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Re-Emergence of Malaria and Dengue in Europe

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1. Introduction
Currently, the emergence/reemergence of several vector-borne diseases in Europe is one of the most important threats for Public Health. In recent years, it is well known that global change have led to drastic modifications in the eco-epidemiology of various tropical and subtropical diseases. Global change can be defined as the impact of human activity on the fundamental mechanisms of biosphere functioning. Therefore, global change includes not only climate change, but also habitats transformation, water cycle modification, biodiversity loss, synanthropic incursion of alien species into new territories or introduction of new chemicals in nature. Consequently a holistic approach is a key factor to assessing the likelihood of vector-borne diseases transmission in Europe. Among these vectors, culicid mosquitoes are probably the most important because of its large vectorial capacity and its high degree of opportunism (Table 1).

| Vector species | Distribution | Indigenous/exotic | Vectorial capacity |
|----------------|--------------|-------------------|-------------------|
| Ae. aegypti    | Madeira (Portugal), The Netherlands | Exotic (recently imported) | Dengue (DEN), Yellow Fever (YF), Chikungunya (CHIK), West Nile (WN), Japanese encephalitis (JE), Saint-Louis encephalitis (SLE), La Crosse encephalitis (LACE), Murray valley encephalitis (MVE), Western equine encephalitis (WEE), Eastern equine encephalitis (EEE), Venezuelan equine encephalitis (VEE), Myxomatosis (MYX), Avian Malaria (AMAL), Dirofilariasis (DF) |
| Ae. albopictus | Mediterranean area, Central Europe | Exotic (first reported in Albania in 1979) | DEN, YF, CHIK, WN, JE, SLE, LACE, WEE, EEE, VEE, Jamestown Canyon (JC), Sindbis (SIN), Tahyna (TAH), DF |
### Table 1. Mosquito vectors in Europe with indication of distribution, indigenous or exotic status and vectorial capacity in each case.

| Vector species | Distribution | Indigenous/exotic status | Vectorial capacity |
|----------------|--------------|--------------------------|--------------------|
| Ae. vexans     | All over Europe | Indigenous | WN, TAH, Tularaemia (TU), DF |
| Ae. vittatus   | Spain, Portugal, France, Italy | Indigenous | DEN, YF, CHIK, AMAL |
| An. algeriensis| Mediterranean area, Eastern Europe, Central Europe, United Kingdom | Indigenous | Malaria (MAL) |
| An. claviger s.l. | All over Europe | Indigenous | MAL, WN, Batai (BAT), TAH, MYX, Anaplasmosis (ANA), Borreliosis (BO), TU, DF |
| An. maculipennis | All over Europe | Indigenous | MAL, WN, BAT, TAH, MYX, DF |
| An. sergentii  | Sicily (Italy) | Indigenous | MAL |
| An. superpictus| Southeastern Europe | Indigenous | MAL, DF |
| Cx. pipiens   | All over Europe | Indigenous | WN, SIN, Usutu (USU), TAH, AMAL, DF |
| Oc. atropalpus| Italy, France, The Netherlands. | Exotic (first reported in Italy in 1996) | WN, JE, SLE, LACE, MVE, WEE, EEE |
| Oc. caspius    | All over Europe | Indigenous | WN, TAH, MYX, TU, DF |
| Oc. japonicus | France, Belgium, Switzerland, Germany | Exotic (recently imported) | WN, JEV, SLE, LACE, EEE |
| Oc. triseriatus| Intercepted in a batch of used tyres imported from Louisiana (USA) to France in 2004 | Exotic (not yet known as established) | DEN, YF, WN, SLE, LACE, WEE, EEE, VEE, JC |
2. Malaria

Malaria was a widespread disease in the whole of Europe until the second half of the 20th century. The anthroponosis, often called "marsh fever" in the past, was particularly devastating between the XVI and XIX centuries in Southern Europe due to the boom of irrigation techniques based on long flooding periods (e.g., rice fields). Several environmental modifications (mainly the drainage of swamps, moats, ditches, and other stagnant waters), but particularly the availability of efficient synthetic antimalarial drugs and improved mosquito control activities including DDT spraying after World War II, have led to the disappearance of malaria from Europe (Bruce-Chwatt & de Zulueta, 1980). However, although Anopheles populations were significantly reduced by different control methods, in most cases, the vectors were not eradicated.

Today malaria annually affects 500 million people and threatens directly or indirectly 40% of the world population (World Health Organization [WHO], 2007). However, it is well known that these morbidity and mortality data show an asymmetric distribution, mainly depending on the economic, social, and sanitary level of each country or region. The disease is endemic in much of Africa and several countries of Asia, Central America, and South America. In Europe, the cycles of malaria transmission are relatively common in Georgia, Azerbaijan, Kyrgyzstan, Tajikistan, Uzbekistan, and Turkey (WHO, 2010). This mosquito-borne parasitaemic disease is caused by protozoa of the genus Plasmodium. Although the simian parasite Plasmodium knowlesi (Knowles and Das Gupta, 1932) has been found recently as a cause of human malaria in Southeastern Asia (Luchavez et al., 2008), other four plasmodia species are the most recognized to infect humans in nature conditions: Plasmodium falciparum (Welch, 1897), Plasmodium vivax (Grassi & Feletti, 1890), Plasmodium malariae (Feletti & Grassi, 1889), and Plasmodium ovale (Stephens, 1922). About 90% of malaria mortality is caused by tropical strains of P. falciparum (most pathogenic species), which is also the species of Plasmodium most frequently imported to Europe (European Network on Imported Infectious Disease Surveillance [TropNetEurop], 2010). Furthermore, P. vivax shows the largest distribution range because it may also develop in temperate climates, being consequently the only species currently present in the cycles of transmission in Europe. Finally, P. malariae and P. ovale are characterized by its narrow distribution range and low parasitemia.

