Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Results of a Mosquito Survey at Onderstepoort during the Summer 1931-32 in connection with the Transmission of Horsesickness.

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Notwithstanding the fact that work on the transmission of horsesickness was commenced as long as 30 years ago, the actual natural transmission of this disease has up to the present not been cleared up.

According to general opinion mosquitoes are strongly suspected of being the transmitting agents, although this viewpoint does not find unanimous support amongst the farming community in South Africa amongst whom a diversity of opinions exists. We will, however, discuss these latter theories more fully in another paper confining ourselves here solely to the main facts.

Horsesickness is not contagious. Its occurrence under natural conditions is restricted normally to the summer months and the severity of an outbreak depends mainly on the amount of rainfall. Infections generally take place only at night, round about sunset or sunrise. Horses kept in screened stables at night escape infection even in known bad horsesickness areas. These facts collectively are to be regarded as strong indications that mosquitoes are involved in the transmission of this disease and when we started our work on the transmission of horsesickness in 1931 mosquitoes received primary attention.*

* This paper constitutes the first of a series dealing with the transmission of horsesickness and bluetongue of sheep, carried out at the Onderstepoort Laboratories.

One of the authors (O.N.) remained in South Africa during the winter 1931 and the summer 1931/32 as a foreign Fellow of the Rockefeller Foundation and continued work during the summer 1933, assisted by a Research Grant of the Rockefeller Foundation and a grant from the South African Government. He wishes to express his deep indebtedness to the Rockefeller Foundation for the generous support granted him, which made it possible for him to study these interesting problems, and to Dr. P. J. du Toit, Director of Veterinary Services and Animal Industry, for the generous hospitality extended to him at the Onderstepoort Laboratories. He also wishes to express his gratitude to Prof. Dr. L. de Blieck, Director of the Institute for Parasitic and Infectious Diseases of Utrecht, who allowed him the long leave, necessary for the journeys to South Africa.

In order to obtain the necessary information about the species of mosquitoes most probably connected with the transmission of the disease under review, a mosquito survey was carried out at Onderstepoort during the summer months of 1931-32. At the same time, however, suitable experimental methods had to be worked out and the transmission experiments themselves conducted. The desired degree of completeness in this survey could, therefore, not be expected.

The original farm on which Onderstepoort stands was known in former years as a particularly bad place for horsesickness. Although subsequently many important alterations have taken place in the neighbourhood of Onderstepoort, notably the construction of the Bon Accord Dam, the site was regarded as suitable for this research, as we considered that the breeding places of the most probable transmitters could quite reasonably still be expected to exist. The success of the work, however, depended largely on climatic factors, namely, as to whether these would be such as to result in a real horsesickness season or not. In this respect we were disappointed.

I. CLIMATIC CONDITIONS DURING THE SEASON 1931-32 AT ONDERSTEPOORT.

As previously stated, all available information tends to indicate that outbreaks of horsesickness depend mainly on the amount of rainfall during the summer months. The rainfall recorded from October, 1931, until April, 1932, at the meteorological post at Onderstepoort is shown in Table 1.

TABLE 1.

	1931.			1932.			
	October.	November.	December.	January.	February.	March.	April.
	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.
1..........	—	—	·14	—	—	—	—
2..........	—	·13	·01	—	·66	—	—
3..........	—	·025	—	·40	·10	—	—
4..........	·70	·28	·44	—	1·05	—	—
5..........	—	—	—	—	·30	—	—
6..........	—	1·55	—	·29	—	—	—
7..........	—	·11	—	—	—	—	—
8..........	—	·12	—	—	—	·07	—
9..........	—	·01	—	—	—	1·58	—
10..........	·10	·53	—	—	—	—	—
11..........	·01	—	—	·03	—	—	—
12..........	—	—	—	—	—	·05	—
13..........	—	—	—	—	—	—	—
14..........	—	—	—	—	·16	—	—
15..........	—	—	—	—	—	—	—
16..........	—	—	—	—	—	—	—
17..........	—	—	·40	—	1·40	—	—
18..........	—	—	·12	—	—	—	—
19..........	—	·02	—	·04	·43	—	—
20..........	·39	—	·05	1·30	·05	·29	—
21..........	·46	·09	·14	1·77	—	·05	—
22..........	—	·65	·01	—	—	—	—
23..........	·09	·64	—	—	—	—	·17
24..........	—	—	—	—	—	—	·23
25..........	—	·24	—	—	·20	—	·04
26..........	—	·30	·07	·02	—	—	·31
27..........	·03	—	—	—	—	—	—
28..........	·22	—	—	·14	·48	—	—
29..........	·45	—	—	—	·02	·04	—
30..........	—	·38	—	—	—	—	—
31..........	—	—	·27	—	—	—	—
Per Month..	2·45	5·075	1·65	3·99	4·85	2·08	·75

The total amount of rain during this period was 20·84 inches, the maximum per month 5·07 inches in November, and the minimum 0·75 inches in April. There were 9 days with rain in October, 15 days in November, 10 days in December, 8 days in January, 11 days in February, 6 days in March and 4 days in April, or 63 days altogether in 7 months. Only those days on which at least $\frac{1}{4}$ inch rain is recorded are of any real value, the soil immediately drying up again with less than this amount. In the season in question, therefore, we were confronted with 3 suitable days in October, 7 days in November, 3 days in December, 3 days in January, 4 days in February, 2 days in March and 1 day in April, giving a total of 23 days which were in any degree suitable.

To be sure of good breeding, at least one inch of rain is necessary to start with, which must be followed by an additional fall during the ensuing 5-7 days in order to ensure complete larval and pupal development. A rainfall of 1 inch or more, with 1·77 as the maximum, was noted on 1 day in November, not at all in December, twice in January, twice in February, and once in March, altogether on 6 out of more than 200 days.

The rainfall actually corresponded with that of a very dry season which was further borne out by observations on plant life and the level of water present in the Bon Accord Dam, lying in the immediate neighbourhood of the Onderstepoort Laboratories, which was far below that of normal seasons. As a result of the dry season, only a few isolated cases of horsesickness occurred at Onderstepoort and the adjoining farms towards the end of the season, and in only one of these cases did no history of a previous immunization exist.

To obtain a clear idea of the rainfall in a bad horsesickness season, we must compare the rainfall in the season under review with that of the summer 1917-18 and 1922-23, when severe outbreaks of horsesickness occurred at Onderstepoort and the adjoining farm Kaalplaas, which also belongs to the Laboratory. This difference is clearly shown in Table 2 and Graph 1.

Graph I.

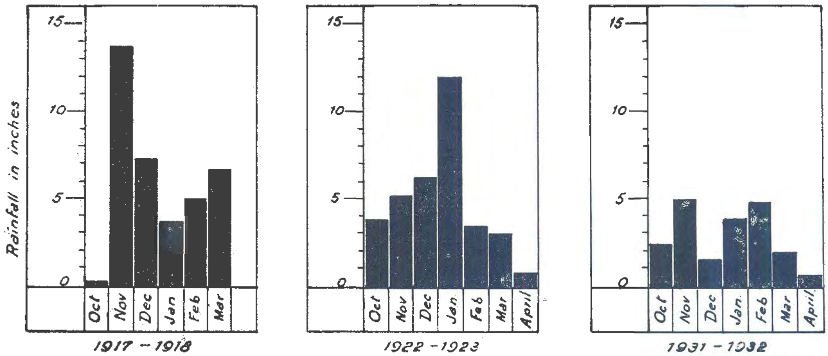

Fig. 1.—Monthly distribution of rain during two good horsesickness seasons (1917-1918 and 1922-1923) and during the season 1931-1932.

TABLE 2.

	1917-1918.	1922-1923.	1931-1932.
July	·40	—	1·43
August	2·07	3·07	—
September	·34	·31	—
October	·35	3·97	2·45
November	13·82	5·26	5·075
December	7·35	6·30	1·65
January	3·74	12·05	3·99
February	5·11	3·58	4·85
March	6·89	3·12	2·08
April	—	·96	·75
May	—	·01	—
June	—	·12	—
JULY–JUNE	40·07	38·75	22·275
NOVEMBER–APRIL	36·91	31·47	18·395

Comparison between the rainfall in 1917-1918 and 1922-1923, two notable horsesickness seasons, with the season under review.

2. SPECIES OF MOSQUITOES RECORDED FROM SOUTH AFRICA.

In South Africa the following 102 species of mosquitoes belonging to 13 different genera have been found up to the present:—

Tribe Anophelini.

1. *Anopheles (Anopheles) mauritianus* Daruty & d'Emmerez.
2. *Anopheles (Myzomyia) ardensis* (Theobald).
3. *Anopheles (Myzomyia) cinereus* Giles.
4. *Anopheles (Myzomyia) cinereus* Giles.
5. *Anopheles (Myzomyia) funestus* Giles.
   Anopheles (Myzomyia) funestus leesoni Evans.
6. *Anopheles (Myzomyia) gambiae* Giles.
7. *Anopheles (Myzomyia) jacobi* (Hill & Haydon).
8. *Anopheles (Myzomyia) listeri* De Meillon.
9. *Anopheles (Myzomyia) longipalpis* Theobald.
10. *Anopheles (Myzomyia) maculipalpis* Giles.
11. *Anopheles (Myzomyia) marshalli* (Theobald).
12. *Anopheles (Myzomyia) natalensis* (Hill & Haydon).
13. *Anopheles (Myzomyia) nili* Theobald.
14. *Anopheles (Myzomyia) pharoensis* Theobald.
15. *Anopheles (Myzomyia) pretoriensis* Theobald.
16. *Anopheles (Myzomyia) rhodesiensis* Theobald.
17. *Anopheles (Myzomyia) rufipes* (Gough).
18. *Anopheles (Myzomyia) squamosus* (Theobald).
19. *Anopheles (Myzomyia) squamosus sydippis* de Meillon.
20. *Anopheles (Myzomyia) theileri* Edwards.
19. *Anopheles (Myzomyi) squamosus cydippis* De Meillon.
20. *Anopheles (Myzomyia) demeilloni* Evans.
   Anopheles (Myzomyia) demeilloni carteri Evans & De Meillon.

