Potential vectors of equine arboviruses in the UK

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# **ABSTRACT**

There is growing concern about the increasing risk of disease outbreaks caused by arthropod-borne viruses (arboviruses) in both humans and animals. There are several mosquito-borne viral diseases that cause varying levels of morbidity and mortality in horses and that can have substantial welfare and economic ramifications. While none has been recorded in the UK, vector species for some of these viruses are present, suggesting that UK equines may be at risk. We undertook, therefore, the first study of mosquito species on equine premises in the UK. Mosquito Magnet traps and red-box traps were used to sample adults, and larvae were collected from water sources such as tyres, buckets, ditches and pools. Several species which are known to be capable of transmitting important equine infectious arboviruses were trapped. The most abundant, with a maximum catch of 173 in 72 hours was *Ochlerotatus detritus,* a competent vector of some flaviviruses; the highest densities were found near saltmarsh habitats*.* The most widespread species, recorded at >75% of sites, was *Culiseta annulata.* This study demonstrates that potential mosquito vectors of arboviruses, including those known to be capable of infecting horses, are present and may be abundant on equine premises in the UK.

# **INTRODUCTION**

Globally, there is increasing concern over emerging infectious diseases, particularly arthropod-borne viruses (arboviruses) affecting humans and livestock (Kilpatrick and Randolph 2012; Durand and others 2013). Examples from the UK include bluetongue and Schmallenberg viruses in ruminants. The introduction of West Nile virus into North America demonstrated the effects of mosquito-borne disease on a naïve host population, both human and equine, and concerns have also been raised over the potential for spread of other mosquito-borne arboviruses affecting horses (Brown and others 2008; Pages and others 2009; Durand and others 2013). Mosquito-borne arboviruses affecting horses include West Nile virus (WNV), Japanese encephalitis virus (JEV), Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), Venezuelan equine encephalitis virus (VEEV), Ross River virus (RRV), Murray Valley encephalitis virus (MVEV) and Getah virus (Table 1).

Further knowledge about potential vector mosquitoes in the UK and their ability to spread arboviruses will play a key role in control and surveillance of disease in the event of an outbreak. Climate change may increase the risk of emergence of arboviral diseases by several mechanisms. Higher temperatures increase the ability of vectors to transmit viruses (Guis and others 2012; MacKenzie-Impoinvil and others 2014) and also have the potential to increase the geographical range of mosquitoes (Elbers and others 2015). Increased winter rainfall may also contribute to increase in mosquito populations, due to creation of more temporary freshwater sites for breeding, and greater abundance of emerging mosquitoes in spring (Vardoulakis and Heaviside 2012).

There are 34 species of mosquito in the UK (Medlock and Vaux 2011)and species that are implicated as vectors of arboviruses of horses elsewhere in the world include *Aedes cinereus, Ae.* *vexans, Anopheles maculipennis s.l*., *Coquilletidia richiardii*, *Culex pipiens, Cx. modestus*, *Culiseta morsitans, Ochlerotatus caspius, Oc. dorsalis and Oc. flavescens* and a number of these are widely distributed and locally abundant across the UK(Table 2)*.* In addition, some mosquito species present in the UK have been shown in the laboratory to be competent vectors of at least one of these viruses including *Oc. punctor, Oc. detritus, Cx. modestus, Ae. vexans, Cx. pipiens s.l.* and *An. plumbeus* (Table 2).

In the UK there have been recent and ongoing sampling and surveillance of mosquito species (Snow and Medlock 2008; Medlock and Vaux 2013, 2014, 2015; Vaux and Medlock 2015; Vaux and others 2015), however there has been no sampling of mosquito species with specific focus on the equine host. Accordingly, we carried out a survey of the mosquitoes present at 32 premises across England (see Figure 1 for approximate locations) to obtain baseline data on the species composition and abundance of mosquitoes that may interact readily with equines. Our results identify which species may play an important role in outbreaks of mosquito-borne equine viruses in the UK and hence contribute to the development of national strategies to monitor and manage this risk.

# **METHODS**

A total of 32 sites were sampled - 8 equine premises in each of North West, North East, South East and South West regions in England (Figure 1). A stratified sampling approach was utilised due to the fact that many mosquito species have a patchy population distribution. In this case four types of mosquito breeding habitat were identified: land associated with drainage ditches (drained farmland) or fenland (site 29), woodland, urban and saltmarsh (Hutchinson and others 2007). We aimed to recruit two equine premises in each of the four habitats in each region (32 premises in total).