Regarding malaria vectors, there are about 40 Anopheles species with an important role in disease transmission (Kiszewski, 2004).

2.1 Malariogenic potential of Europe

The increasing of imported malaria cases in the last decades, together with the high presence of anophelines in many Southern Europe regions (Romi et al., 1997; Ponçon et al., 2007; Bueno Marí & Jiménez Peydró, 2010a), has enabled the appearance of several autochthonous malaria cases, as recently has occurred in countries like Italy (Baldari et al., 1998), Greece (Kampen et al., 2002), France (Doudier et al., 2007) or Spain (Santa-Olalla Peralta et al., 2010). This situation forces us to investigate the possible reemergence of malaria in the current context of global change. One of the best methods to deepen into the knowledge of possible malaria reemergence is the study of the malariogenic potential, which can be analyzed from the study of the receptivity, infectivity, and vulnerability parameters (Romi et al., 2001; Bueno Marí & Jiménez Peydró, 2008).
2.1.1 Receptivity

Receptivity could be analyzed by the presence, density, and biological characteristics of vectors. At respect, the estimation of the Vectorial Capacity (VC) is postulated as a very useful tool to assess the receptivity of a determined territory in a concrete moment (Carnevale & Robert, 2009). The VC could be estimated by the MacDonald formula (MacDonald, 1957) according to the modifications proposed by Garrett-Jones (1964):

\[
VC = \frac{ma^2 p}{n} - \ln p
\]

Where, \(m\) represents the relative vector density (number of vectors per man), \(a\) refers to human-biting frequency (number of human blood meals per vector and per day), \(p\) is the daily survival rate (life expectancy of the female mosquito) and \(n\) alludes to duration of the sporogonic cycle (length in days of the latent period of the parasite in the mosquito, i.e. extrinsic incubation cycle). It is important to note that \(ma\) is usually measured by collecting mosquitoes during an entire night using human bait. Consequently VC could be defined as the future daily sporozoite inoculation rate arising from a currently infective human case, on the assumption that all female mosquitoes biting that person become infected (Githeko, 2006). Of course VC changes from site to site, from vector to vector, and within and between transmission seasons.

2.1.1.1 Malaria receptivity in Southern Europe

Because of climatic conditions, the Southern Europe represents the territory of the Old Continent where disease cycles can be completed more likely. In terms of receptivity, of twenty species of \(Anopheles\) described in Europe twelve are confined in its distribution to Southern areas (Table 2). In the Iberian Peninsula rice cultivation was clearly associated with malaria endemicity until the beginning of the 20th century (Cambournac & Hill, 1938; Cambournac, 1939, Blázquez, 1974; Bueno Marí & Jiménez Peydró, 2010b). In these larval biotopes the species \(Anopheles atroparvus\) and, to a much lesser extent and only in the more arid areas, \(Anopheles labranchiae\) were supposed to be the major malaria vectors (Bruce-Chwatt & de Zulueta, 1977), although some other species, such as \(Anopheles maculipennis\) or \(Anopheles claviger\) may locally also have contributed to disease transmission (Bueno Marí, 2010). Currently \(An. atroparvus\) remains widespread in rice fields and other potential \(Anopheles\) breeding sites of Portugal and Spain (Capinha et al. 2009; Sainz-Elipe et al. 2010), since the most important western Mediterranean malaria vector \(An. labranchiae\) is considered disappeared. \(An. labranchiae\) was found to be abundant in a restricted area of the contiguous Alicante and Murcia Provinces (South-eastern Spain) in 1946 (Clavero & Romeo Viamonte, 1948), but had disappeared by 1973 (Blázquez & de Zulueta, 1980) probably due to abandonment of rice cultivation in this area (Eritja et al., 2000). Recent surveys carried out in this area have revealed again the absence of \(An. labranchiae\) as well as high populations of the secondary vector \(Anopheles algeriensis\) also characterized by high domiciliation degrees (Bueno Marí, 2011). This was the only area where \(An. labranchiae\) has been able to establish itself in the Iberian Peninsula (Blázquez & de Zulueta, 1980). Though abundant along the African coastline between Ceuta and Tangiers, \(An. labrachiae\) has been unable to obtain a foothold in 15 km distant coastal plains of southern Spain, where rice fields support large populations of \(An. atroparvus\) (Ramsdale & Snow, 2000). It is important to note that the most important vector of the Iberian Peninsula \(An. atroparvus\) is suspected of being the vector of an autochthonous case of \(Plasmodium vivax\) which recently occurred in Northeastern Spain (Santa-Olalla Peralta et al., 2010) and even also in other case of \(Plasmodium ovale\) which happened in Central Spain, although airport malaria cannot be discarded in this last case due to the proximity of the patient's residence to two international airports (Cuadros et al., 2002).
An. multicolor

Spain -

An. petragnani

Fra, Cors, Spain, Port, Ital, Sard, Sic -

An. plumbeus

Brit, Ire, Swe, Den, Fra, Cors, Spain, Port, Belg, Neth, Lux, Ger, Aust, Czech, Slovk, Pol, Switz, Ital, Sic, Ser-Mon, Croa, Bosn, Slovn, Mace, Alb, Gree, Turk, Hung, Rom, Bulg, Ukr, Bela, EurRus, Lith, Est

Note 1: Countries with anophelines records considered as doubtful or sporadic were not included. If it is thought that the species has been eradicated, the country is also not listed.

