10. Should we expect Chikungunya and Dengue in Southern Europe?

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Abstract

Chikungunya and dengue arboviral diseases are in expansion throughout the world. *Aedes aegypti,* the principal vector of dengue and Yellow Fever has disappeared in Europe. In contrast, populations of *Ae. albopictus* established in Europe recently and are spreading to most southern regions. In the laboratory, this species has proven competent for transmitting various viruses. At the same time, an increase of virus importation is observed due to increased international travel. A limited Chikungunya outbreak which occurred in August - September 2007 in Italy confirmed that Europe is definitely at risk for *Aedes*-borne diseases. The risk of local transmission of Chikungunya and/or dengue in southern Europe depends on many historical, social, economic, environmental, climatic and entomological factors that we are presenting and discussing.

Keywords: Chikungunya, dengue, Europe, risk, mosquitoes

Introduction

Establishment and spread of the Asian tiger mosquito *Aedes albopictus* in Europe, as well as the steady increase of imported cases of *Aedes*-borne viruses such as Chikungunya and Dengue, raise the question of risk of these tropical diseases becoming established and spreading in Europe.

What are Chikungunya and dengue?

Epidemiology and symptoms

Chikungunya (CHIK) and dengue (DEN) are diseases caused by arboviruses (arthropod-borne viruses) transmitted to vertebrates by mosquitoes. Vectors responsible for transmission to humans are *Aedes aegypti* (which is also the vector of the Yellow Fever virus), and *Ae. albopictus*. Both diseases are in expansion throughout the world. A major outbreak of CHIK virus occurred in the Indian Ocean between 2004 and 2007, causing more than 3 million cases in Kenya, the West Indian Ocean islands, India, Indonesia, etc. (Pialoux *et al.* 2007). DEN has a worldwide distribution and is present in almost all tropical countries (Gubler 2006).

Chikungunya virus (CHIKV) is an alphavirus of the family Togaviridae, which was isolated for the first time in 1952 in Tanzania (Ross 1956). Its natural vectors are African forest *Aedes* mosquitoes of the subgenera *Diceromyia*, *Stegomyia*, and *Aedimorphus* that feed preferentially on wild primates, which represent the natural hosts of the virus (Diallo *et al.* 1999). Autochthonous human cases and outbreaks have been observed in Africa, East African islands and Asia. Three major phylogenetic groups of virus have been described: West African, Central-East-South African and Asian genotypes (Powers *et al.* 2000). The 'domestic' form of *Ae. aegypti* is closely associated with human habitations, readily enters houses, feeds almost exclusively on human beings, and is ubiquitous throughout the tropics. *Ae. albopictus,* the species implicated in the La Réunion outbreak, is sometimes present far from human habitation, and feeds readily on many species of mammals and birds (Reiter *et al.* 2006). CHIKV is responsible for an acute infection, characterised by high fever, arthralgia, myalgia, headache, and rash which last about 10 days. The typical clinical sign of the disease is arthralgia, which may persist for several months (Jupp and McIntosh 1988, Powers and Logue 2007). Despite being considered as slightly pathogenic, some deaths have been directly attributed to the virus, and abnormally high death rates have been observed during the recent outbreak in La Réunion. (Pialoux *et al.* 2007).

Dengue virus (DENV) is a group of 4 flaviviruses of the family Flaviviridae (DEN1, DEN2, DEN3, DEN4). DEN1 serotype was first isolated in 1943 by Hotta and Kimura, and the other serotypes were isolated between 1944 and 1957. The viruses probably originated from Asian non-human primates (see Gubler 1997 for references on dengue), where sylvatic cycles involving forest mosquitoes exist, as is also the case in Africa (Rudnick 1986). However, virus strains which are currently circulating within human populations are distinct from forest strains (Wang *et al.* 2000). Dengue is found in almost all tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. Symptoms of dengue fever vary from a non-symptomatic infection to severe haemorrhagic fever. Normally it involves a few days of flu-like illness with high fever, severe headache, muscle and joint pains, and rash. DENV are responsible for 50-100 million annual infections, 500.000 cases of dengue haemorrhagic fever and more than 20.000 deaths every year, mainly children (Gubler 2006). There has been a dramatic geographical expansion of the disease over the last 50 years, with severe forms like dengue haemorrhagic fever becoming more frequent. Like CHIKV, DENV is transmitted to humans by *Ae. aegypti,* which is the unique and major vector in South America, the Caribbean, and Asia, and by *Ae. albopictus*, which has been implicated in several outbreaks, like in La Réunion in 1978 (Coulanges *et al.* 1979).

