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UNDERSTANDING THE IMPACT ON HUMAN AND WILDLIFE HEALTH OF THE INVASIVE ALIEN MOSQUITO SPECIES AEDES ALBOPICTUS IN NORTHERN ITALY

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Introduction

Aedes albopictus is among the most widespread alien species in the world and its introduction and spread in northern Italy has been documented since 1990 (Dalla Pozza et al., 1994; Ferrarese, 2005; Fontenille et al., 2007; Romi, 2008; Bonizzoni et al., 2013). A map showing the spatial distribution of this species in north-eastern Italy is presented in Figure 1. Referred as being an opportunistic species, depending on the local availability of hosts, it is mainly anthropophilic. However, it can feed also on other hosts, including birds and mammals, as documented in the literature (Richards et al., 2006; Delatte et al., 2010); moreover, the ecological plasticity of this species could lead to the invasion and adaptation to several different ecological niches where temperature acts as major limiting factor (Roiz et al., 2011; Neteler et al., 2011; Bonizzoni et al., 2013). In Italy, its impact on human health is well known not only for its nuisance but also for being the most important vector involved in one epidemics of Chikungunya (CHIKV) occurred in 2007 (Rezza et al., 2007; Tomasello and Schlagenhauf, 2013). In term of veterinary importance, this species act as vector of Dirofilariasis (*Dirofilaria repens* and *Dirofilaria immitis*) (Schafner et al., 2013) and USUTU virus (USUV) (Calzolari et al., 2010, 2012, 2013; Tamba et al., 2011) but the implication for non domestic species is unknown.Therefore, its role as bridge vector of infections impacting both on human health and wildlife is currently under evaluation (Medlock et al., 2012). In fact, extensive epidemics of West Nile virus (WNV) (Rizzo et al., 2012; Barzon et al., 2013; Gobbi et al., 2014) and USUV (Tamba et al., 2011; Vázquez et al., 2011; Calzolari et al., 2013; Gaibani et al., 2013) have been recently documented within the study area. To improve our knowledge about the potential role of this species as vector of pathogens of medical and veterinary importance, we assessed its feeding preferences and infection prevalence with a number of viruses in northern Italy (Trentino and Veneto regions). We following summaries some preliminary findings.

Materials and methods

Sampling and screening of mosquitoes were carried out within two projects: FP7 Eurowestnile (EWN) and Aedespread. EWN Project: Mosquitoes were collected from May to October 2012, using 20 and 10 BG-Sentinel™ traps (BioQuip products, CA, USA) in Veneto and Trentino, respectively, located half in a rural and half in an urban environment, with BG-Lure® attractant and dry ice, checked weekly (Figure 2). Male and non-fed female mosquitoes captured were killed at -80°C, identified to species level and pooled according to date, location, species and gender and stored in EMEM (SafcBiosciences, Hampshire, United Kingdom) supplemented with FBS (Thermo Scientific Hyclone Inc., South Logan, Utah, USA) and a mixture of antibiotics (Penicilin, Streptomicine; Euroclone, Milan, Italy) at -80°C until molecular analysis. After RNA extraction with QIAamp® Viral RNA kit (Qiagen, Hilden, Germany), we used a generic RT-nested-PCR targeted on a region of the NS5 gene for the screening of flaviviruses (Sánchez Seco et al., 2005). The phylogenetic analysis were realized on a fragment of 1000bp (Vázquez et al., 2012). For samples positive at the generic NS5 RT-nested-PCR, virus isolation was attempted in C6/36 cell lines (from *Ae. albopictus* mosquito). Fresh supernatants and cells from cell cultures with evident cytopathic effect, were used for electron microscopy studies. Fed females were stored individually and underwent to blood meal analysis according to Alcaide et al. (2009). Aedespread Project: Mosquitoes were collected in Trentino to study the seasonal variation of feeding preference, from May to October 2013, using 13 BG-Sentinel™ traps with BG-Lure® attractant, located n. 3 in the residential area of a small town with numerous private gardens, common green areas and animals and n. 3 in a residential area with few green areas and animals, but numerous blocks of flats. The traps were checked monthly. Fed female mosquitoes were treated as in EWN protocol. Male and non-fed female mosquitoes captured were counted and used to determine relative density for site and seasonal variations. Further mosquitoes were captured out of the protocols.

Figure 1: Distribution map of *Ae. albopictus* in Trentino and Veneto.

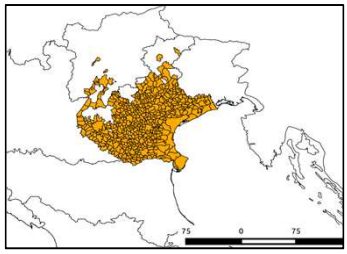

Figure 2: BG-Sentinel™ trap.

