
Gonderia Mutans or Theileria Mutans?

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IN April, 1923, there appeared in the "Annales de Parasitologie" a paper by Brumpt in which he claimed that *Gonderia mutans* and *Theileria annulata* were synonymous. He based his opinion on the fact that by the inoculation of blood, originating from a Tunisian calf that showed small piroplasms in the circulating blood, diagnosed as *P. mutans* in two instances several months after inoculation, the injected cattle died of an acute paroxysm in which the typical plasma bodies were found in great numbers. In October, 1924, Brumpt, in his "Revue Critique" on the piroplasmoses of cattle dealing with "Les Theilerias," came back on the same subject. He re-established the species *Theileria annulata* and ascribed the disease described by him in 1923 to this species. He brings a number of new observations which to our mind leave no doubt that he is dealing with a definite and distinct entity, different from that caused by *P. mutans* and due to the species *T. annulata*. He does, nevertheless, not admit the validity of the genus *Gonderia* and according to him, the species *Gonderia mutans* should be *Theileria mutans*. The characteristic of the genus *Theileria* is the presence of the plasma bodies—the Agamonts and Gamonts—in the evolution of its cycle, which has so far been found in *Theileria parva*, in *Theileria annulata* and more recently in *Theileria dispar*. Although up to the present nobody has seen these plasma bodies in animals infected with *P. mutans*, Brumpt assumes that they are there all the same and may yet be found. His argument is as follows:—Since in *T. annulata* infection we are dealing with a chronic condition in which in some animals plasma bodies are found in the internal organs, so in *P. mutans*, infection, which also is a chronic condition, a similar occurrence may take place, but the plasma bodies have hitherto escaped observation since they are very rare. In support of his views, he quotes Doyle's observations in Egypt, who found that plasma bodies are only found in a small number of cattle imported from Cyprus, that otherwise show small piroplasms in the blood and who also thinks that plasma bodies belong to the cycle of *P. mutans*. It may be stated here that Doyle's evidence cannot be accepted as convincing, since he is probably dealing with the disease Brumpt had under observation, viz., that caused by *Theileria annulata*. According to Brumpt, there would now be four distinct species of the genus *Theileria*, viz., *T. parva*, *T. annulata*, *T. dispar*, and *T. mutans*. Ever since Theiler described for the first time the *Piroplasma mutans*, it was held that the distinguishing characteristic between this infection and that caused by *Theileria parva* was the presence of the plasma bodies in lymphatic glands and spleen, which were sometimes also fairly frequently found in the peripheral blood of sick cattle. Indeed in South Africa, on this

idea was placed the East Coast fever diagnosis in the field and in all cases where plasma bodies were found, the law as existing for the eradication of the disease was applied. Brumpt's article in 1923 did not shake our views. We realized that if really *P. mutans* could produce acute paroxysms in such a high percentage of cases as he shows, then plasma bodies would have occurred at one time or another in all parts of South Africa wherever *P. mutans* has been diagnosed and wherever ticks are present, and these are found not only in the regions in which East Coast fever is known to exist, but also in areas far distant. Besides we also had evidence to show that in a great number of cattle which were inoculated with blood containing *P. mutans*, never such a paroxysm was observed. Since 1909, we maintained the strain *A. centrale* in imported English cattle, using such blood as a vaccine against the Anaplasmosis. The blood contains also the *Piroplasma bigeminum* and *P. mutans*. Thus it acts as a polyvalent vaccine against the Piroplasmoses. Since its inception, the strains *A. centrale* and *P. mutans* have passed through twenty successive generations, involving 143 imported cattle of different breeds, such as Shorthorns, Sussex, Devon, Poll Angus, Hereford, Aberdeen-Angus, and Frieslands, besides 44 South Africans. There was never any paroxysm seen. Since there was never any death, no occasion for autopsies occurred, and thus never any plasma bodies could be found. They are never seen in the blood of the animals that were regularly examined during the fever reaction and subsequent to it, although *P. mutans* was often present in conspicuous numbers. During the same period a total number of 232,000 doses of vaccine were issued and we may state without hesitation that probably the equal number of cattle was vaccinated. We have in no instance heard of a paroxysm to which the animals succumbed, and they should have succumbed in great numbers if Brumpt's percentage of paroxysms, which, together with Stockman's experiment was a very large one, would hold for the *P. mutans* infection. In the experiments of these two gentlemen, out of a total number of 28 head of cattle, 5 died as a result of the inoculation, showing plasma bodies, or, leaving some doubtful cases out, of 24 animals, 5 succumbed, thus over 20 per cent., a mortality which can only be called short of alarming. Our experience thus shows clearly that the disease caused by *T. annulata* is not identical with the *P. mutans* infection, and moreover we can safely conclude that it is not present in South Africa.

A month previous to the appearance of Brumpt's first article, however, a new observation was made in South Africa relevant to the subject. Dr. Viljoen of the Veterinary Research Division was sent to make enquiries into the cause of a mortality amongst calves in the District of Marico, in the Western Transvaal, bordering on to Bechuanaland, a region far away from the East Coast fever infected districts. Viljoen found the calves suffering from acute and chronic anaemias caused by the infection of *P. bigeminum*, *P. mutans* and *Anaplasma marginale*. The farmers called the disease "liver disease," and indeed on post-mortem an icteric liver alongside of a splenic tumour was practically always present. The disease was only met with in calves under three months, and the farmers stated once they had passed this age, they began to thrive. Viljoen had the opportunity of examining a sick calf, showing a high temperature, which died during his presence and in which he found the lesions already mentioned. General icterus was also present. Spleen-smears were

made of this calf and on their examination in the laboratory by Nesor, the diagnosis Piroplasmosis and Anaplasmosis was confirmed, and to the surprise of all concerned, the presence of plasma bodies, indistinguishable from those of East Coast fever, were found. The presence of these bodies could not be explained, hitherto we considered them always typical of East Coast fever, but this diagnosis had to be excluded. The mortality was exclusively occurring amongst calves up to a certain age, an observation never made before in outbreaks of East Coast fever. We were for a time on the horns of a dilemma. Were we dealing with East Coast fever in an enzootic form, or were we dealing with a new and hitherto unknown Theileriosis? In order to settle this point, a new and more extensive investigation was arranged for under Dr. Viljoen, who proceeded immediately to the Marico District and made exhaustive enquiries. He visited 12 farms and examined 27 different calves, making blood and gland smears in all cases and spleen-smears of some dead calves, but he failed to find any plasma bodies. A result of this visit, however, was the detection of the prevalence of the bacillus of paratyphoid in some of the calves, these having shown the characteristic necrotic foci in the liver, and the bacilli could be isolated in a pure culture. Accordingly, it had to be admitted that the mortality in the Marico District was probably due to several causes, and it was thought that the paratyphoid infection was responsible to some extent, complicating the *P. bigeminum*, *P. mutans*, and *A. marginale* infections, or rather these infections favoured the complication with the former. All calves in tick infested areas have to pass the *P. bigeminum*, *P. mutans* and *A. marginale* infection, but the mortality from these two diseases in young calves is not a great one and accordingly the unusual heavy mortality in Marico District could find an explanation by this paratyphoid infection.

An attempt at immunization against this infection was made by means of killed cultures, and in January, 1924, an officer of the Division, Martinaglia, proceeded to inoculate calves of farmers, who volunteered to have the immunisation applied, and to report on the results of the vaccine. He also had to instruct them how to perform the inoculation. In the beginning of February, 1924, Martinaglia made a second visit to the district (9th to 18th February) in order to vaccinate more calves and to make further enquiries into the cause of the mortality. He made a number of autopsies and examined both bacterioscopically and microscopically spleen and blood of calves that had succumbed or were killed for the purpose. In one of these calves, he found numerous defined free and intracellular plasma bodies. This particular calf was three months old. It showed a rough coat, slow gait, marked lachrymation, slight salivation, rhinitis, tucked up appearance, and diarrhoea. Temperature 105° F. The autopsy was made immediately after slaughter; it revealed the existence of a marked anaemia, with a very slight hydrothorax and ascites, marked tumor splenis, enlarged liver with icteric patches and slight enteritis. A bacterioscopic examination of spleen and liver revealed the absence of any bacteria. The blood contained rare anaplasmas and small piroplasmas. In the spleen of a second calf that had died during the night, only one plasma body was found and there were a few small piroplasms in the blood corpuscles of the spleen-smear. These were the only two calves in which Martinaglia found plasma bodies. In some calves he found a pure paratyphoid

infection, in others he found anaplasma, *P. bigeminum* and *Piroplasma mutans*. He was also able to show the presence of coccidiosis (red diarrhoea) in some places, and heartwater in others, so that he came to the conclusion that a number of various causes were responsible for the mortality.

In March, 1926, Dr. Viljoen again paid a visit to the Marico District with a view to ascertain the value of the preventive vaccination which had been carried out there, but more particularly to collect more evidence about the prevalence of the plasma bodies met there in 1923 and 1924, and if he should be successful of finding infected calves to bring them to the Laboratory for further studies. A number of farms were visited and both blood and gland examinations were undertaken on the spot, also some spleens of dead calves, but no plasma bodies could be detected. The conclusion he arrived at was again that a number of diseases were responsible, of which the tick borne ones were the most important.

Paratyphoid was also diagnosed. All attempts to obtain infected living calves in Marico failed, and accordingly the mystery of plasma bodies could not be cleared up in that district.

In April, 1924, there appeared in the "Annales de l'Institut Pasteur" the publication of Edm. Sergent and his co-workers on the bovine piroplasmoses of Algeria, in which they described the new species of *Theileria dispar* that produced a fatal disease in cattle. Our attention was of course directed to this new disease in which anaemia and splenomegalia were distinct symptoms, but since this disease attacks full grown cattle as well, and since the plasma bodies are very numerous in the internal organs, both observations, contrary to those made in the Marico District, the idea of the presence of *Theileria dispar*, had to be discarded. Besides it is hardly likely that such a deadly disease could have existed somewhere in the Union without the Veterinary Division knowing something about it.

In considering the results of the Marico investigations, we find that out of a fairly great number of calves, plasma bodies were found only three times in the spleen, in two cases in fairly large numbers, and in one case only a single one. The mortality was not due to one disease exclusively, but to a number of causes; the finding of Koch's bodies had therefore to be considered in the light of a lucky accident, a coincident in animals that were suffering from multiple infections. Accordingly the idea expressed by Brumpt that in the cycle of *P. mutans* there may be found plasma bodies occasionally, although rarely, appeared to us as an acceptable one. We should, therefore, be in the position to find with good luck and perseverance such bodies in *P. mutans* infection, if we apply our newly gained knowledge to the examination of *P. mutans* infected calves. In accepting Brumpt's theory, we thought it feasible that the infection at its inception in calves might begin with plasma bodies for a while, and only subsequently the multiplication in the blood might continue in the hitherto accepted way. This then would explain our failures with adult cattle in which we never found plasma bodies. This then also gave a new line of research. Another attempt of solving the problem was based on the observation made in this institute by De Kock and Quinlan, who found that after the removal of the spleen of horses, a latent *Nuttalia* infection would relapse, after removal of the spleen of cattle, piroplasmosis and anaplasmosis, the same thing would occur. It might, therefore, also be expected that *P. mutans* infection would relapse and in its course plasma bodies might reoccur.

The experimental research to be undertaken was planned as follows:—

- I. To infect young calves with blood containing *P. mutans*, to examine lymph glands for plasma bodies, eventually to kill the calves and examine the spleen.
 - II. To allow cows to drop their calves into a highly tick-infested area, so that they were exposed from the day of birth to tick infestation. To keep the calves on a somewhat restricted milk supply, imitating the farming conditions in Marico. To examine the blood at frequent intervals and the glands and spleen as soon as *P. mutans* was seen in the blood.
 - III. In case plasma bodies should be found in the spleen of a living calf to kill it and inject the spleen pulp into susceptible young calves kept free of ticks.
 - IV. To expose susceptible full-grown calves to tick infection for a short period, to observe them in the stable and to examine glands irrespective of the presence of parasites in the blood.
 - V. To remove the spleen of calves that show a *mutans* infection in order to cause a relapse and to examine the lymph glands for the presence of plasma bodies as well as the blood.
- I.—To NOTE WHETHER AFTER AN INJECTION OF BLOOD CONTAINING *P. mutans*, PLASMA BODIES CAN BE OBSERVED IN THE LYMPHATIC GLANDS.

(1) 11.6.24. Calf 932, bull, born 10.6.24 on station, injected intrajugularly 5 c.c. blood of vaccine heifer 838. This calf was kept under observation until 13.8.24. The blood was examined every second day and at times every day. There appeared in the blood, as was to be expected, *P. bigeminum*, which was present for some time. Its presence gave rise to hardly any fever, and also the anaemic blood changes were but moderate. Also *Anaplasma centrale* appeared in moderate numbers and about at the same time *P. mutans*, viz. on the 23.7.24. From this date onward the examination was extended to smears of the lymphatic glands. At times *P. mutans* was fairly numerous, but at no time were there seen any plasma bodies in the glands.

(2) 11.6.24. Calf 904, bull, born on 26.4.24 on station, injected 5 c.c. blood of heifer 838. In this case *P. bigeminum* also appeared, but produced but little febrile disturbance. The examination of gland-smears was commenced before the appearance of *Anaplasma centrale* and *P. bigeminum* and continued until 19.8.24. At no time were there seen any plasma bodies in the gland.

(3) 14.8.25. Calf 1193, heifer, born on 19.6.25 on station, injected 5 c.c. blood of vaccine heifer 838. The examination of blood and gland-smears was commenced 5 days after the injection. The temperature on the 5th day was 105° F. but no *P. bigeminum* was seen, which was expected.

This calf was injected 15.9.25 with paratyphoid vaccine, 15 c.c., which gave rise to a fever to 105° F. the same evening. *Piroplasma mutans* appeared late, 1.10.25, and was present only on rare occasions and in rare numbers. *Anaplasma centrale* was never recorded. The calf was examined until 2.11.25, but no plasma bodies were seen in the glands.

