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Investigations into the Transmission of Horse-sickness at Onderstepoort during the Season 1932-1933.

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WE commenced our investigations into the transmission of horse-sickness during the summer of 1931-1932 at the Veterinary Laboratories of Onderstepoort, the reports of which have appeared in this Journal. It has been stressed in these papers that the known epidemiological facts all pointed to some mosquito species as the most probable vector of the disease and the mosquito survey, carried out in the environment of Onderstepoort concurrently with the actual transmission experiments, served to confirm this view and pointed particularly to some species of *Aëdes* as the most probable vector. Our experimental work was based principally on this theory.

In all 35 experiments were carried out in which 1,434 species of *Aëdes caballus*, *A. lineatopennis*, *A. hirsutus*, *A. dentatus*, *A. vittatus*, *A. punctothoracis*, *A. cumminsi*, *Culex theileri* and *Anopheles squamosus* were either injected into or fed on susceptible horses, at intervals of from ½ to 65 days after having fed on experimentally infected horses. Only 3 of these experiments were positive, viz., the first in which 5 *Culex theileri* were injected about ½ day after their original feed, the second in which 85 *Aëdes caballus*, 94 *A. lineatopennis* and 115 *A. hirsutus* were injected after 6 days and the third in which 68 *Aëdes caballus* and 64 *A. lineatopennis* were injected 7 days after having fed. The remaining experiments, including all those in which mosquitoes were fed on suceptible horses, were negative.

These results were not very promising and we were unable to demonstrate that any development or multiplication of the virus took place in the mosquitoes as it was apparently destroyed rapidly in these insects as a rule or only very exceptionally survived 7 days. The virus strains used for infecting the mosquitoes were not very

suitable as most of the experiments were carried out with O. virus which, being a strain that had been isolated 30 years previously and kept alive by direct animal passage, could not be considered as suitable in that it had not been through the natural vector during this period. The other strains at our disposal were all associated with a history of immunisation against O-virus so that we might possibly have been dealing with O-virus in relapse form throughout.

The final conclusion arrived at was, that, taking everything into consideration, *Aëdes* species were probably not the transmitters of horsesickness. This conclusion had to be qualified, however, on account of the nature of the virus strains used.

We continued our experimental work on horsesickness transmission during the summer 1932-1933, the results of which are given in this paper. One of us (R. du Toit) commenced the work in October, 1932, and was joined by the other (O. Nieschulz) from the end of December until the middle of April, 1933. The work was concluded at the end of May.

As this second series of experiments was equally unproductive of positive results, it has not been considered necessary to go into such detail as has been given to the previous work. Our entomological observations will be included in this paper and the experimental work will be added in the form of an appendix at the end of the paper.

I. SCHEME OF EXPERIMENTS.

In this new series of experiments our objective was first of all to ascertain to what extent our negative results with the *Aëdes* species were dependent upon the strains of virus used. With this object in view we therefore made use of fresh virus material, or at any rate early generations, obtained from spontaneous cases of horsesickness in which no history of any reaction set up by the O-virus could be traced. In order to exclude as much as possible any specific undesirable properties of a certain strain, which might deleteriously affect transmission experiments, some of the horses used for feeding mosquitoes on were infected with a large number of different strains at the same time. The *Aëdes* species used were mostly *A. caballus*, *A. lineatopennis* and *A. hirsutus*, which, in the light of our previous experience, were the most likely species. Besides these, *Aëdes argenteus* was made use of in some experiments, as, although a house frequenting species and as such not to be regarded as a transmitter of practical importance, its association with yellow fever and dengue, two human diseases which have much in common with horsesickness, made it a species which promised possible interesting results.

Mucidus mucidus was the next species, the transmission capacity of which had to be tested, as this rather large species lives in close association with *Aëdes caballus*, *A. lineatopennis* and also *A. hirsutus*, feeding upon their larvae. As we had been able to demonstrate towards the end of the previous season that it was a blood-sucking species it had to be looked upon as a potential transmitter of equal standing with the *Aëdes* species mentioned above.

Practically no experiments had been carried out with the Anophelines during the previous season. They had only been present in small numbers and our mosquito survey had not given us sufficient information to allow of us incorporating them amongst the probable transmitters. Their scarcity had undoubtedly been due to the adverse climatic conditions, but as there had also been very few cases of horse-sickness, there may have been some inter-relationship between these two facts. We therefore decided to pay particular attention to the Anophelines during this investigation.

Concurrently with the transmission experiments, a further entomological survey had to be carried out, both within the Onderstepoort area, but especially outside, taking into consideration at the same time insects other than mosquitoes as well. Work outside Onderstepoort was not possible however on account of a serious outbreak of foot and mouth disease within the Union which necessitated the absence of one of us (R. du Toit) during the months of February and March, the most important part of the season for our insect survey. The other, while carrying on the experimental work, was able to make a survey only in the environment of Onderstepoort.

II. CLIMATIC CONDITIONS DURING THE SEASON 1932-1933 AT ONDERSTEPOORT.

The rainfall recorded at the meteorological post at Onderstepoort for the period September, 1932, to April, 1933, is shown in Table 1.

The total amount of rain during this period was 14·07 inches; the maximum for one month was 3·545 inches in December and the minimum ·995 inches in February. During the previous summer the total amount was 20·84 inches which was well below the average, the maximum for one month being 5·07 inches in April.

During the 242 days under consideration there were only 44 days on which rail fell, viz., 2 in September, 4 in October, 10 in November, 9 in December, 6 in January, 4 in February, 6 in March and 3 in April. An amount of $\frac{1}{4}$ inch or more rain fell in 20 days, one inch or more rain per day only on 2 days, viz., 1·3 inches on December 30th and 1·06 inches on January 15th.

The rainfall recorded thus indicates an extremely dry season. From November until April, the season in which horsesickness occurs, the total amount of rain was 11·43 inches, as compared with 18·4 inches in 1931-32, which in itself was looked upon as a very dry year. During very wet summers three times this amount of rain is recorded, e.g. in 1917-18 an amount of 36·91 inches fell during the same months and in 1922-23 31·47 inches. According to the official report of the meteorological office at Pretoria the year under review was the dryest of the previous 29 years. The whole country was affected and the veld very much resembled the winter conditions.

TABLE 1.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.
	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.
1.....	—	0·12	0·03	—	—	—	0·32	—
2.....	—	—	—	—	—	—	—	0·185
3.....	—	0·88	—	—	—	—	0·90	—
4.....	—	0·58	0·06	0·50	—	—	—	0·04
5.....	—	—	—	—	—	—	0·102	—
6.....	—	—	—	—	—	—	—	—
7.....	—	—	0·72	—	—	—	0·06	—
8.....	—	—	—	—	—	—	0·42	—
9.....	—	—	—	—	—	—	—	—
10.....	—	—	0·22	—	—	—	—	—
11.....	—	—	—	—	—	—	—	—
12.....	—	—	—	0·065	—	—	—	—
13.....	—	—	—	0·09	—	—	—	—
14.....	—	—	—	0·56	0·21	—	—	—
15.....	—	—	—	—	1·06	—	0·34	—
16.....	—	—	—	0·35	0·13	—	—	—
17.....	—	—	—	—	0·03	—	—	—
18.....	—	—	—	—	0·08	—	—	0·16
19.....	—	—	0·28	—	—	0·29	—	—
20.....	0·87	—	0·18	0·12	0·03	0·035	—	—
21.....	—	—	—	—	—	0·01	—	—
22.....	0·01	—	0·40	—	—	—	—	—
23.....	—	—	0·04	—	—	—	—	—
24.....	—	—	—	0·54	—	—	—	—
25.....	—	—	0·24	—	—	—	—	—
26.....	—	—	—	1·30	—	—	—	—
27.....	—	—	—	—	—	—	—	—
28.....	—	0·18	—	—	—	0·36	—	—
29.....	—	—	—	—	—	—	—	—
30.....	—	—	0·37	0·02	—	—	—	—
31.....	—	—	—	—	—	—	—	—
Per month..	0·88	1·76	2·54	3·545	1·54	0·695	2·06	1·05

III. HORSESICKNESS DURING THE SUMMER 1932-33.

On account of the dryness of the season the opinion held generally was that little or no horsesickness could be expected to occur under field conditions and that certainly no severe outbreaks could be expected. The reverse, however, was the case, and during the latter part of March reports of several outbreaks were received, whereas in April the position became actually serious in most of those districts where the disease occurs habitually. Such also was the position at Onderstepoort where the laboratories alone lost 9 horses from horsesickness while 3 contracted horsesickness but recovered. Of these 14 horses which contracted the disease, 6 had been exposed day and night to natural infection on the farm Kaalplaats adjoining Onderstepoort, 2 had been used at Onderstepoort for transport purposes but were stabled at night while the remaining 6 horses were stabled in the laboratory stables from the time of their arrival at Onderstepoort which had been prior to the summer months of 1932-33. The latter 6 horses were allowed to run daily in kraals close to the

laboratories from 8 a.m. until shortly after 2 p.m. The stables in which they were kept, although not mosquito proof, were well closed at night and only rarely were mosquitoes found in them. It may be mentioned here that the horses in our experiments never left the stables day or night.

The first death from horsesickness occurred on March 15th, 1933, and the last on April, 28th. In the second half of March 4 horses died, in the first half of April 6 horses died and one animal showed clinical symptoms of horsesickness. In the latter half of April 2 more horses showed clinical symptoms of the disease but recovered.

We may assume under natural conditions horses become infected 10 to 14 days before death occurs. Most of the infections in the above-mentioned cases must have taken place during March or the early part of April. If we accept some mosquito or other insect species as the natural transmitter of horsesickness we must allow 1 to 2 weeks for an extrinsic incubation period of the virus in this insect, if we do not regard the transmission as purely mechanical. The vector must have taken up its infection and been present in sufficient numbers between the middle of February and the end of March, but especially the first half of March, therefore. If one now glances at Table 1, which shows the rainfall at Onderstepoort, it becomes apparent, that, although some rain fell during the first half of March, this, in the light of our previous years experience, could scarcely have been enough to permit of the breeding of adult insects, especially mosquitoes, and that they would therefore have bred out as a result of earlier rains. Furthermore, it seems unlikely that an insect after hatching will take its first blood meal from an infected horse and then engorge on a susceptible animal immediately after the extrinsic incubation period of the virus has been completed. The rains in February would therefore seem to be of principle importance, but this month was exceptionally dry as rain fell on only 4 days with a total rainfall for the month of 0·695 inches. From January 21st until February 18th no rain had fallen at all which indicates that the subsequent rain that fell, under South African conditions, would have been absorbed by the ground almost immediately, leaving practicall no excess water for breeding places. From the above considerations it is clear therefore that the inter-relationship between rainfall and the occurrence of horsesickness, if it should exist, can be quite complicated.

The main conclusions to be arrived at from the occurrence of horsesickness during this season is that there does not necessarily exist a relation between the amount of rain and the extent of the outbreaks of the disease, as severe outbreaks of horsesickness may occur in exceptionally dry seasons as well.

A further consideration is that a number of stabled animals contracted the disease. Stabling of horses at night has always been regarded as a good prophylactic measure in itself as it is believed that infection almost invariably takes place in the open at night or round about sunset or sunrise. *In the above-mentioned cases stabling was not a sufficient means of protection,* so that the infection must have taken place either in the stables at night or outside in the kraals

during the daytime. It may be mentioned here that none of our own susceptible experimental horses, which were stabled in the same stables, but during the day as well, contracted the disease spontaneously. *The possibility exists, therefore, that the horses became infected in the kraals during the day.*

Two important epidemiological facts, on which our experimental work had largely to be based, viz., the relationship between horsesickness and rainfall and the protection afforded by stabling at night, proved to be unreliable. These conclusions were only arrived at at the end of the season however and could therefore not be made of for the experiments under review.

IV. RESULT OF A MOSQUITO SURVEY AT ONDERSTEPOORT.

By making use of the time available between the experiments a mosquito survey was carried out concurrently with the experimental work, but, for reasons already referred to, this had to be limited to the environment of Onderstepoort.

In a previous paper (*Nieschulz, Bedford* and *du Toit*, 1934), the results of a mosquito survey of the same area during the season 1931-32 have been described in full. For a clearer understanding of the character of the area and the situation of the various breeding places the reader is referred to this paper.

1. Species of Mosquitoes found at Onderstepoort.

During the previous season 24 species of mosquitoes were found, most of which were present this summer as well. Only two additional species were found, viz., *Anopheles gambiae* and *A. rufipes* which have however previously been recorded from Onderstepoort (c.f. *Bedford*, 1918).

2. The Use of Mosquito Traps during the Season 1932-33.

Throughout the previous summer two mosquito traps, consisting of tents of thin mosquito hessian protected from the weather by a " fly " of canvas and containing horses as bait in portable wooden horseboxes, had been used nightly between 5 p.m. and 7 a.m. in order to obtain an indication of the mosquito species present. The mosquitoes entered the trap through a vertical slit in the hessian at one end and those engorged specimens which were found resting against the sides and roof of the tent were caught the following morning. These traps however failed to give an accurate indication of the relative abundance of the various mosquito species as they tended to select those species which showed a tendency towards entering stables or rooms at night and to exclude the typical field species which were present in large numbers in the vicinity. We did not expect to obtain much data of value by the use of these traps therefore but on account of the unusual nature of the season wished to collect as much information as possible. Only one trap was erected, which we placed towards the east of the laboratory close to the bank of the Aapies river under some trees at the edge of an area of bushveld.

Between March 17th and April 19th, 1933, 107 specimens of mosquitoes were caught. These mosquitoes belonged to the following 16 species:—

	Specimens.
1. *Aëdes caballus*	1
2. *Aëdes dentatus*	41
3. *Aëdes hirsutus*	1
4. *Aëdes lineatopennis*	4
5. *Aëdes nigeriensis*	2
6. *Aëdes salisburiensis*	1
7. *Anopheles cinereus*	1
8. *Anopheles gambiae*	1
9. *Anopheles mauritianus*	3
10. *Anopheles squamosus*	3
11. *Culex annulioris*	2
12. *Culex decens*	3
13. *Culex fatigans*	1
14. *Culex theileri*	41
15. *Culex univittatus*	1
16. *Lutzia tigripes*	1

The previous season had also yielded 16 species from the traps, and the following 13 species were common to both seasons: *Aëdes caballus, A. dentatus, A. hirsutus, A. lineatopennis, Anopheles cinereus, A. mauritianus, A. squamosus, Culex annulioris, C. decens, C. fatigans, C. theileri, C. univittatus* and *Lutzia tigripes. Aëdes powei, A. punctothoracis* and *Taeniorhynchus africanus* were collected only in 1931-32, whereas *Anopheles gambiae, Aëdes nigeriensis* and *A. salisburiensis* were found only in 1933. The species not common to both seasons were present only in small numbers.

The catch for the summer of 1931-32 may be divided up into 21 specimens or 1·7 per cent. *Anopheles*, 683 or 55·9 per cent. *Aëdes*, 517 or 42·2 per cent. *Culex* and 3 or ·2 per cent *Taeniorhynchus* and *Lutzia*. The 1933 season's catch was made up as follows, 8 specimens or 7·5 per cent. *Anopheles*, 50 or 46·7 per cent. *Aedes*, 48 or 44·8 per cent. *Culex* and 1 or 1 per cent. *Lutzia*. *Aëdes* and *Culex* were about equal in numbers for both these seasons and they formed the majority of mosquitoes caught. *Anopheles* were emparitively rare but were somewhat more plentiful in 1933, reaching 7·5 per cent. of the total catch.

The commonest species in both seasons were *Aëdes dentatus* and *Culex theileri* whereas *Aëdes lineatopennis* were caught in relatively large numbers only in 1931-32.

Except, therefore, for a slight increase in the number of Anopheles *the same information was obtained from our traps as in* 1931-32.

3. Survey of the Breeding Places.

In 1931-32 a thorough survey of the breeding places around Onderstepoort was carried out and the experience obtained proved to be of much help to us this year.

(*a*) *Breeding Places of the Field Species of* Aëdes *and of* Mucidus.

Under the term field species we include those species of *Aëdes* which have their breeding grounds in the more or less open bushveld, not in the vicinity of human habitations or stables. This group comprises the common species, *A. caballus*, *A. lineatopennis*, *A. hirsutus*, *A. dentatus* and a number of relatively rare species, e.g. *A. durbanensis*, *A. nigeriensis*, *A. poweri*, *A. punctothoracis* and *A. salisburiensis*.

Mucidus mucidus may be included in the same biological group as it makes use of the same breeding grounds as *Aëdes caballus*, *A. lineatopennis* and *A. hirsutus* and feeds upon the larvae of these mosquitoes in its larval stage.

The observations made the previous year on the breeding grounds and breeding habits of these species could be confirmed in full and a detailed description is therefore unnecessary. The same breeding places were made use of everywhere except that on account of the limited rainfall there was less chance of the larvae hatching out and completing their development. If we had had to depend on natural breeding conditions, it would have been impossible to obtain sufficient material for our experiments.

The previous season we had found that, by artificially flooding the breeding grounds of certain *Aëdes* species enormous numbers of larvae developed and we made use of this method to great advantage in the case of one particular breeding ground of *Aëdes caballus* and *A. lineatopennis* (marked on the map in a previous article as Nos. 20-21) which had been connected by means of a long ditch to the agricultural irrigation system of Onderstepoort. It may be mentioned that this breeding place had been flooded for the first time this season early in October, long before any adult *Aëdes* had appeared, with the result that large numbers of larvae developed immediately. This would indicate that the eggs must have lain there ready to hatch throughout the whole of the winter.

