



# Bionomics of *Aedes aegypti* subpopulations (Diptera: Culicidae) from Misiones Province, northeastern Argentina

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## ABSTRACT

Life statistics of four *Aedes aegypti* subpopulations from the subtropical province of Misiones were studied during autumn and winter, under semi-natural conditions, coming from the localities of Posadas (SW), San Javier (SE), Bernardo de Irigoyen (NE) and Puerto Libertad (NW). The eastern subpopulations are geographically separated by the central mountain system of the province from the western subpopulations. High percentages of larval and pupal survival (97–100%) were recorded, and no significant differences were detected among the four subpopulations. Larvae and pupae lasted approximately 8 days to complete their development, no significant differences being detected among the four subpopulations studied. Sex ratio recorded did not differ significantly from 1:1. Male longevity did not show difference among the different subpopulations, but female longevity was remarkably different among the four subpopulations ( $F = 16.27$ ; d.f. = (3;8);  $P = 0.0009$ ), ranging among 11.45 days for San Javier and 57.87 days for Posadas. Fecundity also varied considerably among subpopulations, the greatest number (307.44 eggs/female) being recorded for Posadas ( $F = 4.13$ ; d.f. = (3;8);  $P = 0.04$ ). *Ae. aegypti* females of the western subpopulations lived longer than the eastern subpopulations studied, therefore, the risk of dengue outbreak would be greater on the Misiones Province border with Paraguay.

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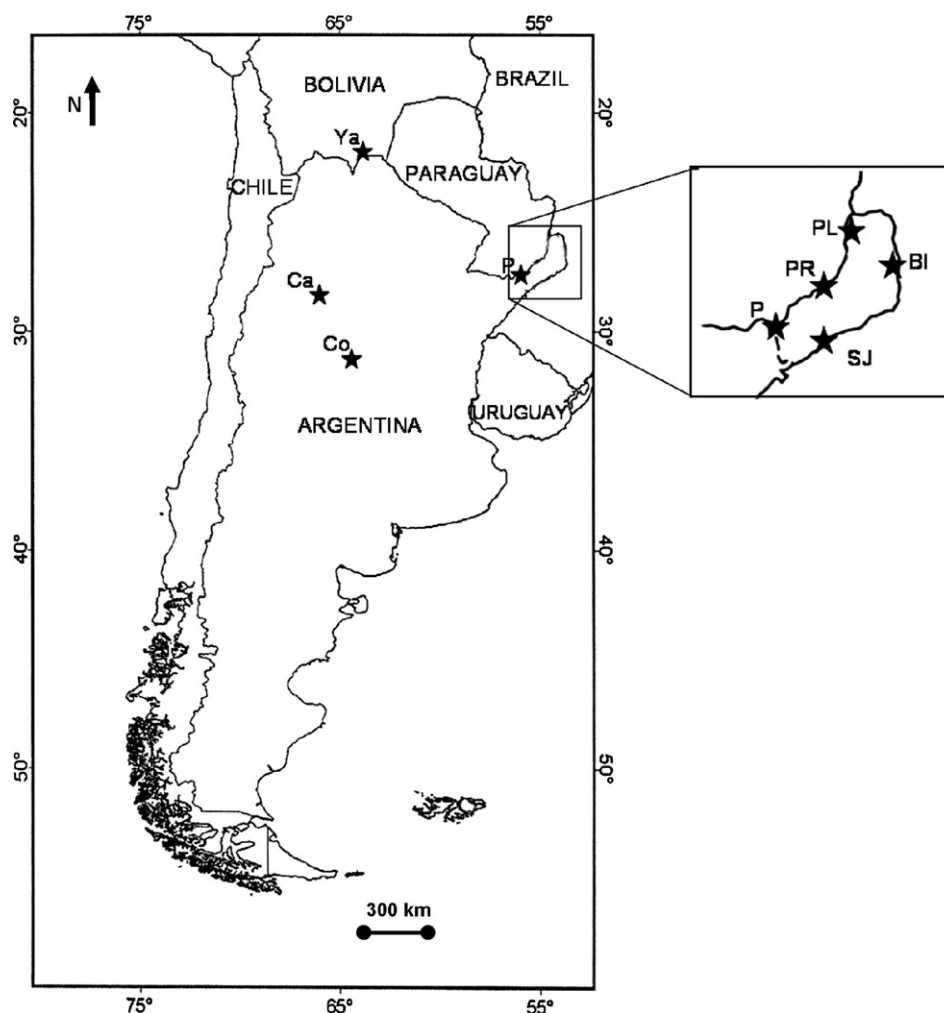
## 1. Introduction

