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9
10 Title: **Protection of horses against Culicoides biting midges in different housing systems in**
11 **Switzerland**

12
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24

25 ABSTRACT

26 Species belonging to the *Culicoides* complexes (*Diptera*, *Ceratopogonidae*), *obsoletus* and *pulicaris*,
27 in Switzerland, are potential vectors of both bluetongue virus (BTV) and African horse sickness
28 virus (AHSV). The epidemic of BTV in 2006 and 2007 in Europe has highlighted the risk of
29 introduction and spread of vector-borne diseases in previously non-endemic areas. As a measure of
30 prevention, as part of an integrated control program in the event of an outbreak of African horse
31 sickness (AHS), it is of utmost importance to prevent, or substantially reduce, contact between
32 horses and *Culicoides*. The aim of the present study was to compare the effect of three protection
33 systems, net, fan, repellent, or combinations thereof, with regard to their potential to reduce contact
34 between horses and *Culicoides*. Three different equine housing systems, including individual boxes
35 (BX), group housing systems (GR), and individual boxes with permanently accessible paddock
36 (BP) were used. The efficacy of the protection systems were evaluated by comparing the total
37 number counts of collected female *Culicoides*, of non-blood-fed and blood-fed *Culicoides*,
38 respectively, with UV black light traps. The study was conducted over 3 summer months during
39 2012 and 2013 each and focused on the efficacy and practicality of the protection systems. The
40 repellent was tested in 2012 only and not further investigated in 2013, as it showed no significant
41 effect in reducing *Culicoides* collected in the light traps. Net protection system provided the best
42 overall protection for the total number of female *Culicoides*, non-blood-fed and blood-fed
43 *Culicoides* in all tested housing systems. The net, with a pore size of 0.1825 mm^2 , reduced the total
44 number of *Culicoides* collected in the housing systems BP, GR and BX by 98%, 85% and 67%,
45 respectively. However, in the GR housing system, no significant difference between the
46 effectiveness of the fan and the net were determined for any of the three *Culicoides* categories. The
47 results of the present study demonstrated that horse owners can substantially reduce their horses'
48 exposure to *Culicoides*, by using net protection in the housing systems BX, BP and GR. In GR
49 housing systems, protection against *Culicoides* using a fan is also recommended.

50

51 **Key Words:** Vector protection; fan, net, repellent, African horse sickness

52

53 1. Introduction

54 African horse sickness (AHS), like bluetongue disease (BT), is caused by an Orbivirus from the
55 family of Reoviridae (Caisher and Mertens, 1998). The unforeseen epidemic of BT in 2006 and
56 2007 in Europe has highlighted the risk of introduction and spread of vector-borne diseases in
57 previously non-endemic areas (Hofmann et al., 2008). African horse sickness is a non-contagious
58 disease, transmitted by blood-sucking Culicoides biting midges (Diptera: Ceratopogonidae), in
59 particular by *Culicoides (Avaritia) imicola* Kieffer and *Culicoides (Avaritia) bolitinos* Meiswinkel
60 (Venter et al., 2000; Harrup et al., 2015). Nine African horse sickness virus (AHSV) serotypes are
61 known (McIntosh, 1958; Howell, 1962).

62

63 African horse sickness outbreaks still regularly occur in parts of Africa, particularly southern Africa
64 (Guthrie and Quan, 2009, Akakpo et al., 2011; Diouf et al., 2013), but have not been observed north
65 of the Sahara since 1991 (Zimmerli et al., 2010). There are no Culicoides-free areas in any of the
66 agriculturally utilized areas of Switzerland (Kaufmann et al., 2009). Although the main implicated
67 vector of AHSV, *C. imicola*, does not occur in Switzerland (Mellor et al., 1990; Casati et al., 2009),
68 it is known that species of the Culicoides complexes, *obsoletus* and *pulicaris*, which are potential
69 vectors of both BTV and AHSV are widely distributed in Switzerland (Racloz et al., 2008;
70 Kaufmann et al., 2012). In addition to their emerging role as vectors, Culicoides are also considered
71 nuisance pest in many parts of the world, and that they can cause insect bite hypersensitivity ('sweet
72 itch'), particularly in horses (Anderson et al., 1996). These Culicoides also transmit a second
73 orbivirus of horses, equine encephalosis virus (Guthrie et al., 2009). African horse sickness virus
74 could potentially be imported into Switzerland by movements of infected equines (Mellor, 1993). In

