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HOST PREFERENCES OF *CULICOIDES* MIDGES (DIPTERA: CERATOPOGONIDAE) IN SOUTH AFRICA AS DETERMINED BY PRECIPITIN TESTS AND LIGHT TRAP CATCHES

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ABSTRACT

NEVILL, E. M. & ANDERSON, DORA. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and light trap catches. *Onderstepoort J. vet. Res.* 39(3), 147–152 (1972).

The host-preferences of a number of South African *Culicoides* biting midges were determined by precipitin tests and by an analysis of catches from light traps set near host animals. Results showed *C. pallidipennis* to feed predominantly on cattle and horses and possibly sheep, *C. schultzei* mainly on cattle, *C. distinctipennis* only on birds (possibly poultry), *C. pycnostictus* chiefly on birds, and *C. milnei* mostly on horses. A further five species gave positive precipitin test reactions to blood of either cattle, horses or birds but too few specimens were available for the significance of these reactions to be evaluated.

These results suggest that *C. pallidipennis* and *C. schultzei* might be involved in the transmission of cattle diseases, *C. pallidipennis* and *C. milnei* of horse diseases, *C. distinctipennis* and *C. pycnostictus* of poultry diseases and *C. pallidipennis* of sheep diseases.

INTRODUCTION

Culicoides midges are proven transmitters of bluetongue virus (BTV) of sheep. *Culicoides pallidipennis* Carter, Ingram & Macfie, 1920 transmits BTV in South Africa (Du Toit, 1944) and *C. variipennis* Coquillet, 1901 was proved to be its vector in the U.S.A., (Price & Hardy, 1954; Foster, Jones & McCrory, 1963). Recently in Kenya, Walker & Davies (1971) isolated BTV from pools of wild-caught *C. pallidipennis, C. milnei* Austen, 1909 and from a new species *C. tororoensis* Khamala & Kettle, 1971. In Israel BTV has been isolated from *C. pallidipennis* by Goldsmit & Braverman (cited by Braverman, Boreham & Galum, 1971).

Culicoides midges are also suspected of transmitting African horsesickness. Du Toit (1944) showed wild-caught midges to be infected and succeeded in transmitting horsesickness with *Culicoides* midges (cited by Wetzel, Nevill, & Erasmus, 1970).

These findings suggest that more than one *Culicoides* species may be involved in the transmission of BTV and possibly horsesickness virus in South Africa. Over 26 *Culicoides* species have been found in the Republic, of which several are still undescribed. However, because of their different modes of life and host preferences it is very unlikely that all of them are involved in disease transmission of domestic animals. Some may not even require a blood meal for the maturation of their ovaries.

A knowledge of the host preferences of South African *Culicoides* midges will help future workers to decide which species should be investigated as possible disease vectors. This information can be obtained by identifying the blood-meals found in the abdomens of wild-caught *Culicoides* and by analysing the *Culicoides* species in catches from light traps set close to different groups of potential hosts.

MATERIALS AND METHODS

Collection of engorged Culicoides *for precipitin tests*

Two 250 volt modified New Jersey light traps were operated almost nightly in the vicinity of the Veterinary Research Institute, Onderstepoort from 1965 to 1969. The first was hung within 15 metres of an open stable housing up to 50 mules and horses nightly. Many different species of domesticated animals were kept in a 100 metre radius of this trap, including cattle, pigs, goats, sheep, poultry, rabbits, guinea pigs, dogs and ferrets.

The second trap was operated about 5 km away at a Government farm, "Kaalplaas". Besides being a stock farm where cattle and horses are grazed, this is a nature reserve where many species of wild birds as well as small mammals such as hares, rock hyraxes and the smaller antelopes occur.

After collection in organdie-covered boxes, the midges were killed with ether vapour and the relatively few engorged specimens present were removed. They were identified according to wing pattern, using the key by Fiedler (1951), after which their abdomens were dissected off and placed individually in round bottom Wassermann glass tubes (75 mm × 10 mm), sealed with a rubber stopper and stored at —10°C to —20°C. Periodically a batch of 20 to 60 tubes was placed in a vacuum flask with "dry-ice" and sent to the Arbovirus Unit of the South African Institute for Medical Research where one of the authors (D.A.) identified the blood by means of the precipitin test.

