Culicoides (Diptera: Ceratopogonidae) associated with livestock in the Onderstepoort area, Gauteng, South Africa as determined by light-trap collections

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ABSTRACT

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In 54 light-trap collections made at 28 sites in the Onderstepoort area a total of 178 941 *Culicoides* midges of 35 species was collected in March 1988; the survey was repeated at 26 sites in September and yielded 19 518 *Culicoides* of 24 species. The number of *Culicoides* species collected totalled 38. *C. imicola* was the most abundant species at 27 of the 28 sites sampled, and accounted for 88% and 67% of all midges collected in the two months respectively. This study not only confirms that *C. imicola* is widespread and abundant in the greater Onderstepoort area, but also that its numbers correlate positively with the historical prevalence of African horse sickness (AHS) and bluetongue (BT) locally.

The high numbers of *C. imicola* make Onderstepoort the ideal site for the study of its laboratory vector capacity. The relatively low numbers of *Culicoides* spp. other than *C. imicola* in the Onderstepoort area, will severely limit studies on their roles in the transmission of arboviruses.

The origin of the blood-meals of 1 338 engorged *Culicoides* belonging to 13 species was determined by means of a cross-over electrophoresis precipitin test; *C. imicola* fed on cattle, horses, sheep and pigs. Four other *Culicoides* species showed a similarly wide host range.

Keywords: Abundance, Ceratopogonidae, *Culicoides, C. imicola,* livestock, Onderstepoort, vector competence

INTRODUCTION

In 1905 the farm "Onderstepoort" was selected by Sir Arnold Theiler as the site for the new Onderstepoort Veterinary Institute (OVI); the high prevalence of African horse sickness (AHS) in the area reputedly influenced his choice (Howell 1975). Since the OVI's establishment in 1908 several researchers there have investigated the role of arthropods in the epidemiology of AHS and bluetongue (BT). Originally mosquitoes were targeted, because of the early views of Nocard (cited in Anonymous 1901), and Watkins-Pitchford (1903): "...that horse-sickness may be caused, like malaria, by the bite of some nocturnal insect..."

This was followed by a detailed series of studies on various genera of mosquitoes at Onderstepoort (Gough 1910; Theiler 1930; Nieschulz, Bedford & Du Toit 1934a, b, c); these authors were singularly unsuccessful and led Nieschulz & Du Toit (1937) to conclude that "...the virus of horse sickness may thus remain alive, in exceptional cases, up to seven days

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in certain *Aedes* and *Anopheles* species. Normally, however, it is destroyed more quickly and, in our experiments, it never could be transmitted by the feeding of infected mosquitoes. Mosquitoes do not appear to be the natural transmitters of horse sickness". To this day data regarding the role of mosquitoes in the transmission of AHS remain inconclusive (Mellor & Boorman 1995).

Only when Du Toit (1944a) started using a modified version of the New Jersey suction light-trap, described by Mulhern (1942), did he become aware that *Culicoides* occurred in great numbers at Onderstepoort. This persuaded him to investigate their role as vectors of AHS and BT. This led to his discovery that both viruses can regularly be isolated from wild-caught *Culicoides* in late summer at Onderstepoort and to his successful biological transmission experiments with *Culicoides* midges and BT virus (Du Toit 1944a). Fiedler (1951) referred to *C. pallidipennis* (= *C. imicola*) as "the most abundant species at Onderstepoort". This was confirmed by later studies (Nevill 1971; Nevill & Anderson 1972; Venter, Nevill & Van der Linde 1996). The foregoing led us to assume that Du Toit's initial 1944 experiments on BT and those on AHS (cited in Wetzel, Nevill & Erasmus 1970) involved *C. imicola*, as he had only broadly concluded "...that certain species of the genus *Culicoides* are capable of becoming infected with ...virus..." (Du Toit 1944a).

Since Du Toit's original findings the viruses of AHS and BT have repeatedly, and most often, been isolated from *C. imicola* throughout its extensive range in the Old World (Davies, Walker, Ochieng & Shaw 1979; Mellor, Osborne & Jennings 1984; Blackburn, Searle & Phelps 1985; Braverman, Barzilai, Frish & Rubina 1985; Mellor, Boned, Hamblin & Graham 1990; Nevill, Erasmus & Venter 1992). Although these two diseases are widespread in South Africa, *Culicoides* surveys have revealed that *C. imicola* can be rare in areas where BT, specifically, remains a problem. These are the colder, high-lying areas of central South Africa (Jupp, McIntosh & Nevill 1980; Venter & Sweatman 1989; Venter & Meiswinkel 1994). The dominant species in these studies were *C. pycnostictus*, *C. zuluensis* and *C. bolitinos* respectively.

Until its recent description (Meiswinkel 1989), the cattle-dung-breeding *C. bolitinos* was for many years misidentified as *C. imicola* (Nevill 1969); its close association with cattle, a reservoir and amplifying host for BT virus, brings *C. bolitinos* strongly into play as a potential BT vector. Elsewhere in South Africa the occasional isolation of BT virus from identified batches of wild-caught *C. gulbenkiani* and *C. pycnostictus* suggests that BT may be vectored by more than one *Culicoides* species (Nevill *et al.* 1992). The same scenario does not seem to apply to AHS as no AHS-endemic area has yet been identified that does not also have significant populations of *C. imicola* (Venter & Meiswinkel 1994).

