The suitability of the Triple trap for the collection of South African livestock-associated *Culicoides* species

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The relatively large number of *Culicoides* midges (Diptera: Ceratopogonidae) that can be collected with a light trap makes it the most widely used tool for this purpose. However, the majority of these traps were originally designed for collecting mosquitoes. The evaluation and improvement of traps to increase their effectiveness in collecting *Culicoides* midges will unavoidably form part of research on these insects. In the present study the efficiency of the Triple trap for collecting livestock-associated *Culicoides* midges was compared with that of the Onderstepoort 220 V, the BG-sentinel and the mini-CDC traps. A unique feature of the Triple trap is that selected surfaces are coated with TiO_2 (titanium dioxide) which, in the presence of ultra violet light, acts as a photo-catalyser to produce CO_2, which in turn may attract blood-feeding insects. Overall, the Onderstepoort trap collected significantly higher numbers of midges than the others. Relative efficiency varied between different occasions and under some conditions, for example periods with low midge abundance during the winter, the mean numbers collected with the Triple trap did not differ significantly from those of the Onderstepoort or BG-sentinel traps. By replacing the collection chamber of the Triple trap with a sock and beaker, similar to that of the Onderstepoort trap, it can effectively be used for the collection of *Culicoides* midges.

Introduction

The effective qualitative and quantitative detection of all potential vectors of African horse sickness, bluetongue and other viruses transmitted by *Culicoides* midges is essential for risk analysis and implementation of integrated control measures. The finding that these viruses can be transmitted by several species in the genus *Culicoides* (Diptera: Ceratopogonidae) (Carpenter, Wilson & Mellor 2009; Mellor, Boorman & Baylis 2000; Mellor, Carpenter & White 2009) and that they are linked to the near-cosmopolitan distribution of bluetongue virus emphasises the need for comprehensive and comparable data on the presence of these vectors. Due to the relatively large species diversity and numbers of *Culicoides* species that can be collected in the vicinity of livestock, light traps have become the most widely used tool for this purpose (Mellor *et al.* 2004). At present, only a limited number of suction light traps are commercially available and most were originally designed for the collection of mosquitoes.

The numbers of *Culicoides* midges collected, and the species diversity that is determined, with light traps are seldom comparable to the *Culicoides* biting rate on the livestock host (Carpenter *et al.* 2008; Gerry *et al.* 2009; Scheffer *et al.* 2012; Viennet *et al.* 2011). The evaluation and improvement of the efficacy of light traps to collect potential vectors of livestock pathogens will therefore be important in *Culicoides* research.

The Triple trap (The Kendal Group, Hubers cc, South Africa) was developed to reduce mosquito numbers in a particular area and is marketed internationally or this purpose. Essentially, it is a 220 V down-draught light trap utilising two 15 cm 4 W parallel ultra violet (UV) light tubes to attract insects, which are then drawn into a container underneath the fan. Unique to this trap are the selected surfaces coated with TiO_2 (titanium dioxide), which with UV light acts as a photo-catalyser and produces heat, CO_2 and H_2O (Fujishima, Rao & Tryk 2000). On its own, or as an additional attractant to a light, CO_2 may increase species diversity and numbers of *Culicoides* midges collected (Harrup *et al.* 2012; Kline, Hagan & Wood 1994; Ritchie *et al.* 1994). Olfactory cues can differ between species (Cilek & Kline 2002), but the role they play in the attraction of Afrotropical *Culicoides* is unknown.

In the present study, the efficiency of the Triple trap for the collection of South African livestock-associated *Culicoides* midges was compared with three other models: the 220 V Onderstepoort down-draught (Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI), Onderstepoort, South Africa); the BG-sentinel (Biogents AG, Regensburg, Germany); and the mini-CDCModel 1212 (J.W. Hock, Gainesville, USA). Whilst all of these traps are routinely used for the collection of *Culicoides* midges (Venter *et al.* 2009), this will be the first time that the Triple trap is evaluated for this purpose. Except for the Onderstepoort trap, all the others were originally designed for the collection of various species of mosquitoes.

Material and methods

Two trials were conducted. In the first trial, the efficiency of the Triple, Onderstepoort, BG-sentinel and mini-CDC traps for the collection of livestock *Culicoides* species was compared. To get an insight into their relative sensitivity, trials were performed for 12 nights in winter (a period with low-midge abundance) between 22 June 2010 and 18 July 2010 in South Africa. The four traps were deployed in three replicates of a 4x4 randomised Latin square design (Perry, Wall & Greenway 1980).

Based on the results of the first trial, the efficiency of the Triple was then compared with that of the Onderstepoort trap for eight nights in summer between 13 January 2012 and 30 January 2012 (when midge populations were known to be high). Two traps of each design were used and the four were deployed in two replicates of a 4x4 randomised Latin square design.

