Mosquitoes in Malta: Preliminary entomological investigation and risk assessment for vector-borne diseases (Diptera: Culicidae)

Francis SCHAFFNER[1*], Paul GATT[2], Sabine MALL[3], Michele MAROLI[4], Gianfranco SPITERI[5], Tanya MELILLO[5] & Hervé ZELLER[6]

ABSTRACT. At the request of Malta's Infectious Disease Prevention and Control Unit (IDCU), the European Centre for Disease Prevention and Control (ECDC) organized an expert mission in Malta in 2009 to provide support for a vector-borne disease (VBD) risk assessment. As these diseases are clearly related to the presence of competent and efficient vectors, and as little information was available on the local mosquito fauna (Diptera: Culicidae), a field study was implemented during the mission. The aim was to determine the presence and relative abundance of potential vectors, and to assess the risk of transmission of mosquito-borne diseases. From April 6th to May 1st, 2009, a total of 114 among 513 investigated putative larval habitat units contained mosquito immature stages. Artificial habitats and coastal saltwater rock pools predominated among the larval habitats. Also, 1,150 female mosquitoes were caught with dry-ice baited light traps. Seven species among the nine known to occur in Malta at the time of the study were observed and *Culex theileri* is reported here for the first time. The most commonly encountered species were *Culiseta longiareolata* and *Culex pipiens*. *Culex hortensis* was only found on the island of Gozo. The mosquitoes of the Mariae complex have been identified in the past as *Aedes zammitii* but all except four of the specimens collected showed morphological characters corresponding to *Aedes mariae*. Neither *Anopheles* spp. nor *Aedes albopictus* were found despite the investigation of suitable larval habitats and the use of CO_2-baited light traps and a few ovitraps. However, these species are known to be more abundant during the summer months and further investigations are needed to confirm their absence or their presence and distribution on the islands. The mosquito-related risk of disease for humans in Malta is discussed and West Nile fever appears to be the most probable mosquito-borne disease that could appear in Malta. The eventual finding of *Aedes albopictus* after this study was completed further strengthens the need to survey both chikungunya and dengue infections.

KEY WORDS. Mosquito, Vector, Mosquito-borne disease, Arbovirus, *Culex theileri*.

[1] Avia-GIS, Zoersel, Belgium / Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland. E-mail: francis.schaffner@access.uzh.ch
[2] Research Associate, International Environment Institute, University of Malta, Msida, Malta. E-mail: paulgatt@onvol.net
[3] NRW Institute of Health and Work (LIGA.NRW), Muenster, Germany / Robert Koch-Institute, Berlin, Germany. E-mail: s.mall@gmx.de
[4] Istituto Superiore di Sanitá, Roma, Italy. E-mail: michele.maroli@iss.it
[5] Infectious Disease Prevention and Control Unit, Department of Health Promotion and Disease Prevention, Msida, Malta. E-mail: gianfranco.spiteri@gov.mt; tanya.melillo@gov.mt
[6] ECDC, Stockholm, Sweden. E-mail: Herve.Zeller@ecdc.europa.eu
*Corresponding author

INTRODUCTION

The picture of vector-borne diseases (VBD) is altering mainly due to environmental changes as well as an increase in trade and tourism (Randolph & Rogers, 2010). Therefore, further knowledge on the risk of the introduction and spread of VBD in Europe has become a critical issue. Considering the location and the climate of Malta, Malta's Infectious Disease Prevention and Control Unit (IDCU) requested the support of the European Centre for Disease Prevention and Control (ECDC) to assess the public health risk posed by VBD. Consequently, the ECDC organized an expert mission to Malta. The Maltese archipelago consists of 7 islands covering 316 km^2 with over 400,000 inhabitants, and attracts more than 1 million (mainly European) tourists every year (1.29 million in 2008) through an increasing number of maritime and aerial connections. As VBD are clearly related to the presence of competent and efficient vectors and as little information is available on the mosquito fauna (Diptera: Culicidae) of the Maltese islands (Gatt, 1996), limited field investigations were also implemented during the mission. The findings and a discussion on the mosquito-related risk of disease for humans in Malta are presented in this article.

The most extensive study on the Maltese mosquito fauna was implemented in the 90s (Gatt, 1996), and five species had at that time already been reliably reported: *Aedes zammitii* (Theobald, 1903) [=*Ochlerotatus zammitii*, see Reinert, 2000], *Anopheles maculipennis* s.l. Meigen, 1818, *Culex pipiens* L., 1758, *Culex laticinctus* Edwards, 1913 and *Culiseta longiareolata* (Macquart, 1838) (da Cunha Ramos *et al.*, 1992; Gatt, 1996). Field data (Gatt, 1996) revealed the presence of four other species: *Aedes [=Ochlerotatus] detritus* (Haliday, 1833), *Aedes [=Ochlerotatus] caspius caspius* (Pallas, 1771), *Culex hortensis hortensis* Ficalbi, 1889, and *Uranotaenia unguiculata unguiculata* Edwards, 1913. Two other species, *Aedes aegypti* L., 1762 [= *Stegomyia aegypti*, see Reinert *et al*., 2004] and *Culex quinquefasciatus* Say, 1823, which had previously been reported were not considered as occurring or having occurred in Malta for several reasons (Gatt, 1996). Finally, recent findings led to the report of a tenth species, *Culex perexiguus* Theobald 1903 (Gatt, 2009) (see Table 2).

