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Movements of adult *Culicoides* midges around stables in KwaZulu-Natal

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Abstract

Preferences of adult *Culicoides* midges (Diptera: Ceratopogonidae) were examined to identify focal spots for vectors of African Horse Sickness (AHS). Five similar regions across five farms were sampled at regular periods over one year. The catches were identified to species level and regression analysis was performed on untransformed data which followed a negative binomial distribution with a log link function. Midges were found to frequent dung heaps and the interior of stable blocks significantly more than any other site. This occurs most markedly during July when temperatures are at their lowest and midges find shelter, warmth and food in these places. Recommendations for vector control with a suitable spray programme are provided.

Keywords: African horse sickness, *Culicoides*, midge, adult, stables
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Introduction

Culicoides midges are the primary vector of African horse sickness (AHS) (Braverman & Chizov-Ginzburg, 1995). Devastating losses occur during epizootics of AHS (Barnard, 1998; Mellor & Hamblin, 2004). In South Africa where the virus is enzootic, losses are experienced every year. In the 2005/2006 season, 844 horses were positively identified with AHS and 148 of them died (AHS website, 2010). Actual losses could be up to five times higher (Mullins, pers. comm.). Only two *Culicoides* midges have been proven as vectors of the AHS virus (Venter, G.J., pers. comm., Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110). They are *C. imicola* (Du Toit, 1944), and *C. bolitinos* (Meiswinkel & Paweska, 2003). These two species are crepuscular (Meiswinkel *et al*., 1994; Mellor *et al*., 2000). It is not known where the insects "roost" when they are not active nor is it known where they are generally most active while they are mobile. *C. bolitinos* has been shown to the endophylic (that is prefer to enter stable blocks to feed) while *C. imicola* is exophylic, preferring to stay outside and attack horses when they are not under cover (Meiswinkel *et al*., 2000). Barnard (1997), however, found that *C. imicola* will actively enter stables to feed. Breeding sites for *Culicoides* midges are small, widely spread and not uniformly chosen by any one species. Increases in moisture, permanence of wetness, and incident radiation all positively increase midge catches (Jenkins, 2008).

Midge numbers are seasonal; low during mid winter, increasing in December, and peaking in February and March (Coëtzer & Erasmus, 1994). A seven year study on adult midge ecology was conducted in Onderstepoort (Gauteng) (Coëtzer & Erasmus, 1994) demonstrated this seasonality and site preference in the northern province of SA. A survey of AHS in South Africa showed KZN to be the second hardest hit region of the country after Gauteng (AHS website, 2010). For this reason, behaviours and habitats of *Culicoides* in KwaZulu-Natal were studied to target zones of preference of the vector of the African horse sickness virus (AHSV) to inform effective prophylaxis.

Materials and Methods

Midges were collected using standard down-draught traps (Van Ark & Meiswinkel, 1992; Barnard, 1997; Rawlings *et al*., 1998; Musuka *et al*., 2001; Meiswinkel & Paweska, 2003; Rawlings *et al*., 2003) at five sites at each of five stable yards across KwaZulu-Natal over the period of a year. Traps were positioned (1) away from the horses in a paddock, (2) near the dung heaps, (3) inside or very close to the stable block, (4) near boggy mud and (5) near open water. Collections were taken over two consecutive nights at each farm, from two hours before sunset to two hours after sunrise. Sites were visited approximately every two months throughout the year in May, July, October, December and February.

Sites were sampled using downdraught light traps equipped with an 8W blacklight (Van Ark & Meiswinkel, 1992; Barnard, 1997; Rawlings *et al*., 1998; Musuka *et al*., 2001; Meiswinkel & Paweska, 2003; Rawlings *et al.*, 2003). The number of midges caught was analysed using generalized linear modelling procedure in Genstatv9© (2006). The data followed a negative binomial distribution, as the data was non-normal count data where the relationship between the variance and the mean was not equal. A log link function was used (McConway *et al.*, 2006) to analyse catch data responses to location, species, sex, and reproductive status of female midges.

