Serological Investigations into some Diseases of Domesticated Animals in South Africa caused by Trypanosomes.

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Most of the experiments in this paper were done at the suggestion of the Director of Veterinary Education and Research and were commenced during the period that the writer was stationed at the Veterinary Research Laboratory, Pietermaritzburg, Natal, between October, 1921, and February, 1923. Originally the intention was simply to apply the complement fixation test in the diagnosis of dourine in horses. This test had not previously been done in this country for the disease. Opportunities, however, presented themselves of applying the complement fixation test in the diagnosis of nagana in cattle and other animals. The results obtained were of considerable value, as the animals in the nagana experiments were all inoculated with pure strains of either *T. congolense* or *T. brucei*. Later, on my return to the Veterinary Research Laboratory, Onderstepoort, Pretoria, the experiments with complement fixation both in dourine and nagana were continued, and the results supported those obtained in Natal.

In addition to the complement fixation test, the Sachs Georgi and Meinicke lipoid fixation reactions were tried both in dourine and nagana.

As regards complement fixation in dourine, this has been tried on a large scale by Watson (1) in Canada, to whom great credit is due for his extensive experiments, and, in particular, for simplifying the technique for producing the antigen. Practically all workers in this field are agreed as to the remarkably uniform results obtained with this test and the small percentage of error, if satisfactory serum samples are obtained.

Bessemans & Leynen (2), experimenting with the different types of trypanosomes as regards their antigenic value when used for the diagnosis of dourine, found that antigens prepared from *T. lewisi* and *T. rhodesiense* were greatly inferior in value to those made from *T. brucei, evansi*, or *equiperdum*. As regards their value, he placed *T. brucei* first, *T. evansi* second, and *T. equiperdum* third. Bessemans (3) found that after trying various methods of making antigens, emulsions of trypanosomes obtained by fractional centrifugalization, as done by Watson, were the most satisfactory. Serum of infected rats proved useless, as did alcoholic or aqueous extracts of organs of these animals. Ethereal extracts of infected rats' blood produced a

weak antigen. An emulsion of the residue obtained by centrifugalizing blood containing trypanosomes and haemolyzing it with 5 per cent. Saponin was very satisfactory, but very anticomplementary.

As regards the lipoid fixation reaction of Meinicke, Dahmen (4) writes very favourably of the results he obtained with it in the diagnosis of dourine, and prefers it to the complement fixation; considering it more reliable. A short description of his technique is given in this paper under the section devoted to the results obtained here with this reaction.

Very little information is available as to the value of the Sachs Georgi reaction in trypanosomiasis. Dahmen (4) states that he found it of no value in the diagnosis of dourine. That definite positive reactions are sometimes obtained in cases of nagana in cattle will be seen from the results obtained in South Africa, but the value of the test in the diagnosis of the disease is very doubtful.

1. The Complement Fixation Test.

(*a*) *Dourine.*

As no strain of *T. equiperdum* from South African horses was available, attempts were made to produce an efficient antigen from strains of *T. brucei*. It was assumed that this species would serve the purpose, as the reaction in trypanosomiasis was considered to be non-specific; that is, an antigen from any species of trypanosome would prove satisfactory. Sufficiently heavy infections with *T. brucei* in the animals used were, however, not obtained, and even in white rats it was found very difficult to get heavy infections in several at the same time.

Soon after the commencement of the experiments, a strain of *T. equiperdum* was obtained from England, and with it a satisfactory antigen was obtained from the blood of white rats. The technique employed for obtaining the antigen and for carrying out the complement fixation test for dourine was essentially the same as that used by Watson (1). In making the antigen, the most important point to remember is that the white rats used should be inoculated from a white rat with a heavy infection of *T. equiperdum*, and not from a guinea-pig or rabbit. If the latter two species be used to inoculate from, the white rats will not show uniformly heavy infections at the same time, and one is, therefore, almost certain to waste the animals. With the strain of *T. equiperdum* used here, it was found that if six white rats were inoculated intra-peritoneally, each with 0.5 c.c. of citrated blood from a heavily infected white rat, four or five of them would show a very heavy infection on the third or fourth day after inoculation. As observed by Watson, it was found that when the rats become heavily infected, it was necessary to watch them very closely, as they died without showing much in the way of symptoms beforehand. Watson used as many as twenty-five rats at a time, but such large numbers were not used here, as they were not available; in fact, for some time great economy in their use had to be practised. If quantities of blood larger than 0.5 c.c. were used for inoculating the white rats, the incubation period was much shortened. If 1 c.c. was used for the inoculation, the rats all had to be killed on the second day after it; that is, within forty-eight hours. The dose used

here now for inoculating rats for antigen preparation is, therefore, 1 c.c., as there is no object in using a smaller dose and waiting another day or two.

When at the height of infection, the rats were bled into 0.85 per cent. salt solution plus 1 per cent. sodium citrate, the quantities of blood and citrated salt solution being roughly equal. The blood was then centrifugalized at a low speed, about 1,500 revolutions per minute for ten minutes. Thick glass test-tubes were used, three inches long by quarter of an inch in diameter. It was found that in these tubes the white layer of tryponosomes over the blood corpuscles, even after one centrifugalization, was about an eighth to a quarter of an inch thick and sharply marked off from the blood corpuscles.

