Onderstepoort J. vet. Res. 38 (2), 65-72 (1971)

CATTLE AND CULICOIDES* BITING MIDGES AS POSSIBLE OVERWINTERING HOSTS OF BLUETONGUE VIRUS

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ABSTRACT

NEVILL, E. M. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort J. vet. Res.* 38 (2), 65-72 (1971).

Light trap catches over 7 years at the Veterinary Research Institute, Onderstepoort showed that *Culicoides* adults are active on occasional winter nights and in some years remain active almost throughout the winter. *Culicoides* were also found to survive refrigerator temperature for up to 53 days and to survive outdoors during winter for up to 51 days. Moreover, day-time temperatures during winter were high enough to allow continued development throughout this season. Bluetongue virus (BTV) may therefore be able to survive short winters in infected *Culicoides* midges while warmer nights in some years may allow midges to fly and feed and so reinfect new hosts.

By testing large numbers of midges BTV has been shown to be present in *Culicoides* adults at the beginning of October. Apparently BTV does not disappear completely in spring but is present in so few midges as to be difficult to detect.

BTV could only be detected in late November or December in sample groups of five cattle at Onderstepoort. If a high percentage of the cattle population could be tested BTV will almost certainly be isolated at an earlier date and even perhaps in winter.

The results of these investigations support the theory that the biological cycle of BTV can continue in *Culicoides* and/or cattle throughout the winter in the Onderstepoort area.

INTRODUCTION

Du Toit (1944) showed bluetongue virus (BTV) to be plentiful in *Culicoides* midges in the late summer at the Veterinary Research Institute, Onderstepoort. He also succeeded in transmitting BTV by the bite of *Culicoides pallidipennis* Carter, Ingram & MacFie, 1920. In the U.S.A., BTV has been transmitted by the bite of *C. variipennis* Coquillett, 1901 (Foster, Jones & McCrory, 1963).

Du Toit (1962) and Howell (1963) noted that BTV had been recovered from cattle by various workers. Du Toit (1962) was, however, the first to investigate the role played by cattle in the epizootiology of bluetongue. He found that at Onderstepoort, cattle readily become infected from late November onwards to reach a peak in incidence in January and February.

Bluetongue cases stop abruptly when the numbers of *Culicoides* midges drop sharply at the onset of winter (Du Toit, 1962). The virus then disappears until about November or December, when it can be recovered in small numbers of cattle and in *Culicoides,* nearly 4 months after these midges resume their activity at the end of the winter period.

Many theories have been advanced to explain this delay but could not be proved because of our lack of knowledge of *Culicoides* activity (especially during winter) and of the incidence and survival of BTV in *Culicoides* and cattle.

The following studies were conducted in an attempt to remedy this need.

SEASONAL INCIDENCE OF *CULICOIDES* MIDGES AT ONDERSTEPOORT

During the period 1963 to 1970 the number of *Culicoides* caught in a single suction-type light trap at Onderstepoort was recorded daily. The trap used was described by Nevill (1967), the two basic components being a 100 watt globe and a large extractor fan. The catches consisted chiefly of *C. pallidipennis,* which at times accounted for up to 97,4 per cent of the catch. A statistical comparison of these catches with climatic conditions will form the subject of another paper.

To get some idea of *Culicoides* activity throughout the year the average catch per night on which they were active was calculated monthly for almost 7 years (Table 1) and was plotted together with the total monthly rainfall and mean monthly maximum and minimum temperatures (Fig. 1).

The number of nights each month on which midges were active and the total number of nights on which the trap was operated were also recorded (Table 1).

(a) *Incidence of* Culicoides *adults during the winter period of minimal activity* (*May to August*)

A detailed summary of flights during this period is given in Table 2. This period started from the time when no *Culicoides* were caught on 7 or more consecutive nights and ended when they were again caught regularly.

Results summarized in Tables 1 and 2 and illustrated in Fig. 1 show that *Culicoides* were absent in catches from May to August in some years and for shorter periods in others. During these periods of minimal activity they were active on 1 to 5 nights each month. The longest period recorded when they were completely absent was 88 days in 1965, while in 1969 they were never absent.