An internet search was conducted using Google Maps and The Phone Book from British Telecom, using the search terms ‘Riding Schools’, ‘Livery’, ‘Stables’, ‘Stud’. BHS Riding Schools and Livery Yard Lists and the British Equestrian Directory and Newmarket Trainers Association lists were also utilised**.** This produced a list of businesses with publicly available contact details.

For each premises the local area was investigated for potential mosquito habitats using Magic ([www.magic.gov.uk](http://www.magic.gov.uk)) and Google Earth. Sites were assigned a category based on habitat (some sites qualified for two categories) and were graded based on area of presumed habitat and proximity of habitat to the premises. We aimed to locate premises within suitable habitats or, if that was not possible, within 500 m (woodland and urban sites), 1 km (urban and drained farmland sites) or 3 km (saltmarsh). A maximum distance of 500 m for woodland sites was selected reflecting the relative ease of finding sites close to woodland. For saltmarsh or grazing marsh it was not possible to find sites in close proximity in many cases, but species associated with floodwater, such as *Aedes vexans* and coastal saltmarsh such as *Ochlerotatus detritus*, tend to have greater dispersal capacity and *Oc. detritus* is capable of flying at least 2.5 miles (Service 1969, 1971b; Snow and Medlock 2008; Becker and others 2010; Verdonschot and Besse-Lototskaya 2014). In order to try and include all four habitat types within reasonable travelling distance, the four areas within the regions were chosen as follows:

Wirral peninsula and Chester (North West); between Scunthorpe, Gainsborough, Doncaster and Goole (North East); within 20 miles of Exeter (South West); a transect from Newmarket to the Wash (South East).

Premises were recruited by sending out either a letter or e-mail to the business, and following up with a telephone call. For sites where there was no response or a negative response, correspondence was then sent to a number of alternative second choice sites, for that category of habitat, until 32 sites (8 in each of four regions) were recruited.

## **Mosquito sampling**

### Host Seeking Adults

Each of the 32 sites was visited three times throughout the summer of 2015, and mosquitoes were trapped continuously for three days. Timing of visits was based on what is presently known about peaks in adult mosquito numbers of different species in the UK, visiting each of four regions within each of 3 seasonal peaks of mosquito activityin the months of May, late June-early July and September (Service 1969, 1977; Medlock and others 2007; Snow and Medlock 2008; Becker and others 2010; Medlock and Vaux 2015).

Trapping at each site was carried out using a Mosquito Magnet, Independence model (Woodstream Europe Ltd.). The mosquito magnet is designed to catch host-seeking mosquitoes by using propane as a fuel source to produce heat, moisture and carbon dioxide. The trap was also baited with 1-octen-3-ol (as supplied by the trap manufacturer). The Mosquito Magnet trap was run continuously for ~ 72 hours starting in the morning and a data logger was placed underneath the body of the trap to record the environmental temperature and relative humidity for this time period.

Attempts were made to catch mosquitoes landing on hosts in order to confirm horse-biting. Four sites in each area were sampled in June/July and September in the mid-late afternoon, and four sites around dusk. For each sampling effort a group of horses was observed for fifteen minutes, to see if any mosquitoes could be identified landing on them. If no mosquitoes were observed, then another group of horses was observed for 15 minutes. Group sizes ranged from 1 to 10, as horses were in their normal grazing environment (with the exception of site 25 where sampling was attempted in the stable as there was no grazing). If no mosquitoes were observed on two groups the attempt was abandoned. If mosquitoes were observed landings were counted for 2 minutes and then mosquitoes sampled from the head and neck of the horse (for reasons of safety) for 30 minutes, to allow for species identification. Some premises could not be sampled at dusk due to access restrictions, so were only sampled in the afternoon. In order to trap mosquitoes feeding on horses, a mechanical pooter (Watkins and Doncaster) was modified with an elongated inlet tube and was muffled, so as to avoid startling the horse. Individual horse behaviour was discussed with the yard owner in advance, and permission to attempt landing catches with each horse or group of horses was obtained.