Historical distribution of Aedes-borne diseases in temperate and tropical regions

Dengue: It is difficult to distinguish DEN and CHIK diseases based only on descriptions of clinical symptoms in old reports. The first description of dengue was provided in a Chinese encyclopaedia published in 992 (Gubler 1997). DEN-like outbreaks were recorded in the Caribbean in 1635, in Panama in 1699, in Egypt and Indonesia in 1779. It seems that DEN was first observed in Europe in the Mediterranean area in 1778, particularly in Cadiz and Sevilla in Spain (Angolotti 1980) as a consequence of the global expansion of ship movements and urbanisation. Outbreaks occurred again in Cadiz in 1860-1868 and in the Southern USA between 1870–1873, where the virus remained established. Dengue was identified in Athens, in Greece, in 1883–1886, and in 1927–1928 when a major outbreak occurred with more than 1000 deaths and one million cases (Copanaris 1928, Gubler 1997).

The current distribution of DEN covers all tropical countries, with regular and major outbreaks occurring in South East Asia, the Caribbean and South America. No indigenous cases have been recorded in European temperate countries in recent years. The last outbreak in Japan was reported during World War II, the vector being *Ae. albopictus*. Sporadic cases occur in sub-tropical Southern USA in Texas and in Queensland in Northern Australia, where *Ae. aegypti* and *Ae. albopictus* are both present.

Chikungunya: Diagnosis of CHIK disease, between outbreak periods, is rarely achieved, and distribution of the disease may be underestimated. Based on historical description of clinical signs, Carey (1971) suggested that CHIK outbreaks may have occurred as early as 1779 in Jakarta (formerly Batavia, in Indonesia) and in Cairo (Egypt), and later on, in Zanzibar (The Republic of Tanzania) in 1823.

Using more specific diagnostic methods, it has been shown that the virus circulates in intertropical Africa from Senegal to Kenya (Thonnon *et al.* 1999, Chretien *et al.* 2007) and in tropical Asia (Pialoux *et al.* 2007). The recent 2005–2007 outbreak occurred in all Indian Ocean islands and surrounding countries (Kenya, Comoros, Madagascar, Mauritius, Seychelles, La Réunion, Maldives, Sri Lanka, India, Indonesia, and Malaysia). For the first time in August 2007, a Chikungunya outbreak has been declared in Europe with more than 150 human cases in two small towns close to Ravenna in the Emilia Romagna province, on the Adriatic coast of Italy. The vector was *Ae. albopictus* which was very abundant in the area. The virus was detected by PCR (polymerase chain reaction) in several *Ae. albopictus* pools. The virus was very probably introduced by a traveller who returned from South-West India where the virus was circulating.

Yellow Fever: Yellow Fever (YF) is mainly transmitted to humans by *Ae. aegypti* (and *Haemagogus* spp. in the Americas). Outbreaks regularly occur in Africa despite a very efficient vaccine, and to a lesser extent, in South America. Paradoxically, while originating from Africa, YF outbreaks were first reported in the Caribbean after the introduction of its vector via the slave trade from West African slave harbours. The first Caribbean outbreaks occurred in Guadeloupe in 1635 and in 1647 in Barbados. Yellow Fever and *Ae. aegypti* were introduced as early as 1701 in Europe, particularly in areas surrounding harbours of Portugal, Spain, France and Italy. Outbreaks occurred in Gibraltar, Sevilla, Cadiz, Malaga, Lisbon, Porto, Barcelona, Marseille, Brest, Saint- Nazaire, Rochefort, Bordeaux, and Livorno during the 19^{th} and beginning of the 20^{th} Century (Bres 1986). More than 5000 people died in Barcelona between 1821 and 1824, and more than 6000 in Lisbon in 1857. Yellow Fever cases remained restricted to main harbour cities. No outbreak was observed in inland cities. Currently, YF occurs mainly in tropical West and Central Africa, and in South America. No case has ever been observed in Asia despite the presence of competent *Ae. aegypti* populations.

Historical and current distribution of potential vectors in Europe

According to current knowledge on dengue and Chikungunya transmission to man, the two potential vectors in Europe are *Ae. aegypti* and *Ae. albopictus*.

Aedes aegypti, described by Linnaeus in 1762, takes its name from specimens collected in Cairo, Egypt. Despite being recorded in mosquito checklists from several European countries such as Albania, Croatia, France, Greece, Italy, Portugal, Spain (Schaffner *et al.* 2001), it seems that this species is no longer present in Europe as well as in neighbouring countries. However, *Ae. aegypti* was very abundant in southern Europe and western Asia at the beginning of 20th century, particularly in Syria, Lebanon, Turkey, Greece, former Yugoslavia, Italy, Corsica (France) and Spain (Curtin 1967, Aitken 1954). *Aedes aegypti* was considered common in Spain until the 1950's (Rico-Avelló y Rico 1953). In Italy, the species was very common up to World War II but most of the official records stated the presence in harbour cities. (Capra 1944, La Face and Raffaele 1928, Piras 1917). The species was active in summer and was probably re-introduced regularly in these cities. The outbreak which occurred in Livorno in 1804 (a harbour city of the central West Coast of Italy, Leghorn in English) lasted about four months, from August to November, raising the question of mosquito overwintering (Levrè 2002). The last record of *Ae. aegypti* in Italy occurred in Desenzano del Garda (Brescia province, northern Italy) in 1971 (Callot and Delecolle 1972). Two major causes have been suggested for installation and spreading of this species since the 15th century: (1) increase of international exchanges and importation of exotic insect species from Africa, the Carribean and Central-South America, and (2) dramatic development of urbanisation and increase of domestic larval development sites. This period was the golden age of *Ae. aegypti* in Europe. Vector populations disappeared after the 1950's for two major reasons. Firstly the development of sanitation and management of urban water collections, which led to almost complete disappearance of domestic water storage practices, and secondly anti-malaria vector control with dichloro-diphenyl-trichloroethane (DDT), which had a collateral effect on other mosquito species.