For the above reasons it is essential that laboratory vector capacity studies on orbiviruses at the OVI be extended to include species other than *C. imicola*. Such studies would be simplified if sufficient wild-caught specimens could be obtained in the immediate surroundings of Onderstepoort. An equally important motivation for the present survey was to establish if *C. imicola* is widespread and common in the greater Onderstepoort area, and so ascertain if its high abundance at the OVI is not simply a local phenomenon. Finally, we also wanted to establish the relative abundance of *C. bolitinos*, as prior to its description in 1989 it had been misidentified as *C. imicola* (= *C. pallidipennis*) in earlier studies on *Culicoides* at the OVI.

MATERIALS AND METHODS

Study area

The entire study area is situated immediately north of the Magaliesberg range, and thus adjoins the major residential areas of the city of Pretoria; it includes most of the Wonderboom magisterial district in which the OVI is located (Fig. 1). The area surveyed covers approximately 920 km^2, between latitudes 25°29'S and 25°42'S and longitudes 27°55'E and 28°24'E. Height above sea level varies from 1 110–1 290 m (excluding isolated hillocks and the Magaliesberg range). Most of the rain falls in summer from November to March, and ranges between 430 and 1 017 mm per annum at the OVI (height above sea level 1 219 m). The annual mean daily maximum temperature as measured at the OVI is 26,3 °C; the annual mean daily minimum is 9,3 °C. Between April and September an average of 32 d of frost occurs per annum at the OVI (Weather Bureau 1986). In the most eastern part of the survey area at Roodeplaat (height above sea level 1 164 m) the annual mean daily maximum and minimum temperatures are 25,8 °C and 10,5 °C respectively, with only 13,5 d of frost per annum (Weather Bureau 1986). As defined by Acocks (1988), the vegetation in the study area falls into Other Turf Thornveld (veld type no. 13) and Sourish Mixed Bushveld (veld type no. 19).

The OVI is situated on the banks of the Apies River near the Bon Accord Dam (Fig. 1). Adjoining the Institute is the farm Kaalplaas (3 000 ha), the only farm in the area where extensive cattle ranching is practised; at least 40 horses are also present. The Bon Accord irrigation scheme along the Apies River serves a number of small farms as one proceeds northwards; these intensively produce vegetables, milk, pigs, poultry and sheep. Horse stables are found on some of the smallholdings that dot the study area.

Light-trap collections

Four 220 volt 8 W ultraviolet down-draught suction light-traps were used. Collections were made directly into phosphate-buffered saline (PBS) to which 0,5% 'Savlon' (manufactured by Johnson & Johnson and containing Chlorhexidane Gluconate and Cetrimide) antiseptic had been added according to the method of Walker & Boreham (1976). Large insects were excluded from the collections by means of mosquito netting placed around each trap.

Light-traps were operated overnight, and as close to livestock as possible. Each morning the collections were transported to the OVI for species identification, and blood-fed females were removed. Each trap was either left for a further night's collection, or moved to a new site. During March traps were usually run for two nights at a site, whereas in September only one catch was made per site. In March 1988, 28 sites were sampled and in September of the same year 26. One site (Sportarena dairy #3) was not re-sampled during September and one site (Hoefyster horse stable #15) had ceased to exist.

The position of each site relative to the OVI is shown in Fig. 1. The trap site numbers in Fig. 1 are the same as those used in Table 1. Details of hosts available at each site, and whether irrigated pastures are present or absent, appear in Table 1. At eight trap sites cattle were the dominant stock type, at a further eight horses, sheep at six, poultry at three and pigs at two (Table 1); at most sites more than one host species were present.

Blood-meal identification

The method used for the collection, storage and identification of blood-meals was the same as that used by Nevill, Venter, Edwardes, Pajor, Meiswinkel & Van Gas (1988), i.e. a cross-over electrophoresis precipitin test.

RESULTS AND DISCUSSION

Light-trap collections

The light-trap collection results are summarized in Tables 2 and 3; the single most abundant species at each site appears in bold type. In the tables where *Culicoides* species numbers appear in lieu of a species name the numbering system of Meiswinkel