Host preferences of Culicoides *as determined by precipitin test*

A total of 657 blood meals from engorged *Culicoides* collected during the summer months of 1965 to 1969 were subjected to the precipitin test to identify the hosts.

The Uhlenhuth - Weidanz capillary precipitin test was used as modified by Schubert & Holdeman (1956) and Anderson (1967). Specific antisera were prepared in adult roosters using the methods of Tempelis & Lofy (1963) for specific mammalian antisera and of Tempelis & Reeves (1962) for specific avian antisera.

Group antisera to mammalian, avian and amphibian-reptilian sera were prepared in rabbits. Only those antisera having titres of over 1/5000 were satisfactory owing to the small volumes of *Culicoides* blood meals; anti-amphibian-reptilian sera failed to reach this level and were not available for inclusion in the tests.

Due to their small volumes *Culicoides* blood meals were suspended in 0,2 ml of diluent instead of the 0,5 ml used previously for mosquito blood meals. This amount of extract only allowed for a maximum of eight

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tests. Extracts were usually clear and did not require centrifugation.

The procedure followed was to test all blood meals against anti-mammalian and anti-avian sera and, if negative, also against an anti-rabbit serum, prepared in roosters. Those positive to anti-mammalian sera were retested against anti-ox and anti-horse sera; if negative, they were then tested against anti-human, anti-rodent and anti-dog sera, and in some cases also against antisera to cat, pig and rock hyrax (*Procavia* sp.).

As the anti-ox serum reacted with sheep blood, antisera specific to ox and sheep were prepared using the absorption method described by Weitz (1956). The absorbed sera, though specific, had a reduced titre.

Those blood meals positive to anti-avian serum were retested against specific antisera to guinea-fowl, wild duck, coot, sacred ibis, pigeon and masked weaver. Tempelis (1962) produced a specific anti-chicken serum in pheasants. As pheasants were unavailable, guinea-fowls and francolins were used in an attempt to produce a specific antiserum but without success. An anti-chicken serum prepared in rabbits reacted to a wide range of avian species. Attempts to increase its specificity by absorption failed because of loss of titre.

These procedures and the sequence followed seemed the best with the available antisera and probable hosts. Owing to the small quantity of blood, negative results do not necessarily exclude the animals in the test, so that only positive results should be considered.

Collection of Culicoides *midges in light traps near bait animals for species analysis*

Portable light traps based on the modified Communicable Diseases Centre trap used by A. L. Dyce (CSIRO McMaster Laboratory, Sydney), were constructed for use near bait animals at Onderstepoort. These traps incorporated a 12 volt, 6 candle power tungsten filament bulb and a 9 volt gramophone turntable motor operating a fan at 1 500 r.p.m. Insects attracted to the light were sucked through the fan blades into a container of 70% alcohol or, if required alive, into a nylon chiffon bag. The traps were operated off 12 volt D.C. car batteries and could run for up to 60 hours on one charge.

Portable traps were hung in a poultry house containing about 50 Leghorn hens; in an open cattle stable containing 13 tethered oxen; in a sheep paddock within 20 metres of about 50 Merino sheep and under a tree in the garden of one of the authors (E.M.N.) at the Veterinary Research Institute at least 150 metres from the nearest stock. This last trap acted as a control as no specific attraction was present other than the trap's light. The relative positions of these traps are given in Figure 1.

