

## **Bovine Theileriasis in South Africa with Special Reference to *Theileria mutans*.**

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EAST Coast fever has now been present in various parts of the Union for over 30 years, during which period active measures for its control have been enforced uninterruptedly. At various periods good progress seemed to be made towards the final eradication of the disease only to be followed later by serious setbacks. This experience has not been confined to the Union of South Africa, because both Southern Rhodesia and Swaziland have on occasions been very close to attaining complete eradication of the disease.

The setbacks which have so frequently occurred have been variously explained, but one of the most common reasons offered has been failure in diagnosis and locating infection commonly due to a scarcity of ticks either naturally or as a result of intensive tick destruction measures. As a result of this only odd cases of the disease occur which may escape diagnosis through failure to obtain a smear for microscopic examination or through the submission of smears which are unsuitable for diagnostic purposes.

In order to overcome this the campaign against East Coast fever was so reorganised as to concentrate largely on the diagnosis of the disease and the early location of infected centres. Accordingly special attention has for some years been paid to the following:—

- (a) *Census of Cattle*.—In all East Coast fever areas an accurate count is made of all cattle at regular short intervals by inspectors appointed for the purpose. The frequency with which these counts are made varies according to the proximity of the area to known infected or alternatively with the likelihood of the disease making

its appearance in any locality. Depending on the circumstances obtaining in any given area the cattle within it are therefore carefully counted every 5, 7, 14 or 21 days. Thus the counts in actually infected areas are made at every dipping (i.e. at 5-day intervals), while in clean areas (i.e. those well removed from known infected centres) dipping is not officially supervised and the inspection of cattle is arranged at convenient centres and at longer intervals as indicated above.

The essence of this system of checking of cattle is that all increases and decreases have to be accurately accounted for, in the first instance to ensure that no dangerous or illegal movements of cattle are effected and secondly to gain accurate information in regard to mortality from any cause.

- (b) All deaths of cattle from any cause whatever within East Coast fever areas must be immediately reported by the owner to an official of the Department and a fresh organ smear must be submitted for microscopic examination. In practice fresh smears are required from all animals that are slaughtered as well as those that die from natural causes and the counting system outlined under (a) provides the Department with a ready check of the deaths against the smears which are received. It is of course not always possible to obtain a smear suitable for diagnostic purposes. For various reasons decomposed smears are frequently submitted or often specimens are unsuitably prepared and at times even, smears are not submitted at all. All such cases in practice mean undiagnosed deaths or rather comprise cases in which East Coast fever as a possible cause of death can not definitely be eliminated.

Owing to the numerous practical difficulties encountered in the course of ordinary farming practice it is quite impossible to ensure that suitable smears are submitted in all cases but through the exercise of reasonable care such missed diagnoses can be reduced to an odd case now and again. In any case the Department is forced to treat with suspicion any farm from which a series of unsuitable smears are submitted. Such farms have therefore perforce to be placed in temporary quarantine and special supervision exercised there until such time as the true disease position can be ascertained.

This system of control requires an efficient organisation which the Department has been at great pains to perfect during the last 10 years or more, and it can be accepted that the organisation now is such that the Department is placed in possession of accurate knowledge in regard to—

- (i) the incidence of infection;
- (ii) subsequent mortality when once the disease has been diagnosed.

Chart I illustrates the increase of smears submitted from the Natal Province during the period 1912-1935 and while the percentage of smears submitted from individual areas (as compared with the deaths which have occurred there) varies, it is safe to accept that as an average over the Province smears are now received from 95 per cent. of animals which die or are slaughtered (exclusive of apparently healthy animals which are slaughtered for human consumption in the abattoirs in Durban, Pietermaritzburg and Johannesburg). The percentage of unsuitable smears varies somewhat from year to year (during 1935, 6.75 per cent. of the total number submitted were unsuitable for definite diagnosis) but making due allowance for all diagnoses missed through various causes it can be accepted that as an average over the Province the presence or absence of East Coast fever infection can definitely be determined in at least 90 per cent. of cattle that die or are slaughtered.

While admittedly the facilities for diagnosing the disease, as outlined above, are not perfect, the likelihood of infection escaping detection for any length of time is reduced to a minimum considering the vastness of the area, the large number of cattle involved and the innumerable practical difficulties which operate against the attainment of complete efficiency.

Under these circumstances the Departmental statistics in regard to the incidence of East Coast fever may be accepted as reliable enough for all practical purposes, especially when it is remembered that the official control on infected and suspected areas is much more intensive than in "clean" areas and that in such areas the facilities for diagnosis are proportionately better, i.e. the percentage of cases in which suitable smears are submitted is considerably higher than the average figure given above (90 per cent.).

The intensive campaign against East Coast fever has resulted in the reduction of the mortality rate to so low a figure that in many quarters farmers have ceased to fear this disease as a disease.

In Natal during the twelve months ended 30th June, 1933, there occurred 29 fresh outbreaks of the disease involving 12 magisterial districts; positive cases of the disease, however, only accounted for 0.4 per cent. of the total number of cattle involved in these outbreaks in native areas and 0.8 per cent. on privately owned farms.

During the year ended 31st August, 1935, East Coast fever outbreaks involved 44,724 head of cattle in native areas and 10,606 head on privately owned farms, among which the average mortality from East Coast fever amounted to 0.59 per cent. and 2.81 per cent. respectively.

On the other hand the mortality in individual outbreaks often reached quite a high proportion of the total number of animals involved as the following instances will show:—

## BOVINE THEILERIASIS IN S. AFRICA.

District.	Area.	Date of Outbreak.	Number of Cattle Involved.	Total Mortality from E.C.F.	Date of Last Case.
NATAL.					
Helpmekaar .....	Dabula .....	20. 3.33	106	12	24. 1.34
Entonyaneni .....	Wonderhoek .....	24. 3.33	136	52	17. 10.33
Lions River .....	St. Johns .....	18. 4.34	64	29	22. 1.35
Pietermaritzburg .....	Binchester Grange	5. 2.34	217	24	31. 1.35
Helpmekaar .....	Dresden .....	8. 6.34	209	60	19. 3.35
New Hanover .....	Duma .....	5. 9.34	1616	105	13. 6.35
Ixopo .....	Alderley .....	19. 1.35	314	29	17. 5.35
Camperdown .....	Burnside .....	17. 2.35	71	22	8. 5.35
Polela .....	Ingelhurst .....	9. 7.35	196	34	9. 3.36
TRANSVAAL.					
Barberton .....	Zeist .....	27. 4.34	366	25	16. 5.34
Pilgrims Rest .....	Spitzkop .....	25.10.34	413	47	20. 1.35
" .....	Hebron .....	14. 1.35	117	11	4. 2.35
Carolina .....	Batavia .....	8. 5.35	196	112	20. 4.36
" .....	Welgevonden .....	15. 4.36	483	56	26. 6.36
Pilgrims Rest .....	West Lodge .....	25. 1.36	198	25	30. 6.36

As against these outbreaks with a comparatively high mortality however there has in latter years occurred a large number of infections diagnosed by smear examination in which only a limited number of animals has succumbed to the disease. Undoubtedly stringent control measures have contributed very largely to the reduction of the mortality figure but in such a large proportion of cases, characterised by the occurrence of Koch's bodies in spleen or gland smears, *the outbreaks were confined to the initial death that further investigation of such cases was indicated.*

There were, however, in addition other factors which induced the authors to undertake a further study of these outbreaks and these observations are dealt with separately below.

