Use of geoprocessing to define malaria risk areas and evaluation of the vectorial importance of anopheline mosquitoes (Diptera: Culicidae) in Espírito Santo, Brazil

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In Brazil, introduced malaria occurs from the flat to the sloping hot areas, predominantly outside the Amazon Region, where endemic malaria has occurred in the past. This is a consequence of human migrations to other Brazilian states, including the state of Espírito Santo (ES). The objective of this study was to use geoprocessing to define the areas at risk of introduced malaria transmission and evaluate the vectorial importance of species of anophelines in ES. Anophelines were sampled from 1997-2005 in 297 rural localities identified or not identified as foci of malaria during the last 20 years. The geoclimatic variables temperature, relief and marine influence were obtained from a database of the ES Natural Units. The 14,663 anophelines captured belonged to 22 species. A significant association was found between the occurrence of malaria foci and the presence of hot, low-lying areas or gently undulating to undulating relief. The occurrence of the disease was associated with the presence of Anopheles darlingi *and* Anopheles aquasalis. *Geoprocessing was determined to be a useful tool for defining areas at risk for malaria and vectors in ES.*

Key words: malaria - *Anopheles* - geoclimatic variables - Espírito Santo - Brazil

Malaria is recognized globally as a grave public health problem. In 2006, there were an estimated 247 million malaria cases worldwide of which 86% occurred in the African region, 9% in South-East Asia, 3% in Eastern Mediterranean Regions and 1% in the Americas (WHO 2008).

According to WHO (2008), an estimated 50% of the human population is exposed to the risk of contracting the disease in more than 100 countries, particularly in tropical and subtropical regions. There were an estimated 881,000 deaths worldwide in 2006, of which 90% were in the African region and 85% in children under five years.

In Brazil, the number of cases reported in 2006 was estimated at 1.4 million, over half of the total for the Americas (WHO 2008). According to the Brazilian Ministry of Health (MS 2007), 99.5% of the notified cases of malaria in Brazil occurred in the endemic region known as the Legal Amazon region and most of the remaining 0.5% represent exports from this region to the rest of the country. Outbreaks of malaria in the extra-Amazonian regions of Brazil frequently occur in low-lying areas throughout the country, associated with the breeding areas of *Anopheles* spp. In recent years, large human migrations from the Amazonian Region to other Brazilian states have produced outbreaks of secondary cases derived from imported cases (introduced malaria),

such as those recorded recently in Ceará during 2002 and Piauí and Espírito Santo (ES) in 2004, 2005 and 2006 (Rey 2001, Forattini 2002, MS 2007).

Within the Southeastern Region of Brazil, ES is noteworthy as the state with the highest malaria potential due to its topographical and climatic characteristics, which favour breeding of the vectors (MS 2003, 2007). Timber extraction was an important economic activity in ES until the 1970s, when deforestation led to the migration of large numbers of families to the Amazon, particularly the states of Pará (PA) and Rondônia (RO). This led to the movement of people between malaria-endemic regions and ES, where the descendants of the migrants still live (Marques 1986, IPEMA 2004).

The first studies on the anopheline fauna of ES were performed by Coutinho (1947), Andrade and Brandão (1957) and Ferreira (1964). These authors identified 21, 22 and 24 species of anophelines respectively, with *Anopheles darlingi* being recorded in all the surveys. These species represent almost half the anopheline fauna of Brazil, which includes 54 species (Deane 1986, Consoli & Lourenço-de-Oliveira 1994, Neves et al. 2000, Neves 2003).

The distribution of insect vectors shows a close relationship with the geographical and climatic characteristics of each region (Forattini 2004). The geographical information system (GIS) is the geoprocessing technique most commonly used to describe events observed in a particular geographical space, such as the presence of vector species and environmental factors that influence their distribution (Margonari et al. 2006).

In Brazil, the use of geoprocessing to identify natural communities that tend to concentrate in ecosystems where the ecology is simultaneously favourable to vec-

tors, hosts and micro-organisms has been used by various authors in studies of malaria in the Brazilian states of Roraima, RO, PA and Mato Grosso (Vasconcelos et al. 2006, Atanaka-Santos et al. 2007, Rosa-Freitas et al. 2007, Zeilhofer et al. 2007, Rodrigues et al. 2008). In ES, studies on the spatial distribution and geographic characteristics of diseases have been performed for visceral and tegumentary leishmaniases (Falqueto et al. 2001).

