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FIRST ISOLATIONS OF ARBOVIRUSES FROM PHLEBOTOMINE SAND FLIES IN WEST AFRICA

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Abstract. For the first time in West Africa, arboviruses were isolated from phlebotomine sand fly pools. One strain of Chandipura virus (a Vesiculovirus), four strains of Saboya virus (a Flavivirus), and one strain of a not yet identified virus were isolated. Three hundred twenty-two pools were established from a population of 33,917 sand flies caught in CO_2 light traps in the Ferlo Sahelian region of Senegal from November 1991 to December 1992. This is the first isolation of Chandipura virus from any arthropod in Africa. Saboya virus has already been isolated from small rodents in Senegal; thus, its transmission cycle probably involves rodentophilic sand flies. No strain of Rift Valley fever phlebovirus, which caused an epizootic in this region in 1987, was isolated. During the same time at the same site, 11 sand fly species were identified from 4,191 specimens caught on sticky traps, including *Phlebotomus duboscqi,* a leishmaniasis vector.

African Rift Valley fever (RVF) virus was responsible for a severe epizootic and epidemic that killing nearly 220 people in 1987 in southern Mauritania.[1] No RVF virus was obtained from more than 490,000 mosquitoes collected in this area, which were suspected vectors after human cases were diagnosed. Since RVF virus is a Phlebovirus (Bunyaviridae), most of the phleboviruses known in the world were isolated from sand flies, and RVF virus can be experimentally transmitted by *Phlebotomus duboscqi,*[2] we investigated the possible natural transmission of RVF virus and other arboviruses by sand flies in Senegal. To date, the only viruses isolated from pools of phlebotomine sand flies in Africa are yellow fever virus in Uganda, Perinet virus in Madagascar, and sandfly fever Sicilian and sandfly fever Naples viruses in Egypt.[3–5] However, in subSaharian Africa, viruses naturally transmitted by phlebotomine sand flies are not associated with known human or animal pathology. This is the first study on virus transmission by sand flies in West Africa, although major research efforts have been conducted on the dipterans and the transmission of cutaneous leishmaniasis.[6]

MATERIALS AND METHODS

Study site

Sand flies were caught around temporary ground pools in the district of Barkedji in the rural Ferlo region of Senegal (15°17′N, 14°53′W) (Figure 1). This area is situated in the Sahelian shrubby savannah. Two ground pools were investigated: Niakha and Beliboda, which are situated 4 km west and 7 km southeast, respectively, of Barkedji village. Average annual rainfall in the study area since 1980 was approximately 300 mm, with 420 mm in 1991 and 285 mm in 1992. The dry season lasts from November to June. The monthly average minimum temperature ranges from 14°C to 22°C and the monthly average maximum temperature ranges from 30°C to 38°C. The temporary ground pools are flooded immediately after the first rains in July and dry up in January, February or March, depending on the pools.

Collection and identification of sand flies

To identify the species present in the area, phlebotomine sand flies were collected from May 1992 to December 1992 on sticky traps. Traps were made of a 20 cm × 20 cm sheet of paper covered on both sides with castor oil and placed at night on the openings of sand flies resting sites such as animal burrows, termite hills, or tree holes. The following morning, the sand flies were removed from the paper and preserved in 70% alcohol. They were mounted on slides using Canada balsam and were identified using the keys of Abonnenc (unpublished data) and Davidson.[7]

