TABLE VII.

Summary of Exposure Experiments at Tzaneen.

Year Animals Exposed.	Origin and Number of Cattle Exposed.		Koch's Bodies.			Number showing Koch's Bodies without *T. mutans*.	Number showing *T. mutans* without Koch's Bodies.	Number showing *T. mutans* Rare.	Number showing *T. mutans* more Frequent.	Number which Died as a sequal of Piroplasmosis.	Number which Died as a sequal of Heartwater.	Number which Died as a sequal of other Causes.	Number which Died or were Killed in Extremis as a sequel to *T. mutans* and Ticks.	Number which showed Hyperin Liver and Kidneys.		
	Vryburg.	Kaalplaats.	Number of Animals showing Koch's Bodies.	Number of Days after Exposure.	On how many Days.									Doubtful.	Slight.	Prominent.
1934	18	—	10	16–22	1–2	—	8	13	4	8	2	3	—	—	—	—
	—	12	7	18–22	1	—	3	6	4	5	—	3	—	—	—	—
1935	12	—	12	14–19	Several days before death	2	1	6	4	-	-	1 Killed for collection of specimens	7 Killed 4 Died	5	3	4
	—	10	8	13–19	1–4	1	2	9	—	—	—	1 Killed for collection of specimens	—	—	—	—
1936	10	—	10	14–18	1–5	10	—	10	—	2	1	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total: Vryburg	40	—	32 (80 %)	—	—	12	9	19	8	10	3	4	11	5	3	4
Kaalplaats	—	22	15 (70 %)	—	—	1	5	15	4	5	—	4	—	—	—	—

TABLE VIII.

Umzinto (Natal) Exposure Experiment, 1934. S. 5375.

Number.	Origin.	Designation.	Koch's Bodies.			*T mutans*.			Temperature Reaction.			Ears Affected.	Reaction.	Etiological Diagnosis.	Lymphoid Hyperplasia.	
			First appearance. (days.)	Frequency.		First appearance. (days.)	Frequency.		Commencement.	Highest Temperature.	Duration. (days.)				Liver.	Kidney.
				Days.	Frequency.		Days.	Frequency.								
1 (579)	Allerton	Inner paddock	—	—	—	—	—	—	5	106·0°	20 irreg.	xxxx	xxx	Septicemia, sequel ticks and malnutrition	—	—
2 (582)	,,	,,	—	—	—	33	33	4	5	106·4°	33	xx	xxx	*P. bigem*.....	—	—
3 (583)	,,	,,	19	19 22 27	xxx xxx x	32	32	x	5	107·0°	32	xx	xxxx	*P. bigem*.....	—	—
4 (588)	,,	,,	—	—	—	—	—	—	6	106·0°	31	xx	xxx	*P. bigem*.....	—	—
5 (66)	,,	,,	—	—	—	39	39	xx	5	106·2°	39 irreg.	xxx	xxx	Sequel ticks (?).....	—	—
6 (77)	,,	,,	—	—	—	—	—	—	5	105·4°	17	xxxx	xx	Septicemia, sequel ticks	—	—
7 (66)	,,	Outer paddock	—	—	—	—	—	—	17 (?) irreg.	105·4°	irreg.	xx	xx	*Anaplasmosis*.....	—	—
8 (77)	,,	,,	—	—	—	—	—	—	26	107·0°	4	x	xx	Sequel *P. bigem*....	—	—
9 (88)	,,	,,	—	—	—	—	—	—	16	106·0°	irreg.	xx	xx	*P. bigem*.....	—	—
10 (99)	,,	,,	—	—	—	—	—	—	17	105·0°	irreg.	x	x	Killed (apparently healthy)	—	—
11 (67)	,,	,,	—	—	—	—	—	—	17	106·4°	14	x	xxx	*P. bigem*.....	—	—
12 (68)	,,	,,	—	—	—	45	45	xx	17	106·2°	irreg.	x	xxx	*Anaplasmosis*.....	—	—
1 (6)	Local	Inner paddock	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2 (7)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3 (8)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4 (9)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 (L)	,,	,,	—	—	—	—	—	—	—	—	—	—	x	—	—	—
6 (6)	,,	Outer paddock	—	—	—	—	—	—	—	—	—	—	x	—	—	—
7 (7)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8 (8)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9 (9)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10 (L)	,,	,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—

In view of the number of Allerton cattle and local cattle exposed and in view of the heavy tick infestation it can be concluded that the Koch's bodies observed at Umzinto were not of the nature of schizonts of *T. parva* but those of *T. mutans*. Nothing of the nature of East Coast fever was observed macroscopically, histologically or in the examination of the smears of those Allerton animals which died at Umzinto.

The extent of infection and mortality attributed to piroplasmosis was again very pronounced and significant. Here again the devastation caused by the effects of ticks and tick-borne diseases is illustrated. Approximately 100 per cent. of the newly introduced animals succumbed, whereas the effects of ticks on the local cattle stand in a most remarkable contrast. What is this resistance observed in the local cattle due to? Is it of the nature of an acquired immunity not only to tick-borne diseases but also to the effects of other agencies for which the ticks may be directly responsible?

Symptomatology.

According to Tables I, II and III it will be seen that in some of the exposure experiments the temperature reaction commenced in some of the animals on the day after exposure. In some it was slightly longer but in the majority of animals the temperature reaction commenced within a week after exposure. From the tables it will become apparent that these temperature reactions were rather severe and continuous and in some instances actually exceeded 108° F. during the evening (see Charts II and III). In the majority it lasted for six weeks and longer. The duration of the temperatures in the different animals is indicated in tables.

On the third day of the 1934 experiment a few ticks were recorded on the animals, although on that date many ticks were identified on the grass. On the fourth day the number of ticks markedly increased and the majority of these belonged to *Rhipicephalus appendiculatus*. A few bont and bont leg ticks were also identified.

The first symptoms were observed in C. 5209 and 5297 on the sixth day. These were observed to be listless and not feeding well. Subsequent to this date loss of condition, signs of cachexia, listlessness, weakness, prostration, slight icterus, besides loss of vigour made their appearance. These depended to a certain extent on the tick infestation, particularly *R. appendiculatus* in and on the ears. Swelling of the ears, especially at the base, was observed shortly after exposure. This was subsequently followed by haemorrhage, more marked swelling, a suppurative dermatitis with necrosis and gangrene. This subsequently led to the sloughing off of portions of the ears. Marked swelling occurred in connection with the regional lymph glands, especially the sub-parotids. These in some instances became markedly enlarged, the size of an orange, painful, not and in some cases fluctuating. In a number of cases brown ticks also attached themselves to the eyelids and in the majority of cases this led to swelling of the region of the eyes and in a few cases to occlusion of the eye and sometimes to blindness. The swelling of the head as described above gave some of the animals a very peculiar appearance

and to the dropping of the ears, head and neck. Some of the animals were killed in extremis to obviate such animals dying overnight and in that way preventing the collection of fresh material for microscopical examination.

In the 1935 experiment these symptoms were aggravated in view of the extremely heavy tick infestation which occurred during that year. On the third day after exposure the cattle were heavily infested with adult ticks, almost all of which belonged to *Rhicephalus appendiculatus*, and on the fourth day the tick infestation was said to be appalling, even the eyelids, and in some cases underneath the tail and over the whithers were simply covered with adult ticks. On the eighth day after exposure the cattle were showing the effects of tick infestation to an enormous extent. The ears were completely filled with ticks, were markedly swollen and bleeding profusely. The heads of most of the animals were very much swollen and some of them were entirely blind owing to the fact that the swollen eyelids had completely obscured vision by covering the eyes. The intermandibular region in most cases were swollen and oedematous, giving the animals the characteristic bottle-jaw appearance. The majority of animals had by that time ceased feeding and stood about listlessly with heads low down. Some showed a tendency towards wandering away alone in the bush and great difficulty was experienced in herding these animals. The ears had a most offensive odour and blowfly eggs were identified in some of the cases.

The majority of the Kaalplaats animals which did not succumb to other causes made an uneventful recovery, whereas all the Vryburg animals were either killed *in extremis* or died as a result of tick infestation.

In the 1936 experiment the exposed Vryburg cattle, besides moderate temperature reactions and the effects of a slight tick infestation, revealed no symptoms of any outstanding importance. Three animals died in this experiment as a result of piroplasmosis and heart water.

PATHOLOGY.

In Appendix II and III the various cases in the three exposure experiments and in the Umzinto experiment are described in respect of the macroscopical and microscopical manifestations. In the text the most important aspects of these cases will be referred to.