Two epidemiological patterns of the disease are recognized in this state: autochthonous malaria (malaria contracted locally) in the mountains, which is restricted to forested areas at elevations of approximately 800 m with a mean annual temperature of 17ºC and lowland malaria in areas below 300 m with a mean annual temperature varying from 21-23ºC, which has been introduced by migrants from endemic areas, principally Amazonia (Feitoza et al. 2001, FUNASA 2004). Studies by Rezende et al. (2005) and Rodrigues (2005) suggest that *Anopheles cruzii* is the vector of the former and *An. darlingi* of the latter. However, little is known about the participation of other anopheline species in the transmission of the disease, not only in ES, but also in other regions of the country. In the present study, geoprocessing was used to define the areas at risk of malaria transmission and to evaluate the vectorial importance of anophelines in ES.

MATERIALS AND METHODS

Study area - ES is located in the Southeastern Region of Brazil between longitudes 17º53'29''S 21º18'03''S and latitudes 39º41'18''W 41º52'45''W. Its geographical area of 46,184 km² represents only 0.58% of the national territory. It is divided into four mesoregions and 13 microregions, administered by 78 municipalities (Fig. 1).

Sampling - The present study was based on secondary data obtained from 297 rural localities, from all 78 municipalities of ES. Malaria foci were characterized from the records of autochthonous cases for the different localities included in the study, according to reports sent to the Brazilian National Health Service (FUNASA) and Secretaryship of Health of ES (SESA) from 1985-2005 (FUNASA 2004).

The malaria foci were established when the presence of the first autochthonous case of the disease was registered in the municipality. Thus, the imported case from an endemic region can transmit malaria to an introduced case and the latter can transmit to an autochthonous case, if the region was vulnerable to this broadcast, because of the presence of biogeographical conditions for the development of vector species of epidemiological importance (FUNASA 2001).

Characterization of the anopheline fauna was based on collections assembled by technicians of the Entomology's Nucleus of SESA/Federal University of Espírito Santo from 1997-2005, in all the municipalities of ES, whether they were malaria foci or not.

The disparity between the historical background of the collection of anopheline mosquitoes and malaria foci does not seem to influence the results presented because there were no significant changes in the environmental characteristics of ES in the last 20 years (IPEMA 2004).

Anophelines were collected by active searches in the peridomicile during the first four hours after sunset, using a Castro aspirator. Insects were captured in the surrounding dwellings, resting on external house walls and domestic animal shelters.

They were identified using a key for adults of *Anopheles* species of Brazil (Consoli & Lourenço-de-Oliveira 1994).
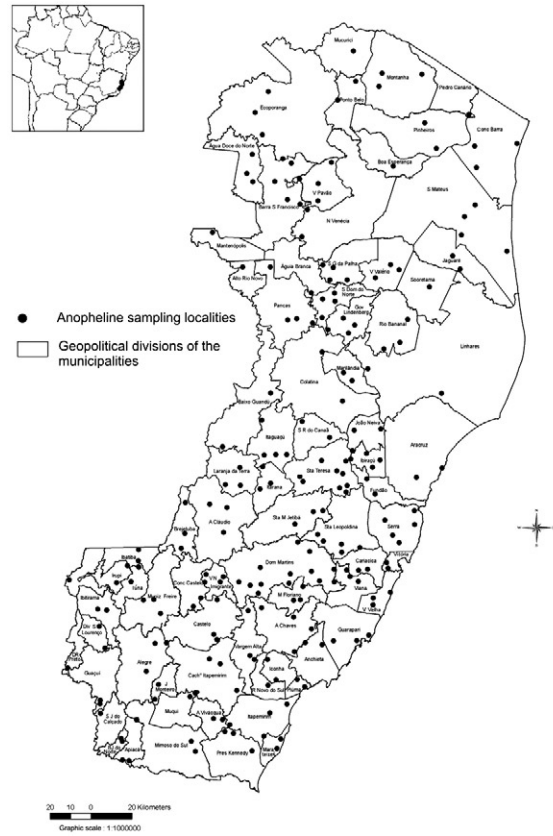

Fig. 1: geographical distribution of anopheline sampling localities in the state of Espírito Santo, Brazil.

