
**Preliminary Report on Investigations
carried out in connection with a
Calf Disease in the Marico District,
with special reference to Para-
typhoid “B. enteritidis” Infection.**

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Introduction.—The main object of this preliminary communication is to report on a severe mortality in calves which has been under investigation by the writers during the last three years and to draw particular attention to infection by organisms of the paratyphoid *B. enteritidis* group. While organisms of this group are recognised in European countries as a fairly common cause of disease in young animals, they have up to quite recently not been paid any serious attention in South Africa.

It is not proposed in a communication of this kind to attempt a review of the literature that has appeared in various countries on paratyphoid infection in animals, but reference should be made to the commonly accepted view that infection by this group of organisms takes place particularly in animals whose vitality and powers of resistance have been reduced by a variety of factors and through different unfavourable circumstances.

Historical.—Under the farming conditions occurring in South Africa, enzootic diseases of calves connected with feeding and housing have not been so common as in European countries; cattle breeding is commonly carried out under ranching conditions, the calves running free on the pasture and obtaining their food direct from their mothers. Digestive troubles arising from improper or irregular feeding and diseased conditions contracted from infected stables and calf pens consequently have been of rarer occurrence. Where these conditions are departed from, as in the case of dairy herds, the same troubles, perhaps even to a worse degree, are experienced as in other parts of the world.

Generally speaking, comparatively few instances of serious calf mortality have been brought to the notice of our veterinary authorities and consequently no special study of calf diseases has been made in this country. Henning in the Cape Colony described a disease in calves called "Yellow Liver," but no bacteriological work was carried out in connection therewith. In the light of our present investigations we are inclined to believe that Henning had to deal with paratyphoid infection as one of the causal factors.

The disease to be described here was first reported to the Veterinary Research Division about five years ago. It was then stated that in certain localities of the North-western Transvaal a rather high mortality in calves resulting from a form of septic pneumonia was occurring. No opportunity for investigation presented itself until the beginning of 1923, when reports of a serious mortality in calves in the Marico District were received and when one of us was instructed to carry out preliminary inquiries on the affected farms.

It was soon found that the condition was not septic pneumonia of calves, but that the mortality was sufficiently serious to warrant further investigation.

During these investigations we were seriously handicapped by the fact that the affected farms were situated long distances from the railway line, which made it difficult to get fresh material for bacteriological work and animal inoculation to the Laboratory. At the same time it has been impossible to procure for experimental work a sufficient number of calves of the susceptible age.

Animals Affected.—As far as our experience goes the disease attacks only calves between the ages of one and four months; there appears to be very little doubt that animals of two to three months old suffer most. The mortality does not appear to be worse in any particular breed, although it should be stated that the cattle which came under our observation were largely of the cross-bred types.

CONDITIONS UNDER WHICH THE DISEASE OCCURS.

These are of the utmost importance and must, therefore, be discussed in some detail. Under this heading will be described also the principal predisposing and contributory factors and for the sake of clarity they will be considered separately.

(1) *Locality.*—As far as our information goes the disease occurs in the bushveld of the Marico district and probably also in other low-lying parts of the Union.

(2) *Climatic Conditions.*—The low veld of the Transvaal and other parts of the Union is well-known for its extremely hot climate; in addition, the localities concerned are thickly wooded, thus making conditions for parasitic life, especially ticks, particularly favourable. Tick life is exceptionally abundant and active during the hot summer months, and it is during this time that the calf disease makes its appearance. It is particularly prevalent during the period January to March, and is exceptionally severe after heavy rains.