The supernatant fluid was removed and discarded. It may contain a fair number of trypanosomes, but is usually not rich enough in them to warrant spending much time in trying to centrifugalize them out. Unless one is working with large quantities of antigen, the trypanosomes obtained from the supernatant fluid make very little difference to the bulk obtained from the blood. The white layers were then pipetted off into fresh tubes of the same size as before, as little blood as possible being removed with the trypanosomes. The tubes were filled to about a third of their capacity with trypanosomes mixed, of course, with some blood corpuscles, and the tubes were then filled up with 0.85 per cent. salt solution. After mixing the emulsion of trypanosomes well with the salt solution, the tubes were recentrifugalized for a quarter of an hour at a high speed. The trypanosome layer in the tubes was then found to be a thick white one with a few red corpuscles in the bottom. The supernatant fluid was again discarded and the trypanosome layers added together. To the total bulk of trypanosomes, twice the amount of Watson's glycerine formalin preservative fluid was added, and the emulsion constituted the antigen. If kept in sealed glass pipettes it would keep for six month in cold storage at 35° F. Formalin is not necessary as a preservative if the antigen is kept at this temperature. If one examines the trypanosome emulsion preserved in this way, even after four or five months one can often find large numbers of well formed trypanosomes in it, most of which stain well.

Before the test was applied to natural cases from the field, the sera of a number of horses infected with dourine and a number of uninfected ones were tested. The serum samples were numbered for subsequent identification, and it was found possible to pick out the sera from the infected animals with great accuracy. After a number of confirmatory tests, samples of serum from known and suspected cases of dourine from the districts where the disease occurs were tested with satisfactory results, and the complement fixation test has now become a routine measure. A fair number of samples of serum from suspected cases have been sent in, of which a good many have proved positive. A number of samples have been unsatisfactory for use owing to having become decomposed in transit. The addition of one-half per cent. carbolic acid to serum samples has usually proved a satisfactory preservative even under South African summer conditions, but samples have often been sent without preservative, or the carbolic acid has been added to the whole blood or to blood-stained serum. In the latter case the serum frequently coagulated when subsequently inactivated.

All sera before use were inactivated at 62° C. for half an hour, as it had been found that many samples heated at a lower temperature gave non-specific reactions. Even after heating to 62° C. a few donkey sera have been found to give non-specific reactions and could, therefore, not be used.

It is doubtful whether the systematic testing of horses in infected districts will be undertaken in South Africa owing to the conditions under which horse-ranching is carried out. It would be very difficult to round up and test the troops of practically wild horses on the ranches, and with the decline of horse-breeding, owners will not go to much trouble over their animals.

(*b*) *Nagana.*

At the time the complement fixation was introduced for dourine, experiments in connexion with the drug treatment for experimental nagana were in progress. This seemed an opportune time to test the value of complement fixation in this disease, using dourine trypanosome antigen. As has been previously stated, it was believed that the test in trypanosomiasis was non-specific and that any species of trypanosome could be used as antigen. Owing to an unavoidable shortage of white rats the facilities for experimenting with antigens from different trypanosomes were limited, and only dourine antigen was used.

As all the animals used in the drug experiments were infected with pure strains of *T. brucei* or *T. congolense*, the results of the tests were much more valuable than if the animals had been natural cases in which one had to rely on the findings in blood smears for the diagnosis of the type of trypanosome infecting them. It is very difficult to find out by smear examination whether an animal has a pure or a mixed infection, as *T. brucei* may be present, but hardly ever be seen in smears.

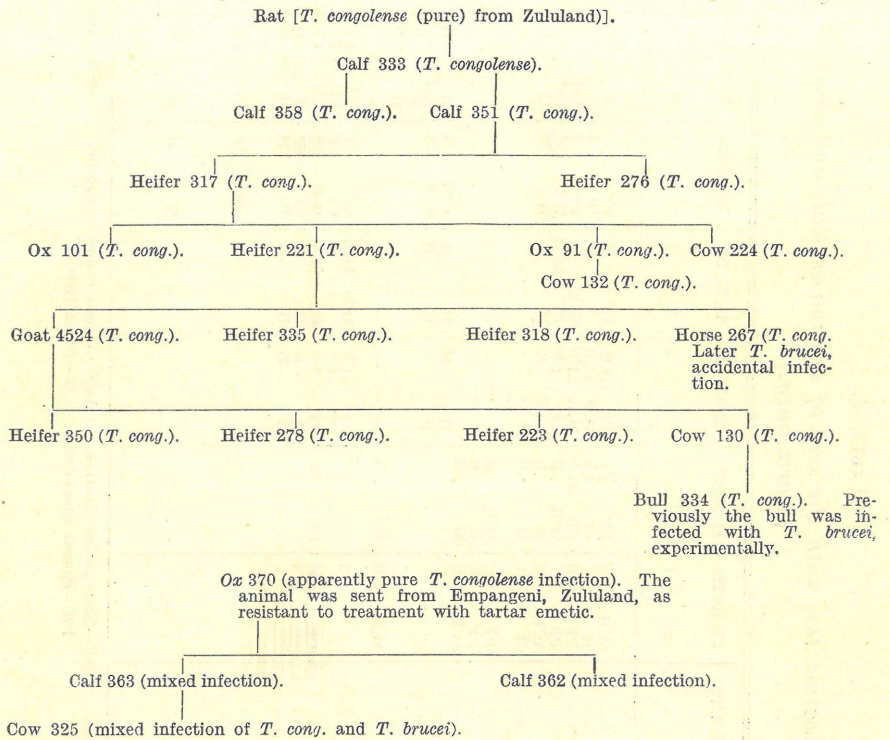
The strains of *T. congolense* and *T. brucei* were both obtained from the Nagana Research Laboratory in Zululand. The strain of *T. brucei* was typically dimorphic and resembled in all ways the classical description of the organism. It was exceedingly pathogenic for the horse, rabbit, guinea-pig, white rat, and white mouse, but only slightly so for the ox. In this animal a mild infection resulted, accompanied by the scanty appearance of trypanosomes as determined by blood-smear examination and absence of definite clinical symptoms. In horses a heavy infection of the blood occurred with marked progressive loss of condition.

