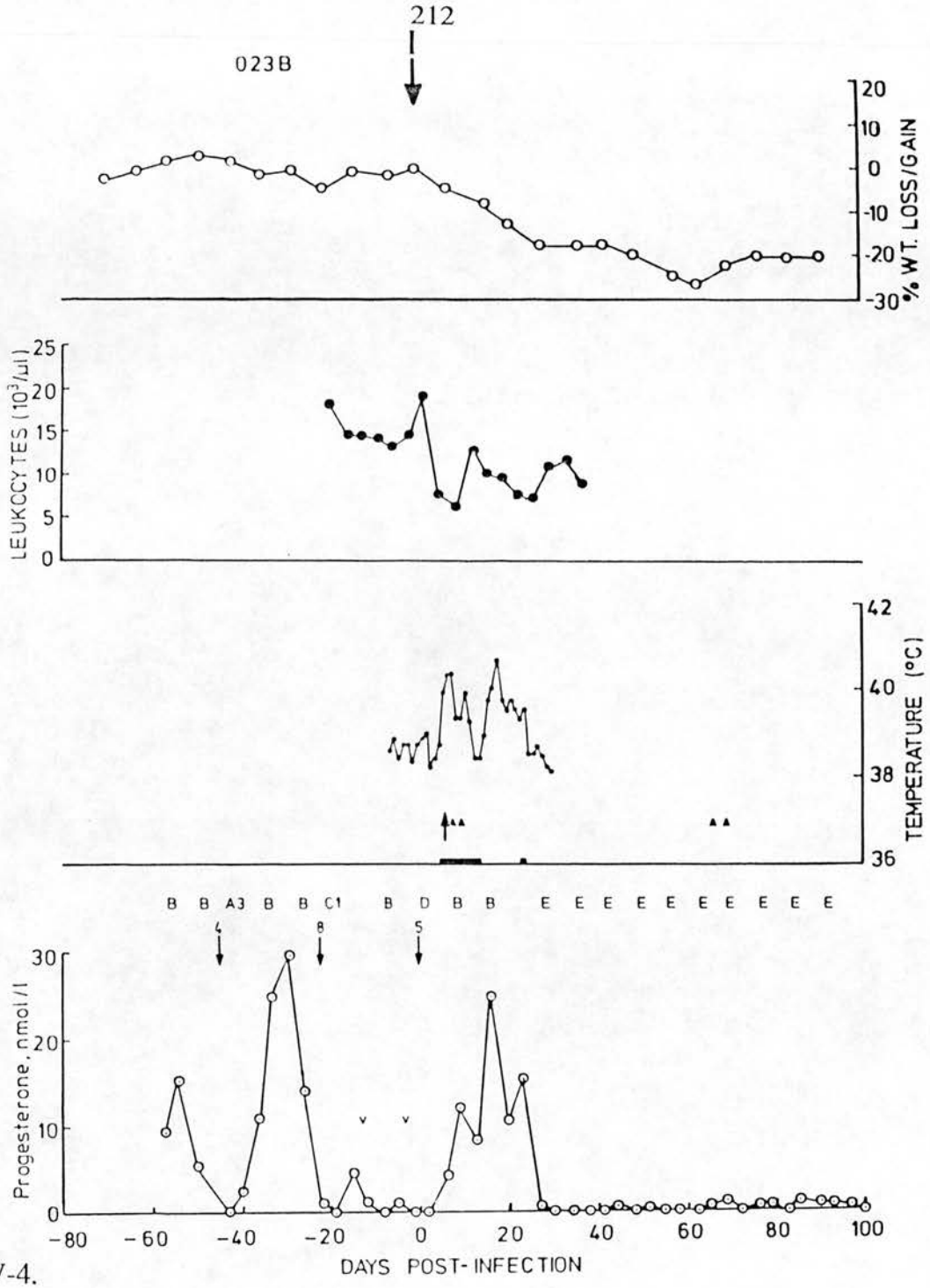


Fig.V-4.

Serum progesterone concentration, ovarian structures, rectal temperature, shizont parasitosis (bar), leucocyte concentration and weight change in heifer 023B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Progressing CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$; ↑ = Medamycin; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

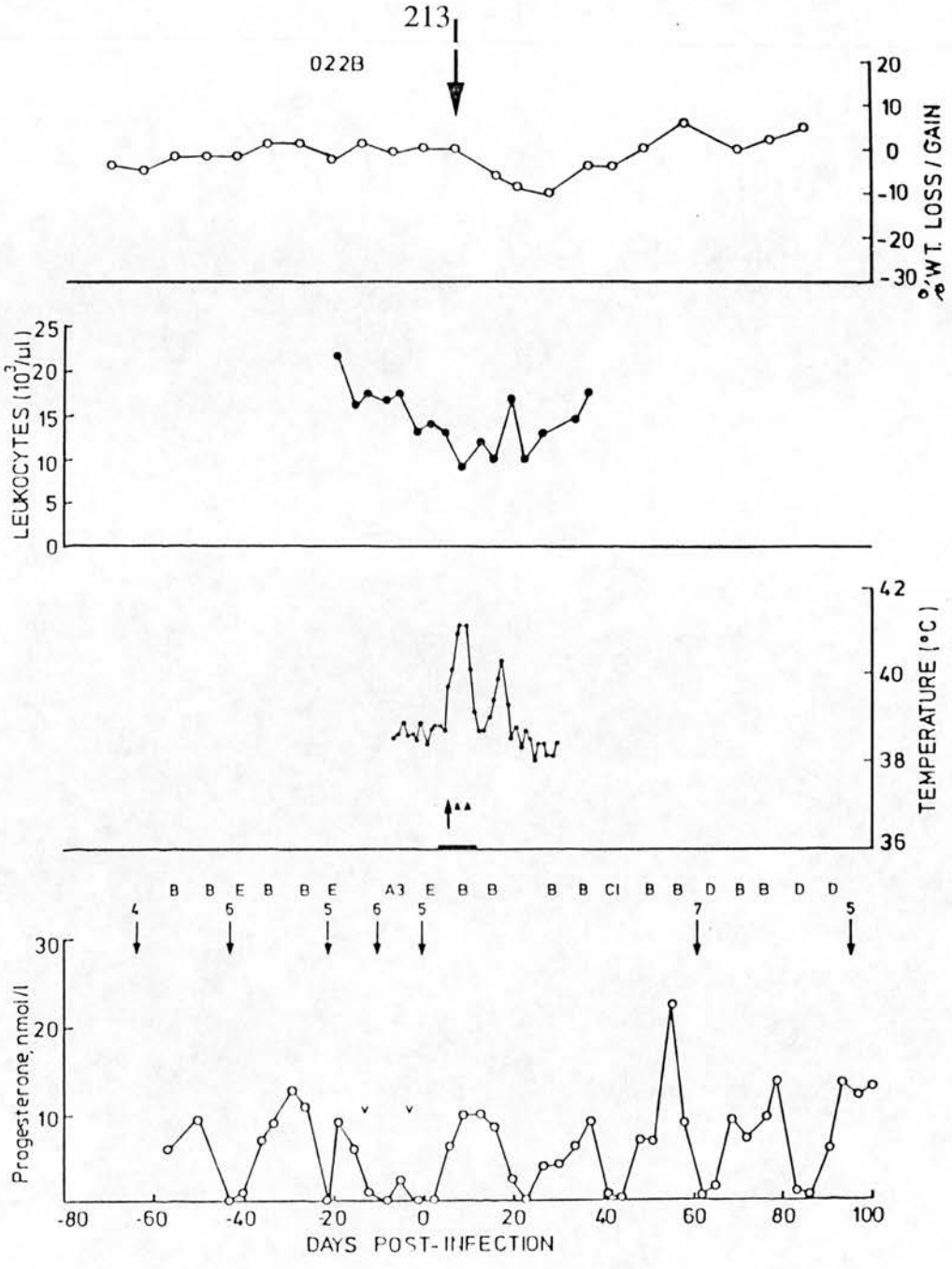


Fig.V-5.

Serum progesterone concentration, ovarian structures, rectal temperature, shizont parasitosis (bar), leucocyte concentration and weight change in heifer 022B infected with undiluted *T. parva* stabilate I13081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$; ↟ = Medamycin; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

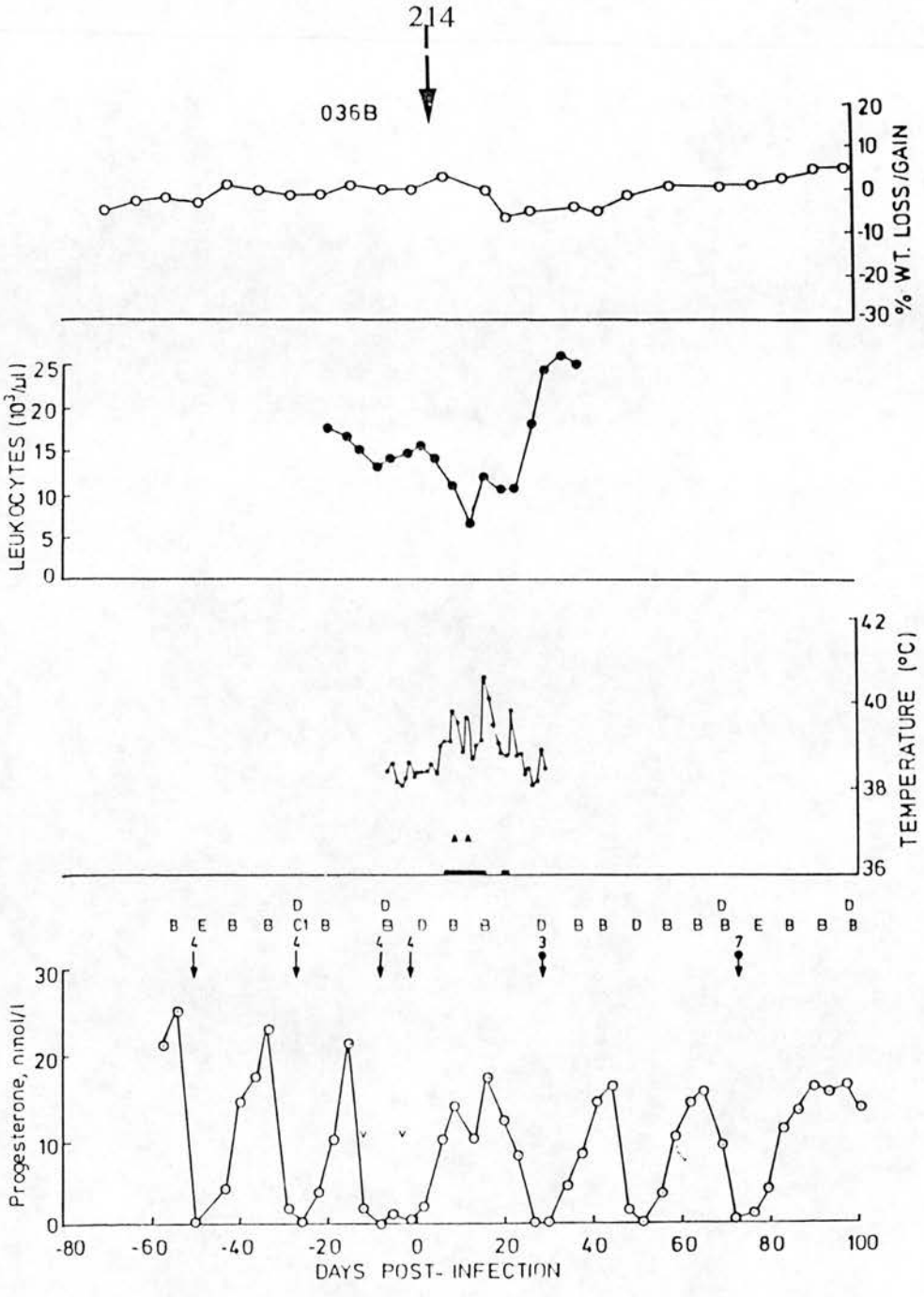


Fig.V-6.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and weight changes in heifer 036B infected with undiluted *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-infection body weight. Ovarian structures are represented by B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. ▼ = Observed mated heats. V = $PGF_2\alpha$; ▲ = Clexon/Medamycin treatments. Arrow [I] = Infection.

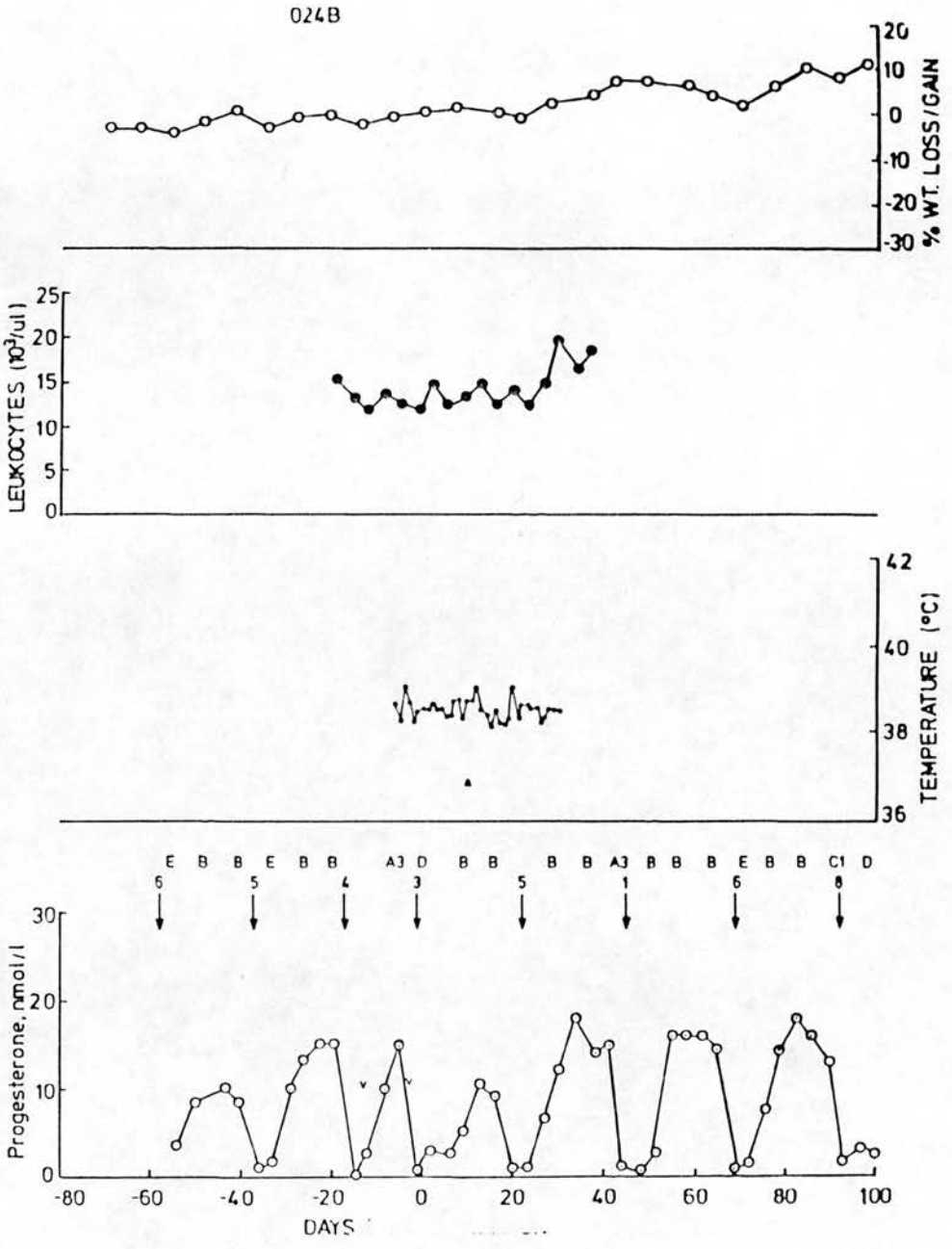


Fig. V-7.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and weight change in control heifer 024B. Weight is expressed as a percentage of initial body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Inverted arrows indicate observed oestrus and oestrous scores in superscript. V = PGFα; ▲ = Clexon/Medamycin treatments.

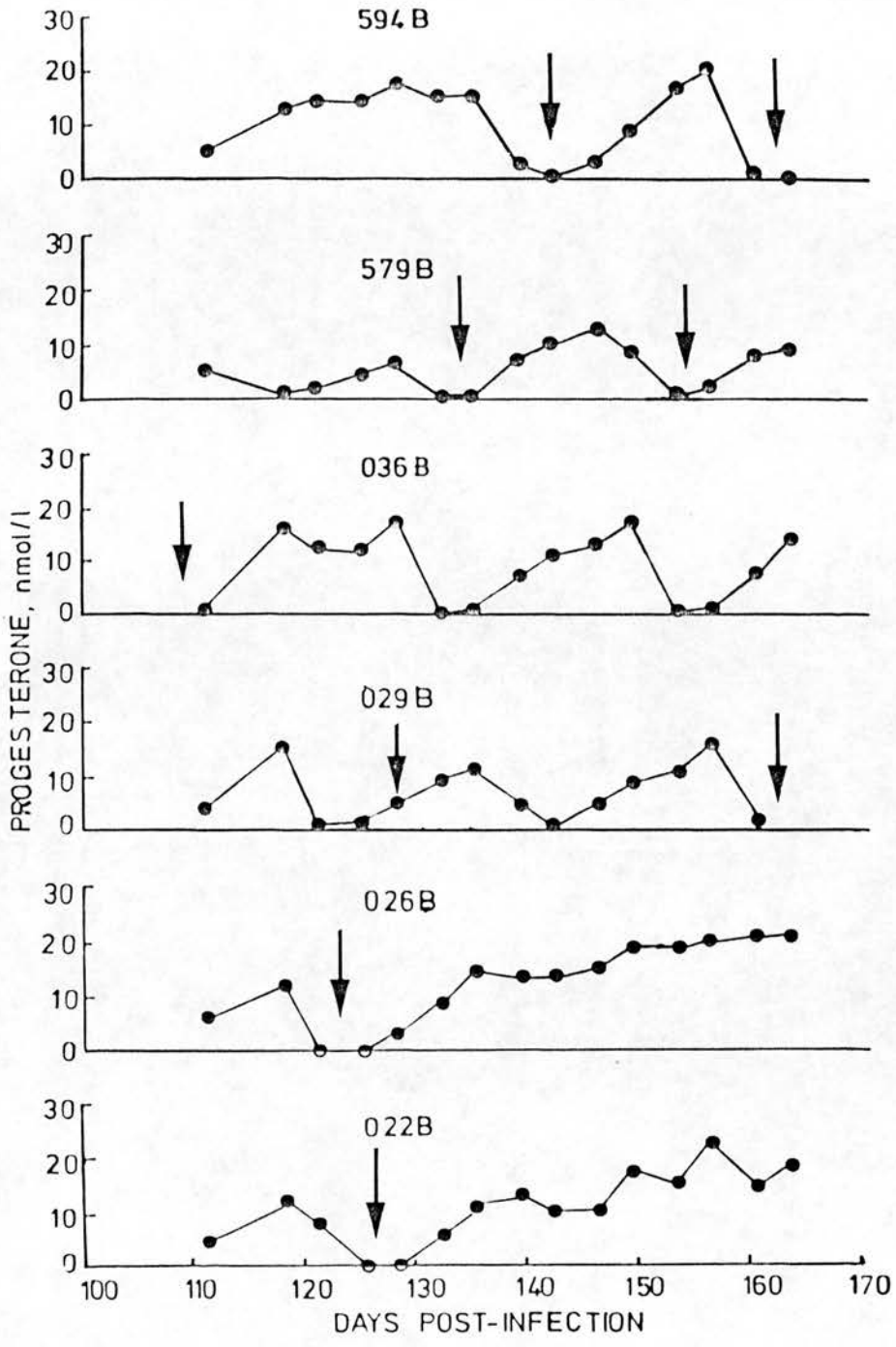


Fig.V-8.

