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

**Authors:**
Gert J. Venter1,2, Karien Labuschagne1,3, Solomon N.B. Boikanyo1, Liesl Morey4

**Affiliations:**
1Agricultural Research Council – Onderstepoort Veterinary Institute, Parasites, Vectors & Vector-borne Diseases, South Africa
2Department of Veterinary & Tropical Diseases, University of Pretoria, South Africa
3Department of Zoology & Entomology, University of Pretoria, South Africa
4ARC-Biometry Unit, Hatfield, South Africa

**Correspondence to:**
Gert Venter
Email: venterg@arc.agric.za

**Postal address:**
Private Bag X05, Onderstepoort 0110, South Africa

**Dates:**
Received: 29 Feb. 2012
Accepted: 18 July 2012
Published: 07 Nov. 2012

**How to cite this article:**
Venter, G.J., Labuschagne, K., Boikanyo, S.N.B. & Morey, L., 2012. ‘The effect of high frequency sound on Culicoides numbers collected with suction light traps’, Journal of the South African Veterinary Association 83(1), Art. #10, 5 pages. http://dx.doi.org/10.4102/jsava.v83i1.10

© 2012. The Authors.
Licensee: AOSIS
OpenJournals. This work is licensed under the Creative Commons Attribution License.

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 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
impacts 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 constitute 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 waves could have a 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 (χ²) 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 pyenostictus* 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.

| Culicoides species | Light trap treatment | Untreated | Fitted with EMR | Statistical significance (p-value) |
|--------------------|----------------------|-----------|----------------|-----------------------------------|
| **Total Culicoides** | Number of collections made | 16 | 16 | - |
| | Number of species collected | 13 | 13 | - |
| | Total Culicoides 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 | - |
| **Culicoides imicola** | 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 | - |
| **Culicoides enderleini** | Total collected | 602 (0.6%) | 837 (1.0%) | 0.248 |
| | Average collected | 37.6 | 52.3 | - |
| | Range in collection size | 0–163 | 0–193 | - |
| **Culicoides imicola 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.

Acknowledgements

The ARC-OVI is thanked for supporting this work, and Truuske Gerdes, Errol Nevill and Arthur Spickett for constructive comments on earlier drafts of this manuscript.

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 – Ondersteypoort Veterinary Institute) was the project leader and was responsible for the project design. S.N.B.B. (Agricultural Research Council – Ondersteypoort Veterinary Institute) was responsible for the collection of the Culicoides midges and the rotation of the light traps. K.L. (Agricultural Research Council – Ondersteypoort 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 – Ondersteypoort Veterinary Institute) wrote the manuscript.

References

Andersen, G.S., Belton, P. & Kleider, N., 1988, ‘The hypersensitivity of horses to Culicoides bites’, Canadian Veterinary Journal 29, 718–723. PMid:17423117

Borkent, A., 2005, ‘The biting midges, the Ceratopogonidae (Diptera)’, in W.C. Marquardt (ed.), Biology of disease vectors, 2nd edn., Elsevier Academic Press, San Diego.

Carpenter, S., Eyres, K., McEndrick, I., Smith, L., Turner, J., Mordue, W. et al., 2005, ‘Repellent efficiency of BayRepel against Culicoides impunctatus (Diptera: Ceratopogonidae)’, Parasitology Research 95, 427–429. http://dx.doi.org/10.1007/s00436-005-1298-6

Carpenter, S., Mellor, P. & Torr, S.J., 2008, ‘Control techniques for Culicoides biting midges and their application in the UK and northwestern Palaearctic’, Medical and Veterinary Entomology 22, 175–187. http://dx.doi.org/10.1111/j.1365-2915.2008.00743.x PMid:18816267

Carpenter, S., Wilson, A. & Mellor, P.S., 2009, ‘Culicoides and the emergence of bluetongue in northern Europe’, Trends in Microbiology 17, 172–178. http://dx.doi.org/10.1016/j.tim.2009.01.001 PMid:19399131

