TABLE 5.

T. vivax—*Artificial Transmission.*

No.	Animal.	Source of Blood.	Date Sub-inoculated.	Tryps. First Appeared.	Initial Symtoms.	Dead.	Further Remarks.
1	Ox 4024	Cow Cry. 1, natural case (only *T. vivax* seen in smears, but apparently *T. congolense* also present)	16/ 3/22	30/3/22 (*T. vivax*)	30/3/22, temp. 103·2°; 1/4/22, *T. congolense* seen; 7/4/22, lachrymation; 13/5/22, eating earth; 20/6/22, purging	28/ 7/22 (nagana)	Infected by 20 c.c. fresh blood intrajugularly.
2	Calf 17	Cow Cry. 1, natural case (only *T. vivax* seen in smears, but apparently *T. congolense* also present)	16/ 3/22	—	28/3/22, lachrymation and both cornea "filmed"	—	Infected by 20 c.c. fresh blood subcutaneously.
3	Goat 7	Cow J. 1, natural case	29/ 4/22	—	14/5/22, temp. 105°	13/ 1/23 (pneumonia)	Infected by 50 c.c. citrated blood subcutaneously.
4	* Cow 343	Ox 4024, artificial case (mixed infection)	27/ 7/22	2/8/22 (*T. congolense*)	2/8/22, temp. 105·2°, smear *B. bigemina*; 10/8/22, oedema of throat	23/ 8/22 (nagana)	Infected by 60 c.c. citrated blood intrajugularly.
5	Bovine 272	Ox 4024, artificial case (mixed infection)	27/7/22	—	5/8/22, temp. 104°; 11/8/22, unable to rise	12/ 8/22 (cause unknown)	Infected by 55 c.c. citrated blood subcutaneously.
6	Dog 48	Ox 4024, artificial case (mixed infection)	26/ 7/22	—	—	—	Infected by 10 c.c. citrated blood subcutaneously.

* Animal received treatment.

TABLE 5—*continued.*

No.	Animal.	Source of Blood.	Date Sub-inoculated.	Tryps. First Appeared.	Initial Symptoms.	Dead.	Further Remarks.
7	Dog 42	Ox 4024, artificial case (mixed infection)	27/ 4/22	See Table 3.		—	Infected by 50 c.c. citrated blood subcutaneously.
8	Goat 9	Ox 4024, artificial case (mixed infection)	27/ 4/22	See Table 3.		—	Infected by 50 c.c. citrated blood subcutaneously.
9	Goat 10	Ox 4024, artificial case (mixed infection)	27/ 4/22	See Table 3.		—	Infected by 50 c.c. citrated blood subcutaneously.
10	Goat 8	Cow J. 4, natural case	29/ 4/22	—	—	—	Infected by 50 c.c. citrated blood subcutaneously.
11	Bovine 342	Cow M. 2, natural case	26/ 6/22	—	13/7/22, temp. 104°; 19/8/22, losing condition	27/ 8/22 (Abscess of liver)	Infected by 250 c.c. citrated blood intrajugularly.
12	Bovine 345	Ox Col. 15, natural case	18/ 8/22	—	1/9/22, blood anaemic, redwater suspected	15/ 9/22 (anaemia following redwater)	Infected by 150 c.c. citrated blood intrajugularly.
13	Donkey 14912	Cow 2646, natural case (mixed infection)	23/6/22	See Table 3.		—	Infected by 300 c.c. citrated blood intrajugularly.
14	Goat 4582	Cow 2646, natural case (mixed infection)	23/ 6/22	See Table 3.		—	Infected by 200 c.c. citrated blood intrajugularly.

TABLE 5.—*continued.*

No.	Animal.	Source of Blood.	Date Sub-inoculated.	Tryps. First Appeared.	Initial Symptoms.	Dead.	Further Remarks.
15	Goat 4757	Warthog Z. 373	20/ 6/22	—	See Table 11.	14/ 8/22 Heart-water)	Infected by 20 c.c. citrated blood subcutaneously.
16	Donkey 14734	Warthog Z. 373	20/ 6/22	—	See Table 11.	—	Infected by 30 c.c. citrated blood subcutaneously.
17	Dog 117	Duiker Z. 570	27/12/22	—	See Table 12.	4/ 1/23 (shock following wounds caused by dogs)	Infected by 25 c.c. citrated blood subcutaneously.
18	Goat 33	Duiker Z. 570	27/12/22	—	See Table 12.	3/ 3/23 (cause unknown)	Infected by 35 c.c. citrated blood subcutaneously.

In addition to the above sub-inoculations, 4 rabbits and 2 guinea-pigs were injected with *T. vivax* blood, but all blood-smears were negative.

4. Influence of Age on Nagana.

Apart from the nutritiousness of the veld herbage as a factor in arresting the course of *T. congolense* disease in bovines, nothing else contributed so much in preventing infection or rather checking the development of the disease as age. Hornby (1921), whose description of nagana corresponds more closely to the state of affairs met with in Zululand than any other author, writes:—"Age confers no immunity; nevertheless when an outbreak of the disease occurs in a herd the adult animals die first." Nagana was rarely encountered in cattle less than eighteen months of age, and only three cases were observed in animals less than one year old. The youngest sufferer appears to have been a calf of six months of age obtained from the tsetse fly research camp in October, 1923, and found by Dr. Berg (who has kindly furnished these details) to be infected with all three species of nagana trypanosomes. Possibly *T. congolense* disease is less frequent in calves owing to the system of management, namely, grazing around the homestead, whereas older cattle wander for miles.

Young dogs experimentally infected with *T. brucei* were no more resistant to infection than older animals.

5. Differential Diagnosis.

Until a positive smear is obtained from a case of nagana or, in the absence of smear examination, until improvement follows an injection of tartar emetic, many maladies if seen for the first time or kept under observation for only a short period might be confused with trypanosomiasis. Some of these conditions in their order of importance are:—

1. Parasitic gastro-enteritis.
2. Loss of condition following—
   (*a*) deterioration of pasture in autumn;
   (*b*) cold spell of weather;
   (*c*) bad management, e.g. overwork and underfeeding.
3. "Styfsiekte."
4. Gall-sickness or Anaplasmosis.
5. Heartwater.