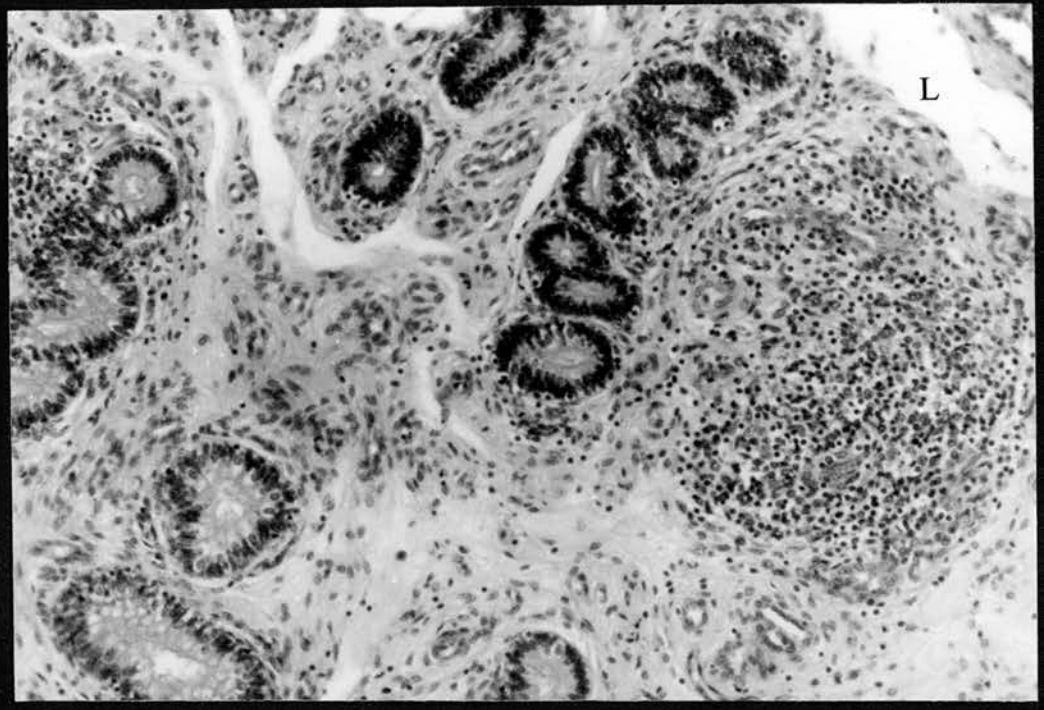
Serum progesterone concentrations between day 112-163 post-infection in group B heifers. Observed oestrus (arrows).

Fig. V-9.

Section of uterus of heifer No.587B killed on day 38 post-infection showing a focal mononuclear cell infiltration in the superficial endometrium. Uterine lumen [L]. H&E, (x140).

Fig. V-10.

Same section as in Fig. V-9 at higher magnification. H&E, (x350).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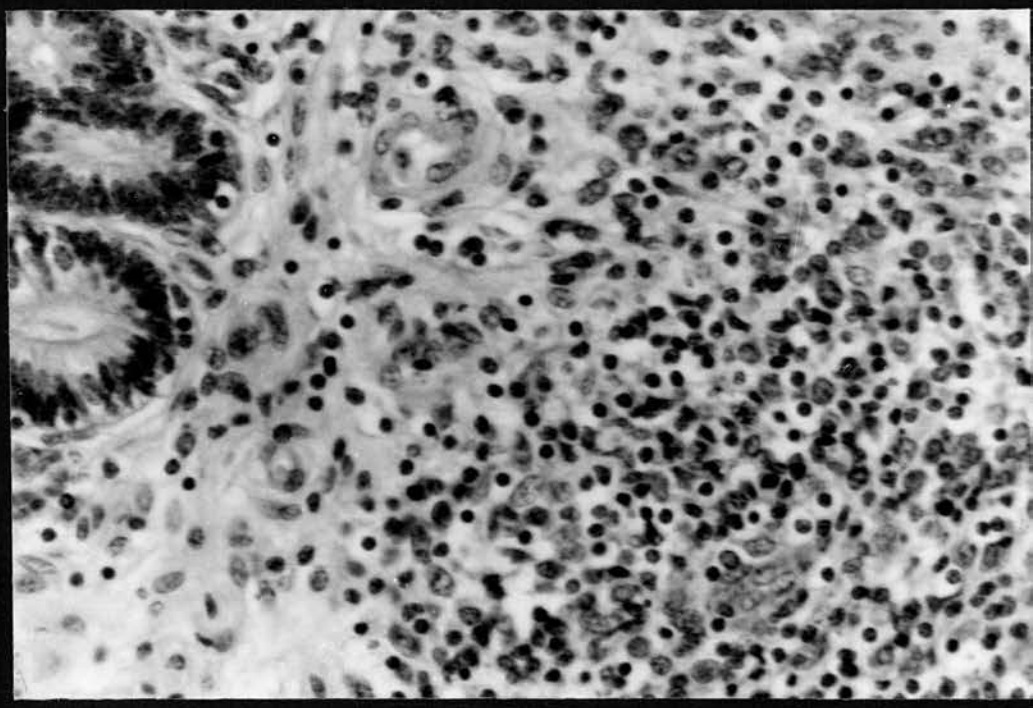
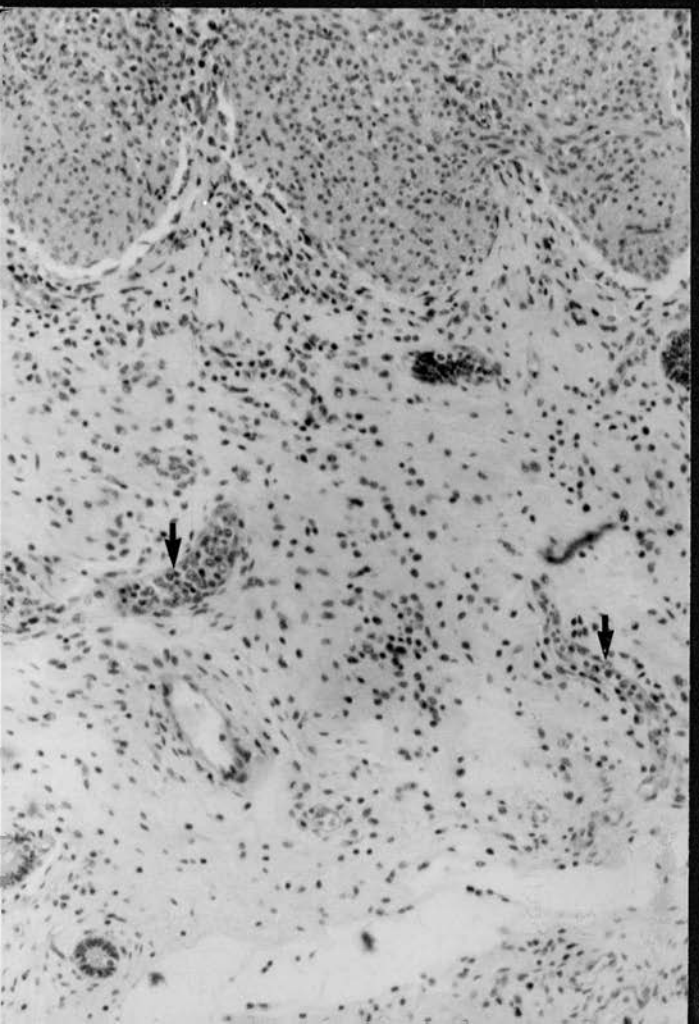


Fig. V-11.

Section of uterus of heifer No. 587B killed on day 38 post-infection showing diffuse mononuclear cell infiltration in the superficial endometrium. H&E, (x350).

Fig. V-12.

Section of uterus of heifer No. 602B killed on day 18 post-infection showing patchy mononuclear cell infiltration in the deep endometrial stroma. Increased numbers of mononuclear cells in vascular lumina [Arrows]. H&E, (x140).



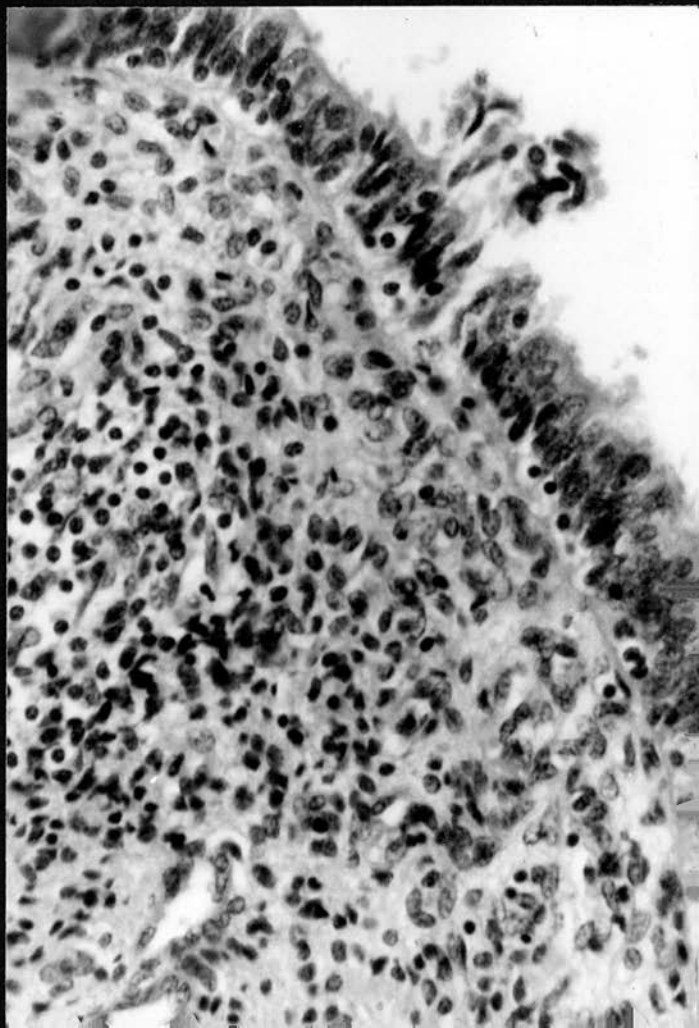
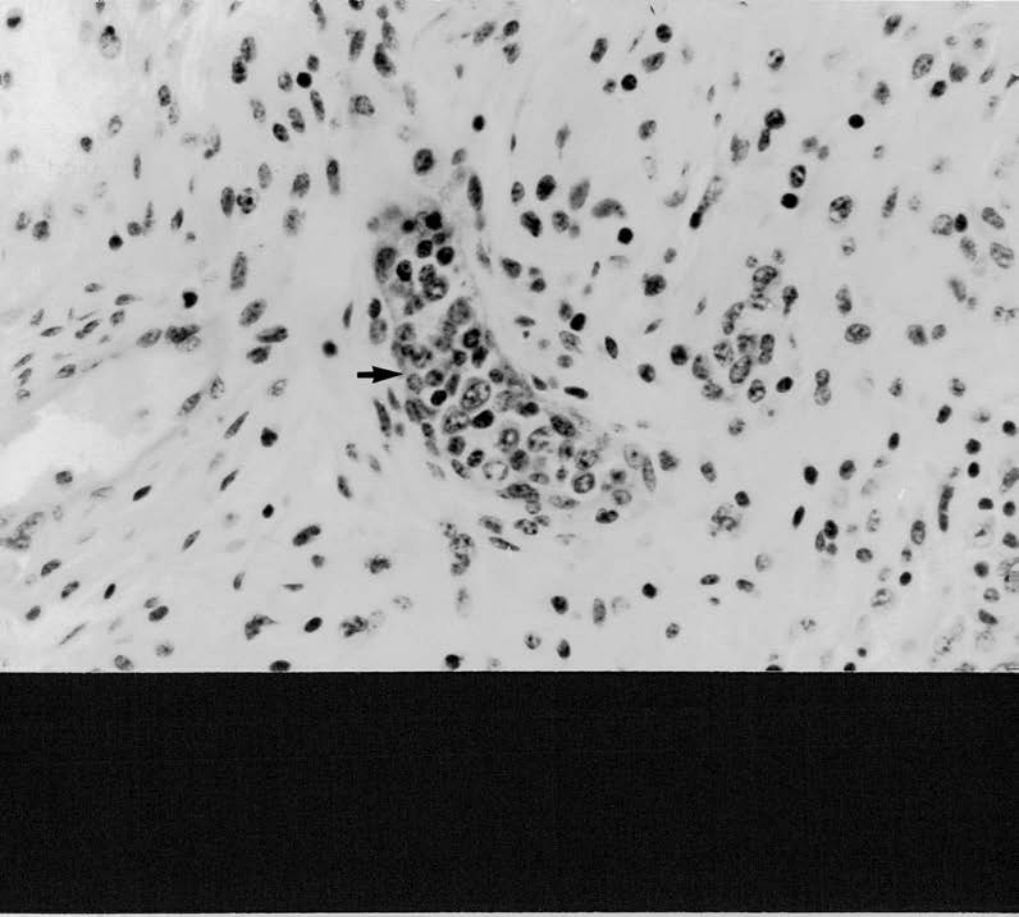


Fig. V-13.

Same section as in Fig. V-12 at a higher magnification. Increased number of mononuclear cells in the lumen of a blood vessel [Arrow]. H&E, (x350).

Fig. V-14.

Section of uterus of heifer No. 023B which died on day 101 post-infection. The superficial endometrium shows infiltration of the stroma by neutrophils and macrophages. Uterine lumen [L]. H&E, (x350).



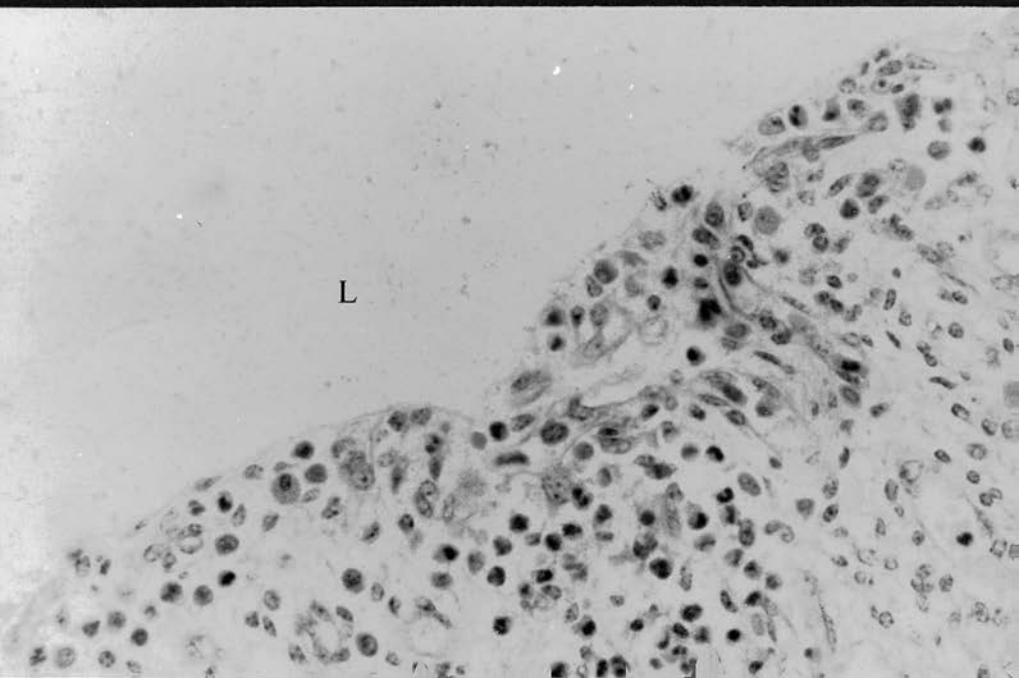
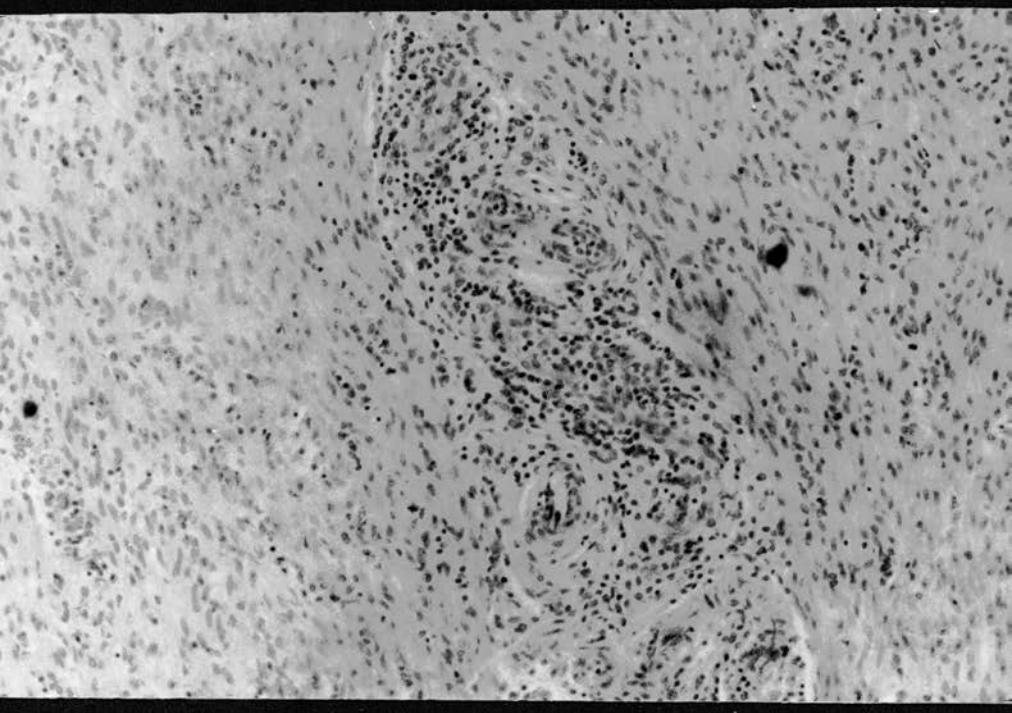

L

Fig. V-15.

