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CULEX SALTANENSIS DYAR, 1928 – NATURAL VECTOR OF *PLASMODIUM JUXTANUCLEARE* IN RIO DE JANEIRO, BRAZIL

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Searching for the natural vector of Plasmodium juxtanucleare *in an enzootic locality: Granjas Calábria (33% of the chickens infected), Jacarepaguá, in Rio de Janeiro, Brazil, 13 comparative captures of mosquitoes were carried out, simultaneously on man (out-doors) and on chicken (in a poultry-yard), between 6 and 9 p.m., from September 1988 to March 1989.*

Culex saltanensis *was the most frequent species in captures on chicken, accounting for 41.7% of the mosquitoes collected on this bait, showing to be highly ornithophilic (90% captured on chicken versus 10% on man). Seven specimens of* Cx. saltanensis *were found naturally infected in Granjas Calábria: five with mature pedunculate oocysts and two with sporozoites (one in the haemocoele and one in the salivary glands). These sporozoites produced an infection by* P. juxtanucleare *in a chick, which had parasitemia on day 41 after inoculation. One* Cx. coronator *was found with mature pedunculate oocysts.*

Culex saltanensis *was regarded as primary vector of* P. juxtanucleare *in Rio de Janeiro for being highly ornithophilic and in enough density to maintain the transmission, having been found with infective sporozoites in its salivary glands, and being susceptible to the parasite and able to transmit experimentally it by the bite.*

Key words: *Culex saltanensis* – *Plasmodium juxtanucleare* – avian malaria vector – ornithophilic mosquitoes – chicken malaria

The causative parasites of the avian malarias were some of the first plasmodia studied and have been found in about 450 species of migratory or non-migratory birds of all continents. They are arranged in four subgenera: *Haemamoeba, Giovannolaia, Novyella* and *Huffia*.

Considering the Brazilian abundant avian fauna, there are few studies on the prevalence of its plasmodia and still less is known about their geographic distribution, biology and transmission.

Up to now, two plasmodia have been described infecting chicken *(Gallus gallus): Plasmodium (Haemamoeba) gallinaceum* Brumpt, 1935 and *Plasmodium (Novyella) juxtanucleare* Versiani & Gomes, 1941. The first one, in spite of being spread out in laboratories of almost all over the world, occurs naturally only in the Orient. Differently, *P. juxtanucleare* has been considered cosmopolitan, being recorded in countries of Latin America, Asia and Africa, chiefly in domestic fowls and sometimes in other wild and domesticated galliforms.

Plasmodium gallinaceum is capable of infecting experimentally several genera and species of Culicini, Aedini and Anophelinae mosquitoes (Huff, 1965; Garnham, 1966), while *P. juxtanucleare* infects only *Culex* species (Paraense, 1944; Akiba, 1959; Dhanapala, 1962; Bennet & Warren, 1966; Bennet et al., 1966; Krettli, 1972a, b).

Although the pioneer investigations on the vectors of *P. juxtanucleare* have been carried out in Latin America, its carrier in this continent remained unknown during almost 50 years. The first experiments were performed by Paraense (apud Versiani & Gomes, 1943) and Beltran (1943), who failed to infect *Aedes aegypti* (Linneus, 1762), *Culex quinquefasciatus* Say, 1823 and *Anopheles aztecus* Hoffman, 1935. In 1944, Paraense tested 158 specimens of *Ae. aegypti*, 15 of *Ae. lepidus* Cerqueira & Paraen-

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se, 1945 and 232 *Cx. quinquefasciatus* and verified that only three specimens of the latter species had become infected: he found one with oocysts after 9 days of infection and two with sporozoites in their salivary glands after 13 and 23 days, showing that although *P. juxtanucleare* did not seem to be adapted to this species of *Culex*, the vectors of this parasite would possibly be among other species of this genus of mosquito. This was confirmed later with the studies carried out in the Old World, mainly by Bennett et al. (1966).

The natural vectors of *P. juxtanucleare* were first discovered in Malaysia by Bennett et al. (1963) who incriminated *Culex sitiens* Wiedmann, 1828 and *Culex vishnui* Theobald, 1901 (= *Cx. annulus*) by finding them naturally infected. The authors also verified that other species of *Culex* belonging to the *pseudovishnui* complex are experimentally susceptible to *P. juxtanucleare*, but their role in nature has not been evaluated. Bennett et al. (1966) described the sporogonic cycle of *P. juxtanucleare* in *Culex* and verified that its oocysts attach to the stomach wall by a characteristic conspicuous peduncle.