75 addition, virus-infected *Culicoides* might be introduced by different means, for example by air
76 transport (DEFRA, 2012). Though *Culicoides* are relative poor flyers, and actively travel only a few
77 kilometres, they can be carried over long distances by wind (Sellers et al., 1977), in some cases up
78 to 700 km (Boinas et al., 2009). Pérez et al., (2006) reported one *Culicoides* in 10 mg/m² of dust
79 deposited by the wind. Global climate change may shift the geographical distribution of equine
80 diseases and their vectors (Timoney et al., 2007). Climate-driven changes in size and activity of
81 specific vector populations as well as replication rate of the virus in the vector can enable virus
82 transmission to become sustainable in previously non-endemic areas (Khasnis and Nettleman, 2005;
83 Haines et al., 2006; Herholz et al., 2006; Gould and Higgs, 2009). In Switzerland, the AHSV would
84 move into a completely naïve horse population (Zimmerli et al., 2010). An outbreak of a highly
85 lethal horse disease, such as AHS, would have serious animal welfare and economic consequences
86 for the horse industry and for national and international equine movements and trade in non-
87 endemic regions (Herholz et al., 2008).

88

89 As part of an integrated control program in the event of an outbreak of AHS, it is of utmost
90 importance to prevent, or substantially reduce, contact between horses and *Culicoides*. Stabling of
91 equids at night, meshing of stables, the use of fans and application of insect repellents or
92 insecticides both to the animal and the stable environment have been recommended as preventive
93 measures (Barnard, 1997; Meiswinkel et al., 2000; DEFRA, 2009, Page et al., 2009). The efficacy
94 of these preventive measures against *Culicoides*, in various equine housing systems, in Switzerland
95 is, however, unknown.

96

97 This study aimed to compare the effects of net, fan and repellent protection systems, or
98 combinations thereof, with regard to their potential to reduce contact between horses and *Culicoides*
99 in different equine housing systems including boxes, group housing, and boxes with paddock. The
100 study focused on the efficacy as well as practicality of the protection systems with the aim of

101 improving recommendations for protection systems against Culicoides in equine holdings in
102 Switzerland and other countries with similar housing systems.

103

104 **2. Materials and Methods**

105 *2.1 Housing systems and study design*

106 The three most common horse housing systems in Switzerland (Bachmann and Stauffacher, 2002),
107 individual box (BX), individual box with permanently accessible paddock (BP) and group housing
108 (GR), were assessed in 2012 and 2013, at the Swiss National Stud in Avenches (46°53'05.533" N,
109 7°0'54.220" E, 480 m above sea level, at the southern edge of the Broye Plane, in the Swiss
110 Midplains Region). A treated stable and an untreated control stable in each housing system, in
111 immediate proximity of each other, were used in a cross-over design for assessment of each
112 protection system. In the middle of each experiment, the treated and control stables were crossed
113 over to avoid the effect of site influence. Climatic variables, temperature and humidity, were
114 monitored with data loggers (iButton, Maxim Integrated, San Jose, CA, U.S.) in the treated and
115 control stables of each housing system. Local weather data were requested (MeteoSchweiz, 2014).

116

117 The BX housing consists of four indoor boxes for one horse of approximately 12 m². Four horses
118 were housed in each of the treated and the control stable during the experiment. The BP housing
119 (box area: 12 m²) was constructed for individual horses with permanently accessible paddocks of 13
120 m² each. A total of four BPs were under one roof, separated by a central hallway. Two BP systems,
121 on each side of the central hallway, were used for the treated and the control group, which
122 comprised two horses each. The GR housing consisted of a tent (24 m²) and a paddock of
123 approximately 40 m² for two horses each. The two GR housing systems were located approximately
124 3 km from the other housing systems.

125 In both years, data was collected in the BX housing in June, in the GR housing in July and in the BP
126 housing in August. In 2012, each protection system was tested during four nights in each housing
127 system (with the exception that the fan with net was applied in the BX housing only). In 2013, the
128 repellent was not re-tested, however the number of nights of data collection was doubled to eight
129 nights per remaining protection system in each housing system.

130

131 *2.2 Horses*

132 A total of 24 horses were used during both years. Sixteen stallions, two geldings and six mares with
133 a mean (range) age 12.8 (4-19) years were included in the study. Five of the horses were Swiss
134 warmbloods and 19 were Freiberger horses. The horses were randomly allocated to a protection
135 group, within each housing system that they were normally resident, prior to the start of the
136 experiment. Equal numbers of each breed were used in the treated and control group in each
137 housing system.