*Aedes albopictus***,** originates from Asia, was first described by Skuse in 1894, and has recently spread to all continents. It was recorded for the first time in Europe in Albania in 1979 (Adhami and Reiter 1998), then in Italy (Sabatini *et al.* 1990, Dalla Pozza and Majori 1992). It is now present in all European countries around the Mediterranean Sea (see Chapter 14). It is particularly abundant in Italy and is becoming a major nuisance in South-East France, close to Italian border, since 2005. *Aedes albopictus* was introduced in Italy through shipments of used tyres from the USA (Dalla Pozza *et al.* 1994). In the USA, the species was imported at the beginning of the 1980's from South-East Asia and/or, more probably from the northern regions of Japan (Moore *et al.* 1988). Despite a specific federal law (the so called 'Tyre act'), that imposed a certificate of disinfestation to tyre containers entering the USA (but not leaving) from endemic *Ae. albopictus* areas, the species was reported across most of the USA within a few years (Moore and Mitchell 1997). In Italy, the species has quickly spread across the country, since the first record of a breeding population in the outskirts of Padova (Veneto Region) in 1991, showing a great ability to adapt to different ecological situations. At present, scattered foci of the species are reported in all the regions of Italy, from coastal plains to low inland hills (up to about 600 m of altitude) with the exception of Valle d'Aosta (North-West Italy). *Aedes albopictus* density depends on rainfall, relative humidity and available breeding sites. In Rome, *Ae. albopictus* has encountered particularly favourable conditions. Since the first record in the capital city in 1997, the species has colonised the entire metropolitan area, despite efforts to block its spread (Romi *et al.* 1999, Romi 2001). In Europe and other temperate regions, *Ae. albopictus* mainly colonises man-made breeding sites such as flower plates and pots, particularly in cemeteries and sometimes tree-holes and rock pools. In Italy, after seventeen years of surveillance, *Ae. albopictus* is confirmed to be a strict 'container breeding mosquito' never found in 'natural' breeding sites, with the exception of tree holes (*Platanus spp., Robinia pseudoacacia)*.

How to assess Dengue and Chikungunya risk in southern Europe?

The risk of local transmission of DEN, CHIK or other mosquito-borne viruses, is the result of simultaneous presence of the virus, competent mosquitoes, susceptible human hosts, and contact between these three entities. DEN and CHIK viruses are not yet endemic in Europe, all reported cases are imported, with the remarkable exception of the August 2007 Ravenna Province outbreak in Italy. *Aedes aegypti* has disappeared, but *Ae. albopictus* is currently invading southern Europe, particularly in urban areas where human-mosquito contact is increasing. Assessing the risk of transmission in Europe requires evaluation of the frequency of virus importation, spreading of *Ae. albopictus*, human-vector contact, and vector competence of anthropophilic mosquito species. A simple approach for assessing such a risk is to evaluate the components of the basic reproductive rate, R_0, which can be summarised by the following model, first developed by MacDonald (1957) for malaria. If the virus is introduced by a viremic host in a location, it will generate secondary cases if R_0 is > 1.

$$R_0 = (m \,.\, a^2 \,.\, p^n / -\ln p) \,.\, b. \, c \,.\, 1/r$$

m: Ratio of mosquitoes to humans

a: Human biting rate (number bites on a human, per mosquito, per day)
p: Daily survival rate of the mosquitoes
n: Incubation period (days); the extrinsic incubation cycle of the virus
b: Transmission efficiency from an infected mosquito to a human
c: Transmission efficiency from an infected human to a mosquito
1/r: Human infectious period (days)

