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A Short Study of Bovine Anaplasmosis, with Special Reference to the Chemotherapy.

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NOTWITHSTANDING that more than twenty years have elapsed since Theiler discovered the causal agent of bovine anaplasmosis, the position in connection with the control of this disease still remains unsatisfactory in those areas where it has not been possible seriously to influence the tick population. Immunisation against the disease by the use of *Anaplasma centrale* has been proved to be of great value and the extension of this means would undoubtedly reduce considerably the losses at present due to *Anaplasma marginale infection*. In this short study is described, in addition to certain observations on the disease, a chemotherapy which seems to be distinctly promising and which, possibly, might serve as a starting point for the development of an efficacious treatment. An efficient method of treatment is an essential requirement for the control of the disease as it occurs in many areas. The losses due to *A. marginale* especially in the better class of stock are considerable, if it be taken into consideration that the disease is widely distributed throughout the world.

It is proposed, in this article, first to deal with the observations made on untreated bovines suffering from the disease and then, when considering the chemotherapy, to study the effect of the treatment on the various major symptoms thus determined.

The strain of *A. marginale* used in these experiments was obtained from a dairy cow in the Pretoria District. In this herd the loss of bovines from anaplasmosis had been severe. The transmission to the various experimental animals was carried out both via the subcutaneous and the intravenous route by the injection of citrated blood. The disease which resulted in susceptible adult bovines was always severe and in only a few cases in such animals did recoveries without treatment occur. In young susceptible bovines recoveries were the rule. Occasionally bovines did not react. This was not unexpected as some of the bovines utilised in the experiment were obtained from areas where anaplasmosis was known to occur. Such resistant bovines and others which reacted only slightly were not considered as suitable for experimental therapy. Only those

which showed an initial severe reaction were treated. The consequence of this arrangement was that all bovines selected for treatment were only utilised after the disease had run several days of its course.

At the commencement of the subinoculations the strain was contaminated with *Piroplasma bigeminum* and *Theileria mutans*. These parasites were lost during the course of transmission from animal to animal. The reservoir of *A. marginale* infection now utilised is bovine 2889. This animal belongs to the eighth generation and for the last two-and-a-half years subinoculations to the number of forty-four have been made into other bovines without the production of a single case of either *P. bigeminum* or *T. mutans* infection. The freedom of bovine 2889 and consequently of the strain from contamination of *P. bigeminum* was further established by the injection, into many of these animals, of blood infected with *P. bigeminum* with positive results. Working with a pure strain of *A. marginale* was of great advantage for, obviously, the complications introduced by a prior reaction to piroplasmosis would possibly affect the subsequent reaction to anaplasmosis especially as the latter would have, at times, occurred within a day or two of the former that is before the bovine could have recovered entirely from the effect, especially on the blood, of the piroplasmosis.

The major manifestations of *A. marginale* infection in untreated bovines will now be dealt with.

The period of incubation after artificial infection is, when judged by the first appearance of the parasites, as short as 11 days in some cases; when judged by the first elevation of temperature the shortest period is 14 days. In all the cases the parasites were determined before the first elevation of temperature, appearing usually three and exceptionally six days earlier. The period of time between the appearance of the parasites and the first elevation of temperature is not characterised by any symptoms which would likely attract attention in other than experimental cases. The first definite signs of illness appear with the temperature elevation. The disease consequently is one which is established for several days before therapeutical interference can be undertaken. A treatment to be of value under natural conditions must be one which can bear the test of late application. The examination of stained blood smears at the commencement of the infection shows the peripherally situated parasites few in number and clearly defined. Within a few days the number of parasites has increased greatly. Thereafter in many cases there is a fairly rapid decrease of parasites. As soon as the polychromasia and punctate basophilia appear—anisocytosis having already been evident for several days—usually at about the third to fifth day of temperature, the parasites are somewhat masked and are more difficult to enumerate. When special measures are taken in the preparation of the blood smears the enumeration is facilitated. The method adopted for this purpose is the preparation of the blood smears directly from the jugular blood, the smear itself being made as if it were going to be utilised for a white cell differential count.

The temperature in most cases shows an abrupt initial elevation reaching approximately the maximum on the first or second day. Thereafter there are more or less marked exacerbations and remissions or a more continuous curve of high temperatures until, in recovery cases, a gradual return to normal occurs. In fatal cases the temperature also often returns to normal and may be associated with a decrease of parasites. It would appear that the animal dies after the activity of the parasites has decreased.

