Onderstepoort Journal of Veterinary Research, 61:327–340 (1994)

# The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses

G.J. VENTER and R. MEISWINKEL

*Entomology Section, Onderstepoort Veterinary Institute*
*Private Bag X5, Onderstepoort, 0110 South Africa*

**ABSTRACT**

VENTER, G.J. & MEISWINKEL, R. 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort Journal of Veterinary Research*, 61:327–340

Altogether 52 078 *Culicoides* biting midges of 35 species were collected during February 1990 and 1993 in 40 light-trap collections made on 17 cattle and/or sheep farms in the Bethlehem and Fouriesburg districts of the colder, high-lying eastern Orange Free State. *Culicoides (Avaritia) bolitinos* was by far the most abundant species, representing 50,9 % of all specimens collected. *Culicoides (A.) imicola*, considered to be the most common stock-associated species in the summer rainfall areas of southern Africa, and the only proven vector of bluetongue virus (BTV) and African horsesickness virus (AHSV) in the subregion, was uncommon, comprising only 1,4 %. While AHS is apparently absent, BT and bovine ephemeral fever (BEF) are endemic in this cooler, high-lying area of South Africa. The virtual absence of *C. imicola* implies that other *Culicoides* species, such as *C. bolitinos* and *C. cornutus*, may be involved in transmitting BT virus (and perhaps BEF) in the eastern Orange Free State, and possibly elsewhere in Africa. Virus isolation attempts made on 45 single species pools of *C. bolitinos, C. pycnostictus, C. milnei, C. leucostictus, C. zuluensis* and *C. gulbenkiani* were, however, negative. Finally, 20 of 28 blood-engorged *Culicoides* of 11 species, which were tested against cattle, sheep, horse, pig and bird antisera, tested only positive against cattle antisera.

## INTRODUCTION

*Culicoides imicola* is widespread in Africa and is one of the most abundant species found in association with livestock in the summer rainfall region of southern Africa (Nevill & Anderson 1972; Braverman & Phelps 1981; Phelps, Blackburn & Searle 1982; Meiswinkel 1989). To a large extent this has been borne out by a light-trap survey of stock-associated *Culicoides* species made at 34 sites throughout the Republic of South Africa; *C. imicola* was found at 33 of these sites, was the dominant species at half of them and represented 71 % of ± 3 000 000

Accepted for publication 12 September 1994—Editor

*Culicoides* captured during this 3-year period (Venter 1991). Based on precipitin tests, *C. imicola* feeds on both cattle and sheep (Nevill & Anderson 1972; Braverman & Phelps 1981; Nevill, Venter, Edwardes, Pajor, Meiswinkel & Van Gas 1988) and still remains the only proven vector of bluetongue (BT) virus and African horsesickness (AHS) virus in South Africa (Du Toit 1944; Du Toit, unpublished data cited in Wetzel, Nevill & Erasmus 1970; Venter, Hill, Pajor & Nevill 1991).

Various serotypes of BT virus have been isolated from *C. imicola* in parts of Africa and the Mediterranean (Davies, Walker, Ochieng & Shaw 1979; Mellor, Osborne & Jennings 1984; Blackburn, Searle & Phelps 1985; Braverman, Barzilai, Frish & Rubina 1985; Nevill, Erasmus & Venter 1992a). AHS virus serotypes 2, 4 and 7 have been isolated from *C. imicola* in South Africa (Nevill *et al.* 1992a), while serotype 4 has been isolated from *C. imicola* in Zimbabwe (Blackburn *et al.* 1985) and Spain (Mellor, Boned, Hamblin & Graham 1990). BEF virus has been isolated from *C. imicola* in Zimbabwe (Blackburn *et al.* 1985). The infection rate for a single population of *C. imicola* from the hotter, low-lying parts of the Northern Transvaal was established as 31 % for BT virus serotype 3 and 24 % for BT virus serotype 6 (Venter *et al.* 1991); and at 67 % for AHS virus serotype 4 for *C. imicola* at Onderstepoort (G.J. Venter & B.J.H. Barnard 1993, unpublished data).

Two previous studies have revealed *C. imicola* to be relatively scarce in the colder, higher-lying areas of southern Africa. In a light-trap survey of sheep farms in the Bethulie district of the drier southern Orange Free State, Jupp, McIntosh & Nevill (1980) found a mere 44 (0,9 %) *C. imicola* amongst a total of 4 964 *Culicoides* of 17 species captured. Similarly Venter & Sweatman (1989) found *C. imicola* to comprise only 1,7 % of 32 819 *Culicoides* captured at Roma, Lesotho. These collections were made over a 12-month period at a small group of cattle, sheep, chickens and pigs. Nevertheless, questionnaires returned by veterinarians show that BT and BEF appear to be common in the cooler areas of South Africa immediately adjacent to Lesotho, including the eastern Orange Free State, and that most sheep and cattle are vaccinated annually. These questionnaires indicated that AHS was absent (Venter 1991). Donkey sera tested with an ELISA for the presence of AHS antibodies (15 donkeys from the Bethlehem magisterial district and 26 from the Ficksburg magisterial district—all negative) indicate that AHS is either of low prevalence or absent (G.J. Venter & R. Williams 1992, unpublished data).

It was therefore decided to determine how widespread and abundant *C. imicola* is, and to investigate whether the studies conducted in the southern Orange Free State and at Roma, Lesotho, represented the general situation or whether it was merely a reflection of the proximity of larval habitats and/or particular host animals near the particular light-trap sites. A study trip was undertaken to the region from 31 January to 8 February 1990, and again from 15 to 19 February 1993. This month was chosen because seasonal studies at Roma had indicated it to be the time of peak *Culicoides* activity (Venter & Sweatman 1989). Furthermore, this coincides with the time of occurrence of BT, BEF and AHS in most of the temperate parts of South Africa (Henning 1956; Verwoerd & Erasmus 1994; Coetzer & Erasmus 1994; St George 1994).

## MATERIALS AND METHODS

### Study area and host animals

*Culicoides* were collected at 23 sites; 17 of these were situated in a rough semi-circle to the east of Bethlehem (28°15′S, 28°20′E), starting from approximately 30 km to the north to about 30 km to the south of the town. One site, on the farm Mariba, was about 18 km to the west of Bethlehem. An additional five sites were on an east-west line between the Golden Gate Highlands Park (28°30′S, 28°35′E) in the east and Clarens (28°32′S, 28° 25′E) in the west.

From west to east, the topography changes from moderately to strongly undulating, extending along the upper western escarpment of the Drakensberg range between 1 600 and 2 500 m altitude. Temperatures are mild to very cold, with an annual average of 12,5–15 °C; frost is severe. Bethlehem annually experiences 79,8 d of minimum temperatures below 0 °C and 47,0 d when they are below –2,4 °C (Weather Bureau 1986). The mean maximum daily temperatures for January 1990 at Bethlehem and Golden Gate were 26,7 °C and 26,6 °C, respectively; the mean daily minimum temperatures were respectively 12,8 °C and 14,4 °C. The total rainfall for Bethlehem for January 1990 was 81,9 mm and 115,2 mm for Golden Gate. In January 1993, the mean daily maximum and minimum temperatures for Bethlehem were respectively 27,7 °C and 12,7 °C. The total rainfall for Bethlehem for January 1993 was 102,8 mm.

The Bethlehem and Fouriesburg districts are described as falling into the pure grassveld types, 56 (Highland Sourveld to *Cymbopogon, Themeda* veld transition) and 58 (*Themeda, Festuca* Alpine Highveld) and the temperate and transitional forest and scrub type, 44 (Highland Sourveld and Dohne Sourveld) (Acocks 1988). Tainton (1981) classifies the region as climactic climax grassland at low and high elevations and fire climax grasslands of potential forest areas at high elevations.

Rainfall in summer averages between 600 and 1 000 mm/annum; downpours can be heavy, runoff high and moisture penetration into the soil, therefore, low. In mid-summer, high evaporation and high transpiration losses lead to moisture deficiency, restricting most plant growth to the early summer and autumn period. As a consequence, the natural pastures have a high nutritive value for only 3–4 months and animals require supplementary feeding. For this purpose, most farmers plant oulandsgras (*Eragrostis curvula*) which they bale for winter supplement; cattle also subsist on maize fodder. Because of the relatively high summer rainfall < 2,5 % of this area is under irrigation (Bonsma & Joubert 1957). As much as 80 % of the sites surveyed had either rivers running through them or earth dams, the marshy backwaters fringed with reeds and grasses. The relatively high rainfall of the region ensures these rivers and dams are filled during the summer, and carry water well into the winter. Potential *Culicoides* larval habitats, for non-dung breeders, are therefore quite plentiful (Table 1).

Owing to the area being climatically suited to most exotic breeds of livestock, it is an important production area with most of the livestock being free-ranging, especially on the larger farms (Table 1). Most of the farms surveyed, had stock-holding areas near the homesteads, which had been planted to kikuyu pasture. The two magisterial districts comprise 0,4 % (4 734 $km^2$) of the Republic of South Africa. The 1991 livestock census of the Directorate of Animal Health returned the following figures for these two districts (the figure in parentheses being the percentage of the total number of a stock species occurring in South Africa): cattle 101 019 (0,9 %); sheep 188 197 (0,7 %); horses 1 993 (1,0 %); donkeys and mules 74 (0,1 %) and goats 3 159 (0,1 %). This is also an important cereal-production area.