The strain of *T. congolense* was first received from Zululand in white rats, but subsequently it was found possible to infect the guinea-pig with it, and the strain was kept going in this species of animal. It may be of interest to note that rabbits could be infected with this strain of *T. congolense*, but only showed a scanty infection of the blood. Infected guinea-pigs usually lived for three weeks to a month and white rats ten to fourteen days. For cattle the strain was very pathogenic, thus contrasting markedly with *T. brucei*. Only one horse was infected, but it showed little in the way of clinical symptoms subsequently, and trypanosomes were always rare when present in the blood. A goat was inoculated, but although it showed numerous trypanosomes in the blood, no clinical symptoms were evident.

Amongst the experimental cattle was one case, that of an ox, D.O.B. 370, which had been sent from Zululand on account of the trypanosomes in its blood being tartar-emetic resistant. This animal was thought to be harbouring *T. congolense* only, but subinoculation made from it showed that *T. brucei* was present as well.

In the following table (1) are shown the subinoculations made in connexion with the *T. congolense* strain and also those made from the ox D.O.B. 370 mentioned in the previous paragraph.

TABLE 1.



Periodical examination of the sera of animals under drug treatment was commenced in September, 1922, and continued until February, 1923, when the writer left Pietermaritzburg for the Veterinary Research Laboratories, Onderstepoort, Pretoria. A large number of tests were carried out, but more would have been done if a larger number of white rats had been available. No success was experienced in attempting to make satisfactory antigens from guinea-pigs' blood, this being more particularly the case with *T. congolense*. Even using white rats, only very small quantities of *T. congolense* antigen were obtained, and only once was enough available to use for a series of tests. In white rats it was difficult to get a number to show heavy infections at the same time, and the tendency of the trypanosomes to attach themselves to blood corpuscles appeared to interfere with their being centrifugalized out. It is possible that in the course of subinoculations the strain may become more acclimatized to white rats and produce heavier and more uniform infections.

TABLE 2.

Showing results of complement fixation test with the sera of nagana animals and using T. equiperdum *antigen (Maritzburg.)*

Animial.	Infection.	Date of Inoculation.	29/9/22.		25/10/22.		31/10/22.		22/11/22.		24/11/22.		12/12/22.		29/12/22.		19/1/23.		21/1/23.		3/2/23.	
			·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1
Calf 358..	*T. congolense*.....	8/8/22.....	—		ACH	ACH	PF	PF	PF	ACH	PF	H	PF	H	H	H	—		H	H	—	
,, 317..	,, ...	8/8/22.....	—		H	H	CF	CF	ACH	ACH	—		H	H	—		ACH	ACH	H	H	H	H
,, 276..	,, ...	8/8/22.....	—		PF	ACH	PF	ACH	ACH	ACH	NSF	—	PF	H	NSF	—	ACH	ACH	ACH	ACH	H	H
Ox 101...	,, ...	14/9/22....	H	H	PF	PF	ACH	H	CF	CF	PF	ACH	ACH	H	NSF	—	NSF	—	NSF	—	H	H
Cow 221..	,, ...	14/9/22....	H	H	ACH	ACH	H	H	ACH	H	H	H	H	H	H	H	—		H	H	H	H
,, 224..	,, ...	14/9/22....	—		H	H	H	H	—		—		—		—		—		—		—	
Ox 91....	,, ...	14/9/22....	H	H	—		—		—		—		—		—		—		—		—	
Cow 132..	,, ...	29/9/22....	—		PF	PF	ACH	H	—		—		—		—		—		—		—	
Calf 335..	,, ...	7/10/22....	—		PF	PF	H	H	—		—		—		—		—		—		—	
,, 318..	,, ...	7/10/22....	—		ACH	ACH	H	H	—		—		—		—		—		—		—	
Heifer 350	,, ...	15/11/22...	—		—		—		H	H	H	H	ACH	H	H	H	—		H	H	H	H
,, 278	,, ...	15/11/22...	—		—		—		H	—	H	H	ACH	H	NSF	—	—		H	H	H	H
Cow 223..	,, ...	15/12/22...	—		—		—		—		—		—		H	H	—		H	H	H	H
,, 130..	,, ...	15/12/22...	—		—		—		—		—		—		H	H	—		H	H	H	H
Ox 370...	*T. congolense* and *T. brucei*	Natural case	—		ACH	ACH	H	H	—		—		—		—		—		—		—	
Calf 362..	,, ,,	20/10/22...	—		—		—		CF	CF	ACH	ACH	CF	CF	PF	PF	—		CF	CF	ACF	ACF
,, 363..	,, ,,	20/10/22...	—		—		—		CF	CF	—		CF	CF	PF	PF	—		—		—	
Horse 262	*T. brucei*.....	5/10/22....	—		CF	CF	CF	CF	CF	PF	CF	PF	ACF	PF	CF	CF	CF	CF	CF	CF	CF	CF
,, 253	,, ...	1/11/22....	—		—		—		H	H	H	H	CF	CF	H	H	—		CF	CF	—	
,, 267	*T. congolense* and subsequently *T. brucei*	2/11/22, *T. brucei* from 7/12/22	—		—		—		H	H	H	H	H	H	H	H	—		—		—	
Bull 334..	*T. brucei* and subsequently *T. congolense*	*T. brucei*, 2/11/22; *T. cong.*, 4/1/23	—		—		—		—		—		CF	CF	CF	CF	CF	CF	CF	CF	CF	CF
Goat 4524	*T. congolense*.....	7/10/22....	—		—		—		—		—		H	H	H	H	H	H	H	H	H	H
Cow 325..	*T. congolense* and *T. brucei*	15/12/22...	—		—		—		—		—		—		—		CF	CF	CF	CF	H	H