(3) *Farming Conditions.*—The bushveld of the Marico district offers ideal conditions for cattle breeding under ranching conditions, provided always measures for tick eradication are actively pursued. The few farmers in that area who have been practising this for the last few years and who have not been trying to combine cattle breeding with dairying have suffered practically no losses from the calf disease. In fact, according to available information, it would seem that their annual loss of calves from all causes (disease, accidents, etc.) does not exceed 5 per cent. In these cases the calves were allowed to run with their mothers during the day, and were paddocked during the night. Only some cows were milked, but this

was done under strict supervision. On the other hand, on the farms where the disease has been so prevalent, entirely different methods of animal management were customary; some or all of the following methods were practised:—

- (a) No attempt was made to keep the animals free of ticks, the result being that there was gross infestation with ticks, among them carriers of Piroplasmosis, Anaplasmosis, Theileriosis, and Heartwater, diseases which are enzootic in that area and which are to be discussed later.
- (b) In most cases cattle breeding and milk or cream production were combined, this necessitating rearing of calves under the most unnatural conditions, of which the following might be mentioned:—The cows are generally milked only once a day, and this means that the calves receive their food at too long intervals, commonly only once in 24 hours. Owing to the extremely hot climatic conditions, calves develop an excessive thirst, the result being that too much milk is taken in at one time or that the rate of suckling is far too rapid.

Cows in that area are not herded and only rarely are there fenced-in camps on the farms, so that there is no way of getting the animals to the homestead for milking at stated times. This again means irregular feeding of calves. Most cows are not of the heavy "milk strains," extra feeding is not resorted to, and yet they are expected to supply sufficient milk for both their young calves and for the creamery.

Besides that, milking is often entrusted to young irresponsible natives who certainly cannot be expected to look after the interest of the young calves. All this must sooner or later lead to digestive troubles, unthriftiness, lowered vitality, etc., with the end-result that the calves fall an easy prey to bacterial or protozoan infection.

- (c) Dirty open calf pens or kraals with, very often, insufficient shade, are found on practically all farms. Sick and healthy calves are usually herded together in such kraals, with the result that ideal conditions for the transmission of bacterial infections are provided. There can be no doubt that millions of organisms of the paratyphoid group must be contained in the soft faeces scattered everywhere in the kraal and on the coats of calves by the diarrhoeic patients. That such calf kraals or pens are grossly infected, can be accepted without any reserve.

(4) *Enzootic Diseases.*—As already mentioned, such infectious diseases as Heartwater, Piroplasmosis, Anaplasmosis and Theileriosis are enzootic in the Marico district. Providing the transmitting ticks are present—and they are present in enormous numbers—every calf born in that area must pass through an attack of all the diseases named. Under normal conditions the large majority of calves contract these diseases during the first two or three months after birth and develop an immunity against them without going through very marked reactions; in fact, in most cases it is unusual for any visible signs of illness to be observed at all. Full grown cattle that have

been born and reared on these farms possess a strong immunity against these diseases and, as a matter of fact, it is rare that an adult animal dies from any of them. If, on the other hand, cattle are brought on to these farms from other parts of South Africa where these diseases do not normally occur, severe losses from Redwater, Anaplasmosis and Heartwater must be expected.

Etiological Factors.—Having mentioned all the conditions under which the disease makes its appearance, we may now enter into a brief discussion of the principal factors involved in its production.

As predisposing causes must be mentioned the digestive disturbances and lowered vitality brought about by faulty animal management.

We have already referred to the infectious diseases which are enzootic in the Marico Bushveld area, and it now remains to see in how far they can be excluded from, or incriminated in, the causation of the mortality.

From our observations it would seem to be unlikely that Heartwater could have played any prominent part. The cases of Theileriosis referred to must remain unexplained but it seems doubtful whether this disease could have been of great importance. It should be mentioned here that so far only one form of Theileriosis has been recognised in the Union, namely, that set up by *Theileria parva*. This latter disease, however, has been unknown in the Marico district for the last 10 years and, as a matter of fact, there are many reasons for excluding its existence there altogether. In a small proportion of cases of the calf disease under discussion typical Koch's granules were observed in smears made from the internal organs, liver, spleen and kidneys. At the same time small intracorpuscular parasites, indistinguishable from *T. parva* and *G. mutans* were seen in the blood, but these parasites were never numerous, not nearly as frequent as one commonly meets with in cases of East Coast fever.