The details of the cases from which the initial observations were made are tabulated in Appendix I.

(a) *Single Death Outbreaks with the appearance of Koch's Bodies in Spleen or Gland Smears.*

The following figures reflect the relationship between the total number of infections of East Coast fever in Natal during the last six years and the incidence of "single death" outbreaks:—

1929-30	... ..	58 outbreaks,	17 of which	single deaths.
1930-31	... ..	42	" 16	" " " "
1931-32	... ..	30	" 24	" " " "
1932-33	... ..	29	" 18	" " " "
1933-34	... ..	40	" 26	" " " "
1934-35	... ..	34	" 22	" " " "

With the system of field control in vogue there becomes available certain information, a full and careful consideration of which, in conjunction with the technical diagnosis arrived at by smear examination, provides a fairly reliable means of gauging the true position in regard to the incidence of East Coast Fever infection.

The data referred to comprise:—

- (1) Smear position over an extended period preceding the death diagnosed as East Coast fever.
- (2) History of the farm or area with special reference to—
  - (a) introductions of cattle from outside.
  - (b) movements of cattle through the farm,
  - (c) previous outbreaks of East Coast fever.
- (3) Relationship of the farm to known or suspected infection.
- (4) Control exercised, both officially and by the owner.
- (5) Mortality.
- (6) Tick life.

From a careful study of these data serious doubts have arisen as to the true etiological diagnosis in many of these "single death" outbreaks, diagnosed by the presence of Koch's bodies.

Thus it is found in many instances that East Coast fever as a possible cause of death can be eliminated in up to 100 per cent. of deaths which have occurred on the suspected farm for up to two years prior to the suspicious case. This would constitute definite evidence against the possibility of the infection having been present undetected on the farm in question for any lengthy period. From this it cannot, of course, be concluded that the suspicious death must of necessity be the first case as it is possible that previous cases could have occurred in animals which had recovered, as smears are not taken as a routine from sick animals, and in this way a case of the disease could easily escape detection.

We know however that while this is possible, the infection cannot escape detection in this way for any length of time as recoveries from East Coast fever occur only in a limited percentage of cases and it is highly improbable, therefore, that the disease could be perpetuated undetected in a sequence of recovered animals.

If now the history of the animal concerned is such as to negate any possibility of it having acquired infection outside the limits of its home farm, one is forced to the conclusion that infection had been picked up on the farm where it must have been introduced either by a movement of cattle or in some mechanical way, e.g. through grass or hides off an infected property.

Through the efficient permit system controlling all movements of cattle into, within and out of East Coast fever areas it is a comparatively simple matter to trace up every movement of cattle involving in any way the suspected farms.

It is, however, a more difficult matter to gain definite information in respect of the movement of animal or vegetable matter which through the presence of infected ticks may have transmitted the disease mechanically. The movements of such material from East Coast fever infected areas is controlled by permit but it is difficult if not quite impossible to be quite sure that grass for example is not occasionally moved off infected farms by natives. While a possible loophole for the conveyance of infection does admittedly exist here it is highly improbable that that infection is conveyed in this way over any very great distances and where suspicious cases occur on farms situated say 50 to 60 miles from the nearest active infection it would seem safe to rule out mechanical transmission as a source of infection.

Furthermore if the mortality remains confined to the initial case in spite of active and prolific tick life there is strong reason to doubt that East Coast fever infection was responsible for the death of the animal in which the suspicious diagnosis was obtained by smear examination.

(b) *Kind of Animal Involved.*

(1) A glance at Appendix I will indicate how frequently these "Koch's body" cases concern *calves*, ranging in age from a week or two to 18 months.

In these instances there is very frequently a history of a previous calf mortality on the farm from such conditions as coccidiosis, chronic scours, paratyphoid, or severe scalding as a result of dipping.

In other instances again the history of the animal concerned almost invariably discloses one or more of the following symptoms for a variable period before death:—

diarrhoea, inappetence, starvation, general unthriftiness,  
nervous disorders, leg weakness or heavy infestation with  
internal parasites.

The duration of these symptoms varies from one day to six weeks and longer and the animal is often slaughtered by the owner because no hope is entertained of its ultimate recovery. Unfortunately the smear examination is usually done some time after the death of the animal when it is too late to make careful observations or conduct a proper post-mortem and one has to rely almost entirely on the observations of laymen for the description of the animal's condition, hence it is impossible to obtain sufficiently detailed information in regard to either clinical symptoms or post-mortem appearances for the purpose of a diagnosis and it is only when some specific condition such as coccidiosis or paratyphoid causes a mild epizootic that the veterinarian is enabled to see cases and collect material.

(2) Next in order of frequency such cases occur in *adult slaughtered animals*. It is common knowledge that natives will often slaughter animals which are sick and are not expected to recover. Such cases are however excluded from consideration under this heading.

Animals are usually slaughtered for human consumption, being apparently healthy and usually nothing abnormal is observed post-mortem. In fact a large proportion of these cases are actually slaughtered at European-owned butcheries for consumption by European customers. Such animals are usually reported to have been in good slaughter condition, having been expressly acquired for slaughter. A remarkable feature of these cases is that there is usually a history of the animal in question having been moved from one farm or area to another shortly before slaughter. While this observation has been made very frequently, it has not so far been possible to attach any significance to the nature of the movement beyond the mere change of environment. It does not appear to matter whether the movement is from a higher to a lower altitude or vice versa, from a colder to a warmer climate or from an area with a lesser to one with more active tick life. The only constant factors in this connection appear to be—

- (i) that a movement has taken place, and
- (ii) that it took place shortly before slaughter usually up to three weeks before, with an average period of a fortnight.

It has been a frequent observation by those who have been intimately associated with the eradication of East Coast fever that the disease shows a proneness to occur on previously infected properties at more or less regular intervals of two to three years. With this in mind the previous histories of the cases tabulated in Appendix I have been carefully checked and going back as far as 1930 a previous history of East Coast fever infection could only be demonstrated in the following instances:—

(1) *Wombat, Pietermaritzburg District.*

On this property an outbreak of East Coast fever occurred on 3.2.33 among 193 head of cattle. The farm was cleared by slaughter of the whole herd on 16.2.33 by which date five positive cases of the disease had occurred. No cattle were permitted on this property again until June, 1935. The animal involved in the outbreak on 26.11.35 was a very old cow (stated to be 14 years of age) which had become involved in a difficult parturition and was destroyed by the owner as she did not appear to be recovering.

(2) *Klipbank 1672, Pietersburg District.*

In this area East Coast fever broke out in a herd of 155 head of cattle on 22.4.32. The farm was cleared by slaughter on 28.5.32 by which date fifteen positive cases of East Coast fever had occurred. No cattle were permitted onto the farm for 18 months, i.e., until November, 1933. On 20.12.33 a 3 months' old calf died, the smears showing Boch's bodies; no small piroplasmids were present but a few *P. bigeminum* were observed.

