EPIDEMIOLOGY OF BOVINE ANAPLASMOSIS WITH SPECIAL
REFERENCE TO AN AREA OF SOUTHERN BRAZIL

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The literature on the epidemiology of bovine anaplasmosis is reviewed on a worldwide basis. An investigation into the epidemiology of the disease in an area of the Rio Grande do Sul state of Brazil is described.

Thin blood smears submitted to the laboratory from clinical cases were examined microscopically after staining with giemsa. A serological survey was carried out. 892 bovine sera from 26 properties were examined for antibodies to Anaplasma marginale using the complement fixation test (CFT). 102 of these sera were further subjected to the rapid card agglutination test (CT). A questionnaire was used to collect relevant data from the survey farms.

Laboratory - confirmed clinical cases showed a seasonal variation in incidence with three times as many confirmed outbreaks occurring during the autumn and winter (18) as during the spring and summer (6).

161 (18.1%) sera were negative to the CFT, 91 (10.2%) gave a suspicious reaction and 640 (71.7%) were positive. Mean herd prevalence of positive reactions was 76.4% but considerable variation occurred from farm to farm. The results of the CT and CFT were in agreement on 90% of 30 sera negative to the CFT while 76% of 72 sera giving a positive or suspicious CFT result were positive to the CT. Overall agreement was 80%. Questionnaire returns indicated a relationship between serological results and managamental practices controlling tick numbers.

It was concluded that the area is enzootically unstable for anaplasmosis and recommendations are made for control measures based on the use of A. centrale for immunoprophylaxis.
INTRODUCTION

Anaplasmosis is a significant cause of loss and major constraint to the expansion and improvement of the cattle industry throughout the tropical and sub-tropical regions of the world. As such it poses a threat to the ambitions of developing nations and to world food supply. An infectious but non-contagious disease of cattle and some other ruminants, it occurs as an acute or sub-acute condition characterised by fever, anaemia, icterus and weakness. Susceptible animals not infrequently die.

Although it is generally accepted that anaplasmosis is widespread in the south of Brazil, basic epidemiological information, an essential prerequisite to the selection of appropriate control measures is deficient. No serological data on anaplasmosis are available for the disease in Brazil while data based on clinical information are frequently confused by lack of differentiation from babesiosis. Throughout much of South America both anaplasmosis and babesiosis are referred to clinically as tristeza which translates as sadness - a reflection on the dejected demeanor of the affected animal. While this confuses treatment it is not totally without justification as the two diseases, because of common factors in their epizootiology, may occur in association in the same animal. Furthermore, similarities in the clinical signs produced by these two diseases makes diagnosis without blood examination indiscreet.

Ecological information on potential biological and mechanical vectors in Brazil is incomplete while attitudes to the disease are based upon broad assumptions which may not hold true under the circumstances which apply in the region.
While responsible for a veterinary laboratory project in the Rio Grande do Sul state of southern Brazil, the author’s attention and interest were drawn to the study of the role of anaplasmosis as a cause of disease in the region. Intriguing differences in the apparent incidence of the disease called for clarification and it was felt that a serological survey, combined with the collection of relevant data would be of value in providing a scientific basis on which to relate appropriate control measures.

The present work presents the results of this survey which are discussed with relevance to control. The literature on the epidemiology of bovine anaplasmosis is reviewed.
4. REVIEW OF THE LITERATURE

(a) Historical Background.

Smith and Kilborne (1893), during their classical studies on Texas fever in the United States of America (U.S.A.) described marginal bodies which, from their size, shape, staining properties and location, leave little doubt as to their true identity as Anaplasma marginale organisms. However, these workers concluded that the marginal bodies were a stage in the development of Babesia bigemina though they described two forms of Texas fever. The acute form developed rapidly and marginal bodies were not seen. In the mild form progression was slower and marginal bodies could be detected.

It was left to Theiler (1910) to propose a separate identity for the peripheral cocci-like bodies which he too had encountered during his earlier work on B. bigemina and Theileria mutans. He proposed the name A. marginale, the generic name in view of its uniform nuclear-like staining properties and apparent absence of cytoplasm and the species name to reflect the predominantly marginal location of the organism in the erythrocyte.

Theiler pointed to similar descriptions by workers elsewhere. Knuth (1904) had seen bodies similar to A. marginale in bovine erythrocytes during his studies on tristeza in the Uruguay region of South America and considered them to be development stages of B. bigemina. In the Transcaucusus, Luhs and Dschunskowsky (1904) described organisms similar to A. marginale which they encountered while working with T. annulata.

(b) Staining Properties

The early descriptions of A. marginale were based on the use
of Giemsa stain (Theiler, 1910; Lignieres, 1914) and this Romanowsky stain continues to be the most widely employed (Bram, 1971; Carmichael & Hobday, 1975) to detect the bodies which appear as bluish-purple, rounded or oval structures with a diameter of 0.3 to 0.6 microns. Some workers have preferred Wrights stain and fluorescent-labelled specific antibody techniques have proved useful as have Auramine O and Acridine orange in the detection of low parasitaemias. (Friedhoff & Ristic, 1966; Hidalgo, 1975; Kreier & Ristic, 1963; Pilcher, Wu & Muth, 1961). Other special purpose stains employed by investigators have included New Methylene Blue (Schalm, Osebold & Murphy, 1962), Feulgen, Celestine Blue with Eosin, Leifson flagellar stain (Pilcher et al., 1961) and Toisson's fluid (Kreier et al., 1963). The organisms are also visible using phase contrast on cells haemolysed by water or sonic disruption (Espana & Espana, 1963).

(c) Structure

The most useful knowledge on the structure of the anaplasma body has come through the application of electron microscopy. In ultrathin sections the marginal body can be seen to be separated from the erythrocyte cytoplasm by a limiting membrane of about 10 milli-microns (Ristic & Watrach, 1961). Inside the membrane is a matrix or envelope material in which are embedded the sub-units of the marginal body, the initial bodies which measure between 300 and 400 milli-microns in diameter. These number between one and eight and have an internal structure visible as aggregates of finely granular material in an electron-lucid background. Earlier reports, (Ristic, 1960) using shadow cast techniques of polyhedral sub units to the initial body have not been confirmed. Recent studies, (Amerault, Roby &
Sealock, 1975) using a freeze-fracture technique, suggest that the internal structure of the initial body consists of dark areas of ribosomes and strand-like material ascribed to D.N.A. The initial body has a trilaminar membrane consisting of two entities; a cell wall and a plasma membrane. Projecting tips may have some feeding function. Others (Simpson, Kling & Love, 1967) have described vacuoles in the matrix substance which could be food vacuoles.

There has been some doubt as to the origin of the outer limiting membrane but the consensus of current thought is that it is of erythrocytic origin. (Ristic, 1967; Simpson et al., 1967. Its thickness and ultrastructure are similar to the erythrocytic membrane and anaplasmas liberated from the erythrocyte by sonic disruption do not possess this membrane, being surrounded by an envelope of the matrix material.

(d) Cycle of Development

Electron micrographs have shown evidence of division of the initial body (Ristic and Watrach, 1963) and it is now widely accepted that this represents a process of growth and multiplication. Entry of the erythrocyte is presumed to be effected by the single initial body. Enzymic processes have been proposed (Ristic et al., 1963) but the similarity of construction between the limiting and erythrocytic membranes would seem to support the hypothesis of Simpson, et al., (1967) that a process of invagination of the erythrocyte membrane occurs, which their electronmicrographs strongly suggest. These workers doubted that a body of 0.3 to 0.6 microns could pass the membrane without severely damaging it, as appears to occur. These workers further proposed that exit from the erythrocyte occurs by a reverse of the invagination process. That inter-erythrocytic transfer
Proposed Developmental Cycle of Anaplasma marginale

1. Extra Erythrocytic Body
2. Newly Arrived Initial Body
3. Binary Fission
4. Reproducing Initial Bodies
5. and 6. Immature Erythrocytic Bodies
7. Mature Inclusion

Initial Body
PLATE 1

From Ristic (1975)
takes place seems evident from the knowledge that the parasitaemia can double on a daily basis during the acute phase, involving the new infection of numbers of erythrocytes beyond the capacity of the haemopoietic system so that active infection of erythrocytes must occur intravascularly.

As a result of electron micrograph and fluorescent antibody studies carried out sequentially through the course of the disease a cycle of development has been proposed (Ristic et al., 1963) on the following lines.

The first stage was characterised by the appearance of initial bodies only. A few days later marginal bodies were seen so that a second stage represented by a mixed population of marginal and initial bodies was recognised. Then came a phase of growth and transfer followed by a stage of massive multiplication when marginal bodies predominated. If the animal survived this challenge, a latent carrier stage supervened where marginal bodies were no longer visible and infection was maintained by the sub-microscopic initial bodies.

(e) Classification

Earlier suggestions that A. marginale should be classified with the protozoa were to a large extent based on clinical and epidemiological similarities with Babesia spp. The possibility that they were products of viral multiplication has also been raised (Dickmans, 1933). The current classification in Bergey's manual (Ristic & Kreier, 1974) of A. marginale into the family Anaplasmataceae of the order Rickettsiales would appear to be well founded on morphological and structural grounds (Ristic et al., 1961; Amerault et al., 1975;) and also respiration rate (Pilcher et al., 1961).
Differentiation from the relatively non-pathogenic *A. centrale* is achieved by a comparison of the frequency of location at the margin or in the centre of the erythrocyte and can be confirmed serologically (Kuttler, 1967).

In North America organisms, indistinguishable from *A. marginale* with Giemsa stain or Acridine orange, but which have appendages or tails visible by phase contrast microscopy and with New Methylene Blue stain or the use of fluorescent antibody techniques have been identified. (Kreier et al., 1963; Schalm et al., 1962; Pilcher et al., 1961). These organisms, named as *Paranaplasma caudatum* and *P. discoides*, were considered to be of low pathogenicity by Kreier et al., (1963) but Taylor (1969), drawing attention to the immunogenic differences between the Florida (pure *A. marginale*) strain and Oregon strain (mixed *Paranaplasma spp.* and *A. marginale*), recommended the use of vaccines capable of giving protection against both isolates, regarding *Paranaplasma spp.* as significantly pathogenic.

(f) Pathogenesis.

The pathogenic manifestations of an infection by *A. marginale* are consequent upon an acute anaemia (oligocythaemia) which is the predominant clinical feature of the disease (Theiler, 1960). After an incubation period frequently of between 15 and 36 days though it may be longer (Ristic, 1960), there is a sharp temperature rise to around 41°C, later, loss of appetite, difficult breathing, weakness and inco-ordination, become evident. In acute cases the disease may run a course of seven days (Theiler, 1910) while in subacute cases it may extend over two to three weeks. During the first few days of sickness the parasites increase rapidly in numbers while the number of erythrocytes falls. If the animal survives for more than about
eight days following the onset of parasitaemia, polychromasias and basophilia becomes evident together with the appearance of normoblasts. Anisocytosis and poikilocytosis are detectable at later stages. In some cases mucus membranes may show signs of icterus. Morbidity is a reflection of the conditions affecting transmission and of the susceptibility of the population but explosive outbreaks can occur. Mortality was found by Lignieres (1914) to be between 40 and 70%.

Although raised values for faecal urobilinogen and serum iron have been noted and increased cell fragility demonstrated, haemoglobinuria is not a clinical sign and haemoglobinaemia has not been demonstrated (Brock, Norman, Kliwer & Jones, 1965).

Eap & Fahrney, (1975) using a continuous flow apparatus, showed a drop in parasitaemia over a two day period of 8% which could not be accounted for by the degree of haemolysis measured. The anaemia characteristic of the disease owes much to the removal of cells from the blood stream by the reticuloendothelial system, especially in the spleen and liver. Anaplasma infected erythrocytes have been observed in the Kupffer cells (Simpson et al., 1967). Workers using erythrocytes labelled with Fe$^{59}$ (Baker & Douglas, 1957) and Cr$^{51}$ (Baker & Douglas, 1959) concluded that the anaemia is not a reflection of reduced bone marrow function but rather of reduced erythrocyte survival. The bone marrow observations made by Kreier, Ristic & Schroeder, (1964) further testify to the lack of involvement of bone marrow depression. They found no change detectable during the incubation period but five days before the peak parasitaemia was reached changes were apparent with the major shift in the myeloid/erythroid ratio occurring after the development of anaemia when increased erythropoiesis attempted to compensate and immature forms
were released into the circulation. They further noted that erythro-
phagocytosis was markedly evident in the bone marrow and that this
coincided with the period of rapid decline in the packed cell volume.
Active proliferation of the macrophages of the splenic cords and
kuppffer cells as observed by Ristic & Sippel (1958) indicates
participation of these cells in the elimination of the organism from
the circulation and such activity no doubt accounts to a large
extent for the marked enlargement of the spleen which occurs in
anaplasmosis.