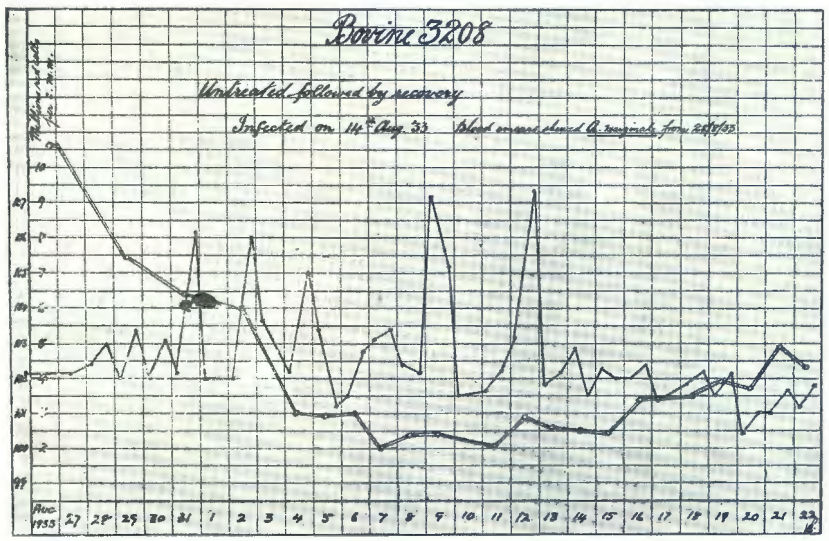
At the onset of the disease there is some constipation, the faeces being in the form of hard pellets, small, smooth-surfaced, and shiny. Later the quantity of faeces becomes less and less until ultimately there may be a cessation of defaecation, indicative of a complete atony of stomach and intestines. No case of diarrhoea has been observed.

The usual changes in connection with rumination, appetite, condition of skin, etc., associated with a high fever are prominent. The animal may not partake of food for days on end. The respirations and pulse are accelerated but, if the bovines are not disturbed, distress does not become a conspicuous feature.

The mucous membranes of the eyes and the mouth are at first slightly reddened but soon become pale. Occasionally, and then only for a short time, is an icterus present. Many cases show practically no yellowish colouration. This comparative absence of icteric mucous membranes in the cases under observation is all the more surprising when it is taken into consideration that the plasma on the same days show the presence of a deep yellow colour. In bovine 3208 the colour of the plasma was subjected to colorimeter observations at frequent intervals over a period of several weeks. The mucous membranes were still of a pinkish colour when the plasma was a deep yellow. The most intense yellow colour was observed in the plasma on about the 5th day of temperature whereas the mucous membranes showed no tendency towards an icteric coloration until about the 10th day after first temperature elevation when the yellow colour of the plasma was already becoming less intense. Two days later the mucous membranes were definitely yellow. By this time the red cell count had dropped to below 3 millions of red cells per c.m.m. On account of such observations of the lateness of the appearance of icterus of the mucous membranes and its indefiniteness, this symptom cannot be regarded as of any great importance or assistance when dealing with the examination of a bovine for anaplasmosis. The plasma colouration, on the contrary, should be regarded as being a sign of some value. No explanation is advanced to account for this absence, in the early stages of the experimental cases, of a definite yellow colouration of the mucous membranes. In the usually accepted description of the disease, icterus as a symptom is given a prominent place. It may be that the description of the symptoms of anaplasmosis has been based largely on observations made towards the end of the course of the disease.

The variations in the red cell counts and the red cell volume (red precipitate percentage) of the various animals observed follows a fairly uniform course. The figures for the red cell count—the

red cell volume follows this fairly closely—of bovine 3208 (Fig. 1), one of the rare recoveries without treatment, are submitted as a typical example together with the temperature curve and the blood smear examination. The chart enables one to compare the relationship of onset of the disease and of the variations of the red cells' count. It will be noted that the decrease of red cells commences fairly early at about the time of the appearance of the parasites. The process, however, is a gradual one, not reaching the minimal determination until the 8th day of temperature. It remains more or less at the same level until the 16th day when a definite upward trend of the curve is observed. Yet the punctate basophilia appeared on the 3rd day and the normoblasts on the 6th day of temperature. It would seem that the late appearance of these argues a sluggish regeneration of red cells due to, possibly, the direct parasitic effect and that the process of regeneration, once the primary parasitic effect