As was the case during the previous season almost all the larvae were *Aëdes caballus* and *A. lineatopennis*. *A. hrisutus* was also present in small numbers, mostly at the beginning of the season, and later on fair numbers of *Aëdes dentatus* made their appearance in a part of the breeding ground which was shaded by trees. Other *Aëdes* species appeared from time to time but only in small numbers, e.g. *A. nigeriensis*, whereas the larvae of *Mucidus mucidus* appeared after every flooding and became quite numerous towards the middle of the season.

(*b*) *Breeding Places of* Aëdes argenteus.

During the previous year a few larvae only of *Aëdes argenteus* had been found in concrete pig troughs near the stables. This year, in March, we found a very good breeding place situated at the side

of a railway embankment near a bridge crossing the Aapies River. The place occupied a position between temporary houses erected for native railway labourers.

(c) *Breeding Places of Anophelines.*

Anopheline larvae had been very scarce the previous year and only a few larvae of *Anopheles squamosus* and *A. pretoriensis* had been found in backwaters of the Aapies River. It must be mentioned, however, that no really extensive search had been made.

This season we paid special attention to the Anophelines. To start with, our routine examinations of all possible breeding places, including those formed after rain, met with no success. Towards the middle of January, however, an extremely dry part of the season, fair numbers of *Anopheles pretoriensis* were found quite unexpectedly in the Aapies River, which runs close to the edges of the irrigated lands of the Laboratories. Most of the larvae were hidden under stones towards the edges of a small backwater just below a concrete weir (Fig. 1). A few days later a fall of rain caused the river to rise with the result that all the larvae were washed away.

A thorough search for larvae was made almost daily but only towards the end of February were we successful in finding any more larvae, this time near the Bon Accord dam. During March and April larvae could be collected in sufficient numbers for our requirements.

On account of the dry season the normal pools and pans in the neighbourhood of Onderstepoort were dry and larval development was limited, therefore, to the following localities: the irrigation channels of the Laboratories, the Aapies River, the Bon-Accord Dam, the continuation of the river beyond the dam and finally a marshy pool formed at the foot of the new native location by leakage from a water pipe (Figs. 2 and 3).

The irrigation channels contained water during the greater portion of the season, but only *Culex* and *Lutzia* larvae were found from time to time.

The Aapies River contained very little water this season and for the greater part what water there was, was mostly underground. Only after rain had fallen in Pretoria, did the river assume its normal aspect for a time and then no larvae were found in the small pools and animal hoof prints along its edges. In the few pools remaining during the drought in the river bed itself larvae were encountered, however, only in those pools which contained large numbers of loose stones along the edges (Fig. 4). Between these stones small pools of clear water with practically no current were found to harbour fair numbers of larvae which could be easily caught when the stones were removed.

Most of the larvae collected were either *Anopheles pretoriensis* or *A. gambiae,* with occasionally a few *A. squamosus.* For the successful development of these Anophelines either a very wet or a very dry season appears to be necessary. Rain of medium amounts

does not create breeding places by allowing the river to rise above its normal level and thereby form pools when it again subsides, but instead destroys the breeding places in the river bed by means of the increased current produced and the varying levels of the water.

The presence of relatively large numbers of *Anopheles gambiae*, one of the two most important malaria carriers of the Union, was unexpected. This species normally breeds only after heavy rains in small pools, animal hoof prints, etc., alongside river beds or roads, in fact, in pools which generally only remain wet long enough to allow of the development of the larvae.

In the Bon-Accord Dam no larvae were found either in the open stretches or in places covered by reeds. A few larvae were found in a furrow overgrown with reeds and grass near the dam. Nomally a small steam of water flows in this furrow but on account of the falling in the water level only a little stationary water was left. Larvae were never numerous in this furrow.

Good breeding places, similar to those found higher up in the Aapies River, were found in the continuation of the river below the Bon-Accord Dam, especially during April and the first portion of May. Larvae were, furthermore, found in pools, caused by seepage from the dam, which were largely overgrown by grass and reeds and which contained slow running water. These larvae were also for the most part *Anopheles gambiae* and *A. pretoriensis*.

The last breeding place found was situated at the foot of the new native compound in a stream which was quite dry at the time but which, in normal seasons, contains a small amount of running water which empties into the Bon-Accord Dam. The stream is crossed by a water pipe at this place and by leakage from this pipe and the wash water from a tap close by a marshy pool largely covered by grass had been formed. Between the grass stems anopheline larvae were found in fair numbers during March and April, most of the larvae being those of *Anopheles rufipes* and *A. mauritianus*. *Anopheles pretoriensis* and *A. squamosus* were present in small numbers whereas *A. gambiae* was absent. Besides these anophelines, *Culex theileri* and *C. annulioris* were present in fair numbers.

As was the case with the breeding places in the Aapies River this site was suitable as a breeding ground only during dry weather as even a moderate rain would be sufficient to wash away all the larvae present.

(d) Blood-sucking Insects other than Mosquitoes.

From time to time a horse was taken out into the veld surrounding Onderstepoort or to the farm Kaalplaas where it was used as a bait animal to attract any blood-sucking insects which might be present.

In and near the stables as well as in the veld *Stomoxys calcitrans* was found but never in very large numbers and frequently it could be regarded as relatively rare. Species of *Tabanus* and *Haematopota* were present in the veld, especially under the trees near the Aapies River, but never in anything but small numbers.

Musca crassirostris, a true haematophagous species of this genus, was present in fair numbers and was at least as common as *Stomoxys* during March and April on horses at Onderstepoort and Kaalplaas. It only occasionally entered stables and generally attacked the animals in the open veld or kraals, showing a definite predilection for the belly and legs of horses. It feeds throughout the day up until sunset (c.f. *du Toit* and *Nieschulz*, 1933).

At Kaalplaas, *Bdellolarynx uniseriatus*, a small haematophagous species belonging to the *Stomoxidinae*, appeared to be quite common in March and April. It attacked our horses and mules in relatively large numbers just before and after sunset, biting by preference on the belly and legs (c.f. *Nieschulz* and *du Toit*, 1933).

No other blood-sucking insects were observed to attack the bait animal while daylight lasted. On two occasions we took a horse out in the late afternoon to Kaalplaas and carefully noted every insect which settled on the animal up until some hours after sunset by the help of electric torches. Round about sunset *Bdellolarynx uniseriatus* was very common but after dark only very few *Culex theileri*, *Aëdes caballus* and *Anopheles squamosus* were collected. No information of importance in relation to the natural transmission of horsesickness could be gathered during these excursions at night.

(*e*) *Summary of the Insect Survey.*

In a tent-like mosquito trap in which a horse was used as a bait animal, 16 species of mosquitoes of the genera *Aëdes*, *Anopheles* and *Culex* were collected. These species were for the most part the same as those obtained the previous year, but the percentage of Anophelines showed a slight increase.

Owing to the limited rainfall suitable breeding places for mosquitoes were scarce. The results obtained during the previous year regarding the breeding habits of *Aëdes caballus*, *A. dentatus*, *A. hirsutus* and *A. lineatopennis* could be confirmed. Temporary water only was used by these species and artificial flooding of suitable places gave good results.

Anopheline larvae were present in fair numbers during March and April and were found in small pools in the bed of the Aapies river when it was at its lowest level and also in a small marshy area formed by leakage from a water pipe. These breeding places were only present during the driest parts of the season as even a moderate amount of rain washed all larvae away. Most of the larvae collected belonged to *Anopheles gambiae*, *A. pretoriensis*, *A. rufipes* and *A. mauritianus*. According to our observations, Anophelines appear to find suitable breeding conditions only in very wet seasons or else during very dry weather.

If we exclude the commoner species of *Culex*, Anophelines were the only mosquitoes present in large numbers during and shortly before the outbreaks of horsesickness. At Onderstepoort, at any rate, their breeding was not prevented by the low rainfall. They have to be regarded therefore as possible natural transmitters of horsesickness, as during the season in question they were the only

species, the appearance of which showed some relation to the appearance of the disease. Apart from mosquitoes *Tabanids*, *Stomoxys calcitrans*, *Musca crassirostris* and *Bdellolarynx uniseriatus* were the only other blood-sucking insects observed at Onderstepoort. All of these feed only during daylight up to shortly after sunset and some of them are especially plentiful at this time. They can only be regarded as possible vectors, however, if we assume that the transmission of the disease takes place during the day.

V. EXPERIMENTAL TECHNIQUE.

During the previous season a method of feeding mosquitoes on horses and keeping these mosquitoes alive under South African climatic conditions had been worked out and described in detail (Nieschulz and du Toit, 1934) so that a further description is necessary here. The results had been satisfactory and the same technique, except for minor details, was adopted during this work.

During the previous winter we had used a room for housing the mosquitoes which was electrically heated. The heat regulation had to be done by hand which was unsatisfactory and large numbers of mosquitoes were frequently lost due to the temperature rising too high, especially at night. One of us had in the meantime devised a control mechanism whereby both temperature and humidity were controlled automatically at any desired degree. The technical construction of this control will be described in another paper. This apparatus worked extremely well, but the practical results were not as good as we had hoped due principally to the fact that we did not know the exact temperature and expecially the humidity requirements of mosquitoes. It appeared, furthermore, that the different species had different optima of temperature and humidity. Further research in this direction will be necessary to overcome these difficulties.

In the type of cage used during the previous year and described in the abovementioned paper, the humidity varied in the different parts of the cage, which afforded the mosquitoes a limited choice of humidity at any rate. When the exact climatic requirements of a species are not known, it would appear that this type of cage is to be preferred, at any rate during summer, when no special degree of temperature has to be provided for.

As was the case last season *Aëdes caballus* was the most difficult species to keep alive for long periods in captivity, whereas *A. lineatopennis* and *A. dentatus* gave little difficulty. *Aëdes argenteus* proved to be very resistant to artificial conditions. In the case of *Mucidus mucidus* the results were very satisfactory as also was the case with the different species of *Anopheles*. There was naturally always mortality especially when the mosquitoes had to be kept alive for long periods but a sufficient number of specimens, which had fed on virus horses, remained alive long enough to enable us to carry out long interval experiments.

VI. STRAINS OF VIRUS AND EXPERIMENTAL ANIMALS.

Last season most of our experiments had been carried out with the O-virus strain, which for reasons already mentioned, could not be regarded as entirely suitable for transmission experiments. This year we used spontaneous strains as far as possible, where no connection with O-virus could be traced. Spontaneous cases were used on which mosquitoes were fed or horses were injected with early generations of preserved material. In certain experiments the pooled blood of a number of different strains was used in order to exclude any undesirable properties which one or other strain might possess. In one case the virus had persisted in mosquitoes for 7 days and in another had been passed through mouse brain. In all the following 9 strains were used:—

1. *Strain* 16989Y.—Second generation of 7.4.1925 for virus horse 4, 3rd generation for virus horse 6 and 4th generation (after one passage through mosquitoes) for virus horse 8.

2. *Strain* 18076.—Second generation 10.8.1926 for virus horse 4, 3rd generation for virus horse 6 and 4th generation (after one passage through mosquitoes) for virus horse 8.

3. *Strain* 18091.—Second generation of 10.8.1926 for virus horse 4, 3rd generation for virus horse 6 and 4th generation (after one passage through mosquitoes) for virus horse 8.

4. *Strain* 18384 W.C. 11.—Second generation of 9.2.1927 for virus horse 4, 3rd generation for virus horse 6 and 4th generation (after one passage through mosquitoes) for virus horse 8.

5. Spontaneous case 19386 of 22.3.1929 for virus horse 4, 1st generation for virus horse 6 and 2nd generation (after one passage through mosquitoes) for virus horse 8.

6. *Strain* 20376.—First generation of 19.5.1932 for virus horses 1, 3 and 4, 2nd generation (after one passage through mosquitoes) for virus horse 8.

7. Spontaneous case 20536 of 14.3.1933 for virus horse 5, 1st generation for virus horse 6 and 2nd generation (after one passage through mosquitoes) for virus horse 8.

8. Spontaneous case 20201 of 21.4.1933 for virus horse 7.

9. *Mouse brain virus.*—November 1932 (see below) for virus horse 2.

With these strains the following 8 horses were infected and used for feeding mosquitoes on:—

Virus horse 1 (20449).—Injected on October 14th, 1932, intrajugularly with 5 c.c. preserved blood of horse 20376, a spontaneous case of horsesickness which had occurred at Kaalplaas in May, 1932.

Result.—Up to October 21st no reaction resulted and the temperature did not exceed 100·2° during these 7 days.

On October 21st the same horse was injected with 5 c.c. preserved blood of horse 20329 which had been infected with the original material of the same strain (Virus strain 6).

Result.—Temperature normal up to October 24th. On 25th (a.m.) 102°, the 26th 103·2° and 104°, the 27th 103° and 103·4°, the 28th 103° and 105°. The horse died during the night.

Virus horse 2 (20337).—Injected on 22nd November, 1932, with brain emulsion of a mouse (generation 5, strain 20449) infected with horsesickness.

Result.—The temperature rose on the 6th day, November 28th p.m. 101·8°, the 29th a.m. 100, p.m. 101·6°, the 30th 100·6° and 103°, the 1st December 101° and 102·2°, the 2nd 100° and 103°, the 3rd 100·6° and 102·2°. Thereafter the temperature returned to normal where it remained until January, 1933, when the horse was injected with 5 c.c. virulent blood (O-virus) to which it succumbed. On 1st December, 1932, blood from this horse, when injected into mice intracerebrally, failed to infect. It is doubtful whether sufficient virus existed in the blood of this animal to infect the mosquitoes. (Virus strain 9.)

Virus horse 3 (20448).—Injected on January 5th, 1933, intrajugularly with 5 c.c. blood collected on May 19th, 1932, from horse 20329, the first generation of the same spontaneous strain as was used for infecting virus horse 1. (Virus strain 6.)

Result.—The temperature rose on the 4th day, December 9th, to 101·4° and 102·4°, the 10th 101·4° and 102·6°, the 11th 101·6° and 104°, the 12th 103·6° and 104°, the 13th 103° and 106·2° and the 14th 103° and 100·4°. The horse died during the night.

Virus horse 4 (20464).—Injected intrajugularly on January 23rd with 5 c.c. blood from horse 18076 (spontaneous strain 2nd generation of August 10th, 1926), 5 c.c. blood of horse 18091 (spontaneous strain 2nd generation of August 10th 1926), 5 c.c. blood of horse 16989Y (2nd generation of April 7th, 1925), 5 c.c. blood of horse 18384 W.C. 11 (2nd generation of February 9th, 1927), 5 c.c. blood of horse 19386 (spontaneous case, original material of March 22nd, 1929), and 5 c.c. blood from horse 20329 (a spontaneous strain, 1st generation of May 19th, 1932). The material contained the pooled preserved blood of 6 different cases of horsesickness, all original material or early generations of spontaneous strains. Virus strains 1 to 6.

Result.—Temperature normal up to January 26th. The 27th 99° and 103·2°, the 28th 100° and 105·2°, the 29th (a.m.) 102·8° and the 30th (a.m.) 104·2°. The horse died the same day.

Virus horse 5 (205363 had been exposed at Kaalplaas to natural infection in the " East Coast Fever " camp. It was brought back to Onderstepoort showing slight clinical symptoms of horsesickness and died 4 days later. Virus strain 7.

The temperature 104·6° on March 14th, the 15th 102·2° and 105°, the 16th 103·2° and 104·2°, the 17th 102° and 106° and the 18th 103·8° and 107°. The horse died during the night.

Virus horse 6 (20577).—Injected on March 31st intrajugularly with 2 c.c. blood from horse 20536 (a spontaneous case of horsesickness, virus horse 5) and 2 c.c. blood from horse 20464 (virus horse 4, which had been injected with a number of spontaneous strains of the first and second generations). Virus strains 1 to 7.

Result.—The temperature remained normal for 3 days. On April 4th (p.m.) it rose to 101·6°, the 5th 101° and 102·6, the 6th 100·4° and 102·8°, the 7th 102·2° and 103·4°, the 8th 102·6° and 104·2° and the 9th (a.m.) 103·8°. The horse died during the day.

Virus horse 7 (20201) came in from Kaalplaas on April 21st showing symptoms of dikkop. No temperatures were taken upon admission. Mosquitoes were fed during the night but it is doubtful whether they ingested infected blood as the temperature taken at the time that the mosquitoes were put on was normal. The horse recovered. Virus strain 8.

Virus horse 8 (20539) became infected by the injection of 8 *Anopheles gambiae*, 8 *A. mauritianus* and 7 *A. pretoriensis* which had fed 7 days before on virus horse 6 (see experiment 24). Virus strains 1 to 7.

The horses used in our experiments consisted of animals of little commercial value, recruited principally from the large towns. They were kept in stables day and night throughout the course of the experiments and were never allowed out. The stables were rendered more or less insect proof by means of wire gauze except the doors which were opened during the daytime but closed at night. At night, at any rate, these stables were thus fairly safe, but not absolutely mosquito-proof. The degree of protection seemed to be sufficient as no spontaneous cases of horsesickness occurred amongst our experimental animals during this or the previous season.

As to the susceptibility or immunity of these horses to horsesickness no guarantees could be obtained as their histories were not known. The general experience at Onderstepoort is, however, that only very occasionally is an immune animal encountered and this state of affairs was confirmed by our experience. All animals infected experimentally by means of virus containing blood proved to be susceptible. Whereas during the previous season most of the horses were tested for immunity, this season only very few were subjected to such immunity tests on account of the expense involved and on account of the fact that all the tests of the previous season had been positive.

