Distance from the stable affects trapping of biting midges (Diptera, Ceratopogonidae)

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Several species of Culicoides biting midges (Diptera, Ceratopogonidae) are competent vectors of important veterinary pathogens in Europe. Bluetongue virus (BTV) has emerged in Europe since the late 1990s and is transmitted by several species of the genus Culicoides (Caracappa et al. 2003, De Liberato et al. 2005). African horse sickness (AHS) was observed at the end of the last century on the Iberian Peninsula (Mellor and Hamblin 2004) and since the end of 2011 there has been evidence that biting midges are important vectors of the Schmallenberg Virus (SBV) emerging in Central Europe (Hoffmann et al. 2012). SBV causes considerable congenital damage, premature births, and reproductive disorders in ruminants.

Monitoring programs for biting midges are generally based on UV-light/suction trap sampling (Mehlihorn et al. 2009, Nielsen et al. 2010). Results of the sampling and trapping protocols could be significantly affected by uncontrollable environmental factors like wind and temperature (Bishop et al. 2000, Carpenter et al. 2008) and/or even the phase of the moon (Bishop et al. 2000). In contrast, there are controllable factors that could be standardized and would result in more comparable results from entomological surveillance programs. Firstly, there is the standardization of the trapping method, which could have different specificity for abundance, taxa, sex, or feeding stadium (Holbrook 1985, Anderson and Linhares 1989, Venter et al. 2009a, Viennet et al. 2011). Secondly, some authors mentioned that the positions of the traps have a significant impact on the sampling results, such as height (Venter et al. 2009b) or habitat (Bishop et al. 1994, Bishop et al. 1995). In this study, we were interested in the impact of the distance to the stable on the trapping result. Due to a positive correlation between biting midge abundance and the presence and abundance of hosts (Bellis and Reid 1996, Baylis et al. 2010, Garcia-Saenz et al. 2011, Viennet et al. 2011), we expected a decreasing number of biting midges to occur in traps with increasing distance to the stable.

The study was conducted between May and June, 2009 at one livestock farm that is surrounded by meadows in northern Germany. This site represents a typical dairy farm with 220 dairy cattle and is located in a typical agricultural region in the marshland of Lower Saxony. The grazing density of cattle was approximately 7.3 individuals per hectare, but the cattle were in the stables during the night.

Sampling of Culicoides spp. was conducted with BG-Sentinel™ light traps fitted with ultraviolet light. Each of the three traps was placed in concentric circles in distances of 25, 50, 100, and 200 m (variable DISTANCE) around one trap in direct proximity to the stable (Figure 1a). All traps were installed at 1.5 m above the ground and operated with one car battery each per sampling period. A photo sensor was installed in the immediate vicinity of each trap. The photo sensor was adjusted to illuminate the ultraviolet light from sunset to dawn, because the activity of Culicoides spp. was expected to be highest in this time period (Kettle et al. 1998). The traps were sampled five times, every seventh day (variable SAMPLINGPERIOD). Insects were collected in 70% ethanol and stored in separate containers. The samples were presorted in order to separate biting midges from other insects and to differentiate members of the C. obsoletus and C. pulicaris group (also C. obsoletus or C. pulicaris in the subsequent text) from other Ceratopogonidae.

All data analyses and graphs were made with the program R (R Development Core Team 2011). Due to overdispersion in the data, collection data of females of the Culicoides obsoletus and Culicoides pulicaris group were analyzed with negative binomial generalized linear models through the function glm.nb from the package MASS (Venables and Ripley 2002). The variables DISTANCE, DIRECTION, SAMPLINGPERIOD, and all interactions between the variables were implemented in the model. The value of the variable DIRECTION for each trap tr was calculated as:

\[ D_{tr} = \frac{\pi a_{tr}}{180} + \frac{5\pi}{4} \]

where \( a_{tr} \) is the compass direction in degree for each trap tr measured from the center of the stable (Figure 1b). We
used a phase shift of $5\pi/4$ in the calculation of DIRECTION because the prevailing wind direction in northern Germany is southwest. The value of the variable DIRECTION is close to 1 if the direction of the trap is southwest relative to the stable and close to -1 if the direction is northeast. DIRECTION is close to zero if the position of the trap relative to the stable is either northwest or southeast.

According to recommendations by Zuur et al. (2009), hypothesis testing for significance of the variables and their interactions were conducted using the likelihood ratio test (function lrtest) from the lmtest package (Zeileis and Hothorn 2002), dropping each term in turn and comparing the full model with a nested model. The non-significant variables with the lowest impact on the deviance of the model were excluded and then the model was refit with the remaining terms until all terms were significant. Z-statistics and graphs of the final models are presented. Correlation between the number of females of the *Culicoides obsoletus* and *Culicoides pulicaris* groups were analyzed using the Spearman’s rank correlation test (function cor.test) from the stats package. Additionally, non-parametric chi-square tests for trend were applied to evaluate the proportion of samples positive for females of the *Culicoides obsoletus* or *Culicoides pulicaris* group using the function prop.trend.test from the stats package.

