

Paper

Repellent effect of topical deltamethrin on blood feeding by *Culicoides* on horses

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African horse sickness (AHS) is a vectorborne disease spread by *Culicoides* biting midges. The UK's Department for Environment, Food and Rural Affairs currently suggests using topical deltamethrin for AHS control; however, no data are available regarding its efficacy in the horse. The aims of this study were to investigate the effect of topical deltamethrin on blood feeding by *Culicoides* on horses and to investigate which *Culicoides* species blood fed on horses. Three pairs of horses were placed in partially enclosed cages that allowed samples representing the *Culicoides* interacting with individual horses to be sampled. Four data collection sessions were completed before one horse from each pair was topically treated with 10 ml of 1 per cent deltamethrin solution and another four sessions were then carried out. Collected *Culicoides* were identified and each biting midge examined to see if it had blood fed. The most abundant species collected were *C. chiopterus*, *C. dewulfi*, *C. obsoletus* and *C. scoticus* (44.3 per cent) and either *C. pulicaris* or *C. punctatus* (34.7 per cent). These species were also more likely to have blood fed than other species, supporting their potential role as AHS vectors if the virus were to reach the UK. There was no significant effect of treatment on blood feeding by *Culicoides*. The results do not support the use of topical deltamethrin to prevent blood feeding by *Culicoides* on individual horses; however, the study does not investigate the effect that the widespread use of topical deltamethrin might have on vector numbers or disease transmission from viraemic individuals during an outbreak of AHS.

Culicoides biting midges are among the world's smallest and most widespread blood-sucking insects, with over 1400 different species identified worldwide (Borkent and others 1997, Mellor and others 2000). Their primary importance to horses is as triggers for insect bite hypersensitivity (IBH) and vectors of infectious viral diseases, including African horse sickness (AHS). AHS is an infectious, non-contagious, viral disease of equids that is associated with mortality rates of up to 95 per cent in naive populations (Mellor and others 2004). The disease is endemic in sub-Saharan Africa, but there have been a small number of epizootics in northern Africa, southern Europe and western Asia (Howell 1960, Gohre and others 1965, Rodriguez and others

1992, Mellor and others 2004, Diouf and others 2013). Historically, the risk to the UK was considered to be very low due to the presumed lack of suitable species of biting midge vector; however, outbreaks of bluetongue (BT) in areas of Europe where the traditional vector was not present challenged this idea. BT virus is closely related to AHS virus and shares the same vector species of *Culicoides* in areas where both diseases are endemic. Before 2006, BT had never occurred in northern Europe, where an important vector species, *Culicoides imicola*, is not present. However, there is substantial evidence that Palaearctic *Culicoides* species including *C. pulicaris*, *C. punctatus* and members of the *Avaritia* subgenus (*C. dewulfi*, *C. obsoletus*, *C. scoticus* and *C. chiopterus*) acted as vectors of BT virus in northern Europe from 2006 (Mellor and others 1990, Mehlhorn and others 2007, Hoffmann and others 2009). These species are therefore considered potential vectors for AHS in the UK. This theory is supported by the fact that African horse sickness virus serotype-4 (AHSV-4) was isolated from non-*C. imicola* mixed pools (containing mainly *C. pulicaris* and *C. obsoletus*) during the 1987–1991 outbreak in Spain (Mellor and others 1990).

In the event of an AHS outbreak, the most effective way of bringing the disease under control is vaccination, which was used successfully in Spain during 1987–1991. Prevention of vector–host interaction is also a key aspect of disease control, and there are several methods described to prevent *Culicoides* from biting horses. These include the removal or insecticidal treatment of midge breeding habitats, treatment of livestock or housing with insecticide to kill adult *Culicoides*, housing of livestock to physically prevent interaction, use of repellents on livestock to prevent interaction and use of kairomones to lure and kill adult *Culicoides* (Carpenter and others 2008a). The only truly

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effective control method for IBH is the complete prevention of interaction between horses and *Culicoides*, which has been known for over 60 years (Riek 1953, Schaffartzik and others 2012). While moving horses to areas without *Culicoides* would be effective for preventing AHS virus transmission, it is often highly impractical and potentially inappropriate during a disease outbreak. Stabling of horses has been shown to reduce the signs of IBH and has long been used to prevent AHS (Paton 1863, Mellor and others 1974, Meiswinkel and others 2000). Insect blankets with both neck cover and hood have been shown to limit the feeding rate of *Culicoides* on horses, and the authors of this study suggested they might be helpful to protect horses from bites of AHS-infected *Culicoides* (De Jong and others 2012). The use of repellents and insecticides is another possible method of controlling the spread of vectorborne disease, either by reducing the biting rate or the population of adult *Culicoides*. One of the control methods for AHS that has been proposed by the UK's Department for Environment, Food and Rural Affairs (DEFRA) is the topical application of deltamethrin to horses, although they emphasise that the drug is not licensed in the horse, or specifically against *Culicoides* in any species (DEFRA 2012).