Dyce, A.L., 1969, ‘The recognition of nulliparous and parous Culicoides (Diptera: Ceratopogonidae) without dissection’, Journal of the Australian Entomological Society 8, 11–15. http://dx.doi.org/10.1111/j.1440-6055.1969.tb00727.x

Enayati, A., Hemingway, J. & Garner, P., 2007, ‘Electronic mosquito repellents for preventing mosquito bites and malaria infection (review)’, Cochrane Database of Systematic Reviews 2, Art No. CD005434. http://dx.doi.org/10.1002/14651858.CD005434.pub2

Goffredo, M. & Meiswinkel, R., 2004, ‘Entomological surveillance of bluetongue in Italy: Methods of capture, catch analysis and identification of Culicoides biting midges’, Veterinaria Italiana 40, 260–265.

Meiswinkel, R., Venter, G.J. & Nevill, E.M., 2004, ‘Vectors: Culicoides spp.’, in J.A.W. Coetzer & R.C. Tustin (eds.), Infectious diseases of livestock, 2nd edn., pp. 93–136, Oxford University Press, Cape Town.

Mellor, P.S., Carpenter, S. & White, D.M., 2009, ‘Bluetongue virus in the insect vector’, Veterinary Record 165, 105–109. http://dx.doi.org/10.1136/vr.165.5.105 PMid:19401372

Paweska, J.T., Prinsloo, S. & Venter, G.J., 2003, ‘Oral susceptibility of South African Culicoides species to live-attenuated serotype-specific vaccine strains of African horse sickness virus (AHSV), Medical and Veterinary Entomology 15, 436–447. http://dx.doi.org/10.1111/j.1365-2915.2003.00467.x PMid:14651659

Payne, R.W., Murray, D.A. & Harding, S.A., 2010, An introduction to the GenStat Command Language, 13th edn., VSN International, Hemel Hempstead.

Perry, J.N., Wall, C. & Greenaway, A.R., 1980, ‘Latin square designs in field experiments involving sex attractants’, Ecological Entomology 5, 385–396. http://dx.doi.org/10.1111/j.1365-2311.1980.tb01162.x
Snedecor, G.W. & Cochran, W.G., 1980, *Statistical methods*, 7th edn., Iowa State University Press, Ames.

Trigg, J.K., 1996, 'Evaluation of a eucalyptus-based repellent against Culicoides impunctatus (Diptera: Ceratopogonidae) in Scotland', *Journal of the American Mosquito Control Association* 12, 329–330. PMID:8827615

Van Ark, H. & Meiswinkel, R., 1992, 'Subsampling of large light trap catches of Culicoides (Diptera: Ceratopogonidae)', *Onderstepoort Journal of Veterinary Research* 59, 183–189. PMID:1437020

Venter, G.J. & Hermanides, K.G., 2006, 'Comparison of black and white light for collecting Culicoides imicola and other livestock-associated Culicoides species in South Africa', *Veterinary Parasitology* 142, 383–385. http://dx.doi.org/10.1016/j.vetpar.2006.07.015, PMID:16934933

Venter, G.J., Labuschagne, K., Boikanyo, S.N.B., Morey, L. & Snyman, M.G., 2011, 'The repellent effect of organic fatty acids on Culicoides midges as determined with suction light traps in South Africa', *Veterinary Parasitology* 181, 365–369. http://dx.doi.org/10.1016/j.vetpar.2011.04.034, PMID:21592665

Venter, G.J., Labuschagne, K., Hermanides, K.G., Boikanyo, S.N.B., Majatladi, D.M. & Morey, L., 2009, 'Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of Culicoides species', *Veterinary Parasitology* 166, 299–307. http://dx.doi.org/10.1016/j.vetpar.2009.08.020, PMID:19758757

Venter, G.J., Wright, I.M., Del Rio, R., Lucientes, J. & Miranda, M.A., 2011, 'The susceptibility of Culicoides imicola and other South African livestock-associated Culicoides species to infection with bluetongue virus serotype 8', *Medical and Veterinary Entomology* 25, 320–326. http://dx.doi.org/10.1111/j.1365-2915.2010.00931.x, PMID:21113962