Besides the general emaciation, serous atrophy of the adipose tissue and general anaemia were identified. A number of animals were destroyed *in extremis* in order to obtain the material as fresh as possible for microscopical examination. The most important lesions as regards the exterior were observed in connection with the ears and their immediate surroundings. The changes varied from oedema and hyperaemia to an acute sanguinous suppurative dermatitis with necrosis and gangrene, as well as infestation with tick and fly larvae. There was extensive phlegmosis of the subcutis and the surrounding tissues which in some cases were complicated with haemorrhages, necrosis and gangrene. The regional lymph glands in some instances

were markedly swollen and in the sub-parotid and in the parapharyngeals hoemorrhages, necrosis and even gangrene were identified in some of the cases. The majority of the remaining lymph glands were swollen and oedematous and in the majority of them the usual " marrow-like " appearance of the lymphoid tissue was not in evidence. Some of the lymph glands were hyperaemic and on a few occasions haemorrhages were also recorded. In a few of the cases the lungs revealed areas of hepatisation, in some associated with necrosis. These were usually localised and these lesions were probably of the nature of an embolism, the sequel of the extensive changes met with in the ears. Such sequelae of emboli were also observed in a few of the kidneys in the 1935 experiment. Hydrothorax and hydropericard were observed in some of the cases. In those animals in which a diagnosis of piroplasmosis or a sequel of piroplasmosis was made the general anaemia and icterus were pronounced, and in the acute cases haemoglobineamia and haemoglobinuria were manifested. It is of interest to note that in C. 5763 (specimen No. 16147) *P. bigem.* were exteremely rare and difficult to find in the spleen smears, whereas in the section of the hippocampus the small blood vessels showed the majority of red cells infected with *P. bigem.* This was the only case in which this was observed although brain sections at various levels were examined from the majority of cases. The regular occurrence of multiple ulcers in the abomasum of the cattle in the 1035 experiment should be recorded. These were very similar to those seen in cases of East Coast fever and varied in size from about $\frac{1}{2}$ cm. to $1\frac{1}{2}$ cm. in diameter. Their edges were usually sharply defined but very irregular in outline. The liver in a number of cases was swollen with slight evidence of icterus, whereas in the kidneys of the cattle in the 1935 experiment macroscopic, transparent foci were identified in a very few of the cases. Only in two cases were isolated hyperplastic lymphoid nodules in the kidneys so characteristic of *T. parva*, identified.

The *microscopic examination* revealed interesting changes especially in respect of the liver, kidney, majority of lymph glands and spleen. It is remarkable, however, how mild the regressive changes were in such organs as the liver, myocard and kidneys. In the liver slight changes and slight pigmentation were observed. In the kidneys slight regressive changes and haemosiderosis associated with piroplasmosis were recorded. On the other hand the liver showed interesting changes of a more progressive nature, namely proliferative changes in the periphery of the lobule and lymphoid hyperplasia which varied in the different cases. In some of the cases the proliferative changes were undoubtedly associated with an increase of collagen fibres. It is difficult to say how far such changes actually antidated the subsequent lymphoid hyperplasia attributed to the *T. mutans* infection. Such proliferative changes with an increase of connective tissue elements in the periphery of the lobule besides the presence of cells of the lymphocytic series are frequently met with to a lesser or greater extent in animals which have died from other causes. At present it is not understood what stimuli are actually responsible for such proliferative changes. From Tables IV, V and VI it will be seen that variations in extent and distribution were manifested in respect of the *lymphoid hyperplasia in the liver and kidney.*

Lymphoid hyperplasia in these organs however only occurred in the Vryburg exposed cattle of the 1935 experiment. In all the other exposed animals at Tzaneen and Umzinto there was no evidence of such lymphoid hyperplasia although Koch's bodies in the majority of the Tzaneen animals had been observed. In these tables an attempt was made to express the degree of lymphoid hyperplasia. These varied not only in the different animals but also in the liver and kidney. In some cases it was present to a very large extent in the liver while in the kidney there was only slight evidence. In other instances the reverse was found. In a minority of cases the liver and kidney were more or less affected to the same extent. This lymphoid hyperplasia in the liver manifested itself in the vicinity of the vessels of the periphery of the lobule, whereas in the kidney this occurred in connection with the vessels of the cortex. In a few instances there was an increase of cells of the lymphocytic series in the adventitia of the larger blood vessels between the medulla and the cortex. How far these latter can be attributed to a Theileriasis is at the present moment not known, because such changes have to a lesser degree observed in cases not associated with a Theileriasis.

With reference to *the lesions observed in the spleen and the lymph glands* it will be noted from these tables that specific lesions only manifested themselves in the 1935 Vryburg cattle. These changes were rather characteristic and varied in degree in the different animals under observation. An attempt was made to indicate by symbols the degree of damage. In the majority of cases both the lymph glands and spleen were more or less affected to the same extent. In C. 5000 and C. 5763 the lesions in the spleen were exaggerated. These lesions mainly affected the lymphoid tissue in the malphighian bodies, which appeared enlarged, due to the presence of a homogeneous material which " permeated " the lymphoid tissue like a reticular network. This homogeneous material stained pink with eosin, yellow with van Giesen and failed to stain for amyloid. It caused a disorganisation of the lymphoid nodules and was responsible for the reduction in the number of cells of the lymphocytic series. The demarcation between primary and secondary nodules was lost. In places this homogeneous material extended into red pulpa of the spleen. Besides this marked alteration so characteristic in these *T. mutans* cases, the spleen also revealed necrobiotic changes to a limited extent in the lymphocytic cells of the malpighian bodies. The red pulpa showed an increase in the number of neutrophiles. Sometimes these were present in fairly large numbers and often in clusters. In some of the cases the haemosiderosis was less prominent.

In the lymph glands more or less similar lesions were observed in the lymph nodules. In the first place a reduction in the number of nodules was noted. The usual demarcation of the lymphoid tissue into primary and secondary nodules was suppressed and markedly so in some of the cases. The extreme cases revealed loss of nodules, especially along the marginal zone, and in their usual positions a similar homogeneous material as described in the spleen could be identified. Besides this " homogeneous material not yet identified " and which also extended into the rest of the lymphoid tissue of the

lymph glands, there was a reduction in the number of small lymphocytes, which enveloped the secondary follicle like a concentric layer. Some of the lymphoid glands, especially in the marginal zone, showed accumulations of neutrophiles. Necrobiotic changes were also in evidence in the region of the nodules.

Further histological studies are being undertaken to ascertain the exact nature and pathogenesis of these lesions in the lymphoid tissue of the lymph glands and the spleen. In some of the cases it would appear as if the reticular cells of the secondary nodules were affected. The impression gained in the present study is that the lymphoid tissue in the spleen and the lymph glands is damaged in acute theileriasis. It is not clear to what the increase of neutrophiles, especially in the lymph glands and spleen should be attributed. Their present were also recorded in the spleen of those animals which died as a result of piroplasmosis. Are they to be considered as a sequel to the inflammatory changes referred to in connection with the external ears?

From Table IX it will be seen to what extent similar changes of a lymphoid hyperplasia in the liver and kidneys and lesions in the spleen and lymph gland occurred in a number of East Coast fever cases from various centres of the Union examined for comparison.

TABLE IX.

No. of Speciman.	Koch's Bodies.	*T. parva.*	Liver : Lymphoid Hyperplasia.	Kidneys : Lymphoid Hyperplasia.	Spleen : Lesions.	Lymph Gland : Lesions.
Sabie : Transvaal.						
15956....	xxx	xxxx	xx	xxx	xxx	xxx
15958....	xxxx	xx	xxx	xx	xx	x
15960....	xxx	xx	xx	—	xxxx	xxxx
15954....	xxx	xxxx	xxx	xx	xxx	xxx
Natal.						
17565....	xxxx	xxxx	xxxx	xxxx	xxx	xxxx
17564....	xxxx	xxxx	xx	xxxx	xx	xxxx
16668....	xxxx	xxxxx	xxx	xx	xxx	xxx
Experimental Cases : Onderstepoort. (Steck).						
3189.....	x (x)	—	xx	x	xxxx	xxxx
3113.....	x (x)	—	x	x	xx	xxx
2578.....	x (x)	—	—	—	xxx	—
2574.....	xxxx	xxx	xxx	xx	xxx	xxx
2405.....	x (x)	—	xx	xx	xxx	xxx

Although a difference of degree in the lesions of *T. parva* and *T. mutans* were noticed especially in respect of the lymphoid hyperplasia in the liver and kidneys the same type of lesions was identified. Differentiation between the lesions of *T. parva* and these cases of *T. mutans* was therefore not possible.

Table X.

Haematology.

