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Arbovirus Surveillance from 1990 to 1995 in the Barkedji Area (Ferlo) of Senegal, a Possible Natural Focus of Rift Valley Fever Virus

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ABSTRACT Surveillance for mosquito-borne viruses was conducted in Barkedji area from 1990 to 1995, following an outbreak of Rift Valley fever (RVF) virus in southern Mauritania. Mosquitoes, sand flies, and midges were collected from human bait and trapped by solid-state U.S. Army battery-powered CDC miniature light traps baited with dry ice or animals (sheep or chickens) at four ponds. Overall, 237,091 male and female mosquitoes representing 52 species in eight genera, 214,967 Phlebotomine sand flies, and 2,527 *Culicoides* were collected, identified, and tested for arboviruses in 9,490 pools (7,050 pools of female and 331 of male mosquitoes, 2,059 pools of sand flies and 50 pools of *Culicoides*). Viruses isolated included one Alphavirus, Babanki (BBK); six Flaviviruses, Bagaza (BAG), Ar D 65239, Wesselsbron (WSL), West Nile (WN), Koutango (KOU), Saboya (SAB); two Bunyavirus, Bunyamwera (BUN) and Ngari (NRI); two Phleboviruses, Rift Valley fever (RVF) and Gabek Forest (GF); one Orbivirus, Ar D 66707 (Sanar); one Rhabdovirus, Chandipura (CHP); and one unclassified virus, Ar D 95537. Based on repeated isolations, high field infection rates and abundance, *Culex* appeared to be the vectors of BAG, BBK, Ar D 65239 (BAG-like), and WN viruses, *Ae. vexans* and *Ae. ochraceus* of RVF virus, *Mansonia* of WN and BAG viruses, *Mimomyia* of WN and BAG viruses, and Phlebotomine of SAB, CHP, Ar D 95537, and GF viruses. Our data indicate that RVF virus circulated repeatedly in the Barkedji area.

KEY WORDS *Aedes, Culex*, Rift Valley fever, arboviruses, Barkedji, Senegal

RIFT VALLEY FEVER is an acute viral illness of humans and domestic ungulates. Human disease initially is characterized by an abrupt onset of high fever, severe headache, myalgia, conjunctival infection, and prostration for several days (Peters and Meegan 1981, Meegan and Bailey 1988). Some patients develop fatal hemorrhagic fever or complications such as encephalitis or ocular disease (primarily retinal vasculitis). Rift Valley fever virus also causes severe disease in domestic ungulates, particularly sheep and cattle (Easterday 1965, Shimshony and Barzilai 1983). Such infection often induces fetal abortion and frequently is fatal to young animals.

Transmission of Rift Valley fever virus (RVF, Phlebovirus: Bunyaviridae) has been documented in at least 24 countries throughout Africa (Easterday 1965, Peters and Meegan 1981, Shimshony and Barzilai 1983, Meegan and Bailey 1988). Even where endemic, high rates of transmission occur sporadically in time and space, and epidemics and epizootics have been recognized since the 1930s in eastern Africa (Davies 1975, Meegan 1981) and since the 1950s in southern Africa (Easterday 1965, Shimshony and Barzilai 1983). In West Africa, large-scale RVF outbreaks were not reported before the 1987 epidemic in southern Mauritania (Digoutte and Peters 1989, Jouan et al. 1989, Ksiazek et al. 1989), despite virologic and serologic evidence of previous virus circulation (Saluzzo et al. 1987), in Nigeria (Fagbami et al. 1973), Burkina Faso (Saluzzo et al. 1984), Guinea (Meegan et al. 1983), Mali (Findlay et al. 1939), and Senegal (Meegan et al. 1983, Guillaud et al. 1988). The 1987 Mauritanian epidemic demonstrated the potential of RVF virus to cause devastating epidemics in West Africa and raised new questions about ecological factors important for transmission in that environment.