Results and Discussion

A total of 27283 midges were caught, identified, sexed and aged at each of five locations across the year (Figure 1). A total of 37 *Culicoides* species were caught (Table 1). There are four predominant species in KwaZulu-Natal are: *C. imicola* (8903), *C. zuluensis* (6650), *C. bolitinos* (5660) and *C. gulbenkiani* (2773). Across all catches, the percentage ratio of male midges to females is 8% : 92%. This difference may be due to UV light preference by female midges but more likely indicates a far greater population of female midges in the environment around stables.

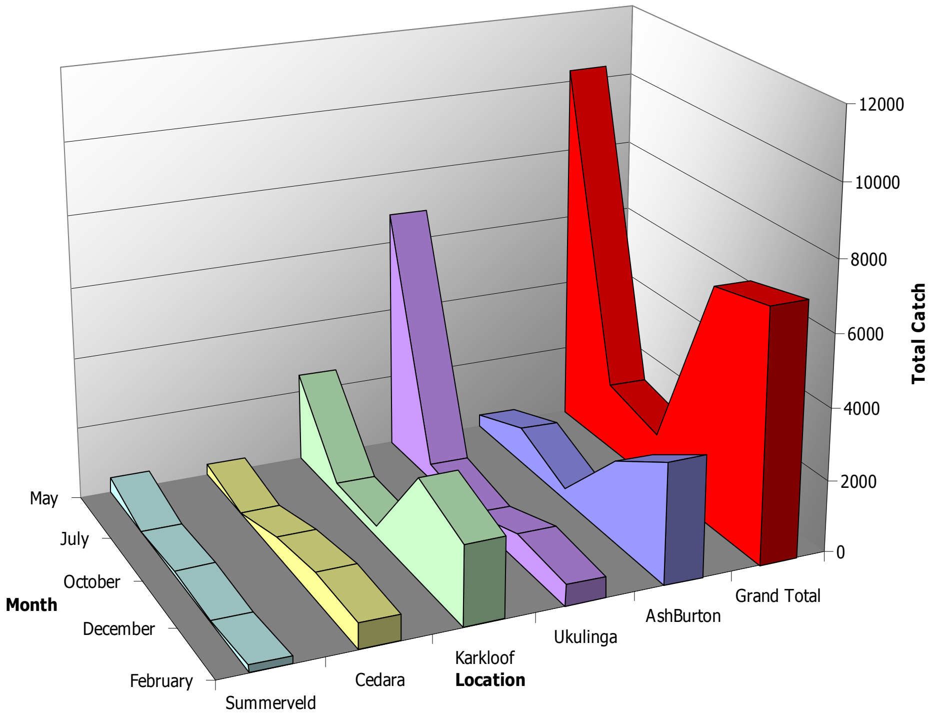

Figure 1 Total catches (grand total and by location) of *Culicoides* spp. across the trial period from May to February of the following year.

Male vectors can be targeted for pheromonal control (Mordue-Luntz, 2003) and so reduce the breeding efficiency of the vector species. The females can be directly targeted at both their larval and adult stages. Most important of the female stages are the nulliparous (virgin females looking for their first blood meal) and parous (second incubation females looking for another blood meal) stages as they both require an immediate blood meal and so are the mechanism by which the disease is vectored. As midges are small and not very robust creatures, the assumption is that not many will survive past their first egg batch to produce a second egg batch. Nulliparous females far outnumbered all other stages (69.3% of all females caught). 23.2% of the females caught were parous, 7.3% were gravid and only 0.2% were blood fed). Venter *et al*.

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Table 1 *Culicoides* species trapped in standard downdraught blacklight traps at five sites at five locations across KwaZulu-Natal over the period of one year

