Veterinary Parasitology 210 (2015) 206–214

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

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

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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 8 April 2015
Accepted 10 April 2015

Keywords:
Vector protection
Fan
Net
Repellent
African horse sickness

ABSTRACT

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

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http://dx.doi.org/10.1016/j.vetpar.2015.04.006
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1. Introduction

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

African horse sickness outbreaks still regularly occur in parts of Africa, particularly southern Africa (Guthrie and Quan, 2009; Akakpo et al., 2011; Diouf et al., 2013), but have not been observed north of the Sahara since 1991 (Zimmerli et al., 2010). There are no Culicoides-free areas in any of the agriculturally utilized areas of Switzerland (Kaufmann et al., 2009). Although the main implicated vector of AHSV, *C. imicola*, does not occur in Switzerland (Mellor et al., 1990; Casati et al., 2009), it is known that species of the Culicoides complexes, obsoletus and pulicaris, which are potential vectors of both BTV and AHSV are widely distributed in Switzerland (Racloz et al., 2008; Kaufmann et al., 2012). In addition to their emerging role as vectors, Culicoides are also considered nuisance pest in many parts of the world, and that they can cause insect bite hypersensitivity ('sweet itch'), particularly in horses (Anderson et al., 1996). These Culicoides also transmit a second orbivirus of horses, equine encephalosis virus (Guthrie et al., 2009). African horse sickness virus could potentially be imported into Switzerland by movements of infected equines (Mellor, 1993). In addition, virus-infected Culicoides might be introduced by different means, for example by air transport (DEFRA, 2012). Though Culicoides are relative poor flyers, and actively travel only a few kilometres, they can be carried over long distances by wind (Sellers et al., 1977), in some cases up to 700 km (Boinas et al., 2009). Pérez et al. (2006) reported one Culicoides in 10 mg/m^2 of dust deposited by the wind. Global climate change may shift the geographical distribution of equine diseases and their vectors (Timoney et al., 2007). Climate-driven changes in size and activity of specific vector populations as well as replication rate of the virus in the vector can enable virus transmission to become sustainable in previously non-endemic areas (Khasnis and Nettleman, 2005; Haines et al., 2006; Herholz et al., 2006; Gould and Higgs, 2009). In Switzerland, the AHSV would move into a completely naïve horse population (Zimmerli et al., 2010). An outbreak of a highly lethal horse disease, such as AHS, would have serious animal welfare and economic consequences for the horse industry and for national and international equine movements and trade in non-endemic regions (Herholz et al., 2008).

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

This study aimed to compare the effects of net, fan and repellent protection systems, or combinations thereof, with regard to their potential to reduce contact between horses and Culicoides in different equine housing systems including boxes, group housing, and boxes with paddock. The study focused on the efficacy as well as practicality of the protection systems with the aim of improving recommendations for protection systems against Culicoides in equine holdings in Switzerland and other countries with similar housing systems.

2. Materials and methods

2.1. Housing systems and study design

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

The BX housing consists of four indoor boxes for one horse of approximately 12 m^2. Four horses were housed in each of the treated and the control stable during the experiment. The BP housing (box area: 12 m^2) was constructed for individual horses with permanently accessible paddocks of 13 m^2 each. A total of four BPs were under one roof, separated by a central hallway. Two BP systems, on each side of the central hallway, were used for the treated and the control group, which comprised two horses each. The GR housing consisted of a tent (24 m^2) and a paddock of approximately 40 m^2 for two horses each. The two GR housing systems were located approximately 3 km from the other housing systems.

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

2.2. Horses

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

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

2.3. Protection systems

2.3.1. Net

The polypropylene nets (Ultravent® Bemisia TIP 250, Texinov, France) had a micrometric mesh of 250 μm × 730 μm (0.1825 mm^2) for protection against insects in accordance with the ISO 9001 2008 standard. Net specifications were mass 52 g/m^2, tensile strength in the machine direction 380 daN/m and 250 daN/m in the transverse direction, 6% light reduction and 49% reduction in air permeability. In the BX housing, the net covered the four open windows and one door while the other door remained closed. In the BP housing, nets were suspended along the hallway adjacent to the box and suspended on wooden frames to cover the paddocks. In the GR housing, a festival tent frame (60 m^2) (Baumann SA, Cudrefin, Switzerland) was used to cover the paddock with the net. All entrances to the three housing systems were cross-covered by the net to facilitate access of personnel and horses.

