Comparison of a mouth aspirator and a mechanical vacuum aspirator for the collection of *Culicoides* midges (Diptera: Ceratopogonidae) in equids

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Original Contribution

In a controlled field trial, a mechanical vacuum and a mouth aspirator were compared with respect to *Culicoides* midge catching results. The two collection methods were equally applied to two Haflinger horses. Once every hour between 2 hr before sunset and sunset, midges were aspirated for 3 min directly off from the coat of each horse at the same time. In total, 16 replicate measurement days were made. To get insight into the number of (blood-fed) midges collected on different body parts, four replicate measurement days were done in which the hourly 3-min samples for each collecting method were separately collected and counted per body part. Mean number of *Culicoides* midges collected on the legs and on the belly of the horse was two times and significantly higher by the mechanical vacuum aspirator compared to the mouth aspirator. Although the mean number of *Culicoides* midges from the other part of the host sampled (head/neck/manes/back/flanks) was comparable between the two methods, the mean number of blood-fed *Culicoides* midges was four times and significantly higher collected by the mouth aspirator compared to the mechanical vacuum aspirator. In conclusion, it can be recommended to use a mechanical vacuum aspirator instead of a mouth aspirator for the legs and belly area, where a systematic, stroke-by-stroke-vacuum procedure is recommended. For the rest of the body, it is strongly recommended to use a targeted procedure by visual guidance of the presence of midges. For now, based on this experiment, we can only recommend using the mouth aspirator for this targeted approach. However, it might be worth assessing the use of the mechanical vacuum aspirator in a targeted way for future studies.

**Keywords**
attack rate, biting rate, blood-fed, horse

**1 Introduction**

Haematophagous biting midges in the genus *Culicoides* (Diptera: Ceratopogonidae) are vectors for viral diseases in livestock and equids such as bluetongue (BT), Schmallenberg virus (SBV), African horse sickness (AHS) and equine encephalosis (EE) (Elbers, Meiswinkel, van Weezep, Sloet van Oldruitenborgh-Oosterbaan, & Kooi, 2013; Mellor, Boorman, & Baylis, 2000). Midge feeding behaviour and host preference are critical to our understanding of how vector-borne diseases are transmitted amongst domesticated...
livestock (Backer & Nodelijk, 2011; Hartemink et al., 2009; Lo Iacono, Robin, Newton, Gubbins, & Wood, 2013). Currently, there are several different field trapping methods for Culicoides, like various models of the light suction trap, the truck-trap, the mouth aspirator (or pooter) and mechanical vacuum aspirator, window-pane trap, sticky trap, the drop-tent bait-trap and the sweep-net (Steykskal, Murphy, & Hoover, 1986). Depending on the research questions to be answered, an appropriate midge collection method should be selected. To estimate Culicoides attack and biting rates on livestock and equids for disease transmission modelling, aspiration off the host animals is a preferred method because you are sure that the midges have landed on—and have potentially bitten and taken a blood meal of—the host (Elbers & Meiswinkel, 2014). Several studies have used the mouth aspirator, which uses visual detection of midges by the collector and subsequent sucking of the midges by the mouth aspirator targeted aspiration (Elbers, van den Heuvel, & Meiswinkel, 2016; Elbers & Meiswinkel, 2014, 2015; Jones, Potter, & Rhodes, 1972; Lillie, Kline, & Hall, 1988; Sholdt & Neri, 1974; White, 1977; Wiens & Burgess, 1972). Other studies used a mechanical vacuum aspirator (in majority an adjusted commercial car vacuum cleaner), which is commonly used in a systematic way by vacuuming the coat stroke by stroke without visual detection of midges (non-targeted aspiration) for the collection of blood-sucking insects from the skin of hosts (Ayllón et al., 2014; Gerry, Sarto I Monteys, Moreno Vidal, Francino, & Mullens, 2009; Mayo et al., 2012; Saenz & Greiner, 1994; Schmidtmann, Jones, & Gollands, 1980; Standfast & Dyce, 1972; Townley, Baker, & Quinn, 1984; Viennet et al., 2011). This might yield better results since it may suck midges from between the hairs of the coat that the collector cannot see and would be missed by the mouth aspirator that is only able to operate on visual detection of midges.

