Study of the malarialogenic potential of Eastern Spain

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Abstract. Recent autochthonous malaria cases which occurred in Spain, France, Greece or Italy have shown the need to delve into the knowledge of potential influence of tropical diseases in Southern Europe. The malariogenic potential of a formerly endemic area of Spain was analyzed in present manuscript according to the epidemiological parameters of receptivity, infectivity and vulnerability. During a five years period (2005-2009) comprehensive larval surveys of anophelines and continuous analysis of imported malaria cases were conducted in a study region of about 23 260 km². The next seven potential malaria vectors were collected: Anopheles algeriensis, Anopheles atroparvus, Anopheles claviger, Anopheles maculipennis, Anopheles marteri, Anopheles petragnani and Anopheles plumbeus. The entomological results conclude that malaria receptivity is still high in different rural and hinterland regions where it is possible to find high densities of An. atroparvus. Moreover An. algeriensis was also commonly found breeding in irrigation channels surrounding urban areas. Although receptivity is relevant in much of the study area, fortunately the vulnerability of the territory is very low. In conclusion, despite our data together with current socio-economic and sanitary conditions of Spain indicate a relatively low malariogenic potential, we must maintain the entomological and epidemiological vigilance in order to prevent the potential appearance of indigenous malaria cases. Therefore, the present Spanish situation can be described as what malariologists of the first half of the last century would have called “anophelism without malaria.”

INTRODUCTION

According to the World Health Organization (WHO), today malaria annually affects 500 million people and threatens directly or indirectly 40% of world population (WHO, 2007). However, it is well known that these morbidity and mortality data show an asymmetric distribution, mainly depending on the economical, 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 parasitic disease is caused by protozoa of the genus Plasmodium. Although the simian parasite Plasmodium knowlesi has been found recently as a cause of human malaria in Southeastern Asia (Singh et al., 2004; Luchavez et al., 2008), other four plasmodia species are the most recognized to infect humans in natural 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 (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 the malaria vectors, there are about 40 *Anopheles* species with an important role in transmission (Kiszewski, 2004).

In Spain, malaria was a highly endemic disease until about the middle of the twentieth century (Pletsch, 1965). Since then, the studies of anophelines biology and distribution have been scanty and limited to certain, mainly central, inland provinces (Zulueta *et al*., 1973; Zulueta, 1974; Encinas Grandes, 1982). In parallel, as in other European countries, an increasing incidence of imported malaria is taking place in Spain in recent years (DGSP, 2010).

The aim of this work was to analyze the malirogenic potential of Eastern Spain according to the parameters of receptivity, infectivity and vulnerability (Romi *et al*., 2001).

**MATERIALS AND METHODS**

**Receptivity**

Receptivity could be analyzed by the presence, density, and biological characteristics of vectors. In Spain, a total of 15 *Anopheles* species are reported (Eritja *et al*., 2000). However, most of data referring to anophelines records are very old and basically confined to the endemic disease period.

Our study area was the Valencian Autonomous Region of Eastern Spain (comprising the Provinces of Castellón, Valencia, and Alicante). Several parts of this area were some of the territories with highest malaria endemicity in Spain (Bueno Mari & Jiménez Peydró, 2010a). To improve and update the knowledge of malaria receptivity, we sampled multiple larval sites of anophelines, using the standard dipping method (Service, 1993) from March – October during 2005-2009. Sampling was also carried out during the winter months of 2008 and 2009 to collect overwintering larval stages of several species. Sampling of small larval habitats such as tree-holes or small containers was done by emptying or pipetting the contents for immature stages. Data were collected from all identifiable aquatic environments across 23 260 km² of the study area. The sampling effort was fixed at ten minutes which included the active search for larvae in each biotope visited (Bueno Mari & Jiménez Peydró, 2011). Anophelines were identified according to the taxonomic keys of Encinas Grandes (1982), Schaffner *et al*., (2001) and Bueno Mari (2011).

