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Eperythrozoonosis in Cattle.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

Introduction.

Adler and Ellenbogen (1934) saw Eperythrozoa in blood smears from a splenectomized calf; these they named *Eperythrozoon wenyoni*. Similar parasites were observed independently by Neitz and Quinlan (1934) in smears from several splenectomized calves. A detailed study of this infection could not be undertaken because under the conditions of the experiments accidental natural infection could not be excluded. Further, the clinical picture in a number of the animals was complicated by concurrent infections of piroplasmosis, anaplasmosis, theileriasis, or spirochaetosis.

Definition.

Eperythrozoonosis of cattle is an infectious disease, caused by *Eperythrozoon wenyoni*, a small supra- or extracellular blood parasite having a ring, rod, irregular or oval shape, and belonging to the family Anaplasmidae. Infection by the microorganisms may result in the production of a mild fever and a slight anaemia. Relapses may occur. The mode of transmission is not known, but it is believed that the vector is a blood-sucking arthropod; probably a louse.

Occurrence.

Little is known about the geographical distribution of *Ep. wenyoni*. Its occurrence has been recorded by Adler and Ellenbogen (1934) in a splenectomized calf in Palestine, by Neitz and Quinlan (1934) in splenectomized and non-splenectomized cattle in South Africa; by Hering* (1934) in California, U.S.A., by Delpy (1936), in Iran, by Donatien and Lestoquard (1937) in Algeria, and by Nieschulz (1938) in Holland, all in non-splenectomized animals. The infection recorded by Nieschulz was produced with blood imported into Holland from Algiers.

* Personal communication.

In South Africa *Ep. wenyoni* has been observed in cattle reared and maintained at Onderstepoort; in cattle bred at Vryburg in the North-West Cape Province subsequent to exposure at Tzaneen in the Northern Transvaal; in a cow at Sabie in the Eastern Transvaal; in a calf at Warmbad in the Northern Transvaal and in an ox at Reitz in the Orange Free State. The latter case was complicated by a concurrent infection of anaplasmosis.

Aetiology.

(*a*) *Morphology.*

Neitz, Alexander and du Toit (1934) suggested that the four genera, *Anaplasma*, *Bartonella*, *Eperythrozoa* and *Grahamella*, should be included in the family Anaplasmidae. Although no morphological differences can be detected between *Ep. ovis* and *Ep. wenyoni* it will be seen from the experiments described below that they must be considered distinct species.

When stained with Giemsa the micro-organisms take on a pinkish purple colour. They are approximately 0·3 to 1·5μ in diameter. The typical form is a delicate ring, but ovoid, comma, rod, dumb-bell and tennis-racket forms are quite common (see photomicrographs, Figs. 1-5). At the thick end of the smear the ring-forms predominate, while towards the thin end the rod and comma forms are more common. This distribution is mechanical, and is brought about during the process of drawing the blood film. Apparently multiplication takes place by budding and by fission.

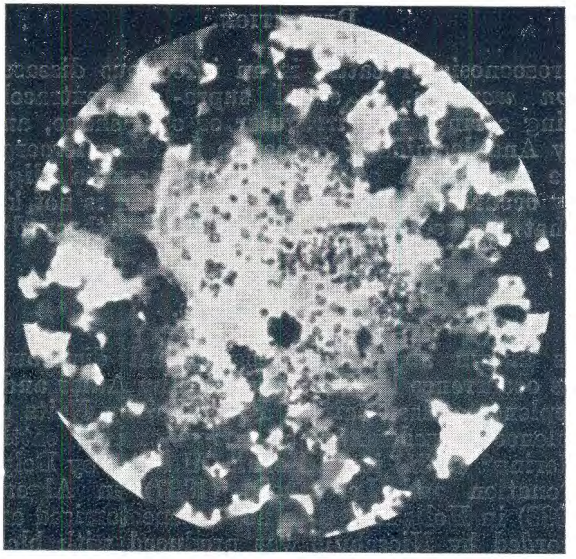

Fig. 1.—*Eperythrozoon wenyoni* in a blood smear from a splenectomized calf; 1,330×.

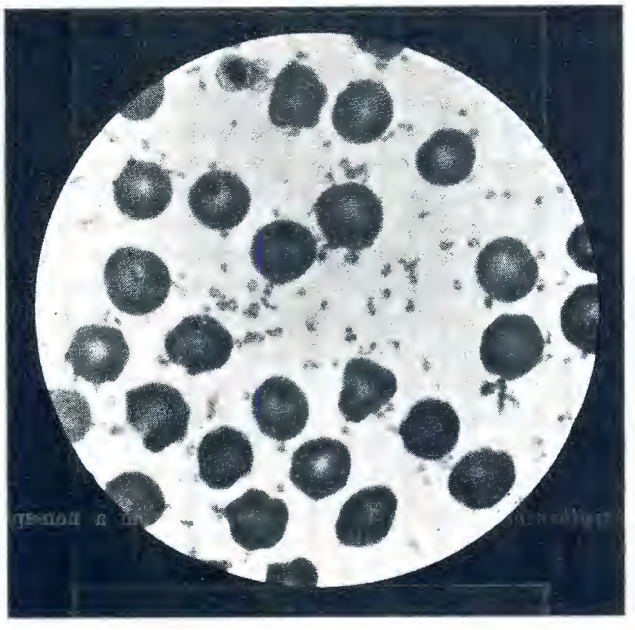

Fig. 2.—*Eperythrozon wenyoni* in a blood smear from a splenectomized calf; 2,000×.