Tiger mosquito (*Aedes albopictus*)

Figure 3: Trapping sites for mosquitoes in Trentino (left) and Veneto (right).

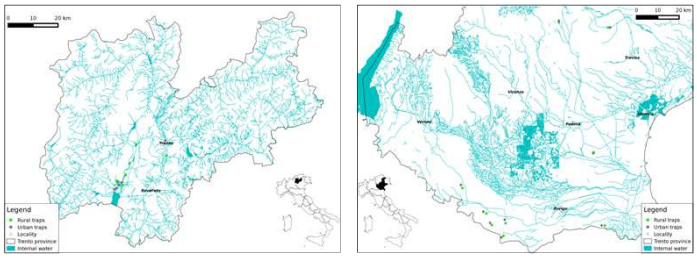

Table 2: AeFV prevalence in *Ae. albopictus*.

Region	AeVF prevalence (%)	Confidence interval (%)
Veneto	3.12	2.07 - 4.5
Trentino	16.84	12.18 - 22.74
Veneto + Trentino	8.07	6.4 - 10.04

Results

Trapping sites for mosquitoes are showed in Figure 3. A summary of the results obtained from the virological and molecular screening are reported in Table 1. Among Flavivirus we detected only Aedes Flavivirus (AeFV) an insect specific flavivirus (ISF), in both Veneto and Trentino regions and the virus of some positive pools was successfully isolated in cell culture C6/36 (from *Ae. albopictus*), with evident cytopathic effect (cell aggregation, cell detachment from the monolayer). Electron microscopy study performed on C6/36 cells infected by AeFV confirmed the presence of flaviviruses. The AeFV prevalence in *Ae. albopictus* found in Veneto and in Trentino in showed in Table 2. AeFV prevalence was influenced by region, having Veneto a lower prevalence despite the higher mosquito density, and did not significantly differ with respect to the type of environment (urban or rural) and the sampling time, in both regions. The phylogenetic analysis of the AeFV sequences detected in this study showed a high percentage of identity between those detected in Veneto, in Trentino and to those previously detected in Italy. Additionally, in Trentino by electron microscopy we also found in one female pool a Rhabdovirus and, with PCR we detected positivity in 4 female pools for n.1 Rhabdovirus/Parvovirus, n.1 Birnavirus/Parvovirus (Espiritu Santo/Negev virus), n.1 Flavivirus (AeFV) and n.1 Rhabdovirus (Flanders/Negev virus), which were also identified by electron microscopy.

Table 1: *Ae. albopictus* mosquitoes captured in EWN and Aedespread Projects and the results of Flavivirus screening and blood meal analysis.

Type of mosquito	Veneto	Trentino
Non- fed female	35 AeFV positive pools/ n. 2708 (181 pools)	49 AeFV positive pools + 1 Rhabdovirus positive pool/ n. 1905 (231 pools)
Fed female	n.3 *Homo sapiens* + n.1 *Er. europaeus*/ n. 4 (4 pools)	n.77 *Homo sapiens* + n.1 *Er. eropaeus* + n.1 *Passer montanus* + n.1 *Turdus merula* + n.1 *Coturnix japonica*/ n. 81 (81 pools)
Male	n. 1220 (91 pools)	49 AeFV positive pools/ n. 574 (123 pools)

Black bird (*Turdus merula*)

European hedgehog (*Erinaceus europaeus*)

Eurasian Tree Sparrow (*Passer montanus*)

Japanese quail (*Coturnix japonica*)

Human (*Femina sapiens*)

Discussion

These preliminary findings indicate the ability of this species to feed also on non-human hosts and thus act as additional potential bridge vector of pathogens among wildlife and humans. However, in this study we could not identify nor West Nile or Usutu virus in our samples. Furthermore, no co-infections with different flaviviruses were detected in this study. The detection of AeFV and, in particular, its higher prevalence in Trentino, arises the hypothesis that the high prevalence of ISFs could damper the transmission of other important pathogenic zoonotic viruses, but more experimental laboratory work is now needed. However, while in Veneto region West Nile virus and Usustu virus are endemically transmitted to humans and animals, in Trentino no clinical cases has so far being reported. Our results confirm the different eco-epidemiological situation of mosquito borne viruses occurring in North-east Italy underlined by previous studies, and report the presence of other important viruses associated with mosquitoes already described in other Countries. Further studies are therefore necessary to better assess the impact of the invasive *Ae. albopictus* on humans, domestic animals and wildlife in Italy.

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