2.3.2. Fan

Six fans (ZOO No. 1400, BM Haus Agrotech, Switzerland) were used. Each 1300 mm × 1380 mm adjustable fan had six belt-driven chrome steel propeller blades encased in protective housing. Fans had a power consumption of 0.75 kW, circulated 39,000 m^3 of air per hour, with wind speed 10–15 km/h generated.

In the treated BX, one fan was placed in each of two diagonally opposite windows (four windows in total). The two fans were suspended outside the windows facing inwards to blow the air into the stable. The two windows without fans were left open at all times. In the treated BP housing, one moveable fan was positioned outside the paddock, facing into the paddock, while one fan was suspended in the hallway facing the boxes. In the treated GR housing, two moveable fans were placed outside the paddock, facing into the paddock. None of the fans positioned outside the BP and GR housing blew directly onto the light traps and depending on their position not directly onto the horses.

2.3.3. Fan with net

This protection system was applied to the BX housing system only. The fan fronts were covered with a net with a pore size of 0.1825 mm^2 (Ultravent® Bemisia TIP 250, Texinov, France) to prevent midges, along with dust and other particles, from being sucked by the airflow into the stable. All other stable openings were closed between dusk and dawn and the windows were covered with nets on the inside.

2.3.4. Repellent

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

2.4. Culicoides collection

Culicoides were collected from dusk to dawn using four (two per stable) Onderstepoort Veterinary Institute (OVI) type 8 W, 220 V ultraviolet (UV) down-draught suction traps (Agricultural Research Council, South Africa). Traps were placed 1.5–2.0 m above ground level and insects collected into 100 ml beakers containing approximately 50 ml of 70% ethanol. The collected insects were transferred into a polypropylene sample jar and covered with 70% ethanol for storage. Culicoides were classified on the basis of their wing patterns into three groups, obsoletus complex, pulicaris complex and other (Goffredo and Meiswinkel, 2004), and the females segregated as blood-fed (BF) or non-blood-fed (NBF) females, using a stereomicroscope. Culicoides males were excluded from the counts used for data analysis.

2.5. Statistical analysis

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

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

3. Results

3.1. Culicoides population and environmental conditions

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

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

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

Weather data showed the highest precipitation in June 2012 and the highest mean value of wind speed (7.2 km/h) in July 2012 (MeteoSchweiz, 2014). The mean temperature and humidity inside the housing systems in 2012 were: BX 20 °C, 74%, GR 17 °C, 83%, BP 17 °C, 84% and for 2013 BX 20 °C, 71%, GR 21 °C, 71%, BP 24 °C, 72%. The mean temperature and humidity in the netted stables compared to the untreated housing systems never differed by more than 0.7 °C and 1%, respectively.

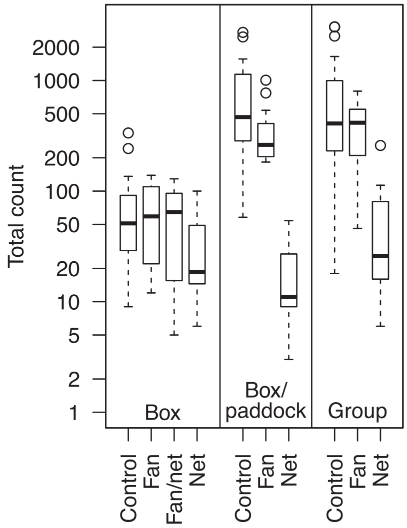

Fig. 1. Boxplots of total Culicoides count collected by four black light traps operated from dusk to dawn for four (2012) and eight nights (2013) in Switzerland, by housing and protection system.

3.2. Protection system efficacy

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

3.2.1. Overall analysis

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

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

3.2.1.2. Female non-blood-fed Culicoides. The female NBF Culicoides counts were close to the total Culicoides counts (Table 1).

3.2.1.3. Blood-fed Culicoides. In every housing system, all protection systems give lower observed median numbers of BF Culicoides than the control (Table 1). In the BX housing, the fan and the net significantly differed from the control (but not from each other), while the fan with net was not different from the control (Table 1). In the BP housing, the net gave the lowest observed BF Culicoides count,

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.

followed by the fan and the control; all these differences were significant. In the GR housing, only the difference between net protection and the control was significant (Table 1).

