STUDIES ON BOVINE ANAPLASMOSIS

THE COMPARATIVE PATHOGENICITY AND IMMUNOLOGICAL RELATIONSHIP OF ANAPLASMA CENTRALE TO ANAPLASMA MARGINALE

KENNETH L. KUTTLER, DVM, MS.

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FACULTY OF VETERINARY SCIENCE

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Kenneth L. Kuttler

Introduction:

Anaplasmosis is an acute or sub-acute, infectious, non-contagious disease of cattle, characterized by anemia, high fever, and icterus. It is caused by the micro-organism, *Anaplasma marginale*, which invades the red blood cell and produces an acute or sub-acute hemolytic anemia. A closely related, sometimes clinically indistinguishable, disease of cattle is produced by the micro-organism *Anaplasma centrale*. This organism, generally considered a sub-specie or varient of *A. marginale*, usually produces a markedly milder clinical course. *A. centrale* is often thought of as an attenuated anaplasm, and the agent of choice in premunizing susceptible cattle against the more virulent *A. marginale* infection.

Anaplasmosis at one time was thought confined to tropical and sub-tropical areas, but now is recognized in most parts of the world where cattle are raised and vectors are present. Veterinarians in East Africa have recognized a high incidence of anaplasmosis among local cattle. A large percentage of local cattle become premunized, by natural exposure, at an early age. Examination of clinic records at the Veterinary College, Kabete, reveal a large number of clinical cases among adult animals which, except for treatment, would result in livestock losses.

The existence of clinical cases of anaplasmosis among adult cattle suggests the presence of a reasonably large number of non-infected cattle in East Africa that reach the age of increased susceptibility without premunizing exposure. This raises the question of possible control measures which may some day be required to contain anaplasma infections, and reduce or eliminate losses from this disease.

The principle objectives of this study are:

1. To survey the incidence of anaplasmosis by means of the complement-fixation test.
2. To measure and compare the relative pathogenicity and pathogenesis of A. marginale and A. centrale.
3. To measure the degree of immunity produced in cattle by A. marginale and A. centrale, to homologous and heterologous challenge.
4. To determine the serological relationship of A. marginale and A. centrale, by means of the complement-fixation test.

Review of the Literature

In their classical publication on Babesia bigemina, Smith and Kilborne (133, 134) in 1893, described a small deep staining, coccus-like body found on the margin of red cells. They considered this parasite as a phase in the life cycle of B. bigemina, but suggested that it might possibly be a separate parasite. Theiler (141, 142), in 1910, recognized these marginal points as the causative agent of an entirely separate disease entity, which he entitled "Anaplasmosis", or, as more commonly referred to in South Africa, "Gall Sickness". He (143) explained his finding as follows "...from the fact that its biology and some of its staining properties considerably resemble that of a protozoon, I concluded that it belonged to this group of parasites (Piroplasms), being a protozoon consisting in the main of a nucleus and devoid of protoplasma. Hence the name 'Anaplasma', which should indicate this condition". Theiler, in his original description, gave what remains even today, a thorough and valid report of the clinical manifestations, the carrier state following recovery, and the transmission of this condition. He classed the organism Anaplasma marginale as a protozoon parasite on the basis of its morphology, staining characteristics, and his inability to pass the causative agent through bacterial filters. Seiber (132), in 1911, published his description of blood changes produced by A. marginale along with drawings of the parasite. Theiler quite accurately recognized ticks as the principle vector in this infectious, but

non-contagious, disease of cattle. Shortly after his description of A. marginale, Theiler recognized a second disease entity caused by a centrally located coccus-like body, closely resembling A. marginale, except for its location in the red cell. Theiler gave the name Anaplasma marginale (variety centrale) to this organism, and recognized it as separate from A. marginale. Theiler states (143) that double infections of A. centrale and A. marginale are commonly seen on the veld. In pure infections, A. centrale is responsible for a disease much milder in character than that produced by A. marginale. Recovery from A. centrale was described as resulting in immunity against future A. centrale infections, but not against A. marginale. Theiler (143) did think, however, that a previous A. centrale infection reduced the severity of a subsequent exposure and attack of A. marginale.

It required a number of years for Veterinarians and Research workers to accept the idea that the marginale bodies described by Theiler were in reality the causal agent of a specific disease and not a pireplasm associated with pireplasmosis (Texas tick fever) or merely an artifact or atypical Howell-Jolly body.(45) The matter was not fully cleared up until the late 1920's and early 1930's when Stiles (137, 138), Boynton (10), and Dikmans (28, 29) reported their work which confirmed Theilers earlier experiments, asserting that the marginal points he saw were a specific pathogen. Penha (88), in 1930, reported that A. marginale and Howell-Jolly bodies had different staining qualities. Following these studies most investigators were inclined to accept Theiler's classification of

A. marginale as a protozoon. However questions were subsequently raised as to whether the anaplasma bodies observed were themselves a protozoon, or whether they were an inclusion body produced by another organism.

Dikmans (29), in 1933, set up a series of filtration experiments in which infected blood was lysed, passed through preliminary coarse filters to remove red cell stroma, then through chamberland filters of known porosity to determine if a filterable infectious agent could be recovered. His experiments failed to show the presence of a filterable agent, although there is some question concerning the methods he used to lyse the red cells, with the suggestion that infectivity had been eliminated before filtration. A check for infectivity of the unfiltered material following coarse filtration was not made. In recent years Ristic (107) succeeded in passing a suspension of infected red cells, which had been hemolysed by distilled water and sonication, through a filter having a porosity size of 650 mu. The filtrate was collected in normal, washed red blood cells. The infectious agent was recovered in this filtrate, and demonstrated by animal inoculation. Allbritton (2) has more recently succeeded in passing lysed cells through even smaller filters. He demonstrated the passage of the infectious agent through a 300 mu filter, but not through a 220 mu filter.

Dikman (28) made a careful and detailed study of the anaplasma body, using Giemsa stained blood smears. In addition

to the characteristic round, deep staining, homogeneous anaplasma body, he observed rods, spherical forms with a filamentous tail, and other bodies with irregular shape. Lotze and Yiengst (71) confirmed Dikmans description, and noted some extracellular bodies which were described as being 1 u. rods with a terminal knob-like structure measuring .2-.3 u. in diameter. These investigators also described a large anaplasma body made up of 8 small sub-units that they termed "sporoid bodies". Franklin (36) observed, with giemsa stain, a projection or tail as part of the anaplasma body, extending beyond the erythrocytic membrane.

The most common technique used to observe anaplasma bodies remains the Romonowsky stains on blood smears, observed with a light microscope. In addition to stained smears, however, a number of other techniques have evolved in recent years, which have been valuable in studies of anaplasma morphology. Ristic (113) in 1957 described the use of serum antibody fluorescent conjugates for staining anaplasma bodies. Fluorescein is attached to the globulin fraction of an anaplasma immune or hyper-immune serum. This material, when placed in contact with an alcohol fixed blood smear containing anaplasma bodies, will selectively fluoresce the anaplasma bodies when viewed under a microscope containing the fluorescent attachment and proper filters. This technique has been used to describe the morphology of anaplasma. Ristic (112, 113) failed to observe organisms other than the coccus-like bodies, and

their sub-units. Madden (73), by the use of fluorescent antibody techniques, observed a variety of appendages attached to the anaplasma body. The use of phase microscopy has added another method for observing the anaplasma body. Phase microscopy, related in principle to the dark field microscope, depends on a phase condensor to alter the light rays. The anaplasma bodies can be seen in some detail in the hemolysed, non-stained blood preparation. This method was used by Pilcher (89), when he described the varied morphological forms of anaplasma. Espana and Espana (32), using phase microscopy, have observed projections attached to the anaplasma bodies. A new methylene blue stain has been developed in recent years, which Schalm et al (124) claims will stain the anaplasma appendages more clearly than Romanowsky's stains. The light microscope is adequate when this stain is used. Gainer (37), in 1961, described an acridine orange stain for anaplasma identification. This method employs the fluorescent microscope, through which the anaplasma bodies are seen to show fluorescence with the acridine orange stain. This method has the dissadvantage that Howell-Jolly bodies also show fluorescence with this stain, and for the inexperienced technician, differentiation becomes a problem.

With the advent of electron microscopy even greater interest has developed in the detailed morphology of the anaplasma body. The first pictures of anaplasma bodies, with the electron microscope, were reported by DeRobertis and Epstein (27). Their observations confirm the description that Lotze and others had made relative

to the small elementary bodies present within the anaplasma body. DeRobertis and Epstein described these elementary bodies, singly in the red cell, and suggested that they may play a role in the life cycle of the anaplasma. Their studies did not reveal the presence of tails or projections attached to the anaplasm. Ristic (106), expressing the idea that a careful study of the anaplasma body would aid in the proper classification of this organism, has pursued with some detail the technique. Using electron microscopy, Ristic (106, 110) has failed to demonstrate projections or tails in association with the anaplasm. He has shown the elementary body described previously, calling them initial bodies. Ristic (111) suggests that these initial bodies are the primary infective units which invade the cell. Once established in the cell they divide by binary fission to form the body visible on light microscopy. In this work he suggests that the causative agent of anaplasmosis is incorrectly classified as a protozoal agent. Foote et al (34), in 1958, examining ultra thin sections of anaplasma infected erythrocytes with the electron microscope, also failed to see the projections previously described and concluded the body being observed was a virus. Scott et al, in collaboration with Foote (126), as the result of electron microscopy, re-examined the earlier virus classification by Foote, and stated that it was neither a protozoon nor a virus, but should rightly be classified as a rickettsial agent. Espana and Espana (33), and later Gates and Ritchie (41), using similar techniques with electron microscopy, have seen tails projections,

crescents etc, supporting the protozoal classification of anaplasma.

Other areas of investigation have been pursued in an attempt to characterize the etiological agent of anaplasmosis. Moulton and Christensen (80) applied histochemical techniques to the anaplasma body in an effort to determine the presence of nuclear material which would assist in the proper classification of the organism. The anaplasma bodies showed positive reactions for deoxyribonucleic acid, ribonucleic acid, protein and organic iron. While this may support the classification of anaplasma as a protozoal agent, the authors state that it does not rule out the possibility of a viral or rickettsial classification. Pilcher et al (89), in a study of the respiratory rate of infected and non-infected blood, showed an increase in the respiratory rate of infective blood, but not of the same order as that occurring with malaria or other protozoon parasites. On the basis of this, Pilcher concluded that anaplasma would be more correctly classified as a rickettsia.

The rather contradictory findings of various workers in morphology description, and other characteristics, led Krier and Ristic (56, 57) to the conclusion that possibly there has been a mixed infection in some instances, or that a second organism is being observed and confused with the primary cause of anaplasmosis. By using a strain which shows tails, and one that does not, fluorescent antibody conjugates were prepared for differential staining. A specific selectivity was noted supporting the theory that two organisms are involved.

Krier (58) has described a difference in the pathogenicity of these organisms. Passage of the tailed variety through deer resulted in the survival of only the typical coccus-like anaplasma organism, indicating the presence of a mixed infection in the original tailed strain. The complement fixations serological relationship has not been described for these two organisms, and until further work is done along these lines, attempts to determine which organism is the true cause of anaplasmosis, if in fact two do exist, and where it should be classified, will be most difficult.

The anaplasma organism has been shown less sensitive to external environment (9) than at one time thought. Methods of preservation have been studied, and freezing with storage at 79°C. has been found satisfactory (7, 8). The survival and multiplication of A. marginale in artificial cultures or in laboratory animals has been very unsuccessful to date. Dikman (28), and later Rossi and Trison (119), reported experiments where multiplication of the anaplasma organism was thought to take place in a suspension of bovine red cells to which glucose was added. The author knows from personal experience of numerous unsuccessful attempts to grow anaplasma marginale on tissue culture, and in laboratory animals, not reported in the literature. Even so, anaplasma infections are not limited entirely to the bovine. Growth of A. marginale and A. centrale are known to occur in African antelope (81, 83). Neitz (81-83) has demonstrated a technique of recovering anaplasms in pure form, from the blood of animals

having B. bigemina and T. mutans, as well as A. marginale. A passage made through a blesbuck, or black wildebeest, resulted only in the survival of A. marginale, as determined by sub-inoculation into cattle. Many workers (12, 20, 21, 85, 114) have recovered anaplasma organisms from American deer. Osebold et al (85) demonstrated tick transmission of anaplasmosis from deer to susceptible cattle, suggesting that deer in his region may play an important part in the spread of anaplasmosis, further complicating the control aspect of the disease. Anaplasma-like bodies have appeared in buffalo blood (15) and apparently have been responsible for inducing mild clinical illness (18, 65). Cases of actual transmission to cattle were not found in the literature, leaving some question as to the relationship of these buffalo anaplasms to A. marginale. An anaplasm found in sheep is quite distinct (Anaplasma ovis) from anaplasmosis in cattle (136), and has been recognized and studied for many years (26). The anaplasms in buffalo, therefore, may be quite different from the anaplasms in cattle. Brien (14) has described a disease in horses associated with anaplasms, (anaplasma equi), but no immunological, or serological relationship has been established with A. marginale.

DeKock and Quinlan (24, 25, 26) in their studies of Anaplasma ovis described the increased susceptibility of sheep to A. ovis as the result of splenectomy. These workers, plus others (66, 98, 99), applied this technique to cattle and calves with similar results regarding their susceptibility to A. marginale. Schmidt (125) records mild cases of anaplasmosis among young animals, with a tendency for infections to become more severe as the animal becomes

older. Artificially induced cases of anaplasmosis were also observed to be more severe during the hot season (125). Roby et al. (116) have studied the severity of A. marginale in different age groups in relation to splenectomy. They found adult splenectomized cattle the most susceptible to anaplasmosis infection. Adult cattle and splenectomized calves had approximately the same degree of susceptibility, with non-splenectomized calves showing the greatest resistance of infection.

Probably the most striking pathogenic change of anaplasmosis as described by the early workers was the acute, often fatal, anemia. (111, 125, 137, 141, 142). Rees (105), made a search for a possible focci of infection by examining blood from numerous organs of animals sacrificed during the course of anaplasmosis. His conclusions were that blood from all organs was equally infective, and that an increase in infective potential was not appreciably altered from one location to another. This confirmed the original assertion that anaplasmosis is essentially a blood disease. Schalm (123) states that this anemia is primarily hemolytic in type, and that a drop of 50 to 80% in the packed cell volume (PCV), over a few days, is not uncommon. PCV may go as low as 7% before death, but a PCV of 10-11%, accompanied by signs of remission such as polychromatophilia, macrocytosis, and basophili stippling, need not be fatal. The occurrance of reticulocytes in the circulating blood at an early stage of the anemia is usually considered an indication of eventual recovery (72, 123). Hansard and Foote (46, 47, 48) have indicated in their publications that the anemia associated with anaplasmosis is due, in large measure, to the suppression of the hemapoietic system. This assumption is

based on iron utilization and tracer studies using the radio active isotope Fe59. In addition to a study of erythrocyte uptake of Fe-59 and plasma clearance, animals were sacrificed at various periods to locate concentrations of Fe-59. These authors showed a decrease of iron concentrations in organs of erythropoiesis before the peak of parasitemia. The low iron in these organs, and a low iron in total blood only 5 days before the peak parasitemia, was considered as evidence of a decreased iron utilization and an initial suppression of the hematopoietic system, as a result of anaplasmosis infection. Baker et al. (6), using radio active cromium, (Cr-51) used a different approach in their study of the anemia of anaplasmosis. Their object was to measure erythrocyte survival time. It was found that during the hemolytic crisis erythrocyte survival was reduced 8 to 10 fold. On the basis of this finding and hypothetical calculations, they concluded that the anemia is primarily, if not entirely, due to hemolysis. More recently Krier (59) et al., in a study of the pathogenesis of anaplasma anemia, conducted a series of myelograms throughout the course of anaplasmosis. These authors demonstrated conclusively that the erythroid elements of bone marrow do not decrease in calves infected with anaplasmosis, either during the incubation period or during the period of rapid increase in parasitized cells. Erythroid elements became much more prominent during severe anemia. Ristic (108) has demonstrated the presence of an auto-antibody which, he suggests, is a factor in the anemia produced in anaplasmosis. He found a relationship between the maximum anemia and the maximum concentration of the auto-antibody.

On the basis of this evidence he theorized that infected erythrocytes become sensitized with the auto-antibody and then are removed from the circulation and broken down in the cells of the reticulo-endothelial system. There was no evidence that this auto-antibody was responsible for intravascular hemolysis. Allbritton (3) confirms these findings by reporting an accelerated removal and destruction of erythrocytes by macrophages of the reticulo-endothelial system.

In addition to the cellular changes occurring in the blood already mentioned, some have described alterations of the leucocyte count and composition. These reports are not always consistent. Sergent et al. (131) observed a leucocytosis, but no change in the differential count. Liwotow (67) also reported a leucocytosis, but indicated a severe eosinophenia appeared at the beginning of infection, persisting for some time following recovery. Arroyo (5) states that a lymphocytosis was seen during the course of the infection, along with a constant shift to the left in the granulocytic series. He was unable to draw any conclusions regarding the actual variation of leucocyte counts because of the great variations among animals and samples. Rees (103) agreed with others that an increase in leucocyte count occurred, but described a mild lymphopenia, marked monocytosis, marked eosinophenia, and a shift to the left with the neutrophils.

Henning (50), in his description of the clinical manifestations of anaplasmosis, other than anemia, describes an early rise in temperature, anorhexia, drop in milk production in lactating animals, suppression of rumination, variable degrees of impaction,

dry muzzle, dullness, depression, and varying degrees of icterus. Even though a severe hemolytic anemia occurs, hemoglobinuria does not occur. Lotse (69), in a study of over 50 cases of anaplasmosis, found a great variation in incubation time following exposure to anaplasmosis, but a reasonaly constant patent period. He also described the occurrence of relapses following the initial response to infection, but found that these are usually less severe than the initial attack.

The diagnosis of anaplasmosis on the basis of symptoms is very difficult because of the large number of other diseases with similar clinical manifestations (50). It is also recognized that blood examination is often ineffective because of the early disappearance of anaplasma bodies in clinical anaplasmosis (50). The persistence of asymptomatic anaplasma carriers was described by Theiler (141, 142), but identification of these animals without a known history, or without animal inoculation was almost impossible. Boynton (13), in 1935, described a serological test for the diagnosis of anaplasmosis. This test depended upon the precipitation in distilled water of euglobulins present in serum of anaplasmosis infected animals. This was admittedly a crude and non-specific test, but, nevertheless, was reported to aid in the diagnosis of most cases. Rees and Mohler (104) were the first to describe the preparation of specific complement fixation (CF) antigens. Two successful antigens were described. One was prepared from laked blood, which was centrifuged and washed, while the other was prepared from extracts of ticks previously fed on anaplasmosis-infected

animals. Consistency in the production of CF antigens was not achieved until some time later when Mott and Gates (79), in 1949, described the production of a CF antigen from the blood of animals acutely infected with anaplasmosis. This technique involved the collection of blood with a high parasitemia, washing the cells in physiological saline, and lysing them in 30 volumes of cold distilled water saturated with CO_2. This produced a precipitate which was collected, washed by centrifugation, suspended in saline and neutralised with sodium bicarbonate. In 1952, Price et al (92) prepared a CF antigen which, in some respects, represented an improvement. Their antigen was prepared from cells highly parasitised. The red cells were washed in saline and lysed in distilled water. The lysed cells were then passed through a refrigerated Sharples centrifuge developing 40,000 X forces of gravity. The sediment thrown down was collected, washed and re-suspended in saline. Present antigens produced by the U.S.D.A. now employ a combination of CO_2 and distilled water antigens. Separate evaluation of these antigens by Gates et al (39) has shown each to be effective. A standardized CF antigen has recently been mass produced and is available to laboratories doing research and conducting control programs. (35).

The CF test has been thoroughly evaluated under many conditions and by many investigators, (40, 42, 43, 44, 77, 78, 91, 93). The general consensus is that this test has an accuracy of about 95-96%. Pearson et al (87) and Merriman et al (74) have demonstrated the value of the CF test in field control programs. In association

with the complement fixation test, Gates et al (38) have shown a direct correlation of CF titers to blood infectivity. Kuttler et al (63) demonstrated CF antibodies in no-infected calves whose dams were carriers of anaplasmosis. This reaction was due to colostral antibodies. By continuous flow paper electrophoresis, Rogers and Dimopoulles (117) demonstrated a relationship between the concentration of globulin components and CF antibody titer. Miller (76) was able to produce specific antibodies against complement fixation antigen in rabbits, supporting the claim of specificity of this antigen as a tool in the diagnosis of anaplasmosis.

Ristic (109), in 1963, reported the development of a new antigen for use in a capillary tube agglutination test (CA). This test is reported to be highly effective in detecting anaplasmosis carriers. The antigen is prepared from heavily parasitized red cells. These red cells are lysed to free the anaplasma bodies by sonic energy, then separated from the stroma by differential centrifugation. Welters and Zuschek (152) described this test as being equal to, or possibly more sensitive and specific, than the CF test, with the advantage of being less expensive and less complicated. Kuttler (61) conducted a series of comparisons between the CF and CA tests, concluding that, when properly conducted, the CA test is equally as reliable as the CF test.

The subject of transmission of anaplasmosis, after over 50 years of investigation, is still one not thoroughly understood. Theiler (141,142)

described tick transmission as the principle source of spread. Since that time many species of ticks and biting insects have been incriminated as possible vectors. Stiles (139), in 1936, indicated that as little as 0.025 ml of blood from an acute case of anaplasmosis could induce the disease. Gates et al (38) showed that as little as 1 ml of a 1/100,000 dilution of blood from an acute case was capable of transmitting the infection. As early as 1932, Sanborn et al (121) were successful in transmitting anaplasmosis by horse flies. Five species of flies, Tabanus gracilis, T. sulcifrons, T. venustus, T. fusciostatus, and Silvius pellinesa, were allowed to feed on an infected animal, then immediately placed on a susceptible host. Transmission of anaplasmosis occurred. At least one, and probably more than one of these flies were then assumed capable of transmitting anaplasmosis. Letse (68) failed to transmit the infection with horse flies when they were first fed on a carrier animal in the quiescent stage of the disease, with no anaplasma bodies being evident. His efforts to transmit anaplasmosis were successful when the flies were first fed on an animal undergoing a mild sub-clinical relapse, in which anaplasma bodies were apparent. Howell (53) reports two cases of positive transmission of anaplasmosis by Psorophora mosquitoes. He states, however, that conditions would have to be just right for natural transmission to occur by this method, and that these conditions are not likely to occur. Roby (115) stated that in some areas blood sucking insects such as horse flies and mosquitoes are more important vectors than are ticks. Hoffman (51) provided considerable

circumstantial evidence to support the theory of insect transmission, during a recent experiment in which he demonstrated that the spread of anaplasmosis can be reduced through intensive insect control. Dikman (30), in discussing the role of biting insects as a means of transmission, stated that it is probably necessary for the insects to move almost immediately from an acutely infected animal, or at least an animal showing anaplasma bodies, to a susceptible animal. The anaplasma organism does not remain viable for any great length of time, and under hot dry conditions the interval is probably a matter of seconds. Anaplasma is not thought to maintain itself in flies such as it does in ticks. The following is a list of flies shown to experimentally transmit anaplasmosis:

Tabanus sulcifrons	(Letse, 70)
Stomoxys calcitrans	(Sanders, 122)
Tabanus fumipennis	(Sanders, 122)
T. americanus	(Howell, 52,54)
T. oklahomensis	(" " ")
T. abactor	(" " ")
T. equalis	(" " ")
T. erythraeus	(" " ")
T. venustus	(" " ")
Aedes Aegypt	(" " ")

Transmission by ticks may occur from stage to stage, trans-ovarian, or from adult to adult in those ticks that go from one animal to another during this stage of their life cycle.(30). The following is a list of ticks capable of transmitting anaplasmosis, and the stage in their life cycle when this transmission occurs:

	Trans-ovarian	Larvae to Nymph	Nymph to Adult	Adult to Adult	Reference
Boophilus decoloratus	✓				144
B. microplus	✓				94, 118 & 16
B. calcaratus	-			✓	130
Hyalomma mauritanicum	-	-	-		130
B. annulatus	✓				100
H. lusitanicum	✓				130
Rhipicephalus simus	✓				144
R. bursa		✓	✓		118
R. bursa	-	-	-	✓	130
R. sanguineus	-	✓	✓		95, 100, 1
Dermacentor andersoni		✓			97
D. andersoni			✓		11
D. andersoni	✓				52, 54
D. andersoni	-	✓	✓		102
D. andersoni	-	-	-		120
D. variabilis	-	✓	✓		96, 100
D. variabilis			✓		122
D. occidentalis	✓				11

	Trans-ovarian	Larvae to Nymph	Nymph to Adult	Adult to Adult	Reference
D. albipictus			/		11
D. nitens	-				102
Ixodes ricinus	/				152, 49
I. scapularis		/			100
I. sculptus			/		100
Amblyoma Americanum	-	-	-		100
A. maculatum	-	-			100
A. cajennense	/	-			100
Omithodoros megnini	-	-			55
D. andersoni				/	120
D. andersoni	-			/	4
D. variabilis	-	/	/		4
Argas persicus			/		55
Boophilus australis	/				82
Haemaphysalis cinnabarina punctata	/		/		82

/: Transmission occurred.

-: No Transmission occurred.

Somewhat paradoxically, Rees (101) failed to transmit anaplasmosis by injecting an emulsion of ticks that had previously fed on an infected animal. Anthony (4) had a similar experience with partially engorged, D. variabilis, female ticks. An emulsion

of these ticks injected into a susceptible animal failed to produce infection.

Early workers with anaplasmosis recognized the need of immunising or premunising susceptible cattle brought into an anaplasmosis endemic area. Schmidt (25) described the technique of premunising susceptible cattle against anaplasmosis. This consisted primarily of injecting blood from a known carrier into young animals. Vaccinations were conducted only during the cool weather. Among more than 1,000 animals premunized at the age of 15 months or under, a mortality of 1.3% was encountered. In older animals the risk of fatal reaction was increased. Cordier (23), in 1932, reported on his attempts to modify the virulence of A. marginale infected blood by storing it with horse serum at various times and temperatures, exposing the organism to serum from horses infected with dourine, using various drugs in contact with the organism, and by irradiating the organism. None of these methods were successful in attenuating the anaplasma, although irradiation did lengthen the incubation time.

Reports of attempted immunisation using material not intended to produce an active infection are rare. Rees (101), after failing to produce infection by injecting an emulsion of ticks that had previously fed on an animal with anaplasmosis, checked the animal for the possible development of immunity, but evidence of an increased resistance or immunity to challenge was not noted.

Pearson et al (86), in 1953, reported testing killed vaccines produced from 8 different tissues, in an attempt to induce a sterile immunity in susceptible cattle. Their efforts were uniformly unsuccessful. Kuttler (60), in 1961, reported the use of a killed adjuvant vaccine on susceptible cattle. Even though this vaccine failed to induce solid protection, it did stimulate an immune response which significantly reduced the severity of infection following challenge.

Theiler (143), in 1911, in his first description of A. centrale, suggested that it may be useful as an immunising tool against A. marginale. Walker (151), in 1915, reported on the use of A. centrale as a vaccine in South Africa. He considered the practice useful in that a prior infection of A. centrale reduced the severity of a subsequent A. marginale infection. In a few years the use of A. centrale as a vaccine had become wide spread throughout Africa(31, 127, 140, 145 ~~106, 109, 110, 112~~) and in other parts of the world (22, 64, 84, 146 ~~107, 108, 111, 113~~). A report from East Africa (140) in 1932 described the use of A. centrale as a practical procedure in the immunisation of European cattle being introduced into this territory. Sergent (127) told of vaccinating 25,000 cattle between 1935 and 1943 with A. centrale in Algeria. Donatien (31) reported that the A. centrale used in Algeria was responsible for a decrease in milk yield in lactating animals, but that this was overcome by vaccinating young animals. Tsur, (146) reported on the use of

A. centrale on 15,000 head of cattle in Israel. Legg (64) has described the introduction of A. centrale into Australia and its routine use on susceptible cattle being introduced into an endemic area. Nothwithstanding the wide spread use of A. centrale, this organism is still prohibited from use in the United States. Christensen (19) discusses some of the reasons for hesitating to introduce A. centrale into the U.S. among which he stated that insufficient is known about the behavior of A. centrale in breeds of cattle found in the U.S.

Even though A. centrale is generally accepted as a vaccine strain of milder virulence than A. marginale, publications describing the virulence, pathogenesis and pathogenicity of this agent are limited (19). Carpano (17), in 1930, concluded that A. centrale should not be considered a variety of A. marginale, because its biological and morphological features are so distinct. He describes A. centrale as being irregular in outline, with proteplasmic projections, and some times a delicate hale appearance, whereas he found A. marginale to be practically constant in size, staining intensely with a homogenous appearance. Carpano believed A. centrale was always found in association with Theileria mutans, and suggests that it may be a stage in the life cycle of T. mutans, or closely related to it. Galin (84) in 1932, reported that he had inoculated a number of susceptible animals with A. centrale, obtained from South Africa. A mild initial reaction was observed,

and when challenged with a Brazilian strain of A. marginale, a high degree of resistance.

Sergent et al (128, 129) (116, 117), in 1933 reported the results of their work on A. centrale. The strain on which they carried out their investigations was obtained from Kenya. It took three weeks to arrive in Algeria from Kenya. The blood was refrigerated during this time. The blood, which was in sodium citrate, was sterile on arrival, but the defibrinated specimen contained viable organisms as determined by animal inoculation. In studying this organism Sergent found that only 8 of 24 calves showed a febrile response, and that generally the infection was very mild. Incubation period ranged from 16 to 45 days, parasites appeared in 16–66 days, and persisted 12 to 57 days. The average parasitemia was 10.8% A. centrale infections produced parasitemias where 90% of the bodies were centrally located, whereas in marginale infections it was found that 80% of the bodies were located on the margin of the cell. Homogeneous challenge among A. centrale and A. marginale carriers was always negative. Heterogeneous challenges were always positive. Sergent reports (128, 129) that A. marginale challenge of A. centrale carriers produced severe reactions. Death did not however, occur; 2 of 12 controls died of the marginale challenge. Cordier (22) in 1932, reported no serious illness among calves first inoculated with A. centrale,

then later given A. marginale. Legg (64), in 1936, described the introduction of A. centrale into Australia and in general terms confirmed the South African description of A. centrale in Australian cattle. A. centrale was thought to possess characteristics that would be useful in an immunization program in Australia. Turner (147) described a technique used for freezing A. centrale for vaccine purposes. Blood from an infected animal was frozen in dry ice and alcohol, and was successfully stored for 254 days at -80 C. One ml. of blood thus frozen was sufficient to induce infection.

Theiler (143) reported that Anaplasma centrale could be transmitted by ticks, and he considered mixed infections of A. centrale and A. marginale as not uncommon. Metianu (75), in 1950, reported two naturally occurring cases of A. centrale infection.

Waddell (149) examined carefully the A. centrale organism as it appeared in two splenectomized calves. Using ordinary light microscopy he observed that a high parasitemia of 12% was developed, 2.8% of the bodies had contact with the periphery of the cell, and 11.3% showed thin projections from the bodies. The size varied from 0.4 to 0.95 u. Using electron microscopy Waddell (150) later described A. centrale as having a very close resemblance to A. marginale.

Theiler (143) described A. centrale transmission by Boophilus decoloratus, and Metianu (75) described its transmission by Haemaphysalis cinnabarina punctata.

Experiment #4: Serological Survey of Anaplasmosis Incidence in East Africa, using the Complement-Fixation Test.

It is possible in an anaplasmosis endemic area to have almost 100% incidence among cattle native to such an area. Under these circumstances clinical cases are rare, due to the early premunition of calves at a time when their resistance is high. On farms where dipping effectively controls the tick population it is likely that the incidence of natural exposure in young cattle may be less than presumed. No previous serological survey of anaplasmosis has ever been undertaken in East Africa, hence it was thought appropriate to test, at random, samples collected from cattle native to the area.

Materials and Method

Complement-Fixation Test:

The complement-fixation test used follows, in general, the outline described by the United States Department of Agriculture, (USDA) (148). Reagents and materials used must be collected, prepared, and standardized before setting up the complement-fixation (CF) test.

Sheep blood was collected regularly each week in equal parts sterile Alsever's solution.

Alsever's solution:	Sodium citrate	6.0 grams
	Sodium chloride	2.1 grams
	Glucose	10.25 "
	Q.S. Distilled water	500.0 ml.

The collection of sheep blood was made on Friday or Saturday, from normal sheep, and held in the refrigerator (5 C.) for use the next week. Sheep cells will remain useful for several weeks in Alsever's solution, but after this time the cells begin to show signs of erratic behavior in our hemolytic system, so, in order to ensure uniformity, fresh cells were collected each week, and used the following week.

Sheep RBC's are washed three times in veronal buffered physiological saline, pH 7.2-7.3. This buffered saline is prepared

from a concentrated stock which is composed of:

Veronal Buffered Stock Solution	
Sodium chloride	42.5 gms.
5,5 diethylbarbituric acid	2.875 gms.
Sodium 5,5 diethylbarbiturate	1.875 gms.
Q.S. Distilled water	1000 ml.

One volume of the stock buffer is mixed with 4 volumes of distilled water to give the final buffered physiological saline solution. This buffered saline is the standard diluent in all aspects of the CF test. Packed, washed RBC's are made into a 2% suspension in buffered saline, and used within 24 hours of preparation.

Complement was obtained by bleeding healthy guinea pigs, collecting serum, freezing the serum in 2.5 ml aliquots, which were thawed and titrated before every test. Generally, complement is not re-frozen, so any remainder not used on a given day's test was discarded.

Complement was titrated, using a hemolytic system consisting of hemolytic amboceptor (2.5 units) and 2% sheep RBC's in equal parts. In the first complement titration, when the amboceptor titer is unknown, a slight excess of amboceptor is used. The hemolytic system is used the day of preparation. Complement (guinea pig serum) was diluted in buffered saline to give a 1/25 dilution, by adding 0.1 ml serum to 2.4 ml saline. The

titration is set up as follows:

1/25	(Quantity in Ml.)								
Complement	.05	.075	.10	.125	.150	.175	.20	.225	.25
Saline	1.45	1.425	1.40	1.375	1.350	1.325	1.30	1.275	1.25
H.S.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Incubate 45 minutes in water bath at 37.5°C.								
Readings:	4+	3+	2+	2+	1+	-	-	-	-

Readings: 4/ — no hemolysis
3/ — 25% hemolysis
2/ — 50% hemolysis
1/ — 75% hemolysis
Tr. — < 100% hemolysis but >85%
\- — 100% hemolysis

Readings are arrived at by visual examination following centrifugation to throw down all non-hemolysed RBC's.

The exact unit in the above complement titration would be:

.175 ml. of a 1/25 dilution

Two units would be .175 X 2 = .35 ml of a 1/25

It is desirable to use 2 U. of complement in 0.5 ml amounts, so:

.35 : 25 :: .5 : X

.35 X = 12.5

Therefore, X = 36, or 1/36 dilution, prepared by adding 1 ml serum to 35 ml saline.

The commercial hemolytic amboceptor available was of such low titer that it was necessary to sensitise rabbits to sheep RBC's and make our own. This was accomplished by injecting two rabbits at 5 day intervals with a given quantity of washed sheep RBC. The following schedule of injections were made:

Day 0 —	2ml.	10% suspension of washed sheep cells.
5 —	4ml.	" " " " " "
10 —	5ml.	" " " " " "
15 —	5ml.	" " " " " "
20 —	5ml.	" " " " " "
25 —	5ml.	" " " " " "

On day 30, both rabbits were bled by cardiac puncture, a total of 16ml. clear serum was harvested from one rabbit, and 23 ml from the other. The rabbit serum was then mixed with equal parts glycerine for preservation, and stored at 5 C. A 10 ml portion of the amboceptor was inactivated 35 minutes at 57 C., then titrated. The hemolytic amboceptor titer has been found in the past to be reasonably constant, so this material was only titrated once.

Hemolytic ambeceptor	Complement	Buffered	2% Sheep	Reading.
.5 ml of each dil.	2 units	saline	RBC	
1/400	.5 ml	1.0 ml	.5 ml	-
1/600	"	"	"	-
1/800	"	"	"	-
1/1200	"	"	"	-
1/1600	"	"	"	-
1/2400	"	"	"	-
1/3200	"	"	"	Tr
1/4800	"	"	"	1
1/6400	"	"	"	2
No ambeceptor	"	1.5 ml	"	4

1 unit = 0.5 ml of a 1/2400 dilution (highest dilution giving complete hemolyses).

2.5 units = 0.5 ml of a 1/960 dilution (this dilution is used in the preparation of hemolytic system)

The antigen used was obtained from the USDA, prepared to meet USDA specifications by Franklin and co-workers (35). This antigen was prepared from infected blood having a high level parasitemia. The CO_2 and distilled water antigens previously described (89) were combined and large batches were standardized and frozen at -79 C. The antigen can be stored at this temperature indefinitely (35). A quantity of this antigen was shipped to Kenya in dry ice containers at the beginning of this work in 1963. It has been stored at -20 C since that time and has maintained its titer for the last 18 months. The antigen titration is performed with normal and anaplasmosis-positive serum to arrive at the dilution of antigen in 0.5 ml quantity that will be used in the test proper. Normal and positive sera are diluted 1 + 4 giving a 1/5 dilution and inactivated 35 minutes at 58 C. The 1/5 serum dilution is the lowest used in screening unknown samples, hence the dilution is used for the antigen titration. The hemolytic system is prepared as previously described, that is, 2.5 units hemolytic amboceptor in buffered saline plus an equal part 2% sheep RBC suspension. The following schedule is followed for the antigen titration:

An initial antigen dilution of 1/6 (1 + 5) is made.

Antigen amount	.05	.10	.15	.20	.25	.30	.35	.40	.45	0
Buff. saline	.45	.40	.35	.30	.25	.20	.15	.10	.05	.50
Pos. Ser. 1/5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Comp., 2 units	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Incubate 1 hour, at 37.5 C. (water bath)										
Hemolytic System	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Incubate 45 minutes at 37.5 C (water bath)										
Reading:	1+	3+	4+	4	4	4	4	4	4	-
Negative Serum Reading:	-	-	-	-	-	-	-	-	-	-

A similar test with normal negative serum will detect anticomplementary antigens. The same materials in identical amounts are tested with negative serum. The results with negative serum are given above.
1 unit of antigen is the smallest amount producing complete fixation of 2 units of complement, with positive serum. In this instance 1 unit = .15 ml of a 1/6 dilution.
2 units which is used in the test = 2 X .15 or .30 ml of 1/6.
The test proper uses antigen in .5 ml. amounts, therefore:

$$.30 : 6 :: .5 : X$$

$$.30X = 3.0 \quad ; \quad X = 10, \text{ or a } 1/10 \text{ dilution of antigen } (1 + 9).$$

This 1/10 antigen dilution, arrived at in this titration, has remained constant, and has been used in all CF tests performed using the USDA antigen.

The CF test proper is conducted following the standardisation of all reagents, on clear serum either freshly collected, or thawed following storage at -20 C. Freezing and storage of serum at this temperature does not impair its complement-fixing properties.
A serum control without antigen is set up for each sera, to test it for possible anticomplementary activity. The following outline describes the test procedure followed:

1/ and 2/ are considered suspicious.

3/ and 4/ are considered positive.

	Test Proper	Test for A.C. properties.
Buffered saline	0.4 ml	0.9 ml
Unknown serum	0.1 ml	0.1 ml
Inactivated 35 minutes at 58 C (waterbath)		
2 units Antigen	0.5 ml	0.0
2 units Complement	0.5 ml	0.5 ml
Incubate 1 hour at 37.5 C. (waterbath)		
Hemolytic system	1.0	1.0
Incubate 45 minutes at 37.5 C. (waterbath)		

Reading of: - and Tr. are considered negative.

Animals Tested:

Two herds of cattle, one from Kenya and one from Uganda, for a total of 334 animals, have been tested. The herd from Kenya was, in fact, several herds that had been newly formed over a period of time, by gathering cattle over a large geographical area, mainly the Rift Valley and Central regions of Kenya. These cattle were brought onto the East Africa Veterinary Research Organisation (EAVRO) estate and held for other experimental purposes. This herd consisted of 234 head. Herd 2 consisted of 100 animals held at the East African Trypanosomiasis Research Organization (EATRO) estate near Tororo, Uganda. This herd has been maintained relatively intact, and did not represent a recent collection of animals, as was the case with the EAVRO herd. The EATRO herd had been subjected to a regular (once a week) dipping program for tick control.

In addition to the 334 cattle samples, sera collected from game animals was also tested. Samples from Tanzania, Kenya, and Uganda were tested. The significance of the CF test in game animals is not fully established, as it has been with cattle, but it is reasonable to surmise that a CF positive reaction in game samples, not anticomplementary, does indicate a specific fixation of anaplasma antigens by serum antibodies formed by an exposure of the individual animal to anaplasma or anaplasma-like antigens in the past. With cattle it has been established that the CF positive reaction is indicative of a carrier state. Post and Thomas (90) showed that a positive reaction in American elk does not necessarily indicate a carrier animal. So little work has been done on the significance of this test in game animals that it is only safe to say that it may indicate either a carrier or a past exposure to the organism at such a level as to produce a detectable serological response. The numbers and types of animals tested are given in Table I. The waterbuck samples were collected in Elizabeth Park[1], Uganda;

buffalo samples were obtained from animals kept at EAVRO for research purposes; 36 wildebeeste samples were obtained from northern Tanganyika[2]; two wildebeeste, Coke's hartebeeste (kongoni), impala, and Grant gazelle samples were obtained in the Masai areas of southern Kenya[3]; gerenuk samples were obtained from the Baragoi area of the N.F.D.[3]; oryx samples were from the Baragoi area of the N.F.D.[1-3] and lesser kudu samples were from the Galana River area of eastern Kenya!

Note: 1—Samples were collected by Norman Smith in connection with carcass evaluation studies of game animals in East Africa.

2—Samples were obtained from the virus section EAVRO, collected as a part of studies of rinderpest incidence in wildebeeste.

3—Samples were obtained as the result of hunting safaris conducted by the author.

Results

Results are tabulated in Table I. Almost 1/3 of the cattle tested classified as negative, or susceptible to infection or reinfection. This number was actually higher than anticipated. In some endemic areas of the U.S. fewer than 10-15% are found susceptible (62). This high number of susceptibles probably reflects the relatively efficient control of tick vectors in the areas where samples were taken. Nevertheless, East Africa, on the basis of this survey, probably should be classed as an anaplasmosis endemic area.

Serum samples from game animals showed a very low percentage of overall infection. There was sufficient response to indicate a possible significant factor in the epizootiology of anaplasmosis in East Africa. This is especially true of wildebeests who showed a fairly large number of clear-cut positive reactions.

Table I

Serological Survey of Anaplasmosis Incidence

	No. tested	Positive	%	Suspicious	%	Negative	%
KAVRO Herd	234	111	47%	43	18%	80	34
KATRO Herd	100	19	19%	44	44%	37	37%
Totals:	334	130	39%	87	26%	117	35%

Table 1 (continued).

Game Animals:

	No. tested	Positive	Suspicious	Negative
Waterbuck	38	1	0	37
Buffalo	6	1	0	5
Wildebeeste	38	4*	5	29
Impala	4	1	1	2
Grant Gazelle	4	0	0	4
Geremuk	3	0	2**	1
Oryx	15	0	0	15
Kongoni	1	0	0	1
Lesser Kudu	8	0	0	8
Totals:	117	7	8	102

* 2 of these 4 animals were located in Kenya, Masai area.

** Blood smears on these two animals revealed several anaplasma like organisms.

Experiment #2: Comparative Pathogenesis of *Anaplasma marginale* and *Anaplasma centrale*

In considering the comparative pathogenesis of *A. marginale* and *A. centrale*, it is necessary to take into account those factors which will influence the course of infection as produced by these organisms. Previously, it has been mentioned that animal age has an important bearing on the relative susceptibility of a given animal. The surgical technique of splenectomy further influences the relative susceptibility of an animal. It was decided, therefore, that a more accurate understanding of the relative pathogenesis and virulence of these organisms could best be obtained by using several age groups.

In early work by the author, it was noted that the strain of *A. marginale* obtained locally appeared far more virulent than the strain commonly used in the U.S. and isolated in Nevada. This suggested the possible variation in virulence of *A. marginale* strains, which could well influence findings of relative virulence between *A. centrale* and *A. marginale*. It was decided therefore, to examine the pathogenesis of more than one strain of *A. marginale* in making these comparisons.

In the past, reports (17, 22, 31, 64, 125, 129, 137, 141, 142, 143, 151) on the pathogenesis of *A. centrale* and *A. marginale* have dealt

mainly with temperature response, and clinical appearance, with erythrocyte counts and the degree of parasitemia having been done on only a limited number of samples. No statistical evaluation of differences in animal response to these pathogens has been reported. This report attempts to increase the number of factors observed, to characterize better the differences and similarities of these two organisms, and to apply statistical analysis to these differences for the determination of significance.

An effort has been made to determine what, if any, alterations occur in the total leucocyte count of animals affected with A. centrale and A. marginale. Differential anaplasma counts have been made of both A. marginale and A. centrale infections to determine what percentage of anaplasma bodies were actually located on the margin or in the center of the infected erythrocyte.

Materials and Methods

Animals:

Anaplasma infections have been induced, by blood inoculation, in the following four groups of animals:

1. Splenectomised calves, 3 to 7 months of age
2. Non-splenectomised calves, 2 to 7 months of age
3. Yearling cattle, 10 to 16 months of age
4. Adult cattle, over 18 months of age, but for the most part young animals.

1. Splenectomised calves:

At the onset of our work, there was some question concerning the availability of adult, anaplasmosis-susceptible cattle. Since Roby et al (116) had reported that splenectomised calves showed a similar degree of susceptibility to adult cattle, these animals were selected as our principle experimental group. Previous experience also indicated that if non-apparent infections of anaplasmosis or piroplasmosis actually existed in these young calves, splenectomy would precipitate an acute relapse within a few weeks of surgery, thus eliminating chronic carriers that might influence a subsequent infection with the agent being tested.

