

Paper

The threat of midge-borne equine disease: investigation of *Culicoides* species on UK equine premises

M. Robin, D. Archer, C. Garros, L. Gardès, M. Baylis

There are concerns that outbreaks of exotic or novel vector-borne viral diseases will increasingly occur within northern Europe and the UK in the future. African horse sickness (AHS) is a viral disease of equids that is transmitted by *Culicoides* and is associated with up to 95 per cent mortality. AHS has never occurred in the UK; however, it has been suggested that appropriate *Culicoides* species and climatic conditions are present in northern Europe to support an outbreak. No data are currently available regarding the *Culicoides* species present on UK equine properties. This study demonstrates the presence of potential AHS virus vector *Culicoides* species on both urban and rural equine properties within the south-east UK. PCR analysis revealed that engorged members of these species contained equine DNA, proving a direct vector–host interaction. It is therefore possible that an AHS outbreak could occur in the UK if the virus were to be imported and, given the severe welfare and economic consequences of AHS, this would have devastating consequences to the naïve UK equine population.

Introduction

It has been suggested that recent changes in the global distribution of several vector-borne viral diseases may be associated with climate change and globalisation (particularly the transportation of animals and animal products), leading to concerns that these diseases will increasingly threaten northern Europe and the UK (Mintiens and others 2008, Guis and others 2012). Indeed, the northern European outbreak of bluetongue virus (BTV) serotype 8, which began in 2006 and affected tens of thousands of farms over the ensuing three years, demonstrates the risk that arboviral diseases may pose in the future for our naïve animal populations.

African horse sickness (AHS) is an infectious, non-contagious, insect-borne viral disease of equids that is classified as a reportable disease by the World Organisation for Animal Health (OIE). The disease is associated with four recognised syndromes; a cardiac form, a pulmonary form, a mixed form and subclinical horse sickness fever. Mortality rates are reported at between 50 and 95 per cent, with the highest rates seen in naïve populations, therefore an outbreak would have devastating effects on animal welfare, as well as major economic consequences (Guthrie 2007). Midges of the genus *Culicoides* are the primary vectors of AHS, and the geographical distribution and

seasonal occurrence of AHS is dependent on those of the species of *Culicoides* involved in transmission (Du Toit 1944, Mellor 1994).

AHS is endemic in eastern and central Africa; however, the furthest north that the disease has occurred is the Iberian Peninsula, with the last outbreak in this region occurring between 1987 and 1991 (Rodriguez and others 1992). In Spain alone, 110 horses died as a direct result of the virus, with a further 900 slaughtered as part of control measures (Rodriguez and others 1992). When considering the risk of AHS virus (AHSV) to the UK, comparisons are often made with BTV, as the two are closely related and share the same vector species in areas where they are endemic (Ortega and others 1998). Following the introduction of BTV-8 to northern Europe in 2006, it spread rapidly to many countries where it had never occurred before, including the UK. There is evidence that Palaearctic *Culicoides* species of the *Obsoletus* and *Pulicaris* groups were acting as vectors at this time, based on viral isolation from *Culicoides* batches caught during the outbreak (Hoffmann and others 2009). It is therefore possible that the appropriate *Culicoides* species and climatic conditions are present in northern Europe to support an outbreak of AHS, even if the traditional vector species in Africa (*Culicoides imicola*, *Culicoides bolitinos*) are not present.

In the Netherlands and France, members of the *Obsoletus* and *Pulicaris* groups have been shown to be the *Culicoides* species most attracted to horses (van der Rijt and others 2008, Viennet and others 2013). In the UK, members of these groups have been shown to comprise 93.5–97 per cent of *Culicoides* caught on farms using light-vacuum traps (Purse and others 2012, Kluiters and others 2013). These investigations were carried out on rural properties housing mostly cattle and sheep. The preferential *Culicoides* landing sites on the horse and peak activity times of several species have been described, with *Obsoletus* group midges noted to be the most numerous (Mellor 1974, Mellor and McCraig 1974). A more recent study on the experimental infection of *Obsoletus* group *Culicoides* with BT virus used *Culicoides* collected from a stable; however, the exact species composition of the catches were not described (Carpenter and others 2008a). To our knowledge, there has been no dedicated light-suction trap-based

Veterinary Record (2014)

doi: 10.1136/vr.102151

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Provenance: not commissioned;
externally peer reviewed

Accepted January 8, 2014

survey of *Culicoides* on equine premises in the UK. This information is essential for modelling the potential spread of disease and in formulating evidence-based disease control strategies.