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

3.2.2. Analysis of the Culicoides count differences

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

3.2.2.1. Total Culicoides. In the BX housing, only the net significantly reduced the number of collected Culicoides (Table 2); with a confidence of 95%, the median reduction by the net was 10.0–131.5 Culicoides. No significant effect of the fan or the fan with net was demonstrated. In the BP housing, only the net significantly reduced the median Culicoides number, no significant effect of the fan could be established. In the GR housing, both the net and the fan significantly reduced the median number of Culicoides. The higher variability of the data for the BP and the GR housing systems compared to the BX housing system is mirrored in the wider confidence intervals. A significant difference between the efficacies of the protection systems was only found in the BX housing system. This is not a contradiction to the above findings: first it is tested whether the confidence intervals contain zero, i.e. whether each protection on its own is effective. A second aspect is addressed by testing how much the distributions overlap for different protection systems.

3.2.2.2. Female non-blood-fed Culicoides. The results and comparisons determined are presented in Table 2.

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 Culicoides	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.

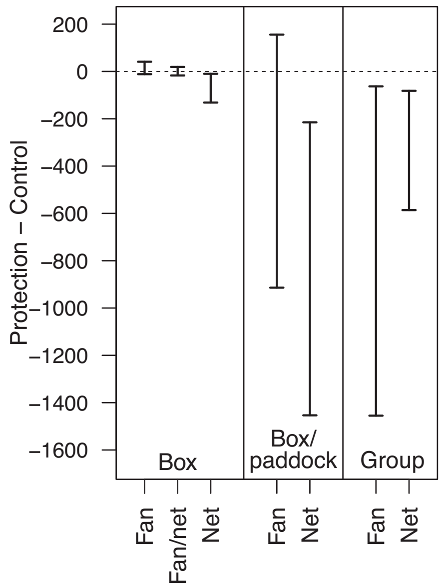

Fig. 2. Graphical presentation of 95% confidence intervals for the median difference. (protection–control) of the total number of Culicoides collected by four black light traps operated from dusk to dawn for four (2012) and eight nights (2013) in three housing systems and three protection systems in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

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

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

4. Discussion

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

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

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

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

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

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

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

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

Compared to South African conditions, the abundance of Culicoides appears to be relatively much lower in Switzerland. For example, Venter et al. (2012) collected a total of 194,684 Culicoides in 32 light traps on 8 consecutive nights in autumn. This is in contrast to the 79,895 female Culicoides which were collected in 4 light traps in 96 light trap-nights in the present study. It has been shown that vector density is a more important indicator for establishment and spread of disease agents, such as AHSV, than host density (Backer and Nodelijk, 2011). However, in areas with a high horse density, AHSV is more likely to spread to a neighbouring herd. In Switzerland, areas with the highest density of equids are the cantons Geneva (18.5 equids per km^2 land use), Ticino ($16.9\,km^{-2}$), Zurich ($15.8\,km^{-2}$) and Jura ($14.7\,km^{-2}$) (Schmidlin et al., 2013). It is postulated that these cantons are at the highest risk for potential spread of AHSV and that in these areas infected horse herds should be kept in meshed housing to prevent virus-infected Culicoides from escaping in the event of an outbreak.

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

5. Conclusion

Although the risk of an AHS outbreak in Switzerland was estimated to be low (Zimmerli et al., 2010), the increasing importance of Culicoides in northern Europe as potential vectors of pathogens has been emphasized (Carpenter et al., 2008). Advance preparation could help to reduce the impact of an AHS disease outbreak and keep as many horses as possible free of disease during an outbreak. The results of the present study showed that owners can substantially reduce their horses' exposure to Culicoides that could potentially carry AHSV in the event of an outbreak by net protection in the housing systems BX, BP and GR. In GR housing systems, which are distant from neighbouring horse farms, Culicoides protection using a fan can also be recommended with regard to both efficacy and practicality.

Conflict of interest statement

No conflict of interest is declared.

Acknowledgements

We thank the Federal Food Safety and Veterinary Office for funding of the study. Furthermore, we thank Mr Samuel Schär and Mr Jean-Pierre Musy for their work and assistance during the experiments at the Swiss National Stud, as well as all horse owners for their co-operation and Christian Kaufmann and Ms Jeannine Hauri, from the Institute of Parasitology, University of Zurich, for their assistance in counting and identifying the Culicoides.

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