The high density of *Aedes aegypti* (L.) in most of the northern Argentine provinces, the introduction of dengue virus to Argentina from bordering countries such as Bolivia, Paraguay, and Brazil, and the low level of immunity of Argentine inhabitants represent significant risk factors for our Country (Avilés et al., 1999), particularly for the northeastern Misiones Province. In 2000, a dengue outbreak by serotype DEN-1, with 218 notified cases, was reported for Misiones Province, outbreak that also affected the neighboring countries Bolivia, Brazil and Paraguay; in 2002, the serotype DEN-3 was detected in Misiones Province (Avilés et al., 2003; PAHO, 2004).

Biological characteristics of *Ae. aegypti* mosquitoes seem to vary depending on particularities of each location (Rodhain and Rosen, 1997). Genetic differences have been found among subpopulations of these mosquitoes throughout molecular marker studies (Gorochotegui-Escalante et al., 2000). Additionally, variability in vectorial competence among different subpopulations has been demonstrated (Beerntsen et al., 2000).

After the eradication of *Ae. aegypti* from Argentina in 1964 (Ousset et al., 1967), the first re-infestation of the Country by these mosquitoes was reported in 1986 for Posadas city, Misiones Province, probably from Paraguay (Boffi, 1998). Currently, the distribution is wider than before the eradication, including the provinces of La Pampa and San Luis where these mosquitoes were not previously listed. The re-infestation would have occurred in the northwestern provinces from Bolivia, and in the northeastern provinces from Paraguay and Brazil (Rondán-Dueñas, 2005). Genetic studies on *Ae. aegypti* subpopulations, from Argentina and Bolivia, have been carried out, and determined a different composition of haplotypes according to the different geographical regions (Rondán-Dueñas, 2005). Different haplotypes were detected for *Ae. aegypti* subpopulations from the provinces of Misiones (Posadas city), Catamarca, and Córdoba (NE, central-western and central Argentina, respectively), and Yacuiba (southern Bolivia, bordering with the NW of Argentina), subpopulations that are geographically separated for at least 300 km (Fig. 1). In addition, different responses of *Ae. aegypti* larvae of these four subpopulations to temephos, the most common insecticide used to control these mosquitoes in Argentina, were observed under laboratory conditions (Biber et al., 2006). According to these observations, the objective of this work was to compare the bionomics of four *Ae. aegypti* subpopulations from Misiones Province (northeastern

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**Fig. 1.** Map of Argentina and bordering countries. Bolivia: Ya = Yacuiba city. Argentina: Ca = Catamarca city (Catamarca Province); Co = Córdoba city (Córdoba Province); PL = Puerto Libertad city, PR = Puerto Rico city, P = Posadas city, BI = Bernardo de Irigoyen city, SJ = San Javier city (Misiones Province).

Argentina), subpopulations separated geographically by shorter distances but that could also show biological differences.

## 2. Materials and methods

### 2.1. Study area

To represent the whole province of Misiones, *Ae. aegypti* subpopulations were sampled from the following localities (Fig. 1): Bernardo de Irigoyen city (26°15'S; 53°38'W) on the northeastern region of the province, at 800 m.a.s.l., and bordering with Brazil, where the passive dispersion of these mosquitoes would be high. Puerto Libertad city (25°54'S; 54°37'W) on the northwestern region of the province, on the Paraná riverside, at 35 km from Iguazú city. Posadas city (27°23'S; 55°53'W), the main city of the province, neighboring the Paraguayan city of Encarnación, with a significant people and vehicular traffic among these cities through the San Roque González de Santa Cruz international bridge. San Javier city (27°52'S; 55°08'W) on the southeastern region of the province on the Uruguay riverside, bordering with the Brazilian city of Porto Xavier. The three latter cities are situated under 300 m.a.s.l.

