

# Notes on *Culicoides* (Diptera, Ceratopogonidae) from the Sudan in relation to the epidemiology of bluetongue virus disease

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## RÉSUMÉ

Notes sur les *Culicoides* (Diptera, Ceratopogonidae) du Soudan relatives à l'épidémiologie de la fièvre catarrhale du mouton

Des captures de *Culicoides* au piège lumineux dans les régions de Khartoum et de Wad Medani au Soudan ont comporté onze espèces. Les références préalables sont discutées, le nombre d'espèces enregistré jusqu'à présent étant seize.

Une analyse des captures a donné un cycle gonotrophique d'une durée de cinq jours pour *C. kingi* et *C. imicola*, ainsi qu'un taux de survie quotidien de 0,86 et 0,72 respectivement.

*C. kingi* et *C. imicola* sont probablement considérés comme les espèces de *Culicoides* les plus vraisemblablement impliquées dans les cycles de transmission virale. Cependant, un examen de 11 193 *C. kingi* et de 848 *Culicoides* d'autres espèces n'a permis aucun isolement du virus. Plusieurs raisons possibles pour ce phénomène sont discutées.

## INTRODUCTION

Although studies have been made of insects of medical importance in the Sudan (e.g. LEWIS, 12, 13, 14, 15), little attention seems to have been given to insects of veterinary significance. For instance, bluetongue virus (BTV) appears to be widespread and infections with this virus are common (7), but there is little information on the presence or distribution of the potential vector species or of their biology in the Sudan. The following notes represent data collected during 3 short visits in October 1979, February 1980 and October 1980 as part of a general study on *Culicoides* and viruses affecting ruminants in the area.

## MATERIALS AND METHODS

Insects were collected in Monks Wood light traps (20) operated from a car battery or from the mains supply via a transformer from dusk until dawn.

Those insects required for identification purposes were collected in saline with a little weak detergent and were preserved in formalin until return to the United Kingdom. *Culicoides* were then sorted under a stereomicroscope and were slide-mounted where necessary. Identifications were made by comparison with specimens in the British Museum (Natural History), by comparison

with descriptions or, where possible, by comparison with types.

The insects required for virus isolation experiments were collected in saline containing 0.1 p. 100 detergent and 100 i.u. of neomycin per ml. *Culicoides* species were sorted under a binocular within 12 h of capture and were distributed into the following groups: *C. kingi* (nulliparous and parous), *C. imicola* (nulliparous and parous), « other *Culicoides* » (nulliparous and parous) and « mixed blood-engorged *Culicoides* ». Nulliparity or parity was gauged by the absence or presence of red abdominal pigment (6).

All *Culicoides* intended to be used in virus isolation experiments were kept at 4 °C for at most 48 h, after which they were transferred to - 70 °C until required for processing. Pools of midges numbering up to 200 were ground in Griffiths tubes containing 3.5 ml of 2 p. 100 bovine albumen in phosphate buffered saline plus 100 i.u. of neomycin per ml. The suspensions were clarified by centrifugation at 2 000 rpm for 5 min and 10-fold dilution series were prepared down to 10<sup>-2</sup>. Virus isolation was then attempted from each pool by intravenous inoculation of 10 day old embryonating chick eggs (9), intracerebral inoculation of 3 day old suckling mice (19) and inoculating BHK 21 cells (18). Eggs and mice dying between 5 and 10 days post infection and tissue cultures showing a cytopathic effect within the same time scale were saved and passed a second time to avoid missing virus strains with a low pathogenicity.

## RESULTS

### Species recorded in previous and present studies

Sixteen species of *Culicoides* have previously been recorded from the Sudan, namely, *C. austeni* CIM 1920 (17), *C. bedfordi* I & M 1923 (17), *C. fulvithorax* (AUSTEN, 1912) (17), *C. fuscicaudae* MACFIE 1947, *C. grahamii* AUSTEN 1909 (1), *C. neavei* AUSTEN 1912, *C. nilogenus* KIEFFER 1921, *C. nilophilus* KIEFFER 1921, *C. pallidipennis* CIM 1920 (17), *C. praetermissus* CIM 1920 (17), *C. schultzei* (ENDERLEIN 1908) (17), *C. pycnostictus* I & M 1925 (17), *C. schultzei* (ENDERLEIN 1908) (17), *C. signatus* KIEFFER 1921 and *C. similis* CIM 1920 (17).