It seems extremely doubtful that the subsequent suspicious cases could in these two instances have been directly connected with the earlier positive outbreaks of the disease. In the first place all susceptible animals were slaughtered so that the infection could only have been perpetuated on the property within the ticks; this would only be possible if the adult tick could live long enough off a host to cover the periods during which these two properties were kept cattle free, i.e., 28 and 18 months respectively. While it is true that ticks have under laboratory conditions been kept live for periods in excess of these, it is extremely doubtful whether under natural conditions a tick would survive so long and even if it should there is every reason to believe that an infected tick would by that time no longer be able to convey its infection to a susceptible host.

A second ground for discounting the possibility of infection having been perpetuated on these two properties is the fact that of a very large number of properties which have during recent years been cleared of cattle on account of East Coast fever infection has in no single case recurred on restocking after 18 months. An experiment (S. 5929) with a heavily infected paddock on the farm Spitzkop, recently gave a negative result on animals being placed in it after 15 months.

From the foregoing it must of course not be assumed that these suspicious cases can be treated lightly or that they do not in some cases develop along more serious lines.

In spite of the numerous cases in which these "single death" outbreaks fail to develop there have been instances where definite *East Coast fever* has followed a "suspicious" case shortly before and a few examples are quoted below:—

*Spitzkop: Pilgrimsrest District.*

On 8.6.34 a smear was submitted from an ox which had died showing at post-mortem evidence of an internal abscess with extensive peritonitis. On examination of the smear numerous small piroplasms were seen and a few bodies which were suspicious of Koch's bodies. The farm was kept under careful observation but nothing further transpired until 23.10.34 when an ox died showing in the smear submitted frequent Koch's bodies and numerous small piroplasms. On the following day two further animals died, the smears from which also showed frequent Koch's bodies and small piroplasms. In spite of a prolific tick life on the farm another two months passed without further deaths, but during December twenty-four positive deaths occurred.

The farm was cleared of cattle by slaughter on 20.1.35, by which date forty-seven deaths from East Coast fever had occurred among the herd of 366 head on the farm.

*Julesburg: Letaba District.*

On 10.3.35 a six-year-old bull died on this farm, showing on smear examination rare Koch's bodies but no small piroplasms. The farm was quarantined and kept under close observation.



On 1.10.35 a young bull died after having been sick for a week. The smear submitted from this animal showed small piroplasms and a few bodies suspicious of Koch's bodies.

There was a total of 1,221 head of cattle on this farm, and between the date of the first suspicious death (10.3.35) and 14.10.35 the smear position was as follows:—

No. of deaths ... ..	147
No. of smears submitted	145
No. smears negative ... ..	130
No. positive ... ..	2 (1 anaplasmosis, 1 suspicious East Coast fever).
No. unsuitable for definite diagnosis ... ..	13

giving 89.78 per cent. of diagnosed deaths. By 27.10.35, two East Coast fever deaths had occurred; the next death was on 9.1.36 and by 7.4.36 when the farm was cleared by slaughter a total of eleven East Coast fever deaths had occurred; the smears in these cases giving the typical East Coast fever picture in which both Koch's bodies and small piroplasms were numerous.

*Tank Area. 90 Location and Ixopo.*

This tank area was infected with East Coast fever during 1930, when four deaths from this disease occurred there, the last of which took place on 22.5.30. No further evidence of infection was found until a smear from a three-year-old bull killed on 3.3.35 showed Koch's bodies, but no small piroplasms.

The area was placed in quarantine and kept under strict observation as an area suspected of being infected with East Coast fever.

There were 2,800 head of cattle in the area and although East Coast fever as a possible cause of death could definitely be eliminated in 96.4 per cent. of cases by smear examination no further trace of infection was found until 3.3.36 when a three-year-old bull died, the smear showing both Koch's bodies and small piroplasms. Another death, a month later, i.e. 2.4.36, also produced a typical East Coast fever smear.

*Albert Falls: New Hanover District.*

The area concerned here consists of a number of small holdings with a common grazing ground.

A smear submitted from a cow which died here on 18.2.33 showed rare Koch's bodies but no small piroplasms.

There was a total of 178 head of cattle running on the suspected area which was placed under close observation. During the subsequent 12 months there occurred 30 deaths, 29 smears being negative and 1 showed anaplasmosis. The next death was that of a heifer on 17.3.34 and this smear showed both Koch's bodies and small piroplasms fairly frequent.

Further deaths, with typical East Coast fever smears, occurred on 15.6.34 and 9.2.35 since which date no further evidence of infection has been traced.

The question naturally arises as to whether the original "suspicious" cases could be ascribed to *T. mutans*, in which case it would be mere coincidence that positive cases of East Coast fever followed subsequently.

In the case of Spitzkop one is inclined to accept, in the light of subsequent developments, that the original death which occurred on 8.6.34 was a positive case of East Coast fever.

In the first place the smear was really typical of the usual East Coast fever picture except for the fact that the Koch's bodies present did not happen to have been well formed. Secondly it is quite certain that the 3 deaths which occurred on 23rd and 24th October 1934 were not the first cases to occur on this farm. Bearing in mind, therefore, that this farm is very high-lying and that the "suspicious" case occurred in midwinter when tick life would normally be at a very low ebb, one would not have expected any serious mortality until the advent of summer.

In the case of Julesburg one favours the view that the suspicious case which occurred on 10.3.35 was an instance of the formation of plasma bodies by *T. mutans*. Firstly the smear was more characteristic of those tabulated in Appendix I and secondly this case occurred at a time of the year when tick life in that locality is most active and any infection introduced there then must inevitably have been reflected in a heavy mortality almost immediately.

Moreover there is every indication that the infection *T. Parva* which was detected on this farm had its origin in the serious outbreak of East Coast fever which was diagnosed on the farm Tours on 10.4.35. These two farms adjoin one another at a corner beacon and in the absence of fencing intermingling of cattle occurred fairly freely, until the disease was detected on Tours.

In the case of Tank Area 90, Location 8, Ixopo, the suspicious smear, though showing Koch's bodies, was not typical of East Coast fever and the history of the animal indicates that it was slaughtered while apparently healthy.

As indicated above the facilities for detecting any infection which may have been present were particularly good (96.4 per cent. suitable smears) and yet it was not until a year later that a positive case of East Coast fever is diagnosed in the suspected area. Furthermore, although the two herds in which these two cases had been diagnosed belonged to the same dipping area they actually had their normal grazing grounds some miles apart and in separate valleys.

The smear from the "suspicious" case on Albert Falls Commonage on 18.2.33, again was similar to those referred to in Appendix I. For thirteen months, during which period 100 per cent. of deaths are accounted for by smear examination, no further trace of the disease was apparent until the positive case diagnosed on 17.3.34.

While there is no proof of the introduction of infection from any of the outbreaks in the vicinity, there still remains that possibility; these outbreaks were:—

	First Death.	Last Death.	Total.
Wombat.....	3. 2.33	16. 2.33	5
Notuli.....	16. 5.33	7. 6.33	5
Binchester Grange.....	5. 2.34	9. 3.34	9

These outbreaks were all in the immediately vicinity of Albert Falls and seemed definitely to have some relationship one with the other.