TABLE I

State of Espírito Santo, Brazil, natural zones and area of coverage

Natural zones[a]		
Denomination	Acronym	Area (%)
Cold, sloping and rainy lands	FAC	8,70
Mild, sloping and rainy lands	AAC	11,80
Mild, sloping and rainy/dry lands	AACS	6,90
Hot, sloping and rainy lands	QAC	4,10
Hot, sloping and rainy/dry lands	QACS	6,70
Hot, sloping and and dry lands	QAS	31,20
Hot, low relief and rainy lands	QPC	3,20
Hot, low relief and rainy/dry lands	APCS	11,20
Hot, low relief and dry lands	QPS	16,20

a: data from SEAMA (2008).

Ecological variables - Based on the Espirito Santo Ecosystems Atlas, nine natural zones occur in ES (Table I) (SEAMA 2008). Since water parameters were excluded from this study, seven zones were redefined. This redefinition was based on the association between the variables of temperature, relief and marine influence (Table IIA) and obtained from the Natural Units database at the zone and subzone level. These were processed using GISUNES, a system that catalogues information for the ecological characterization of regions of the state (Feitoza et al. 2001, Feitoza 2006) (Table IIB).

Statistical analysis - Statistical analysis was performed using software (SPSS version 10) and significance was defined as p < 0.05. In the first stage, univariate analysis was carried out by means of the chi-square test to determine whether there were associations between the geoclimatic characteristics of the 297 localities studied and the presence or absence of foci of the disease, with a view to identifying the areas at risk for malaria transmission in ES. The second stage of the analysis associated the presence or absence of each species of anopheline with areas defined as being propitious for the occurrence of malaria by the chi-square test. The anopheline species that showed significant relationships with the areas where malaria occurred were then submitted to multivariate analysis by means of binary logistic regression to confirm this association. Maps were obtained using GEOBASES and GISUNES, operational in GIS, that served as a basis for the study of associations between the different variables (Feitoza et al. 2001).

TABLE II

Applied criteria for temperature and relief (A) and definition of zones resulting from the environmental variables (B) in the state of Espirito Santo, Brazil

A: applied criteria for temperature and relief used to classify natural units at zone level, excluding water parameters

Temperature

Denomination	Criterion altitude (m)	Frost period (months)	Degree-above (degree days/year)	Mean maximum temperature for the hottest month (ºC)	Cold period (month)	Number of hours with temperature below 7ºC (h)	Mean minimum temperature for the coldest month (ºC)
Cold	> 1200	> 3	< 2,440	< 25.3	5,5	> 150	< 7.3
	850-1200	0-3	2,440-3,190	27.8-25.3	4.5-5.5	50-150	9.4-7.3
Mild	450-850	0	4,040-3,190	30.7-27.8	0-4.5	0-50	11.8-9.4
Hot	0-450	0	5,080-4,040	34-30.7	0	0	18-11.8

Relief

Denomination	Criterion	Relief	Slope (%)
Sloping	Clustering of soil mapping units of components with slope above 8%	Rolling	8- 20
		Hilly	20- 45
		Mountainous	45- 75
		Escarpment	> 75
Plain	Clustering of soil mapping units with at least one component with slope below 8%	Flat	0- 3
		Gently undulating	3- 8

Marine Influence

Criterion: clustering of soil mapping units below 10 m above sea level and situated less than 2 km from saline waters collections

Feitoza et al. (2001).

B: zones resulting from the association between the variables temperature, relief and marine influence obtained from the natural units database.