Mosquitoes are the main RVF vectors to animals and humans (Meegan et al. 1980, McIntosh and Jupp 1981, McIntosh et al. 1983), although human infection by contact also may result during animal slaughter, food preparation, and necropsy or laboratory experimentation (Smithburn et al. 1949, Gear et al. 1951, Van Velden et al. 1977). Potential vectors include >30 African mosquito species from which RVF virus has been isolated (Meegan and Bailey 1988). Experimental and field studies indicate that certain flood water *Aedes* emerging from temporary ponds after heavy rains are important in maintenance and are enzootic vectors (Linthicum et al. 1984a, Davies et al. 1985). In East Africa, epizootic transmission is correlated with

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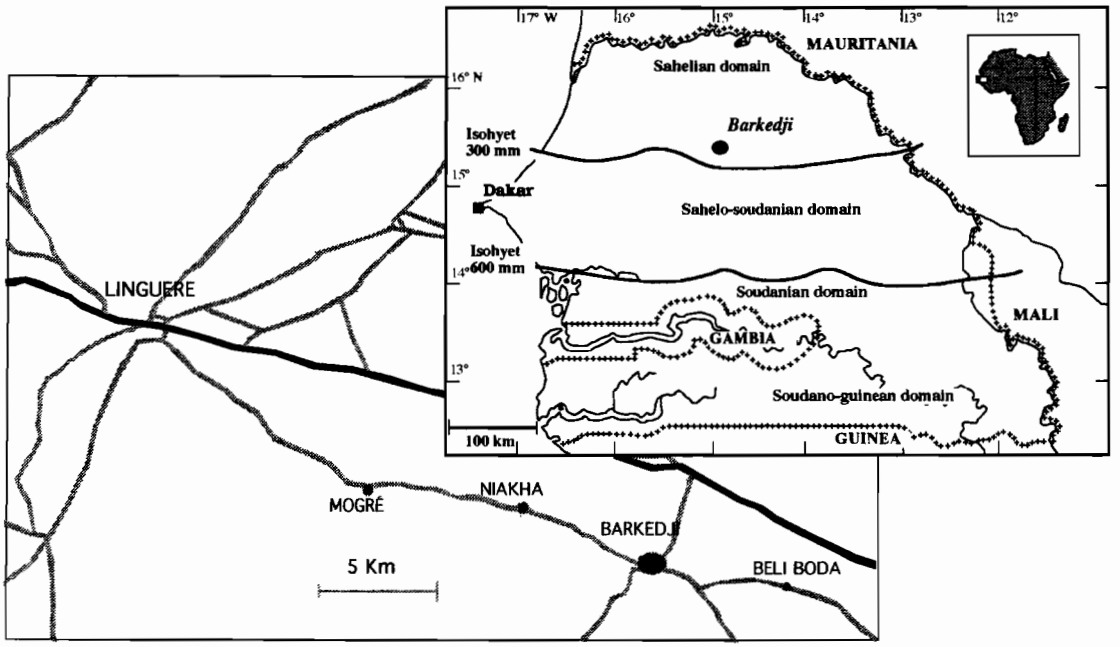

Fig. 1. Map of Senegal with location of Barkedji and mosquito collection sites.

heavy rainfall and the presence of infected *Culex* (McIntosh 1972, Meegan et al. 1980, McIntosh et al. 1983). Transmission to humans depends on species that frequently bite people (Hoogstraal et al. 1979).

After the 1987 RVF epidemic in southern Mauritania, entomological studies were done in western Senegal near Saint-Louis in the Senegal River delta to investigate the enzootic activity of RVF virus; however, RVF virus was not isolated from almost 500,000 mosquitoes tested for infection. In October 1990, a similar study was undertaken in central Senegal (Barkedji area, Ferlo), where temporary ponds similar to the "*dambos*" found in East Africa occur (Ackermann 1936, Linthicum et al. 1983). In addition to mosquito populations, Phlebotomine sand flies also were tested because RVF virus is a Phlebovirus. Most other Phleboviruses have been associated with Phlebotomine sand flies, and *Phlebotomus duboscqi* Neveu-Lemaire has transmitted RVF virus experimentally (Turell and Perkins 1990).

The current report describes the results of the Barkedji arbovirus survey from October 1990 to December 1995. Our specific goal was to identify the vectors of RVF virus and define their importance in virus transmission, persistence, and amplification. Other arboviruses isolated and their relation to human health was presented.