has passed over, takes some considerable time to catch up with the process of destruction. It is during the period of low red cell count that the atony of the digestive system is such a prominent symptom; by this time the percentage of red cells parasitised has dropped to about one-tenth of the maximum figure which occurred, in bovine 3208, on the third day of temperature elevation. From the observations made on the various bovines, it seems that the disease can reasonably be divided into three stages, the first the parasitic stage, the second the stage of severe anaemia and the third, the stage of improvement. In the parasitic stage the parasites reach their maximum count, the red cells decrease in number, the appearance of red cell regeneration is noted in blood smears; the condition is one of high fever with its associated symptoms. The second stage shows a greatly decrease count of parasites and red cells, marked anaemic changes in the blood smears, an atony of stomach and intestines; this is the stage of anaemia and of the changes produced in various systems by the parasites. The third stage reveals an increasing red

cell count, a general improvement, which, however, may be interrupted and arrested by a temporary increase of parasites and, at times even, a temperature elevation.

CHEMOTHERAPY OF ANAPLASMOSIS.

In recent years there has been a large amount of work carried out in an endeavour to establish a specific treatment for bovine anaplasmosis. A number of claims have been advanced for various drugs but of these it is intended to refer to only a few which have been strongly advocated by certain investigators.

Freund (1929) referring to bovine anaplasmosis in Palestine says that the disease, as a rule, runs a prolonged course but does not usually end fatally. He claims that the most satisfactory results in treatment were obtained by combining Atoxyl with either Antimosan or Stibosan and that the Antimosan influences the fever in a favourable manner.

Diebeu (1929) advocates the use of Collargol. He, however, does not supply sufficient data to enable one to form an opinion of its effects on the disease. Collargol was later also used by Lagas and Seijffers (1931) in outbreaks of the disease. The diagnosis was not, in every case, supported by blood smear examinations. Consequently it is not possible to assess the true value of the treatment. In their cases there is, at times, no reduction of the temperatures until 72 hours after the first injections. They stress the advisability of early treatment, and are inclined to ascribe the absence of icterus of the mucous membranes, observed in all but two cases, to their early application of injections of Collargol. In addition to the fact that some of their cases may have been diseases other than anaplasmosis or very mild attacks of this disease, there must also be considered the possibility of the non-development of icterus in even severe cases especially during the early stages of the course of the disease. They administer the Collargol intravenously as a 1 per cent. solution in doses of 2 gm.

Ducloux and Cordier (1930) use quinine associated with certain dyes and arsenicals. Jacotot and Evanno (1931) also claim good results with quinine when associated with an arsenical.

Färber (1930) working in Palestine reports a number of cases treated with trypaflavin but gives details only of a few. The dose of trypaflavin he uses is approximately 2 gm. administered intravenously in a watery solution. His chief claim is that the parasites, in some cases, disappear soon after the first injection. Such a disappearance of parasites would undoubtedly be the best criterion of the specificity of trypaflavin. He claims also an amelioration of the disease with a rapid return of temperatures to normal. Unfortunately he provides temperatures for only a few days and one cannot, therefore, judge whether the reduction in temperatures to normal is temporary or not, although Velu (1933) regards Färber's cases as an illustration of the return to normal of the temperatures due to the use of the drug. As regards Färber's claim of disappearance of parasites it is of interest to compare his findings with those of Picollo reported below.

Velu (1933) is a strong supporter of the use of flavins in anaplasmosis. He claims that Gonacrine is the best drug for anaplasmosis that he has met with during the course of several years although he has not had the opportunity of trying it out on many cases. The dose he recommends is 1 gm. He advocates Gonacrine particularly in those cases where the diagnosis, on account of non-availability of facilities for smear examinations, cannot be definitely made, holding that Gonacrine is efficacious in both piroplasmosis and anaplasmosis.

The extract from the report of the Provincial Veeartsenijkundigen Dienst van Oost-Java (1933) on the use of Trypaflavin does not supply sufficient data to enable one to ascribe to it any definite beneficial effect on the disease.