The conditions under which *single deaths associated with Koch's bodies* occurred in *East Coast fever areas* in Natal were referred to above and stress was laid on the great difficulty experienced in deciding whether these should be attributed either to *T. parva* or to *T. mutans*. From the evidence it would appear that the majority of these cases were undoubtedly associated with *T. mutans* and not with *T. parva*. Their apparent increase in Natal can be attributed to the intensive smear control and in spite of this it can be assumed that many cases are still overlooked.

### KOCH'S BODY CASES IN NON-EAST COAST FEVER AREAS.

In *non-East Coast fever areas*, e.g., Marico, Pretoria, Pietersburg and Zoutpansberg, Koch's body cases have from time to time been recorded without any subsequent mortality.

In *Marico* three Koch's body cases are recorded in three calves, post-mortemed during an investigation by Viljoen and Martinaglia (1928, and file 51/539 of 1934), in respect of a heavy calf mortality in that vicinity. These three cases showed unthriftiness, staring coat, listlessness, unsteady gait, drooping of the ears, cachexia and a sunken in appearance of the eyes. The Koch's bodies varied in number from one to numerous free and intra-cellular forms. Viljoen and Martinaglia were of the opinion that the mortality could be ascribed to bad animal management, poor nutrition, tick infestation, tick-borne diseases, as well as partyphoid, which seemed to have played a very important part. This was the first record of the occurrence of Koch's bodies in an area which had been free of East Coast fever for more than twenty years.

In the Pretoria districts, since the severe and wide-spread outbreaks of East Coast fever of 1922 there have been no further cases of that disease. It was eradicated by means of dipping under close supervision. East Coast fever occurred on 59 farms and on some of them the mortality was extremely high. Since then several Koch's body cases have been observed at Onderstepoort and on the Kaalplaats farm in the Pretoria district. Theiler and Graf (1928) record several cases of Koch's bodies in small numbers with *T. mutans* practically in every case and occurring in the blood for several months. The cases occurred in calves (C. 2063, 2208, 2187 and 2096) exposed to natural tick infestation from date of birth. In some the only symptom recorded was a slight temperature reaction. Reference is also made to an exposure experiment of a number of young Vryburg oxen to heavy tick infestation at Kaalplaats for ten days and then stabled. These animals showed multiple infection of blood parasites, anaemia, but no Koch's bodies. It should, however, be pointed out that the examination of the gland smears in respect of these animals were not carried out regularly and for that reason Koch's bodies may have been overlooked, when considered in the light of our present knowledge and information.

Du Toit (1931) records a few Koch's body cases (C. 1863, 2336 and 2207) in an East Coast fever immunity experiment carried out at Kaalplaats. Susceptible and recovered East Coast fever animals were exposed to heavy tick infestation. From the results obtained *T. mutans* was diagnosed in very many of the animals, but blood and gland smears from all animals were collected only once a week. The possibility that the Theileria observed in these experiments may be regarded as *T. parva* is completely refuted by du Toit although it is true that a distinction between the blood forms of these two parasites is practically impossible. Other factors were considered, especially the fact that *T. mutans* is a mild and perfectly innocuous parasite, whereas *T. parva* is a very deadly parasite, causing a mortality of from 90 to 95 per cent. In these experiments the Koch's bodies were considered as developmental stages of *T. mutans*. Sub-inoculations with blood into six susceptible calves failed to produce any infection.

In March, 1933, another Koch's body case (C. 5385) occurred in one of the Onderstepoort camps. This animal was found dead and in the spleen intra- and extra-cellular agamonts and gamonts were frequent, besides small piroplasms. Unfortunately the specimens collected are no longer available, but in the liver and kidneys of this case there was no evidence of lymphoid hyperplasia.

#### THE TZANEEN (N. TRANSVAAL) EXPOSURE EXPERIMENTS.

Koch's bodies have been reported on at least three occasions in cattle on the Town Lands of Tzaneen. These were respectively seen in three oxen slaughtered in the 6th March, 1933, 3rd April, 1933, and 3rd July, 1933. No small piroplasms were identified and there was no suspicion of East Coast fever at the time of slaughter. The last case of East Coast fever in that vicinity was recorded in April, 1927. From the following smear records of the animals slaughtered

on the Town Lands of Tzaneen and the prolific tick infestation, it was most unlikely that East Coast fever could have "smouldered" there for seven years:—

*Smear Records: Tzaneen Town Lands, 3.3.33-2.3.34.*

<i>(a) Family Butchery: Tzaneen.</i>	
Slaughtered .....	173
Smears .....	169
Negative .....	154
Plasma bodies .....	3
Useless .....	1
Decomposed .....	1
(?) .....	3
7 Smear results not to hand.	
<i>(b) Tzaneen Butchery.</i>	
Slaughtered .....	212
Smears .....	194
Negative .....	178
Useless .....	3
(?) .....	4
9 Smear results not to hand.	
<i>(c) Tzaneen Smears.</i>	
Dead .....	19
Smears .....	14
Negative .....	9
Decomposed .....	3
2 Smear results not to hand.	

The Town Lands of Tzaneen are situated on the foothills of the Drakensberg range, with sour veld and a heavy rainfall, ideal for tick life. Natural grazing, although plentiful, is not of good quality, and stock do not thrive throughout the year. As a rule the management of stock is carried out on very poor lines and dipping, which is left to the owners, is not carried out regularly and in the majority of cases in a very indifferent manner. East Coast fever control on the other hand consists in counting and checking cattle and concentrating on obtaining as many good smears as possible. As can be expected the diseases met with in these areas are the usual tick-borne diseases, for instance, redwater, anaplasmosis and heartwater. In addition paratyphoid, digestive troubles and occasionally sweating sickness manifest themselves.

The Town Lands of Tzaneen, about 100 morgen in extent, is under the supervision of the Tzaneen Health Committee. There are about 50 head of cattle running on the Town Lands, besides the 30 to 40 oxen slaughtered monthly for the meat supply in this rather closely settled area.

In view of frequent occurrence of Koch's bodies in that vicinity, the facilities offered by Tzaneen Health Committee, the prolific infestation of ticks, the fact that local animals could serve as controls, etc., this area was selected to study this condition.

Accordingly an exposure experiment was commenced in 1934. Thirty grade animals varying in age from  $1\frac{1}{2}$  years to 3 years were selected for this purpose. Some of these emanated from Vryburg

where the presence of ticks, especially the brown tick, occurs in very small numbers, whereas the others came from Onderstepoort and the adjoining farm Kaalplaats, where there had been ample opportunity for frequent infestation with ticks. At Kaalplaats brown ticks, during the East Coast fever experiments in 1931 (du Toit) were extremely plentiful. All the exposed animals were in good condition when they left Onderstepoort and as far as possible tick free, although one of us (R. du Toit) records a fair number of *Hyalomma aegyptium* and *Rhipicephalus evertsi* shortly after the arrival of the 1935 batch at Tzaneen. Consequently they must have been picked up at Onderstepoort. There was accordingly a *difference* between these two groups of cattle, namely the Vryburg cattle which had been subjected to minimal brown tick infestation whereas the Onderstepoort-Kaalplaats cattle had ample opportunity of becoming so infested in spite of regular dipping.