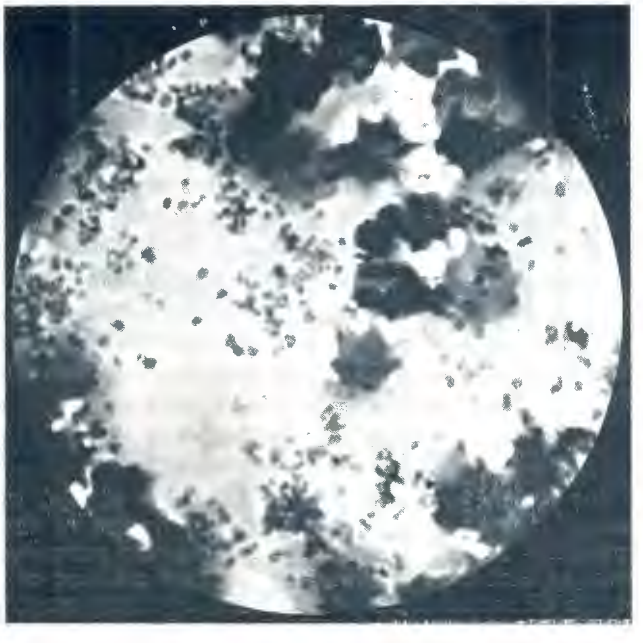

Fig. 3.—*Eperythrozoon wenyoni* in a blood smear from a splenectomized calf; 2,000×.

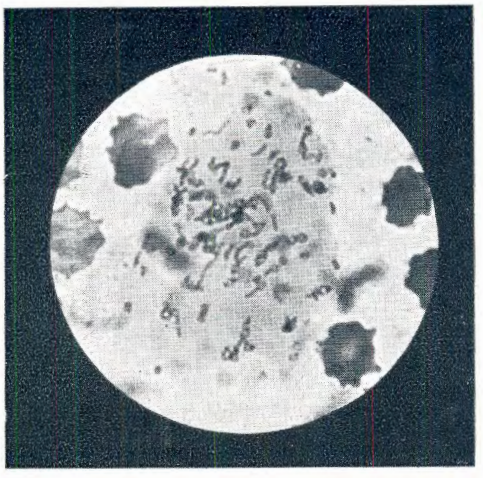

Fig. 4. *Eperythrozoon wenyoni* in a blood smear from a non-splenectomized calf; 1,500×.

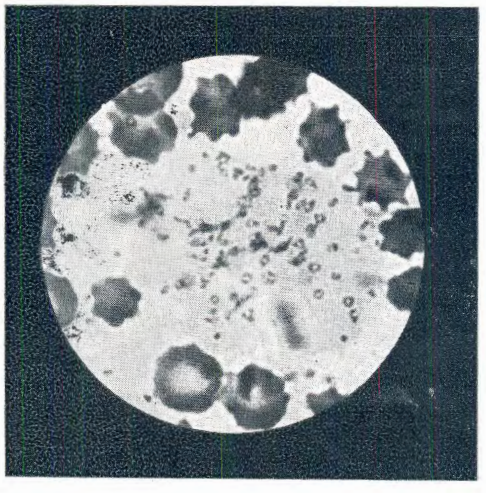

Fig. 5. *Eperythrozoon wenyoni* in a blood smear from a non-splenectomized calf; 1,500×.

(*b*) *Localization.*

It is not known whether the distribution of *Ep. wenyoni* in stained smears is a true picture of the distribution of the parasites in the circulating blood. The union between the parasites and host cells is loose, so that it is possible that a large number of free forms may simply be detached during the process of smear preparation. It has frequently been observed that the proportion of supracellular and extracellular forms varies considerably in different preparations made from the same animal at the same time.

1. *Supracellular Forms.*

A single parasite may be present on an erythrocyte. On the other hand its entire surface may be dotted with parasites varying in number from 20 to 60. The parasites may be superimposed on each other. Most commonly the supracellular forms are to be found in clusters of 3 to 12 aggregated towards the centre, or the periphery or actually around the circumference of the erythrocyte.

2. *Extracellular or Free Forms.*

These forms usually predominate. In thick smears where the erythrocytes are closely packed together the interstices may appear to be filled with a homogeneous mass which stains in a manner similar to the parasites.

Transmission.