1. Parasitic gastro-enteritis.

Synonyms:—"Umuncu," a native term for any disease associated with emaciation, but which in the vast majority of cases is the disease in question. "Swamp disease" or "Mbaswana Swamp disease," since it is common in water-logged areas. The most severe outbreaks have been reported from the vicinity of the Mbaswana Swamp in Ubombo Division. "Wasting disease," so-called from its most prominent symptom.

Course and nature of disease:—Along the grass-covered coastal zone, which is swampy and badly drained, outbreaks of this disease, characterized by loss of condition and scouring, occur almost every year. Until investigated for the first time at Mbaswana in July, 1921, it was believed by stock-owners and others, in spite of blood-smears being constantly negative, that the malady was none other than nagana. Since 1921 further inquiries have been made, and it would appear that the same condition exists in the southern portion of Mozambique; and observations show that the identical malady is prevalent along the whole south-eastern coastal region. The veld along the Zululand portion of the coast is so grossly infected that

it is impossible to raise sheep and goats, and in some years losses among young cattle amount to 50 per cent. Parasitic gastro-enteritis resembles nagana in that it occurs more or less regularly almost every year and is most severe during the winter when the pasturage is at its worst.* The clinical picture is somewhat similar, being characterized by a subacute or chronic course, in which a loss of condition, lachrymation, oedema of throat, weakness, anaemia, and profuse diarrhoea are generally observed. If the disease is not too far advanced, recovery often follows an improvement of pasture. On post-mortem the general appearance is not unlike nagana in that there is oedema and emaciation. Apart from blood-smear examination in which trypanosomes may be seen in nagana, a differential diagnosis may be based on the following:—The topography of the locality, the history of the outbreak, the fact that the majority of the sufferers are young animals under eighteen months of age, that, although lachrymation may be present, corneal opacities are absent. A careful post-mortem examination of the gastro-intestinal tract will also show that nematodes, especially *Haemonchus contortus*, are frequent in the abomasum, the mucosa is marked with numerous minute haemorrhages, the mucosa of the duodenum is thickened, and the inner aspect coated with much mucus. In material submitted to the Director of Veterinary Education and Research from the Mbaswana outbreak of 1921, other nematodes identified were *Cooperia punctata* and *Cooperia pectinata* in large numbers from the small intestines.

2. As would be expected, any loss of condition is viewed by owners of stock in and around fly areas with grave concern. In some cases, e.g. where a span of oxen has been hard at work ploughing, it is not difficult to definitely associate any loss of condition with the circumstances under which they have been working, especially if the remaining cattle are in good condition. Oxen, however, are often the first animals on a farm to contract the disease, as ploughing operations are usually carried out on alluvial land near some bush-lined stream. With regard to the effect of cold weather, cattle are the most susceptible of all domesticated animals to adverse changes of weather, especially if accompanied by cold winds. The two factors above referred to lead only to a temporary loss of condition, and as soon as the cause operating has ceased, improvement rapidly takes place. A loss of flesh is generally noticeable in the autumn, particularly in animals grazing on elevated situations, e.g. high veld. Coupled with the factor of cold is the deterioration of the pasture, and in many cases the diet provided by the herbage is not sufficient as a maintenance ration. Animals thus may lose anything up to 100-200 lb. in weight during the dry season. On the warm, well-drained stretches of coastal veld cattle keep in excellent condition throughout the year, provided overstocking does not take place. Probably the worst offenders in regard to careless management of cattle are cane farmers, who plough up every available acre of land for cultivation, leaving only the badly drained portions for grazing purposes. As a consequence the oxen, which are already overworked

* Apart from the nutritional factor, an important reason is the fact that the only green grass is localized in moist patches (vleis), and at these all the cattle graze until the places must teem with eggs and larvae.

in many cases, do not receive sufficient nourishment and are reduced to mere walking skeletons. Examination of blood-smears microscopically would naturally in all cases prove negative.

3. "Styfsiekte." This term is applied to several affections, all probably differing in their etiology, but characteristic of each is stiffness during progression. The forms of styfsiekte met with were:—(*a*) A stiffness seen in cows chiefly during the early stages of lactation; (*b*) a stiffness seen in young growing animals. Besides the difficulty in gait, affected stock are stunted, anaemic, pot-bellied, and may develop bony enlargements in the region of the lower joints of the limbs. Both conditions are encountered on so-called "Sourveld," or herbage of low nutritional value, and are therefore probably deficiency diseases. A differential diagnosis would be necessary in those grass-covered areas adjoining bushveld, for the conditions described are invariably seen in open grassveld. In both cases, history, symptoms, negative blood-smears, and absence of mortality would rule out nagana.

4. Anaplasmosis, especially in subacute or chronic forms of the disease, possesses symptoms which might be confused with nagana. These are loss of condition, anaemia, dullness, depression, and lack of energy, but smear examination will either show *Anaplasma marginale*, the causal organism, or anaemic changes, e.g. polychromasia and punctate basophilia as a sequel of the disease. The lachrymation and corneal opacities typical of nagana would in addition be absent.

5. In animals that have not been noticed ill, or in fact have not been seen for some time, and are examined at death, a little difficulty has been met with by farmers in differentiating nagana from heartwater. In both diseases accumulation of effusion occurs in the serous cavities, and the spleen is in a condition of tumour splenis. On the other hand the carcass of a nagana animal would be emaciated, and eye lesions might be present. Blood-smear examination and knowledge of history of case, e.g. sudden death in heartwater, would be helpful in arriving at a diagnosis.

Several other conditions may be mistaken for nagana, e.g. tuberculosis of the bovine and biliary fever of the dog; but particular care should be taken lest eye symptoms due to other causes, e.g. foreign body, ophthalmia,* orbital eczema (in dog), be ascribed to trypanosomiasis.

* An ophthalmia of sheep, apparently contagious in nature, was met with on several occasions. Cattle, too, suffer from ophthalmia chiefly during the summer months.

V.—MISCELLANEOUS EXPERIMENTS.

The following experiments, not lending themselves to inclusion in other sections, are described herein. Their titles are—

(1) "To confirm Taute and Huber's (1919) observation that *T. brucei* is not pathogenic for man."
(2) "To ascertain how soon nagana will be contracted by domesticated animals exposed in various parts of the Ntambanana Settlement."
(3) "To investigate what game animals acted as reservoirs of nagana infection."
(4) "To ascertain if the young born of mothers suffering from nagana are infected."