Interesting proposals have been put forward to explain the
mechanism behind the stimulation of phagocytic activity. Mann &
Ristic (1963), using an autolytic test, the Coombs anti-globulin:
globulin lattice test and an auto agglutination test, demonstrated
the presence in the sera of infected calves of an autohaemagglutinin.
They also demonstrated the presence on the erythrocyte surface of an
anti erythrocytic antibody. Though Brock, Kliewer & Pearson (1965)
using the same methods, were unable to confirm this, more recently
Cox & Dimpoullos (1972) corroborated the findings of the earlier
workers by means of a passive haemagglutination test and an immuno-
ferritin test. A positive correlation between the presence and titre
of the free-serum auto haemagglutinin, the erythrocyte-fixed auto
antibody and the period and intensity of erythrophagocytosis led to
the suggestion that these factors were opsonins released by parasitic
damage to the erythrocytes (Kreier et al., 1964). The opsonins were
in fact present in the sera of infected calves at this time was
confirmed by Schroeder & Ristic (1968). Using mouse peritoneal
phagocytes they showed that the opsonins sensitized autologous and
homologous erythrocytes and again correlated the opsonic titres with
the intensity and persistence of anaemia.

It is unlikely that the free-serum auto haemagglutinins and the erythrocyte-bound opsonins are identical as the former have been found to be beta-2-M globulins with non-specific, cold-reacting type antibody requiring tyssinized cells to permit demonstration of haemagglutination (Mann et al., 1963). The opsonins are gamma globulins and react at 37°C with intact erythrocytes (Morris & Ristic, 1970). The opsonins can be eluted from infected erythrocytes and the opsonic activity of the eluate is detectable before its presence in the serum is demonstrable, being present before the packed cell volume begins to fall. The titre of the eluate increases rapidly with the development of anaemia and parasitaemia. It has been proposed (Morris, Ristic & Lykins, 1971) that the erythrocyte is the target cell for the opsonin and it only becomes noticeable in the serum when the erythrocytes are saturated.

Thus the evidence indicates that the anaemia of anaplasmosis is the result of an immunopathological phenomenon triggered off by infection and damage to the erythrocytes resulting in altered antigenic structure.

(g) Antibody production and serology of anaplasmosis

A number of serological tests are available to detect humoral antibody responses in anaplasmosis and are applicable to the diagnosis of individual cases and also the detection of latent carriers of the infection, when the blood remains infective for susceptible animals but the characteristic marginal anaplasma bodies are no longer visible in blood films using standard staining methods. They are also indispensable to epidemiological studies, particularly in the dis-
closure of the prevalence of infection in a population.

It has not been shown that humoral antibodies exert a protective effect and immune sera did not confer protection against anaplasmosis (Ristic & Carson, 1975; Carson, Ristic & Lee, 1976). It would seem that immune mechanisms in anaplasmosis are cell-mediated (Ristic et al, 1975) and for this reason they are reviewed later, together with other aspects of resistance. Non-specific antibody production has been covered under pathogenesis.

Specific humoral antibodies produced in response to infection with A. marginale are active in complement fixing, agglutinating, precipitating and fluorescent antibody reactions.

The complement fixation test (CFT). The CFT was the first to be studied and has for many years been usefully employed in diagnostic research and investigational studies. Rees & Mohler, (1934) were responsible for demonstrating the application of this test to anaplasmosis with an antigen prepared from ticks engorged on the blood of infected animals. However, many difficulties were encountered in the production of adequate antigen and the work was discontinued until Mott & Gates (1949) took up the challenge some ten years later using a method adapted from one used to produce antigen for a CFT for malaria. Bovine blood was used as the source. Large doses of infective blood were rapidly passaged until a high parasitaemia was quickly attained, before antibody production could interfere. The method used to prepare the antigen from blood exhibiting a high parasitaemia involved lysis by carbon dioxide-saturated ice-cold water of the packed and washed parasitized erythrocytes harvested. The antigen produced deteriorated markedly in a few months at refrigerator temperatures but could be
preserved at -50°C to -70°C. Results with this antigen were encouraging (Mohler, Eichhorn & Rogers, 1949) but the margin between antigenic activity and anticomplementary activity was poor and problems of colour and consistency impeded its use for general testing. Improvements were attempted by modifications using distilled water lysis and differential centrifugation with a Servall centrifuge (Price, Poelma & Faber, 1952) or a Sharples centrifuge (Gates, Mohler, Mott & Schoening, 1954) or various combinations of these (Franklin, Heck & Huff, 1963; Rogers, Hidalgo & Dimopoullos, 1964). Use of these antigens in laboratory and field trials demonstrated the possibility of using the CFT for control and eradication of the disease (Price, Brock & Miller, 1954; Merriman, 1962; Price, Poelma, Hastings & Mitchell, 1953; Gates, Mohler, Mott, Poelma, Price & Mitchell, 1954; Pearson, Brock & Kliwer, 1955). The general consensus was that the test had an accuracy of around 96% and was specific in action. Antigen production in the U.S.A. was standardized and centralized as was the method of performing the test by the United States Department of Agriculture (USDA) (Anon., 1958).

As a result of the extensive use of the test in North America its application came to be studied elsewhere. Kuttler (1965) in his East African survey encountered a high percentage of reactions which were classified as suspicious and he speculated upon the possibility of cross reaction with a related haemoprotozoan. However, Rogers (1971) in Australia found there was no interference with the test by infections with B. argentina, B. bigemina, Eperythrozoon teganodes or T. mutans. Kuttler, (1962) also drew attention to the false positive reactions in the first two or three months of life found in calves from infected dams and due to maternal colostral antibody transfer.
At the 1:5 serum dilution recommended in the USDA procedure, antigen prepared from *A. marginale* would not differentiate between *A. marginale* and *A. centrale* antibodies though titration would show a higher titre with the homologous antigen (Kuttler, 1967). This may need to be born in mind in areas where *A. centrale* has been used prophylactically. This could be the case with *Paranaplasma* spp. also.

Further improvements in antigen quality, in particular, the use of the French pressure cell to release the organism from infected erythrocytes have enabled microtechniques to be developed with consequent economies of reagents. (Martin & Ritchie, 1973).

Disadvantages of the test have rested largely with the complexities attached to all CFT techniques which detract from its use in the field. Interpretation of the results of this test on animals other than cattle should be viewed with caution in view of the findings of those workers who have attempted to confirm the infectivity of blood from game animals positive to the CFT. (Osebold, Christensen, Longhurst & Rosen, 1959; Jacobsen, Worley & Hawkins, 1977; Peterson, Kristner & Davis, 1973; Renshaw, Vaughn, Magonigle, Davis, Stauber & Frank, 1977;) Kuttler (1965) was similarly cautious in interpreting his positive findings in several game species in East Africa, although he was inclined to suspect this as indicating multiplication of the organisms in the animals concerned.

One of the consequences of a complex test like the CFT is that it is open to endless variations in the manner in which it is performed and so one must be cautious in comparing the results obtained by different workers. Although the subjectivity of the readings may be avoided by using a spectrophotometer it has been pointed out that the
extra handling and delay that this entails may be counter productive (Price et al., 1964). It is customary to give readings of one to four pluses for 25 to 100% non-haemolysed cells remaining at the end of the test period. However, the interpretation of these readings as suspect or positive is subject to some variation. Similarly, the dilution of serum used in screening tests is sometimes varied. Further difficulties in comparing survey data become apparent when comparisons are made between results obtained using different serological tests.

In spite of the disadvantages outlined, the CFT remains one of the most useful tools in the study of anaplasmosis. It becomes positive at the end of the incubation period and generally remains positive during the persistance of the parasitaemia. Under field conditions this is likely to be lifelong though under experimental conditions, where reinfection is avoided, the CF antibody may begin to fluctuate around detectable levels after some 320 days (Rogers, 1971). However, Murphy, Osebold & Aalund, (1966) found titres of 1:20 545 days following infection. Roby, Rose & Ilemobade, (1974) noted that the test became negative between five and twelve months after sterilization with imidocarb. Where vaccination in one form or another is practised its effects on the CFT will be borne in mind. The use of a dead vaccine such as that described by Brock, et al., (1965) is said to cause a transient CFT response to the first dose but following the recommended schedule of boosters the CFT may effectively be permanently positive. As has been noted above, A. centrale vaccination will cause a CFT response indistinguishable from A. marginale infection at the serum dilution normally employed in screening tests. That the complement fixing antibody is physically
heterogeneous was demonstrated in studies using ion exchange chromatographic separation of the immunoglobulins (Murphy et al., 1966). The sequential changes were followed during the course of infection. For the first four to five days that CFT antibody was detectable it consisted exclusively of gamma M globulins (IgM). Electro-phoretically fast gamma G globulins (IgG) then appeared and by 30 days after the maximal decline in packed cell volume a relatively stable relationship of 25% fast IgG to 75% IgM was reached which persisted throughout the parasitaemia. The rather unusual persistence of IgM was attributed to continued stimulation by the persistence of the organism in the carrier state.

Capillary Tube Agglutination Test (CAT)

The development by Ristic (1962) provided an alternative to the CFT which was not only rapid and simple to perform with inexpensive equipment but also equally as accurate as the CFT and capable of using undiluted serum. It was free of the anti-complementary problems and could even utilize haemolysed serum. Its feasibility was due to the production of a particulate antigen in pure form obtained by a combination of sonication and differential sedimentation of highly parasitised bovine erythrocytes. It could be formalized and, kept at 4°C, maintained its antigenicity well. Its accuracy and specificity was comparable to the C.F.T. (Kuttler, 1963; Rogers, 1971; Hibbs, Weide & Marshall 1966; Kuttler, 1967) and the effects of vaccination similar. It became positive a little later than the C.F.T. The studies of Murphy et al., (1966) indicated that although the distribution of agglutinating activity between the immunoglobulins was basically similar to the C.F.T. antibodies, it seemed probable that the persisting capillary agglutination response in carrier animals was due to electrophoretically fast IgG almost exclusively.
Card Test (CT)

When first introduced, the CT suffered from a lack of sensitivity but in its modified form (Amerault, Rose & Roby 1972) it accurately detected the status of all 26 experimental animals and on 1352 cattle of unknown status an overall agreement of 96% was recorded between the C.F.T. and modified card test (Amerault, Roby, Rose & Frerichs 1976).

The CT is easy and rapid to carry out and, with the use of a commercial kit and some apparatus, can be adapted for use in the field. The purified antigen, released from erythrocytes by a French pressure cell is stained with Fast Green dye for ease of reading. Its use would probably be more widespread if it were not that access to the test is virtually restricted to the purchase of the commercial kit from one source.

Indirect Fluorescent antibody test (IFAT)

Antibodies active in the fluorescent antibody test are present in the sera of infected animals and may be employed to detect low parasitaemias in the direct fluorescent antibody test (Ristic et al., 1963) or to identify infected carriers in the IFAT (McCosker, 1975) though the latter is rather too cumbersome for use on large numbers of samples in serological surveys.

Gel precipitation test (GPS)

Soluble anaplasma antigens have been isolated from serum (Amerault and Roby 1968) and lysed erythrocytes (Ristic and Mann, 1963; Amerault and Roby, 1967) and react with antibody in the serum of acutely infected calves in gel precipitation tests. The antibody appears later than both complement fixing and agglutinating antibodies. The reaction has not been developed for diagnostic purposes.
Mechanisms of resistance

A. marginale occurs in nature in cattle and other domestic and wild species of ruminants (Neitz, 1965) although there is little evidence that it causes disease other than in the former.