(*a*) T. mutans *and Sequel Tick Infestation.*

No. of Bovine.	Date.	Etiology.	R.C.	R.P.	R.P. R.C.	W.C.	S.L.	L.	M.	N.	Remarks.
4872	15.3.35	Killed in extremis.	—	—	—	—	10	46	8	38	The cytoplasm of some of the lymphocytes stain intensely blue with Giemsa. Difficulty experienced in differentiating some of these from certain forms of monocytes.
	18.3.35	Sequel *T. mutans* and tick infestation	2·82	16	5·67	·20	2	30	5	63	
5570	15.3.35	Died. Sequel *T. mutans* and tick infestation	2·60	13	5·00	3·90	5	24	20	51	Do. do. do.
	18.3.35		2·38	16	6·72	4·90	1	20	19	60	
5629	14.3.35	Killed in extremis.	4·33	20	4·61	15·00	7	38	9	46	Difficulty in classifying some of the lymphocytes because they are difficult to distinguish from certain forms of monocytes. Some of the cells resemble lymphoblasts.
	18.3.35	Sequel *T. mutans* and tick infestation	4·20	20	4·76	8·40	5	27	11	57	
	21·3·35		3·61	20	5·54	14·90	4	9	13	74	
5641	18.3.35	Piroplasmosis and sequel tick infestation	2.80	18	6·43	17·70	3	54	20	23	Difficulty in classifying some of the lymphocytes. Lymphocytes also identified as well as lymphocytes with granules. Degeneration of some of the lymphocytes observed in the form of vacuolation and slight disintegration of the nuclei. Slight anisocytosis; Basophilia; slight polychromasia.
	21·3·35		3·98	20	5·02	12·70	1	46	21	32	
5648	14.3.35	Killed in extremis. Sequel *T. mutans* and tick infestation	4·08	18	4·41	14·00	3	32	3	62	In unripe lymphocytes developing agamonts identified. Vacuolation of lymphocytes also observed.
5663	21.3.35	Died. Sequel *T. mutans* and tick infestation	4·26	17	3·99	6·00	—	24	4	72	Slight anisocytosis.
5665	14.3.35	Killed in estremis. Sequel *T. mutans* and tick infestation	3·00	13	4·33	14·50	2	22	11	65	Slight anisocytosis. Degenerative changes observed in lymphocytes in the form of vacuoles and slight disintegration of the periphery of the nuclei.
5726	15.3.35	Killed in extremis. Sequel *T. mutans* and tick infestation	4·52	22	4·86	8·3	3	35	1	52	A few of the lymphocytes difficult to distinguish from monocytes. Degeneration of lymphocytes identified.
5763	15·3·35	Killed in extremis..	3·57	14	3·93	14·80	5	59	2	36	Rieder forms of lymphocytes. Cytoplasms of some intensely stained. Agamont granules in all stages identified. Degenerative changes of nuclei of some lymphocytes observed. Lymphoblasts with agamont granules present. Some lymphocytes vacuolated.

HAEMATOLOGY.

In Table No. X interesting data are given in respect of haematological observations made on a number of cattle, which succumbed to or were killed as a result of the sequelae of *T. mutans* and tick infestation, in the 1935 experiment. Significant is the oliocythaemia revealed in all the cases. This varied from 2·38 million to 4·5 million per cubic mm. This was also associated with morphological changes such as anisocytosis, polychromasia and basophilia. In none of the cases was a *leucopaenia* identified to the extent observed by Steck (1928) in East Coast fever, although there was a reduction in the number of lymphocytes and an increase in the number of neutrophiles. In two of the cases the monocytes were increased, although the one case was complicated with piroplasmosis. The neutrophilia could probably be attributed to the suppurative dermatitis of the ears referred to. The lymphocytes observed in the differential counts revealed interesting changes. There was a dearth of small lymphocytes, the majority being of the medium and especially the large type. In some the cytoplasm of these lymphocytic cells was stained a rather intense blue with the Pappenheim modification of Giemsa. In respect of this, difficulty was experienced in differentiating between some of these and certain forms of monocytes. Some of these cells undoubtedly resembled lymphoblasts and for this reason the enumeration of the cells, especially into lymphocytes and monocytes caused great difficulty. Degeneration of some of the lymphocytes was observed, especially in the blood smear of C. 5641. This was of the nature of a vacuolation of the cytoplasm and slight disintegration of the nuclei.

In these cases the occurrence of granules of various types and sizes is recorded in the cytoplasm of the lymphocytes. The significance of the granules is not clearly understood. Probably these granules stand in relation to the damage of the lymphoid tissue of the spleen and lymph glands (Fig. 10 and 11). In addition *Rickettsia bovis* Donatien and Lestoquard 1936 was demonstrated in the blood and lymphgland smears prepared from some of the animals.

TICK TRANSMISSION EXPERIMENTS.

(See Appendix IV.)

Experiments were undertaken at Onderstepoort in order to ascertain to what extent ticks were responsible for the febrile reactions, apart from the theileriasis, heartwater and piroplasmosis. Adult *Rhipicephalus appendiculatus* were collected at Tzaneen on the grass. Ticks from Tours (Letaba), Rietfontein and Batavia (Carolina) were collected on cattle infected with East Coast fever. On these farms East Coast fever had broken out and was responsible for a very heavy mortality. These latter ticks were collected for the purpose of cross immunity experiments at Onderstepoort. The ticks so collected were fed on cattle and sheep at Onderstepoort and the results of these experiments are detailed in the tables of Appendix IV. From the experiments it will be observed that by excluding factors such as

change of environment, hot climate, natural infection with blue tongue, mass tick infestation, etc., it was *not* possible to bring about a similar severe febrile reaction as observed in those cattle exposed at Tzaneen in 1934 and 1933 respectively. The recovery of blue tongue virus from the cattle exposed at Tzaneen in 1934 and 1935 forms the subject of another paper (De Kock, Neitz, du Toit, 1936). It should, however, be noted that although this blue tongue virus, recovered at Tzaneen produced no febrile reactions in susceptible cattle at Onderstepoort it caused severe acute febrile reactions in sheep with a high percentage mortality.

1. The Feeding of Tzaneen Ticks on Cattle at Onderstepoort in 1935 and 1936.

(Appendix IV.)

In Table I the feeding of the ticks on the cattle is recorded. These bovines, viz., No. 4676 (splenectomized), Nos. 6027, 5523, 6463, and 6291 were infested on the ears with adult *Rhipicephalus appendiculatus*.

Four of these animals, Nos. 2676, 6027, 5523 and 6463, showed febrile reactions after about 10 days and from the 14th day Koch's bodies in rare numbers could be demonstrated in the smears from the prescapular glands. The splenectomized bovine, No. 4676, showed slight clinical symptoms and Koch's bodies frequent and present for a longer period than observed in the other three. Of the two animals that did not react No. 6019 had been exposed at Tzaneen in 1935 and was therefore considered to be immune, while No. 6291 did not react in spite of the fact that numerous ticks engorged on this animal. Blood sub-inoculated at various stages of the febrile reactions observed in No. C. 4676 and No. 6027 into sheep and from No. 6463 into a calf gave negative results.

2. The Feeding of Batavia Veld Ticks on Cattle at Onderstepoort during the Year 1935.

The observations are recorded in Table II, Appendix IV.

Adult *Rhipicephalus appendiculatus* and *R. evertsi* ticks were placed on the ears of C. 4283 on two occasions with negative results.

3. The Feeding of Tours Veld Ticks on Cattle at Onderstepoort during the Year 1935.

C. 4283 (see Table II, Appendix IV) that failed to react after tick infestation with veld ticks from Batavia was infested with *Rhipicephalus appendiculatus* and *R. evertsi* adult ticks collected at Tours. The results were similar to those observed in the cattle infested with the Tzaneen veld ticks referred to above. A febrile reaction was observed and Koch's bodies were identified in the smears made from the prescapular lymphatic glands.

TABLE XI.—TZANEEN DISEASE. TICK INFESTATION.

