Original Article

Anophelism in a Former Malaria Area of Northeastern Spain

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Abstract

Background: A field study on diversity and distribution of anophelines currently present in a past endemic malaria area of Spain was carried out in order to identify possible risk areas of local disease transmission.

Methods: Multiple larval sites were sampled from June to October of 2011 in the Region of Somontano de Barbastro (Northeastern Spain). The sampling effort was fixed at 10 minutes which included the active search for larvae in each biotope visited.

Results: A total of 237 larval specimens belonging to four *Anopheles* species (*Anopheles atroparvus, An. claviger, An. maculipennis* and *An. petragnani*) were collected and identified.

Conclusions: Malaria receptivity in the study area is high, especially in the area of Cinca river valley, due to the abundance of breeding sites of *An. atroparvus* very close to human settlements. Although current socio-economic conditions in Spain reduce possibilities of re-emergence of malaria transmission, it is evident that certain entomological and epidemiological vigilance must be maintained and even increased in the context of current processes of climate change and globalization.

Keywords: Malaria, Mosquitoes, Entomological surveillance, Vector borne diseases, Spain

Introduction

Malaria was a widespread disease in Europe until the second half of 20th century. The parasitosis was particularly devastating between XVI and XIX centuries in Southern Europe due to the boom of irrigation techniques based on long flooding periods, mainly rice cultivation. Although the disease is currently considered eradicated from Europe, it must be noted that cycles of malaria transmission still are relatively common in countries like Georgia, Azerbaijan, Kyrgyzstan, Tajikistan, Uzbekistan and Turkey (WHO 2010). Moreover, the increasing of imported malaria cases in last decades together with the high presence of anophelines in many Southern Europe regions (Romi et al. 1997, Ponçon et al. 2007, Bueno Marí and Jiménez Peydró 2012) has enabled the appearance of few autochthonous or probable autochthonous malaria cases, as recently has occurred in countries like Italy (Baldari et al. 1998, Romi et al. 2012), Greece (Kampen et al. 2002), France (Doudier et al. 2007) or Spain (Santa-Olalla Peralta et al. 2010).

From the fifteen *Anopheles* species reported in Spain (Bueno Marí et al. 2012a), only two were considered primary malaria vectors: *An. atroparvus* Van Thiel, 1927 and *An. labranchiae* Falleroni, 1926. *An. labranchiae* was found to be abundant in a restricted area of the contiguous Alicante and Murcia Provinces (Southeastern Spain) in 1946 (Clavero and Romeo Viamonte 1948), but had disappeared by 1973 (Blázquez and de Zulueta 1980) probably due to abandonment of rice cultivation in this area (Eritja et al. 2000). On the other hand, *An. atroparvus* still is well distributed in different regions of Ibe-

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rian Peninsula (Encinas Grandes 1982, Bueno Marí 2011). *Anopheles labranchiae* and *An. atroparvus* are considered the most important eastern and western Mediterranean malaria vectors respectively.

The aim of this study was to analyze the diversity and distribution of anophelines currently present in a past endemic malaria area of Northeastern Spain in order to identify possible risk areas of local disease transmission.

Materials and Methods

Study area

The study area selected was the Somontano de Barbastro Region (N 42º02’47’’/ E 0º07’23’’), which is situated in Huesca Province (Northeastern Spain) (Fig. 1). This region is a transition zone between high mountain area (Pyrenees) and vast plain area (Monegros) that is characterized approximately by 15 ºC of average annual temperature and 400–700 mm of annual rainfall. In this region malaria was a common disease during XVIII century, even have been described several epidemic episodes as occurred between 1783 and 1785 with hundreds of deaths (Nieto Callén and Bosch Ferrer 1991). When malaria was present, two different areas could be distinguished in terms of disease morbidity and mortality: for one side lowland territories adjacent to Cinca River where the anthroponosis was very intense and on the other side the rest of the region where malaria incidence was very low. The area of Cinca river valley has been traditionally associated with various types of irrigated crops and the presence of *An. atroparvus* was supposed to be very frequent according to old registers of malaria incidence. The study area is also characterized by a mountainous region at north with several small rivers and streams, all of them tributaries of Cinca River, and a dry region dedicated to the development of rainfed crops at south.