(b) *Incidence of* Culicoides *adults during the period of continuous activity* (*August to May*)

In 4 out of the 7 years during which records of catches were kept the *Culicoides* population increased gradually in numbers from August until the period January to March, when the number suddenly increased between 3 and 15 fold (1963 to 1967) (Table 1, Fig. 1). In the year 1967/68, however, peak catches were recorded in the early summer months of September, October and November but decreased to less than a third of that number in the period January to March. During the years 1968/69 and 1969/70, catches varied from month to month with no clear peak period of abundance although the highest catch in both years was recorded in January.

*(Diptera: Ceratopogonidae)

Received 26 March 1971

TABLE 1 *Mean nightly light trap catches of* Culicoides *and number of nights on which* Culicoides *were caught - Onderstepoort, 1963 - 1970*

	June	July	August	September	October	November	December	January	February	March	April	May
1963/64	25* 3/19**	66 4/22	152 10/22	889 17/17	2 090 19/19	3 835 19/19	5 667 13/14	3 387 21/21	13 176 14/14	19 527 17/17	3 072 5/8	1 530 12/14
1964/65	0 0/7	0 0/21	42 6/21	586 21/21	780 19/19	1 856 16/16	1 494 14/16	17 139 19/19	12 154 19/19	14 911 22/22	2 940 15/19	2 298 12/18
1965/66	0 0/22	0 0/21	150 6/22	1 404 16/21	895 17/19	1 877 19/20	3 688 15/15	4 049 16/16	6 552 17/17	28 458 21/21	8 763 18/18	3 670 16/20
1966/67	178 13/21	57 3/19	146 15/23	449 19/19	395 20/20	2 840 21/21	7 605 19/19	21 623 21/21	79 574 19/19	124 267 19/19	17 789 19/19	20 885 20/20
1967/68	1 934 10/22	310 2/20	1 308 8/23	11 548 19/19	17 424 21/21	11 575 21/21	9 754 17/17	4 468 18/18	5 056 21/21	3 776 21/21	5 984 18/20	2 825 19/21
1968/69	0 0/20	69 15/23	258*** 15/15	1 858 18/18	1 873 21/21	4 776 20/21	3 232 18/18	6 421 19/19	2 863 19/19	3 671 20/20	5 103 20/20	2 111 24/24
1969/70	624 18/21	653 18/21	343 20/21	— —	680 22/22	762 19/20	968 19/19	1 789 21/21	1 352 15/15	598 18/18	694 17/17	— —

*Mean calculated for nights on which *Culicoides* were caught, e.g. in June 1963, an average of 25 midges were caught on each of 3 nights
**No. nights on which *Culicoides* were caught/No. nights on which the trap was operated
***Catches estimated from here onwards
— Trap out of order

TABLE 2 Culicoides *incidence during periods of minimal activity - Onderstepoort, 1963 - 1969*

Year	Period of minimal activity			No. of nights when *Culicoides* active ***				
	Onset	Termination	Duration (days)	May	June	July	Aug.	Total
1963	17 May	22 Aug.	97	4	3	4	4	15
*1964	19 June	19 Aug.	61	—	0	0	1	1
1965	24 May	20 Aug.	88	0	0	0	0	0
1966	20 June	10 Aug.	51	—	3	3	2	8
1967	9 June	23 Aug.	75	—	5	2	2	9
1968	1 June	10 July	39	—	0	0	—	0
1969	**	**	0	24	18	18	20	80

*Trap did not operate 29 May to 18 June
***Culicoides* active throughout winter with no period of minimal activity
***Traps were operated on about 20 nights each month (see Table 1)

(c) *The effect of climate on the incidence of* Culicoides *adults*

Except in winter, temperatures do not differ markedly enough from year to year to account for sudden changes in *Culicoides* numbers. However, rainfall does. Nevill (1967) discussed the possible ways in which it may influence *Culicoides* abundance and with further records now available this relationship has become clearer.

These data provide abundant evidence to link increases in catches with good rains the previous month (Table 1, Fig. 1). Good rains in early summer (October-November) did not, however, result in as large an increase in catch as did the same rainfall from December onwards. This could be due to the fact that the early summer catches are possibly composed chiefly of midges which have overwintered as larvae. Good rains from December onwards are apparently essential to provide these midges with suitable breeding sites, which would then give rise to the peak catches recorded from January onwards.