Virus importation

There is no common institutional registration database for detected arbovirus cases in Europe. Sources of information are statistics of Ministries of Health, tropical disease networks such as TropNetEurop (www.tropnet.net; Accessed 28 August 2007), scientific articles, and reports. Regular reports based on cases observed in European hospitals, or from serological surveys in specific populations (troops, travellers, etc.) show that DENV and to a lesser extent, CHIKV, are regularly introduced to Europe (Jelinek 2000, Badiaga *et al.* 2003). Viruses are imported by tourists and professionals visiting tropical countries, European citizens or soldiers living in endemic regions and returning to Europe, and disease-endemic country citizens visiting or immigrating to Europe. Most DEN cases acquired between 1999 and 2002 by 483 travellers living in Europe came from South East Asia, Central America / the Caribbean and Brazil (Jelinek *et al.* 2002, Wichmann *et al.* 2003). Most imported CHIK cases reported by several European countries (Belgium, Czech Republic, France, Germany, Italy, Norway, Switzerland, UK[10], Spain and Italy) are related to the 2005-2006 Indian Ocean outbreak (Depoortere *et al.* 2006). CHIK and DEN fever are generally not easily recognised in European countries, which has led to a huge underestimation of cases. During the 2007 Italian outbreak, first declared cases were initially suspected as Phlebotomus fever. Moreover, asymptomatic or mild cases are not recorded. Notification has been implemented only in 2006 in metropolitan France (i.e. excluding French Caribbean, Indian Ocean and Pacific Ocean overseas departments). A total of 898 CHIK cases were reported between April 2005 and January 2007 in metropolitan France from confirmed laboratory data (Krastinova *et al.* 2006, see www.invs.sante.fr/(Accessed 28 August 2007). Almost all these cases originated from the Indian Ocean, particularly from La Réunion island where more than 266,000 cases were estimated to have occurred. Most imported cases were observed in Paris and in the South East region where *Ae. albopictus* is now established (in the Alpes Maritimes and Corsica departments). There is regular importation of DEN virus in metropolitan France, particularly from overseas French tropical departments. A total of 209 imported DEN cases have been recorded from laboratory data, between January and November 2006 [11]. The real number, including asymptomatic and mild cases, is probably much higher. Dengue is now endemic, with epidemic episodes of the different serotypes, in French Guyana, Martinique, Guadeloupe, Tahiti and New Caledonia. A limited outbreak of DEN1 occurred in La Réunion and Mayotte in 2004. The number of imported cases is lower in other European countries than in France. For example, in Italy the National reference center on arboviruses at Istituto Superiore di Sanita (ISS) confirmed an average of 40 DEN cases per year over the last five years (Nicoletti and Ciufolini, personal communication). A total of 23 CHIK cases have been detected and confirmed in 2006-2007, which were nearly all contracted by tourists visiting Mauritius (Fusco *et al.* 2006, Beltrame *et al.* 2007). The recent CHIKV outbreak near Ravenna also demonstrated that the virus could be introduced from India, and possibly other parts of Asia.

[10] See www.hpa.org.uk/cdr/archives/2006/cdr5006.pdf (Accessed 29 August 2007).

[11] See www.invs.sante.fr/presse/2007/le_point_sur/dengue_cas_importes_250107/dengue_cas_importes_250107.pdf (Accessed 29 August 2007).

Importation of infected mosquitoes seems negligible compared to travel of infected humans. Most aircrafts arriving from tropical regions are disinsectised and *Ae. aegypti* has rarely been found on aircraft (Gratz *et al.* 2000). During the last two decades, airport malaria cases have sometimes been noted, but never DEN or CHIK cases (Tatem *et al.* 2006). Similarly importation of DEN or CHIK infected *Aedes* eggs (through vertical transmission) by ships must be an exceptional event.

Vector competence of European mosquito populations

Vector competence describes the ability of a mosquito to serve as a disease vector. It is governed by genetic factors that influence the ability of a vector to transmit a pathogen (Hardy *et al.* 1983). It is assessed in the laboratory by experimental infections where infection rate corresponds to the proportion of female mosquitoes capable of transmitting the pathogen by bite after a period of virus replication following an infected blood meal. Vector competence experiments are conducted in secure laboratories using standardised protocols. Batches of mosquitoes of the same age from the same population or colony are allowed to feed on blood containing the virus at a given titer. The proportion of infected mosquitoes is calculated after two weeks by indirect immunofluorescence on head squashes (Kubersky and Rosen 1977) or by RT-PCR (reverse transcription Polymerase Chain Reaction) (Lanciotti *et al.* 1992).

Aedes aegypti

Important variations in vector competence have been observed between *Ae. aegypti* populations (Gubler *et al.* 1979). Two major morphological and behavioural forms have been recognised. *Aedes aegypti formosus* originates from tropical African forests, with both domestic and sylvatic populations, the more urban *Aedes aegypti aegypti is* present in the New World, in Asia and the Pacific area. By investigating the susceptibility of *Ae. aegypti* populations to infection with DEN virus, Failloux *et al.* (2002) confirmed recently that the *formosus* form had lower infection rates than the *aegypti* form. The genetic basis of these differences is not yet well understood. Several quantitative trait loci (QTLs) on chromosomes X, II and III were found to affect midgut susceptibility to dengue infection (Bennett *et al.* 2005, Gomez-Machorro *et al.* 2004). *Aedes aegypti* is also the major CHIK vector to humans in West and East Africa, Comoros, India, etc. A wide variation in CHIKV susceptibility to experimental infection has been demonstrated among different strains of *Ae. aegypti* (Banerjee *et al.* 1988). In the same way, the different strains of CHIKV behave differently in *Ae. aegypti* (Mourya *et al.* 1987). Mourya *et al.* (1994) showed that variation in susceptibility to CHIKV was due to genetic factors related to a gene located on chromosome III. Refractoriness is due to a mesenteron barrier since *Ae. aegypti* strains can support viral multiplication after intrathoracic inoculation (Mourya *et al.* 1998). The mesenteron barrier controlled by CHIKV-specific receptors could be one of the important factors determining susceptibility of mosquitoes to the virus. However, other interacting factors may affect this trait.