The most comprehensive and controlled work on the use of flavins in anaplasmosis, available to the writer, is that of Picollo (1928). He divides his work into four sections each of which deals with a number of bovines imported into Brazil from Europe. In the first two sections he administers intravenously Trypaflavin in doses of 0·2 gm. to 0·6 gm. At least two doses are given and, in addition, adjuvants such as oils, ether and serum are used. In the third and fourth sections he uses no adjuvants but increases the doses of Trypaflavin. His largest single intravenous dose is 3 gm. and the greatest quantity given on any one day is 4·8 gm. (in two doses). In many cases the injections are repeated at frequent intervals with the consequence that one case received no less than 16 gm. In no case does he administer less than two doses, some receiving 6 to 8 doses. To one bovine is given 4 gm. intravenously and 13 gm. per os. The injections in some cases are spread over several weeks. One would expect that the use of the drug in such large quantities, if Färber's experience be taken into consideration, would have, in some cases at any rate, resulted in the disappearance of the parasites from the blood smears. Picollo, however, found a persistence of the parasites subsequent to the completion of his injections. If the temperatures of Picollo's cases be examined, it will be seen that these are not reduced to any great extent or with any uniformity by the use of Trypaflavin.

Taking the foregoing into consideration and also a few cases treated, with Trypaflavin in 1931 at this Institution, the writer can come to no other conclusion but that Trypaflavin and possibly also Gonacrine have little, if any, beneficial effect on anaplasmosis: it certainly cannot be regarded as a specific for the disease. The mere recovery of a bovine from anaplasmosis cannot be, with safety, advanced as a proof of specificity or even of being of any beneficial value for the reason that, in anaplasmosis, one is dealing with a widely distributed parasite of considerable variations of virulency and with bovines which apparently have a variable resistance to the disease. Young bovines seem to have a greater resistance to anaplasmosis than older ones. Until one could be certain that, for experimentation, a uniform susceptibility of bovines is available and a uniform virulency of parasites is arrived at, one cannot regard, especially when dealing with small numbers, the recovery of treated bovines as a true index of the efficacy of the drug experimented with.

The possibility of arriving at an opinion of the efficacy or otherwise of a drug could perhaps be best attained by the consideration of the effect of the drug on the parasites and on the various manifestations of the disease.

In the examination of the untreated bovines, the chief points observed were in relationship to the parasites, the temperature, the atony of the stomach and intestines and the length of the course of the disease together with the condition and general behaviour of the animal. If now in each bovine the observations on these manifestations show that the use of a drug has affected them in a beneficial manner, then would it be permissible to advance a claim for the drug, especially if two, or more preferably, of the manifestations be beneficially influenced. As it often requires only some slight beneficial effect to direct a case of anaplasmosis along the road to recovery, even such an influence would be great assistance in combating the disease.

It was with the above idea of the correct line of investigation that the experiments on the chemotherapy were so arranged that the effect of the drug used could be determined on the individuals treated. After the abandonment of the use of trypaflavin and other drugs in the experiments, Mercurochrome 220 soluble was used. This drug gave indications early on of being a drug worthy of some attention.

Mercurochrome-220 soluble, dibrom-oxymercuro-fluorescein, is extensively used as a disinfectant of the skin and genito-urinary tract. It has also been recommended for certain blood infections. Its administration in a watery solution by the intravenous route does not apparently produce any ill-effects if the doses be properly regulated. The writer has given it in this manner to normal horses, cattle and sheep.

The strain used in these experiments was, as has been already mentioned, passed from bovine to bovine by artificial means. It produced in practically all the susceptible adults a severe infection. In the large number of young bovines inoculated the disease was only occasionally of a serious nature.

The dose of Mercurochrome used in these experiments was 0·4 gm. in calves and a maximum of 1·3 gm. in fully grown adults. It was always given in a 2 per cent. saline solution intrajugularly. Only in four cases was a second injection of the drug given, and of these four three died. However, as can be seen on reference to the table, two of these three animals were treated very late in the course of the disease. The information in connection with the third bovine was meagre, due to difficulties in controlling it, but the disease was well established before treatment was applied.

The administration of a drug containing mercury to an animal suffering from a disease which produces marked changes in the liver should, however, be undertaken with care. Only one bovine receiving a single dose of mercurochrome died. This animal received the injection when *in extremis*.