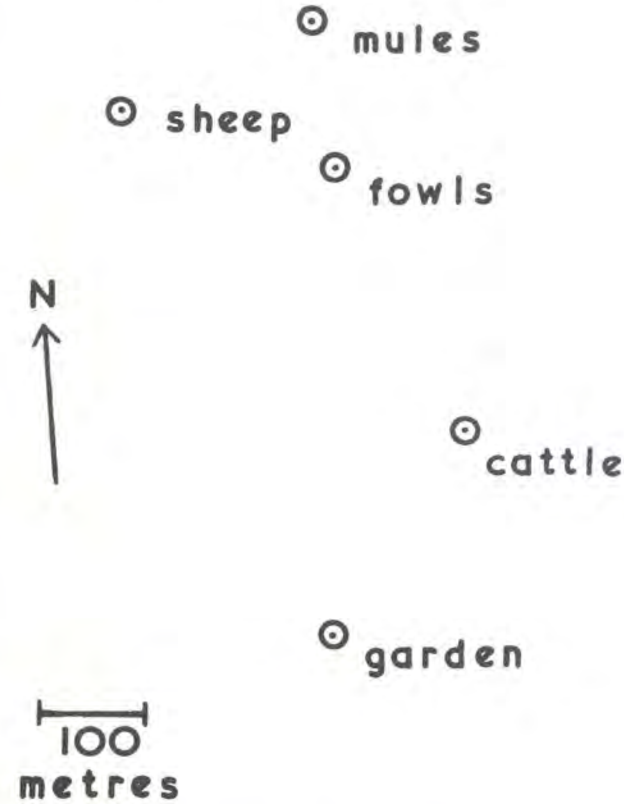

FIG. 1 Relative positions of light traps set near bait animals at the Veterinary Research Institute, Onderstepoort

As a modified New Jersey trap was already in permanent operation 15 metres from an open stable housing 50 mules and horses, a sample of this trap's catch was used for comparison with catches from the portable traps. This was considered permissible as determination of the species composition of the catches was the aim of the investigation, not the absolute numbers trapped.

TABLE 1 Precipitin test identification of *Culicoides* blood meals - Onderstepoort area 1965–1969

Culicoides species	Blood Meals		Antisera						
			Group		Specific mammalian				
	Unidentified	Identified	Mammal	Avian	Ox	Horse	Sheep	Ox or sheep	Host unidentified
C. pallidipennis	152	263	263	0	82	77	1(3)	95(2)	8
C. distinctipennis	51	56	0	56	0	0	0	0	0
C. pycnostictus	32	17	3	14	1	2	0	0	0
C. schultzei	14	15	15	0	13	1	0	1	0
C. milnei	0	15	14	1	2	12	0	0	0
C. nivosus	13	3	2	1	0	0	0	1	1
C. neavei	4	1	0	1(4)	0	0	0	0	0
C. magnus	1	1	1	0	0	0	0	1	0
C. engubandei	0	2	2	0	1	1	0	0	0
C. gulbenkiani	0	1	1	0	0	1	0	0	0
Other species(1)	16	0	0	0	0	0	0	0	0

(1) *C. babrius, C. bedfordi, C. cornutus, C. eriodendroni, C. ravus, C. similis*
(2) 53 from cattle stable (3) From sheep pen (4) From fowl house

The traps were operated on 7 to 14 nights in February and March 1968, and the trap in the cattle stable was also operated in October and November 1968. Catches were identified and the numbers of each species, their sex and whether or not they were engorged, were recorded.

RESULTS

Precipitin tests (Table 1)

A total of 657 specimens belonging to 16 species was tested, of which 374 from 10 species were positive to mammalian or avian antisera. *C. pallidipennis* was by far the commonest species tested (263 positive) and was only positive to mammalian antisera, chiefly cattle and horses plus a few sheep (see Discussion). *C. schultzei* Enderlein, 1908 also fed exclusively on mammals, nearly all cattle (13/15).

Very few *C. magnus* Colaco, 1946, *C. engubandei* De Meillon, 1943 and *C. gulbenkiani* Caeiro, 1959 were tested and these fed either on horses or cattle.

C. milnei preferred to feed on horses (12/15) but fed twice on cattle and once on birds. *C. nivosus* De Meillon, 1937 fed on both mammals and birds but only small numbers were tested so it is impossible to say which they preferred. *C. pycnostictus* Ingram & Macfie, 1925 clearly favoured birds (14/17) although one specimen was positive to cattle and two to horses. The only positive specimen of *C. neavei* Austen, 1912 fed on birds.

An important finding was that *C. distinctipennis* Austen, 1912 fed only on birds, probably poultry, despite the fact that 39/56 were colected in the trap 15 metres from 50 mules and horses (Fig. 1).