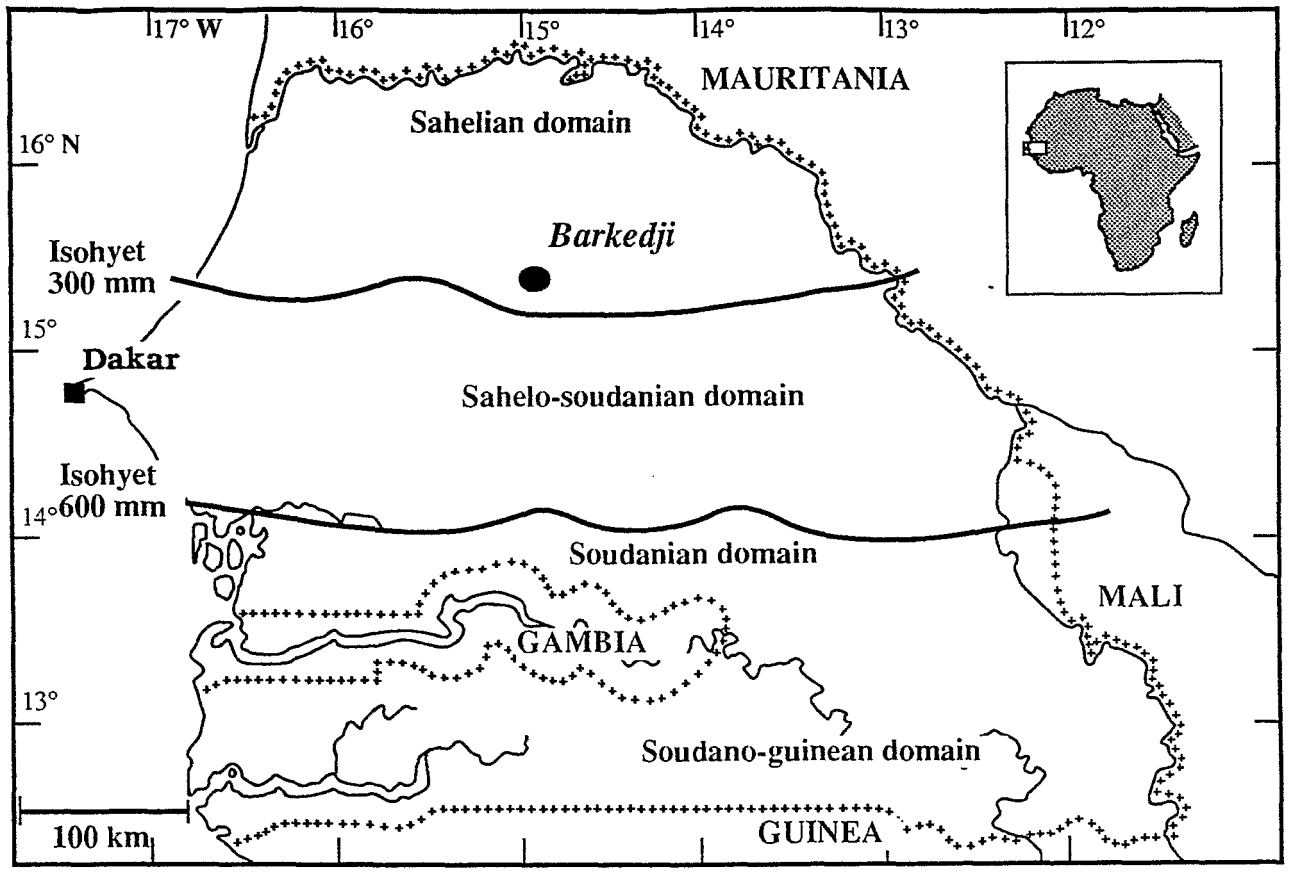

FIGURE 1. Map of Senegal showing the Barkedji study site.

For the arbovirus research, sand flies were caught in fine-mesh bag light traps (Centers for Disease Control and Prevention [CDC], Atlanta, GA) with dry ice (10–15 trap-nights each month) from November 1991 to December 1992. Traps were placed in front of the openings of sand flies resting sites. Sand flies were killed in dry ice the following morning, put in pools of 50–200 specimens, and placed in a cryotube in liquid nitrogen for further analysis.

Isolation and identification of arboviruses

Arbovirus isolations were conducted following the protocols of Sudia and Chamberlain with minor modifications.[8] Briefly, each pool was triturated in Hanks' albumin media, and after centrifugation, it was inoculated into newborn mice and into two continuous cell lines: Vero and AP 61 from *Aedes pseudoscutellaris*. Detection of viruses growing in cell culture was performed by immunofluorescent analysis of reference mouse immune ascitic fluid pools.[9] Identification of the viruses isolated on cell culture and/or suckling mice was made using the complement fixation (CF) technique and confirmation was done using a seroneutralization test.[10] Tests were performed at the World Health Organization Collaborating Center for Reference and Research on Arboviruses (CRORA) at the Pasteur Institut in Dakar.