1. *Object.*—"To confirm Taute and Huber's (1919) observation that *T. brucei* is not pathogenic for man."

Preliminary Remarks.—No record exists of any human being ever having contracted sleeping sickness in Zululand and, in spite of Bruce's (1915) statement that "Many cases of death occurred among hunters and explorers, which were usually put down to malaria, but it is possible some of these may have been due to infection by nagana," it is safe to assert that the disease does not occur in the country. Not only is Zululand well policed (better so formerly than at the present time), but district surgeons, magistrates, and other officials have every opportunity of investigating any report of suspicious disease. Furthermore, thousands of persons, especially natives, are bitten by Glossina annually without untoward results. In order, however, to supply experimental proof in support of the opinion expressed above, a small experiment was undertaken.

Method.—A European (C) and two natives (V) and (C), adults, were inoculated subcutaneously in left forearm with 1 c.c. of mixture of blood ($\frac{1}{4}$ c.c. 1 per cent. solution potassium citrate and $\frac{3}{4}$ c.c. blood) at 8 a.m., 1st February, 1922, taken from Horse 14301 a few minutes earlier. As will be seen from Table 4, a series of animals, ranging from white mice to bovines, were inoculated from the same animal on 28th January, 1922, and the results of the injections are shown therein. Temperatures were taken night and morning until the 12th March, 1922, but in no instance did the temperature exceed 98.8. Blood, either stained smears or hanging-drop preparations, was examined daily, but nothing unusual was noted during the period in question. On the 1st March, 1922, it was decided to test the infectivity of the blood of the European and native (V) Accordingly sub-inoculations were made as follows:—

TABLE 6.

Donor.	Test Animal.	Injection.	Result.
European.....	Dog 33.....	17 c.c. cit. blood subcutaneously	Discharged, well, 4/4/22.
„	White rat 34...	2 c.c. cit. blood intraperitoneally	Discharged, well, 1/6/22.
Native (V).....	Dog 34.....	10 c.c. cit. blood subcutaneously	Discharged, well, 4/4/22.
„	White rat 35...	4 c.c. cit. blood intraperitoneally	Discharged, well, 1/6/22.

Discussion.—Objections to this experiment would naturally be raised, especially by those supporting Bruce's contention that *T. rhodesiense* and *T. brucei* are identical. To those who maintain that the strain of *T. brucei* employed had not become accustomed to human blood (and was, therefore, incapable of setting up disease) it may be answered that opportunities have existed for generations for *T. brucei* to become habituated to human blood throughout the inhabited portions of Glossina country. As mentioned before, natives are bitten daily, giving ample chances for *T. brucei* to become established in the human, but so far, fortunately, without any effect. It may be added that the horse's blood frequently showed posterior nuclear forms, and in the sub-inoculated white mice this type of trypanosome was abundant. Other investigators who have attempted to throw light on the question of pathogenicity of trypanosomes for humans by inoculation are Ascenso (1911)* and, according to Fantham (1914), Todd and Brumpt, who employed *T. brucei.*

2. *Object.*—" To ascertain how soon nagana will be contracted by domesticated animals exposed in various parts of the Ntambanana Settlement." At the time this set of tests was carried out, it was believed that outbreaks of nagana were primarily due to non-Glossina agents, as Glossina were so rarely seen. This being the case, the possibility was investigated as to whether biting-flies, other than Glossina, acted as vectors; but proof of tsetse being present was soon forthcoming after " tethering experiments " were arranged.

Preliminary Remarks.—Since many of the local farmers purchased cattle in Natal, a question frequently asked was with regard to the period after which the disease might be expected to appear in animals that had been recently introduced. This naturally depended directly on the relative abundance of Glossina on the various farms. Indirectly, the following factors were also to be considered:—

(*a*) Time of year cattle brought into the settlement. The chances of infection were a great deal less in the winter than in the summer.

(*b*) Situation and topography of farm. Bushveld farms were more dangerous from a tsetse point of view than mixed bushveld and grassveld farms, and the latter were again more risky than the open grass country characteristic of the high veld.

(*c*) Facilities available for preventing the wandering of cattle to suspicious fly areas. Good fencing was far more effective in checking the wandering of stock from open grassveld farms than herding by native boys.

Several opportunities occurred for making observations with regard to the above matter and the experiments carried out may be briefly summarized as follows:—

Method.—Differed according to experiment. *In Experiment*

* In the Bull. Sleeping Sickness Bureau, III, 26, page 174, appears an extract of a letter written by Dr. Ascenso in November, 1906, who states that on 7th October, 1903, he inoculated himself and two natives each with 1 c.c. blood taken from a cow suffering from nagana (trypanosome?) near Lake Moero. Nothing untoward resulted. This information was supplied by Professor Todd.

No. 32 the cattle were received from Pretoria, and on arrival were immediately driven to the different farms, as follows:—

TABLE 7.

Farm No.	Bovines.	Exposure. From.	Exposure. To.	Type of Infection.	Date.	Further Remarks.
316	40	21/10/22	12/ 1/23	*T. congolense* .	8/1/23	
	96	..	,,	,, .	..	
	4617	,,	,,	,, .	19/1/23	
	3628	,,	,,	,, .	,,	
	100	,,	,,	Negative.....	—	
311	159	21/10/22	25/ 1/23	Negative.....	—	
	150	..	..	,, ...	—	
	133	,,	,,	,, ...	—	
	153	,,	,,	,, ...	—	
	124	,,	,,	*T. congolense*..	2/2/23	
282	141	3/11/22	20/11/22	—	—	Died, 20/11/22, heartwater.
	4649	,,	23/ 5/23	*T. congolense*..	28/5/23	
	149	,,	1/12/22	—	—	Died, 1/12/22, heartwater.
	157	,,	23/ 1/23	Negative.....	—	
	4180	,,	23/ 5/23	*T. congolense*..	21/5/23	
	4539	29/11/22	,,	,, ..	24/5/23	
273 Nagana Research Lab.	4716	21/10/22	26/ 5/23	Negative.....	—	
	4801	,,	,,	,, ...	—	
	4799	,,	,,	,, ...	—	
	4574	,,	,,	,, ...	—	
	126	,,	3/12/22	—	—	Died, 3/12/22, heartwater.
271	257	21/10/22	23/ 3/23	Negative.....	—	
	12	,,	,,	,, ...	—	
	127	,,	,,	,, ...	—	
	140	,,	,,	*T. vivax*.....	30/3/23	
	4557	,,	,,	*T. congolense*..	19/3/23	
262	131	21/10/22	26/ 5/23	Negative.....	—	
	130	,,	,,	,, ...	—	
	128	,,	,,	*T. vivax*.....	22/5/23	
	129	,,	,,	Negative.....	—	
	3773	,,	,,	,, ...	—	

As will be seen from Map 3, the cattle were distributed on farms which extend roughly from north to south across the settlement. The animals were in each case allowed *to graze along with the cattle belonging to the farm* and treated in every way the same. The results, however, of the exposure experiment vary considerably.