Note 2: Brit (Britain), Ire (Ireland), Nor (Norway), Swe (Sweden), Den (Denmark), Fra (France), Cors (Corsica), Spain, Balear (Balearic Islands), Port (Portugal), Belg (Belgium), Neth (Netherlands), Lux (Luxemburg), Ger (Germany), Aust (Austria), Czech (Czech Republic), Slovk (Slovakia), Pol (Poland), Switz (Switzerland), Ital (Italy), Sard (Sardinia), Sic (Sicily), Malt (Malta), Ser-Mon (Serbia-Montenegro), Croa (Croatia), Bosn (Bosnia), Slovn (Slovenia), Mace (Macedonia), Alb (Albania), Gree (Greece), Turk (Turkey), Cypr (Cyprus), Hung (Hungary), Rom (Romania), Bulg (Bulgaria), Moldv (Moldavia), Ukr (Ukraine), Bela (Belarus), EurRus (European Russia), Lith (Lithuania), Latv (Latvia), Est (Estonia).

a Records referred to Anopheles maculipennis s.l.

b Species recently described by molecular and morphological techniques.

c There is confusion with these two species.

d Present in Asiatic Turkey.

Table 2. Anopheles species with endemic presence in Europe and indication of historical data about its vectorial role (Ramsdale & Snow, 2000; Schaffner et al., 2001; Beck et al., 2003; Nicolescu et al., 2004; Linton et al., 2005; Becker et al., 2010; European Mosquito Taxonomists [MOTAX], 2010).

In France, the same two species mentioned above for the Iberian Peninsula, are also considered to be primary malaria vectors because of their abundance and their potential anthropophily: An. atroparvus in continental France and An. labranchiae in Corsica. In a former malaria-endemic area of Southern France, intensive samplings conducted recently in rice fields showed that Anopheles hyrcanus seems to be the only potential vector likely to play a role in malaria transmission in view of its abundance and anthropophily (Ponçon et al., 2007). Since 1994 several cases of vivax and falciparum malaria with no history of international travels, blood transfusion or injection drug use have been reported in Southern France (Delmont et al., 1994; Baixench et al., 1998; Doudier et al., 2007). In Corsica, where An. labranchiae still present in high densities in different regions (Toty et al., 2010), autochthonous P. vivax malaria transmission has been diagnosed, probably via the bite of a local Anopheles mosquito infected with P. vivax from a patient who had acquired infection in Madagascar (Armengaud et al., 2006). The second most important malaria vector of Corsica, Anopheles sacharovi, has not been detected in the island since 2002 (Toty et al., 2010).
If we analyze the VC of European anophelines we can extract several conclusions. In Spain the populations of An. atroparvus were deeply studied by several authors basically during the endemic period (Buen de, 1931, 1932; Buen de & Buen de, 1930, 1933; Torres Cañamares, 1934; Olavarria & Hill, 1935; Lozano Morales, 1946; Zulueta de, 1973; Blázquez, 1974). The estimation of VC shows that An. atroparvus was an important malaria vector in different wetlands of Spain mainly during summer months. The VC was especially high for P. vivax (in August VC=0.7–21.2) which has a shorter sporogonic cycle than P. falciparum (in August VC=0.2-5.3). In September VC values were lower for both P. vivax (VC=0.2–9.2) and P. falciparum (VC=0.04-2.3) and in October VC values were drastically reduced, but still relevant in the case of P. vivax (VC=P. vivax=0.01-2.1 / VC P. falciparum=0.00007-0.02) (Bueno Marí & Jiménez Peydró, 2012). These results are similar to others derivates from different entomological researches carried out in Italy more recently. During August 1994 in Tuscany (Grosseto Province) were reported for An. labranchiae VC values ranging from 8.3-32.5 for P. vivax and 7.3-26 for P. falciparum (Romi et al., 1997). However VC was very low in early July, constituting no real risk for malaria transmission (<0.01 for both P. vivax and P. falciparum). Subsequently during 1998 in the same province but in areas where only natural anopheline breeding sites were reported, the VC of An. labranchiae from mid-July through the end of August ranged from 0.96-3.3 for P. vivax and 0.8-2.9 for P. falciparum (Romi, 1999). In other Mediterranean areas (North of Morocco), VC of An. labranchiae for P. vivax also showed high values during summer months (in July VC=17.2; in August=34; in September=18.3), while values from April to June were lower ranging from 0.5-3.7 (Faraj et al., 2008). On the other hand the average VC of An. sacharovi was found to be 0.22 (VC ranging from 0.63-0.014) in an endemic area of Southeastern Turkey (Tavşanoğlu & Çağlar, 2008). These last low VC values were probably related with very low percentages of human blood meals by anophelines. Accordingly, although of course all these values of VC are purely theoretical, it is important to note that can be numerically shown that summer (from July to September, but especially in August) is an excellent season for malaria transmission, at least at receptivity level, in Southern Europe.