The insecticidal susceptibility of *C. obsoletus* to deltamethrin has been investigated in vitro, with 24-hour LD₉₀ values between 0.00105 and 0.00203 per cent demonstrated, consistent with high susceptibility (Venail and others 2011, Del Río and others 2014). One of these studies went on to investigate the efficacy of topically applying 0.75 per cent deltamethrin to the top line of sheep on the mortality rate of *C. nubeculosus*, another Palaearctic species of *Culicoides* (Venail and others 2011). In this case, despite the theoretical applied dose being around 180 times the *in vitro* LD₅₀, the maximum mortality rate of *Culicoides* feeding on the thigh of the sheep was only 45 per cent and results were highly variable. In contrast, the topical application of 0.75 per cent deltamethrin over all exposed skin of shorn sheep completely prevented biting by *C. obsoletus*, another Palaearctic species, although sample sizes in this study were low (Mullens and others 2010). In a study in horses using another pyrethroid (permethrin), there appeared to be a small reduction in the number of blood fed *Culicoides* caught near horses; however, the result was not significant (De Raat and others 2008). The authors could find no data investigating the efficacy of deltamethrin application to horses to reduce blood feeding by *Culicoides*.

The aim of this study was to investigate the effect of the application of topical deltamethrin solution on blood feeding by *Culicoides* on horses. Additionally, the blood feeding of different *Culicoides* species was examined to investigate potential disease vector roles.

Materials and methods

Animals

Horses were recruited in pairs for the investigation and matched as closely as possible for size and colour. The selection criteria used for recruitment were that the horses must be healthy (with no history of any dermatological condition), have a temperament suitable for placement in the cages and not have been treated with any topical insecticide or repellent in the last six months. Six horses were used in total (three pairs), and these were recruited from three sources: The University of Liverpool teaching herd and two private horse owners who provided informed consent for their horses to be used. The horses had an age range of 7–19 years.

Experimental setting

Investigations were carried out at a single site on the University of Liverpool's Leahurst Campus, Wirral, UK. Two galvanised steel cages were designed and commissioned for the study as shown in Fig 1. Each cage was made out of 3 mm iron grille and measured 2.4 m long×1.2 m wide×2.4 m high. This size was

chosen as it allowed adequate enclosure of a horse, without the animal having to touch the sides of the cage. This was felt to be important as contact with the cage could reduce the available biting sites for *Culicoides* and might also be associated with rubbing off of the insecticide onto the equipment. The two longest sides, rear and top of the cage, were solid and fixed in position. The front of the cages was completely open, apart from a rope restraint at the height of the base of the neck. This design was felt to help reduce anxiety in experimental horses and allow rapid and safe exit from the cages if they panicked. The cages were situated approximately 2 m apart on flat ground. Both cages were securely fixed to the ground using 10 mm diameter nylon rope and grounded fittings. The sides and rears of each cage were lined internally with netting of an aperture size <1.5 mm², to act as a barrier to reduce *Culicoides* exiting the cage following interaction with the horse. A single Onderstepoort black light suction trap (Onderstepoort Veterinary Institute, Onderstepoort, South Africa) was hung within each cage, at a position 0.5 m from the rear netting.

All horses were trained to back into the cage and stand for up to 60 minutes prior to commencing trapping sessions. Sessions occurred during a 60-minute period immediately prior to sunset as this has been shown to be the time period when most feeding of *Culicoides* on horses occurs (Van Der Rijt and others 2008). Data collection was carried out from June to September 2013. Hay-nets were provided to ensure that the horses were relaxed. Handlers remained at a distance of 15 m away throughout. The light traps were activated to start collecting *Culicoides* as soon as both horses were positioned in the cages. After 60 minutes, the light traps were deactivated and the horses were removed from the cages. The collected insects were transferred to a preservative solution of 70 per cent ethanol for storage.