The cattle in this exposure experiment were temperatured from the time of arrival at Tzaneen and smears from the blood and glands were made on alternate days. The symptoms and presence of ticks, etc., were recorded as they occurred. Further exposure experiments were carried out in 1935 and 1936. These experiments were either commenced in February or in March because the largest number of Koch's body cases occurred in that vicinity during that period. There was, however, a variation in the duration of these experiments, which undoubtedly played a very important part, especially in respect of the 1935 experiment. In the 1934 experiment the animals were exposed from the 20th March to the 3rd May, and then returned to Onderstepoort, while in the 1935 experiment they were exposed from the 22nd February to the 11th March and were returned to Onderstepoort at the height of reaction. In the 1936 experiment the animals were only exposed for a short period, namely from the 18th March to the 26th March, in order to allow them to become moderately tick infested. The transport of the animals by train from Onderstepoort to Tzaneen occupied 30 hours. During this period the animals were neither fed nor watered although a certain amount of hay was left in the trucks. The 1935 animals returned at the height of reaction were thus placed under severe conditions and it is believed that this undoubtedly aggravated the condition and was responsible for the high mortality in that experiment.

Ticks during 1935 were extremely plentiful and as early as the third day after exposure the cattle were heavily infested with adult ticks, almost all of which were *Rhipicephalus appendiculatus* and on the fourth day it is recorded that the tick infestation on the exposed cattle was appalling.

In Tables I, II and III the most important observations of the experiments have been charted. In these tables the course of the temperature reaction is indicated as well as the occurrence of the Koch's bodies and *T. mutans*, and whether these were rare or more frequent. The extent to which these bodies and the regularity with which they occurred in the various exposure experiments immediately becomes apparent as well as the difference in mortality between the two groups, Vryburg and Onderstepoort (Kaalplaats) exposed animals.









In Tables IV, V, VI, and VII a summary has been given of the occurrence of Koch's bodies and *T. mutans* before death, as well as the degree of infection, symptomatology and to what extent characteristic lesions in the liver, kidney, spleen and lymph glands occur. It is realized that it is extremely difficult to express by symbols the degree of infestation and lesions as they occurred in these three experiments, but nevertheless this method gives an indication as to what has transpired in these exposure experiments.

Koch's bodies in the 1934 experiment occurred more or less to the same extent in Vryburg and Onderstepoort cattle, and they were observed more or less at the same period after exposure. These bodies were only observed for one or two days and their number varied from cases in which they were extremely rare to those in which they could easily be identified in the gland smears. A number of the animals showed *T. mutans* infection in the blood without the identification of Koch's bodies in the gland smears, but this is no criterion that Koch's bodies were not present. On a few occasions only one definite Koch's body was identified in a gland smear in spite of careful examination of practically every field in the smear. In some cases (C 4872, 5629, and 5641) of the 1935 Tzaneen experiment Koch's bodies varied in number and occurrence in the different lymph glands and in one instance (C 5651) Koch's bodies were identified in only one gland in spite of the fact that smears had been made from several lymph glands besides spleen, liver and kidneys. In view of this irregular distribution of Koch's bodies and the fact that they may sometimes occur in very rare numbers, it stands to reason that such Koch's bodies may not be present in a particular smear or they may be overlooked unless every field in the smear is examined. Another very important point to consider is the period when gland smears should be examined after the exposure of "susceptible animals" to tick infestation. According to the tables this period after exposure varied from the 13th to the 22nd day and the majority of cases occurred in the interval between these extremes. It is therefore more than likely that in observations made here and elsewhere, Koch's bodies were missed in the development of *T. mutans* because the right time was not taken into consideration, not a sufficient number of days were chosen for the making of gland smears, and a sufficient number of fields in a gland smear was not examined.

The occurrence of parasites in the red cells also varied considerably, especially in blood smears of those animals which did not die as a result of tick infestation. Gametocytes continued to occur in the blood after it was no longer possible to identify Koch's bodies. In some instances these small piroplasms only appeared after the disappearance of Koch's bodies and in a few of these cases parasites were frequent. In no instance, not even in those cases which died as a result of the sequelae of ticks and *T. mutans*, were erythrocytes infected to the same extent as in East Coast fever, not only in respect of the number of red cells but especially in connection with the number of parasites in the individual red cells.

No morphological differences could be identified between the various forms of *T. mutans* and *T. parva*. The majority of forms described by Cowdry and Danks (1933) were encountered, in which the spherical-ovoid forms, the tail parasites and plump parasites were more prominent.

In the 1934 experiment a large number of cattle died as a result of infection with *P. bigemium* and this infection occurred to the same extent in Vryburg and Kaalplaats cattle in spite of the fact that the latter had undoubtedly been subjected to such infections on the veld. Most probably the Tzaneen area is infected with a very virulent strain of redwater. On the other hand the resistance of these animals had been so reduced that their disposition to the disease had become enhanced. It should be stressed that this, as an important factor in the mortality of newly introduced cattle, should be seriously considered, especially in view of the fact that in many instances the reaction was more of the nature of a "sequel" than an haemolysis so characteristic of an acute infection. Such "sequel cases" would be difficult for farmers to diagnose and accordingly to deal with satisfactorily. In six cases the cause of death in the 1934 exposure experiment could not be identified. It could probably be attributed to a sequel of heavy tick infestation. These six deaths could not be attributed to *T. mutans* in view of the fact that they did not reveal Koch's bodies in any of the organ smears, nor did the liver and kidneys show any evidence of lymphoid hyperplasia. Two cases of heartwater occurred in this experiment, diagnosed by the identification of *Rickettsia ruminantium* in intima smears. Intima smears were examined in the majority of cases where a definite diagnosis could not be arrived at.

The 1935 experiment is of great interest, especially in respect of the 100 per cent. occurrence of Koch's bodies in the Vryburg exposed animals and the approximately 100 per cent. mortality (a large number was killed in extremis) as a result of tick infestation complicated with *T. mutans* infection. This mortality is in contradistinction to the similarly exposed Onderstepoort cattle in which 8 out of 10 showed the presence of Koch's bodies without any mortality whatsoever from natural causes. The reasons why the course of this experiment was so different to the 1934 experiment were probably due to the fact that these animals were transferred from Tzaneen to Onderstepoort at the height of a severe reaction. As a result of this transfer they were kept in the trucks without water for a period of 30 hours. It would seem as if this played an important part in the mortality and was responsible for "setting up" these unusual lesions of *T. mutans*. A large number of cattle have been imported from Great Britain and brought from the port of landing to Onderstepoort in a tick free condition. These served as vaccine producers after they had reacted to the Onderstepoort strains of redwater and *Anaplasma centrale*. In the greater majority of these cases these animals also became infected with *T. mutans* and in suite of the fact that they are regarded as highly susceptible nothing of the nature observed in the exposed animals at Tzaneen has ever been encountered at Onderstepoort. In fact it has always been accepted at Onderstepoort that *T. mutans* was an absolutely innocuous parasite. In view of the absence of mortality in the Kaalplaats exposed animals, this mortality in the Vryburg animals could not be attributed to East Coast fever. Furthermore, in spite of a very extensive tick infestation, no spread of East Coast fever occurred in respect of the control animals on the Tzaneen Town Lands and the large number of animals introduced into Tzaneen for slaughter or for sale purposes. In these

Vryburg exposed animals *T. mutans* infection was therefore produced with the occurrence of Koch's bodies and post-mortem and histological changes resembling East Coast fever. From the tables it will be seen that lymphoid hyperplasia in liver or kidneys, or both, could be identified besides very interesting lesions in the lymph glands and spleen, similar to those identified in cases of East Coast fever investigated at Onderstepoort.