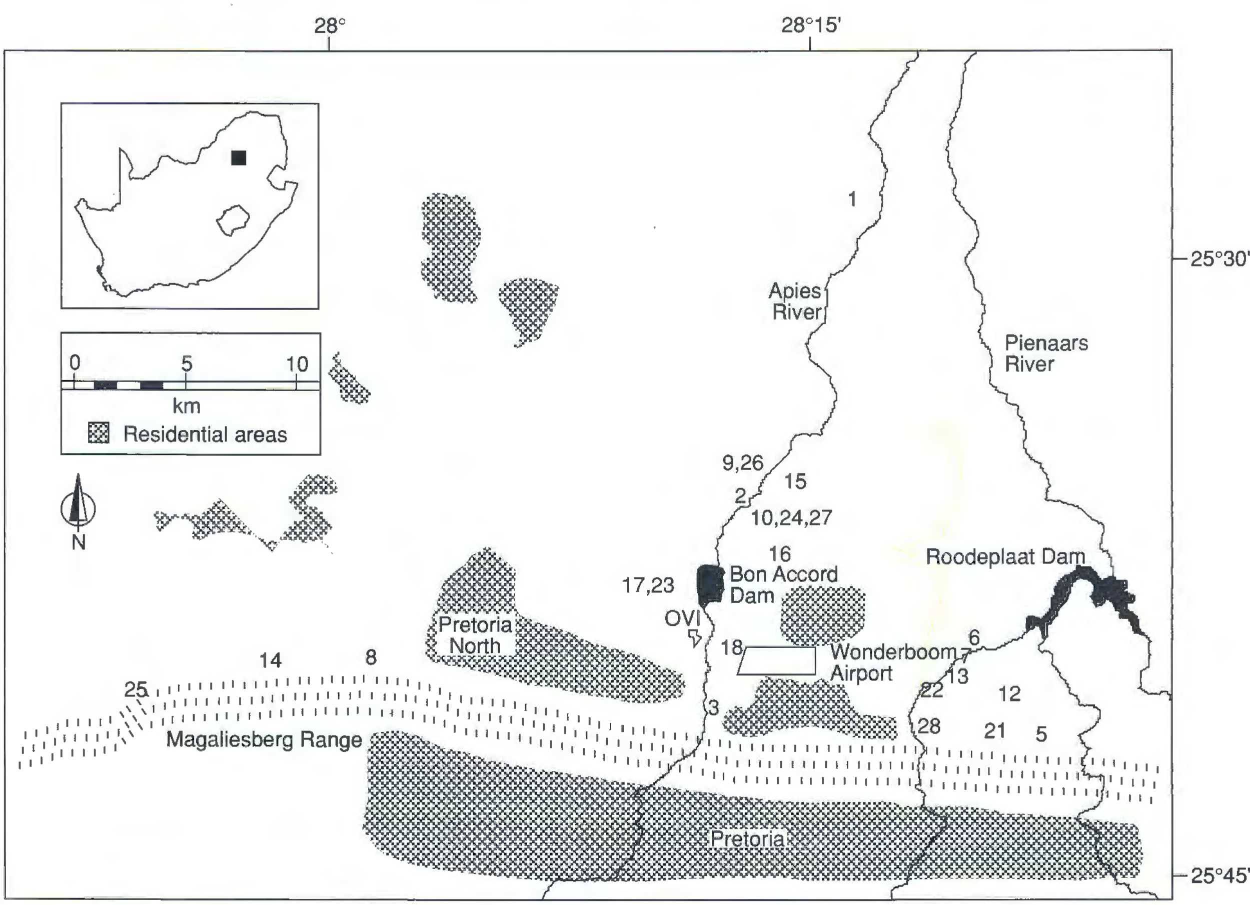

FIG. 1 Map of the study area showing the relative position of the 28 sites sampled: sites 4, 11, 19 and 20 are at the Onderstepoort Veterinary Institute (OVI)

TABLE 1 Details of light-trap locations for two *Culicoides* surveys conducted in the Onderstepoort area, north of Pretoria, 1988

Site no. and farm	Grid reference	Position of trap	Type of farming	Other stock present	Irrigated pastures
1 Rian's	25°27′30′′S,28°16′00′′E	Dairy	Dairy	Sheep	Yes
2 Alfalfa	25°35′06′′S,28°12′36′′E	Dairy	Dairy	Horses and sheep	Yes
3 Sportarena	25°41′09′′S,28°11′18′′E	Dairy	Dairy	–	Yes
4 OVI[1]	25°39′00′′S,28°11′10′′E	Cow shed	Mixed	Mixed	Yes
5 Jonjay	25°40′54′′S,28°20′11′′E	Dairy	Dairy	Horses	Yes
6 Dalrika	25°38′40′′S,28°19′00′′E	Cow kraal	Dairy	Sheep	Yes
7 Bertram's	25°39′32′′S,28°18′38′′E	Cow kraal	Dairy	Sheep and horses	Yes
8 Levonne	25°39′45′′S,28°02′11′′E	Cow kraal	Dairy	–	No
9 North End Farm	25°34′45′′S,28°12′49′′E	Sheep shed	Pigs and sheep	Horses and cattle	Yes
10 Giltford	25°35′37′′S,28°12′51′′E	Sheep shed	Pigs and sheep	Horses and cattle	Yes
11 OVI Camp 102	25°39′00′′S,28°11′10′′E	Sheep shed	Mixed	Mixed	Yes
12 Doman	25°40′54′′S,28°18′07′′E	Sheep shed	Sheep	Cattle	Yes
13 Agridome	25°39′37′′S,28°18′35′′E	Sheep shed	Sheep	Cattle	Yes
14 Jan Smit	25°39′54′′S,27°58′20′′E	Sheep shed	Sheep stud	–	Yes
15 Hoefyster	25°35′00′′S,28°13′05′′E	Horse stable	Riding school	Sheep and cattle	Yes
16 Kunz	25°37′18′′S,28°12′51′′E	Horse stable	Horse stable	Cattle and sheep	No
17 Kaalplaas	25°38′05′′S,28°09′31′′E	Horse kraal	Mixed	Chickens and cattle	No
18 Lustig	25°39′29′′S,28°12′07′′E	Horse stable	Horse stable	Sheep	No
19 OVI Camp 168	25°39′00′′S,28°11′10′′E	Horse kraal	Mixed	Mixed	Yes
20 OVI Transport	25°39′00′′S,28°11′10′′E	Horse shed	Mixed	Mixed	Yes
21 Wilmar	25°41′15′′S,28°18′24′′E	Horse stable	Horse stud	–	No
22 Dannheimer	25°39′57′′S,28°17′42′′E	Horse stable	Horse stable	Cattle	Yes
23 Kaalplaas	25°38′05′′S,28°09′31′′E	Chicken house	Mixed	Cattle and horses	No
24 Giltford	25°35′37′′S,28°12′51′′E	Chicken pen	Pigs and sheep	Horses and cattle	Yes
25 De Wildt	25°40′37′′S,27°55′29′′E	Chicken house	Chickens	–	No
26 North End Farm	25°34′45′′S,28°12′49′′E	Piggery	Pigs and sheep	Horses and cattle	Yes
27 Giltford	25°35′37′′S,28°12′51′′E	Piggery	Pigs and sheep	Horses and cattle	Yes
28 Derdepoort Resort[2]	25°41′11′′S,28°17′18′′E	Farmyard	Mixed	–	No

