

*Anopheles labranchiae*, an important malaria vector in Italy, and other potential malaria vectors in Southern Europe

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In August 1997, an autochthonous case of *Plasmodium vivax* malaria occurred in a rural area in the province of Grosseto (Tuscany): the results of an epidemiological inquiry confirmed it as the first case of introduced malaria in Italy following eradication (Baldari *et al.*, 1998). The area belongs to the so called "Maremma", a large coastal plain located in central Italy, where malaria was endemic up to 50 years ago. The vector species in the area was *Anopheles labranchiae* Falleroni, the most important member of the Maculipennis Complex in south-western Europe (Hackett & Missiroli, 1935). In Maremma, as a result of the eradication campaign, the species disappeared or was greatly reduced in density, but over the past few decades it has recolonised the area. Today, *An. labranchiae* is back to substantial densities and represents the only species of the Maculipennis Complex found in the province of Grosseto (Romi *et al.*, 1997).

As a part of the epidemiological inquiry in 1997, an entomological survey was carried out in the area soon after the malaria case was reported. The investigation covered a 3-km radius around the house where the malaria case occurred. Hand collections of indoor resting adult mosquitoes as well as night mosquito catches on human bait were performed. A search for potential anopheline breeding places, with larval collections, was also carried out. A follow up survey was made in 1998.

*Anopheles* mosquitoes were found in about 80% of the animal shelters examined, with a mean density of 2.25 females/shelter (range 0-10). The human biting rate was 4.5 *Anopheles*/person/night. *Anopheles* were found breeding in isolated pools in a few partially dried irrigation canals (0.5-1 larvae/dip) and in large wells used as water supplies for agricultural purposes (0.1-0.3 larvae/dip). All collected anophelines, adults and larvae, were identified by morphological characters as *An. maculipennis* s.l. About 1/3 of the adult females (n=21/72) laid eggs and were identified as *An. labranchiae* which is the only species of the Maculipennis Complex reported from this area since 1993 (Romi *et al.*, 1997).

Adult females (n=72) were dissected and their salivary glands and midgut examined for the presence of plasmodia. In addition, a molecular study of purified DNA was performed, extracted from the same specimens by PCR technique. In particular two sets of primers specific for 18S rDNA of the genus *Plasmodium* and for the circumsporozoite protein of *P. vivax* were employed in order to detect any *Plasmodium* genome. None of the collected specimens of *An. labranchiae* was found positive for *Plasmodium* species.

In 1998, the vectorial capacity (VC) of *An. labranchiae* for *P. vivax* and *P. falciparum* was estimated in the same area of the Grosseto province. The biting rate on humans (h) was calculated as the mean of indoor plus outdoor landings per human per night. The mean number of bites per mosquito per night (a) was calculated considering a gonotrophic cycle of 3 days and a human blood index (HBI) of 0.5. This index was assessed by analysis of daytime resting blood-fed females collected in the premises near rural houses. The anopheline mosquitoes collected on humans were dissected after each night catch to calculate the parity rate (P). The expectation of infective life of the vector was calculated considering a sporogonic cycle duration of 11 days for *P. falciparum* and 10 days for *P. vivax* at 25°C mean daily temperature.

The values of VC increased during the summer: at the beginning of July the value of VC was low and such as not to constitute a real risk for the transmission of malaria (<0.01 for *P. falciparum* as well as for *P. vivax*); at the beginning and especially at the end of August the values of VC were high especially with respect to *P. vivax* (VC= 0.96-3.3) which has a shorter sporogonic cycle than *P. falciparum* (VC= 0.8-2.9).

These values of vectorial capacity are purely theoretical because of the low vulnerability of the Italian territory (low number of circulating gametocyte carriers). Recently we calculated that the malariogenic potential of the Italian territory is very low and that a recurrence of malaria transmission appears to be unlikely in most of the country (Sabatinelli *et al.*, 1998). However the possibility that sporadic cases of autochthonous malaria may occur in rural areas of central and southern Italy is a real risk. Areas with densities of *An. labranchiae* very much higher than those described above have been reported in southern Italy, Sicily and Sardinia (Romi *et al.*,

Figure 1. Vectorial capacity of *Anopheles labranchiae* in province of Grosseto, Italy, 1998.

| Date                             | h*<br>(human-biting rate) | a**<br>(bites/mosquito/night) | P<br>(parous females/total dissected) | p<br>(vector survival probability/day) | p <sup>n</sup> /-log <sub>e</sub> p <sup>***</sup> |     | VC <sup>Δ</sup> |      |
|----------------------------------|---------------------------|-------------------------------|---------------------------------------|--|--|-----|-----------------|------|
|                                  |                           |                               |                                       |  | Pf   | Pv  | Pf              | Pv   |
| July<br>(2 <sup>nd</sup> week)   | 2                         | 0.16                          | 3/24                                  | 0.49                                   | < 0.01   |     | ---             | ---  |
| August<br>(1 <sup>st</sup> week) | 5                         | 0.16                          | 20/32                                 | 0.85                                   | 1  | 1.2 | 0.8             | 0.96 |
| August<br>(last week)            | 10                        | 0.16                          | 40/59                                 | 0.88                                   | 1.8  | 2.1 | 2.9             | 3.3  |

\* Mean of indoor + outdoor bites per human per night.