20a. *Anopheles (Myzomyia) garnhami* Edwards.
   Anopheles (Myzomyia) garnhami walshi Evans and De Meillon.

Tribe Culicini.

21. *Aëdes (Aëdimorphus) abnormalis* (Theobald).
22. *Aëdes (Rëdimorphus) albocephalus* (Theobald).
23. *Aëdes (Aëdimorphus) alboventralis* (Theobald).
24. *Aëdes (Aëdimorphus) apicoannulatus* (Edwards)
25. *Aëdes (Aëdimorphus) argenteopunctatus* (Theobald).
26. *Aëdes (Aëdimorphus) bevisi* Edwards.
27. *Aëdes (Aëdimorphus) capensis* (Edwards).
28. *Aëles (Aëdimorphus) cumminsi* (Theobald).
29. *Aëdes (Aëdimorphus) dentatus* (Theobald).
30. *Aëdes (Aëdimorphus) durbanensis* (Theobald).
31. *Aëdes (Aëdimorphus) fascipalpis* (Edwards).
32. *Aëdes (Aëdimorphus) filicis* Ingram & De Meillon.
33. *Aëdes (Aëdimorphus) furcifer* (Edwards).
34. *Aëdes (Aëdimorphus) marshalli* (Theobald).
36. *Aëdes (Aëdimorphus) minutus* (Theobald).
37. *Aëdes (Aëdimorphus) ochraceus* (Theobald).
39. *Aëdes (Aëdimorphus)punctothoracis* (Theobald).
40. *Aëdes (Aëdimorphus) quasiunivittatus* (Theobald).

41. *Aëdes (Aëdimorphus) tarsalis* (Newstead).
42. *Aëdes (Banksinella) lineatopennis* Ludlow.
    Aëdes (Banksinella) lineatopennis circumluteola Theobald.
43. *Aëdes (Banksinella) luteolateralis* Theobald.
    Aëdes (Banksinella) luteolateralis flavinervis Edwards.
44. *Aëdes (Finlaya) barnardi* Edwards.
45. *Aëdes (Ochlerotatus) caballus* (Theoabld).
46. *Aëles (Stegomyia) argenteus* (Poiret).
47. *Aëdes (Stegomyia) metallicus* Edwards.
48. *Aëdes (Stegomyia) poweri* Theobald.
49. *Aëdes (Stegomyia)pseudonigeria* (Theobald).
50. *Aëdes (Stegomyia) unilineatus* (Theobald).
51. *Aëdes (Stegomyia) simpsoni* (Theobald).
52. *Aëdes (Stegomyia) subargenteus* Edwards.
53. *Aëdes (Stegomyia) vittatus* Bigot.
54. *Armigeres argenteoventralis* Theobald.
55. *Culex annulioris* Theobald.
56. *Culex aurantapex* Edwards.
57. *Culex bitaeniorhynchus* Giles.
58. *Culex consimilis* Newstead.
59. *Culex decens* Theobald.
60. *Culex duttoni* Theobald.
61. *Culex fatigans* Wiedemann.
62. *Culex laurenti* Newstead.
63. *Culex pallidocephalus* Theobald.
64. *Culex pérengueyi* Edwards.
65. *Culex pipiens* Linné.
66. *Culex pulchrithorax* Edwards.
67. *Culex quasigelidus* Theobald.
68. *Culex rima* Theobald.
69. *Culex salisburiensis* Theobald.
70. *Culex sergenti* Theobald.
71. *Culex simpsoni* Theobald.
72. *Culex thalassius* Theobald.
73. *Culex theileri* Theobald.
74. *Culex trifilatus* Edwards.
75. *Culex univittatus* Theobald.
76. *Culex vansomeri* Edwards.
77. *Culex (Culicomyia) nebulosus* Theobald.
78. *Culex (Micraedes) inconspicuosus* Theobald.
79. *Eretmopodites chrysogaster* Graham.
80. *Eretmopodites quinquevittatus* Theobald.
81. *Eretmopodites silvestris* Ingram & De Meillon.
82. *Harpagomyia trichorostris* Theobald.
83. *Lutzia tigripes* Daruty & d'Emmerez).
84. *Megarhinus (Toxorhynchites) brevipalpis* Theobald.
85. *Mimomyia hispida* (Theobald).
86. *Mimomyia mimomyiaformis* (Newstead).
87. *Mucidus mucidus* Karsch.
88. *Mucidus scatophagoides* Theobald.
89. *Taeniorhynchus (Taeniorhynchus) aureus* Edwards.
90. *Taeniorhynchus (Taeniorhynchus) chubbi* Edwards.
91. *Taeniorhynchus (Taeniorhynchus) fuscopennatus* Theobald.
92. *Taeniorhynchus (Taeniorhynchus) metallicus* (Theobald).

93. *Taeniorhynchus (Mansonioides) africanus* Theobald.
94. *Taeniorhynchus (Mansonioides) mediolineatus* Theobald.
95. *Taeniorhynchus (Mansonioides) uniformis* Theobald.
96. *Theobaldia (Allotheobaldia) longiariolata* Macquart.
97. *Uranotaenia alba* Theobald.
98. *Uranotaenia bilaeniata fraseri* Edwards.
99. *Uranotaenia candidipes* Edwards.
100. *Uranotaenia fusca* Theobald.
101. *Uranotaenia mashonaensis* Theobald.
102. *Uranotaenia montana* Ingram & De Meillon.

3. MOSQUITO SPECIES FOUND AT ONDERSTEPOORT.

It was naturally impossible to conduct transmission experiments with every one of these 102 species and no useful purpose would have been served. Unlikely species had, therefore, to be eliminated. As already noted Onderstepoort could be regarded as a good place for horsesickness work and the natural transmitter of the disease could be expected to occur here in fair numbers at any rate. Species not present at Onderstepoort could therefore be disregarded.

During the course of the work, 24 species of mosquitoes were found towards the end of the winter of 1931 and summer of 1931-32.

In the mosquito traps, which will be described in the next chapter, the following 16 species were caught as adults:—

1. *Anopheles cinereus.*
2. *Anopheles mauritianus.*
3. *Anopheles squamosus.*
4. *Aëdes caballus.*
5. *Aëdes dentatus.*
6. *Aëdes hirsutus.*
7. *Aëdes lineatopennis.*
8. *Aëdes poweri.*
9. *Aëdes punctothoracis.*
10. *Culex annulioris.*
11. *Culex decens.*
12. *Culex fatigans.*
13. *Culex theileri.*
14. *Culex univittatus.*
15. *Lutzia tigripes.*
16. *Taeniorhynchus africanus.*

The following species were found in the larval stage in their breeding grounds:—

17. *Anopheles pretoriensis.*
18. *Aëdes argenteus.*
19. *Aëdes durbanensis.*
20. *Aëdes nigeriensis.*
21. *Aëdes vittatus.*
22. *Culex duttoni.*
23. *Culex salisburiensis.*
24. *Mucidus scatophagoides.*

Of these species, *Aëdes durbanensis* and *A. nigeriensis* were recorded from Onderstepoort and the Transvaal for the first time. The following were collected both as larvae and adults:—

Aëdes caballus.
Aëdes dentatus.
Aëdes hirsutus.
Aëdes linneatopennis.
Anopheles squamosus.
Culex theileri.
Lutzia tigripes.

It must be mentioned here that no special effort was made during this season to procure as many species as possible.

Besides the species mentioned above the following have previously been collected at Onderstepoort (cf Bedford 13th and 14th Rept. Dir. of Vet. Ed. and Res. 1928):—

25. *Anopheles argenteolobatus.*
26. *Anopheles ardensis.*
27. *Anopheles funestus.*
28. *Anopheles gambiae.*
29. *Anopheles maculipalpis.*
30. *Anopheles marshalli.*
31. *Anopheles natalensis.*
32. *Anopheles rhodesiensis.*
33. *Anopheles rufipes.*
34. *Anopheles theileri.*
35. *Anopheles demeilloni.*
36. *Aëdes cumminsi.*
37. *Aëdes quasiunivittatus.*
38. *Aëdes simpsoni.*
39. *Culex bitaeniorhynchus.*
40. *Culex pulchrithorax.*
41. *Culex quasigelidus.*
42. *Culex simpsoni.*
43. *Mucidus mucidus.*
44. *Taeniorhynchus fuscopennatus.*
45. *Taeniorhynchus uniformis.*
46. *Theobaldia longiareolata.*
47. *Uranotaenia candidipes.*

Altogether therefore, 47 species of mosquitoes belonging to the following 8 genera are known to occur at Onderstepoort up to the present:—

Anopheles	15	species.
Aëdes	13	,,
Culex	11	,,
Lutzia	1	,,
Mucidus	2	,,
Taeniorhynchus	3	,,
Theobaldia	1	,,
Uranotaenia	1	,,

The finding of 2 species of *Aëdes* during this survey not previously known to occur in the Transvaal indicates that this list might not yet be complete, although the more common species have certainly all been included.

4. RESULTS OF CATCHING MOSQUITOES BY MEANS OF TRAPS.

The number of mosquito species present at Onderstepoort is still far too large to make it practicable to use them all for transmission experiments. Further selection on epidemiological grounds is, therefore, still necessary.

In order to obtain some information as to the relative abundance of the mosquito species flying around at night, we used specially constructed traps, containing a horse as bait animal. Traps of this sort had already been used in the earlier mosquito work at Onderstepoort.

These traps consist of a wooden framework covered with thin mosquitoproof hessian and protected against rain by waterproof linen. This mosquito tent contains a horsebox into which a horse is put in the late afternoon, at 5 o'clock, the animal remaining there over-night. The entrance is closed in such a manner that a small vertical slit remains open between the two overlapping parts of the hessian forming the entrance. Mosquitoes, attracted by the animal can enter through this slit, but it is difficult for them to find their way out again. When looked for the next morning they are found resting under the roof or at the sides, often in some dark corner behind the wooden poles supporting the tent.