In recent studies that used the mouth aspirator, significantly lower midge biting rates were observed in equids compared to cattle (Elbers, Gonzales, & Meiswinkel, 2018; Elbers & Meiswinkel, 2015). This might be of importance to African horse sickness (AHS) virus transmission modelling: low midge biting rates would decrease transmission rate and intermittent feeding on cattle and sheep pastered nearby—so-called dilution effect—would dampen even more possible transmission (Elbers et al., 2018).

One of the hypotheses proposed to explain the lower proportion of blood-fed midges in equids (Shetland pony, Haflinger) compared to cows (Elbers et al., 2018; Elbers & Meiswinkel, 2015) was that it might be due to the coat of some equids being denser and furrier than that of the cow, thus compelling mides to burrow more deeply, with the result that they are not easily observable and extracted by the person operating a mouth aspirator (Elbers et al., 2018). A mechanical vacuum aspirator might have enough strength to extract these mides, picking-up midges by systematic vacuuming of the skin even if they are not observable between the hairs of the coat.

To our knowledge, no systematic study has been executed to compare the mouth aspirator with a mechanical vacuum aspirator for the collection of Culicoides mides. Therefore, the objective of this study was to establish whether there is a difference in mean number of (blood-fed) Culicoides mides collected by a mechanical vacuum aspirator compared to a mouth aspirator.

2 | MATERIALS AND METHODS

2.1 | Study site and hosts

The study was conducted between 16 August and 27 September 2017 at a horse holding (housing seven Haflinger horses used for pulling a carriage with tourists on trips into the forest) in the city of Staverden, eastern Netherlands (grid-reference: 52°. 17’N; 5°, 44’E). The experiment was conducted in a pasture area (several hectares) surrounded by forest. At a distance of 300–400 m from the experimental site, a total of seven dry cows were pastured. Two Haflinger horses (mother and daughter) were used for the experiment (10 and 5 years old, respectively; height at the withers: 146 and 150 cm, respectively; approximate weight: 475 and 575 kg, respectively; both were about 6 months in pregnancy).

2.2 | Study design

A paired block design was used for the study: the two collection methods were equally applied to the two different horses. Three hours before sunset (SS-3 hr), the two horses were tethered randomly to a wooden shed wall in the pasture (distance between the horses approximately 5 m) and the hide of the horses was thoroughly brushed for about 20 min. Once every hour between SS-2 hr and SS, Culicoides were aspirated directly off from the coat of each animal host at the same time. These collection moments around sunset were chosen because of the commonly known high midge activity at this time period for potential vector-borne disease Culicoides species: peak activity around sunset for most midge species and peak activity for C. chiopterus and C. dewulfi about 2 hr before sunset (Elbers & Meiswinkel, 2015). Mides were collected using a handheld mouth aspirator (Aspirator 1135A by BioQuip Products Inc., http://www.bioquip.com) by one collector and a mechanical vacuum aspirator by another collector. The collecting methods were not randomized amongst the two collectors. Operating the mouth aspirator requires experience with respect to detecting and aspirating the small mides from the coat of the host animal, while the mechanical aspirator can be handled by a more-or-less unexperienced collector because one has to follow an easy-to-learn procedure of systematic vacuuming (stroke by stroke until the complete area is done) the coat of the host animal in which process one does not need to visually detect the small mides.