(4) Calf 1194, heifer, born on 16.6.25 on station, injected as above. The examination was carried out as with calf 1193. Also in this calf a rise of temperature occurred on the sixth day to 105° F., but the expected *P. bigeminum* was not seen. The calf was injected 15.9.25 with paratyphoid vaccine 10 c.c. The temperature on the following day rose to 104.4° F. *Anaplasma centrale* appeared before *Piroplasma mutans*. The former on the 25.9.25, the latter on the 1.10.25. *P. mutans* was present for a considerable time but only in rare numbers. The calf was examined until 3.11.25, but plasma bodies were never seen in the glands.

(5) Calf 1195, heifer, born on 24.6.25 on station, injected 14.8.25 intrajugularly 5 c.c. blood of vaccine heifer 842. There was no temperature disturbance and no *P. bigeminum* were seen. This calf was injected on 15.9.25 with 10 c.c. paratyphoid vaccine. There was a fever reaching 105.8° F. in the evening, and although abating by degrees, continued for three days. *P. mutans* was seen in the blood on 1.10.25. The parasites were present for a considerable length of time until the observations were discontinued on 3.11.25. *Anaplasma centrale* was not recorded. The examination of the smears gave constantly negative results.

(6) Calf 1196, heifer, born on 29.6.25 on station, treated in the same way as calf 1195. It also received an injection of paratyphoid vaccine which gave but a mild fever reaction. *P. bigeminum* and *A. centrale* were not recorded in this case. *P. mutans* was seen for the first time on 1.10.25 and the following day. It soon disappeared again, however. Observations were discontinued on 3.11.25. At no times were plasma bodies seen in the glands.

(7) Calf 1199, heifer, born on 30.6.25 on station, injected 14.8.25 with 5 c.c. blood of vaccine heifer 843. On the 15.9.25 5 c.c. paratyphoid vaccine were injected. There was a rise of temperature to 105.4° F. the same evening. No *P. bigeminum* and no *A. centrale* were recorded in this case. *P. mutans* appeared on the 28.8.25. It was recorded until the day of the discontinuation of the observations, 3.11.25. The gland-smears were constantly negative.

(8) Calf 1223, heifer, born on 14.7.25 on station, injected as above. It also received 5 c.c. paratyphoid vaccine on 15.9.25. It responded with a rise of fever the same evening to 105.2° F. This fever passed, but a febrile condition returned accompanied with symptoms of catarrh and remaining for about six days. No *P. bigeminum* were recorded in this calf, nor *A. centrale*. *P. mutans* appeared on the 28.8.25. It was present until one week before the discontinuation of observations, appearing and disappearing in intervals. The smear examination was constantly negative for Koch's bodies.

Discussion of Results.—The injection of blood was successful in every instance by the appearance of *P. mutans* in the blood. *P. bigeminum* did not appear in every instance, nor did *Anaplasma centrale*. The cases did, therefore, not represent complex blood infections. The injection of paratyphoid vaccine in four cases certainly produced in every case a thermic disturbance, but had no influence on the *P. mutans*. The fact of importance is that in the eight cases no plasma bodies were noted in the lymphatic glands. In six cases the examination was commenced during the incubative period and continued right through the period the parasites were seen in the blood. If plasma bodies were present in the glands, they must have been exceedingly rare.

II.—TO EXPOSE CALVES FROM DATE OF BIRTH TO NATURAL TICK INFECTION AND TO OBSERVE IF *Piroplasma mutans* WILL DEVELOP IN SPLEENS AND GLANDS.

Observations on calves grazing at Kaalplaats from May, 1926, to March, 1927.

The main object was to note whether at any time plasma bodies could be found in blood, gland, and spleen smears in animals undergoing a natural infection of *Piroplasma mutans*. Beginning of 1927, the incidence of paratyphoid infection was also taken into consideration. Throughout this period subinoculations were made in practically every case of rise in temperature over 104° F. into heartwater susceptible sheep, but *very* few reactions in sheep were noted—even in cases where a clinical or post-mortem diagnosis of heartwater was made. This is probably due to method of collecting blood in "oxalate anticoagulant" consisting of a mixture of 5 litres water, 5 litres glycerine, 50 c.c. carbolic acid, and 50 grams Pot. oxalate. Up to 30.9.26 temperatures were only taken every second day, but from 1.10.26 onward daily. Smears were examined as often as possible—especially during temperature reactions. The smears of November are not included in the report. Agglutination tests with various paratyphoid strains were undertaken from 9.2.27 onwards—every calf available being tested and as many of the sheep used for subinoculations as could be traced—which were only about one-third of total number used. In a few cases very marked agglutinations occurred of paratyphoid strains with sheep serum, e.g., 15,845 and S. 51,583 agglutinated up to 1:10,000 dilution—though at this dilution no longer completely. Details of agglutination tests attached. (Page 93.)

Up to 21st September, 1926, calves were allowed to run with cows day and night, but from that date onwards, were separated from the cows, which were partially milked in order to decrease milk supply to calves and thus produce semi-starvation conditions.

N.B.—*Piroplasma mutans* was present in practically every case, usually being very rare and persisting for months. Plasma bodies were found in only four cases and in these in such small numbers that on examination of a whole smear, only three could be found in a single smear.

Group I.—Calves still in Experiment. February, 1927.

(1) 1690, heifer, born 5.9.25, observed since 5.5.26. Showed on 30.6.25 *P. mutans* very rare, *A. marginale* very rare, 23.9.26 *P. mutans* very rare, 11.12.26 *P. mutans* rare, 27.12.26 *P. mutans* very rare, 3.1.27 *P. mutans* very rare. Examined blood-smears 14, gland smears 6.

Slight temperature reaction from 30.6.26 to 9.7.26 with a maximum 103.6° F. on 3.7.26. *A. marginale* very rare 30.6.26. Cause of temperature reaction anaplasmosis [relapse?]. Very irregular temperature curve from 17.11.26 to 14.12.26 with maximum of 103° F. on 18.11.26, 24.11.26, 29.11.26 and minimum 99° F. Cause unknown. 13.2.27 Serum agglutinates Page spleen strain (Marico) and Kaalplaats 2217 up to 1:200 dilution, Paratyphoid B strain up to 1:50 very slightly. Discharged 9.2.27.

(2) C. 1697, heifer, born 1.10.25, observed since 5.5.26. Showed on 6.5.26 *P. mutans* rare, 25.5.26 *P. mutans* fairly rare, 26.5.26 *P. mutans* comparatively numerous, 28.5.26 *A. marginale* rare,

P. bigeminum very rare, 30.6.26 *A. marginale* fairly rare, *P. mutans* very rare, 6.12.26 *P. mutans* very rare, 11.12.26 *P. mutans* rare. Number of blood-smears examined 30, gland 2, spleen 2.

Marked temperature reaction from 16.1.27 to 23.1.27 with a maximum of 106.8° F. on 18.1.27 with blood and gland-smears negative. May be due to paratyphoid infection, since serum of this calf agglutinates strongly various strains. Blood [glyc. oxalate] was subinoculated on 25.1.27 into S. 15795 and S. 15821 with negative results for heartwater although S. 15795 showed an irregular temperature 104-105° F. from 4th to 8th day after inoculation.

Agglutination tests with calf's serum were strongly positive up to 1: 500 dilution against Kaalplaats 2217 and Page spleen (Marico) strain on 12.2.27. Sheep serum (15821 and 15795) did not agglutinate over 1:100 dilution on 19.2.27. Discharged 9.2.27.

(3) C. 1701, heifer, born 15.10.25, observed since 5.5.26. Showed on 30.6.26 *A. marginale* rare, 6.12.26 *P. mutans* rare. Number of blood-smears examined 13, gland 9.

No temperature reactions of any note. No subinoculations. Serum agglutinates Page spleen (Marico) and Kaalplaats 2217 strain up to 1: 500 dilutions partially on 13.2.27. Negative for paratyphoid B. strain. Is a very doubtful reaction. Discharged on 9.2.27.

(4) C. 1800, heifer, born 14.10.25, observed since 5.5.26. Showed on 6.5.26 *P. bigeminum* very rare, *P. mutans* rare, 26.5.26 *A. marginale*, 8.9.26 *P. mutans* very rare, 11.11.26 *P. mutans* very rare, 6.12.26 *P. mutans* very rare. Number of blood-smears examined 16, gland 9.

Temperature reaction from 17.6.26 to 30.6.26 with maximum 105° F. on 21.6.27, blood-smear being negative. Blood (glyc. oxalate) subinoculated intrajugularly on 23.6.26 into S. 10569 with no resultant temperature reaction. Calf's serum agglutinates Page spleen and Kaalplaats 2217 strain incompletely in 1: 100 dilution, i.e. very doubtful reaction 13.2.27, probably negative. Sheep's serum not available for agglutination tests. Discharged 9.2.27.

(5) C. 1801, heifer, born 14.10.25, observed since 5.5.26. Showed on 11.12.26 *P. mutans* rare, 27.12.26 *P. mutans* very rare, 3.1.26 *P. mutans* very rare, 10.1.27 *P. mutans* very rare. Number of blood-smears examined 13, gland 9.

Slight temperature reaction from 18.5.26 to 1.6.26 with maximum 104.2° F. on 20.5.26 blood-smear on 28.5.26 negative. Another slight reaction from 11.6.26 to 21.6.26 with maximum 104.4° F. on 17.6.26. Cause unknown. No subinoculations. Agglutination tests against three strains negative on 13.2.27. Discharged 9.2.27.

(6) 1802, heifer, born 15.10.25, observed since 5.5.26. Showed on 30.6.26 *P. mutans* very rare, *P. bigeminum* very rare, 14.7.26 *P. bigeminum* very rare, 8.9.26 *P. mutans* very rare, 2.11.26 *P. mutans* very rare, 6.12.26 *P. mutans* very rare, 11.12.26 *P. mutans* very rare, 24.1.27 *P. mutans* very rare. Number of blood-smears examined 16, gland 9.

Slight thermal reaction during 1.7.26 to 10.7.26 with maximum 104.2° F. on 6.7.26—probably due to *P. bigeminum* infection as parasite seen in blood on 14.7.26. Agglutination test positive up to 1: 200 for Page spleen and Kaalplaats 2217 strain, negative for paratyphoid B. 13.2.27. Discharged 9.2.27.

(7) C. 1804, heifer, born 19.12.25, observed since 5.5.26. Showed on 30.6.26 *P. mutans* extremely rare, 2.11.26 *P. mutans* rare, *P. bigeminum* very rare, *A. marginale*. Number of blood-smears examined 16, gland 8.

Temperature reaction from 13.5.26 to 30.5.26 with maximum 104.6° F. on 20.5.26 and 22.5.26. Blood-smear negative. Cause uncertain—paratyphoid? Agglutination test positive up to 1:200 for Page spleen strain and 1:500 for Kaalplaats 2217 strain on 13.2.27, negative for paratyphoid B. strain. Discharged 9.2.27.

(8) 1863, heifer, born 27.11.25, observed since 5.5.26. Showed on 6.5.26 *P. mutans*, *A. marginale*, 28.5.26 *P. bigeminum* very rare, 9.6.26 *A. marginale* very rare, 23.6.26 *P. mutans* very rare, 30.6.26 *P. mutans* very rare, 21.7.26 *P. mutans* very rare, 11.8.26 *P. mutans* rare, 14.12.26 *P. mutans* rare, 29.1.27 *P. mutans* very rare. Number of blood-smears examined 21, gland 14. Slight thermal reaction during May, 1926, due to *P. bigeminum* infection. Agglutination test (13.2.27) positive for Page spleen strain up to 1:200, Kaalplaats 2217 1:500 dilution, i.e., taken as a positive reaction—negative for paratyphoid B. No subinoculations. Discharged 9.2.27.

(9) C. 1892, heifer, born 30.12.25, observed since 5.5.26. Showed on 6.5.26 *P. mutans* very rare, 25.5.26 *P. mutans* very rare, 25.6.26 *P. mutans* very rare, 28.5.26 *A. centrale* and *A. marginale* very rare, 9.6.26 *P. bigeminum* fairly rare, *P. mutans* rare, 16.6.26 *A. marginale* fairly rare, *P. mutans* very rare, 23.6.26 *P. mutans* fairly rare, *A. marginale* very rare, 30.6.26 *A. marginale* very rare, 22.9.26 *P. mutans* very rare, 2.10.26 *P. mutans* rare, 28.12.26 *P. mutans* very rare, 1.2.27 *P. mutans* very rare. Number of blood-smears examined 22, gland 14.

Thermal reaction from 29.5.26 to 5.6.26 with maximum 105.2° F. on 1.6.26—due to Anaplasmosis and Redwater infection. Agglutination test 13.2.27 positive up to 1:200 dilution for Page spleen (Marico) and Kaalplaats 2217 strain, i.e. doubtful reaction. Discharged 9.2.27.

(10) C. 1945, heifer, born 26.1.26, observed since 5.5.26. Showed on 30.6.26 *P. mutans* very rare, 1.11.26 *P. mutans* fairly rare, 28.12.26 *P. mutans* very rare, 4.1.27 *P. mutans* very rare. Number of blood-smears examined 14, gland 7.

No temperature reaction. Agglutination test on 13.2.27 positive up to 1:200 for Page spleen and Kaalplaats 2217 strains. Negative for paratyphoid B. strain.

(11) C. 2063, heifer, born 24.2.26, observed since 5.5.26. Showed on 6.5.26 *P. bigeminum* very rare, 13.5.26 *P. bigeminum* very rare, *A. marginale* very rare, 20.5.26 *A. marginale* fairly rare, 26.5.26 *A. marginale* rare, 28.5.26 *A. marginale* numerous, *P. bigeminum* very rare, 2.6.26 *A. marginale* rare, 9.6.26 *A. marginale* fairly numerous, *P. mutans* very rare, 16.6.26 *A. marginale* rare, *P. mutans* numerous, 23.6.26 *P. mutans* numerous, 30.6.26 *A. marginale* fairly rare, *P. mutans* fairly numerous, 8.7.26 *P. mutans* rare, 11.8.26 *A. marginale* very rare, *P. bigeminum* very rare, 28.12.26 *P. mutans* rare, 1.11.26 *P. mutans* very rare, 4.1.27 *P. mutans* rare, 19.1.27 *P. mutans* rare, 25.1.27 *P. mutans* rare. Number of blood-smears examined 26, gland 16.