Discussion.—The series of cattle which suffered most severely was that exposed on Farm No. 316, which is situated in an area which has always been considered dangerous. Although situated on the watershed dividing the White Umfolosi and Enseleni Rivers, the bush growth has encroached along all the valleys, which thus form ideal protection for Glossina. It will be observed that none of the cattle kept on Farm No. 273, Nagana Research Laboratory, contracted nagana. This may be attributed to two factors. In the first place, the bush growth is comparatively scanty, only the valleys containing trees. Then, again, the farm was completely enclosed by a strong barbed-wire fence, which prevented the animals wandering down the valleys to localities where Glossina may have existed.

Method in Experiment No. 33.—Three sets of animals, comprising a horse, bovine, and goat in each group, were exposed at three points away from the Enseleni River (Farm No. 311). The first set was *tethered* to trees on the river bank (left), the second at

the edge of the fringe of river scrub, and the third on an eminence overlooking the stream and free from tree growth. The animals were fed and watered *in loco*, and temperatures and blood-smears were taken regularly. [See (A), Map 3.] The results of the experiment from the nagana aspect are as follows:—

TABLE 8.

Set	Animal.		Exposure. From.	Exposure. To.	Type of Infection.	Date.	Further Remarks.
1.	Horse	13810	25/10/22	15/12/22	—	—	Died, 24/12/22, horse-sickness.
	Bovine	134	,,	,,	*T. congolense*..	3/2/23	10/1/23, symptoms of nagana.
	Goat	16	,,	25/11/22	Negative.....	—	
2.	Horse	13857	25/10/22	10/11/22	—	—	Died, 10/11/22, horse-sickness.
	Horse	14696	13/11/22	15/12/22	Negative.....	—	
	Bovine	4681	25/10/22	,,	,, —	—	
	Goat	17	,,	,,	—	—	Killed, 31/12/22.
3.	Horse	14683	25/10/22	15/12/22	Negative.....	—	
	Bovine	3922	,,	,,	,, —	—	
	Goat	14	,,	25/11/22	—	—	Died, 25/11/22.
	Goat	18	29/11/22	15/12/22	Negative.....	—	

Discussion.—The chances of contracting nagana were obviously less in tethered animals than in those allowed to roam at will.* Nevertheless, the bovine fastened in the dense bush nearest the river became infected. As *Glossina* were captured in this locality, the question of transmission of nagana by other biting-flies need not be considered.

Method in Experiment No. 34.—Goats were omitted from the experiment, as they were not attractive to biting-flies. The series, including a horse and ox in each case, were *tethered* on the right bank of the Ntambanana River (Farm No. 250). [See (B), Map 3.]

The details regarding animals are as follows:—

TABLE 9.

Set	Animal.		Exposure. From.	Exposure. To.	Type of Infection.	Date.	Further Remark.
1.	Horse	8944	28/12/22	22/1/23	Negative.....	—	None of these animals had developed nagana by 16/3/23.
	Bovine	111	,,	,,	,, ...	—	
2.	Horse	10371	,,	,,	,, ...	—	
	Bovine	135	,,	,,	,, ...	—	
3.	Horse	12600	,,	,,	,, ...	—	
	Bovine	99	,,	,,	,, ...	—	

Discussion.—Although the period of exposure was twenty-five days, only one specimen of Glossina was caught during the observations and no animal became infected. In the summer of 1920 over 100 head of cattle died of nagana about the vicinity of the experimental site, and it would appear that at that time either Glossina were more abundant or that infection was spread mechanically by biting-flies other than tsetse. It is safe to assume that every herd

‡ This applies particularly to country where Glossina are few and scattered.

in the settlement contains a few reservoirs of disease, and these naturally constitute a focus from which infection spreads.

Method in Experiment No. 38.—The horses and cattle were *tethered* along the left bank of the Umhlatuzi River on Farm No. 283. It was not possible to place the third set of animals on open rising ground as in the two previous experiments, as the ground was not elevated in any way. As in the previous experiment, no goats were employed. [For situation see (C), Map 3.]

TABLE 10.

Set	Animal.	Exposure. From.	Exposure. To.	Type of Infection.	Date.	Further Remarks.
1.	Horse 13183	11/2/23	14/4/23	Negative.....	—	
	Bovine 146	,,	,,	,, —	—	
2.	Horse 15105	,,	18/3/23	,, —	—	18/3/23, died horse-sickness.
	Bovine 119	,,	14/4/23	Negative.....	—	
3.	Horse 15292	,,	,,	,, ...	—	
	Bovine 123	,,	,,	*T. congolense*..	25/5/23	Symptoms of nagana, 19/3/23.

Discussion.—As shown above, the animals were removed from the Umhlatuzi Valley on 14th April, 1923; but they were kept under close observation until the middle of June, during which period one animal, Bovine 123, was proved to be suffering from nagana.

Results of Experiments.—These are as follows:—

(*a*) It has been shown that introduced animals may become infected within two to three months after arrival in the Ntambanana Settlement, the portion of which south of the Enseleni River had been considered Glossina-free until these observations. From field observations in connexion with cattle belonging to the settlers, cases of clinical nagana have been observed in from one to two months, showing that infection must have occurred soon after arrival.

(*b*) With regard to the entomological side of the experiments, since Glossina were proved to be present, no advance was made in connexion with the view that non-Glossina agents were responsible for propagation of the disease. It was proved that the southern boundary of tsetse, instead of being the Enseleni River, as was originally believed, was at least as far south as the Umhlatuzi Valley. Bearing in mind the topography of the surrounding country, the probable southern limit might be taken as the Ngoye Range in Mtunzini Division. (See Map 1.)