In practically every instance, calves were obtained from local dairies at an average age of 2-3 days. On arrival at EAVRO all calves were sprayed with a pyrethrum mixture* to kill any ticks that might possibly be present. Physical examination of calves treated in this manner has always shown them to be tick free.

The calves were then placed in tick-free isolation quarters. These quarters consisted essentially of a concrete floor surrounded entirely by a water moat, 12 inches wide. Shelters, animal crush, and feeding facilities were all provided within the confines of the moat to properly house and care for the animals, at the same time preventing tick contamination. Bedding and hay were fumigated with methyl bromide gas to kill any vectors possibly present on these materials. This fumigation was done in an air tight room, using one pound methyl bromide gas per 100 cubic feet, for 24 hours. The only other items introduced into this isolation unit were bran, dairy calf pellets, and milk. Such precautions were successful in maintaining tick-free quarters, and under these conditions accidental transmission of anaplasmosis was not observed. European dairy breeds, Holstein, Jersey, and Guernsey, were all represented, with a slight predominance of the Holstein breed being obvious. These calves were reared by bucket feeding, being taken off milk and onto a calf pellet starting at about 3 to 4 weeks of age. Those animals to be used as splenectomized calves were selected for surgery at 6-to 8 weeks of age, depending to some extent on their condition and whether or not they were completely on dry feed. It was desirable that they be weaned at the time of surgery.

* The spray consisted of 2 ounces of a commercial preparation "Pyesita", I.C.I., consisting of 6% pyrethrum diluted in two gallons of water.

Surgical Procedure:

The surgical technique for removal of the spleen was quite simple. The calves were starved 24 hours before surgery. The site of incision in the left para-lumbar fossa was clipped or shaved. The calves were anesthetized with a 20% solution of chloral hydrate to effect. In most instances this required about 30 ml. for a 120 pound calf, but varied considerably. Complete anesthesia was avoided as a safety precaution, with a local anesthetic* being used to infiltrate the incision area. The calves were secured to a metal operating table, with final cleaning and washing of the sight of incision taking place at this time. Although aseptic procedures are not necessary, efforts were made to minimize contamination by careful washing, and the liberal use of antiseptics and alcohol. A 4 to 5 inch incision, about 1½ inches posterior and parallel to the last rib in the left para-lumbar fossa, was made through the skin, abdominal muscles, and peritoneum. The spleen, lying between the rumen and diaphram is then loosened by manually breaking down the connective tissue and fascia holding it in place. Some caution is required to avoid tearing the splenic artery and vein which enter at the hilus on the dorsal third near the anterior border. The spleen is removed through the incision, care being taken to avoid rupturing the splenic capsule, or breaking the splenic vessels. The vessels are ligated with a non-absorbable "Vetafil Bengen" synthetic surgical suture (0.60 mm.), the splenic blood vessels are then severed, and the spleen removed. The peritoneum and internal abdominal (obliquus abdominis internus) muscle are closed

*Xylotox, Veterinary: a 3% w/v solution of N-diethylamino-2,6-dimethylacetanilide with adrenalin 0.00002 grams per cc., caprylhydrocuprein otoxin HCl 0.00002 gm. per cc., and thymol 0.0004 gms. per cc., in a Ringer's solution; Pharmaceutical Manufacturing Co.

with a continuous suture using medium hard No.2 catgut. The external abdominal (obliquus abdominis externus) is closed by a second continuous suture, using medium hard No.2 catgut. The skin is closed with a horizontal interrupted mattress suture with "Vetafil Bengen", synthetic, non-absorbable suture (0.60 mm.).

Post surgical care consits of 10 ml terramycin injectable (Liquamycin 50 mg/ml.) given intraperitoneally, and placing the calf in a dry and well-bedded stall. The animals are generally on their feet within 20 minutes. They are allowed ample hay and water with some bran for the first two days following surgery. Following this, grain and calf pellets are fed in small amounts, this gradually being increased. Some difficulty was encountered with bloating when grain pellets were given free choice following surgery. Dermal sutures were removed 2 to 3 weeks after surgery.

Splenectomized calves were not used for experimental purposes for a minimum of four weeks following surgery, and usually not till after six weeks.

2. Non-splenectomized calves:

Due, to the extreme virulence of East African Anaplasma marginale (EAM), in order to maintain a source of EAM, it was necessary to establish carrier infections in non-splenectomized calves. Also, because young animals are artificially premunized with anaplasma infections in some parts of the world, it was decided to use intact calves of similar age to splenectomized animals as a second group for this pathogenesis study. These calves were obtained from the same source and with essentially the same breeding as previously described.

3. Yearling cattle:

Thirteen yearling cattle were infected with A. marginale and A. centrale. These animals had been purchased at 2-3 days of age, from essentially the same sources as previously described and treated exactly as described before. They were not splenectomized, but were maintained in tick free isolation until reaching the age of 10-16 months, at which time they were artificially infected with anaplasmosis.

4. Adult cattle:

Adult cattle used in these experiments were secured from two sources. Six animals, 9474, 9475, 9476, 9477, 9478 and 9479 were obtained from the Veterinary Laboratory at Kabete, where they had been raised as calves in tick free isolation quarters. These animals were of mixed Zebu breeding, and approximately 18 months of age at the time they were used. All were shown to be susceptible to anaplasmosis by the use of the CF test and later by challenge with anaplasma organisms. The 15 other adult cattle were selected on the basis of negative CF tests from a recently formed herd of cattle on the EAVRO estate. These cattle were of mixed breeding with considerable evidence of Zebu stock. These cattle were subsequently shown anaplasma susceptible by their reaction to challenge.

Anaplasma strains:

Four strains of anaplasma were used in these studies. They are designated as follows:

1. East African A. marginale, EAM.

2. East African A. centrale, EAC.

3. Beltsville, U.S.A. A. marginale, BAM.

4. Nevada, U.S.A., A. marginale, NAM.

1. East African A. marginale:

The East African A. marginale (EAM) organism was obtained from the "Wellcome Research Laboratory", at Kabete, from an infected calf. This organism was known to be a pure strain, of high virulence, used and passaged for several years as a part of the Wellcome Research Laboratory drug screening program. This strain had originally come from South Africa.

2. East African A. centrale:

The East African A. centrale (EAC) was obtained from the Kenya Veterinary Department, Veterinary Laboratory at Kabete, and was their regular vaccine strain. This had originally been obtained in South Africa. The infected blood was frozen in 1 ml. samples at -79 C. where it had been stored seven months. At the time of collection and freezing the blood had shown a 30% parasitemia.

3. U.S. Beltsville A. marginale:

The Beltsville strain of A. marginale (BAM) was originally isolated from Virginia, and found to produce only mild response in susceptible cattle tested at Beltsville, U.S.A. Dr. T.O. Roby hand carried blood from a BAM carrier to Kenya, where a susceptible calf was inoculated. The blood was maintained at 2 to 4 C. during transit for 72 hours.

4. U.S. Nevada A. marginale:

The Nevada, U.S.A., A. marginale strain was originally isolated

from an acute case of anaplasmosis in an adult cow located on pasture in Washoe Valley, Nevada. This has been a standard laboratory strain in use at the University of Nevada Experiment Station for about four years. Blood from a carrier animal was supplied by the University of Nevada, being shipped on ice to Dr. T.O. Roby, who hand carried it to Kenya at the same time he brought the BAM strain. This blood had been held on ice for 96 hours before injection into a susceptible animal in Kenya. In the U.S. this strain produced severe anemias in splenectomized calves, but the majority of these animals recovered.

Because of the shortage of animals, and time, complete comparisons of all the above four strains were not made in all four classifications of animals. All four animal groups were used to compare BAM and BAC, but only splenectomized calves were infected with BAM and NAM.

Clinical and Laboratory Determinations:

The following factors were observed to characterize the anaplasma reaction in each animal tested and recorded in these trials:

1. Incubation time
2. Time required for the low PCV to occur
3. Packed red cell volume (PCV)
4. Persistence of anemia
5. Temperature response
6. Degree of parasitemia
7. Complement-fixation response.

The incubation time was determined in days, measured from the time the animal was exposed until the first evidence of infection occurred. All cases of anaplasma infections observed were induced by the injection of infected blood. No tick transmissions were attempted. In most instances, with the exception of adult cattle, observations were made three times weekly following infection. The first evidence of infection was considered to be the occurrance of a 1% parasitemia, or a 4+ CF reaction, whichever came first.

The time required for the low PCV to occur was measured in days, starting from the first evidence of anaplasma infection, to the time when the lowest PCV occurred. It was hoped that by this observation some conclusions could be drawn concerning the relative rapidity of infection.

Packed red cell volumes (PCV) were determined with capillary tubes in a micro-hematocrit centrifuge. Capillary tubes 1.3 - 1.5 mm. diameter by 75 mm. length were filled to within 10-20 mm. of the top with the blood to be tested. The air end was sealed in a gas flame and the tube placed in the centrifuge. Readings of the percent packed red cell volume were made following centrifugation 5 minutes at approximately 11,000 rpm's.

The persistence of anemia was expressed as days after the onset of infection, when the PCV was 80% or less of the pre-infection normal average. Animals recovering from anaplasma infection frequently show a relapse, during which time the PCV may drop to less than 80% of the normal. An attempt was made to record only the primary anemic

response for comparative purposes.

The degree of parasitemia is determined on thin blood smears stained with Giemsa, and expressed as the percent erythrocytes showing anaplasma bodies, either marginal or central in location.

The CF test using U.S.D.A. antigen was conducted regularly on sera collected from infected animals as previously described. With the occurrance of the first 4+ positive CF reaction, titrations consisting of two-fold serial dilutions of serum, beginning at 1/5 and continuing to 1/2560, were conducted. The high CF titer was noted and recorded for each animal. Rectal temperatures were taken daily in infected animals, with the exception of 15 adult cattle who were not temperatured.

Following infection, degree of parasitemia and PCV were determined three times weekly (Monday, Wednesday and Friday) on calves, two times weekly (Monday and Thursday) on yearlings and the six adult cattle from Kabete, and weekly on the 15 adult cattle. Complement-fixation response was determined weekly (Monday sample) on all animals. Serum was collected each time a blood sample was taken for PCV. In the case of calves, yearlings and the six adult cattle from Kabete, all samples were tested for the week prior to the first 4+ reaction to more accurately fix the earliest evidence of infection.

Standard deviations and an analysis of variance, as described by Snedecor (135), were conducted on all tabulated values to indicate the degree of animal variation occurring within a group, and also to fix confidence limits on average, differences. With splenectomized

calves which were tested with four anaplasma strains, the additional calculation of the least significant difference was included for comparisons of groups within this classification. The complement-fixation titers recorded in group tables are a mode, not a mean, and the analysis of variance was conducted on logrithmic transformation of the dilution. Standard deviations were not conducted on the serological titers.

Tabulated values of reactions to A. centrale and A. marginale (EAM) were so arranged in Table 7 to allow a comparison of relative animal group susceptibility to these infections. These differences were subjected to an analysis of variance and the determinations of least significant differences where indicated.

Experimental Inoculations:

Splenectomized calves:

Four splenectomized calves, 9372, 9549, 9551 and 9562 were injected with blood from a Beltsville A. marginale (BAM) infection. Calf 9372 received 20 ml whole blood subcutaneously from a carrier calf. The inoculum had been refrigerated 72 hours during transit from the U.S. The parasitemia was unknown in this instance. Calves 9549, 9551, and 9562 each received the same inoculum which consisted of 5 ml whole blood SC from a calf showing 8% BAM parasitemia.

Four splenectomized calves, 9455, 9376, 9554, and 9555 were injected with blood from a Nevada A. marginale (NAM) infection. Calf 9455 received 20 ml. whole blood from a carrier calf; 10 ml subcutaneously and 10 ml intravenously. The inoculum had been

refrigerated 96 hours during transit from Nevada where it had been collected. Calves 9376, 9554, and 9555 each received 5 ml whole blood subcutaneously collected from a calf showing a NAM parasitemia of 20%.

Eleven splenectomized calves, 9277, 9278, 9361, 9364, 9457, 9458, 9459, 9460, 9470, 9553, and 9572, received East African A. marginale (EAM) infections. Calf 9277 was inoculated subcutaneously with 5 ml whole blood from a calf showing a 10% EAM parasitemia. This blood was collected from an infected animal at The Wellcome Research Laboratory. Calf 9278 was inoculated subcutaneously with 10 ml washed, packed red blood cells which had been lysed in distilled water and a Vir-Tis tissue homogenizer. The blood had originally been drawn from a calf showing a EAM parasitemia of 45%. Calf 9361 was inoculated subcutaneously with 5 ml whole blood from a calf showing a 12% EAM parasitemia. The infected donor calf was located at the Wellcome Research Laboratory, Kabete. Calf 9364 was inoculated subcutaneously with 5 ml whole blood collected from a calf showing a near 100% EAM parasitemia. Calves 9457 and 9458 were inoculated subcutaneously with whole blood from a calf showing less than 1% EAM parasitemia. Calf 9459 was inoculated subcutaneously with whole blood from a calf showing a 14% EAM parasitemia. The blood had been frozen and stored 193 days at -60 C. Calf 9460 was inoculated subcutaneously with 5 ml whole blood from a calf showing a 5% EAM parasitemia. Calf 9470 was inoculated subcutaneously with 10 ml cell-free plasma from a calf showing a 45% EAM parasitemia. Calf 9553 was inoculated subcutaneously with 10 ml whole blood from a calf showing a 12% EAM parasitemia. The inoculum had been held at 4 C.

24 hours after collection, and before inoculation. Calf 9572 was inoculated subcutaneously with 10 ml whole blood from calf showing a 12% EAM parasitemia. The inoculum had been frozen and stored 30 days at -60 C.

Nine splenectomized calves, 9212, 9279, 9280, 9373, 9375, 9487, 9747, 9751, and 9754 received East African A. centrale (EAC) infections. Calf 9212 was inoculated intravenously with 10 ml. whole blood from an animal showing a 30% EAC parasitemia. The inoculum had been frozen and stored in 1 ml amounts at -79 C. for 210 days. Calf 9279 was inoculated subcutaneously with 5 ml whole blood from a calf showing a 34% EAC parasitemia. Calf 9280 was inoculated SC with 10 ml whole blood from a calf showing a 2% EAC parasitemia. Calf 9373 was inoculated subcutaneously with 5 ml whole blood from a calf showing less than 1% EAC parasitemia, but known to be a EAC carrier. Calf 9375 was inoculated subcutaneously with 5 ml whole blood from a calf showing an 8% EAC parasitemia. Calf 9487 was inoculated subcutaneously with 5 ml whole blood from a calf showing less than 1% EAC parasitemia, but known to be an EAC carrier. Calf 9747 was inoculated intravenously with 100 ml., washed packed RBC's which had been re-suspended in physiological saline, from a calf showing a 4% EAC parasitemia. Calf 9751 was inoculated intravenously with 100 ml washed packed RBC's, which had been re-suspended in physiological saline, from calf 9747, at a time when it was showing a 10% parasitemia. Calf 9754 was inoculated intravenously with 120 ml washed, packed RBC's which had been re-suspended in physiological saline. The donor animal was 9751,

who, at the time was showing a 10% parasitemia.

Non-splenectomized calves:

Four non-splenectomized calves, 9363, 9692, 9693, and 9694 received EAM infections. Calf 9363 was inoculated subcutaneously with 5 ml whole blood from a calf showing a 12% EAM parasitemia. The infected doner calf was located at the Wellcome Research Laboratory, Kabete. Calves 9692, 9693, and 9694 were each inoculated subcutaneously with 5 ml whole blood from a calf showing a 4% EAM parasitemia.

Six non-splenectomized calves 9396, 9370, 9374, 9461, 9462, and 9463 received EAC infections. Calves 9369 and 9370 were inoculated SC with 5 ml whole blood from a calf showing a 34% EAC parasitemia. Calves 9374, 9461, 9462, and 9463 were each inoculated subcutaneously with 5 ml whole blood from a calf showing a 12% EAC parasitemia.

Yearlings:

Six yearling cattle, 9223, 9271, 9557, 9575, 9567, and 9576 received EAM infections. The inoculum was the same for each animal and consisted of 5 ml whole blood from a calf showing a 7% EAM parasitemia, inoculated subcutaneously.

Seven non-splenectomized yearlings, 9218, 9229, 9424, 9565, 9568, 9574, and 9577, received EAC infections. The inoculum was the same for each animal and consisted of 5 ml whole blood, from a calf showing a 6% EAC parasitemia inoculated subcutaneously.

Adult cattle:

Seven young adult cattle, 9380, 9382, 9387, 9388, 9855, 9475, and 9479 received EAM infections. Cattle 9380, 9382, 9387, 9388, and 9855 were each inoculated subcutaneously with 5 ml whole blood from a

calf showing a 15% EAM parasitemia. Cattle 9475 and 9479 were each inoculated subcutaneously with 5 ml whole blood from a calf showing an 18% EAM parasitemia.

Fourteen young adult cattle, 9778, 9785, 9788, 9790, 9791, 9795, 9825, 9833, 9834, 9838, 9474, 9476, 9477 and 9478 received EAC infections. Cattle 9474, 9476, 9477, and 9478 were each inoculated subcutaneously with 5 ml whole blood from a calf showing a 1% EAC parasitemia. Each of the remaining 10 cattle were inoculated subcutaneously with 5 ml whole blood from a calf showing a 5% EAC parasitemia.

More detailed animal descriptions such as age, sex, and breed are given for each animal on the individual animal charts, in appendix I.

Total Leucocyte Counts:

The influence of East African A. marginale (EAM) and East African A. centrale (EAC) infections on total leucocyte counts in splenectomized and non-splenectomized calves was determined. The counts were made using standard white cell diluting pipettes and hemocytometers. The diluting fluid was 3% acetic acid in distilled water. The technique used was essentially the same as described by Schalm(123). In an attempt to detect differences as the result of infection, four white cell counts were made on different days before the animal had shown signs of infection, four counts were made on different days during the acute course of infection at a time when a high parasitemia existed, and in the case of EAC infections, four counts on different days, during the convalescent period, when parasitemia had subsided and

the packed cell volumes were normal or nearly so. The convalescent counts were not made with EAM infections due to the 100% mortality among untreated splenectomized calves. White cell counts were made on nine splenectomized calves with EAC infections, 13 splenectomized calves with EAM infections, 10 non-splenectomized calves with EAC infections, and 5 non-splenectomized calves with EAM infections. Differences were analysed for significance as previously described (135) with the exception that multiple observations on individual animals made it possible to refine the final evaluation.

Location of anaplasma bodies in A. centrale and A. marginale infections:

An arbitrary criterion was established, to determine the percent anaplasma bodies located in the center or periphery of the infected erythrocyte. An anaplasma body located a distance greater than its own diameter from the periphery of the infected erythrocyte was considered central in location. The reverse applied in that any anaplasma body situated a distance equal to or less than its own diameter toward the margin of the infected erythrocyte was considered a marginal body. Using this criteria, differential counts of anaplasma body location in 46 different EAC blood smears and 20 different EAM blood smears from animals showing parasitemias were made to determine how consistant the anaplasma bodies situated themselves in accord to our classification A. centrale and A. marginale infection.

RESULTS:

Splenectomized calves:

The course of Beltsville *A. marginale* (BAM), Nevada *A. marginale* (NAM) East African *A. marginale* (EAM), and East African *A. centrale* (EAC) infections in 28 splenectomized calves is summarized in Table 2, and presented in more detail on the animal charts of appendix I. The average pre-infection PCV in these four groups ranged from 37% to 40%, but considering the normal variation, no significant difference exists. The average incubation time of these four anaplasma strains varied from 15 days to 18 days. Individual variation was such that these differences could not be considered as significant. The size and virulence of the inoculum undoubtedly grossly influences the incubation time, but, as Lotze (69) pointed out, in most instances the patent period or course of the disease was not so greatly altered by this factor.

The average time interval required for the low PCV to occur following the first evidence of infection varied from 9 to 12 days and was shortest in animals infected with EAM, followed by NAM, with BAM and EAC being equal. This trend is that would be expected, but again, because of the high individual variations, it was impossible to ascribe significance to these findings.

All 11 calves infected with EAM died with typical signs of anaplasmosis. A terminal packed cell volume (PCV) was not available in every instance, but autopsy invariably demonstrated the severe

anemia so characteristic of anaplasmosis. For this reason a low PCV of 9% was assigned to those animals where a terminal PCV determination was not made. Using this as a basis for calculating the significance of the variance, it was found that EAM produced a significantly lower PCV than did the other three strains. No deaths occurred in splenectomized calves infected with NAM, BAM, or EAC. This observation alone would easily establish the increased virulence of the EAM strain over the other three. NAM produced the next lowest average PCV, at 14%, with EAC being 16%, and BAM, the mildest in respect of PCV, with an average of 17%. The PCV differences among these three strains failed to reach significance. The relative PCV percent of normal paralleled the actual PCV determinations in differences and significance.

Splenectomized calves infected with EAM showed only an average persistence of anemia of 3.4 days. This reflects the acute nature and rapid course of the infection, which, in every instance, terminated in death. For this reason the calculations of significance for the persistence of anemia did not include these figures. Analysis of variance on the average persistence of anemia, resulting from BAM, NAM, and EAC infections showed highly significant differences. EAC infection resulted in a significantly shorter duration of anemia when compared with NAM infection. The average duration of anemia was shorter with EAC infections than recorded for BAM infection, even though the difference could not be demonstrated to be significant. BAM infection produced a significantly shorter duration of anemia than that observed with NAM infection.

The temperature response to anaplasma infection was significantly higher in EAM infections than observed with BAM. Even though no other significant differences could be demonstrated, a definite trend was observed, which showed that the more severe reactions of EAM and NAM infections produced the higher temperature response.

Without question the average parasitemia (percent erythrocytes showing anaplasma bodies) was very much higher in infections due to EAM. The average parasitemias of the remaining strains, BAM, NAM, and EAC did not differ significantly.

Considerable variations occurred in the CF titers of infected animals, and due to the inherent inaccuracies of a mean average on figures which progress in multiples of two, a mode average was used in tabulating the average CF response. Logrithmic transformations of the reciprocal of the dilution allowed a more reasonable solution for the analysis of variance, which showed the CF response in animals with EAC infections to be significantly lower, using U.S.D.A. A. marginale antigen, than resulted in BAM, NAM, and EAM ifections. No significant difference in CF titer was detectable among the three A. marginale strains, notwithstanding the very obvious difference in virulence.

Figure 1 charts the influence of EAM and EAC infections on PCV and degree of parasitemia in splenectomised calves for a 45 day period following the first evidence of infection. The rapid and fatal course of EAM infection is clearly evident and contrasts markedly with the much milder EAC infection. A very marked anemia and a persistent parasitemia was, however, noticed with EAC infection.

Table 2

Pathogenesis of BAM, NAM, EAM, EAC infections in Splenectomized Calves.

	BAM infection	NAM infection	EAM infection	EAC infection	LSD* and significance
No. of animals	4	4	11	9	
Pre-infection PCV	38% ± 2	37% ± 2	40% ± 3	37% ± 5	N.S.
Incubation Time in days	18 ± 13	15 ± 7	18 ± 7	17 ± 9	N.S.
Time required for low PCV in days	12 ± 2	10 ± 2	9 ± 3	12 ± 4	N.S.
PCV: Low	17% ± 5	14% ± 3	9% ± 0	16% ± 3	3.87 P. < .01
% of Normal	46% ± 13	36% ± 8	23% ± 2	44% ± 8	11.14 P. < .01
Persistence of anemia in days	28 ± 14	51 ± 6	3.4** ± 2	15 ± 7	14.28 P. < .01
High Temperature	103.6 ± 0	104.7 ± .7	105.3 ± 1.1	104.0 ± 1.3	1.6 P. < .05
High Parasitemia	20% ± 14	28% ± 20	80% ± 22	36% ± 19	30.5 P. < .01
High CF (1) titer.	1/640 1/1280	1/640	1/1280	1/160	P. < .05
Deaths	0	0	11	0	(2)

± : standard deviation
N.S.: No significant differences among the group average.
*LSD: least significant difference
** excluded in the analysis of variance due to the 100% mortality in this group.
(1) The mode of CF response is recorded, not the mean.
(2) No significant differences exist between the three AM groups, but the EAC titers were significantly lower (P .05) than the AM groups.

Table 2

PCV - Packed Cell Volume.
CF - Complement-Fixation.
P <.01 - Probability of error less than .01.
P <.05 - " " " " " .05.
BAM - Beltsville A. marginale.
NAM - Nevada A. marginale.
EAM - East African A. marginale
EAC - East African A. centrale.

Non-splenectomized calves:

The average response of four non-splenectomized calves to EAM infection, and six non-splenectomized calves to EAC infection is given in Table 3. Pre-infection PCV showed only a small difference, which was not significant. Infection with EAM resulted in shorter average incubation time, shorter time required to reach a low PCV, a lower PCV and a higher parasitemia, than in EAC infections, but none of these differences reached significance in non-splenectomized calves, due to the high degree of individual animal response, and the small numbers in each group. EAM infection resulted in a longer period of anemia, and a higher temperature response than EAC infection, which proved to be significant. These significant differences, along with the non-significant trends, again clearly establish the increased virulence of EAM when compared with EAC. The relative resistance of non-splenectomized animals to anaplasma infections, make differences in virulence more difficult to demonstrate. A slightly lower CF response was recorded in animals with EAC infections but this average of 1 dilution tube difference was not significant.

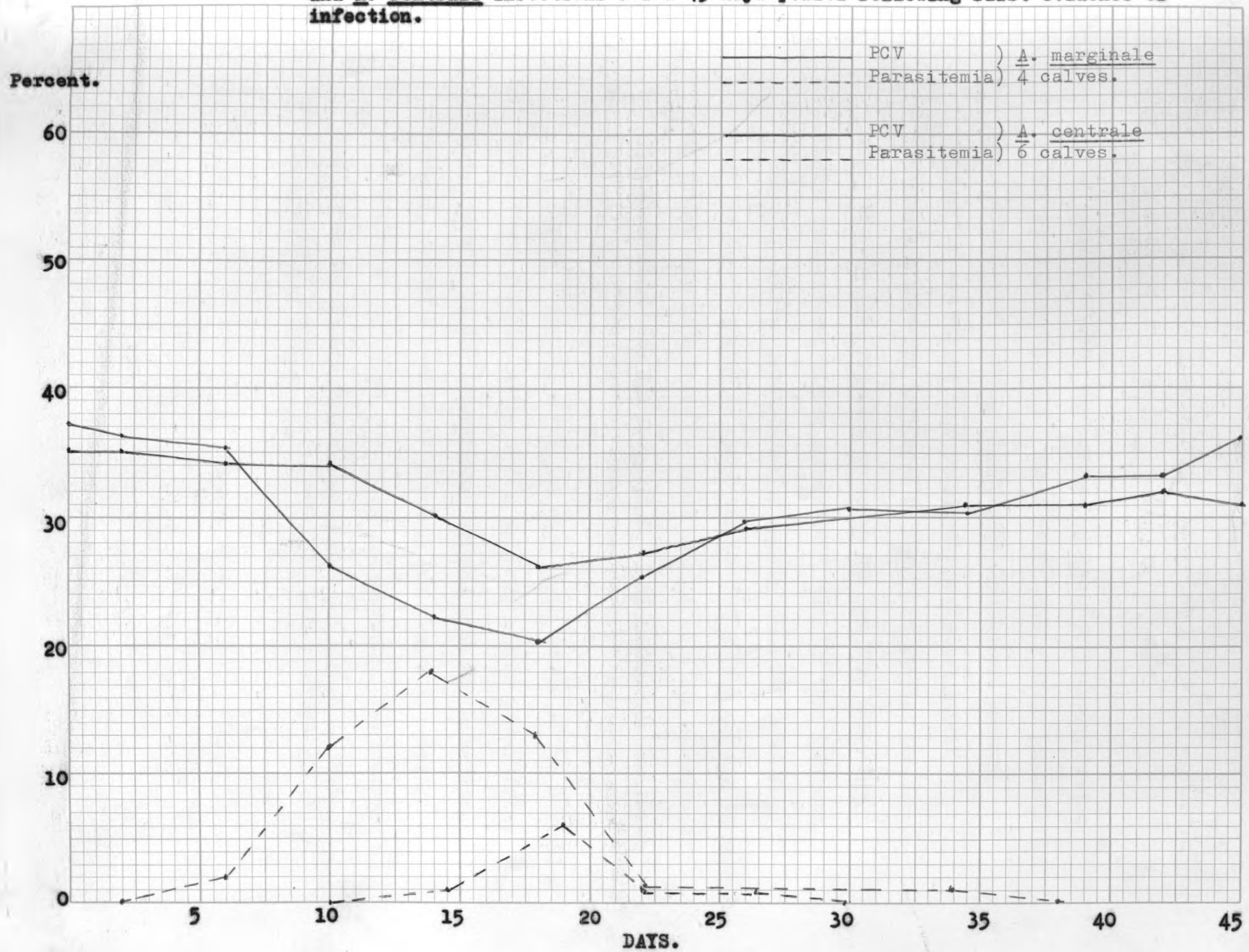
Figure 2, which presents the average response of PCV and degree of parasitemia, for a 45 day period, to EAM and EAC in non-splenectomized calves, confirms Table 3 in that EAM results in higher parasitemia, longer persistence of parasitemia, and a lower PCV than seen with EAC infections. Recovery following both EAC and EAM infections appears complete.

Table 3

Pathogenesis of EAM and EAC infections in Non-splenectomised Calves

	EAM Infection	EAC Infection	Significance
No. of Animals	4	6	
Pre-infection PCV	37% ± 3.6	35% ± 4.0	N.S.
Incubation Time in days	9 ± 2.4	11 ± 2.0	N.S.
Time required for Low PCV in days	13 ± 2.6	29 ± 18	N.S.
PCV: Low	18% ± 3.9	22% ± 7.0	N.S.
% of Normal	49% ± 14.0	62% ± 17.0	N.S.
Persistence of Anemia, in days	24 ± 12.0	7 ± 6.0	$P < .05$
High Temperature	105.0 ± 1.0	103.3 ± 1.0	$P < .05$
High Parasitemia	26% ± 22.0	6% ± 8.0	N.S.
High Titer	1/640	1/320	N.S.

Figure 2. **Average parasitemia and PCV response of non-splenectomized calves to A. marginale and A. centrale infections for a 45 days period following first evidence of infection.**



Yearling cattle:

Table 4 records the average response of yearling cattle to EAM and EAC infections. These young cattle again demonstrated the difficulty of showing significant differences between EAM and EAC infections in a relatively resistant animal. The averages show EAM infection to result in shorter incubation time, shorter time required to reach the low PCV, lower PCV, higher temperature, higher parasitemia, and, in this instance, a slightly shorter persistence of anemia, than EAC infection. Only the time required to reach the low PCV showed any significance. CF titers were much lower in those animals with EAC infections than in animals with EAM infections. This difference was highly significant.

Figure 3 clearly shows the more rapid course of EAM infection among these animals, along with a slightly higher parasitemia, when compared to EAC infections. Animals with EAM infection, however, showed a more rapid recovery and return to normal, with a shorter period of persistent parasitemia. The average low PCV on the 45 day observation period was almost identical between the two infections.

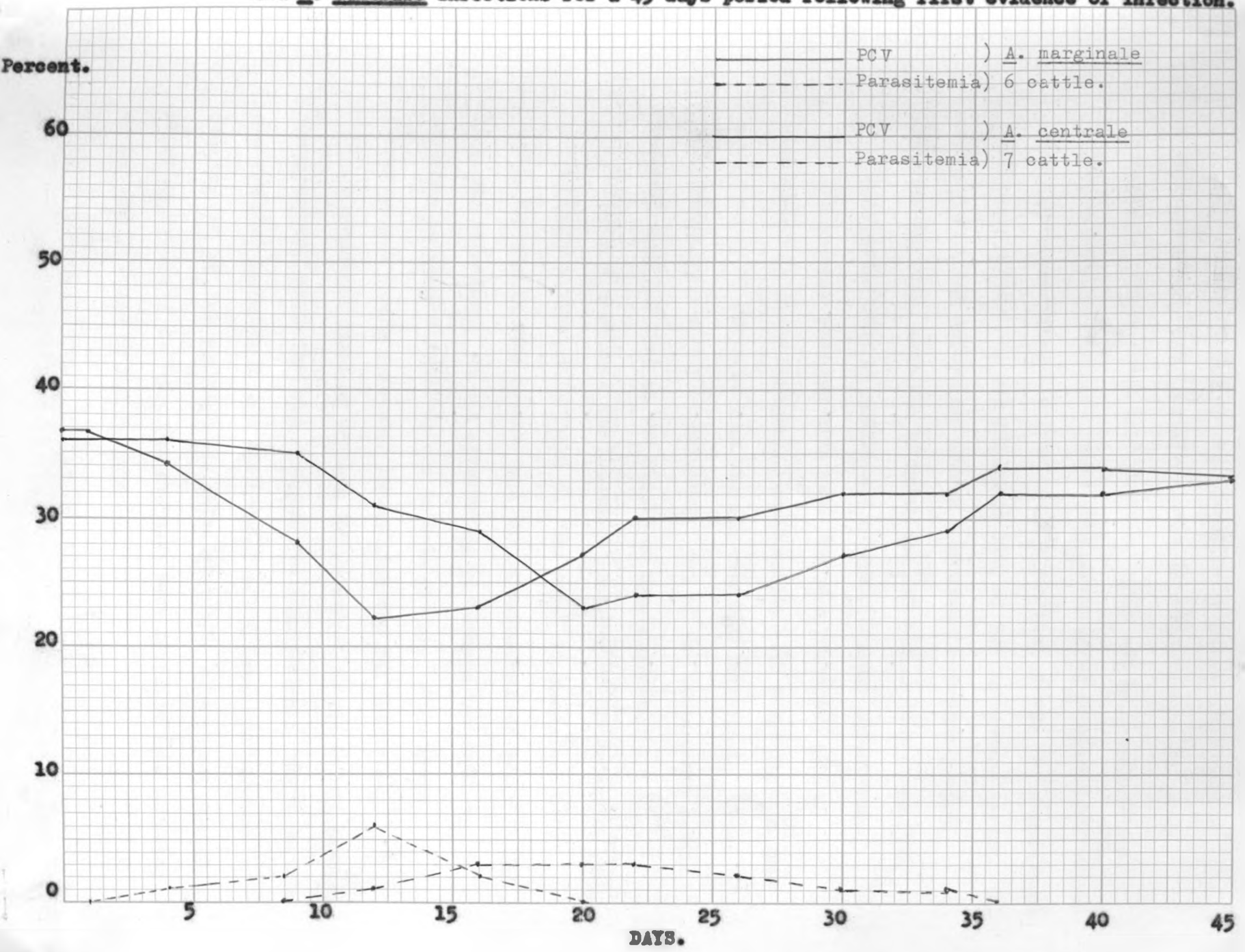
Table 4

Pathogenesis of EAM and EAC Infections in Yearling Calves

	EAM Infection		EAC Infection		Significance
No. of Animals	6		7		
Pre-infection PCV	36	± 2.6	36	± 1.7	N.S.
Incubation Time in days	7.5	± 1.2	9.1	± 2.0	N.S.
Time Required for Low PCV in days	12	± 3.6	19	±1.6	P. <.01
PCV: Low	20%	± 3.5	22	± 3.6	N.S.
% of Normal	56 %	± 9.5	61	±10.5	N.S.
Persistence of Anemia in days	11	± 6.9	13	±5.9	N.S.
High Temperature	104.1	± 1.1	103.3	± .9	N.S.
High Parasitemia	7%	±6.0	4%	±1.9	N.S.
High CF Titer	1/640		1/160		P <.01

Figure 3.

Average parasitemia and PCV response of yearling cattle to A. marginale and A. centrale infections for a 45 days period following first evidence of infection.



Adult cattle:

Tabulation of the average response of seven adult cattle to EAM infection, and 14 adult cattle to EAC infection is given in table 5. These young adult cattle, while not as susceptible as splenectomized calves, did react more sererely to anaplasma infections than non-splenectomized calves and the yearling group. No significant difference existed in pre-infection PCV, of the two groups tested. Animals with EAM infections showed a shorter incubation period, much shorter time required to reach the low PCV, a much lower PCV, a longer persistence of anemia and a higher average parasitemia than did animals infected with EAC. These differences proved significant in every instance. Adult cattle with EAC infection gave a much lower CF response than animals with EAM infections. These differences were highly significant. One of the seven adult cattle infected with EAM died as the result of anaplasmosis.

Figure 4 confirmed the increased severity of EAM infection among adult cattle indicated in Table 5. The onset of anemia was more rapid, the anemia more severe, and the parasitemia more acute in EAM than EAC infection. It was interesting to observe the rapid return of PCV's to almost normal values in both groups. A low grade parasitemia actually persisted longer with the EAC infection.

A composite table of EAM and EAC reactions in all animal groups was prepared, summarized and analyzed for significance. These results are recorded in Table 6, and show highly significant differences between EAM and EAC reactions in splenectomized, non-splenectomized, yearling and adult cattle. The averages were prepared from 28 cases of EAM and 36 cases of EAC infections.

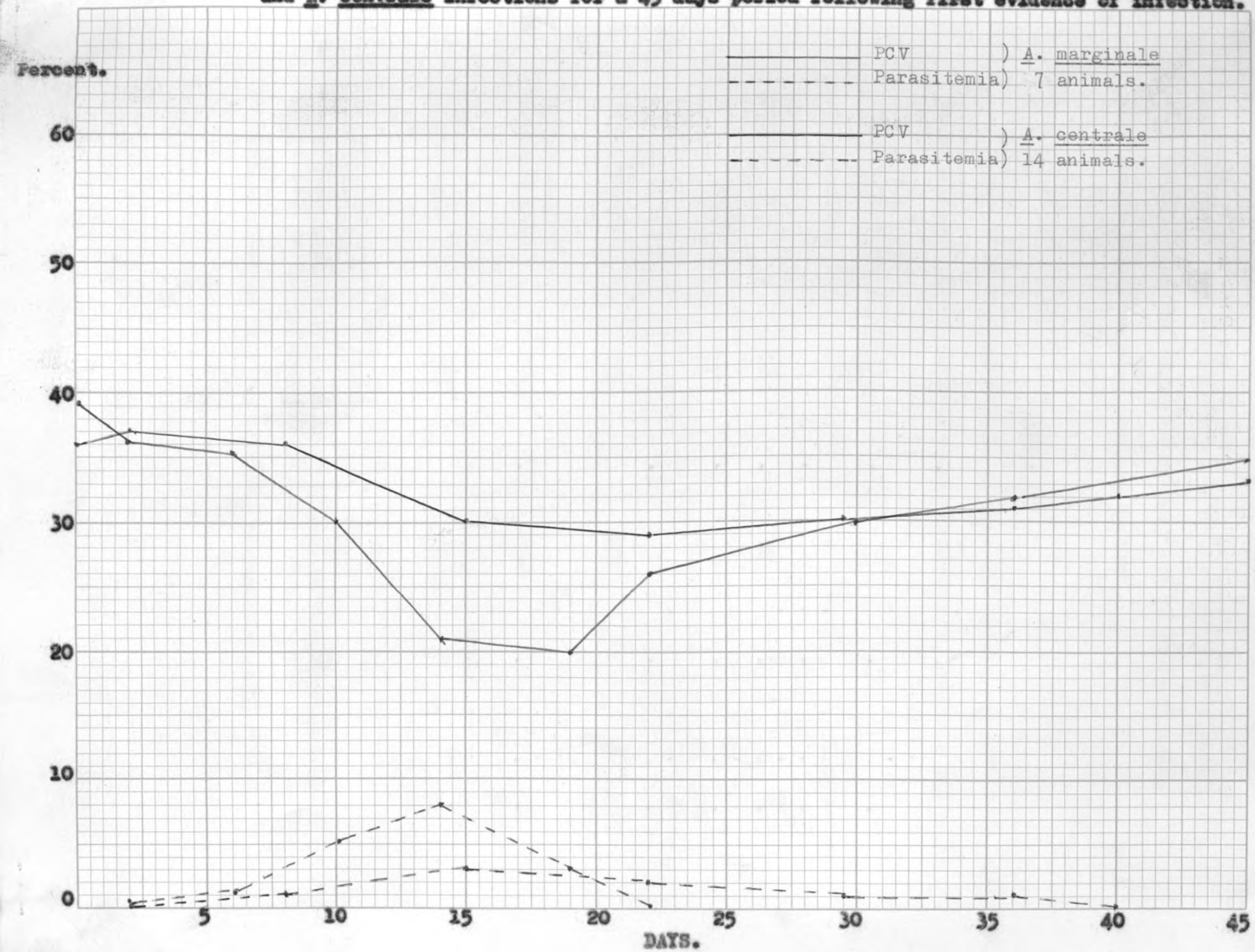
Table 5

Pathogenesis of EAM and EAC Infections in Adult Cattle

	EAM Infection	EAC Infection	Significance
No. of Animals	7	14	
Pre-infection PCV	39% ± 3.6	36% ± 3.5	N.S.
Incubation Time in days	11 ± 2.1	15 ± 4.7	P. < .05
Time Required for Low PCV in days	13 ± 3.7	24 ± 9.9	P. < .05
PCV: Low	18% ± 5.5	26% ± 5.5	P. < .01
% of Normal	46% ± 11.5	72% ± 12.0	P. < .01
Persistence of Anemia in days	21 ± 7.5	11 ± 9.3	P. < .05
High Temperature	NT	NT	NT
High Parasitemia	10% ± 6.8	4% ± 4.1	P. < .05
High CF Titer	1/1280	1/160	P. < .01

NT: No Test.

Figure 4. Average parasitemia and PCV response of adult cattle to *A. marginale* and *A. centrale* infections for a 45 days period following first evidence of infection.



Time required to reach low PCV, low PCV, persistence of anemia and high parasitemia were factors examined, and in every instance more severe reactions were produced by EAM.

Table 6

Summary of Pathogenesis of EAM and EAC Infections to Splenectomized calves, Non-splenectomized calves, Yearling and Adult

	EAM Infection	EAC Infection	Significance
Number of Animals	28	36	
Time Required for Low PCV in days	11	21	P. .01 (F - 20.4)
Low PCV % of Normal	39%	61%	P. .01 (F - 28.5)
Persistence of Anemia in days	18.2*	10.3	P .01 (F - 8.78)
High Parasitemia	39%	12.4%	P .01 (F - 14.63)

* Splenectomized calves are not included in this average because of the 100% mortality produced by EAM.

F: F value obtained from the analysis of variance (135)

Highly significant differences are demonstrated in relative susceptibility of splenectomized calves, as compared to non-splenectomized animals. Age while often an important factor (116) in relative susceptibility to anaplasma infections did not appear responsible for significant differences in these trials.

Considering the total, of both A. centrale and A. marginale (EAM) reactions, the time required for a low PCV to occur following first evidence of infection was significantly less among splenectomized calves than intact calves of comparable age and adult animals, however not significantly different than yearling cattle. No differences in this factor were observed among the three groups of non-splenectomized cattle.

The PCV, expressed as percent of normal pre-infection PCV, again showed splenectomized calves to be significantly more susceptible. PCV's were significantly lower in splenectomized calves than the three other groups. Differences among the non-splenectomized, intact cattle were not significant.

Statistical analysis, of persistence of anemia, was conducted on only the three groups of intact cattle, calves, yearlings, and adults. No significant differences were observed among these three groups of cattle.

High parasitemia, or the percent red cells showing parasitic anaplasma bodies at the height of infection, were significantly greater among splenectomized calves, than animals of the other three intact groups, No differences among non-splenectomized calves, yearlings or adults were observed.

Table 7 also records the reactions in the four groups, produced by A. centrale and A. marginale with the thought that differences in relative group susceptibilities may occur in one infection and not another. Such differences failed to occur. In all instances animal group reactions, even though less severe with A. centrale than A. marginale, followed the same general group susceptibility patterns. Splenectomised animals being the most susceptible, whether they had A. centrale or A. marginale infection, with little if any significant differences existing among the other three groups.

Table 7

Influence of Age and Splenectomy on the Relative Susceptibility to A. centrale and A. marginale Infection

	Splenec-tomised calves	Intact calves	Yearling cattle	Adult cattle	LSD	
Number of Animals						
A. centrale	9	6	7	14		
A. marginale	11	4	6	7		
Total:	20	10	13	21		
Time required for low PCV, in days						
A. centrale	12	29	19	24	12	P .01
A. marginale	9	13	12	13	4	P .05
Total:	10	23	16	20	7	P .01
Low PCV, expressed as % of normal.						
A. centrale	44%	62%	61%	72%	15	P .01

Table 7

	Splenec-tomized calves	Intact calves	Yearling cattle	Adult cattle	LSD	
A. marginale	23%	49%	56%	46%	15	P .01
Total:	32%	57%	58%	63%	9	P .01
Persistence of anemia, in days.						
A. centrale	15	6	13	11	NS.	
A. marginale	*	24	11	21	NS.	
Total:		14	12	14	NS.	
High parasitemia (% RBC'S showing anaplasma bodies)						
A. centrale	36%	6%	4%	4%	13	P .01
A. marginale	80%	26%	7%	10%	26	P .01
Total:	60%	14%	6%	6%	12	P .01

* Death occurred in 100% of these cases.

Total leucocyte counts:

Average total leucocyte counts in splenectomized and non-splenectomized calves infected with EAM and EAC are recorded in table 8. In splenectomized calves EAC infections failed to produce a change in the leucocyte count during the peak of infection, although a slight, but significant, drop in leucocyte counts occurred during the convalescent period. Fatal EAM infections in splenectomized calves, actually, were accompanied by a slight (not significant) drop in leucocyte count, during the acute phase of the disease. Among non-splenectomized calves no alteration in leucocyte count occurred as a result of EAC infection. A small but significant increase in leucocyte count did, however, occur in convalescent sera. EAM infection in non-splenectomized calves did not produce a significant alteration in leucocyte count. A small, but highly significant, difference does exist between splenectomized and non-splenectomized calves with the latter showing lower average counts. The significance of differences detected, was entirely due to the statistical analysis of large numbers of samples, using repeated counts on the same animal to correct for a large animal variation. Based on these results it would seem that it is highly doubtful if any consistant increase or decrease in leucocyte count due to anaplasmosis could be detected in the clinical laboratory where only a limited number of samples would be available. Some animals did demonstrate a marked leucocytosis, apparently associated with anaplasma infection but these observations were sporadic and not consistent.

Table 8.