VII. DISCUSSION OF THE EXPERIMENTAL WORK.

The actual experimental work, which appears as an appendix at the end of this paper, has been grouped into 5 sections according to the species of mosquitoes used and a small sixth section is added which deals with two experiments wherein ticks were utilised. Each section deals systematically with the particular virus horse used, the strains of virus and the histories of the mosquito groups.

1. Discussion of the Results obtained with the Field Species of Aëdes.

Aëdes caballus, A. lineatopennis, A. hirsutus, A. dentatus and *A. nigeriensis,* the typical *Aëdes* species of the Transvaal bushveld were used in 11 experiments but in no case was horsesickness transmitted.

As the source of virus for infecting the mosquitoes 5 horses (virus horses 1-3 and 5 and 6) and the stains 6 to 9 were used. Two of these horses (1 and 3) were injected with the original material or the first generation of a spontaneous case of horsesickness which had occurred in May, 1932, at Kaalplaas (strain 6). Two horses (5 and 6) were cases of spontaneous infection also contracted at Kaalplaas. One of these horses was used for feeding mosquitoes during the 4 days of fever prior to its death (strain 7) and the other, which recovered, shortly after the actual fever reaction (strain 8). The remaining horse (2) was infected by means of a mouse brain strain of virus (strain 9).

In all 454 specimens of the different *Aëdes* species were emulsified and injected after intervals of from 7 to 39 days and 274 specimens refed on susceptible horses after intervals of from 5 to 20 days.

Twenty-nine specimens were injected after 7-9, 15 after 16-17 and 410 after 31-39 days, viz:—

Twenty-one *A. cabullus* after 7 days, 27 *A. lineatopennis,* 15 after 16-17 and 12 after 38-39 days, 398 *A. caballus* and *A. lineatopennis* combined, 50 after 31 days, and 348 after 36-39 days and 8 *A. nigeriensis* after 7-9 days.

Of the 274 specimens used in the feeding experiments 88 refed after 5-9 days, 90 after 11-14 days and 96 after 15-20 days, viz., 137 *A. caballus,* 72 after 5-9 days, 37 after 11-14 days and 28 after 15-20 days, 28 *A. lineatoppenis,* 5 after 11 days and 23 after 16-18 days, 5 *A. caballus* and *A. lineatopennis* combined after 7 days, 88 *A. hirsutus,* 48 after 11 days and 40 after 15-16 days and 16 *A. dentatus,* 11 after 7-9 days and 5 after 17-19 days.

Most of these mosquitoes had fed during the fever reactions of spontaneous cases or cases produced by injections of the first to second generation of spontaneous infections. Five *A. caballus* and *A. lineatopennis* (mixed) had ingested infected blood of a strain of virus fixed for mice and had refed 7 days later. On the spontaneous case of dikkop, which did not show any temperature reaction when used for the feeding of mosquitoes, 46 *A. lineatopennis* had fed which either refed after 17-18 days (19 specimens) or were injected after 16-17 (15 specimens) and 38-39 days (12 specimens). If we exclude these mosquitoes and also those which refed within the first 14 days

of having fed on an infected horse, there remain 427 specimens, which were injected after 7-39 days and 77 which refed after 15-20 days. Arranged according to species this is:—

Injected: 21 *A. caballus* after 7 days.
398 *A. caballus* and *A. lineatopennis* mixed, 50 after 31 and 348 after 36-39 days.
8 *A. nigeriensis* after 7-9 days.

Refed: 28 *A. caballus* after 15-20 days.
4 *A. lineatopennis* after 16 days.
40 *A. hirsutus* after 15-16 days.
5 *A. dentatus* after 17-19 days.

The numbers of *A. caballus*, *A. lineatopennis* and *A. hirsutus* used in these experiments appears to be sufficiently great to make the negative results obtained significant.

2. Discussion of the Results obtained with Aëdes argenteus.

Aëdes argenteus was used in 4 experiments. Fifty specimens were injected, 10 after 7-8 days and 40 after 27-30 days of having fed on infected horses. Both experiments were negative.

Fifty-five specimens were refed on susceptible horses after intervals of from 10-23 days, viz., 12 specimens after 10-12 days, 2 after 14-16 days, 38 after 16-19 days and 3 after 21-23 days. These experiments were equally unproductive of positive results.

The mosquitoes had fed, during the fever reactions, on horses infected spontaneously (virus horse 5, strain 7) or injected experimentally with a mixture of preserved blood from spontaneous cases or first to second generations (virus horse 6, strains 1-7). The virus strains used were, therefore, undoubtedly suitable.

If we exclude those mosquitoes which refed on the susceptible horses within the first 14 days after having fed on the virus horse, there remain 50 specimens which were injected after 7-30 days, and 43 specimens which refed after 14-23 days. The number of mosquitoes used was again large enough to make the negative results obtained significant.

In the light of these results *Aëdes argenteus* is, therefore, not a suitable transmitter of horsesickness. There is no development or multiplication of the virus in this mosquito species and the virus is quickly destroyed.

3. Discussion of the Results obtained with *Mucidus mucidus*.

Mucidus mucidus had to be looked upon as a promising transmitter for reasons discussed previously. We were able to obtain, from the experimentally flooded breeding grounds of *Aëdes caballus* and *A. lineatopennis*, a few small and one large batch of *Mucidus* and were very successful in inducing this species to feed on infected horses.

In all 5 experiments were carried out with this species, using strains 1-7 and 9, in which 16 specimens were injected and 26 refed on normal horses 5-24 days after their infective meal. Five specimens were injected 5 days after having fed, 10 after 10 days and 1 after 20-24 days. One specimen refed after 7 days, another (doubtful) after 11 days and again after 15 days and 25 after an interval of 15-22 days. In none of these experiments did infection result.

In the more important experiments with this species virus horse 4 was used during the 3rd day of its fever reaction. This horse had been infected with a number of different strains, original material or early generations (strains 1-6). One specimen had fed on virus horse 3 [infected with the 1st generation of a spontaneous strain (strain 6)], and the last specimen on virus horse 2 [infected with a strain fixed for mice (strain 8)]. If we, therefore, exclude this specimen and those which fed within the first 14 days of their original feeding there remain 16 specimens which were injected after 5-24 days, and 25 specimens which refed after 15-22 days.

The negative results in these experiments may be looked upon as proving that *Mucidus mucidus* is not a suitable vector of horse-sickness.

4. Discussion of the Results obtained with *Culex annulioris*.

Culex annulioris is not a very common species at Onderstepoort and only relatively small numbers were available. They were found breeding together with certain anophelines which were regarded as potential transmitters. Two experiments were carried out in which 4 specimens were refed after 19 days and 3 specimens injected after a period of 30 days. Virus horse 6 was used which had been infected with strains 1-7.

On account of the very limited amount of material used no definite conclusions could be arrived at.

5. Discussion of the Results obtained with Anopheles Species.

During the season under review 5 species of *Anopheles*, viz., *A. gambiae*, *A. mauritianus*, *A. pretoriensis*, *A. rufipes* and *A. squamosus*, were obtained in sufficient numbers from breeding places described in the section dealing with the insect survey. At least three of these species *A. gambiae*, *A. pretoriensis* and *A. rufipes*, could be regarded as potential carriers and as the most promising species amongst the anophelines.

In all 9 experiments were carried out in which 326 mosquitoes were either refed or injected into susceptible horses at intervals varying between 5 and 54 days after their infective meal.

All the mosquitoes were reared from larvae and the technique which had previously been worked out for *Aëdes* species could be applied with equally satisfactory results to *Anopheles* as well. In order to limit the number of horses required for these experiments

the different species were fed on or injected into the same horse together in most cases. In the event of positive results being obtained it was the intention then to carry out further experiments with the single species only.

Virus horses 6 to 8 were used and virus strains 1 to 8. One of these virus horses had been experimentally infected with early generations of a large number of different strains (virus horse 6, strains 1-7), another with the same strains after one passage through mosquitoes (experiment 24), and the remaining one was a spontaneous case of horsesickness ending in recovery (virus horse 7, strain 8).

Only one of these experiments was positive, viz., 8 *A. gambiae*, 8 *A. Mauritianus* and 7 *A. pretoriensis* were emulsified and injected 7 days after having fed on a virus horse. The incubation period was 18 days and the infection resulted in death. The other experiments were all negative.

An analysis of the negative results is interesting, viz., 45 specimens belonging to *A. gambiae*, *A. mauritianus*, *A. pretoriensis* and *A. rufipens* were injected, 9 after 5 days, 13 after 27-28, 9 after 39 and 15 after 52-54 days. The intervals for the different species were :—

4 *A. gambiae*, 1 specimen after 27-28, 1 after 39 and 2 after 52-54 days; 5 *A mauritanus*, 1 specimen after 39 and 4 after 52-54 days; 19 *A. pretoriensis*, 9 specimens after 5, 3 after 27-28, 6 after 39 and 1 after 52-54 days; 18 *A.rufipens*, 9 specimens after 27-28, 1 after 39 and 8 after 52-54 days.

In the experiments in which transmission was attempted by feeding infected mosquitoes 6 horses were used on which 257 specimens of *A. gambiae*, *A. mauritianus*, *A. rufipes* and *A squamosus* were refed, viz., 21 specimens after 7-8 days, 57 after 11-13, 98 after 15-18, 39 after 24-29 and 42 after 32-36 days. The intervals for the different species were : 36 *A. gambiae*, 4 specimens after 7-8, 20 after 17-18, 7 after 24-29 and 5 after 35-36 days; 60 *A. mauritianus*, 1 specimen after 7-8, 33 specimens after 15-18, 4 after 24-28 and 22 after 32-35 days; 70 *A. pretoriensis*, 5 specimens after 7-8, 23 after 11-13, 23 after 17-18, 14 after 24-29 and 5 after 35-36 days; 76 *A. rufipes*, 10 specimens after 7-8, 33 after 11-13, 12 after 17-18, 12 after 24-29 and 9 after 35-36 days; 15 *A. squamosus*, 1 specimen after 7-8, 1 after 12-13, 10 after 17-18, 2 after 28 and 1 after 35 days.

The one positive result obtained by the injection of emulsified mosquitoes shows that the virus is able to remain alive in Anopheles for 7 days. This is, however, not regularly the case.

The *Anopheles* species used in our experiments cannot be regarded as possible natural transmitters as all the injection and feeding experiments, conducted after intervals which are considered as quite adequate, were negative.

In one experiment (23) 9 specimens, which had fed on a horse during a fever reaction which later appeared as probably not having been caused by horsesickness, were injected. In two experiments (29 and 31) specimens were refed and 37 injected. These mosquitoes

had had their original feed on virus horse 7, a spontaneous case of dikkop which ended in recovery, but only after the temperature had returned to normal. It is quite possible therefore that these mosquitoes did not ingest active virus. If we therefore exclude these mosquitoes and further, to be quite on the safe side, those also which refed on susceptible horses within a period of less than 14 days after their original feed, the negative results obtained were the result of injection and refeeding of the following mosquitoes:—

A. gambiae, 3 specimens injected after 27-54 days and 29 specimens refed after 17-36 days.

A. mauritianus, 4 specimens injected after 52-54 days and 55 specimens refed after 15-35 days.

A. pretoriensis, 4 specimens injected after 27-54 days and 17 specimens refed after 17-36 days.

A. rufipes, 17 specimens injected after 27-54 days and 21 specimens refed after 17-36 days.

A. squamosus, 10 specimens refed after 17-35 days.

There remain therefore 28 specimens which were injected after periods of from 17-54 days and 132 specimens which refed after 15-36 days. This material seems to be sufficiently large to expect transmission in the event of one of the species being a transmitter.

VIII. EXPERIMENTS WITH TICKS.

In the concluding two experiments the eggs, laid by ticks which were collected in the adult stage from a spontaneous case of horsesickness, were injected into susceptible horses in order to determine whether the virus was capable of persisting through the egg and of being transmitted by the ensuing larval generation.

In the first experiment *Rhipicephalus appendiculatus* was used and in the second *Hyalomma aegyptium*. The adult ticks were collected from virus horse 5 (strain 7) on March 19th shortly after its death. This animal had been exposed to natural infection at Kaalplaas and was brought in to Onderstepoort on March 14th showing slight clinical symptoms of horsesickness. The engorged ticks were kept in test tubes in the laboratory until the eggs were laid.

Discussion of Results.

In two experiments portion of the freshly laid eggs of 10 *Rhipicephalus appendiculatus* and 1 *Hyalomma aegyptium* were injected into susceptible horses. No reaction comparable to that of horsesickness fever followed. The adult ticks had been collected from a spontaneous case of horsesickness which had ended in death and they had undoubtedly taken up sufficient virus.

In these two species of ticks, therefore, the virus was not transmitted through the eggs to the next generation.

IX. GENERAL DISCUSSION OF THE HORSESICKNESS WORK CARRIED OUT DURING THE SEASON 1932-1933.

During the latter part of the winter of 1931 and the summer of 1931-32 a series of experiments had been carried out at Onderstepoort with the object of finding the natural transmitter of horsesickness. An insect survey conducted concurrently had pointed out that, taking into consideration the epidemiological evidence generally accepted as correct, certain *Aëdes* species had to be regarded as the most promising amongst the potential transmitters. Almost 1,500 specimens of *Aëdes caballus*, *A. lineatopennis*, *A. hirsutus*, *A. dentatus*, *A. vittatus*, *A. punctothoracis*, *A. cumminsi*, *Anopheles squamosus* and *Culex theireli* were injected into susceptible horses after intervals ranging between ½ and 65 days, and 704 specimens of *Aëdes caballus*, *A. lineotopennis*, *A. hirsutus*, *A. dentatus*, *A. vittatus* and *Culex theileri* were refed after periods of from 1 minute to 63 days. Positive results were obtained in 3 experiments in which the mosquitoes were injected after ½ and after 7 days. These experiments had been carried out with O. virus strain, an old laboratory strain transmitted by means of direct inoculation from horse to horse for over 30 years, or with material from field cases, in which, however, a history of immunisation with O. virus existed. There was, therefore, a possibility that alterations in the biological properties of the strain could have been responsible for the negative results.

The work on horsesickness was continued during the summer 1932-33. On this occasion it was our object to control the results obtained the previous year with *Aëdes species* by making use of fresh strains which could be regarded as entirely suitable for transmission experiments, and to carry out further experiments with other potential transmitters. At the same time a further inspect survey at Onderstepoort and where possible also away from this site in known horsesickness areas was planned.

1. Climatic Conditions.

The summer under review was extraordinarily dry, the total amount of rain from November, 1932 to April, 1933, being only 11·43 inches as compared with 18·4 inches during the summer 1931-1932, which had also been very dry, and 36·91 inches in a wet summer, e.g. 1917-1918. During the best part of the summer the veld looked very much like typical winter veld.

2. Natural Occurrence of Horsesickness.

Notwithstanding the exceptionally dry character of the summer under review, horsesickness was very severe throughout the country during the second half of March and April. The laboratory, for example, lost 14 horses during this period. A number of these animals had remained outside in the field day and night, whereas the others had always been stabled at night. In these latter cases the infection must have occurred during the daytime or at night in the stables. From these observations we may conclude that, in contradistinction to general opinion, there does not necessarily exist an interrelationship between the amount of rain and the severity

of outbreaks of horsesickness, and that stabling at night does not give a sufficient degree of protection against infection. Two important epidemiological facts on which our experimental work had been based to a large extent thus proved to be unreliable. We could not make use of these conclusions for the experimental work under review as they were arrived at only towards the end of the season.

3. Results of a Mosquito Survey.

During the present summer an insect survey was carried out in the environment of the laboratory. The survey planned outside the Onderstepoort area had to be abandoned.

In traps, in which a horse was placed during the daytime representatives of the following species were collected: *Aëdes caballus, A. dentatus, A hirsutus, A. lineatopennis, a nigeriensis, A. salisburiensis, Anaphels cinereus, A. gambiae, A. mauritianus, A. squamosus, Culex annulioris, C. decens, C. fatigans, C. theileri, C. univittatus* and *Lutzia tigripes*. Most of these species had also been collected in 1931-1932. *Aëdes dentatus* and *Culex theileri* were the most common species during both seasons and, except for a slight increase in the percentage of Anophelines, the same information was obtained from the traps as during the previous season.

As often as was possible during the course of the experimental work on investigation of mosquito breeding places around about Onderstepoort was carried out. The results bbtained the previous year regarding the biology of the important fields species of *Aëdes, A. caballus, A. lineatopennis, A. hirsutus* and *A dentatus* were confirmed in full. Regularly, only after a good fall of rain, did the larvae of these species develop in their typical breeding grounds. Larvae of *Mucidus mucidus* always developed in fair numbers in company with the above-mentioned *Aëdes* species. Good breeding pools of *Aëdes argenteus* were also located during the year near certain railway construction works.