Section of uterus of heifer No. 592B killed on day 18 post-infection showing a perivascular mononuclear cell infiltration in the myometrium. H&E, (x140).

Fig. V-16.

Section of the pituitary gland of heifer No. 587B killed on day 38 post-infection showing a focal mononuclear cell infiltration in the adenohypophysis [Arrow]. H&E, (x140).



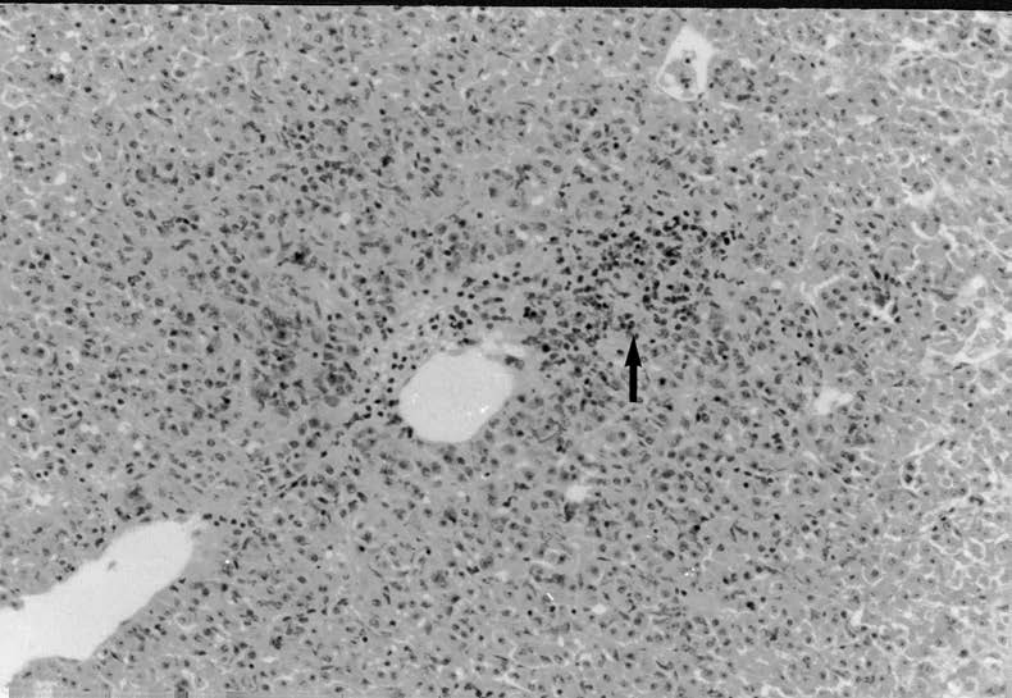
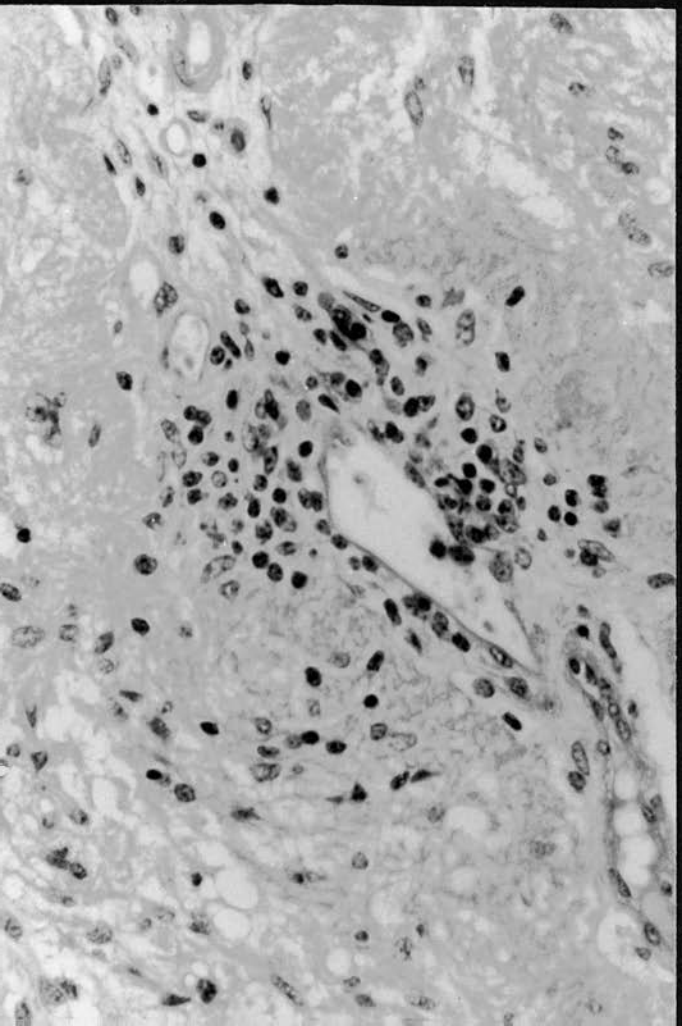


Fig. V-17.

Same section as in Fig. V-16 at higher magnification. H&E, (x350).

Fig. V-18.

Section of pituitary gland of heifer No. 587B killed on day 38 post-infection showing a focal perivascular mononuclear cell infiltration in the neurohypophysis. H&E, (x350).



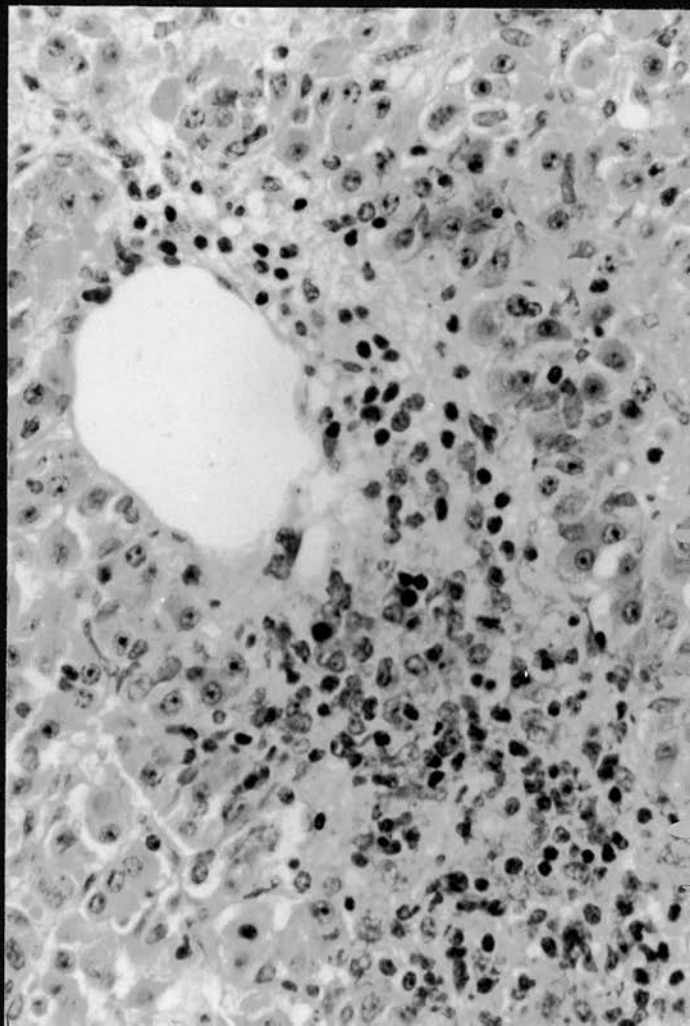


Fig. V-19.

Section of pituitary gland of heifer No. 587B killed on day 38 post-infection showing degenerative changes in the epithelial cell lining and infiltration of the intermediate lobe by mononuclear cells. H&E, (x350)

Fig. V-20.

Section of adrenal gland of heifer No.602B killed on day 18 post-infection showing a scattered mononuclear cell infiltration in the *zona glomerularis*. H&E, (x140).

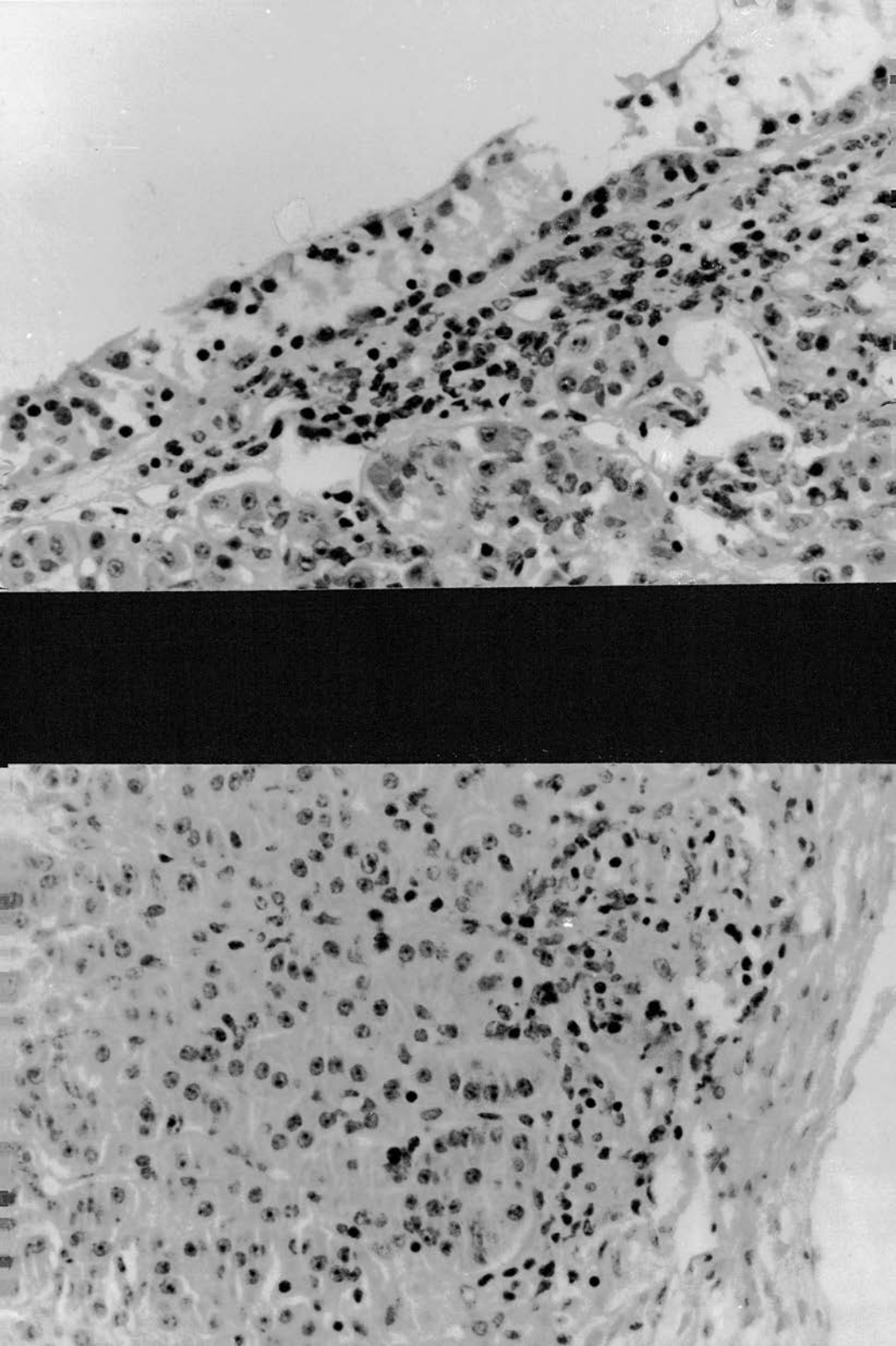
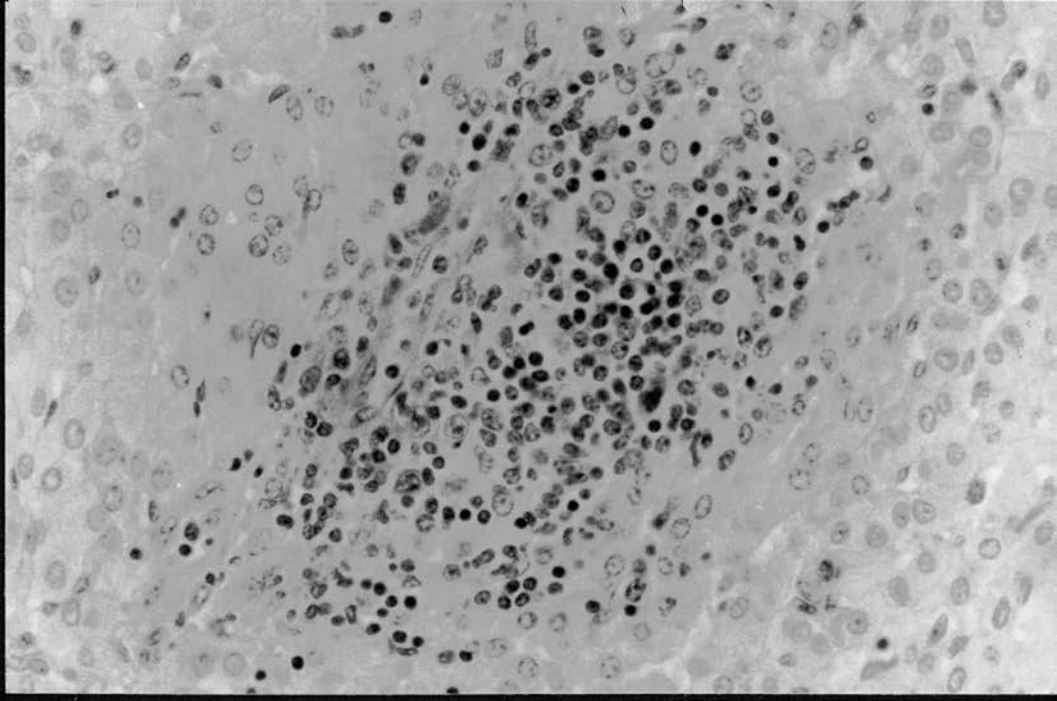


Fig. V-21.

Section of adrenal gland of heifer No. 592B killed on day 18 post-infection showing a focal mononuclear cell infiltration in the *zona fasciculata*. H&E, (x350)

Fig. V-22.

Section of adrenal gland of heifer No 592B killed on day 18 post-infection showing patchy infiltration of the *zona reticularis* by mononuclear cells. H&E, (x140).



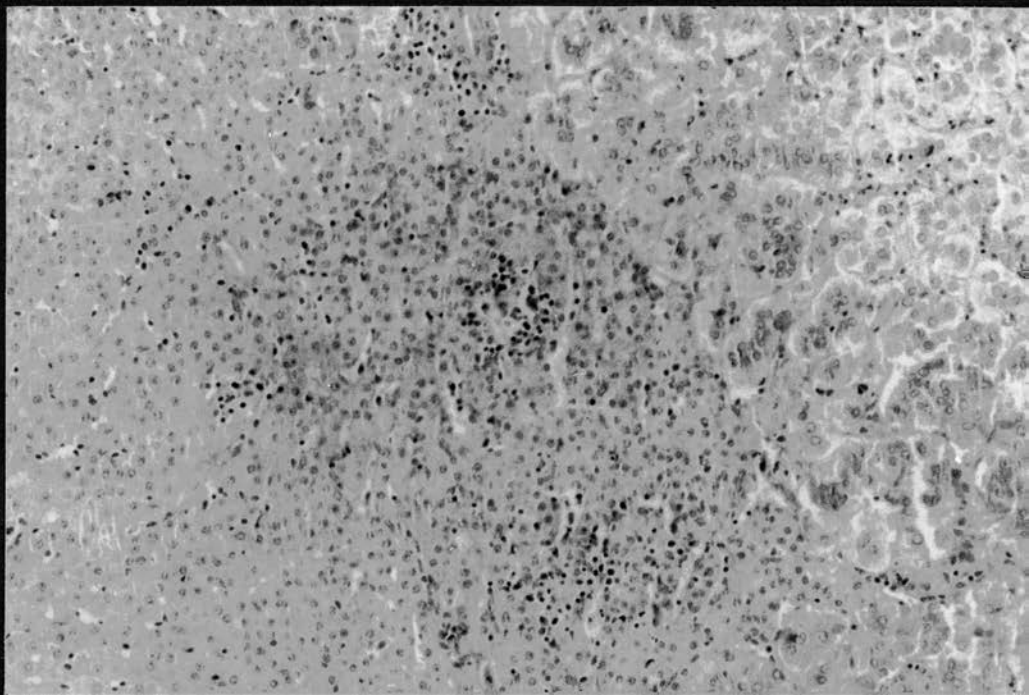
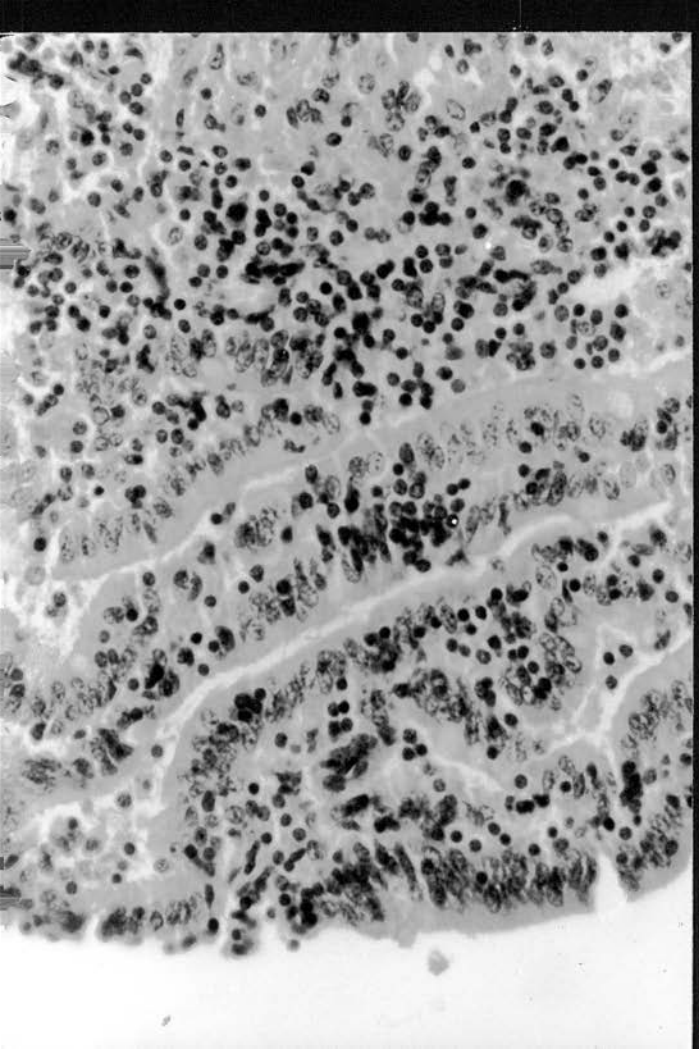


Fig. V-23.