For the purposes of this investigation, special attention was paid to the potential vector species among the known Maltese mosquito fauna as well as to the invasive species *Aedes albopictus* (Skuse, 1894) [= *Stegomyia albopicta*]. The aims of this study were (1) to determine the presence (i.e. introduction and establishment) of exotic species, (2) to determine the relative abundances (in relation to the occurrence of larval habitats) of the potential vectors, and (3) to assess the risk of transmission of mosquito-borne diseases.

MATERIAL AND METHODS

The mosquito fauna was investigated between April 6th and May 1st, 2009. Potential mosquito larval habitats were checked randomly during field trips and in pre-selected places (ports, public parks, cemeteries, farms, nature areas); immature stages were collected (Fig. 3) and some reared until adult hatching. Light traps (similar to CDC miniature light traps) baited with dry-ice were used overnight in selected places for adult capture. Ovitraps (1 litre black plastic buckets with water and a piece of 5x5x2cm of polystyrene as oviposition support) were also placed in some selected places for several days, in order to collect eggs of container-breeding *Aedes* species. Immature stages and adults were identified to species level with identification keys (Harbach, 1985; Schaffner *et al*., 2001). Larval habitats were classified in two categories: artificial (man-made) (Figs 4 and 5b) and natural (Figs 5a,c and 6). The landscape/land cover was divided into 3 zones: nature, rural and urban.

RESULTS

A total of 513 potential larval habitat units were checked. Among them, 114 (22.2 %) had at least one immature mosquito specimen (Table 1, Fig. 1, Fig. 2). Artificial habitats predominated in the urban zone but they occurred in all land cover zones and are disseminated throughout the island (Table 1, Fig. 2). A quarter (26.7 %) of these habitats were positive (23 pos./86 neg.) for *Culex pipiens* and *Culiseta longiareolata* and larvae were found in basins, pools, fountains, and other containers in gardens (5/6) as well as in boats containing rainwater (3/4). A few vases and plastic bottles in the cemetery were also positive (3/60); neither of the two water cisterns inspected on roofs contained larvae. Wells were not accessible for inspection. In the rural and nature zones, only three water cisterns in fields and gardens were found positive whereas seven were negative, probably due to the presence of predatory fish. In the nature zone, saltwater rock pools predominated (326 among 349 larval habitats). Overall, they were frequently colonized (81/295) by *Aedes mariae/ zammitii*. Puddles, ditches, fresh water rock pools and residual puddles in streams and valley beds were positive for *Culex* spp. (10/13).

Table1. – Numbers of positive/negative larval habitats investigated in the different land cover zones on the Maltese islands, April 6th – May 1st, 2009

Type of larval habitat		Urban zone	Rural zone	Nature zone	Total no.
		Pos./Neg.	Pos./Neg.	Pos./Neg.	Pos./Neg.
Artificial	Vase, plastic bottle	3/60	0/0	0/0	3/60
	Plastic container, cask, tyre	5/2	1/0	1/1	7/3
	Old bathtub	1/0	0/0	0/0	1/0
	Basin, fountain, pit, pool	5/7	0/3	0/0	5/10
	Basin with saltwater	1/0	0/0	0/0	1/0
	Water cistern on roof	0/2	0/0	0/0	0/2
	Water cistern in garden	0/0	2/6	1/1	3/7
	Boat	3/4	0/0	0/0	3/4
Natural	Puddle with vegetation	0/0	0/0	1/0	1/0
	Saltwater rock pool	2/30	8/10	71/255	81/295
	Freshwater rock pool	0/0	0/4	5/7	5/11
	Ditch	0/0	0/0	2/2	2/2
	Stream/river with vegetation	0/0	2/3	0/0	2/3
	Pool with vegetation	0/0	0/0	0/1	0/1
	Tree hole	0/1	0/0	0/0	0/1
		20/106	13/26	81/267	114/399

Positive/negative - presence/absence of immature mosquitoes

Table 2. – Relative abundance of mosquito species observed in larval habitats on the Maltese islands, April 6th – May 1st, 2009