During the course of some work on protozoon diseases four calves which had been born and bred at the experimental station Armoedsvlakte, Vryburg, were exposed to tick infestation under natural conditions, three at Tzaneen in the Northern Transvaal, and one at Spitzkop in the Eastern Transvaal. These calves were selected for the following reasons:—(1) Careful observation over a number of years has shown that very few ticks are found at Armoedsvlakte, so that the chance of prior tick infestation was very limited. (2) Extensive experimental work has shown that the great majority of cattle from Armoedsvlakte are fully susceptible to anaplasmosis, piroplasmosis, and other tick-borne diseases of South Africa.

The calves became very heavily infested with ticks, chiefly *Rhipicephalus appendiculatus*, *Boophilus decoloratus*, *Hyalomma aegyptium*, *Amblyomma hebraeum*, and *Rhipicephalus evertsi*. So heavy was tick infestation that the animals suffered severely from the direct effects as evidenced by marked swellings of the ears, eyelids and superficial lymphatic glands and development of abscesses. In addition specific infections of *Theileria mutans* and *Spirochaeta theileri* appeared. Subsequently *Ep. wenyoni* were demonstrated. (For detail see Table 4.) From these observations the tentative conclusion appeared warranted that one or more of the species of ticks had transmitted the Eperythrozoan infection. It was then decided to carry out a series of carefully controlled experiments to throw further light on this point.

It is well known that splenectomy of animals harbouring a latent infection of genera of the family Anaplasmidae is followed by their appearance in large numbers in the blood stream. That this observation holds good for the genus Eperythrozoon is apparent from a consideration of the results of splenectomy of a heterogeneous collection of animals detailed in Table 5. It will be seen that in every instance *Ep. wenyoni* appeared subsequent to splenectomy.

To insure the initial susceptibility of the animals to be used in the experiments, four calves which had been born and bred under carefully controlled tick-free conditions were splenectomized. The subsequent history of these animals is tabulated in Table 1. It is

seen that two of the four showed a relapse of Eperythrozoonosis after an interval of 40 days, while two remained free for a period of over two months. It must be emphasized that the conditions under which these animals were maintained was such that any possibility of accidental tick infestation may be excluded with every confidence. In addition accidental transmission during the process of preparing blood smears was eliminated by using separate sterile instruments for each animal. On the other hand it was noticed that lice *Bovicola bovis* and *Linognathus vituli* were present in fairly large numbers.

It was concluded therefore that rearing calves under tick-free conditions is no guarantee of their freedom from infection with Eperythrozoa and for experimental work of this nature it is essential to use animals whose full susceptibility is proved by the results of splenectomy, if the conclusions drawn for the experiments are to have any value.

At this stage it was decided to modify the programme of work. Previous experience with tick transmission experiments has shown that to work out the cycle in the invertebrate host large numbers of susceptible animals are essential. Rather than use the only two susceptible bovines available, it was thought that if *Ep. wenyoni* of cattle is identical with *Ep. ovis* of sheep, the work could be completed more easily on a larger number of splenectomized sheep which could easily be made available. Consequently transmission experiments with cattle were temporarily abandoned.

Discussion.

Donatien and Lestoquard (1937) claimed that *Ep. wenyoni* is transmitted by ticks. They obtained adult ticks of an undetermined species of *Hyalomma* from Iran. These ticks were fed on two bulls; 14 days later *Th. dispar* appeared and 8 days later *Ep. wenyoni*. From this they concluded that infection had been transmitted by ticks, but from the observations recorded above it is immediately apparent that the conclusion was not justified, since, in the first place, no attempt was made to determine whether the bulls harboured a latent infection or not. The fact that parasites appeared in the blood stream of calves reared under tick-free conditions subsequent to splenectomy indicates strongly that ticks definitely are not the only transmitters.

The ectoparasites which were common to the calves under the conditions of the experiment were lice, so that the rôle of these arthropods as transmitters must be taken into serious consideration.

Elliot 1936 showed experimentally that the vector of *Eperythrozoon coccoides* of the mouse is the louse *Polyplax serrata*. It is considered that this is the only case where the transmission of an *Eperythrozoon* is supported by adequate experimental data.

Relationship of *Ep. wenyoni* and *Ep. ovis*.

It has not been possible to demonstrate any morphological difference between *Ep. wenyoni* and *Ep. ovis*. Neitz (1937) stated that *Ep. ovis* is pathogenic for calves, thus indicating the probable

identity of the two species. In view of the more carefully controlled experiments detailed below that statement must now be corrected. A susceptible splenectomized calf 6289 (see Table 1) was given a subcutaneous injection of 10 c.c. blood from a calf 6049, which was passing through a relapse to *Ep. wenyoni* after splenectomy. Parasites were demonstrated intermittently from the 11-55th day after injection. It was therefore concluded that *Ep. wenyoni* may be transmitted to a susceptible calf from an infected calf by the subcutaneous injection of blood. A second susceptible splenectomized calf 6295 received 10 c.c. of blood from a sheep 41839 which was a known carrier of *Ep. ovis*. Parasites were demonstrated intermittently from the 6th to the 34th day after injection and were associated with a marked anaemia (Table 1).