CF—Complete fixation. PF—Partial fixation. NSF—Non-specific fixation. ACF—Almost complete fixation. ACH—Almost complete haemolysis.
PH—Partial haemolysis. H—Haemolysis.

The sera of all the animals tested in these drug experiments were inactivated at 62° C. for half an hour. A small percentage of sera showing non-specific fixation after this heating was still observed, but it was almost negligible.

Contrary to what was expected, it was found that the sera of animals suffering from a pure *T. congolense* infection did not react with antigen made from *T. equiperdum* even after having been infected for months. In rare cases a partial fixation occurred, but it was not the rule. On the other hand, the sera of animals infected with *T. brucei* gave strong reactions, quite as marked as would be observed in sera from cases of dourine.

It would therefore appear that the complement fixation reaction in trypanosomiasis is a group reaction, not a general non-specific one.

The following table (2) shows the results obtained with the complement fixation test, using sera of nagana animals and antigen prepared from *T. equiperdum*. It will be noticed that the sera were tested in the amounts of 0.2 and 0.1 c.c., and not to the limit of fixation, as should have been done in positive cases. The sole reason for this was shortage of antigen.

Since the writer's return to Onderstepoort a large number of further tests have been carried out with complement fixation for nagana experimentally produced, and the results tend to confirm the previous observations. Most of the series of tests were carried out with antigen made from *T. equiperdum*. A few, however, were made with a *T. congolense* antigen, but only on one occasion was sufficient obtained to make this possible, and the results with it were very interesting, though they must be considered simply as an indication until confirmed by a larger series of tests.

Table 3 shows the results obtained with sera of three horses and a calf inoculated with different species of trypanosomes for demonstration to students and using *T. equiperdum* antigen.

TABLE 3.

Showing result of complement fixation test with sera obtained from undermentioned animals and using T. equiperdum *as antigen.*

Animal.	Infection.	Date of Infection.	Date of Inoculation.											
			11/4/23		18/4/23		8/5/23		17/5/23		29/5/23			
			0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1	0.2	0·1	0·05	
Horse 15183	*T. equiperdum*..	6/4/23	H	H	H	H	—		—		—			
15736	*T. brucei*.....	6/4/23	H	H	H	H	CF	CF	CF	CF	CF	CF	CF	
15125	*T. congolense*...	6/4/23	H	H	H	H	H	H	H	H	H	H		
Calf 331	*T. congolense*...	6/4/23	H	H	H	H	H	H	H	H	H	H		

NOTE.—CF=Complete fixation. H=Haemolysis.

The above results indicate that animals suffering from *T. congolense* infection do not react to *T. equiperdum* antigen. It may be added that some positive dourine sera and negative samples were used as controls.

Table 4 shows the results of the complement fixation test with sera from a series of animals using *T. congolense* antigen.

TABLE 4.

Animal.	Infection.	Date of Infection.	14/6/23.	
			0·2	0·1
Horse 15568.....	*T. equiperdum*.....	Natural case	ACH	ACH
Stallion 15565.....	*T. equiperdum*.....	Natural case	PF	PF
Horse 15125.....	*T. congolense*.....	6/4/23.....	CF	CF
„ 15741.....	Neg. Control.....	—	H	H
„ 15742.....	Neg. Control.....	6/4/23.....	H	H
Calf 331.....	*T. congolense*.....	6/4/23.....	CF	CF

NOTE.—CF=Complete fixation. H=Haemolysis. PF=Partial fixation. ACH=Almost complete haemolysis.

The above results indicate that animals suffering from *T. congolense* infection may react if *T. congolense* antigen is used. It will be noticed that one *T. equiperdum* animal gave a partial fixation but the *T. congolense* animals a complete fixation. This preliminary experiment suggests that it may eventually be possible to diagnose *T. congolense* infection by complement fixation. On account of the occurrence of *T. brucei*, often as a mixed infection with *T. congolense* in the same animal, tests would have to be carried out with both antigens, first one being used, then the other.