The temperature of all the bovines treated was reduced. The reduction at times was spectacular being, in some cases, as much as 5° F. In most cases the drop to normal persisted until complete

recovery resulted. Occasionally there was a slight temperature elevation which, however, never reached as great a height as before the injection. This elevation of temperature did not persist for longer than a few hours and in no such case was it necessary to institute treatment.

No obvious effect on the parasites was observed as the result of an injection of Mercurochrome. In a number of cases treatment was commenced comparatively late, i.e. actually at a time when one would have expected the parasites to show a decrease whether treated or not. Consequently the enumeration of parasites gave no information of a reliable nature. No bovine was treated very early, i.e. before the first elevation of temperature soon after the parasites had appeared. Such an early application of treatment might give further information on the point as to whether the parasites are or are not affected by the Mercurochrome.

The constipation or later the actual cessation of defaecation in anaplasmosis was strikingly affected by the use of Mercurochrome. In normal sheep the action on the defaecation of fairly large doses, given intravenously, was somewhat irregular. In most cases there was purging but some did not react. If sheep were killed a few minutes after the intravenous administration of Mercurochrome, a chrome yellow coloured ingesta was found in the abomasum and small intestines. In cattle affected with anaplasmosis the action of the intravenously administered drug was to produce, in practically every case, free purging, the faeces being fluid in nature, chrome yellow in colour and voided without any marked straining. It has not yet been determined at which point of the course of the disease the Mercurochrome should be administered to produce the best purging effect. It would, however, appear that the effect would be least satisfactory when the red cell count is at its lowest. Uniformity of action will probably depend on the proper regulation of dosage and the selection of the proper time for administration in the course of the disease. For many years the treatment for anaplasmosis has been of a symptomatic nature and has been aimed at, chiefly, the atonic condition of the intestines. In Mercurochrome it would seem there is available a drug which will give the best service in this direction.

Blood examination, i.e. for variations in red cells, the red cell count, and the red cell volume, was carried out in treated bovines. The treatment with Mercurochrome did not stop the decrease in red cell count or red cell volume. This continuation of the drop in the number and volume of red cells was present in all the treated bovines examined. So constant was it that it may be taken as established that the administration of Mercurochrome has no halting effect on the decrease, and as the lowest red cell count of some of the treated bovines went down to nearly 2 millions of red cells per c.mm. it may also be taken as established that it had no effect on the severity of the ultimate anaemia. It cannot be decided whether the return to normal from the lowest point of red cell count was more rapid in the treated than the untreated cases for the reason that it is not possible to say whether the infection in the treated was more severe or not than in the untreated. As far as the question of the effect of Mercurochrome on the anaemia is concerned it can only be said

that there is no obvious or well-marked influence. Tentatively other substances, e.g. liver extract, carotine, etc., were introduced in some cases for the purpose of causing a stop to the decrease of the red cells but without success. The anaemic changes of red cells observed in stained smears did not appear earlier in the treated than in the untreated bovines.

The bovines after treatment with Mercurochrome did not lose condition as rapidly as usually occurs in severe cases of anaplasmosis, even though the red cell count went to below 3 millions per c.mm. This might possibly be ascribed to the elimination of high temperatures and the increased activity of the bowels. The animals in every case were kept quiet and saved from exertion but no provision was made for special diet. The utilisation of green succulent food in place of the somewhat unpalatable routine ration provided would probably have had a favourable influence.

There are submitted a table (Table I) providing information in connection with cases treated at this Institution and graphs of a few cases (*vide* Figs. 2, 3, 4, 5) illustrating treated and untreated cases. A number of cases have been treated away from Onderstepoort with fairly good results. The main difficulty in the treatment of such cases is that of determining from the examination of the bovine whether it is in the early or late stages of the disease. The presence in the blood smear of no or only a few punctate basophilia is taken as an indication that the disease is in the early stages. If there are advanced anaemic changes, the advisability or not of injecting Mercurochrome is a point that has not as yet been decided.

SUMMARY.

1. The description of the major symptoms of bovine anaplasmosis.

2. The utilisation of Mercurochrome-220 soluble in the treatment of bovine anaplasmosis in doses of 0·4 to 1·3 gm. administered intrajugularly.

