

The effect of high frequency sound on *Culicoides* numbers collected with suction light traps

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Culicoides midges (Diptera: Ceratopogonidae), are involved in the transmission of various pathogens that cause important diseases of livestock worldwide. The use of insect repellents to reduce the attack rate of these insects on livestock could play an important role as part of an integrated control programme against diseases transmitted by these midges. The objective of this study was to determine whether high frequency sound has any repellent effect on *Culicoides* midges. The number of midges collected with 220 V Onderstepoort white light traps fitted with electronic mosquito repellents (EMRs), emitting 5-20 KHz multi-frequency sound waves, was compared with that of two untreated traps. Treatments were rotated in two replicates of a 4 x 4 randomised Latin square design. Although fewer midges were collected in the two traps fitted with EMRs, the average number collected over eight consecutive nights was not significantly different. The EMRs also had no influence on any of the physiological groups of *Culicoides imicola* Kieffer or the species composition of the *Culicoides* population as determined with light traps. The results indicate that high frequency sound has no repellent effect on *Culicoides* midges. There is therefore no evidence to support their promotion or use in the protection of animals against pathogens transmitted by *Culicoides* midges.

Introduction

Small blood-feeding flies, 1 mm – 3 mm in size, of the genus *Culicoides* (Diptera: Ceratopogonidae), are associated worldwide with the transmission of several viruses ($n = 66$), protozoa ($n = 15$) and filarial nematodes ($n = 26$) to a diversity of livestock hosts (Borkent 2005; Meiswinkel, Venter & Nevill 2004). At least three orbiviruses, namely, African horse sickness, bluetongue and epizootic haemorrhagic disease viruses, cause diseases of such international significance in livestock that they have been classified as notifiable to the World Organisation for Animal Health (OIE). *Culicoides* midges can also cause an acute allergic dermatitis (sweet-itch) in horses (Anderson, Belton & Kleider 1988).

Despite the availability and wide use of vaccines against the African horse sickness virus, more than 630 horses succumbed to African horse sickness during the 2010/2011 summer season in southern Africa (African Horse Sickness Trust 2012). Effective virus transmission and the rate thereof are dependent on, amongst other factors, the biting rate of a vector population. Under favourable climatic conditions *Culicoides* midges and especially *Culicoides imicola* Kieffer can become super-abundant in the close vicinity of livestock and more than a million females can be collected in one night in a single light trap (Meiswinkel *et al.* 2004). A light trap intercepts only a relatively small portion, < 0.0001%, of the active blood-seeking females (Meiswinkel *et al.* 2004). The implied high attack rate focused attention on the use of insect repellents and/or insecticides in addition to vaccines (if available), to form part of integrated control programmes against African horse sickness and other diseases, for example bluetongue, caused by pathogens transmitted by *Culicoides* midges.

The evaluation of insecticides and repellents against these insects is hampered by their relatively small size and predominantly nocturnal habits. Although it is known that certain species, for example *C. imicola*, are attracted by and prefer to feed on bigger mammals, and that other species prefer birds (Meiswinkel *et al.* 2004), there is almost no information on host detection mechanisms that regulate this.

The use of insecticides for the control and reduction of the biting-rate of *Culicoides* species in Europe has recently been reviewed (Carpenter, Mellor & Torr 2008). Laboratory studies showed a high variability in susceptibility to insecticides across trials (Carpenter *et al.* 2008). This variability was partly ascribed to variations in experimental protocol, although differences in susceptibility between species, laboratory colonies and field populations could also have played a major role (Carpenter *et al.* 2008). It was also shown that midges were able to feed and possibly transmit viruses before being incapacitated (Mullens *et al.* 2000). There are increasing concerns that the

impact of chemicals on the environment coupled with insecticide resistance in the insects will result in a decline in the number of agents available for livestock pest management (Carpenter *et al.* 2008). The use of repellents to decrease the *Culicoides* biting rate could therefore form an essential part of the integrated management of diseases transmitted by these insects.