Leucocyte Response to

EAM and EAC Infections.

	Splenectomised Calves			
	EAM Infection		EAC Infection	
Number of Animals	13		9	
Number of observations for each period	52		36	
	counts recorded in thousands.			
Pre-Infection	12.9	3.9	13.0	3.8
At time of high Parasitemia	11.4	5.3	13.0	5.1
Convalescent Period	NT		10.2	2.5
Significance	NS		P .01 (F - 5.74)	
	Non-splenectomised Calves			
Number of Animals	5		10	
Number of Observations for each Period	20		40	
	counts recorded in thousands			
Pre-Infection	10.2	1.8	10.4	3.5
At Time of High Parasitemia	10.8	2.6	10.2	2.9
Convalescent	NT		12.1	3.0
Significance	NS		P .01 (F - 10.63)	

Table 9 records the results of differential anaplasma counts. In animals showing a parasitemia due to A. marginale infection, 78.8% of the anaplasma bodies were classified as marginal. In animals showing a parasitemia due to A. centrale infection, 82.8% of the anaplasma bodies were classified as central in location.

Table 9

Variation of Anaplasma Location within Erythrocytes of Animals Infected with A. marginale (EAM) and A. centrale (EAC)

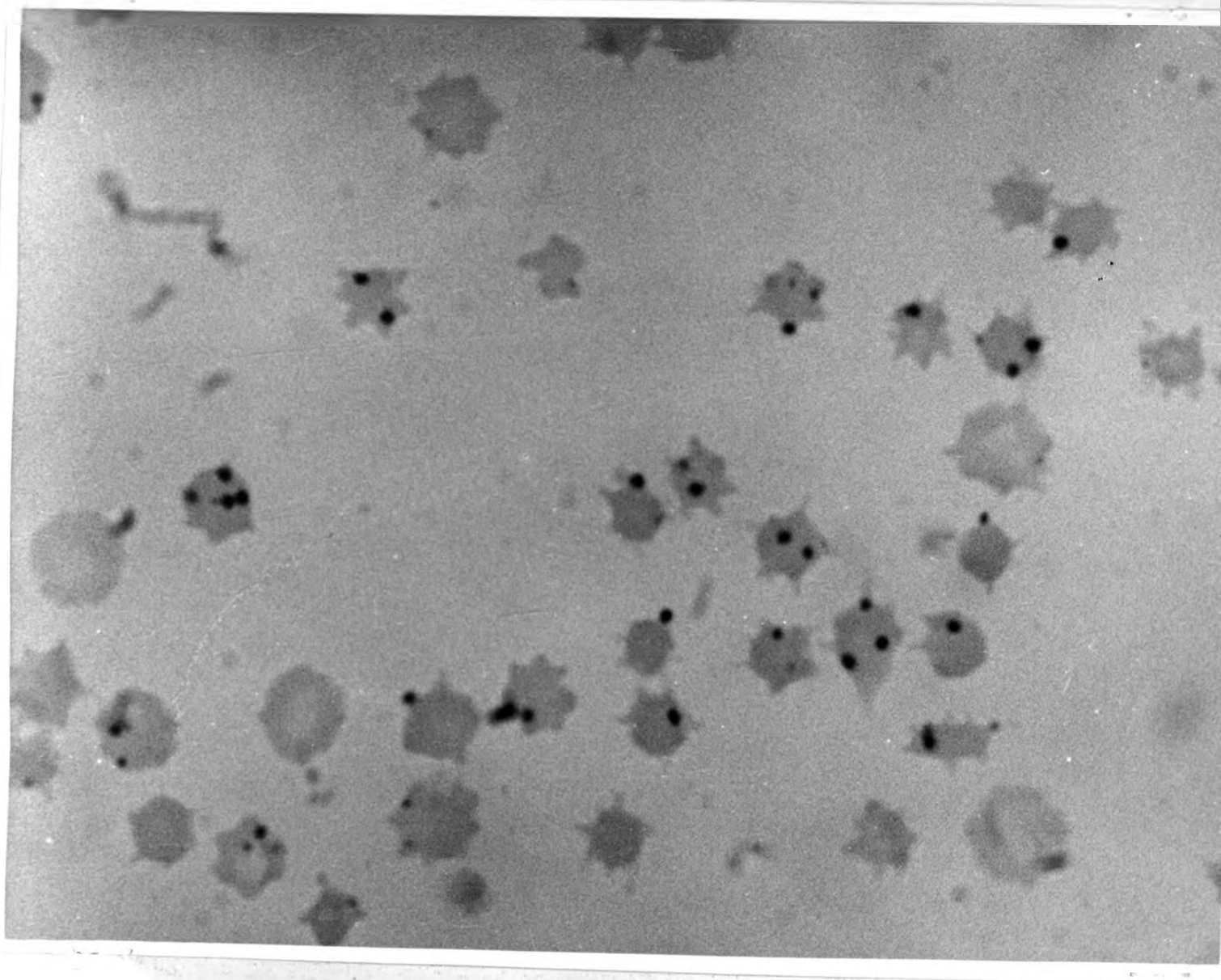
	EAM Infection 46 observations	Standard Deviation	EAC Infection 20 observations
Percent anaplasma bodies showing central location	17.2%	7.4	82.8%
Percent anaplasma bodies showing marginal location.	78.8%	7.5	21.2%

Conclusions:

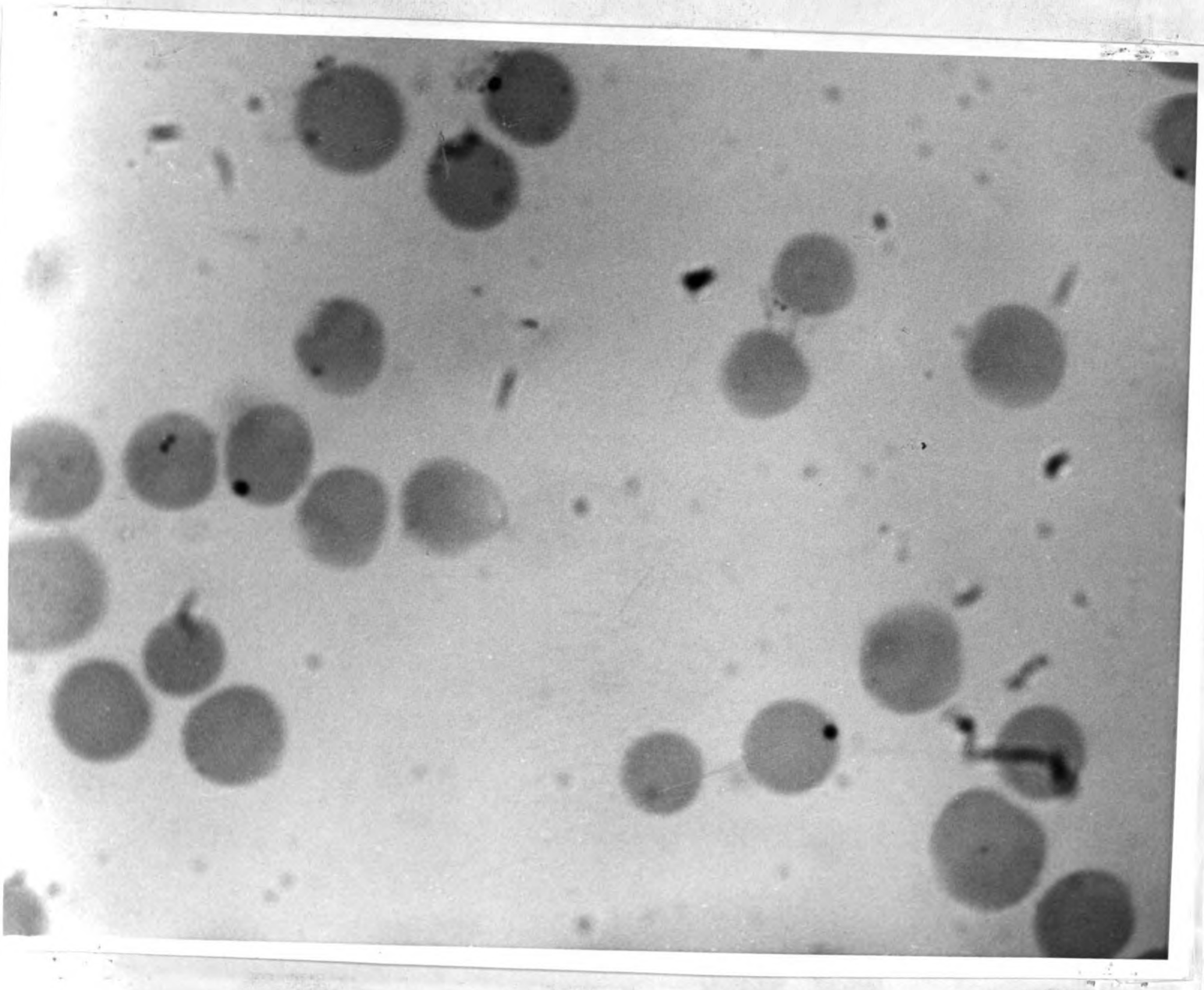
Anaplasma strain differences were best demonstrated among the more susceptible splenectomized calves. Among splenectomized calves, A. centrale proved much milder than the local East African strain of A. marginale (EAM), but it did not produce infections siginificantly less virulent than A. marginale strains obtained from the U.S., which were selected for their mild reaction. Unfortunately these U.S. strains (EAM and BAM) were tested on only a few animals, but their virulence was very nearly the same as A. centrale. The only factor measured, in which the U.S. A. marginale strains showed greater virulence, was the persistence of anemia.

Comparisons in adult cattle showed that A. centrale produces a significantly milder reaction than EAM infection, although not as striking as seen with splenectomized calves. Among young non-splenectomized cattle these differences are less pronounced suggesting that, if artificial premunition is to be followed in this age group, there is not much to be gained from the use of A. centrale.

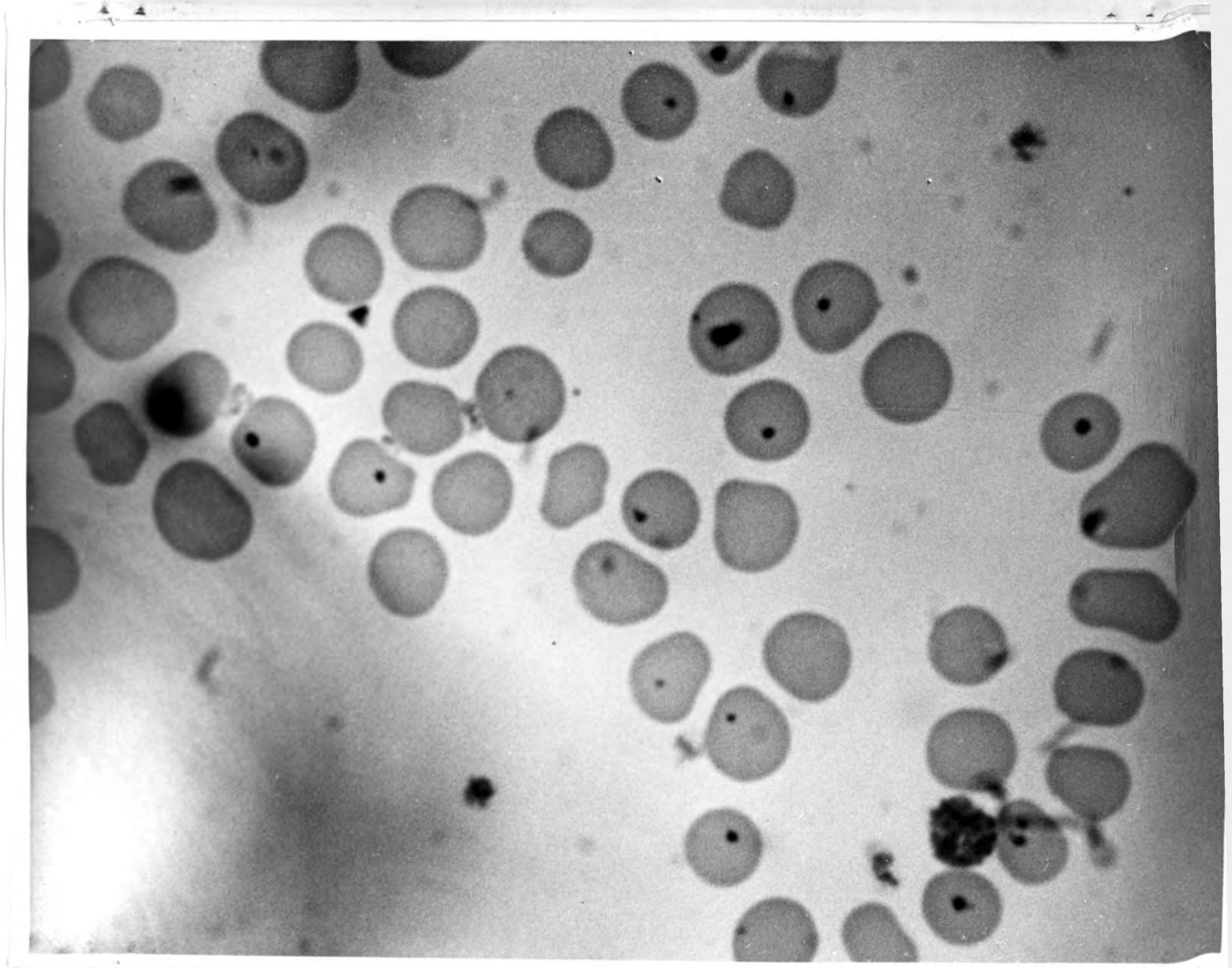
Statistical comparison of the relative susceptibility of splenectomized calves, non-splenectomized calves, yearling cattle, and adult cattle, showed highly significant differences existing. The type of infection, whether A. centrale or A. marginale, did not alter the relative animal group susceptibility. Splenectomized calves were much more susceptible than the three other groups. No significant differences in relative susceptibility were detected among the three other groups.



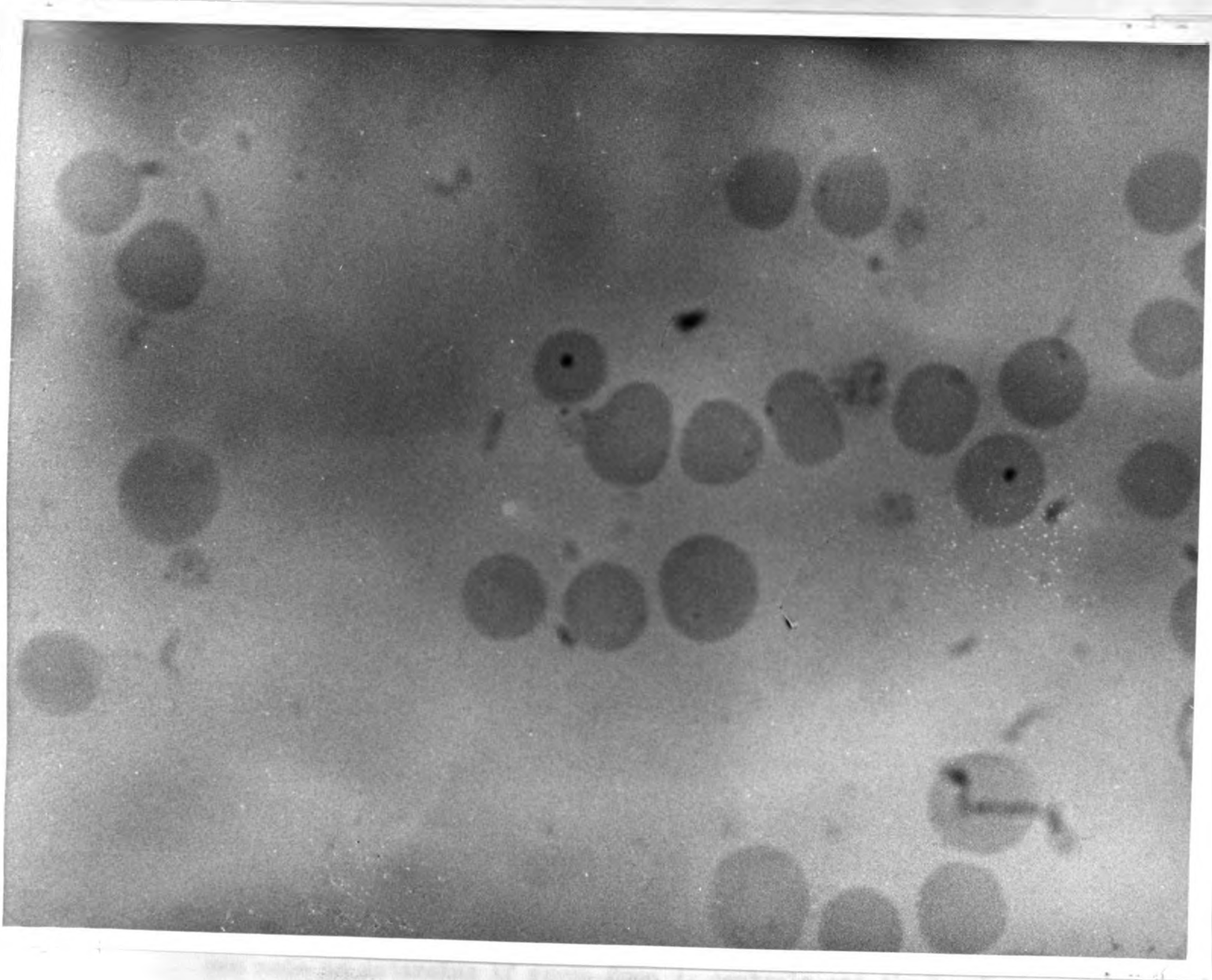
Bovine erythrocytes showing an *Anaplasma marginale* parasitemia, accompanying an acute, fatal case of anaplasmosis.



Bovine erythrocytes showing an *Anaplasma marginale* parasitemia.



Bovine erythrocytes showing an *Anaplasma centrale* parasitemia, accompanying an acute but non-fatal infection.



Bovine erythrocytes showing an Anaplasma centrale parasitemia.

Experiment #3: Serological Relationship of *Anaplasma marginale* (EAM, BAM, NAM) and *Anaplasma centrale* (EAC) as Measured by the Complement-Fixation and Capillary Tube Agglutination Tests.

In the previous experiment measuring the pathogenesis of *A. marginale* and *A. centrale* (EAC) infections, it was noted that animals infected with EAC also developed antibodies capable of fixing complement in the presence of *A. marginale* antigen. The antigen used in these tests was obtained from the U.S.D.A. and is described in Experiment 1. It was noted, however, in Experiment 2, that significantly lower serum titers were observed in animals with EAC infections, as compared to animals with East African *A. marginale* (EAM), Beltsville *A. marginale* (BAM) and Nevada *A. marginale* (NAM) infections, suggesting the possibility that serological differences may occur on serum titrations. In order to investigate more completely this possibility it was decided to prepare complement-fixation (CF) antigens from the local *A. marginale* (EAM) and *A. centrale* (EAC) strains for comparative titrations with the U.S.D.A. antigen, to determine if serological differences do occur, and whether or not these differences may also occur between marginale strains of different origins (EAM vs. BAM and NAM). In this study an attempt was made to determine if serum from *A. centrale* and the local *A. marginale* would produce agglutination of the Capillary Tube Agglutination (CA) antigen, prepared commercially in the U.S., from U.S. strains of *A. marginale*.

The complement fixation test, was conducted as described in Experiment 1 of this paper.

Materials and Methods

Antigen Production:

The preparation of CF antigens from infected blood has been described (39, 44, 79, 92, 104), and these techniques, while not used exclusively, did form the basis of our antigen production method. Generally, in order to produce successful antigens from *A. marginale* infections, very high parasitemias, approaching 100% are required. Such parasitemias rarely occur with EAC infections, suggesting a possible difficulty in producing antigens of sufficient titer to overcome anti-complementary properties of a blood antigen. A basic procedure was developed after a number of attempts, which has resulted in the production of successful CF antigens from both EAM and EAC infections, and even from animals showing a relatively low parasitemia. These general steps will be listed before the discussion of antigen preparation of each individual antigen:

1. Animals infected with anaplasmosis, showing a PCV of over 20% with a maximum parasitemia, were used. In the case of EAM infections, parasitemias of over 70% are common, but with EAC the maximum obtained was 42%.
2. Whole blood was collected in 12% sodium citrate solution (in physiological saline solution), at a ration of .5 ml citrate for 10 ml blood. At least 200 ml of blood is required for satisfactory antigen production using the equipment available. A micro technique could easily be developed.
3. The citrated blood may be refrigerated overnight, but further delays

at this stage are hazardous due to the fragility of infected red cells. This blood is centrifuged, and the red cells washed three times in cold physiological saline (.85% NaCl), using a table top centrifuge at speeds of approximately 2000 rpm's for 10 minutes. The plasma, and later saline, was drawn off with suction, and red cells resuspended in saline for successive washings. It was found that insufficient washing of the red cells at this time was associated with high levels of anti-complementary activity.

4. The volume of packed red cells is then estimated and added to 2.5 - 5.0 volumes of cold distilled water to produce cell lysis. At this step the lysed cells are stored at 5 C. overnight.

5. The lysed cells may show a gel like consistency when only 2.5 volumes of distilled water are used, and when allowed to stand overnight at 5C. This is easily broken up in a Waring blendor, or can be avoided by immediately centrifuging after cell lysis. The use of larger volumes of distilled water will also prevent the formation of this gel.

6. The lysed cells are now centrifuged. A model L-Spinco ultra centrifuge, using rotor #30, at 20,000 RPM's developing a force of 35,000 X gravity, for 45 minutes, is used. Centrifugation is carried out at refrigerator temperatures (2-5 C.). Attempts at centrifugation at ordinary speeds (2,000 - 3,500) resulted in disappointing yields of antigen.

7. The sediments are collected and resuspended in a convenient volume of veronal buffered physiological saline solution, pH7.2 (described in Experiment 1). There is a firm sediment and what

appears to be a colloidal interface just above. Both are collected in the veronal buffer.

8. The supernate is discarded, as being inactive in the CF test. This material was tested for activity and will later be described.

9. The suspended sediments are then ground in a Vir-Tis tissue homogenizer a minimum of two minutes at full speed. The grinding cups are packed with ice and water to prevent overheating during the grinding procedure.

10. This material is again centrifuged in the model L. Spinco ultra centrifuge at 35,000 X gravity for 45 minutes. After grinding, the excessive foam makes immediate centrifugation difficult. No loss of potency or activity has been observed in allowing the material, produced in step 9, stand in the refrigerator overnight.

11. The sediments are again collected. The colloidal interface, while apparently greatly diminished is now separated from the packed sediment. The supernate, which is still highly colored, is poured off. When the colloidal interface starts to come off this is collected separately. The surface of the packed sediment is gently washed with veronal buffer, the washing being combined with the interface sediment. A measurement of interface sediment was not made as it was contaminated with both supernate and washings from the packed sediment. Antigen titration of the interface sediment revealed a low level of

activity, and no anti-complementary activity. The packed sediment was weighed, and prepared as a 2.5% or 5% suspension in veronal buffer. This suspension was mixed in a Vir-Tis tissue homogenizer to ensure a uniform suspension. This preparation was an opaque suspension with only a slight pink color. There was far less suspended matter and color than with the U.S.D.A. antigen, markedly facilitating accurate readings of the CF test proper. This suspension was bottled and frozen at -20 C. for future testing. The sediment antigen was by far the most successful of those produced, and was used on all tests and titrations recorded.

Antigen EAC-9375:

Animal 9375, with a PCV of 22% and a 30% EAC parasitemia, was selected for our first attempt to produce an EAC antigen. In this instance red cells were washed two times, lysed in 5 volumes of distilled water, but otherwise treated in essentially the manner described, with approximately a 5% suspension being prepared as the final antigen. The antigen gave the following results on titration:

EAC-9375, .5 ml quantities.

Antigen dil:	1/1	1/2	1/4	1/8	1/16	1/32	1/64	No antigen
1/5 Pos. serum	4	4	4	2	Tr.	-	-	-
1/5 Neg. serum	4	4	1	Tr.	-	-	-	-
		(control)	U.S.D.A. antigen, .5 ml quantities					
1/5 Pos. serum	4	4	4	4	4	2	Tr.	-
1/5 Neg. serum	-	-	-	-	-	-	-	-

Positive serum used in this titration and all subsequent titrations, unless otherwise specified, was collected from EAM infected animals, and diluted with negative serum so as to give a positive reaction at a 1/10 dilution with U.S.D.A. antigen. Negative serum was a pooled sample from known anaplasmosis negative animals. Both positive and negative samples were phenolized by adding .1 ml of 5% phenolized saline to .9 ml serum, and stored at 3-5 C. These samples were tested for possible anti-complementary activity before use in titrations, and found to be free of any such activity. With the exception of the antigen dilutions used in this titration, the test was conducted as previously described. The readings of Tr., 1, 2, 3, and 4 are to be interpreted as follows:

4: No hemolysis

3: 1-25% hemolysis

2: 26 to 50%hemolysis

1: 51 to 85% hemolysis

Tr.: Less than 100% hemolysis but over 85%

-: 100% hemolysis.

These results clearly indicate that EAC-9375 antigen was anti-complementary, in that complement was fixed in the presence of negative serum and antigen. There was about a one tube difference in complement fixation between positive and negative serum which did indicate some specific fixation. An attempt at reducing the anti-complementary characteristics of this antigen was made by centrifugation, and

re-suspending the sediment in veronal buffer. A 10 ml aliquot was removed from the EAC 9375 antigen pool, and centrifuged 10 minutes at about 2500 rpm's in a small table top centrifuge. Some sediment was recovered wich was re-suspended in 10 ml. veronal buffer and labeled Sed-1. The supernate was collected and labeled S-1. A second 20 ml aliquot of EAC 9375 antigen was centrifuged 30 minutes at 3500 rpm's, with the temperature controlled at 5C. The supernate was collected and labeled S-2, the sediment was collected, resuspended in 15 ml veronal buffer and labeled Sed.-2. These four antigens (Sed.-1, S-1, Sed-2, S-2.) were diluted 1/2, 1/4, 1/8, and 1/16 and tested with negative and positive serum in an attempt to detect evidence of a more specific reaction, or a reduction in anti-complementary properties. The following results were obtained.

	Pos. Serum				Neg. Serum			
Antigen dil.:	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
Sed.-1	4	4	2	Tr.	4	3	1	-
S-1	-	-	-	-	-	-	-	-
Sed.-2	4	4	3	1	4	3	1	Tr.
S-2	-	-	-	-	-	-	-	-

This attempt to remove or reduce anti-complementary properties of 9375 antigen, even though unsuccessful, did show the absence of CF activity in the supernate, following relatively low speed centrifugation.

Antigen EAC-9573:

A second EAC antigen was prepared from 450 ml blood collected

from animal 9573 during an acute infection with anaplasma centrale, when the PCV was 20%, and the parasitemia was 32%. The red cells were washed only one time in physiological saline before lysis in equal parts of distilled water. The other steps in the preparation of this antigen followed those already described. The packed sediment, weighing 4.21 grams was suspended in veronal buffer making a 5% suspension. The antigen was labeled and frozen. A titration was made of this antigen with serum from an A. centrale infection as well as the usual EAM positive. Suspecting an anti-complementary reaction, it was thought that an early reading might show evidence of greater difference in complement fixation between positive and negative serum. The results of antigen titrations against serum from EAM, EAC infections and negative serum, read at 15 minutes and the standard 45 minutes are given below:

Reading after 15 minutes: on Antigen 9573:

Antigen dil.:	1/4	1/6	1/8	1/10	1/16	1/20	1/32	1/40	1/64
Neg. Serum	4	4	3	2	Tr.	-	-	-	-
Pos. Serum (EAM)	4	4	4	4	3	1	-	-	-
Pos. Serum (EAC)	4	4	4	4	3	1	-	-	-

Readings after 45 minutes

Neg. Serum	3	2	1	-	-	-	-	-	-
Pos. Serum (EAM)	4	4	4	3	-	-	-	-	-
Pos. Serum (EAC)	4	4	4	4	-	-	-	-	-

Again the EAC antigen appeared to have anti-complementary properties.

In this instance, however, a sufficient difference exists between positive and negative serum to suggest that this antigen might be useful at a 1/9 dilution (1/8) for comparative testing.

In early efforts at serum titrations with antigen EAC-9375, it was noted that a 3/ positive serum reaction on sera from EAC infected animals was observed with sera diluted as much as 1/12,000,000, even though a 1/5 negative sera only gave a Tr. or 1/ reaction. A saline control was then set up which produced a 3/ reaction in the presence of antigen, complement and hemolytic system. A titration of negative serum produced a 1/ reaction at 1/20 dilution but negative response at 1/5 and 1/10. These results suggested that the addition of normal serum to the diluting fluids used in serum and antigen titrations might result in a reduction of AC activity previously noted. Consequently an antigen titration of EAC-9573 was set up in which .1 ml normal serum was added to each tube in addition to the serum normally required for the test. The titration was set up with a 1/ 4 dilution of antigen EAC-9573 in veronal buffer, using different quantities, with the following results:

	(ml) EAC-9573								
1/4 antigen amount:	.05	.10	.15	.20	.25	.30	.35	.40	.45
Normal serum	.10	.10	.10	.10	.10	.10	.10	.10	.10
Veronal buffer	.85	.80	.75	.70	.65	.60	.55	.50	.45
Positive serum	.10	.10	.10	.10	.10	.10	.10	.10	.10
2 units complement	.50	.50	.50	.50	.50	.50	.50,	.50	.50
Incubate 37.5 C. 1 hour.									
Hemolytic system	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Incubate 37.5 C. 45 minutes.									
Reading:	-	-	2	3	4	4	4	4	4

Exactly the same protocal was followed for the negative serum control. In this instance .10 ml negative serum was substituted for the .10 ml positive serum. This gave a total of .2 ml normal serum in each tube.

Reading: - - - - - - - - -

The addition of normal serum appears responsible for reducing anti-complementary properties of EAC-9573 antigen. Successful serum titrations were made using 1/5 dilutions of EAC 9573 antigen in the presence of .1 ml normal serum, even though in the absence of serum this antigen was anti-complementary.

Antigen EAC-9754:

Because of the AC properties of EAC 9375 and EAC 9573, a third attempt was made to prepare a more satisfactory antigen. The low parasitemia, in animals used to make the two previous antigens, may have been responsible for low titers. An attempt was made in this instance to increase parasitemia by repeated massive intravenous inoculations of infected blood. Calf 9747 (splenectomized) was inoculated intravenously with 100 ml packed, washed red cells, suspended in 250 ml .85% NaCl. The red cells were collected from an EAC carrier showing a 4% parasitemia. Calf 9747 developed an 18% parasitemia in 28 days. At 30 days, with a PCV of 25%, and a parasitemia of 10%, 400 ml of blood was collected for the purpose of infecting a second calf. The red cells were washed one time, resuspended in 250 ml of .85% NaCl, and injected intravenously into splenectomized calf 9751. Calf 9751 showed a high parasitemia of 20%, 11 days after infection. Twelve days after infection when a 12% parasitemia and a 24% PCV existed, 9751 was bled for sub-inoculation into a third splenectomized

calf. Six hundred ml of blood was collected, washed one time, suspended in 300 ml of .85% NaCl, and injected intravenously into splenectomized calf 9754. Nine days after infection 9754 showed a 42% parasitemia and a 22% PCV. At this time 1100 ml of blood was collected in citrate for antigen production.

Red cells were washed in .85% NaCl three times, and the antigen prepared as previously described. Only 180 ml packed cells were used for antigen production. A firm sediment was removed after the last centrifugation, weighing 2.62 grams, and suspended in veronal buffer solution to give a 2.5% suspension. This suspension was ground in the Vir-Tis tissue homogenizer to produce the final antigen. The colloidal interface was collected separately, as previously described, ground in the Vir-Tis with veronal buffer, and tested for antigenic activity. A 1/6 (1/5) dilution of each antigen (sediment and interface) was made and tested using different antigen amounts as described in Experiment #1. Normal serum was not added as described for titrations on EAC-9573. The following results were obtained:

1/6 dilution of EAC-9754 Sediment antigen

	.05	.10	.15	.20	.25	.30	.35	.40	.45
Pos. serum (EAC)	Tr.	2	3	4	4	4	4	4	4
Pos. serum (EAM)	-	1	2	3	4	4	4	4	4
Neg. serum	-	-	-	-	-	-	-	-	-

Two units of EAC-9754 antigen was calculated as follows:

1 unit - .20 of a 1/6 dilution

2 units - .40 of a 1/6 dilution

The CF test requires the use of 2 units of antigen in a .5 ml volume, therefore: .40 : 6 (1/6) :: .5 : X

.40 X = 3.0

X = 7.5, or 1/7.5 (1/6.5), 2 units in .5 ml.

1/6 dilution of EAC-9754, interface antigen.

	.05	.10	.15	.20	.25	.30	.35	.40	.45
Pos. serum (EAC)	-	-	-	Tr.	2	2	3	4	4
Pos. serum (EAM)	-	-	-	Tr.	1	2	3	4	4
Neg. serum	-	-	-	-	-	-	-	-	-

This antigen failed to show any evidence of anti-complementary properties, and was further characterized by specific fixation of complement in the presence of the antibodies produced in both EAC and EAM infections. The interface antigen, while showing activity, was of

such low titer that it was not used. The sediment antigen was successfully used to titrate sera, without the addition of normal serum.

The obvious difference in virulence between infections produced by A. marginale, EAM and A. marginale, WAM, and BAM, suggested the need to prepare a CF antigen from the local anaplasma strain (EAM) for use against all three A. marginale infections, to determine if possible serological differences occur between marginale strains, and to serve as a control for locally produced A. centrale antigens, since our method of production is slightly different from the standard U.S.D.A. antigen.

Antigen EAM-9464:

Splenectomized calf 9464 was selected as a doner animal for antigen production, during the acute phase of anaplasmosis, when the PCV was 24%, and parasitemia was 76%. Five hundred ml. of blood was collected in citrate. Red cells were washed three times and antigen was prepared as previously described. The sediment was not weighed, but an approximate 5% suspension was prepared as the final antigen. The following titration was conducted using positive (EAM) and negative sera.

.5 ml EAM-9464 antigen

Antigen dilution	1/4	1/6	1/8	1/10	1/20	1/30	1/40	1/60	1/80
Pos. serum	4	4	4	4	3	2	Tr.	-	-
Neg. serum	-	-	-	-	-	-	-	-	-

Used at a 1/8 dilution.

This antigen, based on the above results, shows specific fixation of complement, and no evidence of anti-complementary properties.

Antigen EAM-9465:

A second EAM antigen was prepared for the primary purpose of testing several fractions for possible CF activity. It was hoped that if activity occurred in several fractions, these antigens might possess different properties and could then be tested for possible usefulness in differentiating EAC and EAM infections.

Splenectomized calf 9465 was selected at a time when it was showing an acute anaplasmosis reaction, with a PCV of 22% and an 82% parasitemia. Red cells were washed two times, lysed in 5 volumes of distilled water and centrifuged as previously described. Antigen 1 was a 2.5% suspension, in veronal buffer, of the final sediment, prepared as previously described. Antigen 2 was the supernate collected after the first centrifugation of lysed cells described in step 6 of the antigen production outline. Antigen 3 was the supernate collected after the last centrifugation described in step 10 of the antigen production outline. Antigen 4 consisted of a mixture of the firm sediment (#1) and the colloidal interface. This antigen (#4) represents an additional step to the preparation procedure already described. The two sediments were collected together, suspended in veronal buffer, ground in the Vir-Tis at full speed for two minutes, then centrifuged at high speed (35,000 X gravity) for 1 hour. The sediment was weighed and made into a 20% suspension in veronal buffer. Antigen 5 was the supernate collected following the centrifugation to collect sediment for antigen 4. Antigens 2, 3, and 5 are supernate preparations, and antigens 1 and 4 are

sediment preparations. Titrations were conducted on all five antigens using anaplasma positive and negative serum. The sediment antigens were used in .5 ml amounts, varying the dilution as previously described, and the supernate antigens were tested undiluted in .25 ml and .50 ml amounts.

RAM-9465 antigen, .5 ml amounts

Antigen dil.:	1/4	1/6	1/8	1/10	1/20	1/30	1/40	1/60	1/80
Antigen #1									
Pos. serum (RAM)	4	4	4	4	4	3	2	1	-
Neg. serum	1	Tr.	-	-	-	-	-	-	-
Antigen #4									
Pos. serum (RAM)	4	4	4	4	2	1	-	-	-
Neg. serum	-	-	-	-	-	-	-	-	-
Antigen #2		.25 ml		.50 ml.					
Pos. serum (RAM)		-		-					
Neg. serum		-		-					
Antigen #3									
Pos. serum (RAM)		-		-					
Neg. serum		-		-					
Antigen #5									
Pos. serum (RAM)		-		-					
Neg. serum		-		-					

These results indicate antigen #1 and antigen #4 produced specific fixation of complement in the presence of positive serum, with only minimal AC properties. The supernates collected from the various stages of antigen production were uniformly negative.

Several EAM antigens were prepared in which the red cells were washed only one time. In every instance the final antigens were anticomplementary as described for EAC 9375 and EAC 9573.

Antigen EAM-9745:

A final satisfactory EAM antigen was prepared from 9745, a splenectomized calf in the acute phase of EAm infection, showing a 20% PCV and a 70% parasitemia. The preparation of this antigen from 1000 ml bleed followed the procedure previously outlined. The final sediment weighed 4.0 grams, which was made into a 5% suspension. The titration was made on a 1/10 dilution using different amounts of antigen with positive and negative sera.

Antigen EAM 9745. (1/9)

Antigen amounts:	.05	.10	.15	.20	.25	.30	.35	.40	.45 ml.
Pos. serum (EAM)	2	4	4	4	4	4	4	4	4
Neg. serum	-	-	-	-	-	-	-	-	-

1 unit - .10 ml of 1/10

2 units - .20 ml of 1/10

We desire to have 2 units of antigen in .5 ml volume,

Therefore: .2 : 10 :: .5 : X

.2 X = 5.0

X = 25 (1 / 24). A 1/25 dilution contains 2 units antigen in .5 ml.

Test Procedure:

Forty five serum samples from animals of unknown anaplasmosis

history, 4 serum samples from known anaplasmosis carriers, and 6 serum samples from known negative animals were tested at a 1/5 serum dilution with U.S.D.A., EAM 9745 and EAC 9754 CF antigens to calculate the degree of agreement possible using antigens of different origins.

Antigens EAC 9754 and to a lesser extent EAC 9573, EAM 9464, EAM 9745, and U.S.D.A. (A. marginale) were used in titrating sera from animals with East African A. centrale (EAC), East African A. marginale (EAM), Nevada A. marginale (NAM) and Beltsville A. marginale (BAM) infections in an attempt to detect serological differences.

Comparative serum titrations of 26 animals infected with A. marginale have been made using U.S.D.A., EAM and EAC antigens. Of this number, two animals had BAM infections, three NAM infections and the remaining 21 had EAM infections. Of the 21 animals with EAM infections, all were titrated with U.S.D.A. antigen, seven were titrated with EAM 9464 antigen, four were titrated with EAM 9745, three were titrated with EAC 9573, and 14 were titrated with EAC 9745. The five animals with BAM and NAM infections were each titrated with U.S.D.A., EAM 9745 and EAC 9754 antigens.

Comparative serum titrations of 26 animals infected with A. centrale have been made using U.S.D.A., EAM and EAC antigens. Sera from six animals have been titrated with U.S.D.A., EAM 9464, and EAC 9573 antigens, and three others tested with U.S.D.A. and EAM 9464 only. Sera from 10 animals have been titrated with U.S.D.A., EAM 9745 and EAC 9754 antigens, and seven others were tested with U.S.D.A. and EAC 9754 only.

The capillary tube aglutination test (CA) was performed on 37 serum samples from animals infected with anaplasma organisms, and 12 samples from normal animals. Of the 37 sera from infected animals, eight were from EAM, seven from NAM and BAM, and 22 from EAC infections. The CA test used a commercially prepared A. marginale antigen.* following the procedure described in the literature (61, 109, 152).

An analysis of variance as described by Snedecor (135) was used to determine if significant differences in the percent agreement of the CF test using U.S.D.A., EAM and EAC antigens occur. In order to detect possible significant differences between serum titers, with different antigens, averages of the highest serum dilutions producing complete fixation in the CF test were recorded. The differences (paired comparisons) in serum titers produced by two antigens were recorded, and then a logrithmic transformation of these differences was analysed for the determination of "t" values and the possibility of error. This statistical calculation is described by Snedecor (135).

* Diamond Laboratories, DeMoines, Iowa.

Results

Table 10 tabulates the CF response of 55 serum samples tested with U.S.D.A., EAM 9745 and EAC 9754 antigens. No differences occurred in CF reactions using U.S.D.A. and EAM antigens, and only a very slight difference is recorded for the EAC antigen. Numerical values were assigned for the determination of percent agreement, as recorded in Table 11. The following is an example of how these calculations are made:

Antigens:	U.S.D.A.		EAM	Percent agreement
	-	or	-	100%
	4		4	"
	4		3	75%
	4		2	50%
	4		1	25%
	4		Tr.	12.5%
	4		-	0%

The same reading with both antigens regardless of the reaction was considered 100% agreement. Each variation of 1 in reading reduced the percent agreement by 25%. Comparisons are made on only two antigens at a time, and the average agreement recorded in Table 11.

Table 10

CF Reactions of 55 sera with U.S.D.A., EAM-9745 and EAC-9754 antigens

Antigen:	Positive (3+ and 4+)	Suspicious (1+ and 2+)	Negative (Tr. and -)
U.S.D.A.	21	14	20
EAM 9745	21	14	20
EAC 9754	20	16	19

EAM antigen produced a 96.1% agreement with U.S.D.A. antigen. This variation in agreement probably reflects the limitations of the test rather than differences in antigens. A somewhat lower agreement of 94.6% occurred when only those animals showing a 1/ or greater reaction were examined. The agreement between U.S.D.A. and EAC antigens was only 91.5% for all samples and 87.9% among animals with 1/ or greater CF response. This 87.9% proved to be significantly lower than the 94.6% agreement between U.S.D.A. and EAM antigen. No other significant differences occurred. Comparisons between EAM and EAC antigen showed agreement of 92.7% on all samples, and 90.3% on those samples showing a 1/ or greater reaction.

Table 11

Percent Agreement of CF Results Using Different Antigens

	EAM 9745	EAC 9754	Significance
U.S.D.A.:			
All samples	96.1%	91.5%	N.S.
1/ and greater reactions.	94.6%	87.9%	P < .05%
EAM 9745			
All samples		92.7%	
1/ and greater reactions		90.3%	

N.S.: Not Significant

P < .05: Probability of error less than .05

Tables 12 and 13 list the highest serum dilutions producing complete fixation of 2 units of complement in the CF test as previously described. There is considerable range in serum titers since samples were taken at various times in the course of infection. These samples tested are tabulated, as to the type of infection, and the antigen used in the test. Generally, higher titers appeared to occur with the homologous antigen.

The great variation dictates the need of using paired comparisons, and averages, to demonstrate differences. The results of averages and statistical analysis are recorded in Tables 14, 15, 16, and 17.

CF titers of sera from animals with EAM infections are averaged and analysed for significance in Table 14. The average serum titer with U.S.D.A. antigen was 1/80, whereas with EAC antigen it was only 1/34 or 2.34 times less. This difference proved highly significant. The average serum titer of 11 animals tested with U.S.D.A. antigen was 1/83, whereas these same sera tested with EAM antigen showed an average of 1/76 or 1.09 times less. This difference was not significant. The average serum titer of six animals tested with EAM antigen was 1/67. These same sera tested with EAC antigen showed an average of 1/50 or 1.34 times less. This difference was not significant.

Table 12

Serum CF titers of Animals Infected with *A. centrale*, Tested with U.S.D.A., EAM and EAC Antigens.

U.S.D.A.	EAM-9464	EAC-9573
1/40	1/40	1/80
1/80	1/80	1/320
1/80	1/80	1/320
1/80	1/80	1/640
1/20	1/20	1/640
1/20	1/20	1/160
1/5	1/10	
1/160	1/80	
1/20	1/40	
	EAM-9745	EAC-9754
1/80	1/80	1/320
1/40	1/40	1/80
1/5	1/5	1/20
1/160	1/160	1/640
1/20	1/20	1/40
1/40	1/40	1/160
1/5	1/20	1/40
1/80	1/80	1/160

Table 12 contd.

U.S.D.A.	EAM-9745	EAC-9754
1/320	1/320	1/640
1/80	1/80	1/160
1/160		1/320
1/80		1/320
1/160		1/320
1/160		1/160
1/320		1/640
1/320		1/320
1/320		1/320

Table 13

Serum CF Titers of Animals infected with A. marginale, Tested with U.S.D.A., EAM and EAC Antigens.

Type of Infection	U.S.D.A.	EAM-9464	EAC-9573
EAM:	1/80	1/80	1/80
	1/160	1/80	1/160
	1/80	1/80	1/80
	1/40	1/40	
	1/40	1/40	
	1/320	1/320	
	1/640	1/640	
		EAM-9745	EAC-9754
	1/80	1/80	1/40
	1/160	1/80	1/80
	1/40	1/40	1/20
	1/640		1/320
	1/1280		1/640
	1/160		1/80
	1/640		1/320
	1/640		1/320
	1/640		1/320
	1/20		1/5
	1/40		1/20

Table 13 contd.

Type of Infection	U.S.D.A.	EAM-9745	EAC-9754
EAM:	1/40		1/20
	1/40		1/20
	1/320	1/320	1/320
BAM:	1/1280	1/640	1/320
	1/640	1/320	1/160
NAM:	1/80	1/40	1/80
	1/160	1/320	1/160
	1/160	1/160	1/80

Table 14

Average Serum CF Titers of Animals Infected with *A. marginale* (EAM)

No. of Animals	Avg. titer U.S.D.A.	Avg. titer EAM	Avg. titer EAC	Difference	Significance
17	1/80		1/34	2.34 X	P < .001
11	1/83	1/76		1.09 X	N.S.
7		1/77	1/57	1.35 X	N.S.

CF titers of sera from animals with BAM and NAM infections are averaged and analysed for significance in Table 15. Only five such animals were tested and even though the same trends are noticed, with the highest titers occurring with U.S.D.A. and EAM antigens, none of the differences proved significant.