[1] Onderstepoort Veterinary Institute
[2] Derdepoort Municipal Resort keeps a number of animals and poultry in a farmyard situation for the education of city children

(1995) is followed; *Culicoides* spp. 30, 48, 50, 54 df and 75 are all undescribed members of the subgenus *Avaritia* to which *C. imicola* also belongs. Table 4 summarizes the relative abundance of each *Culicoides* species collected.

During March 178 941 *Culicoides* belonging to 35 species were collected in 54 catches made at 28 sites. *C. imicola* was overwhelmingly dominant, i.e. it comprised 88% of the total number of midges collected, and was the dominant species at 27 of the 28 sites sampled. In September, however, *Culicoides* numbers and species diversity were significantly lower; 26 collections yielded a total of 19 518 *Culicoides* belonging to 24 species. At six of these sites *C. imicola* was replaced as the dominant species by *C. pycnostictus*, but the former still accounted for 67 % of all midges collected in September. The collections in March had an average size of 3 314 midges while those in September averaged 751 (Table 4). In the summer rainfall area of South Africa (which includes the survey area) most AHS and BT cases occur in late summer, i.e. from March to May (Verwoerd & Erasmus 1994; Coetzer & Erasmus 1994). Earlier work at the OVI (Du Toit 1962) showed that BT virus only became freely available in wild-caught *Culicoides* in the latter half of summer. The results of this survey confirm that *Culicoides* numbers, both species and individuals, tend to peak towards the end of summer, increasing the chances for virus transmission during this period. This supports the findings of a seven-year study at the OVI by Nevill (1971) that *Culicoides* are most abundant around March. It was also shown by Venter (1991) that the number of parous females at the OVI was above average from October to May; a high parity, coupled to high population levels, increases the potential for virus transmission.

In the Onderstepoort area *C. imicola* has been found to breed in wet (natural or irrigated), organically enriched kikuyu (*Pennisetum clandestinum*) pastures and that these suitable habitats expand during years of exceptional rainfall (Nevill 1967; Meiswinkel, Nevill & Venter 1994). Thus large populations of *C. imicola* occur each summer in this area (probably seeded from permanently irrigated situations) and may increase tenfold during very wet years (Nevill 1971). The exact size of breeding sites utilized by *C. imicola* immatures has never been determined for any given area, but it is likely that *C. imicola* breeds extensively, as suggested for the North American species *C. haematopotus* and *C. crepuscularis* (Jones 1961). This predominance of *C. imicola* in the Onderstepoort

TABLE 2 Numbers and species of *Culicoides* recovered from light-traps in the Onderstepoort area north of Pretoria, during March 1988, expressed as percentage of total catches. At each site the most abundant species is in bold type