The Onderstepoort, BG-sentinel and mini-CDC traps are described in more detail elsewhere (Venter *et al.* 2009). The Triple trap was modified for the collection of *Culicoides* midges by replacing the collection chamber, which is made of plastic, with a metallic grid (mesh size 3 mm x 2 mm), with a sock and beaker similar to that of the Onderstepoort trap (Venter *et al*. 2009). Bigger insects were excluded by polyester netting (mesh size 2 mm) placed around the entrance portals of the trap, as with the Onderstepoort trap.

Trials were conducted at the ARC-OVI (25°39'S, 28°11'E 1219 m above sea level). Traps were operated from approximately 2 h before sunset to about 2 h after sunrise at open-sided barns that house from 20 to 40 cattle each. Traps were hung 1.4 m above ground level as close to the cattle as possible. To minimise interference between traps, sites were located at least 15 m apart (Venter *et al.* 2012).

Insects were collected into water to which 0.5% 'Savlon' antiseptic (Johnson & Johnson, South Africa) (contains Clorhexidinegluconate 0.3 g/100 mL and Cetrimide 3.0 g/100 mL) was added. In the mornings they were transferred into 80% ethanol and stored at 4 °C in the dark until analysed. Large collections were sub-sampled (Van Ark & Meiswinkel 1992). Based on abdominal pigmentation (Dyce 1969), females of all species were categorised into nulliparous (unpigmented), parous (pigmented), gravid or freshly blood-fed. Captured males were also counted.

Data analyses

Analysis of variance (ANOVA) was used to compare trap efficiency at the 5% level. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% significance (VSN International 2012). Proportions of insect counts between traps were compared using Chi-squared (χ^2) tests and species abundance in the different treatments was compared using linear regression GraphPadInStat Version 3.

Ethical considerations

This experiment posed no health risk to researchers and no vertebrate animals were harmed. The study was preformed as part of a project on National Assets at the ARC-OVI (project no. OV 7/03/P002 – Insect Collection).

Results

Comparison of the four traps in winter

Of the 13 469 *Culicoides* midges in the 48 collections between 22 June 2010 and 18 July 2010, 5806 (43.1%) were collected with the Onderstepoort trap and 4640 (34.4%) with the Triple trap (Table 1). Taking into account the substantial day to day variation, the mean number collected with the Triple trap (386.7) did not differ significantly from that of the Onderstepoort (483.8) or BG-sentinel traps (186.9) (Table 1). The mean number of the mini-CDC trap (65.0) was significantly less than that of the others (Table 1).

Culicoides imicola Kieffer was the dominant species in all four traps. Its representation ranged from 97.2% in the mini-

TABLE 1: Culicoides midges collected with four different down-draught light traps during winter (22 June 2010 – 18 July 2010) at the ARC-OVI, South Africa.

Species	Collected	Onderstepoort		Triple		BG sentinel		Mini-CDC		Statistical significance (p-value)
		n	%	n	%	n	%	n	%	
Number of species collected	-	12	-	11	-	10	-	7	-	-
Total *Culicoides* collected	-	5806[c]	43.1	4640[b,c]	34.4	2243[b]	16.7	780[a]	5.8	0.001
Mean number collected	-	483.8	-	386.7	-	186.9	-	65	-	-
Range in number collected	-	22–1230	-	0–1128	-	10–596	-	2–162	-	-
Culicoides imicola	Total collected	5691[c]	98.0	4552[b,c]	98.1	2205[b]	98.3	758[a]	97.2	0.001
	Mean number collected	474.3	-	379.3	-	183.8	-	63.2	-	-
	Range in number collected	20–1217	-	0–1104	-	9–563	-	2–162	-	-
	Physiological status:	-	-	-	-	-	-	-	-	-
	• Nulliparous	3279	57.6	2588	56.9	1275	57.8	416	54.9	0.451
	• Parous	2333	41.0	1877	41.2	894	40.5	332	43.8	0.457
	• Freshly blood-fed	16	0.3	25	0.6	6	0.3	3	0.4	0.132
	• Gravid	15	0.3	29	0.6	15	0.7	1	0.1	0.007
	• Males	48	0.8	33	0.7	15	0.7	6	0.8	0.860

Note: A total of 12 collections were made with each of the traps. Numbers per row followed by a different alphabetical letter (a, b or c) were significantly different at the 5% level.

CDC trap to 98.3% in the BG-sentinel trap (Table 1). The mean numbers in the Triple did not differ significantly from those of the Onderstepoort or BG-sentinel traps (Table 1). All four traps found nulliparous *C. imicola* females to be the most abundant (Table 1). Except for the proportion of gravid *C. imicola* females, no significant differences were found in the sex ratios or other female groupings (Table 1).