CF titers of sera from all animals showing A. marginale infections (BAM, NAM, and EAM) are averaged and analysed for significance in Table 16. These results include the data presented in Tables 14 and 15. The average serum titers of 22 animals tested with U.S.D.A. antigen was 1/90, whereas with EAC antigen it was only 1/41 or 2.19 times less. This difference proved highly significant. The average serum titer of 16 animals tested with U.S.D.A. antigen was 1/100, whereas the same sera tested with EAM antigen showed a titer of 1/87, or 1.15 times less. This difference failed to reach significance. The average serum titer of 12 animals tested with EAM antigen was 1/93. These same sera tested with EAC antigen showed an average of 1/74 or 1.25 times less. This difference was significant with the possibility of error being less than .05%.

CF titers of sera from animals with EAC infections are averaged and analysed for significance in Table 17. The average serum titer of 23 animals tested with U.S.D.A. antigen was 1/31, whereas with EAC antigen it was 1/122 or 3.9 times greater.

Table 15

Average Serum CF Titers of Animals Infected with *A. marginale*, NAM and BAM.

No. of Animals	Avg. titer U.S.D.A.	Avg. titer EAM	Avg. titer EAC	Difference	Significance
5	1/183		1/123	1.49 X	N.S.
5	1/183	1/128		1.43 X	N.S.
5		1/128	1/123	1.04 X	N.S.

Table 16.

Average Serum CF Titers of Animals Infected with *A. marginale*, NAM BAM, and EAM.

No. of Animals	Avg. titer U.S.D.A.	Avg. titer EAM	Avg. titer EAC	Difference	Significance
22	1/90		1/41	2.19 X	p. < .001
16	1/100	1/87		1.15 X	NS
12		1/93	1/74	1.25 X	P < .05

This difference was highly significant. The average serum titer of 19 animals tested with U.S.D.A. antigen was 1/20, whereas the same sera tested with EAM antigen showed an average of 1/27, or 1.35 times greater. This difference was not significant. The average serum titer of 16 animals tested with EAM antigen was 1/29. These same sera tested with EAC antigen showed an average of 1/100 or 3.45 times greater. This difference was highly significant.

Table 17

Average Serum CF Titer of Animals Infested with A. centrale

No. of Animals	Avg. titer U.S.D.A.	Avg. titer EAM	Avg. titer EAC	Difference	Significance
23	1/31		1/122	3.9 X	P < .001
19	1/20	1/27		1.35 X	N.S.
16		1/29	1/100	3.45 X	P < .001

These results, when considered together, clearly show the pattern of serum specificity, notwithstanding the presence of common antigens. Animals with EAC infections show the largest difference between serum reaction to A. marginale and A. centrale antigens. Animals with A. marginale reactions, while showing higher serum titers with the homologous antigen, did not show as great a difference as occurred with homologous antigens in A. centrale infections. An analysis of these differences failed to show any significance. In most instances of A. marginale infections, EAM antigen resulted in slightly lower titers when compared to U.S.D.A. antigen, but in no instance was it possible to show any significance to these differences. It is probable that the much clearer, almost colorless, EAM antigen resulted in the visual

detection of smaller amounts of hemolysis, whereas this degree of hemolysis in U.S.D.A. antigen might have been mistaken for antigen color. No evidence of serological or antigenic difference could be detected between the three A. marginale strains.

Table 18 records the CA results of 49 serum samples classified as EAM, BAM,NAM, EAC infections and normals. All strains of anaplasma infections reacted to the CA antigen, which is a U.S. strain of A. marginale, although different in origin than that strain used in the preparation of CF antigen. It was interesting to note the greater incidence of 3/ and 4/ reactions in animals with A. marginale infections, and the very noticeable shift to 1/ and 2/ reactions in sera from EAC infections. The CA test again clearly demonstrates the common antigenic features of EAC and EAM infections, but these results suggest that CA titrations may well show a pattern of specificity similar to that found in the CF test. Insufficient CA antigen prevented the continuance of this investigation.

Table 18

Capillary Tube Agglutination Test on Sera from EAM, BAM, NAM, and EAC Infections.

Type of Infect.	No. of Animals	CA reaction. Negative	1/	2/	3/	4/
EAM	8	0	0	0	3	5
BAM-NAM	7	1	0	2	2	2
EAC	22	1	7	7	3	4
Neg.(Cont.)	12	11	1	0	0	0

(Exp. 3) Conclusions:

Successful CF antigens from A. centrale, and East African A. marginale strains were prepared, which, in some respects, proved more desirable than the standard U.S.D.A. antigen, used as a basis of much of this work. It was possible by centrifugation to eliminate most of the color and to concentrate the antigen. Antigens were prepared with titers several time higher than U.S.D.A. antigen. The CF activity was found entirely in the sediments with no CF antigen being demonstrable in the supernate, suggesting that the antigen is particulate in nature, and not soluble. Anticomplementary activity, of some A. centrale antigens, was reduced by the addition of normal serum in the test proper. This procedure made it possible to use some A. centrale antigens which would otherwise have been anticomplementary.

CF tests conducted in this section show that the average low serum titers observed in Experiment 2 among animals infected with A. centrale are not necessarily the result of reduced virulence, or a low grade infection, but more probably the result of specific serological differences resulting from exposure to different antigens. These differences suggest that A. centrale should be considered a distinctly separate strain or specie of anaplasma, notwithstanding the admitted close relationship of these two organisms.

Anaplasma marginale antigens prepared both in the U.S., and those produced locally, showed similar reactions with sera collected from animals infected with both U.S. and East African A. marginale. Both the U.S. and East African A. marginale gave evidence of different antigenic components when compared with A. centrale, CF antigens.

Without serum titrations these differences are not easily apparent, with a high percentage agreement existing among all antigens when tested against 1/5 dilutions of negative and positive (both A. marginale, and A. centrale) sera.

The differences in serum titers of animals infected with A. centrale, and A. marginale are generally greater with MAC antigen, than they are with marginale antigen. These observations, while not supported by cross absorption studies, suggest the presence of a more complex or an additional antigen in A. centrale than that which occurs in A. marginale.

Experiment #4: Cross Infectivity Trials using *Anaplasma marginale* and *Anaplasma centrale*.

Theiler (143), in his early work, stated that a previous infection with *A. centrale* did not in all cases prevent a subsequent *A. marginale* infection, but rather, reduced the severity of the infection. Sergent et al (129) recognised *A. centrale* as a distinct pathogen which failed to confer any immunity against subsequent infections with *A. marginale*. Sergent's conclusion was undoubtedly correct if immunity is considered in the strict sense of being a state or condition of complete resistance to subsequent exposure to a specific pathogen. On the other hand, most workers (22, 64, 84, 127, 140, 145, 146) have found that pre-infection with *A. centrale* will reduce the severity of *A. marginale*, thus producing some degree of immunity. Cross infectivity trials previously reported in the literature have been concerned mainly with the *A. marginale* reaction produced in cattle previously premunised with *A. centrale*. The results of these trials have been reported in terms of general clinical response without apparent attempts at statistical evaluation of measured blood changes and serological response to such infections. The general purpose of this experiment is to measure incubation time, packed red cell volume (PCV), parasitemia, temperature, and complement-fixation response in premunized cattle challenged by blood inoculation with homologous and heterologous anaplasma organisms. Cattle originally premunised with each *A. marginale* and *A. centrale* were subsequently infected with homologous and heterologous infections, and the above

factors were recorded and analysed for significance to determine what degree of immunity or resistance might be expected in animals so challenged. These reactions to challenge were observed in four groups of cattle: splenectomized calves, non-splenectomized calves, yearling cattle, and adult cattle.

Materials and Methods

Procedures:

The procedures used in conducting the tests and observations made on animals exposed to a second anaplasma reaction have been previously described in experiments 1, 2, and 3. The average time, expressed as days, following the first evidence of primary infection, to the date when challenge occurred, is recorded under the heading "Days PPI" (days post primary infection). The incubation time in challenged animals is determined by numbering the days after exposure till such time as some evidence of the challenge infection occurs. In some instances, where no apparent reaction occurs, this measurement was not made. All other factors recorded in summary tables are self explanatory, or have previously been discussed. Most of the animals used in these trials have previously been used in experiment #2, and in these experiments were challenged with the heterologous and homologous anaplasma organisms. The initial reactions to the primary infections served as controls for challenged animals, in the statistical comparisons of susceptibility.

Slenectomized Calves:

A total of 17 cross infectivity trials were conducted on 17 splenectomized calves, with 20 splenectomized calves serving as

infected controls.

Twelve splenectomized calves (9279, 9280, 9362, 9365, 9373, 9375, 9552, 9571, 9700, 9747, 9751, 9754) which had previously been exposed to A. centrale and had apparently recovered, were challenged by inoculating, subcutaneously, 5 ml of blood from an A. marginale (East African origin EAM) carrier animal an average of 94 days post primary infection (PPI). Of these 12 calves, one was challenged with blood showing a 1% parasitemia, one with blood showing a 2% parasitemia, two with blood showing a 4% parasitemia, one with blood showing a 7% parasitemia, two with blood showing a 76% parasitemia and five with blood showing an 85% parasitemia.

Four splenectomized calves (9549, 9551, 9554, 9376), which had previously been exposed to A. marginale (the mild Beltsville, and Nevada strains, BAM, and NAM), and had apparently recovered, were given a homologous challenge by inoculating subcutaneously 5 ml of blood from an A. marginale (EAM) carrier animal an average of 74 days PPI. All four animals received a challenge with blood showing less than 1% parasitemia.

Eleven splenectomized calves (9277, 9278, 9361, 9364, 9457, 9458, 9459, 9460, 9470, 9553, 9572) served as controls for these two groups, and were infected by injecting infective material from EAM carriers. Of these 11 calves, one received an infecting inoculum consisting of 5 ml cell free plasma, two were infected by injecting subcutaneously 5 ml blood showing less than 1% parasitemia, one with blood showing a 5% parasitemia, four with blood showing a 12% parasitemia, two with blood showing a 44% parasitemia and one with blood showing

approximately 100% parasitemia.

One splenectomized calf (9555), which had previously been exposed to A. marginale (EAM), and had apparently recovered, was challenged by inoculating subcutaneously 5 ml of blood from an A. centrale (EAC) carrier showing a 5% parasitemia, 81 days PPI. Nine splenectomized calves (9212, 9279, 9280, 9373, 9375, 9487, 9747, 9751, 9754) served as controls to this one animal, and infected by inoculating blood from EAC carriers. Of these nine calves, six received subcutaneously, infections of 5 ml of blood showing EAC parasitemias ranging from less than 1% to 34%. Three of these calves were given intravenous injections of 100 to 120 ml packed red cells showing 4% to 12% EAC parasitemias.

Non-splenectomized Calves:

A total of 10 cross infectivity trials were made on non-splenectomized slaves, with an equal number of non-splenectomized calves serving as infected controls.

Six non-splenectomized calves (9369, 9370, 9374, 9461, 9462, 9463), which had previously been exposed to A. centrale and had apparently recovered, were challenged by inoculating, subcutaneously, 5 ml of blood from A. marginale (EAM) carriers, an average of 120 days PPI. Of these six calves, five were challenged with blood showing a 76% parasitemia, and one was challenged with blood showing a 1% parasitemia. Four non-splenectomized calves (9363, 9692, 9693, 9694) served as infected controls to this group and were injected subcutaneously with 5 ml blood from EAM carriers. Of these four calves, three were infected with blood showing 4% parasitemia and one was infected with

blood showing a 12% parasitemia.

These same four non-splenectomised calves (9363, 9692, 9693,9694), following apparent recovery, were challenged by inoculating, subcutaneously, 5 ml of blood from A. centrale carriers an average of 131 days PPI. Of these four calves, one was challenged with blood showing less than 1% parasitemia, one with a 1% parasitemia, and two with blood showing a 5% parasitemia. Six non-splenectomised calves (9369, 9370, 9374, 9461, 9462, 9463) served as infected controls to this group and were injected subcutaneously with 5 ml blood from EAC carrier. Of these six calves, four received blood showing 12% parasitemia and two were infected with blood showing 34% parasitemia.

Yearling cattle:

A total of 13 cross infectivity trials were made on yearlings, with an equal number of cattle serving as infected controls.

Five yearling cattle (9218, 9565, 9568, 9574, 9577), which had previously been infected with A. centrale and had apparently recovered, were challenged by inoculating subcutaneously,5 ml of blood showing a 3% EAM parasitemia, an average of 57 days PPI. Six yearling cattle (9223, 9271, 9557, 9567, 9575, 9576) served as infected controls for this group. These controls were each infested by injecting subcutaneously, 5 ml of blood showing a 7% EAM parasitemia.

Four yearling cattle (9557, 9567, 9575, 9576), which had previously been infected with A. marginale(EAM) and had apparently recovered, were challenged by inoculating subcutaneously 10 ml blood showing a 3% EAC parasitemia, an average of 59 days PPI. Seven yearling cattle (9218, 9229, 9424, 9565, 9568, 9574, 9577) served as controls to this group and were infected by injecting subcutaneously 5 ml of blood

showing a 6% EAC parasitemia.

Four yearling cattle (9229, 9424, 9223, 9271) were given a homologous challenge following recovery from primary infections. Calves 9229 and 9424, having recovered from an A. centrale infection, were challenged by injecting subcutaneously 10 ml blood showing 3% EAC parasitemia, an average of 56 days PPI. Calves 9223, and 9271, having recovered from an A. marginale (EAM) infection, were challenged by injecting subcutaneously 5 ml blood showing 3% EAM parasitemia, an average of 58 days PPI. The same controls used in heterologous challenge served these two groups.

Adult cattle:

A total of 22 cross infectivity tests were conducted in adult cattle with 21 cattle serving as infected controls.

Fourteen adult cattle (9474, 9476, 9477, 9478, 9778, 9785, 9788, 9790, 9791, 9795, 9825, 9833, 9834, 9838), which had previously been infected with A. centrale and had apparently recovered, were challenged by inoculating subcutaneously 5 ml of blood from A. marginale carriers, an average of 55 days PPI. Of these 14 adult cattle, four were challenged with blood showing an 18% EAM parasitemia, and the remaining ten were challenged with blood showing a 15% EAM parasitemia. Four cattle (9782, 9806, 9822, 9828), having previously recovered from a naturally occurring A. marginale infection, were given a homologous challenge by inoculating subcutaneously 5 ml blood showing a 15% EAM parasitemia. Seven adult cattle (9380, 9382, 9387, 9388, 9475, 9479, 9855) served as controls for these two groups. Two of these cattle were infected by inoculating subcutaneously 5 ml blood showing 18% EAM parasitemia, and the remaining 5 were infected with blood showing a 15% EAM parasitemia.

Four adult cattle (9777, 9811, 9828, 9836), having previously recovered from a naturally occurring A. marginale infection, were challenged by inoculating subcutaneously 5 ml blood showing a 5% EAC parasitemia. Infected controls for this group consisted of the same 14 cattle previously mentioned (9474, 9476 etc.....). These animals were originally infected by injecting subcutaneously 5 ml blood showing a 5% EAC parasitemia.

Individual animal charts on all animals challenged are recorded in Appendix II of this paper. Animal charts on those animals serving as controls are recorded in Appendix I of this paper. These animal charts record individual animal reactions to challenge and initial infection. Tables 18, 19, 20, and 21 summarize and present the statistical evaluation of these reactions.

In the summary tables a mean average of the reciprocal of the CF titers is presented, in contrast to a mode previously used. A logrithmic transformation was used in the analysis of variance to determine significant difference between CF titers of different groups. Procedures for the calculation of standard deviations and analysis of variance are presented by Snedecor (135).

Results:

Splenectomised calves:

The results of cross infectivity trials and those of primary single anaplasma infections in splenectomised calves are summarised in Tables 19 and 20.

Of 12 splenectomised calves with a primary East African A. centrale (EAC) infection, 10 showed some evidence of reacting to the East African A. marginale challenge (EAC X EAM). Three of the 10 reacting animals died as the result of EAM challenge, as compared to 11 deaths out of 11 in the control group receiving only a single EAM infection. Summarised data is presented for the 12 animals composing this group in Table 19.

Four splenectomised calves received essentially a homologous challenge. These calves, having recovered from the mild Beltsville (BAM) and Nevada (NAM) strains of A. marginale, were then challenged with East African A. marginale (NAM-BAM X EAM). One of the four calves failed to show any detectable changes in blood patterns that might indicate infection as the result of the challenging organism. In the other three animals reactions to challenge were questionable, consisting of slightly lower PCV's and a slight increase in parasitemias. No temperature reactions occurred. There is a problem of differentiating a specific reaction due to the EAM challenge and the possibility of natural mild relapses of the original A. marginale infection, which are known to occur, particularly in splenectomised

calves. For this reason, all three calves showing some reaction are considered questionable. Averages for all four animals were included in the statistical analysis comparing the effects of inoculating EAM infected blood in calves previously infected with EAC, BAM, NAM, The control EAM animals had no previous anaplasma infection.

The difference in pre-infection and pre-challenge PCV reflects the influence of anaplasma infections on the two previously infected groups as compared to the controls. The incubation time of 32 days among the 10 reacting animals (EAC X EAM) proved significantly longer than the 18 day incubation time for the EAM controls. It was impossible to determine an incubation time in those animals showing no reaction, or only a questionable reaction, hence the single comparison. An average low PCV of 17.9% occurred in the EAC X EAM group, 25.0% in the BAM-NAM X EAM group, and 9.0% in the control EAM group. These differences proved significant. In addition to the significant difference between the control and the two previously infected groups, the difference between the EAC X EAM and BAM-NAM X EAM groups proved highly significant, falling within the range of the least significant difference. An average of the low percent of the pre-infection and pre-challenge PCV showed that 58.0% occurred in the EAC X EAM group, 80% in the BAM-NAM X EAM group and 23% among the EAM controls. These differences were highly significant, following exactly the same pattern as the PCV differences except that the F values are somewhat higher. The persistence of anemia was calculated only in the EAC X EAM and NAM-BAM X EAM groups, because of the 100% mortality among the EAM controls. It was found that the average

persistence of anemia of 15.0 days in the EAC X EAM group was not significantly greater than the period of 3.7 days among the EAM-NAM X EAM group. An average high parasitemia of 80% occurred among the EAM control group, in contrast to 25.9% among the EAC X EAM group and 4.2% among the EAM-NAM X EAM group. Only the difference between the control EAM and the two previously infected groups was significant. An average high CF titer of 1/666 was observed in the EAM group which proved significantly higher than the average titers of 1/71 and 1/325 observed in groups EAM-NAM X EAM and EAC X EAM respectively. No abnormal temperature response occurred among the four animals with the homologous challenge. Elevated temperatures occurred in the other two groups. It was found that the EAM controls gave a significantly higher temperature response than did the EAC X EAM group.

The one splenectomized calf recovering from an A. marginale (NAM) infection and subsequently challenged with A. centrale (NAM X EAC) showed evidence of a marked reaction. Even though statistical evaluation of the differences between this animal (NAM X EAC) and the controls (EAC) are impossible due to the lack of duplication, the two groups show almost identical patterns of reaction with the exception of incubation time, which appears longer in the animal receiving the EAC challenge (NAM X EAC).

Non-splenectomized calves:

The results of cross infectivity trials and those of primary, single anaplasma infections in non-splenectomized calves are summarized in Tables 21 and 22.

Table 19

A. centrale and *A. marginale* (BAM and NAM) Carriers Challenged with *A. marginale* (EAM) in Splenectomized Calves

	EAC X EAM	NAM-BAM X EAM	EAM	Significance and LSD:
No. of Animals	12	4	11	
Animals Showing Apparent Reactions	10	3(?)	11	
Days PPI	89 (66-234)	74 (68-81)		
Pre-Inf. and Pre-Chall. PCV	30.6% ± 4.2	30.2% ±1.7	40.0% ± 3.0	P <.01
Incubation Time (in days)	32.4 ±15.2	(?)	18.0 ±7.0	P <.05
Low PCV	17.9% ±7.8	25.0% ± 4.6	9.0% ±0.0	P <.01 6.8
% of Pre-inf. and Pre-chall. PCV	58.0% ± 22.1	80.0% ±14.0	23.0% ±2.0	P <.01 -19.2
Persistence of Anemia (in days)	15.0 ± 16.0	3.7 ± 2.4	-	NS.
High Parasitemia	25.5% ± 30.8	4.2% ±3.0	80.0% ± 22.0%	P <.01 31.1
High CF Titers	1/335 ± 1/712	1/71 ± 1/66	1/666 ±1/496	P <.05*
Temperature Response	103.6 ± 1.5	N.R.	105.3 ±1.1	P <.01
Deaths.	3	0	11	

* The difference in CF titers of 1/335 and 1/71 is not significant, whereas the differences between 1/666 and 1/335; and 1/666 and 1/71 are significant

Abreviations used:
EAC X EAM: Animals were premunized with EAC, and challenged with EAM.
LSD: Least significant difference.
Days PPI: Days after (Post) Primary Infection, that challenged took place.
Pre-inf., Pre-chall.: Pre-infection and Pre-challenge.
N.R.: No response.
± : Standard deviation.
(?): Questionable reaction.
NS : Not significant.

Table 20.

A. marginale (MAM) Carrier Challenged with *A. centrale* (EAC) in a Splenectomized Calf

	MAM X EAC	Control EAC
No. of Animals	1	9
Animals showing apparent Anaplasma reactions	1	9
Days PPI	81	
Pre-Inf., Pre-Chall. PCV	31.0%	37.0% ± 5.2
Incubation time (days)	38.0	17.0 ± 8.9
Low PCV	15%	16.0% ± 2.6
% of Pre-inf. & Pre-chall. PCV	48%	44.0% ± 8.5
Persistence of anemia (days)	14	15 ± 6.9
High parasitemia	20%	36.0% ± 18.9
High CF titers	1/160	1/244 ± 1/182
Temperature response	N.R.	104.0 ± 1.3
Deaths.	0	0

Of six non-splenectomized calves with a primary EAC infection, only three showed evidence of reaction, and none died, when an A. marginale challenge was given (EAC X EAM). Because of the possibility of non-detectable reactions, averages for the six animals so challenged were compared with the 4 EAM controls to determine possible significant differences. Pre-infection and pre-challenge PCV's were practically the same. The incubation time was characteristically lengthened to 35 days in the EAC X EAM group, as compared to 9 days in the controls. However, this difference did not prove significant, probably in part, because of the small numbers involved, but also because of the large individual variation. The average PCV of the six animals in the EAC X EAM group was 25.5% whereas a control EAM average in non-splenectomized calves was 18% which was significantly lower, with a probability of error less than .05. A similar finding was observed with the percent of pre-infection, pre-challenge PCV, in that significantly lower values occurred in the control EAM group. The average time in which PCV's remained below 81% was 12 days in the EAC X EAM group, and 24 days in the control EAM group. This difference was significant. An average parasitemia of 1.4% was observed in EAC X EAM group with the control EAM group showing an average of 22% parasitemia. The small numbers, and high variation prevented these differences from reaching significance. The CF titer with the EAM group averaged 1/800, whereas in the EAC X EAM group the average was 1/110 which proved to be significantly lower. No temperature response occurred in the EAC X EAM group, in marked contrast to the high average temperature of 105.0 observed in animals of the control group.

Table 21

A.centrale Carriers Challenged with A. marginale (KAM) in Non-splenectomized Calves

	KAC X KAM	Control KAM	Significance
No. of Animals	6	4	
Animals showing apparent Anaplasma reactions	3	4	
Days PPI	120 (81-206)		
Pre-inf., pre-chall. PCV	36.0% ± 1.7	37.0% ± 3.6	NS.
Incubation time (days)	35.0 ± 13.5	9.0 ± 2.4	NS.
Low PCV	25.5% ± 4.9	18.0% ± 3.9	P.<.05
% of Pre-inf. & pre-chall. PCV	70.0% ± 11.4	49.0% ± 14.4	P. <.05
Persistence of Anemia (days)	8.7 ± 7.8	24.0 ± 11.9	P <.05
High Parasitemia	1.4% ± 2.4	26.0% ±22.0	NS.
High CF titers	1/110 ± 1/162	1/800 ± 1/320	P. <.05
Temperature response	N.R.	105.0 ± 1.0	
Deaths:	0	0	

NS: Not significant.

All four non-splenectomized calves with a primary EAM infection reacted to an EAC challenge(EAM X EAC). Statistical comparisons with 6 animals reacting to a primary EAC infection showed only a very few significant differences. The incubation time was significantly greater in the EAM X EAC group, being 38 days, as compare to 11 days for the controls. All other factors were so nearly the same that significant differences could not be demonstrated, supporting the evidence observed with splenectomized calves, that A. marginale does not give the same protection against A. centrale that A. centrale produces against A. marginale.

Yearling Cattle:

The results of cross infectivity trials and those of primary, single anaplasma infections in yearling cattle are summarized in Tables 23, 24 and 25.

Among this group of animals about the only significant effect produced by either a primary A. centrale or A. marginale infection, when followed by a later heterologous challenge, was a longthening of the incubation time. All five animals challenged with A. marginale after having recovered from A. centrale infection (EAC X EAM) showed some evidence of reaction. The incubation time in the EAC X EAM group was 24 days in contrast to 7.5 days in the control EAM group. This difference was highly significant. Table 23 records all other average values, none of which show any significant differences, indicating that the reactions produced by A. marginale (EAM) challenge were essentially the same in calves previously premunized with A. centrale as in calves with no known previous exposure to anaplasma infections.

Table 22.

A. marginale (EAM) Carriers Challenged with A. centrale (EAC)
in
Non-splenectomised Calves

	EAM X EAC	Control EAC	Significance.
No. of Animals	4	6	
Animals showing apparent Anaplasma reactions	4	6	
Days PPI	131 d. (79-184)		
Pre-inf., Pre-chall. PCV	33.0% ± 1.9	35.0% ± 4.2	NS.
Incubation time (days)	38.0 d. ± 19.6	11.0 d. ± 2.0	P < .01
Low PCV	23.0% ± 3.7	22.0% ± 6.6	NS.
% of Pre-inf. & pre-chall. PCV	69.0% ± 7.8	62.0% ± 16.8	NS.
Persistence of anemia (days).	13.0 d. ± 9.3	6.5 d. ± 5.9	NS.
High Parasitemia	2.75% ± 1.0	6.0% ± 8.3	NS.
High CF titers	1/101 ± 1/74	1/453 ± 1/446	NS.
Temperature response	N.R.	103.3	
Deaths	0	0	

All four yearling cattle reacted to A. centrale challenge after having recovered from a previous A. marginale infection (Table23). The reactions showed no differences from a primary infection with A. centrale, with the exception of a significant increase in incubation time, and a significantly higher temperature reaction. The incubation time in the EAM X EAC group was 25 days as compared to 9.1 days in the control EAC group. The average temperature response in the EAM X EAC group was 104.9 whereas in the EAC control group the average was only 103.3. These differences proved significant.

Two yearling cattle with a primary EAM infection (EAM X EAM) and two yearling cattle with a primary EAC infection (EAC X EAC), were given a homologous challenge (Table 25). Other than a temperature response of 104.5 in the EAM X EAM group, no evidence of specific reaction could be detected in any of the four animals.

Adult Cattle:

The results of cross infectivity trials and those of primary, single anaplasma infections in adult cattle are summarized in Tables 26, 27, and 28.

Of 14 adult cattle challenged with A. marginale after having first experienced an A. centrale infection (EAC X EAM), only five gave detectable evidence of response to challenge. No deaths occurred in the EAC X EAM group whereas one of seven died among the controls (EAM). The average values for these 14 cattle can be compared with the 5 reacting animals, and are examined in Table 26 for statistically significant differences with seven control animals reacting to a primary A. marginale infection (EAM). Pre-infection and pre-challenge PCV's are not significantly different.

Table 23.

A. centrale Carriers Challenged with A. marginale (EAM)
in
Yearling Cattle.

	EAC X EAM	Control EAM	Significance
No. of animals	5	6	
Animals showing apparent Anaplasma reactions	5	6	
Days PPI	57 (55-58)		
Pre-inf., pre-chall. PCV	33.0% ± 1.9	36.0% ± 2.6	NS
Incubation time (days)	24.0 ± 9.2	7.5 ± 1.2	P < .01
Low PCV	21.0% ±7.5	20.0% ±3.5	NS
% of pre-inf. and pre-chall. PCV	63.0% ± 22.5	56.0% ±9.5	NS
Persistence of anemia (days).	12.0 ±7.0	11.0 ± 6.9	NS
High parasitemia	6.0% ± 5.0	7.0% ±6.0	NS
High CF titers	1/384 ± 1/242	1/800 ± 1/392	NS
Temperature response	103.3 ± 1.8	104.1 ± 1.1	NS
Deaths	0	0	

Table 24

A. marginale (EAM) Carriers Challenged with *A. centrale* (EAC)

in

Yearling Cattle

	EAM X EAC	Control EAC	Significance
No. of Animals	4	7	
Animals showing apparent Anaplasma reactions	4	7	
Days PPI	59 (58-61)		
Pre-inf., pre-chall. PCV	31.0% ±.8	36.0% ± 1.7	P < .01
Incubation time (days)	25.0 da. ±1.7	9.1 da. ±2.0	P <.01
Low PCV	22.0% ±2.2	22.0% ±3.6	NS
% of pre-inf. and pre-chall. PCV	71.0% ±5.0	61.0% ±10.5	NS
Persistence of anemia (days).	10.0 da. ±2.5	13.0 da. ± 6.0	NS
High parasitemia	3.5 da. ± .6	4.0% ±1.9	NS
High CF titers	1/240 ±1/92	1/217 ±1/100	NS
Temperature response	104.9 ±1.2	103.3 ± .9	P <.05
Deaths.	0	0	

Table 25.

A. marginale (EAM) and A. centrale (EAC) Homologous Challenge in Yearling Cattle.

	EAM X EAM	EAC X EAC
No. of animals	2	2
Animals showing apparent Anaplasma reactions	0	0
Days PPI	58	58
Pre-inf., pre-chall. PCV	36%	35%
Incubation time (days)	N.R.	N.R.
Low PCV	34%	34%
% of pre-inf. and pre-chall. PCV	92%	96%
Persistence of anemia (days)	0	0
High parasitemia	0	0
High CF titers	1/120	1/22.5
Temperature response	104.5	N.R.
Deaths	0	0

N.R.: No Reaction.

Note: No detectable reactions were produced in these trials.

Primary infection controls listed on Tables 23 and 24

Incubation time, which is calculated only on those animals showing evidence of challenge reaction, is significantly increased in the EAC X EAM group, being 25 days as compared to 11 days for the controls. An average low PCV of 30% in the EAC X EAM group is significantly higher than the 18% occurring among the controls. The percent of pre-infection and pre-challenge PCV's follows the same pattern of difference and significance, being 86% in the EAC X EAM group and 46% in the control EAM group. There was an average 6 day persistence of anemia among animals of the EAC X EAM group and 21 days in the control group which was significantly different, indicating that a longer period of time was required for recovery with a primary A. marginale infection than occurred in the group first premunized with A. centrale then given A. marginale infection. The average parasitemia was relatively low in both the EAC X EAM (,41%) and EAM (4.0%) groups, but a significantly higher parasitemia did occur in the EAM group. Temperatures were not measured in adult animals.

All four animals challenged with A. centrale, after having recovered from a previous A. marginale infection, showed evidence of reaction to challenge (Table 27). When compared with 14 controls reacting to a primary A. centrale infection, it was again noted that the incubation time was significantly increased in the EAM X EAC group, being 37 days, as compared to 15 days for the controls. No further significant differences in the course of A. centrale reactions in adult cattle could be detected among the two groups, although generally the infection appeared slightly milder in the EAM X EAC group.

A homologous challenge (EAM X EAM) of four adult cattle with a naturally acquired A. marginale infection, again showed such animals to be entirely refractory to re-infection, by the same anaplasma.

Table 26.

A. centrale Carriers Challenged with *A. marginale* (EAM)

in

Adult Cattle

	EAC X EAM	Control EAM	Significance
No. of animals	14	7	
Animals showing apparent Anaplasma reactions	5	7	
Days PPI	55 (49-70)		
Pre-inf., pre-chall. PCV	35.0% ± 4.4	39.0% ± 3.6	
Incubation time (days)	25.0 ± 11.3	11.0 ± 2.0	P <.01
Low PCV	30.0% ± 5.6	18.0% ± 5.5	P <.01
% of Pre-inf., and pre-chall. PCV	86.0% ± 11.6	46.0% ± 11.5	P <.01
Persistence of anemia (days).		21.0 ± 7.5	P <.01
High parasitemia	.41% ± .72	4.0 ± 4.1	P <.05
High CF titers	1/65 ± 1/119	1/1005 ± 1/342	P <.01
Temperature response	NT	NT	
Deaths.	0	1	

NT: Not tested.

Table 27.

A. marginale (EAM) Carriers Challenged with A. centrale (EAC)
in
Adult Cattle.

	EAM X EAC	Control EAC	Significance
No. of animals	4	14	
Animals showing apparent Anaplasma reactions	4	14	
Days PPI	NT		
Pre-inf., pre-chall. PCV	36.7% 4.0	36.2% 3.5	
Incubation time (days)	37.0 d. 14.0	15.0 d. 4.7	P .01
Low PCV	31.0% 5.7	26.0% 5.5	NS.
% of Pre-inf., and Pre-chall. PCV	85.0% 12.0	72.0% 12.0	NS.
Persistence of anemia (days).	1.7 d. 3.5	11.0 d. 9.0	NS.
High parasitemia	3.0% 1.4	4.0% 4.1	NS.
High CF titers	1/72.5 1/65	1/208 1/174	NS.
Temperature response	NT.	NT.	
Deaths.	0	0	

Table 28.

A. marginale Carriers given a Homologous Challenge in Adult Cattle

	EAM X EAM	Control EAM	Significance
No. of animals	4	7	
Animals showing Anaplasma reactions	0	7	
Days PPI	NT		
Pre-inf., pre-chall., PCV	38.5% 5.0	39.0% 3.6	NS.
Incubation time (days)	N.R.	11.0 2.0	NT.
Low PCV	35.0% 5.7	18.0% 5.5	P .01
% of Pre-inf., and pre-chall. PCV	91.0% 4.3	46.0% 11.5	P .01
Persistence of anemia (days.)	0	21.0 7.5	NT.
High parasitemia	.52% .55	4.0% 4.1	P .05
High CF titers	1/5	1/1005 1/342	P .01
Temperature	NT.	NT.	NT.
Deaths.	0	1	

Conclusions

Among splenectomised calves, a previous East African *A. centrale* (EAC) infection proved 75% effective in preventing death loss from an East African *A. marginale* (EAM) challenge. Although 83% of the animals showed a marked response to EAM challenge, the reactions were less severe than primary infections. These reactions to EAM challenge again confirm the immunological differences which occur between *A. centrale* and *A. marginale*, as well as demonstrating the close relationship of these organisms. It should be remembered that splenectomised calves are highly susceptible, and because of this would react severely to any anaplasma exposure. Roby et al (57) estimate that splenectomised calves are about equally as susceptible as older adult animals, hence this group might well indicate the probable value of *A. centrale* vaccination in such animals. The one splenectomised calf infected with *A. centrale* after recovery from Nevada *A. marginale* (NAM) would indicate no immunity or resistance was produced by *A. marginale* (NAM) to an *A. centrale* challenge.

Among non-splenectomised calves, evidence was observed to indicate that a preliminary infection of *A. centrale* was responsible for a slight attenuating effect on the course of subsequent *A. marginale* infection. Of equal academic interest was the almost complete absence of any protection or increased resistance in animals first given *A. marginale* and then challenged with *A. centrale*, with the exception that a prior *A. marginale* infection was probably

responsible for an increase in the incubation time.

Cross infectivity tests among yearling cattle failed to indicate any value in previous premunition with either A. centrale or A. marginale when a heterologous challenge was given. The temperature response was actually greater with A. centrale infection in cattle previously premunised with A. marginale. This finding should not be interpreted as meaning that A. marginale would increase the susceptibility to A. centrale, but rather as further evidence that A. marginale does not protect or immunise against an A. centrale infection. The almost complete absence of reaction in yearling cattle premunised with both EAC and EAM, to later homologous challenge, supports the conclusion that specific resistance to infection is possible for homologous strains of anaplasma.

Anaplasma marginale reactions in adult cattle more closely resemble the response seen in splenectomised calves. Significant reduction in A. marginale virulence is noted in adult cattle first premunised with A. centrale. The reverse of this observation did not occur, however, when cattle first premunised with A. marginale were challenged with A. centrale. In this instance A. centrale infection occurred, which showed no significant differences from those observed with a primary A. centrale infection. Animals with A. marginale infections were, however, completely resistant to re-infection by a homologous challenge.

In reviewing the results of 62 cross infectivity trials it appears that premunition with A. centrale is more indicated in highly susceptible adult cattle, and in these animals is capable of

significantly reducing the virulence of subsequent infections with A. marginale. In more resistant young cattle, on the other hand, premunition with A. centrale was of less value. In a group of yearling animals, A. centrale failed to influence a later infection with A. marginale. A. marginale and A. centrale premunition resulted in almost complete protection against a homologous challenge. The use of mild A. marginale (MAM and BAM) strains among highly susceptible splenectomized calves resulted in almost complete protection against virulent A. marginale (EAM) challenge. Only a few animals were tested with these mild strains, however.

These trials do reveal a close immunological relationship between A. centrale and A. marginale, but also show marked differences. While A. centrale will produce a degree of protection against A. marginale infection, the reverse is not true. A. marginale did not, in these trials, produce a demonstrable degree of protection against A. centrale infection. This observation corresponds with serological evidence of different antigenicity between A. marginale and A. centrale. This serological and immunological evidence would suggest that, in addition to a common antigenic component present in both A. centrale and A. marginale, that an additional, distinctly different, antigenic component is present.

General Discussion

Experiment #1 : Serological survey of Anaplasmosis.

Anaplasmosis is often overlooked in areas where the disease is endemic, because of the almost universal exposure of calves at an early age. Anaplasma infection in the young animal is usually accompanied by a mild or inapparent infection, followed by a state of premunition, in which the animal is resistant to future exposure, but is capable of serving as a reservoir of infection to susceptible cattle (45,50). The introduction of adult susceptible cattle into such endemic areas is frequently followed by cases of acute anaplasmosis in these animals, with subsequent losses of the animals are untreated.

Anaplasmosis is generally transmitted by ticks (50). In east Africa, tick control has been introduced in many areas of high livestock production, as an essential measure in the control of such disease as East Coast Fever and Piroplasmosis. Before the introduction of such procedures, it is likely that premunition against anaplasmosis occurred quite naturally in almost all animals, hence the lack of evidence that anaplasmosis constituted a problem or threat to livestock production. As a result of the introduction of exotic breeds into East Africa, and the control of ticks by regular dipping or spraying, it is probable that large numbers of cattle may now be reaching a highly susceptible age without prior contact with anaplasma. Such adult cattle would be expected to develop severe reaction following exposure to *Anaplasma marginale* with an appreciable mortality if untreated.

Such susceptible populations have been shown to exist as a result of a serological survey on 334 cattle. In the East African Trypanosomiasis Research Organisation (EATRO) herd of 100 animals, the overall positive

reaction to the complement-fixation (CF) test was only 19%. These animals were a part of the herd in which regular dipping had been practiced for years, without the introduction of new stock. This low incidence of reactors suggests the possibility that a relationship exists between the management practice of dipping and relative isolation, and the low percentage of positive CF reaction. In contrast to this there were 47% positive reactions among 234 cattle of an East African Veterinary Research Organization (EAVRO) herd, a newly formed herd of highgrade cattle from various locations in Kenya. This 47% incidence is actually low when compared to serological surveys conducted in some endemic areas of the U.S. In one such area of the U.S., where ticks are responsible for transmission, and no attempt is made to control these vectors, incidences of over 90% were recorded in some individual herds, with an average of almost 75% in a large number of herds (62). On the basis of this, the assumption can be made that in both the EATRO and EAVRO herds, the incidence of anaplasmosis reflects dipping practices commonly followed in the more profitable cattle producing areas of East Africa.

The total percentage of animals showing some degree of reaction to the test, is about the same in the two herds tested, with a much larger number of reactions in the EATRO herd being suspicious. A possible explanation for this finding is that unidentified hemoprotozoan or other blood parasites might be present which would either produce a related antibody response in the bovine host, or possibly result in an interference of the specific anaplasma serum antibody titer of a given animal. A second possibility is that tick control was sufficiently effective to

prevent re-infection from animals within the herd, if such a phenomenon does occur. Insufficient information is available to state definitely that antigen variations occur in A. marginale infections. However, if such variations did occur, as they do in some hemoprotozoa, then a constant re-infection may occur among animals of a given herd, providing the vectors are present, thus maintaining a higher level of infection, with more definite serum reactions to the specific A. marginale antigens. In the absence of such re-infection, brought about by the absence of vectors, it might be surmised that the serum titers would gradually drop over a period of time, theoretically returning to normal.

The complement-fixation test in game animals is of unknown reliability. In order to establish confidence that a positive serological reaction to anaplasma antigen is indicative of a carrier state, or a past anaplasma reaction, animal inoculation would have to be carried out to prove the presence of infection. This has not been done due to the lack of isolation facilities to contain possible other infections that game animals might carry. The ability of serum to fix complement in the presence of specific antigen, and to not fix complement in the absence of such antigens, does strongly suggest the presence of an antibody which is specific for the antigen being used. Such specific antibodies can only be produced in a given animal as the result of some past exposure to the antigen being tested, or to antigens closely related. We may thus suppose that the specific antibodies detected in waterbuck, buffalo, wildebeeste, and impala, are probably the result of a previous exposure in these animals to anaplasma antigens. Only after sub-inoculation, however, of blood from these CF positive animals, will it be possible to determine

if the CF test is indicative of an anaplasma carrier or merely a reaction to a passive exposure to anaplasma antigens.

The injection of killed anaplasma antigens does produce a CF response in the recipient animal (60), but generally is of low titer and short duration. It is necessary to use large quantities of such antigens in the presence of oil adjuvants, in order to produce this reaction. Hence it is doubtful that the relatively small naturally occurring antigenic exposure via tick vectors would alone produce such a serological response without multiplication of anaplasma in the wild host. If multiplication does actually occur in these animals showing CF response, then several implications are possible. One is that game animals may, at some future date, act as a reservoir of anaplasma infection potentially dangerous to adult susceptible cattle. A second implication is that, if multiplication occurs in impala, wildebeeste, and waterbuck, possibly artificial infections could be induced in some of the small ruminants of East Africa such as dik-dik, duiker, steinbuck, etc. If this were possible, a convenient, and eventually a cheap experimental animal might be found to facilitate anaplasmosis research, which is now entirely dependent on the use of cattle for maintenance of infection. Previous unpublished experience by the author has indicated that anaplasma isolated from game animals (deer), in some instances, show marked attenuated characteristics, at least in the first or second cattle passage. Serial passages of anaplasma organisms in a host other than cattle, might well be explored as a method of developing attenuated A. marginale strains.

Experiment #2 : Comparative pathogenesis of A. marginale and A. centrale.

A study of the pathogenesis of A. centrale and A. marginale in splenectomized calves, non-splenectomized calves, yearling and adult cattle

shows evidence of differences in relative pathogenicity.

Striking individual variation in susceptibility was noted within all animal groups, creating a situation where reasonably large numbers of animals had to be observed in each group, with statistical evaluation required to determine if the apparent differences in virulence between A. centrale and A. marginale were, in effect, the result of altered virulence, and not the relative susceptibility of a given animal.

Incubation time (or the time, in days, required for the first evidence of infection to occur following exposure) was recorded in our observations as one of the factors possibly related to pathogenicity. It should be recognized that incubation time is extremely variable, and is influenced by the size and route of inoculation, as well as the individual animal susceptibility. It was impossible to use the same inoculum in every animal, hence an inherent source of error in this factor. Large inoculums injected intravenously are generally responsible for reduced incubation time (35). With the exception of three splenectomized calves infected with A. centrale, the injection of large volumes of infected erythrocytes intravenously was avoided, choosing in preference the subcutaneous route and 5-10 ml of whole blood from carrier animals as the standard inoculum. The course of infection measured from the first evidence of infection to the occurrence of the low PCV, and then the time for eventual recovery, were observed by Lotze (69) to be more consistent and to provide a more reliable measure of the comparative pathogenesis.

Packed cell volumes (micro-hematocrit) (PCV), provides the most reliable measure of a hemolytic anemia as is found in anaplasmosis. The results are more reproducible than erythrocyte counts using diluting pipettes and hemocytometers. It does have the draw back that when recovery

begins, an influx of large numbers of macrocytes, reticulocytes, and other immature erythrocytes, results in a marked increase in the mean corpuscular volume, with the PCV returning to normal levels much more rapidly than the corresponding erythrocyte counts. The occurrence of large numbers of these immature erythrocytes is generally a favorable sign, so that it would appear the measurement of duration of anemia, by means of PCV, is a valid observation, since its increase does indicate that recovery from the primary infection is occurring.

Temperature readings were made daily, with the exception of Sundays, and are generally elevated in association with anaplasmosis infections. This reaction, however, was not consistent in all animals. So many other factors are capable of influencing temperature that consideration of this measurement as an indication of relative virulence must be, at best, guarded. Even so, there does appear to be significantly greater temperature response in splenectomized and non-splenectomized calves infected with the more virulent anaplasma strains (EAM).

Parasitemia, or the percentage of erythrocytes having parasitic anaplasma bodies, is considered a good measure of virulence and invasive properties of a given anaplasma strain. One difficulty with this observation is that in peracute cases, the course of infection may be so rapid that the period of peak parasitemia may be missed, due to the intervention of death before the next 24 or 48 hour observation. In most cases observations were made every other day, or, in some cases, daily, during acute A. marginale infections in splenectomized calves. In animals recovering from milder infections it was commonly observed that the parasitemia may build up over a very few days, then almost completely drop to zero during the recovery phase. Because of the presence of artifacts,

Jelly bodies, and remnants of nuclear material in red cells, especially in very young animals, parasitemias of less than 1% were not considered positive evidence of infection. With experience the trained observer can distinguish anaplasma bodies from these other materials, even when found in numbers less than 1%, but to allow a margin of safety this criterion was followed in most cases.

