RESEARCH COMMUNICATION

Culicoides biting midges at the National Zoological Gardens of South Africa

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ABSTRACT

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Culicoides biting midges (Diptera: Ceratopogonidae) are responsible for the transmission of a large number of pathogens to livestock and wild animals. In this study the presence of the genus, using light traps based at four different sites within the National Zoological Gardens of South Africa, was investigated during 2002–2004. In total, 37 species were recorded, including large numbers of *Culicoides imicola* Kieffer, 1913, which is responsible for the transmission of economically important arboviruses in South Africa, Europe, Middle and Far East. These results are discussed with reference to the wider *Culicoides* fauna in the Onderstepoort area of South Africa, their vector competence as well as biosecurity at the National Zoological Gardens.

Keywords: Abundance, biosecurity, *Culicoides*, light trap, National Zoological Gardens, survey

Culicoides biting midges are responsible for the transmission of several globally significant arboviruses of livestock, along with many other pathogens of local importance (Linley 1985; Mellor, Boorman & Baylis*,* 2000; Meiswinkel, Venter & Nevill 2004). In sub-Saharan Africa, they are of interest primarily as vectors of bluetongue virus (BTV) and African horse sickness virus (AHSV), which cause two devastating diseases of ovinae and equidae, respectively. While the impact of both these viruses in South Africa is limited, because of high endemic immunity in those hosts in which they commonly cause disease and the use of polyvalent live attenuated vaccines, sporadic and severe outbreaks of these diseases still occur (Meiswinkel *et al.* 2004). Outbreaks of African horse sickness (AHS) in 1999 and 2004 in the surveillance zone of the declared AHS-free area in the Stellenbosch district had considerable financial implications and on both occasions led to a 2-year embargo on the export of horses from South Africa (Venter, Koekemoer & Paweska 2006). Of the approximately 120 species of *Culicoides* identified in southern Africa (Meiswinkel, unpublished data 1996), the vast majority remain uninvestigated as potential vectors of disease. However, *Culicoides imicola* Kiefer, 1913 and *Culicoides bolitinos* Meiswinkel, 1989 two species that are closely associated with both livestock and game (Meiswinkel 1989, 1995) have been identified as primary candidates for this role. Both AHSV and BTV have been shown to replicate under laboratory conditions to what are presumed to be transmissible levels in these spe-

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cies (Venter, Paweska, Van Dijk, Mellor & Tabachnick 1998; Venter, Graham & Hamblin 2000).

In this paper, the results of a light trap-based survey carried out over 2 years in which the *Culicoides* spp. occurring in the zoo is presented and their biosecurity risk is defined. Given the movement of animals as part of breeding programmes or loans into countries with a greater clinical disease risk from e.g. bluetongue (BT) or AHS than that in South Africa, biosecurity practices are important to minimize the risk of accidentally importing these viruses/ diseases as happened following the importation of AHSV-infected zebras from Namibia into Spain (Lubroth 1988). Additionally, methods to minimize the numbers of *Culicoides* breeding in this habitat are suggested.

The National Zoological Gardens is located in central Pretoria (25°45' S; 28°11' E) and its inhabitants are isolated from other game and large livestock in the general area by the surrounding urban development, although poultry and some small livestock such as goats are kept. The zoo covers an area of approximately 80 ha and houses over 100 exotic and indigenous species of mammals and over 160 bird species. The survey was carried out from August 2002 to August 2004 using 220V black light down-draught light traps (Meiswinkel *et al.* 2004) placed at four sites on the premises of the zoo (Fig. 1). The sites were selected in close proximity of animal species possessing the potential of filarial, protozoa and viral transmission (Karstad 1979; Meiswinkel *et al.* 2004). The sites chosen were at the Cape buffalo (*Syncerus caffer*), African elephant (*Loxodonta africana*), white rhinoceros (*Ceratotherium simum*)/ black-faced impala (*Aepyceros melampus petersi*) enclosures and the walk-through aviary.

Light traps were operated once weekly from dusk until dawn. Collections were made into a 1 % Savlon (containing Clorhexidane gluconate 0.3 g/100 mℓ and Cetrimide 3.0 g/100 mℓ [Johnson & Johnson, SA]) -water solution and transferred the following morning to 80 % alcohol for storage purposes. All the collections containing more than 500 *Culicoides* specimens were sub-sampled using the method of Van Ark & Meiswinkel (1992). *Culicoides* was identified to species level by examination of wing markings (Meiswinkel 1996), with the exception of the *Culicoides nigripennis* Carter, Ingram and Macfie, 1920 and *Culicoides accraensis* Carter, Ingram and Macfie, 1920 species complexes which are extremely difficult to separate accurately based on their morphology. In order to prevent a bias in numbers of the different species at each site, comparisons of the number and species present were made only between those days when all four traps were in operation. Ninety samples from each site were compared and used for analysis.