A

Most abundant host	Cattle								Sheep					
Site no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Culicoides spp.														
C. imicola	**93,5**	**84,1**	**79,8**	**98,7**	**82,4**	**76,7**	**56,8**	**78,1**	**66,4**	**94,8**	**98,6**	**94,9**	**88,2**	**83,6**
C. zuluensis	0,4	2,8	0,4	0,1	1,5	1,2	3,5	3,6	8,1	4,1	0,4	2,0	0,4	1,6
C. bolitinos	0,5	0,3	0,2		2,4	7,7	14,0	9,2			0,2	1,3	2,0	0,8
C. nivosus	0,7	1,6	5,0	0,0**	3,6	7,1	17,6	2,7	0,1		0,2	0,2	5,2	0,5
C. sp.48*	1,0	4,7					0,0	0,2	21,4	0,1				
C. pycnostictus	0,8	1,4	3,8		5,4	1,4	2,0	3,0		0,1		0,0	1,9	6,5
C. enderleini	0,5	0,5	0,4	1,1	0,4	0,3	0,7	0,4		0,2	0,2	1,1	0,3	
C. leucostictus	0,2	0,6	3,3		2,1	4,0	3,2	1,8		0,1	0,3	0,4	1,4	1,6
C. magnus	0,2	0,9		0,0	0,2	0,1	0,0		1,2	0,5		0,1	0,0	
C. brucei	0,4	0,4						0,1	1,9	0,2	0,0	0,1	0,0	3,0
C. bedfordi	0,6	0,5	0,2		1,0	0,4	0,1	0,1		0,0		0,0	0,1	
C. tropicalis	0,9	0,6	0,6			0,4	0,4	0,2	0,1	0,0		0,0	0,0	0,5
C. similis		0,3			0,0		0,3						0,1	
C. exspectator	0,1	0,7	0,4	0,1	0,1	0,1	0,0			0,0				
C. neavei	0,0**	0,1			0,6	0,2	0,4	0,1		0,0	0,1		0,1	
C. subschultzei	0,1	0,2				0,1			0,1	0,0	0,1		0,2	
C. sp. 30					0,1	0,1	0,6	0,2				0,0		
C. coarctatus		0,2			0,0				0,5					
C. ravus	0,2	0,1	0,2			0,1	0,2						0,1	0,3
C. milnei	0,1							0,1						0,3
C. nigripennis grp			4,2		0,2	0,0	0,0						0,0	
C. schultzei			1,3				0,3							
C. gulbenkiani						0,1		0,1						
C. kobae	0,0						0,0						0,0	
Other *Culicoides* spp.	0,0[a]	0,0[b]	0,0[c]			0,0[d]		0,1[a]	0,3[fgh]				0,0[i]	1,3[j]
Total no. of *Culicoides*	9 721	22 606	480	7 462	2 233	2 420	12 679	1 402	1 507	36 526	2 698	31 882	5 438	372
No. of collections	2	2	3	2	2	2	2	2	2	2	3	2	2	2

B

Most abundant host	Horses								Poultry			Pigs		Mixed
Site no.	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Culicoides spp.														
C. imicola	**77,3**	**98,9**	**66,5**	**92,6**	**86,3**	**83,1**	**87,2**	**82,1**	**95,7**	**91,5**	**67,6**	29,5	**91,6**	**80,1**
C. zuluensis	16,1	0,1		1,7	5,5	0,2	0,3	2,0	0,2	4,3	0,1	11,0	0,5	1,3
C. bolitinos	0,4		0,4		0,9		3,4	10,9	0,2	0,0*	1,9			3,4
C. nivosus			4,3	1,7	1,4	3,3	0,1	1,3	0,5	0,1	0,4	0,1	1,9	3,4
C. sp. 48*		0,1	1,3							0,2	0,2	**48,7**	0,2	
C. pycnostictus		0,1	14,1	0,2	1,5	8,5	0,3	0,1	0,9	1,1	9,7	0,1	1,4	0,8
C. enderleini	1,9	0,2	0,4	3,7	3,0	0,9	7,8	2,9	0,1	0,2			0,2	2,5
C. leucostictus	0,4	0,1	4,6		0,7	1,3	0,9	0,6	0,8	0,4	8,6		0,7	2,6
C. magnus	0,9	0,2							1,0	0,1		1,3	0,2	
C. brucei	2,4	0,0				0,1	0,0		0,2	0,9		8,5	1,8	0,2
C. bedfordi	0,4						0,0		0,1	0,4			0,2	0,2
C. tropicalis		0,0	5,4		0,5	1,9	0,0			0,5	4,2	0,1	0,6	0,7
C. similis									0,1	0,2	2,9			1,5
C. exspectator	0,1				0,1	0,0					1,7		0,1	0,2
C. neavei		0,1				0,0				0,0	0,1	0,1		
C. subschultzei		0,1							0,1			0,1		3,0
C. sp. 30							0,1		0,1		2,4			
C. coarctatus														
C. ravus		0,0	1,9		0,2	0,6				0,1			0,1	
C. milnei								0,1	0,1			0,6	0,2	
C. nigripennis grp.			0,7			0,0	0,0	0,1	0,1					
C. schultzei											0,1			
C. gulbenkiani		0,0		0,1				0,1			0,1			
C. kobae							0,0							0,2
Other *Culicoides*														
Other *Culicoides* spp.			0,6[jk]						0,1[g]					
Total no. of *Culicoides*	957	7 110	541	1 861	1 063	2 305	9 666	7 096	1 734	2 534	1227	2 087	836	2 498
No. of collections	2	2	2	1	2	1	2	1	2	2	2	2	1	2

* Where numbers appear in lieu of names the numbering system of Meiswinkel (1995) has been followed

** < 0,05 % representation

a *C. nigeriae*
b *C.* sp. 54 dark form
c *C. trifasciellus*
d *C. olyslageri*
e *C.* sp. 50
f *C. micheli*
g *C. nevilli*
h *C. dekeyseri*
i *C. dutoiti*
j *C.* sp. 75
k *C. eriodendroni*

TABLE 3 Numbers and species of *Culicoides* recovered from light-traps in the Onderstepoort area north of Pretoria, during September 1988, expressed as percentage of total catches. At each site the most abundant species is in bold type

