Infection (2017) 45:111–114
DOI 10.1007/s15010-016-0941-8

CASE REPORT

Source identification of autochthonous-introduced *Plasmodium vivax* Malaria, Spain

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Received: 5 May 2016 / Accepted: 17 August 2016 / Published online: 26 August 2016

Abstract In 2014, an autochthonous case of introduced malaria caused by *Plasmodium vivax* was identified in Spain. The strain that infected this patient was identical to that of a prior imported case from Pakistan. This is the first case where the source of infection could be identified since elimination in Spain.

Keywords Autochthonous · Malaria · *Plasmodium vivax* · Spain

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Introduction

Spain was an endemic area for malaria up to the mid-twentieth century. It was one of the last countries of Western Europe to eliminate autochthonous malaria, and was declared free of malaria by the World Health Organization (WHO) in 1964. Since then, cases of autochthonous malaria have been occasionally identified [1, 2]. We describe the first case of introduced malaria (a case documented to be acquired by mosquito transmission from an imported case in an area where malaria does not normally occur) [3] caused by *Plasmodium vivax* in Spain, in which the possible source of infection was identified.

Case description

On August 27, 2014, a 62-year-old man was admitted to the emergency department at a local hospital in the region of Navarra with fever of 39.5 °C, headache, malaise, and shivering. He received cefuroxime and acetaminophen scheduled by his primary care physician 5 days before. The patient had no underlying diseases and was transferred to the intensive care unit with spiking fever and thrombocytopenia, which increased throughout his hospital stay. Laboratory analysis revealed altered liver enzymes. Blood and urine cultures were sterile. On the third day, parasitic forms of *P. vivax* were observed unexpectedly in a blood smear (parasitemia 2.3 %). An immunochromatography (Binax NOW Malaria, Alere) yielded a positive result for *Plasmodium* spp. (non-*falciparum*). Real-time PCR (multiplex PCR FTD Malaria differentiation, Fast Track Diagnostics) was positive for *P. vivax*. The National Center for Microbiology confirmed by immunochromatography (Immunoquick malaria +4, Byosinex), microscopy and multiplex

PCR the presence of *P. vivax* [4, 5]. The patient was treated with chloroquine phosphate 600 mg (at 0 h) plus 300 mg (at 6, 24 and 48 h) and primaquine 30 mg for 14 days [6, 7]. The clinical outcome was satisfactory.

An epidemiological survey was conducted. The patient had no history of surgeries, blood transfusions, tissue or organ transplantation, or intravenous drug use. There was no travel history except to Cuba in 2004. The patient resided in a small village in Navarra. During the year, the patient had only visited two neighboring regions: the Basque Country, where he stayed in Bilbao for a couple of nights, and La Rioja, which was visited several times in the months before symptom onset but he did not stay overnight. In La Rioja, he used to go for a walk with his grandson most afternoons or after sunset in summertime in a park near the River Ebro, a wetland area that could provide adequate breeding sites for the mosquitoes [8, 9]. An entomological search carried out in this area in September/October did not find presence of anophelines mosquitoes. However, prior entomological studies had documented the presence of *Anopheles atroparvus* [8, 9]. All visited places were more than 50 km away from harbors and from airports with direct flights from endemic countries.

Recent cases of *P. vivax* in Navarra and neighboring regions were looked through. In Navarra, 12 cases of imported malaria by *P. falciparum* (Equatorial Guinea, Cameroon, Mali, and Ghana) had been reported in 2014 but the last *P. vivax* malaria case was diagnosed in 2012. In neighboring regions, only two patients with imported malaria by *P. vivax* had been reported in 2014. Both cases had been diagnosed in July 2014. They affected related Pakistani women with residence in La Rioja close to the River Ebro, and had traveled to Pakistan a year earlier.

Blood samples and/or DNA elutes stored at −20/−80 °C were available from different patients with *P. vivax* infection in the area and in other Spanish regions, including the two Pakistani women from La Rioja. To characterize the strains, genotyping of the *P. vivax* samples was carried out at the National Center for Microbiology by amplification and sequencing of three hypervariable regions of the gene encoding the merozoite surface protein (msp-1) [10], the gene encoding the 18S SSU rRNA [11] and the full mitochondrial DNA [12]. The comparison of the *P. vivax* genotype of the Navarra patient with the two Pakistani women and other imported cases occurred in Spain in close dates showed that the *msp*-1 hypervariable regions, the ribosomal gene and the mitochondrial genome were identical only between the Navarra patient and one Pakistani woman, but different from all other cases. These results suggest with very high probability that one of the Pakistani women living in La Rioja was the source of infection. During the following 6 months, special attention was paid to all patients with febrile syndrome without known cause in the area for early detection of any *Plasmodium* infection.

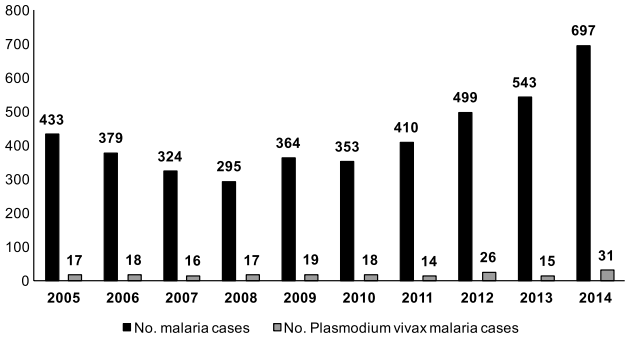

Fig. 1 Malaria cases between 2005 and 2014 in Spain. Source: Epidemiological Surveillance Network of Spain

Discussion

From 2005 through 2014, the number of imported malaria cases annually reported in Spain has ranged from 295 to 697, with an increasing trend since 2008. *P. vivax* was reported in 4 % (191/4297) of cases (Fig. 1).