As is the case with other members of the order Rickettsiales, young calves, while susceptible to infection, develop a mild form of the disease, recovery without treatment being the rule. The animal will then resist further infection; this resistance being maintained into adult life and is associated with persistance of the organism in the blood at a level non-detectable by normal microscopic procedures.

Active proliferation and hypertrophy of the macrophages of the splenic cords as well as the Kupffer cells of the liver and reticular cells of the bone marrow has been noted (Ristic, 1968). The relatively high ratio of reticular-endothelial tissue to body weight of the young animal together with a greater capacity for erythropoiesis may be influential in the resistance of these young animals (Ristic, 1968). Calf resistance is not absolute and deaths in calves do occur. Santos (1967) reports numerous deaths in crossbred native calves two to three months old perhaps indicating a reduced resistance in the very young calf. Kuttler, Marble & Matthews, (1962) produced evidence of maternal transfer of antibodies to the calf and noted that in their study calves in their first summer of exposure to infection did not acquire infection to the same degree as yearlings on the same range during their second summer and suggested an effect of a non-infectious maternal immunity passed to the suckling calf. Ristic (1968), however, notes that although calves from infected dams were positive to the CFT and CAT they were equally as susceptible to infection as calves where such antibodies were absent.
The belief that sterilization of infection by excessive treatment would leave the animal susceptible was not substantiated by Roby et al., (1974) when Friesians of known response to anaplasmosis survived exposure one year after sterilization with imidocarb, showing only subclinical reactions.

There are differing reports on the susceptibility of Zebu breeds in relation to European breeds. Stepanova (1976) regards Zebu as sensitive to anaplasmosis in Russian while in Southern Queensland in a ten year study period when 386 confirmed cases occurred, 90% were in Bos taurus breeds while the rest were crosses. It is not clear how this relates to the breed distribution in the area concerned. Gee (1976) also in Australia infers that susceptibility may build up in Zebu herds in marginal tick areas because their known tick resistance lessens the chances of them contracting the disease while still young. In Colombia there is evidence that this does not occur as a serological survey in an endemic area indicated that 94% of both Zebus and Bos taurus breeds reacted positively. Only 77% of crosses sampled were positive. (Pararroyo, Villa & Diazgranados, 1978).

The relationship between the spleen and resistance is noteworthy. Splenectomy of carrier animals precipitates a recrudescence of disease, regardless of age and also increases the susceptibility of uninfected animals. A cell-mediated immune response has been demonstrated in anaplasmosis (Buening, 1973; Carson, Sells & Ristic, 1976). The leucocyte migration inhibition test has shown a good correlation between cell-mediated immunity and protection with the test becoming positive either prior to or at the onset of parasitaemia. The early development of a positive response indicated a better chance of surviving challenge. The test remains positive throughout the
carrier period. Attenuated *A. marginale* vaccine also induces a cell-mediated response whereas killed antigens do not (Carson, et al, 1976). The lymphocyte transformation test, delayed cutaneous hypersensitivity test and cell-mediated cytotoxicity assay have been used to study the cell-mediated response in anaplasmosis but have not proved to correlate well with protection (Ristic & Carson, 1976; Buening, 1976).

(i) Reservoir hosts

Unquestionably the principal source of infection for maintaining the disease is carrier cattle which have survived infection as calves or later and in which the organism persists for years. In the face of reinfection this is likely to be a lifelong state. Even though the organism is present in the blood at levels below microscopic detection, remarkably little blood is needed to effect transmission to a susceptible animal, possibly as little as 0.01 ml in recently recovered animals or 0.5 - 1.0 ml in a carrier of six years standing. (Ristic, 1968). Any program of control or eradication by separation and slaughter of infected stock would need to consider the role of non-bovine hosts. In some areas, such as the Western parts of the U.S.A., deer have been shown to be important potential sources of infection. (Christensen, Osebold & Douglas, 1962; Christensen & McNeal, 1967).

The inadequacy of serological tests demands reliance on calf inoculation to determine the infective status of deer blood. The expense which this entails has led to the use of pools of blood from varying numbers of animals being used as inocula, complicating calculations of the percentage infection in the deer population. An additional difficulty has been the known fall-off in infectivity of blood on storage, necessitating rapid injection for meaningful results. Where distances have caused this to be difficult to attain, researchers have resorted to transporting calves in fly-proof cages into deer-inhabited country.
These complications may, in part, account for the variation in results obtained. Negative results from calf inoculation have been recorded from Bison (Petersen & Roby, 1975) Elk, (Renshaw et al., 1977) and Pronghorn Antelope (Jacobson et al., 1977), all in areas considered enzootic for anaplasmosis. Peterson et al., (1973) recorded negative results for Mule Deer in Oregon as did Thomas, Rytt & Hancock (1970) in Wyoming, while the results of Renshaw et al., (1977) indicated that some Mule Deer were carriers in Idaho.

The involvement of Columbian black tailed deer in California is more universally supported (Osebold et al., 1959; Christensen et al., 1967) A study by Howarth, Roby, Amerault & McNeal, (1969) showed a rising incidence of infection in this species with age. 31% of fawns, 47% of yearling deer and 92% of adult deer gave positive transmission results. Bearing in mind the 1½ million deer population in California and the evidence that cattle acquire anaplasmosis during winter and spring grazing on deer inhabited range (Christensen et al., 1962), the role of deer under such circumstances cannot be ignored. It has been demonstrated that A. marginale can cycle in deer in the prolonged absence of cattle and it has been suggested that the agent could be a natural parasite of deer, in which species its pathogenicity is negligible. (Osebold et al., 1969). This author also demonstrated that transmission from deer to cattle, back to deer, and again to cattle was possible.

In Africa anaplasmosis has been recorded from a number of wild ungulates. (Neitz, 1965; Kuttler, 1965). In Botswana, anaplasma parasitaemias were detected only in Buffalo, seven other species of wild bovidiae being negative (Carmichael et al., 1975.) In the buffalo the level of parasitaemia was higher in young animals. However Uilenberg (1976) points out that anaplasmosis is equally
common in regions without ungulates, such as Madagascar, as in areas with game.

(j) Transmission

A critical factor in proposing control measures for anaplasmosis is a knowledge of the means of transmission. A review of the literature on this aspect of the epidemiology of the disease reveals that different conclusions have been reached by researchers as to the most important method acting in their study area. Although vertical transmission has been shown under experimental conditions (Swift & Paumer 1976) where the dam is infected in the last trimestre of gestation, there is little evidence that this occurs frequently in nature. The studies of Kuttler (1962) indicated that a compliment fixation titre in newborn calves was due to maternal antibody transfer and that active infection was acquired much later. Even though losses in young calves may be greater than generally realized, at least in Latin America (Santos, 1967) these tend to occur later than would be the case with infection in utero.

In any particular area transmission may be predominantly biological through a tick, mechanical through biting flies or contaminated instruments, or a combination of these. For clarity, these will be dealt with separately.

Biological transmission. Ticks have been implicated in the transmission of anaplasmosis since the days when the early workers described the peripheral bodies in cattle erythrocytes. Knuth for instance (1904) records that he encountered these intraerythrocytic bodies only in animals from tick infested country. Theiler (1910) was able to demonstrate tick transmission by transferring ticks from infected to sound animals. He stated that tranovarial transmission
must occur as the Boophilus decoloratus tick was one host but he did not present evidence that this occurred. Dickmans (1950) reviewed the literature on tick transmission available at that time and notes that 20 species of hard ticks (19 on today's classifications) and two species of soft ticks had been demonstrated experimentally capable of transmission, although host preferences would limit the possible epidemiological importance of many of them.

The eradication of B. annulatus from the U.S.A. left epidemiologists to explain the continued presence of the disease although this tick had been designated as the most likely culprit up to that time. Ticks are still favoured in the Northern and Western States of the U.S.A. but not in the South-eastern states where the disease is also endemic. Epizootological data points to the probable role of at least two tick species in the U.S.A.; Dermacentor occidentalis in California and D. andersoni in the intermontane states (Dickmans, 1950; Long, Stanber & Frank, 1974; McCallon, 1976; Peterson et al., 1977).

D. occidentalis has a slightly stronger case as all development stages are found on cattle whereas only the adults of D. andersoni are normally parasitic on the bovine (Hooker, Bishopp & Wood, 1912). There is experimental evidence to support transmission by these ticks (Bram, 1971; Rees, 1933; Anthony & Roby, 1966). Sanborn, Stiles & Moe (1938) points out the potential of the male D. andersoni in transmission and transovarian transmission has been recorded for these two tick species (Dickmans, 1950) although these ticks are not one host and therefore do not require this attribute to perpetuate the disease.

Elsewhere, when tick transmission is implied, the one host
Boophilus ticks are regarded on epidemiological data to be the principal tick vectors. (Map 1).

Lignieres (1914) found only *Margaropus* (Boophilus) *microplus* on naturally occurring cases in Argentina. Theiler (1910) records an association with *B. decoloratus* in South Africa. More recently Rogers (1978) in Australia confirmed the findings of earlier workers that the disease was closely associated with the distribution of *B. microplus*. Uilenberg (1968) in Madagascar also points to the epizootiological evidence for this association with *B. microplus* as seems to be the case in Uruguay (Canabez & Bawden, 1975) and Colombia (Carrier, 1975). *B. annulatus* in Mexico has been shown to be infected (Kuttler, 1971).

Conclusive evidence of transovarial transmission by these ticks is not abundant. Kuttler (1971) succeeded in infecting two splenectomized calves with *B. annulatus* larvae. There is considerable doubt surrounding the ability of *B. microplus* to transmit through the egg. Most of the successful reports quoted go back to 1931 and beyond and are open to criticism on some counts, particularly the possibility that mechanical transmission may have occurred as this possibility was not widely appreciated. The original articles of Quevedo (1916 and 1929) and Rosenbusch and Gonzales (1927) are not readily available for examination but the report by Brumpt (1931) of successful transmission is, by his own admission, based on somewhat shaky conclusions. The only evidence of infection was the appearance of marginal bodies in the occasional erythrocyte on the 176th day after infection, absent again by the 177th day. Emphasis was placed on the animals subsequent resistance to challenge inoculation with the homologous Brazilian strain of *A. marginale*. Ristic (1968) has pointed out that some animals in a population show innate resistance to anaplasmosis and although this
Shaded area indicates the distribution of ticks of the genus *Boophilus*. Within this area local variations can be expected to result from the influence of geographical features (e.g. high mountains, deserts) or tick control schemes.

Source. Cattle Tick Control, Cooper McDougall & Robertson Ltd.

Berkhamsted, England.
Shaded area indicates areas where anaplasmosis occurs.

cow was from Northern France this possibility exists. One must also note that an outbreak of anaplasmosis was registered in France around this time (Rossi & Simenois, 1952).

Although these are the traditionally quoted references to successful transovarial transmission with *B. microplus*, others can be uncovered in the literature. As recently as 1975, Laranja, Arregui & Arteche, in Brazil record the use of a strain of *B. microplus* of proven capability to transmit *A. marginale* in studies on the effects of feeding on abnormal hosts. A field strain of *B. microplus* was used in these experiments, obtained as engorged females. Oviposition and hatching were allowed to take place under laboratory conditions and some of the larvae so obtained were allowed to attach to an adult Hereford ox from a tick-free zone. Anaplasmosis was confirmed on the basis of temperature rise and blood slide examination. Later descendants of this larval strain, maintained on infected animals, were used to challenge six other similar cattle which had shown no sign of disease when paraistized by larvae from ticks which had completed a parasitic cycle on an abnormal host species. On challenge by these infected larvae clinical and haematological examination confirmed anaplasmosis between the 36th and 55th day following challenge.

Notably, all successful recordings referred to above were made with South American ticks and parasites which could infer a strain difference as the negative records are all of other origins. However, an article by Shaw (1966) recording investigations on an insecticide resistant strain of *B. microplus* of Australian origin presents an exception. Ticks were sent to Britain and reared on calves which could not have previously been exposed to anaplasmosis as the disease
has never been recorded in Britain. The author states that one calf died of anaplasmosis. Precautions against fly transmission were taken in the construction of the building. It was not clear which tick stage or stages were sent from Australia. If the larvae responsible for producing this infection were British bred from the original Australian stock - which seems most probable - then transovarial transmission is clearly implied and in a tick of non-South American origin.