In October 1964, for example, 154,2 mm of rain was recorded and the midge catch only increased from 780 in October to 1 856 in November. However, during the following month (December 1964) nearly the same amount of rain fell (165,0 mm) and was followed by a very sharp increase in catch from 1 494 in December to 17 139 in January.

Evidence that the early summer catches are composed chiefly of midges which have overwintered as larvae can be obtained by a comparison of catches in May, just before low temperatures stopped flight, with catches in October or November. In nearly all the years studied there is a similarity in catch size for these two periods; this is most clearly shown in 1967 when the catch for May averaged 20 885 and that for October 17 424. Thereafter the catches dropped and because of relatively poor rainfall did not rise above 5 984 (April 1968).

(d) *Overwintering of* Culicoides *larvae*

Low temperatures during winter slow down the development both of *Culicoides* larvae and the micro-organisms on which they feed. By placing a culture of *Culicoides* larvae in a refrigerator at 6,5°C for 14 days development was brought to a complete halt, but on returning it to a temperature of 26°C development continued as if there had been no interruption (Nevill, 1967).

Day-time temperatures in winter are, however, sufficiently high for slow but continuous larval development, pupation, and adult emergence so that there is a potential supply of midges which will fly when night-

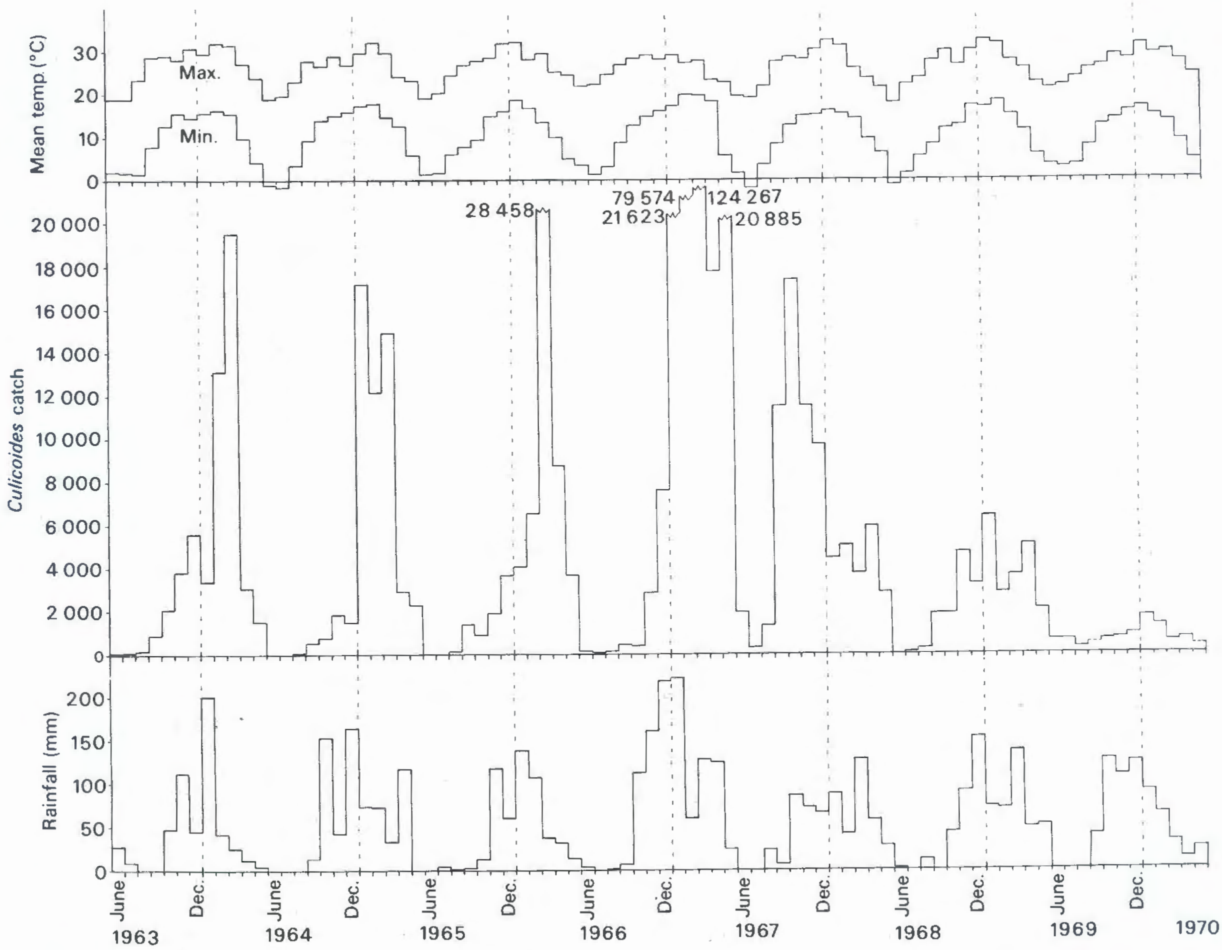

FIG. 1 Mean nightly *Culicoides* light trap catches, mean monthly maximum and minimum temperatures, and total monthly rainfall at the Veterinary Research Institute, Onderstepoort, June 1963 to May 1970

time temperatures are high enough. Once the adults are on the wing they may mate, feed, and oviposit, so that winter breeding is possible in years when temperatures are high enough for flights to take place. For example, during a warm period from 23 June to 4 July 1967, when the average minimum temperature increased from the monthly average of 0,6°C to 4,2°C, a total of 16 667 midges was caught. This period was preceded by 10 nights during which no midges were caught and followed by another 28 nights with nil catches.

The relationship between the seasonal incidence of *Culicoides* midges and the prevalence of BTV will be discussed later.

THE EFFECT OF LOW TEMPERATURES ON THE SURVIVAL OF ADULT *CULICOIDES*

(a) *Refrigerator experiments*

The effect of prolonged exposure to low temperatures was tested on several occasions by transferring adult *Culicoides* taken in light traps to unwaxed 250 ml ice cream cups and placing them in a refrigerator. The humidity was maintained by placing each cup with a wet cotton-wool pad in a closed glass container.