Species	Artificial larval habitats [1]						Natural larval habitats [2]				
	Vase, plastic bottle	Plastic container, cask, tyre	Old bathtub	Basin, fountain, pit, pool	Water cistern in garden	Boat	Puddle with vegetation	Saltwater rock pool	Fresh water rock pool	Ditch	Stream/ river with vegetation
Ae. albopictus											
Ae. caspius											
Ae. detritus											
Ae. mariae/zammitii				+*				+++			
An. maculipennis s.l.											
Cs. longiareolata	+	+++	+	+++	++	++			++		
Cx. hortensis				+					++		+
Cx. laticinctus		+		+	+						
Cx. pipiens	+	+	+	++	+	+	++		+	+++	+
Cx. perexiguus											
Cx. theileri											++
Ur. unguiculata							+				

+ present; ++ common; +++ abundant; * basin with saltwater
[1] No 'water cistern on roof' with larvae was identified
[2] No positive 'pool with vegetation' and 'tree hole' were found

A total of eight species were observed during this study (Tables 2, 3), including *Culex theileri* Theobald, 1903 which has never been observed before on the Maltese islands. One larva of this species was collected in a residual puddle in a stream bed in Gozo. The most commonly encountered species were *Culiseta longiareolata* and *Culex pipiens*. *Culex* and *Culiseta* spp. were found in a large panel of larval habitats, both artificial and natural. Numerous *Aedes mariae/zammitii* immature stages were found in coastal, natural or man-made salt harvest rock pools (locally named saltpans) which are numerous on these islands. The species was also found once in a man-made basin containing saltwater and located close to the shore. *Uranotaenia unguiculata* larvae were found only once in a flooded puddle. A total of 1,150 female mosquitoes were captured during 20 trap-nights. *Culex pipiens* was largely predominant (n=1,122); a single specimen of *Culex laticinctus* was identified whereas 8 specimens could not be attributed to any of these species, as the observed morphological features were not typical. Seventeen *Culiseta longiareolata* were trapped in urban environments and two *Aedes detritus* were caught in a nature area. Neither *Anopheles* spp. nor *Aedes albopictus* were found during larval collection or adult trapping.

Table 3. – Results of adult trapping with CO_2-baited CDC light traps on the island of Malta, April 6th – May 1st, 2009, total numbers

Site localisation: Land cover zone and municipalities	Site	no. of trap/night	*Ae. detritus*	*Cx. laticinctus*	*Cx. pip/lat**	*Cx. pipiens*	*Cs. longiareolata*
Urban (Attard, Marsa, St Julian's, Paola)	Gardens Cemetery Horse stable	3 2 1				37 0 0	1
Rural (Birzebbugia, Luqa, Zejtun)	Farms	10				48	6
Nature (Naxxar, San Pawl)	Salina Għadira	2 2	2	1	8	36 1001	10
no. total		20	2	1	8	1122	17

* Specimens that could not be attributed to either of these species

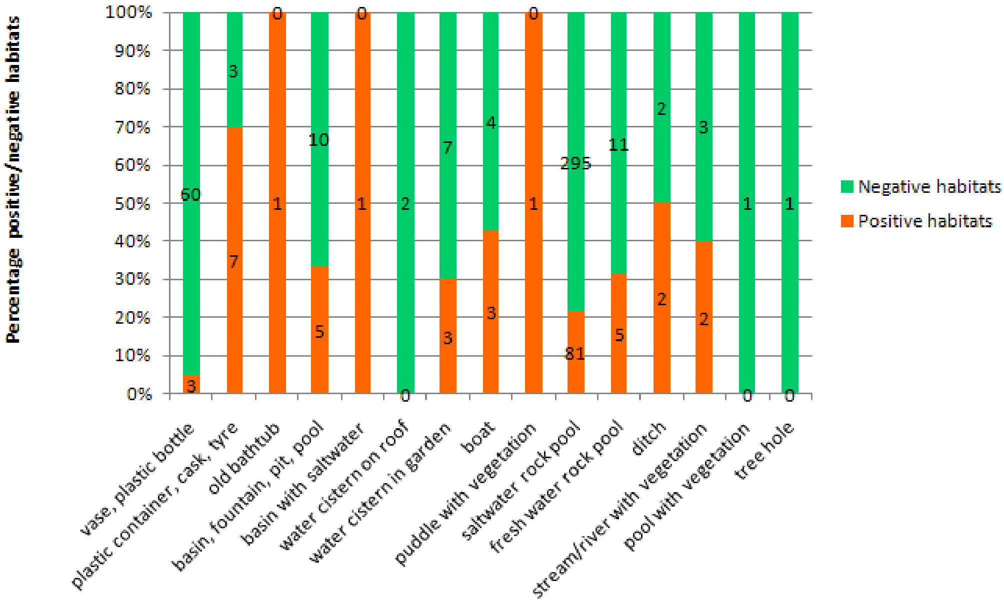

Figure 1: Percentages and numbers of positive/negative larval habitats investigated on the Maltese islands, April 6th – May 1st, 2009