The aims of this investigation were therefore to identify the *Culicoides* species present on urban and rural equine properties in the UK and to confirm which of these had actually fed on horses and could therefore potentially act as disease vectors.

Materials and methods

Trapping was carried out in and around the Newmarket area in south-east UK in May/June 2012. Newmarket was chosen for two reasons; first, it is the focus of the UK thoroughbred racing industry and contains a high density of both urban and rural equine properties; second, the first cases of bluetongue (BT) in the UK also occurred

in the south-east, and wind dispersal of infected *Culicoides* from mainland Europe was hypothesised as the possible source of the virus (Gloster and others 2007, 2008). Two online databases (www.directoroftheturf.com and www.newmarketracehorsetrainers.co.uk. Both accessed in March 2012) were used to identify equine stud farms and racing yards with a CB8 postcode and these were all marked onto an Ordnance Survey map. A 12×12 km-sized area was then divided into 25 squares, and a single property within each square was randomly selected. Property owners were contacted to gain permission to carry out insect trapping on the premises. If permission was not granted, then another property within the square was randomly selected until a suitable property was selected. The properties were allocated as either urban (within Newmarket and surrounded by buildings) or rural (outside the main town and surrounded by vegetation).

A single trap site was selected at each property situated as close to horses as possible. UV light-emitting-diode Centre for Disease Control and Protection (UV LED CDC) traps (Bioquip Products Inc, 2321 Gladwick Street, Rancho Dominguez, CA 90220, USA) were used for *Culicoides* collection (Fig 1). These traps used LED lights to attract insects, before sucking them into plastic beakers situated beneath the lights using fan-generated suction. The beakers contained 100 ml of water, to which a drop of detergent was added to break the surface tension and submerge the insects. The traps were each powered by a 6 V battery. Overnight maximum/minimum temperature and humidity were recorded at all locations on each night using a hygrometer (S. Brannan and Sons Ltd, Leconfield Industrial Estate, Cleator Moor, Cumbria, CA 25 5QE, USA) and wind speed was also measured at the time that each trap was set.

All trapping took place during May/June 2012. Traps were set up and activated between 16:00 and 18:30, and collections were retrieved the following morning between 7:00 and 9:30. Trapping took place on consecutive nights until three successful (more than 10 *Culicoides* in total) catches were obtained. The collected insects were transferred to a preservative solution of 70 per cent ethanol, before being transported to the laboratory for analysis. Individual *Culicoides* species were identified using a dissecting microscope and standard wing-pattern identification keys (Fig 2) (Mathieu and others 2012). As male *Culicoides* do not feed and are therefore unable to act as AHSV vectors, they were not recorded. The females making up the Obsoletus group (*Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides chiopterus* and *Culicoides dewulfi*) cannot be readily identified using microscopic techniques. Therefore, 100 Obsoletus group *Culicoides* were randomly selected and identified



FIG 1: LED-CDC light trap used for collection of *Culicoides* on all properties.