The mechanical vacuum aspirator consisted of a commercially available car wet/dry vacuum cleaner (HP Autozubehör, article code 20,285; 12 V, 90 W, weight: 1.05 kg; Bodenwerder, Germany; http://www.hp-autozubehoer.de), with a wide-mouthed (10 cm wide) flattened attachment (with bristle hair attached to the mouth part), that was connected to the vacuum cleaner core part with a flexible tube (1 m). Before the start of the study, the mechanical vacuum cleaner was tested. We added a false-air valve at the beginning of
the suction tube to reduce the suction power, because in its normal configuration, 10%–15% of the midges caught were damaged (e.g., loss of wings). We adjusted the sucking power of the mechanical aspirator with the valve in such a way, that the number of damaged midges was negligible (Figure 1). The core part of the mechanical aspirator consisted of a suction ventilator and a plastic collection pan, which held for the midge collecting process a soap solution. During the vacuuming, the midges were blown into the soap solution, and after the collection period, the midges were moved from the soap solution to a tube with 70% alcohol for preservation. The mouthpiece of the mechanical vacuum aspirator was equipped with a line of sturdy brush hair of 1 cm length, implicating that the plastic, air-sucking mouthpiece was raised 1 cm above the skin of the host. At the side of the mouthpiece that would first pass over the skin of the host, the brush hair was clipped away, so there was an opening in the line of brush hair and no brush hairs would touch the hide at that spot, only sucking-air would pick up midges. In operation, the mouthpiece of the mechanical vacuum aspirator was passed over the total skin surface in each region in a systematic—stroke—way (not guided by visual presence of midges in a certain spot on the skin), and depending on the total surface area, several times within the time period assigned, as was done in previous studies using a mechanical vacuum aspirator (e.g., Bellis, Melville, Hunt & Hearnden, 2004; Greiner, Fadok & Rabin, 1990; Schmidtmann et al., 1980; Viennet et al., 2011). For vacuuming the manes section, the flattened attachment was detached from the flexible tube. Each collector used the same collection method throughout the study. Three regions of the animals were aspirated (the order in which regions were aspirated randomized each hourly collection; both collection methods aspirated the same body region at the same time) for three minutes each once every hour: (a) head, mane, neck, shoulders, front, back (to tail) and flanks, (b) the belly, and (c) the legs, resulting in one sample for each collecting method per hourly collection. In total, 16 replicate measurements (days) were made. In order to get insight into the number of (blood-fed) midges by different body parts, four replicate measurements (days) were done in which the hourly samples for each collecting method were separately collected and counted per body part. Measurements were done only when the following weather conditions applied: windless, no rain and temperatures >10˚C; most collection days were bright, at most only partly cloudy. Mean temperature (in degrees Celsius) and relative humidity (%) at 1.5 m above ground level was measured in the experimental pasture once every hour between SS-2 hr and SS while Culicoides midges were being aspirated off the animals.

2.3 | Culicoides identification

Culicoides were identified morphologically using primarily the published keys of Campbell and Pelham-Clinton (1960), Glukhova (1989), and the unpublished PhD thesis of Jean-Claude Delecolle (1985). The C. obsoletus complex comprised two species: C. obsoletus and C. scoticus; C. chiopterus and C. dewulfi do not form part of the C. obsoletus complex and so are treated separately (Gomulski, Meiswinkel, Delecolle, Goffredo, & Gasperi, 2005). Female midges were age-graded as either nulliparous, parous, gravid or freshly blood-fed (engorged) (Dyce, 1969).

2.4 | Culicoides attack and biting rate

The Culicoides attack rate equates to the total number of midges aspirated per minute from all three body regions of an animal combined. The Culicoides biting rate equates to the total number of midges found to be engorged aspirated per minute from all three body regions of an animal combined.

2.5 | Statistical analysis

Comparisons between sampling methods were done by fitting generalized linear (GLM) or mixed models (GLMM) with a Poisson or negative binomial error distribution, which were selected based on the best fit to the data. For the statistical analysis, the midge totals of the three collection moments per day were added and used as response variable. To assess whether there were differences between the sampling methods in both total Culicoides counts and blood-fed counts, we fitted GLMM with a Poisson error distribution and corrected for overdispersion by calculating the overdispersion parameter. Based on data exploration and the experimental design, the following model structure was used: total or blood-fed Culicoides counts per experimental day were the response variables. The sampled horse and experimental day were modelled as random variables and sampling method (mechanical vacuum aspirator/mouth aspirator), which is the variable of interest for comparison, was modelled as an explanatory (fixed) variable. Random variables were introduced to account for within- and
Table 1: Number of Culicoides spp. biting midges collected by mouth and mechanical vacuum aspiration (3 min per whole hour) from two Haflinger horses, conducted for three consecutive hours (from 2 hr before to and including official sunset) over a period of 16 days in August–September 2017.