A

Most abundant host	Cattle							Sheep					
Site no.	1	2	4	5	6	7	8	9	10	11	12	13	14
Culicoides spp.													
C. imicola	**46,8**	**64,8**	**98,9**	16,5	**44,0**	**50,2**	33,5	**53,1**	**83,9**	**86,1**	**97,5**	**57,3**	12,1
C. zuluensis	1,1	5,0	0,2			3,0	0,5	13,1	3,5	1,5	0,5	1,2	3,0
C. bolitinos		4,8				3,6	1,6				0,2		
C. nivosus	1,5	2,1		0,5	9,9	9,9	6,4			2,2	0,1	1,7	3,0
C. sp. 48*	0,1	1,2					0,5	11,6					
C. pycnostictus	29,4	11,0	0,2	**49,5**	19,8	23,7	**43,1**	1,2	0,5	9,5	0,3	19,6	**54,6**
C. enderleini	0,2				0,8		0,5	0,4	0,2		0,1		
C. leucostictus	6,1	1,7	0,3	6,8	5,8	6,3	11,2	0,5	1,1		0,1	12,7	18,2
C. magnus	0,5	1,0	0,5			0,3		8,2	7,5	0,7	0,6	0,2	
C. brucei	0,6	0,8		1,0	1,2			4,7	1,3			0,3	
C. bedfordi	10,3	4,3		18,9	17,7	2,1			2,0		0,7	5,0	
C. tropicalis	0,4												
C. similis	1,1	1,0		1,5		0,9	1,6	0,2				1,1	
C. exspectator													
C. neavei	1,6	0,2		4,4									
C. subschultzei	0,1	0,2					1,1	0,3	0,1			0,5	6,1
C. coarctatus	0,2	0,8											
C. ravus								1,1	0,1				
C. milnei												0,3	
C. gulbenkiani							0,5						
C. dekeyseri	0,1	1,2										0,2	
C. onderstepoortensis			1,5	0,8							0,2		0,1
C. engubandei													
C. accraensis grp													3,0
Total no. of midges	1 144	517	649	206	243	333	188	3 184	1 335	137	1 096	667	33
No. of collections	1	1	1	1	1	1	1	1	1	1	1	1	1

B

Most abundant host	Horses						Poultry				Pigs		Mixed
Site no.	16	17	18	19	20	21	22	23	24	25	26	27	28
Culicoides spp.													
C. imicola	**80,7**	8,2	**93,3**	**73,6**	**91,6**	**60,9**	**76,5**	**45,7**	**25,8**	35,3	**61,1**	77,4	33,8
C. zuluensis	1,9		0,4	1,0	0,5	0,4	0,9	0,8	22,6		20,1	8,9	1,0
C. bolitinos		0,7	0,2			4,0	14,9						
C. nivosus	0,6	3,0	0,7	4,0	1,4	0,5	0,2	7,9			0,1		2,6
C. sp. 48*			0,2				1,4				5,4		
C. pycnostictus	4,8	**41,5**	2,3	12,9	4,7	19,1	3,2	18,1	9,7	**41,2**	2,1	0,7	**45,0**
C. enderleini				0,2		0,7	0,4						
C. leucostictus	1,8	23,7	1,2	2,8	0,6	8,7	0,5	13,4		5,9	0,2	2,1	9,3
C. magnus	2,3	0,7	1,1	0,7		0,7	0,4	0,8	3,2	5,9	2,9	2,7	
C. brucei	0,1	0,7	0,1	0,3	0,2			0,8	6,5		6,4	0,7	
C. bedfordi	7,2	14,8	0,1	3,3	1,0	3,2	1,3	11,0	**25,8**	5,9	1,0	6,9	6,4
C. tropicalis													
C. similis	0,1	3,7				0,4		0,8		5,9	0,1		1,0
C. exspectator	0,1												
C. neavei	0,1												
C. subschultzei	0,2	1,5	0,4	1,0	0,1	0,9		0,8	6,5		0,4	0,7	1,0
C. coarctatus													
C. ravus		0,7									0,4		
C. milnei						0,2	0,4						
C. gulbenkiani													
C. dekeyseri						0,2							
C. onderstepoortensis	0,7												
C. engubandei	0,1												
C. accraensis grp													
Total no. of midges	1 033	135	998	599	888	555	558	127	31	17	4 388	146	311
No. of collections	1	1	1	1	1	1	1	1	1	1	1	1	1

* Where numbers appear in lieu of names the numbering system of Meiswinkel (1995) has been followed

area is probably partly due to the high numbers of sedentary livestock available for blood-meals.

As regards other species, the survey showed that although *C. bolitinos* is the third most common species, it only accounted for 2,1 % of all *Culicoides* collected. It does, however, achieve a certain measure of abundance in a few localized situations (sites #7, #22, Tables 2 and 3). The close association between *C. bolitinos* and cattle would seem to favour it as a potential BT vector (Venter & Meiswinkel 1994). However, its low population levels in the Onderstepoort area would require it to have an extremely high infection rate, if it were to come near *C. imicola*'s proven role in BT transmission (Venter, Hill, Pajor & Nevill 1991). As regards numbers, a similar argument would apply to *C. zuluensis* and *C. nivosus*. The latter's role in BT transmission may be weakened further by its suspected ornithophilic host preference (Braverman & Hulley 1979).