Culicoides midges belonging to at least 13 species were collected. Whilst the Onderstepoort trap had 12 species, only 7 were collected with the mini-CDC trap (Table 1). The Triple trap collected 11 species and linear regression showed that the proportion of different species of the Triple trap to be nearly identical (R^2 = 99.99%) to that in the others in all cases. Differences in species diversity between traps were the result of single specimens collected only once or twice. These species represented < 0.1% of the total number collected.

Comparison of the Triple trap with the Onderstepoort trap in summer

In the second trial, 64 262 *Culicoides* midges were present in the 32 collections made between 13 January 2012 and 31 January 2012. Notwithstanding substantial day to day variation in numbers, the larger mean collected with the Onderstepoort trap (2693.9) was significant (p = 0.045), being nearly double that of the Triple trap (1322.4) (Table 2).

Similar to winter collections, *C. imicola* was again dominant (Table 2). Although the mean number of *C. imicola* collected with the Triple trap (1289.3) was significantly (p = 0.048) lower than that collected with the Onderstepoort trap (2623.4), its proportional representation as determined with the Triple trap (97.5%) and Onderstepoort trap (97.4%) did not differ significantly (Table 2).

Culicoides enderleini Cornet and Brunhes was the second most abundant species in the Triple trap, whilst in the Onderstepoort trap it was *Culicoides nevilli* Cornet and Brunhes (Table 2). Relatively small numbers of both species were collected. The mean numbers collected were significantly (p < 0.001) lower for both in the Triple trap (Table 2).

Contrary to the collections made in winter (Table 1), parous *C. imicola* females were dominant in both traps (Table 2). They did not differ significantly (p = 0.550), with 40.1% in the Triple trap and 41.1% in the Onderstepoort trap (Table 2). Relatively small, but statistically significant, differences were found in the proportions of freshly blood-fed (p = 0.009) and gravid females (p < 0.001) as well as of males (p < 0.001) (Table 2). These differences were due to the large sample size and the sensitivity of the statistical test used.

Culicoides midges belonging to 21 species in total were collected. Linear regression showed the proportion of the different species to be nearly identical (R^2 = 99.99%) between the two traps. Differences in species diversity was the result of single specimens collected only once or twice. These species represented < 0.05% of the total number collected.

In summer, the Onderstepoort trap collected 7.4 times more midges than in winter, whilst the Triple trap only collected 4.6 times more (Table 1 and Table 2). This difference in trap efficiency was statistically significant (p < 0.001).

Due to the netting (mesh size 2 mm) around the entrance portals of the Triple and Onderstepoort traps, mosquitoes were not collected. For accurate identification to species level, mosquitoes should preferably not be collected into a liquid medium.

Discussion

Whilst the Onderstepoort (Venter *et al*. 2009), mini-CDC (De Deken *et al.* 2008; Miranda, Ricón & Borràs 2004; Mullens & Schmidtmann 1982) and BG-sentinel (Kiel *et al.* 2009) traps are routinely used for the collection of *Culicoides* midges, this is the first time that the Triple trap has been evaluated for this purpose. Under the test conditions, the mean numbers

TABLE 2: Summary of Culicoides midges collected with the Onderstepoort trap and Triple trap during summer (13 January 2012 – 31 January 2012) at the ARC-OVI, South Africa.

Species	Collected	Onderstepoort		Triple		Statistical significance (p-value)
		n	%	n	%	
Number of species collected	-	20	-	17	-	-
Total *Culicoides* collected	-	43 103	67.1	21 159	32.9	-
Mean number collected	-	2693.9	-	1322.4	-	0.045
Range in number collected	-	260–7140	-	111–4014	-	
Culicoides imicola	Total collected	41 975	97.4	20 628	97.5	0.386
	Mean number collected	2623.4	-	1289.3	-	0.048
	Range in number collected	234–6859	-	102–3889	-	-
	Physiological status:	-	-	-	-	-
	• Nulliparous	17 236	41.1	8418	40.1	0.550
	• Parous	18 756	44.7	9160	44.4	0.516
	• Freshly blood fed	105	0.3	77	0.4	0.009
	• Gravid	488	1.2	110	0.5	<0.001
	• Males	5390	12.8	2863	13.9	<0.001
Culicoides enderleini	Total collected	200	0.5	162	0.8	<0.001
	Mean number collected	12.5	-	10.1	-	-
	Range in number collected	0–68	-	0–48	-	-
Culicoides nevilli	Total collected	263	0.6	47	0.8	<0.001
	Mean number collected	16.4	-	2.9	-	-
	Range in number collected	0–100	-	0–12	-	-

Note: A total of 16 collections were made with each trap.
p-values < 0.05 indicates statistical significance.

collected are not significantly different from the BG-sentinel trap, but the Triple is more effective than the mini-CDC trap. Species diversity in the vicinity of cattle for the Triple do not differ from that of the other three. The proportional representation of *C. imicola* as determined for the Triple trap do not vary significantly from that for the others and it is shown to be the dominant species in all four traps. Differences found in the physiological status of the *C. imicola* females collected and sex ratios are mostly not of statistical significance.