| Species                | Mouth aspirator | Mechanical vacuum aspirator |
|------------------------|-----------------|----------------------------|
|                        | Female age grades |                      | Female age grades |                      |
|                        | Rank | No. | %  | NP | P  | G  | BF | No. | AR | BR   | Rank | No. | %  | NP | P  | G  | BF | No. | AR | BR   |
| C. obsoletus/scoticus  | 1    | 2115| 48.4| 1015| 620 | 2  | 478| 6   | 14.7| 3.3  | 1    | 2207| 58.2| 1257| 739| 0  | 211 | 1  | 15.3| 1.5 |
| C. dewulfi             | 2    | 1910| 43.7| 755 | 335 | 0  | 820| 6   | 13.3| 5.7  | 2    | 1206| 31.8| 726 | 209| 2  | 269 | 3  | 8.4 | 1.9 |
| C. chiopterus          | 3    | 267 | 6.1 | 263 | 0   | 4  | 0  | 1.9 | 0.03| 0.0  | 3    | 274 | 7.2 | 0   | 269| 1  | 4  | 1  | 1.9 | 0.03|
| C. punctatus           | 4    | 33  | 0.8 | 20  | 13  | 0  | 0  | 0.2 | 0.0 | 0.0  | 4    | 47  | 1.2 | 24  | 18 | 0  | 5  | 0  | 0.3 | 0.03|
| C. fascipennis         | 5    | 21  | 0.5 | 9   | 12  | 0  | 0  | 0.15| 0.0 | 0.0  | 5    | 26  | 0.7 | 15  | 8  | 0  | 3  | 0  | 0.2 | 0.02|
| C. pulicaris           | 6    | 14  | 0.3 | 8   | 4   | 0  | 2  | 0.1 | 0.01| 0.0  | 6    | 24  | 0.6 | 12  | 12 | 0  | 0  | 0  | 0.2 | 0.0 |
| C. subfascipennis      | 7    | 7   | 0.2 | 5   | 2   | 0  | 0  | 0.05| 0.0 | 0.0  | 7    | 3   | 0.1 | 3   | 0  | 0  | 0  | 0  | 0.02| 0.0 |
| C. stigma              | 8    | 5   | 0.1 | 4   | 0   | 0  | 0  | 0.03| 0.0 | 0.0  | 7    | 3   | 0.1 | 1   | 1  | 0  | 1  | 0  | 0.02| 0.01|
| C. lupicaris           | 9    | 1   | 0.0 | 0   | 0   | 1  | 0  | 0.01| 0.01| 0.01 | 8    | 1   | 0.0 | 1   | 0  | 0  | 0  | 0  | 0.01| 0.0 |
| C. achrayi             | 9    | 1   | 0.0 | 0   | 1   | 0  | 0  | 0.01| 0.0 | 0.0  | 8    | 1   | 0.1 | 0   | 1  | 0  | 0  | 0  | 0.01| 0.0 |
| C. impunctatus         | 8    | 1   | 0.0 | 0   | 0   | 0  | 0  | 0.01| 0.0 | 0.0  | 8    | 1   | 0.1 | 0   | 1  | 0  | 0  | 0  | 0.01| 0.0 |
| Total                  | 4374 | 1816| 1251| 2   | 1305| 12 | 30.4| 9.1 | 26.3| 3.4  | Total | 3793| 2040| 1257| 3  | 493| 5 | 26.3| 3.4 |

Note: Crude attack rate (AR) in specimens per minute; crude biting rate (BR) in blood-fed specimens per minute. Abbreviations: BF, blood-fed; G, gravid; NP, nulliparous; P, parous.
between-horse variability and daily variability in Culicoides counts. Temperature and relative humidity were also assessed as random variables, but the model including day of sampling alone showed a better fit and day appeared to cover the variability introduced by these environmental variables.