A total of 21,436 biting midges was collected in addition to 297,918 of other arthropods. *C. pulicaris* accounted for 20% with 75.6% females (3,253 females/4,301 ind.), *C. obsoletus* for 53.6% with 76.4% females (8,778 females/11,486 ind.) and other Ceratopogonidae for 26.4% with 66.6% females (3,761 females/ 5,649 ind.) of the samples.

The number of collected females of *C. pulicaris* was significantly affected by the variable DISTANCE ($\chi^2 = 8.9$, $P < 0.01$; Table 1 and Figure 2), while the number of *C. obsoletus* was additionally affected by the variable DIRECTION as we found a significant interaction for the variables DISTANCE and DIRECTION ($\chi^2 = 5.2$, $P < 0.05$; Table 1 and Figure 3). The number of trapped females significantly decreased with increasing distance to the stable for both groups. The *C. obsoletus* group only had higher numbers in the traps southwestwards from the stable relative to the other traps (Figure 3).

Neither the interaction DISTANCE and DIRECTION nor the single variable DIRECTION were significant for *C. pulicaris* ($\chi^2 = 1.2$, $P > 0.05$ and $\chi^2 = 0.8$, $P > 0.05$). The variable SAMPLINGPERIOD was not significant for both groups (*C. obsoletus*: $\chi^2 = 0.3$, $P > 0.05$; *C. pulicaris*: $\chi^2 = 0.8$).

### Table 1. Results of negative binomial generalized linear models in R. Negative estimate values indicate that the abundance of females of the *Culicoides obsoletus* and *Culicoides pulicaris* group decreased with increasing value of the corresponding coefficient.

| Culicoides females       | Coefficients     | Estimate ± SE | z value | Pr(>|z|) |
|--------------------------|------------------|---------------|---------|---------|
| **Culicoides obsoletus** | Intercept        | 5.690 ± 0.333 | 17.099  | <0.001  |
|                          | Distance         | -21.703 ± 3.253 | -6.671  | <0.001  |
|                          | Direction        | 1.954 ± 0.618 | 3.160   | <0.01   |
|                          | Distance:Direction | -15.611 ± 5.922 | -2.636  | <0.01   |
| **Culicoides pulicaris** | Intercept        | 4.589 ± 0.307  | 14.929  | <0.001  |
|                          | Distance         | -9.128 ± 2.980 | -3.064  | <0.01   |

Figure 2. Number of females of the *Culicoides pulicaris* group caught with light suction traps positioned in five different distances from the stable on one farm in northern Germany. The best fit regression line with confidence intervals is shown.

Figure 3. Number of females of the *Culicoides pulicaris* group caught with light suction traps positioned in five different distances from the stable on one farm in northern Germany. The best fit regression lines are shown. Solid line: DIRECTION above zero; dashed line: value below zero.
decreased with increasing distance but did not show a significant trend for *C. pulicaris*. These differences might be caused by different breeding site preferences, but a general knowledge of the breeding sites of most important vector biting midges is still missing (Nielsen et al. 2010). However, surroundings of livestock farms provide an abundance of suitable habitats (Zimmer et al. 2008). Pre-adult stages of *C. obsoletus* are expected to be closely connected to anthropogenic livestock farming substrates (Nielsen et al. 2010). Species were confirmed for dried dung, animal litter, and maize silage (Zimmer et al. 2010), which generally occur close to the farms. With preferences for wet soils and bogs (Purse et al. 2003), *C. pulicaris* is generally expected to be more euryoeciously distributed (Nielsen et al. 2010).

The impact of the variable direction on the number of *C. obsoletus* females has to be evaluated with caution because the position of traps was not uniformly distributed in respect to the compass direction. The main wind direction in northern Germany is southwest, which perhaps resulted in higher values in the traps positioned southwestward from the stable but, as reviewed by Sedda et al. (2012), at present there are no studies about the small-scaled impact of wind on *Culicoides* flight. A much more important factor could be the position of the traps relative to productive breeding sites that cannot be evaluated from our study.

Further studies need to evaluate whether a decreasing trapping success with increasing distance to the stable is a general pattern for other farms and for all species of biting midges. Additionally, we are concerned about the spatial interaction between UV-light/suction traps, as the spatial dependence of the samples is largely unknown. Rigot and Gilbert (2012) demonstrated a significant spatial interaction for Onderstepoort-type blacklight traps in a distance of 50 m. A similar effect may be expected for the BG-Sentinel™ light traps. The impact on our study results is hardly predictable, because we do not know to what extent the traps were affected by interference. Nevertheless, due to the strong decrease of collected females with increasing distance to the stable, at least for *C. obsoletus*, there is probably also a significant impact under the presumption of trap interference. Further studies should study the spatial interaction for BG-Sentinel™ light traps to improve study designs and allow spatial independent samples.

Because of a significant decrease of collected biting midges even in small distances from the stable, sampling in the direct surroundings of stables (< 25 m) probably promises the highest trapping success. However, this suggests that comparative studies on biting midges should be interpreted carefully if trap positions relative to the stable differ. Moreover, it is necessary to standardize trap position as far as possible in order to achieve comparable data.

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