All horses were housed under the same conditions and kept at pasture for the duration of the study. Horses participated in the study in pairs, and each pair completed the entire study prior to starting with the next pair. Trapping sessions took place on consecutive evenings, and if no *Culicoides* were caught, an additional session took place the following evening. After four successful sessions (pre-treatment data), one of the horses in each pair was randomly selected for insecticide treatment, using a 1 per cent solution of deltamethrin ('Spot on', Zoetis; Zoetis UK, London, UK). As this product is not licensed in horses, the manufacturer's instructions on dosage and application to cattle were used and 10 ml of solution were applied to a single location on the dorsal midline, just caudal to the withers. At this time, the horses were moved to separate paddocks, situated to prevent any horse-to-horse contact from occurring. Trapping continued for another four successful sessions (post-treatment data). At this time, the pair of horses was deemed to have completed the study. Data were collected from a total of three pairs of horses.

Culicoides were sexed and individual species identified using a dissecting microscope and standard wing pattern identification keys (Mathieu and others 2012). As male *Culicoides* do not feed and are therefore unable to act as AHS virus vectors, they were not recorded. *Culicoides punctatus* and *C. pulicaris* (these two species will be referred to together as *C. pulicaris/C. punctatus*), as well as *C. obsoletus*, *C. scoticus*, *C. chiopterus* and *C. dewulfi*, were classified as potential vector species. All other species were considered non-vectors. A sample of 100 *Culicoides* individuals from the *Avaritia* subgenus (50 blood fed and 50 non-blood fed) were randomly selected and identified using a species-specific PCR assay previously described (Nolan and others 2007). The PCR was used because the females of the four sympatric species of the *Avaritia* subgenus cannot be easily identified using microscopic techniques. These data were obtained to investigate the relative blood feeding rates of the four species.

Engorged/blood fed individuals were identified based on the presence of blood within the abdomen. To establish if the engorged *Culicoides* had fed on horses, 20 randomly selected



FIG 1: Experimental set-up showing a single pair of horses within cages. Light traps are positioned above the horses within the cages, with netting covering two sides and part of rear of cage

engorged vector *Culicoides* were analysed to identify the origin of their blood meal using PCR assays previously described (Garros and others 2011). Blood meal source was molecularly identified using host species primers, including horse as a simplex PCR and cattle/sheep/goat as a multiplex PCR. Universal primers for vertebrates were also used as a control of host DNA quality and quantity.

Prior to completing the main study described above, a preliminary study was carried out to establish that the *Culicoides* caught within each cage were representative of midge interaction with the horse in the cage and not simply the midge population in the environment. The study was set up in the same way as described previously; however, three catches were obtained during each one hour trapping session. One catch was from a single Onderstepoort Veterinary Institute (OVI) light trap situated within a cage that contained a horse; a second was from a single OVI light trap situated in the adjacent, empty cage; and a third catch was obtained from a single OVI trap hung nearby (to act as a control). Trapping took place over four sessions, with one horse used during sessions 1 and 2 and a different horse used during sessions 3 and 4.

Data analysis

Data were analysed using the SPSS software package (V21.0.0.0). The treatment effect of topical deltamethrin on the relative change in both the total *Culicoides* and the percentage of blood fed vectors caught was investigated using paired-sample *t* tests. The percentages of blood fed vectors data were log transformed prior to analysis. The relationship between blood feeding and *Culicoides* species was investigated using Fisher's exact test. The relationship between vector species and blood feeding was investigated using the χ^2 test. Statistical significance was set at <0.05 .

Results

Preliminary study

The results of the preliminary study showed that the number of *Culicoides* caught in the trap situated in the cage containing the horse was much greater than that from the trap in the empty adjacent cage and the control trap (Table 1). From these data, the trap situated in the cage containing the horse appears to represent the *Culicoides* interacting with the horse, rather than a sample of the *Culicoides* present in the environment. The decision was therefore made to continue using this method for the main study, with the percentage of the potential vector species of *Culicoides* within the light-trap catch that had blood fed being used as an indication of the rate of *Culicoides* feeding on the horse within the cage.