Light trap catch analyses (Table 2) (Fig. 1)

A comparison of the percentage of each *Culicoides* species caught in light traps set near poultry, mules, sheep, cattle and in a garden (the control) showed the following:–

C. pallidipennis accounted for more than 94% of the catches near mules, sheep, and cattle compared with only 47,06% near poultry and 26,20% in the control.

C. distinctipennis represented less than 1,1% of the catches near mules, sheep and cattle but 47,42% of the catch near poultry, indicating a strong preference for poultry. Unexpectedly, it constituted 56,06% of the catch in the garden. However, close inspection of these specimens showed that males and females were present in nearly equal numbers, indicating nearby breeding sites. One such site was in fact found in mud bordering an irrigation furrow 70 metres away.

C. pycnostictus also was mostly caught in the control trap (12,56%) but in its case the females well outnumbered the males (± 21 : 1). This species was otherwise commonest near poultry (1,88%).

C. schultzei was most abundant in the poultry house trap (2,47%) followed by the cattle trap (1,25%); in the other traps they were scarce (± 0,7%).

C. babrius De Meillon, 1943 was most abundant in the control trap (1,98%) and sheep trap (1,63%) but accounted for only 0,21% to 0,23% of the other catches. *C. milnei* was commonest in the sheep trap (1,09%) but made up only 0,23% of the catch in the other traps set near stock.

C. nivosus, C. similis Carter, Ingram & Macfie, 1920, *C. magnus, C. neavei, C. hirtius* De Meillon & Lavoipierre, 1944 (= *C. brucei* Austen, 1909), *C. bedfordi* Ingram & Macfie, 1923, and *C. eriodendroni* Carter, Ingram & Macfie, 1920 were also trapped but only in extremely low numbers.

With regard to *engorged* females of the different species, there were only enough specimens of *C. pallidipennis* and *C. distinctipennis* to make a comparison of the figures from the different traps worthwhile. Less than 0,74% engorged *C. pallidipennis* were collected near poultry, mules and sheep while 3,07% were collected in the trap set near cattle, indicating a preference for cattle. No engorged *C. distinctipennis* was found in the catches from the traps set near mules, sheep or cattle while 9,55% were collected in the poultry house. This is further evidence of this species' strong preference for feeding on poultry.

DISCUSSION OF RESULTS

The high proportion of negative precipitin test results (43%) could be due to the lack of appropriate antisera or more probably, to the small amount of blood in the meals; a possible explanation for the high percentage of identified blood-meals of *C. milnei* could be due to the volume of blood in this larger species.

When the blood meals positive to anti-avian serum were retested against the specific anti-avian sera no host species could be identified. The Onderstepoort light trap was situated near to the poultry section so it would appear likely that the avian blood meals were of domestic fowl origin.

C. pallidipennis fed entirely on mammalian blood - horse and ox and at least in one case on sheep. A further 95 blood samples which were only tested against ox antiserum could have originated from either ox or sheep; however, as the majority were collected near cattle it would seem that these were largely of this species.

A comparison of precipitin test results and light trap analyses shows that both methods agree on the hosts preferred by three *Culicoides* species in the study area. They are:

C. pallidipennis - cattle, horses, sheep
C. distinctipennis - birds (? poultry)
C. pycnostictus - birds (? poultry)

The most interesting findings from these investigations are the absolute preference of *C. distinctipennis* for avian blood, the strong preference of *C. pycnostictus* for birds and the fact that *C. milnei, C. nivosus* and *C. neavei* also feed at times on birds. These findings may be useful to workers on disease transmission in poultry since *Culicoides* spp. have already been shown to transmit various protozoan diseases of birds including *Leucocytozoon caulleryi* in poultry in S.E. Asia and Japan (Kettle, 1965). Those species of *Culicoides* which feed on both birds and mammals may be important when the epizootiology of virus diseases includes both these host groups.

Cattle are excellent reservoirs of BTV in summer (Du Toit, 1962) and overwintering of BTV may possibly take place in them (Nevill, 1971). Thus any of the midges which feed on cattle may be involved in the transmission of BTV, and perhaps of other viruses of cattle. The above results show that *C. pallidipennis* and *C. schultzei* have the strongest preference for feeding on cattle but in addition three other species have also been found to feed on these animals.