Annual average cumulative rainfalls for the subtropical province of Misiones is 1900 mm. The mean of the maximum, medium and minimum temperature are 27.6, 21.5, and 16.6 °C, respectively (Servicio Meteorológico Nacional).

### 2.2. *Ae. aegypti* mass rearing

A colony of each *Ae. aegypti* subpopulation was established in the laboratory from immature stages collected in each locality. Eggs were collected using ovitraps while larvae and pupae were collected from artificial containers, and kept under semi-natural conditions (Gerberg et al., 1994; Domínguez et al., 2000). Eggs were kept on the same absorbent paper used in the ovitraps at least for 4–5 days to ensure the embryogenesis. Larvae and pupae were kept in 750 ml water-filled trays, larvae being fed with liver powder (0.25 mg/larva/day); water surface was daily cleaned with absorbent paper to avoid fungus and bacteria development. Adults were kept in cardboard cylinder entomological cages (30 cm diameter, 50 cm high), and fed with a 10% sugar solution from cotton wick in 70-ml plastic flasks. Blood source was provided placing a restrained mouse into each entomological cage for 2 h twice a week. Plastic flasks containing water and absorbent paper were located inside the entomological cages to obtain eggs.

When there were enough eggs from each locality, they were induced to hatch. Three cohorts of 30 first instar larvae for each subpopulation were simultaneously organized and followed. Each cohort was kept in a 750 ml water-filled tray, and feeding larvae with liver powder as previously was described. Bionomics was estimated through horizontal life tables from these cohorts.

**Table 1**

Mean and standard deviation of *Ae. aegypti* larval and pupal survival, and sex ratio of adults that emerged recorded for four subpopulations from Misiones Province. Three replicates were performed for each subpopulation.

		Posadas	Puerto Libertad	San Javier	Bernardo de Irigoyen
Immature stage survival (%)	Larva	98.9 ± 1.9 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>
	Pupa	97.8 ± 1.9 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>	98.9 ± 1.9 <sup>a</sup>
Sex ratio (%)	Male	55.9 ± 18.9 <sup>a</sup>	53.6 ± 5.2 <sup>a</sup>	44.5 ± 4.9 <sup>a</sup>	48.5 ± 6.5 <sup>a</sup>
	Female	44.1 ± 18.9 <sup>a</sup>	46.4 ± 5.2 <sup>a</sup>	55.5 ± 4.9 <sup>a</sup>	51.5 ± 6.5 <sup>a</sup>

Different letters among columns mean significant differences ( $P < 0.05$ ).

*Ae. aegypti* mass rearing was carried out indoors in a house in Puerto Rico city, Misiones Province (26°48'S; 55°01'W), among April–August 2006 (autumn–winter). During this period, the mean temperature was daily recorded, and varied among 9–32 °C, mean of 20.7 °C (mean maximum = 24.4 °C; mean minimum = 16.7 °C). The relative humidity varied among 70–85%, and the photoperiod did among 11.55–10.4 h light (Comando de Regiones Aéreas, 2005). During larvae and pupae rearing, the mean temperature recorded was 25.6 ± 0.9 °C.

### 2.3. Larval and pupal survival and development time

The number of living and dead larvae (by instar) and pupae, and the number of exuvias were daily recorded. Survival ( $I_x$ ) was expressed as the percentage of individuals that reached the next instar/stage. Larval and pupal mean development time and the sex ratio of emerged adults were also estimated (Gómez et al., 1977).

### 2.4. Adult longevity and fecundity

Adults that emerged from each cohort were placed into an entomological cage. The number of living and dead males and females was daily recorded. Adults were fed with a 10% sugar solution provided daily. After 2 days the first female had emerged, they were provided with blood (restrained mouse) and the process was repeated twice a week. Once the mouse was withdrawn from the entomological cage, a plastic flask with an absorbent paper and water was placed into the cage, as an ovitrap, which was daily replaced. Male and female longevity was estimated as well as fecundity. Daily fecundity was expressed as the number of eggs laid/female/day. The oviposition time was expressed as the mean time while females were laying eggs.

### 2.5. Data analysis

Larval and pupal development time, larval and pupal survival, adult longevity, oviposition time, and fecundity were analyzed by ANOVA, followed by the Duncan's Test, to detect significant differences among the four subpopulations studied. Sex ratio in each cohort was analyzed by the Student *t*-test for differences of pro-

portions (Steel and Torrie, 1988). Sex ratio among subpopulations was analyzed by ANOVA, previous arcsin data transformation. All the data was checked by normality.