There are many records of unsuccessful attempts to demonstrate infection through the egg (Brumpt, 1919, 1920; Lignieres, 1919, Gomes de Faria, 1927; Regendanz, 1933; Rees, 1940; Uilenberg, 1968; Connell & Hall, 1972; Leatch, 1973).

It is difficult to explain these variations in experimental results. It may be that there are strain differences in the ability to transmit hereditarily. Bram (1971) draws attention to the variation in results of experiments working with transtadial transmission in D. andersoni and he attributes negative results to the use of strains of A. marginale which had been maintained by artificial passage for more than one or two passages. He postulates that A. marginale loses its ability for biological transmission under these circumstances. It is tempting to infer that something similar may act in the case of transovarial transmission. However, failures have been recorded with field isolates (Connell, 1974).

Regendanz (1933), faced with this anomaly, suggested that transmission may occur when development stages of B. microplus fall off the host and reattach to another. He noted that the ticks changed position on the host, an observation confirmed by the studies of Bennett (1974). Connell (1974) by artificial transfer showed that intra and inter-
Stadial transmission could occur while Uilenberg (1970) succeeded in producing in contact transmission when a susceptible tick-free animal was penned with an infected animal infested with larvae. Fly transmission was precluded. However, enormous numbers of larvae were required on the infected animal. Trials with 12,000 and 90,000 larvae failed even when the host animal died of anaplasmosis. The successful transfer involved the use of 360,000 larvae. Connell et al., (1972) also succeeded in demonstrating in-contact transmission.

It is not easily accepted that such accidental transfers can be responsible for infecting 75% or more of the calf crop by nine months of age as apparently occurs in endemic areas with regularity (Corrier, 1977). Anaplasmosis is a major problem in extensive cattle raising areas of the tropics and sub-tropics where, even allowing for the gregarious habits of the species, stocking rates are low. Contact experiments have been made using large numbers of infecting larvae under confined conditions and, as the trial by Uilenberg showed, transmission is not readily achieved even under such optimal conditions. Unfortunately, in Bennett’s work (1974) absolute numbers were not possible due to technical difficulties in collecting falling ticks and recoveries were counted only from the anterior portion of the body. However, doubling the numbers he thus obtained and discounting larvae, most of which fell in the first 24 hours and are unlikely to have been infected, an infestation of 40,000 larvae would return less than 350 live nymphs to the ground. In practice the numbers would be even less as grooming was prevented in this study. The dilution of these infected falling instars in the total tick population would make it unlikely that they could achieve the infection rates indicated by serological surveys. Transovarial transmission would seem to be a
pre-requisite to achieve this.

Few studies have been made on the fate of the anaplasma organisms in the vector tick. Conflicting results were obtained using the fluorescent antibody test on D. andersoni. Anthony, Madden & Gates, (1962) could not detect the organism after the second day from removal. During this time they were observed in the gut contents and excreta but not in the salivary glands or reproductive organs. Friedhoff et al., (1966) could detect the organisms in gut tissues, haemolymph and malpighian tubes up to the fifth day. Anthony and his co-workers suggested that failure to detect may be due to antigenic changes in the organism.

Mechanical Transmission - (a) biting insects. The persistence of anaplasmosis in areas of the south eastern states of the U.S.A. after the elimination of B. annulatus from these areas led to the search for other possible vectors. Although other tick species such as Rhipicephalus sanguineus could be shown capable of transmitting anaplasmosis, (Rees, 1930) it was evident that its distribution could not entirely explain the situation and in any case this was primarily a dog tick. Attempts to incriminate Amblyomma americanum and A. cajennense have not been encouraging. (Miller, Price and Kuttler, 1976) Sanborn, Stiles & Moe, (1932) investigated horse-fly transmission with four Tabanus species and one Silvius. One animal bitten 43 times over 14 days developed fatal anaplasmosis 28 days after the last bite. Three other mild cases were produced, one from a carrier cow as source of the interrupted blood meal. Sanders (1933) showed that cattle could be kept anaplasmosis free under the protection of fly screens in an endemic area of Florida, where horse flies, stable flies, horn flies and mosquitoes were prevalent. They were unsuccessful in transmitting the disease
with the last two groups but succeeded with *T. fumipennis* using 100 flies. *Stomoxys calcitrans* succeeded where several hundred flies were utilized. *Anaplasma* organisms were demonstrated on beak-smears from flies. Roberts, Pund, McCrory, Scales & Collins, (1969) noted a seasonal incidence in tick-free Mississippi delta with cases occurring in July and August. His studies eliminated *Culicidae* in addition to *Haematobia irritans*, *S. calcitrans* and several *Culicoides* species. Tabanids were incriminated although the relationship was not entirely conclusive. The close association of the eye gnat, *Hippelates* species, with Tabanids was brought to light by Roberts (1968) and they too, could have been involved in transmission particularly in view of the results of Davies, Dimopoulos & Roby (1970) on infection via the ocular route.

It is widely accepted that successful transmission by biting flies requires almost immediate transfer but it is interesting to note the results of Mazzola, Amerault & Roby, (1976) on the survival of *A. marginale* in mosquito cell cultures for 21 days and of Roberts & Love (1977) that ground up eye gnats and tabanids were infective by innoculation into splenectomized calves up to three days post feeding.

Wilson & Meyer, (1966) believe *T. fuscicostatus* to be important in anaplasmosis transmission in Lousisiana and Pipano (1976) states that, while the field vectors have not been identified in Israel, there is strong evidence for mechanical transmission. In Africa, Wiesenhutter (1975) noted peak tabanid incidence 30 to 60 days before peak anaplasmosis incidence in his study. In South America, Vicvaino Gerdts (1976) accepts a role for biting flies although he believes *B. microplus* to be the prime vector in Colombia.
In Australia seasonal distribution of the disease and transmission trials have not credited biting flies with anything more than an incidental role. (Rogers, Blight & Knott, 1978; Connell, 1974).

Transmission by contaminated instruments. The surprising ease with which anaplasmosis can be transmitted by any procedure which allows the rapid transfer of minute quantities of blood from one animal to another presents an additional and serious hazard to control measures. (Stiles, 1936; Reeves & Swift, 1977). It has been shown that as little as 0.025 ml of acute case blood can result in infection when introduced intradermally. Explosive outbreaks can be produced by mass vaccination or chemoprophyloxis and losses can be considerable (Rossi et al., 1952; Grobov, 1961). In East Africa, Weisenhutter (1975) found that careful attention to the sterilization of needles used to inject trypanoprophylactic drugs on a farm where tick control was already rigorous resulted in a marked drop in case incidence. Corrier (1975) in a Colombian study noted a trend towards a relationship between vaccinations per animal per year and occurrence of anaplasmosis. In this context it is interesting to bear in mind the opportunities for transmission that exist in Latin America where a triannual vaccination against foot and mouth disease is common practice, not infrequently carried out with minimum supervision from trained staff. The frequent use of prophylactic drugs against trypanosomiasis in Africa adds to the opportunities in that continent. Clearly the use of prophylactic treatments to control anaplasmosis itself during seasons of vector activity presents a special hazard.

(k) Epidemiological patterns

Although a broadly similar epidemiological situation may exist over a large geographical area, considerable local or even farm to
farm variation can exist making full investigation into the factors at work in the particular disease situation under review desirable. Correct diagnosis is a basis necessity. Clinical similarities with other arthropod-borne blood parasites which may occur together with anaplasmosis demand that laboratory diagnosis be employed on any critical assessment. Haematological examination and a search of Giemsa-stained blood films for characteristic marginal bodies is normally sufficient to confirm or refute the clinical diagnosis. As Anaplasma spp. may occasionally be encountered in the blood of carrier animals it is usual to adopt a minimum parasitaemic level as a criteria for positive confirmation (McCosker, 1975). Such examinations will allow a record of confirmed cases to be built up over a period of years and seasonal patterns will be identified. These can be related to climatic factors and the activities of potential vectors. More precise information requires the use of a suitable serological test to determine the incidence of positive reactors. This information, related to age, origin, breed and management factors allows an assessment to be made of the risk of disease occurring under the circumstances prevailing. The possibility of non-bovine reservoirs being involved needs to be considered.

By such investigations differing patterns have been brought to light. In Australia the seasonal incidence peaks in the autumn with a significant carry through to the winter whereas in Mississippi, anaplasmosis is restricted to a short period in summer. (Roberts et al., 1969). These periods correspond, with allowance for the incubation period, to the periods of vector activity; in the first case B. microplus and in the second, tabanid flies. In the Australian example the rigorously enforced tick control measures allow comparisons to be made between tick-infested and tick-free zones. (Rogers, et al., 1978).
In the tick-infested zone 42% of cattle had complement fixing antibodies compared with 0.4% in adjacent tick-free zones. The extreme variation in the percentage reactors that Kuttler found in his East African survey (1965) could have been influenced by the relative efficiencies of tick control measures. The pattern in Africa can be extremely complex as brought out by Weisenhutter's study in Tanzania (1975) where, with the elimination of the tick factor, the resulting pattern could only be accounted for by a combination of needle and fly transmission. The role of game in Africa remains speculative.

The possible complexity is evident from the North American situation where Columbian black-tailed deer are strongly implicated as reservoir hosts in California where the Pacific coast tick *D. occidentalis* is the most important vector while the related white-tailed deer is not involved in the South-eastern enzootic area where tabanid transmission is responsible.

In Colombia, while a role has been suggested for mechanical transmission via vaccination needles (Corrier, 1975) and biting flies (Vizcaíno Gerits, 1976) in some areas, it is generally believed that *B. microplus* is the principal vector in the region (Corrier, Cortes, Thompson, Riano, Becerra & Rodríguez 1978). This tick was the only species common to all 37 farms in a serological survey which revealed a mean herd prevalence of 74%. A comparison of anaplasmosis prevalence in the difference climatic zones of Colombia reveals a direct correlation with mean annual temperature. At 2,600 metres above sea level where the mean annual temperature was 13°C, Kuttler, Adams & Zaraza, (1970) reported that anaplasmosis was absent. At 1,200 metres it was 51%, at 1000 metres 63%, at 450 metres 68% and at 13 metres where the mean temperature reached 28°C prevalence was 91%.
Uruguay presents a somewhat similar situation in that, although outbreaks have been associated with mechanical transmission, *B. microplus* is again regarded as the prime vector (Canabez et al., 1975) although there is little critical work to support this. Uruguay marks the southernmost extension of *B. microplus* but the whole country may be regarded as marginal on the basis of the climatic factors elucidated by McCullock & Lewis (1968) for this tick in Australia. Temperature variations are due to latitude rather than altitude and tick control measures reinforce natural limitations so that the tick is largely confined to the northern half of the country where, in fact, most outbreaks of anaplasmosis occur though serological information is, as yet, limited. (Canabez et al., 1975). As mean monthly temperatures in this region are below 16.7°C in winter (which Hitchcock (1955) found to be the threshold temperature for hatching), seasonal variations in tick activity are to be expected. Rainfall, however, is sufficiently abundant and well distributed to remove this as a limiting factor.

Apart from climatic factors and acaricide usage, tick populations can be influenced by local factors. Thus cattle from the islands of the delta of the river Jacui in Brazil are notoriously susceptible to tick-borne disease as their grazing land is frequently flooded. Any ticks engorging drown when they fall from the host animals which are accustomed to grazing knee-deep in water. Similar situations exist in Zambia (Akafekwa, 1976) and Bolivia (Callow, 1974).

As outlined above, calves, if exposed before about nine months of age normally experience a sub-clinical infection and acquire a state of premunity with persistence of the organism which may be maintained by continual exposure. Thus in enzootic situations, provided that vector transmission is maintained at efficient levels disease may
be kept at low levels as the calf population acquires immunity through early infection. On the other hand, if vector density is very low it may be close to the limit for transmission to occur and cattle may remain susceptible for prolonged periods, without the disease reaching serious proportions. Between these two extremes lies a position of enzootic instability, influenced by local fluctuations in vector density and where losses may be expected, particularly in the one to two year old cattle (Callow, 1974). Most of these basic epidemiological ideas have resulted from work on babesiosis in Australia (Mahoney & Ross, 1972) but have been applied to anaplasmosis also (Callow, 1974) Mathematical models have been designed to enable calculation of the risk in any particular situation. Two important factors are the infection rate in the ticks and the number of fresh tick bites per day per animal. It has been calculated (Callow, 1974) that for a stable situation to exist each animal requires an infective tick bite every 90 days. As these are not easy parameters to measure, the risk has been related to the percentage of calves infected by nine months of age which can be determined serologically. Where this percentage is over 90 or under 5 the risk of disease is low.