As mentioned before, the appearance of large immature red cells is generally an indication of a favorable prognosis. It was observed that these large immature cells are rarely, if ever, invaded by the anaplasma parasite. As infected cells are removed from the circulation by the reticulendothelial system the parasitemia falls rapidly to almost zero. The reason why these large immature cells are not found parasitized is not known, but possibly these cells have physical properties which render them less susceptible to parasitemia, or perhaps by the time of their appearance in the circulation an antigen-antibody reaction has occurred invivo, which, at this point, diminishes the invasive properties of the anaplasma organisms. Possibly it is just a matter of time required for the new cells to become visibly parasitized.

In support of the first and last theory, it is very common to observe secondary relapses among splenectomized calves, recovering from a primary infection of A. centrale, or mild strains of A. marginale and in other cattle groups infected with the more virulent A. marginale (EAM). This observation would suggest that as the circulating erythrocytes reached maturity, as evidenced by a decrease in mean corpuscular volume, the anaplasma organism again invades the red cells. These studies have not attempted to analyse these secondary responses as a means of establishing

relative virulence of different organisms, even though such a study could well be useful, and may throw light, not only on the relative virulence of different organisms, but also on the possibility of invivo antigenic variation.

The complement-fixation test, while most useful in the detection of carrier animals, is also of value in measuring the degree of response produced in a given animal to anaplasma infection. High CF serum titers accompany acute anaplasmosis, and Gates et al (38) have reported a direct correlation between these serum titers and blood infectivity titers. On the basis of this finding, it was hoped that the CF test would give a measure of infectivity in the blood, hence another measure of relative pathogenicity.

The relative pathogenesis of East African A. marginale (EAM), Beltsville A. marginale (BAM), Nevada A. marginale (NAM), and East African A. centrale (EAC), was measured in 28 splenectomized calves, considering the factors previously discussed. Among splenectomized calves an increased virulence was detected in EAM infections, when compared to EAC and two U.S. isolates of A. marginale, BAM and NAM. EAM infection produced a 100% mortality, whereas EAC infection resulted in 100% survival.

Perhaps of equal interest, however, is the relatively mild reaction produced in these calves by A. marginale isolates obtained in the U.S. The Beltsville A. marginale isolate, was used in these experiments because it was known to produce reasonably mild reactions in susceptible cattle. The Nevada organism, however, was isolated from a field case in which severe signs of anaplasmosis were present, and in Nevada, produces about 10-15% mortality among splenectomized calves. NAM infections have failed to result in any mortality among the few calves infected in this

study. If the only A. marginale comparison to A. centrale had been between these U.S. strains, the conclusion would have been that A. centrale produces equally as severe a reaction in splenectomized calves as does A. marginale, for little or no difference is detectable between these three anaplasma organisms, EAC, BAM, and NAM. In only one instance, NAM showed significant evidence of greater virulence than EAC or BAM. The persistence of anemia was greater in this instance, which suggests that complete recovery was markedly prolonged in animals with this infection.

In countries where premunition with A. centrale is practiced, often the recommendation is to vaccinate only young animals, (127) which, of course, are intact, and not splenectomized. For this reason a group of non-splenectomized calves was included for comparisons of A. marginale (EAM) and A. centrale (EAC) reactions to see how marked the difference in virulence would be.

The relative resistance to anaplasma infections of young animals resulted in fewer significant differences appearing as the result of infections with EAM and EAC. The only two factors, among all those observed, that showed significant differences, were the persistence of anemia and temperature response. In both instances the evidence pointed to increased virulence with the EAM infection. Even though only these two factors showed statistical significance, it is worthwhile noting that some individuals of this group showed severe reactions to A. marginale infections not noted in animals infected with A. centrale.

A second group of young animals (Yearlings), not splenectomized, was infected with both EAC and EAM infections to further explore the differences in virulence that might be expected with normal premunition

practices. These animals were about 8-10 months older, being designated yearlings, and because of their increased age were expected to show somewhat more severe reactions. This, in fact, failed to occur, and the reactions were almost the same as observed with the younger animals. The only significant difference observed was in the time required for the low PCV to develop after first evidence of infection. This observation indicated that EAM resulted in a more rapid course, with an accelerated onset of anemia, than occurred with EAC infection.

Among adult cattle, definite differences occurred between infection produced by EAM and EAC. Adult animals used in these trials were selected on the basis of a negative CF serum test, from a herd showing 47% serum reactions. The absence of known anaplasmosis-free areas in Kenya required the use of such animals in order to measure A. centrale reactions in adult cattle. It is possible, and even probable, that a small percentage of these CF negative animals were not entirely susceptible to anaplasmosis, but rather, in the past, had some exposure to anaplasma, and had spontaneously lost their serum titers. It was decided, however, that in most cases a negative CF test did reflect a susceptible animal, and this assumption was generally confirmed by reaction to anaplasma exposure. The reaction of these adult cattle to EAC was much milder than expected, although, sufficiently definite to detect and measure. This mild reaction suggests the possibility that a greater tolerance to EAC occurs in adult cattle not observed in other age groups. Before such an assumption could be entirely proven, these EAC reactions should be measured in cattle, not only negative to the CF test, but also from a known anaplasmosis-free area.

The complement-fixation (CF) test in every age group showed lower serum titers accompanying EAC infection, than seen with A. marginale.

A. marginale antigens were used in all these titrations. It seems more likely that the relatively low CF titers among animals infected with EAC, are the result of specific serological differences, and not a reflection of relative virulence, because A. marginale infections produced by BAM and NAM resulted in serum titers equally as high as seen in EAM infection, but a level of virulence equal to that produced by EAC. It would appear doubtful, therefore, that the CF test is a valid measure of strain virulence, at least as applied in these trials. Possibly there may be a valid correlation between blood infectivity and CF titers, but this does not appear to carry over to a correlation of percent parasitemia and CF titers, because high CF titers were observed in connection with fairly low parasitemias in BAM and NAM infections.

There are numerous reports in the literature indicating that increases in total leucocyte counts occur as the result of anaplasmosis (5, 67, 131). Leucocyte counts were therefore made on 22 splenectomized and 15 non-splenectomized calves infected with both A. marginale (EAM) and A. centrale (EAC), with the thought that differences might occur as the result of infections produced by these two anaplasma organisms. Multiple observations were made at various stages of the disease for comparison with the pre-infection, normal leucocyte counts. It was interesting to note that some individual animals showed very marked increases, but these reactions were very sporadic and inconsistant. Statistical analysis of the averages failed to show any significant differences in leucocyte response when comparisons between EAM and EAC infections were made.

A. marginale (EAM) infections failed to produce any significant changes in the averages of either splenectomized calves or non-splenectomized calves, when compared to pre-infection leucocyte counts. Because of the

acute, fatal nature of this infection in splenectomized calves, no convalescent blood was available for leucocyte counts.

The only significant leucocyte changes resulting from A. centrale infections were small differences in the average counts of convalescent animals. In splenectomized calves the leucocyte counts were slightly lower than those observed in the normal, pre-infection samples. The acute phase produced no difference when compared with pre-infection counts. In non-splenectomized calves, A. centrale infection resulted in just the opposite reaction. The counts were slightly higher in convalescent sera, when compared to those made before infection and those during the period of high parasitemia. These results point to the spleen as playing some role in leucocyte production during the recovery period of anaplasmosis, possibly explaining, in part, the increased susceptibility of splenectomized calves to anaplasmosis.

These observations, while confirming that leucocyte changes do occur in association with anaplasma infection, failed to demonstrate a consistant leucocyte pattern that would be useful from a diagnostic point of view.

It is erroneous to assume that all anaplasma bodies found in A. centrale infections are located in the center of the erythrocyte, and that all anaplasma bodies found in A. marginale infections are situated on the margin. Even though the location of these pathogens in the infected red cells is responsible for the names "marginale" and "centrale", considerable variation in location can and does occur. In chronic cases, where only a very few anaplasma bodies are seen, it is sometimes difficult to differentiate the two infections by examining blood smears. Generally in instances where the parasitemia is over 1%, differentiation is not difficult.

In A. centrale infections a majority of the anaplasma bodies are

located in the cell, away from the periphery. In A. marginale infections a majority of the bodies are located on the periphery of infected erythrocytes. Because the central bodies were not always found in the exact center of the cells, an arbitrary criterion was established in designating an anaplasma body as either marginal or central in location. It was decided that an anaplasma body, located within a red cell a distance from the periphery greater than its own diameter, would be considered central in location, whereas an anaplasma body, located on the periphery or a distance from the periphery equal to or less than its own diameter, would be considered marginal in location. Morphologically, using giemsa stain, and a light microscope, no differences could be distinguished between A. centrale and A. marginale. A classification of the anaplasma infection as either A. centrale or A. marginale, on blood smear examination was therefore entirely based on location of the body within the erythrocyte. Micrometer measurements were not made on these designations, but rather visual estimates were made to determine the location of anaplasma bodies. On the basis of this, 83% of the anaplasma bodies in A. centrale infection were classified as central in location and 79% of the anaplasma bodies in A. marginale infection were classified as marginal in location.

Experiment #3 : Serological relationship of A. marginale and A. centrale as measured by the complement-fixation and capillary-tube agglutination tests.

The finding that lower complement-fixation (CF) serum titers occur in animals infected with A. centrale, when tested with A. marginale antigen, suggests that specific serological differences might occur between A. marginale

and A. centrale. It was desirable therefore, to prepare CF antigens from A. centrale and A. marginale (EAM) infections, for the testing of serum from both A. marginale and A. centrale carriers.

With the availability of adequate equipment (centrifuge capable of developing 35, - 40,000 X gravity at 3-5 C.), the production of anaplasma CF antigens proved easier than expected. The East African strain of A. marginale (EAM) was found to produce parasitemias of 60-90% in almost every splenectomized calf, following a challenge with 5 ml whole blood from a carrier animal. These high parasitemias are most difficult to obtain with some U.S. strains, which usually require massive serial intravenous inoculations of packed, washed, infected red cells, in order to produce a parasitemia in excess of 80% for antigen production. Using EAM, these preliminary steps to increase parasitemia were entirely unnecessary. The EAM strains was capable of producing a high titer CF antigen, which, some respects, was superior to the antigen produced by the U.S.D.A. Not only is EAM more virulent than standard strains in the U.S., but it is also highly antigenic.

The production of CF antigens from A. centrale infection, was not nearly as successful, probably due to the usually low parasitemia produced in this infection. This organism behaved more like the mild U.S. strains of A. marginale (MAM and BAM), and required massive, serial intravenous passages of washed, packed cells to achieve the desired level of parasitemia. Even then the most successful antigen was prepared from only a 42% parasitemia. Other antigens were prepared from blood with low parasitemia, resulting in low antigen titers. Some of these low titered antigens were anticomplementary, in that they produced non-specific fixation of complement. The addition of normal serum to the test was found to reduce

the anti-complementary properties of these antigens.

A comparative test, using A. marginale antigen prepared from the local East African strain, A. centrale antigen prepared locally, and A. marginale antigen prepared and issued by the U.S. Department of Agriculture, conducted on 1/5 dilution of bovine sera of unknown anaplasmosis status, showed an extremely high level of agreement. The inherent human error probably accounted for the differences observed in reaction occurring with the two A. marginale antigen, where the percent of agreement was 96.1%. The level of agreement was not so high with the A. centrale antigen, but was over 90% (91.5%), which confirms the close serological relationship between A. centrale and A. marginale.

Notwithstanding this close relationship, serum titrations, using A. marginale (EAM), A. marginale (U.S.), and A. centrale antigens, revealed distinct serological differences between A. centrale and A. marginale infections. Serum titers with different antigens varied greatly. Animals with A. marginale infection showed over a two fold higher titer with the homologous antigen, whereas animals with A. centrale infection showed about a four fold higher titer with the homologous antigen. These differences in specificity suggest antigen differences as well as obvious similarities.

Serum antibody titers produced by East African A. marginale (EAM), Beltsville A. marginale (BAM), and Nevada A. marginale (NAM) are almost identical when tested with A. marginale antigens prepared either locally from the EAM strain or in the U.S. (USDA antigen). From this it is possible to surmise that probably the three A. marginale strains tested are antigenically and serologically the same. Capillary tube agglutination tests (CA) were performed on sera from A. marginale and A. centrale carriers.

Because of the shortage of a commercially prepared antigen, serum titrations were not conducted using this test. Readings of the CA test offer some chance for quantitative determinations because the reactions are easily categorized as 1+, 2+, 3+, or 4+ reactions, all of which are considered positive. The more definite reactions of 4+ are generally observed to occur with higher titered sera, with 1+ and 2+ reactions occurring in sera of low titer. A pattern similar to the complement-fixation test was observed, in that sera from both A. centrale and A. marginale infection produced positive reactions, but sera from A. marginale infections showed the greater number of 4+ reactions. The CA antigen was prepared from an A. marginale strain.

Experiment #4 : Cross infectivity trials using A. marginale and A. centrale.

Four groups of animals, splenectomized calves, non-splenectomized calves, yearlings, and adult cattle, having recovered from anaplasma infections were challenged with heterologous, and in a few instances, with homologous anaplasma organisms.

These challenges were not intended to be massive, but undoubtedly were, in all instances, greater than would be experienced under natural conditions with tick vectors. In all instances challenge consisted of 5 or 10 ml of infected blood injected subcutaneously.

The same general factors were observed following challenge as were recorded during the primary infection when the relative pathogenesis was being studied. These factors, in some instances, are influenced by the primary infection. The results are, however, sufficiently clear to enable tabulation and statistical analysis for the determination of significance.

In most instances, the PCV's were slightly lower at the time of challenge than they were before the initial primary infection. In

view of this change, greater importance is attached to the percent of pre-challenge PCV, as a guide to the severity of the secondary infection rather than rely entirely on the low PCV, although this latter factor is recorded as well. Incubation time in these animals has reference to the time required for the secondary infection to become evident, but in many instances this was not easy to determine. It is not impossible for secondary relapses to occur following the primary infection. It was difficult therefore to rule out the possibility that a reaction following challenge was, due to a secondary relapse of the primary infection rather than a result of challenge. The greatest difficulty was encountered in A. marginale challenge of A. centrale carriers, and in homologous challenge. Strangely enough, all instances of A. centrale challeng to A. marginale carriers, resulted in clear cut reactions to challenge. In homologous challenge it would have been impossible to determine if a reaction were the result of the first or second injection of infected blood. Fortunately, homologous challenge failed to result in any reaction that might be considered the result of challenge. In the case of A. marginale challenge of A. centrale carriers, some animals showed a very mild reaction, and low grade parasitemia, which was difficult to lable as either A. marginale, or A. centrale infection. In most cases however, a clear cut reaction occurred which allowed the calculation of an incubation time. In some of these animals, after a clear cut challenge reaction, it was possible to follow a prolonged course of infection, with parasitemias ranging from less than 1% to 2%, in which the marginal and central bodies were almost equally divided. In these instances it was presumed that a mixed infection was not being observed.

Among splenectomized calves carrying A. centrale, the challenge

dose of A. marginale infected blood varied from 1 to 85% parasitemia. All deaths in this group, as the result of challenge, occurred where the challenge dose was blood with a high parasitemia, but certainly not all animals receiving this high level challenge died, and some showed only minimal reactions. This again emphasized the large variation of individual animal reactions.

Because of the 100% mortality among splenectomized calves infected with EAM, a homologous challenge with this strain was impossible. All calves infected with the mild U.S. strains of A. marginale recovered. Of these animals, four were subsequently given a homologous challenge with EAM, and one was given a heterologous challenge with A. centrale. If a reaction to EAM challenge occurred at all in these animals, it was very mild, whereas A. centrale produced a moderately severe reaction in the one animal so tested.

Among non-splenectomized and adult cattle the general results of challenge were the same. A. centrale carriers challenged with A. marginale failed to react severely to challenge and, in some instances, did not show any detectable reaction whatsoever. Animals, when challenged with A. centrale after having recovered from A. marginale, showed almost the same degree of response to A. centrale as did animals with a primary A. centrale infection. There appeared to be some protection induced by A. centrale infection to a subsequent challenge to A. marginale, but little or no detectable protection induced by A. marginale for A. centrale infection. The one exception to this was in the incubation time which was significantly increased in A. marginale carriers challenged with A. centrale, suggesting a possible interference phenomenon, but once the A. centrale organism began to develop, the course of infection was

essentially unchanged when compared to the primary A. centrale infection.

Among yearling cattle a previous infection, with either A. marginale or A. centrale, failed to alter a secondary infection sufficient for significant differences to occur, again with the one exception that incubation times were increased.. Two animals received a homologous A. centrale challenge, and two received a homologous A. marginale challenge. No reactions occurred with either of these homologous challenges.

The periodic relapses which occur in anaplasmosis, supports the theory that antigenic variations may occur within the animal, resulting in the production of antigens sufficiently different to overcome previous immunity, being accompanied by a sudden influx of anaplasma parasites, mild anemia, and a mild clinical illness, with subsequent remission and return to normal. It was with this thought in mind that some animals were given homologous challenges. It was entirely possible that A. marginale organisms of different origins may be as different in their antigenic makeup as A. centrale and A. marginale would be. These trials failed to show a single marked reaction to a homologous challenge, whereas heterologous challenge resulted in reactions, although, in most instances less severe than a primary infection.

A degree of protection is induced by A. centrale against a subsequent A. marginale challenge, but a reciprocal protection by A. marginale for an A. centrale challenge does not occur. This observation bears out the serological findings, indicating that the two anaplasms have common antigens but also there is an additional antigen component present in A. centrale, not present in A. marginale.

Premunition with a mild A. marginale strain results in a primary

infection no more severe than that produced by A. centrale. Challenge of such animals with A. marginale results in almost complete immunity. This would indicate that if a program of premunition is desired, mild A. marginale strains would be more effective than A. centrale. One very distinct hazard to using mild A. marginale in such a program would be the introduction of a pathogen which, with serial passages either in nature or by artificial means, might regain virulence. A. centrale has the advantage of being readily distinguished in blood smears, because of its characteristic location, and also has not, in the past, shown evidence of increased virulence through successive passages. Premunition with A. centrale has been shown, in this study, to be effective in reducing the severity of A. marginale reactions in adult cattle. In young animals, yearlings and calves, A. centrale has been seen to produce reactions of equal severity as that produced by A. marginale (EAM). In such animals the value of A. centrale would be very questionable. In these instances premunition could be just as easily carried out with A. marginale. The apparent variation of A. marginale virulence might be such that even young animals would be severely affected in some areas. In such instances A. centrale might well be indicated even in young animals.

The introduction of A. centrale into a country, where it does not already exist, seems of questionable value. Vaccines of greater value, and no greater virulence, could probably be developed from existing A. marginale strains, if a premunition policy is to be followed. Notwithstanding its proven value in some situations, A. centrale represents a serologically and immunologically distinct pathogen, capable of inducing a clinical disease in susceptible cattle, for which no effective vaccine is available.

General Summary:

A limited serological survey of the incidence of bovine anaplasmosis in East Africa indicates a high level of infection. It is probable that anaplasmosis is endemic in most areas of East Africa where cattle are produced commercially. The marked reduction of incidence in a well managed herd where regular dipping is practiced does, however, reveal the probable presence of large numbers of highly susceptible adult animals. The presence of susceptible cattle indicate the need for continued tick control to prevent future spread of infection. The presence of specific complement-fixing antibodies in sera of game animals has been demonstrated. This is not necessarily indicative of anaplasma marginale infection, but does suggest the presence of or past exposure to anaplasma antigens, if not identical, at least, resembling A. marginale.

Comparative pathogenesis studies on 72 animals have been complete. A. centrale produced a markedly milder course of infection in splenectomized calves, and adult cattle, than virulent East African A. marginale (EAM). Mild A. marginale strains obtained from the U.S. resulted in infections of approximately equal virulence to A. centrale. Differences in virulence of A. centrale (EAC), and EAM infections in young non-splenectomized animals are not as readily demonstrable.

Complement-fixation antigens were prepared using blood collected from animals showing both A. centrale and A. marginale (EAM) infections. These antigens were used in addition to an antigen obtained from the U.S. Department of Agriculture, prepared from A. marginale infections. Complement-fixation (CF) studies show that the mild strains of A. marginale

are serologically indistinguishable from the East African virulent A. marginale. In low serum dilutions, cross reactions between A. centrale and A. marginale serums and antigens occur, but serum titrations reveal marked serological differences not observed between different A. marginale strains. These findings indicate that A. centrale should not be considered a mild strain of A. marginale.

A total of 62 cross infectivity trials further confirms the distinct nature of the A. centrale pathogen. A. centrale produces a degree of immunity against challenge with A. marginale. In most instances, such a challenge produces an infection of reduced severity. A total of three deaths occurred among 12 splenectomized calves when challenged with EAM, after first having been premunized with A. centrale. Of 11 controls, all died from anaplasmosis, following a similar challenge. A. centrale was responsible for significant protection against EAM challenge in adult cattle, but was of questionable value among non-splenectomized calves and yearling cattle. Animals first infected with A. marginale and then challenged with A. centrale, generally react to the challenge with almost equal severity as animals showing a primary A. centrale infection. No detectable protection is produced by A. marginale to a subsequent A. centrale infection. A homologous challenge of anaplasma carrier cattle failed to produce clear cut evidence of reaction to challenge.

Difference in virulence among A. marginale isolates has been demonstrated. Evaluation of A. centrale or other premunizing vaccines is influenced by the relative virulence of the A. marginale challenge administered.

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References

1. Alcorn, L. M.: Anaplasmosis and Its Spread in Kansas. Vet. Med. 28, (1933), 234-237. (Vet. Bul. 4, (1934), 222.)
2. Allbritton, A. R., and Parker, L. T.: Filtration of the Infective Agent of Bovine Anaplasmosis. Am. J. Vet. Res., 23, (1962): 809-812.
3. Allbritton, A. R., and Sager, C. L.: The Transport and Excretion of Bile Pigments in Anaplasmosis. Am. J. Vet. Res. 23, (Sept. 1962), 1011.
4. Anthony, D. W., Roby, T. O.: Anaplasmosis Studies with Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (D. venustus Marx) as Experimental Vectors. Proceedings of the Fourth National Anaplasmosis Conference, Reno, Nevada, 1962.
5. Arroyo, M. C.: Contribucion al Estudio del Hemograma y de las Variaciones Hemometricas en los Bovinos Durante el Proceso de Premunicion. (The Blood Picture of Cattle Undergoing Premunition). Rev. Grancolomb. Zootec Hig. Med., Vet. 3, (1949), 839-914. (Vet. Bul. 21, (1951), 752).
6. Baker, N. F., Osebold, J. W. and Christensen, J.F.: Erythrocyte Survival in Experimental Anaplasmosis. Am. J. Vet. Res. 22, (May 1961), 590-596.
7. Barnett, S. F.: The Preservation of Babesia bigemina, Anaplasma centrale and Anaplasma marginale by Deep Freezing. Vet. Rec., 76, (1964), 4.
8. Bedell, D. M., and Dimopoullos, G. T.: Biologic Properties and Characteristics of Anaplasma Marginale. I. Effects of Temperature on Infectivity of Whole Blood Preparations. Am. J. Vet. Res.23, (May 1962), 618.

9. Beddel, D. M., and Dimopoullos, G. T.: Effects of Sonic Energy on Infectivity of Anaplasma marginale. Am. J. Vet. Res., 24, (March 1963), 278.

10. Boynton, W. H.: Further Observations on Anaplasmosis. Cor. Vet., 22, (1932), 10-28.

11. Boynton, W.H., Herms, W.B., Howell, D.E., and Woods, G.M.: Anaplasmosis Transmission by Three Species of Ticks in California. J. AVMA, 88, (1936), 500-502.

12. Boynton, W.H. and Woods, G.M.: Deer as Carriers of Anaplasmosis. Science, 78, (1933), 559-560.

13. Boynton, W.H. and Woods, G.M.: A Serum Reaction Observed in Anaplasmosis. J. AVMA, 87, (1935), 59-63.

14. Brion, A.: A New Equine Disease: Anaplasmosis and Its Causal Parasite, Anaplasma equi. C.R. Acad. Sci. Paris 217, (1943), 709-710. (Vet. Bul. 18, (1948), 154)

15. Brocklesby, D. : Transmission of Disease between Buffalo and Cattle. Div. of Protozoal and Arthorpod-Born Diseases, E.A.V.R.O. Report for the period July 1957 - May 1958, p. 18.

16. Brumpt, E.: Transmission d'anaplasma marginale par Rhipicephalus bursa et par margaropus. Ann. de parasitol. 9, (1931), 4-9. (Am. J. of Vet. Res. 11 (1950), 5-16).

17. Carpano, M.: Sur la Nature des Anaplasmoses et en Particulier da l' Anaplasma centrale. (The Nature of Anaplasma and of Anaplasma centrale in particular). Ann. Parasitol. 8, (1930), 231-240. (Vet. Bul. 2, (1932), 140).

18. Carpano, M.: L'infezione da anaplasma del Tipo Marginale dei Bufali in Egitto. (A. Marginale type of Anaplasma infection of Buffaloes in Egypt). Clin. Vet. Milano 57, (1934), 589-592. (Vet. Bul. 5 (1935), 206).

19. Christensen, J. F.: Possibilities of Introducing *Anaplasma centrale* into the United States of Use in the Immunization of Cattle against Anaplasmosis. Proc. Book, American Veterinary Medical Association 91st Annual Mtg., Seattle, August 23-26, 1954, p. 56.

20. Christensen, J.F., Osebold, J.W., Harrold, J.B., and Rosen, M.N.: Persistence of Latent *Anaplasma marginale* Infection in Deer. J.A.V.M.A., 136, (May 1960), 426.

21. Christensen, J.F., Osebold, J.W., Rosen, M.N.: Deer Infected with *Anaplasma marginale*. J.A.V.M.A., 132 , (1958), 289-292.

22. Cordier, G., and Menager, J.: L'*Anaplasmose centrale* Experimentale Peut elle Conferer la Premunition Contre l'*Anaplasmose marginale* Experimentale dans l'espece Bovine. (Can Premunition Against Experimental *Anaplasma marginale* infection be Conferred on Bovines by Experimental *A. centrale* infection?). Bull. Soc. Sci. Vet., Lyons, 35, (1932), 162-170. (Vet. Bul. 4, (1934), 24).

23. Cordier, G. and Menager, J.: Nouveaux Essais de Premunition dans l'*Anaplasmose marginale* Bovine. (New attempts to produce premunition in Bovine anaplasmosis due to *A. marginale*). Bull. Soc. Sci. Vet. Lyons, 35, (1932), 171-179. (Vet. Bul 4, (1934), 25).

24. DeKock, G.: A Study of the Reticulo-endothelial System of the Sheep. 13th and 14th Reports of the Director of Vet. Ed. and Res. Onderstepoort, South Africa, October 1928, p. 647-724.

25. DeKock, G.: A Short Note on Chronic Anaplasmosis and Gonderiosis in Small Ruminants after Splenectomy. 16th Report of the Dir. of Veterinary Services and Anim. Industry, Union of South Africa August, 1930, p. 3-10.

26. DeKock, G., and Quinlan, J.: Splenectomy in Domesticated Animals- Its Sequellae, with Special Reference to Anaplasmosis in Sheep. 11th and 12th Rep. of Dir. of Vet. Res., part I, Onderstepoort, Union of South Africa, (1926), p. 369.

27. DeRobertis, E., and Epstein, B.: Electron Microscope Study of Anaplasmosis in Bovine and Red Blood Cells. Proc. Soc. Exp. Biol. N.Y., 77, (1951), 254-258.

28. Dikmans, G.: The Morphology of Anaplasma. J.A.V.M.A., 83, (1933) 203-213.

29. Dikmans, G.: V. The Nature of Anaplasma. J.A.V.M.A., 83, (1933) 101-104

30. Dikmans, G.: The Transmission of Anaplasmosis. Am. J. Vet. Res. 11, (1950), 5-16.

31. Donatien, A.: Immunisation against Bovine Anaplasmosis. Arch. Inst. Pasteur Alger., 30, (1952), 44-46. (Vet. Bul. 22, (1952), 688).

32. Espana, C., and Espana, E.M.: Further Studies on the Morphology of Anaplasma Marginale with Phase Contrast and Light Microscopy. Proc. of the Fourth National Anaplasmosis Conf., Reno, Nevada, April 1962.

33. Espana, C., Espana, E.M., and Gonsales, D.: Anaplasma marginale. I. Studies with Phase Contrast and Electron Microscopy. Am. J. Vet. Res. 20, (Sept. 1959), 795.

34. Foote, L.E., Geer, J.C., and Strich, Y.E.: Electron Microscopy of the Anaplasma Body: Ultrathin Sections of Bovine Erythrocytes. Science 128, (1958), 147-148.

35. Franklin, T.E., Heck, F.C., and Huff, J.W.: Complement Fixation Antigen Production. Am. J. Vet. Res., 24, (1963), 483-487.

36. Franklin, T.E., and Redmond, H.E.: Observations on the Morphology of Anaplasma marginale with Reference to Projections or Tails. A. J. Vet. Res., 19, (Jan. 1958), 252.

37. Gainer, J.H.: Demonstration of Anaplasma marginale with Fluorescent Dye, Acridine Orange; Comparisons with the CF Test and Wright's Stain. Am. J. Vet. Res., 22, (1961), 882.

38. Gates, D.W., Madden, P. A., Martin, W.H., and Roby, T. O.: The Infectivity of Blood from Anaplasma-Infected Cattle as Shown by Calf Inoculation. Am. J. of Vet. Res., 18, (1957), 257-260.

39. Gates, D.W., Mohler, W.M., Mott, L. W., Poelma L.J., Price, K.E., and Mitchell, J.: A Comparison of Antigen Production Methods and Complement-Fixation Procedures for Diagnosing Bovine Anaplasmosis. Proc. U.S. Live. Stk. Sanit. Assoc. 58th Ann. Meet., Nov. 1954.

40. Gates, D.W., Mohler, W.M., Mott, L. O., and Schoening, H.W.: Complement-Fixation Test as a Tool in the Control of Anaplasmosis. Proc. 91st Ann. Meet. AVMA, Seattle, 51-53, 1954.

41. Gates, D.W., and Ritchie, A.E.: Forms of Anaplasmosis marginale Theileri as Observed by Electron Microscopy. Proc. of the Fourth National Anaplasmosis Conf., Reno, Nevada, 1962.

42. Gates, D.W., Roby, T. O., and Mott, L.O.: Studies on the Specificity of the CF Test for Anaplasmosis. U.S. Livestock San. Assn. 59th Ann. Meet. Nov. 1955, p.89.

43. Gates, D. W., and Roby, T. O.: The Status of the Complement Fixation Test for the Diagnosis of Anaplasmosis in 1955. Ann. N.Y. Acad. Science 64, (1956), 31-39.

44. Gates, D.W., Roby, T. O., and Mott, L.O.: Studies on the Specificity of the Complement-Fixation Test for Anaplasmosis. Proc. 59th Ann. Mtg. U.S. Live Stk. Sanit. Ass., 1955, 89-97.

45. Hagan, W. A., and Bruner, D. W.: The Infectious Diseases of Domestic Animals. 4th Edition, Comstock Publishing Assoc. Ithaca, New York, 1961.

46. Hansard, S. L., and Foote, L. E.: Effects of Anaplasmosis on Physiological Behavior of Chromium 51 and Iron 59 in Young Calves. Fed. Proc. 17, 478. (Vet. Bul. 28 (1958), 432).

47. Hansard, S. L., and Foote, L. E.: Anemia of Induced Anaplasmosis in the Calf. Am. J. Physiology, 197, 711-716. (Vet. Bul. 30 (Mar. 1960), 117).

48. Hansard, S. L., and Foote, L.E.: Absorption and Physiological Movement of Iron in Calves Induced with Anaplasmosis. J. Anim. Sci., 20, (1961), 395.

49 Holm, R.: Beitrag zum Anaplasmen Problem. Ztschr. f. Infektionskr., 25, (1924), 199-226. (Am. J. Vet. Res. 11, (1950), 5-16).

50. Henning, M. W.: Animals Diseases in South Africa, 3rd Edition, Central News Agency Ltd., South Africa, 1956.

51. Hoffman, R. A., Smith, K.O., Collins, J.C., Mott, L.O., and Scales, J. W.: Insect Control Can Reduce Spread of Anaplasmosis. Miss. Farm Res. 24, (April 1961) 4. (Agricultural Experiment Station, Mississippi State University)

52. Howell, D. E., Seaborn, C.E., Roseboom, L.E., Stiles, G.W., and Noe, L.H.: The Transmission of Anaplasmosis by Horseflies. (Tabanidae). Okla. A&M Col. and Agri. Exp. Sta. Tech. Bull. T-11, 1-23, 1941. (Am. J. Vet. Res. 11, (1950), 5-16).

53. Howell, D. E., Stiles, G. W., Moe, L. H.: The Transmission of Anaplasmosis by Mosquitoes. J.A.V.M.A., 99, (August 1941), 107-109.

54. Howell, D. E., Stiles, G. W., and Moe, L. H.: The Hereditary Transmission of Anaplasmosis by Dermacentor andersoni Stiles. Am. J. of Vet. Res., 2, (1941), 165.

55. Howell, D. E., Stiles, G. W., and Moe, L.H.: The Fowl Tick (Argas persicus) a New Vector of Anaplasmosis. Am. J. of Vet. Res., 4, (1943), 73-75.

56. Kreirer, J. P., and Ristic, M.: Anaplasmosis: X.Morphologic Characteristics of the Parasites Present in the Blood of Calves Infected with the Oregon Strain of Anaplasma marginale. Am. J. Vet. Res., 24, (July 1963), 676-687.

57. Kreier, J.P., and Ristic, M.: Anaplasmosis XI. Immunoserologic Characteristics of the Parasites Present in the Blood of Calves Infected with the Oregon Strain of Anaplasma marginale. Am. J. Vet. Res., 24, (July 1963), 688-696.

58. Kreier, J. P., and Ristic, M.: Anaplasmosis XII. The Growth and Survival in Deer and Sheep of the Parasites Present in the Blood of Calves Infected with the Oregon Strain of Anaplasma marginale. A. J. of Vet. Res., 24, (1963), 697-702.

59. Kreier, J.P., Ristic, M., and Schroeder, W.: Anaplasmosis XVII The Pathogenesis of Anemia Produced by Infection with Anaplasma. Am. J. Vet. Res., 25, (1964), 343-352.

60. Kuttler, K.L.: Anaplasmosis Immunization Studies. Proc. 65th Ann. Mtg. U.S. Livestock Sanit. Ass., October, 1961.

61. Kuttler, K.L.: Comparisons of Complement-fixation and Capillary Tube Agglutination Tests for Detection of Bovine Anaplasmosis. J.A.V.M.A., 143, (1963), 729-733.

62. Kuttler, K.L., and Bohman, V.R.: Anaplasmosis. Bull. 219, Agri. Exp. Stat., Max C. Fleischmann Col. of Agri., Univ. of Nevada, 1960.

63. Kuttler, K.L., Marble, D.W., and Matthews, N.J.: Anaplasmosis Complement-fixation Response in Calves from Anaplasmosis Infected Dams. Am. J. Vet. Res., 23, (1962), 1007-1010.

64. Legg, J.: Anaplasmosis: Cross Immunity Tests Between Anaplasma centrale (South Africa) and Anaplasma marginale (Australia). The Aust. Vet. J. 12, (1936), 230-233.

65. Lestoquard, F.: Transmission au Buffle des Piroplasmes du Boeuf. Modifications Subies par Anaplasma marginale. Bull. Soc. Path. exot., 24, (1931), 820-822.

66. Lestoquard, F., Donatien, A., and Salord, C.: Effect de la Splenectomie dans l' Anaplasmose du Mouton. Bull. Soc. Path. exot., 29, 264-266. (Vet. Bull. 7, (1937), 16).

67. Liwotow, N.A.: Kwoprossu ob Izmenenii Krowi Pri Anaplasmose Krupnogo Rogatogo Skota. (The Blood picture in Bovine Anaplasmosis) Arb. Mittelasia. Forsch. Inst. Wiss. Vet., 1, (1933), 22-28. (Vet. Bul. 5, (1935), 414).

68. Lotze, J.C.: Carrier Cattle as a Source of Infective Material for Horse Fly Transmission of Anaplasmosis. A. J. Vet. Res., 5, (1944), 164-165.

69. Lotze, J.C.: Variables and Constants in Experimental Bovine Anaplasmosis and Their Relationship to Chemotherapy. Am. J. Vet. Res. 8, (1947), 267-274.

70. Lotze, J.C., and Yiengst, M.J.: Mechanical Transmission of Bovine Anaplasmosis by the Horsefly, Tabanus sulcifrons (Macquart). A.J. Vet. Res., 2, (1941), 323-326.

71. Lotze, J. C., and Yiengst, M. J.: Studies on the Nature of Anaplasma. Am. J. Vet. Res., 5, (1942), 312-320.

72. Machado, A.V.: Estudo Preliminar Sobre a Ocorrencia de Reticulocitos no Anaplasmose e Piroplasmose. (The occurance of reticulocytes in anaplasmosis and piroplasmosis). Rev. Fac. Med. Vet., S. Paulo 4, (1949), 185-196. (Vet. Bul. 23, (1953), 242).

73. Madden, P.A.: Studies on Anaplasma marginale using direct fluorescent antibody methods. Proc. of the 4th Nat. Anaplasmosis Conf., Reno, Nevada, April 1962.

74. Merriman, G.M., Buckner, C., and Hobbs, C.S.: Field Trial with The Complement-fixation Test for Anaplasmosis in a Herd Free of Clinical Evidence of Infection. J.A.V.M.A., 141, (Dec. 1962), 1335.

75. Motianu, T.: A propos d'un Nouveau Parasite Endoglobulaire (A. centrale) Chis les Bovides de Roumanie. (Anaplasma centrale in Roumania) Ann. Parasit. Hum. Comp., 25, (1950), 8-14. (Vet. Bul, 21, (1951), 668).

76. Miller, J.G.: The Production and Identification of an Anaplasmosis Complement-fixing antibody. Am. J. Vet. Res., 15, (1954), 147-148.

77. Mohler, M.M., Eichorn, E.A., and Rogers, H.: Complement-fixation test for Serum Diagnosis of Bovine Anaplasmosis. Vet. Med. 44, (1949), 155-156. (Vet. Bul. 20, (1950), 201).

78. Mohler, W.M., and Gates, D. W.: Present Status of the Complement Fixation Test for the Diagnosis of Anaplasmosis. Proc. 56th Ann. Meet. U.S. Livestock Sanit. Ass., 1952, 61-64.

79. Mott, L. O., and Gates, D.W.: Production of an Antigen for Anaplasmosis Complement Fixation Tests. Vet. Med., 44, (1949), 296-299.

80. Moulton, J. E., and Christensen, J. F.: The Histochemical Nature of Anaplasma marginale. Am. J. Vet. Res. 16, (July 1955), 377.

81. Neitz, W. O.: Bovine Anaplasmosis: The Transmission of Anaplasma marginale to a Black Wildebeeste (Connochaetes Gnu). Onderstepoort J. of Vet. Sci. and Anim. Ind., 5, (July 1935), 9-11.

82. Neitz, W.O.: A Consolidation of Our Knowledge of the Transmission of Tick-born Diseases. The Onderstepoort J. of Vet. Res., 27, (Oct. 1956), 115.

83. Neitz, W. O., and DuToit, P.J.: Bovine Anaplasmosis: A Method of Obtaining Pure Strains of Anaplasma marginale and Anaplasma centrale by Transmission through Antelopes. 18th Rep. of the Dir. of Vet. Ser. and Anim. Ind., Union of South Africa, August, 1932, 3-20.

84. Omlin, A.: Versuche zur Immunisierung Gegen Die. "Tristesa" Schweis. Arch. f. Tierheilkunde, 74, (1932), 559-569. (Vet. Bul. 4, (1934), 24).

85. Osebold, J. W., Douglas, J. R., and Christensen, J. F.: Transmission of Anaplasmosis to Cattle by Ticks Obtained from Deer. Am. J. Vet. Res., 23 (Jan. 1962), 21.

86. Pearson, C. C., Brock, W. E., and Kliewer, I.O.: Studies on Use of Biologics in Control of Anaplasmosis. Vet. Med., 48, (1953), 435-437.

87. Pearson, C.C., Brock, W.E., and Kliewer, I.C.: A Preliminary Report on the Use of the Complement Fixation Test as an Aid in the Control of Anaplasmosis in Range Cattle. Proc. 59th Ann. Mtg. U.S. Livestock Sanit. Ass., 1955, 98-102.

88. Penha, A.: Comportements des Anaplasmes et des Corpoccles de Jolly vis-a-vis de la reaction nucleaire de Feulgen. Compt. Rend. Soc. Biol., ciii, (1930), 1331.

89. Pilcher, K. S., Wu, W.C., and Muth, O.H.: Studies on the Morphology and Respiration of Anaplasma marginale. Am. J. Vet. Res., 22, (March 1961), 298.

90. Post, G., and Thomas, G.M.: A Study of Anaplasmosis in Elk. J. A. V. M. A, 139, (Aug. 1961), 357.

91. Price, K. E., Brock, W. E., and Miller, J. G.: An Evaluation of the Complement Fixation Test for Anaplasmosis. Am. J. Vet. Res., 15, (Oct. 1954), 511-516.

92. Price, K. E., Poelma, L. J., and Faber, J. E.: Preparation of an Improved Antigen for Anaplasmosis Complement Fixation Tests. Am. J. Vet. Res., 13, (April 1952), 149.

93. Price, K. E., Poelma, L. J., Hastings, J. W., Sr., and Mitchel, J. I.: The Practical Application of the Complement Fixation Test for Anaplasmosis. Proc. 57th Ann. Meet. U.S. Livestock Sanit. Ass., p. 90-97, 1953.

94. Quevedo, J. M.: LaTristeza de los Bovinos, (Piroplasmosis, Babesiellosis, Anaplasmosis), Jesus Menendez, Edit. Buenos Aires, 193, 1929. (Am. J. Vet. Res. 11, (1950), 5-16).

95. Rees, C. W.: The Experimental Transmission of Anaplasmosis by Rhipicephalus sanguineus. No. Am. Vet. 11, (1930), 17-20. (Am. J. of Vet. Res. 11, (1950), 5-16.

96. Rees, C. W.: The Experimental Transmission of Anaplasmosis by Dermacentor variabilis. Science 75, (1932), 318-320.

97. Rees, C. W.: The Experimental Transmission of Anaplasmosis by Dermacentor andersoni . Paras., 24, (1933), 569-573.

98. Rees, C. W.: Some Effects of Splenectomy on the Blood of Carriers of Anaplasmosis. J. of Paras., 18, (1931), 128.

99. Rees, C. W.: Splenectomy in Cattle and Sheep in Relation to Anaplasmosis. No. Am. Vet., 14, (1933), 23-31.

100. Rees, C. W.: Transmission of Anaplasmosis by Various Species of Ticks. U.S. Dept. of Agric. Tech. Bull. 418, Washington, D.C., 1934. (Am. J. of Vet. Res. 11, (1950), 5-16.)

101. Rees, C. W.: The Effects of Injection into Bovidae of Emulsions of Anaplasma infected Ticks. Vet. Med. 35, (1940), 20-21.

102. Rees, C. W., and Avery, J. L.: Experiments on the Hereditary Transmission of Anaplasmosis by Ticks. No. Am. Vet., 20, (1939), 35-36.

103. Rees, C. W., and Hale, M. W.: Morphological and Chemical Studies of the Blood of Cattle in Health and During Anaplasmosis. J. Agric. Res. 53, 477 - 492. (Vet. Bull. 7, (1937), 327).

104. Rees, C. W., and Mohler, W. M.: Preliminary Studies on the Complement-fixation test for the Diagnosis of Bovine Anaplasmosis. J.A.V.M.A., 85, (1934), 669-674.

105. Rees, C. W., Underwood, P.C.: Comparative Counts of Infected and Non-infected Erythrocytes in Bovine Anaplasmosis. Proc. Helminth. Soc. Wash., 6, (1939), 48-50. (Vet. Bull. 10, (1940), 416).

106 Ristic, M.: Structural Characterization of Anaplasma marginale in Acute and Carrier Infections. J.A.V.M.A., 136, (1960), 417.

107. Ristic, M.: Filtration of the Causative Agent in Anaplasmosis. Am. J. Vet. Res., 21, (Sept. 1960), 890.

108. Ristic, M.: Studies in Anaplasmosis III. An Autoantibody and Symptomatic Macrocytic Anemia. Am. J. Vet. Res., 22, (1961), 871.

109. Ristic, M.: A Capillary Tube Agglutination Test for Anaplasmosis - A Preliminary Report. J.A.V.M.A., 141, (Sept. 1962), 588.

110. Ristic, M., and Watrach, A.M.: Studies in Anaplasmosis II. Electron Microscopy of Anaplasma marginale in Deer. Am. J. Vet. Res., 22, (Jan. 1961), 109.

111. Ristic, M., and Watrach, A.M.: Studies in Anaplasmosis VI. Studies and a Hypothesis Concerning the Cycle of Development of the Causative Agent. Am. J. Vet. Res., 24, (Mar. 1963), 267-277.

112. Ristic, M., and White, F.H.: The Detection of an Anaplasma marginale Antibody Complex Formed Invivo. Science, 131, (April 1960), 987.

113. Ristic, M., White, F.H., and Sanders, D.A.: Detection of Anaplasma marginale by Means of Flourescein-labeled Antibody. Am. J. Vet. Res., 18, (Oct. 1957), 924.

114. Roberts, H. H., and Lancaster, J. L.: Determining Susceptibility of White Tail Deer to Anaplasmosis. Ark. Farm Res. 12, #1 (January, February 1963), Agric. Exp. Stat., University of Arkansas.

115. Roby, T.O.: Natural Transmission of Bovine Anaplasmosis. Southwestern Vet. 16, (Fall 1962), 1.

116. Roby, T.O., Gates, D.W., and Mott, L.O.: The Comparative Susceptibility of Calves and Adult Cattle to Bovine Anaplasmosis. Am. J. Vet. Res., 22, (Nov. 1961), 982.

117. Rogers, T. E., and Dimophullos, G. T.: Electrophoretic Studies of Bovine Serum V. Complement-fixation Antibodies in Serum Fractions in Anaplasmosis. Proc. Soc. Exp. Biol. Med. 110, (June 1962), 359.