FIG 2: Close-up view of wing patterns of members of the Palaeartic Obsoletus (top) and Pulicaris (bottom) groups. Images not to scale. www.iikculicoides.net/

TABLE 1: Total number of *Culicoides* females of each species caught at each of the 13 equine properties over three nights

Species/group	Total female <i>Culicoides</i> caught over three nights at each property													Total
	A3 R	A4 R	B3 R	B4 U	B5 U	C1 R	C2 R	C4 U	C5 U	D3 R	D4 R	E2 R	E3 R	
Obsoletus group	400	200	102	2	63	149	13	153	143	5	37	166	18	1451
<i>Culicoides pulicaris</i>				1				1				5	1	8
<i>Culicoides punctatus</i>								1						1
<i>Culicoides circumscriptus</i>	1											2		3
<i>Culicoides festivipennis</i>											1			1
<i>Culicoides pictipennis</i>											2	1		3
Total	401	200	102	3	63	149	13	155	143	5	40	174	19	1467

R, rural properties; U, urban properties

using a species-specific PCR assay previously described (Nolan and others 2007). Females were graded into pigmented, non-pigmented and engorged (Dyce 1969). In order to identify the species origin of the blood within engorged *Culicoides*, all blood-fed Obsoletus group *Culicoides* were analysed following the 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.

A questionnaire was designed to record methods of vector protection used at each of the properties, other species housed nearby and equine movements on and off the premises. Ethical approval was granted by the Veterinary Research Ethics Committee, University of Liverpool.

Results

Trapping data

Permission was obtained to undertake sampling on a total of 14 properties, including 4 urban (B4, B5, C4, C5) and 10 rural (A3, A4, B3, C1, C2, D3, D4, E2, E3). At property B4, all horses were housed indoors and the trap location was also indoors. The trapping initially took place at all 14 properties; however, trapping was discontinued at one premises at the owner's request. The traps had to be run overnight on a total of 16 nights in order to obtain the three sets of catches as planned. On the 13 nights where catches were not used, this was because there were fewer than 10 *Culicoides* caught in total.

Culicoides were caught at least once at each of the 13 locations (Table 1). A total of 1467 female *Culicoides* were caught at the 13 sites over three nights. Of these, 99 per cent (1452) were from the Obsoletus group. The total Pulicaris group catch was 9 (8×*Culicoides pulicaris*, 1×*Culicoides punctatus*). Taken together with the Obsoletus group, this gave a total vector species catch of 99.6 per cent (1461). The mean individual trap catch per night over three nights was 37.6 *Culicoides*; however, there was marked variation in the mean catch per trap between the three nights (2.2, 97.2 and 13.6 *Culicoides* per trap per night, respectively). The mean weather data and average catch per trap are shown in Table 2. The mean individual trap catch per night over three nights was 91.0 at urban properties (or 120.3 when excluding the indoor location) and 122.6 at rural properties.

PCR data

Obsoletus group species

Ninety per cent of the selected Obsoletus group *Culicoides* were successfully amplified using PCR. *C. obsoletus* (52.3 per cent) and *C. scoticus* (38.9 per cent) were the most commonly identified members of the Obsoletus group, with *C. dewulfi* (8.9 per cent) detected far less frequently. No *C. chiopterus* were identified.

Source of blood meals

A total of 10 blood-fed *Culicoides* females were collected, and all were from the Obsoletus group. Species-specific PCR analysis identified the blood meal sources of 3/10 as equine, with an additional blood meal also producing a weak positive equine signal (Table 3). The blood meal of one *Culicoides* contained both equine and ovine DNA.

Questionnaire data

In total, 8/13 (62 per cent) of the properties used some form of housing or management strategy to reduce insect bites. Rugs (5/8) and reduced turnout at dawn/dusk (4/8) were the most commonly used methods. None of the properties had any vector-proofed housing available. International horse movement had occurred on or off of 54 per cent (7/13) of the properties within the month prior to trapping taking place.

Discussion

The present study is, to our knowledge, the first dedicated light-suction trap-based survey of *Culicoides* species present on equine premises in the UK. Ninety-nine point six per cent of the *Culicoides* caught in the Newmarket area of the UK were considered potential vector species, and these were present on all properties with no apparent difference between urban and rural locations. The presence of equine blood within the potential vector species confirms that not only are these species present on equine properties, but they are also blood-feeding on the horses. These findings are of significance when considering the AHS threat to the UK, as vector-host interaction is a key predictor of a vector's capacity to transmit viral disease.