From this experiment it appeared justifiable to conclude that a susceptible calf may be infected by the subcutaneous injection of sheep blood containing *Ep. ovis*. It would therefore appear that the two parasites are species identical. To confirm this conclusion it was decided to transfer the infection from the calf back to susceptible splenectomized sheep bearing in mind that Neitz (1937) had shown that sheep may be infected with *Ep. ovis* by the subcutaneous route. Two splenectomized sheep (Table 2) which had shown no recrudescence of infection after the operation were injected subcutaneously with 5 c.c. of blood from calf 6295. Smears were examined daily for 5 weeks but no parasites were seen. The result of this experiment was so striking that it was deemed essential to continue the investigation.

Three non-splenectomized lambs whose blood had failed to reveal the presence of Eperythrozoa during the previous 7 weeks and 5 splenectomized sheep which remained clean for from 5-10 weeks after splenectomy received injections of cattle blood known to contain large numbers op *Ep. wenyoni* (Table 2). In no single instance did the injection result in infection of the sheep.

The 3 lambs and one of the splenectomized sheep subsequently were found to be fully susceptible to *Ep. ovis;* the remaining 3 sheep were utilized for other experiments. From this it can be concluded that sheep susceptible to *Ep. ovis* are refractory to infection with *Ep. wenyoni.*

Discussion.

Since conflicting results appear to have been the outcome of the experiments recorded, a careful analysis is essential. The appearance of Eperythrozoa in the blood of a susceptible splenectomized calf subsequent to an injection of blood containing *Ep. ovis* warranted the conclusion that calves are susceptible to the sheep parasite. If this actually was the case it should have been possible to transfer infection from the calf back to susceptible sheep. The attempt failed. Further it was found to be impossible to infect 8 other susceptible sheep with *Ep. wenyoni.* The logical explanation would therefore appear to be that the calf became accidentally infected with *Ep. wenyoni* by some unknown vector, certainly not a tick but possibly a louse, and that

the appearance of the parasites in the blood stream happened to coincide with the time that they could have been expected after the injection of the infective blood. It is finally concluded therefore that *Ep. wenyoni* and *Ep. ovis* are distinct species infective for cattle and sheep respectively. The experiments serve to emphasize the necessity of exercising the greatest care in planning work of this nature, and of drawing conclusions only after exhaustive investigation.

Pathogenesis.

The Eperythrozoa parasitize the erythrocytes, but it is not clear how destruction is brought about. In non-splenectomized animals a mild or no anaemia has been observed, whereas in several of the splenectomized calves severe anaemia and icterus resulted from the infection. The blood showed a drop in the red cell precipitate obtained on centrifugation, and the red cell count fell as low as 1,200,000 per c.cm. The decrease in the number of erythrocytes commences at the time of the first appearance of parasites. There is also a rise in the leucocytic count up to 37,000 per c.cm. Degenerative and regenerative processes namely anisocytosis, polychromasia, punctate basophilia, reticulocytes, Jolly bodies and normoblasts are seen in stained smears. Erythrophagocytosis is well marked. Haemoglobinuria has only once been observed, viz., in a non-splenectomized bovine, which was reacting to *Ep. wenyoni*, *Th. mutans* and *Sp. theileri*.

Symptoms.

The symptoms and course of the disease have been studied in a very limited number of non-splenectomized and splenectomized cattle.

A. *Observations in Non-splenectomized Cattle.*

1. In the experimentally infected cattle mentioned in Table 3 it will be seen that the incubation period varied from 16-22 days. The parasites could be demonstrated microscopically for a period of 1 to 8 days. No relapses were observed in any of the animals. A slight anaemia was observed in two of them. Febrile reactions and clinical symptoms were absent. If daily blood smears had not been examined, the infection would have passed unnoticed.

2. In the cattle mentioned in Table 4 the latent infection of *Ep. wenyoni* was activated by the direct effects of the heavy tick infestation and by the severe *Th. mutans* reaction. The parasites could be demonstrated microscopically for a period of 4-13 days, but relapses were not observed. The severe anaemia and the haemoglobinuria were in all probability also partially due to the theileriasis and spirochaetosis present.

Table 1.

Splenectomy of Calves reared under Tick-free Conditions.

Number of calf.	Date of birth.	Date of splenectomy.	*Ep. wenyoni* appeared after.	Remarks.	Subsequent History.		
					Injected from.	Date.	Remarks.
6049	20/3/34	19/11/34	40 days	*Ep. wenyoni* seen for 11 days. A slight anaemia resulted from the infection	—	—	—
6050	27/3/34	14/11/34	40 days	*Ep. wenyoni* seen for 11 days. A slight anaemia resulted from the infection	—	—	—
6289	27/7/34	15/11/34	—	Blood smears were negative for a period of 75 days	Splenectomized calf 6049 harbouring *Ep. wenyoni*	10 c.c. blood subcut.	*Ep. wenyoni* could be demonstrated on the 11th—17th, 20th—21st, 28th, 38th—44th and again on the 52nd—55th days after injection. Anaemic changes, namely basophilia, polychromasia, anisocytosis and Jolly bodies were observed in the blood smears.
6295	17/8/34	15/11/34	—	Blood smears were negative for a period of 75 days	Sheep 41839 harbouring a latent infection of *Ep. ovis*	10 c.c. blood subcut.	Eperythrozoa were seen on the 6th–8th, 14th–15th, 24th, and 26th–34th days after injection. At the time of the first appearance of parasites the red blood count was 10×10^6 and the white blood count 8,400. A progressive anaemia developed and on the 37th day the red blood count dropped to $1{\cdot}2 \times 10^6$ and the white blood count rose to 37,300. The blood showed basophilia, anisocytosis, polychromasia, normoblasts, Jolly bodies and erythrophagocytosis. The anaemia gradually disappeared and on the 60th day the red blood count was $5{\cdot}47 \times 10^6$, and the white blood count 13,400. Blood from this calf was injected into two splenectomized sheep 41473 and 41474 mentioned in Table 2.

