



# Diversity and ecology survey of mosquitoes potential vectors in Belgian equestrian farms: A threat prevention of mosquito-borne equine arboviruses

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## ABSTRACT

Emergence of West Nile Virus was recently recorded in several European countries, which can lead to severe health problems in horse populations. Europe is also at risk of introduction of mosquito-borne equine alphavirus from Americas. Prevention of these arboviruses requires a clear understanding of transmission cycles, especially their vectors. To characterize mosquito fauna, their ecology and identify potential vectors of equine arboviruses in Belgium, entomological surveys of six equestrian farms located in the Wolloon Region were conducted during 2011–2012. The harvest of mosquitoes was based on larval sampling (272 samples from 111 breeding sites) and monthly adults trapping (CO<sub>2</sub>-baited traps, Mosquito Magnet Liberty Plus). Among 51,493 larvae and 319 adult mosquitoes collected, morphological identification showed the presence of 11 species: *Anopheles claviger* (Meigen), *An. maculipennis s.l.* (Meigen), *An. plumbeus* (Stephens), *Culex hortensis* (Ficalbi), *Cx. territans* (Walker), *Cx. pipiens s.l.* L., *Cx. torrentium* (Martini), *Coquillettia richiardii* (Ficalbi), *Culiseta annulata* (Schrank), *Aedes cantans* (Meigen), *Ae. geniculatus* (Olivier). Molecular identification of *Cx. pipiens* species complex allowed the detection of three molecular forms, *Pipiens* (92.3%), *Molestus* (4.6%) and *Hybrid* (3.1%). Larvae of *Cx. pipiens s.l.* and *Cx. torrentium* were omnipresent and the most abundant species. Water troughs, ponds and slurry (liquid manure) were the most favorable breeding sites of mosquito larvae. Based upon behavior and ecology of the identified mosquito species, Studied Belgian equestrian farms seem to provide a suitable environment and breeding sites for the proliferation of potential vectors of arboviruses and those being a real nuisance problem for horses and neighboring inhabitants.

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## 1. Introduction

Viruses transmitted by hematophagous arthropods (arboviruses) represent a threat for animal and human health with the increase of their emergence outside the endemic areas. Environment and climate change, as well as intensification of international trade could favor the re-emergence of vector-borne diseases (Gould and Higgs, 2009). Mosquitoes (Diptera: Culicidae) are considered as the main vectors of some arboviruses that both

affect horses and cause serious diseases in humans during epidemics, i.e., Eastern Equine Encephalitis virus (EEEV, Togaviridae: *Alphavirus*), Western equine encephalitis virus (WEEV, Togaviridae: *Alphavirus*), Venezuelan equine encephalitis virus (VEEV, Togaviridae: *Alphavirus*) and West Nile virus (WNV, Flaviviridae: *Flavivirus*). These arboviruses are responsible for encephalitis, with usually no specific clinical signs, resulting in recovery or death of the host depending on the severity of the disease. No specific treatment is available for such viruses, and the only protection remains vaccination of the potential host and vector control (Davis et al., 2008; Goddard, 2008 and Zacks and Paessler, 2010).

The EEEV is the most pathogenic arbovirus endemic in the Americas and can be distinguished into two strains: South American and North American, the latter being more virulent for humans. This arbovirus has been isolated from 20 mosquito species including

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*Aedes vexans* (Meigen), *Ae. albopictus* (Skuse), *Culex pipiens* s.l. L., *Culiseta morsitans* (Theobald), and *Cx. territans* Walker that can be found frequently in Europe (Scott and Weaver, 1989 and Pfeffer and Dobler, 2010). In America, the majority of mosquito vectors of EEEV have been identified as belonging to the genus *Culex* (*melanocnion*), which has a wider host range (Arrigo et al., 2010).

Western equine encephalitis virus (WEEV) is found in the Western part of North America and South America. The WEEV is a recombinant virus between Sindbis virus and EEEV (Weaver et al., 1997). The WEEV has two transmission cycles; the main transmission cycle (North America) is maintained between *Cx. tarsalis* (Coquillett) and host birds. The secondary cycle of transmission (South America) involves *Ae. melanimon* Dyar and host mammals and/or rodents (Pfeffer and Dobler, 2010). *Aedes melanimon* acts as a bridge vector to transmit the disease to humans and horses, as *Ae. dorsalis* Meigen and *Ae. campestris* (Dyar & Knab). The WEEV could also be isolated from *Cx. pipiens*, and *Ae. vexans* (Pfeffer and Dobler, 2010).

Venezuelan equine encephalitis virus (VEEV) is an important emerging and re-emerging pathogen of horses and humans in South America. Epizootic strains are opportunistic for the choice of potential vectors. A wide variety of vectors may be involved as *Psorophora confinnis* (Lynch Arribalzaga), *Ps. columbiae* (Dyar & Knab), *Ae. sollicitans* (Walker), or else *Ae. taeniorhynchus* (Wiedemann) whose was considered as the primary vector during an epizootic (Weaver et al., 2004 and Aguilar et al., 2011). Enzootic strains are almost exclusively transmitted by mosquitoes of the genus *Culex* subgenus *Melanoconion* (Weaver et al., 2004). *Aedes albopictus* is an efficient vector of VEEV under laboratory conditions (Aguilar et al., 2011).