The first field work performed in order to discover the natural vectors of *P. juxtanucleare* in the Americas was that of Paraense (1949) who dissected 18 *Cx. quinquefasciatus* out of 24 collected in a Shannon's trap with a chicken as bait in an enzootic area in Bambuí, Minas Gerais State, Brazil. All were negative. Krettli (1971) also had no positive result while dissecting 102 mosquitoes belonging to seven different species collected in Belo Horizonte, in the same State. Later, Krettli (1972a) found pedunculate oocysts on the stomach of two out of 476 *Cx. quinquefasciatus* she tried to infect experimentally with *P. juxtanucleare*.

In view of the frequency of *P. juxtanucleare* in Latin America, particularly in southeastern Brazil (Versiani & Gomes, 1941; Paraense, 1944; Franco et al., 1954; Krettli, 1972b; Massard, 1982), we decided to investigate which would be its natural vector in a part of the continent where it was originally described.

MATERIALS AND METHODS

Studied area – The present study was carried out in Granjas Calábria, Jacarepaguá, City of Rio de Janeiro, Rio de Janeiro state (23°00' S 43°26' W). It is a rural area in the western lowlands bordering the city, with characteristic vegetation and tropical climate as described by Abreu (1957), Alonso (1977), Nimer (1977) and Lourenço-de-Oliveira (1984b). This locality was chosen because its mosquito fauna had already been studied by one of us (Lourenço-de-Oliveira, 1984a).

There, chickens are kept free during the day and in a poultry-yard from sunset to sunrise. All chickens were born and raised in this locality and derived from a breeding started about 10 years ago. The poultry-yard is not provided with any anti-mosquito prevention measure. It is less than two meters from the Cortado Canal margin, which is one of the principal waterways in this part of the city. The canal and the abundant permanent and temporary pools are the preferred breeding places of most of the local mosquito species (Lourenço-de-Oliveira et al., 1986).

Search for the natural vectors – Mosquito captures and dissections were performed in order to know the most ornithophilic species and find sporozoite natural infections by *P. juxtanucleare*.

The field work was carried out, fortnightly, from September 1988 to March 1989 and mosquito captures were performed from 6 to 9 p.m., simultaneously on chicken in the poutry-yard and on a human bait in the open country. The procedures used for mosquito collection have been presented previously (Lourenço-de-Oliveira, 1984b). All mosquitoes collected were kept alive, fed on sugar solution and dissected on the following day, to search for oocysts on the stomach and sporozoites in the salivary glands.

Experimental infection of mosquitoes – One batch of 50 *Aedes fluviatilis* Lutz, 1905 and of 50 *Cx. quinquefasciatus* were fed on a cock that had been experimentally infected with *P. juxtanucleare* (Rio de Janeiro strain) 10 months previously. These mosquitoes were dissected from days 3 to 20 after the infective blood meal.

Two batches of 100 *Culex saltanensis* Dyar, 1928 were fed on two infected birds: a chicken and a cock experimentally infected, respectively, 2 and 16 months before, with the same strain of the parasite. Part of each batch was dissected

TABLE I

Mosquito species collected on chicken in an enzootic locality of *Plasmodium juxtanucleare*, Granjas Calábria, Jacarepaguá, City of Rio de Janeiro, Brazil, from September 1988 to March 1989, arranged in decreasing frequency

Mosquito species	Number	%
Culex (Cux.) saltanensis Dyar, 1928	377	41.7
Culex (Cux.) declarator Dyar &Knab, 1906	160	17.7
Culex (Cux.) coronator Dyar &Knab, 1906	159	17.6
Culex (Cux.) quinquefasciatus Say, 1823	74	8.2
Culex (Cux.) bidens Dyar, 1922	55	6.1
Culex (Cux.) spp.	43	4.8
Mansonia titillans (Walker, 1848)	22	2.4
Culex (Cux.) chidesteri Dyar, 1921	4	0.4
Mansonia (Man.) spp.	4	0.4
Aedes (Och.) spp.	3	0.3
Aedes (Och.) fluviatilis (Lutz, 1904)	1	0.1
Aedes (Och.) scapularis (Rondani, 1848)	1	0.1
Culex (Cux.) lygrus Root, 1927	1	0.1
Culex (Cux.) nigripalpus Theobald, 1901	1	0.1
Total	905	100