Anopheline ladvae had been very scarce during the previous season, no extensive search, however, having been made. During this season special attention was paid to them and quite unexpectedly, fair numbers of larvae belonging to *Anopheles gambiae, A. pretoriensis, A. rufipes, A. mauritianus* and *A. squamosus* were located during the second half of the summer. Good breeding places were found in the Aapies River in particular when no rain at all had fallen for a number of days and the river was at its lowest level. Between the stones which covered the river bed in large numbers small pools with practically no current were formed in which larvae of *Anopheles gambiae, A. pretoriensis* and *A. squamosus* were found. The completion of their larval development was only possible in these pools provided no rain at all fell for at least a week. A rain of medium intensity, e.g. in Pretoria, was sufficient to wash away all larvae. Another good breeding place used by larvae of *Anopheles rufipes, A. mauritianus, A. pretoriensis* and *A. squamosus* was located in a dried up furrow in which a marshy area covered by grass and reeds had been found due to leakage from a

water pipe. This place as well only offered suitable breeding conditions in wet or very dry seasons. In view of the unexpected outbreak of horsesickness during the season, Anophelines had to be regarded as good potential transmitters.

Besides mosquitoes, members of the genera *Tabanus* and *Haematopota* and also *Stomoxys calcitrans*, *Musca crassirostris* and *Bdellolarynx uniseratus* were the only other blood sucking insects observed. However, all af them feed only during the daytime and up to shortly after sunset.

4. Experimental Technique.

The experimental technique was practically the same as that worked out during the previous season, the results of which could be regarded as having been satisfactory.

5. Scheme of Experiments.

The experimental work itself was limited during this season almost exclusively to mosquitoes. Notwithstanding the negative results obtained the previous year these insects had still to be regarded as the most promising transmitters. Before taking into consideration any other group of insects as possible vectors the rôle of mosquitoes in a positive or genative sense had to be ascertained in any case. To start with an investigation into the rôle of the more important field species of the genus *Aëdes* had again to be carried out to see whether the negative results obtained the previous year were independent of the nature of the virus strains used. The transmitting capacity of *Mucidus mucidus*, a species biologically closely related to the abovementioned *Aëdes* species, had then to be determined. Only at the end of the previous season had it been ascertained that this species was a voracious bloodsucker. The finding of a good breeding place of *Aëdes argenteus* offered the opportunity of carrying out some experiments with this well known vector of other virus diseases. Our attention was directed towards the Anophelines especially, however, for reasons already mentioned. Five species were obtained in sufficient numbers to make a few experiments possible. A few experiments with ticks were conducted finally by way of a sort of appendix.

As in the first series of experiments already published, the transmission of horsesickness was not regarded as being of a purely mechanical character but as one in which the virus requires a definite period of development or multiplication in the insect vector and, therefore, a certain extrinsic incubation period, the probable duration of which was fixed at 14 days, which should be sufficiently long.

The clean mosquitoes, which were reared mostly from larvae, were infected by feeding them on experimentally infected horses during the fever period at temperatures usually not less than 102°. Only those specimens which had definitely taken up a sufficient amount of blood were used for further experiments. The infected mosquitoes were kept in the laboratory in cages and fed during the intervals on sugar water. From the experiences obtained from other

virus diseases it is known that the duration of the extrinsic incubation period depends upon the outside temperature, the higher the temperature the shorter the incubation period. The infected mosquitoes were kept during the cooler part of the summer in a room electrically heated therefore in order to render the developmental conditions for the virus as favourable as possible.

After different intervals the infected mosquitoes were either emulsified and injected subcutaneously into susceptible horses or fed on them. It is obvious that only by feeding experiments can definite results be obtained regarding the transmitting capacity of the species used. Positive results following upon injection of mosquitoes would be of some value only after a sufficiently long interval had elapsed and these would have to be confirmed by actual feeding experiments. A negative result obtained by injection, on the other hand, would prove that no virus was present in any part of the mosquito and would offer a strong indication to the effect that the species in question was not a suitable transmitter.

Nine different strains of virus were used in these experiments. With the exception of one, used only in a small number of experiments, they were obtained from spontaneous cases or early generations of such cases. In none of these strains could a definite relationship to O-virus be traced. In some of the experiments material from different strains (up to 7) was pooled and injected into the same horse to avoid any possible undesirable properties of a certain strain.

6. Experiments with Field Species of *Aëdes*.

Aëdes caballus, *A. dentatus*, *A. hirsutus*, *A. linneatopennis* and *A. nigeriensis* were used in the following 11 experiments:—

Experiment 1.—17 *A. caballus* fed after 11 days.
6 *A. caballus* fed after 16 days.
5 *A. lineatopennis* fed after 11 days.
4 *A. lineatopennis* fed after 16 days.
49 *A. hirsutus* fed after 10-11 days.
39 *A. hirsutus* fed after 15-16 days.
Result negative.

Experiment 2.—19 *A. caballus* fed after 15 days.
1 *A. lineatopennis* fed after 15 days.
Result negative.

Experiment 3.— 5 *A. caballus* and *A. lineatopennis* (mixed) fed after 5 days. Result negative.

Experiment 4.—*A. caballus* and *A. lineatopennis* (mixed)—
50 injected after 31 days.
348 injected after 36-39 days.

Experiment 5.—21 *A. caballus* injected after 7 days.
Result negative.

Experiment 6.—22 *A. caballus* fed after 5 days.
50 *A. caballus* fed after 7-9 days.
20 *A. caballus* fed after 12-14 days.
3 *A. caballus* fed after 17-20 days.
Result negative.

Experiment 7.—15 *A. lineatopennis* injected after 16-17 days.
Result negative.

Experiment 8.—19 *A. lineatopennis* fed after 17-18 days.
Result negative.

Experiment 9.—12 *A. lineatopennis* fed after 38-39 days.
Result negative.

Experiment 10.—11 *A. dentatus* fed after 7-9 days.
5 *A. dentatus* fed after 17-19 days.
Result negative.

Experiment 11.— 8 *A. nigeriensis* injected after 7-9 days.
Result negative.

These 11 experiments were all negative. 454 Specimens were injected after intervals of from 7 to 39 days, viz. 29 after 7-9, 15 after 16-17 and 410 after 31-39 days, whereas 274 specimens refed after 5-20 days, viz. 88 after 5-9 days, 90 after 11-14 and 96 after 15-20 days.

Arranged according to the species the results were:—

Injected: 21 *A. caballus* after 7 days.
27 *A. lineatopennis*—
15 after 16-17 days.
12 after 38-39 days.
398 *A. caballus* and *A. lineatopennis* mixed—
50 after 31 days.
348 after 36-39 days.
8. *A. nigeriensis* after 7-9 days.

Refed: 137 *A. caballus*—
72 after 5-9 days.
37 after 11-14 days.
28 after 15-20 days.
28 *A. lineatopennis*—
5 after 11 days.
23 after 16-18 days.
5 *A. caballus* and *A. lineatopennis* mixed after 7 days.
88 *A. hirsutus*—
48 after 11 days.
40 after 15-16 days.
16 *A. dentatus*—
11 after 7-9 days.
5 after 17-19 days.

The virus used to infect most of the mosquitoes consisted of spontaneous cases or early generations and was certainly suitable for transmission experiments. A few specimens were fed on a strain fixed for mice or on a spontaneous case showing no fever. If we exclude these specimens and those which refed within the first 14 days, there remain 427 specimens which were injected after 7-39 days, viz., 21 *A. caballus* after 7 days, 398 *A. caballus* and *A. lineatopennis* (mixed) after 31-39 days and 8 *A. nigeriensis* after 7-9 days. Seventy-seven mosquitoes were refed after 15-20 days, viz., 28 *A. caballus* after 15-20 days, 4 *A. lineatopennis* after 16 days, 40 *A. hirsutus* after 15-16 days and 5 *A. dentatus* after 17-19 days.

The number of *A. caballus*, *A. lineatopennis* and *A. hirsutus* used in these experiments was large enough to make the negative results obtained significant.

In the first series of experiments, already published, 1,214 specimens of *Aëdes* had been injected after 5-65 days and 695 mosquitoes had refed on susceptible horses after 13-62 days. In both series of experiments together 1,668 specimens were injected after 5-65 days and 969 specimens refed after 5-62 days.

Two of the experiments of the first series had been positive. In the first experiment 85 *A. caballus*, 115 *A. hirsutus* and 94 *A. lineotopennis*, altogether 294 specimens, were injected after an interval of 6 days; in the second, 68 *A. caballus* and 66 *A. lineatopennis*, altogether 134 specimens, were injected after 7 days.

All the other experiments had been negative. If we combine the negative experiments of the first with this series we find that no infection resulted after: The injection of 1,240 specimens after 5-65 days, viz. 180 after 5 days, 511 after 7-9, 87 after 15-17, 46 after 29-30, 410 after 31-39 and 6 specimens after 63-65 days; the refeeding of 969 specimens after 5-62 days, viz., 88 after 5-9 days, 125 after 10-14, 509 after 14-20 (21), 141 after 20-24, 20 after 25-27, 76 after 33-37 and 10 after 60-62 days.

Arranged according to the species, negative results were obtained by:—

Injection of 353 *A. caballus*—

94 after 5 days.
231 after 7-9 days.
28 after 15-16 days.

184 *A. lineatopennis*—

30 after 5 days.
121 after 7-9 days.
15 after 16-17 days.
12 after 38-39 days.
6 after 63-65 days.

398 *A. caballus* and *A. lineatopennis* mixed after 31-39 days.

213 *A. hirsutus*—
30 after 5 days.
137 after 7-9 days.
46 after 29-30 days.

52 *A. vittatus*—
20 after 5 days.
32 after 15 days.

39 *A. dentatus*—
6 after 5 days.
11 after 8-9 days.
12 after 16 days.

8 *A. nigeriensie* after 7-9 days.

2 *A. punctothoracis* after 8-9 days.

1 *A. cumminsi* after 8-9 days.

Refeeding of 327 *A. caballus*—
72 after 5-9 days.
57 after 11-14 days.
211 after 14-20 days.
7 after 23 days.

235 *A. lineatopennis*—
40 after 11-13 days.
60 after 16-18 days.
59 after 19-22 days.
66 after 33-35 days.
10 after 60-62 days.

5 *A. caballus* and *A. lineatopennis* mixed, 5 after 7 days.

275 *A. hirsutus*—
48 after 11 days.
131 after 15-16 days.
96 after 20-21 days.

118 *A. dentatus*—
11 after 7-9 days.
54 after 15-19 days.
27 after 20-23 days.
20 after 25-27 days.
6 after 35-37 days.

9 *A. vittatus*—
5 after 23 days.
4 after 37 days.

From these experiments we can conclude that the horsesickness virus can remain alive in *Aëdes* species up to 7 days. Ordinarily, however, the virus is destroyed within 5 days. The many negative results obtained with an undoubtedly sufficient number of mosquitoes make it reasonably certain that the species of *Aëdes* used in these experiments cannot be natural vectors of horsesickness.

7. Experiments with *Aëdes argenteus*.

A. argenteus was used in the following four experiments:—

Experiment 12.—10 *A. argenteus* injected after 7-8 days.
Result negative.

Experiment 13.—17 *A. argenteus*—
12 refed after 10-12 days.
2 refed after 14-16 days.
3 refed after 21-23 days.
Result negative.

Experiment 14.—38 *A. argenteus* refed after 16-19 days.
Result negative.

Experiment 15.—40 *A. argenteus* injected after 27-30 days.
Result negative.

In these experiments 50 specimens were injected, viz., 10 after 7-8 and 40 after 27-30. Fifty-five specimens refed on susceptible horses, viz., 12 after 10-12 days, 40 after 14-19 and 3 after 21-23 days. No infection resulted. The virus strains used were certainly suitable and the numbers of mosquitoes large enough to make the results significant.

It is interesting that the virus is quickly destroyed and does not show any tendency towards development or multiplication even in this *Aëdes* species. Owing to its house-frequenting habit, this species does not fall within the range of probable natural transmitters. It is, however, the main vector of some important virus diseases of man, e.g., yellow fever and dengue, which show, at any rate in certain of the biological characters of their viruses, a certain relationship to horsesickness, and the negative results must be of some importance therefore.

8. Experiments with Mucidus.

Mucidus mucidus, an extraordinarily large mosquito shows a systematic relationship to the *Aëdes* group. From a biological point of view it has to be regarded as good a potential transmitter of horsesickness as the common field species of *Aëdes*. Its larvae feed mainly upon the larvae of these *Aëdes* and it has therefore the same seasonal distribution, depends on the same climatic conditions, and, further, lives together with them in the same surroundings.

The following 5 experiments were made with *Mucidus*.

Experiment 16.— 1 *Mucidus* refed after 7 days.
Result negative.

Experiment 17.— 1 *Mucidus* injected after 20-24 days.
Result negative.

Experiment 18.— 5 *Mucidus* injected after 5 days.
Result negative.

Experiment 19.—10 *Mucidus* injected after 10 days.
Result negative.

Experiment 20.—25 *Mucidus*—
19 refed after 15 days.
6 refed after 22 days.
Result negative.

These 5 experiments were all negative. 16 Specimens were injected after 5-24 days, viz., 5 after 5, 10 after 10 and 1 specimen after 2-24 days. 26 Specimens refed on susceptible horses after 7-22 days, viz., 1 specimen after 7, 19 specimens after 15 and 6 after 22 days. Only one specimen had fed on a case in which the virus was not regarded as suitable. During the previous season no experiments had been carried out with this species. The material used was not very large but large enough to expect a positive result, at any rate in the injection experiments, had *Mucidus* been a suitable transmitter. We, therefore, do not regard this species as a possible vector of horsesickness.

9. Experiments with *Culex* Species.

Only one species of *Culex, C. annulioris*, was used this season. This species was found breeding with some Anophelines which were regarded as potential transmitters. The following two experiments were carried out:—

Experiment 21.—4 *C. annulioris* refed after 19 days.
Result negative.

Experiment 22.—3 *C. annulioris* injected after 30 days.
Result negative.

The numbers were very small and no definite conclusions could be arrived at.

In the first series a larged number of experiments were carried out with another *Culex* species, *C. theileri*. Only one experiment was positive, viz., that in which 5 specimens were injected ½ day after having fed. All the other experiments were negative. 240 Specimens were injected after 5-25 days, viz., 50 after 5, 50 after 16 and 140 after 25 days, whereas 9 specimens fed alternately on an infected and a susceptible horse with the object of direct transmission.

The total number of *Culex* mosquitoes used amount to 248 for injection and 12 for feeding experiments.

10. Experiments with Anophelines.

During the previous season only one experiment with *Anopheles squamosus* had been carried out whereas during the summer in question our atteution was specially directed to the Anophelines. For reasons stated previously some *Anopheles* species, and amongst them especially *A. gambiae, A. pretoriensis* and *A. rufipes*, had to be regarded as potential and promising vectors. In all, 9 experiments

were conducted with *A. gambiae, A. mauritianus, A. pretoriensis, A. rufipes* and *A. squamosus*. To limit the number of horses necessary for these experiments different species were fed together on the same horse in most cases.

Experiment 23.— 9 *A. pretoriensis* injected after 5 days.

Result negative.

Experiment 24.— 8 *A. gambiae* injected after 7 days.
8 *A. mauritianus* injected after 7 days.
7 *A. pretoriensis* injected after 7 days.

Result positive.

Experiment 25.—20 *A. pretoriensis* refed after 11 days.
29 *A. rufipes* refed after 11 days.

Result negative.

Experiment 26.—47 *A. mauritianus*—
28 refed after 15-16 days.
19 refed after 32-33 days.

Result negative.

Experiment 27.—18 *A. gambiae* refed after 17-18 days.
3 *A. mauritianus* refed after 17 days.
2 *A. pretoriensis* refed after 17 days.
1 *A. rufipes* refed after 17 days.
7 *A. squamosus* refed after 17 days.

Result negative.

Experiment 28.—11 *A. gambiae*—
6 refed after 28-29 days.
5 refed after 35-36 days.

5 *A. mauritianus*—
2 refed after 28 days.
3 refed after 35 days.

15 *A. pretoriensis*—
10 refed after 28-29 days.
5 refed after 35-36 days.

20 *A. rufipes*—
11 refed after 28-29 days.
9 refed after 35-36 days.

3 *A squamosus*—
2 refed after 28 days.
1 refed after 35 days.

Result negative.

Experiment 29.— 2 *A. gambiae*—
2 refed after 18 days.
1 refed after 24 days.
4 *A. mauritianus*—
2 refed after 18 days.
2 refed after 24 days.
25 *A. pretoriensis*—
21 refed after 18 days.
4 refed after 24 days.
12 *A. rufipes*—
11 refed after 18 days.
2 refed after 24 days.
3 *A. squamosus*—
3 refed after 18 days.
Result negative.

Experiment 30.— 4 *A. gambiae* refed after 7-8 days.
1 *A. mauritianus* refed after 7-8 days.
8 *A. pretoriensis*—
5 refed after 7-8 days.
3 refed after 12-13 days.
14 *A. rufipes*—
10 refed after 7-8 days.
4 refed after 12-13 days.
2 *A. squamosus*—
1 refed after 7-8 days.
1 refed after 12-13 days.
Result negative.

Experiment 31.— 4 *A. gambiae*—
1 injected after 27-28 days.
1 injected after 39 days.
2 injected after 52-54 days.
5 *A. mauritianus*—
1 injected after 39 days.
4 injected after 52-54 days.
10 *A. pretoriensis*—
3 injected after 27-28 days.
6 injected after 39 days.
1 injected after 53-54 days.
18 *A. rufipes*—
9 injected after 27-28 days.
1 injected after 39 days.
8 injected after 53-54 days.
Result negative.

In three of these experiments 69 specimens were injected after 5-54 days, whereas in the remaining 6 experiments 257 mosquitoes were refed on susceptible horses after 7-36 days.

One of these experiments was positive; 8 *Anopheles gambiae*, 8 *A. mauritianus* and 7 *A. pretoriensis*, altogether 23 specimens, were injected in the form of an emulsion 7 days after having fed on a virus horse.