Aedes albopictus

Aedes albopictus has been incriminated as a DEN vector in Japan, Indonesia, the Seychelles, Thailand, Malaysia, Indian Ocean islands (Hawley 1988, Coulanges *et al.* 1979) and more recently, in 2001, in Hawaii (Hayes *et al.* 2006), and China (Xu *et al.* 2007). Similar to *Ae. aegypti*, variations among geographic strains of *Ae. albopictus* have been observed in susceptibility to infection with DENV (Gubler and Rosen 1976). In the Americas, *Ae. albopictus* has never been implicated in a DEN outbreak but it has been found naturally infected with DENV in Mexico and in Brazil

(Ibanez-Bernal *et al.* 1997). *Aedes albopictus* from North America has been shown to be an efficient experimental vector of DEN viruses and the YF virus (Mitchell *et al.* 1987, Boromisa *et al.* 1987). Two Italian populations of *Ae. albopictus*, from Padova and Rome respectively, were infected with DEN2 virus using an Emotec system. More than 30% of females were positive after two weeks (R Romi and MG Ciufolini, unpublished data). Regardless of their geographic origin, strains of *Ae. albopictus* are more susceptible to infection with CHIKV than are the strains of *Ae. aegypti* (Tesh *et al.* 1976, Turell *et al.* 1992, Konishi and Yamanishi 1986). *Aedes albopictus* was reported to be more susceptible to an Asian than an African CHIKV strain (Tesh *et al.* 1976). Like in *Ae. aegypti*, different strains of CHIKV replicate differently in *Ae. albopictus* (Vazeille, unpublished data). In areas of recent colonisation like in the Americas, *Ae. albopictus* may be implicated in the transmission of different viruses like West Nile virus (WNV) (Turell *et al.* 2001), viruses from the *Bunyamwera* group of Bunyaviridae (Francy *et al.* 1990), Potosi virus, Cache valley virus, La Crosse virus (Mitchell *et al.* 1998) and Eastern equine encephalitis virus (Niebylski *et al.* 1992). Under experimental conditions, *Ae. albopictus* is a competent vector of at least 22 arboviruses including DEN viruses, YF, CHIKV and Ross River virus (Gratz 2004). Experimental infections showed that *Ae. albopictus* from the South of France is also highly susceptible to CHIKV. At a viral titer of 10^7 pfu/mL in the artificial blood meal, 56-77% of females were able to transmit after two weeks of incubation at 28°C (Failloux and Vazeille, unpublished data). Finally, the 2007 CHIK outbreak in Italy suggests that Italian *Ae. albopictus* are also competent under field conditions.

Other mosquito species

Several other French mosquito species like *Aedes vittatus* and *Ochlerotatus caspius* are also able to experimentally transmit CHIKV whereas *Ae. vexans* and *Oc. detritus* are less susceptible (Failloux and Vazeille, unpublished data).

Vertical transmission

Several arboviruses might be maintained in vector populations through vertical transmission from the female to her progeny. *Aedes aegypti* has been shown capable of transmitting all four DEN serotypes vertically (Khin and Than 1983, Hull *et al.* 1984, Mourya *et al.* 2001, Joshi *et al.* 1997, Joshi *et al.* 2002), as well as many other viruses such as YF (Fontenille *et al.* 1997), WNV (Baqar *et al.* 1993), etc. CHIKV vertical transmission has not been demonstrated yet for *Ae. aegypti* (Mourya 1987). Vertical transmission of the four DEN serotypes has also been observed in the field and experimentally in *Ae. albopictus* (Mitchell and Miller 1990, Shroyer 1990, Serufo *et al.* 1993, Ibáñez-Bernal *et al.* 1997). Following experimental assays, Mourya (1987) concluded the absence of CHIKV vertical transmission in *Ae. albopictus*. However, the virus was isolated (and PCR detected) in two pools of larvae, out of more than 500 pools tested, during the 2006 CHIKV outbreak in La Réunion, which demonstrates that vertical transmission can occur in the field. Moreover, experimental transmission studies conducted with *Ae. albopictus* colonies from La Réunion showed that ovaries of females were infected with CHIKV only six days after the initial infection (Failloux, unpublished data). Finally, vertical transmission of WNV by *Ae. albopictus* has been experimentally demonstrated (Baqar *et al.* 1993). Moreover, Ross River and Sindbis viruses, which are genetically close to CHIKV, have been isolated from field-collected immature stages of *Ae. camptorhynchus* in Australia, indicating the existence of vertical transmission as a mechanism for arbovirus maintenance in mosquito populations in temperate regions (Dhileepan *et al.* 1996). Epidemiological consequences of such vertical transmission are often difficult to assess, and vary depending on epidemiological context.