TABLE II

Mosquito species captured on chicken and on man in Granjas Calábria, Jacarepaguá, City of Rio de Janeiro, Brazil, from September 1988 to March 1989. Number of specimens collected and average per 10 hours of capture

Mosquito species	Number		Average per 10 hours	
	Chicken	Man	Chicken	Man
Ae. fluviatilis	1	9	0.5	3.1
Ae. scapularis	1	113	0.5	39
Ae. (Och.) spp.	3	–	1.5	–
Cx. (Ads.) amazonensis	–	1	–	0.3
Cx. bidens	55	2	27.5	0.7
Cx. chidesteri	4	–	2	–
Cx. coronator	159	116	79.5	40
Cx. declarator	160	35	80	12.1
Cx. lygrus	1	–	0.5	–
Cx. nigripalpus	1	–	0.5	–
Cx. quinquefasciatus	74	1	37	0.3
Cx. saltanensis	377	61	188.5	21
Cx. (Cux.) spp.	43	2	21.5	0.7
Cx. (Mel.) ribeirensis	–	1	–	0.3
Cq. venezuelensis	–	29	–	10
Ma. titillans	22	846	11	291.7
Ma. (Man.) spp.	4	–	2	–
Ph. davisi	–	26	–	9
Ph. deanei	–	161	–	55.5
Ph. theobaldi	–	12	–	4.1
Total	905	1,415	452.5	487.8
Hours spent	20	29	–	–

between 10 and 30 days after the infective feed. The other part was fed again on five non-infected chicks, 23 days after the infective blood meal on the adult fowls. These birds were kept in the laboratory, protected against mosquitoes and examined 15 days after being bitten by the infected mosquitoes.

All *Ae. fluviatilis*, *Cx. quinquefasciatus* and *Cx. saltanensis* mosquitoes used in these experiments were born and kept in the laboratory with controlled humidity (70-80%) and temperature (27 ± 1 °C) and fed on sugar solution. The presence of gametocytes of *P. juxtanucleare* was confirmed by blood examination previous to the infective feed on the adult fowls.

Aedes fluviatilis (Belo Horizonte strain) and *Cx. quinquefasciatus* (Manguinhos strain) were used in these experiments for being available from colonies kept in our laboratory since 1981 and *Cx. saltanensis* (Granjas Calábria strain) because of its ornithophilic behaviour.

RESULTS

Blood samples of 11 chickens and one cock bred in Granjas Calábria were examined and three hens and the cock were found naturally infected with *P. juxtanucleare* (33.3%), with different degrees of parasitemia.

Thirteen mosquito captures were carried out in Granjas Calábria from September 1988 to March 1989.

The mosquito species collected on chicken is presented on Table I in decreasing order of frequency. A total of 905 mosquitoes belonging to 14 species were obtained, but 91.3% corresponded only to five: *Culex saltanensis* (41.7%), *Culex declarator* (17.7%), *Culex coronator* (17.6%), *Culex quinquefasciatus* (8.2%) and *Culex bidens* (6.1%). The other species captured were scarse, accounting for only 8.7% of the total; 4.8% corresponding to non-identifiable *Culex* specimens.

The results of the comparative captures on avian and human baits in Granjas Calábria are presented on Table II. *Cx. saltanensis*, the most frequent on chicken (188.5 specimens per 10 h of captures) was less numerous on man (21 exemplars per 10 h). It was followed in frequency, but at some distance, by *Cx. declarator* (80 specimens per 10 h of capture on chicken versus 12.1 on man), *Cx. coronator* (79.5 versus 40), *Cx. quinquefasciatus* (37 versus 0.3) and *Cx. bidens* (27.5 versus 0.7).

A total of 1415 mosquitoes were collected on human bait and *Mansonia titillans* (Walker, 1848) being the most frequent with 846 specimens on man versus 22 on chicken, or 291.7 and 11 specimens per 10 h of capture, respectively. This species was followed at some distance by *Phoniomyia deanei* Lourenço-de-Oliveira, 1983 and *Aedes scapularis* (Rondani, 1848) with, respectively, 55.5 and 39 specimens per 10 h on man and none and 0.5 on chicken. These three species accounted for 79.2% of the total collected on man but only 1.6% of the obtained on chicken.