A further series of tests were carried out at Onderstepoort in 1924 on a large number of cattle with experimental nagana. Most of the animals were inoculated with *T. congolense*, but a few with *T. brucei*, and some with both. They were used for experiments with a new series of drugs, whose value as curative agents in nagana had to be tested. Some of these drugs were very toxic, and a few cattle were killed outright, so their reactions could not be observed over any extended period. The following table gives details of the animals' reactions, and it will be noticed that they confirm the previous observations as to the failure of *T. congolense* animals' sera to react with *T. equiperdum* antigen.

Table 5.

Number.	Infection and Date.	Drug Treatment.	19/2/24.		28/2/24.		5/3/24.		13/3/24.				19/3/24.		27/3/24.		4/4/24.		10/4/24.		30/4/24.	
			0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1	0·05	0·02	0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1
355....	*T. congolense*, 14/2/24..	M. 207.....	H	H	*		—		—				—		—		—		—		—	
436....	*T. congolense*, 14/2/24..	M. 207.....	H	H	H	H	H	H	H	H	—		H	H	H	H	H	H	H	H	H	H
440....	*T. congolense*, 14/2/24..	Tryparsamide	H	H	H	H	H	H	H	H	—		H	H	H	H	H	H	H	H	H	H
446....	*T. congolense*, 14/2/24..	Tryparsamide	H	H	H	H	H	H	H	H	—		‡‡		—		—		—		—	
450....	*T. congolense*, 14/2/24..	Control.....	H	H	H	H	H	H	H	H	—		‡‡		—		—		—		—	
456....	*T. congolense*, 14/2/24..	T. 10.....	H	H	H	H	H	H	H	H	—		H	H	H	H	H	H	H	H	H	H
520....	*T. brucei*, 14/2/24.....	Tryparsamide	H	H	PF	ACH	ACH	H	H	H	—		H	H	H	H	PH	H	H	H	H	H
554....	*T. brucei*, 14/2/24.....	Control.....	H	H	H	H	ACF	PH	CF	CF	PH	ACH	CF	ACF	CF	CF	PF	CF	CF	CF	—	
575....	*T. brucei*, 14/2/24.....	T. 12.....	H	H	H	H	ACF	PH	CF	CF	ACF	PH	CF	ACF	CF	CF	CF	CF	CF	CF	CF	CF
599....	*T. cong.* and *T. brucei*, 14/2/24	M. 207.....	H	H	H	H	‡‡			—			—		—		—		—		—	
602....	*T. cong.* and *T. brucei*, 14/2/24	Tryparsamide	H	H	H	H	H	H	H	H	—		H	H	H	H	H	H	H	H	H	H
684....	*T. cong.* and *T. brucei*, 14/2/24	Control.....	H	H	H	H	H	H	H	H	—		H	H	H	H	H	H	H	H	H	H
Horse— 15139	*T. equiperdum*, 20/2/24.	Control.....	H	H	PH	H	CF	ACF	CF	CF	ACF	PH	CF	CF	CF	CF	CF	CF	‡‡		—	
15831	*T. brucei*, 14/2/24.....	Tryparsamide	H	H	PF	H	CF	ACF	CF	ACF	PH	H	CF	CF	‡‡		—		—		—	
15125	*T. equiperdum*, 14/2/24.	Tryparsamide	H	H	ACH	H	ACF	PH	CF	CF	PH	PH	CF	PF	CF	CF	‡‡		—		—	
15739	*T. brucei*, 14/2/24.....	Tryparsamide	H	H	PF	PF	CF	CF	CF	ACF	PH	PH	CF	PF	CF	CF	CF	ACF	CF	ACF	‡‡	
273	*T. congolense*, 13/3/24..	T. 11.....	—		—		—		—				H	H	H	H	‡‡		—		—	
320	*T. congolense*, 13/3/24..	T. 13.....	—		—		—		—				H	H	H	H	H	H	H	H	H	H
321	*T. congolense*, 13/3/24..	Control.....	—		—		—		—				H	H	H	H	H	H	H	H	H	H

NOTE.—All the animals, except those prefixed as horses, were cattle.
‡‡—Dead. CF—Complete fixation. ACF—Almost complete fixation. PF—Partial fixation. ACH—Almost complete haemolysis. H—Haemolysis. Drugs M. 201 and 207 were methylene blue derivatives, the *T.* series being trypan-blue derivatives. Cattle 602 and 684 never showed *T. brucei* in the blood, so the infection probably failed.

It is of interest to mention at this point that there were several cattle in experiment here inoculated with a pure strain of *T. vivax* and showing the parasite in the blood. The sera of these animals when tested by complement fixation, using *T. equiperdum* antigen, did not give any fixation.

Conclusions.

1. The complement fixation test may be used for the diagnosis of dourine occurring naturally in South Africa, provided *T. equiperdum* antigen is employed.

2. Animals infected with *T. brucei* react to the complement fixation test when *T. equiperdum* antigen is used.

3. Animals suffering from a pure *T. congolense* infection do not usually react when *T. equiperdum* antigen is used, though in rare cases a partial reaction may be obtained. In Table 2 it will be observed that cattle 317 and 101 each on one occasion reacted strongly. That this was exceptional is shown by the series of reactions in Table 5.