At 13 of the 23 sites sampled, cattle were the dominant stock species, while sheep and horses were dominant at five and three sites, respectively. At most sites a mixture of stock species including cattle, horses, sheep, pigs, goats and poultry were found (Table 1). In 1993, only those farms on which the livestock composition had remained stable, were resampled.

### Light-trap collections and species identification

At each of the 23 sites a 220-v ultraviolet down-draught suction light-trap, equipped with an 8-W black-light tube, was used. Three to four sites were sampled per night and light-traps were hung as closely as possible to various livestock species (Table 1). A light-trap was usually operated at each site for one night only before being moved. Collections were made directly into phosphate-buffered saline (PBS) to which 0,5 % "Savlon" [ICI (South Africa) Ltd] antiseptic had been added, according to the method of Walker & Boreham (1976). With this method, specimens were not only provided in excellent condition for identification, but they could also be recovered for bloodmeal identification and virus isolation.

Traps were visited daily and the collections returned to the field laboratory, where they were kept at 4 °C until the blood-engorged specimens and pools of identified species for virus isolation were sorted out. The remainder of the collection was transferred to 80 % ethyl alcohol and subsequently identified and sexed with the help of a slide reference collection, preliminary keys and a wing picture atlas of Afrotropical *Culicoides* (R. Meiswinkel 1994, unpublished data). Where possible, species are assigned to subgenera to facilitate discussion and comparison with related species found elsewhere in the world. As the breakdown of the genus *Culicoides* into subgenera is still in a state of flux, those species of uncertain affinity are assigned the nearest subgenus, the doubt indicated by a question mark.

### Virus isolation

As there is no evidence of transovarial virus transmission in *Culicoides*, only parous individuals, with no visible bloodmeal, were sorted out for virus isolation (Nelson & Scrivani 1972; Allingham & Standfast 1990). Abdominal pigmentation was used as an indicator of parity (Dyce 1969). Forty-five groups, each consisting of 50 parous individuals of one species only, were tested for the presence of virus. Twenty of the 45 groups consisted of *C. bolitinos*, nine of *C. pycnostictus*, seven of *C. milnei* and three groups each of *C. leucostictus, C. zuluensis* and *C. gulbenkiani*. *Culicoides* for virus isolation were stored at 4 °C in PBS with antibiotic (Streptomycin and Benzyl penicillin) [Novo Industries (Pharmaceuticals) Pty Ltd].

At the Onderstepoort Veterinary Institute (OVI) the *Culicoides* were homogenized in Eagle's medium. This suspension was centrifuged and the supernatant passaged intravenously in embryonated chicken eggs, intracerebrally in suckling mice and on CER cell-culture on roller tubes.

### Bloodmeal identification

Freshly engorged *Culicoides* were removed from the light-trap collections and identified to species level. The PBS was then absorbed with tissue paper and the dried specimens placed in individual size 1 gelatine capsules. Each capsule was numbered and stored over silica gel. Bloodmeals were identified by means of a cross-over electrophoresis precipitin test (Culliford 1964). Host-specific antisera against cattle, sheep, horse, pig and bird were prepared in rabbits. Non-specific, cross-reacting antibodies were precipitated out by absorbing the antisera with the heterologous serum. This was particularly important in the case of the cattle and sheep antisera which might otherwise have cross-reacted.

## RESULTS

### Light-trap collections

A total of 52 078 *Culicoides* midges belonging to 35 species was collected in 40 light-trap collections made at 23 sites in the study area during February in 1990 and 1993. An analysis of the light-trap collections is summarized separately in Tables 2 and 3. The single most abundant species per site appears in bold type. The total numbers of midges of each *Culicoides* species collected in both years are summarized in Table 4. The most abundant species collected were *C. bolitinos, C. leucostictus, C. pycnostictus, C. magnus, C. nivosus, C. zuluensis, C. milnei, C. gulbenkiani*, C. sp. # 35 (Schultzei group) and *C. cornutus*. Each of these species represented more than 2 % of the total number of midges collected in any one year, and are discussed below. *Culicoides imicola*, though rare, is also discussed because of its importance elsewhere in Africa.

*C. imicola*

This well-known African species represented only 1,4 % of the midges collected in this survey, it being absent from 12 of the 40 collections made (Tables 2 and 3). The highest number of *C. imicola* captured at a site was a mere 128 individuals on the farm Makana (# 7) (Table 2); the same farm had the highest number (95) in 1993; this indicates that its pattern of prevalence is constant.

*C. bolitinos*

The cattle-dung-inhabiting *C. bolitinos* was the most widespread and abundant species, representing 50,9 % of all *Culicoides* collected (Table 4). It was found in all 40 collections made, with numbers ranging from 14–10 039; it was the dominant species in 23 collections and the second most abundant in a further nine (Tables 2 and 3). Four collections deserve mention:

- At the Khotso mutton Merino stud at Koeberg (# 6a), *C. bolitinos* represented 81,9 % of the 12 253 midges collected, a little more than a third of the 30 274 *Culicoides* collected in the 1990 survey (Table 2). The two catches were made at opposite ends of an open-sided stable which housed about 300 sheep. The stable was surrounded by kikuyu and lucerne pastures which were regularly fertilized but not irrigated. In total there were about 5 000 sheep on the farm and, within a 750-m radius, ± 250 cattle (Table 1). While not as abundant, *C. bolitinos* was still the dominant species at Koeberg in 1993 (Table 3).
- In 1990, high numbers of *C. bolitinos* were also collected on the farm Rendevouz (# 5) where it represented 55,0 % of 2 140 midges collected adjacent to a closed stable which housed about 30 sheep (Table 2); other animals in the vicinity included cattle and chickens (Table 1). Two blood-fed females collected here tested positive against cattle antisera. In 1993 it represented 61,3 % of 80 *Culicoides* collected at Rendevouz; the catch was small owing to cold, blustery conditions (Table 3).
- At the Sunnyside Holiday Resort (# 17), *C. bolitinos* represented 44,9 % of 4 256 *Culicoides* collected near horses kept in a treed camp on the edge of a tributary of the Little Caledon River. Over 150 *C. bolitinos* adults were reared from five cattle-dung pads collected in an adjacent camp where 30 cattle, belonging to the farm Madrid (# 16), were kept.
- In a collection made at Stirling (# 13) in 1993, *C. bolitinos* represented 60,1 % of 4 942 *Culicoides* collected near ± 600 cattle kept on the kikuyu pastures of an intensively managed dairy farm (Table 3).

*C. leucostictus* and *C. pycnostictus*

The second and third most abundant species were, respectively, *C. leucostictus* and *C. pycnostictus*, each representing approximately 10 % of all *Culicoides* collected (Table 4). The former was the most abundant species in five collections, the latter, in seven (Tables 2 and 3).

*C. magnus, C. nivosus, C. zuluensis* and *C. milnei*

Each of these species represented 2,5–5,0 % of the total number of midges collected (Table 4); all four species were widespread, being found at 21 or more of the 23 sites sampled (Tables 2 and 3). In the 1990 survey, *C. magnus* was the most abundant *Culicoides* species found at two sites, viz. Weltevreden (# 4) and Uitvlugt (# 12), where cattle were dominant (Table 2). While *C. nivosus* was uncommon (0,3 %) in 1990, it jumped to prominence in 1993 (9,4 %; Table 4). In the same year, *C. zuluensis* was the dominant species on the farm Uitvlugt, in collections made near cattle and poultry (Table 3). *C. milnei* was the dominant species at sheep on the farm Langkloof (# 3) and the second-most abundant species at horses and cattle on the farm Uitzicht (# 10); these two farms were not surveyed in 1993 as they had been abandoned by their owners.

*C. gulbenkiani*

This dung-inhabiting species was found at 16 of the 20 sites which were sampled in 1990, but represented only 2,9 % of all the midges collected. It was not dominant at any site (Table 2), and declined in prominence in 1993 (0,7 %; Table 4).