Vectorial capacity of Aedes albopictus

Very few studies have been conducted on vectorial capacity components (i.e. density, trophic preference, longevity, duration of gonotrophic cycles) of European populations of *Ae. albopictus* (with *Ae. aegypti* having disappeared). *Aedes albopictus* is generally considered to have a wide range of hosts for blood feeding, from birds to humans. Like *Ae. aegypti* (Scott *et al.* 2000), *Ae. albopictus* females probably take several blood meals within the same gonotrophic cycle (Do Si Hien 1976). Eggs from the same batch might be laid over several days and be dispersed over several breeding sites. Gonotrophic cycles estimated in the field and in the laboratory last between 3–5 days (Mori and Wada 1977). Its longevity depends on climate, environment, habitat, and populations. It is widely assumed that longevity is around 4–6 weeks (Hawley, 1988).

In Italy, *Ae. albopictus* represents the major human biting pest throughout much of its range. The species has found a peculiar breeding site represented by the manholes of the rainwater collection system and has easily colonised man-made breeding sites in the peridomestic environment (Knudsen *et al.* 1996). In central Italy, diapausing winter eggs hatch during the second half of February and the first half of March. Larvae are present up to November and some females are active until December. The mean pre-imaginal duration of egg hatching to pupation lasts 6-7 days at 25 °C, which is usual for this species (Hawley, 1988). Adult abundance peaks in late August-September. It has recently been observed in Rome that some females remained active during winter and 30% of a set of 650 ovitraps were positive (Figure 1).

This may suggest a polymorphism in an adaptive physiological mechanism to temperate climate conditions and a selection for populations active all year long (Romi *et al.* 2006). While most females are diurnal and exophagic, mosquitoes might be captured biting at night and/or indoors, even at the higher floors of buildings, where breeding sites for larvae are indoor containers (flower pots, etc.).

Entomological risk related to global changes

Lessons from history teach us that Europe may have major outbreaks of *Aedes*- borne arboviruses. The establishment and spreading of a new competent DEN and CHIKV vector in southern

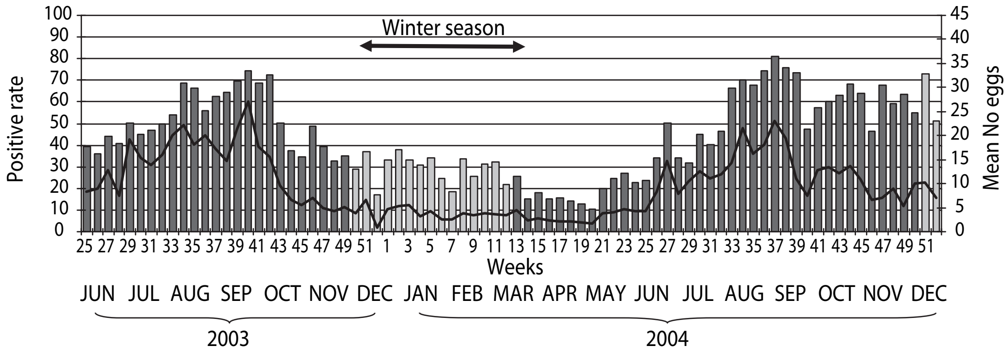

Figure 1. Weekly density of Aedes albopictus in Rome. Percentage of positive traps out of 650 traps (bars, left axis), and mean number of eggs by positive traps (black line, right axis).

Europe creates conditions for establishment of indigenous DEN, CHIK and even YF (witch can be experimentally transmitted by *Ae. albopictus*). Absence of such an outbreak since the time *Ae. albopictus* became established in Europe until 2007 is not an argument for considering this risk negligible. Despite an abundant and very competent vector, the tropical island La Réunion also didn't experience DEN or CHIK outbreaks for more than 36 years, since the last major DEN outbreak in 1978. Indigenous transmission occurred on at least eight occasions between 1980 and 2005 in the USA, following the introduction of the virus by travellers (Gubler 2006). Occurrence of the CHIK outbreak in Italy in August 2007 dramatically demonstrates that Europe must be definitively considered at risk for *Ae. albopictus* borne arboviruses.

Climate

Although temperate climate is less favourable to arbovirus transmission, DEN and YF which are transmitted by *Ae. aegypti* occurred in very temperate regions of Europe (i.e. Glasgow), USA (i.e. Boston), and in South America for example in Argentina: Concordia (18.9 °C; 12.3 °C for average yearly temperature and average coldest month temperature, respectively), Parana (18.2 °C, 11.2 °C) and even in Buenos Aires (16 °C, 10 °C) which experienced several YF outbreaks in the second part of the 19^{th} century (Otero *et al.* 2006). In comparison, several cities in southern Europe have similar temperatures as Buenos Aires; Nice in France (15.6 °C, 9.1 °C), Rome in Italy 17.6 °C, 9.4 °C) and Athens in Greece (18.5 °C, 9.4 °C).

