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Comparative study on the effectiveness of different mosquito traps in arbovirus surveillance with a focus on WNV detection

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ABSTRACT

The selection of the ideal trap for arbovirus surveillance is an issue of primary importance to increase the sensitivity of virus detection and the cost-effectiveness of the entomological surveillance. During the summer 2011, the effectiveness of five types of mosquito traps (CDC gravid trap, CO_2-baited trap, BG-Sentinel™ and two experimental prototypes) to attract females potentially infected with West Nile virus were assessed. The study was carried out in three natural wetland sites located in the Emilia-Romagna Region (Northern Italy), using a Latin square scheme. Single night collections of adult females were performed and determination of species and physiological state (gravid, nulliparous or parous) was made upon return to the laboratory. The species most frequently collected in the gravid trap was *Culex pipiens* sl. L., being gravid females the large majority of the individuals. Species diversity was much higher in CO_2-baited traps, which may therefore enable a more comprehensive description of the vector species composition and their role in arboviruses circulation. Our findings indicate that gravid traps can be a valid tool and should be integrated in the West Nile virus surveillance system in the Emilia-Romagna region, mainly based on collections made with CO_2-baited traps.

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1. Introduction

The increasing international trade and tourism, and the globalization of work are rapidly changing the distribution of arboviruses worldwide, posing new concerns on public health due to the increased risk of mosquito-transmitted arboviruses. In Europe, the most dangerous mosquito-borne viruses are the four dengue serotypes (DENV) causing the dengue fever, and the West Nile virus (WNV) that can cause lethal encephalitis, as well as the Usutu (USUV) and Bagaza viruses (BAGV) (Agüero et al., 2011; Roiz et al., 2012a; Vazquez et al., 2011) all belonging to the Flaviviridae family. WNV lineage 1 has been responsible for repeated disease outbreaks in the Mediterranean basin over the past 50 years. In 2004, and in subsequent years, the WNV lineage 2 appeared to spread throughout Hungary and Austria, and subsequently emerged in Greece in 2010 and in Italy in 2011, involving outbreaks on the Italian mainland and Sardinia. Further spread through the Balkan countries is also suspected (Hernández-Triana et al., 2014). WNV is transmitted in an avian cycle by ornithophilic mosquitoes, chiefly of the genus *Culex*, being mammals dead end hosts because viraemia is generally too low to infect mosquitoes (Reiter 2010). *Aedes albopictus* (Skuse) recently established in Southern Europe, is the vector species of Chikungunya and Dengue viruses (Togaviridae family, *Alphavirus* genus), and it was responsible of an outbreak of chikungunya disease in Emilia-Romagna (Northern Italy) (Rezza et al., 2007), and of several outbreaks in France (Delisle et al., 2015; Semenza et al., 2014). In addition, in 2007, in Northern Italy insect flavivirus DNA sequences integrated in *Ae. albopictus* populations were found by Roiz et al. (2009), while in 2008 a new insect flavivirus was detected in one pool of *Ae. cinereus/geminus* (Meigen) mosquitoes (Roiz et al., 2012a), whose significance for human health has still to be addressed.

This scenario requires the development of effective surveillance programs, where the entomological surveillance should allow detecting emerging viruses in field-collected mosquitoes. It has been demonstrated, both in the US and Europe (Dennet 2007; Hoel et al., 2009; Hubálek et al., 2010; Romi et al., 2004), that regular mosquito surveillance programs can enable to detect virus circulation some weeks before the appearance of human cases (Unlu et al., 2009). Depending on the arboviruses under surveillance, different systems monitoring non-human hosts, vector species or human categories at risk must conveniently be chosen in order to maximize

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virus detection. WNV can spread very quickly and easily, and may threaten human's health before its detection (Kramer et al., 2008). Bustamante and Lord (2010), using a model that simulates the process of mosquito sampling, pooling, and virus testing, found that mosquito infection rates commonly underestimate the prevalence of arbovirus infection in a mosquito population. They conclude that other factors, like mosquito population size, age structure, weather and historical baseline data have to be considered to assess the risk of arbovirus transmission. According to Bellini et al. (2014a), the development of surveillance programs and vector control strategies in European regions based on data obtained from studies performed in North America is not entirely appropriated (and also not feasible due to different legislative frameworks). The diversity of the susceptible bird fauna and the vector species involved in the enzootic and tangential transmission of WNV in Europe, pose a situation remarkably different to that in US (Bellini et al., 2014a). The improvement of entomological surveillance methods and programs can increase the reliability of the risk assessment and contribute to reduce the gap between infection rate estimates and the risk of arbovirus transmission to humans and animals (Gu et al., 2008; Roiz et al., 2012b).