3. *Object.*—"To investigate what game animals acted as reservoirs of nagana infection."

Preliminary Remarks.—Two series of experiments were carried out and these for convenience are designated Series A and Series B. As preliminary tests showed that the solution of potassium citrate employed by Bruce (1896), viz., the addition of "a twentieth of its bulk (i.e. blood) of a 0.5 per cent. citrate of potassium solution," thus corresponding to a 0.025 per cent. solution of potassium citrate, was insufficient to prevent coagulation, a 1 per cent. solution was at first employed. This was obtained by the addition of 90 c.c. of blood to

10 c.c. of 10 per cent. solution of potassium citrate, which is used for redwater (*B. bigemina*) and gall-sickness (*Anaplasma centrale*) vaccine. Later, when further tests showed that 0.2 per cent. solution was generally effective in preventing coagulation, bottles were prepared containing 20 c.c. of 1 per cent. solution of potassium citrate in saline, and to this 80 c.c. of blood was added. In the case of zebra blood, 25 c.c. was necessary to prevent coagulation. Apart from the question of non-clotting of the blood was to be considered the effect of the various solutions on the trypanosome. It was found that, as a rule, with a 0.2 per cent. solution parasites were alive twenty-four hours after collecting of the blood; but in a few cases both *T. brucei* and *T. congolense* were dead after six hours. In this connexion it is noteworthy that no sub-inoculations in respect of *T. vivax* were successful where citrated blood was used (see Section IV). It is interesting to record that sodium citrate was more efficient than the potassium salt.

Series A.

Method.—The game animals used for the purpose of obtaining blood were shot along the White Umfolosi River near its confluence with the Munywane River. Blood was collected from the dead animal as aseptically as possible into bottles containing 10 c.c. of 10 per cent. sterilized solution of potassium citrate in saline. A messenger was then dispatched as soon as possible with the sample of blood to the Nagana Research Laboratory, twelve miles distant, where experimental animals were inoculated with as little delay as possible. The blood on arrival at the laboratory varied in age from three hours to twenty hours, and on one occasion it was twenty-six hours old. From the results of the preliminary citrate tests it was realized that in some cases, after six hours, the trypanosomes in the game blood, if present, might have already died. In the first sample of blood received, viz., that of a warthog, live trypanosomes were discernible twenty-four hours after death of the warthog. In every case hanging-drop preparations were examined from the samples of blood as they were delivered. As will be observed from the particulars detailed in Table 11, dogs and goats were used for sub-inoculation. It was hoped that *T. brucei* infection would develop best in the dog and *T. congolense* and *T. vivax* in the goat; white rats were also employed for this series of inoculations, but in no case were trypanosomes seen.

Table 11.

Serial No.	Zool. Book No.	Game Animal.	Date of Sub-inoculation.	Sub-inoculation made into.	Amount of Blood Injected.	Sub-inoculation made into.	Amount of Blood Injected.	Animals Died.
1	373	Warthog	20/6/22	Donkey -14734	30 c.c. subcut.	Goat 4575	20 c.c. subcut.	Goat, heartwater, 14/8/22.
2	376	Wildebeest	22/6/22	Dog 104	50 ,,	,, 4475	20 ,,	—
3	377	Duiker	23/6/22	,, 105	15 ,,	,, 4467	15 ,,	Goat, heartwater, 22/8/22.
4	378	Zebra	24/6/22	,, 40	50 ,,	,, 4531	50 ,,	Goat, pneumonia and pleurisy, 20/7/22.
5	379	Warthog	24/6/22	,, 43	15 ,,	,, 3219	20 ,,	Dog, 2/9/22, marasmus.
6	380	Waterbuck	24/6/22	,, 21	25 ,,	,, 4528	30 ,,	Dog, 28/9/22, marasmus.
7	385	Bushbuck	28/6/22	,, 46	15 ,,	,, 4910	15 ,,	—
8	386	Kudu	28/6/22	,, 116	30 ,,	,, 4558	30 ,,	Goat heartwater, 27/8/22.
9	388	Kudu	28/6/22	,, 115	30 ,,	,, 4466	50 ,,	Dog killed by mates, 30/7/22.
10	389	Zebra	29/6/22	—	—	,, 4591	30 ,,	—
11	390	Bushbuck	29/6/22	—	—	,, 4911	30 ,,	—
12	397	Cheetah	2/7/22	Dog 119	30 c.c. subcut.	,, 4583	40 ,,	—
13	398	White-tailed mongoose	2/7/22	,, 118	7 ,,	,, 4471	7 ,,	Goat, heartwater, 27/8/22.
14	399	Bushbuck	2/7/22	,, 117	15 ,,	,, 4470	20 ,,	Goat, heartwater, 26/8/22.
15	402	Zebra	4/7/22	,, 54	20 ,,	,, 4556	30 ,,	Dog killed by mates, 16/9/22.
16	403	Vervet monkey	4/7/22	—	—	,, 4560	15 ,,	Goat heartwater 30/8/22.
17	404	Warthog	4/7/22	Dog 44	20 c.c. subcut.	,, 4565	30 ,,	—
18	406	Bushbuck	5/7/22	—	—	,, 4593	50 ,,	—
19	407	Steenbuck	5/7/22	—	—	,, 4585	40 ,,	Goat, heartwater, 18/7/22.
20	408	Bushbuck	5/7/22	—	—	,, 4477	40 ,,	Goat, heartwater, 14/8/22.
21	409	Vervet monkey	5/7/22	—	—	,, 4554	15 ,,	—
22	411	Warthog	8/7/22	—	—	,, 4476	60 ,,	Goat, heartwater, 22/7/22.
23	412	Duiker	10/7/22	—	—	,, 4591	60 ,,	—
24	418	Warthog	11/7/22	Dog 50	20 c.c. subcut.	,, 4510	20 ,,	Goat, heartwater, 22/8/22.
25	419	Warthog	11/7/22	,, 51	15 ,,	,, 4678	20 ,,	Dog, marasmus, 5/9/22.
26	420	Wildebeest	13/7/22	,, 49	20 ,,	,, 4527	30 ,,	Goat, heartwater, 6/10/22.
27	421	Duiker	13/7/22	,, 38	30 ,,	,, 4663	50 ,,	Goat, heartwater, 26/8/22.
28	429	Duiker	14/7/22	,, 47	20 ,,	,, 4675	30 ,,	Goat, heartwater, 30/7/22.
29	430	Warthog	14/7/22	,, 39	20 ,,	,, 4646	20 ,,	Dog, marasmus, 4/8/22.
30	448	Warthog	17/7/22	,, 52	20 ,,	,, 4566	40 ,,	—
31	449	Waterbuck	17/7/22	,, 33	20 ,,	,, 4478	60 ,,	Goat, heartwater, 12/8/22.
32	450	Warthog	17/7/22	,, 57	20 ,,	,, 4518	60 ,,	Dog killed by mates, 7/9/22.
33	451	Warthog	17/7/22	,, 58	20 ,,	,, 4541	50 ,,	Goat, heartwater, 23/8/22.
34	452	Bushbuck	20/7/22	—	—	,, 4545	40 ,,	Dog killed by mates, 22/7/22.
35	453	Warthog	20/7/22	—	—	,, 4536	50 ,,	Goat, heartwater, 25/8/22
36	454	Zebra	20/7/22	—	—	,, 4515	80 ,,	—
37	455	Duiker	20/7/22	—	—	,, 4521	75 ,,	Goat, heartwater, 14/8/22.
38	456	Water tortoise	22/7/22	—	—	,, 4960	50 ,,	—
39	462	Buffalo	24/7/22	—	—	,, 4577	110 ,,	—
								Goat, heartwater, 19/8/22.