138

139 For the BX housing, four horses each were used as control and treated group. In the middle of the
140 first half of the experiment, the control horses were switched to treated group horses and vice versa
141 to avoid individual effects of the horses on the *Culicoides* population. The only exception was for
142 the repellent treatment where the horses were not switched, but replaced with new horses every
143 night to avoid a carry-over effect of the topical treatment. In the second half of the experiment,
144 control and treated stables were rotated, and four new treated and four new control group horses
145 were used. The new group of horses also switched their roles as treated and control horses in the
146 middle of the second half of the experiment. The housing systems BP and GR comprised of two
147 control and two treated group horses. Similarly, the cross-over of control and treated stable as well
148 as the change of the roles as control or treated group horses was done as described for the BX
149 housing.

150

151 2.3 *Protection systems*

152

153 2.3.1 *Net*

154 The polypropylene nets (Ultravent[®] Bemisia TIP 250, Texinov, France) had a micrometric mesh of
155 250 x 730 microns (0.1825 mm²) for protection against insects in accordance with the ISO 9001
156 2008 standard. Net specifications were mass 52 g/m², tensile strength in the machine direction 380
157 daN/m and 250 daN/m in the transverse direction, 6% light reduction and 49% reduction in air
158 permeability. In the BX housing, the net covered the four open windows and one door while the
159 other door remained closed. In the BP housing, nets were suspended along the hallway adjacent to
160 the box and suspended on wooden frames to cover the paddocks. In the GR housing, a festival tent
161 frame (60 m²) (Baumann SA, Cudrefin, Switzerland) was used to cover the paddock with the net.
162 All entrances to the three housing systems were cross-covered by the net to facilitate access of
163 personnel and horses.

164

165 2.3.2 *Fan*

166 Six fans (ZOO No. 1400, BM Haus Agrotech, Switzerland) were used. Each 1300 mm x 1380 mm
167 adjustable fan had six belt-driven chrome steel propeller blades encased in protective housing. Fans
168 had a power consumption of 0.75 kW, circulated 39,000 m³ of air per hour, with wind speed 10-15
169 km/h generated.

170 In the treated BX, one fan was placed in each of two diagonally opposite windows (four windows in
171 total). The two fans were suspended outside the windows facing inwards to blow the air into the
172 stable. The two windows without fans were left open at all times. In the treated BP housing, one
173 moveable fan was positioned outside the paddock, facing into the paddock, while one fan was
174 suspended in the hallway facing the boxes. In the treated GR housing, two moveable fans were
175 placed outside the paddock, facing into the paddock. None of the fans positioned outside the BP and

176 GR housing blew directly onto the light traps and depending on their position not directly onto the
177 horses.

178

179 *2.3.3 Fan with net*

180 This protection system was applied to the BX housing system only. The fan fronts were covered
181 with a net with a pore size of 0.1825 mm² (Ultravent[®] Bemisia TIP 250, Texinov, France) to
182 prevent midges, along with dust and other particles, from being sucked by the airflow into the
183 stable. All other stable openings were closed between dusk and dawn and the windows were
184 covered with nets on the inside.

185

186 *2.3.4 Repellent*

187 A permethrin and DEET containing insecticide (Flymax, 6 mg/ ml permethrin and 20 mg/ml DEET,
188 Audevard Ltd., France) was used on the horses as these substances have a reported repellent effect
189 on *Culicoides* (De Raat et al., 2008; Page et al., 2009). The product also contained piperonyl butox-
190 ide as a synergist. Each animal in the treated group barn was sprayed with the repellent on both
191 sides on the neck, abdomen, flank, back and croup (3 spray bursts of 0.2 ml on each side and loca-
192 tion, respectively 2 spray bursts on the croup) approximately one hour before *Culicoides* collection
193 started. According to the manufacturer's recommendation insecticide treatment should be repeated
194 every second day. As the horses were replaced every night (cross-over design), the repellent was
195 applied only once.

196

197 *2.4 Culicoides collection*

198 *Culicoides* were collected from dusk to dawn using four (two per stable) Onderstepoort Veterinary
199 Institute (OVI) type 8 W, 220 V ultraviolet (UV) down-draught suction traps (Agricultural Research
200 Council, South Africa). Traps were placed 1.5-2.0 m above ground level and insects collected into
201 100 ml beakers containing approximately 50 ml of 70% ethanol. The collected insects were

202 transferred into a polypropylene sample jar and covered with 70% ethanol for storage. Culicoides
203 were classified on the basis of their wing patterns into three groups, obsoletus complex, pulicaris
204 complex and other (Goffredo and Meiswinkel, 2004), and the females segregated as blood-fed (BF)
205 or non-blood-fed (NBF) females, using a stereomicroscope. Culicoides males were excluded from
206 the counts used for data analysis.