The injection of 45 specimens after 5-54 days, viz., 9 after 5, 13 after 27-28, 9 after 39 and 15 after 52-54 days were negative as were also the feeding experiments with 257 specimens after intervals varying between 7 and 36 days, viz., 21 after 7-8, 57 after 11-13, 98 after 15-18, 39 after 24-29 and 42 specmens after 32-36 days. If we take into consideration, further, the one previous experiment with *Anopheles squamosus* (5 specimens injected after 5 days) and arrange the results according to species negative results were obtained by the:—

Injection of 4 *Gambiae*—
1 specimen after 27-28 days.
1 specimen after 39 days.
2 specimens after 52-54 days.

5 *A. mauritianus*—
1 specimen after 39 days.
4 specimens after 52-54 days.

19 *A. pretoriensis*—
9 specimens after 5 days.
3 specimens after 27-28 days.
6 specimens after 39 days.
1 specimen after 52-54 days.

18 *A. rufipes*—
9 specimens after 27-28 days.
1 specimen after 39 drys.
8 specimens after 52-54 days.

5 *A. squamosus*—
5 specimens after 5 days.

Refeeding of 36 *A. gambiae*—
4 specimens after 7-8 days.
20 specimens after 17-18 days.
7 specimens after 24-29 days.
5 specimens after 35-36 days.

60 *A. mauritianus*—
1 specimen after 7-8 days.
33 specimens after 15-18 days.
4 specimens after 24-28 days.
22 specimens after 32-35 days.

70 *A. pretoriensis*—
5 specimens after 7-8 days.
23 specimens after 11-13 days.
23 specimens after 17-18 days.
14 specimens after 24-29 days.
5 specimens after 35-36 days.

76 *A. rufipes*—

10 specimens after 7-8 days.
33 specimens after 11-13 days.
12 specimens after 17-18 days.
12 specimens after 24-29 days.
9 specimens after 35-36 days.

15 *A. squamosus*—

1 specimen after 7-8 days.
1 specimen after 12-13 days.
10 specimens after 17-8 days.
2 specimens after 28 days.
1 specimen after 35 days.

In three experiments (23, 29, 31) mosquitoes were used which had perhaps not taken up sufficient virus. Excluding these mosquitoes and, furthermore, those specimens which refed within the first 14 days after their original feed on an infected horse, there still remain 33 specimens which were injected after 5-54 days, and 132 specimens which refed after 15-36 days.

It may be concluded from these observations that horsesickness virus may remain alive for periods of up to 7 days in some of these Anophelines. The only positive experiment was obtained by the injection of 23 specimens, a relatively small number therefore. Further experiments by injection of mosquitoes especially at longer intervals were negative however. All the experiments in which the infected mosquitoes were fed on susceptible horses were also negative. We may exclude the Anophelines, in all probability therefore from the list of possible vectors of horsesickness.

11. Experiments with Ticks.

In the last two experiments the eggs of ticks which had engorged on a spontaneous case of horsesickness were injected into susceptible horses.

Experiment 32.—Eggs of 10 *Rhipicephalus appendiculatus*, injected. Result negative.

Experiment 33.—Eggs of 1 *Hyalomma aegyptium*, injected. Result negative.

In the case of 10 *Rhipicephalus appendiculatus* and 1 *Hyalomma aegyptium* the virus was not transmitted to the eggs. The material used was not sufficient to make possible any definite conclusion regarding the rôle ticks may play in the transmission of horsesickness. From the epidemiological aspect, viz., the definite seasonal limitation of the disease, ticks do not appear to be very promising vectors.

12. Concluding Remarks.

In all, 31 experiments were carried out at Onderstepoort during the summer 1932-1933 with different species of mosquitoes belonging to the genera *Aëdes*, *Culex*, *Mucidus* and *Anopheles*, those species, which an insect survey in the field had pointed out as the most promising transmitters, being used in particular.

Experiments with Aëdes.—Five hundred and four specimens *belonging to A. argenteus, A. caballus, A. lineatopennis* and *A. nigeriensis* were injected after 7-39 days, and 329 specimens belonging to *A. argenteus, A. caballus, A. lineatopennis, A. hirsutus* and *A. dentatus* were refed after 5-23 days.

Experiments with Culex.—Three *C. annulioris* were injected after 30 and refed after 19 days.

Experiments with Mucidus mucidus.—Sixteen specimens were injected after 5-24 days and 26 specimens refed after 7-22 days.

Experiments with Anopheles.—Sixty-eight specimens of *A. gambiae, A. mauritianus, A. pretoriensis* and *A. rufipes* were injected after 5-54 days and 257 specimens of *A. gambiae, A. mauritianus, A. pretoriensis, A. rufipes* and *A. squamosus* refed after 7-36 days.

In all 591 specimens were injected after 5-54 days and 615 specimens refed after 5-36 days.

During the previous season and in the course of 35 experiments, 1,434 specimens of *Aëdes, Culex* and *Anopheles* had been injected after ½-65 days and 704 specimens of *Aëdes* and *Culex* had been refed after from 1 minute to 62 days. During both seasons together, in a total of 66 experiments, 2,025 mosquitoes had been injected and 1,319 specimens refed.

Of these 66 experiments only 4 were positive. In the first 5 *Culex theileri* had been injected about ½ day after their initial feed, in the second 294 *Aëdes* species after 6 days, in the third 134 *Aëdes* after 7 days, and in the last experiment 23 *Anopheles* after 7 days. The other injection experiments and all the feeding experiments were negative.

The virus of horsesickness may thus remain alive, in exceptional cases, up to 7 days in certain Aëdes and Anopheles species. Normally, however, it is destroyed more quickly and, in our experiments, it never could be transmitted by the feeding of infected mosquitoes. Mosquitoes do not appear to be the natural transmitters of horsesickness.

This conclusion is based upon a fair number of experiments with quite a large number of specimens. We undoubtedly used only a small percentage of the 100 mosquitoe species known to occur in South Africa up to the present. A thorough mosquito survey carried out at Onderstepoort, a well known horsesickness area, enabled us to restrict the possible transmitters to a limited number of species belonging to the genera *Aëdes, Mucidus* and *Anopheles*. The most important of these were tested experimentally but in no case was a tendency for the virus to develop or multiply in the mosquitoes demonstrated. It is unlikely that better results are to be expected with other species not tested in these experiments. We know from the experience obtained with other virus diseases, e.g. yellow fever, that the development of the virus is not limited to one species only. It also takes place in more or less related species under experimental conditions and the virus can at least exist for a considerable period

in species of quite different groups of mosquitoes. The rapid destruction of horsesickness virus in all the mosquito species used is certainly of significance, especially as this virus is extremely resistant to varying degrees of temperature and putrefaction.

Errors in experimental technique may be excluded as being responsible for the negative results and the strains of virus used, especially in the second series of experiments, were undoubtedly suitable as they were derived from a number of spontaneous cases. The mosquitoes were fed for preference during the first few days of fever reactions and they must have taken up sufficient quantities of virus as only fully engorged specimens were used for the subsequent work. The temperature at which the mosquitoes were kept was sufficiently high to allow of rapid development of the virus and during the cooler portions of the season they were always kept in a warm room. There appears to be nothing of any importance so far as technique is concerned to which the consistent failures in transmission can be attributed. So far as we are able to judge the only reason for the negative results obtained must be the fact that mosquitoes are not the natural transmitters of horsesickness.

This conclusion is purely negative in nature as our present knowledge does not permit of our suggesting any other probable vectors with any degree of certainty once mosquitoes have been excluded. Further information will have to be collected particularly by means of insect surveys conducted in the field. In the course of these surveys, it will have to be borne in mind that the epidemiological facts, so far as the propagation of horsesickness are concerned, regarded as significant for many years, proved to be incorrect in two important points during the course of the season; i.e. the relation supposed to exist between rainfall and the severity of outbreaks of horsesickness and, the nocturnal limitation of the transmission of the disease. For future work mosquitoes may be excluded but other insects and arthropoda not entirely dependent upon rainfall and including those of diurnal habits will have to be taken into consideration.

X. SUMMARY.

During the summer of 1932-1933 our work on the natural transmission of horsesickness was continued at Onderstepoort.

The summer was extraordinarily dry, the total rainfall from November, 1932, to April, 1933, being only 11·43 inches.

Notwithstanding the dry character of the season horsesickness was extremely severe throughout the country during the second half of March and in April.

At Onderstepoort, animals which were always stabled at night also contracted the disease.

In contradistinction to the general opinion, therefore, there appears to exist no very close relationship between rainfall and the occurrence of horsesickness and stabling at night does not afford a

sufficient degree of protection against infection. Two extremely important epidemiological facts so far as the planning of experimental work was concerned thus proved to be unreliable.

An insect survey carried out in the field at Onderstepoort confirmed the results obtained during the previous season as to the important field species of *Aëdes*, viz. *A. caballus*, *A. lineatopennis*, *A. hirsutus*, *A. dentatus* and *Mucidus mucidus*.

An extensive search revealed the unexpected result that larvae of Anophelines, especially of *Anopheles gambiae*, *A. pretoriensis*, *A. rufipes* and *A. mauritianus* were present in fair numbers during the dryest part of the season in a river bed and in a marshy area formed by accidental leakage of water. Rain of even medium intensity destroyed the breeding places. Anophelines appear to find suitable breeding conditions in very wet or very dry seasons and had thus to be regarded as potential transmitters.

The occurrence of *Anopheles gambiae*, an important malaria carrier, at Onderstepoort during a very dry season is of special interest.

The experimental technique was the same as that worked out during the previous season and already described in a separate paper.

The strains of virus used for infecting the mosquito were derived from a number of spontaneous cases. Only fresh material or early generations were used. In some experiments the virus horses were infected with different strains at the same time.

In all, 31 experiments were carried out with mosquitoes in which experimentally infected insects were either injected subcutaneously into or refed after varying intervals on susceptible horses. 591 Specimens were injected after 5 to 54 days, and 615 mosquitoes refed after periods of from 5 to 36 days.

One experiment only was positive in which 8 *Anopheles gambiae*, 8 *A. mauritianus* and 7 *A. pretoriensis* were injected in the form of an emulsion 7 days after having fed on a virus horse. The other experiments were all negative. The negative results comprised the injection of: 21 *Aëdes caballus* after 7 days, 27 *A. lineatopennis* after 16-39 days, 398 *A. caballus* and *A. lineatopennis* (mixed) after 31-39 days, 8 *A. nigeriensis* after 7-9 days, 50 *A. argenteus* after 7-30 days, 3 *Culex annulioris* after 30 days, 16 *Mucidus mucidus* after 5-24 days, 4 *Anopheles gambiae* after 27-54 days, 5 *A. mauritianus* after 39-54 days and 19 *A. pretoriensis* after 5-54 days.

The negative results obtained by feeding mosquitoes consisted of the feeding of 137 *Aëdes caballus after* 5-20 days, 28 *A. lineatopennis* after 11-18 days, 5 *A. caballus* and *A. lineatopennis* (mixed) after 7 days, 88 *A. hirsutus* after 11-16 days, 16 *A. dentatus* after 7-19 days, 55 *A. argenteus* after 10-23 days, 3 *Culex annulioris* after 19 days, 26 *Mucidus mucidus* after 7-22 days, 36 *Anopheles gambiae* after 7-36 days, 60 *A. mauritianus* after 7-35 days, 70 *A. pretoriensis* after 7-36 days, 76 *A. rufipes* after 7-36 days and 15 *A. squamosus* after 7-35 days.

During the previous season, in a total of 35 experiments, 1,434 specimens of *Aëdes*, *Culex* and *Anopheles* had been injected after intervals of from ½ to 65 days and 704 *Aëdes* and *Culex* had been refed after 1 minute to 65 days. During the two seasons together 2,025 mosquitoes had been injected and 1,319 specimens refed after intervals of up to 65 days.

Positive results had been obtained occasionally by the injection of relatively large numbers of mosquitoes at intervals of up to 7 days but never after this period or by feeding.

The important species of all the promising groups of mosquitoes, viz., *Aëdes*, *Mucidus* and *Anopheles* have been controlled with sufficient material for transmission purposes to justify the conclusion that, *mosquitoes are not vectors of horsesickness.*

It is of interest to note that even in the case of *Aëdes argenteus*, the vector of yellow fever and dengue, the horsesickness virus displayed no tendency to persist for any length of time.

Finally, in two experiments, the eggs of 10 *Rhicephalus appendiculatus* and 1 *Hyalomma aegyptium*, which had engorged on a spontaneous case of horsesickness, were injected without producing a reaction.

With the present state of our knowledge we are unable to indicate any probable vectors of horsesickness the transmitting capacity of which might be investigated, once mosquitoes have been excluded. Further information will have to be obtained by means of insect surveys in the field and the new epidemiological facts established during this season will have to be taken into consideration.

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APPENDIX.

As outlined in the text this appendix includes the actual experimental work only. For discussions of the actual results obtained and the conclusions drawn the reader is referred to the relative sections of the text which correspond to the numerals heading the different sections of the appendix.

VII.—I. Experiments with the Field Species of *Aëdes*.

(*a*) Experiments with *Aëdes caballus*, *A. lineatopennis*, and *A. hirsutus* mixed.

In four experiments batches of infected *Aëdes caballus*, *A. lineatopennis* and *A. hirsutus* were either fed together after 7-16 days on the same horses or injected together after 31-39 days. On the virus horses 1,940 specimens engorged.

Virus horses 1-3, strains 6 and 9 were used and the following mosquito groups:—

Aëdes caballus group 1. Fed on virus horse 1 from October 24th, 1932, p.m., to 25th, a.m. First day of fever. Temperature 101° and 102°. 72 specimens engorged (caught as adults). Used for experiments 1 and 5.

A. caballus group 2. Fed on the same horse from October 26th to 27th Third day of fever. Temperature 104° and 103°. 61 specimens engorged. Used for experiment 2.

Aëdes lineatopennis group 1. Fed on virus horse 1 together with *A. caballus* group 1. 21 specimens engorged (caught as adults). Used for experiment 1.

Aëdes caballus and *A. lineatopennis* mixed, group 1. Fed during the night of December 1st to 2nd on virus horse 2. Third day of fever. Temperature 101° and 102°. 12 specimens engorged (hatched from larvae). Used for experiment 3.

A. caballus and *A. lineatopennis* mixed, group 2. Fed during the night of December 9th to 10th on virus horse 3. First day of fever. Temperature 102·4° and 101·4°. 518 specimens engorged (hatched from larvae). Used for experiment 4.

A. caballus and *A. lineatopennis* mixed, group 3. Fed during the night of December 10th to 11th on virus horse 3. Second day of fever. Temperature 102·6° and 101·6°. 345 specimens engorged (hatched from larvae). Used for experiment 4.

A. caballus and *A. lineatopennis* mixed, group 4. Fed during the night of December 11th to 12th on virus horse 3. Third day of fever. Temperature 104° and 103·6°. 377 specimens engorged (hatched from larvae). Used for experiment 4.

A. caballus and *A. lineatopennis* mixed, group 5. Fed during the night of December 12th to 13th on virus horse 3. Fourth day of fever. Temperature 104° and 103°. 305 specimens engorged (hatched from larvae). Used for experiment 4.

A. caballus and *A. lineatopennis* mixed, group 6. Fed during the night of December 13th to 14th on virus horse 3. Fifth day of fever. Temperature 106·2° and 103°. 101 specimens engorged (hatched from larvae). Used for experiment 4.

Aëdes hirsutus group 1. Fed on virus horse 1 together with *A. caballus* group 1. 87 specimens engorged (reared from larvae). Used for experiment 1.

A. hirsutus group 2. Fed on the same virus horse from October 25th to 26th, a.m. Second day of fever. Temperature 103·2°. 22 specimens engorged. Used for experiment 1.

A. hirsutus group 3. Fed on virus horse 1 together with *A. caballus* group 2. 18 specimens engorged. Used for experiment 2.

Experiment 1.—119 Aëdes caballus, A. lineatopennis *and* A. hirsutus. *Feeding. Interval* 10-16 *days. Horse* 20298.

November 4th, 1932, p.m., 4 groups of *Aëdes* were put on this horse and during the following night 17 *A. caballus* group 1, 5 *A. lineatopennis* group 1, 39 *A. hirsutus* group 1, and 9 *A. hirsutus* group 2, fed. The same mosquitoes were fed on this horse again during the night of November 9th to 10th and this time 6 *A. caballus* group 1, 4 *A. lineatopennis* group 1, 31 *A. hirsutus* group 1, and 8 *A. hirsutus* group 2 engorged themselves. These mosquitoes had been infected on virus horse 1 (strain 6) on October 24th-25th and 25th-26th during the first to second day of fever.

Altogether 119 mosquitoes fed on this horse. 70 after 10-11 and 49 after 15-16 days, viz.:—

23 *A. caballus*, 17 after 11 and 6 after 16 days.

9 *A. lineatopennis*, 5 after 11 and 4 after 16 days.

87 *A. hirsutus*, 48 after 10-11 and 39 after 15-16 days.

Reaction.—No reaction occurred after the feeding of the mosquitoes. The horse was kept under observation up to December 12th., i.e. 34 days after the feeding of the last group of mosquitoes. During this time the highest temperature recorded was 100·8°.

The same horse was used on January 24th, 1933, in experiment 17 (1 *Mucidus mucidus*, injection, interval 20-24 days), and on March 23rd for experiment 12 (10 *Aëdes argenteus*, feeding, interval 7-8 days). Both experiments were negative.

