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CURRENT KNOWLEDGE ON THE DISTRIBUTION AND BIOLOGY OF THE RECENTLY INTRODUCED INVASIVE MOSQUITO *AEDES KOREICUS* (DIPTERA: CULICIDAE)

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Current knowledge on the distribution and biology of the recently introduced invasive mosquito Aedes koreicus *(Diptera: Culicidae)*

Aedes koreicus, a new species of exotic mosquito, was recorded in 2011 in north-eastern Italy. The aim of this work was to characterize the biology, the environment and the current distribution of this mosquito by preliminary field and laboratory experiments, and to report the findings of the surveillance projects. Different studies were carried out to monitor the distribution of *Ae. koreicus* and to better understand its behaviour and vector competence: breeding sites and larval monitoring, ovitrap collection, adult mosquitoes collection, laboratory and field tests. *Ae. koreicus* was found in 46/105monitored municipalities (43.8%) in a range of altitude from 173 to 1250 m a.s.l. The most positive breeding sites were artificial water containers (41.0%). The period of activity ranged from March to October. Ovitraps and adult mosquito traps were efficient to collect *Ae. koreicus* specimens. Adults were also collected by human landing captures. In the laboratory studies, females fed on blood collected from dogs, cattle, sheep, chickens and humans, while in field conditions they clearly showed a preference for humans. *Ae .koreicus* seems to be a suitable intermediate host for *Dirofilaria immitis*; indeed the infective L3 stage was observed in all body districts including the proboscis showing it may be involved in the natural cycle of *D. immitis.* This work, once again, stresses the importance to know the biology and vector competence of invasive mosquitoes for an early detection in order to control their further spread and minimize the risk of pathogen transmission.

KEY WORDS: *Aedes koreicus*, invasive species, entomological surveillance, exotic mosquito, north-eastern Italy.

INTRODUCTION

Over the last decades the container-breeding mosquito species belonging to the genus *Aedes* (Meigen) were frequently detected out of their place of origin(REITER, 1998). Invasive *Aedes* species may be of public health relevance when they are able to establish in a new area and their competence to transmit disease agents is proven (LUNDSTROM, 1999; MEDLOCK *et al.*, 2007).

Italy is characterized by temperature and environments that offer many suitable habitats to different species of mosquitoes; therefore invasive *Aedes* mosquitoes may have the opportunity to establish after their introduction (ROMI et al., 2009). Since the discovery of the most invasive mosquito species *Aedes albopictus* (Skuse) or tiger mosquito, many local surveillance and control programs were started. In 2011, during a surveillance activity in a tiger mosquito-free area of the Veneto Region, an unexpected mosquito was noticed which clearly did not belong to the recorded Italian fauna. Larvae and adults were then morphologically and molecularly identified as *Aedes* (*Finlaya*) *koreicus* (Edwards) (CAPELLI *et al.*, 2011).

The biology and ecology of *Ae. koreicus* are poorly known. This species is native to the south-east of Asia, is reported to feed on humans and domestic animals and seems to be well adapted to urban environment (MYIAGI, 1971). Larvae develop in all types of artificial containers close to houses and in natural receptacles such as rock pools or tree holes. Adults seem to bite humans

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mainly during the daytime. Like other species of the genus *Aedes, Ae. koreicus* overwinters at the egg stage, hatching in the spring when the snow melts (LACASSE and YAMAGUTI, 1950; MYIAGI, 1971). In the past, *Ae. koreicus* was considered being a variety of *Ae. (Finlaya) japonicus* (Theobald) and was often confused with other similar species (MYIAGI, 1971); for this reason, information on its biology and behaviour is needed, in particular out of its place of origin.

The finding of *Ae. koreicus* in Italy represents the second incursion in Europe (CAPELLI *et al.,* 2011), after a previous report in Belgium in 2008 (VERSTEIRT *et al.,* 2012). After the first Italian finding, a more intensive surveillance was carried out until now to follow the distribution of *Ae. koreicus* and to collect biological data on its life cycle.