### Resting adults

The resting box trap was a 40 x 30 x 20 cm black box (Morris 1981), painted red inside (red box trap) and was designed to aid in the capture of blood-fed mosquitoes (Figure 2). It was set in an open area facing west and was emptied on two mornings, (either at 24 and 72 hours after deployment, or 48 and 72 hours) by placing a perspex cover on the open front of the box and aspirating resting mosquitoes.

### Immature mosquitoes

Larval sampling was undertaken on the equine premises themselves and, where there was access, on neighbouring land within 500 m of the Mosquito Magnet or of grazing horses. The aim was to sample all water sources within the boundary of the premises, including all collections of artificial containers. This was not always possible due to access constraints or on larger premises. Larvae and pupae were sampled using a dipper. This is a 500 ml container with a long handle. Each dip was then emptied into a white tray and searched for larvae. For larger water-bodies 5 x 500 ml dips were used in different parts of the water-body, whereas for small containers only one dip sample or partial dip samples could be obtained.

## **Sample handling and identification**

Mosquitoes were removed from the traps with a mechanical aspirator and ‘Fly-nap’ (Carolina Biological Supply Company) was used to produce knock-down. Adult mosquitoes were stored dry, and identified within 4 days. Blood fed mosquitoes were stored in 90% ethanol immediately.

Larvae were pipetted into universal containers for storage. Fourth instar larvae were killed by gradually adding 90% ethanol. Pupae were allowed to emerge for ease of identification. Live 2nd and 3rd instar larvae were allowed to continue to develop until the end of the fieldwork week for ease of identification. Containers were inspected daily and any dead larvae or pupae were preserved using 90% ethanol for identification (Snow 1991).

Mosquitoes of all stages were identified morphologically as far as possible, to species or species complex using keys of British and European mosquitoes (Marshall 1938; Cranston and others 1987; Snow 1991; Schaffner and others 2001; Becker and others 2010). *Cx. pipiens* was differentiated from *Cx. torrentium* by molecular methods as described by Hesson and others (2010).

Due to the skewed distribution of the catches, data were log-transformed prior to averaging. Means were detransformed (i.e. geometric means) for presentation (Figure 3). Data analysis was undertaken with the R statistical programme (The R Foundation 2016).

# **RESULTS**

### **Host Seeking Adults**

It was not possible to find drained farmland in the South West area sampled, so 2 more exposed hillside sites were chosen as a comparison (sites 18 and 19, at altitudes of 120m and 114m respectively). At one of these hillside locations (location 19, Table 3) trapping was not carried out in September 2015 due to loss of the propane canister. A number of specimens could not be identified positively to species level due to trap damage, and are recorded as unidentified Aedes spp.

A total of 917 adult mosquitoes of 14 species were caught over a total of 285 trapping days over the 32 locations (Table 3). The geometric mean catch for each mosquito magnet trapping period (approx. 72 hours) was 3.7 (SD 3.4), across all locations and seasons. Totals caught were 487, 217, 160 and 53 in the areas sampled in the NW, SE, NE and SW respectively.

For locations given one habitat classification, the geometric mean catch (9 days across 3 sampling periods) from a Mosquito Magnet was 6.9 (SD 5.90), 3.8 (2.5), 6.1 (3.3) and 36.5 (5.2) for premises associated with woodland, urban, drained farmland and saltmarsh habitats respectively (Figure 3).

The most abundantly trapped species was *Oc. detritus* with a total of 499 adults caught. All three sites with total catches > 100 were associated with the saltmarsh habitat of this species.

The second most abundantly trapped species was *Cs. annulata,* with 154 adults caught. Cs. annulata had the highest presence and was trapped on 75% (24/32) of sites.

Total catch was highest in September (Figure 4), and the difference in catch was significantly higher (P<0.005) than that in May and that in June/July in a general linear model with a negative binomial distribution using the MASS package in R (Ripley and others 2016). Thirty one sites were sampled (1 site not sampled) and total mosquito number from all locations was 679 with a geometric mean of 5.6 (SD 5.1) per location.

No mosquitoes were trapped whilst feeding on horses. Only 3 blood fed mosquitoes were trapped, all were part-fed individuals caught in the Mosquito Magnet, of which 2 were *Oc. detritus* and one was *Cs. annulata.* One mosquito *(Oc. caspius)* was sampled landing on a human host. Pilot host-landing catches using horses carried out in September 2014 yielded 20 *Oc. detritus,* 3 *Oc. caspius,* and 2 *Cs. annulata,* in two 15 minute daytime sampling efforts at site 8.