Immunity test.—The horse was tested for immunity in April, 1933. The result of this test, which was somewhat atypical, will be described under experiment 12.

Result.—The experiment has to be regarded as *negative*, as no temperature reaction whatsoever followed the feeding of the mosquitoes.

Experiment 2.—20 Aëdes caballus *and* A. hirsutus. *Feeding. Interval* 15 *days. Horse* 20331.

During the night of November 11th to 12th, 1932, *Aëdes caballus* group 2 and *A. hirsutus* group 3 were allowed to feed on this horse. 19 specimens of the former and 1 specimen of the latter species were found engorged. These mosquitoes had been infected 15 days previously on virus horse 1 (strain 6) during the third day of fever.

Reaction.—The horse was kept under observation up to December 14th, i.e. more than a month after the feeding of the mosquitoes. The temperature remained below 101° except for 2 days (November 25th, December 3rd), when temperatures of 102° and 103° were recorded, which, however, did not last longer than half a day.

The same horse was used on February 4th, 1933, for experiment 18 (5 *Mucidus mucidus*, injection, interval 5 days), on March 24th, in experiment 10 (16 *Aëdes dentatus* feeding, interval 7-19 days) and on May 9th in experiment 8 (19 *Aëdes lineatopennis*, feeding, interval 17-18 days). The results of these experiments were negative or doubtful.

Result.—The experiment has to be regarded as *negative*, as no reaction followed the feeding of the 20 mosquitoes.

Experiment 3.—5 Aëdes caballus *and* A. lineatopennis. *Feeding. Interval* 7 *days. Horse* 20335.

On December 7th, 1932, *Aëdes caballus* and *A. lineatopennis* mixed, group 1 (and *Mucidus mucidus*, *cf.* experiment 16) were put on this horse. 5 specimens fed during the ensuing night. These mosquitoes had infected themselves 7 days before on virus horse 2 infected with a mouse-brain strain of virus (strain 9).

Reaction.—The horse was kept under observation up to January 7th, one month after the feeding of the mosquitoes. The temeprature of the animal was somewhat irregular, often reaching 101°. A definite fever reaction, however, did not follow. Temperatures of 102° up to 102·4° were noticed on 3 occasions (December 22nd, 29th, and 31st), each lasting only half a day.

Result.—The experiment has to be regarded as *negative*, no typical fever reaction resulting after the feeding of the mosquitoes.

Experiment 4.—398 Aëdes caballus *and* A. lineatopennis, *Injection. Interval* 31-39 *days. Horse* 20453.

On January 16th, 1933, 50 specimens of *Aëdes caballus* and *A. lineatopennis* mixed, group 2 were emulsified thoroughly in saline and injected subcutaneously into horse 20453. On January 24th, 348 specimens of *A. caballus* and *A. lineatopennis* mixed, group 2 (31 specimens), group 3 (99 specimens), group 4 (85 specimens), group 5 (89 specimens) and group 6 (44 specimens), emultified in saline, were injected subcutaneously into the same horse. The first group of mosquitoes had fed 31 days previously on virus horse 3 (strain 6) during the first day of fever, the second lot of 348 specimens on the same virus horse 36 to 39 days before during the first to fifth day of fever.

Reaction.—The day after the injection of the first lot of mosquitoes the temperature rose up to 102·8° (p.m.) showing 101° and 101·4° the next day and dropping to normal the day after. The second injection on January 24th was also followed the next day by a rise in temperature, this time up to 103·4°. The day after, however, the temperature dropped to normal again and remained normal for 13 additional days, up to February 8th. A distinct fever reaction lasting 4 days then set in, the temperature being 101° and 102° on the 9th, the 10th, 101° and 103°, the 11th, 102·2° and 105·2°, the 12th, 101·4° and 103°. From the 13th onwards the temperature was normal again. The horse was kept under observation up to March 22nd, i.e. up to 37 days after this short fever reaction. During this period the highest temperature noticed was 101·2°.

The same horse was used on March 22nd in experiment 6 (95 *Aëdes caballus*, feeding, interval 5-20 days) and on May 5th in experiment 15 and 17 (40 *Aëdes argenteus* and 3 *Culex annulioris*, injection, interval 27-30 days). Both experiments were negative.

Result.—The experiment has to be regarded as *negative*. A short, but definite fever reaction commenced 23 days after the injection of the first and 15 days after the injection of the last lot of mosquitoes. This reaction, however, cannot be regarded as an attack on horsesickness as no clinical symptoms of this disease were present during or after the short period of fever.

(*b*) Experiments with *Aëdes caballus.*

Aëdes caballus alone was used in two experiments. In the first experiment 21 specimens were injected after 7 days, in the second the susceptible horse was bitten by 95 mosquitoes 5-20 days after having fed on a virus horse. 197 specimens had fed on the virus horse.

Virus horse 1 and 5 were used, strains 6 and 7 and the following mosquito groups:—

Group 1.—Fed on virus horse 1 from October, 14th, 1932, p.m., to 25th, a.m. First day of fever. Temperature 101° and 102°. 72 specimens engorged (caught as adults). Used for experiment 1 and 5.

Group 3.—Fed on virus horse 5 (spontaneous infection) during the night of March 14th to 15th, 1933. Day of fever unknown. Temperature 104·6° to 102·4°. 22 specimens engorged (reared from larvae). Used for experiment 6.

Group 4.—Fed on the same virus horse during the night of March 15th-16th. Temperature 105° to 103·2°. 18 specimens engorged (reared from larvae). Used for experiment 6.

Group 5.—Fed on the same virus horse during the night of March 16th to 17th. Temperature 104·2° to 102°. 39 specimens engorged (reared from larvae). Used for experiment 6.

Group 6.—Fed on the same virus horse during the night of March 17th to 18th. Temperature 106° to 103·8°. 46 specimens engorged (reared from larvae). Used for experiment 6.

Experiment 5.—21 Aëdes caballus, *Injection. Interval 7 days. Horse* 20334.

Into this horse 21 *Aëdes caballus* group 1, emulsified in serum, were injected subcutaneously on October 31st, 1932. These mosquitoes had fed about 7 days previously on virus horse 1 (strain 6) during the first day of fever.

Reaction.—On the site of the injection (right side of the neck) a painfull, circumscribed swelling began to appear, reaching about 6 inches in diameter and persisting for a day or two. The temperature began to rise the same day after the injection (p.m., 102°), being 102·8° on November 1st, the 2nd, 100° and 104°, the 3rd, 99° and 100·8°, thereafter it remained normal. The horse was kept under observation up to December 6th, i.e. up to 36 days after the injectio nof the mosquitoes. The highest temperature noticed during this period was 100·8°.

Immunity test.—The horse was then transferred to another experiment and injected with a strain adapted to mice. A severe fever reaction lasting 8 days with a temperature maximum of 106° followed the injection. Clinical signs of dikkop were present and the disease ended in recovery.

The Result of the experiment was *negative*. The injection of mosquitoes was followed immediately by a temperature reaction, but thereafter the temperature remained normal for more than a month.

Experiment 6.—95 Aëdes caballus. *Feeding. Interval* 5-20 *days. Horse* 20453.

This horse had been used already in January in experiment 4 (398*Aëdes caballus* and *A. lineatoppeni,s* injection interval 31-39 days(. The result of this experiment was regarded as negative.

On March 22nd, 1932, *A. caballus*, group 6 was put on this horse and 22 (out of 43) specimens engorged themselves. The following night 50 (out of 64) specimens of the combined groups 3-5 took up blood. The same batches (groups 3-5) were fed again on the horse during the night of March 28th to 29th and 12 (out of 39) specimens were found engorged. The following night group 6 was put on the horse and 8 (out of 24) specimens fed. During the night of April 4th to 5th the remaining mosquitoes of groups 3-6 were fed for the last time and 3 (out of 25) specimens took up blood. The mosqutoes had fed for the first time on virus horse 5, a spontaneous case of horsesickness from Kaalplaas (strain 7), 5-20 days before, viz., 22 specimens after 5 days, 50 after 7-9, 8 after 12, 12 after 12-14 and 3 specimens after 17-20 days.

Reaction.—The horse was kept under observation up to May 5th, i.e. 44 days after the feeding of the first batch of mosquitoes and 31 days after the last batch. No fever reaction occurred. The highest temperature noticed during the observation period was 101·8°.

The same horse was used on May 5th for experiments 15 and 22 (40 *Aëdes argenteus* and 3 *Culex annulioris*, injection, interval 27-30 days) with negative result.

Result.—The experiment was *negative*, as no fever reaction followed the feeding of the mosquitoes.

(*c*) Experiments with *Aëdes lineatopennis.*

Aëdes lineatopennis alone was used in three experiments in which mosquitoes were refed after 16-17 days or injected after 38-39 days. These mosquitoes had fed on a spontaneous case of dikkop. At the time of feeding, however, the fever had already passed and, therefore, it must be regarded as somewhat doubtful whether the mosquitoes actually did take up sufficient virus.

Virus horse 7 was used, strain 8 and the following mosquito group:—

Group 2.—Fed during the night of April 21st to 22nd on virus horse 7, a spontaneous case of dikkop ending in recovery. Temperature normal. 7 specimens fed (reared from larvae). Used for experiment 7-9.

Experiment 7.—15 Aëdes lineatopennis. *Injection.*
Interval 16-17 *days. Horse* 20534.

The same horse had been used previously in March in experiment 13 (17 *Aëdes argenteus*, feeding, interval 10-23 days). No infection had resulted.

May 8th, 1933, 15 specimens of *Aëdes lineatopennis* group 2 were emulsified and injected subcutaneously into horse 20534. The mosquitoes had fed 16-17 days before on virus horse 6, a spontaneous case of dikkop ending in recovery, after the temperature had returned to normal again (strain 8).

Reaction.—The horse was kept under observation up to June 8th, a month after the injection of the mosquitoes. There was only a slight rise in temperature up to 101·2° directly after the injection of the mosquitoes. The temperature otherwise remained normal (maximum 100·8°) throughout the observation period.

Result.—The experiment was *negative*, as no reaction at all followed the injection of the mosquitoes.

Experiment 8.—19 Aëdes lineatopennis. *Feeding.*
Interval 17-18 *days. Horse* 20331.

This horse had been used previously in November 1932 in experiment 2 (20 *Aëdes caballus* and *A. hirsutus*, feeding after 15 days), in February, 1932, in experiment 18 (5 *Mucidus mucidus*, injection after 5 days) and in March, in experiment 10 (16 *Aëdes dentatus*, feeding after 7-19 days). The first and third experiments were negative, the second doubtful, but very probably also negative.

On May 9th, the remaining specimens of *A. lineatopennis* group 2, were put on horse 20331 and 10 specimens fed during the ensuing night. These mosquitoes had fed 17-18 days before on virus horse 6 (cf. experiment 7).

Reaction.—The horse was kept under observation up to June 7th, i.e. 29 days after the feeding of the mosquitoes. The highest temperature recorded during this period was 100·2°. The temperature was often very low, below 95°, the anus showing tenesmus.

Result.—This experiment also was *negative*, as no reaction resulted after the feeding of the mosquitoes.

Experiment 9.—12 Aëdes lineatopennis. *Injection.*
Interval 38-39 *days. Horse* 20621.

On May 30th, 1933, the remaining 12 specimens of *A. lineatopennis* group 2 were emulsified and injected together with 37 *Anophelines* (cf. experiment 31) into this horse. The mosquitoes had fed 38-39 days before on a spontaneous case of dikkop, however outside the actual fever reaction (cf. experiment 7).

Reaction.—No temperature reaction.

Result.—Negative.

(*d*) Experiments with *Aëdes dentatus.*

Aëdes dentatus was only used in one experiment, in which 16 specimens fed after an interval of 7-19 days. On the virus horse 22 specimens had fed.

Virus horse 5 (spontaneous infection, strain 7) and mosquito groups 1-3 were used.

Group 1.—Fed on virus horse 5 during the night of March 15th to 16th. Temperature 105° and 103·2°. 10 specimens engorged (reared from larvae). Used for experiment 10.

Group 2.—Fed on the same horse during the night of March 16th to 17th. Temperature 104·2° to 102°. 6 specimens engorged (reared from larvae). Used for experiment 10.

Group 3.—Fed on the same horse one night later. Temperature 106° and 103·8°. 6 specimens engorged (reared from larvae). Used for experiment 10.

Experiment 10.—16 Aëdes dentatus. *Feeding Interval* 7-19 *days. Horse* 20331.

This horse had been used before in November, 1932, in experiment 2 (20 *Aëdes caballus* and *A. hirsutus*, feeding interval 15 days) and in February, 1932, in experiment 18 (5 *Mucidus mucidus*, injected after 5 days). The first experiment was negative, the second doubtful, probably also negative.

On March 24th, 1933, *A. dentatus* groups 1-3 combined were put on this horse and during the following night 11 (out of 19) specimens fed. The same lot of mosquitoes were fed on the same horse for a second time during the night of April 3rd to 4th and 5 (out of 14) specimens engorged themselves. These mosquitoes had been infected on a spontaneous case of horsesickness (strain 7) on March 15th to 17th, and thus the first lot of 11 specimens had refed on the susceptible horse after 7-9 days and the second lot of 5 specimens after 17 to 19 days.

Reaction.—On March 31st, 7 days after the first feeding of the mosquitoes a sudden rise in temperature up to 102° and 103° occurred which, however, lasted only 1 day. Thereafter the temperature remained normal (maximum 100·6°) up to May 10th, more than a month after the second feeding of mosquitoes, when the horse was used for another experiment.

The same horse was used on May 9th in experiment 8 (19 *Aëdes lineatopennis*, feeding, interval 17-18 days) with negative result.

Result.—As no reaction followed the feeding of mosquitoes during an observation period of more than one month, the experiment has to be regarded as *negative.*

(*e*) Experiment with *Aëdes nigeriensis.*

Aëdes nigeriensis was used only in one experiment, in which 8 specimens were injected after an interval of 7-9 days. On the virus horse 10 specimens had fed.

Virus horse 5 (spontaneous case, strain 7) was used and mosquito groups:—

Group 1.—Fed on virus horse 5 during the night of March 16th to 17th. Temperature 104·2° to 102°. 7 specimens engorged (reared from larvae). Used for experiment 11.

Group 2.—Fed on the same horse during the ensuing night. Temperature 106° and 103·8°. 3 specimens engorged (reared from larvae). Used for experiment 11.

Experiment 11.—8 Aëdes nigeriensis. *Injection. Interval* 7-9 *days. Horse* 20463.

This horse had been used before in February in experiment 19 (10 *Mucidus mucidus*, injected after 10 days), which experiment had been negative.

On March 25th, 1933, *A. nigeriensis* groups 1 and 2 were emulsified and injected subcutaneously into horse 20463. These mosquitoes had been infected on virus horse 5, a spontaneous case of horsesickness (strain 7), 7-8 and 8-9 days previously.

Reaction.—The horse was kept under observation up to April 29th. No fever reaction followed the injection of the mosquitoes. The highest temperature noticed during these 35 days was 101·2°.

In May the same horse was used in experiment 29 (*Anopheles* sp., feeding, interval 12-24 days) without showing a reaction.

Result.—The experiment was *negative*, as the injection of the mosquitoes was not followed by any temperature reaction.

VIII.—Experiments with *Aëdes argenteus*.

A. argenteus, a wide-spread house frequenting mosquito species of the tropics and sub-tropics, is not very common at Onderstepoort and we were fortunate in obtaining a sufficient number for a few experiments. On account of the fact that *A. argenteus* is the most important transmitter of yellow fever and dengue we were very interested to see whether a development of the horsesickness virus would be possible in them as well.

In all 4 experiments were conducted in which 50 specimens were injected after 7-30 days and 55 specimens refed on susceptible horses after an interval of 10-23 days. 174 specimens had fed originally on the virus horse. All the mosquitoes were reared from larvae.

Virus horse 5 and 6 were used, strains 1 to 7 and the mosquito groups:—

Group 1.—Fed on virus horse 5 during the night of March 15th to 16th, 1933. Temperature 105° and 103·2°. 5 specimens engorged (reared from larvae). Used for experiment 12 and 13.

Group 2.—Fed on the same horse during the night of March 16th to 17th Temperature 104·2° to 102°. 21 specimens engorged (reared from larvae). Used for experiment 12 and 13.

Group 3.—Fed on the same horse on night later. Temperature 106° and 103·8°. 13 specimens engorged (reared from larvae). Used for experiment 13.

Group 4.—Fed on virus horse 6 on April 5th during daytime. First day of fever. Temperature 101° and 102·6°. 46 specimens engorged (reared from larvae). Used for experiment 14 and 15.

Group 5.—Fed on the same virus horse on day later. Second day of fever. Temperature 100·4° and 102·8°. 17 specimens engorged (reared from larvae). Used for experiment 14 and 15.

Group 6.—Fed on the same virus horse on April 7th. Third day of fever. Temperature 102·2° and 103·4°. 15 specimens engorged (reared from larvae). Used for experiment 14 and 15.

Group 7.—Fed on the same horse on April 8th. Fourth day of fever. Temperature 102·6° and 104·2°. 7 specimens engorged (reared from larvae). Used for experiment 14 and 15.

Experiment 12.—10 Aëdes argenteus. *Injection.*
Interval 7-8 *days. Horse* 20298.