All the traps have UV light sources. Whilst the mini-CDC has a single 15 cm 4 W light tube, the Triple is equipped with two 15 cm 4 W light tubes. The light source in the BG-sentinel is a 30 cm 4.88 W UV light tube whilst in the Onderstepoort trap it is a 30 cm 8 W light tube. Although wave length and light intensity will play some role (Bishop *et al.* 2004), the placement of the light source in relation to its visibility and to the suction provided by the fan will influence the efficacy of the trap.

In the present study, it is impossible to determine how much the apparent heat and CO_2 generated by the Triple trap contribute to its efficiency. Since the proportional representation of species collected does not vary significantly between this trap and the others, it does indicate that these physical factors do not contribute to species diversity. Similarly, these factors do not influence the collection of the different female categories or sex groups. In the present comparison any beneficial effect of the CO_2 generated by the Triple trap could have been masked by the presence of cattle close to the trap and the general dominance of *C. imicola*. It has been shown that the presence of livestock can influence the numbers of midges and especially that of *C. imicola* (Venter *et al.* 2010). In most vector surveillance systems the main aim will be to identify livestock *Culicoides* species and trapping is cerried out close to animals, therefore the CO_2 generated by the Triple trap should not influence numbers.

To accurately determine to what extent the CO_2 and heat of the Triple trap influence composition and numbers, comparisons will have to be carried out in the absence of bigger mammals, and the amounts of CO_2 and heat generated by the Triple trap will have to be accurately measured. It can be mentioned that despite the known host preference of *C. imicola* for bigger mammals (Venter *et al*. 2010) relatively little is known about the chemical factors that will attract this and other Afrotropical *Culicoides* species. Information about this will be imperative to effectively safeguard host animals against *Culicoides* midges.

The Onderstepoort trap indicates that 7.4 times more midges can be collected in summer than in winter; the Triple trap shows this increase to be 4.6. During winter the Onderstepoort trap collected on average 1.2 times more midges than the Triple, but the efficiency does not differ significantly. However, in summer the mean number collected with the Onderstepoort trap was double that of the Triple trap and this is statistically significant.

Discrepancies in relative trap efficiency have also been found in previous studies. In a comparison performed in South Africa, 2.0 to 3.5 times more midges were collected with the Onderstepoort than with the mini-CDC trap (Venter *et al*. 2009), whilst in the present study there are 7.1 times more. In Germany, the Onderstepoort trap collected 41.5 times more midges belonging to the Obsoletus complex than the mini-CDC (Probst *et al.* 2009). Also in Majorca, Balearic Islands, the Onderstepoort collected 3.2 to 3.9 times more midges than the mini-CDC, but the mean numbers were not significantly different (Del Río López 2012). Although not statistically significant, it was shown that the mini-CDC can, under certain conditions, collect 1.6 times more midges than the Onderstepoort trap (Del Río López 2012). Although *Culicoides* species composition and density can have an influence, environmental factors may also play a role. Intrinsic differences between the same model, for example the age of the trap and/or light source and power supply will add to this discrepancy.

Conclusion

Despite being an artificial collection method and having intrinsic limitations, for example diurnal species will not be collected, light traps will remain a rapid and reliable way to determine *Culicoides* midge presence and abundance. The numerous factors that may influence the efficiency of light traps render the interpretation and comparison of exact data sets challenging. This is illustrated by the discrepancy in relative efficiency between trapping occasions. Light trap results need to be used in combination with other collecting methods, such as direct collection on host animals, in order to determine the risk of virus transmission in the area. The results also need to be integrated with what is known about host preference, biting rate, biology, capacity and competence of the *Culicoides* species involved.

The present comparison shows that the Triple trap can, by replacing the collection chamber with a sock and beaker similar to that of the Onderstepoort trap, effectively be used for collections to determine species presence and abundance. Based on larger mean numbers collected, the Onderstepoort trap still remains the trap of choice if large numbers of *Culicoides* midges are needed for virus isolation or studies involving live midges.

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Competing Interests

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

Authors' contributions

G.J.V. (Agricultural Research Council-Onderstepoort Veterinary Institute) was responsible for the project design. K.L. (Agricultural Research Council-Onderstepoort Veterinary Institute) did the species analyses and age grading. 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. L.M. (Agricultural Research Council-Biometry Unit) was responsible for most of the statistical analyses. G.J.V. compiled the data and the draft of the manuscript.

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