From 16.6.26 to 30.6.26 *P. mutans* numerous in blood and on 28.5.26 a true plasma body was found in gland-smear. There was a temperature reaction from 7.5.26 to 1.6.26 with maximum 106.4° F. on 26.5.26 corresponding to maximum anaplasmosis infection.

Temperature returned to normal for a few days but from 8.6.26 to 25.7.26 it was irregular several times reaching 104° F.—rare anaplasmosis and *P. mutans* were found during this time. Subinoculations were made with blood into S. 9284 and S. 10374 on 28.5.26 and again on 9.6.26 into S. 7405 and S. 7716 and again on 23.6.26 into S. 10958 without a typical heartwater reaction in any case resulting, though S. 9284 showed a slightly irregular temperature 103-104° F. from 7th to 13th day—probably negative for heartwater, and in S. 7405 temperature rose 2nd day after injection reaching 106.2° F. on 5th day, dropping to normal on 6th day, but was again 105° F. on 15th day after which it was normal. Serum of this sheep tested (agglutination) on 19.2.27 was negative for Kaalplaats 2217 strain and paratyphoid B. strain. Blood-smear during reaction was negative but animal showed clinically anorexia and dullness. Serum for agglutination tests of other sheep was not obtainable. The long temperature reaction in calf 2063 may be due to prolonged anaplasmosis and *P. mutans* infection, but in view of nature of reaction in two of the sheep it may possibly have been the above, complicated with paratyphoid infection. This view is favoured by agglutination test of calf's serum on 12.6.26 when it agglutinated both Kaalplaats 2217 and Page spleen strain in dilution up to 1:500 and paratyphoid B. up to 1:50 slightly. Discharged 11.2.27.

(12) C. 2072, heifer, born 14.4.26, observed since 5.5.26. Showed on 28.5.26 *P. bigeminum* very rare, 30.6.26 *A. marginale* rare, *P. mutans* rare, 11.8.26 *P. mutans* rare, *P. bigeminum* very rare, 9.12.26 *P. mutans* very rare, 28.12.26 *P. mutans* rare, 6.1.27 *P. mutans* rare, 8.1.27 *P. mutans* very rare, 1.2.27 *P. mutans* very rare, 10.2.27 *P. mutans* very rare. Number of blood-smears examined 22, gland 14.

Slight temperature reaction, maximum 104.2° F., on 26.5.26 due to *P. bigeminum* infection. Slight reaction 5.9.26 to 17.9.26 to 103.2° F. on 11.9.26 with unknown cause. Agglutination test on 12.2.27 very doubtful reaction agglutinating Page spleen and Kaalplaats strain up to 1:100—negative for paratyphoid B. strain. 30.6.26 *A. marginale* very rare. Discharged 11.2.27.

(13) C. 2092, heifer, born on 12.5.26, observed since 25.5.26. Showed on 9.6.26 *P. bigeminum* very rare, *A. marginale* very rare, 23.6.26 *A. marginale* very rare, 30.6.26 *P. bigeminum*, *A. marginale*, *P. mutans* very rare, 9.12.26 *P. mutans* rare, 8.1.27 *P. mutans* very rare, 19.1.27 *P. mutans* very rare, 25.1.27 *P. mutans* very rare, 29.1.27 *P. mutans* very rare, 2.2.27 *P. mutans* rare. Number of blood-smears examined 20, gland 12, spleen 2.

Temperature reaction from 24.5.26 to 30.6.26, maximum 104.2° F. on 18.2.26, due to *P. bigeminum* and anaplasmosis infection. Another reaction from 9.8.26 to 25.8.26 with maximum 104.4° F. on 11.8.26. No smears available. Blood (oxalated) was subinoculated on 23.6.26. into S. 9111 which resulted in rise of temperature from 17th to 27th day after injection 105.8° F. and 105.6° F. on 18th and 20th days respectively then to normal (heartwater). Subinoculation from sheep on 15.7.26 (i.e. 22nd day) into S. 14322 and S.14338 was done. In S. 14322 temperature rose from day of injection (104.4° F.) to 104.8° F. on 4th day, then fell gradually and regularly to 102° F. on 11th day. 14338 showed no reaction. Agglutination test of serum C. 2092 showed agglutination of Kaalplaas strain 2217 up to 1:500 and Page spleen up to 1:200 (slight). Paratyphoid B. strain 1:50.

(14) C. 2093, heifer, born 24.5.26, observed since 1.6.26. Showed on 16.6.26 *P. mutans* rare, *P. bigeminum* very rare, 23.6.26 *P. mutans* numerous, *A. marginale* rare, 30.6.26 *P. mutans* fairly numerous, 11.8.26 *A. marginale* fairly numerous, *P. mutans* rare, 14.8.26 *A. marginale* fairly numerous, 18.8.26 *P. bigeminum* very rare, 8.9.26 *P. mutans* very rare, 22.9.26 *P. mutans* very rare, 2.12.26 *P. mutans* very rare. 29.12.26 *P. mutans* rare, 8.1.27 *P. mutans* rare, 12.1.27 *P. mutans* very rare, 19.1.27 *P. mutans* very rare, 25.1.27 *P. mutans* rare, 10.2.27 *P. mutans* very rare. Number of blood-smears examined 28, gland 19, spleen 1.

Temperature reaction from 13.6.26 to 28.6.26 with maximum 104.6° F. on 23.6.26 due to *P. bigeminum*. Another reaction from 28.7.26 to 14.8.26 due to *A. marginale*. Subinoculation on 30.6.26 of oxalated blood into S. 12937 which subsequently showed a temperature reaction beginning on 4th day reaching 106° F. on 5th and 8th days, returning to 102° F. on 9th day. S. 13969 remained negative. Calf's serum on 12.2.27 agglutinated Kaalplaats 2217 and Page spleen up to 1:200 dilution—negative for paratyphoid B. Discharged 11.2.27.

(15) 2095, bull, born 1.6.26, observed since 8.6.26. Showed on 30.6.26 *P. bigeminum* very rare, *P. mutans* rare, 11.8.26 *A. marginale* very rare, 22.10.26 *P. bigeminum* and *P. mutans* very rare, 2.12.26 *P. mutans* rare, 9.12.26 *P. mutans* very rare, 18.12.26 *P. mutans* rare, 12.1.27 *P. mutans* very rare, 25.1.27 *P. mutans* rare. Number of blood-smears 14, gland 9.

Temperature reaction from 22.6.26 to 3.7.26 with maximum 104° F. on 26.6.26 due to *P. bigeminum*. Another reaction from 18.7.26 to 8.8.26 with maximum 103.8° F. on 2.8.26 probably due to anaplasmosis infection as *Anaplasma marginale* was found rare on 11.8.26. Third reaction an irregular fever with maximum 104° F. lasting from 26.11.26 to 28.12.26—cause unknown—except presence of *P. mutans* very rare (paratyphoid?). Blood (oxalated) subinoculated on 30.6.26 into S. 10725 and S. 13856 with negative results. Serum agglutinated weakly (12.2.26) Kaalplaats 2217 and Page spleen strains up to 1:100—very doubtful reactor. Castrated 7.1.27. Discharged 11.2.27.

(16) 2105, heifer, born 28.6.26, observed since 3.7.26. Showed on 14.8.26 *P. mutans* very rare, *A. marginale* and *P. bigeminum* very rare, 8.9.26 *P. mutans* very rare, 22.9.26 *P. mutans* very rare, 2.12.26 *P. mutans* and *A. marginale* very rare, 2.2.27 *P. mutans* rare, 12.2.27 *A. marginale* and *P. mutans* very rare, 15.2.27 *P. mutans* rare. Number of blood-smears 13, gland 7.

Slight temperature reaction 7.8.26 to 28.8.26 with maximum 103.4° F. on 18.8.26. *P. bigeminum* infection. Diarrhoea 25.8.26. Serum agglutinated (12.2.27) completely Kaalplaats 2217 strain up to 1:200, Page spleen up to 1:500—positive reactor—negative for paratyphoid B.

(17) C. 2120, heifer, born 14.7.26, observed since 21.7.26. Showed on 22.10.26 *P. mutans* very rare, 17.11.26 *P. mutans* and *A. marginale* rare, 2.12.26 *P. mutans* very rare, 16.12.26 *P. mutans* very rare, 18.12.26 *A. marginale* and *P. mutans* very rare, 8.1.27 *P. mutans* rare, 28.1.27 *P. mutans* very rare, 2.2.27 *P. mutans* very rare, 8.2.27 *P. mutans* very rare, 12.2.27 *P. mutans* very rare, 15.2.27 *P. mutans* very rare. Number of blood-smears examined 16, gland 11, spleen 2.

Temperature reaction from 10.11.26 to 23.11.26 with maximum 105.4° F. on 16.11.26—cause unknown (heartwater?). Subinoculations with oxalated blood on 17.11.26, S. 14879 and S. 15518—sheep showing no temperature reaction. Agglutination test with calf serum on 12.2.27—doubtful positive reaction, agglutinated only up to 1:100 and was negative for paratyphoid B.

(18) C. 2138, bull, born 10.8.26, observed since 12.8.26. Showed on 22.10.26 *P. mutans* very rare, 2.12.26 *P. mutans* very rare, 10.12.26 *P. mutans* very rare, 16.12.26 *P. mutans* rare, 2.2.27 *P. mutans* very rare, 12.2.27 *A. marginale* very rare, *P. mutans* very rare. Number of blood-smears examined 9, gland 8.

No definite temperature reaction but showed throughout a very irregular temperature varying between 99 and 103° F. Agglutination test 9.2.27 doubtful—agglutinating *B. enteritidis gärtner* (Bainbridge) 1:100, Page spleen 1:100, Kaalplaats 2226 1:100 weakly Kaalplaats 2217 1:50—negative for paratyphoid B.

(19) C. 2168, bull, born 31.8.26, observed since 4.9.26. Showed on 22.10.26 *P. mutans* rare, 10.12.26 *P. mutans* and *A. marginale* very rare, 13.1.27 *P. mutans* rare, 3.2.27 *P. mutans* rare, 15.2.27 *A. marginale* and *P. mutans* rare. Number of blood-smears examined 8, gland 8.

No definite marked temperature reactions. Agglutination tests 9.2.27. Doubtful positive reactor agglutinating *B. enteritidis* 1:100, Page spleen 1:200, Kaalplaats 2226 1:100 and Kaalplaats 2217 1:100—negative for paratyphoid B.

(20) C. 2169, bull calf, born 31.8.26, observed since 4.9.26. Showed on 22.10.26 *P. mutans* very rare, 3.12.26 *P. mutans* very rare, 4.12.26 *P. mutans* very rare, 16.12.26 *P. mutans* fairly rare, 19.12.26 *A. marginale* fairly rare, 29.12.26 *P. mutans* very rare, 6.1.27 *P. mutans* rare, 13.1.27 *P. mutans* very rare, 18.1.27 *P. mutans* very rare, 28.1.27 *P. mutans* very rare, 3.2.27 *P. bigeminum* rare, *P. mutans* very rare, 9.2.27 *P. mutans* very rare, 15.2.27 *P. mutans* very rare. Number of blood-smears examined 14, gland 11, spleen 2.

Irregular temperature reaction varying from 98-104° F. from 29.11.26 to 16.12.26 due to anaplasmosis. Subinoculations into S. 15769 and S. 15820 with negative results on 25.1.27 using oxalated blood. Agglutination tests 9.2.27 of calf negative for all strains used (5). Serum of sheep tested on 19.2.27 agglutinated weakly Kaalplaats 2217 strain 1:100.

(21) C. 2170, heifer, born 3.9.26, observed since 3.9.26. Showed on 2.12.26 *P. mutans* very rare, 10.12.26 *P. mutans* very rare, 16.12.26 *P. mutans* very rare, 27.12.26 *P. mutans* rare, 7.1.27 *P. mutans* fairly rare, 13.1.27 *A. marginale* very rare, *P. mutans* rare, 18.1.27 *P. bigeminum* and *P. mutans* very rare, 22.1.27 *P. mutans* very rare, 25.1.27 *P. mutans* very rare, 28.1.27 *P. mutans* very rare, 9.2.27 *P. mutans* very rare, 16.2.27 *P. mutans* very rare. Number of blood-smears examined 16, gland 4, spleen 1.

Very severe temperature reaction from 15.1.27 to 27.1.27 being 106° F. on 20.1.27 to 24.1.27 with blood *P. mutans* very rare. *P. bigeminum* and *A. marginale* very rare on 13.1.27 and 18.1.27—cause of reaction heartwater (?). Subinoculated oxalated blood into S. 15814 and S. 15786 on 25.1.27—both sheep showing no temperature reactions. Agglutination test of calf serum negative against five strains on 9.2.27. Sheep serum tested on 19.2.27 also negative. Cause of reaction probably heartwater though sheep negative (oxalated blood used).

(22) C. 2184, bull, born 25.9.26, observed since 25.9.26. Showed on 22.10.26 *P. mutans* very rare, 10.12.26 *A. marginale* very numerous, *P. bigeminum* rare, 17.12.26 *A. marginale* fairly rare, 30.12.26 *A. marginale* fairly numerous, *P. mutans* rare, 7.1.27 *A. marginale* very rare, *P. mutans* very rare. 13.1.27 *A. marginale* fairly rare, 18.1.27 *A. marginale* rare, 28.1.27 *A. marginale* rare, 3.2.27 *A. marginale* very rare, *P. mutans* rare. Number of blood-smears examined 12, gland 12.

Temperature reaction from 29.1.27 to 9.2.27 with maximum 103° F. on 31.1.27 due to anaplasmosis. Agglutination tests 9.2.27 negative against 5 strains.