4. There is some evidence to show that sera obtained from animals with pure *T. congolense* infections may give marked reactions with *T. congolense* antigen. The sera of animals infected with *T. equiperdum* react slightly or not at all to this antigen.

5. The sera of a number of cattle showing pure infections with *T. vivax* did not give reactions to the complement fixation test using *T. equiperdum* antigen.

The Sach's Georgi Test.

The technique employed for this test was the modification introduced by Dreyer (5). The antigen he used consisted of an alcoholic extract of ox heart and an alcoholic solution of cholesterin (Kahlbaum) made by dissolving one gramme of it in 100 c.c. of absolute alcohol. The ox-heart extract is a limpid yellow clear fluid, and is made by extracting ox heart with alcohol (94 to 95 per cent.), filtering off the alcohol, and then, after drying the residue, extracting it with acetone twice. The residue is then again put into a bottle with absolute alcohol for ten days at 20° C. and finally filtered. The filtrate constitutes the antigen.

In preparing the antigen for the actual test, one adds 0.1 c.c. of cholesterin solution to 1 c.c. of ox-heart extract. One then adds 0.85 per cent. salt solution to it by allowing it to drop on to the mixture. To every c.c. of the heart extract cholesterin mixture one adds 34 c.c. of salt solution, which is dropped on to it from a height of sixteen inches. The 34 c.c. should take four minutes to drop. The serum is added to the emulsion in small test-tubes, and is allowed to drop into it as well. Shaking is to be avoided. Sera to be tested are heated to 53-54° C. for one and a half hour before use. The tubes containing the serum and saline suspension of ox-heart cholesterin mixture are incubated for twenty-four hours at 37° C. Positive reactions may often be seen at the fifth or sixth hour. In positive cases a granular precipitate of very fine particles forms, and these subsequently settle out, leaving the fluid clear.

In some cases this granular precipitate is not well marked, and one has to examine the tubes against a strong light to see it. In negative cases the fluid in the tubes remained opaque and translucent.

As a routine measure four dilutions of serum were used, 1-15, 1-20, 1-30, and 1-50. Positively reacting sera would sometimes react in higher dilutions, but rarely beyond a dilution of 1-100.

The first tests were carried out with sera of nagana cattle sent from the Nagana Research Laboratory at Ntambanana, Zululand, and it was found that some gave quite marked reactions, some none at all. A large number of dourine sera were tested, and in addition sera of rabbits and guinea-pigs infected with dourine and nagana, but the results were uniformly negative. With horse sera, whether from dourine or nagana cases, no trace of a reaction has been obtained.

The sera of the cattle in the drug experiments with nagana were unfortunately not tested before inoculation with trypanosomes, as it was not known at the time that some uninoculated cattle might react. The negative controls used were uninoculated cattle, and gave negative results, but later it was found that a small proportion of apparently normal cattle might give positive reactions. The test was applied to a number of sera sent in for the agglutination test for contagious abortion, and known to be from animals in nagana-free areas. Most of these sera gave no reaction, but in one lot of samples two out of fifteen were positive.

A table (6) is given showing the results obtained with the Sach's Georgi Test.

Table 6.

No.	29/9/22.				7/10/22.				25/10/22.				31/10/22.				9/11/22.				16/11/22.				Date of Infection.	Infection.
	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50		
91	+	+	+	+	Dead																				14/9/22.....	*T. cong.*
221	±	±	±	±	++	++	++	++	++	++	++	++	±	−	−	−	−	−	−	−	−	−	−	−	14/9/22.....	*T. cong.*
224	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Dead				14/9/22.....	*T. cong.*
101	++	++	++	++	++	++	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	14/9/22.....	*T. cong.*
333	−	−	−	−	Dead																				14/9/22.....	*T. cong.*
276	−	−	−	−	+	+	+	+	+	+	+	+	++	++	++	+	++	++	++	+	++	++	++	+	8/8/22.....	*T. cong.*
132					−	−	−	−	+	+	±	−	−	−	−	−	+	±	−	−	−	−	−	−	29/9/22.....	*T. cong.*
358					±	±	±	±	+	±	−	−	+	+	±	−	++	+	+	+	+	+	+	±	8/8/22.....	*T. cong.*
317					+	+	+	±					±	±	±	±	±	±	±	±	++	+	+	+	8/8/22.....	*T. cong.*
130					−	−	−	−																	15/12/22.....	*T. cong.*
213					−	−	−	−																	Neg. control..	—
133					−	−	−	−					−	−	−	−					±	−	−	−	Neg. control..	—
262									−	−	−	−					−	−	−	−	−	−	−	−	5/10/22.....	*T. brucei.*
318									±	−	−	−	±	±	±	±	−	−	±	−	Dead				7/10/22.....	*T. cong.*
335									+	±	−	−	±	−	−	−	±	−	−	−	Dead				7/10/22.....	*T. cong.*
370									+	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Natural case..	*T. cong.* and *T. brucei.*
Goat																										
4524																					−	−	−	−	7/10/22.....	*T. cong.*
350																									15/11/22.....	*T. cong.*
362																									20/10/22.....	*T. cong.* and *T. brucei.*
363																									20/10/22.....	*T. cong.* and *T. brucei.*
278																									15/11/22.....	*T. cong.*
334																									2/11/22.....	*T. brucei.*
340																									Neg. control..	Not inoc.
352																									Neg. control..	Not inoc.
328																									Neg. control..	Not inoc.
144																									Neg. control..	Not inoc.
223																									Neg. control..	Not inoc.