We can only conclude that this is a species of Theileria, hitherto not recognised in the Union, and differing markedly from *T. parva* in that it is not so highly pathogenic.

As far as Piroplasmosis and Anaplasmosis are concerned, it must be stated that both these diseases undoubtedly play a considerable rôle in the production of the calf mortality under consideration; in many cases of the disease both the clinical manifestations and post mortem appearances were typical of either or both of these conditions. Moreover, in many cases *B. bigeminum* and *A. marginale* together with marked anaemia, usually associated with Piroplasmosis and Anaplasmosis, could be demonstrated. This is certainly not a common occurrence in this country, calves born from immune parents on an infected farm rarely showing any visible signs of illness while passing through the Redwater and Anaplasmosis reaction. An explanation for this occurrence had to be sought and during the further investigations bacteriological work was undertaken. For the purpose of obtaining the necessary material calves showing well-marked and typical symptoms of the disease were killed and examined post mortem; cultures were made from the heart blood and from the internal organs—liver, spleen and lungs—and the growths obtained after incubation consisted of organisms of the colon-typhoid group.

No difficulty was experienced in obtaining pure cultures from any typical case of the disease, provided always it was in an advanced stage. We could, therefore, come to no other conclusion than that we had to deal with a true bacteraemia, as a result of invasion by an organism of the colon-typhoid group. The organism in question was eventually classified as a member of the paratyphoid *B. enteritidis* group, its pathogenicity being established by tests on animals, to be described later.

Symptomatology.—The first symptoms noticed are drooping of the ears, dullness and loss of appetite, affected calves showing disinclination to drink from their mothers, drinking very slowly or taking in less nourishment than usual. If the temperature is taken at this time, there will be found to be a rise varying from 103° to 107° F. Dullness becomes marked, sick calves lying down a good deal or showing disinclination to move when encouraged to do so. This dullness increases with the progress of the disease, so that in the later stages affected calves remain in the recumbent position. In addition, they commonly show a rough, staring coat, arched backs and tucked up appearance of the abdomen; grinding of teeth can be noted in some cases. At the commencement there may be constipation, but in most cases diarrhoea develops sooner or later; the faeces are fairly soft but rarely liquid or blood stained; they usually have a rather offensive smell, particularly in the later stages of the disease. Dribbling from the mouth, sunken eyes and grinding of teeth are sometimes observed.

Very often slight nasal and lachrymal discharges are present, while the visible mucous membranes are usually pale, but may be icteric. An accelerated pulse is commonly present, while hurried breathing or, more rarely, a cough and dyspnoea, may also be present. The disease may last from a few days to two or three weeks.

The mortality is high in calves developing well-marked symptoms, the estimate by farmers being 30 per cent. or higher.

Post-mortem Appearances.—The most notable alteration was met with in the liver, this organ being enlarged, very often yellowish in colour and sometimes showing a mottled appearance; on section, the affected liver was sometimes found to be softer than normal and friable. On closer inspection small focal necrotic areas could very often be seen through the capsula, and on section, these necrotic areas were found to extend throughout the parenchyma. Owing to the obvious change in the liver, many farmers have designated the condition "liver disease" of calves. The mucosa of the gall bladder is often thickened and the bile syrupy in consistence and of a golden green colour. In most cases the spleen was rather swollen and the pulp softer than normal. A catarrhal enteritis was present in many cases, and in one calf small necrotic foci were found in the mucosa of the intestines.

At first the disease was believed by farmers to be a form of septic or infectious pneumonia, but well marked pneumonic lesions were the exception rather than the rule. In some cases atelectatic areas on the lungs were met with, while in rare instances pneumonic areas particularly of the anterior lobes, with fibrinous pleuritis, were observed. Small abscesses were present in these areas and from them

paratyphoid organisms could be isolated. In some cases necrotic ulcers could be demonstrated on the tongue and gums.

MICROSCOPIC LESIONS.

The most noticeable pathological changes occur in the liver and sometimes in the spleen, lymph glands and lungs.