Moreover, we also analyzed historical data and the results of entomologic studies conducted in Spain (De Buen, 1931, 1932; De Buen & De Buen, 1930, 1933; Torres Cañamares, 1934; Olavarria & Hill, 1935; Lozano Morales, 1946; Zulueta *et al*., 1973; Zulueta, 1974; Blázquez, 1974) in order to estimate the vectorial capacity (VC) of *Anopheles atroparvus* Van Thiel, 1927, which is considered the most important malaria vector in Europe (Schaffner *et al*., 2001; Bueno Mari & Jiménez Peydró, 2008). The VC of some Spanish populations of *An. atroparvus* was also estimated by the MacDonald formula (MacDonald, 1957) according to the modifications proposed by Garrett-Jones (1964):

\[
VC = ma^2p^n / -ln p
\]

Where, \(ma\) represents the relative vector density (number of vectors per man-night), \(a\) 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. The estimation of VC is postulated as a very useful tool to assess
the receptivity of a determined territory in a concrete moment (Carnevale & Robert, 2009).

Infectivity
Infectivity is defined as the degree of susceptibility of *Anopheles* mosquitoes to different *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 the same species but different geographic areas can differ drastically at infectivity level due to genetic reasons (Frizzi *et al*., 1975). To evaluate the infectivity of Spanish vectors we analyzed data in published studies about European strains of *Anopheles* because of the lack of studies carried out in our country.

Vulnerability
Vulnerability is determined by the number of gametocyte carriers (malaria patients) during the suitable period for malaria transmission. To determine the vulnerability of the study area we analyzed in detail all the imported malaria cases reported from the Regional Ministry of Health during the same five years period (2005-2009) in which our *Anopheles* samplings were accomplished.

RESULTS AND DISCUSSION

Receptivity
A total of 1179 exemplars belonging to next seven anopheline species were collected and identified: *Anopheles algeriensis* Theobald, 1903, *An. atroparvus*, *Anopheles claviger* (Meigen, 1804), *Anopheles maculipennis* Meigen, 1818 *Anopheles marteri* Sévenet & Prunelle, 1927, *Anopheles petragnani* Del Vecchio, 1939 and *Anopheles plumbeus* Stephens, 1828 (Fig. 1). It is important to note that our samplings indicate that *An. algeriensis* was the species with higher synanthropic degree, being the only anopheline present in irrigation channels surrounding urban areas. Despite not being regarded as a primary malaria vector, *An.
algeriensis has been shown as an accidental vector in North Africa (Horsfall, 1972). Aquatic stages of An. claviger and An. maculipennis were always found in fresh or slightly brackish water in inland mountainous regions away from anthropised environments. Although these biocological aspects, together with their zoophylic tendency, indicate a minor role in malaria transmission, it should be noted that both species have been related with several malaria outbreaks in some Eastern Mediterranean countries (Gramiccia, 1956; Coluzzi et al., 1964; Schaffner et al., 2001).

Anopheles plumbeus is the only strictly dendrolimnic species of the genus Anopheles in Europe. We usually found larvae of this species on white and black poplar, also occasionally on small artificial containers. Due to its restricted distribution, it is a sporadic malaria vector. However, it is suspected to be responsible for several episodes of malaria in England (Blacklock, 1921; Shute, 1954) and Germany (Krilger et al., 2001). Anopheles petragnani was the most abundant anopheline in our study area. Larvae were found in a great diversity of environments. However, since it occurs with An. marteri, both are zoophilic species which usually breeds in wild areas of low anthropisation. Consequently, they are not considered as important malaria vectors. Moreover, winter samplings have allowed the collection of overwintering larvae of An. algeriensis, An. claviger and An. petragnani at water temperatures varying from 4.2ºC to 8.1ºC. The potential for larval diapause of these species has been suggested by different authors in Southern Europe (Schaffner et al., 2001; Becker et al., 2010).

Anopheles atroparvus, the main malaria vector in Spain, was frequently found in small lagoons, temporary puddles, irrigation channels and river margins, but not in rice fields, where the species is well known to be common in the past (Romeo Viamonte, 1950; Pérez Moreda, 1982; Mateu, 1987). It is suggested that the high eutrophication (due to massive employment of fertilizer with nitrogen compounds) and the presence of large residual amounts of various insecticides in Valencian rice fields (Mendoza, 2002; Tarazona et al., 2003) are two possible drawbacks to the larval development of An. atroparvus. Moreover, potential biotopes of An. atroparvus surrounding rice fields were also drastically modified in the last 50 years. Most of the irrigation channels have been destroyed by the strong urban development around these crops and the scanty waters that are currently present are deeply colonized by eastern mosquito fish - Gambusia holbrooki (Girard, 1859), which was introduced to fight against malaria in 1921 (Bueno Marí & Jiménez Peydró, 2010b).