In areas of enzootic instability, large variations in herd susceptibility can exist between cattle raising units because of the effects of different management practices, making movement for commercial reasons hazardous. Movement along stock routes can present special problems and unusual favourable conditions can result in vector extension with serious consequences.

(1) Geographical spread and economic aspects.

According to the Food and Agriculture Organisation (FAO) handbook Animal Health Yearbook for 1977, 95 countries recorded the
presence of bovine anaplasmosis. Map 2 gives an indication of the widespread nature of this disease. The following countries recorded the incidence as high (+++). Mexico, Guatamala, Trinidad and Tobago, Bolivia, Ecuador, Guinea (Bissau), Ghana, Zaire, Mozambique, Rhodesia, Botswana, Kenya and South Africa. In Brazil the incidence was recorded as moderate. Chile was the only South American country not recording the presence of the disease.

In the U.S.A. the disease is spreading with extension from the endemic areas. It has been considered as the second most important cattle disease in the U.S.A. (Amerault et al., 1976) and estimates of deaths of 50,000 to 100,000 head with total losses reaching 100 million dollars a year, taking weight and milk loss and abortions into account have been made (McCallon, 1976). In many countries it is difficult to separate anaplasmosis from babesiosis when trying to arrive at estimates for losses. However, in Peru it has been regarded as the most important tropical cattle disease in the country. (Lora, 1971). Apart from the obvious losses mentioned above, anaplasmosis is a major constraint on the development of the cattle industry in many countries. It interferes with plans to colonize new areas with cattle raising enterprises and limits the movement of cattle populations between zones of different prevalence and makes up-grading of stock through the importation of exotic animals particularly risky. Premunition under such circumstances is often unsatisfactory and expensive in terms of labour and drugs. (Ranalli & Marchesi, 1974). In Brazil Santos (1967) reports that anaplasmosis constitute one of the most important causes of losses in young calves on farms in the Rio de Janeiro area, and notes that the relative difficulty in treatment in comparison to babesiosis emphasizes the importance of the former. Brazil, Monmary,

(m) Control

There are two distinct and opposing strategies for control of anaplasmosis and the choice of a line of attack depends upon the prevalence of infection in the population to be protected and on available resources. Where infection rates are low, attempts at elimination of the parasite may be considered, especially where geographical factors offer some hope of maintaining lines of defense. Where the parasite is widely disseminated in the population a more promising approach would be to opt for immunoprophylactic measures taking advantage of the natural resistance of the young animal.

1. Vector control. Rigorous tick control in Australia has clearly shown a dramatic and highly significant difference in anaplasmosis prevalence on either side of the control line. (Rogers et al., 1978). Biting flies are obviously more difficult to tackle but Hoffman, Smith, Collins, Mott & Scales (1961) have reported on the benefits of intensive insect control in reducing anaplasmosis spread in a susceptible herd. Mechanical spread through contaminated instruments can be avoided by sanitary measures and Weisenhutter (1975) has demonstrated the benefits obtainable.

2. Treatment. Tetracyclines have proved effective in suppressing the reproduction of the parasite (Foote, Farley & Gallagher, 1951) and can be used effectively in treatment provided they are given early in the course of infection. (Christensen & Harrold, 1956); Millar,
1953). Elimination of carrier infections has proved more difficult and requires prolonged administration of high doses (Brock, Pearson & Kliewer 1953). Chlortetracycline orally at 11 mg/Kg daily for 45-60 days or oxytetracycline intravenously or intramuscularly at 11 mg/Kg daily for 10 to 12 days have been recommended (Kuttler, 1973). A new formulation of oxytetracycline which contains 200 mg/ml has proved efficient in clearing carrier infections with as few as two doses at a one week interval (Kuttler & Simpson, 1978; Roby, Simpson & Amerault, 1978).

Recently, two further compounds, imidocarb and gloxazone have been shown to exert a chemotherapeutic effect on anaplasmosis (Barrett, Beveridge, Bradley, Brown, Bushley, Clarke, Neal, Smith & Wilde, 1965; Joyner & Brocklesby, 1973; Kuttler, 1971; Roby, 1972). Imidocarb is used at 2-5 mg/Kg subcutaneously or intramuscularly while gloxazone is given intravenously at 5 mg/Kg. At these levels they have proved effective in moderating the clinical reaction in premunization experiments (Kuttler & Todorovic, 1973). The toxic effects of these two drugs at higher levels limit their use in eliminating infection from the blood stream (Kuttler, 1971).

3. Immunisation. A killed anaplasma adjuvant vaccine based on that described by Brock et al., (1965) is produced commercially in the U.S.A. and McHurdy & Simpson (1973) have reported on a killed vaccine in Africa.

While these vaccines can be used without risk of clinical reaction in the vaccinated animal, the level of protection given has been found inadequate in some circumstances (Ristic, 1975) and widespread use of these vaccines has led to the appearance of problems of
neonatal isoerythrolytic anaemia in calves born of vaccinated dams (Dennis, Young & Dorris O'Hara, 1970). This would seem to be due to the stimulation of antibody formation by erythrocytic components in the vaccine. The killed vaccines may have some value in the protection of highly susceptible imported bulls but cannot be recommended for general use.

An attenuated \textit{A. marginale} strain produced in sheep has given promising results in trials in the U.S.A. and several Latin-American countries. (Ristic, 1975). However the vaccine is not presently available in Brazil and it is necessary to consider a vaccination programme based upon the use of either virulent \textit{A. marginale} or on \textit{A. centrale}.

Vaccination with virulent \textit{A. marginale}, in its crudest form, may be accomplished in an enzootic area by a transfer of blood from carrier adult cattle to calves. Often the source of blood is the calf's dam. While this method is better than doing nothing at all and may reduce losses to some extent (Gonzalez & Todorovic, 1975) it is uncontrolled, both in respect of the results of innoculation in the calf and of the quality of the donor blood. In Peru, serological tests on 19 cattle considered carriers and selected as donors for pre-munization indicated that only nine of these were infected. (Castillo, Chavez & La Rosa, 1966).

For centralised vaccine production donor animals are often selected from tick-infested individuals in the population at large, tested for resistance to challenge, infectivity to susceptible animals and their resistance challenged regularly by innoculation or by ticks. This is the basis for vaccine production for pre-munization
procedures carried out by various groups is Brazil at the present time. Adult vaccination is almost exclusively of imported stock and may be carried out either at special centres or at the receiving farm. Treatment with tetracyclines is instigated on noting a temperature rise. The expected result of such crude blood transfers is a double vaccination against babesiosis and anaplasmosis with the reaction to the former occurring between the fifth and twenty-fourth day following inoculation and a reaction between the twenty-fifth and forty-fifth day being attributed to anaplasmosis (Brasil et al., 1970). Giemsa-stained thin blood smears may be used to confirm the infective agent present. Modifications include the reduction of infectivity by holding the blood at 4 to 8°C for varying periods before use and the giving of less attenuated booster doses later to reinforce the protection achieved (Brasil, et al., 1970).

This procedure is also employed for calf vaccination in some areas of the country though treatment is usually withheld unless absolutely necessary.

Though representing a step forward from the first method, this procedure also carries with it certain risks. Foremost amongst these are the losses inevitable from the use of a virulent field strain of A. marginale especially with an unquantified inoculum under field conditions. This risk is particularly high with adult imported cattle. Also there is some risk that other, undesirable disease producing agents may be present in the inoculum. Furthermore, the use of whole blood vaccines in breeding females may result in the production of antibodies in these animals to incompatible blood which can lead to a haemolytic syndrome in newborn calves after ingesting colostum containing these antibodies.
A more refined approach is the use of stabilates of highly parasitized blood produced in splenectomized calves. Gonzalez and Todoravic (1975) investigating this method in Colombia found the system economically sound with a production of 2 million doses possible from one calf. Washed and concentrated erythrocytes are mixed with a cryopreservative and held at $-65^\circ$C until use when they are diluted with phosphate buffered saline to contain $10^7$ anaplasma organisms per dose. The use of splenectomized calves offers greater assurance against the introduction of unwanted pathogenic agents and the standardized dose gives a more predictable result and involves a much reduced erythrocytic component. Discontinuous production of batches of vaccine is possible. Even so this stabilate vaccine carries with it the inherent danger of the use of virulent *A. marginale* in adult cattle.

Theiler (1911) pointed out the protective effect of *A. centrale* and for many years this serologically related agent has been employed against anaplasmosis in Africa, Australia and South America, (Lora, 1971; Callow, 1976). While not protecting against subsequent infection with *A. marginale* it gives substantial protection against the clinical effects (Kuttler, 1966). There have, however, been reports that *A. centrale* is capable of producing a more severe syndrome in South American (Lora, 1971) and Australian (Legg, 1936) cattle.

McCosker (1975) in recommending the use of *A. centrale* in Bolivia points out that, from his experience in that country, a three month period is required for the immunity induced by *A. centrale* to reach protective levels and thus protection against natural exposure should extend to this period after vaccination. The vaccine is best produced in splenectomized calves in a manner similar to that outlined above for *A. marginale* stabilates.
5. **EPIDEMIOLOGICAL STUDY - BACKGROUND INFORMATION**

Rio Grande do Sul is the southernmost state of Brazil and borders with Uruguay to the south and Argentina to the west. To the east lies the south Atlantic Ocean. (Maps 3 and 4). The economy is largely agriculturally orientated with the predominant crops and agricultural species varying regionally within the state. The survey was carried out in the north western part of the state in an area lying between 27' and 28'30" South and 54' to 53' West. The undulating countryside is heavily cultivated land interspersed with areas of permanent grassland and natural woodland and lies between 350 and 550 metres above sea level with an annual rainfall of around 1620 mm fairly evenly distributed and monthly mean temperatures are between 25 and 14°C (Table 1). Agricultural holdings tend to be small in comparison to other regions of the state with an average of around 30 hectares. Wheat and soya bean as successive crops provide the major source of income, supported in many cases by pig and cattle units.

The region is an important supplier of milk to the state capital and of dairy products to a wider area. 

Bos taurus breeds predominate in both beef and dairy units, the Friesian being the most common dairy breed. Marketing and the supply of inputs is organised through co-operatives, the larger of which provide advisory services through their agronomists and veterinarians as well as other technical services. Reacting to government incentives, the co-operatives are encouraging their members to expand milk production through more intensive grassland management coupled to genetic improvement and animal health control. The cattle tick, B. microplus is endemic throughout the region and control measures practised are based on dips, sprayraces or hand spraying with the first being common on
TABLE 1
Rainfall and Temperature data for Ijui

<table>
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<th>Month</th>
<th>Rainfall (mm)</th>
<th>Mean Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal* 1978</td>
<td>Normal* 1978</td>
</tr>
<tr>
<td>January</td>
<td>131.0</td>
<td>25.1</td>
</tr>
<tr>
<td>February</td>
<td>134.0</td>
<td>24.1</td>
</tr>
<tr>
<td>March</td>
<td>125.0</td>
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<td>20.3</td>
</tr>
<tr>
<td>May</td>
<td>100.0</td>
<td>18.0</td>
</tr>
<tr>
<td>June</td>
<td>159.0</td>
<td>15.2</td>
</tr>
<tr>
<td>July</td>
<td>94.0</td>
<td>15.5</td>
</tr>
<tr>
<td>August</td>
<td>185.0</td>
<td>16.1</td>
</tr>
<tr>
<td>September</td>
<td>157.0</td>
<td>18.2</td>
</tr>
<tr>
<td>October</td>
<td>132.0</td>
<td>20.3</td>
</tr>
<tr>
<td>November</td>
<td>107.0</td>
<td>23.0</td>
</tr>
<tr>
<td>December</td>
<td>179.0</td>
<td>24.8</td>
</tr>
</tbody>
</table>

*Normal = average over eleven years

Source - Instituto de Pesquises Argronomicas, Area de Ecologia Agricola.
larger units and the last on small ones. The grave difficulties which have arisen in some other parts of the state through the appearance of tick populations resistant to acaricides of one or more of the main chemical groupings have not yet reached unmanageable proportions in this region.
MAP 3

Light shading - Brazil

Heavy shading - Rio Grande do Sul state
MAP 4. Rio Grande do Sul state indicating area covered by survey (shaded area)
PLATE 2  Illustrating typical dairy unit. Note extensive soya bean crops in background. The limited grazing available is supplemented with silage and maize feeding.