RESULTS

Sand fly species

Eleven species were identified from 4,191 phlebotomine sand flies caught with sticky traps (Table 1). Densities of sand flies were much higher in Beliboda than in Niakha, with an average of 660 and 84 sand flies/m^2, respectively, for the entire survey. Captures with CDC light traps at both sites showed a peak of abundance in March and April, one month after the complete drying up of the temporary ground pools (Table 2). During these two months, an average of 661 sand flies per trap per night were caught, compared with 25 sand flies per trap per night at the end of the dry season and during the rainy season.

TABLE 1

Number and density of sand flies caught with sticky traps at two sites at Barkedji according to species, from May to December 1992

Species*	Niakha		Beliboda	
	No. of sand flies collected	Average no. of sand flies/m²	No. of sand flies collected	Average no. of sand flies/m²
Phlebotomus (Phlebotomus) duboscqi	31	1	1	0.4
Sergentomyia (Grassomyia) inermis	5	0.2	2	0.7
S. (Grassomyia) squamipleuris	19	0.7	4	1
S. (Sintonius) adleri	9	0.3	0	0
S. (Sintonius) clydei	386	14	193	71
S. (Sergentomyia) antennata	177	6	104	38
Sergentomyia (Sergentomyia) buxtoni	128	4	23	8
Sergentomyia (Sergentomyia) dubia	353	12	623	229
Sergentomyia antennata or *dubia* (males)†	445	16	692	254
Sergentomyia (Sergentomyia) schwetzi	722	26	123	45
Sergentomyia (Parrotomyia) magna	116	4	35	13
Total number of sand flies	2,391	84.2	1,800	660.1

* *S. ghesquierei* was found in the area in January 1993.
† Males of these two species are indistinguishable.[7]

Virus isolations

A total of 322 pools consisting of 33,917 male and female sand flies were inoculated into mice. Monthly distributions of the inoculation pools and of the viruses isolated are shown in Table 2. A strain of a not yet identified virus (Ar D 88909), which was sensitive to chloroform, was isolated after inoculation into newborn mice only. This isolate was obtained from a pool of 200 sand flies caught in March 1992 in front of a termite hill in Niakha. The newborn mice were paralyzed at day 2, after two passages. In April 1992, 85 pools were formed. One strain (ArD 89384) of Chandipura virus was isolated from a pool of 125 sand flies and four strains (ArD 89351, ArD 89363, ArD 89378, and ArD 89394) of Saboya virus were isolated from pools of 120–134 sand flies. All of these sand flies were caught in Beliboda. Chandipura virus grew well in cell culture and had a cytopathic effect within 24 hr and killed suckling mice in 1–2 days. Saboya virus was isolated only from suckling mice. Table 3 shows the CF identification test results of these viruses, which were confirmed by cross-neutralization tests.

TABLE 2

Number of phlebotomine sand flies caught with light traps in Barkedji (Niakha and Beliboda) on a monthly basis from November 1991 to December 1992, and strains of viruses isolated

Month of capture	No. of pools	No. of sand flies	Virus isolated*	No. of strains
November 1991	25	1,545	–	0
December 1991	31	1,825	–	0
January 1992	70	6,931	–	0
February 1992	6	751	–	0
March 1992	67	8,940	PDI D 88909	1
April 1992	85	10,007	Saboya	4
			Chandipura	1
May 1992	2	295		0
from June to September 1992	0	0	–	0
October 1992	5	465	–	0
November 1992	22	2,610	–	0
December 1992	9	548	–	0
Total	322	33,917		6

* – = None.