In March, at the North End Farm piggery (#26), *C. imicola* was replaced as the dominant species by *Culicoides* sp. 48, an undescribed *Avaritia* resembling *C. glabripennis*. At the sheep shed (#9) on the same farm, *C.* sp. 48 accounted for 21 % of the total midges collected. This localized abundance of *C.* sp. 48 can probably be attributed to the presence of a densely reeded marsh, its suspected breeding site. While the low prevalence of *C.* sp. 48 at all other trap locations suggests that it is unlikely to play a role in the dissemination of AHS or BT within the wider study area, it may be involved in arbovirus transmission in marshy situations where it becomes abundant.

Blood-meal identification

The results of the blood-meal identification for March and September are given in Table 5. Most of the specimens that tested negatively belonged to the small species *C. imicola* that, when only partially engorged, probably contains too little blood for an identification to be made. *C. imicola* accounted for 74 % of all specimens tested. Most of the specimens were positive for the stock species near which they were collected.

This study confirms that at least six species of *Culicoides* do feed on pigs, and this should be borne in mind when arthropod vectors of pig viral diseases are sought in future (Table 5). Of the 13 species tested, *C. imicola, C. zuluensis* and *C.* sp. 48 proved to be catholic in their choice of hosts and included cattle, horses, sheep and pigs. Other species with a wide host range were *C. brucei, C. magnus* and *C. coarctatus* (cattle, sheep and pigs). These six species must therefore also be considered as possible vectors of BT between cattle and sheep.

As in previous studies (Nevill & Anderson 1972; Nevill *et al.* 1988; Meiswinkel *et al.* 1994), *C. pycnostictus* and *C. leucostictus* tested positively against bird antisera.

While this study shows no apparent difference in the *Culicoides* species composition in light-traps, the ubiquitous presence of mixed hosts obscures any trends that may exist as regards host preference (Table 1).

CONCLUSIONS

Culicoides imicola comprised 85,5 % of nearly 200 000 *Culicoides* collected in this survey. Furthermore, it was very abundant in March, at the height of the AHS and BT season. While *C. imicola* was less abundant in September, it was still the dominant species at 19 of the 26 sites sampled. This not only confirms the high abundance and vector status of *C. imicola* as established in earlier studies at the OVI (Nevill 1971; Nevill & Anderson 1972; Venter *et al.* 1996) but also proves that *C. imicola* is widespread and common in the greater Onderstepoort area. Its high abundance at the OVI is thus not simply a local phenomenon.

Vector capacity studies on species other than *C. imicola* could have been facilitated if sufficient wild-caught specimens were to be obtained in the immediate surroundings of the OVI. In artificial infection experiments mortalities of up to 60 % can be expected in the holding phase prior to exposure to virus. In the system used at the OVI (Venter *et al.* 1991), the percentage of insects feeding can vary between 40–80 % depending on the population structure of the insects, and the collection and handling techniques. It is our experience that mortalities of up to 80 % can occur during the 8–12 d post feeding incubation period, similar to the findings of Standfast, Muller & Dyce (1992). These authors determined that at least 1 000 insects must enter the system to yield 24 fed *Culicoides* after an incubation period of eight days. During the present study the average daily catch for *C. imicola* was 2 121 which makes it the ideal species to work with in infection and transmission studies. The second-most abundant species, *C. zuluensis,* averaged a mere 72 insects per light-trap collection; this is clearly below the required number needed for testing. Where the number of any other single species may approach 1 000, these collections are usually overwhelmed by *C. imicola;* separating such species live from *C. imicola* increases the mortality rate and so further complicates attempts to establish their infection rates. For example, at the sheep shed on Giltford farm (#10) the average number of *C. zuluensis* collected in PBS was 1 498. The same collection yielded 34 627 *C. imicola* (Table 2). Live collections would yield, due to mortality during capture, a smaller collection, and separating *C. zuluensis,* which would require an extra hour or two of handling on the chill table, would further increase the mortality. It is therefore very difficult to work exclusively with *C. zuluensis* in laboratory infection studies and it would be preferable to find sites elsewhere in South Africa where *C. bolitinos* and *C. zuluensis* are abundant. It would thus seem that the OVI is the

TABLE 4 The total number and percentages and rank of each *Culicoides* species collected in the Onderstepoort area north of Pretoria during March and September 1988