PLATE 3  Dairy calves are predominantly *Bos taurus* with Freisian being the most common breed.
PLATE 4  Charolais steers on a beef unit where grazing is supplemented by stall-fed home grown maize.

PLATE 5  Spray races of this type are used for acaricide application on some properties.
PLATE 6  Covered dipping baths are restricted to the larger properties.

PLATE 7  Illustrates the common practice of simultaneous application of several injectable products. The use of automatic syringes and the scant attention to hygiene offers ample opportunity for the mechanical transmission of anaplasmosis. The building at the end of the spray race houses a dipping bath.
6. MATERIALS AND METHODS

(a) Collection of material - clinical cases.

Material remitted to the Ijui regional laboratory by field veterinarians from cases suspected as being tristeza consisted of thin blood smears and/or a citrated blood sample, both normally jugular blood. Blood smears were routinely fixed in methanol for two minutes, stained with Giemsa, 10% in buffered distilled water, pH 7.2 for 20 minutes. They were then washed in buffer, dried and examined under oil at 1000 times magnification. *A. marginale* was identified using the criteria of McCosker (1975).

(b) Farm survey.

Farms visited were not randomly chosen but were selected to cover the main cattle-raising areas of the region and different farming systems. Choice was to some extent influenced by the relative willingness of farmers to co-operate and the existence of adequate holding facilities.

On small farms all cattle were sampled if possible and on larger units a percentage, normally over 15%. Where the enterprise was a breeding unit, young stock were included. Approximately 10 ml of blood was drawn from a coccygeal vein using evacuated, siliconized tubes (Vacutainer, Becton Dickinson, New Jersey, U.S.A.) and individual needles for each animal. On return to the laboratory, the serum was separated from the clotted blood by centrifugation at 1000 rpm for 10 minutes in a Clayton-Lane centrifuge (R.B. Turner Ltd., London). Serum for the CFT was phenolized at 0.5%, marked and stored at -20°C until required. Serum for the CT was stored unphenolized and was tested within seven days of collection.
Information was collected from each of the sampled properties by means of a simple questionnaire (translated in Appendix 1) on managemental practices which could influence disease spread. Stocking rate, grazing management, parenteral administrations, presence of ticks and vampire bats were noted as were the tick control measures practised and disease situation. Breed, age and origin of the animals sampled were recorded.

(c) Complement fixation test.

The technique was carried out using standard perspex haemagglutination trays and was essentially that employed by Mahoney (1962). The trays 18 x 14 x 1.5 cm contain 80 round-bottomed cells and were prepared for use by washing in tap water followed by distilled water. They were then dried in a hot air oven, paper-wrapped and stored until used.

Reagents:

1. Veronal Buffer - This was prepared as required from commercial C.F.T. diluent tablets (Wellcome Reagents, Beckenham, Kent) dissolved in sterile distilled water.
2. Serum. - After thawing at room temperature, this was diluted 1 in 5 with veronal buffer and inactivated in a 56°C water bath for 30 minutes. It was then allowed to stand at room temperature for 90 minutes before proceeding with the test.
3. Haemolysin. A commercially supplied rabbit anti-sheep erythrocyte serum (Wellcome Reagents, Beckenham, Kent) was utilised. The serum was stored at 4°C and dilutions prepared as necessary with veronal buffer.
4. Sheep erythrocytes. Jugular blood was withdrawn using aseptic precautions into an equal volume of sterile Alsevers solution.
Collections were at weekly or fortnightly intervals, alternately from one of two Corriedale sheep kept at the laboratory. The blood was stored at 4°C. On each day the test was performed, a suitable amount of the stored blood was washed three times with veronal buffer by centrifugation for 10 minutes at 2,000 rpm in an MSE Super Minor Centrifuge (MSE Ltd., Crawley, Sussex), removal of supernatant and any white cells, reconstitution to initial volume and mixing for two minutes on a rotating blood mixer. After washing, the packed cell volume was determined using a Hawksley microhaematocrit centrifuge (Gelman Hawksley, Lancing, Sussex) and the required amount of buffer determined to make up to a 4% solution.

5. Complement. Lyophilized guinea-pig complement (Wellcome Reagents Ltd., Beckenham, Kent) was used. The manufacturers instructions were followed to prepare a one in ten stock solution and from this, dilutions of 1 in 50, 1 in 70, 1 in 90, 1 in 120, 1 in 160, 1 in 210 and 1 in 280 were prepared with veronal buffer.

Complement titration. Complement was titrated each morning on test days. Doubling dilutions of haemolysin were prepared from 1 in 100 to 1 in 3200. Later, in view of consistent haemolytic titres, this range was reduced to 1 in 400 to 1 in 1,600. Each dilution was mixed with an equal volume of 4% erythrocytes and allowed to sensitise at 37°C for 10 minutes. Each mixture was then added to a row of complement dilutions in a perspex tray using volumes of 0.1 ml of sensitized cells and 0.1 ml of complement. Cell controls for each haemolysin dilution were included, buffer replacing complement. The plate was incubated at 37°C in a water bath, then covered with aluminium foil and held at 4°C for two hours before reading. One unit of complement was taken as the highest dilution giving 100% lysis and the optimum
sensitizing dose of haemolysin as the highest dilution giving lysis with the highest lytic dilution of complement. In the test proper two haemolytic doses of complement were used with the optimum sensitizing dose of haemolysin.

6. Antigen. CFT antigen (U.S.D.A. National Veterinary Services Laboratory, Ames, Iowa) was used at the titre indicated on the bottle following one initial check titration to ensure that deterioration in transit had not occurred. The antigen was tested for activity in doubling dilutions of from 4 times to one quarter of the indicated titre against known positive sera. Antigen was stored at 4°C and dilutions to titre prepared when required.

Test Proper. A four volume test using volumes of 0.1 ml dispersed with an automatic pipette coupled to individual sterile Pasteur pipettes for each serum and reagent. Equal volumes of haemolytic serum, diluted to the optimum sensitizing dose, and 4% sheep erythrocytes formed the haemolytic system. Two haemolytic doses of complement were used and the antigen at the checked indicated titre of 1 in 40. The inactivated serum was used at the single dilution of 1 in 5 with veronal buffer as the diluent throughout.

Three double columns were used on each plate orientated to give eight columns and ten rows. The second column in each double column held the serum control where diluent replaced the antigen volume. The format was found convenient as it made reading the test easier and lessened the risk of error in setting up the plate, although it reduced the maximum number of sera on each plate from 40 to 30. Two plates, i.e. 60 sera, could be processed at one time, with the remaining water bath space being utilised for a control plate. The
control plate was a ten row, six column plate made by sawing off two columns from a standard plate. This was necessary as the water bath would not take three entire plates. Controls used were as follows:

1. Known positive serum.
2. Known positive serum control.
3. Known negative.
4. Known negative serum control
5. Complement controls with four doubling dilutions of complement from two haemolytic doses to ¼ haemolytic dose.
7. Antigen anticomplementary control.
8. Antigen haemolytic control.
9. Sera from previous tests which had given negative results were tested for haemolytic activity of the sera i.e. set up as test but without complement (replaced by diluent). This was employed as being more economical of antigen than the alternative of setting up such a control for each serum in the test proper as the number of negative sera was, overall, much less than the number of positive.
10. Serum anticomplementary controls were run for each serum on the test plate. The test was fitted into the laboratory routine as follows:

7.30 a.m. - 11.30 a.m. Sera for test that day thawed, diluted and inactivated. 4% washed erythrocytes prepared from stock. Complement titrated.
11.30 a.m. - 1.30 p.m. Sera held at room temperature. Complement plates held at 4°C and read at end of period.
1.30 p.m. - 5.30 p.m. Test and control plates set up
without addition of haemolytic system. Shaken and 30 minutes allowed for fixation of the complement to occur in the water bath at 37°C, following which period sensitized erythrocytes added to all cells. Plates incubated in the water bath for 45 minutes with shaking at 15 minute intervals. Plates wrapped with foil and held in refrigerator overnight and read following morning.

Using this system 120 sera could be tested each day though as a general rule the number processed was less than this.

(d) Card Test

The modified card agglutination test (Amerault, Rose & Roby, 1972) was used, employing a commercial kit (Hynson, Westcott and Dunning, Baltimore, Maryland, U.S.A.) The kit provides stained antigen and bovine serum factor with gauged dispensing needles, cards with ten, 18 mm circles, marked capillary tubes for dispensing the serum and wooden stirring sticks. The kit and control sera were provided by the U.S.D.A. Veterinary Services Diagnostic Laboratory (Ames, Iowa) and the test was performed as described in the kit instructions. Cards were rotated for 4 minutes by fixing to the inclined face of blood mixer (Gellmen, Hawksley, Lancing, Sussex) which rotated at approximately 40 r.p.m.
(a) Clinical

The monthly distribution of anaplasmosis cases confirmed by slide examination at the Ijui laboratory is given in Table 2. This depicts a tendency for higher incidence of the disease in autumn and winter months and absence of cases in spring. Herd sizes on the 26 properties visited ranged from as low as six to as many as 800 and the 892 sera suitable for testing represented samples of between 3.9 and 100% of individual herds. Only on two of the largest herds was the sample less than 15% of the herd.

**TABLE 2**

Laboratory Confirmed outbreaks of Anaplasmosis in Ijui area during 1978.

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of Outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3</td>
</tr>
<tr>
<td>February</td>
<td>1</td>
</tr>
<tr>
<td>March</td>
<td>1</td>
</tr>
<tr>
<td>April</td>
<td>2</td>
</tr>
<tr>
<td>May</td>
<td>3</td>
</tr>
<tr>
<td>June</td>
<td>3</td>
</tr>
<tr>
<td>July</td>
<td>2</td>
</tr>
<tr>
<td>August</td>
<td>6</td>
</tr>
<tr>
<td>September</td>
<td>3</td>
</tr>
<tr>
<td>October</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
</tr>
</tbody>
</table>

Outbreak = one or more cases occurring on same property during month.
PLATE 8  Reagents for the complement fixation test.

PLATE 9  Illustrating the layout for titration of the complement.
Illustrating layout for the complement fixation test proper.

Card test. Upper circles contain measured drops of test sera, normal bovine serum and stained antigen. These are mixed together with the wooden spatula (left).
Card test. Illustrating adaptation of a blood mixer for card rotation. Positive results are evident in circles 4, 5, 8 and 9.
(b) Serological (Table 3)

Negative results were obtained with 161 sera (18.1%) while 91 (10.2%) were recorded as suspicious (1+ reading) and 640 sera (71.7%) were positive (2+, 3+ and 4+ reading) to the complement fixation test.

TABLE 3

Serological Survey - Complement Fixation Results

<table>
<thead>
<tr>
<th>Farm Ref.</th>
<th>Negative No.</th>
<th>Negative %</th>
<th>Suspect No.</th>
<th>Suspect %</th>
<th>Positive No.</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI 1</td>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>GI 3</td>
<td>5</td>
<td>24</td>
<td>3</td>
<td>14</td>
<td>13</td>
<td>62</td>
</tr>
<tr>
<td>GI 4</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>31</td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td>TP 1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>7</td>
<td>86</td>
</tr>
<tr>
<td>IJ 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>IJ 2</td>
<td>0</td>
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<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>IJ 3</td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>131</td>
<td>89</td>
</tr>
<tr>
<td>IJ 5</td>
<td>13</td>
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<td>11</td>
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<td>237</td>
<td>91</td>
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<td>IJ 6</td>
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<td>6</td>
<td>3</td>
<td>4</td>
<td>63</td>
<td>90</td>
</tr>
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<td>1</td>
<td>20</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>IJ 9</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>IJ 10</td>
<td>1</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>5</td>
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<td>0</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>IJ 12</td>
<td>5</td>
<td>10</td>
<td>8</td>
<td>15</td>
<td>39</td>
<td>75</td>
</tr>
<tr>
<td>TM 1</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>TM 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>TM 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>TM 4</td>
<td>0</td>
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<td>2</td>
<td>50</td>
</tr>
<tr>
<td>TM 6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>TM 7</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>PM 1</td>
<td>41</td>
<td>71</td>
<td>3</td>
<td>5</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>PM 2</td>
<td>18</td>
<td>35</td>
<td>19</td>
<td>37</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>PM 3</td>
<td>36</td>
<td>72</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>PM 4</td>
<td>19</td>
<td>63</td>
<td>4</td>
<td>13</td>
<td>7</td>
<td>23</td>
</tr>
</tbody>
</table>

Totals: 161 91 640
% of total samples: 18.1 10.2 71.7
Mean herd prevalence: 16.2 7.4 76.4
Table 4 shows the breakdown by age group which clearly shows increasing evidence of exposure with older age groups.