Differences between sampling methods associated with specific body parts of sampled animals were assessed. Visual inspection of the data showed differences between methods dependent on the body parts, and hence, we fitted separate models for total and blood-fed counts for each body part, that is (a) belly, (b) legs and (c) head/neck/mane/back/flanks. GLM models were fitted instead of GLMM because data for this assessment were collected during four days of the study period, limiting therefore sample size and number of variables that can be used in the models. In these models, the sampling method and horse were assessed as fixed variables, but horse showed no effect (no confounding); hence, the final models used sampling method as the only variable. The GLMM were fitted using the lme4 library of the statistical software package R, version 3.5.0 (R Core Team, 2018). Akaike's information criteria (AIC) were used as a model selection criterion. Model fit was assessed by variation explained by the model (R²) and by residual analysis.

3 | RESULTS

Eleven Culicoides taxa representing twelve species were captured: 11 species with the mouth aspirator and 12 species with the mechanical vacuum aspirator, and 10 of these species were collected by both collection methods (Table 1). A single individual of C. achrayi was collected by the mouth aspirator and with the mechanical vacuum aspirator one single C. pallidicornis and one single C. impunctatus specimen was collected. More than 98% of the Culicoides midges aspirated off the two experimental animals comprised five taxa only that are competent vectors of arboviral diseases namely the C. obsoletus complex (two species), C. dewulfi, C. chiopterus, C. punctatus and C. pulicaris. In Table 1, a summary of frequency distribution and the crude attack and biting rates of the captured species is presented.

Variation in total number of (blood-fed) Culicoides midges was observed between mother and daughter Haflinger horse, indicating that there is good reason to take this variation into account in the statistical modelling by adding the horse identification as a random variable in the model.

Mean total number of Culicoides midges collected with the mouth aspirator, and the mechanical vacuum was comparable and not significantly different (p = 0.13; R² of model: 0.83) (Figure 2a). However, mean total number of blood-fed Culicoides midges was 2.8 times higher (95% CI: 1.9–4.0) when the horses were sampled with the mouth aspirator compared to the mechanical vacuum aspirator (p < 0.001; R² of model: 0.92) (Figure 2b). The number of (blood-fed) Culicoides midges collected separately by body parts gives insight if observed differences between the two aspiration methods are consistent for all body parts or not (Table 2). From the raw data on the separate body parts, it is clear that there is no difference in total number of midges collected by mouth aspirator and the mechanical vacuum aspirator. Furthermore, more blood-fed midges of C. obsoletus/C. scoticus and C. dewulfi are collected.

**FIGURE 2** Distribution of daily number of (blood-fed) Culicoides midges collected by mouth and mechanical vacuum aspirator (3 min per whole hour) from Haflinger horses, conducted for three consecutive hours (from 2 hr before to and including official sunset) over a period of 16 days in August–September 2017 (Fat dark line in the box: median; lower end of the box: 25% quantile; higher end of the box: 75% quantile; highest bullet or high end of the vertical line coming out of the box: highest value; lowest bullet or low end of vertical line coming out of the box: lowest value)
by the mouth aspirator compared to the mechanical vacuum aspirator on the head/neck/manes/back/flanks section of the host. Mean total number of Culicoides midges was 2.2 times higher (95% CI: 0.95–4.9) collected on the belly ($p = 0.06$) (Figure 3a) and 1.9 times higher (95% CI: 1.03–3.4) on the legs ($p = 0.04$) (Figure 3c) by the mechanical vacuum aspirator compared to the mouth aspirator. Mean total number of blood-fed Culicoides midges aspirated off the belly was comparable and not significantly different between the mouth and the mechanical vacuum aspirator (Figure 3b). Mean total number of blood-fed Culicoides midges was 3.8 times higher (95% CI: 64.3–76.7) collected on the legs ($p = 0.02$) by the mechanical vacuum aspirator compared to the mouth aspirator (Figure 3d). Mean total number of Culicoides midges was not significantly different ($p = 0.54$) collected from the head/neck/manes/back/flanks by the mechanical vacuum aspirator compared to mouth aspirator (Figure 3e), but mean total number of blood-fed Culicoides midges was 3.7 times higher (95% CI: 1.8–9.1) collected by the mouth aspirator compared to the mechanical vacuum aspirator ($p = 0.0017$) (Figure 3f). The main Culicoides species involved in this were C. dewulfi and midges of the C. obsoletus complex.