207

208 2.5 Statistical analysis

209 Statistical analysis was performed using R, version 3.0.2., (R core team, 2014) in two stages:
210 Kruskal-Wallis tests were used to find effects of the protection systems on Culicoides counts
211 separately within each housing system. These tests were performed separately for the number of BF
212 and female NBF Culicoides and for the total number. In case of a significant result, pairwise
213 comparisons using Wilcoxon rank sum tests were performed and Bonferroni-Holm adjusted exact P
214 values were calculated. Possible differences between the two stables used per housing system were
215 tested using Wilcoxon rank sum tests.

216

217 The above analysis strategy does not take advantage of the paired nature of the data: for every
218 Culicoides count obtained for a particular night, a control measurement from the control stable was
219 available for the same night. Therefore, to quantify the efficacy of the protection in comparison to
220 the control, the number of collected Culicoides in the control stable was subtracted from the number
221 of collected Culicoides in the corresponding protected stable and exact nonparametric 95%
222 confidence intervals for the median of the so-defined differences were computed. The efficacy of
223 the protection systems was compared within each housing system using Kruskal-Wallis tests (BX,
224 three protection systems fan, net and fan with net) or Wilcoxon rank sum tests (BP and GR, two
225 protection systems, fan and net). The significance of stable effects was tested with exact Wilcoxon
226 rank sum tests. Results were considered significant if $P < 0.05$.

227

228 **3. Results**

229 *3.1 Culicoides population and environmental conditions*

230 A total of 79 895 female *Culicoides* in 384 light trap collections on 96 trap-nights (on 92 calendar
231 nights, four of which were used for two protocols) during 3 months in 2012 and 2013. The repellent
232 was used in 2012 only, as no significant difference in the number of BF or NBF *Culicoides* was
233 found for the repellent protection system compared to the control (exact Wilcoxon signed rank test,
234 $P = 0.580$ for BF, $P = 0.380$ for NBF). For all further calculations and discussion in the present
235 study, the repellent treated systems and the corresponding control systems are excluded (reducing
236 the overall total to 52 139 insects in 84 trap-nights).

237

238 The total number of *Culicoides* collected per night and stable for the control stables ranged from 9
239 to 3060 (median 146; mean 477). The total nightly number of *Culicoides* collected in the treated
240 stables ranged from 3 to 1008 (median 61; mean 144). Of the 52 139 *Culicoides* collected, only
241 2038 (3.9%) were BF, and the number of trapped BF *Culicoides* never exceeded 163 (median 5;
242 mean 12). The total *Culicoides* count varied considerably with year of collection. In June 2012
243 (2013, respectively), 29 (68) *Culicoides* were collected per night and stable on average, compared
244 to 304 (521) in July and 707 (166) in August.

245 Microscopic identification of the *Culicoides* revealed that 96% belonged to the *obsoletus* complex
246 and 4% to the *pulicaris* complex.

247

248 Weather data showed the highest precipitation in June 2012 and the highest mean value of wind
249 speed (7.2 km/h) in July 2012 (MeteoSchweiz, 2014). The mean temperature and humidity inside
250 the housing systems in 2012 were: BX 20°C, 74%, GR 17°C, 83%, BP 17°C, 84% and for 2013
251 BX 20°C, 71%, GR 21°C, 71%, BP 24°C, 72%. The mean temperature and humidity in the netted

252 stables compared to the untreated housing systems never differed by more than 0.7°C and 1%,
253 respectively.

254

255 3.2 Protection system efficacy

256 In Table 1, the different protection systems are compared against all controls (overall analysis),
257 whereas in Table 2 (analysis of the differences), they are compared only with matching controls
258 (detailed efficacy comparisons).

259

260 3.2.1. Overall analysis

261 The description and Kruskal-Wallis test results of the numbers of total female, female NBF and BF
262 Culicoides of both experimental years are shown in Table 1, the total distribution is shown in Fig. 1.

263

264 3.2.1.1. Total Culicoides

265 The net protection system gave the lowest observed median number of Culicoides in all three
266 housing systems (Table 1). The difference of the net to the control and to the fan was significant in
267 the BP and GR housing, whereas the Kruskal-Wallis-Test was not significant for the BX. No
268 significant difference between the fan and the control could be found in all three housing systems
269 (Table 1).