**TABLE 4**

Age Relationship of Complement Fixation Reactions

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. Tested</th>
<th>Negative No.</th>
<th>Negative %</th>
<th>Suspect No.</th>
<th>Suspect %</th>
<th>Positive No.</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 12 mths</td>
<td>62</td>
<td>34</td>
<td>54.8</td>
<td>6</td>
<td>9.7</td>
<td>22</td>
<td>35.5</td>
</tr>
<tr>
<td>13 - 24 mths</td>
<td>144</td>
<td>61</td>
<td>52.4</td>
<td>24</td>
<td>16.7</td>
<td>59</td>
<td>41.0</td>
</tr>
<tr>
<td>over 25 mths</td>
<td>686</td>
<td>66</td>
<td>9.6</td>
<td>61</td>
<td>8.9</td>
<td>559</td>
<td>81.5</td>
</tr>
</tbody>
</table>

Tables 5 - 9 show the results when the farms sampled are grouped according to herd size, stocking rate, ratio of permanent pasture to temporary grazing, number of vaccinations per animal per year and into dairy and beef enterprises.

**TABLE 5**

Complement fixation test results related to herd size

<table>
<thead>
<tr>
<th>Herd Size</th>
<th>No. of Herds</th>
<th>Negative No.</th>
<th>Negative %</th>
<th>Suspect No.</th>
<th>Suspect %</th>
<th>Positive No.</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 50</td>
<td>16</td>
<td>24</td>
<td>10.6</td>
<td>6</td>
<td>2.1</td>
<td>85</td>
<td>86.5</td>
</tr>
<tr>
<td>51 +</td>
<td>10</td>
<td>137</td>
<td>24.9</td>
<td>85</td>
<td>14.8</td>
<td>555</td>
<td>60.4</td>
</tr>
</tbody>
</table>

* No. = total animals in herd size group tested giving this reaction.
** MH% = mean of percentage of animals tested in each herd in the group with this reaction.
### TABLE 6

Complement fixation test results related to stocking rate

<table>
<thead>
<tr>
<th>Stocking Rate</th>
<th>No. of Herds</th>
<th>Negative *No.</th>
<th>**MH%</th>
<th>Suspect No.</th>
<th>MH%</th>
<th>Positive No.</th>
<th>MH%</th>
</tr>
</thead>
<tbody>
<tr>
<td>under 1 animal unit/ha</td>
<td>12</td>
<td>71</td>
<td>14.5</td>
<td>52</td>
<td>8.0</td>
<td>451</td>
<td>77.4</td>
</tr>
<tr>
<td>over 1 animal unit/ha</td>
<td>12</td>
<td>49</td>
<td>14.4</td>
<td>35</td>
<td>6.6</td>
<td>168</td>
<td>78.9</td>
</tr>
</tbody>
</table>

* No. = total animals in stocking rate group tested giving this reaction.

** MH% = mean of percentage of animals tested in each herd in the group with this reaction.

+ 1 animal unit = 1 adult or 2 yearlings or 10 calves

ha = hectare.

### TABLE 7

Complement fixation test results related to grazing type

<table>
<thead>
<tr>
<th>% permanent pasture</th>
<th>No. of Herds</th>
<th>Negative *No.</th>
<th>**MH%</th>
<th>Suspect No.</th>
<th>MH%</th>
<th>Positive No.</th>
<th>MH%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 50</td>
<td>11</td>
<td>85</td>
<td>25.3</td>
<td>44</td>
<td>9.6</td>
<td>109</td>
<td>64.7</td>
</tr>
<tr>
<td>51 - 100</td>
<td>14</td>
<td>35</td>
<td>6.8</td>
<td>43</td>
<td>5.4</td>
<td>509</td>
<td>87.8</td>
</tr>
</tbody>
</table>

+ Permanent pasture as percentage of total available grazing.

* No. = total animals tested in herd groupings giving this reaction.

** MH% = mean of percentage of animals tested in each herd in the group with this reaction.
### TABLE 8

Complement fixation test results related to number of vaccinations

<table>
<thead>
<tr>
<th>Vaccinations/animal/year</th>
<th>Herds</th>
<th>Negative *No. **MH%</th>
<th>Suspect No. MH%</th>
<th>Positive No. MH%</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 5</td>
<td>13</td>
<td>60 9.9</td>
<td>33 4.9</td>
<td>394 85.2</td>
</tr>
<tr>
<td>5 or more</td>
<td>12</td>
<td>101 24.2</td>
<td>57 9.7</td>
<td>238 66.1</td>
</tr>
</tbody>
</table>

* Vaccinations here includes parenterally administered anthelmintics

** MH% = mean percentage of animals tested in each herd in the group with this reaction.

### TABLE 9

Complement fixation results grouped according to whether Dairy or Beef enterprise.

<table>
<thead>
<tr>
<th>Herd Type</th>
<th>No. of Herds</th>
<th>Negative *No. **MH%</th>
<th>Suspect No. MH%</th>
<th>Positive No. MH%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>20</td>
<td>70 13.4</td>
<td>28 4.8</td>
<td>198 81.9</td>
</tr>
<tr>
<td>Beef</td>
<td>6</td>
<td>91 25.6</td>
<td>63 16.3</td>
<td>441 58.4</td>
</tr>
</tbody>
</table>

*No.  = Total animals tested in herd grouping giving this result.

**MH% = mean percentage of animals in each herd in the group with this reaction.
The number of pure zebu breed cattle encountered in the survey was very low and precludes any meaningful comparison with *Bos. taurus* breeds. A sample of 102 sera representing cattle of all age groups were tested by both CFT and CT. The results are summarised in Table 10.

**TABLE 10**

Comparative results of card and complement fixation tests

<table>
<thead>
<tr>
<th>CFT Result</th>
<th>No. of Cattle</th>
<th>CT +ve</th>
<th>CT -ve</th>
<th>% agreeing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>30</td>
<td>3</td>
<td>27</td>
<td>90</td>
</tr>
<tr>
<td>Suspect or positive</td>
<td>72</td>
<td>55</td>
<td>17</td>
<td>76</td>
</tr>
</tbody>
</table>

82 sera showed agreement between the two tests when a suspect reaction (+) in the CFT was considered to be in agreement with a positive CT result. Of the 20 sera in disagreement, 13 were CFT suspect reactions leaving seven sera in blatant disagreement. Overall agreement was 80%.
From the examination of slides and blood samples remitted to the Ijui laboratory from cases diagnosed clinically as *tristeza*, it would seem that *A. marginale* is frequently the aetiological agent of the syndrome in this region. It is necessary to qualify the statement by noting that the number of cases referred for laboratory confirmation is small in comparison with the probable number investigated in the field. One typical field veterinarian had records of 52 clinical diagnoses of *tristeza* in a 12 month period. As there were more than 50 field veterinarians operating in the area covered by the laboratory the number of cases occurring in the region must, conservatively, number several hundred. In fact, most cases referred were from veterinarians who covered the area in the immediate vicinity of the laboratory. *B. bigemina* is easier to diagnose clinically, responds better than anaplasmosis to treatment and is therefore less likely to be referred to the laboratory. *B. bovis* is not generally believed to be a significant problem in the region (though this may not be true) and is unlikely to be found in the peripheral blood samples which veterinarians are accustomed to send in to the laboratory.

Thus caution must be exercised in drawing wider conclusions from the small sample available but the tendency for anaplasmosis cases to occur in autumn and winter (Table 2) is of interest in view of the broadly similar distribution in Australia found by Rogers (1978) which he associated with peak activity in autumn of *B. microplus*, the carry over of cases to the winter being due to the longish incubation period. Rainfall distribution in Ijui is even with no month receiving less than 5% of the total rainfall and no three month period less than 20%.
Illustrating the one-host life cycle of ticks of the genus *Boophilus*

1. Larvae hatch from egg and seven to ten days later climb the vegetation and seek a host.

2. Larvae attach and engorge within three to five days. After a two day moulting period nymphs emerge and take a blood meal lasting five to six days.

3. The engorged nymphs pass into a two day moult from which adult males and females emerge. Fertilization takes place. The female then completes a large blood meal.

4. The engorged female drops to the ground. 2,000 eggs are laid in a humid niche on the ground.

A. The engorged female

B. The male

Source: Cattle tick control, Cooper McDougall & Robertson Ltd., Berkhamstead, England.
Temperature is, therefore, likely to be the controlling factor on tick development and as only in the winter months of June to August is the mean temperature below the 16.7°C indicated as necessary for hatching by Hitchcock (1955) the cycle of tick population is likely to be similar to that of Southern Queensland. Interpretation of the data for seasonal incidence is complicated not only by the unrepresentative sample but also by the fact that 1978, to which the data apply, was climatically unusual. (Table 1).

The autumn months were unusually dry with April receiving less than a tenth of normal precipitation. This may have adversely affected hatching and larval survival. July proved to be not only abnormally warm but had twice the normal rainfall. Such a combination of circumstances could have favoured tick development in July which may be related to the turn around in the fall-off of cases that was shown in July resulting in a further peak in August, carrying through to September before falling to zero in October.

An alternative and plausible explanation of the results is that clinical cases in autumn and early winter (April - June) were a reflection on peak tick activity in autumn and provided a pool of active infection which was spread by mass vaccination against foot and mouth disease (carried out in June and July in the region) resulting in the August peak incidence.

The serological results (Table 3) show that the organism is widespread in the region. Taking suspicious and positive together some 80% of the total population showed signs of infection. A proportion of the reactors in the 0-12 month old group, some 45% of the population in this age group, will be due to maternal colostral antibody. The
42% negative in the yearling group indicates a disturbingly high proportion of susceptible cattle in the age group and although this falls to a more stable 10% in the older grouping this could infer considerable losses. Marked variation in the percentage of susceptible animals occurs from farm to farm. For example on farm PM-3 (Table 3) a 50 animal sample showed thirty-six negative and fourteen positive or suspicious. Of 20 calves tested on PM-3 17 (85%) were negative and 3 (15%) positive or suspicious. On another dairy farm, IJ-6, an identical number of calves were sampled but only 4 (20%) were negative and 16 (80%) positive or suspicious. This difference continued to be evident in older stock. On PM-3 57% of 21 animals over 2 years old were still negative to the C.F.T. On IJ-6 none of 46 similarly aged animals were negative. The possibility of disease on PM-3, given an efficient means of transmission is obvious as are the consequences of the owner of IJ-6 purchasing adult stock from PM-3, although he is unlikely to experience problems among home-bred stock.

The importation of high-quality dairy stock from tick-free areas in Argentina and Uruguay is common practice, frequently organised and financed by the co-operatives. The necessity for premunition is appreciated but this is often carried out by lay staff with little knowledge of the pitfalls which lie in stall. Results of premunition are often judged by temperature rises though official premunition stations and some others may follow the course of infection with blood slides. The procedure is sometimes carried out on the farm of destination. Losses are frequent. (Brasil, et al., 1970). The results of this survey reinforce the necessity for the utmost care required when contemplating importation of this nature.
Examination of the results may shed some light on the factors influencing the variations in results obtained from property to property. Questionnaire replies revealed that tick infestations were everywhere light to moderate. No attempt was made to count ticks on the properties though subjective opinions were formed while taking the blood samples which gave opportunity for a cursory examination of the perineal region, a predelection site for B. microplus. It is possible to state that adult ticks were frequently seen on cattle on IJ-6 while none were encountered on cattle of farm PM-3. This may at least indicate that tick counts performed on farms with widely different serological results may repay investigation of the means of transmission in this region. Tick control was most frequently by hand applied acaricide although six properties had dipping baths. There was no rational approach to tick control. Only two farms claimed any sort of programme and in these cases the evidence was that 20 day dipping in the tick season was an ideal to be aimed at rather than rigorously adhered to. Although bat rabies is recorded with some frequency in the survey area only two owners gave any indication that vampires were known to exist on their properties.