Environment

It is very unlikely that *Ae. aegypti* may re-colonise Europe. Its major breeding sites at the beginning of 20th century, the domestic water storage containers, have mostly disappeared and other ecological niches tend to be occupied by *Ae. albopictus* by which it is out-competed (Juliano *et al.* 2004). Distribution models predict that *Ae. albopictus* will continue to expand (Benedict *et al.* 2007, Knudsen *et al.* 1996, Medlock *et al.* 2006) depending on transport, environment and climate changes. Global warming may possibly contribute to increase the vectorial capacity (i.e. shortening virus extrinsic incubation cycle and mosquito pre-imaginal development time), but it is only a small component of the model. To date, most modelling studies focus on seasonality (i.e. correlation between annual variations of rainfall or temperature, and variation of DEN incidence), rather than on medium to long-term variations in climate (Chadee *et al.* 2007).

Contact between humans and *Ae. albopictus* contact will depend on the nature and location of breeding sites, alternative hosts, besides mosquito and human behaviour. In Italy, as well as in some French locations, *Ae. albopictus* is highly abundant in urbanised and populated areas and is now a major nuisance, demonstrating that human biting rates can be high.

It is difficult to estimate R_0 in Europe if viremic patients are bitten by mosquitoes. However, taking the following plausible values (based on published and non published data, see Hawley 1988) for R_0 parameters: m = 20, a =0.25, p = 0.85, n = 5 (for CHIKV), 1/r = 4, and considering that b and c are 0.7 each, one obtains R_0 = 0.96, close to 1, the threshold for having secondary cases. These values are rough estimations. Survival, duration of gonotrophic cycle, human biting rate and the extrinsic incubation period of the virus, may vary a lot depending on human and mosquito populations, virus strains and seasons. R_0 may therefore be much higher at certain times or in certain places. That means that secondary cases may occur if CHIK or DEN viruses are introduced in a given region where *Ae. albopictus* is abundant and anthropophilic during a warm period.

There is experimental evidence that other indigenous European vectors are sufficiently competent for transmission of DEN or CHIKV. However, human-vector contact and mosquito longevity (i.e. parameters of vectorial capacity) of these rural species must be carefully assessed to evaluate their real (and not theoretical) role as vectors under natural conditions.

Globalisation

During the last few years, imported DEN cases increased in Europe, in relation to expansion of DEN in the world and increase of international travel. In 2005, the total number of passengers transported by air in the European Union (EU) rose by 8.5% compared to 2004, to more than 700 million. Passenger numbers rose by 8.8% in 2004 and by 4.9% in 2003. Of these passengers, 35% were carried on external-EU flights (Figure 2) (De la Fuente Layos 2006).

Eurostat (a European Union agency) estimated that in 2004, a total of 1,474,218 people travelled from Madagascar (153,766), Mauritius (657,312), Mayotte (63,372), La Réunion (498,388) and the Seychelles (101,380) to the European mainland. More than 60 million passengers travelled to Europe from tropical countries in 2006, among them 2.5 million travelled from India, including about 120,000 to Italy. Several million passengers arrived in metropolitan France in 2005 from regions where CHIK or DEN were endemic or epidemic (Table 1).

Mosquito control

Mosquito control both at the community and household level, may reduce population density and contact with humans. Nevertheless, such control efforts have proven difficult to implement against *Ae. albopictus*, as observed in Italy and the USA (see Chapter 14).The establishment of *Ae. albopictus* in Italy has caused great concern among public health authorities (Knudsen *et al.* 1996) and a national plan of surveillance was implemented and funded for three years. Since 1991, a reference center (at ISS) is in charge of monitoring *Ae. albopictus,* by studying mosquito

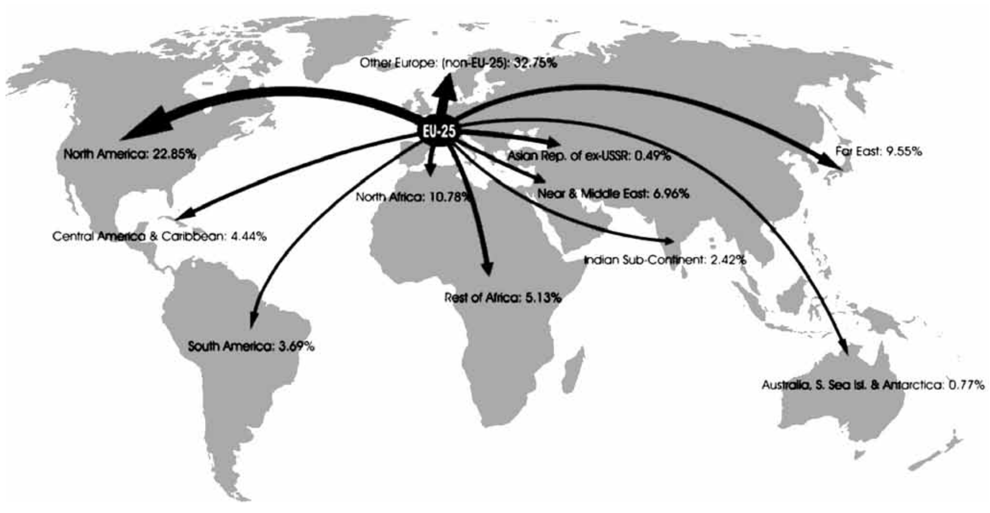

Figure 2. Extra-EU-25 transport of passengers: share in world regions in % of total extra-EU-25 transport in 2005. From De la Fuente Layos (2006).