### Table 2

| Species                  | Body parts                  | Total (%) | Blood-fed (%) | Total (%) | Blood-fed (%) | Total (%) | Blood-fed (%) |
|--------------------------|-----------------------------|-----------|---------------|-----------|---------------|-----------|---------------|
|                          | Legs                        |           |               |           |               |           |               |
|                          | Mouth aspirator             |           |               |           |               |           |               |
| C. obsoletus/scoticus     | 25 (4.6)                    | 3 (1.5)   | 64 (11.9)     | 10 (4.9)  | 449 (83.5)    | 191 (90.5)| 538           | 204           |
| C. chiopterus             | 55 (94.8)                   | 0         | 1 (1.7)       | 0         | 2 (3.5)       | 0         | 58            | 0             |
| C. dewulfi                | 75 (8.8)                    | 1 (0.3)   | 5 (0.6)       | 0         | 772 (90.6)    | 399 (99.7)| 852           | 400           |
| C. punctatus              | 1 (12.5)                    | 0         | 5 (62.5)      | 0         | 2 (25.0)      | 0         | 8             | 0             |
| C. pulicaris              | 0                          | 0         | 0             | 0         | 2 (100.0)     | 1 (100.0)| 2             | 1             |
| C. fascipennis            | 0                          | 0         | 2 (100.0)     | 0         | 0             | 0         | 2             | 0             |
| C. stigma                 | 0                          | 0         | 0             | 0         | 0             | 0         | 0             | 0             |
| Subtotal (%)              | 156 (10.7)                  | 4 (0.7)   | 77 (5.3)      | 10 (1.7)  | 1,227 (84.0)  | 591 (97.6)| 1,460         | 605 (100.0)   |
|                          | Legs                        |           |               |           |               |           |               |
|                          | Mechanical vacuum aspirator |           |               |           |               |           |               |
| C. obsoletus/scoticus     | 120 (16.6)                  | 11 (16.2) | 138 (19.1)    | 11 (16.2) | 464 (64.3)    | 46 (67.6)| 722           | 68            |
| C. chiopterus             | 109 (96.5)                  | 0         | 4 (3.5)       | 0         | 0             | 0         | 113           | 0             |
| C. dewulfi                | 55 (8.4)                    | 4 (3.4)   | 9 (1.4)       | 4 (3.4)   | 576 (90.0)    | 108 (93.2)| 640           | 116           |
| C. punctatus              | 8 (38.1)                    | 0         | 11 (52.4)     | 2 (100.0) | 2 (9.5)       | 0         | 21            | 2             |
| C. pulicaris              | 0                          | 0         | 0             | 0         | 0             | 0         | 0             | 0             |
| C. fascipennis            | 4 (57.1)                    | 0         | 3 (42.9)      | 0         | 0             | 0         | 7             | 0             |
| C. stigma                 | 0                          | 0         | 1 (100.0)     | 0         | 0             | 0         | 1             | 0             |
| Subtotal (%)              | 296 (19.7)                  | 15 (8.1)  | 166 (11.0)    | 17 (9.1)  | 1,042 (69.3)  | 154 (82.8)| 1,504         | 186 (100.0)   |