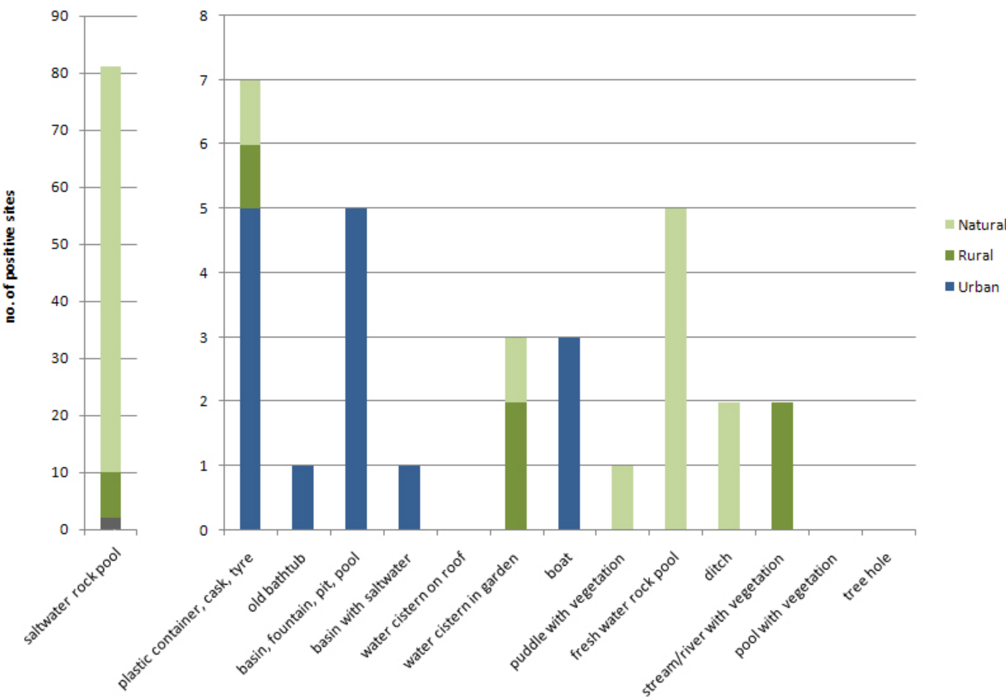

Figure 2: Number of larval habitats in the defined land cover zones on the Maltese Islands, April 6th – May 1st, 2009

DISCUSSION

Because of the risk of introduction and spread of VBD, an expert mission has been organized in Malta (April-May 2009) in order to provide support for a risk assessment on VDB, identify the main VBD with public health relevance occurring on the islands, identify the public health tools in place to ensure timely detection of, and response to, a potential threat from VBD, and propose priorities for public health action in order to ensure optimal preparedness (Mall *et al*., in prep.). The mission was focussed on mosquitoes and included a field entomological investigation. Despite the short duration of this mission and its implementation at a period of the year that was not optimal for summer-breeding mosquitoes, eight mosquito species have been observed. *Culex theileri* is recorded for the first time from the Maltese islands (Gozo). Its presence is not surprising considering its wide distribution in the Mediterranean and the local availability of suitable larval habitats, as previously speculated (Gatt, 1996). Further investigations should be implemented in order to determine its distribution and abundance. The common finding of *Culiseta longiareolata* and *Culex pipiens* is consistent with the numerous artificial breeding sites observed as well as with the historical data (Gatt, 1996). However, the highest adult catch was obtained in a nature area where large larval populations were also observed in ditches (Għadira). The low number of *Culiseta* females caught in the traps may be due to the low attractivity of CO_2 for this species, as dense larval populations were often found close to the trapping points (mainly in urban areas). Larval stages of *Uranotaenia unguiculata* are reported for the first time as only a few adult females of this species had been collected in the past (Gatt, 1996). The mosquitoes of the Mariae complex that breed in saltwater rock pools had been identified in the past as *Aedes zammitii* (Coluzzi & Sabatini, 1968; Coluzzi *et al*., 1974; Gatt, 1996). However, all except four larvae of the collected specimens within this complex (larvae, n>100; emerged adults, n=56) showed morphological characters corresponding to *Aedes mariae.* Further investigation on morphology and genetics will be needed to confirm the identity of the local populations.

Culex hortensis was only found in Gozo, confirming previous findings (Gatt, 1996), and this is surprising considering the availability of similar larval habitats in Comino and Malta and the small distance between these islands and Gozo (1 and 5 km, respectively). The recently recorded *Culex perexiguus* was not observed, but no larval collection or adult trapping was carried out at the site where the species had been reported previously. *Culex laticinctus* has been recorded as widely distributed in the islands in a former study (Gatt, 1996), but we observed only a few specimens in our study. Indeed, populations may still be low in April as shown by all reported collections of immature stages which were made later in the year, from July to November (Gatt, 1996).