Section of adrenal gland of heifer No. 592B killed on day 18 post-infection showing a mononuclear cell infiltration of the adrenal medulla . H&E, (x140).

Fig. V-24.

Section of small intestine of heifer No. 592B killed on day 18 post-infection showing infiltration of the *lamina propria* by mononuclear cells. H&E, (x350).



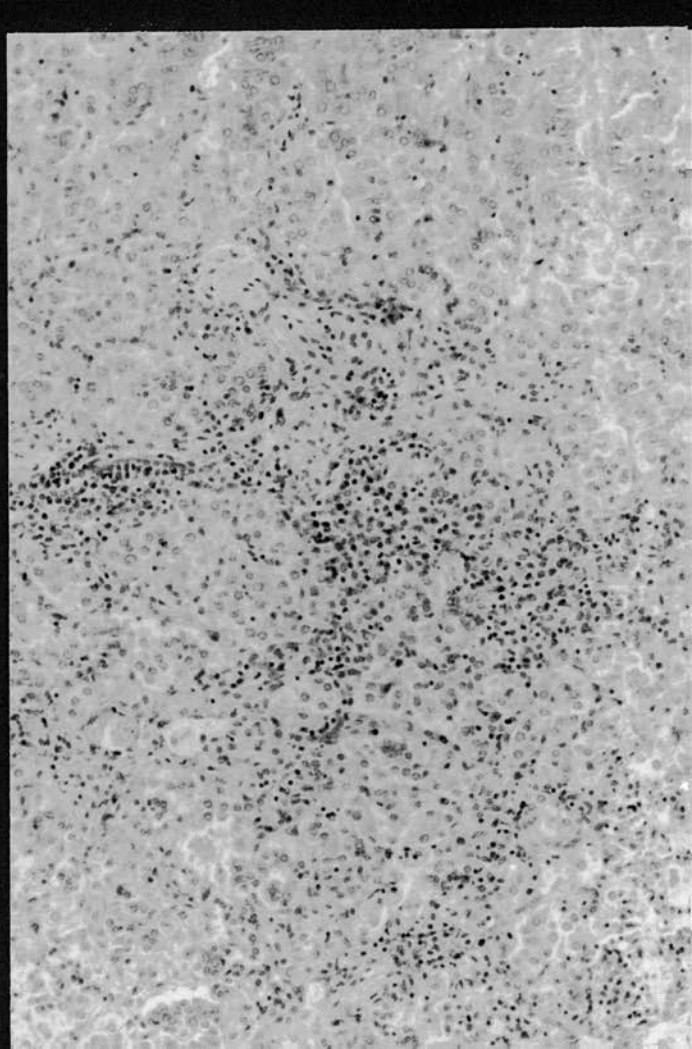
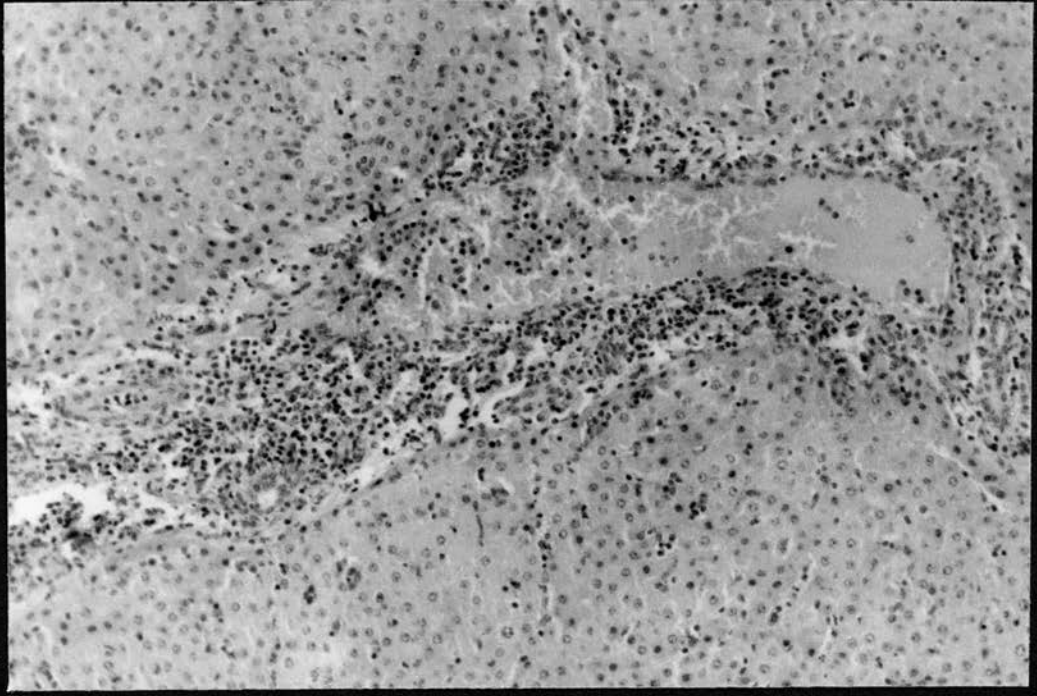


Fig. V-25.

Section of liver of heifer No. 592B killed on day 18 post-infection showing infiltration of the portal triad by mononuclear cells. H&E, (x140).

Fig. V-26.

Section of kidney of heifer No. 023B which died on day 101 post-infection showing a severe mononuclear cell infiltration into the renal cortex. H&E, (x140).



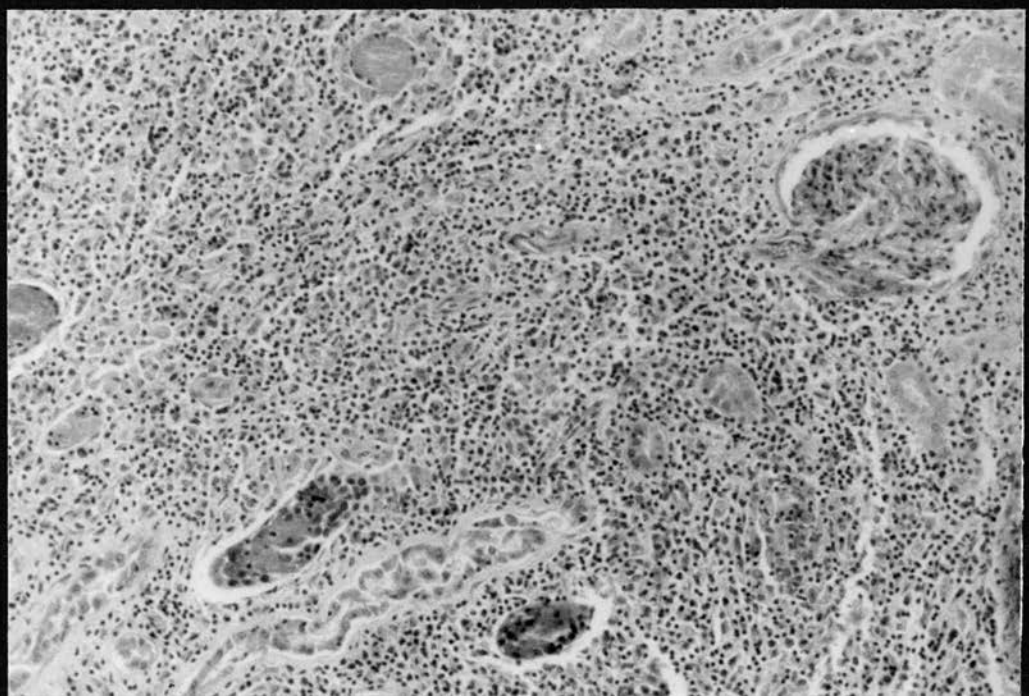
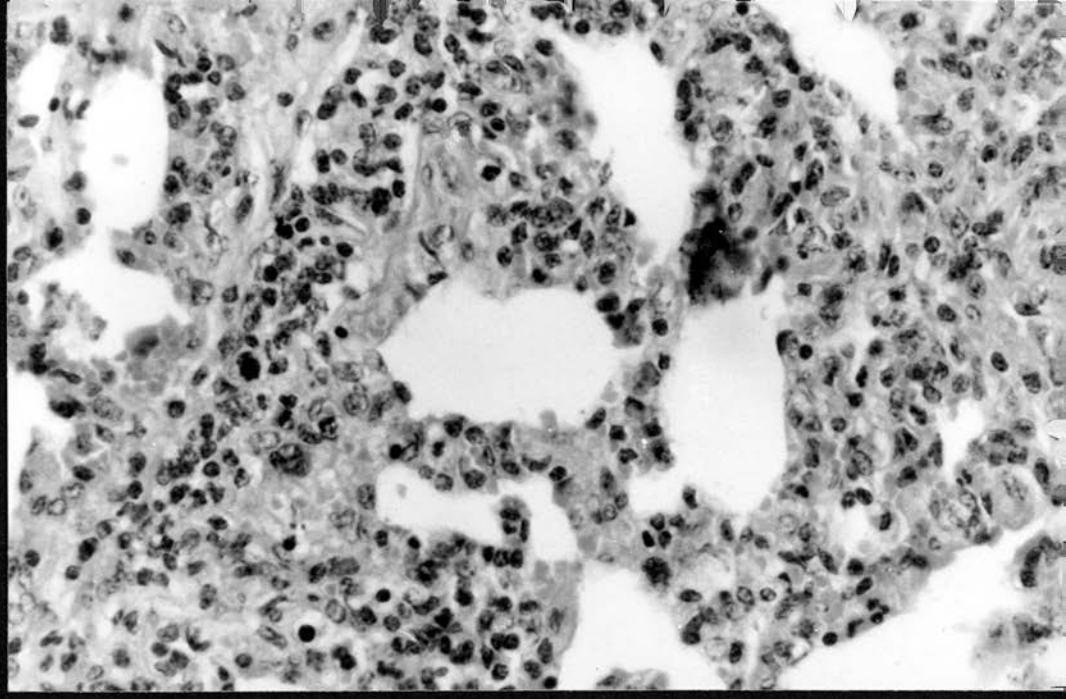


Fig. V-27.

Section of lung of heifer No. 587B killed on day 38 post-infection showing infiltration of alveolar walls by mononuclear cells. H&E, (x350).

Fig. V-28.

Heart section of of heifer No. 592B killed on day 18 post-infection showing infiltration of mononuclear cells between muscle bundles. H&E, (x140).



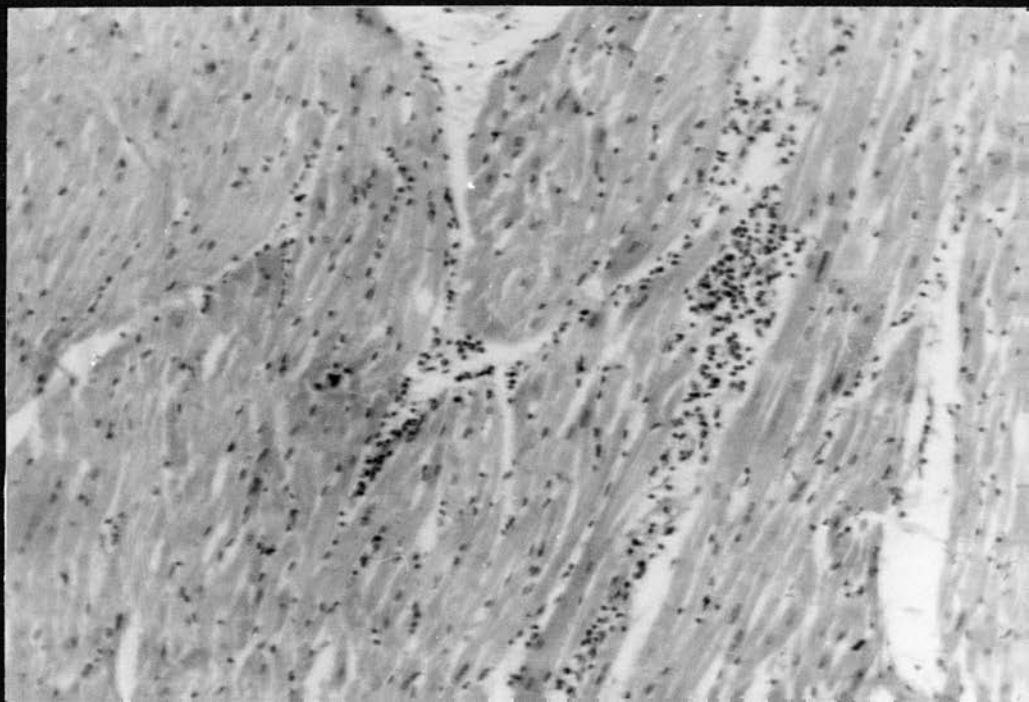
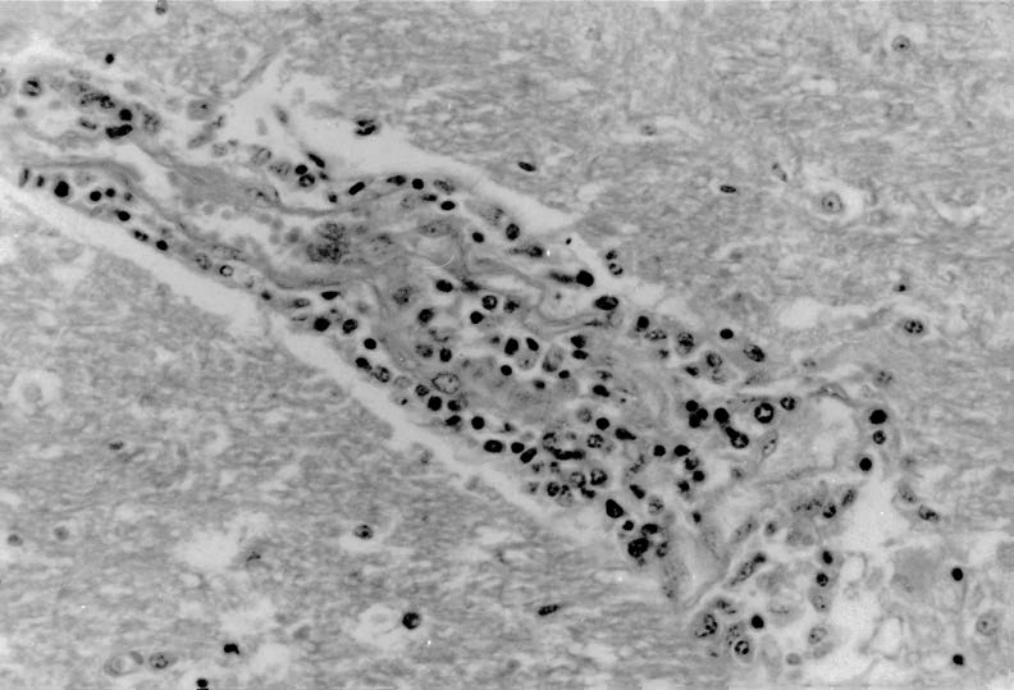


Fig. V-29.

Section from the midbrain of heifer No. 587B killed on day 38 post-infection showing a perivascular mononuclear cell infiltration. H&E, (x350).



CHAPTER SIX

THE EFFECT OF IMMUNIZATION AGAINST THEILERIOSIS ON THE REPRODUCTIVE FUNCTION OF BORAN/FRIESIAN CROSS HEIFERS

6.1 Introduction

When this study was formulated, no reports existed on the effect of immunization against theileriosis on the reproductive function in cattle. Subsequently, Dolan and Mutugi (1989) examined the fertility of Boran heifers following field immunization against buffalo-derived *T. parva* parasites.

In the immunization against theileriosis, the only method developed to a stage where it can be used in the field is the infection and treatment method using *T. parva* sporozoites to infect, and treating with oxytetracyclines (Radley, 1978). Immunity to theileriosis was thought to be sterile (Theiler, 1912) but it was later discovered that recovery from theileriosis is invariably accompanied by a carrier state in which a persistent parasitosis exists (Barnett and Brocklesby, 1966a; Young *et al.*, 1981). Fears have been expressed on the residual effect of *T. parva* on the long term productivity.

It was realized that until another alternative method is developed, reliance would be on infection and treatment as a means of protecting cattle against *T. parva* infections. This study was therefore initiated to study under controlled laboratory conditions the effect of immunization on the reproductive function of cattle. This was achieved by immunizing eight normally cyclical Boran/Friesian cross heifers against a buffalo-derived *T. parva* and monitoring them for cyclical status and/or fertility.

6.2 Materials and methods

6.2.1 Materials

6.2.1.1 Animals

These are described in General Materials and Methods (2.1).

6.2.1.2 Parasite

Details of the *T. parva* stabilate are described in General Materials and Methods (2.2). A 1:50 dilution of stabilate was made by reconstituting 0.5 ml of undiluted stabilate in 24.5 ml of Eagle's Minimum Essential Medium containing 3.5% bovine plasma albumin and 7.5% glycerol as described in General Materials and Methods (2.3).

6.2.1.3 Drugs

1. A synthetic prostaglandin $F_2\alpha$ analogue, Lupriostol, formulated at 7.5 mg/ml (Prosolvin, Intervet International BV, Holland).
2. A long acting formulation of oxytetracycline hydrochloride, formulated at 200 mg/ml of oxytetracycline base (Terramycin LA, Pfizer, UK).
3. Parvaquone, formulated at 150 mg/ml of active base in dimethyl sulphoxide in corn oil (Clexon, Coopers Animal Health, UK).

6.2.2 Experimental design

In this experiment, eight animals were recruited into the experiment three months while four others were recruited three weeks, respectively before the time of immunization. Of the latter, three animals were allocated into the immunization group and one to the control group.

Thirteen days and again three days to the start of the experiment, all the 12

heifers used in this experiment were given 2 ml of Prosolvin intramuscularly to synchronize them into oestrus. Based on behavioural data and P4 profiles, all except one animal (588B) were successfully synchronised to be around oestrus at the time of infection and treatment (referred to as day 0).

The animals were allocated at random into two groups of eight and four animals each. The eight animals were immunized by inoculation of 1 ml of 1:50 *T. parva* stabilate as described in General Materials and Methods (2.3) and concomitantly treated with 20mg/kg bw Terramycin LA through deep intramuscular injection. The immunized animals were divided at random into two groups of four animals each. One group was monitored for cyclical status and was designated as group A and the other was exposed to a bull immediately and designated as group B. Three animals, two in group A and one in group B developed a febrile reaction after infection and were treated with Clexon/Terramycin LA at 10mg/kg bw of each compound in two divided doses 48 h apart. The four animals that acted as controls were injected with Terramycin LA at 20mg/kg bw on day 0.