Zones	Definition
A1	Hot, flat and gently undulating (slope 0-8%), under marine influence lands
A2	Hot, flat and gently undulating (slope 0-8%), excluded marine influence lands
A3	Hot, gently undulating and undulating lands (slope 8-20%)
A4	Hot, hilly, mountainous and escarpment lands (slope > 20%)
B1	Mild, gently undulating and undulating lands (slope 8-20%)
B2	Mild, hilly, mountainous and escarpment lands (slope > 20%)
C	Cold, hilly, mountainous and escarpment lands (slope > 20%)

RESULTS

The 297 sampling localities were distributed evenly throughout all the geoclimatic zones of the state (Fig. 1). In all, 14,663 anophelines were collected belonging to 22 species (Table III). Eighty-one malaria foci were recorded from 1985-2005, divided among 19 municipalities. Significant, positive associations were found between malaria foci and the presence of environmental variables characterizing the following geoclimatic zones: A1 (χ^2 = 16.979; p = 0.000); A2 (χ^2 = 30.403; p = 0.000) and A3 (χ^2 = 18.275; p = 0.000). Zones B2 (χ^2 = 33.533; p = 0.000) and C (χ^2 = 8.352; p = 0.004) presented significant negative associations, indicating areas unfavourable for malaria transmission. Based on the geoclimatic characteristics identified for malaria foci, a map was prepared of the areas considered at risk for transmission of the disease (Fig. 2).

Areas characterized as being at risk for the transmission of malaria presented significant positive associations with the presence of *An. darlingi* (χ^2 = 83.732; p = 0.000), *Anopheles aquasalis* (χ^2 = 17.943; p = 0.000), *Anopheles albitarsis* s.l. (χ^2 = 11.427; p = 0.001), *Anopheles triannulatus* s.l. (χ^2 = 6.504; p = 0.011) and *Anopheles galvaoi* (χ^2 = 5.396; p = 0.020). Significant negative associations were found for *Anopheles lutzi* (χ^2 = 32.470; p = 0.000) and *Anopheles oswaldoi* s.l. (χ^2 = 9.630; p = 0.002). *Anopheles strodei* (χ^2 = 3.370; p = 0.066), *Anopheles evansae* (χ^2 = 1.106; p = 0.293) and *Anopheles argyritarsis* (χ^2 = 0.517; p = 0.472) did not present significant associations with malaria distribution.

Based on multivariate analysis using binary logistic regression, the associations between the occurrence of *An. darlingi* (p = 0.0000) and *An. aquasalis* (p = 0.0024) and the areas at risk for transmission of malaria were significant. A significant correlation between malaria foci and *An. darlingi* occurrence was found in Zones A1 (χ^2 = 7.870; p = 0.005), A2 (χ^2 = 18.980; p = 0.000) and A3 (χ^2 = 35.998; p = 0.000). On the other hand, a negative association was noted between the presence of this species and Zones B2 (χ^2 = 32.018; p = 0.000) and C (χ^2 = 5.852; p = 0.016) (Table IIB). The geoclimatic characteristics of *An. aquasalis* were present only in Zone A1 (χ^2 = 105.087; p = 0.000). It was associated with brackish water in ES and is similar to other studies (FUNASA 2004) (Fig. 2).

DISCUSSION

Previous studies in ES found 17 of the 22 anopheline species identified in the present study (Table III) (Coutinho 1947, Andrade & Brandão 1957, Ferreira 1964).

Hot, low-lying or gently undulating to undulating relief (0-20% slope) were characteristics for the potential risk of introduced malaria, which could lead to autochthonous malaria in ES (Zones A1, A2 and A3). Mountainous areas with moderate or cool temperatures (Zones B2 and C) may be considered as being protected against
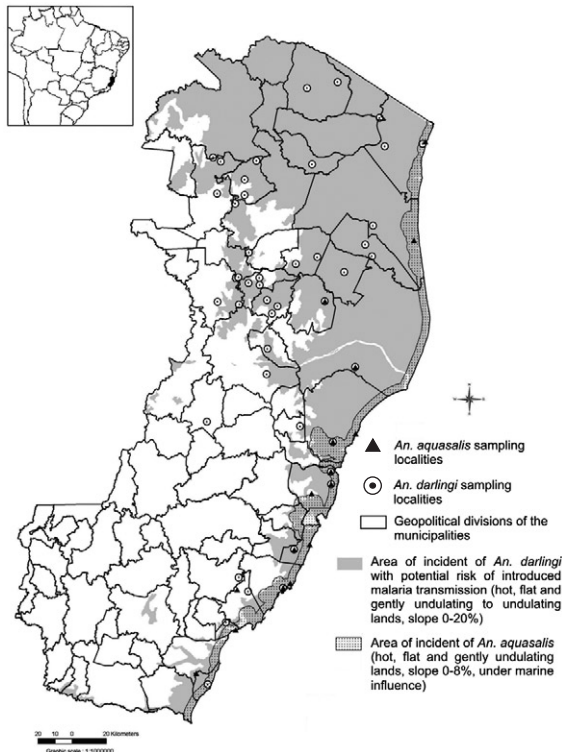

Fig. 2: distribution of *Anopheles darlingi* and *Anopheles aquasalis* and areas at risk for malaria transmission in the state of Espírito Santo, Brazil.