2.1.1.2 Malaria receptivity in Northern Europe

Endemic northern malaria reached to 68°N latitude in Europe during the 19th century, where the summer mean temperature only irregularly exceeded 16°C. It is important to note that precisely 16ºC is considered the lower limit needed for sporogony of P. vivax (Garnham, 1988). In Finland Anopheles beklemishevi has a northern distribution, while the other common species, An. messeae, is dominant in the southern part of the country (Gutsevich et al., 1974; Lokki et al., 1979; Kettle, 1995). Both species are known as an important malaria vectors (White, 1978). Despite other potential vectors, such as An. claviger and An. maculipennis have been observed (Utrio, 1979; Dahl, 1997), it is not possible to define certainly which mosquito species was most important for the malaria transmission in Finland. This is because temperature conditions of Finland, as well as in other northern countries, should have caused that malaria transmission have mainly occurred in indoor conditions due to transmission of sporozoites throughout the winter by semiactive hibernating mosquitoes (Huldén et al., 2005), since it is well known that in warm conditions the overwintering females of Anopheles can take several blood meals (Ekblom & Ströman, 1932; Encinas Grandes, 1982). Therefore, the best malaria vectors in Northern Europe will be those anthropophilic and endophagic anophelines which present hibernating females with...
Re-Emergence of Malaria and Dengue in Europe

...semiactive winter habits but not a complete diapause. In conclusion, northern malaria existed in a cold climate by means of summer dormancy of *P. vivax* hypnozoites in addition to the indoor feeding activity of overwintering *Anopheles* females previously mentioned.

In other Scandinavian countries such as Sweden or Denmark, besides the anophelines which has been mentioned above, there have been described other potential malaria vectors: *An. atroparvus* and *Anopheles plumbeus* (Ramsdale & Snow, 2000). Although *An. messeae* was probably the main vector during the malaria epidemics in Sweden, some authors proposed that *An. atroparvus* may have maintained malaria endemicity in certain coastal localities in the south of the country (Jaenson et al., 1986). Regarding to *An. plumbeus* there are several aspects that should be pointed to understand the increasing epidemiological importance of the species in Central Europe.

*An. plumbeus* is the only hole breeding species of the genus *Anopheles* in Europe. Although it is a strictly dendrolimnic species, during dry periods females can also lay the eggs in small domestic and peridomestic containers, as well as other artificial breeding sites below the ground such as catch basins and septic tanks with water contaminated with organic waste (Bueno Marí & Jiménez Peydró, 2011). There are several reports in Europe about the presence of larvae in a biotope different from the tree cavity (Aitken, 1954; Senevet et al., 1955; Rioux, 1958; Tovornik, 1978; Bueno Marí & Jiménez Peydró, 2010a). Moreover, remarkable populations can also be found in urban situations, where the larvae develop in tree holes in gardens and parks, especially in Central Europe where *An. plumbeus* has increased in numbers during the last decades and can be a major nuisance species (Becker et al., 2010). This is a very important issue, because the continuous development of this species in urban environments could increase considerably the possibilities of interaction between malaria vectors and humans. In fact, *An. plumbeus* has been suspected to be responsible for two recorded cases of locally transmitted malaria in London, United Kingdom (Blacklock, 1921; Shute, 1954) and other two cases recently reported in Duisburg, Germany (Krüger et al., 2001). Of the five *Anopheles* species present in Britain only two, *An. atroparvus* and *An. plumbeus*, have been confirmed as malaria vectors in United Kingdom (James, 1917; Shute, 1954), while *An. messeae* and *An. atroparvus* were the vectors involved in vivax epidemics occurred in Germany during the 20th Century (Kirchberg & Mamlok, 1946).

Therefore, it exists in Europe a latitudinal gradient in relation to the distribution of the *An. maculipennis* complex. Without ignoring the possible participation of several species in malaria transmission cycles, the fact is that in Northern Europe (including European Russia) at 68ºN *An. beklemishevi* prevails as vector, being this species replaced by *An. messae* partially at 63ºN and fully about 59ºN. Around 56ºN *An. atroparvus* begins to acquire an important role in disease transmission and already in Mediterranean countries the situation of malaria receptivity is basically governed by *An. atroparvus*, *An. labranchiae* and *An. sacharovi* in Eastern, Central and Western Mediterranean respectively. As was previously pointed, this situation can be locally modified by the presence of other potential vectors widely distributed in Europe such as *An. claviger*, *An. hyrcanus*, *An. maculipennis* or *An. plumbeus*. Of course climate change could drastically modify not only the distribution of European anophelines, but also their phenology and overwintering patterns. However the changes in agricultural practices have a greater effect on the risk of malaria than an elevation in temperature of approximately 2°C (Becker, 2008), which is considered the average increasing temperature in Europe in next 50 years. Hence habitat modification is probably the factor with more influence in possible changes in malaria receptivity all over Europe.
Infectivity is defined as the degree of susceptibility of the mosquito to become infected through contact with sporozoites of the Plasmodium species. This susceptibility is influenced by several factors that can vary significantly between mosquito species and geographic locations.

Outside the species of the subgenus Cellia, i.e. refers to the possibilities that the sporogonic cycle of parasite could be successfully completed in the same species but different geographic areas can differ drastically at infectivity level due to genetic reasons (Frizzi et al., 1975).

