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ASSESSMENT OF MALARIA TRANSMISSION IN AN AREA WITH VERY LOW MOSQUITO DENSITY

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ABSTRACT: Tests for antibodies against CSP-protein by ELISA were used to assess mosquito mediated malaria transmission in the village of Achada Leite on the archipelago of Cabo Verde, an area with very low mosquito densities where a clonal *Plasmodium falciparum* outbreak occurred in October 1995. Anti-CSP antibodies were found among individuals who had previously been malaria patients, none of whom had travelled out of the outbreak area. The malaria vector *Anopheles arabiensis* was collected in both wet and dry seasons.

KEY WORDS: *Plasmodium falciparum*, *Anopheles arabiensis*, malaria transmission, Cabo Verde, anti-CSP ELISA.

INTRODUCTION

The increase in world travel in recent years, especially to and from areas where vector-borne diseases are endemic, has resulted in a substantial rise in imported cases of those diseases. In particular, malaria is a cause of concern. In those countries at the edge of its distribution, it can be difficult to distinguish between autochthonous and imported cases. However, distinguishing between the two is important because of the different allocation of resources to combat the disease that each requires.

In general, observation of the various stages of parasite development in wild-caught female mosquitoes is considered evidence of autochthonous transmission. Observation of oocysts in the mosquito mid-gut testifies that mosquitoes are susceptible to infection but conclusions cannot be reached about their ability to complete the transmission cycle. Perhaps the best indication of autochthonous transmission is microscopic observation of sporozoites in mosquito salivary glands, since this detects parasites ready to be inoculated (BEIER *et al.*, 1990). Detection of circumsporozoite protein (CSP) (BURKOT, WILLIAMS & SCHNEIDER, 1984) in dry mosquito thoraxes, by Enzyme Linked Immunosorbent Assay (ELISA) is also widely used to determine transmission, especially when large numbers of mosquitoes need to be processed. Such assays provide information about the parasite species infecting the mosquito (BURKOT & WIRTZ, 1986; WIRTZ *et al.*, 1987; BEIER *et al.*, 1990). They are, however, considered less accurate than dissection since sporozoites in salivary glands cannot be distinguished from those in the haemocel (BURKOT & WIRTZ, 1986; BEIER *et al.*, 1990).

In many marginal zones of transmission, the collection of infected mosquitoes is, however, extremely difficult. Under such circumstances alternative measures are required to establish the presence of local transmission. WIJESUNDERA *et al.* (1990), in a study of a malarial outbreak in Sri Lanka, reported that human anti-sporozoite antibodies are of short duration. They can therefore provide an indication of recent exposure, even in the absence of infected mosquitoes.

The archipelago of Cabo Verde, 500 km off the Senegalese coast, has occasional outbreaks of malaria. To date, these have only been reported from Santiago, the main island of the group. In October 1995, a clonal outbreak of *Plasmodium falciparum* Welch, 1897, malaria was recorded in the small village of Achada Leite (AREZ *et al.*, 1997) but entomological studies were unable to fully demonstrate autochthonous malaria transmission. Collections of potential vectors and assessment of anti-sporozoite antibodies in residents was therefore undertaken to confirm that transmission was taking place in the village.

MATERIAL AND METHODS

Achada Leite, 40 kilometers Northwest from the capital, Praia, on Santiago island, has approximately 200 inhabitants living in 35 brick-walled, zinc-roofed houses. These are distributed along the high edges of an isolated deep green valley. A deserted plateau surrounds the valley on three sides with the seashore on the fourth. A freshwater stream flows in the middle of this valley during the rainy season. The climate is characterised by a dry (December to June) and a wet season (July to November) with very low rainfall. Strong winds are common. Two anopheline species have been recorded for the archipelago, *Anopheles pretoriensis* Theobald, 1903

(MEIRA, NOGUEIRA & SIMOES, 1947) and the well-known malaria vector *Anopheles arabiensis* Patton, 1905 (CAMBOURNAC, PETRARCA & COLUZZI, 1982).