There must be some potential for these creatures to be involved in anaplasmosis transmission through interrupted feeds even though the questionnaire results do not lend support to such hypothesis.

It is not proposed to subject the results to statistical examination. This, it should be emphasised, was a positive decision taken in view of the low number of farms sampled and the non random selection of farms for testing. Comparisons between similar types of farming enterprise where the age structures of the populations are similar, such as that already discussed above, are considered valid but the divisions
into farm groups presented in the tables give results which are biased not only by the effect of different age structures but by inter-relationships between the factors chosen. Thus 10 out of 14 farms where permanent grassland accounted for 50% or more of grazing were also included in the group of farms with less than one animal unit per hectare, and this type of farm tended to predominate among the larger properties. Mean herd percentages (MHP) are taken for comparative purposes because, although there is little difference between MHP and percentages of total number of animals sampled for the whole group of 26 properties (Table 3) distortions could be expected in the smaller groupings (Tables 5 - 9) because of large variations in sample numbers between properties of different size.

Nevertheless, and bearing in mind these limitations the tables (5-9) are presented in an effort to seek managemental factors which could be responsible for differences in infection levels between properties. The higher figure for dairy herds (Table 9) over beef herds might be explainable by the more frequent gathering of dairy stock facilitating transmission.

Contrary to expectations, the results suggest that transmission is higher where fewer vaccinations are given (Table 8). A possible explanation would be that farmers not infrequently combine prophylactic administrations with acaricide applications as dipping tanks and spray races are usually sited at the end of holding crushes. Similarly, the larger herds where transmission was lower than on small herds (Table 5) are on properties where dips and spray races are likely to be found.

The effect exerted by grassland management is of interest (Table 7), in that it strongly supports the contention that ticks are the
prime vectors of anaplasmosis in the region.

The intensive use of grassland involving temporary leys of fast growing species for milk and beef production on farms with an arable crop structure, and the use of soya bean and wheat aftermaths supplemented with home grown maize and silage, offers a natural control of tick populations as cattle are rotationally grazed on pastures effectively tick-free. Ticks carried to these pastures by cattle coming from tick infested permanent pasture will contaminate the ground but numbers may not build up to levels required for efficient tick-borne disease transmission. This could explain the high percentage of susceptible stock on farms where permanent pasture is limited.

It is interesting to note that farms in the Table 3 with GI or PM codes tend to the highest percentages of uninfected animals. Though widely separated geographically these two groupings are in the areas of the survey where crop growing is most intensively practised. Further evidence of tick involvement in anaplasmosis transmission may be drawn from the known susceptibility of cattle from the islands of the Jacui in Rio Grande do Sul where they are kept virtually tick-free by the flooded conditions existing there, while biting flies, particularly mosquitoes are abundant.

It is possible to conclude from the mean prevalence levels disclosed by the survey that the cattle population itself forms the main reservoir of infection. Wild deer are a rarity in this region and there are only a handful of buffalo in the state. The role of B. microplus as a reservoir of infection depends upon whether or not transovarial transmission occurs in nature.
If it does not, this tick's role as a reservoir of infection is likely to be negligible. If it does, infection may be maintained in the absence of cattle for as long as the larvae are able to survive on the ground. Such information is not yet available for this region but from studies elsewhere (Hooker et al., 1912) this period is unlikely to extend much beyond four months. Though B. microplus may use alternative hosts such as sheep, horses and dogs, from the available evidence (Laranja et al., 1975) the organism does not survive such feeding cycles.

Although a proper tick survey has not been carried out in Rio Grande do Sul, the information provided by a review of the local literature (personal communication from Dr. D.E. Evans, Institute de Pesquisas Veterinarias Desiderio Finamor, Porto Alegre) does not hold out much promise of an alternative tick vector for anaplasmosis in the state.

21 species of hard ticks have been recorded in the state - 16 different Amblyomma species, 2 species of Haemaphysalis and one each of Rhipicephalus, Ixodes and Boophilus. The three soft tick species are made up of one Argas and two Ornithodorus. However, only B. microplus has been recorded from cattle in the state although eight of the species recorded have been found on artiodactyls outside of Rio Grande do Sul, namely A. cajennense, A. maculatum, A. ovale, A. tegrinum, A. triste, H. juxtakochi, H. leporis-palustris and R. sanguineus.

In the absence of concrete evidence to the contrary, vector control is thus best directed at B. microplus. Seasonal patterns do not fit in well with biting fly transmission and needle transmission is probably of only secondary importance as there was no obvious
relationship discernable between seasonal incidence and mass vaccination campaigns in spring and summer (October and February - Table 2). On some properties, with a low incidence of reactors and an already low tick population held in check by rotational grazing practices, it is tempting to consider eradication as the farms are effectively isolated by cropped lands and the risks entailed in maintaining a susceptible population in an enzootic area are effectively those already faced on these properties. Eradication should be feasible and would involve rigorous tick control and treatment of serologically positive cattle. The absence of acaricide resistant problems would help to ensure success. The need for proper sanitary measures to eliminate mechanical transmission would have to be well understood by owners. However, the desirability of having a uniform approach to anaplasmosis control throughout the region mitigates against implementing such a plan which would apply constraints to the purchase and sale of animals within the region. Also veterinary diagnostic services are not yet capable of instigating widespread serological testing because of antigen supply difficulties.

Control must, therefore, be based on raising the level of immunity in the cattle population generally by immuno-prophylactic means.

Card test results show a good agreement between the two tests at 80% (Table 10). As more than half of the disagreeing results were in the CFT suspect class it is possible that greater experience in the reading and performance of tests could improve on the degree of agreement. The ease of use of the CT would make it the test of choice given adequate antigen supplies.
The mean herd prevalence of 76.4% is broadly similar to those found in Colombia (Corrier et al., 1978) and Botswana (Mehlitz & Ehret, 1974) though higher than those found in Peru (Castillo et al., 1968), East Africa (Kuttler, 1965) and Queensland (Rogers, 1971).
On the basis of clinical cases confirmed by examination of blood slides at the Ijui laboratory it is probable that *A. marginale* is the causative agent of a significant proportion of cases of *tristeza* encountered in the region.

More cases occurred in the autumn and winter than spring and summer.

The serological results indicated a situation of enzootic instability and reveal marked differences in prevalence from farm to farm, emphasizing the dangers that cattle movement into and within the region can involve.

Questionnaire returns suggest that variations in herd prevalence may be related to managemental practices influencing tick populations.

It is recommended that efforts be increased to obtain a laboratory confirmation on a greater proportion of clinical cases over a prolonged period to obtain a move reliable estimate of the prevalence and seasonal incidence of anaplasmosis to aid in decision making on the type and scale of any prophylactic measures envisaged.

There is a need for the wider use of serological tests. Universal testing would be beyond the capacity of present veterinary services. However, testing at cattle sales would be valuable as would serological confirmation of the effect of premunization of imported stock. An extension to the testing of cattle in the nine to twelve months old age group would be useful on deciding on the value of vaccination on individual properties. The modified card test is the most convenient to use with the CFT as an alternative.
The inconveniences of foreign purchase and importation of antigen call for the local production of an antigen for these tests. Though requiring some capital expenditure on equipment, this should be feasible at the central veterinary laboratory at Guaiba.

Eradication by chemotherapy and vector control is unlikely to be a practical proposition in the face of the infectivity levels demonstrated serologically. It is therefore desirable to raise the level of immunity in the population by prophylactic means.

The continued use in some areas of the state of crude, whole blood vaccines containing virulent A. marginale should be reconsidered in view of the dangers of disease transmission, the possibility of causing isohaemolytic disease in calves and on the grounds of the losses incurred by the use of such vaccines. Instead, the production of an A. centrale vaccine in splenectomized calves should be investigated. Present facilities at Guaiba could produce sufficient vaccine for the whole state. To facilitate distribution, frozen vaccine stores could be held at the two regional laboratories. A parallel investigation into babesiosis in the state would be logical and worthwhile as many of the same principles apply and vaccine technology is similar. B. bovis is an unknown quantity but could merit investigation and the inclusion of capillary blood smears in the material submitted to the laboratory from possible clinical cases should be encouraged.

There is a need for controlled exposure experiments under field conditions to resolve the question of transmission. Groups of serologically negative calves would be exposed to either ticks only or biting flies only. Such experiments would be costly to mount and would require external aid and supervision, but would be essential
if vector control is to be envisaged, as well as contributing to the wider knowledge of the subject.

Sequential testing on selected properties would also be valuable, particularly in the evaluation of needle transmission.
ACKNOWLEDGEMENTS

The field work was carried out while in the employment of the Ministry of Overseas Development, London, on attachment to the government of Brazil. My thanks go to the many field veterinarians, farmers and their staff who co-operated in the collection of the blood samples, to the staff of the Ijui regional laboratory, particularly Dr. Luiz Fallavena who, in undertaking an increased work load, allowed me to devote the necessary time to this study. I am grateful to the Director of the Instituto de Pesquisas Veterinárias Desiderio Finamor for permission to publish the results. The serological work would not have been possible without the generous co-operation of the United States Department of Agriculture who kindly donated and shipped to Brazil all the serological reagents.

I would thank particularly Mr. C.G.D. Brown for much helpful advice in the preparation of this dissertation at the Centre of Tropical Veterinary Medicine, University of Edinburgh, where I was in receipt of an in-service training scheme award from the Ministry of Overseas Development. Finally, I would like to record my gratitude to my wife, Virginia, for constant support and encouragement throughout the undertaking and preparation of this study.
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    anaplasmosis. Proceedings of the 91st annual meeting of the


12. **APPENDIX**

A. Questionnaire used in the survey (translated).

<table>
<thead>
<tr>
<th>Farm Reference No.</th>
<th>Date of Blood Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owner</td>
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</tr>
<tr>
<td>Property</td>
<td></td>
</tr>
<tr>
<td>Municipality</td>
<td></td>
</tr>
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</table>

**Stocking Rate**

<table>
<thead>
<tr>
<th>No. of cattle</th>
<th>0 - 12 mths</th>
<th>13 - 24 mths</th>
<th>over 24 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Area available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanant pasture</td>
</tr>
<tr>
<td>Temporary leys</td>
</tr>
</tbody>
</table>

**Vaccinations**

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<th>Age group</th>
<th>frequency</th>
<th>date of last</th>
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<tbody>
<tr>
<td>Foot and Mouth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthrax</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Blackquarter</td>
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<td></td>
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<tr>
<td>Salmonellosis</td>
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</tr>
<tr>
<td>Brucellosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ticks**

Are there ticks on the cattle?

control method: Dip/spray/handspray
frequency of application

**Vampire Bats**

Are vampire bats present on the property?

**General Remarks** - disease situation etc.
B. Complete CFT result for two properties

Herd reference PM-O3

Owner - Hugo Gielow

Date of sampling - 15/2/79

Date of CFT - 15/2/79

No. in herd = 125, No. tested = 50 (40%)

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Breed</th>
<th>Age</th>
<th>Origin</th>
<th>Reading</th>
<th>Result</th>
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</thead>
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<td>4 mths</td>
<td>Home Bred</td>
<td>0</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>5 mths</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>3 mths</td>
<td>&quot;</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>5 mths</td>
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<tr>
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<td>positive</td>
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<td>suspect</td>
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<tr>
<td>16</td>
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</tr>
<tr>
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<td>&quot;</td>
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<td>&quot;</td>
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<td>&quot;</td>
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<tr>
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</tr>
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<tr>
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<td>4</td>
<td>positive</td>
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<td>0</td>
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<tr>
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<td>Imported Uruguay</td>
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</tr>
<tr>
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Owner - IMERAB

Date of sampling 6/12/78

Date of CFT 31/1/79

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