Group A animals and controls were studied to week 16 while the study period for group B was extended to week 20 post-immunization. The monitoring regimen is presented in a summarized form in Table VI-1. The methodologies are described in detail in General Materials and Methods.

Serum P4 concentrations were determined by the WHO Matched Reagent Programme supplied assay as described in General Materials and Methods (2.12.2.2). The mean percentage recovery was 74.4% (mean $\pm$ SD, n=24). Results were not corrected for recovery. The inter-assay coefficients of variation were 18.4% (n=14) at 3.6 $\pm$ 0.7 nmol/L and 10.2% (n=14) at 16.2 $\pm$ 1.7 nmol/L for the low and high quality controls (QCs), respectively. The intra-assay coefficients of variation were 7.2% and 4.6% for the low and high QCs, respectively.

Table VI-1 **Summarized experimental protocol (Experiment 4)**

Parameter monitored	Frequency and distribution		
	Group A	GroupB	Controls
Oestrus behaviour	Daily (x3) : Week -12 to Week 16	Week 20	Week 16
Body weight	Weekly : Week -12 to termination as above		
Serum P4*	Twice weekly : d -57 to d 112	d 139	d 112
Rectal palpations	Weekly : Week -12 to 15		
Rectal temperature	Daily : d -7 to d 35		
Haematology	Twice weekly :Week -12 to 7		
$PGF_{2\alpha}$ 1st	d -13		
2nd	d -3		
Immunization	d 0		ND
Lymph node biopsies	Daily		ND
LEG	d 5 to 28/34	d 28	ND
RPG	d 10to 28/34	d 28	ND
Blood smears	Daily : d10 to d 28/34	d 28	ND
Treatment	588B : d24,26 805B : d 21,23	804B : d19,21	ND
Serology	d 34		
Exposure to bull	ND	d 0 to termination	ND
Cell culture	d 115		ND

* - By WHO Matched Reagent Programme (2.12.2.2)
ND - Not done

6.3 Results

6.3.1 Clinical signs

The results of the animal responses to infection and/or treatment are presented in Table VI-2. Representative temperature changes are presented in Figs. VI-1 to VI-5. Three animals, two in group A and one in group B developed a febrile response 17-20 days after immunization (Fig.VI-2). Temperatures dropped to normal levels after treatment with Clexon/Terramycin LA but two of the three animals had remissions before temperatures fell to and remained at normal levels. The three animals were dull and anorectic during the febrile reaction period. All the other immunized animals and controls remained healthy throughout the study period.

6.3.2 Parasitology

Five of the eight immunized animals developed a patent macroschizont parasitosis (Table VI-2). The prepatent period ranged from 16-23 (mean $\pm$ SD, 19.8 $\pm$ 2.5) days. In two animals (805B and 597B), macroschizonts were detected in the contralateral prescapular lymph node but not in the regional lymph node. Macroschizonts were not detectable following treatment with Clexon/Terramycin LA in 588A. The other two animals (804B and 805B) receiving the same treatment had no detectable parasitosis at the time of treatment. Macroschizont parasitosis was shortlived (1-5 days) with or without treatment (Figs. VI-2 and VI-3 are representative). Six of the immunized animals developed a low grade (<1%) piroplasmaemia between days 16 to 25 post-immunization. The two exceptions without piroplasmaemia were animals 588B and 805B which had been treated with Clexon/Terramycin LA (Table VI-2). Overall, all the eight immunized heifers reacted to the *Theileria* parasite either by the demonstration of macroschizonts, piroplasms or both.

6.3.3 Haematology

6.3.3.1 White blood cell (WBC) concentration

The mean white blood cell concentration for the immunized (groups A and B) and controls are presented in Table VI-3. The immunized animals had a non-significant ($p>0.05$) leucocytosis on day 3 post-immunization and then a significant ($p<0.05$) leucopaenia compared to initial value on all sampled days except on day 31 post-immunization. There was no significant difference between the immunized and controls on all sampled days post-immunization. Leucocyte concentrations were high on day 0 and 3 but did not vary significantly ($p>0.05$) within the control group during the study period.

Representative WBC changes are presented in Figs. VI-1 to VI-4 for the immunized animals. A mild to moderate leucopaenia (Figs. VI-1 and VI-3) developed in five animals and a severe one (Figs. VI-2 and VI-4) in three others following immunization. Leucocyte concentrations did not vary significantly within individual control animals and the data presented for 590B (Fig. VI-5) were typical of the control group.

6.3.3.2 Differential leucocyte count

The mean lymphocyte and neutrophil concentrations are presented in Table VI-4. In the immunized animals (groups A and B), lymphocyte concentrations declined significantly from pre-immunization levels on all sampled days from day 7 to day 38 except on day 31. Lymphocyte concentrations did not differ significantly between the immunized and controls or within the control group over the study period. Lymphocyte concentrations within individual animals paralleled those of white blood cell concentration.

Both immunized and control animals had a non-significant ($p>0.05$) neutrophilia on day 3 post-immunization. A significant ($p<0.05$) neutropaenia

developed between day 21-28 in the immunized animals (Table VI-4). Neutrophil concentrations fluctuated but did not vary significantly in the control group over the study period. There were no significant differences in neutrophil concentrations between the immunized and control groups over the study period. In individual animals, neutrophil concentrations paralleled those of white blood cells.

Eosinophil concentrations were very low and fluctuated very widely within individual animals and within the immunized and control groups to facilitate reasonable interpretation.

6.3.3.3 Red blood cell concentration

The red blood cell concentration during the experimental period is presented in Table VI-5. A highly significant ($p<0.01$) elevation in RBC concentration was seen on day 3 in both the immunized and control groups. Significantly lower RBC concentrations from the pre-immunization levels occurred on days 7 to 10, 24 and 35 post-immunization in the immunized group. RBC concentrations otherwise did not vary significantly within the control group over the experimental period. Red blood cell concentrations were significantly lower in the immunized compared to the control group between days 31 to 38 of the experiment (Table VI-5).

6.3.3.4 Haemoglobin concentration

The haemoglobin concentration change during the experimental period is presented in Table VI-5. Significant and highly significant ($p<0.01$) lower levels to the initial (day 0) concentrations occurred on days 7,10 and 17-28 post-immunization. Haemoglobin concentration in the immunized group approached significantly reduced levels ($p<0.05$) between days 31 to 38 after immunization. Haemoglobin concentration between the immunized and control groups showed significantly lower levels in the immunized group on days 3, 35 and 38. Haemoglobin concentration did not vary significantly within the control group.

6.3.3.5 Packed cell volume (PCV)

The change in packed cell volume in both immunized and control groups during the experiment is presented in Table VI-5. A significantly lower packed cell volume was observed on day 3 and 7 post-immunization in the immunized group. Compared to the controls, the packed cell volume was significantly lower in the immunized group on day 10 and 31 to 38. There were no significant variations in packed cell volume in the control group during the experimental period.

6.3.4 Body weight change

The body weight change within animals is presented in Table VI-6. All group A and two of four group B animals lost weight after immunization. The maximum body weight loss within animals ranged from 1.1-13% of their pre-immunization bw. Two (588B and 805B) of the three animals that reacted clinically to immunization lost more weight compared to the others (Table VI-6). All control animals lost weight ranging from 2.8-3.7% of their initial bw. All immunized and control animals gained weight and were above their initial bw at week 15 except 588B (Table VI-6).

The mean group bw changes are presented in Table VI-7. A non-significant ($p>0.05$) loss in weight occurred in group A and controls initially. At their nadirs, group A and controls had lost 5.6% and 2.9% of their initial bw, respectively. Group B lost no weight. All groups gained weight during the experiment and at 15 weeks post-immunization were each over 10% their initial weight. There were no significant variations in body weight within and among the three groups during the experimental period.

6.3.5 Serology

All the immunized heifers had positive titres to *T. parva* schizont antigen in serum samples collected on day 34 of immunization. Titres ranged from 1:160 to 1:2560 (Table VI-2). All control animals had negative titres to *T. parva* schizont

antigen.

6.3.6 Parasite isolation studies

None of the eight immunized animals demonstrated a carrier status. Cultures of peripheral blood lymphocytes collected on day 115 post-immunization neither showed signs of transformation nor presence of macroschizonts.

6.3.7 Reproductive status

6.3.7.1 Behavioural

A summary of the oestrous signs (scores) recorded during the study is presented in Table VI-8. Within animals, the number of manifested signs ranged from 3-8 per observed oestrus. The major presented signs were restlessness, vulval mucous discharge, swollen vulva, mounting other animals, and standing to be mounted which were observed on 81, 79, 74, 62 and 59 occasions, respectively out of a total of 81 heats. Standing to be mounted as a sign of true receptivity was therefore, seen on 72.8% (59/81) occasions.

Oestrus was observed in 83.2% (79/95) periods of basal P4 concentrations. Based on P4 profiles, 16.8% (16/95) heats were missed while 2.5% (2/81) of the heats were detected during the luteal phase.

6.3.7.2 Serum progesterone concentration

Serum P4 concentrations were used to complement behavioural and rectal palpations in assessing the reproductive status. Representative P4 profiles are presented in Figs. VI-1 to VI-5. Within animals, P4 concentrations varied from undetectable to the highest observed value of 25.9 nmol/L. Luteal phase plateau levels varied widely within and among animals but were mainly above 10 nmol/L.

6.3.7.3 Ovarian structures

A summary of the pooled data on palpated ovarian structures is presented in Table VI-9 where they are related to the cycle based on P4 profiles. Ovarian structures palpated between the two $PGF_2\alpha$ administrations are omitted.

A developing or mature CL was palpated at least once in 75/76 (98.7%) of luteal phases. During the luteal phase, a developing and a mature CL was palpated in 14 and 118 occasions, respectively out of 166 palpations. Agreement between a palpable CL and P4 concentrations during the luteal phase was therefore 79.5% (132/166). Comparative figures for a regressing CL, follicles or no structures were 3% (5/166), 8.4% (14/166) and 9% (15/166), respectively during the luteal phase. A mature CL was palpated on 7.4% (5/68) occasions during the follicular phase Comparative figures for a regressing CL, follicles or no palpable structures were 13.2% (9/68), 36.8% (25/68) and 42.6% (29/68), respectively resulting in a 92.6% agreement between rectal palpation results and P4 concentrations in the follicular period.

The overall accuracy of the rectal palpation results based on P4 concentrations was therefore, 83.3% (195/234) for both phases.

6.3.7.4 Reproductive statuses of various groups

6.3.7.4.1 Group A

This group comprised of three (580B, 584B and 588B) and one (805B) animals that were monitored from three months and three weeks pre-immunization, respectively. From behavioural data, three of the four heifers each had three cycles pre-immunization. Cycle lengths ranged from 10-27 days with individual mean lengths of 20.0 ± 5.7 (mean $\pm$ SD) to 20.3 ± 0.5 days and an overall group mean of 20.1 ± 5.3 days (Table VI-10). P4 profiles from 57 days pre-immunization revealed one cycle in each of the three animals with a mean cycle length of 24.3 ± 2.5 (range, 21-27) days

(Table VI-11). Based on behavioural data, P4 profiles and ovarian structures, all four animals in this group except 588B were successfully synchronized into oestrus at the time of immunization.

From behavioural data post-immunization, animals in this group each had two to four cycles ranging from 14-65 (mean, 28.0 ± 13.6) days (Table VI-10). However, P4 profiles and palpable ovarian structures revealed three to five cycles in each ranging from 17-42 (mean, 23.2 ± 5.9) days (Table VI-11). Of the four animals, three (580B, 584B and 588B) revealed normal cycles post-immunization. Heifer No. 580B was typical of the three and its data is presented in Fig. VI-1. Heifer No. 805B had a long inter-oestrus interval of 42 days which was characterized by high P4 concentrations and a CL indicative of a persistent CL. Regular cycles ranging from 21-28 days were subsequently revealed after the long luteal phase in this animal (Fig. VI-2). It was concluded that three animals in this group cycled normally post-immunization while 805B suffered a transient acyclic period associated with a persistent CL. The latter resolved spontaneously without treatment resulting in subsequent regular cyclicity.

6.3.7.4.2 Group B

In this group, two animals each were monitored from three months (591B and 597B) and three weeks (804B and 806B) before immunization, respectively. Based on behavioural data pre-immunization, the two animals studied from three months prior to immunization each had three cycles ranging from 20-23 (mean, 21.7 ± 0.9) days (Table VI-10). P4 profiles from 57 days before immunization revealed one cycle in each of the two animals ranging from 21-22 (mean, 21.5 ± 0.5) days (Table VI-11). From behavioural data, P4 profiles and ovarian structures, all the four animals were successfully synchronized into oestrus at the time of immunization.

After immunization, animals in this group had each three to five cycles from behavioural data. The cycles ranged from 14-73 (mean, 31.3 ± 14.8) days (Table VI-10). However, P4 profiles and ovarian structures revealed that animals had each three

to seven cycles ranging from 17-29 (mean, 21.3 ± 4.0) days (Table VI-11). Of the observed heats, mating was detected on one to three occasions in each animal representing a range of 16.7% (1/6) to 75% (3/4) of mated observed heats (Table VI-10). Three of the four heifers had repeated heats post-immunization based on behavioural data, P4 profiles and ovarian structures. Heifer No. 591B was typical of the three and its data are presented in Fig. VI-3. Heifer No. 806B ceased cycling after its observed last mated heat 71 days post-immunization. A mature CL was palpated from week 10-16 and P4 concentrations were maintained at an elevated level to week 20 post-immunization in this animal (Fig. VI-4). Rectal palpation of the uterus revealed three animals not pregnant while heifer No. 806B had a soft distended right uterine horn at week 19 post-immunization indicative of pregnancy. At slaughter (day 162), heifer No. 806B had a foetus with a crown-rump length of 19 cm. The uteri of the other three were empty and had no visible gross pathology.

6.3.7.4.3 Controls

Of the four animals in this group, three (590B, 598B and 599B) and one (803B) animal were monitored from three months and three weeks to the start of the experiment, respectively. From behavioural data, all the three animals monitored from three months before the initiation of the experiment had each three cycles ranging in length from 19-23 (mean, 20.4 ± 1.9) days (Table VI-10). P4 profiles from 57 days before the start of the experiment revealed one cycle in each of the three ranging from 19-23 (mean, 20.7 ± 1.7) days (Table VI-11). All four animals were successfully synchronized into oestrus at the initiation of the experiment.

Animals in this group had each four to five cycles ranging from 18-41 (mean, 22.2 ± 5.2) days based on behavioural data after treatment (Table VI-10). However, P4 profiles and ovarian structures revealed five cycles ranging from 18-29 (mean, 20.8 ± 2.3) days in each animal (Table VI-11). All the four controls were therefore cyclical throughout the study period. Heifer No. 598B was typical of the controls and its data

is presented in Fig. VI-5.

6.4 Discussion

This study was undertaken because there was concern that immunization against theileriosis by infection and treatment may result in loss of reproductive efficiency. The stabilate used in this experiment had been found to be highly lethal in undiluted form and to have a mortality rate of 20% (2/10) when used at a 1:20 dilution in a previous experiment (Chapter 3). Stabilate 199 isolated from the same buffalo which was the source of the material used in this experiment was reported to have a mortality rate of 2/3 at 1:1 dilution during immunization trials when using a long acting tetracycline (Terramycin LA) for chemoprophylactic treatment (Mutugi *at al.*, 1988a). In this experiment stabilate IL3081 was used at a 1:50 dilution with one treatment of Terramycin LA on day 0 at a dose of 20mg/kg bw. It was pleasing to note that at 1:50 dilution, this stabilate was highly immunogenic. All infected and treated animals developed antibodies to *T. parva* schizont antigen (Burridge and Kimber, 1972). Although the immunity status was not tested in this study, previous studies had shown that animals that had sero-converted to a 1:10 dilution of a related parasite (stabilate 199) were immune to homologous challenge (Mutugi *et al.*, 1988a).

Following infection and treatment, three animals developed a clinical reaction. Similar findings are reported elsewhere (Mutugi *et al.*, 1988a; Young *et al.*, 1990). Clinical reactions in the infection and treatment methods are dose-related (Mutugi *et al.*, 1988a) and are also dependent on the pathogenicity of the parasite isolate. Morzaria *et al.* (1988) reported no clinical reactions with a 1:50 *T. parva* (Marikebuni) stabilate. On the other hand, reactions to immunization were severe enough to warrant chemotherapeutic treatment (Young *et al.*, 1990). Similar chemotherapeutic intervention was adopted in this experiment since earlier experience with this parasite had revealed its unpredictable pathogenecity in individual animals (Mutugi *et al.*, 1988a,b; Chapter 3, this study). Clexon treatment did not interfere with the

development of antibodies to the parasite. Earlier work had even indicated that when used after day 8 of infection, Clexon can be used in chemoprophylactic treatment (Dolan *et al.*, 1988).

Five of eight animals in this experiment developed a schizont parasitosis. Mutugi *et al.* (1988a) also reported patencies varying from 3/3 to 1/3 with stabilate dilutions of 1:10 and 1:100 stabilate suggesting that establishment of infection is dose-dependent. Piroplasms are rare in buffalo-derived *T. parva* infections (Young *et al.*, 1988). In this experiment, we observed a piroplasmaemia in five of eight immunized animals. Mutugi *et al.* (1988a) also reported presence of piroplasms in two of three animals using a 1:100 but none with a 1:10 stabilate dilution.

We were unable to demonstrate a carrier state by cell culture in this experiment. Mutugi *et al.* (1988a) were also unable to demonstrate a carrier status by cell culture in three animals immunized with a buffalo-derived *T. parva* stabilate and treated with Terramycin LA. However they were able to demonstrate a carrier state in one of the three animals by parasite isolation using uninfected ticks and concluded that all cattle immunized with stabilate 199 and treated with oxytetracyclines were likely to have persistent infections. It is therefore possible that animals in this experiment were carriers but this could not be demonstrated through cell culture.

Leucopaenia is reported to develop following infection and treatment immunization (Dolan *et al.*, 1984a; Mutugi *et al.*, 1988b). In this experiment, all animals developed variable degrees of leucopaenia which was as a result of a lymphopaenia and a neutropaenia. However, leucocyte concentrations for the immunized animals did not vary markedly from those of the controls over the study period suggesting that the response generally was of comparatively mild intensity. Mutugi *et al.* (1988b) reported no changes in the erythrocyte values in buparvaquone treatment immunization. In this experiment, we observed intermittent significant lower values for all erythrocyte values within the immunized animals and between the controls although these values appeared to be close to the pre-immunization levels.