\*\* Human blood index, 0.5; gonotrophic cycle duration, 3 days.

\*\*\* n (sporogonic cycle duration at mean daily temperature of 25°C), 11 days for *P. falciparum* (Pf) and 10 days for *P. vivax* (Pv).

ΔVC was calculated as follows:

- h (biting rate on humans) was calculated through human bait night catches
- a (bites/mosquito/night) was calculated multiplying HBI (=50%; from the bloodmeal analysis of daytime resting samples) and length of the gonotrophic cycle
- P (parous females/total dissected) was calculated by dissection of the females collected on human baits

1997). From what has been said above, it is evident that in theory the vectorial capacity in some areas of Italy is epidemiologically relevant and these areas could be receptive to a resumption of malaria transmission.

It has been demonstrated that Mediterranean populations of *An. labranchiae* can transmit *P. vivax*, as shown by the epidemic which affected Corsica in 1971 (Sautet & Quilici, 1971) and the last event in Italy. As far as *P. falciparum* is concerned, in the 1970s, some samples of *An. labranchiae* collected in Italy were taken on various occasions to Kenya where they were fed on *P. falciparum* carriers (Zulueta *et al.*, 1975; Ramsdale & Coluzzi, 1975). In none of the mosquitoes did plasmodia manage to complete the entire cycle and reach the salivary glands. However these susceptibility tests were carried out with an extremely small number of samples and not sufficient to conclude with certainty that all Italian *An. labranchiae* were refractory to infection with Afrotropical strains of *P. falciparum*.

The increasing presence in rural areas of central and southern Italy of carriers of *Plasmodium* spp., such as people arriving from Africa and Asia, hired as seasonal workers, is particularly worrying. The continuous contact of strains of exotic plasmodia with potential mosquito vectors could lead in the long run to selection and/or adaptation of *P. falciparum* strains capable of developing in Mediterranean populations of *An. labranchiae*.

As far as other European countries are concerned, *An. labranchiae* is still present in Corsica (Schaffner, 1998) and perhaps in Dalmatia (Adamovic, 1985), having disappeared from Spain (Eritja *et al.*, 1998). Other potential vectors belonging to the Maculipennis Complex are present in southern Europe: *Anopheles atroparvus* Van Thiel and *An. sacharovi* Favre. *An. atroparvus* is widely distributed in the central-western countries of the Mediterranean basin (Dahl & White 1978; Eritja *et al.*, 1998; Ribeiro *et al.*, 1988; Schaffner, 1998; Snow & Ramsdale, 1999), but the marked zoophily of the species suggests that it could not represent an important malaria vector, unless under conditions of high density coupled with low standards of living (Zahar, 1990). *An. sacharovi*, the main former vector in the Balkans, disappeared from Romania and Cyprus during

the malaria eradication period (Zahar, 1990); it also disappeared or became very rare in Italy (perhaps present in limited areas at low densities) because of strong environmental changes and modified agricultural practices (Romi *et al.*, 1997). The species is still abundant in many areas of Turkey, the Lamia Plain and northeast Greece (Hadjinicolaou & Betzios, 1973; Ouzounis & Samanidou-Voyadjogou, 1993). The other potential malaria vector in southern Europe, *Anopheles superpictus* Grassi has also been affected by environmental changes which have occurred during the last decades. Scattered foci of this species have been recently reported in Italy (Romi *et al.*, 1997) but it also exists in the Balkan peninsula, in the island of Cyprus and in Turkey (Zahar, 1990). It is worthwhile mentioning that this mosquito could be more susceptible to the infections with Afrotropical *P. falciparum* strains than the species of the Maculipennis Complex, since it is a member of the subgenus *Cellia*, the one to which the principal African malaria vectors belong.

In conclusion, although these results are not particularly worrying, they must urge us to introduce increased epidemiological surveillance, especially considering the fact that the present situation in many countries of southern Europe is extremely dynamic and subject to changes due to socio-political factors. This could lead to substantial changes in the flow of immigrants from endemic malaria areas which, together with changes in environmental factors, could lead to increases in the density and distribution of anopheline populations.

In order to prevent and be able to handle indigenous malaria cases in areas where the density of the vector is high, it is advisable to establish national and regional centres with experts who are competent in epidemiological surveillance and vector control.

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