In the liver there are well-defined circumscribed focal areas. Some foci are as large as a central vein, others occupy over half the liver lobule. Some lobules are unaffected, while others may display several foci. These foci seem to be in various stages of development. In apparent early foci there is an infiltration of cells of which the majority are erythrocytes.

Older foci are made up of invading cells and cellular elements showing necrobiosis. These focal areas are of a reddish pink colour, in sharp contrast with the normal purplish red stain of the intact liver cells.

Further studies are in progress to ascertain the exact nature and origin of the cells in the process of necrobiosis.

See Plates 1, 2 and 3.

Paratyphoid Infection.—As mentioned earlier, organisms of the paratyphoid *B. enteritidis* group could be isolated without any difficulty from the blood and internal organs of calves suffering from the disease in the Marico district. At the Onderstepoort Laboratories a few more cases of multiple necrosis of the liver in calves have been brought to our notice, and in these cases we were able to isolate similar organisms in pure culture from the liver, spleen and kidneys. It is quite likely, therefore, that paratyphoid infection of young animals may be much more widespread than is generally believed.

The following is a short history of some of the strains of paratyphoid organisms with which we have worked recently. Some strains are named after the owner of the farm where the disease occurred:—

- (a) *Geel Strain I.*—Isolated in pure culture in the Marico Bushveld from spleen, liver, kidney, and blood stream. Smears from blood and spleen revealed, microscopically, Koch's bodies and *Anaplasma marginale*. This calf was killed for post-mortem examination.
- (b) *Page Strain I.*—Obtained from a sick calf killed for post-mortem investigation. Organisms isolated in pure culture from the liver and spleen. Microscopic examination of blood and spleen smears revealed the presence of Koch's bodies and Anaplasmosis.
- (c) *Robertson Strain.*—Obtained in pure culture from the blood and internal organs. Microscopic examination of blood and spleen smears showed a heavy infection of *Anaplasma marginale*.
- (d) *Rich Strain.*—Obtained from a calf killed while in advanced stage of the disease. Isolated in pure culture from the blood stream and internal organs. Microscopic examination of spleen and blood smears showed heavy infection with *A. marginale*.

- (e) *Du Toit Strain*.—Isolated in pure culture from a calf soon after it died. Slight Anaplasmosis was present.
- (f) *Calf 73 Strain*.—This animal had been in *T. congolense* experiments and finally died, showing general cachexia and multiple necrosis of the liver. The blood showed anaemia but no protozoa were present.
- (g) *Calf 903 Strain*.—This calf was splenectomised and then artificially infected with *A. marginale*. After death multiple necrosis of the liver was present, and the organisms obtained in pure culture from this organ.
- (h) *Page Strain II*.—Obtained from a calf which after death was found to have been suffering from a septic pneumonia and pleurisy. Organisms obtained in pure culture from the spleen.
- (i) *Pretorius Strain*.—Obtained in pure culture from a sick calf which was still alive 24 hours later. Microscopic examination of blood and spleen smears did not reveal the presence of any abnormalities.
- (j) *Geel Strain II*.—Obtained in pure culture from the blood, liver and spleen of a calf soon after death. No blood changes were present.
- (k) *Snyman Strain*.—Obtained from a calf which was killed while in extremis. Isolated in pure culture from the blood and internal organs. Blood and spleen smears showed the presence of *P. bigeminum* and *A. marginale*.

Remarks.—Analysing these cases one finds that the vast majority were not pure paratyphoid infections but were complicated with other pathological conditions. In many cases a protozoan infection was also present and in these cases it is difficult to say whether the paratyphoid infection was of a primary or secondary nature. In cases (f) and (g) the bacterial infection was undoubtedly of a secondary nature, having taken place in animals whose vitality had become lowered very markedly. As far as one could judge cases (i) and (j) were uncomplicated and have to be considered pure paratyphoid infections. The vitality of these animals might, however, have become lowered by means other than protozoan infection. It would appear therefore that in most cases of the natural disease a serious paratyphoid infection takes place only when the vitality of calves has been lowered by some predisposing factor.