Dolan *et al.* (1984a) reported a loss of 6.6% of the initial body weight by day 22 in cattle infected with *T. parva* and treated with oxytetracycline. Animals in the above study recovered gradually and regained their starting weight by day 60. Young *et al.* (1990) also reported an initial body weight loss followed by recovery in cattle immunized against a combination of cattle-derived and buffalo-derived *T. parva*. In our study, only two clinically reacting animals had a moderate weight loss (9.3-13.0%) suggesting that the weight loss is disease related. Two immunized animals did not lose weight while the rest lost less than 5.3% of their initial weight. Control animals also lost 2.8-3.7% of their starting weight. The mild weight losses could be due to normal fluctuations or to the stress of increased handling after immunization. All immunized and control animals recovered their starting weight at the termination of the experiment. Recovery was slowest in one immunized animal (588B) that had lost most weight. Maximum weight loss was noted during the third week in the immunized group not exposed to a bull (group A) and in controls. The immunized group exposed to a bull (Group B) did not suffer loss in weight. This could be due to a difference in the management practice since this group was kept in a small group of eleven animals as opposed to the others which comprised of over twenty animals in one paddock. Our findings agree with those of Dolan *et al.* (1984a) and Young *et al.* (1990) but differ with those of Morzaria *et al.* (1988) who found that weight gains were overall better in the immunized than in the nonimmunized control groups.

Assessment of the cyclical status based on P4 profiles was the most reliable method. Measured against P4 profiles, behavioural oestrus detection correctly identified only 83.2% (79/95) of the expected heats. At the same time, standing to be mounted as the only true sign of oestrus (Whitmore, 1980) was observed in 72.8% (59/81) of the observed heats. Of the oestrous signs considered non-specific for true oestrus, restlessness and vulval mucous discharge were the most frequent, being observed in 100% (81/81) and 83.2% (79/81) of the heats, respectively and therefore gave the best indication that the animal was around oestrus. Llewelyn *et al.* (1987)

also found restlessness to be the best non-specific indicator of oestrus. P4 profiles also provided information in diagnosing two false heats observed during the luteal phase in this experiment. Palpation of ovarian structures to assess the cyclical status gave an overall accuracy of 83.3%. This compared favourably to our earlier results (Chapter 3) and to those reported elsewhere (Boyd and Munro, 1979; Watson and Munro, 1980). Rectal palpation of the reproductive tract for pregnancy diagnosis was mainly accurate for the non-pregnant status but only sensitive later than day 44 of expected conception in heifer No. 806B. In contrast, P4 profiles were superior in the assessment of pregnancy in this animal.

This study demonstrated that infection and treatment immunization had no long term adverse effects on the cyclical status of Boran/Friesian cross heifers. Of the four group A animals, only one ceased to cycle temporarily due to a persistent CL post-immunization. The persistent CL resolved spontaneously without treatment and regular cyclicity was restored although the cause was not established. In the bovine, involution of the CL is facilitated by $PGF_2\alpha$ of uterine origin (Hansel *et al.*, 1973) and persistence in this case could therefore have been due to impaired uterine synthesis of this hormone. However, there was no clinical evidence of endometritis or other conditions which may have affected prostaglandin synthesis and assessment of uterine status by way of uterine endometrial biopsies was not done. Finally, although this luteal problem occurred during the period of clinical and parasitological reactions following immunization it seems unlikely that disease had any influence on the condition since this feature was not seen in any other animal showing parasitosis and/or fever in this study.

In the fertility studies, one of the four immunized animals was confirmed pregnant. P4 profiles and ovarian structures revealed that the three animals that were not pregnant at the termination of the study cycled throughout. The number of mated observed heats in these three non-pregnant animals varied from one out of six to three out of four. Added to these, all three had basal P4 periods when neither oestrus nor

mating were observed. However, the accuracy of detecting oestrus based on standing to be mounted was only 72.8% in this experiment suggesting that about 27% of the matings went unobserved. Compounded with this, seventy percent of mounting activity is reported to occur between 18.00 h and 6.00 h (Whitmore, 1980) thus suggesting that the number of matings observed in this study may not reflect the total services since behavioural oestrus was only monitored during daylight hours. Increasing the frequency of observations plus use of heat-mount detectors would have increased the mating detection rate (Whitmore, 1980; Llewelyn *et al.*, 1987). That the bull was fertile is unquestionable since one animal in this experiment and two others in a previous one (Chapter 5) were confirmed pregnant. However, the bull's breeding efficiency was not tested. Dolan and Mutugi (1989) reported an incalf rate of 70% and 86% after five months and seventeen months, respectively in Boran heifers immunized against a buffalo-derived *T. parva*. These figures were comparable to those of the entire herd suggesting that immunization had no adverse effect on fertility. Although it is not stated, it is assumed that their heifers were exposed to many bulls and coupled with the longer time of exposure to the bull, their studies achieved a higher in-calf rate. In this study, one bull was used on ten heifers. Although the heifer/bull ratio was considered adequate (Plasse *et al.*, 1970) it is not known whether the bull had certain preferences for individual animals thus affecting the overall mating efficiency.

Assuming that all observed and unobserved heats were mated, then failure to conceive could be attributed to several factors. These would include ovulatory failures, fertilization failures or embryonic mortalities among others. The infrequency of rectal palpation and its sensitivity in assessment of ovarian changes does not allow for interpretation of ovulatory failures. At slaughter, no abnormal lesions were observed that would have interfered with egg or sperm transport. Early embryonic death is reported to be the major cause of bovine infertility (Lamming and Bulman, 1976) and when it occurs early, it may go unnoticed since the animals return to oestrus with no obvious change in cycle patterns (Hafez, 1974). Two animals in this

experiment had cycle lengths of 29 days following mating and although this may suggest embryonic death, this can only be speculative since the range of the cycle lengths pre-immunization extended to this limit. Ultrasound scanning of the uterus could have provided evidence of early conception (Griffin and Ginther, 1992) but was not done in our study.

In conclusion, this study demonstrated that immunization against theileriosis does not have long-term adverse effects on the reproductive function of the Boran/Friesian cross heifers. Although a luteal dysfunction was observed in one of four animals, the disturbance was only temporary and resolved spontaneously. One of four animals exposed to a bull became pregnant. Elsewhere, higher pregnancy rates are reported after longer and possibly more intense exposure to bulls. The short time in which our animals were exposed to a bull may have resulted in the low rate of pregnancy. A number of areas not studied that would have improved our fertility studies include the incorporation of a control group, prior testing of candidate females for reproductive disorders and testing the bull for its breeding efficiency and freedom from genital infections.

Table VI-2 **Reactions of Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Animal #	Days to					Ab. response (titre)
	Schizonts	Fever	Treatment*	Piroplasms	Recovery	
Group A						
580B	.	.	.	16	.	1:640
584B	.	.	.	25	.	1:160
588B	21	17(10)	24,26	.	26	1:640
805B	18	20(2)	21,23	.	19	1:2560
Group B						
591B	21	.	.	24	22	1:160
597B	23	.	.	24	24	1:640
804B	16	19(2)	19,21	24	18	1:640
806B	.	.	.	16	.	1:640
Controls						
590B	.	.	.	.	.	.
598B	.	.	.	.	.	.
599B	.	.	.	.	.	.
803B	.	.	.	.	.	.

* - Clexon/Terramycin-LA

Table VI-3 **Leucocyte concentration in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Day post-immunization	Leucocyte concentration (10^3 μl^{-1})	
	Group A & B(n=8)	Controls(n=4)
0	17.5 ± 4.6	17.6 ± 6.6
3	20.9 ± 2.1	21.5 ± 1.8
7	12.8 ± 3.8*	11.5 ± 3.8
10	12.2 ± 2.3*	11.8 ± 2.8
14	12.8 ± 4.2*	13.1 ± 5.5
17	11.0 ± 3.3*	11.3 ± 3.2
21	10.4 ± 4.1*	11.5 ± 3.2
24	11.0 ± 4.3*	12.6 ± 3.4
28	11.2 ± 4.3*	12.5 ± 3.3
31	14.8 ± 8.6	14.1 ± 3.9
35	12.0 ± 3.1*	12.2 ± 2.8
38	10.5 ± 3.5*	13.3 ± 3.5

* - Significantly lower than initial levels ($p<0.05$)

Table VI-4 **Lymphocyte and neutrophil concentrations in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Day p-i	Lymphocyte concentration 10^3 μl^{-1}		Neutrophil concentration 10^3 μl^{-1}	
	Group A & B	Controls	Group A & B	Controls
0	13.6 ± 3.9	10.8 ± 2.0	4.7 ± 1.8	4.5 ± 1.7
3	13.4 ± 3.0	13.0 ± 2.3	6.0 ± 2.8	8.3 ± 1.2
7	9.1 ± 2.9*	8.5 ± 3.3	3.4 ± 1.1	2.7 ± 0.5
10	8.3 ± 2.4*	6.9 ± 2.2	3.7 ± 0.9	4.7 ± 1.4
14	8.7 ± 2.8*	7.8 ± 2.6	4.0 ± 1.6	5.2 ± 3.2
17	7.5 ± 2.9*	7.7 ± 2.1	3.2 ± 0.7	3.3 ± 1.4
21	7.5 ± 3.3*	7.8 ± 2.7	2.7 ± 1.3*	3.6 ± 1.7
24	8.4 ± 3.6*	9.1 ± 2.2	2.4 ± 0.9*	3.4 ± 1.4
28	8.0 ± 3.7*	9.1 ± 2.6	2.9 ± 1.2*	3.2 ± 1.0
31	10.2 ± 6.4	9.5 ± 3.0	3.8 ± 2.3	4.2 ± 1.2
35	7.6 ± 2.6*	7.3 ± 1.8	4.3 ± 2.3	4.6 ± 0.8
38	7.1 ± 2.9*	8.6 ± 2.4	3.2 ± 0.9	4.6 ± 1.4

* - Significantly lower than initial levels ($p<0.05$)
p-i -post-immunization

Table VI-5 **RBC, Hb and PCV in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Day p-i	RBC concentration (10^6 μl^{-1})		Hb. concentration (gm dl^{-1})		PCV (%)	
	Gp. A&B	Controls	Gp.A&B	Controls	Gp.A&B	Controls
0	8.8 ± 0.3	9.3 ± 1.0	12.3 ± 0.8	12.8 ± 1.3	34.3 ± 2.3	36.8 ±3.8
3	13.9 ± 0.6**	14.3 ± 1.0**	12.8 ± 0.5	13.7 ± 0.7	31.6 ± 1.8**	32.7 ± 1.2
7	8.1 ± 0.6*	9.0 ± 0.9	10.7 ± 0.6**	11.4 ± 1.1	31.6 ± 1.8*	34.5 ± 3.0
10	8.0 ± 0.8*	9.0 ± 0.7	10.6 ± 0.7**	11.4 ± 0.7	32.6 ± 1.8	35.5 ± 2.6
14	9.4 ± 1.0	10.4 ± 0.9	11.9 ± 0.8	12.6 ± 0.9	34.4 ± 1.8	35.5 ± 1.3
17	9.3 ± 1.3	9.9 ± 1.4	10.4 ± 0.5**	12.0 ± 2.1	33.3 ± 1.3	34.8 ± 0.8
21	8.3 ± 1.1	9.6 ± 1.3	10.4 ± 0.8**	11.0 ± 0.6	32.6 ± 2.2	34.0 ± 1.4
24	7.9 ± 0.8*	8.9 ± 0.5	10.0 ± 1.1**	10.8 ± 0.9	31.8 ± 3.4	36.3 ± 2.9
28	8.3 ± 0.7	9.1 ± 0.9	10.2 ± 0.8**	11.1 ± 0.8	33.3 ± 2.1	36.8 ± 2.2
31	9.1 ± 0.8	10.3 ± 0.6	11.3 ± 1.0	12.4 ± 0.9	34.6 ± 2.1	38.8 ± 3.1
35	8.4 ± 0.2**	9.9 ± 0.8	11.4 ± 0.9	12.8 ± 1.0	36.5 ± 2.3	40.5 ± 2.4
38	9.0 ± 0.9	10.5 ± 0.6	11.4 ± 0.8	12.6 ± 0.5	36.0 ± 1.5	39.8 ± 1.5

p-i - Post-immunization

* - Significant as compared to initial level ($p<0.05$)

** - Highly significant as compared to initial levels ($p<0.01$)

Underlined : Indicates significant difference between groups ($p<0.05$)

Table VI-6 **Weight change in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Animal #	Time to (weeks)		Max. bw loss (%)	bw change at Week 15**
	Max. bw loss	Recovery of initial bw		
Group A				
580B	4	5	4.8	124.2
584B	2	4	4.9	109.9
588B	6	15	13.0	99.9
805B	4	10	9.3	110.3
Group B				
591B	.	.	.	120.7
597B	1	2	1.1	103.7
804B	.	.	.	104.0
806B	2	5	5.3	107.0
Controls				
590B	3	4	2.8	122.0
598B	9	10	3.2	107.3
599B	2	3	3.2	123.7
803B	4	6	3.7	110.0

* - Weight loss expressed as a percentage of bw at Week 0

** - bw at Week 15 expressed as a percentage of bw at Week 0

Table VI-7 **Body weight change in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Week post-immunization	Mean body weight (kg)*		
	Group A	Group B	Controls
0	309.9 ± 7.6	321.8 ± 22.8	307.8 ± 36.4
1	301.0 ± 7.3	322.8 ± 7.5	299.3 ± 37.6
2	301.3 ± 15.4	328.5 ± 28.4	303.8 ± 36.7
3	292.0 ± 20.9	331.8 ± 39.2	298.8 ± 41.1
4	299.8 ± 24.4	328.3 ± 26.0	305.0 ± 36.2
5	299.3 ± 29.0	335.0 ± 29.8	314.8 ± 24.0
6	305.0 ± 26.3	337.5 ± 26.4	312.8 ± 23.9
7	306.8 ± 19.3	341.3 ± 29.1	322.3 ± 19.2
8	313.0 ± 23.9	341.8 ± 25.6	326.5 ± 14.1
9	307.0 ± 25.1	338.0 ± 18.7	329.0 ± 23.8
10	316.5 ± 27.7	345.3 ± 18.1	335.8 ± 16.4
11	337.3 ± 27.4	355.3 ± 20.6	348.0 ± 17.4
12	338.5 ± 28.6	351.5 ± 26.1	357.3 ± 18.9
13	337.5 ± 28.0	353.3 ± 22.9	353.5 ± 21.2
14	345.0 ± 33.6	355.5 ± 24.3	351.8 ± 30.7

* - Mean ± SD

Table VI-8 **Oestrus signs (scores) in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Oestrus sign	Pre-imm.	Post-immunization			
	Entire group (n=30)	Group. A (n=15)	Group. B (n=15)	Controls (n=21)	Entire group (n=51)
Mucous discharge	29	15	15	20	50
Restlessness	30	15	15	21	51
Swollen vulva	28	14	14	18	46
Mounting others	27	9	12	14	35
Allowing mounting	24	7	11	17	35
Being sniffed	6	9	7	17	33
Reduced appetite	7	12	15	18	45
Bellowing	8	5	4	7	16
Front mounting	4	2	1	3	6
Head butting	4	4	4	8	16
Raised tail	2	.	.	.	.

n - Number of heats

Table VI-9 **Distribution of palpable ovarian structures in relation to the oestrous cycle in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Palpable structure	Number of observations	Structure present in	
		Luteal phase	Follicular phase
Group A			
Developing CL (A1-3)	6	6	.
Mature CL (B)	46	43	3
Regressing CL (C1-2)	4	.	4
Follicles (D)	12	5	7
NDS (E)	9	2	7
Group B			
Developing CL (A1-3)	5	5	.
Mature CL (B)	40	38	2
Regressing CL (C1-2)	4	2	2
Follicles (D)	15	4	11
NDS (E)	11	5	6
Controls			
Developing CL (A1-3)	3	3	.
Mature CL (B)	37	37	.
Regressing CL (C1-2)	6	3	3
Follicles (D)	12	5	7
NDS (E)	24	8	16

NDS - No detectable structures

Table VI-10 **Cycle lengths in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Animal #	Pre-immunization		Post-immunization		Day to last detected		Pregnancy
		Mean		Mean	Heat	Mating	
Gp. A							
580B	20,20,21	20.3 ± 0.5	42,65,22, 22	37.8 ± 17.7			
584B	20,13,27	20.0 ± 5.7	17,21,20, 14	18.0 ± 2.7			
588B	25,10,25	20.0 ± 7.1	39,17	28.0 ± 11.0			
805B			42,22,21, 28	28.3 ± 8.4			
Mean		20.1 ± 5.3		28.0 ± 13.6			
Gp. B							
591B	23,20,21	21.3 ± 0.5	73,29 14,26	35.5 ± 2.4	132	132	NP (3/4)*
597B	22,22,22	22.0	42,22,29, 43	34.0 ± 8.9	138	66	NP (1/5)
804B			34,21,17, 21,48	28.2 ± 11.4	142	98	NP (1/6)
806B			42,18 21	27.0 ± 10.7	71	71	P (2/3)
Mean		21.7 ±0.9		31.3 ± 14.8			
Controls							
590B	20,19,23	20.7 ± 1.7	20,21,20, 41	25.5 ± 9.0			
598B	20,20,20	20.0	20,22,23, 20,21	21.2 ± 1.2			
599B	22,21,19	20.7 ±1.2	18,20,19, 19,19	19.0 ± 0.6			
803B			28,21,20, 21,20	23.8 ± 3.9			
Mean		20.4 ± 1.3		22.2 ± 5.2			

P = Pregnant; NP = Not pregnant

* - Numbers in parenthesis represent number of observed matings out of observed heats

Table VI-11 **Cycle lengths in Boran/Friesian cross heifers immunized against *T. parva* stabilate IL3081 or in controls**

Animal #	Cycle length in days*		
	Pre-immunization	Post-immunization	
			Mean
Group A			
580B	21	21,23,21,23,22	22.0 ± 0.9
584B	27	17,21,20,21	19.8 ± 1.6
588B	25	21,17,31	23.0 ± 5.9
805B		42,22,21,28	28.3 ± 8.4
Mean	24.3 ± 2.5		23.2 ± 5.9
Group B			
591B	21	18,24,20,29,14,26	21.8 ± 5.0
597B	22	21,18,22,29,24,19	22.2 ± 3.6
804B		19,19,21,17,21,21,21, 17	19.3 ± 1.7
806B		29,18,23	23.3 ± 4.5
Mean	21.5 ± 0.5		21.3 ± 4.0
Controls			
590B	23	20,21,20,21,23	21.0 ± 1.1
598B	20	20,22,23,20,21	21.2 ± 1.2
599B	19	18,20,19,19,19	19.0 ± 0.6
803B		19,21,20,21,29	20.3 ± 0.8
Mean	20.7 ± 1.7		20.8 ± 2.3