270

271 3.2.1.2. Female non-blood-fed Culicoides

272 The female NBF Culicoides counts were close to the total Culicoides counts (Table 1).

273

274 3.2.1.3. Blood-fed Culicoides

275 In every housing system, all protection systems give lower observed median numbers of BF
276 Culicoides than the control (Table 1). In the BX housing, the fan and the net significantly differed

277 from the control (but not from each other), while the fan with net was not different from the control
278 (Table 1). In the BP housing, the net gave the lowest observed BF Culicoides count, followed by the
279 fan and the control; all these differences were significant. In the GR housing, only the difference
280 between net protection and the control was significant (Table 1).

281

282 3.2.1.4. Stable differences

283 Although the cross-over design to balance out stable effects was used, it should be noted that a
284 significant difference (Wilcoxon rank sum test, $P = 0.043$) in the number of BF Culicoides between
285 the two stables used in the GR housing system was found. Detailed examination showed that one of
286 the stables only had a median BF Culicoides count of 7 and the other of 3. No other significant
287 stable effects were found.

288

289 3.2.2. Analysis of the Culicoides count differences

290 To quantify protection system effectiveness, 95% confidence intervals for the median of the
291 difference defined as “difference = protection – control” were computed for every housing and
292 protection system (Table 2) and plotted for the total count (Fig. 2). In case of a significant reduction
293 of the number of Culicoides, the entire confidence interval falls below zero.

294

295 3.2.2.1. Total Culicoides

296 In the BX housing, only the net significantly reduced the number of collected Culicoides (Table 2);
297 with a confidence of 95%, the median reduction by the net was 10.0 - 131.5 Culicoides. No
298 significant effect of the fan or the fan with net was demonstrated. In the BP housing, only the net
299 significantly reduced the median Culicoides number, no significant effect of the fan could be
300 established. In the GR housing, both the net and the fan significantly reduced the median number of
301 Culicoides. The higher variability of the data for the BP and the GR housing systems compared to
302 the BX housing system is mirrored in the wider confidence intervals. A significant difference

303 between the efficacies of the protection systems was only found in the BX housing system. This is
304 not a contradiction to the above findings: first it is tested whether the confidence intervals contain
305 zero, i.e. whether each protection on its own is effective. A second aspect is addressed by testing
306 how much the distributions overlap for different protection systems.

307

308 3.2.2.2. *Female non-blood-fed Culicoides*

309 The results and comparisons determined are presented in Table 2.

310

311 3.2.2.3. *Blood-fed Culicoides*

312 The number of BF Culicoides collected was generally much smaller than for the NBF counts. In the
313 BX housing only the net provided a significant median reduction of BF Culicoides (Table 2). In the
314 BP housing both the net and the fan significantly reduced median BF Culicoides numbers (Table 2).
315 In the GR housing only the fan significantly reduced median BF Culicoides numbers (Table 2).
316 Differences between protection systems were only significant in the BX housing, as above.

317

318 3.2.2.3. *Stable effects*

319 In the BX housing no significant effects of the stables on the differences calculated were found
320 ($P > 0.189$ for all three differences). In the BP housing a significant effect of the stable on the
321 differences was found for the total and the NBF differences (total: $P = 0.017$, NBF: $P = 0.021$), but
322 not for the BF difference ($P = 0.173$). In the GR housing the stable had a significant effect on all
323 three differences (all $P < 0.012$).

324

325

326

326

327 **4. Discussion**

328 Based on the raw Culicoides counts, the net provided the best overall protection with regard to total
329 number of female Culicoides, female NBF and BF Culicoides. The reduction (compared to the
330 untreated control) was significant, with the exception of the total and the NBF insect counts in the
331 BX housing system, and observed in particular for the BF Culicoides in all three housing systems.
332 The confidence intervals computed for the differences to the respective control stables confirmed
333 the efficacy of the net for every housing system, except for the BF Culicoides count in the GR
334 housing where the fan provided better protection than the net. The fan was more effective in the GR
335 housing system than for the other housing systems.

336

337 The net used was densely meshed so that it was not considered necessary to apply any additional
338 repellent to the net. The use of repellent-treated nets or untreated nets to protect stabled horses was
339 reported to be an efficient protection system (Meiswinkel et al., 2000). In contrast, Rohrmann
340 (2009) did not reduce the number of Culicoides trapped by using an insecticide-treated net with a 2
341 mm mesh size, nor did Geerike (2010) in calf stables. Larger mesh sizes may also not allow
342 sufficient contact with insecticide used (Del Río et al. 2014). Porter (1959) reported a strong
343 reduction of Culicoides by using a fine mesh of < 1 mm pore size, where meshes with pore areas of
344 1.6 mm^2 resulted in 56% reduction and smaller pore areas of 0.9 mm^2 in even better, 95% reduction.
345 In the present study, with a pore area of 0.1825 mm^2 , the mean of the total number of Culicoides
346 under net protection was reduced by 67% in the housing system BX, 98% in the housing system BP
347 and 86% in the housing system GR, compared with the respective control measurements. These
348 results need also to be interpreted with regard to the month the housing system was tested: In June
349 (BX) the lowest Culicoides counts were detected, whereas in the months July (BP) and August
350 (GR) higher Culicoides counts were recorded.