TABLE 1 Host animals and farming conditions at 20 light-trap sites in the eastern Orange Free State, February 1990

| Site no. | Farm name | Animals in immediate vicinity of light-trap | Animals within a radius of 100 m | Animals within a radius of 1 000 m | Main type of farming | Irrigation | Standing water in vicinity of light-trap |
|---|---|---|---|---|---|---|---|
| 1 | Lacornel | Nil | 200 cattle, 1 000 sheep | Nil | Merino stud | Nil | Nil |
| 2a | Cyferlaagte | Cattle, horses | Nil | Nil | Sheep | Nil | Dam |
| 2b | Cyferlaagte | Nil | 6 horses, 2 cattle, 11 sheep | Nil | Sheep | Nil | Dam |
| 2c | Cyferlaagte | 50 sheep | 1 000 sheep | Nil | Sheep | Nil | Dam |
| 2d | Cyferlaagte | 100 sheep | 1 000 sheep | Nil | Sheep | Nil | Dam |
| 3 | Langkloof | 50 sheep | 50 sheep, 3 cattle, poultry | 100 sheep | Sheep | Nil | Dam and marshy area |
| 4 | Weltevreden | Nil | Cattle | Cattle, sheep | Sheep | Nil | Dam |
| 5 | Rendevouz | 30 sheep, poultry | 30 sheep | 150 cattle | Mixed farming on smallholding | Nil | Dam |
| 6a | Koeberg | 300 sheep | 5 000 sheep | 250 cattle | Merino stud | Nil | River |
| 6b | Koeberg | 1 cow | 15 cattle, 100 sheep | 30 goats, 200 sheep, 20 cattle | Merino stud | Nil | River |
| 7 | Makana | 15 cattle, 2 pigs, poultry | 15 cattle | Cattle, 5 donkeys | Cattle and maize | Nil | Dam |
| 8 | Geluk | 12 cattle, 9 sheep | Nil | Cattle | Landskaap stud and dairy | Nil | Dam |
| 9 | Montana | 15 cattle | 15 cattle | Cattle | Dairy | Nil | Dam |
| 10a | Uitzicht | 92 cattle, poultry | 14 horses | 80 sheep | Horse and dairy | Irrigated kikuyu pasture | Dam |
| 10b | Uitzicht | 14 horses, poultry | 92 cattle | 80 sheep | Horse and dairy | Irrigated kikuyu pasture | Dam |
| 11 | Tammy | 6 cattle | Nil | 60 cattle | Dairy | Nil | Treed rivulet |
| 12 | Uitvlugt | Nil | 200 cattle, poultry | 200 cattle, poultry | Cattle | Irrigated kikuyu pasture | Nil |
| 13 | Stirling | 570 cattle | 23 sheep | Nil | Cattle feedlot | Irrigated kikuyu pasture | Nil |
| 14 | Mariba | Nil | 160 cattle | Nil | Dairy | Nil | Big dam (0,5 $km^2$) |
| 15 | Stefanium | Nil | 50 cattle, 17 horses | 300 cattle | Dairy and mixed potatoes, maize and asparagus | Nil | Nil |
| 16 | Madrid | 100 cattle, 5 horses | 100 cattle | 300 cattle | Cattle | Nil | River |
| 17 | Sunnyside | 11 horses | 13 cattle | 30 cattle | Holiday resort | Nil | River |
| 18 | Golden Gate | 10 horses | Nil | Small game | National Park | Nil | River |

TABLE 2 Identification and abundance of the *Culicoides* species collected with light-traps in the eastern Orange Free State during February 1990

| *Culicoides* species | Site no. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2a | 2b | 3 | 4 | 5 | 6a | 7 | 8 | 9 |
| *C. bolitinos* | **19,7** | 24,3 | 13,4 | 20,1 | 24,6 | **55,0** | **51,9** | 6,4 | 5,0 | 16,3 |
| *C. leucostictus* | 18,4 | 7,6 | 8,9 | 9,7 | | 9,7 | 0,6 | 21,3 | 15,7 | **34,9** |
| *C. pycnostictus* | **19,7** | **33,9** | **32,2** | 0,8 | 3,8 | 0,8 | 0,2 | **45,5** | **64,1** | 22,8 |
| *C. magnus* | 5,1 | 7,3 | 10,0 | 13,6 | **37,9** | 1,5 | 9,0 | 3,1 | 1,4 | 0,9 |
| *C. zuluensis* | 15,7 | 3,3 | 17,6 | 0,7 | 5,7 | 4,2 | 4,4 | 0,6 | 3,6 | 0,3 |
| *C. milnei* | 2,7 | 8,3 | 5,7 | **21,4** | 23,2 | 3,1 | 0,8 | 8,4 | 0,4 | 1,4 |
| *C. gulbenkiani* | 0,3 | 5,0 | 0,5 | 1,3 | 1,4 | 19,8 | 1,3 | 0,4 | | 0,3 |
| *C. neavei* | 8,7 | 10,3 | 4,3 | 7,1 | | 0,4 | 0,1 | 1,0 | 1,4 | 3,0 |
| *C. engubandei* | 0,5 | | | 0,7 | | 0,2 | 1,0 | 0,2 | 0,4 | 0,4 |
| *C. bedfordi* | | | 0,5 | | 1,4 | | 0,1 | | | 0,1 |
| *C. imicola* | 1,4 | | 3,2 | | | 0,8 | 0,1 | 9,1 | 3,6 | 5,3 |
| *C. onderstepoortensis* | | | | 11,0 | | 0,1 | 0,1 | | | |
| *C. micheli* | | | | | | 3,4 | | 1,2 | | 3,7 |
| *C. dutoiti* | | | | 1,3 | | 0,7 | 0,0* | 0,4 | 1,1 | 6,4 |
| *C. similis* | 0,8 | | 1,4 | | | 0,1 | | 1,1 | 1,8 | 3,7 |
| *C. nivosus* | 5,7 | | 0,2 | 0,7 | | 0,1 | | 0,4 | 1,8 | |
| *C. schultzei* s.l. | | | 1,8 | | | 0,1 | 0,1 | 0,1 | | |
| *C. huambensis* | | | | | 0,5 | | 0,2 | 0,1 | | |
| *C.* sp. nr. *angolensis* | | | | 3,3 | 1,4 | | 0,1 | 0,4 | | 0,3 |
| *C. cornutus* | 1,1 | | | | | | 0,0 | | | |
| *C. brucei* (pale form) | | | | | | | 0,0 | | | |
| *C.* sp. 69** | | | | | | | | | | |
| *C. coarctatus* | | | 0,2 | | | | 0,0 | | | |
| Other *Culicoides* spp. | 0,3[a] | | 0,2[a] | 0,7[d] | | 0,2[b] | | 0,1[i] | | 0,3[dfh] |
| Total no. of midges | 370 | 301 | 562 | 154 | 211 | 2 140 | 12 253 | 1 400 | 281 | 1 464 |
| No. of collections | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |

| *Culicoides* species | Site no. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10a | 10b | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| *C. bolitinos* | 17,1 | 11,3 | **45,9** | 20,9 | **32,0** | 9,8 | **52,5** | **72,9** | **44,9** | **34,2** |
| *C. leucostictus* | 15,0 | 14,3 | 3,5 | 4,4 | 14,0 | **37,8** | 5,7 | 2,3 | 30,5 | 0,5 |
| *C. pycnostictus* | **30,9** | **36,2** | 15,5 | 7,0 | 13,5 | 26,4 | 6,9 | 6,3 | 7,3 | 0,2 |
| *C. magnus* | 2,4 | 2,8 | 10,2 | **27,0** | 2,7 | 3,0 | 5,2 | 1,1 | 0,3 | 13,0 |
| *C. zuluensis* | 6,0 | 6,8 | 1,4 | 19,1 | 21,4 | 10,4 | 3,7 | 3,2 | 0,5 | 1,7 |
| *C. milnei* | 16,7 | 19,4 | 2,2 | 4,4 | 0,1 | 6,1 | 2,2 | | 0,1 | 9,8 |
| *C. gulbenkiani* | 1,4 | 2,6 | 11,2 | 7,8 | | | 4,4 | | 0,0 | 11,1 |
| *C. neavei* | 2,8 | 2,8 | 7,5 | 3,5 | 3,4 | 0,8 | 2,7 | 0,7 | 1,7 | |
| *C. engubandei* | 2,0 | 0,7 | 0,2 | | 0,4 | | | | 0,1 | 23,0 |
| *C. bedfordi* | | 0,1 | 0,8 | 1,7 | | | 0,3 | 4,5 | 8,2 | 0,2 |
| *C. imicola* | 2,8 | 2,1 | 0,2 | | | 1,4 | 0,5 | 0,2 | | |
| *C. onderstepoortensis* | | | 0,2 | 1,7 | | 0,2 | 1,0 | 7,5 | 4,5 | 1,9 |
| *C. micheli* | | | | | | | | | | |
| *C. dutoiti* | 0,3 | 0,1 | | 0,9 | 0,5 | | 1,2 | | 0,0 | 0,2 |
| *C. similis* | 0,3 | 0,1 | | 0,9 | 0,5 | 0,4 | | | 0,2 | |
| *C. nivosus* | 0,7 | 0,3 | | | 1,0 | 3,5 | 3,0 | | 0,1 | |
| *C. schultzei* s.l. | 0,8 | 0,0 | | | 5,1 | | | | 0,0 | 0,1 |
| *C. huambensis* | | 0,1 | 1,0 | | | | | | 0,3 | 1,5 |
| *C.* sp. nr. *angolensis* | | | | | | | 0,3 | 0,7 | 0,2 | 0,9 |
| *C. cornutus* | 0,3 | | | 0,9 | 4,2 | 0,2 | | 0,2 | | |
| *C. brucei* (pale form) | | | 0,2 | | | | | 0,5 | 0,5 | 1,6 |
| *C.* sp. 69** | | | | | | | | | 0,6 | |
| *C. coarctatus* | 0,5 | 0,1 | | | 0,4 | | | | | 0,3 |
| Other *Culicoides* spp. | | | | | 0,9[ab] | | 0,5[e] | | 0,2[acg] | |
| Total no. of midges | 883 | 2 235 | 492 | 115 | 787 | 508 | 405 | 443 | 4 256 | 1 014 |
| No. of collections | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |

[a] *C. brucei*
[b] *C. stercorarius*
[c] *C. eriodendroni*
[d] *C. tropicalis*
[e] *C.* sp. 108
[f] *C. macintoshi*
[g] *C. dekeyseri*
[h] *C. ravus*
[i] *C.* sp. nr. *glabripennis*

* 0,0 < 0,05 % representation
** According to Meiswinkel (1993, unpublished data)
The most abundant species per site appears in bold type
All figures are percentages

TABLE 3 Identification and abundance of the *Culicoides* species collected with light-traps in the eastern Orange Free State during February 1993

| *Culicoides* species | Site no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2c | 2d | 4 | 5 | 6a | 6b | 7 |
| *C. bolitinos* | **58,8** | **33,9** | **68,2** | **61,3** | **34,5** | **62,9** | 9,0 |
| *C. leucostictus* | 2,6 | 14,2 | 1,9 | 8,8 | 17,2 | 3,8 | **30,8** |
| *C. pycnostictus* | 14,1 | 21,2 | 3,3 | 18,8 | 6,8 | 8,4 | 5,0 |
| *C. magnus* | 4,6 | 6,4 | 13,1 | 3,8 | 1,8 | 4,2 | 1,2 |
| *C. zuluensis* | 0,7 | 0,8 | 3,0 | 2,5 | 0,2 | 0,5 | 0,8 |
| *C. milnei* | | 0,1 | 2,4 | | | 0,1 | 0,4 |
| *C. gulbenkiani* | 0,8 | 0,3 | 2,9 | 1,3 | | 0,8 | 0,6 |
| *C. neavei* | 1,1 | 3,5 | 0,2 | 1,3 | 0,4 | 0,3 | 1,4 |
| *C. engubandei* | 0,1 | 0,1 | 0,1 | | 2,7 | 0,5 | |
| *C. bedfordi* | 0,8 | 1,3 | 0,5 | | 13,4 | 12,8 | |
| *C. imicola* | 2,6 | 2,0 | 0,6 | | 3,9 | 0,4 | 18,9 |
| *C. onderstepoortensis* | 0,1 | 0,1 | 1,3 | 4,4 | 5,3 | 0,2 | 0,2 |
| *C. micheli* | 0,3 | 0,7 | | | | | 8,0 |
| *C. dutoiti* | 2,8 | 0,9 | | | 0,6 | 1,2 | 2,2 |
| *C. similis* | 4,7 | 3,7 | | | 0,2 | | 1,8 |
| *C. nivosus* | 3,8 | 9,2 | 0,5 | | 0,4 | 1,4 | 15,3 |
| *C. schultzei* s.l. | 1,3 | 2,0 | 3,1 | 1,3 | 11,1 | 4,4 | 0,2 |
| *C. huambensis* | | | | | 0,1 | | |
| *C.* sp. nr. *angolensis* | | | 0,1 | | 1,5 | 2,7 | |
| *C. cornutus* | 0,3 | 0,5 | 0,2 | | 0,2 | 1,1 | 4,0 |
| *C. brucei* (pale form) | | | 0,1 | | 0,1 | 1,3 | |
| *C. coarctatus* | 0,4 | 0,3 | 0,2 | | 0,3 | 0,1 | 0,2 |
| *C. macintoshi* | 0,1 | | | | | | |
| *C. sp. nr. glabripennis* | | | | | | | 0,0* |
| *C. hildae* | | | | | | | 0,0 |
| *C. galliardi* | | | | | 0,2 | | |
| *C. exspectator* | | | | | | | |
| Total no. of midges | 1 794 | 2 156 | 3 146 | 80 | 1 364 | 1 778 | 503 |
| No. of collections | 1 | 1 | 1 | 1 | 1 | 3 | 1 |

| *Culicoides* species | Site no. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8 | 9 | 11 | 12 | 13 | 15 | 16 | 17 |
| *C. bolitinos* | **40,4** | 19,2 | **72,7** | 29,9 | **60,1** | **66,0** | 28,6 | 22,1 |
| *C. leucostictus* | 21,5 | **31,3** | 1,7 | 1,9 | 1,5 | 3,6 | 4,4 | **44,3** |
| *C. pycnostictus* | 11,9 | 4,4 | 9,1 | 0,8 | 3,4 | 5,1 | 8,4 | 8,4 |
| *C. magnus* | 2,0 | 0,3 | 0,8 | 0,3 | 0,2 | 1,1 | | 0,6 |
| *C. zuluensis* | 0,6 | | 1,7 | **31,0** | 0,6 | 5,3 | 0,4 | |
| *C. milnei* | 0,1 | | | | 0,0 | 0,1 | | |
| *C. gulbenkiani* | 0,1 | 0,3 | 13,2 | | 0,1 | 0,2 | | 0,1 |
| *C. neavei* | 1,7 | 1,9 | 0,8 | 0,1 | 0,2 | 0,1 | | 0,1 |
| *C. engubandei* | 0,1 | 0,3 | | | 0,0 | 0,2 | 0,6 | 0,9 |
| *C. bedfordi* | 0,5 | | | 0,8 | 1,1 | 1,3 | 0,6 | 4,2 |
| *C. imicola* | 4,1 | 5,2 | | 0,6 | 0,5 | 0,2 | | 0,2 |
| *C. onderstepoortensis* | 0,2 | | 0,1 | 0,3 | 0,4 | 4,6 | 11,4 | |
| *C. micheli* | 0,3 | 12,3 | | | 0,0 | | 0,2 | |
| *C. dutoiti* | 1,2 | 5,8 | | 0,4 | 0,7 | 0,1 | | 0,2 |
| *C. similis* | 3,0 | 7,9 | | 0,1 | 1,7 | 0,1 | | |
| *C. nivosus* | 9,5 | 7,6 | | 3,6 | 26,7 | 7,8 | 4,6 | 0,2 |
| *C. schultzei* s.l. | 1,1 | 0,6 | | 13,7 | 1,4 | 3,7 | 6,1 | 7,2 |
| *C. huambensis* | | | | | | | | |
| *C.* sp. nr. *angolensis* | | 0,2 | | | | | | |
| *C. cornutus* | 0,8 | 0,5 | | 16,6 | 1,6 | 4,9 | **41,2** | 0,1 |
| *C. brucei* (pale form) | | | | | 0,0 | | 0,2 | |
| *C. coarctatus* | 0,7 | 0,2 | | | 0,0 | 0,1 | | |
| *C. macintoshi* | 0,4 | | 2,1 | | | | | |
| *C. sp. nr. glabripennis* | | | | | 0,1 | | | |
| *C. hildae* | | | | | | | | |
| *C. galliardi* | | | | | | | | |
| *C. exspectator* | | | | | | | | 0,16 |
| Total no. of midges | 1 729 | 620 | 121 | 723 | 4 942 | 1 113 | 476 | 1 259 |
| No. of collections | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

* 0,0 < 0,05 % representation    The most abundant species per site appears in bold type    All figures are percentages

TABLE 4 The total number and percentage of each *Culicoides* species collected in the eastern Orange Free State during February 1990 and 1993