Studies in Europe showed that compounds such as p-menthane-3,8-diol (PMD), N,N-diethyl-m-toluamide and KBR3023 (Picaradin) will reduce the *Culicoides* biting rate on humans (Carpenter *et al.* 2005; Trigg 1996). In South Africa 15% DEET (Page *et al.* 2009) and a 15% (w/w) mixture of octanoic, nonanoic and decanoic acids (C9810) in light mineral oil (Venter *et al.* 2011) was shown to have a significant repellent effect against *Culicoides* species when applied to polyester mesh. The efficacy and safety of these products for use on livestock still need to be determined.

Electronic mosquito repellents (EMRs) are designed to repel blood-feeding female mosquitoes and a variety of other insects by emitting high-pitched sounds, almost inaudible to the human ear. For more than 60 years, EMRs have been advertised and widely sold for their apparent ability to repel mosquitoes as well as a wide range of other insects. In most instances, though, the use of EMRs to repel insects is not supported by scientific research. All of the devices tested were found to be relatively ineffective in repelling mosquitoes under controlled conditions (Enayati, Hemingway & Garner 2007) and can even increase the biting rate in *Aedes aegypti* (Andrade & Cabrini 2009). Despite the lack of scientific evidence, a vast number of different EMR models are commercially available in South Africa for the control of mosquitoes and a variety of insect pests.

Electronic mosquito repellents, if effective against *Culicoides* midges, would provide a cheap, environmentally safe and practical means to protect animals against the bites of *Culicoides* midges. This would be especially useful in the case of valuable stud animals and race horses. The objective of this study was to determine whether high frequency sound has any repellent effect on *Culicoides* midges. These results will help to generate the data needed to provide scientific advice to stock owners and veterinarians about the usefulness of EMRs for the control of *Culicoides* midges and the viruses transmitted by them.

Materials and methods

To determine the potential repellent effect of high frequency sound on the numbers of *Culicoides* midges collected as well as species composition, the results obtained with two Onderstepoort light traps fitted with a commercially available EMR were compared with those of two traps without any repellent device. The EMRs used emitted 5-20 KHz multi-frequency sound waves and are claimed to give at least 3 m coverage. The 220 V down-draught Onderstepoort trap has been described previously (Venter *et al.* 2009). The EMRs were placed directly on top of the light traps. To reduce the

relatively strong attraction of the light trap, the black light tubes in the Onderstepoort trap were replaced by 8 W 23 cm white light tubes (Venter & Hermanides 2006).

Light traps were suspended 1.5 m above ground level next to open-sided barns, each housing between 20 and 40 cattle, at the ARC-Onderstepoort Veterinary Institute (25°39'S 28°11'E, 1219 m a.s.l.). Trapping was conducted from dusk to dawn on eight consecutive nights in autumn from 12 to 20 April 2011. Traps were hung as close to the cattle as practically possible. To ensure that treatment means were independent of any effects due to site or trapping occasion, treatments at the four sites were alternated in two replicates of a 4 x 4 randomised Latin square design (Perry, Wall & Greenway 1980). To minimise interference between traps, the sites were out of direct sight of each other and were located at least 15 m apart. Light trap operating procedure was conducted as described previously (Goffredo & Meiswinkel 2004; Venter *et al.* 2009).

After retrieval in the morning, the captured insects were transferred to 80% ethanol. Large collections were subsampled (Van Ark & Meiswinkel 1992) and *Culicoides* midges were identified to species level by examination of wing patterns. The females of all species were classified according to the abdominal pigmentation method (Dyce 1969) into unpigmented (nulliparous), pigmented (parous), gravid females (with eggs visible in the abdomen) and freshly blood-fed females. The numbers of males and all other insects collected were also recorded.