In this work we report the results of four years of surveillance on the presence and spreading of *Ae. koreicus* in Italy. Preliminary results on the biology, behaviour and vector competence for human and animals diseases are also described. Different studies were carried out to monitor the distribution of *Ae. koreicus* and to better understand its behaviour and vector competence: breeding sites and larval monitoring, ovitrap collection, adult mosquitoes collection, laboratory and field tests.

MATERIALS AND METHODS

Breeding sites and larval monitoring

The monitoring started in 2011 from Valbelluna, Province of Belluno, northern Italy, where the first *Ae. koreicus* mosquitoes were found. Then, it was extended in the whole Province of Belluno and in the neighbouring Provinces of Vicenza, Treviso and Trento from 2012 until now. All possible breeding sites, such as catch basins, man-made containers, fountain basins, tires, vases and natural mosquito larval habitats (tree holes, water in plants, puddles) were checked. The places visited included private and public places, i.e. private gardens, garden centres and florists, tire markets, cemeteries, farms and houses. Larvae were collected using a standard dipper. The specimens were morphologically identified (MYIAGI, 1971; ROMI *et al.,* 1997) in case of doubtful morphological identification or findings in new areas, a molecular confirmation was carried out using a PCR (CAMERON *et al.,* 2010).

Ovitrap collection

Aedes eggs were collected with standard ovitraps: black vases of 8 cm upper diameter filled with 300 ml of water, with strips of masonite™ as support for oviposition. Surveillance was provided by Local Health Units of Belluno Province in 2011-2012 using 34 ovitraps, then a new investigation started as part of the Lexem project (www.lexem.eu) setting 332 ovitraps in three provinces (Trento, Belluno and Vicenza).

Adult mosquitoes collection

Adults were collected using BG-Sentinel™ traps (Fig. 1, a) baited with CO_2 and BG-lure™ testing the ability of these two different traps to catch this species. Adults were also collected by manual aspiration (Fig. 1, b) and using sticky traps (Fig. 1, c). During larval collection, adults trying to land on the personnel were also hand-picked up. Adults and larvae obtained from the hatching of eggs were examined.The end of *Ae. koreicus* activity was defined when there were no more adults, larvae or positive ovitraps.

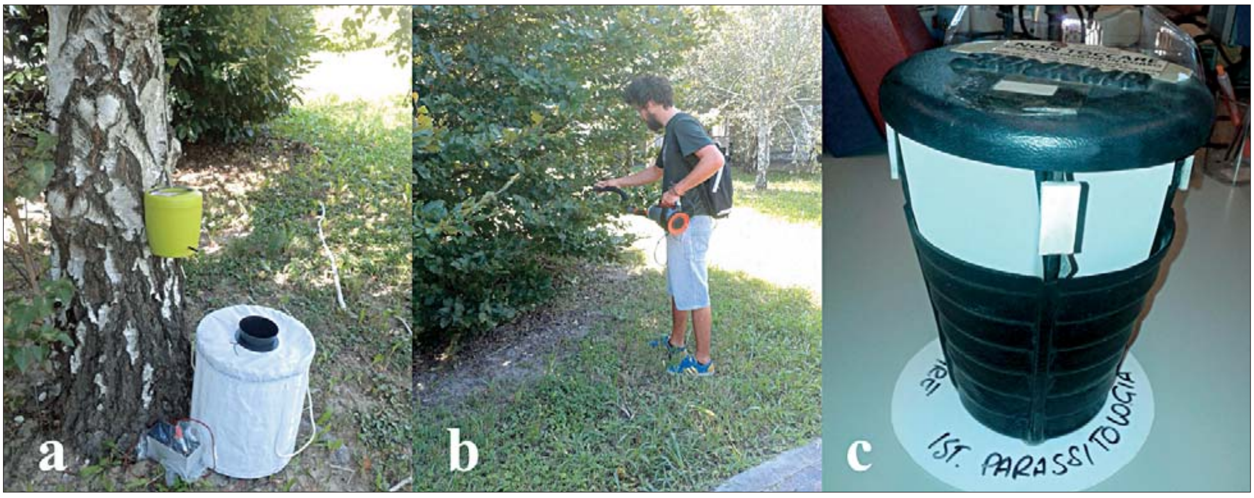

Fig. 1 – Traps used to collect adult mosquitoes. a) BG-Sentinel, b) manual aspiration, c) sticky traps.