| *Culicoides* species | 1990 | | | 1993 | | | Grand total | % |
|---|---|---|---|---|---|---|---|---|
| | Range in numbers | Total | % | Range in numbers | Total | % | | |
| *C. bolitinos* | 14–10 039 | 15 651 | 51,7 | 45–2 969 | 10 851 | 49,8 | 26 502 | 50,9 |
| *C. leucostictus* | 0–1 298 | 3 406 | 11,3 | 2–585 | 2 147 | 9,9 | 5 553 | 10,7 |
| *C. pycnostictus* | 2–810 | 337 | 11,0 | 6–456 | 716 | 7,9 | 5 053 | 9,7 |
| *C. magnus* | 4–1 106 | 1 766 | 5,8 | 0–411 | 809 | 3,7 | 2 575 | 4,9 |
| *C. nivosus* | 0–21 | 88 | 0,3 | 0–1 317 | 2 052 | 9,4 | 2 140 | 4,1 |
| *C. zuluensis* | 1–539 | 1 353 | 4,5 | 0–224 | 469 | 2,2 | 1 822 | 3,5 |
| *C. milnei* | 0–434 | 1 194 | 3,9 | 0–74 | 83 | 0,4 | 1 277 | 2,5 |
| *C. gulbenkiani* | 0–424 | 877 | 2,9 | 0–91 | 159 | 0,7 | 1 036 | 2,0 |
| *C. bedfordi* | 0–348 | 401 | 1,3 | 0–183 | 424 | 1,9 | 825 | 1,6 |
| *C. schultzei* s.l | 0–40 | 75 | 0,3 | 0–151 | 723 | 3,3 | 798 | 1,5 |
| *C. imicola* | 0–128 | 352 | 1,2 | 0–95 | 397 | 1,8 | 749 | 1,4 |
| *C. onderstepoortensis* | 0–192 | 279 | 0,9 | 0–143 | 347 | 1,6 | 626 | 1,2 |
| *C. neavei* | 0–74 | 422 | 1,4 | 0–75 | 174 | 0,8 | 596 | 1,1 |
| *C. cornutus* | 0–33 | 44 | 0,2 | 0–196 | 531 | 2,4 | 575 | 1,1 |
| *C. engubandei* | 0–233 | 422 | 1,4 | 0–37 | 71 | 0,3 | 493 | 1,0 |
| *C. similis* | 0–54 | 107 | 0,4 | 0–85 | 356 | 1,6 | 463 | 0,9 |
| *C. dutoiti* | 0–93 | 139 | 0,5 | 0–51 | 206 | 0,9 | 345 | 0,7 |
| *C. micheli* | 0–73 | 144 | 0,5 | 0–76 | 144 | 0,7 | 288 | 0,6 |
| *C.* sp. nr. *angolensis* | 0–12 | 50 | 0,2 | 0–21 | 39 | 0,2 | 89 | 0,2 |
| *C. brucei* (p/f) | 0–21 | 42 | 0,1 | 0–23 | 28 | 0,1 | 70 | 0,1 |
| *C. coarctatus* | 0–4 | 16 | 0,1 | 0–12 | 41 | 0,2 | 57 | 0,1 |
| *C. huambensis* | 0–18 | 54 | 0,2 | 0–1 | 1 | 0,0 | 55 | 0,1 |
| *C.* sp. 69* | 0–26 | 26 | 0,1 | | | | 26 | 0,1 |
| *C. macintoshi* | 0–2 | 2 | 0,0 | 0–13 | 23 | 0,1 | 25 | 0,1 |
| *C. brucei* | 0–5 | 11 | 0,0 | | | | 11 | 0,0 |
| *C. stercorarius* | 0–4 | 7 | 0,0 | | | | 7 | 0,0 |
| *C.* sp. nr. *glabripennis* | 0–1 | 1 | 0,0 | 0–1 | 6 | 0,0 | 7 | 0,0 |
| *C. exspectator* | | | | 0–3 | 5 | 0,0 | 5 | 0,0 |
| *C. tropicalis* | 0–1 | 2 | 0,0 | | | | 2 | 0,0 |
| *C.* sp. 108* | 0–2 | 2 | 0,0 | | | | 2 | 0,0 |
| *C. eriodendroni* | 0–2 | 2 | 0,0 | | | | 2 | 0,0 |
| *C. hildae* | | | | 0–1 | 1 | 0,0 | 1 | 0,0 |
| *C. galliardi* | | | | 0–1 | 1 | 0,0 | 1 | 0,0 |
| *C. dekeyseri* | 0–1 | 1 | 0,0 | | | | 1 | 0,0 |
| *C. ravus* | 0–1 | 1 | 0,0 | | | | 1 | 0,0 |
| Total *Culicoides* | | 30 274 | | | 21 804 | | 52 078 | |
| Total no. of collections | | 23 | | | 17 | | 40 | |
| Average | | 1 316,3 | | | 1 282,6 | | 1 302,0 | |
| % males | | 12,5 | | | 21,2 | | | |

* According to Meiswinkel (1993, unpublished data)

### *C. cornutus*

This species was relatively scarce in 1990 (0,2 %; Table 2), but it showed a significant increase during 1993 (2,4 %), it being the dominant *Culicoides* species (41,2 %) on the farm Madrid (# 16) (Table 3).

### *C. schultzei* s.l. (= Schultzei group)

This group was represented in the area by two undescribed species, viz. *C.* sp. # 9 and *C.* sp. # 35 (R. Meiswinkel 1993, unpublished data), with the latter accounting for 95 % of all specimens collected. In 1990 this group was found at only seven sites and accounted for < 0,3 % (range 0–40) of all *Culicoides* collected (Tables 2 and 4). The highest numbers captured were on the farm Stirling (# 13) where it represented 5,1 % of 787 *Culicoides* collected (Table 2). In 1993 it was found at 14 of the sites sampled and accounted for 3,3 % (range 0–151) of all *Culicoides* collected (Table 4), and for more than 5 % of the specimens found on the farms Koeberg (# 6a), Uitvlugt (# 12), Madrid (# 16) and Sunnyside (# 17) (Table 3).

TABLE 5 Identification of the bloodmeals of 28 *Culicoides* collected in light-traps in the eastern Orange Free State during February 1990

| *Culicoides* species | No. pos. (cattle) | No. pos. (sheep, pig horse, bird) | No. neg. (against anti-sera used) |
|---|---|---|---|
| *C. leucostictus* | 4 | 0 | 2 |
| *C. zuluensis* | 3 | 0 | 0 |
| *C. milnei* | 3 | 0 | 0 |
| *C. pycnostictus* | 2 | 0 | 4 |
| *C. bolitinos* | 2 | 0 | 0 |
| *C. engubandei* | 1 | 0 | 0 |
| *C. micheli* | 1 | 0 | 0 |
| *C. dutoiti* | 1 | 0 | 2 |
| *C. neavei* | 1 | 0 | 0 |
| *C. gulbenkiani* | 1 | 0 | 0 |
| *C. brucei* | 1 | 0 | 0 |
| Total | 20 | 0 | 8 |

Each *Culicoides* bloodmeal was tested against cattle, sheep, horse, pig and bird antisera

### Virus isolation

No virus was isolated from any of the 45 single species groups of *Culicoides* assayed.

### Bloodmeal identification

Only 28 blood-engorged *Culicoides*, representing 11 species, were collected; each specimen was tested against cattle, sheep, horse, pig and bird antisera. Only 20 were positive, all against cattle, the main host species in the area (Table 5).

## DISCUSSION

This study supports the findings of Jupp *et al.* (1980) in the southern Orange Free State and those of Venter & Sweatman (1989) in Lesotho, and gives further evidence that *C. imicola* is virtually absent in the cooler central highlands of South Africa. As rainfall is adequate and stock animals are plentiful, indications are that *C. imicola* is restricted by low temperatures. The mean minimum temperature line of 12 °C embraces this central plateau and, significantly, is the same isoline that appears to mark the northernmost records of *C. imicola* in the Mediterranean, i.e. 41 °N (Capela, Sousa, Pena & Caeiro 1993). While AHS is absent, BT and BEF appear to be endemic in this cooler, high-lying area of South Africa. The virtual absence of *C. imicola* implies that other *Culicoides* species may be involved in transmitting BT virus, and perhaps BEF, in the eastern Orange Free State, and elsewhere in Africa.

A remarkable feature of the collections is that more than half (52,9 %) of the 52 078 *Culicoides* collected have animal dung as their exclusive larval habitat, and belong to two species of the subgenus *Avaritia*, i.e. *C. bolitinos* and *C. gulbenkiani*. The remaining 47,1 % are groundwater breeders that utilize leaking troughs and contaminated hoofprints in farmyard situations, and the edges of rivers, dams or marshy areas. The biologies of ten of the commonest species are discussed below, and their potential to transmit viruses, evaluated.

### *C. (Avaritia) bolitinos*

This species, like *C. (A.) imicola*, is widespread in the Afrotropical Region. The larval habitat, i.e. cattle dung, is the immediate cause for it being rare to quite common, but most often localized, in its distribution (Meiswinkel 1989). Only once previously, in a light-trap survey conducted in the Western Cape winter rainfall region, has *C. bolitinos* been found to be an abundant species comprising 15,2 % of 33 564 *Culicoides* captured (Nevill *et al.* 1988). It has been shown that *C. bolitinos* can feed on both cattle and horses (Nevill *et al.* 1988). As mentioned above, over 150 specimens of *C. bolitinos* were reared from cattle dung at Sunnyside (# 17); its dominance at the Golden Gate horse-stable (# 18) suggests that it may also utilize horse dung as a larval habitat, as no cattle are kept in this National Park. However, cattle are found on the neighbouring farm, 2 km away. Owing to *C. bolitinos* being only recently recognized as a species separate from *C. imicola* (Meiswinkel 1989), little virus isolation work has been done on this species. An undescribed orbivirus, provisionally named Letsitele virus, has been isolated from *C. bolitinos* (Nevill *et al.* 1992a).

In partial support of the argument that *C. bolitinos* may be an orbivirus vector, it is relevant to note that in Australia the very closely related Oriental/Australian *C. brevitarsis* has been implicated in the transmission of BT virus and Akabane virus (Muller, Standfast, St George & Cybinski 1982; Murray 1987), while BEF has once been isolated from it (Muller & Standfast 1986). *Culicoides brevitarsis*, like *C. bolitinos*, also uses the dung of cattle as a larval habitat and, over the past 200 years since the introduction of cattle and the water buffalo (*Bubalus bubalis*), has spread widely in Australia (Standfast, Dyce & Muller 1985).