TABLE III

Total number collected and percent of different *Anopheles* species in the state of Espírito Santo, Brazil, 1997-2005

Species	Absolute freq.	%
Anopheles evansae[a]	3,470	23.66
Anopheles strodei[a]	3,347	22.83
Anopheles albitarsis s.l. [a]	2,601	17.74
Anopheles argyritarsis[a]	1,851	12.62
Anopheles lutzi[a]	999	6.81
Anopheles galvaoi[a]	605	4.13
Anopheles darlingi[a]	503	3.43
Anopheles triannulatus s.l. [a]	425	2.90
Anopheles aquasalis[a]	228	1.55
Anopheles oswaldoi s.l. [a]	178	1.21
Anopheles minor[a]	152	1.04
Anopheles nunestovari	120	0.82
Anopheles lanei[a]	33	0.23
Anopheles sp.	33	0.23
Anopheles parvus[a]	31	0.21
Anopheles cruzii[a]	28	0.19
Anopheles tibiamaculatus	19	0.13
Anopheles costai	11	0.08
Anopheles benarrochi	10	0.07
Anopheles bellator[a]	9	0.06
Anopheles maculipes[a]	5	0.03
Anopheles intermedius[a]	3	0.02
Anopheles gilesi[a]	2	0.01
Total	14,663	100

[a]: anopheline species identified in the present study that were found previously in state of Espírito Santo by Coutinho (1947), Andrade and Brandão (1957) and Ferreira (1964).

the risk of introduced malaria transmission since the suspected vector for autochthonous malaria in mountainous regions, *An. cruzii*, has been collected almost exclusively in the forest canopy after sunset (Rodrigues 2005). This result explains the occurrence of isolated human cases of autochthonous malaria, probably originated from simians. *An. cruzii* behaviour seems to be different from that observed in other states of Southeast and South Brazilian Regions, where *Kerteszia* has been occurring frequently at ground level (Deane 1986, Ueno et al. 2007).

All of the evidence incriminates *An. darlingi* and *An. aquasalis* as vectors of introduced malaria in ES. *An. darlingi* occurred in hot, flat to undulating areas in the littoral and the interior regions of the state (Zones A1, A2 and A3), while *An. aquasalis* was restricted to hot, low-lying areas under marine influence (Zone A1).

The results of the present study support the data in the scientific literature on this topic. According to Forattini (2002), *An. darlingi* is the principal South American vector of human malaria. Man-made alterations to the environment appear to influence the abundance of this species. The substitution of natural vegetation by plantations, pastures and other activities may create conditions favourable to its development. *An. aquasalis* is considered an important vector at high density, as commonly occurs in coastal areas during the rainy season (Forattini 1962, 2002). Moreover, a relationship has been demonstrated between vectors and specific ecoregions (Rubio-Palis & Zimmerman 1997).

The results did not demonstrate any association between the presence of *An. lutzi* and *An. oswaldoi* s.l. and the occurrence of malaria foci in ES, since these species predominated in areas considered to be free from the risk of transmission. Considering the abundance of *An. strodei*, *An. evansae* and *An. argyritarsis* in most of the localities studied, there is no indication that these species are important in malaria transmission.

GEOBASES and GISUNES were found to be efficient tools for discrimination of malaria risk areas in ES, demonstrating the influence of geoclimatic factors on vector distribution. Such association allows for predicting the occurrence of possible foci in indene areas.

Although the analysis did not emphasize the geopolitical divisions of the municipalities, it was considered relevant to show these in the maps in order to provide the municipal administrations with scientific bases for the identification of risk areas for malaria transmission, leading to the adoption of preventative and control measures against the disease.

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