Main study

A total of 2534 *Culicoides* were caught over the 24 sessions, giving a mean *Culicoides* catch per session of 105.6 (range 10–587) and a mean catch per cage of 52.8 (range 1–321). The most abundant species were *C. chiopterus*, *C. dewulfi*, *C. obsoletus* and *C. scoticus* (44.0 per cent) and *C. pulicaris/C. punctatus* (34.2 per cent), giving a total potential vector species percentage of 78.3 per cent. Sixteen per cent of vector species and 2.2 per cent of non-vector species caught had blood fed (Table 2). Vector species were significantly more likely to have blood fed than non-vector species ($p<0.001$, $\chi^2=72.889$, degrees of freedom=1).

Ninety-eight per cent of the selected *Avaritia* subgenus *Culicoides* were successfully amplified using PCR (Table 3). *Culicoides dewulfi* (46.9 per cent) and *C. obsoletus* (44.9 per cent) were the most abundant species, with *C. scoticus* (7.1 per cent) and *C. chiopterus* (1.0 per cent) both caught in much lower numbers. The data suggest that *C. obsoletus* was more likely to have blood fed and *C. dewulfi* was less likely to have blood fed.

TABLE 1: *Culicoides* female catches from light suction traps located either in cages (empty or containing a horse) or hung nearby as a control

Location of trap	<i>Culicoides</i> catches											
	Night 1			Night 2			Night 3			Night 4		
	Total	BF	% BF	Total	BF	% BF	Total	BF	% BF	Total	BF	% BF
Cage, with horse	26	3	10.3	10	4	28.6	40	28	41.2	70	40	36.4
Cage, no horse	9	0	0	0	0	0	2	0	0	25	0	0
Control	5	0	0	1	0	0	5	1	16.7	16	0	0
BF, blood fed												

TABLE 2: Total amount of blood fed (BF) and non-blood fed (NBF) vectors (*C. chiopterus*, *C. dewulfi*, *C. obsoletus*, *C. scoticus* and *C. pulicaris/C. punctatus*) and non-vectors (all other species) caught over 24 trapping sessions

Species	Number of <i>Culicoides</i> (%)	
	BF	NBF
Vectors	318 (16.0)	1666 (84.0)
Non-vectors	12 (2.2)	538 (97.8)

There was a significant relationship between blood feeding and individual species of the *Avaritia* subgenus ($p=0.034$).

All 20 of the samples analysed using the blood meal origin species-specific PCR were successfully amplified using the equine-specific primers.

The percentages of vector species caught that had blood fed during each session are shown in Fig 2a–c. The relative change in the percentage of blood fed vectors caught before and after treatment was compared between the treated and untreated horses. The application of deltamethrin had no significant effect on blood feeding ($p=0.862$). The mean treatment effect was 0.94 (representing a 6 per cent reduction in the percentage of blood fed vectors caught after treatment), with the 95% CI from 0.24 (a 76 per cent reduction) to 3.6 (a 260 per cent increase). Deltamethrin application also had no significant effect on the total number of *Culicoides* collected before and after treatment ($p=0.493$).

Discussion

The data did not show a significant treatment effect of topical deltamethrin on the blood feeding of *Culicoides* on horses. The most abundant species collected during the study were the four members of *Avaritia* subgenus and *C. pulicaris/C. punctatus*. These species were also more likely to have blood fed. All engorged *Culicoides* assessed by PCR had blood fed on horses. These data provide important evidence of vector–host interaction and support the theory that these species have the potential to act as AHS vectors.

The implications of the results of the present study for the control of vectorborne disease must be carefully considered. Deltamethrin is a synthetic pyrethroid whose mechanism of action involves interference with sodium channels of *Culicoides* nerve axons that results in delayed repolarisation and paralysis (Casida and others 1983). The application of topical deltamethrin to horses could be expected to have three possible effects on *Culicoides*–horse interaction: a repellent effect that prevents blood feeding; *Culicoides* death after blood feeding that prevents onward spread of virus from a viraemic horse and/or reduces the population of available vectors; and, finally, no effect at all. The results of the present study do not support a repellent effect of deltamethrin to protect an individual horse from virus inoculation; however, no conclusions can be drawn on the onward spread of the virus or the vector population.