351

352 The temperatures and humidity recorded inside the net-protected housing systems never differed
353 greatly from the controls and the horses showed no signs of discomfort. In contrast, experiences
354 during the BT outbreak in Switzerland have shown that ventilation was a problem in netted stables
355 of reproduction centres, as the steers started to sweat (Perler, personnel communication). From a
356 practical aspect, to move the horses into the netted-tent in the GR housing system, two persons were
357 required, at least until the horses were used to the tent. Once inside, the horses tolerated their
358 environment very well. The use of fine-meshed net overall offered excellent mechanical protection,
359 though the use of nets in bigger housing systems can be difficult to set up, and it is recommended
360 that nets should not be installed too tightly, especially in windy locations.

361

362 The larger 0.3 mm mesh placed on UV black light traps by Page et al. (2014) brought about a
363 significant reduction in both the number of Culicoides in treated and untreated conditions. This
364 indicates that it may be advantageous to use nets with a finer mesh size than to use treated nets with
365 a greater mesh size, with consideration of the effect of mesh size on ventilation of the housing
366 system. In OIE regulations it is recommended that openings should be vector-screened with mesh of
367 appropriate gauge impregnated regularly with an approved insecticide according to the
368 manufacturers' instruction, to protect animals from Culicoides attack (World Organisation for
369 Animal Health, 2014). Considering the results of Page et al., (2014) and of the present study, the
370 effect of the more finely meshed net, either untreated or treated with insecticide or repellent, on
371 AHSV sero-conversion rates requires further investigation.

372

373 The fan resulted in a significantly lower BF Culicoides count than the combined control
374 measurements in all three housing systems. The confidence intervals calculated suggest that the fan
375 provides an effective protection for the GR housing system with respect to all three Culicoides
376 categories, and additionally for the BF differences in the BP system. As in the GR housing system

377 no significant difference between the efficacy of the fan and the net could be established for any of
378 the three *Culicoides* categories, the fan can specifically be recommended with regard to practicality,
379 because installation of a net tent in GR housing systems is time and labour intensive. Furthermore,
380 *C. obsoletus* was reported to be highly exophilic (Anderson, 1993) and in rural areas with group
381 housing systems, where stabling at night may not be an option, easily applicable and effective
382 protection methods such as fans are advantageous, although horses may need some time to
383 acclimatise to their use. *Culicoides* are relative poor flyers, therefore directed air movement from
384 fans may make it difficult for them to enter stables, to land on the animal and/or to stay immobile
385 long enough to feed. Another advantage of fans may be that it will disperse the odour plumes from
386 animals and make it difficult for blood feeding insects to locate the host. Meiswinkel et al., (2000)
387 did not report a significant reduction in *Culicoides* when using ceiling fans inside stables, indeed the
388 total number of *Culicoides* in the stables was higher when the fans were operated. Likewise, the
389 same observation was made in the present study where the closed housing system BX showed
390 higher total and female NBF *Culicoides* numbers than the control when protected with a fan,
391 although this difference was not significant. Additionally, Meiswinkel et al. (2000) could not
392 reduce the number of *Culicoides* trapped by using ceiling fans alone or in combination with various
393 layers of gauze net. However, it may be an advantage when a net keeps *Culicoides* trapped inside a
394 stable, in particular *Culicoides* potentially infected with AHSV, to avoid onward transmission of
395 virus.

396

397 The protection system fan with net, used exclusively for the housing system BX, had no significant
398 reduction on any *Culicoides* count. To achieve a positive pressure in the stable compared to the
399 exterior the housing system should be tightly sealed and closed, for example with a double door
400 entry system. Shutting the doors and windows only, as it was done in the present study, seems to be
401 insufficient.

402

403 A limitation of the present study is that the female Culicoides were not segregated into nulliparous,
404 parous, and gravid (Dyce 1969). Nevertheless, significant differences were detected in the total
405 number and number of BF Culicoides for the various protection systems and are of practical use to
406 formulate scientific recommendations for equine holdings.