D.O.B.	Date.	Origin.		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
4676	11.3.35	O.P.	Temp.											T +++	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
			Symp.											M +	M +	M +		M +			M(B) + +	M(B) + +	(B) +		M(B) + +	M +	M(B) + +	M +	M + +				
6027	25.3.35	K.	Temp.														T + +	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
			Symp.				M +			M P + +	M P + ++				P +				(B) +			P +	M +		M P + +		P +	M P + +					M +
5523	24.4.35	V.	Temp.								T +	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...							
			Symp.																				M(B) + +					M +		M +			
4283	9.8.35	O.P.	Temp.																	T + +	...	...	...	...	...	...	...	...	...	...	...		
			Symp.																		A M + +	A M(B) + + +			A M(B) ++++			A M + +	A + +	A + +	A M + + +	A M + + +	
6019	4.3.36	K.	Temp.																														
			Symp.		M +	M +	M +	M +	M +	M +	M +	M +	M +	M +	M +	M +								M +		M +							
6291	4.3.36	O.P.	Temp.							T +	...	...	...	...	...	...	...	...	...	...	...	...	...										
			Symp.																														
6463	4.3.36	O.P.	Temp.														T + + +	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
			Symp.																(B) +		(B) +		M + + +		M +			M +					
6407	26.3.36	O.P.	Temp.								T + + +	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
			Symp.																	(B) + +						M +							

D.O.B.	Date.	Origin.		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
4676	11.3.35	O.P.	Temp.	...	...	...	...	...	...	...	...	...	...	...	...																	
			Symp.		M +	M +	M +		M + +		M + +	M + +					M + +	M + +	M +	M + +	M + +		M + +	M + +		M +			M +	M +		
6027	25.3.35	K.	Temp.																													
			Symp.	M +	M +	M +	M P + +			M +			M +	M +			M +															
5523	24.4.35	V.	Temp.																													
			Symp.																													
4283	9.8.35	O.P.	Temp.																													
			Symp.																													
6019	4.3.36	K.	Temp.																													
			Symp.																				P +									
6291	4.3.36	O.P.	Temp.																													
			Symp.	M +																												
6463	4.3.36	O.P.	Temp.	...	...	...	...	...	...	...	...																					
			Symp.																													
6407	26.3.36	O.P.	Temp.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...												
			Symp.			M +	M +	M +					M +		M +																	

K. = Kaalplaats.
O.P. = Onderstepoort.
A.V. = Armoedsvlakte.
T. = Temperature.
B. = Koch's Bodies.
P. = P. bigeminum.
A. = Anaplasma.
M. = T. mutans.
+++ = Acute or Frequent.
++ = Moderate.
\+ = Slight or Rare.
† = Died or Killed.
D. = Dermatitis.

4. The Feeding of Rietfontein Veld Ticks on Cattle at Onderstepoort during the Year 1936.

The result of feeding these ticks is recorded in Table II, Appendix IV. Calf No. 6407 was infested with *Rhipicephalus appendiculatus* ticks. It developed a febrile reaction and the smears from the prescapular gland showed the presence of Koch's bodies. Three months after the reaction the calf was splenectomized. The animal showed a relapse to *P. bigeminum*. *T. mutans* appeared in large numbers in the blood smears and ten days after splenectomy. Koch's bodies in rare numbers were found in the smears from the prescapular lymph gland. No abnormal temperature was recorded in this animal. On the 14th day after splenectomy the animal was killed in order to ascertain whether other organs were responsible for the occurrence of parasites in a large number of red cells. Smears were prepared from all the organs and the presence of Koch's bodies, again in rare numbers, could be identified in the smears from the prescapular lymph gland and the haemo-lymph gland, situated in the vicinity of the precrural lymph gland. The only abnormal change at post-mortem was the slight swelling of the lymph glands and enlargement of the haemo-lymph glands, especially those situated in the vicinity of the superficial lymph-glands. Microscopically an extensive haemosiderosis was seen in the sections from the kidney. There was no evidence of lymphoid hyperplasia either in the kidney or in the liver (see Table XI).

5. The Feeding of Tzaneen Veld Ticks on Sheep at Onderstepoort during the Years 1935 and 1936.

In Table III, Appendix IV, the feeding of ticks on sheep is recorded.

Eight sheep, No. 35004 (splenectomized), Nos. 42330, 42829, 39361, 45738, 44907, 45756 and 42882, were infested on the ears with adult *Rhipicephalus appendiculatus* ticks. It will be seen from the table that seven of the sheep showed a febrile reaction almost similar to that observed in cattle although no Koch's bodies could be identified in the smears from the glands. From Sheep No. 42330, with a severe febrile reaction, blood was inoculated into four other sheep and one bovine on different days of the reaction but no reactions were produced. Sheep No. 39361 was destroyed at the height of the reaction. Smears were examined from the various organs and in the spleen and lymph gland there was an apparent reduction in the amount of lymphoid tissue.

6. The Feeding of Larvae bred from Tzaneen Veld Ticks that had Engorged on Bovine No. 4676.

Since the cause of the febrile reaction referred to above had not been identified an experiment was carried out to ascertain whether this agent would pass through the egg. Four sheep, Nos. 43491, 43310, 43484 and 42706, were infested on the ears with *Rhipicephalus appendiculatus* larvae. The larvae fed well and from 1,500 to 3,000 were collected. In none of these was a reaction observed and it was concluded that the agent did not pass through the egg of the tick. The details of this experiment are set out in Table IV, Appendix IV.

7. The Feeding of Nymphae and Adult *Rhipicephalus appendiculatus* Ticks that had Fed on the Previous Stage on Sheep, reacting to Tzaneen Blue Tongue Virus.

The details are given in Table V, Appendix IV.

Two sheep, Nos. 44987 and 43771, were infested on the ears with *Rhipicephalus appendiculatus* nymphae and adults respectively, that had fed in the previous stage on Tzaneen blue tongue reacting sheep No. 43484. The results were negative. The experiment was repeated, in which sheep Nos. 44276 and 44971 were infested with ticks that had been fed on sheep that had reacted to the Tzaneen blue tongue virus. The results were entirely negative and it would appear that Tzaneen blue tongue virus could not be transmitted by ticks.

From the foregoing it would appear that the feeding of adult *Rhipicephalus appendiculatus* ticks collected at Tzaneen produced a febrile reaction when infested on cattle, with the development of Koch's bodies in the smears from the lymph glands in five out of seven cattle infested for the first time at Onderstepoort. One animal had been previously exposed at Tzaneen and showed no reaction. It was considered to be immune.

Similar results were obtained when *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi* ticks collected at Tours and at Rietfontein were fed on cattle at Onderstepoort. No mortality was observed in any of the experimental animals on which ticks were fed. Clinical symptoms, however, occurred in the splenectomized bovines. Swelling of the ears was noticed in practically all the animals. Nothing that could in any way be confused with East Coast fever was identified in any of the animals so infested.

Febrile reactions in sheep similar to those observed in cattle occurred when infested with Tzaneen adult ticks but on no occasion were Koch's bodies identified in the blood or in the gland smears.

The sub-inoculation of blood from either cattle or sheep during the febrile reactions into either cattle or sheep gave negative results. From this observation it may be conclued *that the agent responsible for this febrile reaction is probably only transmissible by ticks.* Further experiments are indicated to ascertain whether ticks fed on such reacting sheep or cattle will be able to transmit the agent, which apparently also does not pass through the egg of the tick.

No case of East Coast fever occurred in those cattle at Onderstepoort fed on ticks from the badly infected East Coast fever animals of Tours, Batavia and Rietfontein. The Koch's bodies observed as a result of these tick transmissions are probably of the nature of the schizonts of *T. mutans.*

At the present moment it is not understood why we were not able to reproduce East Coast fever by ticks collected on these badly infected East Coast fever farms. This greatly interfered with the immunity experiments planned at Onderstepoort, especially in respect of those Tzaneen animals which had shown Koch's bodies in the Tzaneen exposure and Onderstepoort experiment.

An endeavour was made however to expose a number of such recovered Koch's body cases and controls from Onderstepoort on what was regarded as a badly infected East Coast fever farm. On the 19th March, 1936, such an exposure experiment was commenced on East Coast fever infected Tank Area No. 176, Inanda Location, New Hanover, Natal. The records of East Coast fever since the outbreak on 1.5.34 were as follows:—

Number of animals at the Tank Area	1,050
Number of deaths	68
Deaths during February, 1936	2
Date of last death: 6.3.36.	

These exposed Onderstepoort animals were temperatured twice daily and blood and gland smears were regularly examined. The following animals, No. 4676 (splenectomized), Nos. 5469, 5523, 6072, 6033, 6252, 6288 and 6297, had shown Koch's bodies either when exposed at Tzaneen or during tick transmission experiments at Onderstepoort. The following animals had shown no Koch's bodies and served as controls to the above: animals Nos. 5474, 5563, 5587, 5626, 5717, 5739, 5750, 5754, 5757, 6365, 6391, 6411, and 7130. Up to the 31st August, 1936, there has been no case in which Koch's bodies could be identified in spite of the regular and frequent examination of the gland and blood smears.

The following animals died:—

4676	died on the 22.3.36.	Redwater.
5474	died on the 11.6.36.	Etiology not determined.
5587	died on the 24.4.36.	Etiology not determined.
5626	died on the 15.6.36.	Etiology not determined.
6252	died on the 23.6.36.	Etiology not determined.
6365	died on the 14.6.36.	Piroplasmosis.
6411	died on the 10.4.36.	Piroplasmosis.
7130	died on the 20.4.36.	Etiology not determined.