Studies in other species suggest that, while the application of topical insecticides does not protect the treated animal directly

from vectorborne diseases, it instead reduces the abundance and life expectancy of the vector population (Carpenter and others 2007). Non-AHS-susceptible hosts (mostly sheep and cattle) have a significant effect on the amount of *Culicoides* within an area, and effective use of insecticides to minimise *Culicoides* numbers would rely on the continuous and effective treatment of all animal hosts within an area (Iacono and others 2013). Unfortunately, the efficacy of such use has not yet been definitively demonstrated and the 2-weekly application of topical permethrin to cattle did not reduce seroconversion to BT virus (Mullens and others 2001). However, the viral challenge was very high in this study, with many more untreated cattle nearby, and the regimen used may simply have been overwhelmed.

Up to 80 per cent *Culicoides* mortality has been demonstrated within an hour following exposure to the plucked hair of horses treated seven days previously with another pyrethroid, cypermethrin (Papadopoulos and others 2010). As the extrinsic incubation period (time for viral amplification and migration within *Culicoides*) of AHSV is far greater than one hour, 80 per cent of *Culicoides* contacting the hair would be unable to act as onward virus vectors. Regarding the UK equine population and AHS transmission, it would theoretically be the case that treatment of every single horse and donkey could break the cycle of transmission by preventing onward spread from viraemic individuals, although a similar study using deltamethrin instead of cypermethrin is required to support its use in this way.

Possible explanations for the deltamethrin having no effect include resistance and failure to achieve required concentrations for toxicity at biting locations. Insecticide resistance among vectors of many arboviral diseases (particularly malaria) is well established. The susceptibility of *Culicoides* species to insecticides is relatively poorly documented, although a standardised susceptibility test has been described (Venail and others 2011). Given the high susceptibility of *Culicoides* generally reported to deltamethrin, it seems unlikely that resistance would have been a significant problem in the present study (Venail and others 2011, Del Río and others 2014). Our results are in agreement with a previous study showing a lack of efficacy of another pyrethroid, permethrin, in horses (De Raat and others 2008). In that study, post-treatment samples were taken at a single time point 24 hours after application of insecticide and the authors recommended repeated measures on subsequent days to see if any effect was observed. Despite the repeated sampling for four days after application of the deltamethrin used in this study, no significant effect of the treatment was detected. In sheep, the reported efficacy of topical deltamethrin varies greatly, with effective *Culicoides* mortality occurring for between 10 days to 5 weeks (Mullens and others 2010, Venail and others 2011, Weiher and others 2014). The peak mortality rate has been reported to occur between zero and four days after application of deltamethrin, depending on the method of application, and the present study should therefore have detected an effect (Mullens and others 2010, Venail and others 2011). The product used was selected based on legislative provisions that allow the use of unauthorised medicines by veterinary surgeons (the ‘cascade’) and is a 1 per cent solution of deltamethrin licensed for livestock use in the UK. The dose used was the equivalent of the licensed cattle dose, and the product was applied to a single area behind the withers as per the manufacturer’s instructions for usage on cattle. There are concerns that lack of spread of compounds applied in this way results in lack of efficacy in distant parts of the body (Carpenter and others 2007). Following the application of deltamethrin to the backs of sheep, *Culicoides* exposed to the hair collected from primary biting regions (belly, face, legs) showed lower mortality than those exposed to hair from the backline, although results were variable (Carpenter and others 2007, Carpenter and others 2008a). These results were not significantly improved by applying the products to the flanks of the animals, rather than just along the back line (Carpenter and others 2007). In cattle, deltamethrin has been shown to reach

TABLE 3: Results of PCR analysis to identify species of 50 randomly selected blood fed (BF) and non-blood fed (NBF) *Avaritia* subgenus *Culicoides*

Species	Number of <i>Culicoides</i> (% of species)	
	BF	NBF
<i>C. dewulfi</i>	18 (39.1)	28 (60.9)
<i>C. obsoletus</i>	29 (65.9)	15 (34.1)
<i>C. scoticus</i>	3 (42.9)	4 (57.1)
<i>C. chiopterus</i>	0 (0)	1 (100)