(23) C. 2194, heifer, born 8.10.26, observed since 8.10.26. Showed on 22.10.26 *P. mutans* very rare, 2.12.26 *P. mutans* very rare, 30.12.26 *P. bigeminum* very rare. Number of blood-smears examined 9, gland 8.

Temperature reaction from 20.11.26 to 10.12.26 with maximum 105° F. on 1.12.26 (blood *P. mutans* very rare, gland negative), cause of reaction unknown (paratyphoid?). Somewhat irregular temperature during January, 1927, to 5.2.27 never above 103.4° F.—no smears available—probably redwater and anaplasmosis. Agglutination tests 9.2.27 strongly positive up to 1:500 dilution against all 4 strains used, negative for paratyphoid B.

(24) 2195, heifer, born 9.10.26, observed since 13.10.26. Showed on 10.12.26 *P. mutans* very rare, 17.12.26 *P. mutans* very rare, 30.12.26 *A. marginale* rare, *P. mutans* very rare, 6.1.27 *P. mutans* very rare, 13.1.27 *A. marginale* very rare, 10.1.27 *P. mutans* rare, 4.2.27 *A. marginale* very rare, *P. mutans* rare, 7.2.27 *P. mutans* fairly rare, 9.2.27 *A. marginale* and *P. mutans* rare, 16.2.27 *P. mutans* very rare. Number of blood-smears examined 15, gland 13, spleen 2.

Irregular temperature reaction from 28.1.27 to 9.2.27 with maximum 103.6° F. on 4.1.27 and 5.1.27—anaplasmosis. Agglutination tests 9.2.27 negative against 5 strains.

(25) C. 2202, heifer, born 13.10.26, observed since 14.10.26. Showed on 17.12.26 *A. marginale* numerous, 30.12.26 *P. mutans* and *A. marginale* and *P. bigeminum* rare, 6.1.27 *A. marginale* fairly rare, *P. mutans* rare, 13.1.27 *A. marginale* and *P. mutans* very rare, 13.1.27 *A. marginale* fairly numerous, 17.1.27 *A. marginale* rare, *P. mutans* very rare, 18.1.27 *P. mutans* rare, *A. marginale* most numerous, 27.1.27 *P. mutans* very rare, 2.2.27 *A. marginale* and *P. mutans* rare, 4.2.27 *A. marginale* fairly rare, 8.2.27 *A. marginale* very rare, 16.2.27 *P. mutans* very rare. Number of blood-smears 15, gland 14, spleen 1.

Temperature reaction from 14.12.26 to 3.1.27 with maximum 104.6° F. on 17.12.26 followed by normal temperature up to 10.1.27 when on 11.2.27 temperature was again irregular with maximum 105° F. on 2.2.27—*Anaplasma marginale* infection and probably paratyphoid as agglutination tests performed on 9.2.27 were strongly positive for four strains used in dilutions up to 1:500. Negative for paratyphoid B.

(26) C. 2206, bull, born 20.10.26, observed since 22.10.26. Showed on 28.10.26 *A. centrale* rare, 2.12.26 *P. mutans* very rare, 30.12.26 *A. marginale* most rare, *P. mutans* very rare, 6.1.27 *P. mutans*, *A. marginale* very rare, 7.1.27 *A. marginale* moderately

numerous, *P. mutans* rare, 14.1.27 *P. mutans* numerous, *A. marginale* rare, 18.1.27 *P. mutans* very rare, 27.1.27 *A. marginale* very rare, *P. mutans* not rare, 4.2.27 *P. mutans* rare, 8.2.27 *P. mutans* not numerous, 17.2.27 *A. marginale* and *P. mutans* very rare. Number of blood-smears examined 13, gland 11, spleen 1.

Temperature reaction from 30.12.26 to 12.1.27 very mild with maximum 103° F. *A. marginale* moderately rare. Temperature then normal till 23.1.27 when it began rising again to 105° F. on 26 and 27.1.27, dropping to 102° F. on 30.1.27 and then continuing slightly irregular to 1.3.27 but never over 103° F. On 27.2.27 *A. marginale* very rare. Agglutination tests showed on 9.2.27 positive against *B. enterid.*, Bainbridge and Kaalplaats 2236 up to 1:200 dilution and against Page spleen and Kaalplaats 2217 up to 1:500 dilution. Temperature reaction described may be due to an initial anaplasmosis infection superimposed by paratyphoid infection.

(27) C. 2208, heifer, born 23.10.26, observed since 24.10.26. Showed on 2.12.26 *P. mutans* very rare, 17.12.26 *P. mutans* rare, 18.12.26 *Sp. theileri* very rare, 30.12.26 *A. marginale* moderately numerous, *P. mutans* and *P. bigeminum* very rare, 6.1.27 *P. mutans* fairly rare, plasma bodies in spleen, 7.1.27 *A. marginale* rare, 14.1.27 *A. marginale* and *P. mutans* rare, 18.1.27 *A. marginale* fairly rare, *P. mutans* very rare, 27.1.27 *P. mutans* very rare, 4.2.27 *A. marginale* very rare, *P. mutans* moderately numerous, 8.2.27 *P. mutans* very rare, 17.2.27 *P. mutans* very rare. Number of blood-smears examined 12, gland 12, spleen 2.

Irregular temperature from 25.11.26 to 18.12.26 with maximum 103° F. on 9.12.26. *Sp. theileri* very rare on 18.12.26—spirochaetosis. Another reaction from 22.12.26 to 9.1.27, with maximum 103° F., due to anaplasmosis. A third reaction from 28.1.27 to 10.2.27 with maximum 106° F. on 5.2.27—no smears. Cause uncertain—paratyphoid (?). Agglutination test 9.2.27 positive up to 1:500 dilution for *B. enterid.*, Bainbridge, Kaalplaats 2226 and 2217, up to 1:200 for Page spleen strain—negative for paratyphoid B. *Typical plasma bodies found in spleen on 6.1.27.*

(28) C. 2209, heifer, born 27.10.26, observed since 29.10.26. Showed on 2.12.26 *Sp. theileri* very rare, 17.12.26 *A. marginale* and *P. mutans* very rare, 30.12.26 *A. marginale* very rare, 12.1.27 *P. mutans* very rare, 13.1.27 *P. mutans* rare, 14.1.27 *P. mutans* rare, 15.1.27 *A. marginale* very rare, *P. mutans* rare, 17.1.27 *A. marginale* and *P. mutans* very rare, 19.1.27 *A. marginale* very rare, 4.2.27 *A. marginale* very rare, 17.2.27 *P. mutans* very rare. Number of blood-smears examined 13, gland 12.

Temperature reaction 10.1.27 to 27.1.27 with maximum 106.6° F. on 16.1.27, *A. marginale* doubtful—if present, very rare—cause of reaction doubtful. Agglutination tests 9.2.27 against four strains negative.

(29) C. 2218, bull, born 12.11.26, observed since 19.11.26. Showed on 11.1.27 *A. marginale* numerous, 14.1.27 *A. marginale* very numerous, 15.1.27 *A. marginale* numerous, 23.1.27 *A. marginale* rare, 25.1.27 *A. marginale* very rare, 26.1.27 *A. marginale* moderately numerous, *P. mutans* very rare, 17.2.27 *A. marginale* very rare. Number of blood-smears examined 11, gland 11.

Temperature reaction from 5.1.27 to 24.2.27 with maximum 106° F. on 24.1.27—due to anaplasmosis infection complicated by

paratyphoid or heartwater? Subinoculations into S. 15768 and S. 15796 on 25.1.27 with negative results. Sheep's serum negative—agglutination test 19.2.27. Calf's serum agglutination partially up to 1:100 *B. ent.* Bainbridge, 1:500 Page spleen, and Kaalplaats 2217 and 2226, i.e. positive.

(30) C. 2220, bull, born 17.11.26, observed since 23.11.26. Showed on 2.1.27 *A. marginale* moderately numerous, 14.1.27 *A. marginale* moderately numerous, 15.1.27 *A. marginale* very rare, *P. mutans* very rare, 17.1.27 *A. marginale* and *P. mutans* very rare, 4.2.27 *A. marginale* fairly rare. Number of blood-smears examined 7, gland 6.

Temperature reaction from 6.1.27 to 6.2.27, with maximum 104° F. on 14.1.27 and 15.1.27—anaplasmosis. Agglutination tests 9.2.27 negative.

(31) C. 2221, born 16.11.26, observed since 23.11.26. Showed on 3.12.26 *P. mutans* very rare, few *A. centrale*, 30.12.26 *P. bigeminum* very rare, 12.1.27 *A. marginale* moderately numerous, 13.1.27 *A. marginale* numerous, *P. mutans* very rare, 15.1.27 *A. marginale* numerous, 5.2.27 *A. marginale* rare, 17.2.27 *P. mutans* very rare. Number of blood-smears examined 7, gland 7, spleen 1.

Temperature reaction from 8.1.27 to 17.1.27 and again from 25.1.27 to 8.2.27 with maximum 105° F.—anaplasmosis. Agglutination tests 9.2.27—negative. Subinoculations into S. 15775 and 15778 on 7.2.27 with negative results. Agglutination test negative.

(32) C. 2225, heifer, born 27.11.26, observed since 1.12.26. Showed on 30.12.26 *P. mutans* very rare, 12.1.27 *A. marginale* numerous, 15.1.27 *A. marginale* moderately numerous, 18.1.27 *P. bigeminum* very rare, *A. marginale* rare, 27.1.27 *A. marginale* rare, *P. mutans* fairly rare, 31.1.27 *A. marginale* fairly rare, 3.2.27 *A. marginale* rare, 4.2.27 *A. marginale* rare, 5.2.27 *A. marginale* rare, 7.2.27 *A. marginale* rare, 8.2.27 *A. marginale* very rare, 9.2.27 *A. marginale* very rare, 18.2.27 *A. marginale* moderately numerous. Number of blood-smears examined 14, gland 13, spleen 1.

Temperature reaction from 8.1.27 to 14.2.27 with maximum 106.4° F. on 5.2.27—redwater and anaplasmosis. Agglutinated 9.2.27 slightly up to 1:100—probably negative.

(33) C. 2244, bull, born 24.12.26, observed since 30.12.26. Showed on 18.2.27 *A. marginale* fairly rare. Number of gland-smears examined 5, blood 5.

Temperature reaction 27.1.27 to 10.2.27 with maximum 106° F. on 1.2.27, anaplasmosis. Agglutination tests 9.2.27, negative.

(34) C. 2263, born 14.1.27, observed since 25.1.27. Blood negative. No positive smears. Blood-smears examined 5, gland 5. Agglutination tests 9.2.27 negative.

(35) C. 2297, heifer, born 31.1.27, observed since 1.2.27. Agglutination tests 9.2.27 negative. Blood-smears negative. Blood-smears examined 2, gland 1.

Group II.—Animals which Died during Period of Observation (15).

(1) C. 1933, heifer, born 18.1.26, observed since 5.5.26, died on 8.5.26—probably heartwater. Subinoculated post-mortem blood into S. 12008 with negative results on 10.5.26.

Post-mortem.—Hyperaemia and oedema of lungs, atrophy of spleen, gastro-enteritis, post-mortem changes.

(2) C. 1934, heifer, born 20.1.26, observed since 5.5.26. Died 25.5.26. Temperature rose gradually from 12.5.26 to 106° F. on 20.5.26, then dropped to 105° F. on 22nd.

Post-mortem.—Subepicardial and subendocardial haemorrhages, slight fatty degeneration, abomasitis, slight hyperaemia of intestinal tract. Post-mortem diagnosis heartwater. Smear examination of all organs negative. 20.5.26 blood (oxalated) subinoculated into S. 12473 with negative results. 24.5.26 (at post-mortem) subinoculated into S. 14279 and S. 13754. S. 14279 negative. S. 13754 died of heartwater on 9.6.26. Diagnosis confirmed on post-mortem. Cause of death of C. 1934, heartwater.

(3) 1827, bull, born 10.11.25, observed since 5.5.26. Showed on 6.5.26 *P. mutans* rare, *P. bigeminum* very rare, 20.5.26 *P. mutans* very rare, *P. bigeminum* very rare, 26.5.26 *P. mutans* very rare, 16.6.26 *P. mutans*, 30.6.26 *P. mutans* fairly numerous. No temperature reactions of note except four days before death (31.10.26), temperature suddenly within four days rose to 106° F. Blood and gland 30.10.26 negative. Number of blood-smears examined 14, gland 10.

Post-mortem.—Too decomposed. Cause of death heartwater.

(4) 1875, bull, born 11.12.25, observed since 5.5.26. Showed on 23.6.26 *P. mutans*, 26.6.26 *P. mutans*. Number of blood-smears examined 4, gland 3, spleen 1. Temperature reaction beginning 16.6.26 and rising to 106.4° F. on 22.4.26, then dropping to 102° F. on 26.6.26, on which day it died. 23.6.26 oxalated blood subinoculated S. 9100 with negative results. Sheep serum 9.2.27 negative agglutination.

Post-mortem.—Subendocardial and subepicardial haemorrhage, slight hydropericard, slight acute catarrhal gastro-enteritis, stasis liver and kidney. Diagnosis heartwater.

(5) C. 2217, heifer, born 7.11.26, observed since 12.11.26. Showed on 2.12.26 *A. marginale* rare, 30.12.26 *A. marginale* numerous, 14.1.27 *A. marginale* and *centrale* moderately numerous, *P. mutans* very rare, 25.1.27 *P. mutans* very rare. Number of blood-smears examined 10, gland 8.

Slight temperature reaction from 23.12.26 to 7.1.27 with maximum 104° F. on 30.12.26 corresponding to maximum anaplasma infection. Temperature again rose from normal on 17.1.27 to 107° F. on 24.1.27 and 25.1.27, and then dropped to 103° F. on 28.1.27, and death of calf on 29.1.27. Blood-smears except for *P. mutans* negative during this period. Oxalated blood was subinoculated into S. 15789 and S. 15848 on 25.1.27 with negative results. Serum of sheep gave negative agglutination against Paratyphoid strains.