It will therefore be seen that two animals which had previously shown Koch's bodies succumbed, whereas six which had not previously shown Koch's bodies died. Sections from the liver and kidney failed to reveal any evidence of a lymphoid hyperplasia. The result of the experiment was therefore most unsatisfactory and this could probably be attributed to the fact that ticks were unfortunately not prevalent.

Further attempts will be made to establish East Coast fever infection in animals at Onderstepoort in order to carry out cross immunity experiments, especially in respect of those cases which had shown Koch's bodies as a result of *T. mutans* infection.

The splenectomy of two calves that had shown a febrile reaction and the development of Koch's bodies in the smears from the prescapular lymph gland brought about a relapse of *T. mutans*. In one of these *T. mutans* was present in extremely large numbers, besides the presence of Koch's bodies in lymph and haemo-lymph glands.

In the other *T. mutans* were rare and no Koch's bodies developed. This is the first occasion on which such Koch's bodies have been recorded at Onderstepoort in gland smears made from animals showing a relapse of T. mutans as a result of splenectomy. In view of the irregular distribution of Koch's bodies in the organs in some of the Tzaneen cases it is more than likely that in previous experiments these bodies have been missed because smears from the affected organs were not examined, especially where parasites were frequent in the blood.

In two of the animals, C. 6463, infested with ticks, and C. 6462, sub-inoculated with blood, *Rickettsia bovis* was observed in the lymphocytes of blood and gland smears. Although they somewhat resembled the shape and size of agamonts, yet in view of the staining and character of the granules a differentiation could easily be made. At the present moment their true nature is not understood, nor in what relation, if any, they stand to the febrile reactions in the animals in which they were found. A similar body was recorded in the 1935 Tzaneen exposure experiment. Figure 12 illustrates such a body, especially as regards its size, shape, position in the cell and the character of its granules. Its granules are somewhat smaller than those of agamonts, fairly regular in size and seem to stain a reddish brown colour with the Pappenheim modification of Giemsa. The blood smear of C. 6463 of the 6.4.36, in which these bodies were identified showed interesting changes in respect of the leucocytes. Erythrophagocytosis as well as vacuolation could be identified in the monocytes which were present in fair numbers. Significant is the large number of lymphocytes of the medium and large sizes which show the presence of granules. These granules with the Giemsa stain varying in character from a light red to a slightly darker red and a great deal of variation occurred in respect of their number, size and distribution. Weil-Felix tests were carried out in a number of animals with negative results.

Owing to the positive agglutination of O.P. bovines sera against Proteus Ox strains the positive agglutination obtained with the Tzaneen bovine sera did not appear to be significant. The following bovines were tested by Dr. Mason of this Institute (D.R.C. 14562) on 30.8.34:

4984	5526	5225
5198	4954	5210
5200	4987	4988.

On 27.6.35 the following sheep in the Tzaneen experiment were tested. All negative 1 in 40 against 0 X 2, 0 X 19 and K. strains.

41566	35004	41002
40955	41330	41121
40943	40934	

BLOOD AND ORGAN TRANSMISSION EXPERIMENTS.

Several attempts were made in 1934 and 1935 to reproduce the febrile reactions observed in the Tzaneen exposed cattle by sub-inoculation of blood and organ emulsions into susceptible bovines and sheep at Onderstepoort. In none of these animals was a reaction reproduced resembling that seen at Tzaneen. Some of the cattle developed piroplasmosis, anaplasmosis and heartwater, whereas in some of the sheep blue tongue was transmitted. These sub-inoculation experiments were carried out at Onderstepoort and the details are fully recorded in Appendix V, Table I.

1. C. 5214, 5224 and 4980 of the 1934 experiment were killed at Tzaneen. These animals had shown severe febrile reactions and the presence of Koch's bodies. The blood of these animals was pooled and injected intravenously into the prescapular lymph gland. In addition emulsions were made from the spleen, liver and lymph glands and after pooling the emulsion was injected into the same two calves, namely 5409 (a carrier of *T. mutans*) and 5454 (a calf reared under tick-free conditions). Both these animals contracted anaplasmosis, from which they recovered after treatment. Subsequently both these animals contracted heartwater and died. On the 19th day after injection of calf 5454, blood from it was injected into calf 5188, a carrier of *T. mutans*, *Anaplasma centrale* and *P. bigem.* This calf reacted to *P. bigem* and died on the 6th day in spite of treatment. Two days after the arrival of the survivors of the 1934 Tzaneen exposure experiment at Onderstepoort, blood of twelve of these was collected, pooled and injected into calf 5306 (a *T. mutans* carrier). The animal reacted to piroplasmosis and anaplasmosis and *T. mutans* were also identified.

2. From several cattle in the 1935 exposure experiment at Tzaneen, and which showed the characteristic febrile reactions with the development of Koch's bodies, blood and organ emulsions were injected into animals at Onderstepoort. The material was fresh and the smears from the organs at the time of the preparation of the emulsions showed the presence of Koch's bodies. The material was given either intravenously, intracutaneously, intramuscularly, subcutaneously or directly into the lymph gland. Altogether ten animals were utilized and apart from febrile reactions of a somewhat doubtful nature no symptoms were recorded.

3. At the time when the sub-inoculations in cattle were carried out in 1934 similar injections were made into sheep. The sheep in the 1934 experiment reacted to blue tongue and blood from three of these was injected into three calves (two carriers of *T. mutans*, and one which was fully susceptible). No reactions were observed in the cattle so injected. Blood from ten of the blue tongue reacting sheep in 1935 was injected into eight cattle, which were either fully susceptible or carriers of *T. mutans*. The doses given varies from 5 c.c. to 250 c.c. No reactions were noticed in any of these inoculated cattle. From one of the cattle, 5563, which had not reacted, blood was sub-inoculated 16 days later into C. 5651, that is, the period coinciding with the appearance of Koch's bodies as observed in the Tzaneen cattle. A further sub-inoculation from C. 5651 on the 8th day was carried out into C. 5653, also with negative results.

From the above it will be seen that no febrile reaction was produced in either inoculated cattle or sheep which could be attributed to a specific virus other than blue tongue. In view of the fact that a large number of cattle were utilized for these transmission experiments and that the material was introduced either subcutaneously, intravenously, intraperitoneally or even directly into the lymph gland nothing of the nature of East Coast fever was reproduced in spite of the fact that the material emanated from animals which in the majority of cases were showing the presence of Koch's bodies.

TRANSMISSION EXPERIMENTS IN LABORATORY ANIMALS WITH MATERIAL EMANATING FROM CATTLE EITHER EXPOSED AT TZANEEN OR IN EXPERIMENT AT ONDERSTEPOORT.

(See Appendix V, Table II.)

In conjunction with the sub-inoculation experiments carried out in cattle and sheep at Onderstepoort, similar experiments were undertaken to study the reactions observed in the cattle by the injection of blood, etc., into laboratory animals. The details of these experiments are fully recorded in Table II, Appendix V. Blood from sheep 40994, reacting to blue tongue virus recovered at Tzaneen at the height of reaction was injected into six guinea pigs. Of these three showed no reaction while in three febrile reactions were observed. From the latter three brain emulsions and blood were injected into sheep and guinea pigs. Some of the sheep showed febrile reactions but on testing their immunity with the original virus they were still found to be susceptible. In other sub-inoculated guinea pigs reactions were observed but after further passage no reactions were reproduced.

From the above it would appear that up to the present it was not possible to infer guinea pigs with the Tzaneen strain of blue tongue virus.

Blood from eight reacting cattle exposed at Tzaneen in 1935 was collected from the 5th until the 19th day after exposure. Two animals that were bled on the 19th day showed Koch's bodies. This blood was respectively injected into guinea pigs, rats and mice. The results were entirely negative. Peritonitis occurred in six of the guinea pigs, while two mice died of other causes. Emulsions prepared from the brains of these latter mice were injected into further mice with negative results.

The injection of emulsified unengorged adult *R. appendiculatus* ticks into laboratory animals was undertaken to ascertain whether such ticks collected at Tzaneen harboured a virus transmissible to guinea pigs and mice. An emulsion of ticks in saline was centrifuged and the supernatant fluid injected intraperitoneally into guinea pigs and intracerebrally into mice. Of the three guinea pigs two showed no reactions, while the third died of peritonitis. Of the four mice one died. Its brain was injected intracerebrally into other mice with negative results.

From this experiment it would appear that it was not possible to recover a virus from emulsified ticks injected as indicated above.

SUMMARY.