Materials and Methods

Study Area. Our investigation was conducted in north central Senegal within the village of Barkedji (15° 17 N, 14° 17 W) from October 1990 to December 1995. The region, identified as the Ferlo, is predominantly a grass, thornbush habitat classified as Sahelian savannah (Fig. 1). Annual rainfall averages 500 mm, but varies considerably from year to year, and occurs mainly during July–September (Ndiaye 1983). During the typical 9-mo dry season, no standing water is available and a large well serves as the primary water source for people and animals within a 25-km radius. In addition to the permanent residents of the Barkedji village, nomadic Mauritanian herders usually migrate through the region each year, moving tens of thousands of domestic ungulates southward during the dry season and returning northward during the rainy season (Mbow 1983, Toure and Arpaillange 1986).

Residents grew millet during the rainy season and herded sheep, goats, and cattle year-round. Animal husbandry practices varied seasonally. During the rainy season, when forage and standing water were readily available, herds were kept in close proximity ($\leq$5 km) to their compounds. As the dry season progressed, local forage became sparse and animals were driven over increasingly greater distances, often covering 10–30 km/day. Movement generally was restricted by the distribution of wells; family groups and their herds may become seasonal nomads, temporarily relocating for a few months during which they use grass huts for shelter (Toure and Arpaillange 1986).

Mosquito and Phlebotomine Collection. The variety and seasonal abundance of mosquitoes were studied during 16–19 October and 25–30 November 1990, five nights per month in 1991, and three nights per month from 1992 to 1995. Mosquitoes were collected at the edge of three temporary ponds: Niakha (4 km W from Barkedji), Beli Boda (7 km SE) and near Barkedji village (Fig. 1). In 1993, Maugre pond was

added, because this site remained flooded 1 or 2 mo after the other three ponds dried. Mosquitoes were collected from human bait from 1730 to 2230 hours. Five U.S. Army battery-powered CDC miniature light traps baited with dry ice and animals (one sheep or five chickens) were operated from 1900 to 0600 hours. Traps operated over animal bait ran intermittently (1 min light on, 7 min light off) (H.J.-P., unpublished data).

Mosquitoes were killed by freezing at −18°C, sorted to species using available identification keys (Edwards 1941, Harbach 1988), and pooled on an chill table by species, sex, location, date, and trap or capture method. Pools containing 30–50 mosquitoes were stored in liquid nitrogen. After identification, abdomens from engorged females were removed for later blood meal identification studies, and the head/thorax was used for virus isolation attempts.

Two methods were used for Phlebotomine collection from May 1992 to December 1995. Sticky traps made of a 20 by 20-cm sheet of paper and covered on both sides with castor oil were placed from 1800 to 0700 hours at the openings to sand fly resting sites such as animal burrows, termite hills, or tree holes. Sand flies were removed from the paper, preserved in 70% alcohol, mounted on slides using Canada basalm, and identified using the key of Abonnenc and Minter (1965) and Davidson (1990). For arbovirus isolation attempts, sand flies were collected from November 1991 to December 1995, in fine-mesh bag CDC light traps baited with dry ice (10 trap-nights each month), placed at sand fly resting places. Sand flies were killed on dry ice the following morning, and pools containing 50–200 specimens were stored in liquid nitrogen.

Mosquito Dissection. *Aedes vexans* were age-graded by counting the number of dilatations on a sample of ovariole tubules, with parity in unfed females confirmed by the degree of tracheole coiling in one ovary (Detinova 1962).

Human Blood Sampling. Blood samples from two staff members who became febrile on their way back from the field station in November 1990 were tested for virus and antibody.