BACTERIOLOGY.

Morphology.—Small gram negative rods of irregular size with rounded ends measuring from 0·6–0·8 by 1–3 microns. They vary from almost coccal to bacillary forms. Occasionally a long one is seen in the field of observation.

Motility.—The organisms are actively motile and possessed of peritrichous flagella.

Staining Reactions.—They stain well with all the aniline dyes in common use in the laboratory. In blood or organ smears stained with Giemsa the organisms may be seen singly or in clumps.

CULTURAL FEATURES.

Plain Agar.—On this medium there is a good growth after 16 hours. Individual colonies are of moderate size and show no tendency to spread. They are greyish opalescent, smooth, entire, glistening and translucent.

Gelatin.—On this medium colonies develop with less vigour than on agar. Four days old colonies have a greyish-white appearance. The margin is slightly wavy and centre slightly raised. Gelatin stab shows growth all along the line of inoculation. The medium is not liquefied.

Potato.—After 24 hours incubation on this medium there is a slight, shiny growth of a greyish-yellow hue.

Plain Broth.—This medium becomes turbid within 12 hours, and as the culture becomes older a thin fragile pellicle develops, followed by a greyish-white sediment.

Life Conditions and Properties.—The organisms grow readily on all the ordinary media at a temperature of 25-38° C. They are aerobes and facultative anaerobes. All strains are destroyed by a temperature of 60° C. for one hour. A temperature of 55° C. for one hour fails to destroy them. They are still viable in sealed tubes on plain agar after fifteen months at room temperature. The optimum temperature is 37° C.

(a) *Biochemical Features.*—The following are the more important:—

Indol is not produced. Nitrates are reduced to nitrites. On lead acetate agar there is slight blackening along the needle stab after six hours, and pronounced blackening after 24 hours.

All strains are negative to the Vogis-Proskauer reaction. On litmus milk there is at first a slight initial acidity lasting for about three days, followed in most strains by a total litmus reduction lasting for three to four days; then the medium gradually turns blue again, showing progressive alkalinity with age. After several months the medium becomes semi-translucent.

Milk is not conglutated.

In the following table are shown the reactions obtained with different sugars:—

TABLE I.—CARBOHYDRATE REACTION AFTER 14 DAYS INCUBATION.
 In media consisting of 2 per cent. peptone in distilled water, with 1 per cent. Andrade's indicator, + 1 per cent. of the test substance.

		Saccharose.	Dextrin.	Irethrite.	Inulin.	Raffinose.	Salicin.	Isonite.	Nutrose.	Lactose.	Arabinosc.	Rhamnose.	Galactose.	Glucose.	Mannite.	Sorbito.	Maltose.	Mannose.	Xylose.	Dulcite.	Laevulose.
Geel.....	Acid gas.....	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Page.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Robertson.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Rich.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Du Toit.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Calf No. 73.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Calf No. 903.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Pretorius.....	” ”	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Snyman.....	” ”	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+

It will be seen that all strains, with the exception of Snyman, gave similar reactions and that they are non-isonite and slow arabinose fermenters.

The Snyman strain (*B. aertrycke* group) differs from the other strains (*B. enteritidis* group) in the following respects:—

- (1) It ferments sugar with greater rapidity, especially arabinose.
- (2) It is a constant isonite fermenter.
- (3) It shows slight (if any) reduction of litmus milk.

(b) *Serological Differences*.—To arrive at a final grouping of these organisms, agglutination tests were carried out in connection with typical cultures of *B. enteritidis* and *B. aertrycke*; the latter were very kindly supplied by Dr. E. Jordan of the Chicago University.

Rabbits were immunised with these strains respectively, each rabbit receiving four injections; one intra-peritoneal injection of a killed culture, a week later a subcutaneous injection of a killed culture, and subsequently at a weekly interval two intravenous injections of living culture.