For the sake of clarity and in order to stress the more significant aspects in the above study of Theileriasis with special reference to *T. mutans*, it was thought advisable to briefly summarise the most important points. This would further facilitate the discussion, especially with reference to the literature, which is to follow.

1. The East Coast fever campaign in South Africa has been reorganised in order to concentrate largely on the diagnosis of the disease and on the early location of infection. It mainly involved (*a*) a system of checking cattle in East Coast fever areas by means of which all increases and decreases are accurately accounted for and (*b*) obtaining suitable smears from all deaths of cattle. The Department has taken great pains to perfect this system of control during the last ten years, with the result that it has been placed in possession of accurate data in regard to the incidence of East Coast fever and subsequent mortality when once the disease has been diagnosed. The likelihood of infection escaping detection for any length of time has been reduced to a minimum. The extensive campaign has resulted in the reduction of mortality from East Coast fever to a very low figure. Evidence was however submitted to show that *T. parva* has on the other hand not in any way become modified in Natal, because mortality from East Coast fever, when once established, may reach a very high proportion so characteristic of this disease.

There has been a large number of infections diagnosed by gland or spleen smears with the demonstration of Koch's bodies, where an outbreak has been confined to the *initial* death. From a careful study of the various data as a result of this system of control serious doubts have arisen as to the true nature of the etiological significance in many of these " single death " outbreaks. Various reasons are given why these single deaths showing Koch's bodies could not be attributed to *T. parva*. It is on the other hand remarkable how frequently they occur in calves with a history on the farm of such diseases as coccidiosis, chronic scours, paratyphoid, severe scalding as a result of dipping, multnutrition and verminosis. What is more significant is the fact that a large number of cases were observed in slaugter animals usually in good condition with a history that the animal prior to slaughter was moved from one area to another.

The proneness of such cases to occur on previously infected East Coast fever properties has been referred to but in some instances there was no evidence to show that it could be connected with the previous outbreaks of East Coast fever. On the other hand a number of cases are cited to show that some of these single death cases with the presence of Koch's bodies in the smears were followed by outbreaks of East Coast fever with the characteristic high mortality.

2. The occurrences of Koch's body cases in *non-East Coast fever areas* in the Transvaal were briefly referred to. The conditions under which these occurred in the Marico, Pretoria and Pietersburg districts are fully dealt with. In some instances, e.g. Marico, these cases were associated with such diseases as malnutrition, bad farm management, mass tick infestation, paratyphoid and the usual tick borne diseases. On the other hand cases (e.g. Onderstepoort) are recorded where these

bodies were observed either in newly born calves reared on the veld or more adult animals, without showing any distinct signs of disease. The occurrence of Koch's bodies in slaughter animals (e.g. Tzaneen) under similar conditions as described in the East Coast fever areas in Natal is also referred to.

3. Exposure experiments were respectively carried out in 1934, 1935 and 1936 on the Tzaneen Town Lands. In view of the frequency of Koch's bodies occurring in the slaughter animals on the Tzaneen Town Lands, the prolific manifestation of ticks, the facilities offered, etc., were considered most suitable to study the nature of these Koch's bodies. Variations were made in the duration of the three experiments. In the 1934 experiment, animals were exposed from 20.3.34 to 3.5.34, in the 1935 experiment from 22.2.35 to 11.3.35 and in 1936 from 15.3.36 to 26.3.36. In 1935 the animals were returned to Onderstepoort at the height of the reaction, the journey occupying about 30 hours. This transport of the animals undoubtedly played a very important part and was probably the main factor in bringing about the great variation which occurred in the course and mortality of this experiment. The results obtained in the *two groups of animals exposed*, 1935 (*Vryburg and Kaalplaats*) are very significant. It is maintained that this may stand in relation to the extent such animals had previously been subjected to tick infestation. In the Vryburg animals it was known that they were subjected to minimum tick infestation. Another important point raised was the fact that ticks were more prolific at Tzaneen in 1935 than in 1934 and in 1936 the number of ticks were markedly reduced.

4. The occurrence of Koch's bodies after *definite periods of exposure*, their brief sojourn in the gland smears, the presence and frequency of *T. mutans* simultaneously or after the disappearance of Koch's bodies, etc., are significant. The failure to find Koch's bodies in a gland smear of cattle after exposure is no criterion that Koch's bodies are not present in the body, in view of their rare occurrence and irregular distribution in the different organs of the body. Moreover the regularity with which such bodies appear after exposure to tick infestation forms an important criterion in the examination of suitable gland smears. In no instance were the red cells and especially individual cells infected to the same extent as in the case of East Coast fever. The persistence of parasites in the red cells of some of the cases, sometimes for considerable periods, is recorded. No morphological difference between *T. parva* and *T. mutans* forms could be established.

5. The significance of the prevalence of piroplasmosis in these exposure experiments, either direct or as a sequel, was stressed in contrast to the few cases of heartwater which occurred.

6. The 1935 experiment was characterised by the following in the exposed Vryburg group of animals:—

(*a*) the production of 100 per cent. Koch's bodies;

(*b*) approximately 100 per cent. mortality (including those cases killed in extremis) as a result of tick infestation; and

(*c*) the causation of pathological lesions indistinguishable from East Coast fever.

This stood in remarkable contrast to the Onderstepoort exposed animals in 1935, and the Onderstepoort and Vryburg exposed animals in the 1934 and 1936 experiments. The mortality in the 1935 Vryburg exposed animals must be attributed to the effects of the mass tick infestation and the subjection of these animals at the height of the febrile reaction to a severe and prolonged railway journey from one centre to another. This provoked in these animals an abnormal reaction of *T. mutans*, which has so far not been observed in South Africa, and which it is believed very seldom occurs under natural conditions. The occurrence and nature of the disease in such *T. mutans* cases resemble East Coast fever almost in every respect. In view of the results obtained in both groups of animals of the 1934 experiment in the Onderstepoort animals, of the 1935 experiment, in the Vryburg animals of the 1936 experiment, and the Umzinto experiment, in which no mortality of such a nature occurred, and in view of the extensive tick infestation and the absence of deaths in any of the local cattle utilized as controls, this mortality could in no way be regarded as of the nature of East Coast fever.

7. Koch's bodies in all stages of development occurred as in East Coast fever, except that the impression was gained that the gamonts were less prevalent in the *T. mutans* cases. The frequent occurrence of Koch's bodies in all stages, free and intracellular, in the blood smears was significant in these *T. mutans* cases which died or were killed in experiments.

8. The Umzinto exposure experiment was characterized by the occurrence of only one Koch's body case. In spite of heavy tick infestation and the fact that the mortality in two cases could be directly attributed to mass tick infestation the difference in the onset and course of the febrile reactions manifested in the exposed cattle respectively in the two paddocks was stressed. The importance of piroplasmosis, the approximte 100 per cent. mortality in newly introduced animals and the absence of any mortality in the local exposed cattle were recorded. Is this difference to be attributed to some unknown agent besides the mere mechanical effects produced by the ticks?

9. The symptoms in the exposed animals were characterised by an early onset of an acute febrile reaction with a prolonged course, the early presence of mass tick infestation (practically a pure *R. appendiculatus* infection) with swelling of the ears, vicinity of the ears, cranial aspect of the neck, face, intramandibular space, eyes, followed by haemorrhage, dermatitis, necrosis, gangrene, sloughing off of portions of the ears, all stages of emaciation, cachexia, general anaemia, enlargement of the lymphatic glands, particularly extensive swelling of the subparotids, etc.

10. The pathology of these *T. mutans* cases in the 1935 Vryburg animals was very significant. Besides the extensive changes observed in connection with the ears. eyes, face, regional lymph glands, emaciation, cachexia, general anaemia, very interesting and specific alterations were observed in the *liver, kidney, spleen, lymph glands, and abomasum.* In the latter they were of the nature of multiple ulcers, closey resembling those met with in East Coast fever. On the other hand isolated lymphoid hyperplastic nodules in the kidneys

(so characteristic of East Coast fever) were observed in only two cases. Besides vascular and regressive changes usually met with in infectious diseases, in the liver, kidneys, lungs, myocard, etc., great varation occurred in the extent and distribution of *lymphoid hyperplasia in the liver and kidneys.* None of the exposed animals other than the 1935 *Vryburg cattle* showed anything of this nature. Very characteristic lesions in the follicles of the spleen and lymph glands were observed, in which a homogeneous substance was formed and caused disorganisation of the " germ centre " and necrobiosis to a lesser extent. These resulted in a reduction of the number of follicles and a disorganisation of primary and secondary nodules. The signicance of a neutrophilia to a greater or lesser extent in the spleen and lymph glands was not clear. The impression again in the present study is that the lymphoid tissue in the lymph glands and spleen was damaged as a result of an acute theileriasis. Further studies have been undertaken to explain not only the pathogenesis of the lesions in the spleen and lymph glands, but also the nature of the accumulations of lymphocytic cells in the liver and kidneys. Except for a difference in degree it was not possible to differentiate between the lesions of *T. parva* and those cases of *T. mutans* infection in the 1935 Vryburg exposed cattle.