Of these, the record of *C. bedfordi* cannot be confirmed (MACFIE's specimen in the British Museum is not *bedfordi*) and the descriptions of *C. nilogenus*, *C. nilophilus* and *C. signatus* are too vague for definite identifications to be made (the types of these species have been lost). *C. fuscicaudae* is a synonym of *C. ravus* (3), *pallidipennis* = *imicola* (11) and *praetermissus* = *leucostictus* (24).

During the present study, 11 species of *Culicoides* were recorded: *C. circumscriptus* KIEFFER 1918, *C. distinctipennis* AUSTEN 1912, *C. imicola* KIEFFER 1913, *C. kingi*, *leucostictus* KIEFFER 1911, *C. moreli* CLASTRIER 1959, *C. moucheti* CORNET and KREMER 1970, *C. pycnostictus*, *C. ravus* DE MEILLON 1936, *C. schultzei* and *C. similis*.

The list of species so far recorded can therefore be amended to:

*C. austeni*, *C. circumscriptus*, *C. distinctipennis*, *C. fulvithorax*, *C. grahamii*, *C. imicola*, *C. kingi*, *C. leucostictus*, *C. milnei*, *C. moreli*, *C. moucheti*, *C. neavei*, *C. pycnostictus*, *C. ravus*, *C. schultzei* and *C. similis*.

In October 1979, catches were made at Khartoum (in a garden near the airport and 2 km from the Blue Nile), at Soba (Central Veterinary Laboratory, near cattle and horse pens), at Wad Medani (in town, hotel balcony) and at Nishishiba (University of the Gezira Experimental Farm, 5 km north of Wad Medani, near cattle pens). In 3 catches from Khartoum (total 139 midges), *C. kingi* and *C. imicola* were most abundant (77 p. 100 and 5 p. 100). Other species taken were *C. similis*, *C. ravus*, *C. leucostictus*, *C. pycnostictus* and *C. circumscriptus*. In 2 catches from Soba (total 1,174 midges), *C. kingi* and *C. imicola* accounted for 87 p. 100 and 12 p. 100 of the catch. In Wad Medani, only 2 *C. kingi* were taken. At Nishishiba, the proportions of these species were 81 p. 100 and 18 p. 100 (total midges 14,959). A single *C. moreli* was taken at Nishishiba.

In late January and early February 1980, in addition to catches at these localities, a series of 11 catches was made at a small farm 7 km south of Khartoum, about 150 metres from the bank of the Blue Nile. The number of male and female *C. kingi* and *C. imicola* taken, with other *Culicoides* species, are shown in table I.

The *C. imicola* and *C. kingi* were sorted into 6 age-grades on the basis of presence or absence of red abdominal pigment (6): nulli-

TABLE 1 - The numbers of *Culicoides* species taken at light in eleven catches near Khartoum, January-February 1980

Date	<i>C. imicola</i>		<i>C. kingi</i>		<i>C. circumscriptus</i>		<i>C. similis</i>		<i>C. foveus</i>		<i>C. moucheti</i>		<i>C. pycnostictus</i>	<i>C. distinctipennis</i>
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♀	♀
Jan. 27-28	3	41	433	952	0	1	2	1	236	432	2	9	0	0
28-29	16	148	566	4383	3	6	5	9	486	776	2	8	0	0
29-30	3	54	555	1815	0	5	3	1	198	475	0	6	0	0
30-31	1	19	163	551	0	3	0	0	90	205	1	1	0	0
Jan. 31-Feb. 1	9	34	238	1008	3	6	1	2	51	142	1	2	0	0
Feb. 1-2	7	50	445	1835	0	1	1	2	133	202	1	8	1	1
2-3	16	119	930	6903	3	3	7	3	346	304	1	9	0	1
3-4	5	45	941	1994	6	6	1	3	235	249	0	4	0	0
4-5	0	1	357	335	1	0	0	0	23	33	0	0	0	0
5-6	3	26	555	439	2	2	0	0	0	0	1	0	0	0
6-7	6	13	331	759	2	6	1	3	31	86	0	1	0	0

parous (without pigment); nulliparous, blood-fed; ovarian stages II-IV (with some pigment developing and some blood in the gut); parous empty (with red pigment); parous blood-fed. These stages were easily distinguished in formalin-preserved midges at low magnification without dissection.