Laboratory and field tests

Blood-fed females were isolated from mosquitoes collected by aspiration, BG-Sentinel traps and sticky traps. DNA was extracted from the blood meal of the engorged femalesto assess the feeding preference. All samples were analysed with a specific set of primers described by ALCAIDE *et al.*(2009). *A. koreicus* were also fed in the laboratory using an artificial feeding system (Hemotek^{TM}) providing blood from different hosts (dogs, cattle, sheep, chickens and humans).Human landing captures (HLC) were performed to assess the human attractiveness and the biting activity of *Ae. koreicus*. The tests were set in three sites in Belluno province running before the dusk, approx. from 17:00 to 20:30 hours; each collections lasted 30 min.

A. koreicus specimens were experimentally infected with *D. immitis* to evaluate the development of filarial larval stages in different body parts.They were fed by the artificial feeding system using naturally infected dog blood (3000 microfilariae/ml).Mosquitoes naturally dead and specimens killed at fixed days post infection were dissected; head, thorax and abdomen were examined separately by microscopic observation, and in case of filariae larvae observation a molecular confirmation by PCR was performed (LATROFA *et al.,* 2012).

RESULTS

Distribution, habitat and period of activity

Aedes koreicus was discovered on May 24, 2011 in the Sospirolo village (BL). The monitoring run from the beginning of June to the beginning of November in 2011, from the end of March to the end of September in 2012 and in 2013, and from mid-March to mid-November in 2014. In 2011, the beginning of the *Ae. koreicus* activity remained unknown as the survey started after the first identification at the end of May; the last adults were collected on September 13, but ovitraps remained positive until October. In 2012 the first larvae were caught on March 29,adults were active until the end of September, and larvae were observed until the end of October. In 2013 the first larvae were observed on April 04, and adults were active until the end of September. In 2014 the first larvae were collected on March 13, and adults and larvae were observed on November 17.

A total of 105 municipalities were monitored between 14-1645 m a.s.l., and 46 (43.8%) were positive for the presence of *Ae. koreicus* (Tab. 1) in a range of altitude between 173 and 1250 m a.s.l. Overall,259different types of potential larval habitats were monitored (Tab. 2), and the most positive breeding sites were artificial water containers (41.0%) (Tab. 3).

Table 1 – Prevalence values of positive municipalities for *Aedes koreicus* recorded during a four years period (2011-2014) in north-eastern Italy.

Province	Municipalities monitored/present (%)	Municipalities positive/monitored (%)
2011		
Belluno	23/69 (33.3)	17/23 (73.9)
Treviso	2/95 (2.1)	0/2 (0.0)
2012		
Belluno	45/69 (65.2)	23/45 (51.1)
Treviso	18/95 (18.9)	4/18 (28.6)
Vicenza	13/121 (10.7)	3/13 (25.0)
Trento	8/217 (3.7)	0/8 (0.0)
2013		
Belluno	52/69 (75.3)	34/52 (65.4)
Treviso	21/95 (22.1)	6/21 (28.6)
Trento	9/217 (4.1)	2/9 (22.2)
2014		
Belluno	17/67 (24.6)	11/17 (64.7)
Vicenza	5/121 (4.1)	3/5 (60.0)
Trento	15/217 (6.9)	3/15 (20.0)
2011-2014		
Total monitored	105	46/105 (43.8)

Table 2 – Results of positive and surveyed habitat for the presence of larval breeding sites for *Aedes koreicus* in 2011-2014 in Veneto region.

Habitat	Positive/monitored (%)
Cemeteries	20/101 (19.8)
Garden centers	13/19 (68.4)
Private gardens	32/49 (65.3)
Public parks	2/9 (22.2)
Tiremarkets	4/8 (50.0)
Streets/squares	28/48 (58.3)
Depots	4/5 (80.0)
Buildings	6/11 (54.5)
Farms	2/6 (33.3)
Forests	2/3 (66.7)
Total	113/259 (43.6)

Table 3 – Results of positive and surveyed larval breeding sites for*Aedes koreicus* in 2011-2014 in Veneto region.