11. A reduction of small lymphocytes and " degenerative " changes in respect of the cells of the lymphocytic series were revealed in the various smears. In the acute *T. mutans* cases there was a reduction in the number of lymphocytes and an increase in the number of neutrophiles probably associated with the suppurative changes described in connection with the ears. The presence of an increased number of lymphoblasts in the blood smears was also noted. These " *mutans* " cases moreover showed a definite oligocythaemia. The frequent occurrence of granules in lymphocytes was referred to which in many instances had no resemblance to the ones usually met with in normal lymphocytes.

12. Apart from the mass tick infestation at Tzaneen, environmental conditions, the presence of blue tongue virus, etc., it was not possible in the tick transmission experiment at Onderstepoort to bring about febrile reaction with the same onset, course and severity as observed at Tzaneen. Febrile reactions with a longer incubation period, shorter duration, slight symptoms and the development of Koch's bodies in the majority of animals were produced by ticks emanating either from *East Coast fever* or from *non East Coast fever* areas. Similar febrile reactions were transmitted by ticks to sheep. It was not possible to reproduce these febrile reactions by sub-inoculation of blood into either sheep or cattle.

13. Difficulties in establishing at Onderstepoort East Coast fever with infected ticks and the unsatisfactory course of the cross immunity East Coast fever experiments at the Inanda Location are briefly referred to.

14. A case is recorded in which Koch's bodies in lymph gland and haemo-lymph glands were observed as a result of a " relapse " due to splenectomy of a *T. mutans* carrier. As usual parasites in the red cells were frequent in the blood smears shortly after the operation.

15. *Rickettsia bovis* was noted in the lymphocytes of some of the cattle utilized for the transmission experiments but at present their true nature is not understood nor in what relation they stand to some of the febrile reactions produced.

16. In the blood and organ transmission experiments into cattle, sheep, guinea-pigs, rats and mice, with material emanating from the *T. mutans* cases the results were entirely of a negative character.

DISCUSSION.

T. mutans infection with the appearance of Koch's bodies in organ smears occur in South Africa in East Coast Fever and non-East Coast Fever areas. Such bodies occur in the course of the development of *T. mutans*. That they have not been demonstrated in the greater portion of *T. mutans* infections is no criterion, for reasons given above, that they were not present.

Under certain conditions enumerated above, particularly change of environment, *T. mutans* infection may assume a pathological picture which cannot be distinguished from that of *T. parva*. It is believed, however, that such cases occur rarely in South Africa under natural conditions. *T. Parva* on the other hand causes mortality direct without the operation of other agencies, without which *T. mutans*, remain a harmless parasite.

Similar cases of T. *mutans* infection have been recorded by Doyle (1924) in the Mediterranean Littoral, by Turnbull (1926) in Northern Rhodesia, and by Ajwani and Subbarayudu (1934), who record a fatal case in a bull in India with gametocytes in almost all corpuscles and blue bodies free in the plasma in large numbers. It would appear that Lawrence (1934) has probably been dealing with a similar condition in Southern Rhodesia. Reference is made by him to an undiagnosed disease of cattle in a certain apparently well defined area with heavy annual mortality. The first cases occurred three weeks after the introduction of suitable animals on the grazing area on which the disease is known to occur. Plasma bodies have been encountered sometimes in large numbers in gland smears and still more frequently in spleen smears. The presence of *Koch's* bodies in such numbers in smears is attributed to *T. mutans*, the suggested explanation being that owing to the variation of factors probably associated with the undiagnosed disease, the resistance to the usual latent infection with *T. mutans* drops down and allows this organism to develop rapidly in the lymphocytic tissues of the animal.

Doyle maintains that Koch's bodies as a developmental stage of *T. mutans* differed from East Coast Fever in many respects and above all in the mortality which it caused. Significant is the fact that the Cyprus cattle harboured *T. mutans* when they arrived in Egypt and that the sea voyage and the damp, hot climate in summer are the principal factors in causing a recrudescence of the disease. Blue bodies were recovered in 7·5 per cent. of the Cyprus cattle infected with *T. mutans* out of 200 observations. With the exception of ulcers in the abomasum nothing diagnostic is to be seen in the post mortem. However, in one cyprus bull " necrotic infarcts " were

found quite indistinguishable from those of East Coast Fever. Cyprus cattle are more susceptible than the native Indian breed but even amongst the former the mortality does not rise above 1 per cent. The extent of tick infestation of the grazing is another factor to which Doyle refers.

East Coast Fever had never been reported as existing in the Fort Jamieson district of north-eastern Rhodesia. Turnbull is of opinion that the plasma bodies found by him there represented developmental stages of some parasite other than *T. parva*. The exclusion of East Coast Fever was based on the periodical presence of plasma bodies in the superficial lymph glands of animals in which recrudescences of the disease had occurred as a result of the adverse influence of certain debilitating diseases. He refers to the more frequent scanty presence of intracorpuscular parasites and plasma bodies as compared with East Coast Fever. He was not able to appreciate any morphological differences between the plasma bodies and the two parasites. The parasites in these infections usually occur singly in the red cells, not infrequently two and exceptionally three. Anaemic and haemorrhagic infarcts are sometimes present in the kidneys.

The observations of Cooper (1926) with reference to *T. mutans* infection at Muktesar would seem to indicate that all cattle in India are affected. Although the infection did not appear to be productive of ill effects upon the host, even if organisms occur in large numbers under certain conditions, *T. mutans* may be so exalted in virulence to produce an infection similar to East Coast Fever. During the last two years at the Muktesar Institute microscopic examination of lesions found at post mortem of animals infected with *T. mutans* revealed structures indistinguishable from East Coast Fever.

An ardent attempt was made to follow the position of *T. mutans* in East and Central African literature, but great difficulty was experienced, probably due to the fact that earlier workers had not yet recognised the occurrence of Koch's bodies in the development of *T. mutans*. Formerly all cases of small piroplasms with the presence of Koch's bodies in organ smears were probably diagnosed as East Coast Fever as in South Africa. Interesting therefore are the recent findings of Mettam and Carmichael (1936), who state that *T. mutans* and *T. parva are* immunologically and pathogenetically different parasites. There is no proof and certainly no indication that *T. annulata* or *T. dispar*, as described by French workers of Algiers or other workers in the Mediterranean region, exist in this part of Africa. It is maintained that Theileriasis of Uganda as also of Kenya and East Africa generally resemble the Theileriasis of Southern Africa more than those of Northern Africa. Their reference to the benign form in about 20 per cent. of the cases is of interest. In the veld these mild cases are often overlooked but when temperatures of calves are taken daily from the time of birth the reaction is readily recognised. The lymphatic glands are never seriously affected; they slightly increase in size during the rise of temperature but never return to pre-febrile dimensons. The mortality is negigible. During the reaction *T. parva* is very rare in the blood, while schizonts are only exceptionally found in spleen or lymphatic

glands. Of further interest is the fact that mortality varies within wide limits, depending on the way the sick animals are treated. If they are nursed and given plenty of milk it is low, but neglect, exposure to inclement weather, the presence of intercurrent diseases, unhygienic conditions and lack of milk increases the death rate. The lymphatic system is always involved but in some cases not severely. Renal infarcts or white foci are much rarer, being observed in between 10 to 20 per cent. of post mortem cases at the laboratory. If these data, recorded by Mettam and Carmichael, are carefully viewed in the light of the observations made at Tzaneen the question is asked whether they were actually dealing with infections of *T. parva*. Can this benign form under the abnormal conditions enumerated not be of the nature of *T. mutans* rather than of T. *parva*, which in South Africa is always associated with a high mortality when once it has established itself?

Incidentally it may be stated that *Turning Sickness* has not been observed in South Africa since Mettam's preliminary communication. A circular (May, 1935) was issued to all Government Veterinary Officers in East Coast fever areas in Natal to carry out investigations. In spite of careful examination of the central nervous system of a number of East Coast fever and *T. mutans* cases nothing of this nature has so far been observed. Numerous sections from the central nervous system at various levels have so far been examined but the only abnormality recorded in one case is the occurrence of *P. bigeminum* in practically 75 per cent. of the red cells in the smaller blood vessels of the hippocampus, when in the spleen they could only be found with great difficulty.