* - Cycles adjusted using P4 profiles

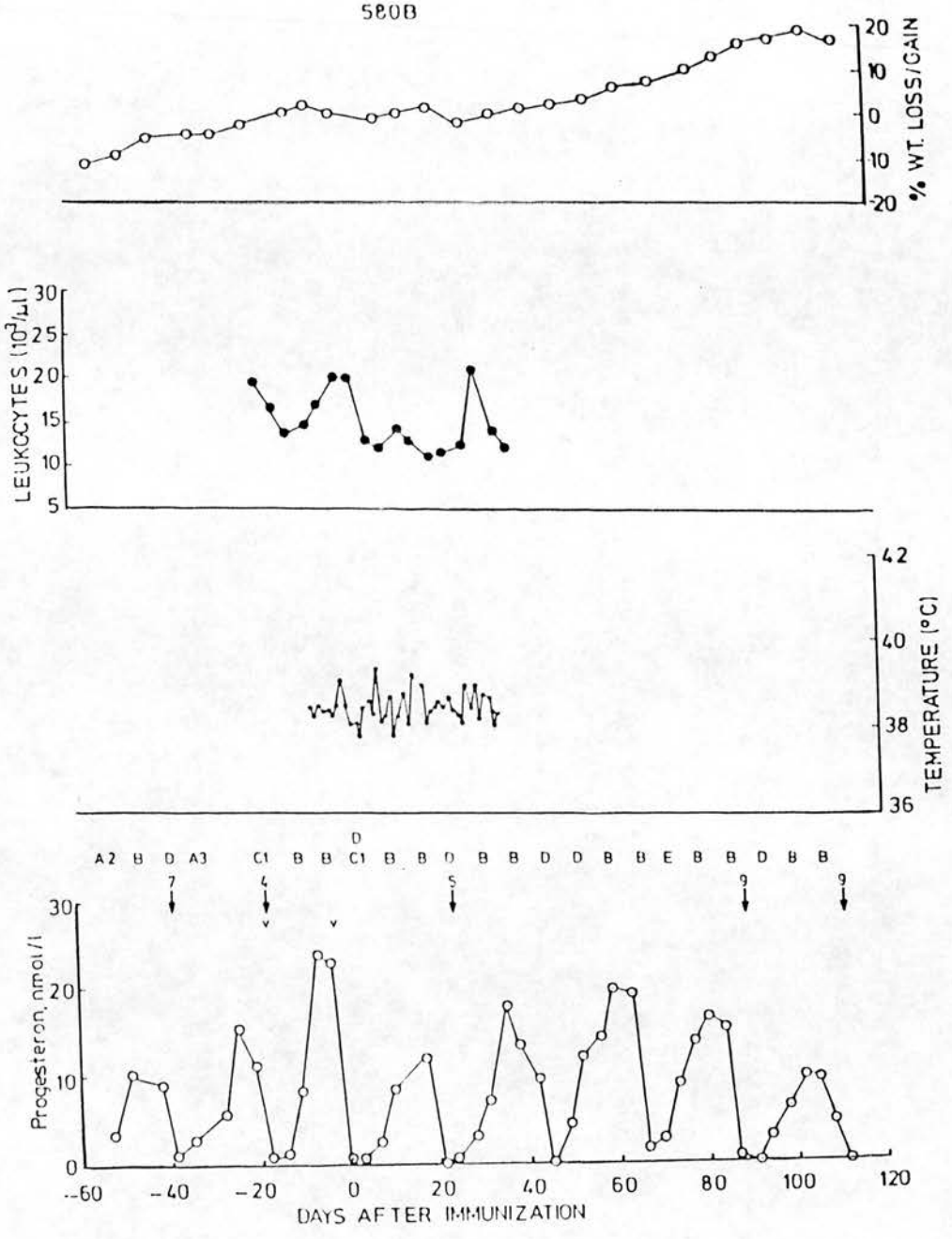


Fig.VI-1.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and weight changes in heifer 580B immunized with *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-immunization body weight. Ovarian structures are represented by A2-3 = Developing CL; B = Mature CL; C1 = Regressing CL; D = Follicles; E = No detectable structures. Arrows = observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$.

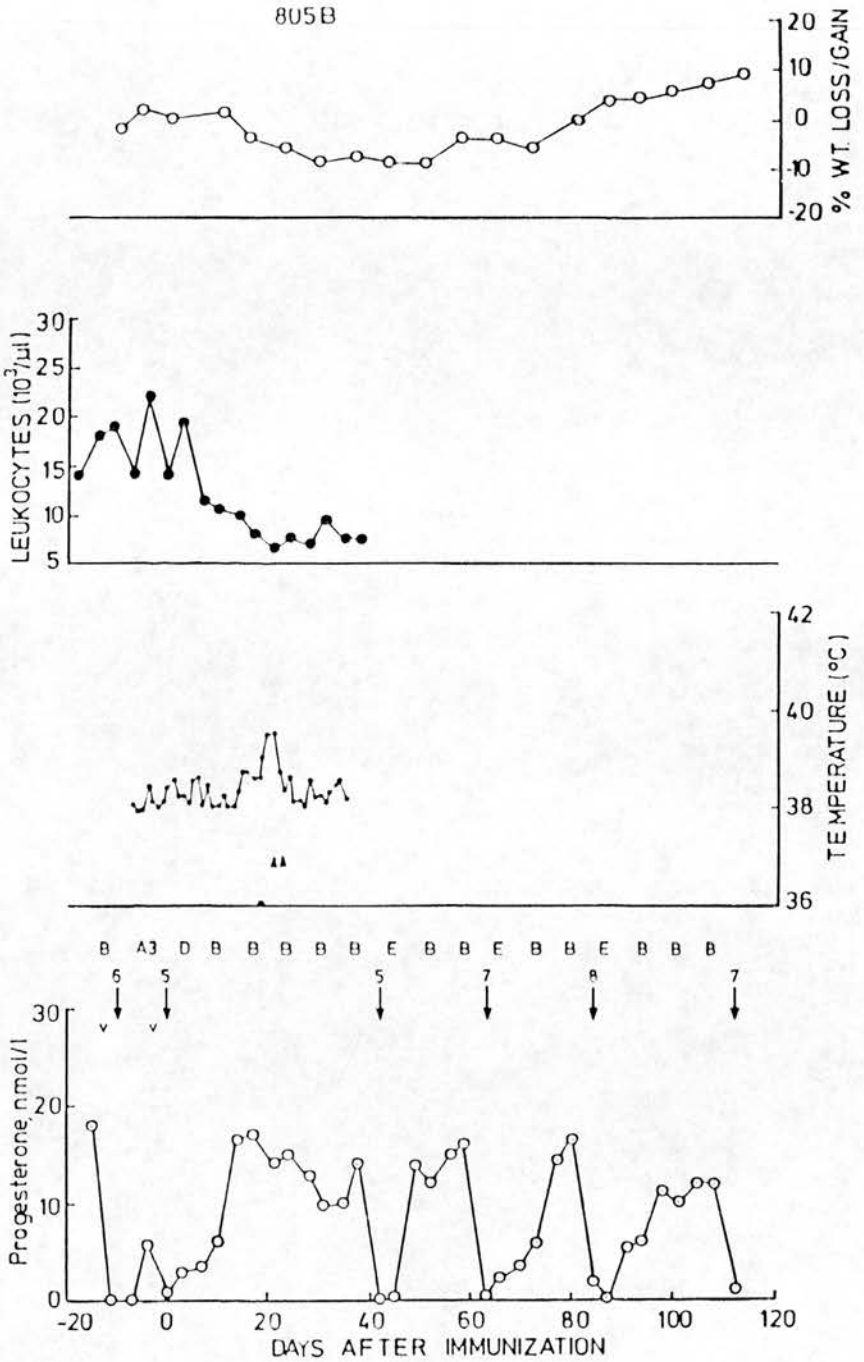


Fig. VI-2.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and weight change in heifer 805B immunized with *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-immunization body weight. Ovarian structures are represented by A 3 = Developing CL; B = Mature CL; E = No detectable structures. Arrows = observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$; ▲ = Clexon/Medamycin treatments.

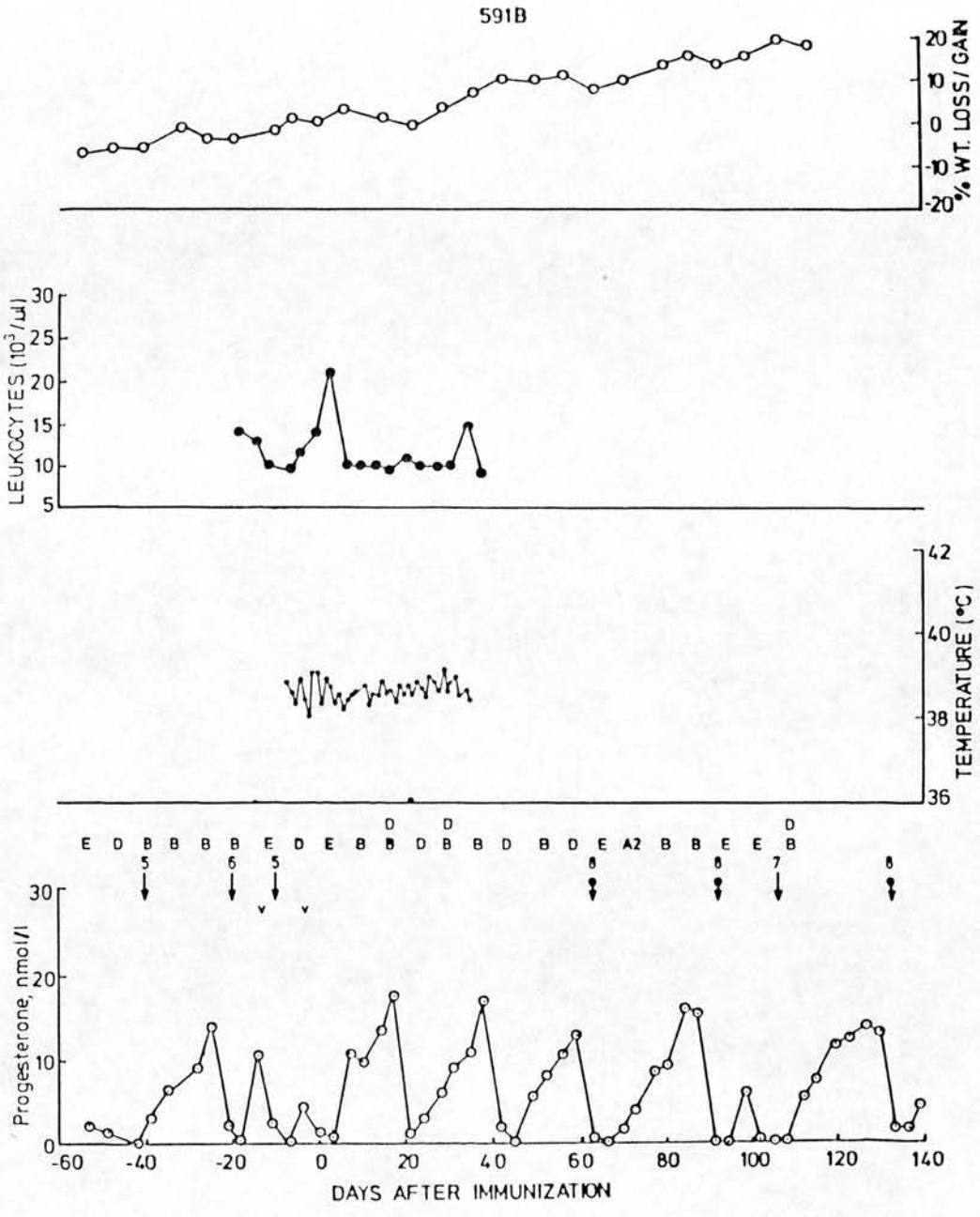


Fig. VI-3.

Serum progesterone concentration, ovarian structures, rectal temperature, schizont parasitosis (bar), leucocyte concentration and weight change in heifer 591B immunized with *T. parva* stabilate I13081. Weight is expressed as a percentage of pre-immunization body weight. Ovarian structures are represented by A2 = Developing CL; B = Mature CL; D = Follicles; E = No detectable structures. Arrows = observed oestrus and oestrous scores in superscript; ↕ = observed mated heat. V = $PGF_2\alpha$.

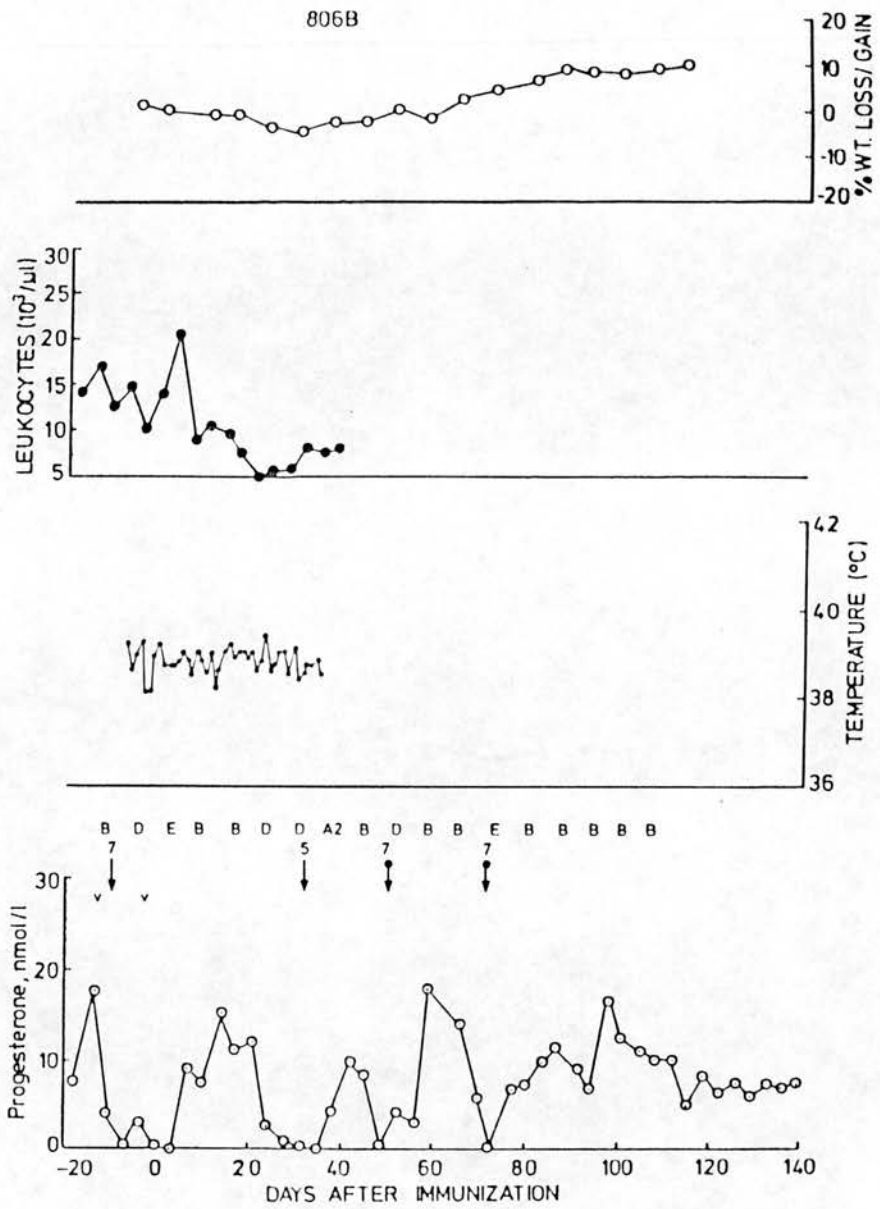


Fig.VI-4.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and weight change in heifer 806B immunized with *T. parva* stabilate IL3081. Weight is expressed as a percentage of pre-immunization body weight. Ovarian structures are represented by A2 = Developing CL; B = Mature CL; D= Follicles; E = No detectable structures. Arrows = Observed oestrus and oestrous scores in superscript; ↓ = Observed mated heat. V = $PGF_2\alpha$.

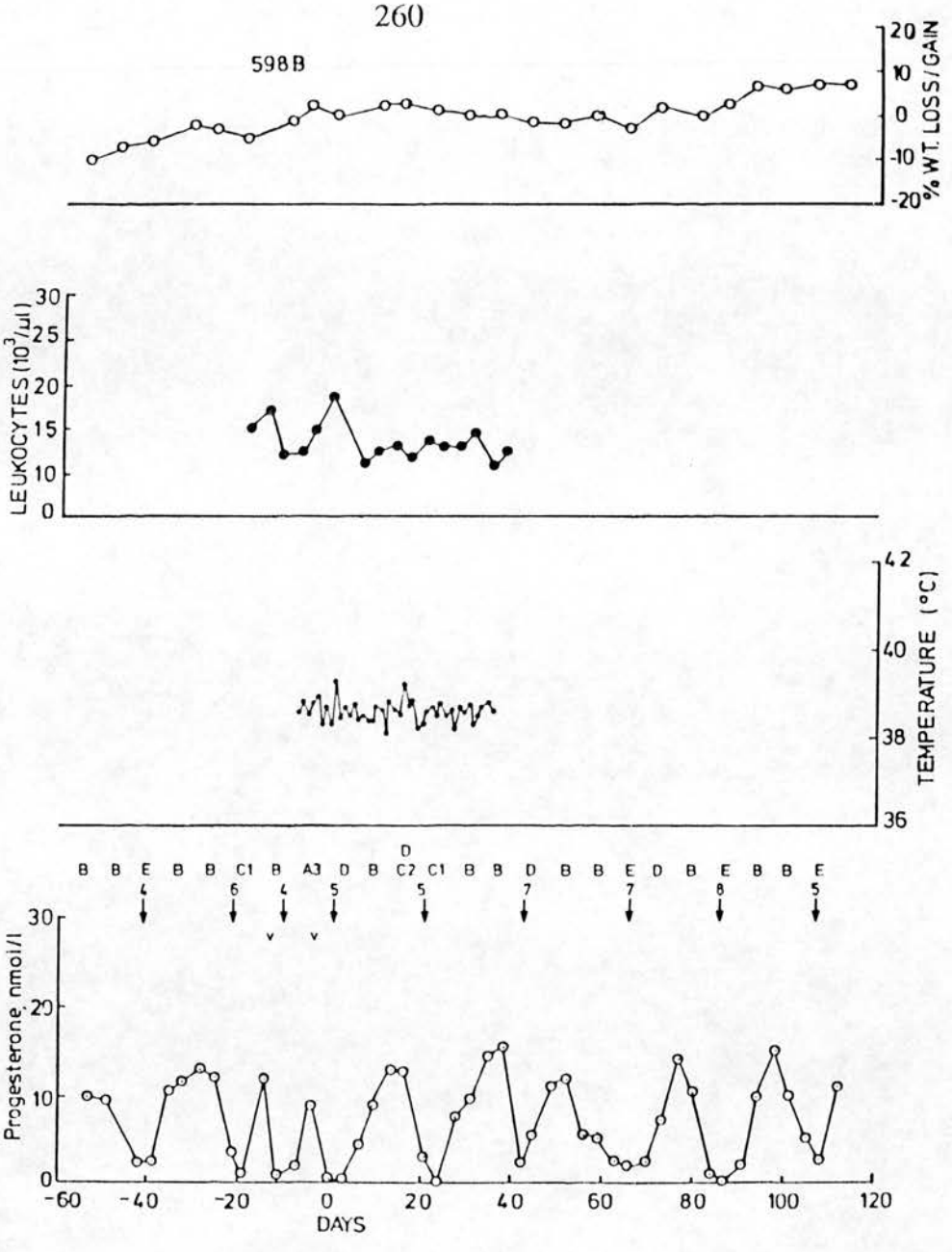


Fig. VI-5.