Breeding sites	Positive/monitored for *Ae. koreicus* (%)
Vases	16/93 (17.2)
Water container	57/139 (41.0)
Catch basins	18/47 (38.3)
Puddles	3/6 (50.0)
Tires	4/9 (44.4)
Basin of fountains	6/9 (66.6)
Dunghill	0/1 (0.0)
Treeholes	0/1 (0.0)
Total	104/305 (34.1)

Mosquito collections by traps

As for *Ae. albopictus*, ovitraps were attractive for adult females resulting in the high rate of positivity 15/21 (71.4%) from 2011 to 2013, and 18/52 (34.6%) in 2014 (part of the Lexem project). *Ae. koreicus* was prevalent in sites between 400-600 m a.s.l. (71.4%) whereas *Ae. albopictus* was prevalent in locations under 200 m a.s.l. (Fig. 2). *Ae. koreicus* was also well represented at an altitude between 800-1000 m a.s.l. (28.1%) and was found in two sites above 1000 m a.s.l., where *Ae. albopictus* was absent. In total 32 CO_2 baited BG-Sentinel traps run in Veneto region in 2014 but only few specimens (15.9%) were caught. Adults were also capture inside a house and while they were trying to bite the collectors. Overall, 239 mosquitoes were collected by aspiration from 13 sites. Twenty-five samples (10.4%) were found positive to blood meal analysis. Sequencing of the PCR products identified human (*Homo sapiens*) blood in 23 samples, and cattle (*Bos taurus*) and dog (*Canis lupus familiaris*) blood in one sample each. In the laboratory, engorgement and completion of the life cycle was successfully achieved with dog (85%), human (76.5%) and chicken (65.4%) blood. During HLC *Ae. koreicus* were captured 12 times out of 18 collections (mean 0.6) mainly around 18:00 h.

Vector competence test for Dirofilaria immitis

A total of 46 mosquitoes were fed with dog blood infected with microfilariae (85%) and 31 mosquitoes (67%) were infected. The mosquito mortality rate was 52% during the first nine days. The average of microfilariae, L1 (sausage stage) and L3 larval stage observed into the mosquitoes was 14.67, 8.56 and 3.15, respectively. Second stage larvae were observed only once. First stage larvae were first observed at 3 days post infection (dpi) whereas L3 were observed at 8 dpi. The latter were found in salivary glands and proboscis starting on 16 till 28 dpi.

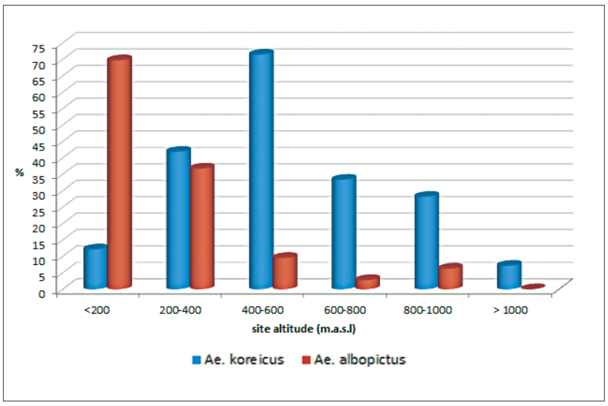

Fig. 2 – Distribution of *Aedes koreicus* and *Aedes albopictus* according to altitude in north-eastern Italy, 2011-2012.