The immune sera so obtained were then tested against emulsions prepared from the following organisms:—

- (1) Two typical non-isonite fermenters, namely our strains (*h*) and (*i*).
- (2) Our Snyman strain which is a constant isonite fermenter.
- (3) The *B. enteritidis* and *B. aertrycke* strains used for immunising the rabbits.
- (4) A *B. enteritidis* strain obtained from Dr. Park-Ross, Durban.

The results are shown in Table No. 2.

TABLE II.—RESULTS OF AGGLUTINATION TESTS WITH SERUM FROM RABBITS IMMUNISED AGAINST *B. aertrycke* AND *B. enteritidis* RESPECTIVELY.

Derivation Strain Emulsion.	Serum.	Serum Dilutions.									
		1-20.	1-40.	1-100.	1-200.	1-400.	1-600.	1-800.	1-1,000.	1-2,000.	1-4,000.
Snyman (calf).....	Rabbit 52.....	-	-	-	-	-	-	-	-	-	-
	„ 244.....	+++	+++	+++	+++	+++	+++	+++	++	++	++
	„ control.....	-	-	-	-	-	-	-	-	-	-
52 (<i>Enteritidis</i>).....	Rabbit 52.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	„ 244.....	-	-	-	-	-	-	-	-	-	-
	„ control.....	-	-	-	-	-	-	-	-	-	-
244 (<i>Aertrycke</i>).....	Rabbit 52.....	-	-	-	-	-	-	-	-	-	-
	„ 244.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
	„ control.....	-	-	-	-	-	-	-	-	-	-
Pretorius (calf).....	Rabbit 52.....	+++	+++	+++	+++	+++	++	++	++	++	++
	„ 244.....	-	-	-	-	-	-	-	-	-	-
	„ control.....	-	-	-	-	-	-	-	-	-	-
Durban (<i>Enteritidis</i>).....	Rabbit 52.....	+++	+++	+++	+++	+++	+++	++	++	++	++
	„ 244.....	-	-	-	-	-	-	-	-	-	-
	„ control.....	-	-	-	-	-	-	-	-	-	-
Page II (calf).....	Rabbit 52.....	+++	+++	+++	+++	+++	+++	+++	++	++	++
	„ 244.....	-	-	-	-	-	-	-	-	-	-
	„ control.....	-	-	-	-	-	-	-	-	-	-

It will be seen that our strains (*h*) and (*i*), non-isonite fermenters, definitely fall in the *B. enteritidis* group and that our Snyman strain corresponds with the *B. aertrycke* type derived from America.

It would seem therefore that Salmonellosis occurring in calves in South Africa is mainly due to *B. enteritidis*.

Pathogenic Properties.—These vary greatly, depending on the age of the strains; when freshly isolated from calves suffering from the natural disease, all strains are pathogenic for small laboratory animals, while older strains that have been kept in the laboratory and sub-cultured for some time, lose their virulence very rapidly.

In small Laboratory animals the following observations have been made:—

Guinea-pigs are susceptible, one c.c. of a 24-hour culture administered subcutaneously proving fatal in from six to ten days and intraperitoneally in from one to nine days. We were not able to kill guinea-pigs by giving cultures per os. All the strains were also proved to be pathogenic for rabbits and white rats. On post-mortem examination of small laboratory animals killed by the organism the following changes are commonly observed: hyperaemia of the subcutis, omental suppuration, fibrinous coagulum on the liver, peritonitis, and very often an excess of fluid in the peritoneal cavity. Necrotic areas in the liver so commonly met with in calves suffering from the natural disease, are only rarely seen in laboratory animals, a few cases of this kind having been observed in guinea-pigs after subcutaneous injection of culture.

In calves of the susceptible age marked disturbances, that may even lead to death, are observed in many cases, provided always freshly isolated strains are used in the experiments.