Table 2.

Attempted Transmission of Eperythrozoon wenyoni *to Sheep.*

		Eperythrozoon wenyoni infection.				*Eperythrozoon ovis* infection.			
Number of sheep.	Date of birth.	History.	Injected from.	Dose of blood i.v.	Result.	Injected from.	Dose of blood i.v.	Incub. period.	Result.
41214	9/8/34	Susceptible nonsplenectomized lamb	Splenectomized calf 5425 actively infected with *Ep. wenyoni* and *Anaplasma centrale*	5 c.c.	Blood smears were examined daily for a period of 7 weeks but *Ep. wenyoni* did not appear	Sheep 41588, a carrier of *Ep. ovis*	5 c.c.	4 days	*Ep. ovis* present from 4th–15th day. Parasites were very frequent. Lamb died on the 17th day, and at autopsy there was a generalized anaemia and icterus, a tumour splenis, an acute fibrinous pericarditis and pleuritis.
41592	10/10/34	Susceptible nonsplenectomized lamb	Splenectomized calf 5425	5 c.c.	Blood smears were examined daily for a period of 7 weeks but *Ep. wenyoni* did not appear	Sheep 41588, a carrier of *Ep. ovis*	5 c.c.	4 days	*Ep. ovis* present from 4th–10th day, i.e., until death. Parasites were frequent. Lamb died on the 10th day and at autopsy there was a hydropericard, subepicardial haemorrhages, oedema of the pharangeal region, tumour splenis, and hyperplasia of the malpighian corpuscles.
41593	10/10/34	Susceptible nonsplenectomized lamb	Splenectomized calf 5425	5 c.c.	Blood smears were examined for a period of 7 weeks but *Ep. wenyoni* did not appear	Sheep 41588, a carrier of *Ep. ovis*	5 c.c.	4 days	*Ep. ovis* present from the 4th–8th day, i.e., until death. Lamb died on the 8th day and at autopsy there was a hydropericard, hydrothorax, ascites, fibrinous peritonitis and a catarrhal enteritis.
41475	10/9/34	Susceptible splenectomized sheep	Splenectomized calf 6289 actively infected with *Ep. wenyoni*	5 c.c.	Daily blood smears were examined for 5 weeks but *Ep. wenyoni* did not appear	—	—	—	—
41476	10/9/34	Susceptible splenectomized sheep	Nonsplenectomized calf 7049 actively infected with *Ep. wenyoni*	10 c.c.	Daily blood smears were examined for 11 weeks but *Ep. wenyoni* did not appear	Sheep 47040, carrier of *Ep. ovis*	10 c.c.	8 days	*Ep. ovis* appeared in large numbers, and marked anaemic changes were observed in the blood smears.

TABLE 2 (*continued*).

Eperythrozoon wenyoni infection.						*Eperythrozoon ovis* infection.			
Number of sheep.	Date of birth.	History.	Injected from.	Dose of blood i.v.	Result.	Injected from.	Dose of blood i.v.	Incub. period.	Result.
41479	10/9/34	Susceptible splenectomized sheep	Nonsplenectomized calf 7075 actively infected with *Ep. wenyoni*	20 c.c.	Daily blood smears were examined for 10 weeks but *Ep. wenyoni* did not appear	—	—	—	—
41486	13/9/34	Susceptible splenectomized sheep	Nonsplenectomized calf 7075 actively infected with *Ep. wenyoni*	20 c.c.	Daily blood smears were examined for 10 weeks but *Ep. wenyoni* did not appear	—	—	—	—
41473	10/9/34	Susceptible splenectomized sheep	Splenectomized calf 6295 that reacted to Eperythrozoa after receiving blood from a sheep harbouring *Ep. ovis*. (See Tbl. 1.)	5 c.c.	Daily blood smears examined for a period of 5 weeks but no parasites appeared	—	—	—	—
41474	10/9/34	Susceptible splenectomized sheep	Splenectomized calf 6295 that reacted to Eperythrozoa after receiving blood from a sheep harbouring *Ep. ovis*. (See Tbl. 1.)	5 c.c.	Daily blood smears were examined for a period of 5 weeks but no parasites appeared	—	—	—	—

Table 3.

Observations in Non-splenectomized Cattle.