### **Resting Adults**

Sampling of resting mosquitoes was unsuccessful. No mosquitoes were found in the red-box traps.

### **Immature mosquitoes**

Immature mosquitoes were recovered by dipping of water sources on 23 of 32 premises. A total of 61 samples containing mosquito larvae or pupae were collected from a variety of water sources including ditches, buckets and water butts, tyres, ruts, muck heaps, pools and ponds.

*Cx pipiens s.l., Cx. torrentium, Cs. annulata/alaskaensis/subochrea, Cs. fumipennis, Cs. morsitans, Oc. caspius, An. claviger* and *An. maculipennis s.l.* were captured using dipping techniques.

The majority of samples were from artificial containers with small amounts of water, such as tyres. Therefore on most occasions, samples from each container were less than 500 ml, so it was not considered appropriate to state the numbers sampled, nor possible to compare larval numbers across sites. Larval samples were used to identify the presence of a species rather than its relative abundance.

A selection of larvae identified morphologically as *Cx. pipiens/torrentium* were further identified by molecular methods for each location. Of the 23 sites from which samples were obtained, *Cx. pipiens* larvae were identified from 15 (65.2%) of locations, *Cx. torrentium* from 11 (47.8%). Both species were found on 5 (21.7%) of these 23 locations. Both *Cx. pipiens* and *Cx. torrentium* larvae were obtained from at least 2 sites in all four regions.

*Cs. annulata/alaskaensis/subochrea* larvae cannot be differentiated morphologically, and were obtained at 9 (28.1%) of the 32 sites. Due to the rarity of *Cs. alaskaensis* and the relative abundance of *Cs. annulata* it is likely that these are *Cs. annulata*. Considering both juveniles and adults, *Cs. annulata* were present at 27 (84.4%) of the 32 sites.

# **DISCUSSION**

This study is, to our knowledge, the first survey of mosquito species on equine premises in the UK. This work has demonstrated the presence of several mosquito species which are candidate vectors of pathogens affecting horses. Commonly found mosquito species on equine premises during this study included *Oc. detritus*, *Oc. caspius,* *Cs. annulata, Cx. pipiens s.l*., *Cx torrentium, An. claviger, An. plumbeus* and *Oc. punctor.* Although mosquito density could be considered low at most of the sites sampled, this can be partly explained by the fact that the spring of 2015 was relatively dry for all of the regions except the North West (Met Office 2016). Climate change predictions suggest increased temperature and potential for flooding events in the UK (Met Office 2010; Caminade and others 2012; Medlock and Leach 2015) which are likely to increase the abundance of native mosquito species. It therefore seems likely that in the future there may be significantly increased horse-vector interaction, particularly with mosquito species which thrive in warmer regions of Europe, such as *Cs. annulata, Oc. caspius, Cx. pipiens s.l. Oc. detritus, An. plumbeus, Cq. richiardii, An. maculipennis and Ae. vexans* (Balenghien and others 2008)*.* The species trapped in the current study are all considered mammalophilic or bite both birds and mammals, with the exception of *Cx. torrentium* which is strongly ornithophilic (bird-biting). Three European studies provide evidence that *Cx. pipiens s.l*. found in rural areas will bite mammals, including horses (Balenghien and others 2008; Börstler and others 2016; Schönenberger and others 2016). Although not all of these studies differentiated *Cx. pipiens* form *pipiens* from *Cx. pipiens* form *molestus* the study of Börstler and others (2016) records a significant number of *Cx. pipiens* form *pipiens* with mammalian blood meals.