118. Rosenbusch, F., and Gonsales, R.: Die Tristeza Uebertragung durch. Zecken und Dessen Immunitatsprobleme. Arch. f. Protistenk., 58, (1927), 300-320. (Am. J. Vet. Res., 11, (1950), 5-16).

119. Rossi, P., and Trioson, F.: Essais de Culture d'une Souche Bovine Francaise d'Anaplasma marginale. (Attempted cultivation of a French strain of A. marginale). Bull. Soc. Path. Exot., 46, (1953), 312-315. (Vet. Bull., 24, (1954), 178).

120. Roseboom, L. E., Stiles, G.W., and Moe, L.H.: Anaplasmosis Transmission by Dermacentor andersoni Stiles. J. Paras., 26, (1940), 95-100.

121. Sanborn, C. E., Stiles, G. W., and Moe, L.H.: Preliminary Experiments in the Transmission of Anaplasmosis by Horse Flies. Okla. Agric. Expt. Stat. Bull. No. 204, (1932). Vet. Bull., 4, (1934), 25).

122. Sanders, D.A.: Notes on the Experimental Transmission of Bovine Anaplasmosis in Florida. J.A.V.M.A., 88, (1933), 799-805.

123. Schalm, O. W.: Veterinary Hematology. Lea and Febiger, Philadelphia, 1961.

124. Schalm, O. W., Osebold, J. W., and Murphy, F.A.: Observation with New Methylene Blue Stain as Applied to Anaplasma marginale. Calif. Vet., (May-June, 1962), p. 36.

125. Schmidt, H.: Anaplasmosis in Cattle. J.A.V.M.A., 90, (1937), 723-736.

126. Scott, W.L., Geer, J.C., and Foot, L.E.: Electron Microscopy of Anaplasma marginale in the Bovine Erythrocyte. Am. J. Vet. Res., 22, (1961), 877.

127. Sergent, E.: Piroplasmoses Bovine (Anaplasmosis). Arch. Inst. Pasteur, Alger., 31, (1953), 1929-1930. (Vet. Bull., 24, (1954), 63).

128. Sergent, E., Donatien, A., Parrot, L. and Lestoquard, F.: Etude Experimentale de Anaplasma centrale Theiler, du Boeuf. Bull. Soc. Path. Exot. 26, (1933), 922-924.

129. Sergent, E., Donatien, A. Parrot, L. and Lestoquard, F.: L'anaplasmose Bovine a Anaplasma centrale. Archiv l'Inst. Past. d'Algerie, 11, (1933), 526-569. (Vet. Bull., 5, (1935), 483-484).

130. Sergent, E., Donatien, A., Parrot, L., and Lestoquard, F.; (In memoriam): Estudes sur les Piroplasmoses Bovines. Inst. Pasteur d'Algierie, Alger, 816, 1945. (Am. J. Vet. Res., 11, (1950), 5-16).

131. Sergent, E., Donatien, L., Parrot, L., Lestoquard, F., and Charpin, A.; Modifications de la Formule Leucocytaire Chez Les Bovins Infectes de Theilerose ou D'anaplasmose Experimentales. Compt. Rend. Soc. de Biol., 100, (1929), 1016.

132. Sieber, M.: Anaplasma marginale (Theiler), Report of the Government Vet. Bact. for the Year 1909-1910, Dept. of Agric. Union of So. Africa, 1911.

133. Smith, T., and Kilborne, F.L.: U.S. Dept. of Agric. 8th and 9th Annual Rep. of the B.A.I., 1891-1892.

134. Smith, T., and Kilborne, F.L.: Investigations into the Nature Causation, and Prevention of Texas or Southern Cattle Fever. U.S. Dept. of Agric., BAI, Bull. 1, (1893).

135. Snedecor, G.W.: Statistical Methods. 5th Edition, The Iowa State College Press, Ames Iowa, 1957.

136. Splitter, E.J., Anthony, H.D., and Twichaus, M.J.: Anaplasma ovis in the United States, Experimental Studies with Sheep and Goats. Am. J. Vet. Res., 17, (July 1956), 487.

137. Stiles, G.W.: Investigations on Anaplasmosis in Cattle. J.A.V.M.A., 27, (1929), 704-723.

138. Stiles, G.W.: Anaplasmosis of Cattle. U.S. Dept. of Agric. Circular 154, 1931.

139. Stiles, G.W.: Mechanical Transmission of Anaplasmosis by Unclean Instruments. No. Am. Vet., 17, (1936), 39-41.

140. Tanganyika Territory: Report of the Work of the Veterinary Laboratory, Mpwapwa. Ann. Rep. Dept. Vet. Sci. and Anim. Husb., 1932, p. 13-71, Dar es Salaam.

141. Theiler, A.: Anaplasma marginale (Gen. and spec., nov.) The Marginal Points in the Blood of Cattle Suffering from a Specific Disease. Rot. Gov. Vet. Bact. Transvaal, Dept. Agric. (1908-1909), 7-64, 1910.

142. Theiler, A.: Gall-sickness of South Africa (Anaplasmosis of cattle). J. of Comp. Path. and Therap., 23, (1910), 98.

143. Theiler, A.: Further Investigations into Anaplasmosis of South African Cattle. So. Afr. Dept. Agric., First Report of the Dir. of Vet. Res., 1911, p. 7-46.

144. Theiler, A.: Uebertragung der Anaplasmosis mittels Zecken Ztschr. f. Infektionskr., 12, (1912), 105-116. (Am. J. of Vet. Res., 11, (1950), 5-16),

145. Tsalacopoulos, A.: Premunition des Bovins Contre l'Anaplasmose Premunition par Anaplasma centrale Realisees par le Service Veterinaire du Comite Special du Katanga au Lomami. (Immunisation

of Cattle against Anaplasmosis using A. centrale). Bull. Agric. Congo Belge., 45, (1954), 721-730. (Vet. Bull. 24, (1954), 651).

146. Taur, I.: Experiments in Immunisation with Anaplasma. Refuah Vet., 10, (1953), 51-53. (Vet. Bull,, 24, (1954), 369).

147. Turner, A. W.: The Successful Preservation of Anaplasma centrale at the Temperature of Solid Carbon Dioxide. Aust. Vet. J., 20, (1944), 295-298.

148. United States Department of Agriculture: A Manual of Conducting the Complement-Fixation Test for Anaplasmosis. August, 1958.

149. Waddell, A.H.: Observations on the Morphology of Anaplasma centrale. Aust. Vet. J., 40, (Feb. 1964), 53.

150. Waddell, A.H.: Electron Microscopy of Anaplasma centrale. Aust. Vet. J., 40, (June, 1964), 233.

151. Walker, J.: Some Observations in Connection with the Immunisation of Cattle Against South African Red Water and Genuine Gall-sickness (Anaplasmosis). The 3rd and 4th Rep. of the Dir. of Vet. Res., Nov. 1915.

152. Welter, J.C., and Zuschek, F.: Properties of Anaplasma marginale Antigen Used in a Capillary Agglutination Test. J.A.V.M.A., 141, (Sep. 1962), 595.

153. Zeller, H., and Helm R.: Versuche zur Frage der Uebertragbarkeit des Texasfievers auf Deutche Rinder Durch die bei uns Vorkommenden Zecken Ixodes ricinus und Haemaphysalis punctata cinnabarina. Berl. Tierarztl. Wchnschr., 39, (1923), 1-4. (Am. J. of Vet. Res., 11, (1950), 5-16).

STUDIES ON BOVINE ANAPLASMOSIS

THE COMPARATIVE PATHOGENICITY AND IMMUNOLOGICAL RELATIONSHIP OF *ANAPLASMA CENTRALE* TO *ANAPLASMA MARGINALE*

Appendix I

Animal Charts for Experiment II.

Abreviations used on animal charts in Appendix I and II.

AB: Anaplasma bodies, variety undetermined

AC: *Anaplasma centrale*, central bodies predominating.

AM: *A. marginale*, marginal bodies predominating

EAM: East African *Anaplasma marginale*

BAM: Beltsville *A. marginale*

NAM: Nevada *A. marginale*

EAC: East African *A. centrale*

BB: *Babesia bigemina*

Epy: *Eperythrozoon wenyonii* : 1 light infection
2 moderate infection
3 heavy infection
4 very heavy infection.

TM: *Theileria mutans*

CF: Complement-fixation

PCV: Packed cell volume

SC: Subcutaneous

IV: Intravenous

NT: No test

PPI: Post primary infection

> : Greater than

< : Less than

9372: Splenectomized calf, Age: 8 Mo. Male - Shorthorn

A. Marginale, Beltsville, U.S.A., BAM.

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	-	37	-	101.4	101.4	101.0	101.4
-3	-	37	-	102.6	101.4	101.0	101.8
0	20 ml blood (refrigerated 72 hours) from BAM carrier inoculated SC.						
3	-	37	-	101.0	101.0	101.0	100.4
7		38	-	101.2	104.4		102.6
12		35	-	102.2	102.0	101.8	101.0
17	-	31	-	101.0	101.8	101.6	101.0
21		33	-Epy 3	102.2	102.0		102.0
26		27	-Epy 2	102.2	105.4		
31	-	27	-	100.6	102.6	99.6	100.6
35		28	AM < 1%	100.4	101.6		
38	> 1/40	30	AM < 1%	100.0	100.0		
40		30	AM 2%	101.0	101.4		
42		28	AM 2%	101.0	101.6		
45	1/640	24	AM 8%	102.6	101.0		
47		20	AM 6%	103.6	102.6		
49		19	AM 8%	103.6	103.0		
53	1/1280	23		100.0	101.0	101.0	
56		27	AB < .5%	100.8	102.0		
59	1/320	31	-	101.0	100.0	100.0	102.4
64		33	AB < .5%	102.0			
66	1/80	34	AM < .5%	102.0			

9549: Splenectomized calf, Age: 118 days. Male Zebu Type

A. marginale, BAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	41	-		101.2		101.0
-1	-	39	-		101.0	100.8	101.4
0	5 ml blood injected SC, from BAM carrier showing 8% parasitemia.						
7	-	41	-	100.6	103,6	101.6	
10	3	37	AM <.5%	102.0	102.0		
13	1/80	38	AM 5%	101.6	102.4		
15		34	AM 10%	102.0	103.2		
18		25	AM 32%	103.4			
20	1/640	16	AM 15%	103.0	103.0		
22		13	AM 5%	102.6	103.6		
24		16	AM 2%	102.0	103.6		
27	1/640	25	AB 1%	102.6	102.0		
31		31	- Epy 4	102.0	99.8		
34	1/320	33	AM. 5% Epy 4	100.0	102.0	101.0	101.4
38		32	AB 4%	101.0	101.2		
41	1/80	31	AM 20% Epy 2	103.0	101.8		
43		22	AM 35%	102.6	103.8		
45		13	AM 3%	103.6	101.0		
48	4	22	AM 2%	101.6	102.6	101.6	102.0
52		27	AM 1%	101.6	101.0		
55	4	31	AM 1%	99.2	100.4		
57		34	AM 1%	99.2	97.0		

9551: Splenectomized calf, Age: 118 days, Male, Zebu type

A. Marginale, BAM

Day	CF	PCV	Smear	0	Temperature, day / 1	2	3
-8	-	36	-		101.8		101.8
-1	-	39	-		101.0	101.4	101.6
0	5 ml blood injected SC, from BAM carrier showing 8% parasitemia.						
7	-	41	-	102.6	101.6	102.0	
10	4	41	AM 1%	102.6	100.8		
13	1/80	39	AM 2%	102.0	102.0		
15		35	AM 6%	101.6	103.0		
18		29	AM 6%	103.6			
20	1/640	25	AM 4%	103.0	102.0	102.0	101.6
24		23	AM 3%	102.0	103.2		
27	1/640	23	AM 4%	102.6	100.6		
31		24	AM 2%		102.0	99.8	
34	1/1280	25	AM < .5%	100.8	102.0		
36		27	-	101.6	101.2		
38		26	AM < .5%	101.6	101.2		
41	1/160	30	AM < .5%	101.6	100.8		
45		29	-	102.0	101.4		
48	4	31	-	101.6	101.8		
50		28	-	103.0	101.0		
52		32	AM < .5%	101.6	101.0		
55	4	32	-	100.6	101.6	101.0	101.4

9562: Splenectomized calf, Age: 118 da., Male Guernsey

A. marginale, BAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	35	-		101.4		102.0
-1	-	35	-		101.2	101.0	101.6
0	5 ml blood injected SC, from BAM carrier showing 8% parasitemia.						
7	-	41	-	101.6	99.6	101.0	
10	1	43	-	102.0	104.8		
13	1/40	40	AM < .1%	101.6	102.0		
15		30	AM < .5%	101.6	101.0		
18		33	AM 6%	101.4			
20	1/640	32	AM 10%	102.0	101.6		
22		28	AM 16%	101.4	101.8		
24		19	AM 32%	101.0	103.6		
27	1/640	14	AM 26%	102.0	100.0		
29		18	AM 6%		102.0		
31		22	AM 5%	102.0	101.4		
34	1/320	26	AM 3%	102.0	102.0	101.0	100.6
38		26	AM 2%	101.2	101.0		
41	1/320	19	AM 10%	101.4	102.0		
43		17	AM 28%	102.4	103.0		
45		14	AM 3%	103,0	101.0		
48	1/160	20	-	101.0	102.2	102.0	101.6
52		22	-	101.2	102.6		
55	4	26	-	101.6	101.4	102.0	101.2
59		29	AM 1%	101.6	102.2		
62	4	34	AM <1%	102.8	102.0		

9376: Splenectomized calf, Age: 8 Months. Female Guernsey.

A. marginale, NAM.

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-10	-	36	-	101.0	100.0	100.0	100.6
-3	-	35	-	102.4	101.8	101.0	
0	5 ml blood injected s.c. from NAM carrier showing 20% parasitemia.						
0		33	-	100.4	101.0	101.6	
4	-	32	-	102.4	102.8	102.2	102.0
8		31	-	101.8	102.0		
11	2	32	AM < .5%	101.6	101.6		
13	4	32	AM 3%	101.8	102.0		
15	4	33	AM 3%	102.0	103.4		
19	1/40	26	AM 18%	103.0	104.0	104.0	
22		14	AM 32%	104.8	103.8		
25	1/80	15	AM 12%	104.8	102.2		
27		18	AM 7%	102.2	102.4		
30		23	AM 3%	101.8			
32	1/80	25	AM 1%	101.6	101.4	102.0	101.0
36		25	AM 8%	101.0	102.0		
39	1/40	27	AM 6%	102.6	102.8		102.6
43		23	AM 8%	102.6	102.0		
46	1/40	25	AM 6%	101.0	101.6	101.0	101.6
50		25	AM 6%	102.0	102.6		
53	4	26	AM 4%	101.4	101.8	101.6	101.6
57		25	AM 6%	101.0	101.4		
60	4	25	AM 6%	102.0	102.4	101.8	101.0
67	4	27	AM 6%	101.0	101.6	101.4	101.4
74	4	33	AM 3%	101.0	101.0	101.0	101.6

9455: Splenectomized calf, Age: 6 Months. Male Holstein.

Anaplasma marginale, Nevada, U.S.A. (NAM)

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-11	-	38	-	101.0	100.0	99.8	100.0
-3	-	38	-	101.4	99.6	99.0	101.0
0	20 ml bleed from NAM carrier (refrigerated 96 hours) injected subcutaneously (SC). Parasitemia unknown.						
3	-	39	-	99.8	99.4	100.4	99.4
7		37	-	100.0	100.0		100.8
12	-	37	-	100.4	100.8	99.8	100.2
17	-	38	-	100.0	100.4	100.2	99.0
21		37	-	99.6	99.6		
24	-	37	-	100.0	100.4		
26	4	35	AM < 1%	99.0	100.0		
29		36		100.6			
31	4	35	AM 8%	100.4	100.4		
33		31	AM 14%	101.0	103.0		
35		22	AM 54%	102.6	104.4		
38	1/640	11	AM 18%	102.2			
39		13		101.0			
40		15	AM 8%	100.0	99.8	100.4	98.0
45	1/640	20	AM 1%	100.0	99.8	101.4	101.4
49		27	AM 2%	101.6	102.0		
53	1/640	29	AM 3%	101.0	101.4	100.6	
56		26	AM 7%	103.0	103.0		
59	1/320	25	AM 25%	102.2	100.4	103.2	102.4

64		21	AM 10%	100.6		101.6	101.4
68		24	AM 4%	102.0	100.4	99.0	100.4
73	4	26	AM 8%	103.0	101.0		102.4

9554: Splenectomized calf, Age: 105 days. Male Holstein.

A. marginale, NAM.

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-10	-	39	-		101.4		102.0
-3	-	39	-		101.8		
0	5 ml blood injected SC, from NAM carrier shewing 20% parasitemia.						
0		35	-	101.4		103.2	
4	-	44	-	101.8	102.6	102.2	102.4
8		41	-	102.4	102.8		
11	1/80	43	AM 1%	102.4	103.0	103.2	105.0
15		40	AM 8%	104.6	103.8		
19	1/320	26	AM 12%	103.6	105.6	104.2	
22		18	AM 3%	103.0	103.2		
25	4	19	AM 8%	102.8	101.8		
27		24	AM 2%	102.2	101.8		
30		27	AM < 1%	103.0			
32	1/640	29	AM 1%	101.6	100.6	102.8	102.2
36		32	AM 4%	102.8	101.6		
39	1/320	32	AM 14%	102.8	101.2		101.6
43		23	AM 7%	103.6	103.0		
46	1/160	23	AM 2%	101.6	101.0	102.0	102.4
53	4	25	AM 2%	102.0	102.0		
60	4	27	AM 1%	102.0	102.6	101.0	102.0
67	4	29	AM 1%	101.4	101.6	101.6	101.6

9555: Splenectomized calf, Age: 105 days - Male Holstein.

A. marginale, NAM.

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-10	-	35	-		102.0		101.0
-3	-	37	-		102.0		
0	5 ml blood injected SC, from NAM carrier showing 20% parasitemia.						
0		35	-	99.0		103.2	
4	-	39	-	101.4	101.0		
6		40	-	101.0	99.0		
8	-	39	-	101.8	102.0		
11	4	39	AB 2%	101.4	99.8		
13		38	AM 8%	101.8	102.0		
15		32	AM 12%	102.0	102.0		
19	4	11	AM 2%	101.6	101.8	104.0	
22		21	AM 2%	102.0	102.2		
25	1/1280	25	AB < .1%	102.0	101.4	102,0	102.4
30		31	AM 1%	100.6			
32	1/640	32	AM 3%	100.0	100.0	102.6	101.4
36		27	AM 12%	102.8	102.4		
39	1/320	19	AM 8%	102.4	99.0	102.0	
43		23	AM 1%	102.0	100.4		
46	1/320	23	AM 1%	99.0	100.0		
48		26	AM 2%	103.0	100.6	101.6	101.4
53	4	27	AM 6%	101.0	101.4		
60	4	28	AB 3%	100.6	102.4	101.8	100.8
67	4	24	AM 3%	101.0	100.0	99.4	100.0
74	4	28	AB < 1%	101.0	101.0	99.8	99.8

9277: Splenectomized calf, Age: 87 days, Male, Holstein

A. Marginale, EAM

Day	CF	PCV	Smear	Temperature, Day / 0	1	2	3
-5	-	43	-	99.6	100.4	100.8	
-2	-	43	-	99.8	102.0	99.8	
0	5 ml blood injected SC, from EAM carrier showing a 10% parasitemia.						
2	-	43	-	101.0	103.4		
4		43	-	102.0			
6	-	43	-	101.0	101.6	102.0	99.8
10	2/	40	-	100.0	100.6		
12	-	39	-	100.0	101.2		
14	-	39	-	101.8			
16	-	36	-	102.4	102.4		
18	1/10	38	AM < 1%	101.6	101.4		
20	1/20	36	AM 3%	103.0	103.2	101.4	
23	1/80	32	AM 13%	103.0	104.4		
25	1/80	25	AM 53%	104.4	104.0		
27		16	AM 57%	103.6			
28		9		102.0			

28: Died acute anaplasmosis.

9278: Splenectomized calf, Age: 220 days, Male, Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-15	-	39	-	102.0	100.8	101.0	100.6
-8	-	37	-	101.6	101.6	100.6	100.4
-	-	38	-	101.6	101.0		
0	10 ml lysed, washed erythrocytes injected SC, from EAM carrier showing a 45% parasitemia.						
1	-	33	-	101.4	101.2		
3		33	-	102.0	101.8		
6	3/	33	-	101.4	101.0		
8		32	-	101.8	100.0		
10		31	-	101.8	101.4		
13	4/	33	-	101.0	101.2		
15	4/	31	-	100.4	101.8		
17	3/	31	AM < 1%	100.4	101.4		
20	4/	30	AM <1%	102.4	101.8		
22		30	AM 8%	102.2	103.4		
24		28	AM 31%	104.0	104.4		
27	1/160	11	AM 95%	106.2			

28: Died acute anaplasmosis.

9361: Splenectomized calf, Age: 70 days, Male, Guernsey

A. marginale EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-5		40	-	102.2	100.4	101.8	
-1	-	40	-	100.4	101.4		
0	5 ml blood injected SC, from EAM carrier showing a 12% parasitemia.						
1	-	41	-	102.8	102.6		
3	-	39	-	103.0	103.0		
6	-	36	-	102.0	102.0		
8	-	38	AM <1%	102.0	102.6		
10	1/40	37	AM 1%	102.4	102.4		
13		34	AM 80%	103.4	104.6		
15		22	AM 100%	105.0			
16		16		106.0			
17	1/1280	9	AM 100%	101.6			

17: Died, acute anaplasmosis.

9364: Splenectomized calf, Age: 90 days, Male, Jersey

A. marginale, RAM

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-12	-	36	-	101.4	101.0	101.2	101.4
-5	-	35	-	102.2	101.6	102.0	101.6
0	5 ml blood injected SC, from RAM carrier showing approximately 100% parasitemia.						
1	-	37	-		101.8	102.4	102.6
6		34	AM < 1%	102.0	101.8		
8	2∕	36	AM < 1%		101.4	102.0	
11	4∕	34	AM 1%	101.8	102.6	102.4	102.6
16	1/640	23	AM 95%	104.8	105.6		
18	1/640	9	AM 100%	100.2			

18: Died acute anaplasmosis.

9457: Splenectomized calf, Age: 114 days, Female Guernsey

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-11		38	-		102.0		102.0
-4	-	42	-		99.8		101.8
0	5 ml blood injected SC, from EAM carrier showing < 1% parasitemia.						
3	-	46	-	102.6	101.4	101.4	101.4
10	-	43	-	100.6	102.4	101.4	102.0
17	-	47	-	102.0	100.0		
19		46	-	100.8	101.0		
21	-	47	AM <.1%	100.0	101.4		
24	1/320	49	AM 1%	100.6	100.8		
26		45	AM 10%	100.8			
27		46	AM 8%	102.4			
28	1/320	41	AM 16%	102.8			
29		34	AM 31%	104.2			
31	1/320	22	AM 50%	106.0			
32		14	AM 45%	105.0			
33		9	AM 38%	103.2			

33: Died, acute anaplasmosis.

9458: Splenectomized calf, Age: 127 days, Male Guernsey

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day + 0	1	2	3
-9	-	38	-	101.4	101.8	101.2	
-4	-	37	-	101.4	101.8	101.6	102.0
0	5 ml blood injected SC, from EAM carrier, showing < 1% parasitemia.						
3	-	42	-	101.6	99.6		
5		42	- TM 1%	99.0	101.0		
7		40	- TM 2%	100.4	101.4		
10	-	39	- TM 2%	101.4	101.4		
12		38	- TM 2%	101.0	101.2		
14		38	- TM 2%	101.0	101.4		
17	1	38	- TM 2%	102.0	100.2		
19		38	AM 1%	101.8	102.0		
21		33	AM 2%	102.2	102.8		
24	3+	24	AM 26%	103.8	104.2		
26	4+	10	AM 56%	105.0			
27: Died, acute anaplasmosis.							

TM: Theileria mutans

9459: Splenectomised calf, Age: 127 da., Male Guernsey

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-12	-	36	-		101.2		101.8
-5	-	40	-		101.6		100.0
0	24 ml blood (frozen 193 days at -60 C.) injected SC, from EAM carrier, showing 14% parasitemia.						
2	-	39	-		101.6		
4		42	-	101.6	100.6		
6		41	-	101.6	102.0		
9	-	44	-	101.6	100.4		
11		44	-	101.0	102.2		
13		44	-	101.8	101.4		
16	-	46	-	101.8	102.2		
18		45	-	102.8	102.0		
20		42	-	102.0	101.0		
23	-	47	AM < .5%	102.0	101.6		
25	2/	42	AM < 1%	102.4	101.6		
27	3/	41	AM 3%	101.4	102.6		
30	4/	34	AM 18%	104.0	106.0		
32		28	AM 48%	104.0			
33	NT	11%		105.4			

34: Died, acute anaplasmosis.

9460: Splenectomized calf, Age: 200 days, Male Shorthorn

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-9	-	36	-	101.4	101.0	100.8	102.4
-2	-	36	-	102.0	100.8	101.0	100.6
0	5 ml blood injected SC, from EAM carrier, showing 5% parasitemia.						
2		34	-	102.2	100.4		
5	-	35	-	101.0	101.4		
7		33	-	100.4	100.4		
10		33		102.4			
12	-	34	-	101.4	100.8		
14		32	-	100.6	103.0		
16		32	AM 12%	102.8	106.2		
19	4	23	AM 58%	106.0			
20		15	AM 88%	106.4			
21	1/320	10		100.4			

22: Died, acute anaplasmosis.

9470: Splenectomised calf, Age: 130 days, Male Guernsey

A. marginale, EAM

Day	CF	PCV	Smear	0	Temperature, day / 1	2	3
-8	-	39	-		101.2		99.8
-1	-	39	-		101.4		
0	10 ml. cell free plasma injected SC., from EAM carrier showing a 45% parasitemia.						
1		38	-	101.2	100.8		
3		39	-	101.6	101.4		
6	-	40	-	102.0	102.0		
8	-	42	-	102.4	101.8		
10	-	42	-	101.6	100.8		
13	-	48	-	100.8	101.0		
15	-	41	-	101.4	102.0		
17	-	40	-	102.8	100.6		
20	-	38	-	101.8	102.0		
22		41	AM <1%	100.4	101.6		
24		40	AM 2%	101.0	100.0		
27	1/160	34	AM 21%	103.8	105.2		
29		25	AM 64%	104.8	105.0		
31	1/640	9	AM 92%	99.8			

31: Died, acute anaplasmosis.

9553: Splenectomised calf, Age: 105 days, Male Guernsey

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	-	43	-		101.8		101.4
-4	-	46	-		100.8		101.4
0	10 ml blood (refrigerated 24 hours) injected SC, from EAM carrier showing 12% parasitemia.						
1	-	47	-	102.0			
3	-	50	-	102.8	100.8		
5		50	-	101.0	102.4		
7		50	-	101.8	102.2		
10	2/	52	AM 2%	103.4	104.6		
12		53	AM 5%	103.4	103.0		
14		48	AM 34%	103.8	104.2		
17	1/1280	21	AM 100%	105.0			
18		9	AM 100%	99.2			
19: Died, acute anaplasmosis.							

9572: Splenectomized calf, Age: 102 days, Male, Guernsey

A. marginale. EAM

Day	CF	PCV	Smear	Temperature, day # 0	1	2	3
-12	-	44	-		101.2		102.2
-4	-	46	-		101.2		
0	10 ml blood (frozen 30 days at -60 C) injected SC, from EAM carrier showing 12% parasitemia.						
2	-	49	-	102.6	101.6		
4		51	-	102.0	101.8		
7		51	-	99.6		101.6	101.4
11		53	-	102.0	101.0		
13		55	-	101.6	102.2		
16	-	54	-	101.6	99.8		100.0
20		51	-	100.0	102.0		
23	-	52	-	99.8	98.4	101.6	101.0
27	-	52	AM < 1%	101.6	99.4		
30	4	53	AM 2%	100.0	100.0		
32	1/80	52	AM 10%	99.8	100.0		
34		47	AM 38%	101.0	102.6		
37	1/1280	15	AM 98%	100.2			

38: Died, acute anaplasmosis.

9212: Splenectomised calf, Age: 90 days, Female Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-5		29	-	99.0	99.4		
-1	-	30		99.8			
0	10 ml blood (Frozen at Kabete for 7 months) injected intravenously, from EAC carrier.						
2		30		101.2	100.2	99.2	
6		36		99.8	100.4		
8		35		100.8	100.4	101.4	101.2
14		24	-	100.8	101.0	100.8	99.4
18	-	26	-	100.2		99.4	100.2
22	1∕	26	AC < 1%	100.4	100.0	101.0	100.4
27	1/20	28	AC 3%	101.2	102.0		
29	1/40	26	AC 12%	102.2	102.6		
31	1/40	23	AC 21%	104.2	101.4		
34	1/80	17	AC 34%	103.8	102.8		
36	1/320	14	AC 31%	103.0	103.0		
38	1/320	16	AC 17%	101.0	101.0		
41	1/320	19	AC 6%	101.0	103.6	102.4	101.2
45	1/160	26	AC 1%	101.0	102.0		
48	1/80	27	AC 1%	101.4	101.4	101.0	100.0
52	1/80	29	AC 1%	100.4	101.0		
55	1/80	31	AC 5%	98.4	101.2	101.2	101.0

20b.

59	1/80	27	AC 12%	101.8	100.4		
62	1/80	21	AC 10%	102.0	101.4	101.8	101.4
66	1/80	23	AC 8%	103.0	102.0		102.0
71		27	AC 1%	101.2	102.0		
73	1/80	30	AC 1%		100.6		

9279: Splenectomized calf, Age: 60 days, Female, Holstein

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-8	-	32	-	101.2	102.4	103.2	102.2
-1	-	32	-	101.4	100.6		
0	5 ml blood injected SC, from EAC carrier with 34% parasitemia.						
1	-	35	-	101.4	101.2	104.2	104.0
6	-	32	-	101.2	102.4		
8	1∕	32	-	102.0	102.8		
10	3∕	34	-	102.0	100.8		
13	3∕	34	-	101.8	101.4		
15	1/5	31	AC < 1%	102.0	101.8		
17	1/20	36	AC 2%	101.8	101.2	101.4	
20	1/80	37	AC 7%	101.4	102.2		
22	4∕	31	AC 21%	103.2	102.8		
24	1/80	28	AC 50%	103.2	104.2		
27	1/640	18	AC 14%	104.0	103.4		
29	1/640	18	AC 10%	101.4	102.4	102.2	101.6
33	1/640	23	AC 2%		101.6	102.6	
36	4∕	27	AC 1%	102.8	101.6	102.0	102.6
41	1/640	32	AC 2%	101.6	101.8	102.6	102.6
45	4∕	33	AC 3%	102.4	101.4		
48	1/160	30	AC 6%	102.8	102.8	102.4	103.2
52	4∕	23	AC 9%	102.4	103.8		
55		17		101.4	102.0		
57		22	AC 1%	103.0	101.6	103.0	102.6

57		22	AC 1%	103.0	101.6	103.0	102.6
62	–	27	AC < 1%	101.0	102.6	102.8	
66		30	–	102.4	103.0		
69	1/80	34	AC < 1%	102.6	101.4	101.6	

9280: Splenectomized calf, Age: 60 days, Female Guernsey

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-5		33		101.0	101.2	101.8	
-2	-	35		101.4	102.0	101.8	102.2
0	10 ml blood injected SC, from EAC carrier, showing 1% parasitemia.						
2		32		102.4	102.8	102.4	102.8
6	-	32		103.6	106.0		
9	-	25		101.8	102.8	102.0	102.0
13	-	26		101.2	102.2		101.2
20	-	29		101.8	102.0		
23	2∕	32		102.0	102.2	102.2	102.0
27	3∕	34	AC<1%	101.6	101.0		
30	3∕	34	AC 3%	101.0	102.2		
32	1/5	32	AC 13%	102.0	102.6	102.6	103.6
37	1/40	26	AC 13%	103.4	103.6		
39	1/20	23	AC 27%	103.4	103.6		
41		18	AC 9%	104.2	103.2		
43		19		102.8			
46	1/80	22	AC 13%	101.0	102.0		
48		25	AC 6%	102.0	102.4		
51	1/320	27	AC 11%	102.0	102.2	103.4	
55		24	AC 9%	102.4	103.0		
58	1/80	23	AC 15%	102.0	102.0	102.0	102.8
62		18	AC 15%	102.8	103.8		

65	1/160	18		101.6	101.4	102.2	102.4
69		24	AC 3%	102.8	103.0		
72	1/80	25	AC 1%	102.8	103.0	102.6	
76		29	AC 2%	101.4	102.0		
79	1/80	31	AC 2%	101.8			

9373: Splenectomized calf, Age: 110 days, Female Jersey.

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day + 0	1	2	3
-11	-	34	-	102.8	102.2	101.6	102.2
-4	-	36	-		102.0		102.8
0	5 ml blood injected SC, from EAC carrier, showing < 1% parasitemia						
3	-	30	-	101.6	101.4	102.0	102.2
7	-	32	-	102.0	101.6		
10	-	35	-	102.6	102.6		
12	1/5	36	AC < 1%	102.4	102.8	102.4	102.0
17	1/40	38	AC 1%	102.6	100.0	102.0	
20		33	AC 8%		103.0	101.8	
24	1/320	28	AC 35%	102.4	103.6		
26		20	AC 40%	104.2	103.8		
28		15	AC 22%	104.8	102.4		
31	1/320	20	AC 3%	100.0	101.6		
34		24	AC 5%	101.2	101.0	101.8	
38	1/80	27	AC 1%	101.6	102.6		
41		31	AC 2%	101.4	101.2	100.8	
45	1/40	30	AC 10%	100.6	101.6	102.6	101.8
49		28	AC 13%	101.6	102.0		102.0
54		23	AC 17%	102.0	101.6	101.0	102.4
59	1/20	23	AC 13%	102.0	102.0	102.2	102.0
63		26	AC 7%	101.8	102.0		
66	1/10	28	AC 10%	102.4	101.0		
68		28	AC 6%	101.4	102.2	102.0	101.4

9375: Splenectomised calf, Age: 110 days, Female Jersey

A. centrale EAC

Day	CF	PCV	Smear	0	Temperature, day / 1	2	3
-11	-	44	-	102.0	101.6	101.2	102.2
-4	-	41	-		102.2		101.6
0	5 ml blood injected SC, from EAC carrier, showing 8% parasitemia.						
0	-	42	-	102.6	102.8		
3	-	36	-	102.0	102.0	102.4	102.4
7	-	41	-	102.6	102.4		
9	2/	42	AC<1%		101.6		
11	2/	41	AC 1%	102.2	101.8	101.4	
14		41	AC 1%	102.2	102.8		
17	1/20	43	AC 7%	102.8	100.0	104.0	
20		34	AC 45%		104.2	103.2	
24	1/160	22	AC 75%	103.0	103.8		
26		14	AC 55%	104.6	105.2		
28		16	AC 12%	104.0	103.4		
31	1/160	22	AC 1%	100.6	101.2		
34		30	AC 1%	101.2	101.4	101.2	
38	1/40	38	AC < .5%	100.8	101.4		101.2
45	1/20	44	AC<1%	101.6	101.8	102.4	101.4
52	1/20	44	AC<1%	100.4	101.0	101.6	100.6
59	1/20	34	AC 20%	102.4	101.0	102.8	102.8
63		23	AC 30%	102.6	102.8		

66	1/40	22	AC 4%	102.4	100.8		
68		26	AC 1%	100.2	100.6	101.6	102.0
73	3/		AC<1%	101.6	101.6		
80	2/	39	AB <.5%	101.4			

9487: Splenectomized calf, Age: 120 days, Female Jersey

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-9	-	34	-	101.8		101.8	
-3	-	34	-	101.8			101.6
0	5 ml blood injected SC., from EAC carrier, showing <1% parasitemia.						
4	-	39	-	102.8	102.4	101.4	101.8
8		40	-	102.8	102.0		101.8
13		36	-	102.2	102.0	102.0	101.4
20		33	-	101.2	101.6		
23		32		102.0			
25	1/10	39	-	101.4	101.8	101.4	102.6
29		35	AC 4%	102.0	103.0		
32	1/20	30	AC 16%	101.4	102.8	101.6	102.0
36		21	AC 12%	101.8	105.0		
39	1/160	13	AC 8%	105.6	105.4		
41		11	AC 4%	103.8	101.0		
43		13	AC 2%	100.4	100.8		
47	4	18	AC < 1%	102.6	102.0		
50		23	AC < .5%	101.6	102.0		
53	4	26	AC < .5%	103.6	99.6	102.0	100.0
58	4	28	AC < 1%	100.6		101.4	100.4
62		32	AC < 1%	102.0	102.0	102.0	102.0

500 ml blood removed by venepuncture.

../25b.

67	4	30	AC 5%	101.2	100.0		101.0
74	4	19	AC 10%	100.2	101.0		
76		16	AC 7%	101.6	102.0	102.0	99.6
81	4	21	AC 2%	101.2	100.0	99.8	100.0
88	4	30	AC 3%	99.0	101.0	100.8	101.4
95	4	32	AC 4%	100.0	100.4	100.6	

9747: Splenectomized calf, Age: 75 days, Male, Holstein

A. centrale EAC

Day	CF	PCV	Smear	0	Temperature, day / 1	2	3
-12	-	43	-		101.4		102.8
-5	-	42	-		99.8		101.0
0	100 ml packed, washed erythrocytes injected intravenously, collected from EAC carrier, showing a 4% parasitemia.						
2		47	-		100.6	100.0	100.8
6		50	-	102.2	98.4	101.0	
9	Tr.	50	AC < .5%		100.0	100.8	
12		46	AC < 1%	101.2	101.2	100.4	
16	3/	43	AC 1%	101.0	100.0	101.0	
19	2/	45	AC 3%	101.2	99.8	100.0	
23	1/160	46	AC 10%	100.8	101.0	101.0	
26	4/	38	AC 15%	100.0	100.0		
28		32	AC 18%	100.6			
30	1/160	25	AC 10%	101.0	100.6	101.6	
33		19	AC 5%		101.0	101.6	
37		23	AC 3%	99.4	101.0	101.4	
40	4/	29	AC 2%		102.4	101.6	101.4
44	4/	30	AC 1%	101.4	101.0	101.2	
47	4/	30	AC 1%	101.4	102.0	101.0	
51	1/40	32	AC 1%	97.4	101.4	101.4	
58	4/	30	AC 2%	99.8	102.0	101.0	

../26b.

65	4/	23	AC 2%	102.6	101.0	101.0	101.6
72	4/	21	AC 3%	101.6	101.6	101.4	101.6
79		25	AC 1%	99.8		100.4	102.8
86	4/	27	AC 1%	101.6	101.6	101.6	101.0

9751: Splenectomized calf, Age: 90 days, Male, Jersey

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-7	-	37	-	100.4	100.0	101.0	100.8
0	100 ml. washed, packed erythrocytes injected intravenously, collected from EAC carrier, showing a 10% parasitemia. (Second serial passage of large quantity of infected blood).						
0	-	35	-	100.6			
3		32	AC 1%		102.0	102.0	
7	-	36	AC 1%	102.2			
8		34	AC 7%	101.0			
9		32	AC 6%	100.0			
10	4/	29	AC 14%				
11		27	AC 20%	101.0			
12		24	AC 12%	104.0			
14	4/	18	AC 12%	102.0	102.6	103.6	
17	4/	23	AC 3%	101.6	103.0	102.0	
21	1/80	29	AC 2%	102.6	102.6	102.6	
24		34	AC <.5%		101.6	102.0	
28	4/	40	AC 2%	102.4	102.0	102.0	
35	4/	39	AC 6%	102.0	102.0	102.4	103.0
42	4/	21	AC 8%	102.4	102.0	102.0	101.6
49		29	AC 2%	102.2		102.0	101.8
56	4/	33	AC 2%		101.6	102.0	102.0

9754: Splenectomised calf, Age: 100 days, Male Holstein

A. centrale. EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-12	-	34	-	101.0	100.2	101.0	
-5	-	32	-	102.0	101.2	102.0	101.6
0	120 ml. washed, packed erythrocytes injected intravenously, collected from EAC carrier showing 12% parasitemia. (3rd serial passage of large quantity infectious blood).						
2	-	34	AC 4%	101.4	102.6	100.4	
5	-	28	AC 16%	101.4	100.4		
7		27	AC 19%	99.8			
9	1/40	22	AC 42%	101.0	101.6	103.4	
12		15	AC 6%	100.0	100.4	98.6	
16	4/	21	AC 2%	98.4	100.6	100.4	
19		26	-	101.0	100.6	102.0	
23	4/	28	AC < .2%	102.0	101.4	100.0	
26		28	AC < 1%	102.0	101.6	100.0	
30	4/	30	AC < 1%	102.2	102.0	102.0	101.0
37		33	AC 2%	101.0		101.6	101.6
44	4/	29	AC 6%	102.6	101.6	100.4	101.0
51		23	AC 12%	99.0	101.0	100.0	
54		20	AC 8%	100.0	100.6	102.7	

9363: Non-splenectomized calf, Age: 75 days, Female Guernsey

A. marginale EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-8	-	38	-	102.8	102.8	102.4	102.4
-1	-	40	-	101.6	101.8		
0	5 ml blood injected SC, from EAM carrier, showing 12% parasitemia.						
1	-	36	-	102.0	101.8		
3	-	38	-	102.2	103.4		
6	1/40	35	-	102.2	102.0		
8	1/320	38	-	102.0	103.0		
10	1/640	33	AM < 1%	102.0	102.8		
13	1/320	34		103.0	102.6	101.8	102.6
17		29	AM 5%	102.0	102.6		
19	1/640	24	AM 35%		102.6	104.0	
22		19	AM 56%	103.6	104.0	103.0	
27	1/1280	22	AM 3%	102.8	103.8	101.6	101.6
31		29	AM 3%	102.4	102.2		
34	1/640	28	AM 2%	102.6	102.8	103.0	102.0
38		33	AM 2%	102.4	103.0		
41	1/320	35	AM 1%	102.8	103.0	102.6	102.8
45		34	AM 1%	102.4	102.0		
48	1/320	35	AM < 1%	102.6	100.0	102.4	102.4
52		41	AM < 1%	102.6	102.0		
57		40	AM < 1%	102.4	102.2	102.0	102.4
62	1/160	42	AM < 1%	101.8	101.6	100.4	
69	4/	38	-	102.6	102.0	101.6	102.4

9692: Non-splenectomized calf, Age: 45 days, Male Holstein

A. Marginale, EAM

Day	CF	PCV	Smear	Temperature, day # 0	1	2	3
-3	-	36	-		102.4		
-1		35	-	102.8	102.8		
0	5 ml blood injected SC, from EAM carrier, showing 4% parasitemia.						
1		34	-	102.8	101.8		
4	-	37	-	102.2	101.2	102.0	101.4
8	-	35	-	103.0	102.2		
11	1/160	34	AM < 1%	101.6	101.6	102.4	102.4
15		31	AM 3%	102.6	101.6		
19	1/320	18	AM 18%	103.6	105.6	104.0	
22		14	AM 15%	104.2	104.8		
25	4	18	AM 2%	103.0	102.2	100.8	101.2
30		25	AB 1%	99.8			
32	1/640	25	AM 1%	102.0	101.4	101.6	101.0
36		29	-	102.2	102.0		
39	1/320	32	AM 1%	102.0	100.4		
43		30	AB <.5%	100.6	100.6		
46	4/	32	-	99.0	99.2	102.0	99.4
53	4/	35	-	99.8	99.8	100.2	100.8
60	3/	36	AM <.5%	98.4	101.0	101.0	100.6
67	3/	38	AM <.5%	98.0	101.0	99.2	100.2
74	2/	31	AM <.5%	101.6	99.8	99.0	100.2
81	2/	34	-	100.0	101.0	101.6	103.0

9693: Non-splenectomized calf, Age: 45 days, Male Holstein

A. marginale EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-3	-	33	-		101.4		
-1		33	-	102.8	102.2	102.8	102.6
0	5 ml blood injected SC., from EAM carrier, showing 4% parasitemia.						
4	-	34	-	102.0	101.6		
6	-	34	-	101.0	102.0		
8	4/	35	-	102.6	102.2		
11	1/320	34	AM < 1%	102.0	102.2	102.6	102.0
15		25	AM 4%	101.8	103.2		
19	1/640	23	AM 2%	102.0	100.4	100.6	
22		24	AM 1%	103.6	104.4		
25	4	26	AM < 1%	101.6	102.6	101.6	102.0
30		29	AB < 1%	102.6			
32	1/640	30	AM < .5%	102.0	101.4	102.0	101.0
36		30	AB < .1%	99.8	100.8		
39	1/160	29	AM < .5%	101.6	100.0		101.6
43		35	AM < .5%	102.0	101.0		
46	4/	32	-	100.6	100.6	101.6	99.4
53	4/	33	-	101.0	100.0	100.0	100.4
60	4/	33	-	99.0	100.0	101.0	101.0
67	4/	35	-	101.0	99.6	99.2	99.6

9694: Non-splenectomized calf, Age: 45 days, Male Holstein

A. marginale EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	-	42	-		101.8		
-1	-	41	-	102.0	103.0	102.0	102.6
0	5 ml blood injected SC; from EAM carrier, showing 4% parasitemia.						
4	-	43	-	101.8	102.6		
6	-	39	-	101.4	102.0		
8	3/	40	-	102.4	102.4		
11	1/160	40	-	101.4	101.6		
13		39	AM 1%	102.8	102.0		
15		34	AM 3%	102.0	101.4		
19	1/640	28	AM 24%	104.2	105.6	104.8	
22		18	AM 30%	106.0	104.8		
25	4/	16	AM 18%	103.0	102.8		
27		20	AM 2%	102.0	101.8		
30		23	AM <1%	101.6			
32	1/1280	28	AM <1%	102.0	101.6	102.0	100.0
36		29	AM 2%	101.4	102.4		
39	1/640	30	AM 2%	102.6	102.2		102.0
43		30	AM 3%	103.6	103.6		
46	4/	30	AM <.5%	102.0	103.0	102.4	100.2
53	4/	32	-	101.0	101.2		
60	4/	35	-	99.4	102.0	103.0	103.6
67	4/	35	-	100.4	100.0	99.2	100.4
74	4/	35	-	101.0	98.4		

9369: Non-splenectomised calf, Age: 45 days, Male Guernsey

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day # 0	1	2	3
-6		41		100.0	100.4	99.6	
-1		38		100.0	100.6	99.0	101.2
0	5 ml blood injected SC., from EAC carrier, showing 34% parasitemia.						
3		36	-	102.0	102.0		
6		36	-	101.2	101.4	100.0	100.0
10	3/	36	-	101.0	100.4		
13	3/	38	-	101.4	99.0		
15	1/5	37	-	99.6	100.6	101.4	101.4
20	1/10	40	-	98.8	100.8	101.0	101.6
24	1/10	38	-	101.4	100.6		101.2
29	1/10	39	-	101.6	101.0	102.4	101.4
34	1/20	33	AC < 1%	102.0			
36		36	AC < 1%	102.8	103.0	101.6	102.0
41	1/80	32	-	101.4	100.6	100.4	101.2
45		36	AC < 1%	100.4	100.4		
48	1/160	34	AC < 1%	101.0	100.4	101.4	101.8
52		31	AC < 1%	102.0	103.4		
55	1/160	31	-	102.6	103.2	102.4	102.4
59		32	-	101.8	102.2		
62	1/160	29	-	102.4	102.6	101.4	
66		29		101.6	102.6		
69	1/80	32	-	101.8	103.0	102.0	101.8
76	1/80	31	-	101.8			

9370: Non-splenectomized calf, Age: approx. 45 days, Male, Guernsey

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-6	-	35	-	101.2	100.4	100.2	
-1	-	37	-	100.0	100.4	100.2	101.6
0	5 ml blood injected SC., from EAC carrier showing 34% parasitemia.						
3	-	36	-	99.8	101.6		
6	-	38	-	99.2	101.0		
8	2/	39	-	102.4	101.2		
10	1/80	41	-	101.8	101.4		
13	1/80	39	-	101.4	99.2	100.0	100.8
17	1/160	41	AC < 1%	101.2	101.0		
20	1/160	37	AC 1%	100.2	99.8	100.0	102.0
24	1/80	30	AC 17%	102.2	101.4		
27	1/80	19	AC 21%	102.2	101.2		
29	1/80	16	AC 12%	101.0	101.8		
31		19	AC 4%	101.6	101.4		
34	1/80	23	AC<1%	99.0	101.2		
36		25	AC < 1%	101.2	101.0	101.8	100.2
40	1/80	26	-		101.0	100.4	
43		29	AC < 1%	100.8	100.8	100.4	101.0
48	1/80	30	-	101.0	101.4		
50		29	-	102.4	102.6	101.8	102.2
55	1/20	25		101.4	101.4	102.0	102.0
59		24	-	102.4	102.2		
62	1/10	25	-	102.0	102.4	101.0	
66		29	-	101.8			

9374: Non-splenectomized calf, Age: 120 days, Male, Guernsey

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-4	-	36	-		101.6		
-1	-	36	-	101.4	102.0	101.8	
0	5 ml blood injected SC., from EAC carrier, showing 12% parasitemia.						
3	-	35	-	102.2	102.0		
5	-	37	-	101.4	101.0		
7	3/	39	-	101.0	102.0		
10	4/	35	-	101.2	102.0	102.2	101.6
14		32	AC < 1%	102.0	101.8		
17	1/320	32	AC < 1%	102.2	101.4		
19		32	-	101.4		101.6	99.6
24	1/320	32	-	101.6	100.2	99.6	101.4
28		32	AC < 1%	99.8	99.6		
31	1/160	30	AC < 1%	100.6	101.6		
34		35	AC 1%	100.8	100.8	100.0	
38	1/160	30	AC < 1%	100.6	101.2		
41		27	AC <.5%	102.0	100.0	101.4	
45	1/160	31	-	101.8	99.8	102.0	99.8
49		37	AC < 1%	100.8	100.2		
52	1/80	35	-	101.6	99.4	101.6	100.6
56		37	AC <.1%	100.0	100.0		
59		40	-	102.2	101.6	101.0	101.4
63		39	AC<.1%	101.6	100.2		
66	1/80	43	-	101.8	100.4	101.8	101.8

9461: Non-splenectomized calf, Age: 60 days, Female Holstein

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-4	-	40	-		101.4		
-1	-	37	-	102.2	104.0	102.0	
0	5 ml blood injected SC., from EAC carrier, showing 12% parasitemia.						
3	-	35	-	102.0	103.0		
5	-	35	-	101.6	102.2		
7	-	37	-	103.0	102.8		
10	4∕	34	-	102.6	103.2	103.4	103.0
14		32	-	103.4	103.0		
17	1/40	32	AC < 1%	102.8	103.2		
19		35	-	102.2		102.6	101.4
24	1/160	36	-	101.6	102.6	102.6	102.2
28		37	-	102.0	101.4		
31	1/80	36	AC <.5%	101.6	101.6		
34		39	AC <.5%	101.2	101.4	101.4	
38	1/80	32	AC <.5%	99.8	103.0		
41		32	AC <.5%	101.6	101.4	102.0	
45	1/40	32	AC <.5%	101.4	101.6	101.6	102.0
49		31	-	101.8	101.0		
52	1/40	32	-	101.0	101.2	101.0	101.0
56		31	AC < .1%	100.8	101.8		
59	1/80	31	-	102.0	102.0	101.2	101.4
63		24	AC < 1%	101.4	100.8		

...36b.