Post-mortem 29.1.27.—Slight anaemia, slight tumor splenis haemorrhagic foci in spleen, pigmentation and necrotic foci in liver. Cultures were made from spleen, and a pure growth obtained which on testing against sixteen different sugars and carbohydrates, proved to be of paratyphoid group. Microscopically gram negative coliform organisms. Cause of death, anaplasmosis complicated by paratyphoid infection.

(6) C. 2226, bull, born 28.11.26, observed since 1.12.26. Showed on 8.1.27 *A. marginale* numerous, 11.1.27 *A. marginale* moderately numerous, 15.1.27 *P. bigeminum* very rare, *A. marginale* moderately

rare, 20.1.27 *A. marginale* moderately numerous, 27.1.27 *A. marginale* fairly rare, *P. mutans* very rare, 29.1.27 *A. marginale* rare, 30.1.27 *A. marginale* rare. Number of blood-smears examined 9, gland 7, spleen 1, kidney 1, liver 1.

Temperature during December, 1926, irregular, varying between 101° F. to 104° F. Mild temperature from 2.1.27, reaching 104° F. on 7.1.27 and 11.1.27, due to anaplasma infection. Temperature then suddenly rose on 26.1.27 to 106° F., dropped during next two days to 103.8° F., rose again to 106° F., on 29.1.27, death on 30.1.27, *A. marginale* during this time being moderately numerous. At post-mortem, two sheep were inoculated, S. 15830 and S. 15845, on 30.1.27. S. 15830 showed a temperature reaction on third day and going over 107° F.—reaction lasted from 1.2.27 to 5.2.27, hanging drop and blood-smear remaining negative for spirochaetes. S. 15845, second day, after injection temperature 106° F. to 107° F., from where it gradually dropped to normal during the following fourteen days. Cultures made from blood of both sheep into bouillon on 4.2.27 remained negative. Agglutination tests on 13.2.27 with both sheep's serum were strongly positive up to 1:500 for Page spleen and Kaalplaats 2217 strain, also positive but less complete for paratyphoid B. at 1:500. On 26.2.27 agglutination positive up to 1:1000 dilution against Kaalplaats 2217—very slight against paratyphoid B. in 1:200 dilution. On 16.2.27, agglutination test positive in 1:500 dilution, against paratyphoid B. and 1:200 against *B. enterit-gärtner* (Bainbridge) and Kaalplaats 2217. Died 29.1.27.

Post-mortem.—Anaemia, general icterus, fatty degeneration and hyperaemia of liver. Tumor splenis and foreign body (seed of *Heteropogon contortus*), penetrating from reticulum into liver where a small abscess was formed. Spleen cultures gave a pure growth of gram-negative coliform organisms, shown by fermentation and agglutination tests to belong to paratyphoid group. Cause of death, anaplasmosis, complicated by paratyphoid infection. Numerous coliform organisms in liver and spleen smears.

(7) C. 2181, heifer, born 18.9.26, observed since 19.9.26. Showed on 3.12.26 *P. mutans* very rare, *P. bigeminum* very rare, 10.12.26 *P. mutans* very rare, 17.12.26 *P. mutans* rare, 30.12.26 *P. mutans* very rare, 6.1.27 *P. mutans* and *A. marginale* very rare, 10.1.27 *A. marginale* rare, 11.1.27 *P. mutans* very rare, *A. centrale* rare, 12.1.27 *A. marginale* rare. Number of blood-smears examined 11, gland 10.

Temperature reaction from 3.1.27, rising gradually to 106.8° F. on the 11.1.27, then dropping to 105.4° F. on day of death, 13.1.27. *A. marginale* very rare. Blood subinoculated at post-mortem into S. 15510 and S. 15532. S. 15510 temperature rose gradually to 106.4° F. on fourth day (16.1.27), then dropped with marked morning and evening exacerbations to normal after thirteen days—blood and hanging drop negative on 17.1.27. S. 15532—temperature reached 106° F. on fourth and fifth days after injection, and then gradually dropped to normal after eleven days. Blood-smear and hanging drop negative. Agglutination test serum S. 15510 on 26.2.27 was negative for paratyphoid B., positive up to 1:100 against Kaalplaats 2217 strain. S. 15532, 26.2.27, negative for paratyphoid B., positive up to 1:200 against Kaalplaats 2217, is probably positive reactor.

Post-mortem.—Anaemia, slight hydropericard, oedema of lungs, bile stasis and degeneration of liver, petechiae peritoneum and small intestines, echymosis serosa of bladder, slight atelectatic foci right lung, submucous haemorrhage at ilio-caecal valves, slight tumor splenis. Diagnosis paratyphoid and anaplasmosis.

(8) C. 2091, heifer, born 5.5.26, observed since 25.5.26. Showed on 9.6.26 *P. bigeminum* fairly rare, 30.6.26 *A. marginale* fairly rare, *P. mutans* fairly numerous, 25.8.26. *A. marginale* very rare, 23.9.26 *P. bigeminum* very rare, 22.10.26 *P. mutans* rare, 15.12.26 *P. mutans* very rare, 5.1.27 *A. marginale* and *P. mutans* very rare, 6.1.27 *P. mutans* very rare, 7.1.27 *P. mutans* rare. Number of blood-smears examined 18, gland 11.

Slight temperature reaction from 28.5.26 to 8.6.26, maximum 103.2° F. due to *P. bigeminum* infection. Another reaction from 12.6.26 to 14.7.26 due to anaplasmosis with maximum 105.2° F. on 26.6.26. From 11.9.26 to 8.10.26 temperature irregular (*P. bigeminum* very rare) with maximum 105° F. on 6.10.26. On 3.1.27 temperature suddenly rose reaching 104.6° F. on 5.1.27 and 6.1.27, then dropping to 99° F. on morning of 8th January when animal was killed after blood for subinoculation was taken (oxalated). Showed clinical symptoms of heartwater. Subinoculation of oxalated blood on 30.6.26 into S. 14307 and S. 14309 with negative results. Subinoculation again on 7.1.27 (oxalated) blood into S. 15649 and S. 15577—both sheep showing a reaction after third day and lasting seven and four days respectively. Not a heartwater reaction—cause unknown—no spirochaetes found. Agglutination test with serum of S. 15649 and 15577 on 19.2.27 was negative.

Post-mortem.—Anaemia, slight hydropericard, endocardial haemorrhages, degeneration liver, tumor splenis, catarrhal enteritis—abscessation of liver, taeniasis. Post-mortem diagnosis, probably heartwater, though not verified by subinoculation.

(9) 2137, heifer, born on 10.8.26, observed since 15.8.26. Showed on 22.10.26 *P. mutans* rare, 3.12.26 *P. mutans* rare, 4.12.26 *P. mutans* rare, 10.12.26 *P. mutans* very rare, 13.12.26 *P. mutans* fairly numerous. *True plasma bodies in spleen*, 13.12.26.

Showed no definite temperature reactions except irregular temperature reactions from 13.11.26 onwards. *P. mutans* very rare. On 13.12.26 *typical plasma bodies extra and intracellular* were found and on 15.12.26 calf was killed. Post-mortem showed distinct tumor splenis and swelling of subcutaneous lymph glands. 6-12 smears were made of all organs for further examination. Three calves injected intrajugularly on 15.12.26 with spleen pulp (C. 2104, 2119, 1759) and three with blood (C. 2102, C. 1773, C. 2098) (*vide* this report, pages 101 and 102).

(10) C. 2067, bull, born 8.4.26, observed since 5.5.26. Showed on 6.5.26 *P. bigeminum*, 13.5.26 *A. marginale*. Number of blood-smears examined 3, gland 2. Died 15.5.26.

Temperature reaction from beginning of experiment 5.5.26 to date of death (15.5.26) rising to 106.6° F. on 12.5.26 and dropping to 105° F. on 15.5.26.

Post-mortem.—Subendocardial haemorrhages, localized oedema and hyperaemia of lungs, liver enlarged, pigmentation, bile stasis, tumor splenis, hyperaemia kidneys, very acute catarrhal gastro-enteritis. Diagnosis redwater and anaplasmosis [and heartwater?]. Cultures of heart blood, spleen, liver negative, i.e., apparently not paratyphoid.

(11) C. 2075, bull, born 24.4.26, observed since 5.5.26. Showed on 20.5.26 *A. marginale* very rare, 26.5.26 *A. marginale* fairly rare, 28.5.26 *P. bigeminum* fairly rare, *A. marginale* very rare. Number of blood-smears examined 5, gland 1, spleen 1, kidney 1. Temperature reaction from 8.5.26 to 7.6.26 with maximum 105.2° F. on 12.5.26. On 20.5.26 blood (oxalated) injected into S. 12201 with negative result.

Post-mortem.—Kachexia, marked anaemia, redwater and anaplasmosis caused death.

(12) C. 2070, bull, born 12.4.26, observed since 5.5.26. Showed on 6.5.26 *Sp. theileri* very rare, 25.5.26 *P. bigeminum* very rare, 28.5.26 *P. bigeminum* very rare, 8.6.26 *A. marginale* rare, 16.6.26 *A. marginale* rare, 23.6.26 *A. marginale* rare, 30.6.26 *A. marginale* very rare, 11.7.26 *P. mutans* very rare, *A. marginale* very rare, 30.10.26 *P. mutans* rare. Number of blood-smears examined 18, gland 8.

Temperature reaction maximum 104.2° F. during May—spirochaetosis and redwater. Severe temperature reaction from 9.6.26 rising gradually to 106.2° F. on 15.6.26, remaining at about 106° F. till 21.6.26, dropping to 104.2° F. on 23.6.26 and rising again to 106.2° F. on 26.6.26, dropping to 101.4° F. by 30.6.26, rising again to 104.2° F. by 3.7.26, then returning to normal—anaplasmosis. Temperature during following months was irregular but never above 103° F. From 8.10.26 temperature rose to 104° F. on 14.10.26, dropped by 23.10.26 to 98° F., rose again to reach 105° F. on 30.10.26, animal died 31.10.26. Blood-smear, except for few *P. mutans*, was negative. Subinoculated 16.6.26 into S. 10531 and S. 10645 (oxalated blood). In S. 10531 temperature rose to 105° F. by eighth day, returning to normal by twelfth day. S. 10645 from fourth to fifteenth day p.i. temperature was very irregular reaching 106° F. on ninth day p.i. Cause possibly paratyphoid bacteraemia. Serum for agglutination test not available. S. 9574 injected with oxalated blood 23.6.26—gave no temperature reaction.

Post-mortem.—Smear negative for *B. anthracis*. Fibrinous pleurisy, slight tumour splenis, hyperaemia and fatty degeneration liver, oedema of lungs. Cause of death paratyphoid infection with fibrinous pleurisy as sequel.

(13) 2097, bull, born 8.6.26, observed since 10.6.26. Showed on 30.6.26 *A. marginale* very rare, 11.8.26 *A. marginale* fairly numerous, 22.10.26 *P. mutans* rare. Number of blood-smears examined 5, gland 2.

Temperature reaction from 14.8.26 to 21.8.26 with maximum 105° F. on 18.8.26—anaplasmosis. From 9.10.26 temperature irregular, rising gradually to 104.8° F. on 20.10.26 then collapsing to below 98° F. on 23.10.26, blood negative except for very rare *P. mutans*. Subinoculated on 24.10.26, into S. 15468 and S. 15471. No definite reaction, temperature varying 102-104° F., though animal dull and not feeding well on fifteenth to eighteenth day p.i. In S. 15468 temperature for first ten days not over 103° F., then rising within four days to 106.4° F. with dullness and anorexia, dropping to normal (102° F.) in four days. At height of reaction 7.11.26 two sheep subinoculated S. 15148 and S. 15520. In S. 15148 no definite temperature reaction though temperature rose to 105.6° F. on fourth day p.i. and several times reached 104° F. before dropping to normal

(102° F.) on thirteenth day. In S. 15520 also an indefinite irregular temperature resulted with maximum 105.4° F. on ninth day—normal on twelfth day. Serum not available for agglutination tests—cause hence not definitely ascertainable. Heartwater (?) or para-typhoid (?).

Post-mortem.—Cachexia and marked acute catarrhal enteritis.

(14) C. 2229, bull, born on 2.12.26, observed since 6.12.26. Showed on 6.1.27 *A. marginale* very rare, *P. mutans* rare, 8.1.27 ditto, 15.1.27 *A. marginale* rare, 1.2.27 *P. bigeminum* very rare, *A. marginale* rare, 2.2.27 ditto, 3.2.27 *P. mutans* and *A. marginale* very rare, 4.2.27 *A. marginale* rare, 5.2.27 *A. marginale* rare, 16.2.27 *A. marginale* fairly rare. Died 17.2.27. Number of blood-smears examined 11, gland 11, spleen 1, liver 1.

Temperature reaction from 12.12.26 to 15.2.27—irregular fever varying between 102° F. and 104° F. for most part, with 105° F. on 7.12.26, 16.12.26, 31.12.26, then sudden rise to 107° F. on 2.2.27, followed by return to normal on 7.2.27, another slight rise to 104° F. on 12.2.27, followed by death on 17.2.27. Serum of C. 2299 negative agglut. on 9.2.27. Cultures made at post-mortem from gall-bladder (bile) and spleen gave a growth of coliform organisms (and a few putrefactive organisms) suspensions of which agglutinated practically completely with serum S. 15830 in 1:500 and 1:1,000 dilutions, i.e. paratyphoid infection.

Post-mortem.—Anaemia, ecchymosis left endocard, tumor splenis, marked pigmentation of liver.

(15) 2236, heifer, born 20.12.26, observed since 23.12.26. Showed on 15.1.27 *A. marginale* very rare, 13.2.27 *A. marginale* rare, *P. mutans* very rare, very rare coliform organisms liver. Number of blood-smears examined 6, gland 6, spleen 1.