407

408 The repellent applied to the horses in 2012 did not prove to be effective in reducing the number of
409 Culicoides collected in the light traps in all three housing systems. In fact, it appeared to increase
410 the number of Culicoides collected in the traps. The most likely reason for these results is that the
411 relatively strong attractant effect of the UV black light trap was not counteracted by the repellent
412 applied to the horse (Page et al. 2009; Venter et al., 2009). Similarly, a recent study with repellent/
413 insecticide applied to mesh placed on a UV light trap by Page et al., (2014) did not demonstrate a
414 significant repellent effect against Culicoides. However, good results with a permethrin-treated net
415 were shown by Griffioen et al., (2011) where the number of Culicoides trapped near sheep was
416 reduced by 50% when Culicoides were collected with a mechanical aspirator. De Raat et al., (2008)
417 also achieved a reduction in the number of Culicoides trapped near horses using insecticide with
418 permethrin and mechanical aspirator to collect the insects. The cited studies support the use of a
419 mechanical aspirator as a more reliable method to evaluate experimental treatment applied to
420 animals, or protective mesh (Mullens, et al., 2010).

421

422 Compared to South African conditions, the abundance of Culicoides appears to be relatively much
423 lower in Switzerland. For example, Venter et al., (2012) collected a total of 194 684 Culicoides in
424 32 light traps on 8 consecutive nights in autumn. This is in contrast to the 79 895 female Culicoides
425 which were collected in 4 light traps in 96 light trap-nights in the present study. It has been shown
426 that vector density is a more important indicator for establishment and spread of disease agents,
427 such as AHSV, than host density (Backer and Nodelijk, 2011). However, in areas with a high horse
428 density, AHSV is more likely to spread to a neighbouring herd. In Switzerland, areas with the

429 highest density of equids are the cantons Geneva (18.5 equids per km² land use), Ticino (16.9/km²),
430 Zurich (15.8/km²) and Jura (14.7/km²) (Schmidlin et al., 2013). It is postulated that these cantons
431 are at the highest risk for potential spread of AHSV and that in these areas infected horse herds
432 should be kept in meshed housing to prevent virus-infected Culicoides from escaping in the event of
433 an outbreak.

434 According to the Swiss law, AHS is classified as an infectious disease and official measures in case
435 of a suspicion or an outbreak of the disease are described. For example, the application of a
436 movement ban to and from an infected farm and the establishment of a protection zone 100 km
437 around the farm would be applied. In endemic areas, AHS is primarily controlled by vaccination
438 with live attenuated polyvalent AHS vaccines (Coetzer and Guthrie 2004). These are generally
439 prohibited in Switzerland, but might be used in a strictly controlled manner in the event of an
440 outbreak (Sánchez-Vizcaíno, 2004). As has been highlighted before, it is thus important that
441 additional methods to prevent or reduce contact between Culicoides and horses will be investigated
442 (Carpenter et al., 2008). The present results indicate that "mechanical" systems (fans and netting)
443 might be sufficient to control/reduce biting rates. This finding can be highlighted by mentioning
444 that there are increasing concerns that the impact of chemicals on the environment and coupled with
445 insecticide resistance in the insects will result in a decline in the number of agents available for
446 livestock pest management. Furthermore, with some adjustments, the protection systems and
447 principals involved in the present study can be applied to protect other farm animals against the
448 attacks from Culicoides.

449

450 **5. Conclusion**

451 Although the risk of an AHS outbreak in Switzerland was estimated to be low (Zimmerli et al.,
452 2010), the increasing importance of Culicoides in northern Europe as potential vectors of pathogens
453 has been emphasized (Carpenter et al., 2008). Advance preparation could help to reduce the impact

454 of an AHS disease outbreak and keep as many horses as possible free of disease during an outbreak.
455 The results of the present study showed that owners can substantially reduce their horses' exposure
456 to Culicoides that could potentially carry AHSV in the event of an outbreak by net protection in the
457 housing systems BX, BP and GR. In GR housing systems, which are distant from neighbouring
458 horse farms, Culicoides protection using a fan can also be recommended with regard to both
459 efficacy and practicality.

460

461

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468

469 **Conflict of Interest Statement**

470 No conflict of interest is declared.

471

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617

618 **Tables**

619 **Table 1:** Median (interquartile range) number of Culicoides from all experiments collected by four
620 black light traps operated from dusk to dawn for four (2012) and eight nights (2013) in three
621 housing systems and three protection systems in Switzerland.

622

623 **Table 2:** 95% confidence intervals for the median of the difference (protection – control) of the
624 number of Culicoides collected by four black light traps operated from dusk to dawn for four (2012)
625 and eight nights (2013) in three housing systems and three protection systems in Switzerland.

626

627 **Figures**

628 **Fig. 1:** Boxplots of total Culicoides count collected by four black light traps operated from dusk to
629 dawn for four (2012) and eight nights (2013) in Switzerland, by housing and protection system.