Two of these traps were placed some distance outside the laboratory. The first trap was erected towards the east near the Aapies river under some trees at the edge of an area of bushveld. The second trap was placed about two-thirds of a mile to the north of the laboratory on a small road sheltered by trees between the native location and a large portion of open bushveld surrounding the Bon Accord dam. In the neighbourhood, breeding places of *Aëdes caballus*, *A. lineatopennis* and *A. dentatus were* present.

On some occasions lights were kept burning all night in the traps with the intention of making them more attractive to mosquitoes. However, no constant differences between dark and illuminated traps were observed.

The first trap was ready for use on September 24th, and the second in the beginning of November, 1931. The use of both traps was discontinued on February 11th, 1932. A list of mosquitoes collected in these traps is given in Table 3.

Altogether 1,224 mosquitoes were caught, representing 16 species. Of these 21 specimens belonged to *Anopheles*, 683 to *Aëdes*, 517 to *Culex*, and 3 to *Taeniorhynchus* and *Lutzia* together.

The *Anophelines* were very rare, only 21 specimens belonging to 3 species, *A. cinereus*, *A. mauritianus* and *A. squamosus*, being found. The season, therefore, must have been very unfavourable for *Anopheles* breeding. Furthermore, *A. squamosus*, to which belonged two-thirds of the total number, is a river breeding species not depending directly on rainfall.

Of the 5 *Culex* species present amongst our material, *C. theileri* was the only common species. Nearly three-quarters of them were caught in October in the trap alongside the Aapies river, where they were breeding in large numbers. It is a species common throughout the year with no special summer preference.

TABLE 3.

List of Mosquitoes collected in Mosquito Traps at Onderstepoort between 24th September, 1931, and 11th February, 1932.

Month.	September.	October.	November.		December.		January.		February.		Total.
Trap.	I	I	I	II	I	II	I	II	I	II	I II
1. *An. cinereus*.......	—	3	—	—	—	—	1	—	—	—	4
2. *An. mauritianus*...	—	—	—	—	2	—	2	—	—	—	4
3. *An. squamosus*.....	1	9	1	—	—	1	1	—	—	—	13
4. *Aëdes caballus*......	—	—	1	13	—	5	—	1	1	—	21
5. *Aëdes dentatus*.....	—	—	77	107	17	65	10	11	62	96	455
6. *Aëdes hirsutus*......	—	—	—	—	—	2	1	1	—	—	4
7. *Aëdes lineatopennis*.	—	2	49	28	8	11	4	10	30	44	186
8. *Aëdes poweri*.......	—	—	—	—	—	4	1	1	—	—	6
9. *A. punctothoracis*...	—	—	—	1	1	8	—	—	—	1	11
10. *C. annulioris*.......	—	—	—	—	3	—	—	1	—	1	5
11. *C. decens*..........	—	—	—	—	4	1	2	2	2	1	12
12. *C. fatigans*.........	—	—	—	—	3	4	1	—	1	—	9
13. *C. theileri*..........	9	327	13	15	48	29	25	8	—	14	488
14. *C. univittatus*......	—	—	—	—	—	—	3	—	—	—	3
15. *L. tigripes*.........	—	1	—	—	—	—	—	—	—	—	1
16. *T. africanus*.......	—	—	—	—	—	—	2	—	—	—	2

Taeniorhynchus africanus was represented by 2 specimens, and *Lutzia tigripes* only by one. This latter species so far as we know, is not a bloodsucker.

The *Aëdes* species were the most numerous. In September 0 out of 10 belonged to them; in October, 2 out of 342; in November, 276 out of 305; in December, 121 out of 216; in January, 40 out of 77; and in February, 234 out of 253 specimens.

This distribution of the *Aëdes* species coincides very closely with the rainfall (cf. Table 1). The largest numbers were caught in November, this month, especially during its first 10 days, having had a good and well-distributed rainfall. December was very dry, only 1·65 inches of rain having fallen. The number of mosquitoes was reduced, but still remained fairly large, a fair number of those which hatched out in November undoubtedly still being alive. In January the effect of the drought in December was well demonstrated by the extremely small number of specimens collected. On 20th and 21st January, more than 3 inches of rain fell and in February large numbers of mosquitoes re-appeared again. In 11 days' time nearly 6 times as many mosquitoes were caught as during the whole of January.

Of the 6 *Aëdes* species found, A. dentatus, with 455 specimens, was by far the most common. Then followed *A. linneatopennis* with 186 specimens, whereas of *A. caballus*, 21, of *A. punctothoracis*. 11, of *A. poweri*, 6, and *A. hirsutus*, 4 specimens were collected. This numerical distribution of specimens over the different species, however, could not coincide with the actual composition of the mosquito fauna in the field. On several occasions we were breeding out thousands and thousands of mosquitoes, especially *A. caballus* within a few hundred yards of our second trap, but notwithstanding this, only very few specimens could be recovered from the trap. *The traps therefore failed to give us an accurate indication of the relative abundance of the mosquitoes present in the field*, which was the main object of their use and that is the reason why further work with them was discontinued in February.

It is a well-known fact, that some species of mosquitoes regularly enter houses or stables at night, whereas others are only exceptionally found inside rooms. Our mosquito tent seemed to have the same effect on these insects as a house or stable. Some species were attracted by it and the bait animal inside and consequently were caught in large numbers. Other species only occasionally entered the traps and some others not at all. Horsesickness, however, is a disease transmitted in the field and only very exceptionally in stables. Thus, instead of giving us the information desired, *the traps merely showed a tendency to select those species, which were less likely to be connected with horsesickness.* This negative information, however, was also of some importance as we will see in the final section of this paper.

5. BREEDING PLACES AND BREEDING HABITS OF SOME MOSQUITO SPECIES FOUND AT ONDERSTEPOORT.

The most valuable information, so far as this could be expected in a not very favourable season, we hoped to obtain from a survey of the breeding places in the neighbourhood of the laboratory. To this we devoted quite an important part of our time.

The Onderstepoort Laboratories are built on what was formerly a farm about 8 miles distant from Pretoria near a small river, the Aapies river, which enters the Bon Accord dam. The Laboratory buildings, houses, stables, farm yard and some areas of cultivated land form a centre, which for a large part is surrounded by typical Transvaal bushveld, more or less untouched except by the grazing of animals. Just at the edge of this veld in particular we searched for breeding places and were fortunate in finding all the different conditions of the veld to be present within a few hours' walk, so that regular visits were possible without interfering too much with the experimental work.

In the accompanying map (Fig. 2) the site of the main breeding places in the neighbourhood of the Laboratory is shown.

A. Breeding Places of *Anopheles* Larvae.

Anopheline larvae were very scarce but were found from time to time together with those of *C. theileri* in backwaters of the river from October onwards. Most of them proved to be *Anopheles squamosus* and later in the summer a few larvae of *A. pretoriensis*

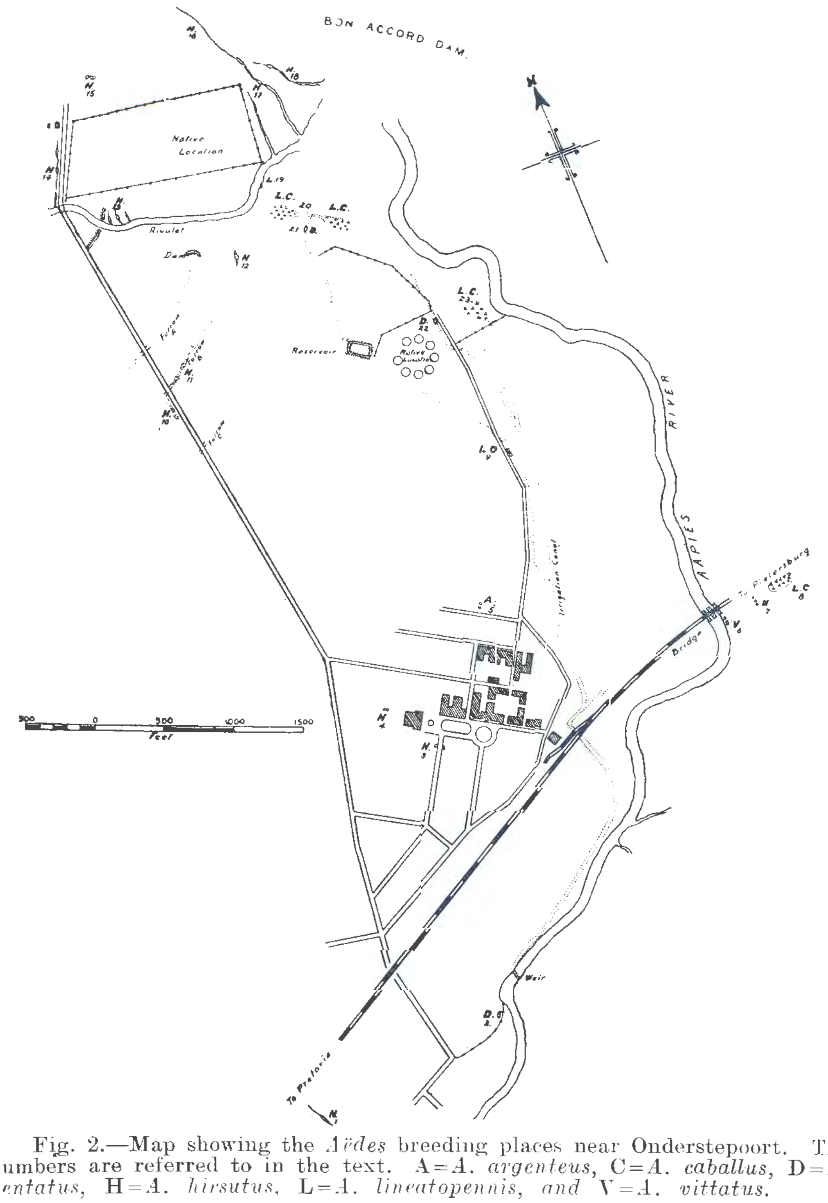

Fig. 2.—Map showing the *Aëdes* breeding places near Onderstepoort. The numbers are referred to in the text. A=*A. argenteus*, C=*A. caballus*, D=*A. dentatus*, H=*A. hirsutus*, L=*A. lineatopennis*, *and* V=*A. vittatus*.

were also collected. Small pools and animal hoof prints alongside the Aapies river and another small rivulet running at the foot of the new native location were investigated on different occasions but except for a few *Culex* larvae nothing was found.

