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Entomological surveillance in a recent autochthonous malaria area of Spain

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Flooding practices, mainly rice cultivation were clearly associated with malaria endemicity in Mediterranean countries until the beginning of the 20th century. In Spain, the disease was officially eradicated in 1964 and was historically eliminated by a mixture of vector control, disease treatment, habitat modification, and improvements in general living standards. *Anopheles atroparvus* Van Thiel 1927 (Diptera: Culicidae) and *An. labranchiae* (Falleroni 1926) were supposed to be the major malaria vectors in Spain, although some other species, such as *An. maculipennis* (Meigen 1818) or *An. claviger* (Meigen 1804) might have locally contributed to the disease transmission[1]. Currently, *An. atroparvus* remains widespread in rice-fields and other potential breeding sites such as irrigation channels or river margins, since the most important western Mediterranean malaria vector, *An. labranchiae* is considered to have disappeared. *Anopheles labranchiae* was found to be abundant in a restricted area of the contiguous Alicante and Murcia Provinces (south-eastern Spain) in 1946[2], but had disappeared by 1973[3] probably due to abandonment of rice cultivation in this area[4]. Recent surveys carried out in this area have revealed again the absence of *An. labranchiae* as well as high populations of the secondary vector *An. algeriensis* (Theobald 1903) also characterized by high domiciliation degrees[5].

The increase of imported malaria cases in last decades, together with the high prevalence of anophelines in many southern Europe regions has enabled the appearance of several autochthonous malaria cases, as recently has occurred in countries like Italy[6], Greece[7], France[8] and Spain[9]. According to the Spanish National Surveillance Network, an average of 400 imported malaria cases are yearly reported and *Plasmodium falciparum* (Welch 1897) and *P. vivax* (Grassi & Feletti 1890) are the more frequent species diagnosed. In October 2010, one case of autochthonous malaria due to *P. vivax* infection was reported in a small village of about 350 inhabitants called Cartuja de Monegros (N 41º 46′ 29.3″ /W 0º 16′ 49.8″) in the Province of Huesca (north-eastern Spain)[9]. The epidemiological investigation was conclusive, since the patient did not have any travel history to endemic or epidemic areas, never was an injecting drug user or had any treatments involving injections, and lived far from international airports. Consequently, transmission may have occurred through local *Anopheles* species after infection from people coming from endemic areas carrying gametocytes in their blood. This could be a proof that malaria is an infradiagnosed disease in Spain, since there have been no report of imported malaria cases from this area (Province of Huesca) in recent years, including 2010. Larval surveys over a period of three months (July to September 2011) were conducted in this area to define which *Anopheles* species could be implicated in disease transmission and also to identify main breeding sites in order to advise local authorities in mosquito control programs.

Larval samplings were done in all the identifiable aquatic habitats of Cartuja de Monegros and the neighboring town of San Juan de Flúmen (N 41º 45′ 35.3″/ W 0º 12′ 6.8″). Both the localities have large areas under rice cultivation. Immature stages were collected using the dipping method of Service[10] and each larval biotope was characterized quantitatively by means of a portable multiparameter for studying the chemical proprieties of water collections. The sampling effort was fixed at 10 min which included the active search for larvae in each biotope visited. Finally, mosquitoes were identified according to the taxonomic keys of Schaffner *et al*[11].

A total of 781 exemplars belonging to six species were identified (Table 1), namely *An. atroparvus*, *Culex modestus* (Ficalbi 1889), *Cx. pipiens* (Linnaeus 1758), *Culiseta annulata* (Schrank 1776), *Cs. longiareolata* (Macquart 1838) and *Ochlerotatus caspius* (Pallas 1771). The only potential malaria vector collected, *An. atroparvus,* was also most abundant species in our study, being detected in high densities during summer months in rice fields and surrounding irrigation channels. The tolerance of *An. atroparvus* to brackish environments[11] was also corroborated by our results, being the species collected up to 4.1% of salinity. Other anthropophilic spe-

Table 1. Abundance of species collected in different biotopes and chemical analyses of water collection

Species	Exemplars	Biotope	pH	Conductivity (mS)	TDS (g/l)	Salinity (%)
An. atroparvus	320	RF, IC	7.56–8.75 (8.1)	0.38–8.7 (3.31)	0.17–4.6 (1.81)	0.2–4.1 (1.7)
Cx. modestus	42	RF	7.8–8.75 (8.21)	0.97–8.7 (4.19)	0.48–4.6 (2.26)	0.5–4.1 (2.2)
Cx. pipiens	288	RF, IC, AC	6.92–8.44 (7.79)	0.22–8.7 (2.14)	0.1–4.6 (1.12)	0.1–4.1 (1)
Cs. annulata	12	IC	7.33–7.97 (7.65)	0.8–1.55 (1.17)	0.41–0.89 (0.65)	0.4–0.8 (0.6)
Cs. longiareolata	44	AC	6.92–7.5 (7.32)	0.22–0.8 (0.41)	0.1–0.49 (0.21)	0.1–0.4 (0.2)
Oc. caspius	75	RF	7.89–8.44 (8.1)	1.79–6.5 (5.2)	0.96–3.21 (2.62)	0.9–3.1 (2.5)

RF—Rice field (RF), IC—Irrigation channel; AC—Artificial container; TDS—Total dissolved solids; *Note:* Figures in parentheses indicate average values calculated for each chemical parameter.

cies such as *Cx. modestus* or *Oc. caspius* were also collected in rice fields, while in domestic and peridomestic containers only *Cx. pipiens* and *Cs. longiareolata* were found. *Ochlerotatus caspius* was only found abundantly in rice-fields that were freshly flooded. This is because the oviposition of *Oc. caspius* takes place in a dry environment (directly on soil) and once the eggs are rehydrated after flooding, if thermal conditions are appropriate, hatching occurs quickly and synchronously.

In conclusion, our data indicate that autochthonous malaria case must have been transmitted by *An. atroparvus*, since this was the only anopheline detected in the surrounding areas. However, the possible participation of other vectors with high receptivity to *P. vivax,* but not recorded in the present work such as *An. plumbeus* (Stephens 1828), cannot be excluded due to its difficult catch related with its strictly dendrolimnic behaviours[12]. It must be noted that *An. atroparvus* was also suspected of being the vector of an autochthonous case of *P. ovale* (Stephens 1922) which occurred in central Spain in 2001, although airport malaria cannot be ruled out in this case due to the proximity of the patient's residence to two international airports[13]. The high densities of *An. atroparvus* in large and abundant breeding sites like rice-fields, must lead to maintain certain epidemiological vigilance and to intensify the mosquito control campaigns in these areas. We can conclude that a combination of incidental human activities, active intervention by the National Epidemiological Surveillance Network and awareness of the human population all contribute in minimizing risks of a return of vector-borne disease[14]. Although malariogenic potential of Spain is very low[15] and the present situation can be described as what malariologists of the first half of the last century would have called "anophelism without malaria", it is important to note the presence, distribution, behaviour and abundance of *Anopheles* species and should be further investigated in order to deepen knowledge of the vector potential of Spanish anopheline fauna[16].

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