Sampling methods and species identification

Multiple larval sites were sampled using the standard dipping method (Service 1993) from June to October of 2011. The sampling effort was fixed at 10 minutes which included the active search for larvae in each biotope visited (Bueno Marí 2010). For small larval habitats such as tree holes or small containers, the sampling was done by emptying or pipetting the contents for immature stages. Tree holes are particularly interesting since *An. plumbeus* Stephens, 1828 is a strictly dendrolimnic species frequent in the Palearctic Region and has been recognized as a minor vector for human malaria in Europe since the beginning of the 20th century (Shute 1954, Krüger et al. 2001, Bueno Marí and Jiménez Peydró 2011a, Schaffner et al. 2012).

Data were recorded from all identifiable aquatic environments across the three different regions (eastern region of Cinca river Valley, northern mountainous region and southern region of rainfed crops) of the study area. The sampling effort was fixed at 10 minutes, which included the active search for larvae in each biotope visited (Bueno Marí 2010). Larval exemplars collected were identified at specific level according to the taxonomic criteria of Schaffner et al. (2001) and Bueno Marí (2010).

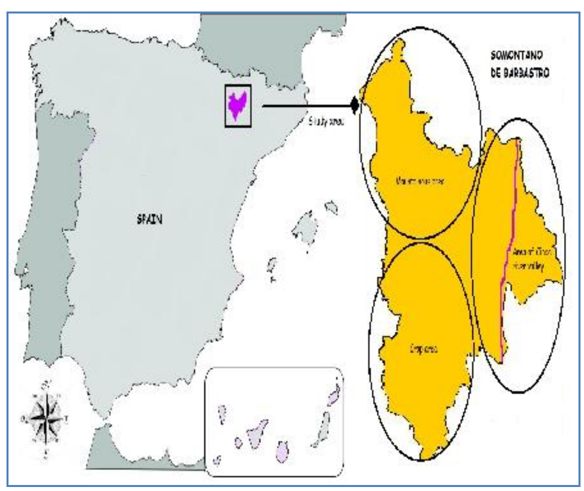

Fig. 1. Situation of study area

Results

A total of 237 *Anopheles* larval specimens were collected and identified (Table 1). Four anophelines were recorded: *An. atroparvus*, *An. claviger* (Meigen 1804), *An. maculipennis* Meigen, 1818 and *An. petragnani* Del Vecchio, 1939. Besides these anophelines collections, nine species of mosquitoes were also caught in the study area, namely *Culex hortensis* Ficalbi, 1889 (n= 23), *Cu.impudicus* Ficalbi, 1890 (n= 27), *Cu. modestus* Ficalbi, 1889 (n= 11), *Cu. mimeticus* Noè, 1899 (n= 67), *Cu. pipiens* Linnaeus, 1758 (n= 481), *Cu. territans* Walker, 1856 (n= 85), *Culiseta annulata* (Schrank 1776) (n= 5), *Cu. longiareolata* (Macquart 1838) (n= 133) and *Ochlerotatus caspius* (Pallas 1771) (n= 48). None mosquito exemplar was collected in tree holes.