Few mosquitoes were caught with the CO_2-baited traps in the urban and rural zones, suggesting that the urban mosquito populations (mainly *Culex* and *Culiseta* spp.) remain quite low here, despite the presence of numerous larval habitats. This could be due to the generally small size of the available larval habitats and to the period of the year in which this investigation was carried out: these species have several generations and continue to breed until fall, increasing the size of the populations. In the nature zone, the mosquito populations were quite large (i.e. more than 500 *Culex* females/trap/night), probably due to the occurrence of large flooded ditches producing large cohorts. If these sites should dry out and then be flooded again, they will 'produce' first *Aedes* spp. cohorts (in more or less brackish water) and then *Culex* spp. cohorts again (in fresh or slightly salted water).

Neither *Anopheles* spp. nor *Aedes albopictus* were found, despite the investigation of suitable larval habitats and the use of CO_2-baited light traps and a few ovitraps. However, these species are known to be more abundant during summer months. Further investigations are needed to confirm their absence or their presence and distribution on the islands.

Figure 3: Investigation of larval habitats. **a**, sample collected from a basin; **b**, samples from different larval habitats; **c**, sampling site (basin with, in the back, some members of the risk assessment team). **Figure 4**: Examples of artificial larval habitats suitable for *Aedes albopictus*, *Culex* spp. and *Culiseta* spp. **a**, old bathtub; **b**, boat; **c**, basin; **d**, water cisterns on roof.

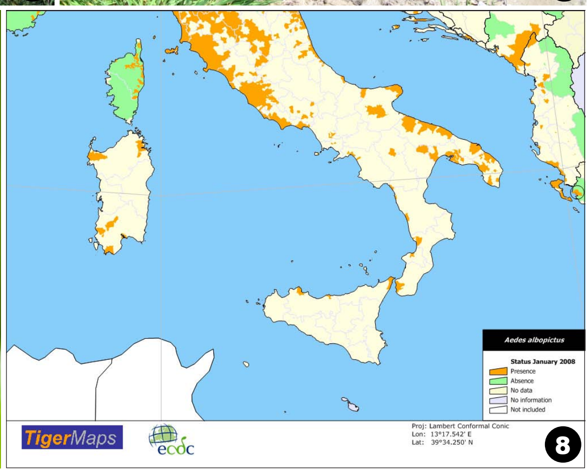

Figure 5: Larval habitats. **a**, fresh water rock pools suitable for *Culex hortensis*, *Culex pipiens* and *Culiseta longiareolata*; **b**, basin with saltwater; **c**, saltwater rock pools suitable for *Aedes mariae/ zammitii*. **Figure 6**: Larval habitats. **a**, Residual puddles in stream bed, suitable for *Culex theileri*; **b**, Puddle suitable for *Uranotaenia unguiculata*. **Figure 7**: Female specimen of *Aedes albopictus*. The medio-longitudinal white line on the thorax is a morphological characteristic of the species. **Figure 8**: Distribution of *Aedes albopictus* in Southern Europe by municipality units, as known by January 2008.

The mosquitoes of Malta and their vector potential

Observed species

With the addition of *Culex theileri* in this paper, a total of eleven mosquito species are now known to occur or to have occurred in Malta.

***Aedes caspius*: ** This species is one of the main pest species in Europe, mainly in coastal areas as it preferably breeds in brackish water. It is recognized as an efficient vector of Tahyna virus and has been found infected in nature by West Nile virus (summarized in Schaffner *et al*., 2001). Under experimental settings, this species was found to be receptive to Chikungunya virus (Vazeille *et al*., 2008) but appeared as an inefficient vector of West Nile virus (Balenghien *et al*., 2008). It is considered as an efficient vector of the tularaemia bacterium *Francisella tularensis* in Russia as well as of myxomatosis (poxvirus) and the canine filariasis nematodes *Dirofilaria immitis* and *D. repens* (summarized in Schaffner *et al*., 2001).

***Aedes detritus* s.l.:** This mosquito could also be a temporary pest species in coastal areas, mainly in spring during the few weeks following the flooding of coastal brackish water marshes. Two sibling species have been described, *Aedes detritus* s.s. and *Aedes coluzzii* Rioux, Guilvard & Pasteur, 1998 based on molecular evidence (Pasteur *et al*., 1977). The latter prefers more saline water. Females are receptive to Chikungunya virus experimental infection (Vazeille *et al*., 2008), are recognized as vectors of myxomatosis and have been found infected by *D. repens* in nature (summarized in Schaffner *et al*., 2001).

***Aedes mariae/zammitii*:** This species could be responsible for a biting nuisance close to its breeding sites (saltwater rock pools) which are abundant on the coast including urban areas. It is not known as a vector for human infectious diseases but females can transmit the avian malaria species *Plasmodium relictum* (summarized in Schaffner *et al*., 2001).