The AHS Regulations 2012 states that 'where a control notice has been served, a veterinary inspector must as far as is reasonably practicable assess places likely to facilitate the survival of the vectors, or to accommodate them, and the practicality of using appropriate vector control measures in such places and begin an epidemiological inquiry to try to establish at least . . . the presence and distribution of vectors' (DEFRA 2012). Such an investigation would most likely be

TABLE 2: Average number of *Culicoides* and average meteorological data (mean of all 13 sites) obtained during *Culicoides* collection

	Night 1	Night 2	Night 3
<i>Culicoides</i> per property	2.2	97.2	13.6
Wind speed (m/s)	1.48	0.04	0.09
Maximum temperature (°C)	18.7	21.3	20.6
Minimum temperature (°C)	11.2	12.2	10.2
Maximum humidity (%)	85.5	83.4	83.1
Minimum humidity (%)	41.2	49.8	49.7

TABLE 3: Results of PCR identification of origin of blood meals in 10 engorged *Culicoides*

Sample	Simplex PCR Horse	Simplex PCR All vertebrates	Multiplex PCR Cattle, sheep, goats
1	Weak signal	Negative	Negative
2	Negative	Negative	Negative
3	Negative	Negative	Negative
4	Positive	Negative	Positive for sheep
5	Positive	Positive	Negative
6	Negative	Negative	Negative
7	Negative	Negative	Negative
8	Negative	Positive	Negative
9	Positive	Negative	Negative
10	Negative	Negative	Negative

conducted using light traps in a similar way to our study. Light traps are the standard sampling method for *Culicoides* midges when conducting epidemiological investigations and have long been used in surveillance programmes throughout the world. They provide an indication of the *Culicoides* population and abundance in an area, although it has always been unclear how the numbers, species composition and physiological status of catches relate to the *Culicoides* actually feeding on a natural host (Carpenter and others 2008b). 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 (Carpenter and others 2008c, Viennet and others 2011). This has not been investigated for the horse, and further work into the association between light-suction traps and animal-baited methods is required. The type of light trap used also has an effect on the catch (Venter and others 2009). The mains-powered Onderstepoort-style black-light trap (OVI) is the most commonly used model; however, we chose to use battery-powered traps as they could be quickly set up and no mains cables were required on the busy equine yards. No specific information is available regarding their performance compared with the OVI, although the OVI trap appears to be more sensitive than other light-suction traps at collecting less abundant *Culicoides* species, which provides important information, as the most abundant species are not necessarily the most competent vectors (Venter and others 2009). The importance of all of these factors is very difficult to determine, particularly as much of the evidence supporting the BTV and AHSV vector roles of certain *Culicoides* species is based on associations between disease occurrence and species abundance as measured by light trapping (Baylis and others 1997, Rawlings and others 1997, Meiswinkel 1998, Capela and others 2003). Given that the ability of many *Culicoides* species to act as effective vectors appears primarily related to abundance, it seems appropriate to consider the 99.6 per cent potential vector catch as significant (Mellor and Wittmann 2002).

There were marked differences in the *Culicoides* catches at each property over the three nights of catching. *Culicoides* counts are known to vary significantly according to climate and weather changes, and higher wind speeds and lower ambient temperatures have been associated with lower Obsoletus group catches (Mellor and others 2000, Baylis and others 2010). Over the three nights, there were variations in the temperature, wind speed and humidity at each of the sites, and this may well have accounted for some of the differences in catch numbers. Night 2 had by far the highest mean *Culicoides* count per property and also the highest mean max/min temperatures and lowest mean wind speed. Additionally, the nights when no *Culicoides* were caught were subjectively all associated with rainfall and increased wind speeds (no data presented). The low total catch at property B4 is likely explained by the indoor location of the trap, if we assume that the majority of the *Culicoides* species caught are exophilic, although there are limited data describing the behaviour of Palaearctic *Culicoides* species. A study from Canada suggests that *C. obsoletus* are exophilic (Anderson and others 1993); however, more recently, *C. obsoletus* have been shown to enter stables to feed (Viennet and others 2013). Local-scale modelling of *Culicoides* on non-equine properties has demonstrated significant variation in the abundance and species counts between neighbouring farms, and this also appeared to be the case in Newmarket (Kluiters and others 2013). These variations in catches would have to be taken into account when conducting an epidemiological enquiry in the face of a disease outbreak.