It may be mentioned here that during the latter part of the summer *A. squamosus* was occasionally found breeding in a typical *hirsutus* pool (*vide* breeding place 11, next chapter).

B. Breeding Places of *Aëdes hirsutus*.

The first larvae of *Aëdes hirsutus* were found at the beginning of November, 1931, opposite the new native compound in breeding place 14 (cf. the map). A small furrow made by rain water runs down a slope alongside the road. After a rain, water remains for some time in a number of depressions between stones, and in these the larvae were caught. There were no trees or other vegetation except grass in the neighbourhood. After continued regular inspections this breeding place was not found to be too good.

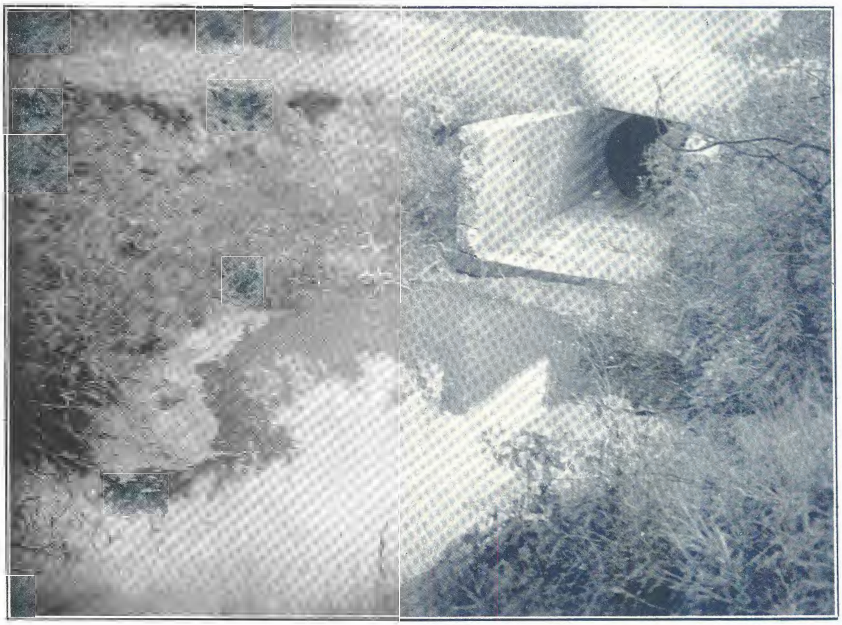

Fig. 3.—Breeding place of *Aëdes hirsutus* in a furrow near a road. At Onderstepoort.

Quite a number of other breeding places were found in the vicinity of this first one, alongside the road between the laboratory and the new native location, and on each side of this location.

Breeding place 11 was situated alongside the road mentioned above in a depression immediately below a concrete culvert passing beneath the road (Fig. 3). It was surrounded by typical bushveld and could be regarded as a good breeding place. A water pipe running over this pool to which a tap was fitted enabled us to fill the pool with water at will.

On the 2nd of January, 1932, e.g. we filled the pool with tap water. On the 4th, 0·4 inch rain fell. On the 6th large numbers of small larvae were seen. The pool depended in the meantime practically solely on tap water. The first pupae appeared on 11th January in large numbers. They were frequent the next day, but thereafter their number decreased, on the 15th the last ones being collected. On that date the breeding place almost dried up but was immediately filled again. Small numbers of larvae reappeared on 21st January, but did not become numerous. On later occasions we were also able to produce larvae by filling the place with tap water and sometimes, but not regularly, obtained them in quite large numbers.

The eggs must have been present in the dried up pool for when larvae appeared at all, they did so after a few days and were nearly all of the same size. When water remained in the pool for some time *Culex* species deposited their eggrafts on the surface of the water, their larvae hatching out, but being quite small still at the time that the *Aëdes* were pupating. This fact also serves to indicate that the *hirsutus* eggs must have been present in some dormant state in the dried pool. Whenever we kept this under water for a longer period than sufficed for one generation of *hirsutus* to breed out no more larvae appeared, or at any rate only very small numbers. As it was not possible to keep the water level in the pool constant, these larvae might have originated from eggs lying in parts of the pool that had been dried up for some time. Occasionally some larvae of *Aëdes lineatopennis* and *Anopheles squamosus* were found breeding in the same pool besides the *Culex* already mentioned.

A few yards from this pool another breeding place of *A. hirsutus* (No. 10) was situated in the same furrow. It was very small and shallow, however, and only occasionally retained sufficient water to produce adults. On one occasion it was filled with clear water by a leakage from a cattle drinking trough and this also caused eggs to hatch out. In this pool the larvae were sometimes found associated with those of *Mucidus*.

Some really good breeding places were located between the native location and a small spruit or stream (No. 13). They con-consisted of a few medium-sized, elongated, shallow pools alongside a few large boulders. They were unsheltered although there were a few low shrubs in the vicinity, the surrounding veld consisting of sparse bushveld. After a good rain these pools became filled with muddy water and, provided they remained full for a sufficiently long period, regularly yielded a good supply of larvae.

Two other breeding places, situated close together, were found on the other side of the native location (No. 15, map). One consisted of a fairly deep old quarry, in which, however, larvae were only occasionally present. The other lay a few yards away in a very shallow depression covered for the most part by short grass. A tree standing in the vicinity afforded the only protection, otherwise the surroundings were quite bare. After a good rain the place would be filled with muddy water and fair numbers of *A. hirsutus* were frequently present. On 20th and 21st January, e.g. heavy rain fell, and on the 25th numerous small larvae were observed. Compared with others, this place, on account of its vegetation, was not a typical *hirsutus* breeding ground.

Typical breeding places were encountered again to the east of the native location in depressions formed in a number of small gulleys, conducting rainwater to the main stream, which traversed a slope covered with sparse bush (Nos. 16-18 of the map and another further south not shown). One of these pools in particular (Fig. 4) regularly produced larvae in fair numbers after every rain. Breeding places of this type, depressions in gulleys or furrows filled with water only after rains, were also found to the south-west of the laboratory (breeding place 1).

Another breeding place, which, during its short existence, supplied us with large numbers of larvae, lay near the main buildings of the laboratory (breeding place 3), alongside a road on either side of which depressions existed which occasionally became filled with rain water. Another similar place was situated behind the library (breeding place 4).

Fig. 4.—Breeding place of *Aëdes hirsutus*. A small pool in the veld filled with muddy water. At Onderstepoort.

A different type of breeding place was found near the Aapies river (breeding place 7), alongside an irrigation furrow which was generally full of water. This consisted of a number of small pools of the size of animal hoofprints and crab holes in which on one occasion a fair number of *hirsutus* were found.

At the beginning of March, 1932, we paid a short visit to the Government Irrigation Settlements at Losperfontein, about 40 miles west of Pretoria, where horsesickness had appeared amongst immunized mules at that time. Near Losperfontein *Aëdes hirsutus* was found breeding in a very remarkable manner. Water for irrigation and drinking purposes was conducted by a small furrow constructed alongside a road. This furrow was provided with concrete intersections at regular intervals along its length. Under these intersections pools (Fig. 5) had formed by the washing out of the ground by the falling water, and in these pools, containing clear water, *A. hirsutus* was breeding in large numbers independent of the rainfall. This was a good example illustrating the necessity of taking precautions when water is led over open ground.

Fig. 5.—Breeding place of *Aëdes hirsutus.* Below concrete intersections in an irrigation furrow. Near Losperfontein.

Aëdes hirsutus *depends principally on rain for its larval hirsutus generally selects as breeding places,* at any rate in the Pretoria district, *small or medium-sized pools of muddy water without any vegetation, frequently lying in depressions in furrows made by rainwater. The pools are not sheltered but lie in more or less open veld.* The water in them remains muddy with a reddish or grey colour until it has practically dried up.

Pools with vegetation, mainly grass, are not disregarded entirely, although they certainly only constitute occasional breeding places.

Aedes hirsutus *depends principally on rain for its larval development. The eggs must be present in the pools before they are filled with rainwater. Furthermore, practically only one generation will hatch out in a breeding place if it remains flooded over a longer period than that necessary for the development of this one generation.*

When the breeding places are filled with clear water, larvae will hatch out in them, but clear water does not seem to be very attractive to the females as it was difficult to create new breeding places by filling suitable spots with tap water and allowing them to dry up from time to time.

The larval development is short, the first adults appearing about 8-10 days after the filling of the breeding places. The rate of larval development depends on the outside temperature and the size of the breeding places, small and shallow pools being warmer than larger and deeper places.

Adults were not observed in the neighbourhood of the breeding places except only directly after hatching from pupae.

C. Breeding Places of *Aëdes caballus* and *A. Lineatopennis*.

The first larvae of *A. caballus* were found quite unexpectedly in October, 1931. On that date larvae were observed in shallow water covering quite a large piece of open veld situated between the Aapies river and the old native location, just below some fields of maize (breeding place 23). The following day a small amount of water only was left which had completely dried up the day thereafter.

A week or so later larvae of *A. caballus* were found, again quite unexpectedly, in a furrow covered with grass situated near a plantation of trees at the edge of open veld, between the Aapies river and the new native location (breeding place 20-21). On returning the next day all the water had disappeared.