The pathology of these *T. mutans* cases will be more fully dealt with under the section on pathology.

Koch's bodies occurred in the organ smears to the same extent as that observed in East Coast fever. In the majority of the cases all stages in the development of agamonts could be encountered from single granules in lymphocytes as described by Cowdry and Danks to well-formed circumscribed agamonts in the cytoplasm of lymphocytes. In the majority of smears free agamonts could be identified in all sizes and stages. It should, however, be stressed that the gamonts were observed to a much lesser degree and in some cases definite gamonts such as those described by Cowdry and others could not be identified. The frequent occurrence of Koch's bodies in all stages of development in the blood smears were significant in these *T. mutans* infections.

The 1936 experiment showed 100 per cent. Koch's bodies without mortality but in this particular instance the animals were only subjected to tick infestation for a comparatively short time while the ticks were decidedly fewer in number as compared with the 1935 experiment. The three deaths which occurred in this experiment were the result of either piroplasmosis or heartwater. In the rest of the animals nothing abnormal besides slight temperature reactions was identified.

#### THE UMZINTO (NATAL) EXPOSURE EXPERIMENT, 1934.

(See Appendix III.)

The Umzinto area was considered to be free from East Coast fever since June, 1932. Recently, however, Koch's bodies occurred in the vicinity of Umzinto town. From Butcher A of Umzinto twelve spleen smears, said to have emanated from twelve slaughter oxen, showed the presence of Koch's bodies rare, whereas no small piroplasms could be identified. There was a strong suspicion that all twelve smears were made from the same spleen. The Government Veterinary Officer who investigated the matter could not ascertain when the smears were taken or in which batch of cattle the animals occurred from which these smears were taken. Of four cattle belonging to butcher B, Umzinto, slaughtered in February, 1934, one showed Koch's bodies, whereas of those slaughtered in the same month one showed Koch's bodies and redwater and in the three others Koch's bodies were identified. In view of the absence of East Coast fever in that vicinity and the frequent occurrences of Koch's bodies in cattle introduced for slaughter purposes, it was considered extremely advisable to undertake an exposure experiment in a district where East Coast fever had a few years ago occurred in order to compare it with the results of the exposure experiment undertaken at Tzaneen.

On the 14th March, 1934, twelve cattle from the Allerton Laboratory, Pietermaritzburg, and ten local cattle were respectively divided into two groups and placed into two paddocks at Umzinto. Each paddock therefore contained six Allerton cattle and five local cattle. These paddocks will be referred to as the *outer* and the *inner* paddocks. The Allerton cattle were comparatively free of ticks, probably as a result of regular dipping. All cattle were temperatured every morning between 8 and 9 o'clock and gland and blood smears were regularly taken and examined at Allerton.

At the end of seven days the Allerton cattle were badly infested with ticks and looked emaciated, unthrifty and listless, with a staring coat and were disinclined to move. The local cattle on the other hand were not anything like so badly infested and maintained good health until the beginning of May, when the experiment was discontinued. On the 8th day after introduction some of the Allerton cattle were so badly tick infested that it was considered advisable to treat the ears and rid them of some of the ticks because abscesses had formed in the ears. It was feared that, if treatment was not undertaken, they would die of the sequelae of tick infestation. In some of the cases besides swelling of the ears, haemorrhages, a suppurative dermatitis, necroses, etc., the whole face, eyes and intermandibular space were swollen.

In Table VIII the results of the 1934 Umzinto experiment are briefly summarized. Significant is the fact that *only one Koch's body case* occurred amongst the twelve exposed Allerton cattle to what was believed a heavy tick infestation and in which two cases of death occurred, directly attributed to the effects of mass tick infestation. The Koch's bodies manifested themselves at the usual time after exposure and were observed for only a comparatively short time. This single case amongst the twelve newly introduced animals is remarkable in view of the previous history of the frequency of Koch's bodies. There is definitely a difference between the exposure experiments carried out in the two paddocks. All the animals in the inner paddock showed marked thermal reactions five to six days after exposure, whereas in the outer paddock the febrile reactions, except those infected with *P. bigemium*, were very irregular, less pronounced and only commenced about the 17th May. Unfortunately the preponderance of *Rhipicephalus appendiculatus* on the animals in these two camps is not recorded, because the difference in the febrile reactions is undoubtedly very significant. The regular occurrence and the degree of the febrile reactions in the inner paddock resembles very closely those observed in the Tzaneen exposure experiment. Is the difference observed in the cattle in the two paddocks at Umzinto to be attributed to some agent so far not yet determined?

Sub-inoculations of blood and gland and spleen emulsions from the Koch's body case at Umzinto were carried out in susceptible cattle at Allerton and, except for the one case of piroplasmosis controlled by trypan blue, nothing unusual was observed. Blood from one of those cattle (C. 578) was sent to Onderstepoort and injected into two immune and two susceptible blue tongue sheep (Experiment S. 5405) with negative results. It would therefore appear that blue tongue virus was not responsible for this difference referred to above.

TABLE IV.  
*Tzaneen Exposure Experiment, 1934. S. 5326. 20.3.34.*

D.O.B.	Year.	Origin.	Koch's Bodies.		<i>T. mutans.</i>			Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death, (days after Exposure.	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance, (days.)	Frequency. Days.	First Appearance, (days.)	Day.	Frequency.						Koch's Bodies.	Piros. <i>T. mutans.</i>				
4829	1934	Vryburg.....	—	—	—	29 40	x x	19	xxx	xxx 107.8°	41	Seq. <i>P. bigem.</i>	—	—P	—	—	—	—
4832	1934	Vryburg.....	19	19	x	28	x	26	xxxx	xxxx 104.8°	28	Killed extremis, seq. ?	—	T	—	—	—	x
4849	1934	Vryburg.....	—	—	—	30 43 47	x x xx	49	xxx	xxx 107.8°	49	Seq. <i>P. bigem.</i>	—	P/Tx	—	—	—	x
4937	1934	Vryburg.....	22	22	x	25 29	x x	30	xxx	xxx 107.6°	37	Seq. <i>P. bigem.</i>	—	P/Tx	—	—	—	x
4954	1934	Vryburg.....	—	—	—	29 35 47 64 107	x x xxx x x	55	—	xx 107.4°	—	—	—	—	—	—	—	—
4957	1934	Vryburg.....	19	19	x	25	x	35	xx	xxx 107.0°	47	Seq. <i>P. bigem.</i>	—	P/Tx	—	—	—	x
4967	1934	Vryburg.....	16	16 19	x x	16 19 25 34	x x x x	25	xx	xxx 107.8°	34	Seq. <i>P. bigem.</i>	—	P	x ?	x ?	—	—
4980	1934	Vryburg.....	—	—	—	10	x	22	xxx	xxx 107.6°	31	Killed extremis, seq. <i>P. bigem.</i>	—	P	—	—	—	—
4984	1934	Vryburg.....	19	19	x	25 41 47 69 93 107	x x x x x x	44	—	xx 107.8°	—	—	—	—	—	—	—	—
4985	1934	Vryburg.....	—	—	—	25	x	41	—	xxx 107.8°	53	Heartwater, seq. <i>P. bigem.</i>	—	P	—	—	—	—
4986	1934	Vryburg.....	20	20	x	26 35 44	x x x	48	xx	xxx 107.0°	58	Heartwater	—	HW	—	—	—	—

TABLE IV.—(continued).