No. of animal.	Expt. No.	Date of birth.	Date of injection.	Injected from.	*Ep. wenyoni* seen on.	Remarks.
7049	S 5856	4/1/36	27/11/36	Cow 6440.....	20th–26th day.	Parasites fairly frequent and a mild anaemia developed. A febrile reaction was not observed.
7075	S 5856	14/2/36	14/1/37	Calves, 7007, 7009 and 7010	16th–19th day.	Parasites very rare. No anaemia and no febrile reaction was observed.
7191	S 6544	10/6/36	23/5/37	Heifer 7168....	19th day only.	Parasites very rare. No anaemia and no febrile reaction was observed.
7367	S 6544	3/4/37	7/10/38	Cow 6438.....	19th–20th day.	Parasites very rare. No anaemia and no febrile reaction was observed.
7411	S 6544	5/7/37	7/10/38	Cow 6438.....	19–22nd day...	Parasites very rare. No anaemia and no febrile reaction was observed.
7468	S 5946	10/11/37	26/9/38	Heifer 7009....	22nd day only.	Parasites very rare. No anaemia and no febrile reaction was observed.
7467	S 5856	8/11/37	9/5/38	Heifer 7170....	20th–27th day.	Parasites rare. Slight anaemia but no febrile reaction was observed.

Table 4.

Observations in Non-splenectomized Cattle exposed to Tick Infestation.

No. of calf.	Date of birth.	Origin.	Period of exposure.	Locality.	Species of ticks.	Period of febrile reaction.	Koch's bodies.	*Th. mutans* seen on.	*Ep. wenyoni* seen on.	Remarks.
6901	16/2/37	Armoedsvlakte	21/2/38–7/3/38	Tzaneen	Chiefly *Rh. appendiculatus*, and to a lesser extent *B. decoloratus*, *Hyal. aegyptium*, *Amb. hebraeum*, *Rh. evertsi*	1/3/38–12/3/38	9/3/38–11/3/38	If animal had not died *Th. mutans* would have appeared	16/3/38–19/3/38	A second febrile reaction was seen from 18/3/38–24/3/38. On 24/3/38 ox showed a marked haemoglobinuria and icterus. No blood parasites other than *Ep. wenyoni* and *Sp. theileri* were seen during the second febrile reaction. Calf received 50 c.c. of a 1% solution of Trypan blue on the 24/3/38, but died the same day.
6725	23/11/36	Armoedsvlakte	21/2/38–7/3/38	Tzaneen	As in the case of 6901	2/3/38–31/3/38	10/3/38–17/3/38	28/3/38	11/3/38–23/3/38	*Spirochaeta theileri* was seen on 28 and 29/3/38. Anaemia, i.e., basophilia, polychromasia anisocytosis was observed. Animal recovered.
6732	30/11/36	Armoedsvlakte	21/2/38–7/3/38	Tzaneen	As in the case of 6901	1/3/38–7/3/38	Although the reaction was similar to that of 6725 Koch's bodies were not seen	18/3/38	16/3/38–26/3/38	Anaemia, i.e., basophilia, polychromasia, anisocytosis was observed. Animal recovered.
5794	22/11/34	Armoedsvlakte	22/4/36–26/9/37	Spitzkop	Chiefly *Rh. appendiculatus*	No febrile reaction	–		23/11/36	Parasites very frequent. Smears examined on one day only, and nothing can, therefore, be said as to whether an anaemia developed or not.

B. *Observations in Splenectomized Cattle.*

1. In the four calves which reacted to a pure infection of *Ep. wenyoni* the following symptoms were noticed:—

A relapse to *Ep. wenyoni* was seen 40 days after splenectomy in calves 6049 and 6050 mentioned in Table 1. The parasites were demonstrated microscopically for a period of 11 days. A slight anaemia resulted from the infection, but no febrile reaction and no clinical symptoms were observed.

In the artificially infected calf 6289, *Ep. wenyoni* appeared on the 11th day and was demonstrated microscopically for a period of 8 days. Four parasitic relapses were observed after the primary reaction. Anaemia, icterus and a mild febrile reaction but no other clinical symptoms were observed.

In the naturally infected calf, 6295, mentioned in Table 1, *Ep. wenyoni* produced severe anaemia and icterus. Three relapses followed the primary appearance of parasites. A febrile reaction, inappetence and general weakness were observed, but the animal recovered without treatment.

2. The three cattle mentioned in Table 5 were inoculated with blood, which at the time of bleeding contained *Piroplasma bigeminum* and *Anaplasma centrale*, but which had been stored at room temperature for periods of 48 and 72 hours. Reactions due to both the mentioned parasites were observed in ox 5435 which had received 48-hour old blood, whereas the two heifers 5425 and 5432 which received 72-hour old blood reacted only to *A. centrale*. The splenectomy caused a recrudescence of the mentioned parasites, as well as that of *Ep. wenyoni*, which had not been previously observed in any of the animals. Severe anaemia was noticed in all the animals. It was possible by timely intervention to control the reactions of *P. bigeminum* with trypan blue and acaprin, and that of *Ep. wenyoni* with neosalvarsan.