Discussion.—The only sample of blood which revealed trypanosomes was that taken from the first warthog shot. The organisms were markedly translatory and suggested *T. vivax;* they were not seen in stained blood-smears, nor did they appear in the sub-inoculated animals. As will be seen from the description of *T. vivax,* Section III, the only case where transmission of the parasite succeeded was where fresh blood was employed. The goats used for experiment had been specially introduced from the Transvaal, as it was considered local goats possessed a high degree of resistance. Unfortunately, however, these animals were extremely susceptible to heartwater, 50 per cent. dying in the six weeks following the first spring rains. Incidentally, this fact demonstrates the difficulties attending the introduction of good class stock into sub-tropical areas. Stained smears from the blood and organs of all game animals shot proved negative* on microscopic examination.

Series B.

Method.—Since the results of the former series of inoculations had been a failure, it was decided to repeat the investigations during the summer. In order to reduce the period from collection of blood to inoculation of same, the experimental animals were sent in fly-proof cages to the White Umfolosi Valley, where they were kept in a specially constructed shelter suitably protected with wire gauze. As soon as a game animal was shot and the blood collected into a bottle containing 1 per cent. sterilized solution of potassium citrate in saline, the assistant hurried back to camp and made the necessary injection and examination of hanging-drop preparation of blood. The inoculated animals were then returned to the Nagana Research Laboratory for observation, whereupon a fresh batch of animals was dispatched to the temporary camp. As a rule, the blood was half an hour to one hour old at time of inoculation, so in no instance should the trypanosomes, if present, have died. The following. Table 12, shows how the work was carried out:—

* The fact that trypanosomes were not seen in any of the experimental animals does not preclude the possibility that some of the specimens of game blood were infected: for, as shown previously, dogs and goats may be refractory to some strains of *T. congolense,* and further, with one exception, it was not possible to transmit *T. vivax* artificially.

TABLE 12.

Serial No.	Zool. Book No.	Game Animal.	Date of Sub-inoculation.	Sub-inoculation made into.	Amount of Blood Injected.	Sub-inoculation made into.	Amount of Blood Injected.	Animals Died.
1	560	Warthog	19/12/22	Dog 104	35 c.c. subcut.	Goat 24	40 c.c. subcut.	Dog, 5/1/23. Goat, 20/2/23.
2	561	Bushbuck	19/12/22	,, 103	30 ,,	,, 23	40 ,,	Goat, 1/2/23.
3	562	Bushbuck	20/12/22	,, 102	35 ,,	,, 22	30 ,,	—
4	563	Bushbuck	20/12/22	,, 39	35 ,,	,, 26	40 ,,	Goat, 31/1/23.
5	563A	Bush-baby	21/12/22	,, 115	10 ,,	,, 25	30 ,,	Goat, 6/2/23.
6	564	Warthog	21/12/22	,, 105	30 ,,	,, 27	40 ,,	Goat, 10/2/23.
7	565	Buffalo	24/12/22	,, 129	30 ,,	,, 29	45 ,,	Dog, 13/4/23. Goat, 26/1/23.
8	566	Bushbuck	24/12/22	,, 128	30 ,,	,, 34	50 ,,	Dog, 11/5/23. Goat, 14/1/23.
9	567	Buffalo	25/12/22	,, 125	35 ,,	,, 21	45 ,,	Goat, 3/2/23.
10	568	Zebra	26/12/22	,, 44	30 ,,	,, 28	40 ,,	Goat, 21/2/23.
11	569	Wildebeest	26/12/22	,, 62	30 ,,	,, 30	40 ,,	Goat, 10/1/23.
12	570	Duiker	27/12/22	,, 117	25 ,,	,, 33	35 ,,	Dog, 4/1/23. Goat, 3/3/23.
13	571	Zebra	1/1/23	,, 71	35 ,,	,, 13	50 ,,	—
14	572	Bushbuck	2/1/23	,, 70	30 ,,	,, 11	40 ,,	—
15	573	Warthog	3/1/23	,, 52	35 ,,	,, 35	45 ,,	Dog, 4/6/23. Goat, 23/1/23.
16	574	Warthog	5/1/23	,, 126	40 ,,	,, 32	55 ,,	Goat, 22/1/23.
17	575	Bushbuck	6/1/23	,, 118	40 ,,	,, 38	45 ,,	Goat, 19/1/23.
18	576	Warthog	8/1/23	,, 48	30 ,,	,, 31	40 ,,	Goat, 22/2/23.
19	577	Zebra	12/1/23	,, 46	40 ,,	,, 43	50 ,,	Dog, 23/2/23. Goat, 22/2/23.
20	578	Bushbuck	13/1/23	,, 65	35 ,,	,, 44	45 ,,	Goat, 6/3/23.
21	579	Bushbuck	15/1/23	,, 67	40 ,,	,, 42	50 ,,	Dog, 19/1/23. Goat, 9/5/23.
22	580	Bushbuck	16/1/23	,, 69	35 ,,	,, 4959	45 ,,	Goat, 19/1/23.
23	581	Warthog	14/1/23	,, 66	50 ,,	,, 41	50 ,,	Goat, 18/2/23.
24	582	Zebra	19/1/23	,, 68	40 ,,	,, 37	50 ,,	—

Further sub-inoculations were made as follows:—

Goat 30 was killed on account of weakness and 300 c.c. citrated blood injected into Bovine 171.