In both cases it was difficult to trace any connection between the appearance of the larvae and rainfall but on going into the matter we found that the ground had most probably been flooded by an overflow of water used for irrigating maize fields. On 4th November we once more saw larvae in the first breeding place and were then able to trace definitely the source of the water to the irrigation operations. Having established this fact, we connected both breeding places with the irrigation system of the farm lands belonging to the laboratory by means of small furrows (cf. map), in order to enable us to fill the breeding places at will and thus be independent of rain to some extent. One difficulty which confronted us, however, was to get sufficient water to the breeding spots during the spells of dry weather. The distance from the point where the water had to be pumped out of the Aapies river to our breeding places was nearly $1\frac{1}{2}$ miles, and it took more than 24 hours for the water to reach its destination as very large quantities were absorbed by the dry ground of the furrows. On account of the high evaporation rate, water had to be led to the places over a period of several days, which would frequently interfere with the farming operations. As an example of the extreme dryness of the ground we may mention the following observation. On one occasion we endeavoured to fill one of the smaller breeding places (No. 15) with water by means of a water cart. 6,000 gallons of water were poured on to the same spot during one day but this was immediately all taken up by the ground. We were therefore forced to give up this idea.

Reverting back to the breeding places themselves, the best were the three marked in the map as 20-21 and already partially described (Fig. 6 and Fig. 7). The centre was formed by an old furrow covered with grass which, since the middle of January, had been connected with the main irrigation canals. The water coming down this furrow was stopped by the remains of an old road slightly raised above the ground level. At this point the water flowed to either side of the furrow into open pans covered with grass but not sheltered in any way. Small dams and furrows were constructed so that we could dry up these pans alternately. A number of holes were dug so as to concentrate the water and larvae in them while the pans were drying up.

On 21st January the larger of the pans was filled by rainwater and during the following days irrigation water was added to prevent it from drying up. On the 26th very numerous larvae and pupae, about equal in number, were observed. Later on we found that 5-6 days was the ordinary period for the larval development during warm weather.

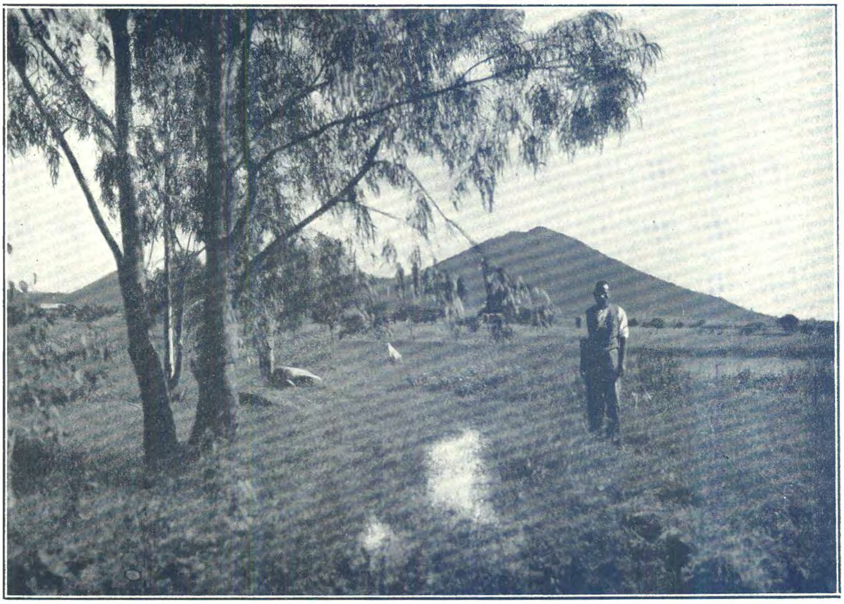

Fig. 6.—Breeding place of *Aëdes caballus* and *Aëdes lineatopennis*. An old furrow covered with grass. At Onderstepoort.

We were now able to expect enormous numbers of larvae with absolute certainty a few days after the place had been filled by rain or irrigation water. However, when the place was kept filled with water over a longer period than that necessary for the development of one generation of *Aëdes*, never would a second generation appear and only *Culex* would then continue breeding thereafter. Eggs of *Culex* were often observed on the water surface. By the time their larvae hatched out, the *Aëdes* were almost ready to pupate. The same holds good for *Lutzia tigripes*, whereas the larvae of *Mucidus* appeared together with those of *Aëdes*. There is no doubt that, as is the case with *hirsutus*, the eggs ready to hatch out are present in large numbers in the dried up pools.

The *Aëdes* larvae accumulated near the edge of the water especially between the grass stems. All the breeding places were covered with grass but in the deeper parts, where the grass was totally immersed, the larvae were less frequently found.

The duration of the larval period was nearly the same for the majority of the specimens, although variations of a few days occurred. The length of the pupal period seemed to be 1-2 days.

When the adults hatched out in these breeding places they appeared simultaneously in thousands so that, when walking alongside the edge of the water at that time, large swarms of mosquitoes, males and females together, were disturbed. They would fly for a short distance and then settle down again in the grass. Numerous visits showed us that these adults would remain at their breeding spots even after the water had disappeared, as the grass of these

Fig. 7.—Breeding place of *Aëdes caballus* and *Aëdes lineatopennis*. A pan in the veld covered with grass. At Onderstepoort.

breeding places retained moisture for a much longer time than the other parts of the veld. It is quite a simple matter to ascertain the presence of these mosquitoes by just walking through the grass, as large numbers are disturbed and will fly up, many settling down for a time on the body or clothes. Inside the comparatively small breeding area we were always able to find large numbers for at least a week, whereas outside this area, except when the whole veld was wet, very few if any were ever present. When the breeding ground itself commenced to dry out completely, the mosquitoes disappeared more or less slowly as the ground no longer offered them the humid conditions required. Some may have found other more suitable places, but the majority, during a prolonged period of drought, will undoubtedly have perished.

In these pans and in the furrow *Aëdes caballus* and *A. lineatopennis* always bred out together. *A. caballus* was the more prevalent species from the beginning, and towards the end of the season the percentage of *A. lineatopennis* still further diminished, so that eventually only very few specimens could be obtained, whereas the numbers of *A. caballus* remained practically unchanged. This may have been due to a seasonal adaptation of the species or to changes in the breeding places, produced by the repeated floodings with irrigation water.

Besides these two species and the other species previously mentioned *Aëdes nigeriensis* and *A. durbanensis* were forund in the same place towards the end of the season. The adults of *A. caballus* and *A. lineatopennis* would frequently attack us in broad daylight although they were definitely more active in the late afternoon. The mosquitoes did not really fly around during the day, but on being disturbed and settling down thereafter on the body, they immediately commenced feeding. The same observation was made with donkeys that frequently grazed over the breeding grounds.

The second place already mentioned (breeding place 23) also regularly yielded a large number of larvae, mainly *A. caballus* mixed with a smaller percentage of *A. lineatopennis*, when it was filled with irrigation water. It was, however, very shallow and dried up quickly and was therefore not as suitable for our purpose as the places described above. The adults behaved in the same manner. After hatching out they remained at their breeding place for a considerable time. Up to the middle of February a mosquito trap containing a horse as bait animal was used a few hundred yards away, but, as has been stated already, only very few *A. caballus* entered it. It would seem, therefore, that this species remains on or near its breeding grounds even at night.

A place, which produced *A. lineatopennis* exclusively, was found not far distant alongside the small stream running at the foot of the new native location and later joining the Aapies river (breeding place 19). Just at the edge of this stream a depression in the form of a small backwater had been formed. It was covered with fairly thick grass and after a rain would be filled with rainwater, but at the same time it became connected with the stream by the rising water. It was remarkable that no *caballus* were found there as conditions appeared to be favourable for this species.

In the water reservoir lying between the old native location and the open fields north of the first described breeding places, larvae of *Aëdes* were occasionally found. This reservoir was only filled with water for irrigation purposes from time to time. The first larvae were observed in it at the beginning of November, most of which belonged to *Culex* and a small number were *Aëdes caballus, lineatopennis* and *dentatus*. The dam did not prove to be a good breeding place for larvae of the latter genus however.

One of the breeding places first located by us lay between the road and an irrigation furrow (breeding place 9). A row of trees stood there and thick grass covered the whole area. After heavy rains the irrigation canal overflowed and pools were formed in the grass under the trees. In November, when the place was first noticed, principally larvae of *A. lineatopennis* were found although besides them a few *A. hirsutus, caballus, dentatus, Mucidus, Lutzia tigripes* and large numbers of small *Culex* larvae were also discovered. The place was very small and dried up on most occasions before the larval development was accomplished, and apart from this, it did not produce larvae after every rain.

The last breeding place of this series (No. 8) was found at the beginning of January on the other side of the Aapies river. It consisted of a small clearing in fairly dense bushveld at the foot of a railway enbankment and lay at a somewhat deeper level than the surrounding ground. After heavy rains it became filled with rain-water, augmented perhaps by an overflow from an irrigation furrow situated close by, and on several occasions contained fair numbers of larvae. These belonged mainly to *A. lineatopennis* and only a small percentage of *A. caballus*. The usual small *Culex* larvae were also present.

Conclusions.

From these observations the following conclusions may be drawn:—

Aëdes caballus and *A. lineatopennis* show a definite preference for certain types of breeding places. *They select furrows, small or medium-sized pans or slight depressions forming part of the veld, i.e. marshy spots covered with grass and filled with rainwater.* Contrary to *A. hirsutus* they prefer places with vegetation.

The larvae of both species develop equally well in rain or irrigation water. *On no account do they make use of permanent water for breeding and should the water in their most suitable breeding places become permanent, not more than one generation will appear therein. If, however, the place dries up for some time and becomes filled with water again, another generation immediately starts to develop.*

The eggs, ready to hatch, must be present in the dried breeding places. The contact with water probably provides the stimulus necessary for the hatching process. Not all the eggs seem to respond to this stimulus at the same time, however, as a number appears to remain unhatched when the place dries up again and perhaps the next contact with water will cause them to hatch. If this were not so, it would be difficult to explain the constant large numbers of larvae which hatch out every time the place is flooded, even when the intervening dry period was very short. This fact is of great biological importance to the species. If all the eggs were to hatch out after the first contact with water, practically all the larvae would be destroyed after some rains insufficient to permit of the adults emerging.