In the majority of studies in connection with *T. mutans* the course of Koch's bodies has been recorded as more or less of the nature of a relapse as a result of debilitating diseases, change of environment, malnutrition, etc. Recently at Onderstepoort a relapse was definitely brought about by splenectomy with the usual frequent occurrence of parasites in the red cells in the blood stream and Koch's bodies in lymph glands and haemo-lymph glands to a lesser extent. The question is asked whether the Tzaneen and Umzinto experiments can be considered in the light of a " relapse " or a " re-infection ". Is it a relapse as the result of the effects of mass tick infestation? This does not seem to be the case in the 1936 exposure experiments where 100 per cent. Koch's bodies occurred after the usual incubation period without extensive impairment of health as a result of a brief exposure to ticks and a moderate degree of tick infestation. How is the infection to be explained in the tick transmission experiments, where Koch's bodies and febrile reactions occurred after a definite incubation period? What is the nature of the infection in slaughter animals moved from one area to another? Du Toit (1931) refers to the fact that partically 100 per cent. of the cattle in South Africa become infected with this ubiquitous, harmless parasite. Is this of the nature of a re-infection with different strains of *T. mutans*, similar to what we believe occurs in the case of *P. bigeminum* in South Africa? In analysing the temperature reactions produced in the exposed Tzaneen cattle, and the tick transmission experiments in cattle and sheep, it would appear as if certain reactions overlap. Can

the sudden onset of high temperature in the exposed cattle be attributed to the blue tongue virus which was recovered but did not produce any febrile reactions when injected into susceptible cattle at Onderstepoort? Does this virus have a more virulent effect on cattle which have been transported over long distances, partially starved, and then subjected to a different environment, especially in respect of grazing and climatic conditions? This does not seem to be the case because it is not borne out by the observations at Tzaneen, where no such reactions were observed in the cattle identically treated but left in the trucks and not exposed to tick infestation. Can the sudden onset of the febrile reaction be attributed to *T. mutans* infection? This also seems to be unlikely because in the tick transmission experiments the moderate febrile reactions and the Koch's bodies were only observed at the earliest ten days after tick infestation. Is seems therefore more than likely that these early acute febrile reactions observed seem to be related to the direct effects of the ticks. The observations of Cooper are interesting in this respect. He states that in addition to the diseases enumerated in his report there is evidence on record to indicate that certain ill defined fevers may be introduced by toxins or poisons injected by ticks at the site of puncture, while excessive parasiticism may occasion serious mechanical injury to the host by setting up wounds which may become subsequently infected by maggots or lead to suppurating sores due to secondary infection by organisms.

It is significant that these early temperatures could not be reproduced by ticks in susceptible cattle. In view of the febrile reactions with a definite incubation period obtained in sheep during the tick transmission experiments it would appear therefore that similar reactions obtained in bovines could not be attributed to the *T. mutans* infection and the appearance of Koch's bodies. The question therefore, is asked whether these light, less acute febrile reactions observed in these tick transmission experiments can be attributed to a " virus " whose identity and nature has so far not been disclosed. Further investigation in respect of this problem is therefore essential before finality can be given to these questions of " relapse " and " re-infection " referred to above.

In dealing with the immunity of cattle inoculated with piroplasma (*T. mutans*) Theiler (1907) refers to an interesting observation at Nelspruit where in 1903 East Coast fever experiments were conducted. Of ten calves introduced from the High Veld early in 1908 the majority died and the microscopical examination of the smears showed the presence of small piroplasms to such an extent that for a short time the diagnosis was doubtful. An investigation was immediately made and it was thus clearly proved that the calves were susceptible to piroplasma (Theileria) *T. mutans* infection and piroplasma (Theileria) *T. Parva* had to be excluded. Animals immune against heartwater, redwater and *T. mutans* as well as controls were exposed. All the exposed animals showed reactions a certain time after exposure. These reactions cannot be determined with absolute certainty although inoculation of the blood collected during the reactions were made into various sheep. All the sheep with the exception of one showed reactions but since none died and heartwater

is usually fatal for sheep it was concluded that not all the reactions given by the exposed cattle were due to heartwater, but that there was *some other agency* responsible, of which we have no knowledge at present. With regard to the reaction which cannot be definitely determined, it must be remembered that the animals had been for a considerable length of time away from tick infestation or at least were exposed to minimal tick infestation and running on a veld in which blue and red ticks were present and the brown ticks were hardly ever noticed. In view of the transmission of these reactions by blood inoculation into sheep it is possible that the reactions in the sheep were due to blue tongue, in the light of the transmission experiments carried out at Onderstepoort. Apart from this however it is possible that blue tongue may have been concurrently present and that the febrile reactions after all have been due to a virus or other agency similarly to what has been suggested above.

The suggestion that the infection set up in the 1935 Vryburg exposed cattle at Tzaneen should be attributed to a new species of Theileria so far not recognised in South Africa has been considered. In view, however, of the recommendations of du Toit (1930) it is felt that the adoption of the more conservative attitude should be favoured at present, viz., that only four species be recognised, namely *T. parva*, *T. dispar*, *T. annulata* and *T. mutans*. In South Africa it is therefore suggested that we continued to recognise only *T. parva* and *T. mutans*. According to du Toit *T. mutans* no longer kills its host, except when the resistance of the host is lowered and the balance between the parasite and host is disturbed. As a result of this many gradations between the virulent *T. parva* parasite and the usual innocuous parasite *T. mutans* may occur.

It is significant how the pathology as discussed by Cowdry and Danks in cases of East Coast fever coincides with the observations made in the Tzaneen exposure experiments, especially in respect of the following:—

(*a*) Developing Koch's bodies have only been found in cells of the lymphocytic series;

(*b*) *small lymphocytes* become progressively less numerous in the course of the disease, both of the lymph glands and the circulating blood;

(*c*) the tissue between the germinal centre and the sinus is greatly reduced in extent and contains few if any lymphocytes;

(*d*) the lymph glands so swollen are oedematous and very poor in lymphocytes.

According to Cowdry and Danks the available evidence seems to indicate that the swelling of lymph glands, which is so characteristic a symptom of East Coast Fever, is not caused by hyperplasia but rather by the accumulation of oedema. From their results and the studies in South Africa it would appear as if acute Theileriasis as set up by either *T. parva* or *T. mutans* is in some way associated after damage to the lymphoid tissue. Cowdry and Danks are of the opinion

that the parasites probably do not act as a powerful stimulant to the multiplication of lymphocytes. They maintain that there is little if any anaemia, but they are not in a position to say which vital activity is so impaired that death results, although the wasting probably may be occasioned partly or wholly by the progressive failure of numerous lymphocytes to perform their proper function. It is doubtful, however, whether the emaciation and cachexia following mass tick infestation in cattle only stand in a definite relation to the damage done to the lymphoid tissue by acute Theileriasis. In the Tzaneen (with the exception of 1935 Vryburg batch) and Umzinto exposed cattle that died or were killed *in extremis* showing pronounced emaciation no such damage to the lymphoid tissue was identified. Damage only occurred in those cases in which there was a definite development of Koch's bodies. At the present moment it is therefore not yet possible to state how these pathological changes ultimately lead to death and in what exact relation they stand to tick infestation. Further studies are also indicated in respect of the nature of the changes and granules observed in the lymphocytes of ruminants more or less on the lines carried out by Wiseman (1931) especially in respect of supervital staining. Furthermore, it would also be interesting to determine the changes brought about in lymph glands by various degrees of radiation of the lymph system of ruminants. How far would this affect the course of *T. mutans* infection, more or less on the lines of the investigations of Murphy (1926), who studied the lymphocytes in relation to tissue grafting in malignant diseases and tubercular infection?

As a result of the above studies and our present knowledge of the development and course of *T. mutans* under various conditions, it is felt that the quarantine periods in East Coast fever areas, especially in respect of contact farms, could materially be shortened and relief granted in this respect, provided however—

(*a*) full co-operation of all farmers concerned is obtained for the accurate census of cattle, checking of all mortality, and the submission of suitable smears from the spleen and superfiicial lymph glands (prescapular);

(*b*) a careful checking up is carried out by the staff in respect of the history of the outbreak, type of animal involved, whether newly introduced, or whether it comes from a locality where ticks are few or dipping carefully carried out, the extent of tick infestation on the farm on which the case occurs, time of the year the condition is diagnosed, any possibility of an introduction of East Coast fever on the farm (i.e. proximity of this farm to outbreaks in the vicinity), the presence of intercurrent diseases such as paratyhoid, malnutrition, cachexia as a result of bad farm management, etc.;

(*c*) that there has been no previous history of East Coast fever on the farm.

Such control and co-operation of the farmer will bring to light what one of us (G. de K.) believes to be the tail end of the East Coast fever infections, especially in Natal.