***Anopheles maculipennis* s.l.:** This species was considered to be a secondary vector of malaria in several countries in the past (summarized in Schaffner *et al*., 2001). It is a complex of 8 currently recognized Palaearctic species, and the one that was previously present in the Maltese islands was never precisely identified (Gatt, 1996). However, old reports of malaria transmission in Salina suggest the presence of *Anopheles atroparvus* Van Thiel, 1927, a competent vector species which can breed in brackish water. *Anopheles maculipennis* s.l. was eradicated from the islands in the middle of the last century. It has not been observed since 1943 but was never actively searched for. Members of the Maculipennis complex may also transmit canine filariasis, tularaemia and myxomatosis and have been found to be naturally infected by Batai, Tahyna and West Nile viruses (summarized in Schaffner *et al*., 2001).

***Culiseta longiareolata*:** This mosquito does not bite humans but has been proven to be an experimental vector of West Nile virus and is recognized as a vector of avian malaria (summarized in Schaffner *et al*., 2001).

***Culex hortensis hortensis*:** The vectorial risk to humans for this species is null, as it only feeds on batrachians and reptiles.

***Culex laticinctus*:** Females of this species do not bite humans and therefore do not represent any risk for vectorial transmission to man.

Culex pipiens**:** This mosquito is a nocturnal pest indoors. Two 'forms' are commonly recognised, an anthropophilic (biting preferentially humans) form which breeds in urban areas, mainly in polluted water or underground (flooded basements), and an ornithophilic (biting birds) form which breeds in clear water mainly in rural and nature areas. Most probably both forms occur in Malta but the specimens caught during this study could not be characterized (e.g. host preferences) and attributed to one of these forms. The species is considered as an efficient vector of West Nile and Sindbis viruses as well as of avian malaria; it is also an experimental vector of Tahyna virus and canine filariasis (summarized in Schaffner *et al*., 2001).

Culex perexiguus**:** Females seems to feed mostly on birds, but they sometimes enter dwellings and bite humans at night. The species is suspected as a vector of West Nile and Sindbis viruses in the Middle East (summarized in Schaffner *et al*., 2001).

Culex theileri**:** Females feed on mammals and are know to bite humans, also indoors. They have been found naturally infected by West Nile and Sindbis viruses in South Africa (summarized in Schaffner *et al*., 2001) and are suspected to be the main vector of *Dirofilaria immitis* on Madeira Island (Santa-Ana *et al*., 2006).

Uranotaenia unguiculata**:** The vectorial risk to humans for this species is null, as it does not bite humans.

Other species

Culex quinquefasciatus (main vector of human filariasis) and *Aedes aegypti* (main vector of dengue) have been reported in the past as abundant on the Maltese islands but these records could not be substantiated (Gatt, 1996).

Aedes albopictus is an invasive species (Fig. 7) which is rapidly extending its range in Europe. For several years it has been present in Italy, including Sicily (Fig. 8). It is an important pest species in urban areas and it also represents a high vectorial risk, as it is receptive to experimental infections with 24 arboviruses (reviewed in Gratz, 2004) and it is an efficient vector of Chikungunya and dengue viruses (Reiter *et al*., 2006). It was responsible for the chikungunya outbreak that affected around 250 people in Northern Italy in 2007 (Angelini *et al.*, 2007). It is also recognized as an efficient vector of canine filariasis (summarized in Schaffner *et al*., 2001).

Mosquito-borne diseases risk assessment for humans

West Nile fever: West Nile (WN) virus mainly infects birds but also humans, horses, some other mammals and reptiles. Human infections are often asymptomatic but can lead to a mild febrile syndrome or neuroinvasive encephalitis. Mosquito vectors of WN virus are abundant on Malta, including in nature areas where migrating birds could stay for some time. Therefore, viral amplification cycles involving birds and mosquitoes could occur, as well as occasional transmission to horses or humans. Introductions of the virus are likely through migrating birds but the development of any local transmission cycle is more dubious as it requires a congruence of numerous factors (i.e. coincidence of presence of infective hosts, competent vectors and susceptible hosts, in favourable climatic conditions). A monitoring of *Culex pipiens* populations (locations, abundance) in nature areas would allow assessment of the risk for local transmission if vector activity coincides with presence of migrating birds. Also a serological surveillance of wild and/ or indigenous birds, more difficult to perform, would be helpful for WN virus risk assessment.