Both species must overwinter in the egg stage. In winter adults of the different *Aëdes* species were never encountered. When, however, at the beginning of October (before the adults appear), one of the breeding places was filled with irrigation water, large numbers of larvae appeared directly. The place dried up before the larvae could complete their development, but on being flooded again, without any adults being present, larvae started to develop again. This can only be explained by the fact that the eggs were present in the places throughout the whole winter.

The larval development was very short, usually occupying 5-6 days in the summer months. With a pupal period of 1-2 days the adults therefore hatched out within a week after a good rain.

The adults, after hatching out, *remained at their breeding ground sheltering in the grass for a considerable period, even when the place had dried up in the meantime*. They were strictly confined to their breeding spots which retained a sufficient degree of humidity for a longer time than the rest of the veld. When, in cases of no further rain, the breeding grounds became absolutely dry, the mosquitoes disappeared from them as well.

Both species fed in broad daylight, if they were disturbed, and had settled down on the body. They did not, however, actually fly around hunting for food.

A. caballus and *A. lineatopennis* usually bred together. In some places one species was more prevalent than the other while in other places the reverse was the case. These differences remained constant throughout the season.

The larvae were more or less regularly associated with those of *Mucidus* and those of *Culex* and *Lutzia*, which, however, generally appeared later. On some occasions we encountered a few larvae of *Aëdes durbanensis, nigeriensis, hirsutus* and *dentatus* breeding with them.

D. Breeding Places of *Aëdes dentatus*.

The best breeding place for this species was found east of the railway line near the Aapies river (breeding place 2). It consisted of a small pond-like depression, a few feet in diameter under some trees, surrounded on all sides by thick bush. The place was very well sheltered. Apart from dead leaves the pool contained no vegetation, its bottom consisting of fine mud. After good rains the pool was filled with water and when the water remained long enough quite large numbers of *A. dentatus* bred out.

Another good place was found near the old native compound (breeding place 22). An old furrow of the irrigation system, which was no longer used, situated alongside an old road, was occasionally filled with waste water from the maize fields. In parts this furrow was well shaded by trees and bushes. The bottom consisted of fine mud mixed with fallen leaves. In these parts, together with the usual *Culex*, larvae of *A. dentatus* were found on several occasions in fair numbers.

Besides these places larvae of *A. dentatus* were found together with those of some other *Aëdes* species in a small shady place near one of the irrigation canals (breeding place 9), and in the water reservoir near the old native location. These places have already been described in the preceding chapter.

Conclusions.

According to our observations *Aëdes dentatus seems to prefer well sheltered and shaded pools without any vegetation.* We have never succeeded in finding its larvae in the open veld.

This species also depends on rain for its larval development, as permanent water was never found to be used.

We did not observe the adults near the breeding places. As stated before, they entered the mosquito traps in relatively large numersb at night, in fact they were the most abundant *Aëdes* species caught in them.

It seems, therefore, that A. dentatus *is a shadow or shelter liking species both in its larval and adult stages.*

E. Breeding Places of *Aëdes vittatus.*

Aëdes vittatus, a species belonging to the same subgenus as the yellow fever mosquito, was found in three places.

The first place (breeding place 6), was situated almost under a railway bridge crossing the Aapies river. On the top of a rock some 10 feet wide and 5 feet high, situated towards one side of the bed of the river, a depression, about 10 inches in depth, existed. After a fair rain this depression became filled with water which remained there for a number of days. From October we kept this place filled with river water for the greater part of the season, as it was known by one of us, from former observations, to be a suitable breeding place for *Aëdes vittatus.* At first *Culex theileri* only were present, but in December, larvae of *Aëdes vittatus* also appeared which were later sometimes associated with those of *Culex salisburiensis.*

The larvae of *A. vittatus* occurred more or less regularly in this pool. Periodical drying up and refilling of the place did not seem to be as necessary for them as for the other *Aëdes* species although hatching condition may possibly be more favourable when the place is regularly dried up.

The number of *A. vittatus* obtained from this place was not very great. From January onwards dragon-fly larvae, which preyed upon the mosquito larvae, were always present in this pool and it was practically impossible to get rid of them notwithstanding daily destructions, as the adult females continued laying eggs. The semi-permanent nature of the pool may possibly have attracted the dragon flies more than would have been the case of a pool under natural conditions.

Another breeding place of *A. vittatus* was found on the farm Kaalplaas near Onderstepoort. In this case the breeding place was of the same nature as the one described above, and consisted of a shallow depression containing rainwater on a large flat rock situated halfway up one of the hills. Quite a number of fullgrown larvae and pupae were present.

The third and last breeding place discovered was also formed by a rock pool. It was located, during a short mosquito survey carried out in March, 1932, on the Government Irrigation Settlements at Losperfontein. On one of the kopjes, which was fairly densely covered with thorn trees, a shallow depression filled with rainwater was observed on a medium-sized rock (Fig. 8). It contained a fair number of *vittatus* larvae and pupae. In the two latter cases no larvae of other species were present.

Fig. 8.—Breeding place of *Aëdes vittatus*. A rock pool on a kopje near Losperfontein.

Conclusions.

Aëdes vittatus appears to breed only in rock pools containing no vegetation. The larvae were never found associated with other *Aëdes* larvae, although occasionally *Culex salisburiensis* were present.

The larvae of *A. vittatus* must be capable of withstanding relatively high temperatures as, on a summer's day, these rock pools become extremely warm. A few preliminary experiments also showed us that the thermal deathpoint of these larvae is higher than that of other *Aëdes* and *Culex* species.

F. Breeding Places of *Aëdes durbanensis* and *A. Nigeriensis*.

Larvae of these two species were only found in breeding places 20 and 21, where they were associated as has been previously mentioned, with larvae of *Aëdes caballus* and *A. lineatopennis*. They appear to have the same breeding habits as these two species.

The larvae began to appear towards the end of the season but never became common.

G. Breeding Places of *Aëdes argenteus*.

Larvae of this species were found in some concrete pig troughs near the stables of the laboratory (breeding place 5). They appeared there in April, towards the end of the season, but were not at all common.

H. Breeding Places of *Mucidus scatophagoides*.

The larvae of *Mucidus*, a remarkably large mosquito in both its larval and adult stages, are carnivorous, preying upon other mosquito larvae. They do not seem to attack other aquatic insects or at any rate mosquito larvae are their preferential food, and they have therefore to breed in association with other mosquito species.

The largest numbers of *Mucidus* larvae were found in the main *caballus* and *lineatopennis* breeding place (Nos. 20-21). There their numbers increased gradually and towards the end of the season they became quite common.

Apart from this, larvae of *Mucidus* were located in three other places (breeding place 1, 9 and 10), twice associated with *A. hirsutus* only (1 and 10), and in the other place (9), with *A. caballus, dentatus, hirsutus* and *lineatopennis*.

In all cases the larvae of *Mucidus* appeared together with those of the *Aëdes* species, whereas the larvae of *Lutzia*, which also lives on mosquito larvae, appeared later. The eggs of *Mucidus* must therefore have been present in the dried pools, and in its breeding habits this species is closely allied to the typical *Aëdes* species. It also does not breed in temporary water and should its breeding pool not dry up one generation only appears. In the main *caballus-lineatopennis* place, where most of the *Mucidus* larvae were found, conditions were extremely favourable on account of the continued experimental flooding, and it is therefore difficult to decide whether, if under natural conditions *caballus* places are more favourable for *Mucidus* development than *hirsutus* pools. That the *caballus* places are very suitable is certain and further, that *hirsutus* pools also accurately fulfil the larval requirements of this species is beyond doubt.

Adults of *Mucidus* were occasionally observed on their breeding grounds, but these had probably hatched out recently, as it would seem that they do not remain there as regularly or as long as the adults of *A. caballus* and *A. lineatopennis*.

Conclusions.

Mucidus scatophagoides *is a larvivorous mosquito resembling in its breding habits the typical* Aëdes *species.* It is often associated with the larvae of *A. caballus, A. lineatopennis* and with those of *A. hirsutus*, thus breeding in marshy spots of the veld covered with grass, and in pools of muddy water containing no vegetation.

As is the case with the typical *Aëdes*, this species breeds only in temporary water and the eggs are present in the dried places ready to hatch after the first rain.

I. Breeding Places of *Lutzia tigripes*.

Lutzia tigripes is another species predatory in its larval stage. It was found fairly often in a large variety of breeding places; in marshy spots with dense vegetation, in pools without vegetation, in concrete basins, in the open field and in sheltered places, in clear, muddy or quite dirty water. It makes use of stagnant water, permanent as well as temporary, and was found together with *Aëdes caballus*, *A. lineatopennis*, *A. dentatus*, *Culex theileri*, *C. duttoni* and *Mucidus*.

In temporary pools the larvae of *Lutzia* always appear a few days later than those of *Aëdes* and therefore together with the *Culex* larvae. The eggs are therefore not present in the dried pools but are deposited after the places have been filled with water. In its breeding habits *Lutzia* resembles the true *Culex*.

Lutzia tigripes does not appear to be a bloodsucking species according to the literature, and in our experiments it could also never be induced to feed on horses. In connection with the transmission of horsesickness this species was therefore of no practical importance.

H. Breeding Places of *Culex theileri*.

Culex theileri is probably the most common mosquito species at Onderstepoort throughout winter and summer.

When we commenced our search for breeding places, at the beginning of September, 1931, the bed of the Aapies River was the only place where larvae of mosquitoes could be expected. Larvae of *Culex theileri* were found in fair numbers at the edge of the water, mainly in backwaters or in pools alongside the river. They were present quite regularly, excepting after heavy rains when they were washed away by the rising water. In the river the larvae seemed to be more abundant towards the end of winter than in summer.

Later on in the summer larvae were also found in a large variety of pools of a permanent or temporary nature. Larvae frequently appeared in typical *Aëdes* places, always later, however, than the latter species.