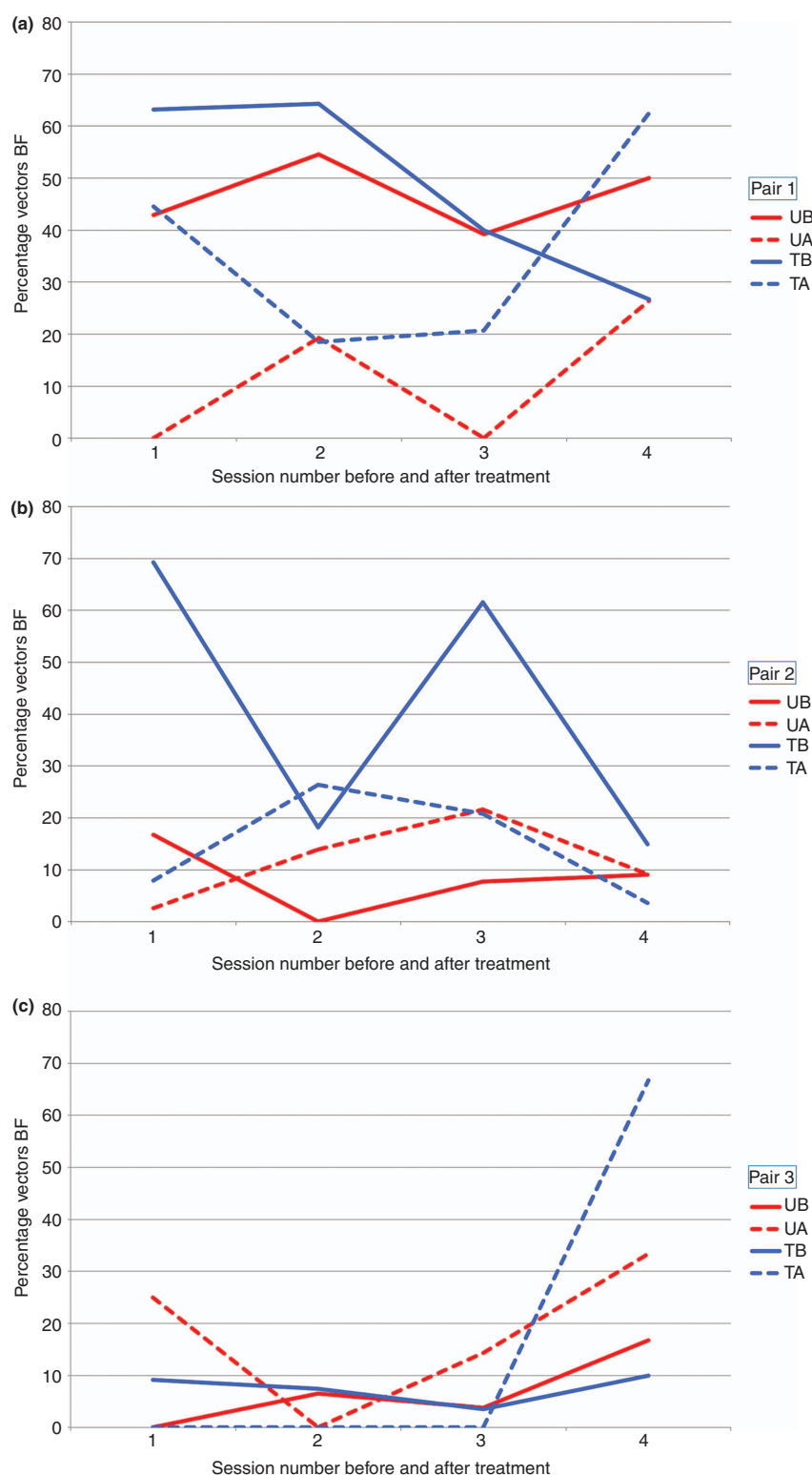


FIG 2: (a–c) Line graphs showing percentage vectors blood fed (BF) for each pair of horses during four sessions before treatment and four sessions after treatment of horses with deltamethrin. Red lines represent the untreated horse (U) in each pair, and blue lines represent the treated horse (T) in each pair. Solid lines represent data during the first four sessions before treatment (B), and dotted lines during the four sessions after treatment (A)

the feet following topical treatment along the spine (Mehlhorn and others 2008). *Culicoides* appear to feed on all parts of the horse, with some species showing apparent predilection sites. In one study involving a single horse, the majority of *C. scoticus* and *C. dewulfi* were collected from the back (main site) and head, whereas *C. obsoletus* had no preferential sites (Viennet and others 2013). Another study showed that the hindquarters and mane were *Culicoides* predilection sites (Townley and others 1984). Following the application of cypermethrin to the face, legs, back

and hindquarters of horses, the toxicity of collected hair to *Culicoides* was consistently lower from the legs and highest from the back (Papadopoulos and others 2009). A further study using an *in vitro* assay to assess the efficacy of 1 per cent deltamethrin applied in a single area of the back would help to determine whether or not lack of compound spread contributed to the results of the present study. In particular, this may explain the higher blood feeding rate of *C. obsoletus* observed as these *Culicoides* would have been more likely to feed elsewhere if the

insecticide was only present at required concentrations on the back of the horse.