Anopheles atroparvus breeding sites have been always situated in rural areas remote from centers of human population. Nevertheless, several decades ago, the high degree of rurality of human population enabled the continuous contact of An. atroparvus with humans. If we analyze the VC of An. atroparvus during this past epoch, we could extract several conclusions (Table 1). The values of VC were high in August especially for P. vivax (VC=0.7–21.2), which has a shorter sporogonic cycle than P. falciparum (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). 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). These results are similar to others derivates from different entomological researches carried out in Italy more recently. During August 1994 in Tuscany (Grosseto Province), VC values ranging from 8.3-32.5 for P. vivax and 7.3-26 for P. falciparum were reported for Anopheles labranchiae Falleroni, 1926, the other most important vector of the Maculipennis Complex (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).
### Table 1. Estimation of *An. atroparvus* vectorial capacity (VC) with indication of region, season, year and main types of *Anopheles* breeding sites in each entomological study conducted

<table>
<thead>
<tr>
<th>Region</th>
<th>Months²</th>
<th>Year</th>
<th>Characteristics of study area</th>
<th>Vector density (Vd) and Vectorial capacity (Vc)</th>
</tr>
</thead>
</table>
| Ebro Delta (Tarragona, NE Spain) | September | 1926 | Rice fields                            | $V_d = 122$ per animal shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.4$  
|                               |          |      |                                        | $V_c P. vivax = 1.38$  |
| Ebro Delta (Tarragona, NE Spain) | October  | 1926 | Rice fields                            | $V_d = 101$ per animal shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.0003$  
|                               |          |      |                                        | $V_c P. vivax = 0.04$  |
| Campo Arañuelo (Cáceres, W Spain) | September | 1929 | River margins, irrigation channels     | $V_d = 50$ per animal shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.04$  
|                               |          |      |                                        | $V_c P. vivax = 0.2$  |
| Campo Arañuelo (Cáceres, W Spain) | September | 1929 | River margins, irrigation channels     | $V_d = 14$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.6$  
|                               |          |      |                                        | $V_c P. vivax = 2.5$  |
| Campo Arañuelo (Cáceres, W Spain) | October  | 1930 | River margins, irrigation channels     | $V_d = 78$ per animal shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.00007$  
|                               |          |      |                                        | $V_c P. vivax = 0.01$  |
| Campo Arañuelo (Cáceres, W Spain) | October  | 1930 | River margins, irrigation channels     | $V_d = 20$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.0006$  
|                               |          |      |                                        | $V_c P. vivax = 0.08$  |
| Campo Arañuelo (Cáceres, W Spain) | October  | 1934 | River margins, irrigation channels     | $V_d = 168$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.02$  
|                               |          |      |                                        | $V_c P. vivax = 2.1$  |
| Campo Arañuelo (Cáceres, W Spain) | August    | 1934 | River margins, irrigation channels     | $V_d = 64$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 5.3$  
|                               |          |      |                                        | $V_c P. vivax = 21.2$  |
| Campo Arañuelo (Cáceres, W Spain) | October  | 1934 | River margins, irrigation channels     | $V_d = 225$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.01$  
|                               |          |      |                                        | $V_c P. vivax = 1.5$  |
| Condado de Jaén (Jaén, SW Spain) | September | 1934 | River margins, lagoons, reservoirs   | $V_d = 34$ per person/night$^c$  
|                               |          |      |                                        | $V_c P. falciparum = 0.4$  
|                               |          |      |                                        | $V_c P. vivax = 1.6$  |
| Campo Arañuelo (Cáceres, W Spain) | August    | 1973 | River margins, irrigation channels     | $V_d = 75$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 1.9$  
|                               |          |      |                                        | $V_c P. vivax = 7.8$  |
| Campo Arañuelo (Cáceres, W Spain) | August    | 1973 | River margins, irrigation channels     | $V_d = 182$ per animal shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.2$  
|                               |          |      |                                        | $V_c P. vivax = 0.7$  |
| Ebro Delta (Tarragona, NE Spain) | September | 1973 | Rice fields                            | $V_d = 35$ per human shelter$^b$  
|                               |          |      |                                        | $V_c P. falciparum = 0.3$  
|                               |          |      |                                        | $V_c P. vivax = 1$  |
| Ebro Delta (Tarragona, NE Spain) | September | 1973 | Rice fields                            | $V_d = 81$ per person/night$^c$  
|                               |          |      |                                        | $V_c P. falciparum = 2.3$  
|                               |          |      |                                        | $V_c P. vivax = 9.2$  |