Data analyses

Analysis of variance (ANOVA) was used to compare the numbers of *Culicoides* midges collected in the traps fitted with EMRs and the control traps. Treatment means were separated using Fisher's protected *t*-test least significant difference (LSD). Comparisons were done at the 5% level of significance (Snedecor & Cochran 1980). Data were analysed using the statistical programme GenStat (Payne, Murray & Harding 2010). Proportions of insect counts between treated and untreated traps were compared using Chi-squared (χ^2) tests. Evenness in distribution of species abundance as determined at the different sites was compared using linear regression GraphPadInStat Version 3. Species diversity at each trapping site was calculated with the Shannon Wiener index, which describes the evenness in distribution of species abundances taking sample size into account (Al Young Studios 2012).

Ethical considerations

These experiments, focusing on insect collection, did not involve mammals or humans. The study was done as part of a project on National Assets at the ARC-OVI (project no. OV 7/03/P002 – Insect Collection).

Results

A total of 194 684 *Culicoides* midges were collected in the 32 light trap collections made from 12 to 20 April 2011. Of this

total 55.8% were collected in the control traps and 44.2% in the traps fitted with EMRs (Table 1). In the control traps, the number of midges collected per night ranged from 164 to 17 750 midges per trap (Table 1). In the traps fitted with EMRs, the numbers ranged from 443 to 15 300 (Table 1). Taking into account the considerable day to day variation in the numbers collected there was no significant difference ($p = 0.100$) in the average numbers of midges collected by the two trapping systems (Table 1).

The dominant species collected in both trapping regimens was *C. imicola* (Table 1). It was also the only species present in all 32 collections (Table 1). It constituted 98.6% of the midges collected in the control traps and 98.1% of the midges collected in the traps fitted with EMRs (Table 1). Like the total number of midges collected, the average numbers of *C. imicola* collected was not significantly different ($p = 0.092$) (Table 1).

The second most dominant species was *Culicoides enderleini* Cornet and Brunhes. This species represented 0.6% of the midges collected in the control traps and 1.0% of the midges collected in the traps fitted with EMRs (Table 1). As for *C. imicola* no significant difference was found in the average numbers collected ($p = 0.248$) (Table 1).

Overall, *Culicoides* midges belonging to 15 species were collected. Whilst *Culicoides leucostictus* Kieffer ($n = 3$) and *Culicoides similis* Carter, Ingram and Macfie ($n = 1$) were only collected in the control traps, *Culicoides nivosus* de Meillon ($n = 1$) and *Culicoides pycnostictus* Ingram and Macfie ($n = 28$) were only present in the traps fitted with EMRs. These species represented < 0.05% of the total number of midges collected. A good linear correlation ($R^2 = 100\%$) was found in the proportion of different species collected with the control traps and that of traps fitted with EMRs. Differences

in species diversity and evenness as reflected by Shannon-Weiner index between treatments were the result of single specimens of some species which were collected on only a few trapping occasions.

As is typical for light trap collection of *Culicoides* midges, females were the most abundant sex to be collected (Table 1). A comparison of the physiological status of *C. imicola* indicates that there were no significant differences in the proportion of nulliparous ($p = 0.186$), parous ($p = 0.290$), freshly blood engorged ($p = 0.184$) and gravid ($p = 0.599$) females collected in the control traps and traps fitted with EMRs (Table 1). Similarly, no significant difference ($p = 0.974$) was found in the proportion of males collected (Table 1). There was also no significant difference ($p = 0.896$) in the numbers of other insects collected (Table 1).

Discussion

The results obtained with light traps fitted with EMRs indicated that high frequency sound has no repellent effect on *Culicoides* midges. Although fewer midges were collected in the traps fitted with EMRs, the average number of midges collected over eight nights was not significantly different. Furthermore it was apparent that EMRs have no effect on any of the age groups of *C. imicola*. The use of EMRs also did not influence the species composition of the *Culicoides* population as determined by the light traps, and no influence was found in the average numbers of other night active insects collected. Owing to the design of the Onderstepoort trap (Venter *et al.* 2009) the effect of EMRs on the numbers of mosquitoes collected could not be evaluated.

The strong attraction of the light traps may decrease their effectiveness for the evaluation of repellents against *Culicoides* midges and these results are not directly applicable

TABLE 1: Summary of *Culicoides* midges collected with white light suction traps in a trial at the Agricultural Research Council - Onderstepoort Veterinary Institute from 12 to 20 April 2011 to determine the effect of high frequency sound on *Culicoides* numbers, species composition, age grading results and physiological status of the population.