D.O.B.	Year.	Origin.	Koch's Bodies.		T. multans.		Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death (days after Exposure.)	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance (days.)	Frequency. Days.	Frequency. Day.	Frequency. Fre- quency.						Koch's Bodies.	Piros. T. multans.				
4987	1934	Vryburg.....	—	—	—	27	52	x	xxx 107.0°	—	—	—	—	—	—	—	—
4988	1934	Vryburg.....	—	—	—	25	39	x	xx 107.2°	—	—	—	—	—	—	—	—
5502	1934	Vryburg.....	18	x 24	x x	24	24	x x	xxx 107.0°	33	Seq. P. bigem.	P	—	—	—	—	—
5510	1934	Vryburg.....	20	xxx	xxx	24	34	x x x	xxx 107.0°	36	Seq. ?	Txxx	—	—	—	x	x
5520	1934	Vryburg.....	20	xxx x	xxx x	24	46	x x	xxx 107.4°	56	P. bigem.	P	—	—	—	—	—
5524	1934	Vryburg.....	—	—	—	19	41	xxx x xx x	xxx 107.0°	49	Seq. ?	—	—	No specimens collected.	—	—	—
5526	1934	Vryburg.....	19	xxx	xxx	19	47	xxx x x x x x	xx 107.2°	—	—	—	—	—	—	—	—
5198	1934	Kaalplaats.....	20	x ?	x ?	23	38	x x x x x	xx 107.2°	—	—	—	—	—	—	—	—
5200	1934	Kaalplaats.....	20	x	x	23	20	x x x x x x	xx 107.0°	—	—	—	—	—	—	—	—

TABLE IV.—(continued).

D.O.B.	Year.	Origin.	Koch's Bodies.			<i>T. mutans</i> .			Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death. (days after Exposure.)	Etiological Diagnosis.	Organ Smeears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance. (days.)	Days.	Frequency.	First Appearance. (days.)	Day.	Frequency.						Koch's Bodies.	Piros. <i>T. mutans</i> .				
5209	1934	Kaalplaats.....	—	—	—	8	8	x	xx	xxx 107.6°	30	Seq. ?	—	Pxx	—	—	—	—	
5210	1934	Kaalplaats.....	20	20	x ?	27	27 23 27 30	x x xxx x	xx	xx 106.8°	—	—	—	—	—	—	—	—	
5214	1934	Kaalplaats.....	—	—	—	—	—	—	xxxx	xxx 108.4°	36	Seq. <i>P. bigem.</i>	—	FTxx	—	—	—	—	
5224	1934	Kaalplaats.....	—	—	—	24	24	x	—	xx 108.2°	32	Killed, observ. (wild)	—	—	x ?	—	—	—	
5225	1934	Kaalplaats.....	20	20	x	27	27 49 64 93	x x x x	xx	xx 106.4°	—	—	—	—	—	—	—	—	
5228	1934	Kaalplaats.....	28	28	x	24	24 36 42	x xxx x	—	xx 105.4°	42	<i>P. bigem.</i>	—	P	x ?	—	—	—	
5272	1934	Kaalplaats.....	—	—	—	31	31 37	x x	xxx	xxx 108.6°	39	Seq. <i>P. bigem.</i>	—	PTx	—	—	—	—	
5297	1934	Kaalplaats.....	20	20	x ?	28	28 38	x x	xxx	xxx 108.2°	39	Seq. <i>P. bigem.</i>	—	PxTx	—	—	—	—	
5386	1934	Kaalplaats.....	18	18	xxx	24	24 28 38	x xx xx	xx	xx 107.0°	46	Seq. <i>P. bigem.</i>	?	P	x ?	x ?	—	—	
5370	1934	Onderstepoort.....	—	—	—	—	—	—	xxx	xxx 108.4°	28	Killed specimens	—	—	x ?	x ?	—	—	



TABLE V.  
*Tzaneen Exposure Experiment, 1935. S. 5616. 22.2.35.*

D.O.B.	Year.	Origin.	Koch's Bodies.			<i>T. mutans.</i>			Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death (days after Exposure).	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance (days.)	Frequency.	Days.	Frequency.	Day.	Frequency.						Koch's Bodies.	<i>T. mutans.</i>				
4872	1935	Vryburg.....	15	xxx xxx xx	15 19 22	19	x	14	xxxx	xxxx 108.0°	24	Killed extremis, seq. <i>T. mutans</i>	xx (irreg.)	xxx	x	—	xxx	xxx	
5000	1935	Vryburg.....	14	x x xxx	14 15 19	19	x	17	xxxx	xxxx 107.6°	21	<i>T. mutans</i>	xxxx	xx	xxxx	x	xxxx	xxx	
5570	1935	Vryburg.....	15	x x xxx x	15 19 20 21 26	20	xxx x x x	27	xxxx	xxxx 108.0°	27	<i>T. mutans</i>	xxx	—	—	xxx	xxx	xx	
5629	1935	Vryburg.....	15	xxx x xx x x	15 17 18 20 23	18	x x xx xx xx	27	xxxx	xxxx 108.0°	26	Killed, extremis, seq. <i>T. mutans</i>	xx (irreg.)	x	—	—	x	—	
5641	1935	Vryburg.....	14	x x x xxx xxx xx xx xxx	14 15 17 19 21 22 26 28 31	19	x x x xx xx xx xx	28	xxxx	xxxx 108.2°	33	<i>T. mutans</i>	xxx (irreg.)	—	x	xxxx	xx	xx	
5648	1935	Vryburg.....	15	x xxx	15 19	19	x	19	xxxx	xxxx 108.0°	20	Killed, extremis, <i>T. mutans</i>	xxxx	x	xxx	xxx	xxx	xxx	
5663	1935	Vryburg.....	14	x x x	14 15 18	18	x x	27	xxxx	xxxx 108.2°	28	<i>T. mutans</i>	xxx (irreg.)	xx	—	—	xxx	xx	
5665	1935	Vryburg.....	—	—	—	19	x	19	xxxx	xxxx 108.4°	20	Killed extremis, <i>T. mutans</i>	xxx (irreg.)	—	x	—	xxx	xxx	
5676	1935	Vryburg.....	16	xxx	16	—	—	18	xxx	xxxx 108.8°	19	Killed, collection of specimens	xxx	—	xx	xx	xx	xx	
5726	1935	Vryburg.....	14	x xxx x	14 16 18	18	x	20	xxxx	xxxx 108.0°	21	Killed extremis, seq. <i>T. mutans</i>	xx	—	x?	x?	xxx	xxx	
5742	1935	Vryburg.....	14	x xxx	14 16	—	—	17	xxx	xxxx 107.6°	19	Killed, seq. <i>T. mutans</i>	xxxx	—	xx	x	xx	xx	

TABLE V.—(continued).