However, three facts seem to mitigate against *C. bolitinos* being an efficient BT virus vector. Firstly, Jupp *et al.* (1980) recorded *C. imicola* from the southern Orange Free State as comprising only 0,9 % of all *Culicoides* sampled. This agrees well with our data, and even though *C. bolitinos* had not been recognized as separate from *C. imicola* at that time, the low numbers strongly indicate that *C. bolitinos* was absent. This is to be expected in a sheep-farming area where no cattle dung would be available for *C. bolitinos*. However, Jupp *et al.* (1980) note that BT is present in the region; this implies that *Culicoides* species other than *C. bolitinos* or *C. imicola* may be locally involved in transmission. Secondly, its sister species, *C. brevitarsis*, has extremely low infection rates for BT virus serotypes 1, 20 and 21, these ranging between 0,2 and 0,3 % (Standfast *et al.* 1985). This species can therefore act as a vector, but only if it occurs in high numbers. A third factor mitigating against *C. bolitinos* as an efficient vector of BT virus, is that this survey reveals it to be very stable in its abundance and prevalence—a direct result of the consistent availability of cattle and dung. Why then are outbreaks of BT irregular in this area and epizootics usually associated with higher rainfall? Is this pattern due to lapses in the vaccination programme or is the disease being transmitted by other *Culicoides* species with differing life-strategies? Before any assumptions can be made on the vector status of *C. bolitinos* for BT virus, and perhaps BEF virus, its infection rates for these viruses must first be determined.

### *C. (A.) gulbenkiani*

This species is restricted to the cooler and wetter areas of South Africa. Only in the Western Cape winter rainfall region has *C. gulbenkiani* been found to be common, comprising 7,9 % of 33 564 *Culicoides* collected (Nevill *et al.* 1988). It is known to feed on cattle and sheep (Nevill *et al.* 1988) and pigs (Braverman & Phelps 1981). It utilizes old cattle and horse dung as a larval habitat, and seems to succeed *C. bolitinos* in these media (Nevill *et al.* 1988). While its low prevalence suggests a weak potential to transmit viruses, it is important to note that in Kenya, Walker & Davies (1971) isolated BT virus serotype 1 and Nairobi sheep disease (NSD) from the closely related *C. tororoensis*. Indeed, there is some confusion about the species status of *C. gulbenkiani* and *C. tororoensis*; Glick (1990) synonymized them, whereas Meiswinkel (1992) indicated that they are "two valid species that need reappraisal both biologically and taxonomically". For example, the rearing by Dyce & Marshall (1989) of *C. gulbenkiani* from elephant (*Loxodonta africana*) and zebra (*Equus burchellii*) dung in the hotter, low-lying Kruger National Park, appears to be a misidentification of *C. tororoensis*. Although *C. gulbenkiani* is rare in northern Zimbabwe (Braverman & Phelps 1981), it is likely that it occurs throughout the higher-lying areas of East Africa where it may be found in sympatry with, and be confused with *C. tororoensis*. Whatever the reality, the close circle of association between these two species, an animal and its dung, appears suited to the transmission of orbiviruses. For this reason, all dung-inhabiting *Avaritia* species deserve consideration as potential vectors.

### *C. (Monoculicoides) cornutus*

Prior to this survey, *C. cornutus* had rarely been collected in South Africa. According to Nevill *et al.* (1988), it is absent in the south-western Western Cape, while Venter (1991) found it comprised a mere 0,02 % of ± 3 000 000 *Culicoides* captured at 34 sites sampled throughout South Africa. In Kenya and Tanzania, however, *C. cornutus* was a dominant species, found especially in dense, localized pockets near domestic stock (Walker & Davies 1971). Here the larvae occur in high concentrations in the mixture of fine mud and dung surrounding animal pens, and in associated effluent ditches. The significant increase in *C. cornutus* numbers in the eastern Orange Free State in 1993, was apparently due to muddier conditions following a better rainy season. A similar positive correlation between rainfall and a rise in *C. cornutus* was noted by Walker & Davies (1971), and seems to explain its disappearance during the drier parts of the season and in years of drought. Such a variable occurrence may have a direct link with the intermittent nature of BT outbreaks which are usually associated with rainier years. While rainfall was uniform in the eastern Orange Free State during 1993, and significant numbers of stock occurred at all sites sampled, the prevalence of *C. cornutus* was patchy. In this area, most stock animals are either free-ranging or maintained, in rotation, in large pastured paddocks. Only at dairies where cattle are held intensively on bare ground, do muddy conditions develop, and soon become polluted because of poor drainage and waste disposal. Such conditions prevailed at Madrid (# 16), Uitvlugt (# 12) and Makana (# 17) where *C. cornutus* was dominant or abundant. It is relevant here to note that in the Ruiru cattle pen in Kenya, Walker (1977) found that 100 % of the *C. cornutus* captured were at the cattle pen and none in the pasture.

In Kenya precipitin tests of blood-engorged females revealed that *C. cornutus* had fed in almost equal numbers on cattle and sheep (Walker & Davies 1971; Walker & Boreham 1976). Although no viruses have yet been isolated from *C. cornutus*, the former authors considered it to have a high vector potential as it was quite dominant in the BT enzootic area. Later, however, Walker & Boreham (1976) were more cautious, ascribing to *C. cornutus* "an incidental role when it occasionally increases greatly in numbers at some sites".

It is notable that *C. cornutus* is the only African member of the subgenus *Monoculicoides* which embraces *C. variipennis*, the most dominant and important BT virus vector in North America (Jones 1985). Furthermore, *C. variipennis*, like *C. cornutus*, also predominates in habitats where the substrate is heavily polluted with animal manure and where waste management is poor (Kline & Greiner 1992).

Although *C. cornutus* accounted for only 1,1 % of all the *Culicoides* species collected, its numbers can escalate under intensive farming conditions. As BT outbreaks are usually associated with increased precipitation, we are inclined to rate *C. cornutus* to be as important as *C. bolitinos* as a potential vector of BT virus.

### *Culicoides (Beltranmyia) pycnostictus*, *C. (B.) nivosus* and *C. (Meijerehelea) leucostictus*

(In most southern African studies *C. leucostictus* has been misidentified as *C. distinctipennis*)

All three species are widespread in South Africa and can become relatively abundant (Venter 1991; Nevill, Venter & Edwardes 1992b). *Culicoides pycnostictus* was the dominant species in light-traps in the southern Orange Free State (Jupp *et al.* 1980), while in the adjoining Lesotho highlands it was second in abundance, comprising 11,7 % of 32 819 *Culicoides* collected (Venter & Sweatman 1989). In Harare, Zimbabwe, Braverman & Phelps (1981) found all three species to comprise a mere 0,1–0,2 % of 43 202 *Culicoides* collected near poultry. Similarly, low numbers were found at cattle and horses (Phelps *et al.* 1982). While it is generally accepted that these species are bird-associated (Nevill & Anderson 1972; Braverman & Rubina 1976; Jupp *et al.* 1980; Braverman & Phelps 1981; Nevill *et al.* 1988), all have been found to feed on cattle as well (Table 5; Walker & Boreham 1976); BT virus serotypes 6 and 24 have been isolated from *C. pycnostictus* (Nevill *et al.* 1992a). This involvement with mammals requires further investigation. Their tolerance of a great variation in larval habitat (Glick 1990), coupled to the omnipresence of wild birds and poultry, probably ensures their wide distribution in southern and eastern Africa. Walker & Davies (1971) rated all three species as having no vector potential, whereas Jupp *et al.* (1980) and Nevill *et al.* (1992b) rated the potential of *C. pycnostictus* as high.

### *Culicoides (Culicoides) magnus*

In South Africa, *C. magnus* appears to be largely restricted to the cooler central plateau and winter rainfall areas; it is very weakly represented in the hotter, lower-lying areas of the Northern Transvaal, Eastern Transvaal and Kwazulu/Natal. It has been found to be abundant in the Western Cape winter rainfall region (Nevill *et al.* 1988); it is moderately abundant in Lesotho (Venter & Sweatman 1989) and the escarpment areas of Kwazulu/Natal (Nevill *et al.* 1992b). It will feed on sheep, cattle, horses and pigs (Walker & Davies 1971; Nevill & Anderson 1972; Braverman & Phelps 1981; Nevill *et al.* 1988; Nevill *et al.* 1992b). Nevill *et al.* (1992b) rated *C. magnus* as having a high vector potential—a view shared by Walker & Davies (1971). Later, however, Walker & Boreham (1976) did not rate *C. magnus* as important in the epidemiology of BT and BEF in Kenya.

*Culicoides magnus* is one of three known species in Africa belonging to the subgenus *Culicoides*; this subgenus is temperate in origin, being well-represented and abundant in the Northern hemisphere. One of its member species, *C. pulicaris*, is considered to have the potential to vector BT virus, and has been successfully infected in the laboratory (Mellor 1992).