In the first test all but one *Culicoides* adult died after 30 days when kept at 3° to 6°C. In a later test at temperatures as low as —1,5°C mortality amongst the various species was recorded at intervals (Table 3).

TABLE 3 *The effect of refrigeration on the survival of wild-caught* Culicoides *adults*

Date	Days in refrigerator	Minimum Temp. °C	No. of *Culicoides* surviving				
			C. pallidipennis	*C. pycnostictus*	*C. schultzei* Enderlein, 1908	*C. hirtius* De Meillon & Lavoipierre, 1944	*C. magnus* Colaço, 1946
21.8.69	0	0	269	19	2	4	3
5.9.69	15	—1,5	39	19	2	3	2
15.9.69	25	0	0	10	0	1	0
22.9.69	32	0	0	6	0	1	0
29.9.69	39	—1,1	0	2	0	0	0
6.10.69	46	—1,1	0	1	0	0	0
13.10.69	53	—1,1	0	1*	0	0	0

*This specimen died shortly after inspection

Only *C. pallidipennis* and *C. pycnostictus* Ingram & Macfie, 1925 were present in sufficient numbers for a comparison of the effect of these low temperatures on different species. Many *C. pallidipennis* died within the first 15 days whereas no *C. pycnostictus* died. Within 25 days all *C. pallidipennis* had died while many *C. pycnostictus* were still alive. The last adult *C. pycnostictus* died after 53 days. Thus this species can apparently withstand low temperatures far better than *C. pallidipennis* and is a better candidate for survival throughout the winter.

(b) *Field experiments*

To determine if *Culicoides* adults could in fact survive fluctuating winter temperatures, and for how long, adult *Culicoides* were exposed outdoors during the winter and the mortality recorded. *C. pycnostictus* adults were reared from mud collected near a leaking water trough and 1 to 10 midges per cup were allocated to a series of 250 ml cardboard cups. Due to a scarcity of laboratory-reared *C. pycnostictus* only a limited number of cups could be used. Sucrose crystals were placed in the cup and it was covered with nylon chiffon, held in position with an elastic band. A pad of cotton wool saturated with water or sugar solution was placed on the gauze cover of some cups and was replaced daily. The cups were fixed with adhesive tape at heights of from 1 to 2 metres above ground level to the branches of various evergreen trees including a loquat (*Eriobotrya japonica*), avocado (*Persea americana*) and a privet (*Ligustrum lucidum*). Evergreen trees were chosen as it was felt that they provided a microclimate similar to the sites where *Culicoides* adults could be expected to rest. A thermohygrograph was operated in a loquat tree close to some of the cups. In two cups trap-caught *Culicoides* adults were used. A control group of *C. pycnostictus* was kept in the insectary at 21,1°C. Longevity results are given in Table 4.