The ability of the *Culicoides* species identified in this study to transmit AHSV is currently unknown. Members of the Obsoletus and Pulicaris groups have been identified as potential vectors of arboviral disease in northern Europe, and our findings show that these are the most abundant species on equine properties in the UK (Hoffmann and others 2009). The use of the species-specific PCR allowed accurate identification of members of the Obsoletus complex that was previously based on highly specialised microscopic examination of morphological features of males only, very few of which were caught in the present study. Individual species identification is essential due to the variations in vector capacity that occur in individual species, and accurate epidemiological investigation and risk assessment both require accurate knowledge of the geographic distributions of individual

members of the groups. *C. obsoletus* and *C. scoticus* were the most common members of the Obsoletus group identified, making up 52.3 and 38.9 per cent, respectively, and future studies should focus on the individual roles of *C. obsoletus* and *C. scoticus*. No *C. chiopterus* were identified, and other studies using light traps have also found relatively low numbers of this species compared with the other Obsoletus group members (Carpenter and others 2008c, Kluiters and others 2013). It has been shown that light traps significantly underestimate the biting rate of *C. chiopterus* in sheep, and the results of the present study should therefore be interpreted with caution (Carpenter and others 2008c).

The findings of the PCR analyses of the blood meals provide definitive proof that potential vector species are blood-feeding on horses in the Newmarket area; however, some of the results require further discussion. Samples 4 and 9 produced a positive result for the horse PCR, but not for the vertebrate PCR as would be expected. The most likely reason for this is related to degeneration or digestion of the host DNA within the engorged *Culicoides*. The length of the DNA sequence amplified in the vertebrate PCR is longer than that amplified in the equine-specific PCR. Therefore, it is possible that sufficient DNA degeneration may have occurred to prevent detection of the vertebrate sequence, without affecting the equine-specific sequence. Degeneration is also likely to be responsible for the negative results of all PCR tests on samples 2, 3, 6, 7 and 10, as well as the reduced signal on sample 1. Interestingly, the blood meal origin from sample 4 was identified as both equine and ovine, suggesting that the midge had fed on both species.

The reasons for selecting the geographical location of the trapping area (400 km² in Newmarket, southeast UK) have been described. Relatively, this is a very small area and it is unclear how accurately the data could be used to describe *Culicoides* distribution in other parts of the UK. There is known to be significant local-scale variation in Obsoletus Group abundance in the UK and, although satellite data can be used to explain much of this variation, it is unclear if this would be accurate enough to model disease vector abundance on a national scale (Kluiters and others 2013). Catching on several more equine premises throughout the UK would be required to determine if this is the case.

In conclusion, this study demonstrates the presence of potential AHSV vector *Culicoides* species on both urban and rural equine properties within the southeast UK. Engorged members of these species were found to contain equine DNA, proving a direct vector-host interaction. Additionally, none of the properties investigated had any form of vector-proofed housing to prevent vector-feeding from occurring. It is therefore possible that an AHS outbreak could occur in the UK if the virus were to be imported and, given the severe welfare and economic consequences of AHS, this would have devastating consequences to the naïve UK equine population.

Acknowledgements

The authors would like to extend their thanks to the owners of the properties used in this study, as without their cooperation it could not have taken place. Thanks also to Mr Hugo Martin (Cirad) for the PCR analysis and Mr Ken Sherlock for his assistance with sample processing. Matthew Robin's clinical training scholarship is funded by the Horserace Betting Levy Board and they also provided the funding for this study.

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Veterinary Record 2014 174: 301 originally published online February 6, 2014

doi: 10.1136/vr.102151

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