Virus Testing. All pools were stored at −70°C. Mosquitoes and sand flies were tested for arboviruses by cell culture or suckling mice (Digoutte et al. 1992). Pools were triturated in chilled tissue-grinders with 1 ml of Hank's balanced salt solution. Suspensions were clarified by centrifugation, inoculated into *Aedes pseudoscutellaris* (AP-61) cell cultures, and screened for virus after 7–8 d by immunofluorescence assay, using various monovalent and polyvalent hyperimmune mouse ascitic fluids (Digoutte et al. 1992). Presumptively positive pools were confirmed by inoculation into suckling mice. Isolates from mouse brain tissue were identified by complement fixation and neutralization tests. Pools of phlebotomines also were triturated in Hank's albumin media, centrifuged, and inoculated into suckling mice, Vero and AP-61 continuous cell lines. Viruses were detected by immunofluorescent assay with mouse immune ascitic fluid pools. Viruses isolated on cell culture or in suckling mice were identified by complement fixation (CF) and confirmed by seroneutralization at the World Health Organization Collaborating Center for Reference and Research on Arboviruses at the Pasteur Institute in Dakar (Center Collaborateur OMS de reference et de Recherche pour les Arbovirus, CRORA). Positive pools and virus isolates were stored at the CRORA. Selected virus identifications were confirmed by the U.S. Communicable Disease Center.

Results

Weather Patterns. Average monthly air temperature peaked in May during1990–1995; the lowest temperatures were observed in December and January and then increased progressively. Seasonal patterns of precipitation from May to October varied markedly among years. Maximum rainfall was observed in August, except in 1992 (September). Annual rainfall was 681.3 mm in 1990, 214.7 mm in 1991, 346.4 mm in 1992, 335.2 mm in 1993, 300.6 mm in 1994, and 404.6 mm in 1995.

Mosquito Collections. Overall, 237,091 mosquitoes, including 227,490 females and 9,601 males, belonging to eight genera and 50 species or species groups (Table 1), and 217,494 other Diptera, consisting of 214,967 Phlebotomines and 2,527 Culicoides (Table 1), were collected, identified, and tested for virus in 9,490 pools (7,050 pools of female and 331 pools of male mosquitoes, 2,059 pools of sand flies, and 50 pools of *Culicoides*). *Culex* mosquitoes represented 46.6% of the total mosquitoes collected, followed by *Aedes* 20.3%, *Mimomyia* 16.3%, *Mansonia* 8.0%, *Aedeomyia* 4.7%, *Anopheles* 4.0%, *Uranotaenia* 0.1% and *Ficalbia* <0.1%. Fourteen species of *Aedes* were identified: six belonging to the subgenus *Aedimorphus* comprised 93.7% of the total *Aedes* captured; four species were *Stegomyia;* and one each were *Diceromyia, Mucidus,* and *Neomelaniconion.* Twelve species were *Culex,* with 11 in the subgenus *Culex* (98.2% of the total specimens) and one in *Lutzia;* 11 species were *Anopheles,* with two species in the subgenus *Anopheles* and nine in *Cellia* (64.1%), six species were *Mimomyia,* three *Uranotaenia* and two *Mansonia. Culex poicilipes* Theobald represented 34.8% of the total mosquitoes collected, and 74.7% of the total *Culex, Aedes vexans* Meigen 17.3 and 85.2%, *Mimomyia splendens* Theobald 14.5 and 89.1%, *Mansonia africana* Theobald 5.2 and 64.8%, respectively. All other species represented <5% (Table 1).

Aedes dominated mosquito collections during the early part of the rainy season, and *Ae. vexans* was most abundant at that time, followed by *Culex (poicilipes), Anopheles (An. ziemanni), Mansonia, Mimomyia,* and *Aedeomyia.*

The parity rate of *Aedes vexans* increased from 1% (two parous females of 297 dissected) in July, to 12% (287/2,388) in August, to 78% (413/529) in September, to 83% (985/1,187) in October, and to 96% (34/38) in November, after which this species disappeared. The occurrence of only one *Aedes* generation per year is an important observation with respect to

Table 1. Female mosquitoes species collected and virus isolated in Barkedji arbovirus surveillance in 1990 to 1995