Infectivity tests carried out on European populations of species of the subgenus Anopheles to their zoophylic behaviour and rural distribution. However, more recent studies have shown the ability of An. algeriensis and An. petragnani to transmit Plasmodium species, i.e. refers to the possibilities that the sporogonic cycle of parasite could be completed within a concrete vector species. It is well known that mosquito populations of An. atroparvus poorly represented in Europe, such as An. claviger and An. daciae can produce sporozoites of tropical strains of Plasmodium species but is refractory to African strains of Plasmodium ovale (Ramsdale & Coluzzi, 1975; Zulueta de et al., 1975). Nevertheless, An. maculipennis complex is remarkable that European populations have not been tested, although this anopheline is probably sensitive, as it belongs to the subgenus An. algeriensis, to which the principal African malaria vectors also belong. Another species of the same species but different geographic areas can differ drastically at infectivity level due to their zoophylic behaviour and rural distribution.

Anopheles multicolor and An. sacharovi in natural conditions has been involved in transmission of autochthonous vivax malaria cases and in Corsica (Kasap, 1990) as well as also Eurasiatic strains of Plasmodium falciparum (Marchant et al., 1998), but not to complete sporogony. Information about Plasmodium vivax strains of An. superpictus has been reported, not only as being the main vector of Plasmodium vivax and Plasmodium malariae in laboratory conditions (Becker et al., 2010). Furthermore, An. daciae can transmit Asian strains of Plasmodium falciparum yet must be tested on its susceptibility to Plasmodium ovale (Constantinescu & Konstantinov, 1990). Finally, there is no infectivity information about An. sergentii, to which the principal African malaria vectors also belong. Another species of the same species but different geographic areas can differ drastically at infectivity level due to their zoophylic behaviour and rural distribution.

Further research is needed to fully understand the role of these species in malaria transmission.
Re-Emergence of Malaria and Dengue in Europe

2.1.3 Vulnerability

Vulnerability is determined by the number of gametocyte carriers (malaria patients) during the suitable period for malaria transmission. If we analyze the data about imported malaria in Europe in recent years we can extract several conclusions. Malaria represents about 77% of tropical diseases imported in Europe (TropNetEurop, 2010). A total of 65,596 cases were reported in Europe between 2000 and 2009 (Table 3). However this number is clearly underestimated, since in last years the number of malaria reporting sites in Europe has increased significantly. Most of these cases are referred to immigrants (48.5%), and *P. falciparum* (81%) was the dominant species in analytic results. A high percentage of malaria cases in immigrants correspond to Visiting Friends and Relatives (VFR). This group of special epidemiological significance refers to those people who, once are established in their host countries, often travel to their origin countries to visit family or friends. Travels that these people can do to their origin countries exponentially increase the chances of disease contracting, since usually these areas are endemic regions and the stay within resident population and their customs is often long and intense (Gascón, 2006). Therefore this is an important collective to promote the need to take appropriate prophylactic measures during travels to endemic areas. Several studies have revealed that only 16% of VFR search for medical advice pre-travel, being malaria prophylaxis practically nonexistent in this collective (Leder et al., 2006). The European countries with higher number of imported malaria cases reported yearly are France and Germany, usually followed by other like Spain, Italy or Belgium. As it was shown before, malaria receptivity is remarkable in concrete regions of these countries.

| Year | Cases (sites reporting) |
|------|------------------------|
| 2000 | 1120 (32)              |
| 2001 | 3313 (38)              |
| 2002 | 4555 (47)              |
| 2003 | 5561 (44)              |
| 2004 | 6536 (47)              |
| 2005 | 7411 (50)              |
| 2006 | 8544 (50)              |
| 2007 | 8904 (52)              |
| 2008 | 9509 (57)              |
| 2009 | 10,143 (59)            |
| Total| 65,596                 |

*Note 1: Unkn./Coinf. (Plasmodium species unknown or coinfection of various species), Imm./Refu. (Immigrants/Refugees), For. Vis. (Foreign Visitors), Eur. E.C. (Europeans living in EC), Eur. Exp. (European Expatriates).*

Table 3. Imported malaria in Europe between 2000-2009 (TropNetEurop, 2010).
The temporal distribution analysis of imported malaria cases indicates that high-risk months for disease transmission (between July and September) also coincides with the period of the most cases reported in Europe. Therefore most of cases occur during the epoch theoretically favorable for malaria transmission. In regard to the diagnostic delay, i.e. the average time between appearance of symptoms and malaria diagnosis (when therapy began), it shows disparate values according to each country. For example, in Eastern Spain the diagnostic delay of imported malaria was estimated in 13.7 days (Bueno Marí & Jiménez Peydró, 2012), while in other European countries like Sweden, France or Italy values are clearly lower, ranging from 3 to 8.2 days (Romi et al., 2001; Askling et al., 2005; Chalumeau et al., 2006). From an epidemiological point of view it is very important to reduce the diagnostic delay, because this is the period when malaria patients could be a source of infection for Anopheles females. Additionally, from an exclusively clinical perspective, delay to diagnosis leads of course to high parasitemia, which itself leads to severe forms of malaria.