66	1/40	31	-	102.4	101.4		
68		31	AC <1%	101.0	101.0	101.2	101.6
73	1/	32	-	101.8	101.6		

9462: Non-splenectomized calf, Age: 60 days, Female Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	-	42	-	103.0	102.6	102.0	102.4
-4	-	29	-		101.2		101.8
0	5 ml blood injected SC., from EAC carrier, showing 12% parasitemia.						
0	-	29	-	102.0	103.0		
3	-	28	-	101.4	101.0		
5	-	29		102.2	103.2		
7	2/	28		103.6	102.6		
10	4/	27		102.2	103.4	103.0	103.2
14		27	AC <1%	103.2	103.2		
17	1/160	25	AC <.1%	103.8	104.6	104.8	
20		23	AC <1%		103.2	101.0	
24	1/640	27	-	101.0	101.2	101.8	101.6
28		26	-	101.8	101.6		
31	1/640	27	-	100.6	101.2		
34		31	-	101.4	102.0	101.4	
38		30	AC <1%	101.2	101.6	102.0	
41		29	-	102.0	102.2	101.8	
45	1/80	32	-	101.4	101.8	103.0	101.4
49		32	AC <.5%	102.0	102.2		
52	1/40	30	AC <1%	102.0	102.2	102.0	101.6
56		31	-	101.4	101.2		
59	1/40	31	-	101.8	101.8		
61		31	-	101.4	102.0	101.8	101.4
66	1/40	33	-	101.4			

9463: Non-splenectomized calf, Age: 60 days, Male Guernsey

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day + 0	1	2	3
-11	-	32	-	101.4	101.6	101.0	102.0
-4	-	30	-		102.0		102.0
0	5ml blood injected SC., from EAC carrier, showing 12% parasitemia.						
0	-	32	-	102.0	101.6		
3	-	31	-	102.0	103.0		
5	-	32		102.2	102.4		
7	-	31	AC < 1%	102.0	103.0		
10	4/	37	AC < .5%	101.6	101.6	100.8	101.8
14		32	AC < 1%	101.6	101.6		
17	1/320	33	AC 1%	101.6	102.4	103.2	
20		23	AC 6%		104.0	102.6	
24	1/640	12	AC 10%	102.2	103.6		
26		14	AC 2%	102.0	101.6		
28		17	AC 1%	100.8	99.0		
31	1/1280	21	AC < 1%	100.4	101.6		
34		26	AC 1%	101.0	101.2	101.6	
38	1/320	30	AC < .5%	101.0	101.6	101.6	
41		29	-	100.4	101.0	101.4	
45	1/320	31	AC < .5%	101.4	101.6	100.4	100.2
49	3/	35	AC < 1%	101.0	101.4		
52	1/160	31	AC 1%	99.6	101.4	98.8	101.6
56		30	AC < 1%	101.0	101.2		
59	1/160	32	AC < 1%	101.4	100.4	101.4	101.8

.../38b

[illegible] Yearling, Female Holstein

[illegible]

				Temperature, day #			
63	[illegible]	31	40 < 1%	100.8	102.6	2	3
66	1/80	33	48 < 1%	100.0	98.4	101.6	101.8
73	2/	38	-	100.6	101.6		

9 5 cc. blood injected No., from BH carrier, showing 1% parasitemia.

5	-	35	-	100.4	101.6	100.8
8	4/	38	-	102.2	100.4	102.0
12	1/320	37	2K < 1%	102.6	102.2	102.0
15		29	2K 0%		102.6	102.0
19	1/640	26	2K < 1%	102.4	101.0	101.6
22		32	-	101.8	101.4	102.0
26	1/640	33	-	102.6	101.6	102.0
29		38	-	102.0	101.4	101.6
33	1/320	33	2K < .1%	102.6	101.6	102.0
36		38	2K < .1%	102.0	101.4	101.6
40		34	2K < 1%	102.0		102.0
43		36	2K < 1%	101.8	101.6	101.4
47	1/640	35	2K < .1%	102.0	102.0	102.4
50		32	2K < .1%	102.0	101.6	101.0
54		33	2K < .1%	102.2	102.6	102.4
57		34	-	101.6	102.0	102.4
61		34	-	101.8	100.8	101.6
64		34	-	102.0		

9271: Yearling, Female Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-8	-	39	-				
-2	-	42	-				
0	5 ml blood injected SC., from EAM carrier, showing 7% parasitemia.						
5	-	40	-	101.4	101.6	101.6	
8	4/	39	-	101.4	101.0	101.2	
12	1/320	38	AM <1%	102.0	102.0	101.4	
15		33	AM 2%		102.4	104.4	
19	1/1280	26	AM 12%	105.6	103.6	102.0	
22		20	AM 2%	102.8	102.0	101.6	
26	1/1280	25	-	102.0	101.2	101.8	
29		29	AC <.1%	102.0	101.4	101.0	
33	1/640	29	AM <.1%	102.6	101.6	101.8	
36		33	-	101.6	100.0	101.4	
40		34	-	102.0		101.8	
43		39	-	101.4	101.2	101.6	
47	1/320	38	-	101.6	101.0	101.4	
50		39	-	101.6	101.0	101.6	
54		38	-	102.0	100.8	101.4	
57		39	AB <.1%	101.6	100.8	101.0	
61		43	-	101.6	101.2	101.6	
64		39	-	101.8	101.0	102.0	

9557: Yearling, Female Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day + 0	1	2	3
-8	-	35	-				
-2	-	37	-				
0	5 ml blood injected SC., from EAM carrier, showing 7% parasitemia.						
5	2/	35	-	101.2	101.4	101.6	
8	4/	32	AM < 1%	103.4	101.2	102.0	
12	1/320	32	AM 1%	103.0	102.0	102.2	
15		22	AM 3%		101.4	102.2	
19	1/320	26	AM <.1%	104.2	102.2	101.4	
22		32	-	102.0	101.6	101.0	
26	1/160	32	-	103.4	101.2	101.8	
29		34	-	102.2	101.0	101.8	
33	1/80	35	-	102.6	101.6	101.0	
36		37	-	101.6	101.4	102.0	
40		38	AM <.1%	102.6		102.0	
43		38	AB < .1%	102.4	100.8	100.4	
47	1/80	33	AB <.1%	101.6	101.0	100.0	
50		30	AM 1%	102.6	101.2	101.6	
54		35	AM 1%	102.0	101.8	102.8	
57		29	-	106.0	103.0	102.0	
61		32	-	102.4	101.8	101.6	
64		31	-	101.8	101.0	102.0	

9567: Yearling, Male, Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-8	-	34	-				
-2	-	36	-				
0	5 ml blood injected SC., from EAM carrier, showing 7% parasitemia.						
5	-	35	-	102.2	101.6	100.0	
8	4/	33	-	101.0	101.4	100.4	
12	1/320	33	AM <.2%	101.8	100.0	101.2	
15		29	AM 1%		102.2	102.2	
19	1/640	20	AM 5%	103.4	102.0	102.6	
22		18	AM <.5%	102.0	102.0	101.6	
26	1/640	27	-	102.6	101.4	102.0	
29		31	-	103.0	101.4	101.2	
33	1/320	30	-	102.0	102.0	100.4	
36		30	-	102.0	101.4	102.0	
40		31	AM <1%	102.4		101.8	
43		32	-	100.6	100.0	100.8	
47	1/80	32	-	102.6	101.6	102.6	
50		31	-	102.6	101.6	101.4	
54		33	AM <.1%	101.6	100.8	102.0	
57		31	-	101.6	102.0	101.6	
61		30	-	102.0	101.6	101.0	
64		30	-	102.6			

9575: Yearling, Male Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	37	-				
-2	-	37	-				
0	5 ml blood injected SC., from EAM carrier, showing 7% parasitemia.						
5	-	36	-	102.2	100.4	100.0	
8	4/	37	AM <.5%	101.4	101.4	101.6	
12	1/320	34	AM 2%	102.4	101.6	102.0	
15		26	AM 3%		102.0	102.2	
19	1/1280	17	AM 3%	103.2	102.0	101.6	
22		20	-	102.4	101.6	100.8	
26	1/640	26	-	102.2	101.6	101.0	
29		28	-	102.0	101.4	101.6	
33	1/320	30	-	101.6	100.8	101.0	
36		30	AM <.5%	102.0	101.0	101.8	
40		30	-	102.0		102.0	
43		34	-	101.6	100.8	100.4	
47	1/160	35	-	102.4	101.2	101.0	
50		33	AB <.1%	102.0	102.2	101.4	
54		31	AM <1%	102.0	101.6	101.6	
57		33	AM <1%	102.0	101.6	101.6	
61		33	AB <1%	101.8	102.2	101.6	
64		30	AB <.1%	102.0	102.0	101.6	

9576: Yearling, Female, Holstein

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	32	-				
-2	-	32	-				
0	5 ml blood injected SC., from EAM carrier, showing 7% parasitemia.						
5	4/	36	-	101.0	102.4	101.4	
8	4/	31	AM <.5%	101.8	102.0	102.0	
12	1/320	26	AM 3%	105.8	102.8	103.2	
15		19	AM 17%		105.0	104.0	
19	1/640	18	AM 8%	104.0	103.0	102.6	
22		17	AM 2%	102.0	101.8	102.6	
26	1/640	22	AM 2%	102.8	102.0	102.2	
29		22	AM 2%	101.6	102.4	100.6	
33	1/640	25	AM 1%	102.6	101.6	102.2	
36		24	AM 1%	102.2	101.4	101.4	
40		26	AM <.4%	102.6		102.0	
43		32	-	102.4	102.0	102.8	
47	1/160	33	-	102.0	102.0	101.6	
50		32	-	102.0	101.4	100.4	
54		32	-	102.6	100.0	101.0	
57		31	-	101.6	102.0	102.2	
61		31	-	102.4	102.2	101.6	
64		31	-	102.0			

9218: Yearling, Female Holstein

A. centrale EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	31	-				
-2	-	36	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	-	31	-	101.4	101.4	100.6	
8	4/	31	-	102.0	101.0	101.0	
12	1/40	32	-	100.4	101.4	101.0	
15		32	AC <.2%		101.6	102.0	
19	1/160	30	AC 1%	102.6	102.0	103.0	
22		26	AC 4%	101.8	102.0	101.8	
26	1/160	18	-	104.0	103.0	103.0	
29		18	AC 2%	102.6	101.4	102.6	
33	1/160	22	AC 1%	103.0	101.2	101.4	
36		24	-	102.0	101.6	101.6	
40	4/	27	-	102.0	102.0	102.0	
43		31	-	102.0	102.0	101.6	
47	1/40	31	-	102.0	101.2	101.0	
50		32	AC <.1%	102.4	101.0	100.4	
54		32	-	102.6	102.0	101.4	
57		32	-	102.0	101.6	101.4	
61		31	-	101.4	101.0	102.0	
64		31	-	102.0	100.0	101.6	

9229: Yearling, Female Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	32	-				
-2	-	36	-				
0	5 ml blood injected SC., from EAC carrier, shewing 6% parasitemia.						
5	-	35	-	101.4	100.4	101.2	
8	3	33	-	102.0	101.0	100.6	
12	1/20	35	AC <.1%	102.6	101.4	102.0	
15		36	-		101.4	102.0	
19	1/80	32	-	102.6	101.4	100.4	
22		30	AC 1%	100.4	100.0	101.0	
26	1/80	27	AC 2%	103.0	101.0	101.6	
29		28	AC 2%	102.4	101.0	102.0	
33	1/80	26	AC 2%	102.6	102.0	102.0	
36		27	AC 2%	102.6	101.6	101.6	
40		34	AC <.5%	102.0		101.4	
43		32	AC <.1%	101.8	102.0	102.0	
47	1/40	32	-	102.8	101.6	101.0	
50		30	-	102.4	101.6	101.6	
54		33	AB <.1%	102.2	102.0	99.0	
57		35	AC <.2%	102.0	101.0	102.2	
61		34	AC <.2%	101.4	101.0	101.4	
64		34	AC <.2%	102.4			

9424: Yearling, Female Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	38	-				
-2	-	38	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	-	40	-	102.2	101.6	101.6	
8	4/	39	-	101.4	101.6	101.0	
12	1/80	40	AC <.2%	101.4	101.6	102.0	
15		36	AC <.5%		101.8	101.4	
19	1/160	33	AC 2%	103.0	101.4	101.4	
22		33	AC 3%	101.8	100.0	102.6	
26	1/160	25	AC 3%	104.0	101.6	101.8	
29		24	AC 2%	102.6	101.6	101.4	
33	1/160	27	AC <1%	102.0	102.4	102.0	
36		30	AC <1%	102.8	101.2	102.6	
40		31	-	102.0		101.8	
43		34	-	100.6	102.0	102.0	
47	1/80	35	AC <.2%	102.0	101.0	100.6	
50		35	-	102.6	101.6	100.0	
54		36	AC <1%	102.0	101.6	100.8	
57		36	-	101.6	102.0	102.0	
61		36	-	102.0	101.4	101.0	
64		36	-	101.6			

9565: Yearling, Male, Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	36	-				
-2	-	35	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	-	38	-	101.2	101.0	100.0	
8	3/	38	-	102.0	100.6	101.4	
12	1/80	35	-	101.6	100.0	100.0	
15		34	-		101.8	100.4	
19	1/160	34	-	102.0	101.0	100.6	
22		33	AC <1%	100.0	101.0	101.4	
26	1/160	32	AC 1%	102.6	101.8	101.0	
29		30	AC 7%	102.0	101.8	101.6	
33	1/160	24	AC 4%	102.2	101.6	101.4	
36		24	AC 5%	102.2	101.2	101.6	
40		25	AC 4%	103.0		101.4	
43		28	AC 2%	101.0	100.8	101.0	
47	1/320	28	AC 2%	102.0	102.0	100.4	
50		28	AC 1%	101.6	101.6	101.0	
54		30	AC 2%	102.0	100.6	101.0	
57		32	AC <.2%	101.2	100.0	101.0	
61		33	-	101.2	102.0	101.0	
64		34	AC <.5%	101.0	101.6	101.0	

9568: Yearling, Male, Jersey

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	36	-				
-2	-	37	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	1/	40	-	101.0	101.4	101.0	
8	4/	39	-	101.2	101.2	102.0	
12	1/40	39	AC <.5%	102.6	101.0	100.0	
15		36	AC <.5%		101.4	101.6	
19	1/80	32	AC 2%	103.0	103.0	101.4	
22		25	AC 4%	101.0	101.6	101.8	
26	1/320	16	AC 4%	104.0	102.0	101.8	
29		20	AC 2%	102.4	101.4	100.8	
33	1/320	21	AC 2%	102.0	101.4	100.0	
36		25	AC <1%	102.2	101.0	102.0	
40		27	AC <1%	102.2		102.0	
43		31	AC <1%	101.2	101.6	101.0	
47	1/320	29	-	102.0	101.0	100.6	
50		27	AC <.3%	101.6	101.0	101.8	
54		32	AC <1%	102.0	102.0	101.8	
57		31	-	101.6	101.0	101.6	
61		32	-	102.0	101.8	101.0	
64		31	-	101.6			

9574: Yearling, Female, Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	-	35	-				
-2	-	38	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	-	36	-	101.0	101.4	101.6	
8	4/	36	-	102.4	102.4	102.0	
12	1/320	36	AC <.1%	102.6	102.6	101.0	
15		38	AC <1%		102.4	101.4	
19	1/320	23	AC 2%	103.0	102.4	102.0	
22		27	AC 4%	102.0	101.8	102.0	
26	1/320	21	AC 4%	103.0	101.4	101.8	
29		24	AC 2%	102.6	101.8	101.4	
33	1/320	27	AC 2%	102.0	101.6	101.0	
36		29	AC <1%	102.0	102.?	101.2	
40		32	AC <.5%	101.8		102.0	
43		35	-	102.0	101.6	102.0	
47	1/160	36	AC <.1%	102.2	101.0	100.6	
50		33	-	102.4	101.6	101.0	
54		36	-	101.0	101.0	100.0	
57		36	-	101.2	101.0	101.0	
61		36	-	102.4	102.4	102.0	
64		36	AB <.3%	102.4	101.0	102.0	

9577: Yearling, Female, Holstein

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day of 0	1	2	3
-8	-	36	-				
-2	-	37	-				
0	5 ml blood injected SC., from EAC carrier, showing 6% parasitemia.						
5	-	34	-	101.0	101.4	101.0	
8	4/	37	-	101.4	101.0	101.4	
12	1/320	35	-	103.0	102.4	101.8	
15		33	-		100.8	102.0	
19	1/160	32	AC 1%	103.0	102.2	101.8	
22		27	AC 4%	101.4	101.0	102.2	
26	1/320	22	AC 7%	103.4	102.4	100.2	
29		22	AC 2%	102.5	102.0	101.8	
33	1/320	24	AC <.5%	102.2	101.8	101.0	
36		27	AC <1%	102.0	101.6	101.0	
40		28	AC <.2%	102.0		101.8	
43		30	-	101.6	101.6	101.0	
47	1/160	33	-	102.4	101.6	101.4	
50		32	-	102.0	101.2	102.0	
54		32	-	101.6	101.4	101.2	
57		33	-	102.0	101.8	101.6	
61		33	-	102.0	101.8	102.0	
64		34	-	101.6			

9380: Adult Steer

A. marginale. EAM

Day	CF	PCV	Smear
-14	-	42	-
-7	-	44	-
0	-	43	-
0	5 ml blood injected SC from EAM carrier, showing a 15% parasitemia.		
7	1/	42	-
11	4/	40	-
14	4/	42	AM <.1%
18	1/160	37	AM 3%
21	4/	32	AM 3%
25	1/640	28	AM <1%
32	4/	32	-
39	4/	36	-
46	4/	37	-
53		37	AB <.1%

9387: Adult Steer

A. marginale. EAM

Day	CF	PCV	Smear
-14	-	38	-
-7	-	40	-
0	-	37	-
0	5 ml blood injected SC., from EAM carrier, showing a 15% parasitemia.		
7	2/	37	-
11	3/	37	-
14	4/	37	AM <1%
18	1/640	35	AM 7%
21	4/	27	AM 8%
25	1/640	19	AM 5%
32	4/	27	-
39	4/	30	-
46	4/	33	AM <.1%
53		34	-

9382: Adult Steer

A. marginale, EAM

Day	CF	PCV	Smear
-14	Tr.	42	-
-7	-	42	-
0	Tr.	40	-
0	5 ml blood injected SC., from EAM carrier, showing a 15% parasitemia.		
7	1/	38	-
11	4/	38	AM <.1%
14	4/	37	AM <.1%
18	4/	37	AM 1%
21	4/	32	AM 6%
25	1/640	22	AM 16%
32	1/320	19	AM 1%
39	4/	26	AM <.1%
46	3/	31	AM <.2%
53		32	AM <.5%

9388: Adult Steer

A. marginale, EAM

Day	CF	PCV	Smear
-14	-	38	-
-7	-	40	-
0	-	42	-
0	5 ml blood injected SC., from EAM carrier, showing a 15% parasitemia.		
7	1/	39	-
11	4/	35	AM 1%
14	4/	33	AM 5%
18	1/1280	19	AM 4%
21	4/	17	AM 1%
25	1/640	26	-
32	4/	27	-
39	4/	30	AM <.5%
46	4/	36	-
53		36	-

9475: Adult Steer

A. marginale, HAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	-	33		102.2		100.4	
-4	-	31		101.0	99.6		102.2
0	5 ml blood from HAM carrier injected SC., parasitemia 18%						
3	-	30	-		100.4		
6		28	-	102.0		101.2	
10	1/20	30	-	101.2	101.6		
12		30	AM <1%	101.0			
13		29	-	101.2	104.4	101.2	
17	1/1280	27	AM 4%	103.4	104.8	104.4	
20		19	AM 22%	106.4			
22		11		105.8			

24: Died, acute anaplasmosis.

9479: Adult Steer

A. marginale, EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	-	42	-	102.4		100.8	
-4	-	40	-		101.6		101.6
0	5 ml blood injected SC., from EAM carrier, showing 18% parasitemia.						
3	-	37	-	100.4			
6		37	-	101.8		101.2	
10	1/640	37	-	101.6	101.8		
12		37	AM 1%	102.0			
13		33	AM 1%	101.4	103.0	101.4	
17	1/1280	29	AM 8%	103.4	105.8	104.6	
20		19	AM 12%	105.8			
22		13		104.6			
24	1/1280	15	-	104.0	101.8	101.8	
27		19	AM 1%	102.4	102.0	101.6	
31	4/	25	-	101.0	101.0	102.0	
34		29	-	101.0	101.0	100.6	
38	4/	28	AM <.5%	101.4	99.8	102.0	
41		27	AB <.5%	101.4	101.2	100.6	
46	4/	32	-	100.0	100.6	101.6	101.0
52		38	-	100.6	100.6	101.6	101.6
59	4/	37	AM <.1%	101.8			

9855: Adult

A. marginale. EAM

Day	CF	PCV	Smear
-14	-	36	-
-7	-	38	-
0	-	38	-
0	5 ml blood injected SC., from EAM carrier showing 15% parasitemia.		
7	4/	36	-
11	4/	33	AM 3%
14	4/	28	AM 6%
18	1/1280	20	AM 2%
21	1/320	26	-
25	4/	29	-
32	4/	30	AM <.5%
39	4/	31	-
46	4/	33	-
53		35	AM <.2%

9474: Adult Steer

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-12	-	38	-		101.4		100.2
-5	-	40	-		101.0		100.6
0	5 ml blood injected SC., from EAC carrier, showing 1% parasitemia.						
2	-	41	-		102.0	101.0	
5	-	37	-	101.4	100.8	101.4	
9	1/40	44	-	101.6	103.0	102.2	
12		45	-	101.0	99.8		
16	1/640	42	-	101.6	101.2	101.8	
19		40	-	101.6	100.8	101.0	
23	1/160	40	AC < 1%	100.4	102.2	101.0	100.2
27		37	AC 1%	100.6	100.0		
30	1/80	32	AC < .5%	101.6	102.2	100.8	
33		29	AC 1%	101.0	101.4	101.4	
37	1/10	36	-	101.8	101.0	101.4	
40		30	-	100.6	101.4	101.4	
44	2∕	35	-	102.0	101.0	102.4	
47		34	-	101.4	101.0	101.6	
51	Tr.	37	-	100.4	101.0	99.8	
54		33	-	102.0	102.4	102.2	
58	1∕	36	-	101.4	101.2	100.0	101.4
65	1∕	34	-	102.0			

9476: Adult Steer

A. centrale. EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-12	-	33	-		101.0		101.0
-5	-	35	-		101.6		101.6
0	5 ml blood injected SC., from EAC carrier, showing 1% parasitemia.						
2		35	-		101.6	101.0	
5		31		101.6	102.0	101.8	
9	3/	34	-	102.0	102.0	102.2	
12		33		101.6	100.8	100.0	
16	1/80	31	-	101.8	101.2	101.0	
19		32	-	102.0	101.6	101.4	
23	1/320	30	AC <.5%	102.2	101.0	101.0	100.6
27		29	AC 1%	101.4	100.0		
30	1/160	24	AC 1%	102.4	102.2	102.2	
33		19	AC 3%	102.4	102.2	102.4	
37	1/160	22	AC 2%	102.8	101.4	101.4	
40		21	AC <1%	102.2	102.0	102.4	
44	1/80	23	AC <1%	102.0	101.6	102.0	
47		23	AC <.5%	101.6	100.2	101.4	
51	1/80	27	AC 2%	101.8	101.4	101.0	
54		24	AC 1%	102.0	101.4	101.4	
58	1/80	27	-	101.6	101.0	101.4	100.6
65	2/	30	AC <.5%	102.0	102.0	101.8	101.0
73	Tr.	34	-	101.6	100.6	102.4	101.0

9477: Adult Steer

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-12	-	37	-				
-5	-	36	-				
0	5 ml blood injected SC., from EAC carrier, showing 1% parasitemia.						
2	-	37	-		101.2	101.0	
5	-	37	-	101.4	102.2	100.6	
9	1/20	39	-	101.6			
12		39	-	101.6	101.2		
16	1/320	36	-	101.8	102.0	101.8	
19		36	-	102.0	102.0	101.0	
23	1/160	34	AC <1%	101.8	101.6	101.4	101.6
27		29	AC 1%	100.4	101.0		
30		27	AC 1%	102.4	100.6	101.0	
33	1/80	26	AC <1%	101.6	101.8	101.6	
37	1/20	30	-	102.6	101.8	101.4	
40		26	AC <.5%	102.0	102.0	102.2	
44	1/	27	-	102.4	102.2	101.0	
47		25	AC <.5%	102.0	101.4	101.4	
51	2/	35	-	101.8	102.0	101.6	
54		30	AC <.5%	102.2	101.6	102.0	
58	4/	25	-	102.0	101.4	101.4	101.0
65	3/	29	-	102.0	101.0	101.0	
73	2/	34	-	101.6			

9478: Adult Steer

A. centrale, EAC

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-12	-	38	-				
-5	-	41	-				
0	5 ml blood injected SC., from EAC carrier, showing 1% parasitemia.						
2	-	39	-		101.6	101.4	
5	-	36	-	101.8	101.2	101.6	
9	Tr.	36	-	101.8	101.2	100.8	
12		38		100.8	101.2	100.6	
16	1/160	37		101.6	102.0	101.4	
19		36	-	101.8	101.6	101.0	
23	1/160	40	AC < 1%	101.6	101.4	102.6	101.6
27		32	AC 2%	101.0	101.0		
30		23	AC 2%	102.4	100.8	101.6	
33	1/80	24	AC 2%	104.2	102.8	103.0	
37	1/40	30	AC 3%	102.2	102.2	103.0	
40		24	AC 1%	101.8	101.4	102.0	
44	1/40	23	-	102.4	101.8	102.8	
47		25	AC < 1%	101.6	102.2	101.6	
51	1/80	36	AC < 1%	102.0	102.4	102.2	
54		27	AC < .5%	101.6	102.0	101.4	
58	1∕	28	-	101.8	102.4	101.6	
65	2∕	29	-	102.2	102.4	101.8	101.2
73	2∕	35	-	102.0	100.0	101.4	

9778: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	37	-
0	5 ml blood injected SC., from EAC carrier, showing 5% parasitemia.		
7	-	40	-
14	3+	41	AB <.5%
21	4+	38	AC 4%
28	4+	23	AC 1%
35	1/80	30	AC 1%
42	4+	32	AC 1%
49	1/20	34	AC 2%
56	4+	35	AC 1%
63	4+	33	AC <.5%
70	4+	36	AC <.5%

9785: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	36	-
7	-	36	-
14	2+	37	-
21	4+	36	AC 2%
28	1/160	27	-
35	1/160	24	AC 4%
42	4+	30	AC 1%
49	-	36	AC 2%
56	-	34	AC 1%
63	-	35	AC 1%
70	3+	33	AC <.

9788: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	36	-
0	5 ml blood injected SC., from EAC carrier, showing 5% parasitemia.		
7	-	36	-
14	4/	37	-
21	4/	36	AC 6%
28	3/	25	AC 15%
35	1/320	27	AC 4%
42	1/80	29	AC 1%
49	4/	22	-
56	4/	29	AB < .5%
63	2/	31	AC < .2%
70	3/	30	AC < 1%

9790: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	28	-
7	-	30	-
14	4/	31	AC < 1%
21	4/	28	AC 8%
28	1/80	19	AC 10%
35	2/	23	AC 1%
42	2/	24	AC 1%
49	1/20	27	AC 1%
56	3/	28	AC 2%
63	-	27	AC 1%
70	-	25	-

9791: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	39	-
0	5 ml blood injected SC., from EAC carrier, showing 5% parasitemia.		
7	-	40	-
14	1/	38	AC 1%
21	1/20	40	-
28	1/	42	-
35	1/5	38	AC 1%
42	1/	37	AC < 1%
49	-	39	AC <1%
56	-	38	-
63	-	40	AC <.1%
70	-	40	-

9795: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	34	-
7	-	35	-
14	4/	35	-
21	4/	36	AC < 1%
28	1/320	37	AC 2%
35	1/80	32	AC 2%
42	4/	30	AC 1%
49	4/	30	AC 2%
56	3/	32	AC <.1%
63	3/	30	-
70	3/	33	-

9825: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	38	-
0	5 ml blood injected SC., from EAC carrier, showing 5% parasitemia.		
7	-	39	-
14	-	40	-
21	-	40	-
28	1/10	40	AC < 1%
35	-	41	-
42	-	36	AC <.1%
49	-	37	-
56	-	38	-
63	-	40	AC <.1% (TM)

9833: Adult

A. centrale, EAC

Day	CF	PCV	Smear
0	-	33	-
7	-	32	-
14	1/80	33	-
21	-	31	AC 2%
28	1/20	26	AC 1%
35	2/	32	AC <.1%
42	-	35	-
49	-	40	AC <.5%
56	-	39	-
63	1/	40	-

9834: Adult

A. centrale EAC

Day	CF	PCV	Smear
0	-	35	-
0	5 ml blood injected SC., from EAC carrier, showing 5% parasitemia.		
7	2/	35	-
14	3/	34	- (TM)
21	4/	36	-
28	1/320	37	AC 1%
35	1/160	24	AC 8%
42	4/	23	AC 2%
49	4/	29	AC 1% (TM-2%)
56	4/	30	AC 1% (TM-1%)
63	4/	33	AC < 1%
70	2/	33	AC 1%

9838: Adult

A. centrale EAC

Day	CF	PCV	Smear
0	-	42	-
7	3/	43	-
14	4/	43	-
21	3/	40	AC 2%
28	1/80	35	AC 2%
35	3/	35	AC 2%
42	1/10	39	AC 1%
49	2/	41	AC 1%
56	2/	42	AC < 1%
63	2/	42	AC 1%
70	1/	45	AC 1%

STUDIES ON BOVINE ANAPLASMOSIS

THE COMPARATIVE PATHOGENICITY AND IMMUNOLOGICAL RELATIONSHIP OF *ANAPLASMA CENTRALE* TO *ANAPLASMA MARGINALE*

Appendix II

Animal Charts for Experiment IV.

9279: Splenectomized calf
Female Holstein.

Primary EAC infection, Challenged with EAM (EAC X EAM)

Day	CF	PCV	Smear	Temperature day 0	1	2	3
-2	1/40	30	AC 2%	103.2			
0		34	AC 4%	104.6			
0	Challenged:	70 days post primary infection (70-PPI) with 5 ml					
		blood injected SC from EAM carrier showing 1% parasites					
3	1/40	27	AC 1%	103.0	102.4	101.6	102.2
7		31	AC < 1%	102.4	102.2		
10	1/40	31	AC < 1%	106.0	106.2	104.8	102.4
	Babesia infection occurred, followed by apparent recovery						
24	1/320	30	AC < 1%	102.6	102.0		
26		29	AB < 1%	103.0	102.4		
28		24	AC 1%	102.4	102.0		
31	1/320	30	AM 2%	102.8	102.2		
34		32		104.0	104.6	104.8	
38	1/640	37	AM 85%	104.0	104.6		
41		16	AM 25%	102.0	102.4	101.6	
45	1/2560	27	AM < 1%	103.2	102.0		
47		29	AM 1%	103.0	101.2		
49		30	AM 2%	101.0	102.0		
52	1/1280	32	AM 7%	101.0	101.6	102.6	103.0
59	1/1280	17	AM 5%	103.0	101.6	101.4	101.4
66	1/1280	29	AB < 1%	101.0			

9280: Splenectomized calf
Female Guernsey

EAC X EAM

Day	CP	PCV	Smear	Temperature day / 0	1	2	3
-10	2/	31	AC 1%				
-3	2/	31	AC 2%	100.6	102.0	101.6	
0	Challenged:	234-PPI, with 5 ml blood injected SC from EAM carrier showing 2% parasitemia.					
4	1/5	30	AC 1%	102.0	101.6	101.8	102.4
8		30	AC < .5%	101.0	100.4		101.6
13		30	AC 1%	101.4	102.6		
15		29	AC 1%	101.6	102.0		
19	1/40	25	AM 2%	103.6	104.6	104.2	
22		17	AB 4%	104.6	102.0		
25	1/80	15	AM 1%	101.6	100.6		
27		21	AB 1%	101.2	101.6		
30		26	AB < 1%	100.4			
32	1/80	26	AM 2%	100.8	103.6		
34		26	AM 3%	105.0	103.6	104.0	104.8
41		19	AB 2%	102.0	101.0	101.0	100.4
48	1/160	25	AM 1%	101.0	100.6	101.6	100.2
53	1/80	29	AM < 1%	101.4	100.4	101.2	100.4
60	4/	28	AM < .1%	101.6	101.2	101.0	

9362: Splenectomized calf
Male Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-3	4/	26	AC 4%	101.4	100.0	99.4	
0	1/40	24	AC 2%	101.0	101.0	103.0	
0	Challenged:	66-PPI, with 5 ml blood infected SC from EAM carrier showing 4% parasitemia.					
4	1/40	23	AC 2%	102.0	101.0	101.6	101.4
8		25	AC 2%	102.4	101.8		101.6
13		28	AC 4%	101.8	102.0	101.2	101.2
19	1/160	28	AB 4%	101.6	100.4	101.4	100.8
25	1/640	29	AB 3%	100.6	100.6	101.2	101.6
32	1/320	33	AC 3%	101.0	100.6	100.2	101.4
39	3/	31	AB 3%	99.8	100.0		101.0
44		25	AM 9%	105.4			
46	3/	23	AM 6%	103.4	103.0		
48		18	AM 7%	103.0	101.4		
50		18	AM 1%	101.6	100.4		
53	2/	21	AM < 1%	101.0	100.8		
55		23	AM <.5%	100.6	101.4		
57		26	AM 2%	100.2	101.2		
60	4/	27	AM 8%	100.6	102.0	103.6	105.4

9365: Splenectomized calf
Female Holstein.

RAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-10	3/	37	AC < 1%				
0	1/40	31	AC <.1%	102.0	101.4	101.4	
0	Challenged:	57-PPI, with 5 ml blood infected SC from EAM carrier showing 4% parasitemia.					
4	1/40	34	AC < 1%	101.6	101.0	100.6	101.8
8		33	-	101.2	101.6		
11	1/40	34	-	102.0	103.2		
13		32	AM <.1%	102.2	102.6	101.6	101.2
19	1/20	35	AB <.5%	100.6	101.0	101.4	101.2
25	4/	35	AB 1%	102.0	102.2	101.4	102.0
30		32	AC <.5%	101.6		101.4	100.4
34		32	AB <.1%	102.0	100.8	101.2	101.4
39	1/20	35	-	101.6	101.0		
46	1/20	38	AC 1%	100.6	100.4	101.2	100.6
53	4/	34	AB 1%	100.8	101.4	100.2	101.2
60	4/	33	AB <.5%	102.0	101.6	101.0	101.6

9373: Splenectomized calf
Male Jersey

EAC X EAM

Day	CF	PCV	Smear	Temperature day ⁄ 0	1	2	3
-3	2⁄	35	AC 3%				
0	Challenged: 101-PPI, with 5 ml blood injected SC from EAM carrier showing 7% parasitemia.						
6		35	AC 1%	102.0	102.2	101.4	102.0
12	2⁄	38	AC 2%	102.0	102.0	102.0	
15		33	AC 5%	102.2	102.2		
18	2⁄	29	AB 4%	103.6	102.4		
20		23	AM 6%	102.6	103.0		
22		17	AM 18%	104.4	103.6		
25	2⁄	17	AM 12%	103.4	103.0		
27		19	AM 10%	102.0	102.0	102.0	101.6
32	1/80	28	AB 3%	101.0	101.6	102.0	101.0
36		29	AB 2%	101.6	101.2		
39	1/80	30	AM 4%	102.4	102.0		
41		28	AM 7%	102.2	102.8	103.0	
46	1/80	17	AM 6%	102.0	104.0	104.4	103.0
53		26	AB 2%	102.0	101.4	101.0	102.0
60	4⁄	30	AM 2%				

9375: Splenectomized calf
Female, Jersey

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-3	1/	35	AC 1%				
0	Challenged: 107-PPI, with 5 ml blood injected SC from EAM carrier showing 76% parasitemia						
4	1/	42	AC 1%	102.4	101.0	101.6	102.0
8		40	AC 1%	101.6	101.8		
12	Tr.	41		102.4	102.0		
15		37	AC 4%	102.2	102.2		
18	Tr.	33	AC 8%	102.2	101.8	101.4	100.6
22		28	AC 5%	102.2	100.8		
25	-	30	AC 2%	103.0	102.0	101.8	102.0
29		28	AC 2%	102.2	101.2		
32	1/	29	AB 4%	101.4	101.2	102.0	
36		27	AM 1%	101.4	101.6		
39	1/	35	AM 1%	101.0	102.2	102.0	102.0
46	2/	42	-	101.0	101.8	102.0	
49		41	AM 1%	102.0	103.4	101.4	
53	3/	43	-	101.0	101.4	101.6	101.4
60	2/	47	AM .5%	102.0	100.8	102.0	101.6

9552: Splenectomized calf
Male Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-7		30	AC 3%	101.6	101.0	101.4	
-3	1/20	30	AC 4%	102.2	99.0	100.8	
0	Challenged: 66-PPI, with 5 ml blood injected SC, from EAM carrier showing 85% parasitemia.						
4		27	AC 4%	102.4	100.4	102.4	
7	1/40	25	AC 5%	100.0	102.4	101.0	
11	3/	28	AC 3%	101.0	101.0	98.4	
14		30	AB 2%	100.8		102.2	
18		27	AM 12%	101.0	101.4	102.2	104.2
21		15	AM 72%	102.2			
22	1/80	9	AM 70%	101.8			

22 - Died, Acute Anaplasmosis.

9571: Splenectomized calf
Male Guernsey

RAC X EAM

Day	CF	PCV	Smear	Temperature day ⁄ 0	1	2	3
-6		27	AC 3%		101.2	99.8	
-2	1/40	29	AC 2%	98.2	99.2	100.4	
0	Challenged:	72-FPI, with 5 ml blood injected SC from EAM carrier showing 7% parasitemia.					
5	4⁄	24	AC 3%	102.6	101.4	100.4	100.4
12	4	23	AC 4%	100.2	102.0	101.0	
19	1/40	21	AC 2%	100.6	101.4	102.0	101.8
26	1/40	24	AC 2%	101.0	99.2	101.0	101.0
33	1/40	25	AC 4%	100.0	101.6	101.4	
36		26	AC 4%	102.0	100.6	101.4	
40		26	AC 1%	99.8		101.0	101.8
47	4⁄	25	AC 2%	102.0	102.0	101.4	
50		25	AC 2%	100.0	101.0	101.6	
68	1/40	22	AM 2%	100.8	101.0	102.0	98.4
72		22	-	100.0	98.4		
75	1/40	25	-	100.0	101.2	100.4	
78		19	AB .5%	101.6	98.8	100.0	
82	4⁄	27	AC .1%	99.2			

9700: Splenectomized calf
Male, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-7		32		100.0	100.0	101.4	
-3	1/80	36	AC 1%	101.0	99.0	100.4	
0	Challenged: 66-PPI, with 5 ml blood injected SC from EAM carrier showing 8% parasitemia.						
4		32	AC 1%	101.0	101.0	101.4	
7		30	AC 1%	100.8	101.4	101.4	
11	4/	32	AC 2%	101.8	101.4	101.0	
14		33	AC 2%	100.0	102.4	101.0	
18	1/40	34	AC 2%	101.0	101.0	102.0	
21		32	AC 1%	101.0			
22		31	AC 1%	101.4	101.2	101.8	100.0
26	1/40	34	AC 2%	101.2	100.8	101.2	
29		30	AC 1%	101.6	101.6		
32		29	-	101.6	101.6	101.4	
35		26	-	100.0	100.4	100.0	
39	1/160	19	AM 3%	102.6	102.0		
42		20	AM 6%	101.6	102.0	104.0	
46	4/	20	AB 3%	104.0	103.4		
49		20	AM 2%	102.0	104.2		
51		21		102.0			
53		22	AM 3%	104.6	102.4	104.2	
56		21	AM 10%	104.0	103.8		
58		19	AM 10%	103.8			
60	1/160	16	AM 10%	103.4			

9700: Splenectomised calf
Male, Holstein.

EAC X EAM

Day	CF	PCV	Smear	Temperature day ⁄ 0	1	2	3
67		21	AM 3%				
74	3⁄	24	AM 4%				
81	4⁄	25	AM 2%				
84		27	-				
88	4⁄	30	-				

9747: Splenectomized calf
Male, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-24	4/	27	AC 1%	101.6	101.6	101.6	101.0
-3	1/20	32	AC 1%	101.0	99.0	101.4	100.6
0	Challenged:	94-PPI, with 5 ml blood injected SC from EAM carrier showing 8% parasitemia.					
1		28	AC 2%	100.8			
4		31	AC 1%	101.8	100.8	100.8	
7		29	AC 2%	101.0	102.0	101.4	
11	3/	30	AC 1%	100.8	101.4	99.0	
14		32	-	99.0	101.2	100.4	
18	1/20	31	AM 1%	101.0	101.0	101.6	
21		30	AM 3%	100.4			
22		27	AM 10%	102.6	102.0	102.4	102.6
26	1/40	14	AM 40%	101.0	101.0		
28		8					

28: Died, Acute Anaplasmosis.