Table 1. Information about anopheline records

Species	Coordinates N	Coordinates W/E	Altitude (m)	Biotope	Region	Nº exemplars
An. atroparvus	41°58' 12.6"	0°10' 15.3"E	273	River margin	Cinca valley	6
An. atroparvus	41°58' 11.1"	0°10' 12.4"E	266	River margin	Cinca valley	3
An. atroparvus	42°03' 54.3"	0°12' 58.5"E	323	River margin	Cinca valley	13
An. atroparvus	42°07' 09.5"	0°13' 47.1"E	355	River margin	Cinca valley	9
An. atroparvus	42°07' 10.8"	0°13' 49.5"E	372	River margin	Cinca valley	7
An. atroparvus	42°11' 28.9"	0°09' 14.6"E	568	River margin	Cinca valley	10
An. atroparvus	42°11' 11.4"	0°09' 15.1"E	557	River margin	Cinca valley	11
An. atroparvus	42°08' 23.3"	0°01' 31.3"E	447	River margin	Mountainous	5
An. atroparvus	42°08' 59.1"	0°01' 40.7"E	463	River margin	Mountainous	14
An. atroparvus	42°15' 01.2"	0°05' 13.2"W	637	River margin	Mountainous	8
An. atroparvus	42°10' 26.2"	0°04' 26.5"W	628	Container	Mountainous	2
An. atroparvus	41°56' 49.8"	0°02' 05.2"W	409	Container	Crops	5
An. atroparvus	41°55' 22.7"	0°05' 37.8"W	348	Irrigation channel	Crops	9
An. atroparvus	41°57' 42.2"	0°04' 03.6"E	315	Temporal puddle	Crops	7
An. atroparvus	41°56' 35.6"	0°03' 36.6"E	301	Rice field	Crops	45
An. atroparvus	41°56' 56.9"	0°03' 42.8"E	306	Temporal puddle	Crops	12
An. claviger	42°14' 09.9"	0°02' 37.5"O	672	Temporal puddle	Mountainous	2
An. maculipennis	42°03' 54.3"	0°12' 58.5"E	323	River margin	Cinca valley	4
An. maculipennis	42°07' 10.8"	0°13' 49.5"E	372	River margin	Cinca valley	2
An. maculipennis	42°04' 39.1"	0°02' 14.6"E	375	River margin	Crops	9
An. maculipennis	42°08' 23.3"	0°01' 31.3"E	447	River margin	Mountainous	6
An. maculipennis	42°08' 59.1"	0°01' 40.7"E	463	River margin	Mountainous	5
An. maculipennis	42°09' 54.4"	0°01' 36.3"E	479	River margin	Mountainous	7
An. maculipennis	42°15' 01.2"	0°05' 13.2"O	637	River margin	Mountainous	3
An. maculipennis	42°08' 47.9"	0°03' 34.2"O	565	River margin	Mountainous	6
An. petragnani	42°10' 28.1"	0°03' 25.1"E	574	Container	Mountainous	12
An. petragnani	42°09' 54.0"	0°1' 59.1"O	372	Container	Mountainous	6
An. petragnani	42°14' 09.9"	0°02' 37.5"O	672	Temporal puddle	Mountainous	9

Discussion

The presence of *An. atroparvus* in the Cinca river valley has been confirmed decades after. The occurrence of *An. atroparvus* is particularly interesting not only all along Cinca River, but also in irrigation channels and flooding areas such as small plots transformed in rice fields where high densities of *An. atroparvus* were found. It is important to note that in the neighboring Region of Monegros, where large territories are flooded artificially and used for rice cultivation, a probable autochthonous case of malaria was diagnosed in 2010 presumably transmitted by *An. atroparvus* (Bueno Mari et al. 2012b). This is probably the first indigenous case of the disease in Spain since 1961.

It is very difficult to compare the entomological data obtained in the study area with other neighboring regions, since current researchers about mosquito fauna in these regions are practically nonexistent. However, if we compare the epidemiological situation of the Region of Somontano de Barbastro with the available information of other bordering territories such as the Region of Monegros, we can conclude that *An. atroparvus* densities are clearly higher in the second one, mainly because rice fields are strongly colonized by the species. Despite both regions are characterized by high extensions of dry environments, the abundance of rice fields in Monegros Region makes this area more suitable the development of *An. atroparvus*.

At phenological level, it is important to note that larval exemplars of *An. atroparvus* were collected during all the months of the study (June-October) which means that the species has a multivoltine cycle and overlapping of generations is quite frequent as has been suggested by other authors for Northern Spain areas (Encinas Grandes 1982). Moreover, also following the criteria of Encinas Grandes (1982) it is likely than our last collections of October corresponds to future over wintering females, since the activity of the species has been traditionally circumscribed between April and September months for Northern Spain, although local climatology could obviously alter this situation.