Discussion and conclusions

The results of our survey show that *Ae. koreicus* was well established in an area of about 3000 km^2 of north-eastern Italy till 2013, but recent findings point out that its spreading is still ongoing reaching other areas of North Italy (Verona province and Lombardia region) (Fig. 3). It was not possible to clearly demonstrate the time of arrival and the route of entry of this mosquito in this part of Italy, but this species was likely introduced at least 3-4 years ago. The way of introduction was likely through eggs within small containers, tires or plants, as happened in the past for *Ae. albopictus* (Reiter, 1998; Romi *et al.,* 2009; Medlock *et al.,* 2012). *Ae. koreicus* larvae were present in the spring, much earlier than the similar species *Ae. albopictus*. Unlike *Ae. albopictus*, the cold season is not a limiting factor to the establishment and spread of *Ae. koreicus* all over the central Europe.

Our observations confirmed that *Ae. koreicus* was well adapted to urban settlements. The main sites colonized were urban and private areas (gardens, streets, squares, etc.) where it bred in a variety of artificial man-made containers. Larval coexistence of *Ae. koreicus* and *Ae. Albopictus* or other species were observed but not common. The standard ovitraps used for tiger mosquito monitoring and the CO_2 baited BG-sentinel traps showed to be suitable for *Ae. koreicus* collection. Accordingly, he surveillance by ovitraps is now complicated by the presence of two similar species in the same environment involving time-consuming in the laboratory and requiring well trained personnel for identification.

In the laboratory studies *Ae. koreicus* demonstrated its ability to complete the life cycle feeding in a wide range of animal blood. However, in field conditions it clearly showed a preference for humans. The anthropophilic behaviour of this species is confirmed by HLC and by the nuisance reported by the residents in areas where only *Ae. koreicus* occurs (Montarsi *et al.,* 2013). Further studies are needed to assess if humans are preferential hosts or if the anthropophily is opportunistic.

Aedes koreicus seems to be a suitable intermediate host for *D. immitis*. Despite the low initial number of microfilariae ingested, the infective L3 stage was observed in all body parts including the proboscis. This results show that *Ae. koreicus* may be involved in the natural cycle of *D. immitis*, increasing the risk of exposure for dogs and humans.