3. Dengue fever and yellow fever

There are many similarities between dengue fever and yellow fever:
- Both are viruses of the genus Flavivirus (family Flaviviridae) and are strictly primatophilic, infecting only primates, including man.
- In their original habitat, both are zoonotic infections transmitted by forest mosquitoes.
- Their importance as human pathogens can be related with two forest mosquitoes characterized by high ecological plasticity that have become closely associated with the peridomestic environment.
- Both diseases have a history of transmission in temperate regions, including Europe, and share essentially the same selvatic and urban vectors.
- Transovarian transmission in female mosquitoes has been demonstrated for both viruses.
- The viruses and their urban vectors have a worldwide distribution due to transportation of goods and people.
- Both arboviruses are characterized by short incubation period and can provoke similar clinical symptoms, including hemorrhagic illness in humans, often with fatal consequences. However mortality rate is higher in yellow fever (20%) than in dengue (5%).

In the case of dengue fever its annual incidence has increased dramatically around the world in recent decades. It is estimated that over 2500 millions people who live in over 100 tropical and non-tropical countries, are currently at risk from dengue viruses globally. The rise in dengue incidence has been marked by geographic expansion of the virus and the vectors due to globalization, habitat modifications, lack of effective mosquito control programs and climate change. Although the major disease burden occurs in South East Asia, the Americas and the western Pacific, dengue was also a common disease in Europe in the past centuries. Large epidemics of dengue and yellow fever occurred in European ports of Spain, Portugal, France, Italy and even Wales and Ireland as the more northern countries of the continent (Eager, 1902; Monath, 2006). Last dengue epidemic in Europe, estimated at one million cases, occurred in Greece in 1927-28 (Papaevangelou & Halstead, 1977; Rosen, 1986).

Dengue is the most frequent tropical arbovirus imported in Europe and together with schistosomiasis both are considered, after malaria, the most important tropical diseases in Europe.
Re-Emergence of Malaria and Dengue in Europe

Quantitative terms in Old continent. Of the hundreds of dengue imported cases reported yearly in Europe (Table 4), the vast majority are represented by tourists (about 84%). A difference to what happens with malaria, immigration (9%) seems to have comparatively little influence on dengue importation. This could be explained, of course, by distinct perspectives and approaches of European tourists (e.g. travels to urbanized areas) and immigrants who come to Europe (e.g. Africa, where malaria is much more prevalent than dengue, is the main origin from immigrants who arrive to Europe), but also by differences between incubation periods and existing prophylactic measures in both diseases. All dengue cases reported have shown the typical symptomatology of the disease, including febrile symptoms in more than 90% of cases (TropNetEurop, 2010). However, it is important to note that the majority of imported dengue infections remain undiagnosed, with a ratio between symptomatic and asymptomatic travelers estimated in 1/3.3 (Cobelens et al., 2002). In general terms, it is estimated that about 80% of all dengue infections are asymptomatic (Farrar, 2008). This high asymptomatic, added to the fact that dengue is not a notifiable disease in much of European countries (Bueno Marí & Jiménez Peydró, 2010c; 2010d), allows us to consider that the knowledge of dengue virus circulation is very limited.

Table 4. Imported dengue in Europe between 2001-2009 (TropNetEurop, 2010).

Aedes aegypti is the primary urban vector of dengue and yellow fever basically because it exist a 'domesticated' form of the species that is rarely found more than 100 m from human habitation and feeds almost exclusively on human blood (Reiter, 2010). Both factors allow that Aedes aegypti will be considered as an excellent urban vector of viruses. Its distribution was traditionally limited by latitude between 45º N and 35º S according to the existence of January and July 10° C isotherms. Although records out of this latitude range are very rarely, it must be pointed that European northernmost collection of the species occurred in Brest (France) at 48º N (Christopher, 1960). Moreover, recent studies have demonstrated that Aedes aegypti larvae can withstand temperatures of 2.5° C (Chang et al., 2007). In Eastern Europe it was also seen at its temperature limit at Odessa (Ukraina) at 46º N. (Korovitzkyi and Artemenko, 1933). Despite the species was relatively common in Mediterranean countries, it disappeared from the entire region in the mid-20th century, for reasons that currently are not clear but probably related with thermic tolerance and intensive mosquito control.
control campaigns with the employment of DDT. Ae. aegypti was common in the Iberian Peninsula mainly introduced from North Africa and was present in this Southern European region up to 1956 (Ribeiro & Ramos, 1999). Since the eradication of the species in Europe, its sporadic presence has been recognized in several countries, namely Britain, France, Italy, Malta, Croatia, Ukraine, Russia and Turkey (Snow & Ramsdale, 1999). However it must be pointed that the species has been reported in Madeira (Portugal) in 2005 (Margarita et al., 2006) and it seems that Ae. aegypti is now deeply established in this region because of continuous collections in later years (Almeida et al., 2007). This is the first report of the establishment of the species in Europe since mid-20th century. More recently Ae. aegypti has also been captured in The Netherlands (Scholte et al., 2010). In summary, we must pay some attention to surveillance and behavior of Ae. aegypti because globalization is provoking the arrival of the species to Europe and global warming could allow the definitive establishment of the species again in Southern areas.