This horse had been used previously in November, 1932, in experiment 1 (119 *Aëdes caballus*, *A. lineatopennis*, and *A. hirsutus*, refed after 10-16 days) and in January, 1933, in experiment 17 (1 *mucidus*, refed and injected after 11 and 24 days). Both experiments had been negative.

On March 23rd, 1933, 10 *A. argenteus* of groups 1 and 2 combined were emulsified in saline and injected subcutaneously into horse 20298. The mosquitoes had fed 7-8 days previously on a spontaneous case of horsesickness (virus horse 5, strain 7).

Reaction.—The horse was kept under observation for a month, up to April 24th. Temperatures exceeding 101°, viz.: 101·4° and 103° were observed twice (April 9th and 11th), however, only for half a day.

Immunity test.—On April 24th the horse was injected subcutaneously with 2 c.c. blood of virus horse 5, the same horse as that used for feeding the mosquitoes on. However, no temperature reactions were noticed over a period of more than a month. On June 2nd the horse was injected intrajugularly with 10 c.c. blood of horse 20329, the first generation of a spontaneous case. The temperature began to rise on June 11th and the horse died 3 days later. The temperature reaction was not very marked (103·2° maximum), but the post mortem findings confirmed the diagnosis horsesickness, dunkop.

Result.—The experiment has to be regarded as *negative*, as no fever reaction followed the injection of the mosquitoes, although the result of the immunity test was somewhat atypical.

Experiment 13.—17 Aëdes argenteus. *Feeding.*
Interval 10-23 *days. Horse* 20534.

During the night of March 27th to 28th the combined groups 1-3 of *Aëdes argenteus* were put onto th is horse and 12 (out of 19) specimens fed. The same mosquitoes were fed 4 days later (March 31st to April 1st) and 2 (out of 14) were found engorged. These mosquitoes were put on to the same horse for the third time during the night of April 7th to 9th and 3 (out of 11) specimens took up blood. In all, the horse was bitten 17 times. The mosquitoes had infected themselves on a spontaneous case of horsesickness (virus horse 5, strain 7), i.e. 12 specimens 10-12 days, 2 specimens 14-16, and 3 specimens 21-23 days previously.

Reaction.—The horse was kept under observation for one month, up to May 8th. There were some variations in temperature. On April 12th a temperature of 102·6° was noticed, the 15th 102° and the 20th 104°. These elevations, however, did not last longer than half a day.

On May 8th the horse was used for experiment 7 (15 *A. lineatopennis*, injected after 15-17 days). This experiment was negative.

Result.—The experiment has to be regarded as *negative*, no typical fever reaction following the feeding of the mosquitoes.

Experiment 14.—38 Aëdes argenteus. *Feeding.*
Interval 16-19 *days. Horse* 20579.

During the night of April 24th to 25th 38 *Aëdes argenteus*, groups 4-7, were fed on this horse together with some *Culex annulioris* (cf. experiment 21), *Anopheles gambiae*, *A. mauritianus*, *A. pretoriensis*, *A. rufipes*, and *A. squamosus* (cf. experiment 27). These mosquitoes had infected themselves 16-19 days before, during the first to fourth day of fever, on a horse injected with a number of spontaneous strains of horsesickness (virus horse 6 strains 1-7).

Reaction.—At the time of feeding the mosquitoes the horse was just developing a temperature reaction of unknown origin. The temperature on April 24th was 101·6° and 102·8°, the 25th, 101·2° and 103°, the 26th, 102° and 104·2°, the 27th, 102° and 104·2°, the 28th, 100° and 102·2°, and the 29th, 99·8° and 100·8°. Thereafter the temperature returned to normal where it remained until June 6th, 43 days after the feeding of the mosquitoes, a temperature exceeding 101° only being noticed once.

Result.—The experiment was *negative*. No reaction followed the feeding of the mosquitoes. There is no reason to regard the temperature reaction during the feeding of the mosquitoes as being connected with a spontaneous horsesickness reaction, which could have conferred an immunity horsesickness to this horse.

Experiment 15.—40 Aëdes argenteus. *Injection.*
Interval 27-30 *days. Horse* 20453.

This horse had been used previously in January, 1933, in experiment 4 (398 *Aëdes caballus* and *A. lineatopennis*, injected after 31-39 days) and in March in experiment 6 (95 *Aëdes caballus*, feeding, interval 5-20 days). The result of both experiments were negative.

On May 5th, 40 *Aëdes argenteus* groups 4-7, the remaining specimens of the previous experiment were emulsified and injected together with some *Culex annulioris* (cf. experiment 22) subcutaneously into horse 20453. The mosquitoes had fed 27-30 days before, during the first to fourth day of fever, on a horse infected with a number of spontaneous strains of horsesickness (virus horse 6, strains 1-7).

Reaction.—The horse was kept under observation for a month, up to June 6th. A slight temperature reaction was noticed 10 days after the injection of the mosquitoes, the temperature being 102° and 103° on May 15th, the 16th 100·8° and 101·8°. Apart from this the highest temperature recorded during the observation period was 101·6°.

Result.—This experiment also was *negative.* The slight temperature reaction noticed could certainly not be regarded as caused by horsesickness fever.

IX.—Experiments with *Mucidus mucidus.*

In all 5 experiments were carried out with this species, in which 16 specimens were injected and 26 refed on normal horses after 5-24 days. 41 specimens engorged on the virus horses.

Virus horse 2, 3 and 4 were used, strains 1-7 and 8 and the following mosquito groups:—

Group 1.—Fed during the night of December 1st to 2nd on virus horse 2. Third day of fever. Temperature 101° and 102·2°. 1 specimen engorged (hatched from larvae). Used for experiment 16.

Group 2.—1 specimen (hatched from larvae) fed for the first time during the night of January 9th to 10th on virus horse 3. First day of fever. Temperature 102·4° and 101·4°. The same specimen was fed for the second time on the same virus horse during the night of January 13th to 14th. Fifth day of fever. Temperature 106·2° and 103°. Used for experiment 17.

Group 3.—Fed on virus horse 4 on January 30th during daytime. Third day of fever, shortly before the death of the horse. Temperature 104·2°. 39 specimens engorged (hatched from larvae). Used for experiment 18-20.

Experiment 16.—1 Mucidus mucidus. *Feeding.* *Interval* 7 *days.* *Horse* 20335.

On December 7th, 1932, 1 *Mucidus* group 1, was fed on this horse together with some *Aëdes caballus* and *A. lineatopennis* (experiment 3). This specimen had fed 7 days before on virus horse 2 injected with a mouse strain of virus (strain 9).

The *reaction* has been described already under experiment 3.

The *result* of the experiment was *negative.*

Experiment 17.—1 Mucidus mucidus. *Injection (and feeding).* *Interval* (11 *and*) 20-24 *days.* *Horse* 20298.

This horse had been used before in November, 1932, in experiment 1 (*Aëdes caballus, A. lineatopennis* and *A. hirsutus.* Feeding. Interval 10-16 days). No temperature reaction had been noticed.

On January 24th, 1933, during the daytime 1 *Mucidus* group 2, was put on to this horse, but it was not absolutely certain if it really had taken up blood. On February 2nd the same mosquito was emulisfied in saline and injected subcutaneously into the same horse. The mosquito had fed twice on virus horse 3 (strain 6) during the night of January 9th-10th and 13th-14th, the first and fifth day of fever. The mosquito had refed after an interval of 11-15 days and was injected after 20 and 24 days.

Reaction.—The (uncertain) feeding of the mosquito was not followed by any reaction. 5 days after the injection a rise of temperature up to 103° was noticed, followed, however, by another period of normal temperature. On February 17th, 15 days after the injection, 99·4° and 102·4° were recorded, the 18th, 101° and 103°, the 19th, 101·2° and 104·2°. 31 days of normal temperature followed, interrupted only by one rise to 103°, lasting, however, only half a day. The horse was kept under observation up to March 22nd, i.e. 48 days after the injection of the mosquitoes.

The horse was used again with negative results on March 23rd in experiment 12 (10 *ëAdes argenteus*, feeding interval 7-8 days).

Immunity test.—The horse was tested for immunity in April. The result of this test was somewhat atypical and has been described under experiment 12.

Result.—The experiment has to be regarded as *negative*, as only a short reaction without clinical symptoms followed the feeding and injection of the mosquitoes.

Experiment 18.—5 Mucidus mucidus. *Injection Interval* 5 *days. Horse* 20331.

Horse 20331 had been used before in November, 1932, in experiment 2 (*Aëdes caballus* and *A. hirsutus*, feeding, interval 15 days) without showing any reaction.

On February 4th, 1933, 5 *Mucidus* of group 3 were injected subcutaneously into this horse. These mosquitoes had fed 5 days before, during the third day fever shortly before death, on virus horse 4 which had been injected with a number of spontaneous strains, original material or 17st to 2nd generations (strains 1-6).

Reaction.—Two days after the injection of the mosquitoes a fever reaction set in. The temperature on February 6th was 102·4° and 104°, the 7th, 99° and 105°, the 8th, 99° and 102·2°, the 9th, 98° and 104°, and the 10th, 100° and 105°. Thereafter some days of subnormal temperatures followed (anus open). The horse was kept under observation up to March 24th, i.e. 48 days after the injection of the mosquitoes. After the reaction already noticed, the temperature remained normal except for two rises to 103° and 102° (February 22nd, and March 9th), which, however, did not last longer than half a day.

On March 24th the same horse was used in experiment 10 (16 *Aëdes dentatus*, feeding, interval 7-19 days) and on May 9th, in experiment 8 (19 *Aëdes lineatopennis*, feeding, interval 17-18 days). Both experiments were negative.

Result.—The experiment has to be regarded as *doubtful*, very probably *negative*. A temperature reaction followed almost immediately upon the injection of the mosquitoes but owing to the strong daily remissions this reaction did not show very much resemblance to horsesickness fever.

Experiment 19.—10 Mucidus mucidus. *Injection. Interval* 10 *days. Horse* 20463.

On February 9th, 10 *Mucidus* of group 3 were emulsified and injected subcutaneously into this horse. These mosquitoes had infected themselves 10 days bofore on virus horse 4 during the third day of fever (strains 1-6).

Reaction.—Shortly after the injection the temperature rose to 102° and the following 3 days was p.m. 101·6°, 101·6° and 102·2°, whereas on each occasion in the morning normal temperatures were recorded. The horse was kept under observation up to March 25th, i.e. 44 days after the injection of the mosquitoes. Except for the first fed days already mentioned, the temperature remained normal (maximum 101·2°).

In March the same horse was used in experiment 11 (*Aëdes nigeriensis*, injection, interval 7-9 days) and in May in experiment 29 (*Anopheles* spp., feeding, interval 18-24 days) without showing a reaction.

Result.—The experiment has to be regarded as *negative*, as no temperature reaction followed the injection of the mosquitoes.

Experiment 20.—25 Mucidus mucidus. *Feeding.*
Interval 15-22 *days. Horse* 20507.

On February 14th, *Mucidus mucidus* group 3 was put on to this horse and during the following night 18 specimens took up blood. The same mosquitoes were fed again during the night of February 21st to 22nd and this time 6 specimens engorged. The mosquitoes had fed originally on a horse injected with different spontaneous strains during the third day of the fever reaction (virus horse 4, strains 1-6). The interval for the first 19 specimens was 15 days, for the second lot of 6 specimens, 22 days.

Reaction.—The horse was kept under observation for 50 days, up to April 5th. Temperatures slightly exceeding 101° were noticed 3 times and on March 23rd, 103·2° was recorded. These elevations, however, were only of half a day's duration.

The same horse was used on April 5th, in experiment 33 (*Hyalomma aegyptium* injection of eggs). No infection resulted.

Result.—The experiment has to be regarded as *negative*, as no real temperature reaction followed the feeding of the 25 *Mucidus.*

X.—Experiments with *Culex annulioris.*

Two experiments were carried out with this species in which 4 specimens were refed and 3 injected later after 19-30 days. 10 specimens were fed on the virus horse.

Virus horse 6 was used, strains 1-7 and the mosquito groups:—

Group 1.—Fed on virus horse 6 on April 5th, during daytime. Temperature 101° and 102·6°. First day of fever. 2 specimens engorged (reared from larvae). Used for experiment 21 and 22.

Group 2.—Fed on the same virus horse during the following night. Temperature 102·6° and 100·4°. First to second day of fever. 8 specimens engorged (reared from larvae). Used for experiment 21 and 22.

Experiment 21.—4 Culex annulioris. *Feeding.*
Interval 19 *days. Horse* 20579.

During the night of April 24th to 25th, 4 *Culex annulioris*, group 1 and 2 combined, engorged on this horse. At the same time *Aëdes argenteus* (cf. experiment 14) and some specimens of different *Anopheles* species (cf. experiment 27) fed on this horse. The *Culex* had infected themselves 19 days previously on a virus horse injected with different spontaneous strains.

Reaction.—The experiment has been described already in full under experiment 14.

Result.—Although a fever reaction was noticed at the time the mosquitoes were feeding, the experiment has to be regarded as *negative.*

Experiment 22.—3 Culex annuelioris. *Injection.*
Interval 30 *days. Horse* 20453.

On May 5th, 3 *Culex annulioris* groups 1-2 were injected subcutaneously together with 40 *Aëdes argenteus* (experiment 15) into horse 20453. The *Culex* had infected themselves 30 days before on a horse injected with material from a number of spontaneous strains.

Reaction.—This experiment has been described already in experiment 15.

Result.—This experiment was also *negative.*

XI.—Experiments with *Anopheles* Species.

In all 9 experiments were carried out in which 326 mosquitoes were either refed on or injected into susceptible horses after intervals varying between 5 and 54 days.

All specimens were reared from larvae. The technique worked out previously for *Aëdes* species could be applied with satisfactory results for these *Anophelines*, as well. To limit the number of horses necessary in these experiments in most cases different species were fed on or injected together into the same horses. In the event of positive results being obtained it was intended to carry out further experiments with single species only.

Virus horses 6 to 8 were used, strains 1 to 8 and the following mosquito groups:—

Anopheles gambiae group 1.—Fed on virus horse 6 during the night of April 5th to 6th, 1933. First days of fever. Temperature 102·6° to 100·4°. 1 specimen engorged (reared from larvae). Used for experiment 24.

Group 2.—Fed on the same virus horse on April 6th, during daytime. First day of fever. Temperature 100·4° to 102·8°. 7 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 3.—Fed on the same horse during the ensuing night. Second day of fever. Temperature 102·8° to 102·2°. Second day of fever. Temperature 102·8° to 102·2°. 18 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 4.—Fed on the same horse during daytime on April 7th. Second day of fever. Temperature 102·2° to 103·4°. 4 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 5.—Fed on the same virus horse during the following night. Third day of fever. Temperature 103·4° to 102·6°. 7 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 6.—Fed during the night of April 21st to 22nd on virus horse 7, a spontaneous case of dikkop ending in recovery. Temperature normal. ? specimens fed (engorged from larvae). Used for experiments 29 and 31.

Group 8.—Fed on the same virus horse one night later. Third day of fever. Temperature 104·8°. 1 specimen fed (reared from larvae). Used for experiments 30 and 31.

Anopheles mauritianus group 1.—Fed on virus horse 6 on April 5th during daytime. First day of fever. Temperature 102·6°. 6 specimens engorged (reared from larvae). Used for experiment 24.

Group 2.—Fed on the same horse the following night. First day of fever. Temperature 102·6° to 100·4°. 10 specimens engorged (reared from larvae). Used for experiments 24, 26 and 31.

Group 3.—Fed on the same horse on April 6th during daytime. Second day of fever. Temperature 100·4° to 102·8°. 5 specimens engorged (reared from larvae). Used for experiments 26 and 31.

Group 4.—Fed on the same horse during the ensuing night. Second day of fever. Temperature 102·8° to 102·2°. 19 specimens engorged (reared from larvae). Used for experiments 26 and 31.

Group 5.—Fed on the same horse one more night later, April 7th to 8th. Third day of fever. Temperature 103·4° to 102·6°. 8 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 6.—Fed during the night of April 21st to 22nd on virus horse 7, a spontaneous case of dikkop ending in recovery. Temperature normal. 7 specimens engorged (reared from larvae). Used for experiments 29 and 31.

Group 7.—Fed during the night of May 2nd to 3rd on virus horse 8, which was infected by injection of mosquitoes. Temperature 105·2° to 103·4°. Second day of fever. 2 specimens fed (reared from larvae). Used for experiment 30.

Anopheles pretoriensis group 1.—Fed on horse 20331 (cf. experiment 20) during the night of February 7th to 8th. Temperature 105° to 99°. 9 specimens engorged (reared from larvae). Used for experiment 23.

Group 2.—Fed on virus horse 6 on April 5th during daytime. First day of fever Temperature 102·6°. 3 specimens engorged (reared from larvae). Used for experiment 24.

Group 3.—Fed on the same virus horse the ensuing night. First day of fever. Temperature 102·6° to 100·4°. 9 specimens engorged (reared from larvae). Used for experiment 24.

Group 4.—Fed on the same horse during the night of April 6th to 7th. Second day of fever. Temperature 102·8° to 102·2°. 20 specimens engorged (reared from larvae). Used for experiments 25 and 31.

Group 5.—Fed on the same horse one night later. Third day of fever. Temperature 103·4° to 102·6°. 7 specimens engorged (reared from larvae). Used for experiemnts 27 and 31.

Group 6.—Fed during the night of April 21st to 22nd on virus horse 7, a spontaneous case of dikkop ending in recovery. Temperature normal. ? specimens engorged (reared from larvae). Used for experiments 29 and 31.