In the present study, the treatment effect was expressed as the relative change in the percentage of blood fed vectors after treatment compared with before treatment. The 95% CI tells us that the treatment effect of deltamethrin on the pre-treatment BF rate lies between a reduction of 76 per cent and an increase of 260 per cent. This 95% CI is very wide, and additional pairs of horses could be used to give a more accurate estimate of the treatment effect. A crossover design could not be used due to the unknown residual effect of the deltamethrin. When considering future study refinements, it is important to bear in mind what treatment effect would be required for the treatment to be recommended clinically as a repellent. This is particularly difficult to define as there appears to be no accepted value and the variation in methodology between studies makes comparisons difficult. In human trials investigating the efficacy of repellents, the dosages that repel 50, 90 and 95 per cent of mosquitoes are quoted; however, most seem to provide at least 90 per cent protection from biting for a period of time (Lupi and others 2013).

Several studies investigating the interaction between horses and *Culicoides* have examined catches obtained from aspiration of *Culicoides* caught within a drop trap around the horse (De Raat and others 2008, Van Der Rijt and others 2008, De Jong and others 2012). Our decision to use light traps to sample *Culicoides* within a similar space was made to increase the number of *Culicoides* sampled, and therefore the analytical power of the study. It has been stated that UV light traps cannot be used directly to assess *Culicoides* biting rates and the collection of a species in a light trap placed near an animal does not prove that it was feeding on it (Viennet and others 2011). The information obtained from light traps does not always appear to correlate well with either the overall biting rate or the species composition found feeding on sheep when sampled by direct aspiration from the host (Carpenter and others 2008b, Viennet and others 2011). Indeed, a study that compared *Culicoides* catches from sticky covers/blankets on a single horse with those in a nearby light trap showed that *C. scoticus*, *C. dewulfi* and, to a lesser extent, *C. obsoletus* were the species most commonly attracted to the host, with the importance of *C. obsoletus* overestimated by UV light trapping (Viennet and others 2013). The results of the current study appear to be in agreement with this, although the methodologies (Viennet and others used a trap hung out of direct sight of the host at a distance of around 50 m compared with trap hung directly above the host within a semi-closed environment) are substantially different. This might also explain the lower blood feeding rate seen in the previous study (0.5 per cent) compared with the average of 16 per cent in the present study.

It has been shown that proximity to cattle increases the total numbers of *Culicoides* and blood fed females caught using light traps (Bellis and others 1996, Baylis and others 2010). In a study where light traps were positioned directly above sheep (but not within a midge-proofed enclosure), the female *C. obsoletus* catch increased linearly with host number (Garcia-Saenz and others 2011). These results help support the assumption made in our study that the *Culicoides* caught in the light traps were attracted by the horse directly below each trap in the cages and therefore represent the specific population of *Culicoides* feeding on them. The addition of partial enclosure of the trap and host by the midge-proof netting should have helped to further isolate the relevant population of *Culicoides*. This is supported by the PCR results that showed 100 per cent of the engorged *Culicoides* tested contained equine DNA. This could be further improved by the use of individual horse-specific primers to establish if the *Culicoides* had fed on the specific horse in the same cage, rather than the adjacent one.

The results of the present study do not support the use of topical deltamethrin solution as a method for preventing *Culicoides* from biting horses during an outbreak of AHS. However, it does not investigate the possible effects of

deltamethrin in reducing onward transmission of disease from viraemic horses or the numbers of adult *Culicoides* within an area. Further studies are required to identify a more suitable repellent product to prevent *Culicoides* blood feeding on horses. *Culicoides pulicaris*, *C. punctatus* and the four members of the *Avaritia* subgenus were the most likely species to blood feed on horses, supporting their potential role as vectors of AHS virus if the disease were to reach the UK.

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Repellent effect of topical deltamethrin on blood feeding by *Culicoides* on horses

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