Eleven of the sixteen species found on equine premises during this study are laboratory competent vectors of, or are implicated in, naturally occurring disease cycles for at least one arbovirus affecting horses (Table 2). An important aspect of this study is that we trapped very few blood fed mosquitoes: just three in the Mosquito Magnet and none by other methods. This begs the question of whether the mosquitoes present at equine premises in the UK only rarely feed on equines, or whether they feed but were not caught. A number of factors suggest that the latter is the most likely explanation: (i) the Mosquito Magnet is designed to trap host-seeking rather than blood fed adults; (ii) many of the premises had other potential hosts present (humans, cattle, small mammals) indicating that the low number of trapped blood-fed mosquitoes cannot be attributed to the specific avoidance of equids; (iii) in pilot work in September 2014, mosquitoes *Cs. Annulata, Oc. Caspius and Oc. detritus* were directly observed by the author feeding on horses; and (iv) most of the species caught in this study have been reported, in other studies, to feed on horses and/or transmit arboviruses to horses. Nevertheless, and probably due to the inherent difficulties in trapping blood-fed mosquitoes in the UK (Brugman and others 2015) blood-feeding on horses has not been confirmed in this study. A large sampling effort and high mosquito densities are required to maximise trapping of blood-fed mosquitoes. The number of sites included in this study dictated that sampling effort on each site was necessarily lower than that of other recent studies (Brugman 2016), and seasonal variation in abundance due to climatic conditions, for example a dry early summer period (Met Office 2016) may have supressed mosquito density. However, all of the species sampled in this study, with the exception of *Cx. torrentium*, and *Cs. morsitans* have been shownto bite equines (Table 2), and four of the six most abundant species in adult catches have been shown to bite horses in the UK either in previous studies, or in pilot work for this study. Further work would be required to investigate the feeding rate of UK populations of these mosquitoes on horses, and host bait catches (Schönenberger et al., 2016) would seem most likely to provide useful information.

The comparatively high numbers of *Oc. detritus* and *Oc. caspius* caught on some saltmarsh associated sites are consistent with previous studies and reports of significant nuisance biting (Clarkson and Setzkorn 2011; Medlock and others 2012; Medlock and Vaux 2013) and confirms that there is significant potential for host-vector interaction between these species and horses. These two species are competent vectors of WNV (Vermeil and others 1960, Blagrove and others 2016). Detailed, high resolution information regarding horse and mosquito species distributions is lacking (Iacono and others 2013). However, using previously published horse distribution data at postcode scale (Boden and others 2012; Iacono and others 2013) and saltmarsh distribution (Adnitt and others 2007), in combination with mosquito species records, several coastal areas of England appear worthy of further investigation for host-vector interaction potential. These areas have high horse density, saltmarsh presence and records of *Oc. detritus* and *Oc. caspius,* (The Walter Reed Biosystematics Unit 2014; National Biodiversity Network 2016a,b) and include the Severn estuary, South Devon coast, the South coast of England from Swanage to Chichester and the Dee and Mersey estuaries. Two of these areas were sampled during this study: Wirral (Dee estuary) and the South Devon coast.

The finding that the WNV vector *Cx. pipiens* was common on equine premises with suitable water sources is expected, as this species has a widespread distribution (Medlock and others 2005; Medlock and Vaux 2011), but this study confirms that suitable container habitats are commonplace on equine premises. *Cx. torrentium* is a major enzootic (wildlife) vector of Sindbis virus in Scandinavia (Hesson and others 2015) and may therefore be capable of a similar role in transmission of other arboviruses. *Cx. pipiens* and *Cx. torrentium* were found on a number of occasions in all four regions, suggesting that *Cx. torrentium* may be more prevalent in the North of England than previously recognised (Medlock and others 2005).

One of the most interesting results to emerge from the current study was the presence of *Cs. annulata* on the majority of sites (27/32). It was also the second must abundant species in Mosquito Magnet samples. Whilst *Cs. annulata* is known to have a widespread distribution in the UK (Medlock and others 2005) this study provides evidence of the potential for host–vector interaction with UK equines. *Cs. annulata* has recently been demonstrated to be vector competent for WNV (M. Blagrove, unpublished observations) and as the species bites both birds and mammals including horses (Schönenberger and others 2016), it therefore has potential to transmit arboviruses from avian reservoirs and hence serve as a ‘bridge vector’. Combined with its ability to breed in a variety of water sources and presence on most sites sampled, this makes it an important species for further study.

Our results suggest that mosquito species presence is determined mainly by local mosquito breeding habitat, rather than equine host availability or management factors. However biting of horses may be affected by practices such as use of repellents, rugs and masks, building design, and duration and timing of grazing.