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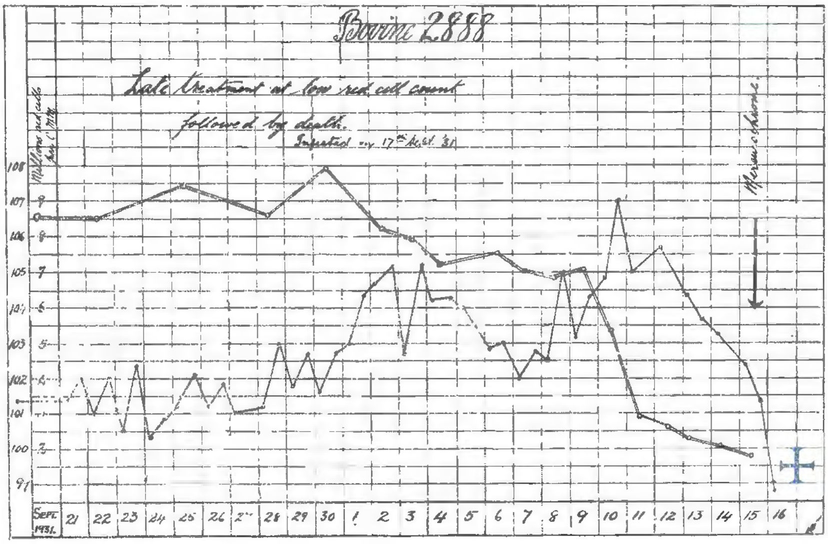
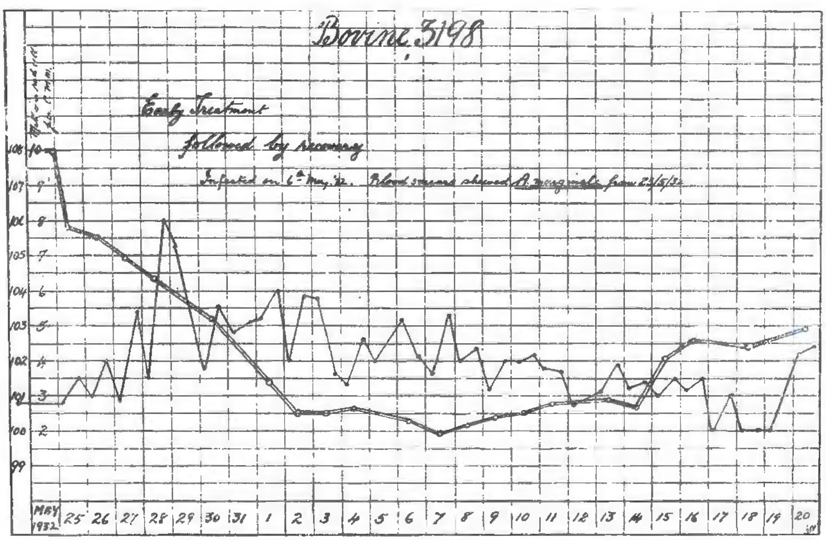
TABLE I.

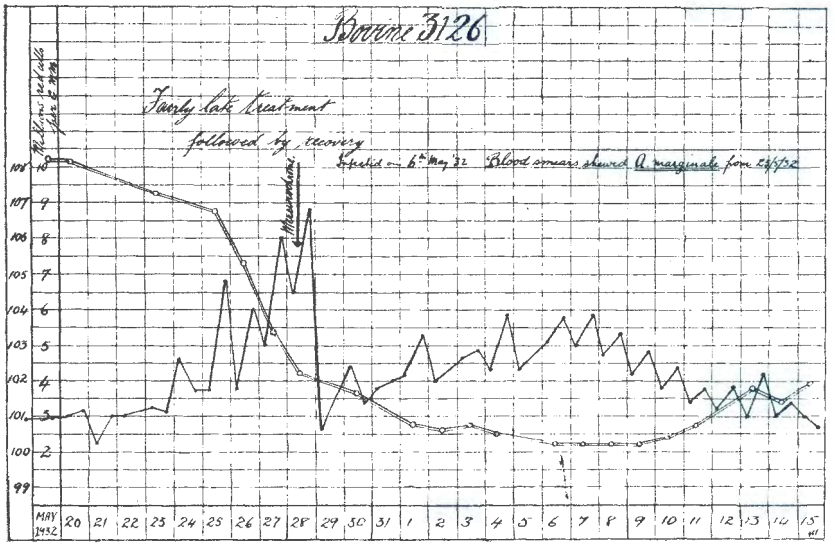
Cases of Bovine Anaplasmosis Treated with Mercurochrome.