Died 12.2.27. Temperature reaction from 20.1.27 to 8.2.27 irregular with maximum 106° F. on 3.2.27—blood examined negative. Another slight reaction from 29.12.26 to 10.1.27 probably due to anaplasmosis since *A. marginale* found 15.1.27. Very rare coliform organisms found in liver-smears. Agglutination test on 9.2.27 negative. Using organisms grown from cultures of spleen and liver, they were agglutinated by serum of two sheep which strongly agglutinated paratyphoid strains (S. 15830 and S. 15845). Cause of death probably paratyphoid infection. Subinoculated blood on 14.2.27 into S. 8529 and S. 8545 with negative results (up to 27.2.27). Using serum S. 15830 in dilutions 1:500 and 1:1,000 and suspension of coliform gram negative organisms isolated from heart blood of C. 2236, agglutination was complete in both tubes, i.e. organisms of paratyphoid group present.

Post-mortem.—Purulent foci in lungs yellowish red, enlarged liver, slight tumor splenis, catarrhal conditions of bowels.

Group III.—Animals transferred to Onderstepoort and taken out of *P. mutans* Experiments on 22nd January, 1927.

(1) C. 1803, bull, born 19.10.25, observed since 5.5.26. Showed on 2.11.26 *P. mutans* very rare, 6.12.26 *P. mutans* very rare. Number of blood-smears examined 12, gland 6. Castrated 7.1.27. No thermal reaction during observation. Doubtful positive agglutination test 16.2.26.

(2) C. 1894, bull, born 8.1.26, observed since 5.5.26. Showed on 6.5.26 *A. marginale* very rare, 23.6.26 *P. mutans* fairly rare, *P. bigeminum* very rare, *A. marginale* rare, 2.11.26 *P. mutans* very rare, 7.12.26 *P. mutans* very rare. Number of blood-smears examined 16, gland 10. No temperature reaction of note. Doubtful positive agglutination test 16.2.26.

(3) C. 1944, bull, born 6.2.26, observed since 5.5.26. Showed on 6.5.26 *P. bigeminum* fairly rare, *P. mutans* very rare, 13.5.26 *P. bigeminum* and *A. marginale* very rare, 20.5.26 *P. mutans* comparatively fairly numerous, *A. marginale* rare, 26.5.26 *P. mutans* fairly rare, *A. marginale* very rare, 28.5.26 *A. marginale* rare, 9.6.26 *A. marginale* rare, 16.6.26 *P. mutans* fairly rare, *P. bigeminum* very rare, *A. marginale*, 23.6.26 and 30.6.26 *P. mutans* very rare, *A. marginale* rare, 1.11.26 *P. mutans* rare, 7.12.26 *P. mutans* very rare, 14.12.26 *P. mutans* very rare, 4.2.27 *P. mutans* very rare.

Temperature reaction during May, 1926, with maximum 106° F. on 11 and 12.5.26 due to anaplasmosis and redwater. Slight reaction from 9.8.26 to 23.8.26 maximum 104° F. on 11 and 18.8.26 with blood negative. Number of blood-smears examined 25, gland 15. Agglutination test 16.2.27 doubtful positive. Castrated 7.1.27. Diarrhoea and slight swelling of subcutaneous glands 4.5.26.

(4) C. 2054, bull, born 17.3.26, observed since 5.5.26. Showed on 6.5.26 *P. mutans* rare, *A. marginale* fairly rare, *P. bigeminum* very rare, 13.5.26 *P. mutans* rare, 20.5.26 *P. mutans* fairly rare, *A. marginale* rare, 26.5.26 *A. marginale* fairly numerous, *P. mutans* very rare, 28.5.26 *A. marginale* moderately numerous, 2.6.26 *A. marginale* fairly rare, *P. bigeminum* rare, 16.6.26 *P. bigeminum* very rare, *P. mutans* numerous, 26.6.26 *P. mutans* comparatively numerous, 8.7.26 *P. mutans* very rare, 30.6.26 *A. marginale* very rare, 2.11.26 *P. mutans* very rare, 4.1.27 *P. mutans* very rare. Number of blood-smears examined 24, gland 16, spleen 2.

Mild temperature reaction May-June, 1926. Anaplasmosis and redwater. 20.5.26 marked diarrhoea (23.6.26) oxalated blood injected into S. 10864 with negative results. 7.1.27 castrated. Discharged 23.1.27.

(5) 2066, bull, born 3.4.26, observed since 5.5.26. Showed on 6.5.26 *P. bigeminum* very rare, 28.5.26 *A. marginale* fairly numerous, 2.6.26 *A. marginale* moderately numerous, 16.6.26 *A. marginale* fairly rare, *P. bigeminum* very rare, 23.6.26 *P. mutans* numerous, *P. bigeminum* very rare, 30.6.26 *P. mutans* numerous, 8.7.26 *A. marginale* fairly rare, *P. mutans* rare 11.8.26 *P. mutans* very rare, 14.8.26 *P. mutans* very rare, 18.8.26 *P. mutans* very rare, 7.12.26 *P. mutans* very rare, 28.12.26 *P. mutans* very rare, 4.1.27 *P. mutans* very rare, 8.1.27 *P. mutans* very rare, 19.1.27 *P. mutans* very rare. Number of blood-smears examined 30, gland 18, spleen 1.

Irregular temperature May-June with maximum 105° F. on 15.6.26 due to anaplasmosis and redwater. Mild temperature reaction 4.8.26 to 28.8.26 with maximum 105° F. on 11.8.26. Subinoculated oxalated blood into S. 8435 and 9567 on 16.6.26 with negative results. Castrated 7.1.27.

(6) 2096, bull, born 7.6.26, observed since 12.6.26. Showed on 16.6.26 *A. marginale* very rare, 30.6.26 *A. marginale* rare, 11.8.26 *P. mutans* fairly rare, *A. marginale* fairly numerous, *P. bigeminum*

very rare, 18.8.26 *A. marginale* rare, 25.8.26 *P. mutans* rare, 1.9.26 *P. mutans* very rare, 8.9.26 *P. mutans* very rare, 1.11.26 *P. mutans* rare, 1.12.26 *P. mutans* very rare, 9.12.26 *P. mutans* very rare, 29.12.26 *P. mutans* fairly rare, 8.1.27 *P. mutans* rare, 12.1.27 *P. mutans* rare, 19.1.27 *P. mutans* very rare. Number of blood-smears examined 17, gland 10, spleen 1. Slight temperature reaction during August—anaplasmosis and redwater. Two sheep subinoculated 18.8.26 S. 6642 and S. 2799—both remained negative. 29.12.26 a typical plasma body found in gland-smear. Castrated 7.1.27.

SUMMARY OF GROUPS.

Group I.

Number of animals in experiment	35
Number of blood-smears examined	487
Number of gland-smears examined	345
Number of spleen-smears examined	16
Plasma bodies found in C. 2208 and C. 2063 of Group I.	
Plasma bodies found in C. 2137 of Group II.	
C. 2208 Spleen 6/1/27 2 months 13 days	Age when Plasma bodies found.
C. 2063 Gland 28/5/26 3 months 4 days	
C. 2137 Spleen 13/12/26 3 months 3 days	
C. 2096 Gland 29/12/26 6 months 21 days	
Plasma bodies found in C. 2096 of Group III.	
<i>Piroplasma mutans</i> established in	32 calves.
<i>Anaplasmosis marginale</i> established in	17 calves.
Redwater (<i>P. bigeminum</i>) established in	12 calves.
Spirochaetosis (<i>Sp. theileri</i>) established in	1 calf.
Heartwater established in	3 calves.
Paratyphoid infection (positive agglutination) established in	8 calves.
Paratyphoid infection (doubtful positive) established in	7 calves.

Group II.

Number of animals	15
Number of blood-smears examined	119
Number of gland-smears examined	83
Number of spleen-smears examined	11
Plasma bodies found in C. 2137.	
<i>Piroplasma mutans</i> established in	11 calves.
<i>Anaplasma marginale</i> established in	9 calves.
<i>P. bigeminum</i> established in	5 calves.
Spirochaetosis established in	1 calf.
Heartwater (1 only by subinoculation) established in	5 calves.
Paratyphoid infection (4 strongly positive) established in	5 calves.

Group III.

Animals transferred to Onderstepoort, i.e. taken out of experiments.	
Number of animals	6
Number of blood-smears examined	124
Number of gland-smears examined	75
Number of spleen-smears examined	4
Plasma body 29/12/26 in C. 2096.	
<i>Piroplasma mutans</i> established in	6 calves.
<i>Anaplasma marginale</i> established in	5 calves.
<i>Piroplasma bigeminum</i> established in	5 calves.
<i>Spirochaeta theileri</i> established in	0 calves.
Heartwater established in	0 calves.
Paratyphoid infection (agglutination tests positive) established in	0 calves.
Paratyphoid infection (agglutination tests doubtful positive) established in	3 calves.

Summary of all three Groups.

Number of animals used	56 calves.
Total number of blood-smears examined	730
Total number of gland-smears examined	503
Total number of spleen-smears examined	31

Number of animals in which Plasma Bodies found—4.

C. 2208 spleen 6/1/27.	
C. 2096 gland 29/12/26.	
C. 2137 spleen 13/12/26.	
C. 2063 28/5/26.	
<i>Piroplasma mutans</i> established in	49 calves.
Anaplasmosis established in	31 calves.
Redwater established in	22 calves.
<i>Spirochaeta theileri</i> established in	2 calves.
Heartwater (only 1 certain by subinoculation) established in	8 calves.
Paratyphoid infection (agglutination and P.M. positive) established in	12 calves.
Paratyphoid infection (agglutination and P.M.) (doubtful positive) established in	11 calves.

DETAILS OF AGGLUTINATION TESTS OF SERUM OF CALVES AND SHEEP USED FOR SUBINOCULATIONS.

NOTE.—Strains of paratyphoid organisms used:—

- (1) Page spleen strain was isolated by Martinaglia from the spleen of a calf which had died from paratyphoid infection in Marico in 1923.
- (2) *B. enteritidis gärtner* (Bainbridge), obtained from the Medical Research Institute, Johannesburg, in 1926.
- (3) Paratyphosus B., obtained from the Lister Institute in 1923.
- (4) Kaalplaats 2217, isolated from spleen of C. 2217 on 29.1.27.
- (5) Kaalplaats 2226, isolated from spleen of C. 2226 on 30.1.27.

Rapid agglutination test:—

A twenty-four-hour-old agar surface culture of the Kaalplaats 2217 strain was washed off with a few cubic centimetres of a 12 per cent. sodium chloride (C.P.) and 0.5 per cent. phenol solution, filtered through filter-paper and standardized to a density of 4 mm. of the Gates nephelometer, PH being adjusted to 6.8. Quantities of 0.08, 0.04, 0.02, 0.01, and 0.005 c.c. of the sera to be tested were put out on a glass plate ruled into inch squares. To each of these amounts of sera 0.04 c.c. of the standardized suspension of paratyphoid organisms was added and then mixed with the serum by means of stirring with a platinum wire. The plate was then passed rapidly over a flame to bring the temperature up to slightly more than blood-heat. In strong positive cases flocculation occurs immediately, but in doubtful cases only after about five minutes' interval. No flocculation occurs with negatively reacting sera. The flocculation is very easily observed by reflected light over a dark surface.

Reference to the method:—

- (1) I. F. Huddleson and E. R. Carlson, J.A.V.M. Association, Vol. 23, No. 2, November, 1926.
- (2) Vet. Record, No. 4, Vol. VII, p. 88, 22nd January, 1927.

Date of Test, 9/2/27.

Calf No.	Strain. <i>Paratyphosus B.</i>					Strain. <i>B. enteritidis</i> (Bainbridge).					Strain. Page Spleen (Marico strain).					Strain. Calf 2226 (Kaalplaats).					Strain. Calf 2217 (Kaalplaats).					Result.	
	1 10	1 50	1 100	1 200	1 500	1 10	1 50	1 100	1 200	1 500	1 10	1 50	1 100	1 200	1 500	1 10	1 50	1 100	1 200	1 500	1 10	1 50	1 100	1 200	1 500		
DOB 2206.....	++	++	0	0	0	++	++	++	++	0	++	++	++	++	++	++	++	++	++	++	0	++	++	++	++	++	Positive.
DOB 2229.....	++	0	0	0	0	+	0	0	0	0	+	++	0	0	0	+	0	0	0	0	0	++	0	0	0	0	Negative.
DOB 2263.....	++	0	0	0	0	+	0	0	0	0	+	0	0	0	0	++	0	0	0	0	0	+	0	0	0	0	Negative.
DOB 2170.....	+	++	0	0	0	++	+	0	0	0	++	++	0	0	0	++	++	0	0	0	0	++	+	0	0	0	Negative.
DOB 2244.....	+	0	0	0	0	++	0	0	0	0	++	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0	Negative.
DOB 2297.....	++	++	0	0	0	++	+	0	0	0	+	0	0	0	0	+	0	0	0	0	0	++	++	0	0	0	Negative.
DOB 2202.....	++	0	0	0	0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Positive.	
DOB 2221.....	++	0	0	0	0	+	0	0	0	0	++	0	0	0	0	+	0	0	0	0	0	++	0	0	0	0	Negative.
DOB 2236.....	++	0	0	0	0	+	0	0	0	0	+	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0	Negative.
DOB 2169.....	++	0	0	0	0	++	++	0	0	0	++	0	0	0	0	++	++	0	0	0	0	++	+	0	0	0	Negative.
DOB 2168.....	+	0	0	0	0	++	++	++	++	+	++	++	++	+	0	++	++	+	0	0	0	++	++	+	0	0	Positive?
DOB 2209.....	+	++	0	0	0	++	++	0	0	0	++	+	++	0	0	++	++	0	0	0	0	++	++	0	0	0	Negative.
DOB 2194.....	++	+	0	0	0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Positive.	
DOB 2184.....	+	++	0	0	0	++	+	0	0	0	++	+	0	0	0	++	+	0	0	0	0	++	+	0	0	0	Negative.
DOB 2218.....	+	++	0	0	0	++	+	++	0	0	++	++	++	++	+	++	++	++	++	++	++	++	++	++	+	Positive.	
DOB 2220.....	++	0	0	0	0	++	++	0	0	0	++	++	+	0	0	++	++	+	0	0	0	++	++	+	0	0	Positive?
DOB 2138.....	+	0	0	0	0	++	++	+	0	0	++	++	+	0	0	++	++	+	0	0	0	++	+	0	0	0	Positive?
DOB 2208.....	++	0	0	0	0	++	++	++	+	++	++	+	++	0	0	++	++	++	++	++	++	+	++	++	++	Positive.	
DOB 2225.....	+	0	0	0	0	++	++	++	++	0	++	+	0	0	0	++	++	++	0	0	0	++	++	++	0	0	Positive?
DOB 2195.....	++	0	0	0	0	++	++	0	0	0	++	++	0	0	0	++	+	0	0	0	0	++	++	++	0	0	Positive?
Control suspensions	Negative					Negative					Negative					Negative											

Symbols.—Complete agglutination = ++, decreasing +++, +, ++, +?, to negative = 0.