The percentages of these age-grades found over the series of 11 catches were: *C. kingi* 43.9, 3.3, 2.4, 8.8, 39.4 and 2.3 p. 100 (total 20,975); *C. imicola* 18.4, 4.7, 1.1, 54.4, 19.8 and 1.6 p. 100 (total 550). The numbers of nulliparous (first 4 grades) and parous (last 2 grades) midges were analysed by the method of BIRLEY and BOORMAN (2) to obtain estimates of survival rates and lengths of the gonotrophic cycle. For a gonotrophic cycle length of 5 days a daily survival rate of 0.72 was obtained for *C. imicola* (correlation index 0.79); for *C. kingi* a cycle length of 5 days corresponded to an estimated daily survival rate of 0.86 (correlation index 0.79).

#### Virus isolation experiments

All insects for virus isolation were collected at Nishishiba (University of Gezira Experimental Farm), using a light trap positioned at the edge of a cattle pen. A total of

12,041 nulliparous and parous female *Culicoides* were collected over 7 nights (table II). More than 93 p. 100 were *C. kingi*, while *C. imicola* comprised less than 2 p. 100 of the catch. At least 6 other species of midge were collected: *C. circumscriptus*, *C. neavei*, *C. schultzei*, *C. distinctipennis*, *C. similis*, and one or more clear-winged species, but together these made up only 0.4 p. 100 of the total catch. Blood-engorged *Culicoides* comprised the final 4.6 p. 100.

The *Culicoides* were divided into 74 pools during the virus isolation experiments but, although several pools were blind-passaged twice in mice and BHK cells, no viral agent was recovered.

#### DISCUSSION

Of the species so far recorded from the Sudan, only *C. imicola* is known to be involved in the transmission of bluetongue in Africa. The virus has been isolated from *C. milnei* in Kenya (23) and therefore this species, together with the closely related *C. austeni* and *C. moreli*, must be regarded as potential vectors, particularly as this group of midges is often found in close association with cattle (5). *C. austeni* and *C. moreli* may be more abun-

TABLE II - Parous and nulliparous female *Culicoides* collected at Nishishiba for virus isolation experiments

Night of	<i>C. kingi</i>	<i>C. imicola</i>	Other <i>Culicoides</i>	Blood-engorged <i>Culicoides</i>
Oct. 30, 1980	485	5	0	25
31	1004	18	3	62
Nov. 1	3000	47	19	46
2	353	4	5	40
3	1755	50	5	89
4	3425	85	7	236
5	1121	14	4	84
Totals	11193	223	43	582

dant in other regions or in other seasons of the year but from our present evidence of only a single specimen they do not seem likely to be vectors in the Khartoum or Wad Medani area. *C. grahamii* is closely related to *C. imicola* but WALKER and DAVIES (23) regarded this species, together with *C. austeni*, to be of low potential in bluetongue virus transmission. Bovine ephemeral fever virus has been isolated from a pool of midges containing *C. kingi* in Kenya (although in this case the species was *C. kingi* sensu KHAMALA and KETTLE (10) not *C. kingi* sensu AUSTEN (1). Palyam virus has been isolated from pools of midges containing *C. schultzei* and *C. pallidipennis* (22). *C. kingi* was found in large numbers in association with cattle in the present study but its capacity to transmit virus is unknown. Over 11,000 *C. kingi* were examined for viral agents during the course of this work but with completely negative results. There are several explanations, in addition to the possibility of *C. kingi* not being a vector, which might account for this. It may be that the conditions under which the insects were kept after collection were not conducive to virus survival, although BTV is usually highly stable at 4 °C (16). It may be that virus was not circulating in the *Culicoides* population at the time when the insect trapping was carried out, although at this time BTV antibodies were recovered from 9 out of 9 calves at Nishishiba aged between 3 and 6 months (JEGGO, pers. comm.). This indicates virus transmission in the preceding 3 months, since maternal antibody in calves wanes within 3 months of birth. Nevertheless, other information (HERNIMAN, pers. comm.) indicates that different