Dog 117 died from shock and 100 c.c. fresh blood were injected into Dog 119.

Goat 38 was killed on account of weakness and 450 c.c. of citrated blood injected into Bovine 336.

All sub-inoculated animals were eventually discharged, being healthy and having shown no signs of trypanosomiasis.

Discussion.—The only positive sample of game blood was that of the duiker (serial No. 12), and here again the trypanosomes were detected in a hanging-drop preparation. The sub-inoculated dog, No. 117, died eight days after injection, but the goat, No. 33, lived sixty-six days. The trypanosomes, seen by the lay-assistant, Mr. W. W. Davies, were described as *T. vivax*. The stained smears, made from blood and organs of the shot animals, were in all cases negative. No sign of nagana was noted in any of the experimental animals and the result of the whole experiment, apart from the positive duiker blood, was entirely negative. The chief objection to the crowding of experimental animals in cages while in fly-country, was the difficulty in keeping them clean. As a result, the slightest abrasion became infected, leading to the formation of suppurating wounds. It should be borne in mind that trypanosomes may be present in the blood of an animal and yet produce no effects, either thermally or clinically. Native goats, which are highly resistant to nagana, were used in this series of tests, and although no deaths took place from heartwater, the mortality which followed was as a result of debility. *Haemonchus contortus* could, as a rule, be demonstrated in the abomasum, but as these nematodes are more or less present in all caprines, no undue importance was attached to their presence. Some of the dogs also became unthrifty in appearance after their return from the White Umfolosi Camp, and the cause of this was not evident. Many of the deaths, however, were due to the fighting of the dogs among themselves.

Conclusion.—In comparing the arrangements made in the carrying out of the two series of game blood transmission experiments, it will be seen that in Series A (1) the work was done in the winter; (2) goats for injection were introduced from the Transvaal, but with disastrous results in consequence of heartwater; (3) white rats were employed; and (4) the inoculations were made at the Nagana Research Laboratory from five to twenty-six hours after collection of blood. In Series B (1) the experiment was carried out in the summer; (2) native goats were employed for sub-inoculation; (3) white rats were not used; and (4) the inoculations were made *in loco* within an hour of collection of blood.

Mitchell,* it might be added, was also unsuccessful in obtaining positive results by blood inoculations. During September and October, 1915, 43 head of game were shot and their blood inoculated into white rats, but with negative result.

* Mr. Mitchell informed the writer that he later obtained one positive result on sub-inoculation, namely, the blood of a young kudu. In 1914, Mitchell showed by inoculation tests that three game animals out of 48 were infected (Transvaal Museum Expedition).

These negative results are described at length in order to demonstrate what precautions were taken and that, in spite of these, negative results were obtained. It is hoped that other investigators will, when opportunity permits, repeat this class of work in order to show what faults, if any, in technique existed.

4. *Object.*—" To ascertain if the young born of mothers suffering from nagana are infected."

Preliminary Remarks.—Although the Sergent brothers and Lhéritier (1919) and Bassett-Smith (1919) have demonstrated that intra-uterine infection is possible in trypanosomiasis, and Lanfranchi (1919) has shown that ingestion of milk from an affected mother may convey infection, direct proof of conveyance of infection from parent to offspring is limited. In the case of *T. congolense* and *T. vivax*, positive evidence is entirely wanting. During two years' sojourn in Zululand, less than a dozen cases of abortion in bovines belonging to settlers were reported, five occurring on one farm where a particularly virulent strain of *T. congolense* existed. In experimental animals only one case of abortion was noted, and that in a rabbit affected with *T. brucei* disease. Microscopic examination of smears from the foetus was negative. Observations were made on all young animals whose mothers were suffering from nagana, as will be seen in the following table:—

TABLE 13.

Young Animal.	Date of Birth.	Mother.	Date of Infection.	Type of Disease.	Experiments and Observations.
Lamb.....	21/2/22	Sheep 2.....	28/1/22	*T. brucei*...	Lamb very weak so 1 c.c. blood drawn and injected intraperitoneally into White Rat 30. Result negative. Lamb died of malnutrition.
Donkey foal...	16/2/22	Donkey 14730.	28/1/22	*T. congolense*	Normal. Foal healthy at birth. A white rat, No. 29, and Dog 36 inoculated with blood, 1 c.c. and 180 c.c. blood respectively, but with negative result.
Pups (three)...	18/2/22	Dog 26.....	28/1/22	*T. congolense*	Pups healthy at birth. A white rat (Nos. 31, 32, and 33) inoculated from each pup with negative result.
Lamb.....	13/4/22	Sheep 4.....	28/1/22	*T. congolense*	Lamb very weak and died 12 days later from malnutrition. No sub-inoculations made.
Pups (twelve)..	23/5/22	Dog 20.....	28/1/22	*T. congolense*	Pups healthy at birth. No sub-inoculations made.

Discussion.—As will be seen from Table 13, the sub-inoculations were negative, even in the case of the donkey foal, where 180 c.c. of blood were drawn and injected into a dog. All sub-inoculations, it may be added, were made on 28th February, 1922. Blood-smears, stained and moist, were examined daily, not only from all young animals, but also from sub-inoculated animals, in every case with negative result. Regarding the five cows which, as mentioned above, aborted as a result of nagana (*T. congolense*), serum was sent to the Veterinary Research Officer, Maritzburg, for agglutination test in order to exclude contagious abortion. This, as was expected from history and symptoms of sufferers, was negative.

VI.—CHEMOTHERAPY.

1. The Use of Tartar Emetic.

Since a study of the literature showed that the only drug which offered any hope of success, when applied on a large scale, to be tartar emetic, this agent, after preliminary observations had been made, was recommended to the farmers for use as a curative or rather palliative remedy. Three articles have been published (Curson, 1922, 1924, and 1926) giving particulars regarding the method of preparation of tartar emetic solution, method of securing the infected animal, and the effects of the drug, but it is proposed to refer briefly to the subject here.