### Discussion

The objective of this study was to establish whether there is a difference in mean total number of (blood-fed) Culicoides midges collected by a mouth aspirator compared to a mechanical vacuum aspirator. It is shown that the mechanical vacuum aspirator collects approximately two times more midges from the legs and the belly of the horse compared to the mouth aspirator. Although the mean total number of Culicoides midges from the third and main other part of the host sampled (head/neck/manes/back/flanks) is comparable between the mouth and the mechanical vacuum aspirator, the mean total number of blood-fed Culicoides midges was approximately four times higher collected by the mouth aspirator compared to the mechanical vacuum aspirator. That the mechanical vacuum aspirator collects about two times more midges from the legs and the belly is significant, and may be explained by the fact that visual detection and aspiration of the midges on these parts by the mouth aspirator is physically difficult for the collector because he/she has to basically crawl under and beside the horse. Handling the mechanical vacuum aspirator in a systematic way (vacuuming stroke by stroke till complete area is done),
there is no need for visual detection of the midges on these body parts, and the results show that by operating the mouth aspirator, you miss about 50% of the midges present. For the area covering the head, neck, manes, back and flanks, there is another mechanism that explains why the mouth aspirator collects significantly more blood-fed midges compared to the mechanical vacuum aspirator. When operating the mechanical vacuum aspirator, systematically vacuuming this large area, the collector will "waste" time at places where no or relatively low numbers of midges are present. On the contrary, the collector using the mouth aspirator, guided by visual large scale appearance of the midges in specific areas of the host like, for example the manes, will collect midges predominantly from those areas and this is valid because that is where the midges are present and have the ability to take a blood meal. In this study, the manes of the horse was a "hot spot" area for the presence of midges. It was visually obvious that there was a high abundance of blood-fed midges in the manes’ area because the blood-fed midges, heavy by a belly full of host blood, were slow in their movements and had difficulties with crawling out of the manes. As a result, these blood-fed midges were easy to detect and being aspirated by the mouth aspirator. Lower midge biting rates were observed in equids compared to cattle in recent studies in the Netherlands (Elbers et al., 2018; Elbers & Meiswinkel, 2015), and one of the hypotheses proposed to explain the lower midge biting rates observed in equids compared to cows was that it might be due to the use of a mouth aspirator instead of a mechanical vacuum aspirator when collecting midges. The results of the present study refute the above-mentioned hypothesis: the mechanical vacuum aspirator does not collect effectively more blood-fed midges compared to the mouth aspirator. The crude Culicoides attack rates observed in this study were comparable with an earlier study in the same region with Fjord horses (Elbers et al., 2018). In horses, the proportion of blood-fed midges ranges commonly between 0.5% and 10% (Ayllón et al., 2014; Gerry, Nawaey, Sanghrájka, Wisniewska, & Hullinger, 2008; Jones, Hayes, Potter, & Francy, 1977; Mullens, Owen, Heft, & Sobeck, 2005; Page, Labuschagne, Venter, Schoeman, & Guthrie, 2015; Scheffer et al., 2012; Viennet et al., 2011). The proportion of blood-fed midges in this study collected with the mechanical vacuum aspirator using a systematic vacuum process was 13%, which is comparable with the above-mentioned investigations. The proportion of blood-fed midges in this study collected with the

**FIGURE 3** Mean number of (blood-fed) Culicoides midges (with standard error bars) collected by mouth and mechanical vacuum aspirator (3 min per whole hour) on the belly (a and b), legs (c and d) and head/neck/manes/flanks/back (e and f) of Haflinger horses, conducted for three consecutive hours (from 2 hr before to and including official sunset) over a period of 4 days in September 2017.
mouth aspirator was 30%, and this is more in the exceptional range reported by Townley et al. (1984) and Greiner, Fadok & Rabin (1990). Maybe this is affected by the circumstance that our study was done in Culicoides peak season (August and September), while our earlier studies with mouth aspiration of equids (with observed lower proportion of blood-fed midges) were done in May–July.

There are some limitations to consider in our study. Firstly, we only used one experienced sampler for the mouth aspirator to reduce variance and improve power for statistical analysis. This means that our results would only apply to experienced collectors able to promptly detect and collect midges. Secondly, we acknowledge that using the mechanical vacuum aspirator in a targeted way, particularly at the head/neck/manes/back/flanks area, might possibly lead to similar results as the mouth aspirator.

In conclusion, it can be recommended to use a mechanical vacuum aspirator instead of a mouth aspirator for the legs and belly area, where a systematic, stroke-by-stroke-vacuum procedure is recommended. For the head, neck, manes, flanks and back, it is strongly recommended to use a targeted procedure by visual guidance of the presence of midges.

For now, based on this experiment, we can only recommend using the mouth aspirator for this targeted approach. However, it might be worth assessing the use of the mechanical vacuum aspirator in a targeted way for future studies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS’ CONTRIBUTIONS

ARWE designed the study, conducted the experiment, analysed the data and wrote the draft manuscript. EP and JLG analysed the data and conducted the statistical analysis, and helped with finalizing the manuscript. All authors read and approved the manuscript.

DATA ACCESSIBILITY

Data collected in this study are available upon reasonable request to the first author.

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