Mosquito collections took place in two surveys, one in the dry season (3-18 February 1996) and the other in the wet season (22-31 October 1996). In the dry season, six CDC light traps were run from 19,00-07,00 hr indoors and outdoors for six nights. When used indoors, the traps were hung close to an unprotected sleeper. The outdoor traps were hung close to windows or by animal shelters. Indoor resting captures with mechanical aspirators (09,00-10,00 hr, 5 collectors) were also used to sample from 26 of the 35 houses of the village. In the wet season, in addition to the above methods, pyrethroid indoor spraying in 7 houses, four all-night (19,00-07,00 hr) indoor and outdoor landing captures on sentinel individuals (4 collectors per night) and one landing capture close to breeding sites (4 collectors, 17,00-19,00 hr), were also used. In both surveys, immature forms were captured by the standard methods and reared in the local laboratory. All identified *Anopheles gambiae s.l.* Giles, 1902, mosquitoes were kept dried at room temperature in individual silica gel-filled tubes for Polymerase Chain Reaction (PCR) species identification. In the second collection, a sub-set of the lab-reared *An. gambiae s.l.* were blood-fed on a human arm twice, in order to obtain half-gravid females for cytogenetical analysis.

PCR identification of members of the *An. gambiae* complex members was performed according to SCOTT, BROGDON & COLLINS (1993) and as described in PINTO *et al.* (1997). Primers used were 5 ng of UN (universal), 2,5 ng of GA (*An. gambiae s.s.*) and 7,5 ng of AR (*An. arabiensis*), in 9 µl of PCR mix. DNA from individual mosquito abdomens was extracted according to COLLINS *et al.* (1988) and eluted in 50 µl of sterile dH_2O. Each PCR sample was loaded with 2 µl of DNA template prepared by a 1:50 dilution in sterile dH_2O, from original DNA extraction tubes. Positive controls for *An. gambiae s.s.* and *An. arabiensis*, and one negative control (no DNA template) were used in each PCR reaction.

Polytene chromosomes from alcohol-acetic Carnoy's preserved ovaries were prepared following the method by COLUZZI (1968) and HUNT (1973). Paracentric inversion karyotypes were scored following the nomenclature by COLUZZI *et al.* (1979).

ELISA for detection of antibodies to *P. falciparum* sporozoites was performed in 14 serum samples from individuals who had been positive for malaria infection, either by PCR or by optical microscopy (OM), at least once in earlier blood collections. The humoral response against the CSP antigen of *P. falciparum* was evaluated with a Sclavo (Siena, Italy) ELISA kit based on (NANP)40 antigen (HABLUETZEL, ESPOSITO & LOMBARDI, 1989). Each sample was tested twice. The cut-off value for seropositivity was the mean plus three standard deviations of the values obtained on eight negative Italian sera. Four positive controls from high *P. falciparum* positive Burkina Faso patients were added as reference.

RESULTS

Only 1 adult *An. gambiae s.l.* was captured, resting indoors, in the dry season survey and 3 (from indoor CDC and outdoor human-bait collection) in the wet season survey. Four adult *An. pretoriensis* were obtained in both surveys, all being caught by outdoor CDC light traps, close to a donkey shed. None of the adult specimens collected were infected with *Plasmodium*.

Despite the dearth of adult mosquitoes, larvae were relatively common. Six positive habitats were identified in the dry season and three in the wet season. Altogether, 674 *An. pretoriensis* and 211 *An. gambiae s.l.* were obtained from larval collections. *Culex ethiopicus* Edwards, 1912, *Culex pipiens s.l.* Linnaeus, 1758, *Culex tigripes* De Grandpre et De Charmoy, 1900, *Aedes aegypti* Linnaeus, 1762, and *Culiseta longiareolata* Macquart, 1838, were also collected from these larval sites. In both seasons, *An. gambiae s.l.* eggs, all larval stages and pupae, were found together in the same breeding sites.

Sample No.	Age	Month of first parasite detection (OM/PCR)	Mean absorbance (SD)
1	2	November 1995 (OM/PCR)	**0,526 (+/–0,012)**
2	25	November 1995 (OM)	0,021 (+/–0,003)
3	3	November 1995 (OM/PCR)	0,009 (+/–0,0005)
4	5	November 1995 (OM/PCR)	0,011 (+/–0,001)
5	58	February 1996 (PCR)	**0,650 (+/–0,003)**
6	61	February 1996 (PCR)	0,044 (+/–0,002)
7	50	February (1996) (PCR)	**0,562 (+/–0,006)**
8	61	November 1995 (PCR)	**0,368 (+/–0,039)**
9	7	February 1996 (OM/PCR)	0,015 (+/–0,002)
10	23	February 1996 (OM/PCR)	0,025 (+/–0,002)
11	23	November 1995 (PCR)	**0,224 (+/–0,0005)**
12	4	November 1995 (OM/PCR)	**1,341 (+/–0,119)**
13	37	November 1995 (PCR)	0,024 (+/–0,0005)
14	7	May 1996 (PCR)	0,059 (+/–0,000)

Table 1.– Anti-Pf. CSP antibodies detection by ELISA. Cut-off value of 0,09, positives in bold. Age and month in which malaria infection was first detected, either by PCR or Optical Microscopy (OM), are shown.