² In August and September, assuming a sporogonic cycle of 12 days for *P. falciparum* and 10 days for *P. vivax* (mean temperature of 25°C). In October, assuming a sporogonic cycle of 23 days for *P. falciparum* and 16 days for *P. vivax* (mean temperature of 20°C). Information about duration of the sporogonic cycle extracted from Boyd (1949)

³ Females of *An. atroparvus* collected in animal or human shelters. According to Kroeger & Alarcón (1993), the human-biting frequency ($a$) was calculated as human hematophagy ($h$) / duration of gonotrophic cycle ($g$); $a = h / g$.

⁴ Mosquitoes collected during an entire night using human bait ($noa$).
Although, of course all these values of VC are purely theoretical, it is important to note that it 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.

**Infectivity**

Infectivity tests carried out on European populations of *An. atroparvus* showed that this species can transmit Asian strains of *P. vivax*, but is refractory to African strains of *P. falciparum* (Ramsdale & Coluzzi, 1975). However, more recent studies have shown the ability of *An. atroparvus* to generate oocysts of *P. falciparum* (Marchant et al., 1998), but not to complete the sporogony.

Furthermore, European populations of *An. plumbeus* can produce sporozoites of tropical strains of *P. falciparum* (Marchant et al., 1998; Eling et al., 2003), as well as Eurasiatric strains of *P. vivax* (Shute & Maryon, 1974). Even some authors suggest that *An. plumbeus* is capable of transmitting the four *Plasmodium* species (Shute & Maryon, 1969). However, this hypothesis should be confirmed with modern molecular techniques. With respect to *An. algeriensis* and *An. claviger*, it is important to note that in natural populations the presence of oocysts of *P. vivax* at intestinal level has been shown (Blacklock & Carter, 1920; Horsfall, 1972). In the case of *An. algeriensis*, even has been successfully tested the transmission of *P. falciparum* in laboratory conditions (Becker et al., 2010). With regard to *An. maculipennis*, it is known that in certain coastal areas in the Balkans, Asia Minor and Northern Iran (Postiglione et al., 1973; Zaim, 1987; Manouchehri et al., 1992), the species has participated actively in malaria transmission cycles. Finally, there is no infectivity information about *An. marteri* and *An. petragnani*. Anyway, the epidemiological role of both species seems secondary due to their zoophylic behaviour and rural distribution.

**Vulnerability**

Of 1729 imported malaria cases reported in Spain during 2005-2009, 294 (17% of total) were declared in the study area (Table 2). Detailed analysis of malaria cases in Valencian Autonomous Region reveals that most cases were related to immigrants from Africa and *P. falciparum* was the species most frequently diagnosed (Table 3). 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 countries of origin to visit family or friends. Such visits exponentially increase the chances of contracting the disease, since usually these areas are endemic regions and the stay within resident population and their customs is often long and intense (Gascón, 2006). Therefore, it is important to promote the need to take appropriate prophylactic measures during travel to endemic areas. Several studies have revealed that only 16% of VFR seek medical advice pre-travel, because malaria prophylaxis is practically nonexistent in this collective (Leder et al., 2006). The results of malaria prophylaxis in the 294 cases reported in the study area also showed worrying data, since only 16.2% took prophylactic measures and just 34.5% (5.6% of total) did it correctly.

The temporal distribution 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 (36.1%). Therefore, most cases occur during the epoch, which is 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), was estimated to be 13.7 days. 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. In other European countries like Sweden, France or Italy, diagnostic delay of imported malaria is clearly lower, ranging from 3 to 8.2 days (Romi et al., 2001; Askling et al., 2005; Chalumeau et al., 2006). Moreover, it also showed once more the need to carry out a
Table 2. Imported malaria cases declared by the Network of Surveillance and Epidemiological control of the Regional Ministry of Health (DGSP-GVA, 2010). SPN. = Spain, VAR. = Valencian Autonomous Region