<i>Culicoides</i> species	Light trap treatment	Untreated	Fitted with EMR	Statistical significance (p -value)
Total <i>Culicoides</i>	Number of collections made	16	16	-
	Number of species collected	13	13	-
	Total <i>Culicoides</i> collected	108 721 (55.8%)	85 963 (44.2%)	0.100
	Average collection size	6795.1	5372.7	-
	Range in collection size	164–17 750	443–15 300	-
	Shannon-Weiner index	0.12	0.09	-
	Species evenness	0.05	0.04	-
	Total other insects collected	3873	3721	-
<i>Culicoides imicola</i>	Total collected	107 251 (98.6%)	84 327 (98.1%)	0.092
	Average collection size	6703.2	5270.4	-
	Range in collection size	155–17 500	434–15 032	-
<i>Culicoides enderleini</i>	Total collected	602 (0.6%)	837 (1.0%)	0.248
	Average collected	37.6	52.3	-
	Range in collection size	0–163	0–193	-
<i>Culicoides imicola</i> physiological status	Nulliparous†	4406.3	3401.5	0.186
	Parous†	2160.5	1748.4	0.290
	Freshly blood fed†	27.7	31.8	0.184
	Gravid†	9.1	9.7	0.599
	Males†	99.6	79.1	0.974

EMR, electronic mosquito repellents.

†, Average collected.

p -values < 0.05 indicate a statistically significant difference.

to animals. Light traps are, however, much less efficient in attracting *Culicoides* midges than the odour plume and other stimuli generated by hosts in attracting *Culicoides* midges. Odour plumes can be distributed more widely by air currents and will potentially attract midges over a much larger distance than a fixed light source.

Outbreaks of bluetongue in northern Europe have indicated that bluetongue virus can be transmitted effectively by several species in the genus *Culicoides* (Carpenter, Wilson & Mellor 2009; Mellor, Carpenter & White 2009). A similar multi-vector potential has been demonstrated for bluetongue virus (Venter *et al.* 2011) and African horse sickness virus (Paweska, Prinsloo & Venter 2003) in South Africa. To be effective, measures to reduce the biting rate need to be based on knowledge of the basic biology of the particular species being investigated, for example breeding site, host preference and vector competence. The involvement of a variety of *Culicoides* species, each with a unique biology, will increase the complexities in the implementation of effective integrated control measures against these vectors.

In an endemic situation, such as occurs in South Africa, the use of vaccines will be the primary measure to control African horse sickness and bluetongue. However, measures that will reduce the *Culicoides* midge attack rate and limit the dissemination of viruses amongst animals during high risk periods are essential. They would be helpful to reduce biting rates during outbreak situations of the viral diseases when the use of live attenuated vaccines may be inappropriate (Mellor & Hamblin 2004). Under these conditions, the use of effective *Culicoides* repellents for animals and their stable environment would be of primary importance. Attractants and/or repellents for different *Culicoides* species need to be determined on a scientific basis for their inclusion in vector monitoring and control programmes.

Conclusion

In the present study, no evidence could be found to support any repellent effect of high frequency sound for *Culicoides* midges. There is therefore no evidence to support their promotion or use in the protection of animals against pathogens transmitted by *Culicoides* midges.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors' contributions

G.J.V. (Agricultural Research Council – Onderstepoort Veterinary Institute) was the project leader and was

responsible for the project design. S.N.B.B. (Agricultural Research Council – Onderstepoort Veterinary Institute) was responsible for the collection of the *Culicoides* midges and the rotation of the light traps. K.L. (Agricultural Research Council – Onderstepoort Veterinary Institute) did all the *Culicoides* species analyses and age grading of the collections. L.M. (ARC-Biometry Unit) was responsible for most of the statistical analyses. G.J.V. (Agricultural Research Council – Onderstepoort Veterinary Institute) wrote the manuscript.

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