On the other hand the situation is clearly divergent in regard to the secondary vector of dengue and yellow fever, Aedes albopictus, usually known as Asian tiger mosquito, due to its quick expansion in Europe in last years. There are several ecological factors that can help us to understand the different importance of Ae. aegypti and Ae. albopictus as primary and secondary vectors of human viruses respectively. Unlike patterns of oviposition and feeding exhibited by Ae. aegypti, Asian tiger mosquito is often abundant in the peridomestic environment, particularly in areas with plentiful vegetation, and feeds freely on humans and other animals. Consequently Ae. albopictus can also exist far from human habitation. Additionally Ae. aegypti has been globally dispersed from Africa by humans activities since several centuries ago while Ae. albopictus was firstly reported out of its original Asiatic distribution range in 1979 in Albania (Adhami & Reiter, 1998). Current data indicate that Ae. albopictus has been detected much farther north than Ae. aegypti and one major difference between both species is that Asian tiger mosquito has the ability to adapt to cold temperatures by becoming dormant during the winter of temperate regions. The ability of Ae. albopictus to resist cold temperatures is partially related with its ability to synthesize a high amount of lipids, especially to produce larger amounts of yolk lipid in cold temperatures. At respect, it was demonstrated that larval lipogenesis of Ae. albopictus is much more efficient than that of Ae. aegypti (Briegel & Timmermann, 2001). Although Ae. albopictus occurs in both temperate and tropical areas, only temperate population, but not tropical ones, show a photoperiodic diapause (Hawley, 1988). During the shortening daylight hours in late summer/early autumn, the reduced photoperiod stimulates the females of Ae. albopictus to produce eggs that enter facultative diapause (Estrada-Franco & Craig, 1995). These eggs can resist hatching stimuli until the following spring and remain in a state of reduced morphogenesis as fully formed first instar larvae, exhibiting increased resistance to environmental extremes. Although the diapause occurs in the egg stage, only adults and pupae are known to be photoperiodically sensitive stages (Wang, 1966; Imai & Maeda, 1976; Mori et al., 1981). Ae. albopictus has been found to be capable to transmit 26 viruses (Moore & Mitchell, 1997; Gratz, 2004; Paupy et al., 2009) and to be experimentally susceptible to several filariasis of veterinary interest (Cancrini et al., 1995; Nayar & Knight, 1999). Globalization has allowed the arrival of this species to Europe, mainly through the transport of eggs and larvae in used tires and gardening products (Reiter & Sprenger, 1987; Madon et al., 2002). The presence of Asian tiger mosquito has been confirmed in 16 European countries, but only in Southern ones the species is deeply established. Particularly interesting is the situation of Italy, where...
Re-Emergence of Malaria and Dengue in Europe

The species was first detected in 1990 (Sabattini et al., 1990) and nowadays has colonized more than 2/3 parts of the territory, even having different areas of the country with mosquito densities in considerable epidemiological levels. Precisely these locally high densities have allowed the appearance of first cases of human viruses in Europe transmitted by *Ae. albopictus*. Specifically, in the province of Ravenna (Northeastern Italy) occurred an outbreak of Chikungunya virus in 2007. This virus is very similar to dengue and yellow fever (same vectors, bioecology and symptoms), but much less pathogenic. Just in two and a half months, a total of 205 cases of Chikungunya were reported in two small towns of Ravenna where the infection of *Ae. albopictus* was also confirmed (Rezza et al., 2007). This outbreak of Chikungunya infection, outside a tropical country, was probably begun by a man from India, country that previous year had suffered an epidemic with more than 1 million cases (Ravi, 2006). The Indian man developed a febrile syndrome two days after his arrival in Italy and also had high titres of antibodies against Chikungunya. The phylogenetic analysis showed that the strain that caused Italian outbreak was similar to the strains detected on the Indian subcontinent (Yergolkar et al., 2006), showing in all cases a better adaptation to *Ae. albopictus* than other variants. However most worrying scenario took place in 2010 with the reappearance of first autochthonous cases of dengue in Europe transmitted by *Ae. albopictus*. In this year, two cases of autochthonous dengue fever were diagnosed in Nice (Southeast France) (Laruche et al., 2010), region where *Ae. albopictus* is established at least since 2004 (Delaunay et al., 2007). Just days after two indigenous cases of Chikungunya in the districts of Alpes-Maritime and Var (also in Southeastern France) were detected through a routinely surveillance of dengue and Chikungunya (ECDC, 2010), which is yearly conducted since 2006 due to the establishment of *Ae. albopictus* in this region. In Greece, other Mediterranean country where *Ae. albopictus* is established at least since 2004 (Klobucar et al., 2006), two cases of indigenous dengue were diagnosed also in 2010 (Schmidt-Chanasit et al., 2010; Gjenero-Margan et al., 2011). The identification of these cases of dengue fever and Chikungunya occurred in 2010, which were in all cases well clustered in space and time, is strongly suggestive that autochthonous transmission of tropical viruses in Europe is ongoing.

According to these epidemiological perspectives it seems evident that there is a need to be able to predict the potential distribution and activity of *Ae. albopictus* in Europe to assess about possible re-emergence of dengue and other tropical arboviruses. At respect several Geographic Information Systems (GIS) have been developed in order to predict the number of weeks of activity of *Aedes albopictus* in Europe to assess about possible re-emergence of dengue and other tropical arboviruses. At respect several Geographic Information Systems (GIS) have been developed in order to predict the number of weeks of activity of *Aedes albopictus* (ECDC, 2009). These GIS models have revealed that throughout much of Europe, more than 23 weeks are predicted to elapse between egg hatching in spring (in response to at least 11.25 hours of daylight and 10.5° C of mean temperature) and adult die-off in autumn (below critical temperature threshold of 9.5° C). Assuming that immature development takes about 2–4 weeks, this constitutes more than 20 weeks of adult activity in Central Europe and Southern United Kingdom, even increasing this activity to more than 40 weeks in southern areas (mainly Greece, Turkey and south of Iberian and Italic Peninsula), depending on availability of surface water for breeding. If these predictions would be fulfilled in Southern Europe, consequently could increase the speed of spread of the species, could also extend the episodes of medical and social alerts derivates from its feeding behavior in urban areas, and even could change the eco-epidemiology of viruses that *Ae. albopictus* can transmit.