I. Breeding Places of *Culex duttoni*.

Larvae of *Culex duttoni* were observed in only one place near the Aapies River. Waste water from the laboratory, which usually ran into the river, was diverted and used for flooding a small piece of ground covered with grass on which a few adults of *A. lineatopennis* had occasionally been found. This latter species, however, did not develop there. Besides *Culex theileri* and large numbers of *Lutzia tigripes* larvae of *Culex duttoni* appeared and were quite plentiful from January onwards. The water filling this place was very dirty.

6. RESULTS OF THE MOSQUITO SURVEY IN CONNECTION WITH THE TRANSMISSION OF HORSESICKNESS.

Before going into a discussion of the results of our mosquito survey it would seem advisable to try and obtain some information from other diseases related to horsesickness itself or showing similarities in the seasonal distribution. There are four diseases which may be of use for comparative purposes, viz., bluetongue of sheep, yellow fever, dengue and malaria.

Bluetongue certainly shows affinities to horsesickness. Both are non-contagious virus diseases and have a similar distribution in South Africa. The transmission of this disease is, however, not yet known.

Yellow fever and *dengue* are virus diseases of man. The viruses, especially in the case of yellow fever, are very similar to that of horsesickness in quite a number of respects. Points of dissimilarity also exist, but of all the diseases we know, yellow fever seems to be the most closely related to horsesickness. Yellow fever and dengue are transmitted mainly by *Aedes argenteus*. Besides this species a number of other mosquitoes, chiefly belonging to the *Aëdes* group, has been found to be capable of transmitting yellow fever experimentally in recent years, whereas dengue is transmitted by at least one other *Aëdes* species.

The seasonal appearance of *malaria* in the Union shows a definite correlation with that of horsesickness. Bad malaria years are bad horsesickness years as well, both diseases being limited to the summer months and depending in their severity on the amount of rainfall. It must be mentioned, however, that geographically the two diseases differ, malaria being more restricted.

Horsesickness cannot, however, be transmitted by the same species of mosquitoes that carry malaria and yellow fever. The two malaria transmitting *Anopheles* species of the Union, *A. gambiae* and *A. funestus*, are house-frequenting species, whereas horsesickness is not generally contracted in stables but in the field. Apart from this, the geographical distribution of the two diseases, as previously mentioned, is not entirely the same. *Aëdes argenteus*, the yellow fever mosquito, can also not play an important part in the transmission of horsesickness. It lives in close association with houses and being mainly a tropical and sub-tropical species its distribution in the Union is more restricted than that of the horsesickness areas.

In analogy with malaria and yellow fever, we can therefore expect a species of Anopheles *or* Aëdes, *but a true field variety of these species, to be the transmitter of horsesickness and bluetongue.*

In trying to obtain information as to the probable transmitter of horsesickness from the results of our *mosquito survey*, we must not lose sight of the fact that *the season under review was not a typical horsesickness season at all.*

The *Anophelines* were very scarce this season. Only a few adults were obtained in the mosquito traps and only a small number of larvae could be collected. In fact, out of the 13 species of *Anopheles* known to occur at Onderstepoort, only 3 were found this season. *A. squamosus*, the only species of which a somewhat larger number and of which both larvae and adults were collected, is not limited to the summer months. It is, therefore, not likely to play any rôle in the transmission of horsesickness.

We were not able, by the experimental flooding of places regarded as possible breeding spots or by any other means, to create conditions favourable for the breeding of *Anopheles*.

The lack of information in our mosquito survey regarding the Anophelines *certainly presents a flaw*, but we had very little chance of getting more complete information.

Of the Culicine tribe, species of *Aëdes*, *Mucidus*, *Culex*, *Lutzia*, *Taeniorhynchus*, *Theobaldia* and *Uranotaenia* are present at Onderstepoort.

L. tigripes, representing the genus *Lutzia*, does not appear to be a bloodsucker and is, therefore, of no further interest in connection with this work.

Regarding the biology of the *Taeniorhynchus* species, *Theobaldia longiareolata* and *Uranotaenia candidipes*, we were able to obtain practically no data from Onderstepoort. During this season only a few adults of *Taeniorhynchus africanus* were caught in the mosquito traps. It seems rather unlikely that one of these 4 species would be connected with the transmission of horsesickness.

The genus *Culex* is represented at Onderstepoort by 11 species of which 7 were collected as larvae or adults during this survey. Of only 3 of the species, viz., *C. theileri*, *C. duttoni* and *C. salisburiensis*, were the breeding places located. *Culex theileri* was very common in the adult and larval stages, but as it was also present in large numbers in the winter, it can therefore not possibly belong to the list of the possible transmitters. The other species appeared in December, but as regards their distribution, not sufficient information was obtained. The larvae of *C. duttoni* became more common towards the end of the summer but they were only found in an artificial breeding place, which had not existed throughout the greater portion of the season.

According to our observations the *Culex* species made free use of permanent water for breeding purposes, possibly even in preference to temporary water. As the eggs of *Culex* cannot withstand complete dessication, and, on account of the embryonic development of the larvae always occupying a number of days, many temporary water pools are not suitable for their breeding as these places dry up before the larval development can be completed.

Our information was certainly far from complete, but nothing pointed to a *Culex* species as being probably involved in the transmission of horsesickness.

Of the 13 *Aëdes* species, known to occur at Onderstepoort, 10 were found during this survey, viz., *A. argenteus*, *A. caballus*, *A. dentatus*, *A. durbanensis*, *A. hirsutus*, *A. lineatopennis*, *A. nigeriensis*, *A. poweri*, *A. punctothoracis* and *A. vittatus*. In the case of 8 of these species the breeding places were located. We had thus gained quite valuable information so far as the *Aëdes* species were concerned.

A number of these Aëdes *species fitted in very well with the epidemiology of horsesickness.* To start with, all the *Aëdes* species studied, *depended entirely upon rainfall.* If there is no rain no mosquitoes will develop, and their numbers increase in accordance with the amount of rain. They do not make use of permanent water for breeding purposes, not even during a very dry part of the season when no temporary water is available. The eggs are present in the dried up pools ready to hatch when these pools become filled with rain-water. Larval development is accomplished within a week during the warm summer months. Should a breeding place dry up for a short time and become flooded again, another generation immediately commences to appear, but it is never followed by a second generation without intermediate drying up.

Of the 8 *Aëdes* species found this year, *A. argenteus* may not be regarded as a transmitter on account of its restricted distribution and special habits, as has been mentioned already.

Aëdes hirsutus has to be reckoned amongst the potential carriers. It was quite common throughout the season and bred mainly in small to medium-sized pools of muddy water containing no vegetation. After a good rain plenty of these pools are formed along the sides of the roads and in the veld. In practically all the temporary water courses formed after good rains pools of this nature remain, filled with sufficient water for breeding purposes. Water may also be added artificially in certain circumstances and in this way adults may be obtained independent of the rain (cf. Losperfontein). This is, however, rather the exception than the rule.

On account of the nature of its breeding places *A. hirsutus* is bound to be one of the most common of the *Aëdes* species. A very wet season is not necessary for its development as even a moderate rain is generally sufficient. This fact may therefore, to some extent, militate against the importance of its rôle as a probable transmitter.

Aëdes caballus and *A. lineatopennis* may be considered together. Although belonging systematically to two different sub-genera, they are biologically closely related to one another. They breed in marshy spots in the veld, by preference in small or medium-sized pans, in small depressions or temporary furrows, provided these are covered with grass.

We were able to breed these two species, especially *A. caballus*, in very large numbers, but only under semi-artificial conditions by flooding their ordinary breeding grounds regularly with irrigation water. Normally these pans are filled with water only after heavy rains, and more rain is required later in order to enable them to retain sufficient water until the larval development is accomplished. We feel convinced that without these artificial floodings *A. caballus* especially, would have been rare. *A. linneatopennis*, which is able to make use of small breeding places as well, is therefore able to breed with less rain, but heavy rains extend its breeding places considerably.

In order that *Aëdes caballus* and *A. lineatopennis* may become really common large amounts of rain are required. A really wet summer is therefore necessary, so that in this respect these two species conform with the requirements of the probable transmitter.

Both species are not exclusively dependent upon rain, however. They may hatch out equally well, as we have seen, in the waste water from irrigation systems. Places filled in this way seldom remain filled long enough, however, to make the completion of larval development possible. In this way, outbreaks during dry seasons may possibly be explained. Further, in the behaviour of the adults of these two species, certain points exist which tend to incriminate them in the list of possible transmitting agents. We have seen that these species, especially *A. caballus*, remain, to a very great extent, in the immediate vicinity of their breeding places day and night. On farms where horsesickness occurs, often only certain parts of such farms, mainly vleis, where the important breeding places are to be expected, are regarded as dangerous, whereas on other parts of these same farms horses may be allowed to graze almost with impunity.

On account of their breeding habits, their dependence on large amounts of rain, and the behavious of the adults, Aëdes caballus *and* A. lineatopennis *must be seriously regarded as potential transmitters of horsesickness.*

Aëdes durbanensis and *A. nigeriensis* have the same breeding habits as the two former species but are less common. They will also therefore belong to the list of potential transmitters.

Aëdes vittatus has also to be reckoned amongst the possible transmitters. It only breeds in rock pools, of which quite a number must exist in most of the horsesickness districts. Such pools may be present in the vleis as well but are, however, more common on or near the kopjes. This fact, to some extent, goes against their rôle as possible transmitters, but they can certainly not be excluded from this list.

Aëdes dentatus is one of the common species of this genus. It breeds in sheltered pools without any vegetation. On account of its breeding habits it may be regarded as nearly as good a possible transmitter as *A. hirsutus*. It entered our mosquito traps relatively frequently in this way showing a preference for stable-like buildings. This fact is not in favour of its being an important transmitting agent, and for this reason this species must be regarded as only of secondary importance.

Aëdes cumminsi, *A. poweri*, *A. punctothoracis*, *A. quasiunivittatus* and *A. simpsoni*, the remaining species known to occur at Onderstepoort, seem to be more or less rare species and will probably not rank amongst the important potential transmitters.