Table 1. Example of passenger traffic in metropolitan France in 2005 (from Chikungunya or Dengue-endemic regions). From Ministère Français de l'Équipement des Transports, de l'Aménagement du territoire du Tourisme et de la Mer (2006).

Region, country	Number of passengers arriving in France in 2005
Africa, Cameroon	98,364
Africa, Gabon	56,310
Africa, Mauritius	222,215
Africa, Kenya	29,335
Asia, India	278,453
Asia, Malaysia	72,408
Asia, Thailand	189,861
Asia, Vietnam	133,678
South America, Brazil	419,944
South America, French Guyana	95,342
All French overseas departments	1,538,294

bionomics and producing guidelines for vector control. As, up to August 2007, despite its potential role as a powerful vector of arboviruses, *Ae. albopictus* in Italy has represented only a source of nuisance biting, the problem of its presence has been shifted from a health problem to an environmental issue. However, the recent Italian outbreak has led the health authorities to reconsider its vectorial status. Monitoring and control are currently carried out by municipal governments, by following the guidelines provided by the ISS. Control activities mainly consist of source reduction through community education and larvicidal treatment of breeding sites located in public areas, with temephos up to 2006, and with diflubenzuron, pyriproxyfen and *Bacillus thuringiensis israeliensis* formulations at present (Romi *et al.* 2003, Toma *et al.* 2003). Focal adulticide space spraying is used in case of local emergences due to massive biting activity of the species (usually carried out with mixtures of fast knock-down synergised pyrethrins and second-third generation pyrethoids). A plan of action in case of an outbreak had recently been implemented by the Ministry of Health. It involved all the local health structures (ASL, Azienda Sanitaria Locale), the departments of infectious diseases of the hospitals of reference, and the ISS (both MIPI Dept-the national centre of reference for aboviruses – and the Unit of vector control for entomological surveillance) but without specific financial support yet. This plan was activated in August 2007. Vector control consisted of five cycles of early morning outdoor spraying with pyrethroid insecticides immediately after the outbreak was detected. Larvicides (Diflubenzuron, an insect growth regulator, and *Bacillus thuringiensis israeliensis*) have also been used both in the public domain and in all private houses after a door to door inspection.

In metropolitan France, a surveillance system in high-risk areas for *Ae. albopictus* has been implemented since 1999, and successfully detected several *Ae. albopictus* importations, which have been controlled. However, in 2005 despite the French Ministry of Health monitoring system, newly established *Ae. albopictus* populations were observed in South-East France. Attempts to control these populations failed and distribution of the species expanded in 2006 to neighbouring regions and Corsica. Following CHIK outbreaks in French overseas departments, a new plan has been developed in 2006 which includes reinforcement of epidemiological surveillance, CHIK and

DEN case declaration, *Ae. albopictus* population monitoring with a dense network of ovitraps, implementation of mosquito control and a strong public communication plan.

At the European level, the European Centre of Disease Prevention and Control (ECDC) has organised a special meeting (ECDC 2006, Depoortere *et al.* 2006) and international consultations for assessing the risk of CHIK in Europe, after the Indian Ocean and Italian outbreaks. The main recommendations were the improvement of information by airline companies to passengers, by National public health authorities and by ECDC itself; the constitution of a group of experts and European reference centers; the development of research on vector biology; the improvement of measures for monitoring *Ae. albopictus* distribution, and for preventing its importation and spreading, and the improvement of information among health services and general physicians on Chikungunya disease and preventive measures.

Conclusions

The answer to the question 'Should we expect Chikungunya and dengue in Southern Europe?' is 'Yes, definitely'. Current data including the recent Italian outbreak clearly show that receptivity and infectivity of mosquito populations (i.e. vectorial capacity and competence), as well as vulnerability of southern Europe (i.e. virus importation) make indigenous transmission of CHIK and DEN possible. Based on current knowledge, it is impossible to predict the time of occurrence and level of a hypothetic outbreak, from localised secondary cases (as it was probably the case in the Italian province of Ravenna) to huge outbreaks similar to historical DEN or YF in Greece and Spain. A rebound of CHIK transmission had been predicted in La Réunion in 2007 after the 2006 episode yet only few cases have been observed without any clear explanation. Vigilance must, however, be maintained as the risk of an outbreak is definitely present, particularly in regions with well-established *Ae. albopictus* populations and mobile human populations. Unfortunately, ongoing global changes will render many urban areas in southern Europe prone to such conditions within the next few years (Sutherst 2004). Europe should consider establishing permanent surveillance and an adequate response strategy, should a new arbo-virus outbreak be discovered.

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