types of BTV pass through populations of cattle (and so, presumably, *Culicoides*) in the Sudan regularly but that gaps of several weeks or months may occur between the successive waves of virus. It is possible that our 7 nights' trapping at Nishishiba may have fallen into one of these gaps. Clearly, further work is necessary before the role of *C. kingi* as a possible vector of BTV in the Sudan can be understood. BTV has not been isolated from *C. kingi* but the large numbers of this midge at Nishishiba compared to those of a known vector, *C. imicola*, (table II) suggest that *C. kingi* must still be strongly suspected of involvement with bluetongue transmission in the Sudan. EL SINNARY and HUSSEIN (8) have shown that *C. kingi* is involved in the transmission of *Onchocerca gutturosa* in the Sudan and that this species bites Sudanese cattle in the region of the hump.

The figures deduced for daily survival and gonotrophic cycle for *C. imicola* and *C. kingi* compare well with those obtained elsewhere. WALKER (22) deduced a survival rate of 0.8 for *C. imicola* and *C. schultzei* in Kenya, with gonotrophic cycle lengths of 4 days and 5 days, respectively. Although the biology of *C. kingi* is unknown, it is closely related to *C. schultzei* and might be expected to have somewhat similar habits. Given the cycle length and survival found in the present series of catches, both *C. imicola* and *C. kingi* are sufficiently long-lived to be considered as potential vectors of viruses. Both species have been noted to bite cattle, here and elsewhere, and in both the proportion of freshly blood-fed midges was about 6 p. 100. The considerable difference in proportions of nulliparous and gravid indivi-

duals taken for the two species suggests that either their response to light at different phases of the gonotrophic cycle is different or their activity patterns are different. Alternatively, the higher proportion of nulliparous *C. kingi* taken might have been due to the trap being placed nearer a breeding site for this species; this is supported by the greater proportion of *C. kingi* males taken (21 p. 100 for *C. kingi*, 11 p. 100 for *C. imicola*) and by the emergence of several *C. kingi* from larvae and pupae collected in a muddy area within 20 feet of the light-trap.

Of the other *Culicoides* species taken, *C. rarus* was the most abundant. Most of the females taken were nulliparous; of a sample of 481 females, 10.5 p. 100 were nulliparous,

15.0 p. 100 gravid and 2.5 p. 100 blood-fed. The source of the blood meal is unknown but these midges are closely related to *C. similis* and *C. moucheti* and they could be bird-biters.

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### SUMMARY

Light-trap catches of *Culicoides* in the Khartoum and Wad Medani areas of the Sudan yielded eleven species. Previous records are discussed and the number of species so far recorded is sixteen. Analysis of the catches gave a gonotrophic cycle length of five days for *C. kingi* and *C. imicola* and a daily survival rate of 0.86 and 0.72, respectively.

*C. kingi* and *C. imicola* are the species of *Culicoides* thought most likely to be involved in bluetongue virus transmission cycles, although the examination of 11,192 *C. kingi* and 848 other *Culicoides* has failed to produce a single virus isolate. Several possible reasons for this situation are discussed.

### RESUMEN

Notas sobre *Culicoides* (Diptera, Ceratopogonidae) en el Sudan en relación con la epidemiología de la lengua azul

Se obtuvieron once especies de *Culicoides* por captura con trampas de luz, en las áreas de Khartoum y Wad Medani del Sudan. Previos recuerdos son analizados y diez y seis el número de especies hasta entonces registradas. El análisis de las capturas dió un ciclo gonadotrófico de cinco días para *C. kingi* y *C. imicola* y una razón de supervivencia diaria de 0.86 y 0.72 respectivamente.

*C. kingi* y *C. imicola* son las especies de *Culicoides* que más parecen ser responsables del ciclo de transmisión viral, aunque el examen de 11,193 *C. kingi* y 893 de otros *Culicoides* no produjo ningún aislamiento viral. Son discutidas varias posibles razones de este fenómeno.

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