Culicoides spp.	Catch	*Culicoides* spp.	Catch
C. bedfordi	17	*C. neavei*	148
C. bolitinos	5660	*C. nevilli*	81
C. brucei	72	*C. nivosus*	260
C. coarctatus	4	*C. onderstepoortensis*	16
C. cornutus	2	*C. pycnostictus*	246
C. dutoiti	1	*C. ravus*	1
C. enderleini	133	*C. schultzei*	1
C. engubandei	675	*C. similis*	9
C. expectator	3	*C. Sp 107*	7
C. glabripennis	22	*C. sp 51*	2
C. gulbenkiani	2733	*C. sp 54*	56
C. huambensis	178	*C. sp near angolensis*	5
C. imicola	8903	*C. subschultzei*	7
C. kibatiensis	21	*C. trifasciellus*	103
C. krameri	66	*C. tropicalis*	3
C. leucostictus	459	*C. tuttifruiti*	6
C. magnus	702	*C. unknown*	25
C. michelli	3	*C. zuluensis*	6650
C. milnei/kram	3		

(1996) suggested that the attraction of some *Culicoides* midges may change as they age or experience different physiological conditions. This theory would account for the very low numbers of bloodfed and gravid females caught.

Figure 1 shows the seasonal variation in catches across the five locations. In season (summer) catches (December, February and May) are higher (P <0.001) than winter catches (July and October). Midge numbers were found to be significantly higher at Ashburton, Ukulinga and Karkloof than at Cedara and Summerveld (P <0.001). Cedara has sandy soils and no standing water, while the Summerveld stables are on a wind-exposed ridge. The other locations provided standing water and accumulated dung piles to attract midges. Dung heaps and sites under eves or in stables yielded better catches than any other sites (P <0.001). Open field sites yielded less midges (P = 0.05), and sites near stables and those near open water were no difference to the reference level (Table 2).

Table 2 Parameter estimates, standard errors and T- probabilities of adult *Culicoides* midge catches at six locations produced by generalized linear modelling using a log link function (Genstat, 2006) at six different trap sites at five different farms across KwaZulu-Natal

Site	Estimate	Std. error	T. Prob.
Dung Heaps [a]	0.695	0.133	<0.001**
Under In stables [a]	0.515	0.134	<0.001**
Near Stables [b]	0.022	0.248	0.928
Boggy Mud [b]	reference level		
Open Water [b]	-0.204	0.173	0.240
Open Field [c]	-0.412	0.147	0.005*

Means with a different superscript differ significantly.
* P <0.05; ** = P <0.01.

The proportion of males and females at each site is not the same (P <0.01) (Table 3). Proportions of midges frequenting the dung sites are similar, but far more females entering the stable structure than males for their blood meal. Male midges do not bite but suck nectar (Mullen, 2002) and so would not be attracted into a stable block. No species sex interaction exists, but highest catches were from traps set on the dung piles, and then from sites located either under the eaves or inside the stable.

Table 3 Percentages of total collections of males and female midges made at different selected sites in KwaZulu-Natal

Site	Female %	Male %
Boggy Mud	8.9	15.3
Dung	41.9	44.1
Near Stables	1.4	1.3
Open Field	5.3	10.2
Open Water	4.4	10.9
Under in Stables	38.1	18.1

The dung piles provide an ideal microclimate for midges, with moisture, humidity, heat and a possible source of nutrients. Female midges will drink honey when raised in the laboratory (Edwards, 1982) and may subsequently go on to produce up to nine batches of eggs, and Lindley (1966) suggested that females may take in some type of carbohydrate source to help them survive till they are mature enough to have their first blood meal. Such nutrients could be derived from the dung. Inside the stables, a favourable microclimate is created that is free of wind, rain and extreme cold, and large catches of midges were made in winter at all sites. The migration of the majority of the midge population to dung heaps and inside stable blocks seems to only really occur during mid-winter. While most midges tend to be exophylic, 50.3% of *C. bolitinos* was caught in stables. 39.5% and 38% of *C. imicola* and *C. zuluensis*, respectively, were caught indoors respectively. *C. gulbenkiani* was the most exophylic with only 7.2% of specimens caught indoors.

Conclusion

For effective vector control, stables need to cleaned, and dung skips emptied even throughout winter. A simple spray program can be used weekly in stable blocks to eradicate any midges that may have entered during the cold winter months. Where prophylaxis is usually concentrated in the months of peak seasonal incidence of the midge vector, this study serves to highlight that vector control in the winter months should not be neglected. Making conditions unfavourable to midges will reduce the bite load on the horses and may assist in reducing the incidence of African horse sickness.

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