This is the first case of autochthonous-introduced malaria in Spain since 1964 in which the possible source of infection was identified. In 2001, *Anopheles atroparvus* was suspected of being the vector of an autochthonous case of *P. ovale* malaria in the Madrid region in a person without travel history to endemic areas, although airport malaria cannot be ruled out due to the proximity of the residence to two international airports [1]. In 2010, one case of *P. vivax* malaria was described in an adult patient with no history of travel to endemic areas. The patient lived in an area where the vector *Anopheles atroparvus* has been identified and where entomological research found no presence of *Plasmodium* in mosquitoes [2].

In the European Union, where almost all malaria cases are imported, 5124 cases were notified in 2012 [13]. In recent years, autochthonous transmission has been reported in France, Germany, Greece, Italy and Spain [1, 2, 14–17]. Nevertheless, such cases never resulted in established local transmission, except in Greece where focal transmission has involved several tens of cases since 2009 [18, 19].

Two criteria must be met for malaria transmission: anopheline vectors capable of transmitting malaria and gametocytemic persons [20]. The transmission of *P. vivax* by indigenous vectors in Spain, a country with appropriate environment and climatic conditions, could potentially favor the re-emergence of malaria.

Anopheles atroparvus (Van Thiel 1927) is a potential vector of malaria in Spain, since it is widely distributed

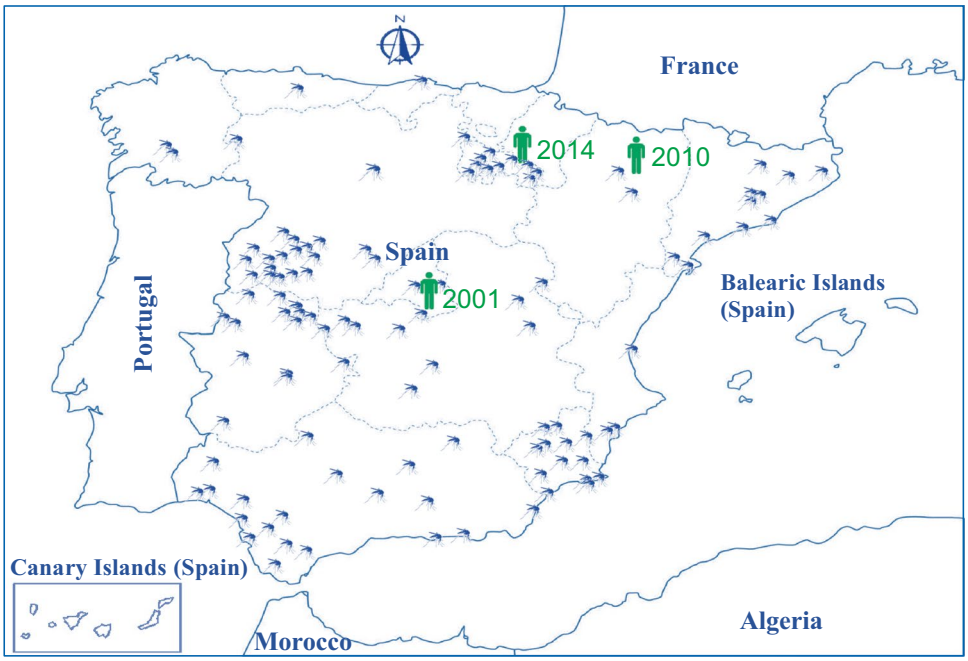

Fig. 2 Map of Spain where the geographical distribution of *Anopheles atroparvus* is represented by blue mosquitoes and three autochthonous malaria cases by *green person figures*. Source: References [2, 8, 9]

(Fig. 2) [2, 8, 9], and it is capable of transmitting Asian strains of *P. vivax*, but is refractory to African strains of *P. falciparum* [21]. Moreover, *Anopheles atroparvus* together with *Anopheles labranchiae* (Falleroni 1926) were supposed to be the major malaria vectors in Spain; however, this last important western Mediterranean malaria vector is considered to have disappeared [8, 9]. The number of imported cases by *P. vivax* is very small (15–31 per year) (Fig. 1) and, therefore, the likelihood of local transmission of the infection is in principle very low. However, *P. vivax* gametocytes develop within the first few days of infection and, therefore, a person may be infective early in the course of the illness. In addition, *P. vivax* may form dormant liver stages, which may become active and cause a relapse of the infection and gametocytemia months to years after a person has left a malaria endemic area [20].

Hypothesis to explain malaria infection acquired in areas without ongoing transmission includes importation of infective anophelines either on airplanes, ships or baggage, as well as transfusion-transmitted malaria. Rarely are cases classified as cryptic or introduced [3], but they should be suspected and a blood smear performed in patients with febrile syndrome without known cause and an abnormal white blood cells scattergram with thrombocytopenia and/or anemia [22, 23].

Conclusions

To our knowledge, this is the first case of autochthonous-introduced malaria in Spain, in which the source of infection was identified. The strain that infected our patient was identical to that of a prior imported Pakistani case, which was probably the index case. It was likely transmitted by local vectors fitting the criteria for an introduced case.

Genetic techniques are important complementary components in the epidemiological investigation of introduced malaria cases. Environmental and entomological studies should be improved to assess the risk of possible malaria transmission in vulnerable areas of non-endemic countries.

Acknowledgments The authors thank Elena Rodríguez Valín and Oliva Díaz García of the National Center for Epidemiology for supplying the annual number of cases of malaria reported in Spain.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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