Lastly, *Mucidus scatophagoides* is regarded in the literature as being a non-bloodsucking mosquito. Only towards the end of the season did we discover that this statement was not correct, as adults fed readily on horses and took up a surprisingly large quantity of blood.

Mucidus uses the breeding places of *Aëdes caballus*, *A. lineatopennis* and *A. hirsutus* and has to rank with these species amongst the potential transmitters. As it feeds on the larvae of these species it is usually less common. Large numbers of *Mucidus* can only be expected from the larger *caballus* pools after heavy rains, and it appears to become frequent only towards the end of the season, after a sufficient number of generations of the species on which it preys have developed. It must, therefore, certainly be regarded as one of the important potential transmitters.

The transmitters of horsesickness must of necessity have a fairly wide *geographical distribution* as the disease itself occurs over the larger part of Africa.

We will give here the geographical distribution of the species of *Aëdes* known to occur at Onderstepoort, so far as it is known in the countries where horsesickness is endemic.

A. argenteus.—Transvaal, Natal and Zululand, Rhodesia, Tanganyika Territory, Uganda, Nyassaland, Belgian Congo, Nigeria, Sudan and Egypt.

A. caballus.—Transvaal, Natal and Zululand, and Kenya.

A. cumminsi.—Transvaal, Natal and Zululand, Kenya, Uganda, Nyassaland, Belgian Congo (var.).

A dentatus.—Transvaal, Natal and Zululand, Kenya, Uganda, Belgian Congo and Sudan.

A. durbanensis.—Transvaal, Natal and Zululand, Kenya and Uganda.

A. hirsutus.—Transvaal, Natal and Zululand, Rhodesia, Kenya and Uganda.

A. lineatopennis.—Transvaal, Natal and Zululand, Rhodesia, Kenya, Uganda, Nyassaland, Belgian Congo, Nigeria and Sudan.

A. nigeriensis.—Transvaal, Kenya, Uganda, Nyassaland and Nigeria.

A. poweri—Transvaal, Natal and Zululand, Rhodesia, Kenya, Belgian Congo.

A. punctothoracis.—Transvaal, Natal and Zululand, Rhodesia and Belgian Congo.

A. quasiunivittatus.—Transvaal, Natal and Zululand, Rhodesia, Tanganyika Territory (? var.), Kenya, Uganda, Nyassaland and Nigeria.

A. simpsoni.—Transvaal, Natal and Zululand, Tanganyika Territory, Kenya, Uganda, Nyassaland, Belgian Congo and Sudan.

A. vittatus.—Transvaal, Natal and Zululand, Rhodesia, Uganda, Belgian Congo, Nigeria and Sudan.

Besides these species the following have a wider distribution over Africa: *Aëdes africanus* was found in Rhoedsia, Uganda, Belgian Congo and Nigeria *A. apicoargenteus* in Uganda, Belgian Congo and Nigeria. *A. argenteopunctatus* in Rhodesia, Uganda, Nyassaland, Belgian Congo, Nigeria and Sudan. *A. metallicus* in Natal and Zululand, Tanganyika Territory and Sudan. *A. ochraceus* in Rhodesia, Kenya, Nigeria and Sudan. *A. tarsalis* in Natal and Zululand, Uganda and the Belgian Congo.

From this list it is obvious that *those species regarded* as potential transmitters have a wide distribution over Africa.

SUMMARY.

Our present knowledge of the epidemiology of horsesickness strongly suggests that mosquitoes are the probable transmitters of the disease.

In order to obtain preliminary information as to the mosquito species most probably involved in its transmission and that of bluetongue in sheep, a mosquito survey was carried out at Onderstepoort during the summer 1931-1932.

The summer in question did not represent a good horsesickness season on account of the extremely dry weather conditions which prevailed. The total amount of rain from November to April was 18·40 inches, compared with 36·91 and 31·47 inches in good horsesickness years (cf. Graph 1).

In South Africa up to the present 102 species of mosquitoes, belonging to 13 different genera, have been found. At Onderstepoort 47 species are known to occur. During this survey 24 of these species were found, 16 as adults, 7 both as larvae and adults, and 8 only in the larval stage.

Using tent-like traps with horses as bait animals, between the end of November, 1931, and the middle of February, 1932, 1,224 mosquitoes were caught, representing 16 species. Of these 21 specimens eblonged to *Anopheles*, 683 to *Aëdes*, 51 7to *Culex* and 3 to *Taeniorhynchus* and *Lutzia*.

The *Anophelines* were exceptionally rare. Amongst the *Culex*, *C. theileri* was the only common species, most of the specimens of this species being caught in October.

The *Aëdes* appeared in October and were common, especially in November and February. There existed a definite relation between their numbers and the amount of rain.

The traps, however, failed to give correct data as to the relative abundance of the *Aëdes* species present in the field, as they were selective for certain species (*A. dentatus*) and unattractive for others (*A. caballus*). The use of the traps was consequently discontinued later.

A considerable time was spent in a survey of the *breeding places* of mosquitoes near Onderstepoort.

Larvae of *Anopheles squamosus* and *A. pretoriensis* were found in backwaters of the Aapies River, the former species sometimes being encountered in one of the *Aëdes hirsutus* pools as well. Anopheline larvae were exceptionally scarce throughout the whole season.

All the *Aëdes* species studied depend mainly on rainfall for their larval development. They make use only of temporary water for breeding purposes and even in a suitable place one generation only will develop so long as the place remains flooded. When such a place has dried up and becomes filled again, another generation appears. Permanent water is never used by them for breeding even should there be no temporary water available for a long time.

The eggs are present in the dried-up pools and the larvae hatch out within a very short time after such pools become filled with water. It appears that on contact with water only a certain percentage of the eggs hatch out, the remainder lying dormant until the place has dried up and is filled again.

The *Aëdes* species pass the winter in the egg stage. In the early spring and summer, without adults being present, large numbers of larvae were observed developing after their breeding places had become filled with water.

Larvae of *Aëdes hirsutus* were quite common after good rains and were found mainly in small to medium-sized pools of muddy water containing no vegetation. Pools of this nature are present in large numbers after good rains.

The larvae of *Aëdes caballus* and *A. lineatopennis* live for the most part associated with one another. They select marshy spots in the veld covered with grass, small to medium-sized pans, furrows or slight depressions in the veld. Most of these places are filled with sufficient water only after heavy rains. Larval development will also take place, however, when the breeding places are filled with irrigation water. By this means we were able to breed these species in large numbers independent of the weather conditions.

The adults of both species remain for a considerable period, even during the day, at their breeding grounds, sheltered in the grass. They are strictly confined to these places which usually retain a sufficient degree of humidity for a longer time than the rest of the veld.

Both species, when disturbed, bite in broad daylight, but do not actually fly around during the day hunting for food.

A. caballus in particular, was not attracted to any extent during the night by horses kept in tent-like stables erected in the neighbourhood of one of its breeding places.

Aëdes dentatus prefers well sheltered pools under trees and bushes with decomposed vegetation for breeding. In the open field its larvae were never found.

Aëdes vittatus breeds only in rock pools which do not contain any vegetation. The larvae are capable of withstanding relatively high temperatures.

Aëdes durbanensis and A. nigeriensis were found associated with *A. caballus* and *A. lineatopennis*. They are comparatively rare species at Onderstepoort.

Larvae of *Aëdes argenteus* were found in concrete pig troughs near stables. They appeared only towards the end of the season and were not at all common.

Mucidus scatophagoides, whose larval prey upon other mosquito larvae, resembles in its breeding habits the typical *Aëdes* species.

It was found associated with the larvae of *A. caballus*, *A. lineatopennis* and *A. hirsutus*, thus breeding in marshy spots covered with grass in veld, and in pools of muddy water containing no vegetation. It only uses temporary water for breeding and therefore also depends upon rainfall.

Culex theileri was common during both winter and summer. Larvae were found in backwaters of the Aapies River and also in a large number of places with permanent and temporary water.

Larvae of *Culex duttoni* were observed in one more or less permanent pool covered with grass.

Larvae of *Lutzia tigripes*, another species predatory in its larval stage, were found quite often in a large variety of breeding places with temporary and permanent water. It resembles *Culex theileri* in its breeding habits.

From the results of this survey we are able to draw the following conclusions:—

Anophelines may be connected with the transmission of horsesickness, their scarcity, during the season under review, indicating their dependence upon rain. Information as to the species probably involved could not be obtained.

The seasonal (not geographical) distribution of malaria in South Africa suggests that the transmitters of both diseases depend on the same climatic conditions. The malaria carrying species themselves cannot, however, be transmitters on account of their house frequenting habit.

A number of *Aëdes* species fit in very well with the epidemiology of horsesickness. Most of the species depend upon rainfall and breed only in temporary pools and their numerical appearance is thus proportionate with the amount of rain. Should any of these species be involved in the transmission the most probable transmitters would be *A. caballus*, *A. lineatopennis* and *A. hirsutus*. The two former species, the adults of which remain throughout their lives near the breeding grounds in the vleis, coincide most accurately with the epidemiology of horsesickness and bluetongue.

Aedes vittatus, *A. nigeriensis*, *A. durbaneneis* and *A. dentatus* must be regarded as potential transmitters of secondary importance.

These species all show a fairly wide geographical distribution over Africa.

The disease which seems to be most closely related to horse-sickness is yellow fever in man, and it is transmitted chiefly by *Aëdes* argenteus, which fact is a further indication that *Aëdes* are good potential carries of the disease under review.

Mucidus is biologically very closely related to the *Aëdes* species and must also be regarded as an equally good potential carrier as *A. caballus* and *A. lineatopennis.* Contrary to statements in the literature this species is a voracious bloodsucker.

It is unlikely that *Culex* species would be involved in the transmission of horsesickness and bluetongue as they make frequent use of permanent water for breeding purposes. Furthermore, the most important species of this genus are not restricted to the summer months.