TABLE 6—(*continued*).

No.	28/11/22.				1/12/22.				19/12/22.				29/12/22.				25/1/23.				Date of Infection.	Infection.
	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50	1/15	1/20	1/30	1/50		
91																					14/9/22.....	*T. cong.*
221					+	+	+	±	−	−	−	−	+	+	±	−	+	+	±	−	14/9/22.....	*T. cong.*
224																					14/9/22.....	*T. cong.*
101	++	++	++	+	++	++	++	+	++	++	++	++	++	++	++	++	+	+	±	−	14/9/22.....	*T. cong.*
333																					14/9/22.....	*T. cong.*
276	++	++	+	+	+	+	±	±	++	+	+	+	+	+	+	±	+	+	+	±	8/8/22.....	*T. cong.*
132	Dead																				29/9/22.....	*T. cong.*
358	±	±	−	−	+	+	+	±	±	±	−	−	++	+	+	±					8/8/22.....	*T. cong.*
317	++	+	+	±	+	+	+	±	++	++	+	+	±	±	−	−	+	+	+	+	8/8/22.....	*T. cong.*
130																					15/12/22.....	*T. cong.*
213																					Neg. control..	—
133	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−					Neg. control..	—
262	−	−	−	−					−	−	−	−	−	−	−	−					5/10/22.....	*T. brucei.*
318																					7/10/22.....	*T. cong.*
335																					7/10/22.....	*T. cong.*
370	Dead																				Natural case..	*T. cong.* and *T. brucei.*
Goat																						
4524					−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	7/10/22.....	*T. cong.*
350	±	±	−	−	+	±	−	−	+	+	+	+									15/11/22.....	*T. cong.*
362	+	+	+	±	+	+	+	±	+	+	±	±	+	+	±	−					20/10/22.....	*T. cong.* and *T. brucei.*
363	++	++	++	+	+	+	+	±	+	±	−	−	++	++	+	+	Dead				20/10/22.....	*T. cong.* and *T. brucei.*
278	±	±	−	−	++	++	+	+	++	++	+	+	++	++	++	++					15/11/22.....	*T. cong.*
334					+	+	+	±	+	+	+	±	+	+	±	−	−	−	−	−	2/11/22.....	*T. brucei.*
340									±	±	−	−									Neg. control..	Not inoc.
352									−	−	−	−									Neg. control..	Not inoc.
328													+	+	±	−					Neg. control..	Not inoc.
144									±	±	−	−									Neg. control..	Not inoc.
223													++	++	++	++					Neg. control..	Not inoc.

From this table it will be seen how the sera of some animals are positive continuously, others intermittently, and some remain negative. The cattle 133, 144, 213, 223, 328, 340, and 352 were not infected with nagana.

In the table—

++ means strongly positive.
+ means definitely positive but not markedly.
± means doubtful (some granules in fluid).
— means negative.

The Meinicke Lipoid Fixation Reaction.

The technique employed in carrying out this reaction was that described by Dahmen (4), and it is as follows:—Alcoholic horse-heart extract is added to half its bulk of distilled water. After standing for two hours, seven times its bulk of distilled water is added to it. To this mixture dourine antigen is added in the same proportion as would be used in the complement fixation reaction. A quantity of 0.2 c.c. serum from a suspected case is added to 1 c.c. of the horse-heart-antigen mixture. As a control some other extract such as a bacterial one is used instead of horse-heart.

One incubates the serum horse-heart plus antigen mixture for sixteen to twenty-four hours at 37° C. The tubes will be found flocculated out. By careful shaking, the clumps are distributed through the fluid in the tubes. One then adds 3 per cent. salt solution to the tubes, after which shaking must be avoided. Incubate again for one hour at 37° C. The clumps in the negative and control tubes will be found to have disappeared, or do so on gentle shaking. In positive cases the clumps do not break down even on shaking. Inactivation of sera to be tested is not necessary, but they can be heated to 50° C. for half an hour without interfering with the test.

In some of the tests several different concentrations of salt solution were used to see what the salt deficiency of the clumps was. The following table (7) gives the results of the tests carried out here:

Table 7.