To develop an efficient vector surveillance system it is necessary to gain knowledge about the following issues: (i) which environmental conditions are necessary for an outbreak to occur; (ii) biology and ecology of the main vector species in the areas potentially at risk; (iii) the efficiency of the different types of trap in attracting the vector species; (iv) the physiological age of the captured females (i.e. nulliparous and parous females) (Hugo et al., 2008). Recognition of parous females is important because it implies females had completed at least one gonotrophic cycle and had taken a blood meal, with the chance to be infected in case the host is viraemic. In addition, the identification of gravid females is also important, because having a batch of eggs ready to be laid implies they already took a blood meal, independently of the physiological age. In the same way, the presence of blood in the stomach is an indication of a potentially infectious female. Thus, mosquito traps capturing a higher proportion of potentially infectious females will enhance the probability to predict a disease outbreak (Bellini et al., 2014a,b). Concerning the efficiency of different models of traps in attracting mosquito females, Kesavaraju et al. (2011) and Allan and Kline (2004) compared some commercial models of gravid traps with structural differences and found that several characteristics significantly affect mosquito collection efficacy. Commercial gravid traps differ in basic design, color and size of the tank that contains the infusion, giving different capture results (Allan and Kline, 2004, Dennet, 2007; White et al., 2009). Moreover, different infusions (aquatic grasses like *Juncus effusus* L., *Rhyncospora corniculata* (Lamarck) and *Typha latifolia* L., cow manure, mix of grass clippings, wheat straw, rabbit chow) can attract different mosquito species depending on the season (Burkett and Mullen 2008; Jackson et al., 2005; McPhatter et al., 2009). According to Williams and Gingrich (2007), the use of gravid traps could give better results for West Nile virus surveillance over light traps or resting boxes. Many types of traps use carbon dioxide as the primary attractant. The produced plume of CO_2 mimics human exhalation and thus makes these traps quite specific for capturing blood-seeking females. CO_2 traps allow the collection of large numbers of mosquitoes and appear to be highly attractive to a wide variety of mosquito species. CO_2 traps are widely used in Italy for vector's monitoring and surveillance (Bellini et al., 2003; Calzolari et al., 2010). Traps called 'resting boxes' are passive devices that serve as shelters for mosquitoes during the day (Crans 1995). They have been used to sample mosquito populations since the time of the malaria's control programs, and still they are successfully used for monitoring many *Anopheles* species (Kweka et al., 2010; Pombi et al., 2014). However, they perform very differently depending on the technical aspects of construction and on the richness of resting sites in the study area (L'Ambert et al., 2012; Panella et al., 2011). The BG-Sentinel mosquito trap mimics convection currents created by a human body and it is widely used in many parts of the world especially for the collection of *Aedes* mosquitoes (Maciel de-Freitas et al., 2006; Bhalala and Arias 2009; Farajollahi et al., 2009; Bhalala et al., 2010). Different variations of the BG-Sentinel can be used according to the target species. The trap can be used with or without carbon dioxide, and with Biogents' proprietary attractant for anthropophilic mosquitoes (such as *Aedes aegypti* L. or *Culex quinquefasciatus* Say).

Our study was designed to compare the effectiveness of five mosquito traps in measuring species abundance and composition in wetland habitats, and to analyze the attractiveness towards potentially infectious females. The work was performed in the perspective of a wide entomological surveillance program, with a focus on *Cx. pipiens* s.l. L. being WNV the most widely distributed arbovirus in Northern Italy.

2. Materials and methods

2.1. Study period and study areas

The study was run from June, 14 to September, 16, 2011 in three wetland sites in the Emilia-Romagna region: La Rizza (44°39′41.82″N - 11°26′19.55″E), Le Vallette (44°44′33.18″N - .11°57′19.95″E) and Oasi Val di Sole (44°56′28.09″N - 11° 2′24.44″E) (Fig. 1).

La Rizza is situated in the municipality of Bentivoglio (BO); it is a natural protected area of about 1,500 ha. The dense vegetation hosts many aquatic bird species, such as ducks, cormorants and herons. This area includes permanent wetlands, wet meadows, reed beds, copses and hedges, but also wetland tanks, fishing lakes and two observation sheds located in an expansion of the Navile canal. A white stork *Ciconia ciconia* (L.) conservation center is also present.

Le Vallette is in the municipality of Ostellato (FE). It is a wetland area of approximately 300 ha located between two canals, which act as its boundaries. At least 150 bird species, mainly aquatic, can be observed. Reeds are the predominant species, but trees like poplar, elm and willow are also present.

Oasi Val di Sole is a natural protected area located in Concordia sulla Secchia (MO) originated from the excavation of clay which began in the '80s. It extends for an area of approximately 25 ha between the Po and the Secchia rivers and consists of four main basins, two ponds, ridges and canyons that make up a rest and nesting area for several bird species. More than 200 bird species have been observed, including some quite rare species like the ferruginous duck *Aythya nyroca* (Guldenstadt), which is the symbol of the oasis.

2.2. Mosquito traps

Five types of traps were compared in this study: the CDC Gravid trap (John W. Hock Company, Gainesville, Florida, model 1712) (Fig. 2A), the CO_2-baited trap (CAA, Crevalcore, Italy, model CAA2004) (Fig. 2B), BG-S trap (BG-Sentinel™, Biogents GmbH, Regensburg, Germany) (Fig. 2C), and two experimental prototype traps specifically designed and manufactured for this study by the authors (Fig. 2D–E).

Gravid trap. The infusion was prepared as follows: 5 L of tap water with 2.5 g of dry brewer yeast and 30 g of dry grass hay. The preparation was kept at 26 ± 1 °C in dim light inside a wide mouth open tank for 3 days. The infusion was stirred once a day to enhance the fermentation (Burkett 2005; Irish et al., 2012).

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