To illustrate the pathogenic effects of some of the strains of the paratyphoid organisms which we had under observation, the following details of a few experiments are given:—

Case No. 1.—Calf No. 722 was injected subcutaneously on the 16th May, 1923, with $2\frac{1}{2}$ c.c. of a 24-hour old culture. Result: The next day the temperature was up and continued to rise until it reached 107 on the 2nd day (for further details see Chart No. 1). It appeared dull on this day, lying down most of the time. At the point of injection a hard swelling developed, measuring 3 cm. in diameter. On the 3rd day there was also slight rhinitis, but the dullness was less pronounced. No marked diarrhoea was ever observed. From now onwards the symptoms gradually disappeared; the animal was discharged from experiment in apparently normal health.

Case No. 2.—Calf No. 726, received per os on the 17th May, 1923, an emulsion made from a full slant of a 24-hour old culture. Result: The temperature reaction is given in Chart No. 2. On the 18th the calf showed marked dullness, walking with an arched back. On the 19th its condition became very much worse; it showed marked dullness, lying down most of the time; its coat was rough and staring, while the muzzle was very dry; the respiration was hurried and abdominal; the abdomen had a tucked-in appearance and there was a profuse yellowish diarrhoea. During the following few days the same symptoms, in a rather more aggravated form, continued. From the 23rd signs of improvement could be observed, the calf being

brighter and the diarrhoea much less. Improvement continued until the 29th when the diarrhoea was only slight and the calf was sucking from its mother fairly regularly; it was then killed for post-mortem investigation and collection of material. There appears to be no doubt that in spite of the severe illness the calf would have made a complete recovery.

Case No. 3.—Calf No. 583, received per os on the 17th May, 1923, an emulsion of a full slant of a 24-hour old culture. Result: The temperature reaction is shown on Chart No. 3. Symptoms of illness could be observed on the day after treatment, and after that the disease developed rapidly, showing essentially the same symptoms as were described under Case No. 2, namely, marked dullness and depression, staring rough coat, increased pulse and respiration, tucked-up abdomen, profuse foetid diarrhoea, the faeces being of a yellowish colour. The calf died in convulsions on the 10th day after drenching with pure culture. The most marked pathological changes were multiple necrosis in the liver, catarrhal enteritis and atelectatic areas in the lungs. The causal organisms were recovered in pure culture from the various internal organs.

Case No. 4.—Calf No. 545, received per os on the 18th May, 1923, half a slant of a 24-hour old culture. Result: The temperature record is shown in Chart No. 4. From the 19th the calf showed dullness, a partial loss of appetite which lasted for only a few days. Complete recovery took place.

Case No. 5.—Calf No. 706 received per os a full agar slant of a 24-hour culture. The organisms were in their second generation, and isolated from calf 583. The temperature Chart No. 5 shows a typical reaction. The calf made a good recovery.

Owing to the difficulty of obtaining freshly isolated strains, no further transmission experiments could be carried out until March, 1926. This time each calf received 5 c.c. subcutaneously + 20 c.c. per os. Two out of the three died with typical symptoms of the natural disease, while the third recovered after passing through a severe illness. In both fatal cases a well-marked general icterus was present, which may be due to the bile stasis so common in this disease. In one case tiny necrotic areas could be seen in the liver.

The temperature reactions of calves Nos. 1939, 1950 and 1951 are shown in Charts Nos. 6, 7 and 8.

From the results of these experiments it becomes quite clear that *B. enteritidis* organisms of calves can produce marked pathogenic effects even when other factors are absent. From all fatal cases these organisms were recovered again in pure culture.

These calves were in splendid condition and kept under ideal hygienic conditions. Had there been other factors concerned in reducing their vitality the results might have been even more conclusive.

Immunity against Calf Paratyphoid Infection.

As soon as it was realised that organisms of the paratyphoid *B. enteritidis* group were the main causal factor of the calf disease under discussion, it was thought advisable to attempt the preparation of an efficient vaccine for prophylactic use in the infected area. It

was hoped by eliminating at least one factor the mortality might be reduced to a great extent. The usual technique for preparing anti-typhoid vaccine was employed in this case.

The safety and efficacy of the vaccine were first of all tested out on small laboratory animals. By two subcutaneous and one intraperitoneal injections of a dead vaccine, with an interval of a week between each injection, we were able to immunize rabbits against a fatal dose of Calf *B. enteritidis*. Control rabbits receiving the same dose died in all cases.