Number.	27/11/23. Salt. sol. 7·5%	5%	3%	1%	24/1/24. Salt. sol. 7·5%	5%	3%	1%	21/2/24. Salt. sol. 7·5%	5%	3%	1%	29/2/24. Salt. sol. 7·5%	5%	3%	1%	Infection.	Date of Inoculation.
H. 15139											±		±	±	±	±	*T. equiperd.*	14/2/24.
H. 15565			++				++				++		++	++	++	++	*T. equiperd.*	Natural case.
H. 15569			++				++				++		+	++	++	++	*T. equiperd.*	Natural case.
H. 15125							++				++		+	++	++	++	*T. congolense*	6/4/23.
																	T. equiperd.	14/2/24.
H. 15831													−	+	+	+	*T. brucei*	14/2/24.
H. 15852			−														Neg. control	—
H. 15844			−														Neg. control	—
C. 320																	*T. congolense*	13/3/24.
C. 321																	*T. congolense*	13/3/24.
C. 331							++			‡‡							—	6/4/23.
C. 395											−		±	+	+	+	*T. congolense*	13/3/24.
C. 436											−		−	±	±	±	*T. congolense*	13/3/24.
C. 440							−		±	+	+	+	−	−	−	±	*T. congolense*	13/3/24.
C. 446											−			‡‡			*T. congolense*	13/3/24.
C. 450											−		±	+	+	+	*T. congolense*	13/3/24.
C. 456											−		−	−	±	+	*T. congolense*	13/3/24.
C. 520											−		±	±	±	±	*T. brucei*	13/3/24.
C. 554											−		±	+	+	+	*T. brucei*	13/3/24.
C. 575											−		±	±	±	±	*T. brucei*	13/3/24.
C. 602											−		±	±	±	±	*T. congolense* and *T. brucei*	13/3/24.
C. 684											+		−	±	±	±	*T. congolense* and *T. brucei*	13/3/24.
C. 722																	*T. congolense* and *T. brucei*	13/3/24.
H. 15739											±		−	±	±	±	*T. brucei*	13/3/24.

H. Horse. C. Cattle. ++ Marked flocculation. + Definite flocculation. ± Slight flocculation. − No flocculation. ‡‡ Dead. Horses 15565 and 15569 were used as positive controls.

TABLE 7 (*continued*).

Number.	7/3/24. Salt. sol. 7·5%	5 %	5 %	1 %.	15/3/24. Salt. sol. 7·5%	5 %	3 %	1 %	21/3/24. Salt sol. 7·5%	5 %	5 %	1 %	Infection.	Date of Inoculation.
H. 15139	−	−	±	±	±	+	+	+		‡‡			*T. equiperd*	14/2/24.
H. 15565													*T. equiperd*	Natural case.
H. 15569													*T. equiperd*	Natural case.
H. 15125													*T. congolense*	6/4/23.
													T. equiperd	14/2/24.
H. 15831	−	±	+	++	+	+	++	++		‡‡			*T. brucei*	14/2/24.
H. 15852													Neg. control	—
H. 15844													Neg. control	—
C. 320													*T. congolense*	13/3/24.
C. 321									−	−	−	−	*T. congolense*	13/3/24.
C. 331									−	−	−	−	—	6/4/23.
C. 395	±	+	+	+	−	±	±	±					*T. congolense*	13/3/24.
C. 436		‡‡											*T. congolense*	13/3/24.
C. 440	−	−	−	−	−	−	−	−					*T. congolense*	13/3/24.
C. 446													*T. congolense*	13/3/24.
C. 450	±	+	+	+	−	−	±	+					*T. congolense*	13/3/24.
C. 456	−	−	−	±	−	−	−	±	±	±	±	±	*T. congolense*	13/3/24.
C. 520	−	±	+	+	−	±	+	++					*T. brucei*	13/3/24.
C. 554					−	+	+	+					*T. brucei*	13/3/24.
C. 575	−	−	±	±	±	±	±	+					*T. brucei*	13/3/24.
C. 602	−	−	±	±	−	−	−	±	±	±	±	+	*T. congolense* and *T. brucei*	13/3/24.
C. 684	−	−	−	±	−	−	−	±	−	−	−	−	*T. congolense* and *T. brucei*	13/3/24.
C. 722									±	±	±	±	*T. congolense* and *T. brucei*	13/3/24.
H. 15739	−	−	±	+					±	+	+	++	*T. brucei*	13/3/24.

H. Horse. C Cattle. ++ Marked flocculation. + Definite flocculation. ± Slight flocculation. − No flocculation. ‡‡ Dead.
Horses 15565 and 15569 were used as positive controls.

It will be seem from Table 7 that animals infected with *T. equiperdum* usually gave a strongly positive reaction with this test. Antigen made from dourine trypanosomes was used for the whole series. Animals infected with *T. congolense* might or might not react positively, but most *T. brucei* animals gave positive reactions. In trypanosomiasis the lipoid fixation reaction appears to be less specific than the complement fixation one. As to whether it is more reliable for dourine than the complement fixation test as stated by Dahmen, a definite opinion cannot be expressed. A sufficient number of tests have not been done here for comparison. Sera from cases in the field usually arrive slightly turbid and often blood-stained, so can only be used for the complement fixation test, very clear serum being required for lipoid fixation.

LITERATURE.

(1) A. E. Watson, " Dourine in Canada," Bulletin of Dept. of Agric., Dominion of Canada, 1920.
(2) Bessemans and Leynen, Bulletin Inst. Pasteur. No. 23. 15th December, 1922.
(3) Bessemans, C. R. de la, Soc. Biol. t. LXXXVI, p. 289, 1922.
(4) H. Dahmen, Berl. Tierarztl. Wochenschr. 41, 1920.
(5) G. Dreyer and H. K. Ward, " A simple quantitative serum reaction for the diagnosis of syphilis," Lancet, Vol. 1, No. 19, 7th May, 1921.