Similar results with precipitin tests have been obtained in Kenya by Walker & Davies (1971), who showed that, of the species they tested which also occur in South Africa, *C. cornutus, C. magnus, C. milnei,* and *C. pallidipennis* all fed on cattle and sheep. In Israel, Braverman *et al.* (1971) by the use of precipitin tests showed *C. pallidipennis* to feed on both cattle and sheep with a distinct preference for cattle.

TABLE 2 Analysis of *Culicoides* spp. in light trap catches near different animals - Onderstepoort, 1968

Trap locality	No. of nights	Category	*C. pallidipennis*		*C. distinctipennis*		*C. pycnostictus*		*C. schulzei*		*C. babrius*		*C. milnei*		*C. nivosus*		*C. similis*		Other species*	
			Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
Poultry House	14	♀	394	46,24	335	39,32	16	1,88	16	1,88	2	0,23	2	0,23	4	0,47	1	0,12	2	0,23
		♂	7	0,82	69	8,10	0	0	5	0,59	0	0	0	0	0	0	0	0	0	0
		Engorged	1	**(0,25)	32	(9,55)	1	(6,25)	0	0	0	0	0	0	1	(25,00)	0	0	0	0
		Total	401	47,06	404	47,42	16	1,88	21	2,47	2	0,23	2	0,23	4	0,47	1	0,12	2	0,23
Mule Stable	13	♀	7828	96,79	53	0,66	52	0,64	49	0,61	17	0,21	19	0,23	1	0,01	6	0,07	3	0,03
		♂	26	0,32	21	0,26	1	0,01	11	0,14	0	0	0	0	0	0	0	0	0	0
		Engorged	24	(0,31)	0	0	0	0	1	(2,04)	0	0	0	0	0	0	0	0	0	0
		Total	7854	97,11	74	0,92	53	0,65	60	0,75	17	0,21	19	0,23	1	0,01	6	0,07	3	0,03
Sheep Paddock	7	♀	1369	93,00	14	0,95	11	0,75	7	0,48	24	1,63	16	1,09	3	0,20	2	0,14	3	0,21
		♂	18	1,22	2	0,14	1	0,07	2	0,14	0	0	0	0	0	0	0	0	0	0
		Engorged	10	(0,73)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Total	1387	94,22	16	1,09	12	0,82	9	0,62	24	1,63	16	1,09	3	0,20	2	0,14	3	0,21
Cattle Stable	7	♀	6716	97,49	12	0,17	11	0,16	81	1,18	13	0,19	15	0,22	1	0,01	0	0	5	0,06
		♂	23	0,33	2	0,03	2	0,03	5	0,07	2	0,03	1	0,01	0	0	0	0	0	0
		Engorged	206	(3,07)	0	0	1	(9,09)	1	(1,23)	0	0	0	0	0	0	0	0	0	0
		Total	6739	97,82	14	0,20	13	0,19	86	1,25	15	0,22	16	0,23	1	0,01	0	0	5	0,06
Garden	13	♀	421	23,72	550	30,99	213	12,00	10	0,56	31	1,75	3	0,17	8	0,45	31	1,75	13	0,74
		♂	44	2,48	445	25,07	10	0,56	1	0,06	4	0,23	0	0	3	0,17	4	0,23	2	0,12
		Engorged	7	(1,66)	5	(0,91)	0	0	0	0	0	0	0	0	1	(12,50)	0	0	1	(7,70)
		Total	465	26,20	995	56,06	223	12,56	11	0,62	35	1,98	3	0,17	11	0,62	35	1,98	15	0,86

*Other species include *C. magnus*, *C. neavei*, *C. bedfordi*, *C. hirtius*, & *C. eriodendroni*

**The number within brackets ( ) indicates percentage females engorged

The present results also show that *C. pallidipennis* and *C. milnei* have a strong preference for feeding on horses and there is evidence that four other species had also fed on horses. The *Culicoides* species involved in horsesickness transmission may be found amongst these.

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