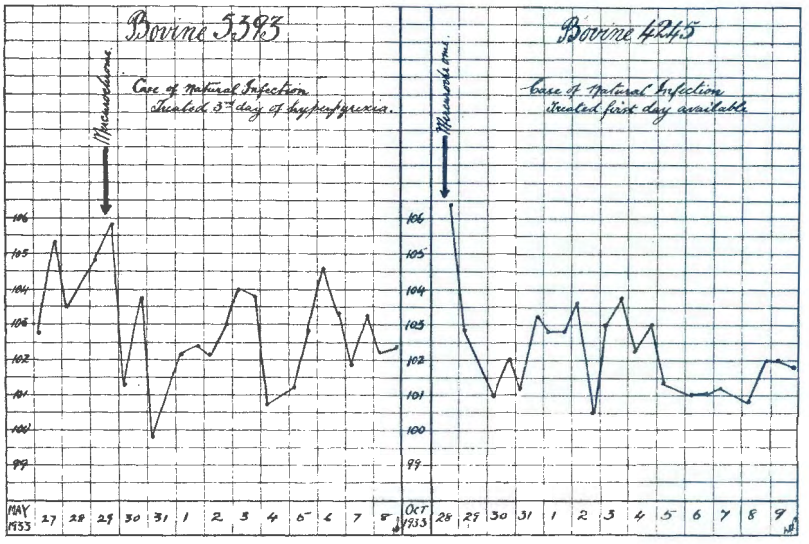
Bovine.	Dose of Mercuro-chrome in gm.	Date of Treatment.	Result.	Remarks.
3518	0·75	15/5/31	Recovery....	Treated on 6th day of hyperpyrexia.
3011	0·75	5/6/31	✱ 11/6/31..	No smears or temperatures available.
	1·0	6/6/31		
3019	0·5	7/7/31	Recovery....	Treated on 5th day of hyperpyrexia.
3020	1·0	18/8/31	,, ...	Treated on 2nd day of hyperpyrexia.
3056	1·0	18/8/31	,, ...	Treated on 9th day of hyperpyrexia.
2889	1·0	3/9/31	,, ...	Treated on 3rd day of hyperpyrexia.
2888	1·3	15/10/31	✱ 16/10/31.	Treated on 15th day of hyperpyrexia.
3547	1·0	28/1/32	Recovery....	Treated on 6th day of hyperpyrexia.
	1·0	30/1/32		
3132	1·0	6/5/32	✱ 7/5/32...	First treated on 5th day of hyperpyrexia and 11th day of parasites.
	1·25	7/5/32		
2269	1·0	14/5/32	✱ 20/5/32..	First treatment on 2nd day of hyperpyrexia and 8th day of parasites.
	1·0	19/5/32		
3126	1·2	28/5/32	Recovery....	Treated on 4th day of hyperpyrexia.
3198	1·2	28/5/32	,, ...	Treated on 2nd day of hyperpyrexia.
3514	1·0	1/7/32	,, ...	Treated on 4th day of hyperpyrexia.
3112	1·0	27/7/32	,, ...	Treated on 3rd day of hyperpyrexia.
3129	1·0	1/10/32	,, ...	Treated on 4th day of hyperpyrexia.
5393	0·8	29/5/32	,, ...	Treated on 3rd day of hyperpyrexia.
5214	0·4	29/5/33	,, ...	Treated on 4th day of hyperpyrexia.
4245	0·9	28/10/33	,, ...	First temperature elevation not known.

✱ Denotes Death.


Bovine 2888
Late treatment at low red cell count
followed by death.
Infected on 17th Aug. '31
Millions red cells per c. mm.
108
107
106
105
103
102
101
100
SEPT. 1931.
21 22 23 24 25 26 28 29 30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16


Bovine 3198
Early Treatment
followed by recovery
108
107
105
104
103
102
101
100
99
MAY 1932.
25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20


Bovine 3126
Fairly late treatment
followed by recovery
Macerandum.
MAY 1932
20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


Bovine 5393
Case of Natural Infection
Treated 3rd day of hyperpyrexia.
Macerandum.
Bovine 4245
Case of Natural Infection
treated first day available.
Macerandum.
MAY 1933
27 28 29 30 31 1 2 3 4 5 6 7 8
OCT 1933
28 29 30 31 1 2 3 4 5 6 7 8 9