DISCUSSION

To date, 29 species of phlebotomine sand flies from Senegal have been identified.[11] While little information is available on the feeding preferences of African sand flies, five of the 11 species collected in the Barkedji region were observed feeding on mammals: *P. duboscqi*, the vector of cutaneous leishmaniasis in Senegal, *Sergentomyia adleri, S. clydei, S. magna*, and *S. schwetzi* (Abonnenc E, unpublished data).[12,13] The six other species are known to feed preferentially on amphibians and/or reptiles as reported by Abonnenc (unpublished data). The viral strains were isolated from 150 pools during the dry season (March and April) when the peak of sand fly abundance occurs. However, no virus was isolated from 170 pools of sand flies collected during the other months. Because it is usually accepted that vertical transmission of arboviruses is frequent in sand flies, males and females were not separated in the present experiment.[14] Since there is no identification key based on external characteristics for African sand flies, all pools were polyspecific.

Chandipura virus, described by Bhatt and Rodrigues in 1967,[15] is a member of the family *Rhabdoviridae* of the vesicular stomatitis virus group of the genus *Vesiculovirus*. It was first isolated in India from patients with febrile illness and from a fatal case in a child with an encephalitic syndrome. It was also found in a pool of 253 unidentified phlebotomine sand flies (*Phlebotomus* sp.) caught in human dwellings and cowsheds in the Maharashtra State of India.[16] Transovarial transmission of Chandipura virus was demonstrated in *P. papatasi;* 8% of the F_1 offspring of intrathoracically infected females were infected with the virus.[17] Experimental transmission from mouse to mouse was obtained with *Aedes aegypti, Ae. albopictus, Anopheles stephensi*, and *Culex tritaeniorhynchus*.[18] In Africa, Chandipura virus has been isolated in Nigeria from hedgehogs (*Atelerix spiculus*)[19] and from humans (Virus Research Laboratory, University of Ibadan, Nigeria, eighth annual report, 1971—1972, unpublished data). The Senegalese strain is the first isolation of Chandipura virus from arthropods in Africa. Perinet virus, another *Vesiculovirus*, was isolated on Madagascar from a sand fly identified as *Sergentomyia berentiensis* by Clerc and others.[5]

Saboya, a Flavivirus, was first isolated in 1968 from a gerbil (*Tatera kempi*) in Saboya village in Senegal[20] and then recovered from other rodent species (*Mastomys* sp., *Arvicantis niloticus*, and *Mus musculus*), especially in the Sahelian region. Two strains were also obtained from *Ae. vittatus* and one was obtained from *Ae. africanus* sp. in the Central African Republic in 1981 (Institut Pasteur, Dakar, Senegal, unpublished data). The isolation of Saboya virus from sand flies strongly suggests a transmission cycle between rodentophilic sand flies and small rodents, of which at least five species are present in the Barkedji area (Duplantier JM and others, unpublished data). Disease associated with Saboya virus in humans or animals is unknown.

TABLE 3

*Antigenic relationships of ArD 89384 virus with Chandipura (IbAn9978) and ArD 89351 virus with Saboya (AnD 4600) by complement-fixation (CF) test**

Virus	Titer of antibody to virus			
	ArD 89384	Chandipura	ArD 89351	Saboya
ArD 89384	512	256	–	–
Chandipura	256	128	–	–
ArD 89351	–	–	2,048	2,408
Saboya	–	–	1,024	512

* The CF results were confirmed by cross-neutralization test. – = not tested.

The third virus isolated (ArD 88909) has not yet been identified. It is different from the only two Phleboviruses previously isolated from *Culicidae* in east and central Africa (Perinet and Arumowot), from Phleboviruses recovered from rodents in Africa (Arumowot, Gabek Forest, Gordil, and Saint Floris), and from 80 other African arboviruses registered at the CRORA.

Although we failed to isolate RVF virus from 322 pools of sand flies, the study site is being closely monitored for RVF virus transmission. The recent RVF outbreak that occurred during the dry season in the Aswan Gouvernorate in Egypt after a 13-year interepizootic period (Arthur RR, unpublished data) should stimulate studies on vectors other than mosquitoes for virus maintenance.

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