During the summer 1923-1924 over a thousand calves in the Marico district received an injection of the vaccine. We thus had the opportunity of studying the immediate effects of the vaccine and found the position to be as follows:—

The majority of calves tolerate the bacterin very well, no serious disturbance being set up in any way. In these cases there is a slight local swelling lasting for a day or two, as well as a mild temperature reaction persisting for a few days. On the other hand, a small percentage (about six) of calves react more severely, apparently due to a well-marked hypersensitiveness to the bacterin. In these cases even such a small dose of bacterin as 1 c.c. may produce grave symptoms, while the less sensitive calves will tolerate as much as 10 or 20 c.c. of the same vaccine without showing any visible reaction. In hypersensitive animals the symptoms come on within a few minutes after injection of the bacterin; the calf shows an anxious expression, unsteady gait, accelerated breathing and rapid pulse, commonly diarrhoea; there may be quivering of the muscles, particularly in the gluteal region. Very often the animal goes down, lying on its side with its legs stretched out, or on its sternum with the head between the fore legs. In the majority of cases these rather alarming symptoms pass off within an hour or so, and usually recovery is complete in about three hours. In two cases out of about 1,000 calves inoculated fatal results were obtained, death taking place within half an hour after the onset of symptoms, and apparently due to oedema of the lungs.

During the summer 1924-1925 several thousand doses of anti-paratyphoid vaccine have been distributed amongst the farmers in the affected area, but up to the time of writing it has not been possible to collect the results obtained from its use.

It is hoped to discuss the question of immunity against Paratyphoid more fully in a later paper, when the antibody production in calves injected with bacterins will be discussed in detail.

The use of anti-sera is also under consideration and will be reported on at a later date.

SUMMARY AND CONCLUSIONS.

1. It would appear that organisms of the Paratyphoid *B. enteritidis* group are of pathogenic significance in South Africa, and deserve serious consideration when the study of diseases involving the alimentary tract of young animals is undertaken.

2. These organisms are probably widespread in South Africa, although it should be stated that up to now no definite observations in connection with their distribution have been made.

There appears to be no doubt that they possess a very marked variation in virulence and that their virulence can be exalted by passage through susceptible animals under certain favourable conditions.

3. These conditions seem to be connected with the climate of the locality, farming methods, and other factors fully discussed elsewhere in this report.

4. It has been shown that organisms recently isolated from calves can be highly pathogenic for this class of animal, but that fatal results are not usually produced in calves that are strong, healthy and well nourished.

It would appear, therefore, that the paratyphoid organisms are often *secondary invaders*, exerting their pathogenic effects in calves whose vitality has been lowered by other factors. Among these factors must be mentioned:—

- (a) Improper and irregular feeding and bad hygienic conditions which predispose or lead to digestive disturbances.
- (b) Infection with Piroplasms and Anaplasmas. In the Marico district Paratyphoid infection was commonly found as a complication to Anaplasmosis and Piroplasmosis. At Onderstepoort Paratyphoid infection was found in two cases of Anaplasmosis artificially produced in splenectomised animals.
- (c) Infection with Trypanosomiasis. At Onderstepoort one such case was met with in a calf that had been infected artificially with *T. Congolense*.
- (d) Infection with *Coccidia* and *Gardia bovis* have also been diagnosed in Marico in a few cases of red scour by us, but Paratyphoid infection has so far not been established definitely in these infections.

5. Incidentally these observations led to the discovery of Koch's bodies not belonging to the developmental cycle of *Theileria parva*. This parasite may not be of any great pathogenic significance, but its occurrence in the Union constitutes a complicating factor in so far as the microscopic diagnosis of East Coast fever is concerned.

6. Apart from anti-paratyphoid vaccination, preventive measures against this calf mortality consist in practising sound methods of animal husbandry and adopting general hygienic principles. Among the latter must be mentioned tick eradication by regular short interval dipping continued over a long period.

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