During the period of these tests minimum temperatures ranged from —1,1°C to 11,1°C and maximum temperatures from 11,1°C to 26,7°C. Temperatures were unexpectedly high in July and August 1968 and *Culicoides* were caught in the light trap from 11 July onwards. Relative humidity readings fluctuated widely from a mean daily minimum of 35,0 per cent in the early afternoon to a mean daily maximum of 88,5 per cent at dawn. Without access to water *Culicoides* adults lived a maximum of 4 to 17 days while those provided with sugar solution or sugar crystals plus water survived a maximum of 36 to 51 days. Some may have lived longer had they not drowned in excess moisture (Table 4). In the control group the last midge died after 63 days, which compares well with those kept outdoors.

Water is thus essential for adult survival under conditions of fluctuating relative humidity as experienced during the course of this experiment. Cups with a wet cotton-wool pad resting on the nylon gauze cover were apparently affected very little by changes in relative humidity since tests showed that the evaporation rate in these cups was much lower than in cups having no wet pads.

INCIDENCE OF BTV IN *CULICOIDES* ADULTS

Du Toit (1962) stated that "from the experience gained of injecting emulsions of *Culicoides* caught in nature by means of light traps into bluetongue susceptible sheep the virus is generally not recoverable from those insects until December when only occasionally a sheep will react to such an injection. From about the middle of February, however, injections of emulsified *Culicoides* produce cases of bluetongue in susceptible sheep with absolute regularity until the first frosts in May when the insects disappear almost entirely". The writer has recently confirmed the presence of BTV in *Culicoides* in May just prior to their winter disappearance (Table 5).

Adult *Culicoides* are, however, already active from the end of August (Table 1). Why then is there a delay of 3 to 4 months before BTV is isolated from *Culicoides*? In his attempts to isolate BTV from *Culicoides*, R.M. du Toit (formerly of the Veterinary Research Institute, Onderstepoort, personal communication, 1964) used batches of approximately 1 000 midges each. To determine whether BTV could be isolated earlier if larger numbers of midges were tested, catches of *Culicoides* were stored at —20°C, or snap frozen in alcohol and dry-ice and stored at —20°C, or stored on dry-ice. When about 10 000 *Culicoides* had been accumulated they were emulsified with a mortar and pestle and about 12 ml of stabilizing solution, prepared from 0,066 M phosphate buffer, pH 7,4, plus 2 per cent peptone and 10 per cent lactose, was added. After centrifuging at ± 2 100 rpm until the supernatant was clear, 5 ml of the supernatant was injected subcutaneously in the inguinal region of bluetongue-susceptible sheep. The sheep were thereafter kept in a closed stable, their temperatures recorded daily and they were examined for other clinical signs of bluetongue.

TABLE 4 *Adult* Culicoides *survival periods out of doors - Onderstepoort, July 1968*

Date	No. of *Culicoides*	Tree	Provided with:		Maximum survival time (days)
			Sugar crystals	Sugar solution	
5.7.68	11 wc	Avocado	+	+	37
,,	12 wc	Privet	+	+	40
4.7.68	1 lr	Loquat	—	+	51
9.7.68	5 lr	Loquat	—	—	10
,,	5 lr	Loquat	+	—	17
,,	4 lr	Loquat	+	+	51**
24.7.68	10 lr	Loquat	+	—	4
,,	9 lr	Loquat	+	water*	36**
,,	10 lr	Loquat	+	+	36**
Control	4 lr	Insectary (21,1°C)	+	+	63

*No sugar solution
**Last 2 or 3 surviving *Culicoides* drowned in excess sugar solution or water
wc Wild-caught
lr Laboratory-reared
+ Present
— Absent

Early in the summer of 1964/65 and 1965/66 it took 2 to 4 weeks to accumulate about 10 000 *Culicoides* adults, but these periods shortened as summer progressed and more *Culicoides* became active. In 1967/68, however, vast numbers of *Culicoides* were caught early in the summer so that in 2 weeks from 80 000 to 150 000 midges were accumulated, while in one 6-day period in October 1967, 174 000 midges were caught. Attempts to isolate BTV during the periods of continuous *Culicoides* activity in the years 1964/65, 1965/66 and 1967/68 are summarized in Table 5.