TABLE III

Mosquito species dissected and examined for the presence of oocysts and sporozoites of *Plasmodium juxtanucleare*, captured in Granjas Calábria, Jacarepaguá, City of Rio de Janeiro, Brazil, from September 1988 to March 1989. Number of stomachs and salivary glands examined and number of positive in parentheses

Mosquito species	Stomachs	Salivary Glands
Ae. fluviatilis	1	1
Ae. scapularis	1	–
Cx. bidens	52	40
Cx. chidesteri	4	4
Cx. coronator	150 (1)	120
Cx. declarator	152	114
Cx. lygrus	1	1
Cx. nigripalpus	1	1
Cx. quinquefasciatus	69	52
Cx. saltanensis	351 (5)[a]	284 (1)[a]
Cx. (Cux.) sp.	1	–
Ma. titillans	15	9
Total	798	626

a: besides these, one more *Culex saltanensis* was found naturally infected with sporozoites in the haemocoele.

Stomach of 798 mosquitoes and 626 salivary glands were dissected to search for sporozoites and oocysts of *P. juxtanucleare* (Table III). Seven *Cx. saltanensis* were found naturally infected, five with several pedunculate oocysts in different degrees of development, including some mature ones and one with sporozoites in the salivary glands but with no oocyst on the stomach. Besides these, an additional *Cx. salta-*

TABLE IV

Summary of attempts to infect experimentally three mosquito species with *Plasmodium juxtanucleare*

Mosquito species	Animal infected (months of infection)	Number of mosquitoes Blood fed	Dissected (positive)
Ae. fluviatilis	Cock (10)	50	50 (–)
Cx. quinquefasciatus	Cock (10)	50	50 (–)
Cx. saltanensis	Chicken (2)	100	21 (17)
	Cock (16)	100	68 (55)

nensis was found with free sporozoites in the haemocoele. Pedunculate oocysts and free sporozoites in the haemocoele were also seen in one *Cx. coronator*. The free sporozoites in the haemocoele were probably from oocysts ruptured during the dissection. Only the sporozoites from the salivary glands of that *Cx. saltanensis* were inoculated intramuscularly in a one month old chick which showed patent parasitemia by *P. juxtanucleare* from day 41 on.

In the mosquito infection experiments (Table IV) all 50 *Ae. fluviatilis* and 50 *Cx. quinquefasciatus* were negative for oocysts and sporozoites. On the other hand, several *Cx. saltanensis* became infected with pedunculate oocysts and sporozoites. Mature oocysts were found 14 days after the infective blood meal and sporozoites in the salivary glands after 16 days. Twenty one out of the 100 *Cx. saltanensis* fed on the experimentally infected chicken (two months of infection) and 68 out of those 100 fed on the cock (16 months of infection) were dissected and, respectively, 17 (80.9%) and 55 (80.9%) were positive with both pedunculate oocysts and sporozoites. The other experimentally infected *Cx. saltanensis* alive were fed on five non-infected chicks and all of these became parasitized by *P. juxtanucleare*. Blood examination of these chicks was started 15 days after being bitten by the infected mosquitoes and these birds have been positive for 10 months. The presence of fertile gametocytes in these chicks was also confirmed by infecting another clean batch of *Cx. saltanensis*.

DISCUSSION

The ornithophilic behaviour of the mosquitoes belonging to genus *Culex* has been recorded for a long time. Although they bite other animals, including man, they show a clear preference for feeding on birds (Davis, 1944, 1945; Tempelis, 1975; Gabaldon et al., 1977b; Lourenço-de-Oliveira & Heyden, 1986; Forattini et al., 1987).

The presence of *Culex* mosquitoes in foci of *P. juxtanucleare* in Latin America was first verified in 1949 by Paraense. The author observed that *Cx. quinquefasciatus* was the only species collected with Shannon's trap using chicken as bait in Bambuí, Minas Gerais state, Brazil. Later, Krettli (1971) found that *Cx. quinquefasciatus* and *Cx. bidens* were the most common mosquitoes in a focus studied in Belo Horizonte, in the same state, using also chicken as bait. Gabaldon et al. (1977 a, b) also found a high frequency of *Culex (Culex)* biting birds in some areas in Venezuela, although these were not foci of *P. juxtanucleare* but of other malaria parasites belonging to subgenera *Giovannolaia* and *Novyella*.

According to our present observations, *Culex (Culex)* mosquitoes are the most frequent Culicidae in captures on chicken on a focus of *P. juxtanucleare* in Rio de Janeiro. Among the species of subgenus *Culex* we emphasize *Cx. saltanensis*, the most common on avian bait, with 188.5 specimens collected per 10 h and representing 41.7% of the total mosquitoes captured on chicken.