In this subset of 360 samples a total of 478 040 midges was collected and identified, representing a total of 33 species (Table 1). The trap based at the elephant enclosure caught the greatest total number of *Culicoides*, representing some 28 different species, followed by the white rhinoceros/black-faced impala site (29 species), the buffalo site (17 species) and the aviary (12 species) (Table 1). Proportionately, *C. imicola* was by far the most commonly trapped species, constituting more than 95 % of all midges caught at the buffalo and elephant sites and 67 % at the white rhinoceros/black-faced impala site, where *Culicoides cor-*

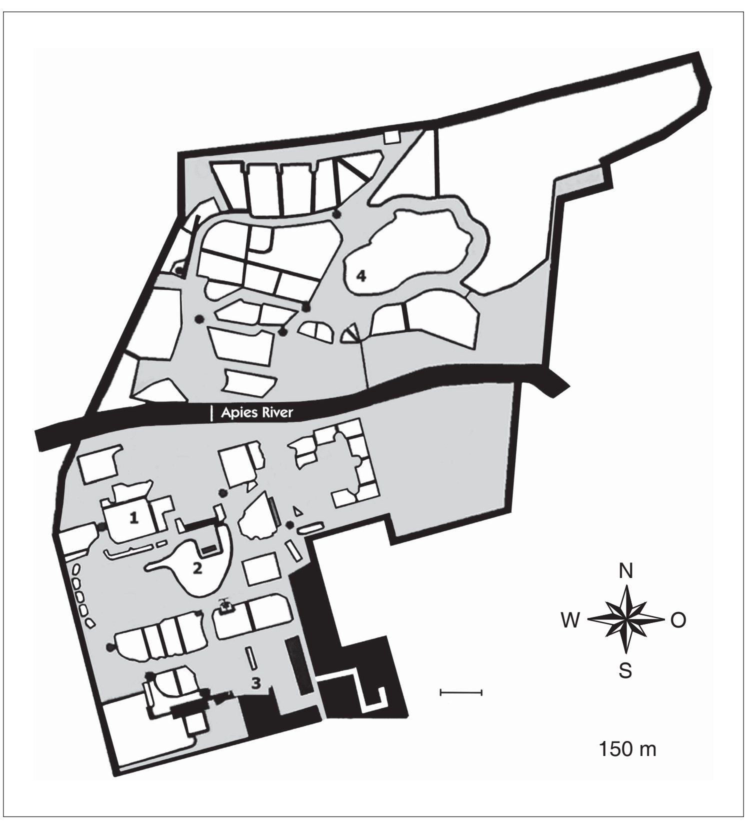

FIG. 1 Plan of the National Zoological Gardens of South Africa, Pretoria, South Africa showing placement of light traps. (1) Cape buffalo enclosure, (2) African elephant enclosure, (3) walk-through aviary and (4) white rhinoceros/black-faced impala enclosure

TABLE 1 *Culicoides* spp. recovered from 360 black light trap collections in the National Zoological Gardens, South Africa during August 2002 to August 2004

Species	Mean (maximum) no. *Culicoides* caught/trap night			
	Buffalo	Elephant	Aviary	Rhinoceros/impala
C. imicola	203.7 (8 027)	3 033.0 (65 520)	0.1 (10)	1 272.4 (18 288)
C. enderleini	9.1 (462)	47.4 (1 386)		91.8 (1 703)
C. bolitinos	3.9 (165)	9.5 (86)	< 0.1 (1)	5.1 (103)
C. cornutus	1.1 (29)	4.3 (54)	0.1 (2)	363.3 (4 699)
C. zuluensis	1.0 (35)	9.9 (75)		18.2 (184)
C. nigripennis s.l.	0.8 (24)	9.7 (109)	3.8 (268)	40.2 (650)
C. nivosus	0.5 (7)	11.3 (126)	0.2 (5)	21.9 (490)
C. leucostictus	0.3 (4)	33.3 (325)	4.3 (38)	58.7 (755)
C. pycnostictus	0.3 (8)	20.2 (254)	0.3 (6)	19.5 (209)
C. magnus	0.1 (3)	0.9 (15)		0.1 (4)
C. subschultzei	0.1 (3)	0.2 (10)		2.1 (54)
C. brucei	0.1 (3)	0.6 (10)		0.2 (22)
C. gulbenkiani	0.1 (5)	< 0.1 (2)		< 0.1 (1)
C. nevilli	0.1 (2)	0.6 (33)		0.5 (31)
C. engubandei	< 0.1 (1)	< 0.1 (1)		0.3 (8)
C. huambensis	< 0.1 (1)			
C. tropicalis	< 0.1 (1)	0.3 (10)		0.1 (5)
C. sp. # 54		0.3 (8)		0.2 (14)
C. sp. # 94		< 0.1 (3)		< 0.1 (3)
C. accraensis s.l.		0.9 (52)	0.1 (1)	< 0.1 (5)
C. albopunctatus			< 0.1 (2)	< 0.1 (5)
C. nr. angolensis				< 0.1 (3)
C. bedfordi		0.4 (13)	< 0.1 (1)	0.5 (10)
C. coarctatus		0.2 (14)		0.1 (8)
C. dekeyseri		< 0.1 (4)		
C. eriodendroni		< 0.1 (2)		
C. exspectator		0.4 (7)		0.4 (15)
C. sp. # 107				< 0.1
C. loxodontis		< 0.1 (1)		
C. neavei		0.5 (10)		0.4 (14)
C. ravus		0.1 (10)	< 0.1 (3)	0.3 (31)
C. schultzei				< 0.1 (1)
C. similis		0.5 (10)	< 0.1 (2)	0.6 (15)
Total	**221.0**	**3 184.5**	**8.9**	**1 897.1**