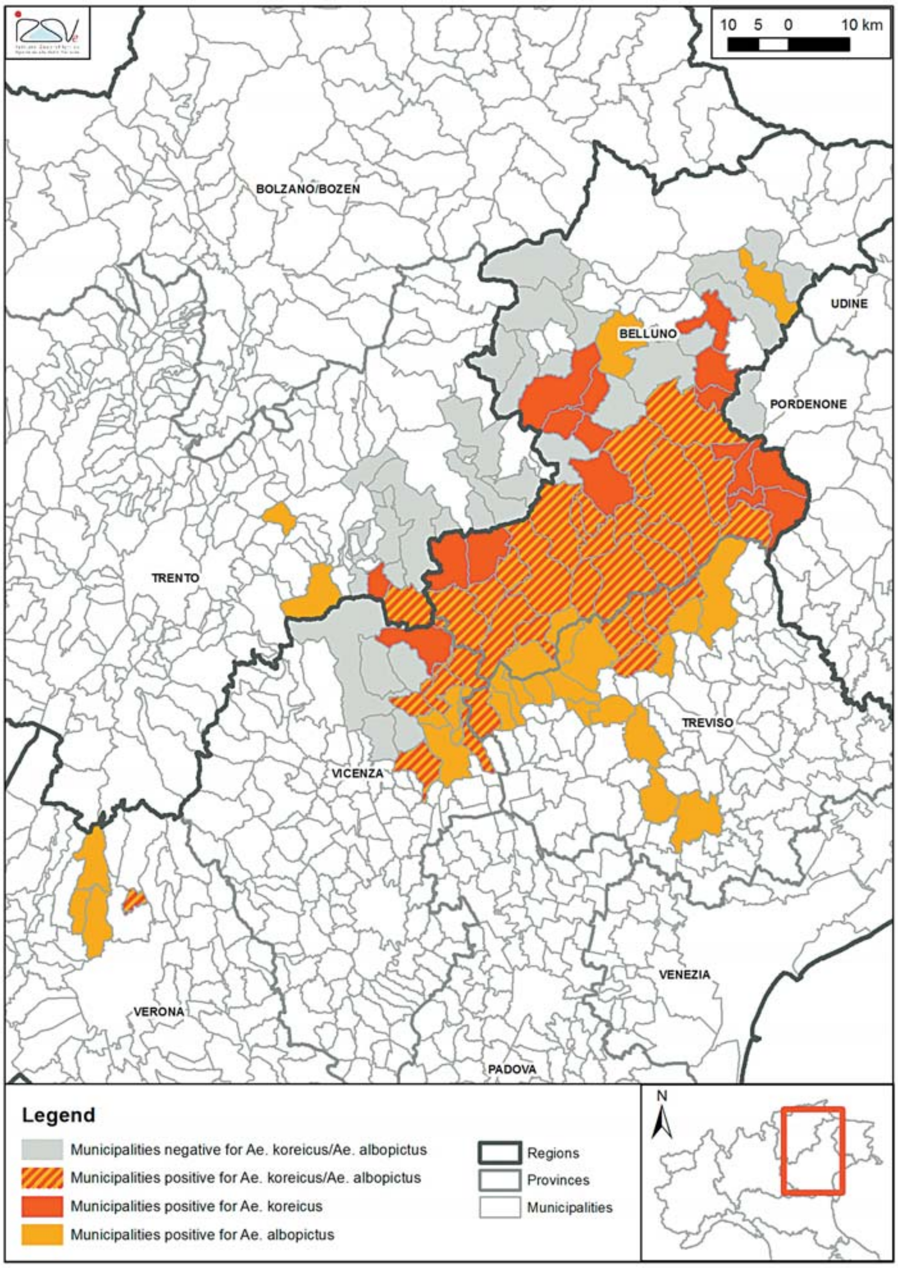

Fig. 3 – Map of monitored municipalities in north-eastern Italy, 2011-2014.Legend: municipalities positive for the presence of *Aedes koreicus*, *Aedes albopictus* and their overlapping areas.

Other aspects of the biology of *Ae. koreicus* need urgently to be clarified, such as the life cycle, the coexistence with other mosquito larvae and the vector competence for viruses. Little and old information on *Ae. koreicus* vector competence are available; experimental transmission of Japanese encephalitis virus (JEV) has been proven, but the virus has not been isolated from wild-caught mosquitoes (SHESTAKOV and MIKEEVA, 1966; GUTSEVICH *et al.,* 1971). Other arboviruses, such West Nile and Usutu viruses, are endemic in Veneto region (BUSANI *et al.,* 2011; CAPELLI *et al.,* 2013) and others, such as Chikungunya and Dengue viruses, are regularly introduced by infected humans every year (GOBBI *et al.,* 2012). If *Ae. koreicus* will be demonstrated to be competent for some of these viruses, the risk of transmission will be extended in this new colonized area, especially at high altitudes, i.e. in places previously regarded as a negligible risk of animal and human outbreaks.

This work, once again, stresses the importance to know the biology and vector competence of invasive mosquitoes for an early detection in order to control their further spread and to minimize the risk of pathogen transmission.

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RIASSUNTO

Aedes koreicus, una specie di zanzara esotica, è stata reperita per la prima volta nel nord-est d'Italia nel 2011. Dalla prima segnalazione sono stati condotti diversi studi sia di laboratorio che di campo con lo scopo di monitorare la sua diffusione e la sue competenze vettoriali. Dagli studidi sorveglianza risulta che attualmente *Ae. koreicus* è presente in ben 46/105comuni monitorati (43,8%) localizzati ad una altitudine compresa fra i 173-1250 m s.l.m. I siti di riproduzione più idonei alla sua riproduzione sono i contenitori di acqua artificiali (41,0%). Il periodo di attività degli adulti è compreso tra marzo e ottobre. In laboratorio, le femmine possono essere nutrite di sangue raccolti da cani, bovini, pecore, galline e uomo, mentre in condizioni di campo hanno chiaramente mostrato una preferenza per gli esseri umani. *Ae. koreicus* sembra essere un idoneo ospite intermedio per *Dirofilaria immitis*; in infezioni sperimentali di laboratorio, la fase infettiva L3 della filaria è stata osservata in tutti i distretti del corpo della zanzara, tra cui la proboscide.

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