Where batches of approximately 10 000 captured *Culicoides* were accumulated and tested at the start of summer, BTV was isolated for the first time on 28 December 1964 and on 24 November 1965. This agrees with the statement by Du Toit (1962) that virus is generally not recoverable until December.

Excellent catches made immediately after winter in 1967 (Table 1) provided the large number of *Culicoides* needed to test the suggestion that BTV is present in a very small percentage of the midges at this time of the year. After the injection of massive numbers of midges, BTV was first isolated from 153 682 *Culicoides* collected between 28 September and 11 October and again in the beginning of November. The fact that other large catches in September and October were negative further supports the suggestion that BTV incidence at this time is extremely low.

Incidence of BTV in cattle

Du Toit (1962) noted that "virus may be recovered from cattle in November in a small percentage of cases, - but this becomes a more regular feature from the end of December to reach a peak in incidence in January and February. Hereafter the incidence drops but odd occurrences may be encountered in May after the onset of cold weather and even into June. The virus may persist in a bovine for a period of over 3 months -".

This statement sums up our existing knowledge. Since both cattle and *Culicoides* are involved in the bluetongue cycle it was considered essential that the incidence of BTV in cattle be studied simultaneously with its incidence in *Culicoides* midges.

In each of the years 1964/65, 1965/66, and 1967 5 head of cattle were kept outdoors where they were exposed to insect bites. They were bled weekly or bi-weekly in oxalate-citrate-glycerine (O.C.G.) (Edington, 1900) and attempts were made to isolate BTV in sheep by intravenous injection of the cattle blood. In the first 2 years of the study the sentinel cattle at Onderstepoort were in close proximity to the light trap and each animal was tested individually for the presence of BTV. In the last year (1967) the cattle were on a Government experimental farm 3 miles from Onderstepoort and a sample of their pooled blood was tested in a single sheep each week. The results of these tests are given in Table 6.

In 1964 and 1965 BTV appeared in one or more of the 5 head of cattle on 30 and 15 December respectively. In 1967 the first isolation was made on 22 November. These results, although from only 5 head of cattle in each case, agree with the findings of Du Toit (1962).

It was at first thought that BTV might show up earlier in cattle in 1967 since it was recovered in early October from *Culicoides*. However, this recovery required the use of vast numbers of midges. Similarly, large numbers of cattle would probably have to be tested to reveal earlier bluetongue infection. Since 5 head of cattle were used in each of the experiments and similar results were obtained in each case, BTV can be assumed to have been equally prevalent in the 3 years studied.

TABLE 5 *Isolation of BTV from wild-caught* Culicoides *midges - Onderstepoort, 1964 - 1968*

Date	No. *Culicoides** injected	Reaction in sheep	Date (continued)	No. *Culicoides* injected (continued)	Reaction in sheep (continued)
23.9.64	10 143	0	26.1.66	568	0
13.10.64	9 664	0	9.2.66	4 500	0
4.11.64	9 678	0	16.2.66	10 365	+
18.11.64	13 819	0	21.2.66	5 551	+
24.11.64	3 610	0	24.2.66	12 657	+
27.11.64	11 377	0	4.3.66	22 995	+
28.12.64	9 185	+	14.3.66	11 815	+
6.1.65	11 100	+	28.3.66	28 830	0
20.1.65	10 024	+	12.4.66	2 119	0
3.2.65	10 290	+	22.4.66	12 161	0
17.2.65	** 3 000	+	3.5.66	8 300	+
9.4.65	** 4 022	+	4.5.66	7 321	+
11.2.65	*** 783	0	9.5.66	17 407	0****
10.3.65	*** 1 800	0	26.5.66	18 799	0****
22.9.65	12 631	0	13.9.67	107 081	0
20.10.65	11 021	0	27.9.67	88 919	0
3.11.65	16 928	0	11.10.67	153 682	+
17.11.65	11 506	0	25.10.67	174 032	0
24.11.65	13 336	+	8.11.67	56 525	+
25.11.65	3 547	0	15.11.67	36 435	0
1.12.65	2 513	0	23.11.67	62 354	+
15.12.65	7 870	+	30.11.67	76 534	+
23.12.65	6 000	0	7.12.67	57 576	+
4.1.66	5 500	+	15.12.67	71 457	+
14.1.66	7 343	0	30.5.68*****	11 452	+

* = A variety of *Culicoides* spp., except where otherwise indicated
** = *C. pallidipennis* only
*** = *Culicoides* spp. excluding *C. pallidipennis*
**** = Tested in sheep in August 1968 after storage at —20°C
***** = *Culicoides* spp. caught from 20.5.68 to 30.5.68
0 = No reaction
\+ = Positive reaction i.e. BTV present.