Date of Test, 12.2.27.

Calf No.	Strain. Parat. B.					Strain. Kaalplaats 2217.					Strain. Page Spleen (Marico).					Result.
	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	
D0B 2093.....	++	+	0	0	0	++	++	++	+	0	++	++	++	+	0	Positive.
D0B 2120.....	+	+	0	0	0	++	++	+	0	0	++	++	+	0	0	Positive?
D0B 2092.....	++	+	0	0	0	++	++	++?	+	+	++	++	++?	+	0	Positive.
D0B 2105.....	++	+	0	0	0	++	++	++	++	0	++	++	++	++	+	Positive.
D0B 1697.....	++	+	0	0	0	++	++	++	++	+	++	++	++	++	+	Positive.
D0B 2063.....	++	+	0	0	0	++	++	++	++?	+	++	++	++	++?	++?	Positive.
D0B 2072.....	+	0	0	0	0	++	++	+	0	0	++	+	+	0	0	Positive?
D0B 2095.....	++	+	0	0	0	++	++	+	0	0	++	++	+	0	0	Positive?
Control suspensions.....	Negative					Negative					Negative					

Symbols.—Complete agglutination = ++, decreasing ++?, +, +?, +??, to negative = 0.

Date of Test, 13.2.27.

Calf No.	Strain. Parat. B.					Strain. Page Spleen (Marico strain).					Strain. Kaalplaats 2217.					Result.
	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	
D0B 1945.....	+	0	0	0	0	++	++	++	++	0	++	++	++	+	0	Positive.
D0B 1863.....	++	++?	0	0	0	++	++	++	+	0	++	++	++	++	++?	Positive.
D0B 1701.....	++	0	0	0	0	++	++	++?	0	0	++	++	++?	++?	++?	Positive?
D0B 1800.....	+	++?	0	0	0	++	+	++?	0	0	++	++?	++?	0	0	Positive?
D0B 1690.....	++	++?	0	0	0	++	++	++	+	0	++	++	+	++?	0	Positive.
D0B 1802.....	++	++?	0	0	0	++	++	++	+	0	++	++	++?	+	0	Positive.
D0B 1892.....	++	++?	0	0	0	++	++	+	++?	0	++	++	++?	++?	0	Positive?
D0B 1804.....	++	++?	0	0	0	++	++	++	++	0	++	++	++	++?	++?	Positive.
D0B 1801.....	++	0	0	0	0	++	+	0	0	0	++	+	++?	0	0	Negative.
Sheep No.																
Sheep 15830.....	++	++	+	+	++?	++	++	++	++	++	++	++	++	++	++	Positive.
Sheep 15845.....	++	++	++	++	++?	++	++	++	++	++	++	++	++	++	++	Positive.
Control suspensions.....	Negative					Negative					Negative					

Symbols.—Complete agglutination = ++, decreasing ++?, +, ++?, +??. to negative = 0.

-Date of Test, 16.2.27.

Calf No.	Strain. <i>Parat. B.</i>					Strain. <i>B. ent. gärt. (Bain.).</i>					Strain. Kaalplaats 2217.					Result.
	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{10}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	
D0B 2066.....	++	++	+?	0	0	++	++	++	+	0	++	++	++	++	+	Positive.
D0B 1894.....	+	+?	0	0	0	+++?	+?	+?	0	0	++	++	++	+	0	Positive?
D0B 2236.....	++	+?	0	0	0	++	++	+++?	+?	+	++	++	++	++	+++?	Positive.*
D0B 2054.....	++	+?	0	0	0	++	+++?	+	0	0	++	++	+	0	0	Positive?
D0B 1803.....	++	+?	0	0	0	++	+++?	+?	+++?	0	++	++	+++?	+++?	0	Positive?
D0B 1944.....	++	0	0	0	0	++	++	+	+?	0	++	++	+++?	0	0	Positive?
D0B 2096.....	++	0	0	0	0	++	+?	0	0	0	++	++	0	0	0	Negative.
	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	
D0B 1697.....	++	+	0	0	0	++	++	+	0	0	++	++	+++?	0	0	Positive.
Sheep No.																
Sheep 15830.....	++	++	+++?	+?	0	++	++	++	++	++	++	++	++	++	+++?	Positive.
Sheep 15845.....	++	++	+?	0	0	++	++	++	++	++	++	++	++	++	++	Positive.
Sheep 8545.....	0	0	0	0	0	++	++	0	0	0	++	++	0	0	0	Negative control.
Sheep 5529.....	0	0	0	0	0	+	0	0	0	0	+++?	0	0	0	0	Negative control.
Suspension controls.....	Negative					Negative					Negative					

* Calf 2236 tested 9.2.26, with definite negative result; died on 15.2.27, and paratyphoid organisms isolated from heart-blood and spleen.
 Symbols.—Complete agglutination = ++, decreasing +++?, +, +?, +++?, to negative = 0.

Date of Test, 19.2.27.

Sheep No.	Strain. Parat. B.					Strain. Kaalplaats 2217.					Result.
	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{1000}$	
D0B 15821.....	0	0	0	0	0	++	++?	0	0	0	Negative.
D0B 15769.....	0	0	0	0	0	++?	++?	0	0	0	Negative.
D0B 15848.....	0	0	0	0	0	++?	++?	0	0	0	Negative.
D0B 15789.....	0	0	0	0	0	++?	0	0	0	0	Negative.
D0B 15820.....	0	0	0	0	0	++	++?	+	0	0	Negative?
D0B 15795.....	0	0	0	0	0	++?	+	0	0	0	Negative.
D0B 9100.....	0	0	0	0	0	++?	++?	0	0	0	Negative.
D0B 15796.....	0	0	0	0	0	++	++?	++?	++?	0	Probably negative.
D0B 15649.....	0	0	0	0	0	0	0	0	0	0	Negative.
D0B 15768.....	0	0	0	0	0	++?	+	0	0	0	Negative.
D0B 17405.....	0	0	0	0	0	0	0	0	0	0	Negative.
D0B 15577.....	0	0	0	0	0	+	0	0	0	0	Negative.
D0B 15814.....	0	0	0	0	0	+	+	0	0	0	Negative.
D0B 15786.....	0	0	0	0	0	++?	++?	0	0	0	Negative.
Positive control 15830...	++	++	++	+	0	++	++	++	++	++	Positive.
Suspension control.....	Negative					Negative					

Agar slope culture from bile of C. 2229—agglutinated completely with serum of S. 15830 in dilutions 1:100, 1:500, 1:1000, i.e. paratyphoid.
 Symbols.—Complete agglutination = ++, decreasing ++?, +, ++?, +??, to negative = 0.

Date of Test, 26.2.27.

Sheep No.	Strain. Parat. B.					Strain. Kaalplaats 2217.					Strain. Kaalplaats 2217. Rapid Agglut. Method.					Result.
	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{25}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{500}$	
Sheep 8545.....	0	0	0	0	0	+	+	+	0	0	+	+	+	0	0	Positive?
Sheep 15775.....	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	Negative.
Sheep 8529.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Negative..
Sheep 15778.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Negative.
Sheep 15510.....	0	0	0	0	0	++	++	0	0	0	+	+	+	+	0	Positive? (probably positive).
Sheep 15532.....	0	0	0	0	0	++	++	+	0	0	+	+	+	+	0	Positive?
Sheep 15830.....	++	+	+	0	0	++	++	++	++	++	+	+	+	+	+	Positive.
Sheep 15845.....	+	0	0	0	0	++	++	++	++	++	+	+	+	+	+	Positive.
Suspension control.....	Negative					Negative					Negative					

Symbols.—Complete agglutination = ++ decreasing ++?, +, +?, +??, to negative = 0

Repeat Test to determine Highest Dilution in which S. 15845 and S. 15830 will agglutinate paratyphoid organisms.

Sheep No.	$\frac{1}{100}$	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{5000}$	$\frac{1}{10000}$	$\frac{1}{20000}$	
15845	++	++	++	++	++	++	0	Kaalplaats 2217.
15830	++	++	++	++	++	+	0	Kaalplaats 2217.
15845	++	++	++	++	+	+	0	<i>B. ent. gärt.</i> (Bain.).
15830	++	++	++	++	+	+	0	<i>B. ent. gärt.</i> (Bain.).

No agglutination occurred in testing out fourteen coliform cols. isolated from faeces of C. 1697 and C. 2194 (seven cols. from each), using serum of S. 15830 in dilution of 1:500 and 1:1000, i.e. no paratyphoid organisms found in faeces examined.

Using serum S. 15830 in dilutions of 1:500 and 1:1000 and suspension of coliform col. isolated from heart-blood of C. 2236 (taken from post-mortem), agglutination was complete in both tubes, i.e. paratyphoid infection established.

Seventeen cols. of coliforms isolated from a plate made from washed agglutinated organisms from an agar slope made from faeces of C. 2194 and C. 1697, when tested with serum of S. 15830 in dilutions of 1:500 and 1:1000, gave negative results in each case. As controls, pure cultures from heart-blood of C. 2236 reacted completely, and a mixed culture from bile of C. 2229 almost completely (23.2.27).

Acknowledgment.—All the above agglutination tests and cultures were made by Mr. D. A. Lawrence, to whom we wish to gratefully express our thanks for his aid.

Discussion of Results.—The conditions observed in this experiment coincide with those reported to obtain in the Marico District, viz., an infection with a number of tick-borne diseases and complicated with paratyphoid. It is under such conditions that in the Marico District the plasma bodies were found, and under similar conditions in a different locality we have shown the presence of plasma bodies in spleen and glands of calves that showed *P. mutans* in the blood. We have, therefore, no doubt to identify the plasma bodies with the presence of *P. mutans*, in whose cycle of evolution it forms a stage, as do the representatives of the genus *Theileria*. These plasma bodies were, however, very rare in the experiments undertaken by us. They were also rare in the cases of Marico, with one exception, in which they were fairly numerous. We have no hesitation to interpret the findings in Marico in the same way as we interpreted those in Kaalplaats, viz., that the plasma bodies are stages of *P. mutans*. We conclude from the observation in Marico that, notwithstanding the rare occurrence which is the general rule, there may occur in the evolution of *P. mutans* a still rarer and occasional paroxysm of plasma bodies formations as have been seen in *T. annulata*.

III.—INJECTION OF BLOOD AND OF SPLEEN PULPA OF A CALF IN WHOSE BLOOD *P. mutans* AND IN WHOSE SPLEEN PLASMA BODIES WERE SEEN, INTO SUSCEPTIBLE CALVES.

A.—Injection of Spleen Pulpa.

Calf 2137, heifer, born on 10.8.26, Kaalplaats. 13.12.26 showed rare plasma bodies in the spleen and was killed 15.12.26. The spleen

was emulsified and 20 c.c. were injected into three calves; at the same time other calves received 50 c.c. blood. The spleen of C. 2137 showed a slight tumor ($23 \times 11 \times 3\frac{1}{2}$ cm.) and the mesenteric lymph glands were markedly swollen.

Microscopical examination of a spleen smear showed besides the *scanty plasma bodies*, also *P. bigeminum* in rare numbers.

(1) 15.12.26: Calf 1759, bull, born on 30.1.25 at Armoedsvlakte, injected 20 c.c. spleen pulp.

There was a fever reaction six days later to 105° F., lasting twenty-four hours, and *P. bigeminum* was seen during and after this period. Subsequently *P. bigeminum*, *P. mutans*, and *A. marginale* appeared at the same time (3.1.27), and the temperature rose to a paroxysm of 105.8° F. and returned slowly to normal within the following fortnight. This fever was caused by *A. marginale*, which was present at one time in fair numbers and the blood showed marked anaemic lesions. During all this period, and after the return of the temperature to normal, *P. mutans* was registered in the blood at regular intervals of two or three days. Gland and spleen smears were examined, but constantly with negative results.

(2) 15.12.26: Calf 2104, bull, born on 26.6.26 on station, injected as indicated above.

There was a fever from the fourth to the ninth day with a paroxysm of 107° F. on the sixth day, and during this time *P. bigeminum* was seen in the blood. *P. mutans* appeared on the 3.2.27, and subsequently there was a reappearance of *P. bigeminum*. Also, *A. marginale* appeared, but was never in great numbers. There was but a slight fever reaction. Anaemic blood changes were moderately marked. *P. mutans* was present during the whole period, but never in great numbers. Repeated examination of glands and spleen were made; but plasma bodies were never found.

(3) 15.12.26: Calf 2119, heifer, born on 13.7.26 on station, injected as described above. There was a very slight febrile condition during which an individual *P. bigeminum* was seen. *A. marginale* appeared on 3.1.27 and *P. mutans* on the 6.1.27. *A. marginale* increased rapidly and reached its maximum on the 10.1.27, coinciding with a fever paroxysm of 107.8° F. on the same date. Subsequently *A. marginale* subsided and marked anaemic blood changes appeared. Also, *P. bigeminum* reappeared. There was a febrile condition present all the time with occasional fever to 104° F. at times. *P. mutans* was constantly present in the blood, although in rare numbers. Examinations of spleen and gland smears were carried out at regular intervals, all with negative results.