All of the *An. gambiae s.l.* identified by either PCR or cytogenetical means were *An. arabiensis*. Three mosquitoes were negative by PCR. These were further analysed with a primer specific for *Anopheles melas* Theobald, 1903, but remained negative. The chromosomal inversions recorded in 6 readable specimens of *An. arabiensis* were 2Ra and 2Rb, both found as inverted homozygotes, and 3Ra, which was polymorphic.

Six (43%) of the 14 former malaria patients tested for anti-CSP Ab were positive. The cut-off value of the ELISA assay was 0,09 and the absorbances of the positive controls ranged from 0,556 to 0,897 (Table 1). Both children and adults were positive (< 5 years, n = 2; 15-49 years, n = 1; and 50 years, n = 3). Three of the positive cases belonged to the same family (subjects 1, 5 and 12) but there was no evidence of family ties amongst the other positive cases and there was no history of any of these subjects travelling out of the area.

DISCUSSION

Malaria transmission in the area was difficult to assess, since even though a variety of collection methods were employed, only a small sample of adult mosquitoes was obtained.

According to GILLIES & DE MEILLON (1968), very few *An. pretoriensis* have been found infected with malaria parasites and, considering the zoophilic habits of this species, those were more likely to be of non-human origin. Although unlikely, available data are not enough to completely exclude *An. pretoriensis* from playing a role in transmitting malaria in the study area.

Nevertheless, entomological data, although scarce, support the idea that *An. arabiensis* should be the only potential vector of malaria in the area. The fact that larvae were collected in both wet and dry seasons also indicates that low-level malaria transmission is possible throughout the year.

Previously, RIBEIRO *et al.* (1980) concluded that the characteristic unstable malaria of the archipelago of Cabo Verde was due to a low vectorial capacity of the local *An. arabiensis* populations, rather than to the presence of a lesser effective malaria vector, *An. pretoriensis*.

Adult mosquito densities are likely to remain low throughout the year and human blood meal sources may be difficult to obtain, due to inclement weather conditions (strong winds are common), altitude and well-built houses.

An. arabiensis, together with *An. gambiae s.s.*, are the most anthropophilic and widespread members of the *An. gambiae* complex and constitute with *Anopheles funestus* Giles, 1900, the most efficient vectorial system available in the world for *P. falciparum* (COLUZZI, 1994). *An. arabiensis* is known to successfully exploit dry habitats, such as arid savannahs and steppes (COLUZZI *et al.*, 1979). Indeed, the capacity of *An. arabiensis* to persist and maintain malaria transmission during the dry season in hot-dry savannah regions and even to maintain a certain level of malaria transmission has been stressed by several authors (OMER & CLOUDSLEY-THOMPSON, 1970; TAYLOR *et al.*, 1993; TOURE *et al.*, 1996).

Although the small number of chromosomally processed specimens does not allow a thorough analysis, the *An. arabiensis* karyotypes recorded in this study were similar to those described by CAMBOURNAC, PETRARCA & COLUZZI (1982) in various islands of the archipelago during July-October 1981. Thus, the *An. arabiensis* population of these islands is apparently very stable. It has been proposed that *An. arabiensis* was introduced to the islands from the nearest continental area, Dakar-Senegal (CAMBOURNAC, PETRARCA & COLUZZI, 1982); and samples from the area of St. Louis, Senegal had a karyotype composition which is very similar to those of Cabo Verde, the main difference being the higher degree of polymorphism of the continental *An. arabiensis* (PETRARCA, VERCRUYSE & COLUZZI, 1987). The lower degree of polymorphism in Cabo Verde is presumably the result of the founder principle and/or canalising selection (MAYR, 1970). However, the high frequency of the inverted arrangement 2Ra in Cabo Verde suggests that selection and adaptation are involved, since it has been shown that the frequency of the carriers of this arrangement increases in more arid sampling sites (COLUZZI *et al.*, 1979; PETRARCA, VERCRUYSE & COLUZZI, 1987).

In conclusion, this study suffered from a very small entomological sample, which did not allow determination of the intensity of malaria transmission based on standard measurements in the local mosquito population. Therefore, entomological inoculation rates or the vectorial capacity could not be estimated. However, the presence of *An. arabiensis*, one of the most powerful African malaria vectors, with apparently perennial breeding, together with the finding of antibodies to circumsporozoite protein among the inhabitants, are indicators of malaria transmission in a non-endemic area where a clonal *P. falciparum* outbreak occurred and persisted for a year.

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