D.O.B.	Year.	Origin.	Koch's Bodies.			<i>T. mutans</i> .			Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death. (days after Exposure.	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance. (days.)	Frequency.	Days.	Frequency.	Day.	Frequency.						Koch's Bodies.	<i>T. mutans</i> .				
5763	1935	Vryburg.....	19	xxx xxx	19 21	x xx	19 21	22	xxxx	xxxx 108.4°	23	Killed extremis, seq. <i>T. mutans</i>	xxx	x	xx	x	xxxx	xxxx	
5469	1935	Kaalplaats.....	13	xx x xxx x	13 14 15 19	x x x x x x x x x x x x x	19 24 31 40 42 50 53 55 60 62 66 83 92	21	xxx	xx 106.8°	—	—	—	—	—	—	—	—	
5474	1935	Kaalplaats.....	—	—	—	x x x x x x x x x x x x x	19 21 24 28 33 35 42 46 50 53 55 60 62 66 69	42	x	xx 107.0°	—	—	—	—	—	—	—	—	
6019	1935	Kaalplaats.....	19	x	19	x x x x x x x x x x x x	24 31 38 40 42 46 50 53 55 62 66 69 83 92	32	—	xx 107.0°	—	—	—	—	—	—	—	—	

TABLE V.—(continued).

D.O.B.	Year.	Origin.	Koch's Bodies.		First Appearance (days.)		Frequency.		First Appearance (days.)	Frequency.		Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death (days after Exposure.	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.					
			First Appearance (days.)	Days.	Frequency.	Frequency.	Day.	Frequency.		Koch's Bodies.	<i>T. mutans</i> .																
6033	1935	Kaalplaats.....	16	16	x	19	x	x	19	x	x	21	x	xx	—	—	—	—	—	—	—	—	—				
				19	x					24	x																
										30	x																
										32	x																
										34	x																
										38	x																
										40	x																
										42	x																
										46	x																
										50	x																
										53	x																
										54	x																
										60	x																
							62	x																			
							66	x																			
							69	x																			
							83	x																			
6252	1935	Kaalplaats.....	16	16	x	19	x	x	19	x	x	35	xxx	xx	—	—	—	—	—	—	—	—	—				
										24	x																
										31	x																
										34	x																
										38	x																
										40	x																
										42	x																
										46	x																
										50	x																
										53	x																
										55	x																
										62	x																
			6254	1935	Kaalplaats.....	—	—	—	19	—	—	19	x	x	32	x	xx	—	—	—	—	—	—	—	—	—	
										24	x																
										31	x																
										33	x																
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							69	x																			
							83	x																			
							92	x																			

TABLE V.—(continued).

D.O.B.	Year.	Origin.	Koch's Bodies.		First Appearance (days.)		Frequency.		Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death. (days after Exposure.)	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.	
			First Appearance (days.)	Days.	Frequency.	Frequency.	Koch's Bodies.	<i>T. mutans</i> .												
6255	1935	Kaalplaats.....	13	13	x ?	19	19	x	35	xx	107.6°	—	—	—	—	—	—	—	—	—
							24	x												
							31	x												
							35	x												
							38	x												
							40	x												
							42	x												
							50	x												
							55	x												
							60	x												
							62	x												
66	x																			
69	x																			
83	x																			
6287	1935	Kaalplaats.....	13	13	xx	—	—	—	14	—	xx	106.8°	15	Killed, clinically healthy	xxxx	—	—	—	—	—
				14	xxx															
6288	1935	Kaalplaats.....	13	13	x	19	19	x	28	x	xx	107.6°	—	—	—	—	—	—	—	—
				16	x															
				19	x															
				24	x															
				31	x															
				33	x															
				35	x															
				38	x															
				40	x															
				42	x															
				46	x															
50	x																			
53	x																			
55	x																			
60	x																			
62	x																			
66	x																			
69	x																			
83	x																			
6297	1935	Kaalplaats.....	13	13	x	19	19	x	28	xx	107.0°	—	—	—	—	—	—	—	—	—
				16	xx															
				19	xxx															
				24	x															
				31	x															
				33	x															
				35	x															
				38	x															
				40	x															
				42	x															
				46	x															
50	x																			
53	x																			
55	x																			
60	x																			
62	x																			
66	x																			
69	x																			
83	x																			

TABLE VI.  
*Tzaneen Exposure Experiments, 1936. S. 5909. 17.3.36.*

D.O.B.	Year.	Origin.	Koch's Bodies.			<i>T. mutans.</i>			Duration of Reaction.	Ears Affected.	Reaction and Highest Temperature.	Death. (days after Exposure.)	Etiological Diagnosis.	Organ Smears.		Liver Lymphoid Hyperplasia.	Kidney Lymphoid Hyperplasia.	Spleen Lesions.	Lymph Gland Lesions.
			First Appearance. (days.)	Days.	Frequency.	First Appearance. (days.)	Day.	Frequency.						Koch's Bodies.	<i>T. mutans.</i>				
5542	1936	Vryburg.....	15	15 20	x x	25	25	x	29	—	xx 106.4°	29	Heartwater....	—	—	—	—	—	—
5620	1936	Vryburg.....	17	17 20	x xxx	59	59	x	31	—	xx 107.0°	—	—	—	—	—	—	—	—
5649	1936	Vryburg.....	17	17 20 22	x x x	20	20 28	x x	30	—	xx 106.0°	—	—	—	—	—	—	—	—
5651	1936	Vryburg.....	17	17 20 22 24 25	x xx xxx xx x	13	13 24 28	x x xx	36	—	xxx 107.4°	39	Seq. <i>P. bigem.</i>	x?	—	—	—	x	x
5653	1936	Vryburg.....	17	17 20 22	xx xx xx	20	20 21 28 36	x x x xx	38	—	xxx 107.6°	38	<i>P. bigem.</i>	—	—	—	—	—	x
5664	1936	Vryburg.....	18	18 20 22	x x x	28	28 36 42	x x x	39	—	xx 107.0°	—	—	—	—	—	—	—	—
5674	1936	Vryburg.....	18	18 22	x x	20	20 22 24 28	x x x x	32	—	xxx 107.4°	—	—	—	—	—	—	—	—
5684	1936	Vryburg.....	17	17	x	28	28 36	x x	27	—	xx 105.8°	—	—	—	—	—	—	—	—
5747	1936	Vryburg.....	17	17	x	36	36 42	x x	32	—	xx 106.4°	—	—	—	—	—	—	—	—
5765	1936	Vryburg.....	14	14 18 20	x x x	13	13 22 28 36	x x x x	37	—	xx 107.2°	—	—	—	—	—	—	—	—