Discussion of the Results.—The injection of fresh spleen pulp that contained the plasma bodies gave rise to the appearance of *P. mutans* in the blood in all three cases. It does not follow of necessity that the plasma bodies are responsible for their appearance, but the evidence may be taken to prove that another disease due to niroplasms, in whose development plasma bodies are seen, can with all likelihood be excluded, and that accordingly we were only dealing with a *P. mutans* infection. As could be expected also, other blood infections such as *Piroplasma bigeminum* and *Anaplasma marginale* were transmitted, which of course influenced the course of the reaction to some degree. It is of importance to point out that in the course of gland and spleen examinations no plasma bodies were found. They may

have been there and may have been missed, but should this have been the case, the interpretation would nevertheless be justified that they were exceedingly rare.

B. Injection of Blood.

(4) 15.12.26, Calf 1773, bull, born on 14.2.25 at Armoedsvlakte, injected intrajugularly 50 c.c. blood of calf 2137.

There was never any febrile reaction and examination of blood-smears at all times were negative. The calf was discharged from observation on 24.2.27.

(5) 15.12.26. Calf 2098, bull, born on 9.6.26 on station, injected as above. There were but very mild febrile disturbances during the first week and similarly very mild ones subsequently during which *A. marginale* was present in scanty numbers. These were also slight anaemic blood changes. There were never seen any *P. mutans*. The calf was discharged 24.2.27.

(6) Calf 2102, heifer, 15.12.26, born on 21.6.26 on station, was injected as above.

There was a febrile disturbance culminating on the 6th day to a temperature of 105° F. Blood examination during this time gave negative results. There was a marked fever from the 24th to the 30.1.27 with evening exacerbations to 107° F. on the 26th and 27th, a typical fever of an infectious disease. Glands and blood-smears taken during this period showed no parasites, likewise the examination up to that period gave negative results. This fever could not be explained. *P. mutans* was not seen during the time the calf was under observation. It was discharged on 24.2.27.

Discussion of Results.—The results were negative as far as *P. mutans* was concerned. In one case *A. marginale* was noted and *P. bigeminum*. These results are unusual and unsuspected and can only be explained on the assumption that a technical error was made by defibrinating the blood with a solution of oxalate that contained a large amount of glycerine and so destroyed the less resistant *P. mutans*.

IV. EXAMINATION OF GLANDS OF SUSCEPTIBLE YOUNG OXEN, BRED IN ARMOEDSVLAKTE, THAT WERE EXPOSED TO NATURAL TICK INFESTATION FOR A PERIOD OF TEN DAYS AND THEN STABLED. THE BLOOD AND GLANDS WERE EXAMINED AT INTERVALS AND THE LATTER IRRESPECTIVE OF THE PRESENCE OF *P. mutans* IN THE BLOOD. THE ANIMALS WERE KEPT FOR TWO MONTHS UNDER OBSERVATION.

(1) Ox 1076. A fever reaction at the conclusion of which *P. bigeminum* appeared. Subsequently it was again seen. No *P. mutans* was noted. Examination of glands constantly negative.

(2) Ox 1077. Fever reaction. *Spirochaeta theileri*, *P. bigeminum*, and *Anaplasma marginale*. Lesions of anaemia. No *P. mutans* was noted. Examination of gland-smears constantly negative.

(3) Ox 1078. Fever reactions with *P. bigeminum* and *P. mutans* and *Spirochaeta theileri*. Slight lesions of anaemia. Examination of gland-smear negative.

(4) Ox 1079. Fever reaction without any parasites in the blood but distinct anaemic changes. Examination of gland-smears negative.

(5) Ox 1080. Marked fever reaction with *Spirochaeta theileri* and slight anaemic changes. No other parasites. Examination of glands negative.

(6) Ox 1081. Slight fever reaction with *P. bigeminum* on one occasion. Slight anaemic changes. No other parasites. Examination of gland-smears negative.

(7) Ox 1082. Slight fever reaction with *P. bigeminum* and *P. mutans*. Slight anaemic changes. Gland examination negative.

(8) Ox 1083. Slight fever reaction with *P. mutans* and very slight anaemic changes. Examination of glands negative.

(9) Ox 1084. Marked fever reactions with *P. bigeminum* and *P. mutans* fairly numerous. Slight anaemic changes. Examination of glands negative.

(10) Ox 1085. Slight fever reaction with *P. mutans* and subsequently *P. bigeminum*. Marked anaemic changes. Gland examination negative.

(11) Ox 1086. Marked and continuous fever reactions with paroxysm to 107° F. due to *P. bigeminum* and *P. mutans* appearing at the same time. Marked anaemic changes with normoblasts. Reappearance of *P. bigeminum* subsequently and presence of *Spirochaeta theileri*. Gland-smears constantly negative.

(12) Ox 1088. Slight fever reaction and *P. mutans*. Subsequently *P. bigeminum*. Gland examination negative.

(13) Ox 1091. Marked fever reaction with *P. mutans* and *Spirochaeta theileri*, subsequently *P. bigeminum*. Slight anaemic changes. Examination of glands negative.

Discussion of Results.—The oxen were about 18 months old, they were reared on the farm Armoedsvlakte, District Vryburg, Bechuanaland, a country with low rainfall and consequently little tick life. Cattle born in these regions are known to be susceptible for all tick-borne diseases. The experiment has shown this to be the case. In addition to these thirteen oxen, there were even more in the experiment that contracted one or other of *P. bigeminum* or *P. mutans* or *Spirochaeta theileri* infection but whose glands were not examined. Some of the fever reactions were also due to the presence of heartwater as a subinoculation at the death of one of the oxen showed.

These oxen thus showed a multiple infection of blood parasites, all tending to produce anaemic changes, which were diagnosed in most animals. The conditions thus resembled those that were found in the Marico District in animals in whose spleens plasma bodies had been found, except that in Marico, only young calves were infected.

The examination of gland-smears, which were not carried out constantly but at intervals of several days, in no instance revealed the presence of plasma bodies.

The net result of this experience thus indicates that if plasma bodies were present in the glands of those oxen that showed *P. mutans* in the blood, they must have been so rare that they escaped detection.

V.—TO NOTE WHETHER AFTER THE REMOVAL OF THE SPLEEN A RELAPSE OF *P. mutans* WILL OCCUR AND WHETHER DURING THIS RELAPSE PLASMA BODIES CAN BE TRACED IN BLOOD AND GLANDS.*

(1) 15.12.24. Calf 893, heifer, born on 2.3.24 on station. It was injected with blood of vaccine heifer 728 that contained *A. centrale* and *P. mutans*, but it only showed *A. centrale*. The animal was

* The operation was carried successfully out by Quinlan of this Station, and we wish to record our thanks to him.

exposed to tick infection. The calf was splenectomised on the 15.12.24 at a time when *P. mutans* was still in the blood in rare numbers. There was a reappearance of *P. bigeminum* on the third day after the operation. *P. mutans* appeared in increased numbers. There was also a slight fever reaction beginning on the fourth day. *P. bigeminum* disappeared temporarily. *P. mutans* was increasing in numbers and remained in considerable numbers over a period of eight weeks, when it decreased in numbers. One month after the operation, *P. bigeminum* reappeared. Also *A. marginale* appeared. The calf showed most of the time a febrile disturbance, but the temperature paroxysms were never high. There were also the changes of acute anaemia, about eighteen days after the operation fairly marked with basophilia coinciding with the appearance of the anaplasma.

During a period of eight weeks, the glands were examined, at times every day, but no plasma bodies were ever seen.

(2) 24.12.24. Calf 1027, bull, born at Schoombie, 1 year old. It was injected with blood of a bovine 755 which contained *P. bigeminum* and *P. mutans*, which both appeared in the injected animal. The spleen was removed on the 24.12.24. Three days later *P. bigeminum* appeared in great numbers and the temperature rose to 104.6° F. and it was thought necessary to inject trypan blue, whereupon *P. bigeminum* disappeared. *P. mutans* also made its appearance on the same day and also in fairly large numbers. It increased the following day and remained in the blood constantly in fairly large numbers. *A. marginale* appeared on the 2.1.25 and remained for some time although in rare numbers. On the 10.1.25 *P. bigeminum* appeared and again in considerable numbers, so that a second injection of trypan blue was considered necessary, when 24 hours later, it disappeared. The calf showed for the first four weeks a temperature disturbance, the paroxysms reaching 105° F. with the second appearance of the *P. bigeminum*. Lesions of an acute anaemia with marked basophilia were present, mostly marked a few days after the second appearance of *P. bigeminum*. The glands were examined from the third day after the operation and then regularly every second day and frequently every day until the parasites began to disappear, viz. over a period of six weeks. At no time were plasma bodies seen.

(3) Calf 1034, bull, born at Schoombie, 1 year old. This calf was injected 31.10.24 with blood of bovine 424, which was a carrier of *P. bigeminum* and *P. mutans*. Within the first week, *P. bigeminum* appeared and *A. marginale* coinciding with a rise of temperature. About four weeks later *P. mutans* made its appearance and in the fifth week *A. marginale*. During this period there was a febrile disturbance with moderate exacerbations not exceeding 104° F. The animal recovered.

20.1.25. The examination of the blood revealed the presence of *P. mutans* in rare numbers. It was splenectomized on this date. *P. bigeminum* appeared three days later and remained for a considerable length of time, being present one day and absent the next. *P. mutans* was found in fair numbers on the fourth day. It increased in numbers and at one time was present in large numbers. In this case there was for over three weeks a marked febrile condition, the evening temperature almost daily at 105° F., and there was a paroxysm to 107° F. on the 4.1.25. The lesions of anaemia were

also marked. The examination of the glands was commenced three days after the operation and during the first few days carried out daily, and subsequently every second day until *P. mutans* became reduced in numbers. At no time were plasma bodies seen in the blood.

Discussion of the Results.—In all three cases there was an immediate relapse of *Piroplasma bigeminum* and of *P. mutans*. These relapses were severe and gave rise to febrile disturbances and marked anaemic changes of the blood. Of interest is the relapse of *P. mutans* which in all cases was very marked, the parasites appearing in the blood soon after the removal of the spleen, increased rapidly in numbers and remained in large numbers during a considerable length of time during which the calves were under observation. A careful and continued search for plasma bodies was made in all cases and in all with negative results.

The influence of the removal of the spleen was a very marked one as explained above. No agamonts and gamonts were seen in the lymphatic glands, where in the absence of the spleen, their formation could be expected. It is possible that some other internal organ took over this rôle, but it is difficult to conceive that such should have been the case, since the lymphatic system is the predilection seat for agamonts and gamonts to form in all theilerioses. It must be concluded that the multiplication of *P. mutans* in these relapse cases did not occur by the indirect process but by the direct one and in the blood itself.

Discussion of a Recent Publication.—

Recently Turnbull (*Journal of Comparative Pathology and Therapeutics*, Vol. XXXIX, Part 4, page 307, Dec. 1926) has published some observations in the Fort Jameson District of North-Eastern Rhodesia, concerning the appearance of plasma bodies in cattle suffering from a disease that cannot be diagnosed as African Coast fever. The disease is mainly found in young calves in which it most often assumes a chronic form, although acute cases may be met with and even in adult animals. He finds small piroplasms of the type *T. parva* in the blood and plasma bodies in the lymphatic glands, and also in the spleen and other organs. He describes lesions in the kidney, the so-called infarct, which resemble in every respect those described in East Coast fever. Turnbull does not draw any definite conclusion as to the exact nature of the parasite, but from the sub-title of this paper, it appears that he considers them morphologically similar to *Gonderia mutans*, referring to Doyle's observation, who considered the plasma bodies he found in Egypt to be developmental stages of *Theileria mutans*. We have pointed out before that Doyle's observations refer most likely to *T. annulata*, in which such plasma bodies are definitely known to exist.

Turnbull, unfortunately, was not able to carry out transmission experiments and to study the disease so produced. We are, therefore, lacking some of the information necessary to interpret the nature of the small piroplasms. Comparing his observation with ours in *P. mutans* infection, it at once is evident that he apparently found the plasma bodies with the greatest ease, and moreover he found lesions in calves and adult cattle that we have never come across as yet. In this respect, attention must be drawn to the existence of "infarcts" in calves that are not of piroplasma origin and unless they can be shown to contain plasma bodies can not be accepted as

evidence of theileria infection. We refer to the "Fleckniere" of calves, a nephritis which Pfenninger has traced to bacillary infection. Nevertheless, it is evident that Turnbull has been dealing with a theileriosis and it remains yet to be seen to which type it belongs. Turnbull was good enough to submit some of the smears to our Laboratory for examination. Dr. Du Toit, who controlled them, was not in the position to interpret all the bodies that were marked as plasma bodies as such. However, there were some that undoubtedly were plasma bodies, but they were rare, as rare as those in the smears which we have examined. Thus giving due allowance to faulty diagnosis, it is possible that after all Turnbull has had to deal with the disease which was met in Marico District, and which we are now inclined to consider to be due to *Piroplasma mutans*, now to be considered as *T. mutans*. After all due consideration must be given to conditions not as yet sufficiently known, that have a bearing on the virulency of the *T. parva*, such as susceptibility of cattle and outside influences. *T. mutans* accordingly may be the cause of a serious disease in some parts and is of little consequence in others.

SUMMARY OF CONCLUSIONS.

(1) Accidental observation in the field (Marico) and experimental exposure of calves to tick infestation (Kaalplaats) have shown that in some calves of early age plasma bodies can be found that resemble in every respect those found in the cycle of *T. parva*, *T. dispar* and *T. annulata*.

(2) In the animals in which plasma bodies were seen, such infections as *Theileria parva*, *Theileria dispar*, and *Theileria annulata* could definitely be excluded, partly for epizootic and enzootic, partly for experimental reasons.

(3) The interpretation of the presence of plasma bodies in the instances described is that they belong to the cycle of *P. mutans*, alias *Gonderia mutans*, which parasites were found in the blood at the same time.

(4) The suggestion of Brumpt as indicated in the introduction to this paper, viz. that *Gonderia mutans* should in reality be *Theileria mutans*, finds an actual support by our investigations and accordingly the genus *Gonderia* (Du Toit) should be sunk in favour for the genus *Theileria* (Franca).