West Nile virus (WNV), which is worldwide distributed, is maintained in an enzootic cycle with birds that serve as amplification reservoir hosts and mosquitoes as the principal transmitting vectors. Humans and horses are considered dead-end hosts because they do not frequently produce sufficient viremia to infect mosquitoes (Campbell et al., 2002 and Hayes et al., 2005). However, the majority of equines are very susceptible to WNV infection, which can be responsible for encephalomyelitis in a fraction of infected animals, and may evolve into fatal encephalitis (Campbell et al., 2002 and Venter et al., 2009). Since that WNV was first detected in New York in 1999, more than 26,581 horses (period, 1999–2013) were affected in the United States ([http://www.aphis.usda.gov/vs/nahss/equine/wnv/wnv\\_distribution\\_maps.htm](http://www.aphis.usda.gov/vs/nahss/equine/wnv/wnv_distribution_maps.htm)), with many horses being euthanized because of grave prognosis indicating that several infections were fatal (Sebastian et al., 2008). A strong resurgence of horse and human cases of WNV was recorded in Europe in the last decade, including Hungary, Italy, southern France, Portugal, Romania, Spain and more recently Greece (Engler et al., 2013 and Di Sabatino et al., 2014). The main route of introduction of WNV in the regions of Europe with a temperate climate is most likely by infected migratory birds from Africa (Hubálek and Halouzka, 1999 and Calistri et al., 2010). Moreover, WNV could be introduced in Western Europe by birds arriving from central Europe (Koopmans et al., 2007). Mosquitoes in the genus *Culex* have been widely implicated as primary vectors of WNV (Becker et al., 2010 and Calistri et al., 2010). Despite the low vector competence of these species to transmit the WNV, other factors such as mosquito density, biting preference and seasonal activity makes *Culex* species the most important mosquito genus in WNV transmission (Kilpatrick et al., 2005). *Culex univittatus* (Theobald) is considered the primary vector in Africa (Hubálek and Halouzka, 1999) and *Cx. vishnui* Theobald in Asia. In the northeast America, *Cx. pipiens* s.l. is regarded as the most important vector of WNV and in the southeast the subspecies *Cx. quinquefasciatus* is considered as an important vector. *Culex tarsalis* takes place as the primary vector in the west American (Campbell et al., 2002 and Kilpatrick et al., 2005). In Europe, WNV

has been detected from largely ornithophilic mosquitoes especially *Cx. pipiens* s.l., *Cx. modestus* (Ficalbi), *Cx. torrentium* (Martini), *Coquillettia richiardii* (Ficalbi), and occasional vectors such as *Ae. cantans* (Meigen) and *Anopheles maculipennis* s.l. (Meigen) (Engler et al., 2013). *Aedes albopictus* is also a competent vector of WNV and has been demonstrated experimentally that can infect horses (Bunning et al., 2002).

Environmental factors, including human activities that enhance vector population densities (irrigation, heavy rains followed by floods, higher than usual temperatures, and formation of ecological niches enabling the mass breeding of mosquitoes) allow the reemergence of this mosquito-borne disease (Hubálek and Halouzka, 1999). An understanding of arboviral transmission cycles begins with the correct identification of potential vectors and thorough knowledge of their bioecology in the environment. The goals of this work were to determine diversity, abundance, and seasonal dynamics of potential mosquito vectors for equine arboviruses in equestrian farms in the Walloon Region of Belgium. Additionally, this study is intended to be useful in assessing the risk that those mosquito species and their breeding sites represent for transmission of equine arboviruses in Belgium.

## 2. Material and methods

### 2.1. Study sites

The present study was performed in 2011–2012, with the aim to investigate the mosquito fauna species and their bioecology in the equestrian farms. The study was conducted in six locations in Belgium belonging to Namur and Liège Provinces (Fig. 1). Each of the six equestrian farms selected contain a hundred horses and had two types of environments: horse livestock in the buildings of the farms (stables) and surrounding grasslands. In addition to the presence of many breeding larval sites in and around stables studied, the farms were also close to forests and streams, which give humid environment and provide suitable conditions for the proliferation of mosquito species. Localities of the equestrian farms inspected are: Gembloux (50°34'40"N, 4°44'40"E; 33 m above sea level), Malonne (50°27'06"N, 4°48'15"E; 85 m a.s.l.), Warsage (50°43'38"N, 5°47'57"E; 223 m a.s.l.), Chênée (50°37'00"N, 5°37'58"E; 151 m a.s.l.), La Reid (50°28'51"N, 5°46'38"E; 372 m a.s.l.), and Sprimont (50°32'46"N, 5°40'16"E; 274 m a.s.l.). Weather data (precipitation and temperature) were obtained from local meteorological stations of Royal Meteorological Institute (RMI®) and were presented by Ombrothermic diagram for each of the localities of equestrian farms (Fig. 2).

### 2.2. Mosquito collection

#### 2.2.1. Larvae sampling

Larvae sampling is realized in all potential larval habitats reported after inspection of each study site and its surroundings (a circle of radius of  $250 \pm 50$  m). All sites have been investigated 4 times/year, in 2011 (June, July, August and October) and 2012 (June, July, August and September). Larvae were collected by using the dipper method made-up by a metal pan (500 ml). However, the smallness of some breeding sites such as the temporal puddles and the inaccessibility of others sites like used tires had necessitated the use of an aquarium net  $12 \times 10$  cm to inspect them and collect immature stages. We collected in several places of breeding sites to obtain homogeneous samples (4–10 times). In the case of breeding sites which are formed by tires, the sample is composed by the sum of 4–10 tires selected on all tires placed in the same location (heap of tires). Data sheets of physical and biological characteristics were designed for each breeding

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