### *Culicoides (Hoffmania?) zuluensis* and *C. (H?) milnei* (= Milnei group)

*C. zuluensis* was the dominant species in Lesotho, comprising 74 % of > 30 000 *Culicoides* collected in a year-long survey (Venter & Sweatman 1989), and it was a co-dominant in the Western Cape winter rainfall area (Nevill *et al.* 1988). Its absence in the hotter and drier low-lying Northern Transvaal (Venter 1991), clearly indicates that *C. zuluensis*, like *C. magnus*, prefers cooler, wetter conditions. These same studies point to *C. milnei* being restricted to even higher, colder elevations, and being rarer than *C. zuluensis*. At Harare, Zimbabwe, Braverman & Phelps (1981) found *C. milnei* and *C. zuluensis* to be the second (27 %) and third (14 %) most dominant species amongst ± 395 000 *Culicoides* collected at cattle and horses. In South Africa, Nevill *et al.* (1992b) rated *C. zuluensis* as having a high vector potential. Walker & Davies (1971) found *C. milnei* to be the commonest species in the BT enzootic zone of Kenya, and accordingly nominated it as the species with the highest vector potential; BT virus serotype 1 was also isolated from it. However, Cornet, Nevill & Walker (1974) and Walker & Boreham (1976) later observed that not only had *C. zuluensis* been misidentified as *C. austeni* in Kenya, but that it could also occur in sympatry with *C. milnei*, just as noted in the above-mentioned South African and Zimbabwean studies. In his review of Kenyan *Culicoides*, Glick (1990) summed up the situation by saying that "most existing literature on the biology of the Milnei complex must be regarded with caution because of the possibility of misidentified material". This later led Walker & Boreham (1976) to regard *C. zuluensis* and *C. milnei* as unlikely to be as important as *C. imicola* and members of the Schultzei group in the epidemiology of BT and BEF "because of their more limited distribution and abundance", but they conceded that these midges "could play an incidental role". Braverman & Phelps (1981) classed *C. zuluensis* and *C. milnei* as opportunistic feeders as they feed not only on large mammals, but also on birds. This may be of epidemiological significance as "many birds are known to carry arboviruses". As regards opportunism, White (1977) recorded *C. milnei* as biting man; attack rates were highest where man lived in company with his domestic animals. Up to 35 000 could be collected per trap-night at two cows and a mule; bloodmeal analyses showed that *C. milnei* fed on these animals.

The large Milnei group, which comprises some 15 species in Africa, is an important and well-represented group in tropical Asia. It is closely related to, if not inseparable from, the subgenus *Hoffmania* of the tropical Americas, which includes the species *C. insignis*, probably the main vector of BT virus in southern Florida, the Caribbean region, and parts of South America (Mellor 1992).

### *Culicoides (Remmia) schultzei* s.l. (= Schultzei group)

In the eastern Orange Free State, *C. schultzei* s.l. is represented by two undescribed species: *C.* sp. # 9 and *C.* sp. # 35. The latter comprised 95 % of the specimens collected; it has been found to be relatively abundant in Lesotho (Venter & Sweatman 1989), but rare in the south-western Western Cape Province (Nevill *et al.* 1988). Unpublished collection records indicate *C.* sp. # 35 to be restricted to the colder, higher-lying central plateau, and the winter rainfall region of South Africa.

As regards their role as arbovirus vectors, various authors (cited below) rate the Schultzei group as second in importance to the Imicola group. One species is suspected of transmitting BT virus in Australia, while epizootic haemorrhagic disease (EHD) and Palyam (PAL) viruses have been isolated from species of this group in Africa and Japan (Mellor 1992). Seven species of the Schultzei group occur in the western and northern parts of South Africa and some species can become plentiful under hot and dry conditions. This led Nevill *et al.* (1992b) to assign a high vector potential to this group, echoing the view of Walker & Boreham (1976). The latter authors noted that this group was sufficiently widespread in Kenya to act as vectors, especially with regard to BEF virus, as it was "the commonest feeder on cattle at disease sites". Though not particularly abundant, *C.* sp. # 35 thus deserves investigation as a potential vector of BEF in the eastern Orange Free State.

The Schultzei group is found throughout Africa, into the eastern Mediterranean across the Middle and Far East into northern Australia. It is taxonomically complex; at least four of the African species remain undescribed, a situation currently being reviewed by Cornet & Brunhes (personal communication 1993).

### Implications for arbovirus transmission

Essential factors contributing to arbovirus transmission are:

- A source of the particular virus must be available.
- Susceptible animals must be present.
- An efficient vector must also be present.

To transmit a virus, an insect must feed on a viraemic animal or suitable reservoir, become infected, survive the 4–8-d incubation period, and then feed on a second receptive host. The probability of it transmitting the virus is therefore low; this suggests that rare insects are unlikely to be important vectors (Standfast & Dyce 1972). A high infection rate can, however, make a less abundant species an effective vector.

Bluetongue occurs regularly in the eastern Orange Free State (Venter 1991). As indicated by Du Toit (1962) cattle now act as a reservoir of BT virus in those areas of Africa where game has been eliminated. Both cattle and sheep were found on more than 50 % of the farms surveyed and on four occasions the light-trap was operated within 100 m of cattle and sheep housed together. The only proven vector of BT virus, namely *C. imicola*, is virtually absent in this area, therefore other species are very likely involved in BT virus transmission. The close association between cattle and *C. bolitinos*, the former providing blood, dung and virus, has implications for virus transmission, not only between cattle, but also from cattle to sheep. Other *Culicoides* species such as *C. cornutus* and *C. pycnostictus* may be involved as well. Under intensive livestock-management situations, they can escalate sharply, and during high-rainfall years coincides with the occurrence of BT.

The absence of AHS in the eastern Orange Free State probably has a multiple cause: the virtual nonexistence of a suitable reservoir host such as the zebra (Barnard 1993), coupled to extremely low numbers of its only currently known vector *C. imicola*. Although the Golden Gate Highlands Park has both zebra and unvaccinated horses, the two species have minimal contact, and no cases of AHS have ever been noted. The AHS sero-status of these zebra is unknown. While there is still doubt about the involvement of donkeys in the epidemiology of AHS, these animals appear to be too rare and patchy in their prevalence to ensure continuous cycling of the virus. This study seems to indicate that none of the other species encountered are important for AHS virus transmission, either because they cannot become infected with the virus or because they occur in too low numbers.

Like BT, BEF occurs regularly over a wide area in South Africa, including the eastern Orange Free State. Too little is known about the epidemiology of this disease to speculate further on its possible vectors.

## CONCLUSIONS

*Culicoides imicola* is rare in this cold, high-lying area. This indicates that it may not be the sole vector species for BT virus and BEF in South Africa. It is therefore important to repeat this kind of study in other climatic regions to develop a more complete understanding of the epidemiology of *Culicoides*-transmitted viruses.

Both cattle and sheep were found on more than 50 % of the farms surveyed. Although cattle, owing to their larger biomass, can serve as a decoy to deflect *Culicoides* away from sheep and thereby reduce the likelihood of transmission (Nevill 1978), BT still occurs.

Furthermore, as cattle are recognized to be a primary reservoir host for BT virus, the risk of BT virus transmission can be increased if cattle and sheep are run together. It is for this reason that the majority of stock farmers in the eastern Orange Free State attempt to keep these two stock species separate as far as is practicable (A.E. Fair, veterinarian, Bethlehem, personal communication 1990). However, the constant rotation of animals for pasture-utilization purposes could aid in the dispersion of *Culicoides*, which would increase the likelihood of transmission. Alternatively, even if the animals were to be maintained separately, the distances might be too small to overcome the dispersal range of *Culicoides*. For farmers it would be difficult to circumvent these various difficulties, but if both cattle and sheep were immunized against BT, this would reduce the source of the virus and the number of susceptible animals, and thus help to suppress BT virus transmission.

Before it can be assumed other *Culicoides* species such as *C. bolitinos*, *C. cornutus* and *C. pycnostictus* play an important role in the epidemiology of BT and other arboviruses, their ability to multiply these viruses must first be investigated and compared with that of *C. imicola*.

## ACKNOWLEDGEMENTS

We wish to thank the following:

Dr Alan Fair for information regarding the incidence of diseases in the area, and for supplying the names and addresses of stock farmers; all farmers on whose properties we made our collections and who provided those vital cups of tea; Dr B.J.H. Barnard of the OVI and his personnel for the virus isolation attempts; Mr J.H. van Gas for the bloodmeal identifications; Mrs Ina Hermanides and Marinda Smith for technical assistance; Dr E.M. Nevill, veterinary entomologist at the OVI, for his support and Prof. G. Bath for his constructive editing of this paper.

## REFERENCES

ACOCKS, J.P.H. 1988. Veld types of South Africa. *Memoirs of the Botanical Survey of South Africa*, 57.

ALLINGHAM, P.G. & STANDFAST, H.A. 1990. An investigation of transovarial transmission of Akabane virus in *Culicoides brevitarsis*. *Australian Veterinary Journal*, 67:273–274.

BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in zebra (*Equus burchelli*), in the Kruger National Park, South Africa, as measured by the prevalence of type antibodies. *Onderstepoort Journal of Veterinary Research*, 60:111–117.

BLACKBURN, N.K., SEARLE, L. & PHELPS, R.J. 1985. Viruses isolated from *Culicoides* (Diptera: Ceratopogonidae) caught at the veterinary research farm, Mazowe, Zimbabwe. *Journal of the Entomological Society of Southern Africa*, 48:331–336.

BONSMA, F.N. & JOUBERT, D.M. 1957. *Factors influencing the regionalisation of livestock production in South Africa*. Department of Agriculture, Republic of South Africa. *Science Bulletin*, 380.

BRAVERMAN, Y. & RUBINA, M. 1976. Light-trapping of biting insects in poultry houses in Israel. *Israel Journal of Zoology*, 25: 95–101.