## 3. Results

### 3.1. Larval and pupal survival and development time

Larval and pupal survival was 100% in cohorts from Puerto Libertad and San Javier, and lightly lower in cohorts from the other two localities, no significant differences being detected among the four subpopulations studied ( $F = 1.19$ ; d.f. = (3;8);  $P = 0.374$ ) (Table 1).

The proportion of males and females that emerged did not differ significantly from 1:1, although more males were recorded in cohorts from Posadas and Puerto Libertad, females were more numerous in cohorts from San Javier and Bernardo de Irigoyen (Table 1).

Larvae and pupae lasted approximately 8 days to complete the development, no significant differences being detected among the four subpopulations ( $F = 0.28$ ; d.f. = (3;8);  $P = 0.8401$ ) (Table 2). Males emerged approximately 1 day before females in all subpopulations.

### 3.2. Adult longevity and fecundity

Male longevity varied among 7.33 and 8.8 days, no significant differences being detected among the four subpopulations ( $F = 0.95$ ; d.f. = (3;8);  $P = 0.4596$ ) (Table 3). Female longevity varied markedly among subpopulations from 11.45 days (San Javier) to 57.87 days (Posadas), significant differences being detected among both subpopulations ( $F = 16.27$ ; d.f. = (3;8);  $P = 0.0009$ ). Oviposition time varied according to female longevity, being longer in long-lived cohorts since females could develop more gonotrophic cycles and laying eggs during more time ( $F = 9.3$ ; d.f. = (3;8);  $P = 0.0055$ ) (Table 3).

Fecundity also varied considerably among subpopulations, the greatest number (307.44 eggs/female) being recorded for Posadas ( $F = 4.13$ ; d.f. = (3;8);  $P = 0.04$ ) (Table 4). The lowest number of eggs was recorded for San Javier (47.14 eggs/female) and Bernardo de Irigoyen (54.48 eggs/female). In the same way, differences

**Table 2**

Mean and standard deviation of *Ae. aegypti* larval and pupal development time, and emerging time for males and females of four subpopulations from Misiones Province. Three replicates were performed for each subpopulation.

		Posadas	Puerto Libertad	San Javier	Bernardo de Irigoyen
Development time (days)	Larva 1 + 2	2.17 ± 0.06	1.18 ± 1.08	1.13 ± 0.12	1.37 ± 0.09
	Larva 3	1.30 ± 0.17	1.45 ± 0.07	1.26 ± 0.04	1.39 ± 0.08
	Larva 4	2.43 ± 0.62	3.10 ± 0.66	3.21 ± 0.22	3.18 ± 0.32
	Total larva	5.90 ± 0.7	5.72 ± 0.56	5.60 ± 0.15	5.93 ± 0.2
	Pupa	2.47 ± 0.03	2.80 ± 0.77	2.37 ± 0.12	2.46 ± 0.1
	Larva + Pupa	8.37 ± 0.73	8.52 ± 1.33	7.97 ± 0.26	8.39 ± 0.16
Emerging time (days)	Male	8.09 ± 0.3	7.29 ± 0.06	7.23 ± 0.04	8.06 ± 0.4
	Female	9.23 ± 0.7	8.13 ± 0.4	8.57 ± 0.4	8.66 ± 0.2

No significant differences were detected ( $P < 0.05$ ).

**Table 3**

Mean and standard deviation of *Ae. aegypti* male and female longevity, and oviposition time for four subpopulations from Misiones Province. Three replicates were performed for each subpopulation.

		Posadas	Puerto Libertad	San Javier	Bernardo de Irigoyen
Longevity (days)	Male	8.8 ± 0.69 <sup>a</sup>	8.56 ± 1.85 <sup>a</sup>	7.71 ± 0.74 <sup>a</sup>	7.33 ± 1.27 <sup>a</sup>
	Female	57.87 ± 4 <sup>a</sup>	31.24 ± 16.92 <sup>b</sup>	11.45 ± 1.13 <sup>c</sup>	15.58 ± 4.72 <sup>bc</sup>
Oviposition time (days)		39.47 ± 6.67 <sup>a</sup>	24.70 ± 12.4 <sup>b</sup>	11.16 ± 3.06 <sup>b</sup>	13.28 ± 3.16 <sup>b</sup>

Different letters among columns mean significant differences ( $P < 0.05$ ).