It must be pointed that *Ae. albopictus* and *Ae. aegypti* are not the only aedine vectors with invasive behavior in Europe. Other exotic mosquitoes, such as *Ochlerotatus japonicus* and

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Current Topics in Tropical Medicine

Ochlerotatus atropalpus, have been also reported. Oc. japonicus is an Asian species and a competent vector of several arboviruses, including West Nile virus and Japanese encephalitis virus and is considered a significant public health risk (Sardelis & Turell, 2001; Sardelis et al., 2002a; 2002b; 2003).

Ochlerotatus atropalpus has been collected only in France, Belgium, Switzerland and Germany (Schafher et al., 2003; 2009; Becker et al., 2011). On the other hand Oc. japonicus has been observed in Italy, France and Netherlands (Romi et al., 1997; Adege-EID Mé diterranée, 2006; Scholte et al., 2009).

Although in the field, Oc. atropalpus has not been evidenced as an important vector of infectious diseases, under laboratory conditions, the species has been proven as a competent vector for West Nile virus, Japanese encephalitis virus, Saint-Louis encephalitis virus, among other arboviruses (King, 1960; Turell et al., 2001).

Globalization, especially traffic of used tires, has led the arrival of Oc. japonicus and Oc. atropalpus to Europe. Out of these exotic vectors, we can not forget or ignore the presence of potential indigenous vectors of dengue and yellow fever in Europe. For example, Aedes vittatus is an important vector of yellow fever in different parts of Africa (Lewis, 1943; Satti & Haseeb, 1966) and also a potential vector of Chikungunya and four dengue serotypes (Mourya & Banerjee, 1987; Mavale et al., 1992). Although the species is deeply distributed in Mediterranean region (Spain, Portugal, France and Italy), the studies about its biology and phenology have been scanty in Europe. Anyway it seems unlikely that Ae. vittatus could start a cycle of virus transmission to humans because of its high degree of ruralism.

Moreover Ochlerotatus geniculatus is a dendrolimnic species endemic to Europe that can efficiently transmit yellow fever, but this possibility has been evidenced only in laboratory conditions (Roubaud et al., 1937).

3.1 New challenges: The development of dengue vaccines

Although a vaccine based on live attenuated virus of the strain 17D is available for yellow fever since years, currently we haven’t any vaccine to be used with full warranty against dengue. However, the need for a dengue vaccine is clear. The most effective measures of an integrated mosquito control program (including changes in human habitation and behavior, the use of insecticides, and long-lasting modification of natural and man-made mosquito habitats) are difficult to implement and largely unsuccessful in most poverty-stricken settings, and consequently have not been carried out comprehensively enough to limit dengue’s spread. While vector control is an integral part of any dengue prevention strategy, it is not enough on its own.

In recent years it has been obtained a better understanding of the disease and its etiopatogenicity, as well as of the necessary aspects to develop a vaccine that provides an effective and lasting protection against the virus. Dengue vaccine development is a very difficult task due to the possible participation of four related serotypes, since immunity to one serotype does not confer immunity to the remaining three. Complicating the scenario further is immune enhancement, which can result in severe dengue hemorrhagic fever or dengue shock syndrome in anyone who has been infected with one of the serotypes and subsequently becomes infected with another. Most of researchers agree that only effective solution is a tetravalent vaccine that simultaneously protects against all four serotypes. Regarding to this, it must be noted that tetravalent vaccines against dengue are currently in last phases of trials and is expected to be available for human population in the next following years.

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4. Conclusions

Although malaria's receptivity is still high in different parts of Europe, we may conclude that the malariogenic potential of the Old Continent is low. Fortunately socio-economic and sanitary conditions of most European countries also support this assertion. While it is true that infectivity studies should be further promoted, percentages of imported malaria cases remain very low. However we must pay some attention to the increasing trend of malaria importation in last years, as well as also awareness among tourists and VFR's for to take corresponding prophylactic measures during their travels to endemic areas.

Anyway, sporadic and local cases of autochthonous transmission mainly transmitted by An. atroparvus, An. labranchiae, An. sacharovi and/or An. plumbeus, can not be discarded in next years.

On the other hand, the answer to the question about if should be expected the re-emergence of dengue and other mosquito-borne tropical viruses in Europe in next years is indubitable: definitively yes. The arrival, establishment and expansion of dengue urbanite vectors due to global changes such as globalization, climate change and the lack of effective mosquito control programs, together with the increasing of imported cases in humans provokes that local and intense transmission of dengue could be a reality in next years in Southern Europe.

To cope this possibility is necessary to enhance the entomological surveillance in potential areas of mosquitoes importation, such as airports or seaports, strength the monitoring of tropical viruses imported and awareness among citizens about their role in mosquito control and best prophylactic measures to take during the travels to tropical regions.

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