TABLE 6 *The isolation of BTV from cattle kept outdoors - Onderstepoort, 1964 - 1968*

1964 - 1965		1965 - 1966		1967	
Date	cattle positive/ cattle sampled	Date	cattle positive/ cattle sampled	Date	cattle positive/ cattle sampled
23.9.64	0/5	17.11.65	0/5	20.9.67	0/5
7.10.64	0/5	24.11.65	0/5	27.9.67	0/5
21.10.64	0/5	8.12.65	0/5	4.10.67	0/5
4.11.64	0/5	15.12.65	2/5	11.10.67	0/5
18.11.64	0/5	22.12.65	2/5	18.10.67	0/5
2.12.64	0/5	29.12.65	4/5	25.10.67	0/5
15.12.64	0/5	5.1.66	4/5	1.11.67	0/5
30.12.64	3/5	11.1.66	0/3	8.11.67	0/5
6.1.65	2/2	19.1.66	0/2	15.11.67	0/5
13.1.65	2/3	2.2.66	0/1	22.11.67	5/5**
20.1.65	3/3	16.2.66	1/2	29.11.67	5/5**
		2.3.66	0/2		
		16.3.66	1/2		
		30.3.66	1/1*		

*Two head of cattle were inadvertently stabled at night from ± 12.12.65 onwards thus reducing their chances of becoming infected with BTV and it took until 30.3.66 before the last animal circulated BTV.

**Since the blood samples were pooled in this year the absolute number of bovines circulating BTV could not be determined.

Discussion

The mechanism whereby BTV survives the winter has still to be explained. Many theories have been advanced, amongst them the following:

(1) *Culicoides* midges may transmit BTV transovarially. If this were so then BTV would overwinter in the larvae and could be expected to be found in emerging adults directly after the winter. However, BTV can seldom be isolated from midges before December, 4 months after the start of *Culicoides* activity, so that transovarial survival of the virus seems unlikely.

(2) A complicated overwintering cycle similar to that of Western Equine Encephalitis (WEE) may be required. In the case of WEE, overwintering of the virus has been found to occur in certain snakes (Gebhardt, Stanton, Hill & Collett, 1964). In the following spring infected snakes give birth to infected offspring on which the vector mosquito, *Culex tarsalis* Coquillett, 1896 feeds and thus continues the cycle.

In Saskatchewan WEE virus has also been isolated from naturally infected leopard frogs (*Rana pipiens*) and garter snakes (*Thamnophis* spp.) (Burton, McLintock & Rempel, 1966). Since both frogs and garter snakes can be infected by the oral route and since frogs form a large part of the natural diet of garter snakes, it is possible that these snakes may also become infected after feeding on infected frogs.

Reeves (1961) has recovered WEE virus from experimentally infected wild birds at intervals up to 10 months after the original infection.

These possibilities have not yet been investigated thoroughly for BTV. Since South Africa experiences relatively mild winters compared with the areas of North America where the WEE studies in snakes and frogs have been conducted, it was felt that a less complicated overwintering mechanism may suffice.

(3) Depending on their ability to survive winter conditions, the length of winter, their longevity and the period they remain infective, infected adult *Culicoides* may be able to survive through the winter and continue the virus cycle the following spring. Jochim & Jones (1966) showed that at 24 ± 2°C bluetongue-infected *C. variipennis* remained infective for the 26 day test period. This period will conceivably be longer at lower temperatures, such as those recorded during winter, when BTV may possibly persist in *Culicoides* until death.

The foregoing investigations have shown that *Culicoides* adults can survive cold periods for up to 53 days and that they may be infected with BTV just prior to the beginning of the cold period. *Culicoides* are active on isolated nights during most winters and they continue to emerge throughout the winter so that adult midges are always available. BTV can be recovered from adults at the beginning of October if large numbers are tested, but only in late November-December if small numbers are involved. These observations strongly support the above theory. A qualification is, however, that the winter must not be severe. Where *Culicoides* are completely absent for 88 days, as in 1965 (Table 2), a more complicated overwintering mechanism is probably necessary.