Mosquito Magnets are a commonly used trap in Europe for surveillance. They catch almost all mammalophagic species of mosquito, catch more species than other systems and in greater numbers. BG sentinel and CDC traps were not used in this study as they were considered less suitable, due to the risk of unpredictable precipitation damaging samples, and because for wide scale trapping in the UK, it may prove more practicable to use propane vendor’s delivery services than to transport large amounts of dry ice or carbon dioxide. Red box traps were used in the current study to attempt to trap blood-fed mosquitoes, however no mosquitoes were captured. Similar but larger red box traps have been successful in capturing *An. maculipennis s.l*., *Culiseta annulata* and *Culex spp*. in England (Brugman 2016). Surveillance on equine premises in the UK should be based around the use of Mosquito Magnets and larval sampling.

Mosquito populations often have a patchy distribution (Medlock and others 2005; Snow and Medlock 2008; Golding 2013) and many are considered uncommon or rare. Simple random sampling of equine premises may have resulted in very low catches. It is also almost impossible to prove species absence, so using random sampling risked obtaining poor quality data. Stratified sampling is an alternative method, commonly used by ecologists studying rare species (Thompson 2012). Using the data obtained under this sampling regime it is not possible to estimate the risk of equine-mosquito interaction across the UK, but more accurate assessment of risk at individual sites based on local habitat is achievable.

There are a number of introduction pathways which could conceivably be involved in importation of arboviral disease to the UK. One is introduction of WNV by migratory birds, but trade and transport of exotic birds and pets, and inadvertent vector transportation are also relevant risks. There is some recent evidence that human populations may continue epidemic transmission of VEEV in urban environments (Bowen and Calisher 1976; Watts and others 1998; Morrison and others 2008). Therefore in the event of an outbreak in the Americas, human movements as well as horse movements may constitute a risk (Adams and others 2012). Livestock transport, human transport and possibly mosquito eggs may present risk of RRV introduction (Harley and others 2001). Due to the complexity of the transmission cycles, virus introduction may not result in autochthonous (in-country) transmission.

In conclusion, the current study has highlighted a number of mosquito species which should be investigated with regards to vector competence and effectiveness of protection measures for equines. Our work has shown that horses in the UK are at risk of attack from a wide variety of mosquito species, several of which are known to be vectors of equine arboviruses in affected countries.

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Figure Legends

Figure 1. Map of study areas.

Figure 2. Red box trap.

Figure 3. Geometric mean of total catch per location for each habitat type (locations only included if given 1 habitat).

Figure 4. Total adult catches by season for each of 6 most abundant species.

Table 1: Mosquito-borne viruses affecting horses and known morbidity and mortality information

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Virus |   |   |   |   |   |   |   |
|  | JEV | WNV | EEEV | WEEV | VEEV | MVEV | RRV | Getah Virus |
| Inapparent infections common | Yes9 | Yes7 | Yes2 | Yes | No9 | Yes13 | Yes12 | Yes |
| Morbidity  | 0.03-1.4% of horses in a region3 | 1 in 11-12 infections7 | 61%1 of horses on some farms  | low | 10% of regional population (estimated)10,11 | Low | Low | Unknown |
| Case Mortality  | 5-40%4,5,6 | 38-57%7 | Up to 73%1 | 20-30%8 | 40-90%10,11 | Low | Low | Not fatal |
| Vaccination available | Y | UK licensed | Y | Y | Y |  |  | Y |

Y – Available in affected countries

1. (Silva and others 2011)
2. (Pauvolid-Corrêa and others 2010)
3. (Spickler 2010)
4. (Ellis and others 2000)
5. (Hale and Witherington 1953)
6. (Nakamura 1972)
7. (Sellon and Long 2013)
8. (Long and Gibbs 2007)
9. (Rico-Hesse 2000)
10. (Sudia and others 1975)
11. (Zehmer and others 1974)
12. (Vale and others 1991)
13. (Holmes and others 2012)

Table 2: Mosquito species present in the UK, horse and mammal biting, and vector status for arboviruses of horses