The behaviour of the various parasites in the animals is extremely interesting. It will be seen from Table 5 that the calves were re-injected at a later date with blood which contained *P. bigeminum* *A. centrale* and *Th. mutans*. Daily blood smears of cattle 5425 and 5435, were examined for a period of 5 years after splenectomy.

(*a*) *P. bigemium* was harboured by cattle 5425 and 5435 for a comparatively short period. Ox 5435 was reinfected and it was necessary to control the reaction with acaprin. This animal lost its infection again.

(*b*) *A. centrale* and *Th. mutans* were demonstrated regularly in blood smears for a period of 5 years. It appears that cattle remain carriers of these parasites indefinitely.

(*c*) In heifer 5425 thre relapses of *Ep. wenyoni* were observed up to 61 days after the primary reaction, but no further relapses were observed during the subsequent 5 years. In ox 5435 on the other hand, *Ep. wenyoni* was seen again 1,456 days after the primary reaction, but it is not clear whether this finding was a relapse, or whether it was due to a reinfection.

Prognosis.

In the limited number of animals under observation it has been found that the course of the disease is extremely mild in non-splenectomized cattle. There was no mortality.

Pathological Anatomical Changes.

As no animals died or were slaughtered, an opportunity to study the pathological changes did not present itself.

Diagnosis.

The diagnosis is dependent upon the microscopic demonstration of the parasites in the blood smears. In cases of anaemia and icterus the possibility of Eperythrozoonosis must be taken into consideration.

The presence or absence of a latent infection can be determined by subinoculating blood into susceptible animals.

Treatment.

Since this infection produces mild or no symptoms in non-splenectomized animals treatment is unnecessary. Occasion, however, may arise where treatment has to be undertaken. In such cases the use of neosalvarsan and the arseno-stibio compound Std. 386 B, which have been shown to act specifically on *Ep. oris* by Neitz (1937) are recommended.

Neosalvarsan has been used in two cattle mentioned in Table 5. In heifer 5425, weighing approximately 150 Kg., 0·9 gms. of neosalvarsan caused the parasites to disappear after 30 minutes. A reappearance of the parasites was observed 15 days later, which indicates that sterilization was not brought about. In the other heifer, 5432, weighing approximately 180 Kg., 1·35 gms. of neosalvarsan were administered. The parasites in this animal could still be demonstrated in small numbers after 24 hours, and a second dose of 0·9 gms. was given. The blood smears which were examined subsequently after a period of 112 days were free of parasites. No attempts were made to ascertain whether sterilization resulted from the treatment in either of the above cases.

Immunity.

From the observations on splenectomized and non-splenectomized calves, it appears that the immunity is due to a "labile infection" or an "immunitas non sterilisans", which leads to an equilibrium between parasites and the host.

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TABLE

Observations in Splen

No. of calf.	Date of birth.	Date of injection.	Injected from.	Blood stored for.	Incubation period.		Remarks.	Date of splenectomy.	Total period of observation.	
					Parasite.	No. of days.				Parasite.
5425	10/7/33	11/11/33	5 c.c. blood subcut. of bovine 5279 a carrier of *P. bigeminum* and *A. centrale*	72 hours	*A. centrale*	38	*A. centrale* produced a very mild reaction. Daily blood smears were examined until 6/9/34 but no other parasites were seen besides *A. centrale*	6/9/34	5 years	*A. centrale* *Ep. wenyoni*
5432	22/7/33	11/11/33	5 c.c. blood subcut. of bovine 5279, a carrier of *A. centrale* and *P. bigeminum*	72 hours	*A. centrale*	31	*A. centrale* produced a very mild reaction. Daily blood smears were examined until 11/9/34 but no other parasites besides *A. centrale* were noticed	11/9/34	106 days	*A. centrale* *Ep. wenyoni*

25-26b →

TABLE 5.

ations in Splenectomized Cattle.