Chikungunya and dengue fevers: Even if monkeys are involved in the sylvatic transmission cycles of Chikungunya (CHIK) and Dengue (DEN) viruses, only humans are commonly infected by these viruses which cause acute febrile illnesses. Although infections may be asymptomatic (<15% for CHIK, >80% for DEN), others may be severe, with a prolonged arthralgic phase which can persist for weeks or months for CHIK, and a haemorrhagic fever or a shock syndrome for DEN. Both viruses are mainly transmitted by *Aedes aegypti* and *Aedes albopictus*. At the time of the present study, there was no evidence of the presence of *Aedes albopictus* in the Maltese islands. Nevertheless, considering the important traffic of ferry boats from continental Italy (e.g. Genoa, Civitavecchia) and from Sicily (e.g. Catania, Palermo, Pozzallo) to the Maltese islands, it was suggested to implement a routine entomological surveillance to confirm the absence of this species and to detect its presence if introduced as soon as possible. Indeed, in fall 2009, the mosquito was reported from Malta (Buhagiar, 2009; Gatt *et al.*, 2009). This stresses the necessity for a rapid implementation of a field surveillance of this vector in order (1) to identify its current distribution and possible establishment, (2) to be able to implement focal control measures and (3) to re-assess the risk areas and periods for local CHIK and DEN virus transmission, given the virus is introduced. There is some possibility to eradicate *Aedes albopictus* if appropriate control measures are taken soon after introduction of the mosquito combined with an appropriate monitoring for assessing presence and absence of the mosquito. Since the species is now known to be present in Malta, transmission of DEN and CHIK can no longer be considered as null, as numerous outbreaks still occur in America, Africa and Asia, allowing worldwide dissemination of the viruses by infected travellers. Further, other indigenous *Aedes* species are receptive to CHIK virus but they would probably not be able to develop and maintain a transmission cycle although this cannot be excluded.

Malaria: Malaria is due to a sporozoan protozoan that parasitises particularly red blood cells, causing mild to severe sickness with many symptoms including fever, arthralgia, vomiting, anaemia, retinal damage, hepatomegaly, renal failure, up to coma and death. The four classical human malaria *Plasmodium* spp. do not apparently affect other animals and are transmitted to humans by the bloodsucking bite of the *Anopheles* mosquito female. Although malaria has been transmitted locally in the past, there is no current evidence of the presence of any suitable vector on Malta as *Anopheles maculipennis* s.l. is still considered as extinct (Gatt, 1996). Therefore, despite occasional introduction of parasites by immigrants and tourists (during the period 1991-1999, 17 imported cases have been reported (Savona-Ventura, 2002)), there is no risk of local transmission. Nevertheless, the absence of indigenous malaria vectors (i.e. *Anopheles* spp.) should be confirmed by monitoring potential larval and adult habitats (CO_2-baited light traps around streams with standing water and emergent vegetation, animal shelters). Special attention should be paid to the surroundings of the international airport as there is the possibility of introducing exotic vectors considering some direct flights from malaria-endemic areas.

Sindbis and Tahyna fevers: Humans infected by Sindbis and Tahyna viruses show symptoms like rash and arthralgia for the former, and fever, headache, nausea, myalgia, and aseptic meningitis for the latter. Vectors of both viruses are present in Malta. As for West Nile virus, the Sindbis virus amplification cycle involves birds and mosquitoes. Therefore, it might occur only in some nature areas after introduction of the virus by migratory birds, which has a low probability. The introduction of Tahyna virus is close to null, as the current circulation of the virus seems to be very low, and the virus could only be introduced with infected humans or other mammals (i.e. horses, rabbits).

Rift Valley fever: This zoonosis affects primarily livestock but can be passed to humans who may be asymptomatic or else exhibit a mild illness with fever, headache, myalgia and liver abnormalities but which could progress to haemorrhagic fever or meningoencephalitis in a small percentage of cases. Humans may become infected by direct exposure to infected animals or tissues but are mainly infected by bites of mosquitoes or other insects. The local mosquito species have some level of vectorial capacity for Rift Valley virus transmission. However, so far no imported or local cases in humans or animals have been reported in Malta. The monitoring of *Culex pipiens* populations in nature areas would provide essential data (i.e. seasonal dynamics) for a risk assessment. Surveillance of animal trade and possible local animal cases of Rift Valley fever would be the main tool for risk assessment, the risk for humans being very low considering the local conditions.

Filariasis: Heartworm infections due to *Dirofilaria immitis* or *D. repens* affect dogs and other carnivores and may result in serious disease for the host. Humans may become infected by mosquito bites as aberrant hosts but it is of negligible public health risk as such infection is highly unusual. Furthermore, human infections are usually of little or no consequence, although infected humans may show signs of respiratory disease with *D. immitis* or subcutaneous nodules or rare organ manifestations with *D. repens*. Considering the presence of several competent mosquito vectors on the islands, it would be relevant to collect data on *Dirofilaria* prevalence in dogs in order to assess the risk to humans.

ACKNOWLEDGMENTS

The authors and IDCU thank ECDC for funding this mission and helping to undertake such a risk assessment. They also warmly thank Dr David Mifsud, the staff of the Għadira Nature Reserve, Marzena Ratynska and Fabienne Schaffner for their support and patience during field work, as well as Prof. Alexander Mathis for his constructive review of the manuscript. The authors acknowledge useful comments and constructive criticism from two anonymous referees. They also thank Dr Roger Eritja (nature photographer) and Els Ducheyne (Avia-GIS) for providing figures 7 and 8 respectively.