**Table 4**

Mean and standard deviation of *Ae. aegypti* daily fecundity, fecundity and total number of eggs recorded for four subpopulations from Misiones Province. Three replicates were performed for each subpopulation.

	Posadas	Puerto Libertad	San Javier	Bernardo de Irigoyen
Daily fecundity	7.84 ± 2.95 <sup>a</sup>	5.67 ± 3.17 <sup>a</sup>	4.54 ± 1.97 <sup>a</sup>	3.9 ± 1.84 <sup>a</sup>
Fecundity	307.44 ± 86.36 <sup>a</sup>	159.69 ± 116.35 <sup>ab</sup>	47.14 ± 14.23 <sup>b</sup>	54.48 ± 35.96 <sup>b</sup>
Total number of eggs	3999.33 ± 2184.11 <sup>a</sup>	1941.67 ± 1352.23 <sup>ab</sup>	583.33 ± 153.79 <sup>b</sup>	792.33 ± 613.24 <sup>b</sup>

Different letters among columns mean significant differences ( $P < 0.05$ ).

were detected in the total number of eggs, since subpopulations with long-lived females could lay more eggs ( $F = 4.20$ ; d.f. = (3;8);  $P = 0.0464$ ), but daily fecundity did not differ significantly among the four subpopulations ( $F = 1.38$ ; d.f. = (3;8);  $P = 0.3167$ ).

#### 4. Discussion

Larval and pupal survival values obtained here were higher than those recorded for larva (76.75%) and pupa (96.4%) reared under semi-natural conditions during summer in Córdoba city, a temperate zone of central Argentina (Domínguez et al., 2000).

A sex proportion of 1:1 was also reported for *Ae. aegypti* adults emerged from larvitrap in the temperate province of Buenos Aires (central-western Argentina) (Campos and Maciá, 1996). In Córdoba city, the proportion of females emerged was lightly higher with relation to males but only when mosquitoes were reared during the summer time (Domínguez et al., 2000).

Bar-Zeev (1958) recorded the larval development time as 9.65 days under laboratory conditions (20 °C). In Córdoba Province (temperate central Argentina), it was estimated as 8.91 days under semi-natural conditions (22.13 °C) (Domínguez et al., 2000). In comparison with the previous reports, at 25.6 °C (Table 2), the larval development time we estimated in Misiones Province was shorter (5.8 days). In the temperate locality of La Plata (Buenos Aires Province, Argentina), the larval development time varied between 4 and 42 days for both sexes, time that was recorded during the spring–summer period and under field conditions (26 °C), with a mean of 8.33 and 10.51 days to pupation for males and females, respectively (Maciá, 2006). Values recorded in La Plata are also longer than the time we estimated in the subtropical province of Misiones.

At 22.13 °C, under temperate semi-natural conditions, Domínguez et al. (2000) estimated the pupal development time as 2.9 days, lightly longer than the 2.5 days we estimated at 25.6 °C in the subtropical province of Misiones. Temperatures lower than 20 °C, even for a few hours a day, interfere in the normal development of larvae (Bejarano, 1956). During January (winter) in Taiwan, Chang et al. (2007) recorded a development time from the first instar to adulthood of 31 days (water temperature of 14–15 °C) under field conditions.

Female longevity was remarkable different among the subpopulations studied. *Ae. aegypti* subpopulations from the eastern region of Misiones Province (Bernardo de Irigoyen and San Javier), with the shortest longevity, are geographically separated from the western subpopulations by the central mountain system (500–800 m) of the province. In addition, the main communication routes in the

province are parallel to the Paraná and Uruguay rivers, as well as, to the mountain system.

Under laboratory conditions, at 25 °C and 80% RH, mean longevity of females was among 70 and 116 days (Christophers, 1960), longer than our results under semi-natural conditions. Female life duration varied from 1 to 76 days in the same cage with all environmental conditions being equal, however, in the field, under the equatorial climate of French Guiana, varied from 2.1 to 28.98 days with a mean value of 14.49 days (Fouque et al., 2006). Evidently, with constant conditions of laboratory female longevity can be over estimated, therefore, not to reflect what really happens in nature.