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Host Biting5,7** | **Evidence of Equine Biting** | **Vector Status** |
| *Ae. cinereus / geminus* | M31, 32 B31, 32 | Morocco1 Switzerland32 | EEEV [I]18 |
| ***Ae. vexans*** | **M31, 32 B31,32** | **France2 Switzerland32** | **WNV [I]5 EEV [I L”]18,,19,20,21,22,**  |
| *An. algeriensis* | M |   |  |
| ***An. claviger*** | **M32** | **Switzerland32** |  |
| ***An. maculipennis s.l.*** | **M31,32 B31** | **UK4,8 Switzerland32** | **WNV [I]5** |
| ***An. plumbeus*** | **M31** | **France2** | **WNV [L]14** |
| ***Cq. richiiardii*** | **M31,32 B32** | **France2 Switzerland32** | **WNV [I]5** |
| *Cs. alaskaensis* | M  |   |  |
| ***Cs. annulata*** | **M14,32 B32** | **UK3, France2 Switzerland32** | **WNV [L]16** |
| *Cs. fumipennis* | B |   |  |
| *Cs. litorea* | M B |   |  |
| *Cs. longiareolata* | B |   |  |
| ***Cs. morsitans*** | **M31 B31** |  | **EEV [Z]17, 19** |
| ***Cs. subochrea*** | **M2** | **France2** |  |
| *Cx. europaeus* | A R B |   |  |
| *Cx. modestus* | M2 B2 | France2 | WNV [V L]2.5 |
| ***Cx. pipiens s.l.*** | **M31 B31,32** | **France2** | **WNV [V L]23, 27  JEV [L\*]23 EEV[N]26 WEEV [N]24,25 VEEV [N]27** |
| ***Cx. torrentium*** | **B31 M31** |  |  |
| *Oc. annulipes* | M9,31 B | France2 |  |
| ***Oc. cantans*** | **M31 B32** | **UK9 Switerland32** |  |
| ***Oc. caspius*** | **M2 B2** | **UK3, France2** | **WNV [I L\*]2,5** |
| *Oc. communis* | M31 |   |  |
| ***Oc. detritus*** | **M2 B** | **UK3, France2** | **WNV [L]16 JEV [L]16**  |
| ***Oc. dorsalis*** | **M6** | **UK6** | **WEEV[I L]28,30** |
| *Oc. flavescens* | M11,12 | Denmark, Canada11, 12 |  |
| *Oc. geniculatus* | M2,31 | France2 |  |
| *Oc. leucomelas* |  |  |  |
| ***Oc. punctor*** | **M10 B** | **UK10** | **WNV [L]14** |
| ***Oc. rusticus*** | **M31, 32 B**  | **Switzerkland32** |  |
| *Oc. sticticus* | M31, 32 B31 |  Switzerland32 |  |
| *Or. pulcripalpis* | B |   |  |

Species in bold were sampled during the present study.

A- amphibians

B – birds

M – mammals

R – reptiles

L – Laboratory competent vector

I – Implicated in disease transmission worldwide

N – Non-competent as laboratory vector

V – Ecologically significant bridge vector worldwide

Z – Ecologically significant enzootic vector worldwide

\* -Relatively inefficient laboratory vector

“ – Variable laboratory competence in a number of studies

1. (Faraj and others 2009)
2. (Balenghien and others 2006)
3. Pilot work for this study - site 8, 2014
4. (Danabalan 2010)
5. (Medlock and others 2005)
6. (Service 1971a)
7. (Becker and others 2010)
8. (Hutchinson 2004)
9. (Medlock and Vaux 2011)
10. (Service and others 1986)
11. (Service and Smith 1972)
12. (Rempel and others 1946)
13. (MacKenzie-Impoinvil and others 2014)
14. (Vermeil and others 1960)
15. (Balenghien and others 2008)
16. Marcus Blagrove, unpublished observation
17. (Andreadis and others 1998)
18. (Armstrong and Andreadis 2010)
19. (Centers for Disease Control and Prevention (CDC) 2006)
20. (Vaidyanathan and others 1997)
21. (Davis 1940)
22. (Chamberlain and others 1954)
23. (Turell and others 2006)
24. (Aviles and others 1990)
25. (Hammon and Reeves 1943)
26. (Merrill and others 1934)
27. (Turell 2012)
28. (Kramer and others 1998)
29. (Vaux and others 2015)
30. (Zacks and Paessler 2010)
31. (Börstler and others 2016)
32. (Schönenberger and others 2016)