Group 7.—Fed on virus horse 8 (infected by injection of mosquitoes) during the night of May 2nd to 3rd. Second day of fever. Temperature 105·2° to 103·4°. 10 specimens fed (reared from larvae). Used for experiments 30 and 31.

Group 8.—Fed on the same virus horse one night later. Third day of fever. 1 specimen fed (reared from larvae). Temperature 104·8°. Used for experiments 30 and 31.

Anopheles rufipes group 1.—Fed on virus horse 6 during the night of April 6th to 7th. Second day of fever. Temperature 102·8° to 102·2°. 29 specimens engorged (reared from larvae). Used for experiments 25 and 31.

Group 2.—Fed on the same horse during the following night. Third day of fever. Temperature 103·4° to 102·6°. 7 specimens engorged (reared from larvae). Used for experiments 27 and 31.

Group 3.—Fed during the night of April 21st to 22nd, on virus horse 7, a spontaneous case of horsesickness ending in recovery. Temperature normal. ? specimens fed (reared from larvae). Used for experiments 29 and 31.

Group 4.—Fed during the night of May 2nd to 3rd on virus horse 8, which was infected by injections of mosquitoes. Second day of fever. Temperature 105·2° to 103·4°. 12 specimens engorged (reared from larvae). Used for experiments 30 and 31.

Group 5.—Fed on the same horse one night later. Third day of fever. Temperature 104·8°. 3 specimens engorged (reared from larvae). Used for experiments 30 and 31.

Anopheles squamosus group 1.—Fed on virus horse 6 on April 7th during daytime. Second day of fever. Temperature 102·2° to 103·4°. 12 specimens engorged (reared from larvae). Used for experiment 27.

Group 2.—Fed on the same horse during the ensuing night. Third day of fever. Temperature 103·4° to 102·6°. 4 specimens engorged (reared from larvae). Used for experiment 27.

Group 3.—Fed during the night of April 21st to 22nd on virus horse 7, a spontaneous case of dikkop ending in recovery. Temperature normal. 7 specimens engorged (reared from larvae). Used for experiment 29.

Group 4.—Fed during the night of May 2nd to 3rd on virus horse 8, which was infected by injection of mosquitoes. Second day of fever. Temperature 105·2° to 103·4°. 3 specimens engorged (reared from larvae). Used for experiment 30.

Group 5.—Fed on the same horse one night later. Third day of fever. Temperature 104·8°. 1 specimen engorged (reared from larvae). Used for experiment 30.

Experiment 23.—9 Anopheles pretoriensis. *Injection.*
Interval 5 *days.* *Horse* 20512.

On February 13th, 9 *A. pretoriensis* group 1 were emulisfied and injected subcutaneously into this horse. The mosquitoes had fed 5 days before on horse 20331 from experiment 20, which had been injected with 5 *Mucidus*, infected 5 days before. At the time of feeding the mosquitoes we regarded the temperature reaction occurring in this horse as probably due to horsesickness fever. This opinion, however, was not confirmed by the subsequent course of the temperature reaction.

Reaction.—The horse was kept under observation for 44 days, up to March 30th, when it was transferred to another experiment.

No temperature reaction followed the injection of the mosquitoes. Only twice, on March 21st, and 23rd, were temperatures exceeding 101° noticed.

The same horse was used on March 30th in experiment 32 (*Rhipicephalus appendiculatus*, injection of eggs). No infection resulted.

Result.—The experiment was *negative.* However, the importance of the result is very limited as the mosquitoes probably took up no horsesickness virus at all.

Experiment 24.—23 Anopheles gambiae, A. mauritianus *and* A. pretoriensis.
Iujection. Interval 7 days. Horse 20539.

On April 12th, 8 *Anopheles gambiae* group 1, 8 *A. mauritianus* groups 1 and 2 and 7 *A. pretoriensis* groups 2 and 3 were emulsified in saline and injected subcutaneously into horse 20539. These mosquitoes had fed 7 days before, during the first day of fever, on a horse infected with early generations of a number of different spontaneous strains (virus horse 6, strains 1-7).

Reaction.—The temperature remained normal, ranging between 98·8° and 101·8°, during the 18 days up to April 30th. On May 1st, a temperature of 101·8° was noticed, the 2nd, 102·2° and 105·2° and the 3rd, 103·4° and 104·8°. The horse died the following morning. The temperature reaction was quite typical for horsesickness and this diagnosis was confirmed by the post mortem findings, which showed typical dikkop lesions.

Result.—This experiment was certainly *positive.* In 23 specimens belonging to three different *Anopheles* species sufficient virus was present after 7 days to cause an infection with horsesickness followed by death. The incubation period was relatively long, amounting to 19 days.

Experiment 25.—49 Anopheles pretoriensis *and* A. rufipes. *Feeding.*
Interval 11 *days.* *Horse* 20591.

During the night of April 17th to 18th, 20 *A. pretoriensis* group 4 and 29 *A. rufipes* group 1 were fed on this horse. These mosquitoes had infected themselves 11 days previously during the second doy of fever on a horse injected with different spontaneous strains (virus horse 6, strains 1-7).

Reaction.—The horse was too wild to take its temperature regularly. It was kept under observation for 46 days, up to May 29th. No symptoms of horsesickness were noticed during this period.

Result.—Although the development of the temperature could not be followed the experiment has to be regarded as *negative.* Symptoms of horsesickness certainly would have been noticed if they had occurred during this observation period.

Experiment 26.—47 Anopheles mauritianus. *Feeding. Interval* 15-33 *days. Horse* 20578.

During the night of April 21st to 22nd, *Anopheles mauritianus* groups 2 to 4 were put on to this horse and 28 specimens took up blood. The same mosquitoes were fed again during the night of May 8th to 9th, and this time 19 specimens engorged. The horse was thus bitten 47 timese. The mosquitoes had infected themselves on April 5th, and the following two nights on a horse injected with different spontaneous strains during the second to third day of fever (virus horse 6, strains 1-7). For the first feeding of 28 specimens the interval was 15-16 days and 32-33 days for the second feeding of 19 specimens.

Reaction.—The horse was kept under observation for 49 days, up to June 9th. There was no temperature reaction at all following both feedings of mosquitoes, the highest temperatures noticed did not exceed 101°.

Result.—The experiment has to be regarded as *negative*, as no temperature reaction at all followed the feeding of the mosquitoes.

Experiment 27.—31 A. gambiae, A. mauritianus, A. pretoriensis, A. rufipes, *and* A. squamosus. *Feeding. Interval* 17-18 *days. Horse* 20579.

During the night of April 24th to 25th *Anopheles gambiae* groups 2 to 5, *A. mauritianus* group 5, *A. pretoriensis* group 5, *A. rufipes* group 2 and *A. squamosus* groups 1 and 2 were put on to this horse and 18 *A. gambiae*, 3 *A. mauritianus*, 2 *A. pretoriensis*, 1 *A. rufipes* and 7 *A. squamosus* engorged themselves. These mosquitoes had infected themselves on a horse injected with different spontaneous strains (virus horse 6, strains 1-7). The interval, in the case of *A. gambiae* was 17-18 days, in the other species, 17 days.

Ttogether with these Anophelines 38 *Aëdes argenteus* and 4 *Culex annulioris* were fed on the same horse (cf. experiments 14 and 21).

The *reaction* has already been described under experiment 14 (*Aëdes argenteusi*.

The *result* has to be regarded as *negative* for reasons discussed under experiment 14.

Experiment 28.—54 Anopheles gambiae, A. mauritianus, A. pretoriensis, A. rufipes *and* A. squamosus. *Feeding. Interval* 28-36 *days. Horse* 20622.

During the night of May 5th to 6th, the remaining specimens of *Anopheles gambiae* groups 2 to 5, *A. mauritianus* group 5, *A. pretoriensis* groups 4 and 5, *A. rufipes* groups 1 and 2 and *A. squamosus* groups 1 and 2 were put on to this horse and 6 *A. gambiae*, 2 *A. mauritianus*, 10 *A. pretoriensis*, 11 *A. rufipes* and 2 *A squamosus*, together 31 specimens, engorged. These mosquitoes had fed previously in experiment 25 or 27. The same mosquitoes were fed again on the same horse during the night of May 12th to 13th, and this time 23 specimens, 5 *A. gambiae*, 3 *A. mauritianus*, 5 *A. pretoriensis*, 9 *A. rufipes* and 1 *A. squamosus* fed. The mosquitoes had originally infected themselves on a horse injected with different spontaneous strains (virus horse 6, strains 1-7). The interval in this experiment was—

for *A. gambiae*: 6 specimens 28-29 days, 5 specimens 35-36 days;
for *A. mauritianus*: 2 specimens 28 days, 3 specimens 35 days;
for *A. pretoriensis*: 10 specimens 28-29 days, 5 specimens 35-36 days.
for *A. rufipes*: 11 specimens 28-28 days, 9 specimens 35-36 days;
for *A. squamosus*: 2 specimens 28 days, 1 specimen 35 days.

Reaction.—The horse was kept under observation up to June 15th, i.e. for 41 days. On May 18th and 19th, 13 days after the first and 6 after the last feeding of the mosquitoes a slight reaction was noticed, the temperatures being on the 18th (p.m.), 101·8°, the 19th, 100·4° and 102·2°. The next day, however, the temperature was almost normal again, not xeceeding 101·2° until the end of the observation period.

Result.—The experiment has to be regarded as *negative*, as no real temperature reaction followed bith feedings of the mosquitoes.

Experiment 29.—47 Anopheles gambiae, A. mauritianus, A. pretoriensis, A. rufipes, *and* A. squamosus. *Feeding. Interval* 18-24 *days. Horse* 20463.

The same horse had been used previously in February in experiment 19 (*Mucidus mucidus*, injection, interval 10 days) and in March in experiment 11 (*Aëdes nigeriensis*, injection, interval 7-9 days). No reaction had resulted from these experiments.

During the night of May 9th to 10th, *Anopheles gambiae* group 6, *A. mauritianus* group 6, *A. pretoriensis* group 6, *A. rufies* group 3 and *A. squamosus* group 3 were put on to this horse and 2 *A. gambiae*, 2 *A. mauritianus*, 21 *A. pretoriensis*, 11 *A. rufipes*, and 3 *A. squamosus* engorged. The same mosquitoes were fed once more during the night of May 15th to 16th, and this time 1 *A. gambiae*, 2 *A. mauritianus*,, 4 *A. pretoriensis*, and 1 *A. rufipes* took up blood. These mosquitoes had been fed originally, during the night of April 21st to 22nd, on a horse recovering from a natural attack of horsesickness (virus horse 7, strain 8). The interval for the two lots of mosquitoes was:—

A. gambiae: 2 specimens 18 days, 1 specimen 24 days.

A. mauritianus: 2 specimens 18 days, 2 specimens 24 days.

A. pretoriensis: 21 specimens 18 days, 4 specimens 24 days.

A. rufipes: 11 specimens 18 days, 1 specimen 24 days.

A. squamosus: 3 specimens 18 days.

Reaction.—The horse was kept under observation up to June 15th, i.e. 36 days after the feeding of the first lot of mosquitoes. No reaction resulted, the highest temperature recorded being 101°.

Result.—The experiment has to be regarded as *negative*, no reactions following both feedings of the mosquitoes.

Experiment 30.—29 Anopheles gambiae, A. mauritianus, A. pretoriensis, A. rufipes, A. squamosus. *Feeding. Interval* 7-18 *days. Horse* 20623.

During the night of May 10th to 11th, *Anopheles gambiae* groups 7 and 8, *A. mauritianus* group 7, *A. pretoriensis* groups 7 and 8, *A. rufipes* groups 4 and 5, *A. squamosus* groups 4 and 5 were put on to this horse and 21 specimens, 4 *Anopheles gambiae*, 1 *A. mauritianus*, 5 *pretoriensis*, 10 *A. rufipes*, 1 *A. squamosus* engorged. The remaining specimens of the same mosquito groups were fed again during the night of May 15th to 16th, and this time 8 specimens, 3 *Anopheles pretoriensis*, 4 *A. rufipes*, and 1 *A. squamosus* engorged. These mosquitoes had been fed originally, during the night of May 2nd to 3rd, and 3rd to 4th on a horse which had acquired an infection by injection of Anophelines infected 7 days previously (experiment 24). The intervals for the two lots of mosquitoes were:—

A. gambiae: 4 specimens 7-8 days.

A. mauritianus: 1 specimen 7-8 days.

A. pretoriensis: 5 specimens 7-8 days, 3 specimens 12-13 days.

A. rufipes: 10 specimens 7-8 days, 4 specimens 12-13 days.

A. squamosus: 1 specimen 7-8 days, 1 specimen 12-13 days.

Reaction.—On May 23rd, 13 days after the feeding of the first and 8 days after the second batch of mosquitoes, the temperature rose up to 103°, remaining at 102·6° the ensuing morning. The temperature was irregular during the following days, varying between 98° and 102°. On June 6th, the temperature was 102·8° and 104·8°, the 7th, 101·4° and 103°, the 8th, 102·8° and 100·2°. A few days of normal temperature followed. The horse, however, was in a very poor condition and could not rise any more and was killed in extremis on June 12th, 28 days after the feeding of the last and 33 days after the first batch of mosquitoes. The temperature elevation and the death of the horse was not due to horsesickness. The animal had a nephritis together with pneumonia and the post mortem revealed an intoxication following on pneumonia as the cause of death.

Result.—The experiment has to be regarded as *negative*. The animal died of pneumonia although the observation period was long enough for a horsesickness infection to have become established.

Experiment 31.—37 Anopheles gambiae, A. mauritianus, A. pretoriensis *and* A. rufipes. *Injection. Interval* 27-54 *days. Horse* 20621.

On May 30th, 37 Anophelines of different groups were still living and to conclude this series of experiments they were emulsified in saline and injected subcutaneously into horse 20621 together with some *Aëdes lineatopennis*. The lot of Anophelines comprised 4 *Anopheles gambiae*, 2 of groups 2-5, 1 of group 6 and 1 of groups 7 and 8, 5 *A. mauritianus*, 4 of groups 2-5 and 1 of group 6, 10 *A. pretoriensis*, 1 of groups 4 and 5, 6 of group 6 and 3 of groups 7-8, and 18 *A. rufipes*, 8 of groups 1-2, 1 of group 3 and 9 of groups 4-5. These mosquitoes had fed between April 6th, and May 3rd, on three different virus horses. One of these horses was injected with early generations of a number of spontaneous strains (virus horse 6, strains 1-7), the second was a natural case of dikkop ending in recovery but showing no fever at the time of the feeding of the mosquitoes (virus horse 7, strain 8), and the third horse was infected by injection of Anophelines, which had fed a week previously on the first of these virus horses (experiment 24). The length of the interval for the different groups of mosquitoes was:—

A. gambiae: 1 specimen 27-28 days, 1 specimen 39 days, 2 specimens 52-54 days.

A. mauritianus: 1 specimen 39 days, 4 specimens 52-54 days.

A. pretoriensis: 3 specimens 27-28 days, 6 specimens 39 days, 1 specimen 53-54 days.

In all 37 specimens were injected, 13 after 27-28 days, 9 after 39 and 15 specimens after 52-54 days.

Reaction.—The reaction has been described already under experiment 9 (*Aëdes lineatopennis*, injection, interval 38-39 days).

Result.—Negative.

XII.—Experiments with Ticks.

In the last two experiments eggs of ticks, which were collected in the adult stage from a spontaneous case of horsesickness, were injected into susceptible horses, to see if the virus could pass into the eggs and so be transmitted in this way to the next generation.

Experiment 32.—*Eggs of* Rhipicephalus appendiculatus. *Injection. Horse* 20512.

The horse had been used previously on February 13th in experiment 23 (9 *Anopheles pretoriensis*, injection, interval 5 days). The result was negative.

On March 30th, part of the eggs of 10 specimens of *Rhipicephalus appendiculatus*, which had been collected on March 14th, from virus horse 5, were emulsified in serum and injected subcutaneously into horse 20512.

Reaction.—The day after the injection, on March 31st, the temperature rose up to 103·4° and remained during the following day, April 1st, between 102·6° and 103·6°. On April 2nd, 102° and 101° were registered, the 3rd, 100·4° and 101·8°. Thereafter the temperature remained normal except for one rise up to 101·2° on April 9th. On May 5th, the horse was discharged after an observation period of 36 days.

Result.—The experiment has to be regarded as *negative*. Only immediately after the injection of the eggs was a rise in temperature recorded, which however was caused by the injection itself.

Experiment 33.—*Eggs of* Hyalomma aegyptium *Injection. Horse* 20507.

This horse had been used on February 14th, in experiment 20 (25 *Mucidus mucidus*, feeding, interval 15-22 days), the result of which had been negative.

On April 5th, part of the eggs of one specimen of *Hyalomma aegyptium*, which had been collected on March 14th, from virus horse 5, were emulsified in serum and injected subcutaneously into this horse.

Reaction.—The day after the injection the temperature began to rise. On April 6th it was 102·2° and 101·4°, the 7th, 103·6° and 101·2°, the 8th, 102° and 103·4°, the 9th, 100°. The following 4 days evening temperatures of 102°, 101·4°, 101·8° and 102·2° were registered. Thereafter the temperature became normal, not exceeding 101°. The horse was discharged on May 5th, 30 days after the injection of the eggs.

Result.—This experiment has also to be regarded as *negative*. The injection was immediately followed by a reaction whereafter the temperature reurned to normal.

FIGS. 1-4.—Breeding places of Anophelines near Onderstepoort.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.