BRAVERMAN, Y. & PHELPS, R.J. 1981. Species composition in samples of *Culicoides* (Diptera: Ceratopogonidae) collected near Salisbury, Zimbabwe in 1976–1977. *Journal of the Entomological Society of Southern Africa*, 44:315–323.

BRAVERMAN, Y., BARZILAI, E., FRISH, K. & RUBINA, M. 1985. Bluetongue virus isolation from pools of *Culicoides* spp. in Israel during the years 1981 to 1983, in *Bluetongue and related Orbiviruses*, edited by T.L. Barber, & M.M. Jochim. New York: Alan R. Liss.

CAPELA, R., SOUSA, C., PENA, I. & CAEIRO, V. 1993. Preliminary note on the distribution and ecology of *Culicoides imicola* in Portugal. *Medical and Veterinary Entomology*, 7:23–26.

COETZER, J.A.W. & ERASMUS, B.J. 1994. African horsesickness, in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:460–475.

CORNET, M., NEVILL, E.M. & WALKER, A.R. 1974. Note sur les *Culicoides* (Diptera, Ceratopogonidae) du groupe de *C. milnei* Austen, 1909, en Afrique orientale et australe. *Cahiers de l'office de la Recherche Scientifique et Technique Outre-Mer, Série Entomologie médicale et Parasitologie*, 12:231–243.

CULLIFORD, B.J. 1964. Precipitin reaction in forensic problems. *Nature*, 201:1092–1094.

DAVIES, F.G., WALKER, A.R., OCHIENG, P. & SHAW, T. 1979. Arboviruses isolated from *Culicoides* midges in Kenya. *Journal of Comparative Pathology*, 89:587–595.

DIRECTORATE OF ANIMAL HEALTH, 1991. *Livestock census*. Directorate of Agricultural Information, Pretoria.

DU TOIT, R.M. 1944. The transmission of blue-tongue and horse sickness by *Culicoides*. *Onderstepoort Journal of Veterinary Science*, 19:7–16.

DU TOIT, R.M. 1962. The role played by bovines in the transmission of bluetongue in sheep. *Journal of the South African Veterinary Medical Association*, 33:483–490.

DYCE, A.L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society*, 8:11–15.

DYCE, A.L. & MARSHALL, B.D. 1989. An early record of *Culicoides* species (Diptera: Ceratopogonidae) developing in the dung of game animals in southern Africa. *Onderstepoort Journal of Veterinary Research*, 56:85–86.

GLICK, J.I. 1990. *Culicoides* biting midges (Diptera: Ceratopogonidae) of Kenya. *Journal of Medical Entomology*, 27:85–195.

HENNING, M.W. 1956. *Animal diseases in South Africa,* 3rd ed., South Africa: Central News Agency.

JONES, R.H. 1985. Vector research with the orbiviruses, in: *Bluetongue and related orbiviruses*, edited by T.L. Barber, & M.M. Jochim. New York: Alan R. Liss.

JUPP, P.G., MCINTOSH, B.M. & NEVILL, E.M. 1980. A survey of the mosquito and *Culicoides* faunas at two localities in the Karoo region of South Africa with some observations on bionomics. *Onderstepoort Journal of Veterinary Research*, 47:1–6.

KLINE, D.L. & GREINER, E.C. 1992. Field observations on the ecology of adult and immature stages of *Culicoides* spp. associated with livestock in Florida, USA, in: *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton, & B.I. Osburn. Boca Raton: CRC Press.

MEISWINKEL, R. 1989. Afrotropical *Culicoides*: A redescription of *C. (Avaritia) imicola* Kieffer, 1913 (Diptera: Ceratopogonidae) with description of the closely allied *C. (A.) bolitinos* sp. nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

MEISWINKEL, R. 1992. Afrotropical *Culicoides: C. (Avaritia) loxodontis* sp. nov., a new member of the Imicola group (Diptera: Ceratopogonidae) associated with the African elephant in the Kruger National Park, South Africa. *Onderstepoort Journal of Veterinary Research*, 59:145–160.

MELLOR, P.S., OSBORNE, R. & JENNINGS, D.M. 1984. Isolation of bluetongue and related viruses from *Culicoides* spp. in the Sudan. *Journal of Hygiene, Cambridge*, 93:621–628.

MELLOR, P.S., BONED, J., HAMBLIN, C. & GRAHAM, S. 1990. Isolation of African horse sickness virus from vector insects made during the 1988 epizootic in Spain. *Epidemiology and Infection*, 105:447–454.

MELLOR, P.S. 1992. *Culicoides* as potential orbivirus vectors in Europe, in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osborn. Boca Raton: CRC Press.

MULLER, M.J., STANDFAST, H.A., ST. GEORGE, T.D. & CYBINSKI, D.H. 1982. *Culicoides brevitarsis* (Diptera: Ceratopogonidae) as a vector of arboviruses in Australia. *Proceedings of the 3rd Symposium of Arbovirus Research in Australia, 1982*: 43–49.

MULLER, M.J. & STANDFAST, H.A. 1986. Vectors of ephemeral fever group viruses. Arbovirus research in Australia. *Proceedings of the 4th Symposium of Arbovirus Research in Australia, Brisbane, 1986*: 295–298.

MURRAY, M.D. 1987. Akabane epizootics in New South Wales: evidence for long distance dispersal of the biting-midge *Culicoides brevitarsis*. *Australian Veterinary Journal*, 64:305–308.

NELSON, R.L. & SCRIVANI, R.P. 1972. Isolation of arboviruses from parous midges of the *Culicoides variipennis* complex and parous rates in biting populations. *Journal of Medical Entomology*, 4:277–281.

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

NEVILL, E.M. 1978. The use of cattle to protect sheep from bluetongue infection. *Journal of the South African Veterinary Association*, 49:129–130.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J. H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 55: 101–106.

NEVILL, E.M., ERASMUS, B.J. & VENTER, G.J. 1992a. A six year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae), in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn, Boca Raton: CRC Press.

NEVILL, E.M., VENTER, G.J. & EDWARDES, M. 1992b. Potential *Culicoides* vectors of livestock orbiviruses in South Africa, in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn. Boca Raton: CRC Press.

PHELPS, R.J., BLACKBURN, N.K. & SEARLE, L. 1982. *Culicoides* (Diptera: Ceratopogonidae) catches and virus isolations from them in the Mukwadzi Valley, Zimbabwe. *Journal of the Entomological Society of Southern Africa*, 45:195–200.

STANDFAST, H.A. & DYCE, A.L. 1972. Potential vectors of arboviruses of cattle and buffalo in Australia. *Australian Veterinary Journal*, 48:224–227.

STANDFAST, H.A., DYCE, A.L. & MULLER, M.J. 1985. Vectors of bluetongue in Australia, in: *Bluetongue and related orbiviruses*, edited by T.L. Barber & M.M. Jochim. New York: Alan R. Liss.

ST GEORGE, T.D. 1994. Bovine ephemeral fever, in: *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press: 1:553–562.

TAINTON, M.M. 1981. The ecology of the main grazing lands of South Africa. *Veld and Pasture Management in South Africa*. Pietermaritzburg: Shuter & Shooter in association with University of Natal Press.

VENTER, G.J. & SWEATMAN, G.K. 1989. Seasonal abundance and parity of *Culicoides* biting midges associated with livestock at Roma, Lesotho (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 56:173–177.

VENTER, G.J. 1991. 'n Studie van die verspreiding en seisoenale voorkoms van *Culicoides* spp. (Diptera: Ceratopogonidae) en gekoppelde virussiektes in die R.S.A. M.Sc. thesis, University of the Orange Free State.

VENTER, G.J., HILL, ELAINE, PAJOR, I.T.P. & NEVILL, E.M. 1991. The use of a membrane feeding technique to determine the infection rate of *C. imicola* (Diptera, Ceratopogonidae) for 2 bluetongue virus serotypes in South Africa. *Onderstepoort Journal of Veterinary Research*, 58:5–9.

VERWOERD, D.W. & ERASMUS, B.J. 1994. Bluetongue, in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:443–459.

WALKER, A.R. & DAVIES, F.G. 1971. A preliminary survey of the epidemiology of bluetongue in Kenya. *Journal of Hygiene, Cambridge*, 69:47–60.

WALKER, A.R. & BOREHAM, P.F.L. 1976. Saline as a collecting medium for *Culicoides* (Diptera: Ceratopogonidae) in Kenya in relation to the epidemiology of bluetongue and ephemeral fever. *Bulletin of Entomological Research*, 66:181–188.

WALKER, A.R. 1977. Adult lifespan and reproductive status of *Culicoides* (Diptera: Ceratopogonidae) in Kenya, with reference to virus transmission. *Bulletin of Entomological Research*, 67: 205–215.

WEATHER BUREAU. 1986. *Climate of South Africa,* WB 40. Weather Bureau, Department of Environment Affairs, Pretoria.

WETZEL, H., NEVILL, E.M. & ERASMUS, B.J. 1970. Studies on the transmission of African horsesickness. *Onderstepoort Journal of Veterinary Research*, 37:165–168.

WHITE, G.B. 1977. Man-biting species of *Chrysops* Meigen, *Culicoides* Latreille and *Simulium* Latreille in Ethiopia, with discussion of their vector potentialities. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71:161–175.