Table 3: Adult mosquito species and number trapped in Mosquito Magnet trap

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  | **Most abundantly caught mosquito species** |  |  |
| **Location Number and Region** | **Habitats** | *Anopheles claviger* | *Anopheles plumbeus* | *Culiseta annulata* | *Ochlerotatus caspius* | *Ochlerotatus detritus* | *Ochlerotatus punctor* | **Other** | **Total** |
| NW 1 | D | 0 | 0 | 12 | 0 | 0 | 0 |  | 12 |
| NW 2 | U | 6 | 0 | 7 | 24 | 1 | 0 | *UA – 5* | 43 |
|  NW 3 | D | 5 | 0 | 0 | 0 | 0 | 0 |  | 5 |
| NW 4 | U,S | 0 | 1 | 12 | 3 | 53 | 0 | *UA - 5* | 74 |
| NW 5 | W | 0 | 1 | 5 | 0 | 2 | 0 | *OCA* - 3 | 11 |
| NW 6 | W | 0 | 0 | 8 | 3 | 17 | 0 |  | 28 |
| NW 7 | S | 0 | 0 | 14 | 1 | 176 | 0 | *UA - 4* | 195 |
| NW 8 | W,S | 3 | 11 | 12 | 4 | 85 | 0 | *UA - 4* | 119 |
| NW 9 | U | 0 | 0 | 1 | 0 | 0 | 0 |  | 1 |
| NW 10 | W,D | 16 | 0 | 15 | 2 | 0 | 10 | *OCA – 3, CR – 2, UA- 9AV – 3,* | 60 |
| NE 11 | W | 0 | 0 | 6 | 0 | 0 | 0 |  | 6 |
| NE 12 | W,U | 1 | 0 | 20 | 0 | 0 | 0 | *CuS– 1, CR - 1* | 23 |
| NE 13 | S | 8 | 0 | 2 | 19 | 0 | 0 |  | 29 |
| NE 14 | S | 5 | 0 | 0 | 3 | 1 | 0 | *CR - 3* | 12 |
| NE 15 | D | 6 | 0 | 15 | 0 | 0 | 0 | *AnM – 1, CR – 1UA- 1* | 24 |
| NE 16 | U | 3 | 0 | 2 | 0 | 0 | 0 |  | 5 |
|  SW 17 | W | 1 | 0 | 2 | 0 | 0 | 0 | *AnM - 1* | 4 |
|  SW 18 | H | 0 | 0 | 0 | 0 | 0 | 0 |  | 0 |
|  SW 19 | H | 0 | 0 | 0 | 0 | 0 | 0 | *CxP – 1* | 1 |
|  SW 20 | W,S | 3 | 0 | 2 | 0 | 4 | 0 | *CxP – 1* | 10 |
|  SW 21 | S,U | 0 | 1 | 0 | 8 | 0 | 0 | *CxP – 1, UA- 8* | 18 |
| SW 22 | W,U | 0 | 0 | 0 | 0 | 1 | 2 |  | 3 |
| SW 23 | W | 0 | 13 | 1 | 0 | 0 | 0 |  | 14 |
| SW 24 | W | 0 | 1 | 1 | 0 | 0 | 0 | *CxP – 1* | 3 |
| SE 25 | U | 0 | 0 | 0 | 0 | 0 | 0 | *CxP – 1* | 1 |
| SE 26 | W | 0 | 0 | 1 | 0 | 0 | 0 | *OD* - 1 | 2 |
| SE 27 | W,U | 0 | 0 | 0 | 0 | 0 | 0 | *CR - 1* | 1 |
| SE 28 | W | 0 | 0 | 6 | 0 | 0 | 0 | *OR* - 3 | 9 |
|  SE 29 | D | 0 | 0 | 3 | 1 | 0 | 0 | *OR* - 2 | 6 |
| SE 30 | S | 0 | 0 | 4 | 33 | 155 | 0 |  | 192 |
| SE 31 | S | 1 | 0 | 2 | 0 | 2 | 0 |  | 5 |
| SE 32 | D | 0 | 0 | 1 | 0 | 0 | 0 |  | 1 |

W – Woodland

D - Drained Farmland

S – Saltmarsh

U – Urban

*UA – Unidentified aedes spp.*

*OCA – Oc. cantans*

*CuS – Cs. subochrea*

*CR – Cq. richiardii*

*AnM – An. maculipennis*

*CxP – Cx. pipiens s.l.*

*AV – Ae. vexans*

*OD – Oc. dorsalis*

*OR – Oc. rusticus*