630

631 **Fig. 2:** Graphical presentation of 95% confidence intervals for the median difference
632 (protection – control) of the total number of Culicoides collected by four black light traps operated
633 from dusk to dawn for four (2012) and eight nights (2013) in three housing systems and three
634 protection systems in Switzerland.

635

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Editorial Board
VETERINARY PARASITOL-
OGY

7. April 2015

**Protection of horses against Culicoides biting midges in different housing systems
Switzerland**

661 **Highlights**

- 662 • We examined 3 protection systems against Culicoides using light traps
- 663 • We assessed the efficacy of a net, fan and fan with net in 3 equine housing systems
- 664 • Net protection was effective in all tested housing systems in reducing Culicoides number
- 665 • In outdoor group housing the fan was also protective against Culicoides

666

667

668

Table 1: Median (interquartile range) number of Culicoides from all experiments collected by four black light traps operated from dusk to dawn in four (2012) or eight nights (2013) in three housing systems and three protection systems in Switzerland.

Feeding status of female Culicoides	Housing system	Protection system			Control	P value
		Fan	Net	Fan with net		
Non-blood-fed and blood-fed (NBF, BF)	Box (BX)	59 (83)	19 (30)	65 (77)	51 (61)	0.121
	Box, Paddock (BP)	262 (134) a	11 (11) b	n.a.	467 (834) a	< 0.001
	Group (GR)	415 (262) a	26 (59) b	n.a.	409 (738) a	< 0.001
Non-blood-fed (NBF)	Box (BX)	56 (79)	18 (30)	62 (66)	48 (55)	0.172
	Box, Paddock (BP)	254 (138) a	11 (10) b	n.a.	434 (826) a	< 0.001
	Group (GR)	410 (263) a	26 (55) b	n.a.	400 (728) a	< 0.001
Blood-fed (BF)	Box (BX)	2 (3.75) b	2.5 (3.0) b	3 (9.0) a, b	5 (9.5) a	0.002
	Box, Paddock (BP)	8 (5.75) b	1 (5.0) c	n.a.	24 (34.3) a	< 0.001
	Group (GR)	5 (3.75) a, b	1 (3.75) b	n.a.	9.5 (10.75) a	0.017

For each protection system, the medians are based on twelve observations for each housing system (the fan with net was only used in the box, n.a. = not applied). The control medians are based on 36 (BX housing) or 24 (BP, GR) observations. The rightmost column contains the P value of a Kruskal-Wallis test to find differences between protection systems. Only in case the result was significant, Wilcoxon rank sum tests were then used for pairwise group comparisons. Due to ties, exact P values were computed and subsequently adjusted for multiple testing with the Bonferroni-Holm method. Protection groups within each row sharing a letter did not differ significantly.

Table 2: 95% confidence intervals for the median of the difference (protection – control) of the number of *Culicoides* collected by four black light traps operated from dusk to dawn for four (2012) and eight nights (2013) in three housing systems and three protection systems in Switzerland.

Feeding status of female <i>Culicoides</i>	Housing system	Protection system			P value
		Fan	Net	Fan with net	
Non-blood-fed and blood-fed (NBF, BF)	Box (BX)	[-11.5, 41.0]	[-131.5, -10.0]	[-17.0, 19.0]	0.012
	Box, Paddock (BP)	[-914.0, 155.5]	[-1453.5, -215.0]	n.a.	0.347
	Group (GR)	[-1455.0, -215.0]	[-586.0, -82.0]	n.a.	0.590
Non-blood-fed (NBF)	Box (BX)	[-8.0, 41.5]	[-109.0, -4.5]	[-12.0, 15.5]	0.015
	Box, Paddock (BP)	[-862.0, 159.5]	[-1353.0, -212.0]	n.a.	0.319
	Group (GR)	[-1426.5, -60.5]	[-579.5, -81.0]	n.a.	0.561
Blood-fed (BF)	Box (BX)	[-7.0, 1.0]	[-23.5, -5.0]	[-8.0, 5.5]	0.042
	Box, Paddock (BP)	[-37.0, -2.5]	[-78.0, -5.0]	n.a.	0.370
	Group (GR)	[-22.0, -2.0]	[-10.5, 2.5]	n.a.	0.484

The rightmost column contains the P value of a Kruskal-Wallis test (for the Box) or of an exact Wilcoxon rank sum test (for Box with paddock and Group) to find differences between the effectiveness of the protection systems. The fan with net was only used in the Box housing system.

n.a. = not applied.