Relapse.		Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which parasites could be seen.	Nature of infection.	Remarks.
		SPLENECTOMY.					
Appeared after.	Seen for.						
5 days	18 days	*A. centrale* and *Ep. wenyoni* produced a very severe anaemia. Before splenectomy the blood count was erythrocytes 7 million and leucocytes 16,700. After 21 days the count was, erythrocytes 1·76 million and leucocytes 16,300. The animal was very weak and 3,000 c.c. blood was transfused into the calf. A gradual improvement was noticed and the calf appeared quite normal several weeks later	3	7	3	2+	Anaemia.
				7	21	5+	Anaemia.
				7	1,775	2+	At first there was anaemia but then blood became normal.
9 days	10 days	*Ep. wenyoni* was extremely numerous from the 7th to 9th day after first appearance of parasites. On the 10th day calf received 0·9 gm. neosalvarsan intravenously, which caused the parasites to disappear after 30 minutes. Parasites reappeared again 15 days later	3	15	17	3+	
				2	1	2+	
				14	2	2+	
		On 15/1/35 calf received 25 c.c. blood intravenously of heifer 5276 harbouring *A. centrale*, *P. bigeminum*, and *Th. mutans*. No reaction to *P. bigeminum* resulted. On 7/2/35 calf received 10 c.c. blood of calf 6289 harbouring *P. bigeminum*, *A. centrale* and *Th. mutans*. Four days later *P. bigeminum* appeared and the reaction was controlled by the use of Trypan blue and Acaprin. Parasites disappeared for 12 days. Animal was treated again with Acaprin but parasites did not disappear, and could still be demonstrated microscopically for a period of 77 days. *Th. mutans* appeared 34 days after the injection of blood of 5276 and could be demonstrated daily for a period of 1,680 days					
25 days	15 days	At the time of splenectomy the blood count was: erythrocytes 7 million and leucocytes 10,400. *Ep. wenyoni* infection was not allowed to run its full course but was controlled with neosalvarsan. *A. centrale* caused a severe anaemia. And 14 days after its first appearance the blood count was: erythrocytes 2 million and leucocyte count 19,600.	3	40	5	3+	Anaemia.
				24	1	2+	
				10	4	2+	
6 days	12 days	Animal was very weak but gradually recovered					
		Ep. wenyoni was extremely numerous from the 7th to 11th day after the first appearance of the parasites. On the 11th day calf received 1·35 gm. neosalvarsan intravenously. As the parasites had not disappeared a second dose 0·9 gms. of neosalvarsan was given on the 12th day, and 24 hours later parasites had completely disappeared, and did not reappear	—	—	—	—	—
		On 2/1/35 calf received 50 c.c. blood of heifer 5276 which harboured *A. centrale*, *P. bigeminum* and *Th. mutans*. On the 8th day *P. bigeminum* appeared in small numbers. The next day the temperature rose and *P. bigeminum* was present in large numbers. Haemoglobinuria developed, animal was treated with Acaprin, a blood transfusion of 3,000 c.c. given, but animal died the next day	—	—	—	—	—

No. of calf.	Date of birth.	Date of injection.	Injected from.	Blood stored for.	Incubation period.		Remarks.	Date of splenectomy.	Total period of observation.	
					Parasite.	No. of days.				Parasite.
5435	26/9/33	24/10/33	5 c.c. blood subcut. from bovine 5277, a carrier of *P. bigeminum* and *A. centrale*	48 hours	*P. bigeminum* *A. centrale*	15 34	The parasites produced a very mild reaction. Daily blood smears were examined until 20/9/34 but only the mentioned parasites were seen	20/9/34	5 years	*P. bigeminum* *A. centrale* *Ep. wenyoni*

NOTE.—2+ indicates parasites very rare. 3+ indicates parasites rare. 4+

TABLE 5 (*continued*).

SPLENECTOMY.							
Relapse.		Remarks.	No. of relapses.	Interval in days of parasite free period.	No. of days during which parasites could be seen.	Nature of infection.	Remarks.
Appeared after.	Seen for.						
30 days	1 day	The *P. bigeminum* reaction was controlled with Acaprin, and 24 hours later no parasites were seen. On the 31/3/36 blood of this animal was injected into heifer 6438, which reacted to *A. centrale* but failed to react to *P. bigeminum*. It was then considered that 5435 had lost its *Piroplasma* infection and on the 14/4/36 it was injected with blood of 6289 which harboured *A. centrale*, *P. bigeminum* and *Th. mutans*. On the 8th day the animal reacted to *P. bigeminum*, and on the 9th day it was treated with Acaprin, which caused the parasites to disappear after 48 hours. 30 Days later this animal was injected again from calf 6289, and 7 days later it reacted again to *P. bigeminum*, but it was not necessary to treat. The parasites could be demonstrated microscopically from time to time up to 62 days after the second injection. *Th. mutans* appeared 42 days after receiving the blood from 6289, and could be demonstrated daily for a period of 1,212 days.					
40 days	18 days	*Ep. wenyoni* and *A. centrale* produced anaemia. Before splenectomy the blood count was: erythrocytes 7 million and leucocytes 11,000. 17 Days after the first appearance of *Ep. wenyoni* the blood count was: erythrocytes 3·2 million and leucocytes 10,800. At the time when *A. centrale* appeared the blood count was: erythrocytes 6 million and leucocytes 7,500. 16 Days after the appearance of *A. centrale* the blood count was: erythrocytes 3 million and leucocytes 11,000. A gradual improvement was noticed and after several weeks the blood count returned to normal	5	51 22 8 34 21	4 2 7 8 1,428	2+ 2+ 2+ 2+ 2+	
8 days	16 days	*Ep. wenyoni* was extremely numerous from the 6th to the 11th day after the first appearance of the parasites. No treatment was undertaken and a marked anaemia developed. *Ep. wenyoni* disappeared for a period of 1,456 days. It is not clear whether in this case it was a relapse, or whether the appearance of parasites was due to a re-infection	1	1,456	5	4+	During the time that the parasites were present a febrile reaction (105° F.) was observed.

s rare. 4+ indicates parasites frequent. 5+ indicates parasites very frequent.