It has been demonstrated the ingestion of blood can increase female survival, therefore, the possibility of pathogen transmission. Female longevity was estimated as 6–8 days at 25 °C and 70% RH, at most 12 days, feeding females only with sugar solution, but in the same environmental conditions, and providing a source of blood, females lived up to 93 days (Christophers, 1960). In our study, females were offered blood meals and the longest longevity recorded (57.87 days) was for females from Posadas city. In addition, it has been demonstrated that mortality was significantly lower in females fed daily on blood than those fed every other day (Styer et al., 2007).

According to Harrington et al. (2001), *Ae. aegypti* female fed human blood exclusively had the highest age-specific survival, followed by those fed human blood plus sugar, mouse blood plus sugar, and mouse blood purely. The difference between mosquitoes fed exclusively on human blood and mouse blood was significant, although mosquitoes fed mouse blood plus sugar produced significantly more eggs (Harrington et al., 2001). Based on these findings, we could consider that female survival in our work could have been longer since they were fed on mouse blood plus sugar.

Under natural conditions, in temperate regions of the northern hemisphere, mean longevity of females was estimated as 15 days, at most 42 days (Korovitzky and Artemenko, 1933). Based on these data and the outbreaks registered at the beginning of the 20th century in Argentina, *Ae. aegypti* mean longevity was estimated as 15–20 days for temperate regions of the Country (Carbajo et al., 2001). With a higher longevity, dengue transmission might be possible southwards in Argentina (Carbajo et al., 2001).

The risk of dengue outbreaks would be higher in areas where *Ae. aegypti* female longevity is longer, since the possibility of transmission is higher. Therefore, the cities of Posadas and Puerto Libertad (Misiones Province), bordering with Paraguay, would be areas of greater risk considering only female longevity, where it was longer, although several factors are involved in the transmission.



In the temperate city of La Plata (Buenos Aires Province, Argentina), Maciá (2006) recorded a mean fecundity of 59.8 eggs/female for *Ae. aegypti*. This value is quite similar to those we recorded for the eastern subpopulations (San Javier and Bernardo de Irigoyen) from Misiones Province (subtropical climate), but quite lower than those reported here for Posadas subpopulation.

In Thailand, Strickman (2006) recorded a daily fecundity for *Ae. aegypti* of 16.4 eggs/female during the coolest period (November–December; mean temperature of 32.8 °C), and 27.7 eggs/female during the warm period (March–April; mean temperature of 37.6 °C). These values are quite higher than those recorded in Misiones Province (subtropical climate) at a lower temperature.

In the Caribbean countries, the history of *Ae. aegypti* control is a cyclic succession of successful control programs, lost of money, cessation of control effort and explosive re-infestation by these mosquitoes. These sporadic cycles of control plus the changes associated with *Ae. aegypti* population size, provide ideal conditions to the rapid genetic changes of these mosquitoes. Small populations offer excellent conditions to prove new adaptations by the intensification of interactions among selection and random effects (Tabachnick, 1991; Liu et al., 2004). Gene selection was determined in *Ae. aegypti*, from Trinidad and Tobago (Yan et al., 1998), and *Drosophila melanogaster* Meigen, from Japan (Miyo and Oguma, 2002), due to the pressure of insecticides.

In Posadas city (Misiones Province), on the Paraná riverside, cypermethrin was applied to control *Ae. aegypti* adults during the last decade, as well as in other cities along this river shore, where most cases of dengue were reported (Boletín Epidemiológico Nacional, 2000–2001). *Ae. aegypti* female longevity from Posadas subpopulation was remarkably longer than other subpopulations, where the insecticide pressure was high.

Possibly the differences in *Ae. aegypti* life statistics observed here, among the subpopulations studied, are based on genetics differences which must be demonstrated. The differences in *Ae. aegypti* subpopulations here reported are epidemiologically important, indicating a higher risk for human populations settled along the shores of the Paraná River, where the constant vehicular traffic among Argentina and Paraguay probably contribute to the passive dispersion of *Ae. aegypti* in the area, as well as, the circulation of the different serotypes of dengue virus.

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