Dose of Drug Employed.—As a routine measure the following amounts are employed:—

Class of Animal.	Approximate weight in kgm.	Tartar Emetic in gm.	Mgms. per kgm. Bodyweight.	Clean Water.
Donkey.....	125–200	1	5 – 10	20 c.c.
Yearling bovine.....	Up to 200	1	Up to 5	20 c.c.
Adult bovine, horse and mule	200–450	1·5	3·3–7·5	30 c.c.
Small dog.....	12	0·100	8·3	2·5 c.c.

The powdered tartar emetic is dissolved in water which has been raised to boiling point. After cooling to body temperature the solution is ready for injection.

Site of Inoculation.—The safest and easiest channel is the intravenous, the jugular in large animals and the saphena vein in dogs.

Method of Inoculation of Drug.—Having cast the patient, place a log under the neck at the upper third, and then thoroughly wash the seat of injection with brush, soap, and water. The vein is then made prominent by pressure with the finger of an assistant, and the needle of the syringe (which is kept aseptic in a dish of boiling water) is introduced into it. If successful, blood issues at once. The syringe, preferably of 50 c.c. capacity, containing the tartar emetic solution is next attached to the needle and the contents *slowly* injected into the vein. When the syringe is empty remove it, and again cause blood to flow from the needle. This has the effect of removing all traces of tartar emetic from the lumen of the needle, so that when it is withdrawn no material is deposited under the skin.

Effects of the Drug.—These may be conveniently classed under the following heads:—

(*a*) Immediate effect on patient.
(*b*) Local effects, including complications.
(*c*) Effect on the trypanosomes.
(*d*) General effect on patient.

(*a*) Immediate effect includes the symptoms, sometimes alarming, seen immediately after inoculation. They occur, however, in less than 1 per cent. of the injections made, and usually occur in the following order:—Hastened and shallow respiration, irregular breathing, rapid and feeble pulse, trembling of muscles, sweating, lowering of head, difficulty in progression, and in extreme cases collapse. Recovery as a rule takes place within 5–10 minutes.

(*b*) Local effects,* including complications. These depend chiefly on the escape of tartar emetic into the subcutaneous tissues during inoculation, although in many cases dirty needles are responsible. The swellings which result may be classed as follows:—

(1) Swellings which disappear in 3–4 days.

(2) Swellings which persist for months, leaving a thickening about the seat of inoculation.

(3) Swellings which develop into abscesses and require opening.

(4) Swellings associated with necrosis and phlebitis. If fresh solutions of the drug are employed, swellings are less likely to occur.

(*c*) Effect on the trypanosomes. Under this heading may be discussed (1) speed at which trypanosomes disappear from the peripheral circulation and (2) interval between disappearance and reappearance of trypanosomes. In regard to (1), observations made in nineteen cases showed that trypanosomes disappeared in from 10–85 minutes after injection. In one case where the drug was administered intramuscularly the parasites disappeared in 95 minutes. Concerning (2), trypanosomes were not observed in the blood circulation at intervals varying from 6 to 414 days.

(*d*) General effect on patient. Invariably marked improvement followed within a week after injection, but frequently the good effects were noticeable within two days. The improvement was shown chiefly as a marked invigoration of the system and a renewed desire to eat.

Number of Injections Recommended.—In some early cases one dose appeared sufficient, but as a routine measure five injections on consecutive days are advised. For a few animals that have survived, without treatment, one or more nagana seasons, as many as ten inoculations have proved ineffective. Temporary improvement of short duration sometimes followed, by a return of symptoms finally ended in death. *The most reliable guide as to whether treatment has been effective or not is the condition of the animal.* So long as there is improvement all is well; but reinoculations are necessary as soon as one of the many early symptoms are recognized.

It should be emphasized at this stage that tartar emetic appeared of little or no value in *T. brucei* infections, which are of a particularly serious nature in equines and canines. Cattle, however, in the vast majority of cases were infected with *T. congolense*, and it is the effects

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* Scars are often seen along the jugular furrow of inoculated animals, thus enabling one who is purchasing stock to judge whether an animal is likely to have suffered from nagana.

of tartar emetic on this parasite that are referred to in the preceding pages. *T. vivax*, of comparatively minor importance, appeared amenable to treatment in the same way as *T. congolense*.

Chart showing relation between cases of Nagana diagnosed in Lower Umfolosi Division and issue of Tartar Emetic to farmers in the same Division.

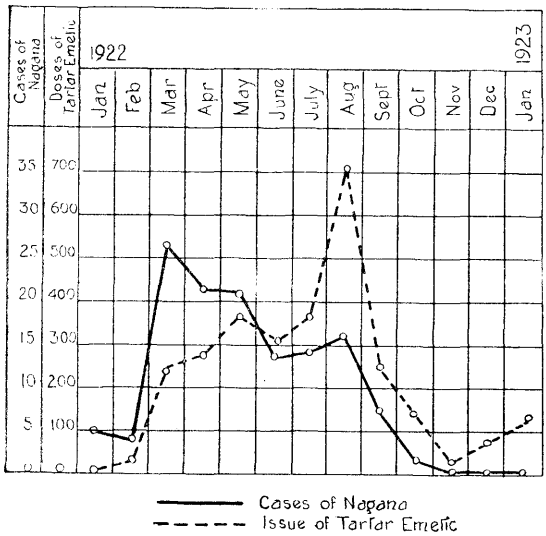

DIAGRAM 8.

Results of Tartar Emetic as Used by Farmers.—During the period October, 1921, to January, 1923, 4,078 doses of tartar emetic were issued gratis to farmers in Zululand, chiefly to settlers in the Ntambanana Settlement. It is estimated that the mortality among cattle as a direct result of this campaign was reduced from 95 per cent. to 20 per cent.

Results of Tartar Emetic as Employed at the Nagana Research Laboratory.—Not only tartar emetic but other agents were tried in the efforts made to combat nagana. As will be seen from the following tabular statement, the drug was given (*a*) alone, (*b*) preceding other medicinal agents, and (*c*) following other medicinal agents. Drugs, other than tartar emetic, were also used, but to a very small extent, as the results with tartar emetic alone were so satisfactory.