(4) Du Toit (1962) showed that when cattle and sheep were kept for 5 months in summer in camps within 59 yards of each other, the cattle all contracted bluetongue while the sheep did not. This suggests that bluetongue vectors (probably *Culicoides* spp.) prefer to feed on cattle. Nevill (1968) showed that a proven transmitter of BTV at Onderstepoort, namely *C. pallidipennis,* breeds in cattle dung so that cattle provide both blood and breeding material for the vector.

Cattle are an excellent source of BTV in summer and could perhaps also be the winter reservoir host. It has been argued that development of immunity after about 2 weeks would rule out cattle as reservoir hosts. However, there is evidence to show that in certain cases BTV can be detected at intervals in the peripheral blood over a period of several months. Du Toit (1962) found that 1 animal at Onderstepoort circulated BTV for 16 weeks. Owen, du Toit & Howell (1965) subsequently showed that only one strain of BTV was present in this animal over the period concerned, so that reinfection with another strain was not the explanation. In another of the 5 head of cattle tested, the same strain was reisolated after 55 days. The possibility of BTV overwintering in cattle has recently been raised in the U.S.A. Bowne, Luedke & Jochim (1967) mention cases where BTV has been recoverable at intervals up to 3 months after experimental infection of cattle. In 1968, Bowne, Luedke, Jochim & Metcalf discussed cases of clinical bluetongue in cattle. Bluetongue virus was isolated from the blood of an acutely ill cow and 5 months later it was placed in insect-free isolation. Bluetongue virus was again demonstrated in the blood after 60 to 64 days of isolation and on death, after 105 days of isolation, BTV was recovered from some internal organs. In 1969 Bowne (cited by Gee, 1970) showed that "carrier cattle can maintain high blood levels of virus (with antibody) up to one year after infection".

In 1967, due to the large numbers of *Culicoides* midges available for testing, BTV was isolated from midges as early as October. The number of cattle tested to date has been too small to trace the odd animal which might be circulating BTV. It is felt that if a large number of cattle could be tested regularly BTV will almost certainly be recovered at a far earlier date, and even perhaps in winter.

(5) The BTV overwintering mechanism which appears to offer the most likely explanation for the situation at Onderstepoort would be a continuation of the summer cycle between cattle and *Culicoides* midges, but on a lower level. This would be due to the smaller number of midges available, fewer nights on which they are active, and the fact that usually only the early part of a winter's night is warm enough for flight. During the years when *Culicoides* are absent for up to 88 days, BTV could have the opportunity of surviving in cattle until conditions for transmission improve.

Summary

Light trap catches over 7 years at the Veterinary Research Institute, Onderstepoort showed that *Culicoides* adults are active on occasional winter nights and in some years remain active almost throughout the winter. *Culicoides* were also found to survive refrigerator temperature for up to 53 days and to survive outdoors during winter for up to 51 days. Moreover, day-time temperatures during winter were high enough to allow continued development throughout the winter. Bluetongue virus (BTV) may therefore be able to survive short winters in infected *Culicoides* midges while warmer nights in some years may allow midges to fly and feed and so reinfect new hosts.

By testing large numbers of midges BTV has been shown to be present in *Culicoides* adults at the beginning of October. Apparently BTV does not disappear completely in spring but is present in so few midges as to be difficult to detect. BTV could only be detected in late November or December in sample groups of 5 head of cattle at Onderstepoort. If a high percentage of the cattle population could be tested BTV will almost certainly be isolated at an earlier date and even perhaps in winter.

The results of these investigations support the theory that the biological cycle of BTV can continue in *Culicoides* and/or cattle throughout the winter in the Onderstepoort area.

Acknowledgements

I wish to thank Dr. P. G. Howell for his helpful criticism of this manuscript, and Messrs. D. W. du Plooy, R. B. Weber, M. C. de Lange and Misses K. Roos and H. Hoogenhout for their invaluable assistance.

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Printed in the Republic of South Africa for the Government Printer, Pretoria, by Heer Printing Co. (Pty) Ltd., Pretoria.