nutus de Meillon, 1937 was also comparatively abundant (19 %) (Table 1). The aviary site, however, was dominated by both *Culicoides leucostictus* Kieffer, 1911 (48 %) and *C. nigripennis sensu lata* (43 %) with *C. imicola* comprising only 1.5 % of the total catch. This highlighted the host preferences of *C. imicola* for larger mammals and increases the capacity of this species being a vector of AHS and BT viruses.

The abundance and diversity of the *Culicoides* fauna found at the National Zoological Gardens closely correlates with the results of a similar earlier study near livestock in the Onderstepoort area (25°39' S; 28°11' E) north of Pretoria (Venter, Meiswinkel, Nevill & Edwards 1996a). As in the case at Onderstepoort, the presence of substantial populations of *C. imicola* is likely to pose the most easily identifiable threat from a biosecurity point of view, especially as light-trap catches tend to represent only a small proportion of the populations sampled. As larvae, this species primarily utilizes a wide range of organically enriched habitats and population numbers could potentially be reduced by chemical treatment (Apperson 1975; Holbrook & Agun 1984), or rapid removal (Bishop, Mckenzie, Spohr & Barchia 2005) or dilution of animal waste. The efficiency of habitat removal techniques for African species of

Culicoides is unknown. In the case of this isolated habitat, modification to the habitat could be considered. This must, however, be an ongoing process given that re-colonization of areas from other parts of the zoo, or from beyond the zoo's boundaries (suburban areas), would appear to be likely given the similarities between the *Culicoides* fauna in the zoo and that of the local (Onderstepoort) area. Chemical treatment can be effective but unfortunately non-target organisms are often also killed (Holbrook & Agun 1984).

The biosecurity risk posed by species of *Culicoides* other than *C. imicola* present in the zoo remains unclear. While *C. bolitinos* is known to possess a substantially greater vector competence for both BTV and AHSV than *C. imicola* (Venter *et al.* 1998, 2000), it was found in relatively low abundance, and should be relatively easy to control by more rapid removal (Bishop *et al.* 2005) or treatment of dung (Standfast, Muller & Wilson 1984) from animal holding areas as it is known to breed directly in the dung of large herbivores (Meiswinkel 1989).

The vector competency for arboviruses of *Culicoides enderleini* Cornet & Brunhes, 1994, the second most common species at the buffalo and elephant sites and *Culicoides zuluensis* de Meillon, 1936, is currently unknown though it was shown that *C. zuluensis* is susceptible to oral infection with the live attenuated vaccine strains of AHSV (Paweska, Prinsloo & Venter 2003). These species are common and widespread across South Africa and although the former appears to utilize a wide range of hosts (Braverman & Phelps 1981; Meiswinkel *et al*. 2004), which may mitigate against it being an efficient vector, it was identified as a potential risk in a previous study (Venter, Nevill, Van der Linde 1996b). Also of interest are the large numbers of *C. cornutus* at the white rhinoceros/black-faced impala enclosure. Little is known of this species' vector competence for arboviruses or other pathogens, its host preference or its larval habitat requirements. However, *C. cornutus* is very closely related to *Culicoides sonorensis* Wirth and Jones, 1957, the vector of BTV in North America and *C. cornutus* is often the dominant species collected in the BT enzootic area of Kenya (Glick 1990). Therefore, this species remains an unknown risk in terms of disease transmission. Of the remaining species, *C. nigripennis sensu lata* dominated the aviary fauna, but was also found in smaller numbers around all the mammal-based traps. Again, little is known regarding this group's competency for transmitting arboviruses. However, their apparent host preference for birds (or at least a very wide host preference) would appear to preclude them from representing a major biosecurity risk for the transmission of mammalian arboviruses, although not of protozoa. At least ten species of protozoa are transmitted by *Culicoides* spp. to birds (Linley 1985).

Imports of sub-clinically infected zoo animals into areas with susceptible hosts for arboviruses have caused outbreaks of a wide range of diseases caused by arboviruses, e.g. the outbreak of AHS in Spain from 1987–1990, which was initiated by the importation of infected zebras (Lubroth 1988). The difficulty of detecting diseases, such as BT and AHS at ports of entry into countries has emphasized the need for routine screening and quarantine. However, these procedures are expensive but quarantine procedures could possibly be reduced, especially when captive bred stock is being relocated by reducing the chances of infection with certain arboviruses in the first place, through the methods suggested in this communication. The major difficulty in accomplishing this process is ensuring that the methods employed in vector control remain ongoing, which, barring the most isolated cases, will require considerable effort in endemic areas of arbovirus distribution.

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