REFERENCES

Angelini, R., Finarelli, A.C., Angelini, P., Po, C., Petropulacos, K., Silvi, G., Macini, P., Fortuna, C., Venturi, G., Magurano, F., Fiorentini, C., Marchi, A., Benedetti, E., Bucci, P., Boros, S., Romi, R., Majori, G., Ciufolini, M.G., Nicoletti, L., Rezza, G. & Cassone, A. (2007) Chikungunya in north-eastern Italy: a summing up of the outbreak. *Eurosurveillance*, **12**(47). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3313

Balenghien, T., Vazeille, M., Grandadam, M., Schaffner, F., Zeller, H., Reiter, P., Sabatier, P., Fouque, F. & Bicout, D.J. (2008) Vector Competence of Some French *Culex* and *Aedes* Mosquitoes for West Nile Virus. *Vector-Borne and Zoonotic Diseases*, **8**(5): 589-596.

Buhagiar, J.A. (2009) A second record of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Malta. *European Mosquito Bulletin*, **27**: 65-67.

Coluzzi, M. & Sabatini, A. (1968) Divergenze morfologiche e barriere di sterilita' nel complesso *Aedes mariae* (Diptera, Culicidea). *Rivista di Parassitologia*, **29**(1): 49-70.

Coluzzi, M., Sabatini, A., Bullini, L. & Ramsdale, C. (1974) Nuovi dati sulla distribuzione delle specie del complesso mariae del genere *Aedes*. *Rivista di Parassitologia*, **35**: 321-330.

da Cunha Ramos, H., Novo, M.T. & Pires, C.A. (1992) *Culex laticinctus* Edwards, 1913 (Diptera, Culicidae), um mosquito novo para a rep. de Malta. *Boletin de Sociedade Portuguesa de Entomologia* **1**(suppl. 3): 253-260.

Gatt, P. (1996) Mosquitoes from the Maltese Islands: New records and observations. *Bollettino della Società entomologica italiana*, **128**(1): 77-84.

Gatt, P. (2009) *Culex* (*Culex*) *perexiguus* Theobald (Diptera: Culicidae), a mosquito species new to the Maltese fauna. *European Mosquito Bulletin*, **27**: 7-9.

Gatt, P., Deeming, J.C. & Schaffner, F. (2009) First record of *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) in Malta. *European Mosquito Bulletin*, **27**: 56-64.

Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, **18**: 215-227.

Harbach, R. (1985) Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex (Culex)* occurring in southwestern Asia and Egypt, with a note on the sub-generic placement of *Culex deserticola* (Diptera: Culicidae). *Mosquito Systematics*, **17**(2): 83-107.

Pasteur, N., Rioux, J.A., Guilvard, E., Pech-Perieres, M.J. & Verdier, J.M. (1977) Existence chez *Aedes* (*Ochlerotatus*) *detritus* (Haliday, 1833) (Diptera-Culicidae) de Camargue de deux formes sympatriques et sexuellement isolées (espèces jumelles). *Annales de Parasitologie humaine et comparée*, **52**: 325-337.

Randolph, S.E. & Rogers, D.J. (2010) The arrival, establishment and spread of exotic diseases: patterns and predictions. *Nature Reviews Microbiology*, **8**: 361-371.

Reinert, J.F. (2000) New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. *Journal of the American Mosquito Control Association*, **16**(3): 175-188.

Reinert, J.F., Harbach, R.E. & Kitching, I.J. (2004) Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zoological Journal of the Linnean Society*, **142**: 289-368.

Reiter, P., Fontenille, D. & Paupy, C. (2006) *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *The Lancet Infectious Diseases*, **6**: 463-464.

Santa-Ana, M., Khadem, M. & Capela, R. (2006) Natural infection of *Culex theileri* (Diptera: Culicidae) with *Dirofilaria immitis* (Nematoda: Filarioidea) on Madeira Island, Portugal. *Journal of Medical Entomology*, **43**(1): 104-106.

Savona-Ventura C. (2002) Parasites and pests of medical significance in the Maltese environment - a historical review of culprit species. *Central Mediterranean Naturalist*, **3**(4): 149-152.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.-P., Rhaiem, A. & Brunhes, J. (2001) *The Mosquitoes of Europe / Les moustiques d'Europe*. An identification and teaching software, IRD Editions & EID Méditerranée, Montpellier, France. CD-Rom.

Vazeille, M., Jeannin, C., Martin, E., Schaffner, F. & Failloux, A.B. (2008) Chikungunya: a risk for Mediterranean countries? *Acta Tropica*, **105**: 200-202.

ISSN : 2070-4526
Date of Publication : 31st October 2010

Received: June 2, 2010
Accepted: July 10, 2010

Note added in proof: Since this manuscript was submitted for publication, adult specimens of *Aedes albopictus* have been recorded from several towns and villages located along the northern and north-eastern coast of Malta, and the species seems to be spreading rapidly southwards. No sightings have so far been reported from the nearby island of Gozo.