Although it has been shown that adults of *An. atroparvus* can migrate distances close to 12 km in search of optimal hosts for feeding (Kaufmann and Briegel 2004), we must highlight that breeding sites of the species detected in our study have been mainly situated near from human settlements. From an epidemiological point of view, of course the proximity of vectors and humans is a very important factor in order to evaluate the possibilities of a disease outbreak. Infectivity tests carried out on European populations of *An. atroparvus* showed that this species can transmit Asian strains of *Plasmodium vivax* (Grassi and Feletti 1890), but is refractory to African strains of *Pl. falciparum* (Welch 1897) (Ramsdale and Coluzzi 1975). However, more recent studies have shown the ability of *An. atroparvus* to generate oocysts of *P. faliciparum* (Marchant et al. 1998), but not to complete the sporogony. Despite *An. atroparvus* was the main responsible in the maintenance of malaria endemicity in much of Europe during centuries, the vectorial role of the species was particularly interesting in northern countries due to its endophagic behavior and semiactive overwintering females (Huldén et al. 2005).

Endemic northern malaria reached to 68°N latitude in Europe during the 19th century, where the summer mean temperature only irregularly exceeded the lower limit of 16 °C needed for sporogony of *P. vivax* (Garnham 1988). This temperature conditions 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 *An. atroparvus* can take several blood meals (Encinas Grandes 1982). Therefore 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 females of *An. atroparvus* and also probably other species of maculipennis complex (Huldén et al. 2005, Bueno Marí and Jiménez Peydró 2011b).

With regards to the collections of *An. claviger* and *An. maculipennis,* their aquatic stages were mainly found in fresh or slightly brackish water in the mountainous area away from anthropised environments. Although these bioecological aspects, together with their zoophilic tendency, indicate a minor role in malaria transmission, it must be noted that both species have been related with several vivax malaria outbreaks in some Eastern Mediterranean countries (Gramiccia 1956, Coluzzi et al. 1964, Schaffner et al. 2001). Respect to *An. petragnani*, the species was only found in small biotopes distributed in the mountainous area. *An. petragnani* is a strictly zoophilic species very frequent in low anthropised environments of southern Europe (Bueno Marí and Jiménez Peydró 2011c). Consequently, *An. petragnani* is not considered as an important malaria vector. This information coincides with an absence of data about the vectorial status of the species in scientific literature.

Ultimately we can conclude that malaria receptivity is high, especially in the area of Cinca river valley, due to the abundance of breeding sites of *An. atroparvus* very close to human settlements. Although *An. atroparvus* is also present in the other areas, the shortage of suitable larval biotopes for the species in the dry area of crops and the low percentage of humans that lives and can serve consequently as a regular hosts for *An. atroparvus* in the mountainous area, provokes that the epidemiological interest of these areas would be less in comparison with the area of Cinca river Valley. All this information fit perfectly with old descriptions of malaria outbreaks in the Region (Nieto Callén and Bosch Ferrer 1991). In any case we must highlight that malariogenic potential of the study area is low due to the low vulnerabity of the country. Vulnerability is a key factor to assess about possible re-emergence of disease and can be defined by the number of gametocyte carriers (malaria patients) during the suitable period for malaria transmission. In Spain the imported malaria cases (mainly tourists and immigrants arrived form endemic regions) reported yearly by National Ministry of Health are around 400 cases. Moreover, malaria is a Notifiable Disease in Spain, so these patients are quickly identified and treated in order to minimize the possible dispersion of parasite by anophelines.

Despite current socio-economic conditions in Spain which reduce possibilities of re-emergence of malaria transmission (Bueno Marí and Jiménez Peydró 2008), it is evident that certain entomological and epidemiological vigilance must be maintained and even increased in the context of current processes of climate change and globalization.

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