9751: Splenectomized calf
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-24	1/80	33	AC 2%	101.6	101.6	102.0	102.0
-3	4+	32	AC 1%	101.6	101.6	101.0	101.6
0	Challenged: 77-PPI, with 5 ml blood injected SC from EAM carrier showing 85% parasitemia.						
1		32	AC 3%	102.0	101.2		
4		35	AC 2%	101.6	101.0	101.6	
7			AC .5%	100.0	101.6	101.6	
11	3+	33	AC 4%	103.0	101.6	100.2	
14		32	AC 3%	101.0	102.0	101.0	
18	1/80	32	AC 2%	101.0	101.0	102.0	
21		30	AC 2%	101.6	102.0	101.6	102.0
25	1/40	35	AC 1%	101.6	100.0	100.8	
28		30	AC 1%	101.0	102.0		
31		29	AC .1%	101.6	100.8	101.4	
34		32	-	101.6	101.5	101.6	
38	1/80	31	AC 1%	102.0	101.6		
41		31	AC 1%	102.0	102.2	101.0	
45	3+	34	AC 2%	101.0	101.4		
48		33	AM 2%	101.6	101.4	102.0	

9754: Splenectomized calf
Male, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-6		20	AC 3%				
-3	1/160	21	AC 1%	98.4	99.0	101.0	101.6
0	Challenged: 96-PPI, with 5 ml blood injected SC from EAM carrier showing 85% parasitemia.						
1		23		101.8	101.4		
4		28	AC 2%	101.0	100.6	100.6	
7		28	AC 4%	100.0	101.8	100.4	
11	4/	30	AC 4%	99.0	101.8	100.4	
14		29	AC 9%	100.6	102.2	101.8	
18	1/160	27	AC 3%	100.0	101.0	102.0	
21		24	AB 8% (4% AM)	100.2			
22		24	AM 10%	100.0	100.0	99.8	100.0
26	1/160	10	AM 62%	101.6			
27		9		95.2			
28: Died Acute Anaplasmosis.							

9376: Splenectomized calf
Female Guernsey

MAM X MAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	1/80	28	AM 2%	101.6	101.8	101.4	101.0
-4		32	AM 1%		100.6	100.6	100.4
0	Challenged:	79-PPI, with 5 ml blood injected SC from MAM carrier showing .1% parasitemia.					
3	4/	36	AM 1%	100.0	100.6		
6		34	AB 1%	101.0	102.0	103.0	
10	2/	35	AM .5%	102.6	101.8	102.6	
13	3/	33	AM 2%	101.0	101.4	100.2	
17	1/80	36	AM 2%	100.0	101.0	100.4	
20	4/	30	AM 2%	101.0	100.8	101.0	
24	1/80	25	AM 8%	100.0	102.0	101.6	
27		20	AM 4%		102.2	101.0	
31	1/160	26	-	101.0	100.8	100.4	
34	4	28	-		101.2	100.0	
38	4	36	-	101.4	101.0	100.0	
45	1/40	37	AM 1%	101.6	101.0	101.0	
52	4	30	AM 5%	100.6	101.4	101.4	100.8
59	4	30	AB .1%	102.0	100.6	101.4	101.6
66	4	31	AM 1%	101.0	101.6		

9554: Splenectomized calf
Male, Holstein.

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-18	1/80	25	AM 6%	103.2	102.8	103.2	103.4
-4		31	AM 1%		100.6	99.4	100.0
0	Challenged:	81-PPI, with 5 ml blood injected SC from EAM carrier showing .1% parasitemia.					
3	1+	34	-		101.0	100.8	
6		32	AM 2%	100.6	100.6	102.0	
10	2+	34	AM 4%	102.4	101.6	102.0	
13	3+	29	AM 5%	102.4	100.6	101.0	
17	3+	27	AM 2%	101.6	102.6	100.0	
20	3+	23	AM 1%	100.6	101.6	100.8	
24	3+	26	AM 1%	101.0	101.6	101.6	
27		23	AM 2%		102.4	102.8	
34	1/80	25	AM 1%		101.8	102.0	
41	4+	25	AM 2%	101.4	100.6	102.2	
45	1/80	30	AM 2%	101.0	101.4	100.4	
52	4+	31		102.0	102.0	102.2	
59	4+	34	AM .5%	101.6	100.4	99.0	100.0
66	4+	33	AM .1%	100.0			

9549: Splenectomized calf
Male, Zebu

BAM X BAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	3/	29	AM 8%				
-4	2/	27	AM 2%		100.0	101.0	100.0
0	Challenged: 68-PPI, with 5 ml blood injected SC from BAM carrier showing .1% parasitemia.						
3	1/	33	AM .1%		101.0	101.6	
6		31	-	101.0	100.6	102.0	
10	-	35	AM .5%	102.6	101.6	102.0	
13	-	34	AB .5%	101.6	100.6	100.0	
17	1/	38	AM 1%	101.0	101.6	101.6	
20	1/	34	AM 1%	101.0	102.0	102.6	
24	2/	37	AM 1%	102.0	100.6	100.0	
27		38	-		102.6	102.0	
31	1/	40	AB 1%	100.4	100.0	101.4	
38	1/	34	AM 1%	101.4	101.0	99.8	
45	1/	37	-	101.0	101.0	100.6	
52	2/	37	AM 1%	101.6	100.2	101.0	
59	1/	36	AB .1%	101.0	100.2	100.4	100.8
66	2/	34	AM 1%	101.0			

9551: Splenectomized calf
Male, Zebu

BAM X BAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-11	1/5	31	-	100.4			
-4	4/	32	-		100.0	100.6	
0	Challenged:	70-PPI, with 5 ml blood injected SC from BAM carrier showing .1% parasitemia.					
3	4/	35	AM .5%		101.0	101.4	
6		30	AM .5	100.0	101.6	102.6	
10	3/	33	AM .5%	102.4	101.0	101.6	101.8
17	3/	33	AM 2%	101.6	100.6	101.6	101.6
24	4/	30	AM 2%	101.6	101.2	101.6	
27		28	AM 2%		102.0	102.0	
31	3/	30	AM 1%	101.2	99.0	101.4	
34	1/10	28	AM 1%		100.4	101.6	
41	4/	25	AM 1%	100.6	101.6	101.4	
45	4/	29	AM 3%	102.0	101.4	100.4	
52	1/40	25	AM 1%	101.6	101.0	101.0	101.4
59	4/	30	AB .1%	100.8	100.4	101.4	101.4
66	4/	30	AM 1%	101.6	101.8	100.6	

9355: Splenectomized calf
Male, Holstein

HAM X HAC

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-4	1/40	31	AB 3%		99.2	99.2	100.2
0	Challenged: 81-PPI, with 5 ml blood injected SC from HAC carrier showing 5% parasitemia.						
3	4+	30	AB 1%		101.0	101.6	
6		28	AM 2%	101.0	100.2	104.6	
10	4+	27	AM 2%	102.0	102.0	102.2	
13	4+	25	AM 2%	101.0	99.0	100.0	
17	4+	25	AM .5%	101.0	101.0	99.6	100.0
24	4+	30	-	100.0	101.0	101.4	
31	4+	33	-	101.0	99.0	101.0	
34	1/160	29	AB 1%		100.6	101.0	
38	4+	31	AC 1%	102.0	101.2	100.0	
41	4+	25	AC 6%	100.0	101.6	100.0	
45	1/80	22	AC 10%	101.0	102.0	102.6	
48		15	AC 20%		100.9	100.2	
52	1/160	15	AC 3%	101.0	102.0	101.4	
55		19	AC 1%	100.0	99.4	100.0	
59	4+	24	-	100.2	99.0	100.0	100.0
66	4+	26	AC 1%	101.0	101.6	100.2	
73		28	AC 3%	100.0		101.6	102.2

9369: Non-splenectomized calf
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature day / 0	1	2	3
-4	1/40	31	-	101.4	101.8	102.0	101.6
0	Challenged: 81-PPI, with 5 ml blood injected SC from EAM carrier showing 1% parasitemia.						
3	1/40	30	AC .1%	102.4	102.0		
7	1/40	30	-	101.6	103.0		
10	1/40	30	-	102.6	101.4		
12		29	AC 1%	101.6	101.0		
14		31	-	102.2	102.2		
17	1/80	32	-	101.2	101.6	102.8	
20		32	AC 2%		102.6	102.0	
24	1/160	28	AM 4%	101.6	103.0		
26		27	AM 6%	103.6	103.2		
28		22	AM 6%	103.2	103.2		
31	1/320	21	AM 4%	103.0	103.2	102.8	102.6
38	1/160	34	-	101.8	100.6		
41		33	AM 1%	102.0	102.4	101.8	
45	1/80	37	AM .1%	102.0	101.6	102.0	101.8
49	1/80	37	-	101.6	101.0	101.6	101.0
56	1/80	41	-	101.6	100.8	101.8	101.4
63	1/40	42	-	101.8	101.4		

9370: Non-splenectomized calf
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-10	2∕	37	-				
-3	2∕	37					
0	Challenged: 206-PPI, with 5 ml blood injected SC from EAM carrier showing 76% parasitemia.						
4	1∕	28	-	102.2	103.4		
6		33	AC .5%	102.8	103.8		
8		31	AC .1%	103.0	101.0		
12	1∕	33	AC .5%	101.6	100.4	100.0	101.2
18	1∕	30	-	102.0	100.0	100.8	
22		27	-	101.0	99.8		
25	Tr.	36	AC .5%	100.4	100.0	101.0	101.0
29		29	-	101.4	100.4		
32	1∕	32	AC .1%	101.0	100.6		
34		31	-	100.8	100.4	101.6	100.0
39	1∕	30	-	101.0	100.0	100.8	99.6
46	2∕	28	-	101.0	99.0	101.2	
49		31	-	100.6	101.0	100.0	
53	2∕	31	-	101.0	100.0	100.8	
56		32	-	100.6	101.4	101.6	
81	1∕	33	-	100.0	99.8	99.8	

9374: Non-splenectomized calf
Male Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-10		40	AC .5%				
-3	3/	35	-				
0	Challenged: 108-PPI, with 5 ml blood injected SC from EAM carrier showing 76% parasitemia.						
4	4/	38	-	99.8	99.8		
6		34	AC .5%	100.8	100.4		
8		34	-	99.8	99.8		
12	1/10	35	-	102.8	99.6	100.4	
15		32	-	99.0	100.8		
18	1/10	33	-	99.0	99.4	100.0	99.8
22		33	-	99.4	101.2		
25	1/5	32	-	99.6	100.4	99.6	100.8
29		33	-	100.4	100.4		
32	1/5	33	-	100.0	101.0	100.8	100.4
36		34	-	102.0	100.4		
39	1/	33	-	101.0	99.8	100.4	101.0
46	3/	33	-	100.4	101.0	99.4	
49		30	-	100.4	100.0	99.4	
53	4/	31	-	99.8	101.0	100.8	100.0
60	3/	31	-	101.0	101.2	99.0	100.0

9461: Non-splenectomized calf
Female, Guernsey

RAC X EAM

Day	CF	PCV	Smear		Temperature, day ∕ 0	1	2	3
-17	-	34	-					
-3	1∕	40	AC	.5%				
0	Challenged: 108-PPI, with 5 ml blood injected SC, from EAM carrier showing 76% parasitemia.							
4	Tr.	31	-		101.0	102.0	100.6	101.0
8		33	-		101.0	101.0		
12	-	39	-		102.8	100.0	100.8	101.2
18	1∕	35	AC	1%	102.0	100.0	100.6	100.4
22		31	-		100.8	102.6		
25	-	32	-		101.0	100.6	100.8	102.2
29		30	-		101.0	100.8		
32	1∕	31	-		101.4	101.8	102.0	100.6
36		34	-		101.2	100.4		
39	Tr.	35	-		100.0	100.4	101.0	101.4
46	1∕	34	-		100.0	100.6	101.0	
49		31	-		100.8	100.6	100.0	
53	1∕	30	-		102.2	100.4	101.0	
60	2∕	28	AM	.5%	101.0	101.2	102.6	
63		27	AM	.1%	102.0	102.0	100.2	
68	1∕	32	-		101.6			

9462: Non-splenectomised calf
Female, Holstein.

RAC X RAM

Day	CF	PCV	Smear	Temperature day ⁄ 0	1	2	3
-10	1⁄	34	-				
-3	3⁄	37	-				
0	Challenged: 108-PPI, with 5 ml blood, injected SC from RAM carrier showing 76% parasitemia.						
4	4⁄	30	-	101.8	101.0	102.0	100.8
8		32	-	100.6	101.2		
12	1/10	35	-	102.4	100.4	101.4	101.8
18	4⁄	32	-	103.0	101.6	102.0	102.0
22		24	AM 1%	101.6	100.0		
25	1/160	21	AM 2%	101.2	101.0		
27		20	AM 2%	100.6	102.6		
29		18	AM 1%	100.8	102.6		
32	1/80	21	AM 1%	101.6	101.4	102.0	100.6
36		22	AB 2%	101.6	101.2		
39	1/160	25	AM 2%	101.8	101.0	101.6	100.4
46	1/320	27	AB 1%	101.0	100.6	100.0	99.6
53	1/320	32	-	101.4	100.6	101.6	102.0
60		30	-	102.0	101.0	102.4	102.0

9463: Non-splenectomized calf
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day of 0	1	2	3
-10	2+	35	AC .5%				
-3	3+	40	-				
0	Challenged: 108-PPI, with 5 ml blood injected SC from EAM carrier showing 76% parasitemia.						
4	3+	33	-	101.2	100.4	100.0	101.0
8		34	-	100.0	99.6		
12	3+	34	-	101.0	100.4	100.2	100.4
18	3+	32	-	100.4	101.2	102.0	101.2
22		30	-	101.0	101.4		
25	2+	31	-	100.4	101.4	101.0	100.8
29		32	-	101.4	100.4		
32	2+	31	-	100.6	101.2	101.6	100.4
36		31	-	101.4	101.2		
39	1+	31	AC .5%	99.6	100.4	100.8	100.4
46	3+	31	-	101.4	101.0	101.2	
49		31	-	100.4	100.4	100.6	
53	3+	32	-	101.4	101.8	100.4	100.8
60	2+	29	-	100.6	101.4	101.0	101.0

9363: Non-splenectomized calf
Female, Guernsey

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3	
-5		36	-					
-2	1/	36	-	102.0	101.4			
0	Challenged: 184-PPI, with 5 ml blood injected SC from EAC carrier showing 1% parasitemia.							
0		35	-	100.6	101.6			
2		35	-	102.0	101.2			
5	1/	36	-	101.2	101.2			
7		35	-	101.0	101.0	101.4	100.0	
12	1/	36	-	100.0	102.4	100.4	101.6	
19	-	33	-	101.4	101.0	101.0		
22		29	-	101.8	100.8	100.4		
26	-	33	AM 1%	100.8	101.0	101.8		
29		28	AM .1%	102.8	101.0	100.4		
33	-	32	-	101.4	101.6	101.4		
36		30	-	102.6	102.0	101.4		
41		32	-	100.6	101.6	103.0	101.8	103.0
47	2/	34	-	100.6	100.6	101.3	100.6	
54	1/	28	AC .1%	101.0	101.8	101.6	100.4	
61	2/	32	AC 2%	101.0				

9456: Non-splenectomized calf
Male, Zebu.

EAM X EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8	1/40	32	-				
-1	4/	32	-				
0	Challenged: 183-PPI, with 5 ml blood injected SC for EAC carrier showing 1% parasitemia.						
7	4/	35	- (TM)	100.6	101.6	100.8	101.6
13	4/	35	-	101.0	99.6	101.2	101.2
20	4/	37	- (TM)	101.0	100.6	100.2	101.4
27	4/	35	- (TM)	101.0	99.4	101.2	101.2
34	1/10	32	AB < .1% (TM)	101.0	100.6	101.0	103.2
41	1/80	32	- (TM)	101.0	101.0	100.6	101.6
48	4/	26	AC 2%	101.0	101.0	101.8	100.8
56	1/80	22	AC 1%	101.6	100.4		
58		24	-	101.0	100.0		
60		25	-	101.6	101.0		
63	1/160	27	AC < 1% (TM)	101.6	101.0		
65		32	AC 1%	100.0	101.6		
67		29	-	101.6	100.0		

9692: Non-splenectomised calf
Male, Holstein

EAM X EAC

Day	CF	PCV	Smear	Temperature, day ƒ 0	1	2	3
-11	2ƒ	34	-				
-4	1/40	30	-	100.0			
0	Challenged: 79-PPI, with 5 ml blood injected SC from EAC carrier, showing 5% parasitemia.						
3	3ƒ	34	-		101.0	100.8	
6		31	-	100.0	101.0	100.4	
10	2ƒ	33	AB < .1%	101.4	100.0	100.8	
13	2ƒ	31	-	101.0	99.8	100.0	
17	2ƒ	35	AC < 1%	100.6	100.0	99.0	
20	2ƒ	30	AC 1%	100.0	102.0	102.0	
24	2ƒ	30	AC 1%	101.8	100.6	101.0	
27		26	AC 2%		102.6	100.4	
31	2ƒ	22	AC 4%	99.0	100.0	100.6	
34	3ƒ	19	AC 3%		101.6	100.0	
38	1/160	20	AC 2%	101.6	100.6	98.4	
42	3ƒ	21	AC 2%	101.0	102.0	101.0	
46	3ƒ	26	AC < 1%	99.0	99.2	100.0	
49		25	AC < .1%		99.8	100.2	
53	> 1/40	30	-	98.6	100.6	100.0	
56		30	AC 1%	100.4	100.0	102.0	
60	3ƒ	29	AC < 1%	101.0	101.6	100.6	
63		27	AC < 1%	101.6			

9693: Non-splenectomized calf
Male, Holstein

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-11	4/	35	-				
-4	1/40	32	-	100.0			
0	Challenged: 79-PPI, with 5 ml blood injected SC from EAC carrier showing 5% parasitemia.						
3	4/	32	-	99.0	100.6		
6		31	-	100.6	102.0	100.6	
10	2/	31	-	101.8	100.8	101.0	
13	3/	30	-	100.8	100.0	101.0	
17	2/	33	AC < .5%	100.0	100.8	100.0	
20	4/	30	AC < .2%	101.0	100.8	101.0	
24	4/	31	AC 2%	100.0	101.0	100.8	
27		29	AC 2%		103.0	100.0	
31	4/	27	AC 3%	102.0	101.6	100.2	
34	1/80	23	AC 3%		100.6	101.0	
38	1/80	23	AC 1%	101.4	100.6	100.6	
42	4/	24	AC 1%	101.0	103.0	103.0	
46	4/	27	-	99.8	99.2	101.0	
49		28	-		100.4	100.2	
53	1/80	30	AC < .1%	100.0	100.6	101.0	
56		30	AC < .5%	100.0	100.6	102.0	
60	4/	29	AC < 1%	101.0	100.6	100.6	
63		26	AC 1%	101.0			

9218: Yearling
Female, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature, day + 0	1	2	3
-11		32	-				
-4	1/40	31	-	101.0			
0	Challenged: 58-PPI, with 5 ml blood injected SC, from EAM carrier showing 3% parasitemia.						
3	1/20	32	-	102.4		101.6	
6		32	-	102.0	101.0	101.2	
10	1/10	31	-	102.0	101.0	101.6	
13		31	-	103.2	101.6	101.0	
17	< 1/40	32	AM < 1%	104.0	105.0	104.8	
20		33	AM 2%	104.0	101.8	100.4	
24		20	AM 8%	102.4	102.0	102.6	
27		14	AM 5%	103.6			
28		13	AM 3%	101.8	103.0	103.6	103.0
32	1/320	19	AB < 1%	102.6	101.4	100.0	
35		22	AC < .2%	101.6	103.0		
38	1/320	23	AM < .1%	100.0	101.0	101.4	
41		26	-	100.6	101.0	101.4	
45	1/160	27	-	101.0	100.0		
48		29	-	100.4	101.2	100.8	
52	4+	30	-	101.4	100.2		
55		31	-	100.8	101.0	101.0	
59	4+	30	-	101.4	101.0	101.0	
62		32	-	100.0			

9565: Yearling
Male Jersey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-8		32	AC <.2%	102.0	100.6	101.0	
-1	1/160	34	AC <.5%	101.0	101.6	101.0	
0	Challenged: 55-PPI, with 5 ml blood injected SC from EAM carrier, showing 3% parasitemia.						
3	1/80	34	AC <.2%	102.2	101.4	100.0	
6		40	-	100.6	101.0	100.2	
10	1/40	37	-	101.6	99.0	100.0	
13		38	AB <.1%	102.0	100.0	100.4	
17	1/80	39	-	102.6	99.4	100.0	
20		39	-	101.0	101.4	100.8	
24		36	AM 1%	101.4	100.8	101.0	
27		32	AM 3%	101.2			
28		33	AM 5%	100.8	101.4	102.0	102.2
32	1/80	18	AM 14%	103.6	102.8	103.2	
35		17	AM 9%	103.6	100.6		
38	1/80	21	AM 1%	102.2	100.0	100.6	
41		27	AB <.1%	100.6	100.4	100.2	
45	1/160	20	-	103.0	102.4		
48		18	-	101.0	101.2		
52	4/	24	-	101.6	100.0		
55		29	AM <.1%	101.6	100.8	100.8	
59	4/	32	- (BB)	100.8	100.4	102.0	
62		29	AM <.1% (BB)	101.6			

BB: Babesia bigemina.

9568: Yearling
Male, Jersey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/160	32	-	102.0	101.8	101.0	101.6
0	Challenged: 58-PPI, with 5 ml blood injected SC from EAM carrier showing 3% parasitemia.						
3	1/160	32	-	101.4	101.0	102.0	
6		33	AC <.2%	101.6	101.0	100.8	
10	1/80	38	-	101.6	99.8	99.0	
13		34	-	101.6	100.6	100.4	
17	1/160	33	AC <.2%	102.0	101.0	100.0	
20		35	-	100.6	102.2	100.8	
24		43	-	101.8	101.0	101.0	
27		34	AC <.1%	101.6			
28		33	-	100.0	101.2	101.6	
32	1/80	34	-	102.0	101.4	100.6	
35		36	-	100.6	100.6		
38	1/40	34	-	101.2	103.0	105.0	
41		36	-	103.2	102.6	102.2	
45	1/80	36	-	101.6	100.4		
48		34	-	100.0	100.0	99.8	
52	4/	36	-	101.0	101.0		
55		35	-	102.2	102.0	101.6	
59	4/	35	-	102.0	100.0	101.4	
62		35	-	100.2			

9574: Yearling
Female, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/40	36	-	102.4	102.4	102.0	
0	Challenged: 58-PPI, with 5 ml blood injected SC from EAM carrier showing 3% parasitemia.						
3	1/20	36	-	102.6	101.6	100.0	
6		36	-	101.6	100.0	100.0	
10	1/80	35	-	102.4	100.4	100.4	
13		38	-	101.6	100.6	101.4	
17	1/320	38	AM <.5%	102.6	102.4	104.2	
20		38	AM 1%	102.0	101.8	101.4	
24		26	AB 3%	102.6	101.0	100.6	
27		20	AM 3%	102.6	101.0		
28		21	AM 4%	101.0	100.8	100.0	100.0
32	1/640	24	AM 1%	103.0	101.0	101.6	
35		28	AM < 1%	100.0	101.2		
38	1/640	33	AM <.2%	101.6	101.0	100.2	
41		34	AC <.1%	101.0	101.4	101.0	
45	1/640	35	AB <.1%	100.4	101.6		
48		35	AB <.3%	100.6	100.6	101.0	
52	4/	36	-	101.0	102.0		
55		39	-	100.0	101.0	101.6	
59	4/	39	-	101.2	102.0	101.4	
62		40	AM <.2%	101.2			

9577: Yearling
Female, Holstein

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/160	33	-	102.0	101.8	102.0	101.6
0	Challenged: 58-PPI, with 5 ml blood injected SC from EAM carrier showing 3% parasitemia.						
3	1/80	33	-	102.0	100.8	101.6	
6		32	-	101.6	101.4	100.0	
10		33	AM < .2%	102.0	101.0	100.0	
13		32	AM < 1%	102.6	101.6	101.6	
17	1/160	28	AM 2%	106.0	104.6	104.0	
20		22	AM 4%	103.6	103.8	102.4	
24		22	AM 2%	102.4	100.2	102.6	
27		24	AM 2%	101.8			
28		23	AM 2%	101.0	100.4	100.0	102.0
32	1/320	22	AM 2%	103.6	101.0	100.6	
35		23	AM 1%	100.8	102.0		
38	1/640	27	AM 1%	101.6	101.0	100.2	
41		29	AM < .2%	101.0	101.4	101.6	
45	1/640	33	-	103.0	100.6		
48		32	-	101.8	102.4	101.0	
52	4/	33	-	100.4	100.6		
55		37	-	101.6	100.6	100.6	
59	4/	37	-	101.4	101.0	101.0	
62		37	AM < 1%	100.0			

9557: Yearling
Female Holstein

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/320	32	-	102.4	101.8	101.6	
0	Challenged: 58-PPI, with 10 ml blood injected SC from EAC carrier showing 3% parasitemia.						
3	1/160	33	-	102.4	101.4	101.6	
6		34	AM <.3%	102.6	101.6	101.0	
10	1/80	34	AM <.1%	102.0	101.0	101.4	
13		38	AB <.1%	100.0	101.0	100.4	
17	1/80	39	AM <.2%	102.6	100.8	101.0	
20		34	-	101.8	101.8	101.8	
24		36	-	101.8	100.8	101.6	
27		36	AC <.2%	102.0			
28		38	AC <1%	101.0	102.0	102.6	103.0
32	1/80	28	AC 2%	103.2	101.4	102.0	
35		26	AC 2%	101.4	102.0		
38	1/160	25	AC 2%	101.4	101.0	101.4	
41		25	AC 2%	102.2	102.0		
45	1/160	28	AC 2%	102.4	102.6	103.0	103.0

Babesia bigemina infection was noted Day 48, which resulted in the death of the animal on day 54. Labor difficulties resulted in the removal of all yearling cattle (except 9271) from the tick free unit for 18 hours on day 20. This was one of several animals infected because of this action, but the only one to develop a fatal reaction. In every instance, fortunately, the specific anaplasma reaction occurred prior to the onset of the Babesia infection.

9567: Yearling
Male, Jersey

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/80	30	-	102.0	101.6	101.0	
0	Challenged: 58-PPI, with 10 ml blood injected SC from EAC carrier showing 3% parasitemia.						
3	1/80	31	-	101.6	100.4	101.0	
6		33	-	102.0	101.4	100.8	
10	1/80	31	-	102.2	99.8	100.6	
13		36	-	101.8	101.6	99.8	
17	1/160	33	-	103.0	99.0	100.4	
20		34	-	101.6	101.4	100.2	
24		34	AC <.3%	102.0	100.8	100.6	
27		31	AC 1%	102.0			
28		29	AC 1%	100.6	100.8	100.6	102.4
32	1/160	24	AC 3%	103.0	105.6	103.0	
35		20	AC 2%	102.0	102.0		
38	1/160	22	AC 1%	101.6	101.6	101.0	
41		21	-	101.4	101.0	100.8	
45	1/160	27	AC <.1%	99.8	100.2		
48		28	AC <.4%	101.0	100.0	101.0	
52	3/	21	AC < 1%	101.2	101.6		
55		22	-	101.0	100.6	101.4	
59	4/	26	-	101.0	99.8	101.2	
62		33	AB <.3%	100.0			

9575: Yearling
Male, Holstein

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/160	33	AB 1%	101.8	102.2	101.6	
0	Challenged:	58-PPI, 10 ml blood injected SC from EAC carrier					
		Showing 3% parasitemia.					
3	1/160	30	-	102.4	99.8	101.6	
6		32	-	101.6	100.0	100.0	
10	1/160	34	-	101.6	100.4	100.0	
13		33	-	101.6	100.4	99.4	
17	1/320	33	-	102.0	99.4	101.6	
20		32	AC <1%	102.0	101.2	101.0	
24		31	AC <1%	102.2	100.0	101.0	
27		28	AC 2%	101.6			
28		26	AC 4%	101.2	102.0	102.4	102.0
32	1/320	22	AC 4%	102.6	101.0	101.4	
35		22	AC 2%	101.0	100.6		
38	1/320	25	AC 2%	101.8	101.6	101.6	
41		28	AC 2%	104.6	105.2	105.0	
45	1/320	29	AC 2%	101.6	100.4		
48		26	AC <1%, BB <1%	103.2	105.2	103.6	
52	3/	17	AC <.2, BB.	102.2	100.8		
55		24	-	100.0	101.6	102.4	
59	4/	25	-	101.4	101.6	102.4	
62		31	-	101.6			

9576: Yearling
Female, Holstein

EAM X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-1	1/320	31	-	102.6	102.0		
0	Challenged: 61-PPI, 10 ml blood injected SC from EAC carrier showing 3% parasitemia.						
3	1/160	31	-	102.4	99.8	101.6	
6		32	-	103.0	101.0	101.6	
10	1/320	32	-	102.4	101.0		
13		33	+	101.6	100.4	100.6	
17	1/320	33	-	102.6	101.0	102.0	
20		33	AC <.5%	101.8	102.4	102.0	
24		30	AC 3%	102.0	101.2	102.6	
27		27	AC 3%	102.6			
28		25	AC 4%	101.4	101.6	102.2	102.2
32	1/320	21	AC 5%	103.6	102.6	105.0	
35		21	AC 3%	105.6	105.6		
38	1/160	23	AC 1%	104.8	103.8	105.4	
41		26	AC <1%	102.6	102.0	102.4	
45	1/160	25	AC <.2%	101.0	100.4		
48		20	AC <.1%	100.0	101.0	100.8	
52	4/	23	AC <.2%	101.6	101.0		
55		28	AC <.5%	101.6	102.6	102.8	
59	4/	25	AC 1%	101.4	101.4	102.0	
62		23	AC 4%, BB.	101.2			

9223: Yearling
Female, Holstein

EAM X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-1	1/160	34	-	102.0	101.6	101.6	
0	Challenged: 58-PPI, with 5 ml blood injected SC from EAM carrier showing 3% parasitemia.						
3	1/160	35	AM <.1%	101.4	101.0	101.6	
6		37	-	101.0	100.4	100.2	
10	1/160	35	-	101.6	101.0	101.0	
13		37	-	101.6	100.6	100.4	
17	1/80	36	-	102.6	100.6	100.8	
20		37	-	101.6	101.6	100.8	
24		33	-	101.6	100.4	101.6	
27		33	-	101.0	100.8		
28		34	-	100.8	100.8	100.0	101.0
32	1/80	34	AB <.2%	102.6	103.2	102.0	
35		30	-	104,0	100.0		
38	1/160	30	AM <.5%	100.4	100.6	100.4	
41		35	-	101.0	101.4	101.6	
45	1/160	30	-	100.0	100.6		
48		32	-	100.6	101.4	102.4	
52	4/	28	-	103.0	102.2		
55		24	- (BB)	102.0	101.6	102.8	
59	4/	26	-	101.2	101.4	101.4	
62		29	AC <.2% (BB)	101.2			

9271: Yearling
Female, Holstein

EAM X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/160	43	-	101.6	101.2	101.6	
0	Challenged: 58-PPI, with 5 ml blood injected SC from EAM carrier showing 3% parasitemia.						
3	1/80	39	-	101.4	101.4	101.6	
6		38	-	101.6	100.8	101.2	
10	1/40	38	-	102.0	101.4	101.4	
13		38	-	102.0	100.4	100.0	
17	1/40	38	-	103.0	105.0	101.0	
20		37	-	101.6	101.0	101.0	
24		38	-	102.0	100.8	101.2	
27		39	-	101.6			
28		39	-	101.6	102.6	102.8	101.4
32	1/80	38	-	102.0	101.0	101.6	
35		37	-	101.2	103.0		
38	1/80	38	-	101.0	100.6	101.2	
41		38	-	102.6	101.0	101.2	
45	1/80	35	-	103.0	101.0		
48		35	AM <.2%	100.2	101.0	101.8	
52	4/	37		101.2	101.0		
55		39	-	101.0	101.6	101.6	
59		40	AB <.3%	102.0	102.0	101.2	
62		37	AB<1%	101.0			

9229: Yearling
Female, Holstein

EAC X EAC

Day	CF	PCV	Smear	Temperature, day 0	1	2	3
-4	1/10	34	AC <.2%	101.4	101.0	101.4	
0	Challenged: 54-PPI, with 10 ml blood injected SC from EAC carrier showing 3 % parasitemia.						
3	1/5	33	-	102.0	101.2	101.0	
6		32	AC <.3%	101.6	100.0	100.0	
10	1/5	32	-	102.2	100.4	102.0	
13		33	-	102.0	101.6	101.4	
17	1/5	34	-	102.6	100.0	100.4	
20		35	AC <1%	102.6	101.0	101.6	
24		32	-	103.0	101.0	101.6	
27		35	AC <.3%	101.0			
28		36	-	101.2	101.8	102.2	102.4
32	<1/5	33	-	103.0	101.0	100.4	
35		33	-	100.4	100.8		
38	<1/5	35	-	101.0	101.0	101.0	
41		37	-	100.0	100.4	100.0	
45	<1/5	35	-	103.4	100.4		
48		34	AB <.1%	100.4	101.4	101.0	
52	4/	34	-	102.0	102.4		
55		32	-	102.4	102.2	102.4	
59		27	- (BB)	103.6	102.0	102.0	
62		27	-	101.6			

9424: Yearling
Female, Holstein

EAC X EAC

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-1	1/80	36	-	101.6	100.0	101.0	
0	Challenged: 58-PPI, with 10 ml blood injected SC from EAC carrier showing 3% parasitemia.						
3	1/40	32	AC <.2%	102.0	100.6	101.6	
6		36	-	102.6	101.6	101.4	
10	1/20	36	-	102.2	101.0	100.4	
13		39	-	102.0	101.0	101.0	
17	1/40	35	AC <1%	102.4	100.6	101.0	
20		35	AC <1%	102.6	101.6	102.4	
24		41	-	102.0	101.0	101.6	
27		37	AC <.3%	101.4			
28		36	AC <.2%	101.4	101.6	102.0	100.0
32	1/40	36	AC <.1%	103.0	101.6	101.8	
35		35	-	101.0	101.2		
38	1/40	40	AC <.3%	101.2	100.4	101.4	
41		41	-	101.0	101.0		
45	1/20	33	AC <.4% (BB)	101.0	102.6	102.4	
48		28	- (BB)	104.0	102.4	103.4	
52	4/	27	-	101.2	100.4		
55		32	-	101.0	101.0	101.6	
59	4/	25	-	101.4	102.0	101.0	
62		34	-	101.2			

9474: Adult
Male, Guernsey

RAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-4	1/	38	-				
0	Challenged: 70-PPI, with 5 ml blood injected SC from EAM carrier showing 18% parasitemia.						
3	-	40	-	101.0	100.4	100.4	
6		35	-	100.4	101.4	102.0	
10	-	37	-	101.8	101.4	101.6	
13		37	-	100.8	101.0	100.8	
17	1/	38	-	101.6	101.2	101.4	
20		40	-	101.4		101.0	
24	1/	38	-	101.4	100.6	101.6	
27		33	-	101.6	100.8	101.4	
31	1/	34	-	101.8	101.4	101.6	101.4
38	1/	35	-	101.0	100.4	102.0	102.0
46	1/	39	-	101.0	100.4	101.0	101.0
51		43	-	101.6	101.0	102.0	101.0
58	1/	40	-	101.6	101.0	101.0	

9476: Adult
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day ∕ 0	1	2	3
-10	Tr.	34	-				
-4	1∕	32	AC <.5%				
0	Challenged:	70-IPI, 5 ml blood injected SC, from EAM carrier showing 18% parasitemia.					
3	1∕	32		101.6	101.2	100.4	
6		30	-	100.4	101.4	102.0	
10	2∕	32	AC < 1%	101.6	101.2	101.0	
13		30	AC <.5%	101.0	101.0	101.6	
17	3∕	34	-	102.0	101.2	101.0	
20		28	AC<1%	101.4		100.9	
24	1/160	23	AM 2%	102.8	101.6	101.4	
27		21	-	101.8	101.8	101.6	
31	1/160	25	-	101.0	101.4	102.0	
34		27	-	101.0	101.0	101.6	
38	1/320	31	-	101.2	100.6	102.0	101.8
46	4∕	36	-	100.6	100.4	101.2	100.8
51	4∕	34	-	100.0	100.8	101.6	102.0
58	4∕	34	-	101.8	101.0	101.4	

9477: Adult
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day 1	2	3	
-10	2+	34	-				
-4	2+	31	-				
0	Challenged: 70-PPI, with 5 ml blood injected SC from EAM carrier showing 18% parasitemia.						
3	-	33	-	101.0	101.0	101.6	
6		30	-	102.0	102.0	101.4	
10	2+	33	AC <.5%	101.0	102.0	101.6	
13		31	-	101.0	101.6	100.4	
17	1+	33	-	101.2	100.8	101.4	
20		32	-	101.4		101.8	
24	-	33	-	101.8	101.0	100.6	
27		33	-	102.4	101.6	101.4	
31	-	34	AC <1%	101.8	101.4		
34		30	-	101.4	101.0	101.0	
41		36	-	102.0	102,2	100.6	
46	Tr.	37	-	101.2	101.6	104.2	101.6
51		38	-	101.6	100.0	102.0	102.0
58	1+	33	-	102.0	100.8	101.6	

9478: Adult
Male, Guernsey

EAC X EAM

Day	CF	PCV	Smear	Temperature, day / 0	1	2	3
-10	2/	35	-				
-4	2/	34	-				
0	Challenged: 70-PPI, with 5 ml blood injected SC, from EAM carrier showing 18% parasitemia.						
3	-	38	-	101.4	100.4	100.2	
6		32	-	101.6	101.4	101.0	
10	2/	37	-	101.6	101.4	101.6	
13		32	-	100.4	102.0	101.4	
17	1/	37	-	101.0	101.0	101.2	
20		34	-	102.0		101.4	
24		35	-	101.8	100.8	100.6	
27		39	-	101.4	102.0	101.6	
31	1/	35	-	101.8	101.8	101.0	
34		31	-	101.6	102.0	101.6	
41		34	-	102.0	102.0	100.8	
46	Tr.	40	-	101.8	101.6	101.6	101.6
52		43	-	100.6	100.6	102.0	100.8
59	1/	39	-	102.0	100.8	102.0	

9778: Adult

EAC X EAM

Day	CF	PCV	Smear
-7	4/	33	AC <.5%
0	1/40	36	AC <.5%
0	Challenged:	57-PPI	
7	3/	35	-
11	2/	37	-
14	1/	42	AC <.5%
18	1/	37	AC <.5%
21	2/	36	-
25	2/	37	AC <.2%
32	<1/5	36	-
39	2/	38	-
46	1/	38	-
53		39	AB <.2%

9785: Adult

EAC X EAM

Day	CF	PCV	Smear
	-	35	AC 1%
	1/80	33	AC <.5%
	Challenged:	49-PPI	
	1/	32	-
	1/	31	AC <.1%
	1/	34	-
	1/	32	AM 2%
	1/	26	AM <1%
	2/	24	AB <1%
	1/160*	30	-
	2/	32	-
	1/	32	AB <.2%
		32	AC <.3%

Challenge: 5 ml blood was injected SC, from EAM carrier showing 15% parasitemia.

* This was a prozone reaction with 4/ not showing up till the 3rd tube (1/20 dilution).

9788: Adult

EAC X EAM

Day	CF	PCV	Smear
-7	2+	31	AC <.2%
0	1/40	30	AC 1%
0	Challenged:	56-PPI	
7	1+	30	AC 2%
11	1+	28	AC 1%
14	1+	30	AC <1%
18	1+	29	AC 1%
21	1+	29	AC <.2%
25	3+	30	AC <.1%
32	1/10	33	-
39	3+	33	-
46	2+	34	AC <.2%
53		34	-

9790: Adult

EAC X EAM

CF	PCV	Smear
-	27	AC 1%
1/5	25	-
Challenged:	49-PPI	
-	25	-
-	26	-
-	30	AC <.1%
1+	27	AM <.2%
1+	27	-
3+	27	AM <.1%
1/5	27	-
2+	28	-
1+	26	AM <1%
	25	AC <.1%

Challenge: 5 ml blood was injected SC, from EAM carrier showing 1% parasitemia.

9791: Adult

EAC X EAM

Day	CF	PCV	Smear
-7	-	40	AC <.1%
0	-	40	-
0	Challenged:	56-PPI	
7	-	38	AC <.1%
11	-	37	-
14	-	39	-
18	-	38	-
21	-	37	-
25	-	39	-
32	-	39	-
39	-	38	-
46	-	39	-
53		40	-

9795: Adult

EAC X EAM

CF	PCV	Smear
3/	30	-
1/40	33	-
Challenged:	56-PPI	
1/	32	-
2/	34	-
3/	32	-
4/	31	AB <.5%
3/	31	-
4/	33	AC <.2%
1/40	33	AC <.5%
3/	35	AC <.2%
2/	35	AC <.2%
	34	-

Challenge: 5 ml blood was injected SC, from EAM carrier showing 15% parasitemia.

9825: Adult
EAC X EAM

Day	CF	PCV	Smear
-7	-	40	AC <.1% (TM)
0	Tr.	31	AC <.1%
0	Challenged:	42-PPI	
7	Tr.	32	-
11	-	32	-
14	-	35	-
18	-	34	-
21	-	34	-
25	1/	33	-
32	1/	32	AC <.2%
39	1/	36	-
46	-	36	-
53		37	-

9833: Adult
EAC X EAM

CF	PCV	Smear
1/	40	-
-	37	-
Challenged:	56-PPI	
-	38	-
-	39	-
1/	39	AC <.2%
4/	39	AM <.2%
3/	32	AC <.1%
3/	34	-
1/40	36	-
2/	39	-
2/	45	-
	42	-

Challenge: 5 ml blood was injected SC, from EAM carrier showing 15% parasitemia.

9834: Adult

EAC X EAM

Day	CF	PCV	Smear	
-7	4/	33	AC < 1%	
0	1/160	33	AC 1%	
0	Challenged:	49-PPI		
7	1/	30	-	
11	1/	32	AC < .5%	
14	4/	32	AC < .5%	
18	3/	29	AB < .5%	
21	2/	22	AM < .5%	
25	3/	25	AM < .2%	
32	1/320	31	-	
39	3/	32	AM < .1%	(TM)
46	4/	30	AB < .2%	(TM)
53		30	-	(TM)

9838: Adult

EAC X EAM

CF	PCV	Smear
2/	42	AC 1%
< 1/5	45	AC 1%
Challenged:	56-PPI.	
3/	43	-
1/	43	-
1/	48	-
2/	43	AM < .1%
2/	43	-
3/	40	AM < .1%
1/5	41	AC < .2%
3/	44	AC < 1%
3/	45	-
	46	-

Challenge: 5 ml blood was injected SC, from EAM carrier showing 15% parasitemia.

9777: Adult
EAM X EAC

Day	CF	PCV	Smear
0	2/	37	-
0	Challenged.		
7	1/10	39	-
14	4/	38	AB ∠.5%
21	4/	35	AC 4%
28	1/10	25	-
35	4/	34	AC 1%
42	4/	35	AC 1%
49	2/	34	AC 3%
56	2/	35	AC 1%
63	1/	36	AC ∠.5%
70	3/	35	AC ∠.1%

9811: Adult
EAM X EAC

CF	PCV	Smear
4/	40	-
Challenged.		
1/40	38	-
4/	41	- (TM)
4/	39	-
1/160	40	AC 4%
4/	39	AC 1% (TM)
2/	40	AC ∠1%
1/	39	- (TM)
3/	35	-
2/	37	AC ∠.2% (TM)
2/	38	AC ∠1% (TM)

Challenge: 5 ml blood was injected SC, from EAC carrier showing 5% parasitemia.

9828: Adult

EAM X EAC

Day	CF	PCV	Smear
0	3+	39	AM <.1%
0	Challenged:		
7	1/20	40	-
14	4+	40	-
21	4+	41	-
28	2+	37	-
35	1+	38	-
42	-	39	- (TM)
49	1/40	42	AC <1%
56	-	41	-
63	-	43	-
70	Tr.	44	-

9836: Adult

EAM X EAC

CF	PCV	Smear
4+	31	AM 25%
Challenged:		
1/320	23	AM 3%
4+	36	AM 3%
4+	33	AM 2%
4+	31	AM 2%
4+	30	AM 2%
4+	31	AM 1%
4+	35	AC 2% (TM)
1/80	28	AC 2%
2+	31	AC 3%
1+	32	AC 2%

Challenge: 5 ml blood was injected SC, from EAC carrier showing 5% parasitemia.

9782: Adult
EAM X EAM

Day	CF	PCV	Smear
0	1/	32	AM ∠.1%
7	Tr.	32	-
11	-	33	-
14	-	34	- (TM)
18	-	36	- (TM)
21	-	33	AB ∠.1%
25	Tr.	33	-
32	1/	28	AB 1%
39	1/	28	AB ∠1%
46	Tr.	30	AB ∠.1%
53	1/	32	AM ∠.5%

9806: Adult
EAM X EAM

CF	PCV	Smear
1/	38	AM ∠.5%
1/	38	-
Tr.	37	-
1/	39	AB ∠.1%
1/	36	-
1/	37	AB ∠.1%
2/	37	-
1/	38	-
1/	39	-
1/	38	AB ∠1%
1/	35	AB ∠1%

On day 0 both animals were challenged with 5 ml blood injected SC from EAM carrier showing 15% parasitemia.

9822: Adult EAM X EAM				9828: Adult EAM X EAM		
Day	CF	PCV	Smear	CF	PCV	Smear
0	3+	40	-	Tr.	44	-
0	Challenged:			Challenged:		
7	3+	35	-	-	42	-
11	3+	35	-	1+	43	-
14	3+	37	-	1+	44	-
18	2+	36	-	1+	42	-
21	2+	37	-	-	45	-
25	1+	36	-	2+	44	-
32	1+	36	-	1+	44	AB $<$ .1%
39	1+	35	-	1+	45	-
46	-	36	-	1+	46	-
53	-	35	-	1+	45	-

Challenge: 5 ml blood was injected SC from EAM carrier showing 15% parasitemia.