Month	March 1988			September 1988			Total		
Culicoides spp.	Number collected	%	Sites positive (out of 28)	Number collected	%	Sites positive (out of 26)	Number collected	%	Sites positive (out of 28)
C. imicola	156 621	87,5	28	13 030	66,8	26	169 651	85,5	28
C. zuluensis	4 324	2,4	27	1 475	7,6	22	5 799	2,9	27
C. bolitinos	4 026	2,3	20	150	0,8	8	4 176	2,1	21
C. nivosus	3 675	2,1	25	193	1,0	20	3 868	2,0	26
C. sp. 48*	2 548	1,4	12	624	3,2	7	3 172	1,6	14
C. pycnostictus	1 538	0,9	24	1 551	8,0	26	3 089	1,6	27
C. enderleini	1 995	1,1	24	27	0,1	9	2 022	1,0	25
C. leucostictus	1 373	0,8	24	467	2,4	24	1 840	0,9	28
C. magnus	517	0,3	16	565	2,9	20	1 082	0,6	23
C. brucei	504	0,3	18	474	2,4	16	978	0,5	23
C. bedfordi	267	0,2	16	710	3,6	22	977	0,5	25
C. tropicalis	474	0,3	21	5	0,0	1	479	0,2	21
C. similis	181	0,1	8	56	0,3	14	237	0,1	15
C. exspectator	219	0,1	14	1	0,0	1	220	0,1	11
C. neavei	105	0,1	14	98	0,5	19	194	0,1	23
C. subschultzei	153	0,1	11	2	0,0	2	155	0,1	11
C. sp. 30	122	0,1	8				122	0,1	8
C. coarctatus	51	0,0**	3	58	0,3	6	109	0,1	7
C. ravus	89	0,1	13	5	0,0	3	103	0,1	15
C. milnei	26	0,0	7	16	0,1	1	42	0,0	8
C. nigripennis grp.	42	0,0	10				42	0,0	10
C. schultzei	39	0,0	3				39	0,0	3
C. gulbenkiani	10	0,0	6	1	0,0	1	11	0,0	7
C. dekeyseri	1	0,0	1	9	0,1	4	10	0,0	5
C. kobae	9	0,0	4				9	0,0	4
C. onderstepoortensis				8	0,0	5	8	0,0	5
C. sp. 75	7	0,0	2				7	0,0	2
C. nevilli	3	0,0	2				3	0,0	2
C. micheli	3	0,0	1				3	0,0	1
C. nigeriae	3	0,0	2				3	0,0	2
C. sp. 54 df	2	0,0	1				2	0,0	1
C. trifasciellus	1	0,0	1				1	0,0	1
C. engubandei				1	0,0	1	1	0,0	1
C. accraensis grp.				1	0,0	1	1	0,0	1
C. sp. 50	1	0,0	1				1	0,0	1
C. dutoiti	1	0,0	1				1	0,0	1
C. olyslageri	1	0,0	1				1	0,0	1
C. eriodendroni	1	0,0	1				1	0,0	1
Total *Culicoides*	178 941	100		19 518	100		198 459	100	
Total no. of collections	56			26			80		
Average catch size	3 313,7			750,6			2 480,7		
No. of species	35			24			4		

* Where numberts appear in lieu of names the numbering system of Meiswinkel (1995) has been followed

** < 0,05 % representation

ideal site for testing *C. imicola* against several arboviruses rather than comparing different *Culicoides* species against a single arbovirus.

Even if *C. imicola* were to have a low vector capacity for AHS and BT viruses, its superabundance in the Onderstepoort area makes it the strongest vector candidate for these viruses in this area. On the other hand the extremely low numbers of the other *Culicoides* species, even were they to have a high vector capacity, make it unlikely that they could play a significant role in virus transmission at Onderstepoort. This, however, does not exclude the possibility that these species may act as vectors in areas where they are more abundant, e.g. *C. bolitinos* in the eastern Free State (Venter & Meiswinkel 1994).

TABLE 5 Identification by means of cross-over electrophoresis precipitin of the blood-meals of 13 *Culicoides* species collected in light traps in the Onderstepoort area, north of Pretoria during March and September 1988

Culicoides spp.	Host					Total positive	Total tested
	Cattle	Horse	Sheep	Chicken	Pig		
March 1988							
C. imicola	129	108	352		12	601	967
C. sp. 48	9		83		31	123	166
C. zuluensis	5	3	12			20	30
C. brucei	1	2	2		1	6	9
C. bolitinos	5	1				6	8
C. leucostictus				3		3	5
C. magnus	2		3			5	5
C. enderleini	3					3	5
C. pycnosticus				1		1	3
C. nivosus	1					1	1
C. similis			1			1	1
Total	155	114	453	4	44	770	1 200
September 1988							
C. imicola	25	28	59		254	366	448
C. sp. 48*	7	2	56		27	92	118
C. bedfordi			40			40	49
C. zuluensis		3	2		31	36	38
C. brucei					19	19	24
C. magnus			5		6	11	12
C. coarctatus	1		1		2	4	8
Total	33	33	163		339	568	697
Grandtotal	188	147	616	4	383	1 338	1 897

* Where numbers appear in lieu of names the numbering system of Meiswinkel (1995) has been folllowed

It also seems indisputable that the dominant *Culicoides* species used in Du Toit's (1944a) BT and AHS transmission experiments was *C. imicola*; his use of a photograph of *C. enderleini* in a popular article (Du Toit 1944b) was likely intended to illustrate the genus *Culicoides* rather than the particular species used in his trials. It also seems reasonable to conclude that earlier work done at the OVI involving *C. imicola* (Nevill 1971; Nevill & Anderson 1972; Venter 1991; Venter *et al.* 1996) did not include significant numbers of *C. bolitinos*.

In the past (Howarth 1985; Nevill *et al.* 1988; Meiswinkel 1989) attention has been drawn to the apparent escalatory effect that man's husbandry practices have on the numbers of midges found in a given area. The results of this survey seem to support the hypothesis that intensively farmed areas, especially irrigated pastures upon which stock are raised, can sustain artificially large populations of *C. imicola*.

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