One may think of opportunism in view of the abundance of *Cx. saltanensis* biting chickens in Granjas Calábria because of the proximity of Cortado Canal to the poultry-yard where these animals rest during the night. However, this mosquito has never been found breeding in this waterway during two years of larva and pupa survey carried out by Lourenço-de-Oliveira et al. (1986). Its preferred breeding-places are transitory natural pools, from where its females come to feed on the sheltered chickens at night.

Although the natural vectors of *P. juxtanucleare* had been discovered 27 years ago in Malaysia (Bennett et al., 1963), they remained unknown outside Asia. In the present study we found, for the first time in the Americas, mosquitoes infected with *P. juxtanucleare*, and in one species – *Cx. saltanensis* – sporozoites detected in its salivary glands produced patent parasitemia when inoculated into a chick. Seven out of 377 dissected were found infected.

Other species have been found naturally infected with pedunculate oocysts in foci of *P. juxtanucleare*, but none with sporozoites in the salivary glands: one out of 155 *Cx. coronator* in the present study and one out of 17 *Cx. nigripalpus*, in a similar survey carried out by Lourenço-de-Oliveira & Castro (1989) in Campo Grande, Rio de Janeiro. These two species are not considered primary vectors of *P. juxtanucleare* in the two areas, since *Cx. saltanensis* was present in both of them and carrying sporozoites in Granjas Calábria. Lourenço-de-Oliveira & Castro (1989) also dissected 255 *Cx. quinquefasciatus*, all being negative.

Culex saltanensis showed to be highly susceptible to *P. juxtanucleare*. About 81% of the specimens became experimentally infected. The other mosquito species tested – *Ae. fluviatilis* and *Cx. quinquefasciatus* – were non-susceptible in the present study, but other workers did infect few specimens of the latter species (Paraense, 1944; Kretlli, 1972a).

In the former studies on mosquito ecology performed in Granjas Calábria using human bait, *Cx. saltanensis* was much more frequent in secondary residual forest but also fed in the open areas with anthropic modifications. It is essentially a night feeder in nature, starting to attack in larger numbers at sunset, sustaining a high biting activity during the first hours of the night and at sunrise. In more protected environments, such as in residual forest, *Cx. saltanensis* will also attack by the end of the morning and throughout the afternoon. Even in the open country it prefers to bite in nights of waning and waxing moons. Although it may be collected continuously during the whole year, its density increases in the spring – September, October and November – and with a smaller peak in December (Lourenço-de-Oliveira, 1984b; Lourenço-de-Oliveira et al.; 1985; Lourenço-de-Oliveira & Silva, 1985).

Considering its habits, we may surmise that in Granjas Calábria malaria due to *P. juxtanucleare* is transmitted by *Cx. saltanensis* chiefly during the first hours of the spring nights. Paraense (1949) had already suggested that transmission of this plasmodium would not occur from December to February (summer) because all 100 birds born in these months he examined in March, in Bambuí, Minas Gerais state, Brazil, were negative, while 21.3% of the older animals were positive.

It is known that several factors of ecological and physiological origin influence the ability of one mosquito species being or not a malaria carrier. A vector must be susceptible to the plasmodium species, must feed mainly on hosts of this hemoparasite and have a considerable density to mantain the transmission, beside longevity.

The results of the present field observations, in Granjas Calábria, show that *Cx. saltanensis* is there the primary vector of *P. juxtanucleare*, because it was found in considerable density, was highly ornithophilic and was detected carrying sporozoites in the salivary glands. *Cx. saltanensis* was able to transmit experimentally *P. juxtanucleare* to chicks by the bite and this, together with the field observations performed here, has provided overwhelming proof that it is a primary vector in the region. This mosquito has been recorded in Brazil (states of São Paulo, Rio de Janeiro and Bahia), in Argentina, Venezuela and Panamá (Lane, 1953; Bram, 1967; Cotrim et al., 1974; Lourenço-de-Oliveira, 1984b). Taking into consideration its wide geographic distribution in South America and part of Central America, we may suppose that this mosquito should have important role in *P. juxtanucleare* transmission in the Neotropical Region. Besides, *Cx. saltanensis* can also be infected with the sparrow malaria *P. (Haemamoeba) cathemerium* (Gabaldon et al., 1988).

The newly discovered vector of *P. juxtanucleare* is easily reared in the laboratory. Moreover, unlike other *Culex* species, it sucks blood indistinctly during the day and at night in artificial conditions, and has very peculiar "pepper-and-salt" gray eggs.

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