<table>
<thead>
<tr>
<th>YEAR</th>
<th>SPN.</th>
<th>VAR.</th>
<th>YEAR</th>
<th>SPN.</th>
<th>VAR.</th>
<th>YEAR</th>
<th>SPN.</th>
<th>VAR.</th>
<th>YEAR</th>
<th>SPN.</th>
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<td>141</td>
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<td>1987</td>
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<td>12</td>
<td>1997</td>
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<td>2007</td>
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<td>0</td>
<td>1989</td>
<td>116</td>
<td>13</td>
<td>1999</td>
<td>392</td>
<td>38</td>
<td>2009</td>
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<table>
<thead>
<tr>
<th>Plasmodium</th>
<th>Temporal distribution</th>
<th>Origin</th>
<th>Human population</th>
<th>% of recidives cases</th>
<th>Diagnostic delay</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>71.3%</td>
<td>First quarter (18.1%)</td>
<td>Africa</td>
<td>96.8%</td>
<td>Immigrants (VPR)</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>5.2%</td>
<td>Second quarter (13%)</td>
<td>Asia</td>
<td>2.1%</td>
<td>Tourists</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>2.9%</td>
<td>Third quarter (36.1%)</td>
<td>America</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>1.6%</td>
<td>Fourth quarter (32.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coinfections</td>
<td>19%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

comprehensive monitoring of patients after treatment due to the high percentage of malaria cases associated with recidivism (25.7%).

In regard to the spatial distribution of imported malaria in the study area, 77.2% of the cases were diagnosed in the two most important urban cores (Fig. 2). In the urban area of Valencia, 63.2% of such cases occurred and fortunately, the absence of *An. atroparvus* in Valencian rice fields reduces the malarigenic potential of this area. Alicante urban area (14%) also highlights the absence of anophelines, but we should focus some attention due to the high presence of *An. algeriensis* in the surrounding areas. The rest of the studied region showed very low rates. Furthermore, there were even some inland rural areas with high density and diversity of anophelines (high receptivity territories) where there has not been any case of imported malaria declared in last five years.

The receptivity of malaria is still high in different rural areas of the study area. Besides the collection of seven potential malaria
vectors, we must remark the absence of records of *An. labranchiae*. This absence is not surprising because it is considered disappeared since 1973 (Blázquez & de Zulueta, 1980) probably due to abandonment of rice cultivation in its restricted distribution area (Eritja *et al*., 2000). The southernmost Province (Alicante) of our study area together with its contiguous Province of Murcia were the only territories where *An. labranchiae* has been able to establish itself in the Iberian Peninsula. Currently, the most relevant epidemiological situations in the study area can be summarized by the high density of *An. atroparvus* in inland rural areas and the presence of *An. algeriensis* around urban areas.

Fortunately, the vulnerability of the territory studied, although has increased in recent years, still remains relatively low. The reduction of the malaria diagnostic delay and the awareness of tourists in making prophylactic measures are two of the most important issues to enhance.

In conclusion, our data indicates a relatively low malariogenic potential for the Valencian Autonomous Region, thus, supporting the hypothesis pointed by other authors for the whole country (López Vélez & Molina Moreno, 2005). Though current socio-economic conditions in Spain reduce possibilities of re-emergence of malaria transmission (Bueno Mari & Jiménez Peydró, 2010c), it is evident that certain entomological and epidemiological vigilance must be maintained. The most obvious evidence of this need is the fact that the first case of autochthonous malaria in the last 50
years in Spain has been detected recently (Santa-Olalla Peralta et al., 2010), affected by *P. vivax* and presumably transmitted by *An. atroparvus*. Furthermore, airport malaria has also been reported at various times in our country (Blázquez, 1986; Cuadros et al., 2002). Therefore, it is recommended the establishment of regional centers with experts competent in medical entomology, epidemiologic surveillance and mosquito control, in order to prevent the appearance of indigenous malaria cases in high receptivity areas.

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**REFERENCES**


De Buen, E. (1931). Algunos estudios sobre biología del Anopheles maculipennis en lo que se refiere a la casa habiteda por el hombre o animales. Medicina de los Países Cálidos 4: 400-414.


Dirección General de Salud Pública (DGSP) Conselleria de Sanitat de la Generalitat Valenciana, Sistema de Notificación Obligatoria, Área de Epidemiología. Available online in [accessed 14th March 2011]:
http://www.sp.san.gva.es/DgspPortal/docs/epidemiologia/PALUDISM.htm


http://www.tropnet.net/reports_friends/reports_friends_index.html