Serum progesterone concentration, ovarian structures, rectal temperature, leucocyte concentration and weight change in control heifer 598B. Weight is expressed as a percentage of initial body weight. Ovarian structures are represented by A3 = Developing CL; B = Mature CL; C1-2 = Regressing CL; D = Follicles; E = No detectable structures. Arrows = observed oestrus and oestrous scores in superscript. V = $PGF_2\alpha$.

CHAPTER SEVEN

GENERAL DISCUSSION

This study has demonstrated that infection with *T. parva* may result in various disease manifestations. Survival depends upon many factors which include the quantity of the infecting parasite (Cunningham *et al.*, 1974; Radley *et al.*, 1974; Dolan *et al.*, 1984b), the virulence of the parasite isolate, the rate of development of infection (Jarrett *et al.*, 1969), the relative susceptibility of the host and the integrity of its immune response at the time of infection. Animals from a naive population vary in susceptibility to infection (Dolan *et al.*, 1984b) and in time to death following a standard infective dose. In this study, the infective inoculum dose varied and the resultant responses were consistent with the above observations.

The first evidence of infection was the detection of macroschizonts in the regional lymph node to the site of parasite inoculation. Occasional exceptions to this general observation were however noted when parasites were first detected in the contralateral prescapular lymph node. Our results showed that the prepatent period increased as the dose of parasite decreased and agrees with the observation that the prepatent period in *T. parva* infection is dose-dependent (Radley *et al.*, 1974; Dolan *et al.*, 1984b). The prepatent period in this study compares favourably with that reported elsewhere (Jura and Losos, 1980; Mutugi *et al.*, 1988a,b). The earliest parasites were detected was on day 5 in this study. Following infection, even with massive doses of infective material, Radley *et al.* (1974) were unable to detect macroschizonts for five days leading them to speculate that there may be another parasitic stage between the sporozoite and the macroschizonts not demonstrable by the usual diagnostic methods. However, four day prepatent periods have been reported (Purnell, 1977).

The antitheilerial properties of parvaquone have been reported severally

(McHardy *et al.*, 1976; Dolan, 1981; Morgan and McHardy, 1982; McHardy *et al.*, 1983; Dolan *et al.*, 1984a). *In vivo*, when used after the development of parasitosis and clinical reactions, parvaquone was reported to induce the degeneration of macroschizonts which were eliminated within 2-14 days. In this study, parvaquone treatment resulted in the disappearance of macroschizonts in 2-4 days although recrudescences were observed. Dolan *et al.* (1984a) reported similar observations.

Piroplasms are rare with the buffalo-derived *T. parva* (Young *et al.*, 1988). Although piroplasmaemia was demonstrated in 50% of the infected animals in this study, the parasitaemia was low and transient. Mutugi *et al.*(1988a) also reported a piroplasmaemia using a related parasite. It is not clear whether the low and transient piroplasmaemia accounted for our failure to transmit infection in the parasite isolation studies.

All animals that survived beyond three weeks of infection developed a positive antibody response. This is in agreement with the observation that antibodies to *T. parva* are not detectable on IFAT until after 20 days of infection (Burridge and Kimber, 1972). Mutugi *et al.* (1988a) were also unable to demonstrate positive titres in animals that died early following infection with a related parasite.

Clinically, fever was the first sign of disease. The incubation period increased as the infective dose decreased, confirming that the the time to febrile response was dose-dependent (Radley *et al.*, 1974; Dolan *et al.*, 1984b). In this study, although the clinical reactions were influenced by the dose of *Theileria* parasite used, the within animal responses to a standard dose of infective material varied reflecting differences in host responses in a naive population. The severity of the manifested syndromes varied from inapparent to mild, moderate and severe with recovery or death (Anon, 1989b). The clinical signs were typical of theileriosis as described in the literature (Henning, 1956; Neitz, 1957; Jura and Losos, 1980). Treatment with Clexon cured all animals that were not sacrificed during the reaction period. Fever declined to normal levels within 2-4 days of treatment but was followed by remissions in the majority of animals.

Similar responses to treatment with Clexon are reported elsewhere (Morgan and McHardy, 1982; Dolan *et al.*, 1984a, 1988). However, one animal developed a clinical syndrome typical of chronic theileriosis similar to that reported by Dolan (1981).

Several reports associate ECF with unthriftiness (Henning, 1956; Neitz, 1957; Oteng, 1977; Young *et al.*, 1981; Dolan, 1986a,b). Weekly weight measurements in this study revealed wide variations in the magnitude of weight loss in a group of animals infected with the same dose of stabilate suggesting there was no obvious relationship between the dose of infective stabilate and the degree of weight loss. However, animals that lost most weight were observed in experiments where the concentration of infective material was high. The magnitude of the weight loss and the rate of regain appeared to be related to the severity of clinical reaction in recovering animals. Dolan *et al.*(1984a) reported higher weight losses and lower gains in animals demonstrating a carrier status. The results in this study were at variance with the above findings. The four animals with a demonstrable carrier status in this study had only a moderate weight loss. However it is possible for an animal with persistent infection to act either as an initial, sporadic or a continuous carrier (Dolan, 1981) and therefore it is not possible to comment accurately on the carrier status since parasite isolation was not attempted at several stages in our studies.

The most common haematologic change observed in all infected animals was a leucopaenia which was a result of a concomitant lymphopaenia, neutropaenia and eosinopaenia. Our findings agree with those reported elsewhere (Steck, 1928; Barnett, 1960; Maxie *et al.*, 1982). There was no obvious correlation between the degree of leucopaenia and the severity of clinical reaction. The white blood cell decline was arrested followed by a leucocytosis in animals recovering after or without treatment. Similar leucocyte responses are reported in the literature (Steck, 1928; Barnett, 1960; Morgan and McHardy, 1982; Dolan *et al.*, 1984a, 1988). The cause of leucopaenia in ECF is not known but it has been ascribed to an arrest in maturation of granulocytes (Wilde, 1966). The lymphopaenia is more difficult to explain but it has been suggested

that it is due to a cell-mediated cytotoxic response which results in lymphocytolysis (Eugui *et al.*, 1981; Morrison *et al.*, 1986).

Anaemia is not a major feature of ECF and when present is slight and non-regenerative (Maxie *et al.*, 1982). No changes were reported in the PCV, haemoglobin concentration or red blood cell concentrations with the buffalo-derived parasite (Maxie *et al.*, 1982). In contrast, these were found to be significantly lower to their pre-infection levels intermittently in all our four experiments. The cause of the anaemia remains unknown but may be related to the loss in condition following infection since animals that lost more weight had comparatively lower erythrocyte values.

The gross lesions observed in fatal cases were similar to those described in the literature (Steck, 1928; De Kock, 1957; Neitz, 1957; Munyua *et al.*, 1973). Animals that recovered naturally or after treatment either had few or no lesions characteristic of theileriosis. Histologically, animals that died of the disease had more marked changes than the ones that recovered. However, there were exceptions where the histological changes were either absent or mild inspite of distinct gross changes. Mononuclear cell infiltration which is characteristic of ECF (De Kock, 1957; Barnett, 1960; Munyua *et al.*, 1973) was also the most consistent finding in this study. The reduction in both gross and histological lesions in the recovered animals suggests either an arrest or reversal in the pathogenesis of the disease. Primarily, the parasitised lymphoid cells are responsible for the lesions observed in ECF. Treatment with Clexon leads to degeneration of macroschizonts and therefore, curtailment of further proliferation and dissemination of parasitized lymphocytes. Under natural recovery, the removal of parasitized and transformed cells by immune mechanisms (Morrison *et al.*, 1986) probably contains and removes traces of the changes associated with the disease.

Three approaches were used to assess the reproductive status in this study. Of the three, measurement of serum progesterone concentrations was found to be the most reliable. Observation of the animals for twenty minutes three times a day resulted

in an oestrus detection rate ranging from 54% to 72.8% (mean, 62.5%) based on standing to be mounted as the true sign of oestrus (Whitmore, 1980). This suggests that although standing to be mounted is the only oestrus sign recognized for receptivity, it does not identify all animals that are on heat. The oestrus detection rate may have been improved by increasing the observation time and use of heat detectors.

In this study, eleven behavioural traits were used to monitor the occurrence of oestrus. Ten of these were not specific for true oestrus (Whitmore, 1980). Of the ten non-specific parameters, vulval mucous discharge and restlessness were good indicators that the animal was around oestrus, being observed in 97.5% (278/285) and 85.6% (244/285) of observed heats, respectively.

Overall, oestrus was detected in 87.4% (326/373) of all basal P4 concentration periods based on specific and non-specific oestrus signs. Five heats were detected during elevated P4 concentrations giving false positives of 1.3%. False positives were also reported during the luteal phase (Williamson *et al.*, 1972; Appleyard and Cook, 1976), during pregnancy, or in association with cystic ovaries (Williamson *et al.*, 1972). Of the 42 periods of basal P4 concentrations when oestrus was not detected, 76.2% (32/42) were in the infected animals post-infection suggesting that infection subdued oestrus manifestation. Alternatively, all the missed heats could be attributed to management errors since animals were not observed continuously for 24 h.

A developing or mature CL was detected at least once in 97.2% (278/286) periods of plateau P4 concentration. However, of the palpable structures, developing and mature CL were consistent to the luteal phase in 81.9% (502/613) occasions. The accuracy of detecting these structures during the luteal phase ranged from 78.4% to 87.1%. Agreement of structures palpated during the follicular phase to P4 concentrations ranged from 75.7% to 98.9% (mean, 89.7%). The overall agreement of the palpable structures to P4 concentrations for both the luteal and follicular phase was 84%. These findings, taken together with those reported elsewhere (Boyd and Munro, 1979; Watson and Munro, 1980; Pathiraja *et al.*, 1987) indicate that rectal

palpation *per se* is fairly but not completely accurate in assessing cyclical status.

The measurement of P4 concentrations afforded the most reliable method of assessing the reproductive status when compared to rectal palpation of the reproductive tract or behavioural oestrus detection. We were able to detect unobserved heats, determine the cyclical status as well as confirm pregnancy status in mated heifers. Both assay methods adopted in this study were considered adequate for the assessment of P4 concentrations.

From the results gathered in our four experiments, it was obvious that the clinical and pathological manifestations in *T. parva* infections were qualitatively similar but were modified either through the dose of the infective material, host resistance and iatrogenic interventions. Although the recovered animals were the primary subjects of study, the examination of changes in the target endocrine organs in fatal cases also gave an opportunity to establish a relationship between impaired function and pathology. From behavioural data, rectal palpation of the reproductive tract and P4 profiles, this study demonstrated that a proportion of animals underwent some disturbance in reproductive function. These were three animals in the 1:20 *T. parva* stabilate experiment, two animals recovered from severe ECF, two animals infected and treated at the height of clinical disease and one animal following immunization by infection and treatment. In addition, two other animals in the late treatment experiment (Ch. 5) had each a period of luteal dysfunction characterised by short periods (11-14 days) of low P4 concentrations after infection. Three animals (two in Ch.4 and one in Ch.5) ought to be excluded from this discussion since they were observed to be acyclic at the start of their experiments. All the controls used in this study cycled regularly.

After exclusion of the three animals that showed impairment of luteal function at the time of infection, our results showed that in 94.9% (37/39) of the animals there was an initiation of/and maintenance of luteal function during the early infection period when the animals were reacting clinically and parasitologically to infection. Six of the

37 animals subsequently underwent variable periods of acyclicity in the absence of detectable parasitosis and clinical evidence of disease apart from loss in condition which could have been due to infection. These results therefore, suggest that there was no direct evidence linking parasitosis and its associated clinical pathology and symptoms on the acyclical status of most animals irrespective of the size of the infective dose. However, behavioural oestrus was detected in only 33.3% (9/27) animals that completed a first post-infection cycle suggesting that the general malaise due to infection probably subdued oestrus manifestation. Of the other two animals, one animal in the late treatment experiment revealed basal P4 concentrations from the time it was infected until it died while the other from the immunization experiment revealed a persistent CL for 42 days after infection and then subsequently cycled normally. The impaired luteal function in the two animals immediately after infection were diametrically opposite and it is not clear whether the impairment was a direct effect of the infection since these were rare findings not observed in the other animals.

Gross and histological lesions were found in animals that cycled and did not cycle. Of the studied endocrine organs which are known to play a role in reproductive function, there did not appear to be any relationship between their involvement and impaired reproductive function. In the pituitary gland, a patchy and mild focal mononuclear cell infiltration of the anterior and posterior lobes was the main finding which suggested no serious anatomical basis of its impairment. Of the animals that were acyclic at some stage after infection, only two animals were acyclic to time of death and none had histopathological changes in the pituitary gland. On the other hand, cases were observed where lesions were present in the adenohypophysis without a concomitant adverse effect on reproductive function suggesting that the lesions had no direct effect on gonadotrophic hormone secretion.

Histological changes in the ovaries included mononuclear cell infiltration of the ovarian cortex, ovarian medulla and the CL and degeneration of luteal cells. At post-mortem examination, primordial to tertiary follicles were observed suggesting that

gonadotrophin release was not interfered with and that the ovaries were responsive to its stimulation. Evidence of luteal function further suggested that LH secretion and LH receptors in the CL were normal. The high levels of serum P4 observed even in the presence of moderate to severe mononuclear cell infiltration, luteal cell degeneration and vascular occlusion in the CL suggested that there was no functional luteal impairment associated with the pathology. In contrast, the CL of one of the acyclic animals (021B) had no detectable lesions despite P4 concentrations having remained at basal levels for more than a hundred days suggesting that luteal dysfunction in this study was due to factors other than pathology.

Histological changes in the uterine endometrium were patchy suggesting that there was enough residual functional tissue. In this study only one animal had a transient persistent luteal tissue suggesting either disturbed uterine secretion of or transport of $PGF_2\alpha$ which is the natural luteolysin in the cow (Hansel *et al.*, 1973). However, there was no clinical evidence in this animal to suggest that this was due to endometritis. CL regression was revealed in other animals which cycled but had histological lesions in the uterus suggesting that the secretion and transport of $PGF_2\alpha$ was not impaired.

Since there did not appear to be a clear evidence linking the parasitosis or pathology of the reproductive endocrine organs to the acyclicity, other mechanism(s) therefore seemed to be involved. Alterations in nutritional status, body condition and liveweight may be important factors in inducing acyclicity through their influence on the hypothalamo-pituitary-ovarian axis (Bond *et al.*, 1958; Beal *et al.*, 1978; Richards *et al.*, 1989). By restricting food intake so that cows lost weight and condition gradually, Richards *et al.* (1989) found that reproductively sound animals became acyclic after losing about 24% of their initial body weight. In this study, it was similarly found that animals that became acyclic lost the most weight. Of the nine animals that had prolonged basal P4 concentrations, six had a maximum body weight loss ranging from 15.9-29% of their initial body weight. Comparative figures for the

three others with transient basal P4 concentrations were 6.7-12.5%. The degree of weight loss at the initiation of luteal dysfunction in this study ranged from 7-13.5% and was lower than reported elsewhere (Imakawa *et al.*, 1986; Richards *et al.*, 1989). However, there were exceptions to this general observation since one infected animal (324A, Ch. 3) lost 19.6% of her initial weight and cycled throughout.

Luteal function was reinitiated in seven of the nine animals as they regained weight. At the reinitiation of luteal function, the animals weighed between 89-101% of their pre-infection weights and were heavier at the time of initiation of luteal function than at initiation of luteal dysfunction suggesting that greater body reserves are required to reinitiate cyclicity than to maintain cyclicity in animals that are losing weight. Our results are similar to those reported by Richards *et al.* (1989).

The physiological mechanisms whereby feed restriction and by implication, loss in condition causes anoestrus in cattle is little understood. This was partly attributed to a decrease in frequency of LH pulses (Richards *et al.*, 1989). In his review, Schillo (1992) suggested that one of the ways dietary feed restriction impairs pulsatile LH secretion is by decreasing GnRH secretion by the hypothalamus. Although he advanced a number of theories for this impairment, he suggested that the most plausible cause was a reduction in availability of oxidizable metabolic fuels.

Contrasting evidence was however presented to indicate that LH concentrations increased during feed restriction (Gombe and Hansel, 1973). Based on evidence of lowered P4 concentrations, these authors suggested that dietary restriction resulted in a reduced abilility of the ovarian tissue to respond to LH. Due to technical problems encountered in LH assays, our study did not confirm whether there was a functional impairment of the gonadotrophin secretion or release.

Behavioural oestrus was observed on one or more occasions during the transient or extended basal P4 concentration periods in four of nine animals. These heats were not associated with subsequent development of luteal tissue suggesting either lack of ovulation or inadequate LH and/or LH receptors in a ruptured follicle.

Our findings are in contrast to those where oestrus was not detected during luteal dysfunction (Richards *et al.*, 1989). It is however reported that the levels of oestrogens are similar in energy maintained or deprived animals either during anoestrus or at reinitiation of oestrus (Richards *et al.*, 1989). Whether there was adequate oestrogen levels to initiate oestrus but there was inadequate LH to induce luteogenesis is not known. Alternatively, oestrus could have been detected erroneously.

The long term effect of infection on fertility was examined in six treated and four immunized animals. Two of six and one of four animals in the treatment and immunization experiments, respectively became pregnant. Our in-calf rate of 30% compares poorly with 86% in immunized animals reported by Dolan and Mutugi (1989). However, in their study, animals were exposed to a bull for 17 months compared to between 3.5-6 months in this study, thus explaining partly their higher in-calf rate. Another possibility for their higher in-calf rate although not verified is the assumption that many bulls were used in their study since it was under ranching management. In this study, one bull was used on ten heifers and although it was considered that the bull:cow ratio was adequate (Daly, 1971), it is was not established whether the bull had psychological preferences to mate particular females.

In conclusion, this study has achieved the main objectives that had been set. It confirmed the anecdotal evidence that impairment of reproductive function may be linked to theileriosis. Further, it demonstrated that the impaired reproductive function was primarily due to loss in condition associated with infection and not due to a direct effect of theileriosis.

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