Species	1990		1991		1992	
	No. collected	Virus isolated	No. collected	Virus isolated	No. collected	Virus isolated
Anopheles coustani	0	0	0	0	45	0
Anopheles ziemanni	1,155	0	459	0	1,661	1 BAG
Anophele sp.	35	0	73	0	0	0
Anopheles domicola	0	0	1	0	0	0
Anopheles flavicosta	0	0	0	0	1	0
Anopheles freetownensis	0	0	0	0	0	0
Anopheles gambiae	0	0	118	0	58	0
Anopheles hancocki	0	0	1	0	0	0
Anopheles pharoensis	181	0	441	0	384	0
Anopheles pretoriensis	5	0	0	0	0	0
Anopheles rufipes	51	0	76	0	31	0
Anopheles squamosus	0	0		0	4	0
Aedeomyia africana	963	1 BGA, 1 WN-BAG	793	0	3,133	0
Aedes argenteopunctatus	0	0	0	0	8	0
Aedes dalzieli	0	0	0	0	0	0
Aedes fowleri	0	0	0	0	2	0
Aedes minutus	0	0	26	0	53	0
Aedes ochraceus	3	0	282	0	634	1 WSL
Aedes vexans	245	0	10,247	0	4,204	0
Aedes vittatus	0	0	0	0	1	0
Aedes furcifer	0	0	0	0	125	0
Aedes sudanensis	5	0	344	0	252	0
Aedes mcintoshi	0	0	281	0	258	0
Aedes aegypti	0	0	0	0	10	0
Aedes luteocephalus	0	0	0	0	0	0
Aedes metallicus	0	0	17	0	101	0
Aedes unilineatus	0	0	36	0	134	0
Culex antennatus	24	0	1,177	0	478	0
Culex bitaeniorhynchus	335	0	216	0	178	0
Culex decens	1	0	17	0	2	0
Culex ethiopicus	62	0	641	1 WN	213	0
Culex gr, ethiopicus	0	0	46	0	0	0
Culex neavei	199	3 WN 1BGA	756	1 BBK, 1 WN	1,946	3 BAG, 3 WN, 1 KOU
Culex gr, sitiens	37	0	0	0	0	0
Culex perfuscus	2	0	134	0	239	1 BAG
Culex poicilipes	21,668	5 BGA, 23 WN, 16 WN-BGA	7,363	1 WN	11,151	4 BAG, 1 NRI
Culex quinquefasciatus	0	0	339	0	0	0
Culex tritaeniorynchus	98	0	137	0	1,242	0
Culex univittatus	77	0	313	0	25	0
Culex sp.	0	0	296	0	0	0
Culex tigripes	0	0	0	0	15	0
Ficalbia uniformis	0	0	0	0	0	0
Mansonia africana	225	0	1,559	0	4,524	4 BUN
Mansonia uniformis	192	0	156	0	703	1 BUN
Mansonia sp.	0	0	114	0	0	0
Mimomyia mediolineata	0	0	1	0	0	0
Mimomyia hispida	1,195	1 BBK, 9 WN	615	0	0	0
Mimomyia lacustris	767	4 WN	341	0	0	0
Mimomyia mimomyiafor	0	0	0	0	51	0
Mimomyia plumosa	0	0	0	0	62	0
Mimomyia splendens	3,789	1 BBK, 3 WN	7,05	1 WN	8,446	0
Mimomyia sp.	136	2 WN	0	0	3	0
Uranotaenia sp.	6	0	48	1 BBK	2	0
Uranotaenia balfouri	2	0	31	0	0	0
Uranotaenia bilineata	39	0	12	0	0	0
Uranotaenia mayeri	0	0	0	0	0	0
Phlebotome sp.	0	0	3,37	0	30,547	0
Phlebotomus dubosqi	0	0	0	0	0	0
Ceratopogonide sp	0	0	15	0	170	0
Total	31,497	1 BBK, 8 BGA, 44 WN, 17 WN-BGA	37,942	2 BBK, 4WN	40,379	9 BAG, 3 WN, 1 WSL, 4 SAB, 5 BUN, 1 NRI, 1 CHP

Table 1. Continued.

1993		1994		1995		Total no. collected
No. collected	Virus isolated	No. collected	Virus isolated	No. collected	Virus isolated	
2	0	0	0	0	0	47
1,416	0	596	0	800	0	6,087
0	0	0	0	0	0	108
0	0	0	0	0	0	1
0	0	0	0	0	0	1
0	0	1	0	0	0	1
334	0	130	0	265	0	943
0	0	0	0	0	0	1
240	0	266	0	231	0	1,745
0	0	1	1 NRI	0	0	6
341	0	87	0	17	0	606
3	0	9	0	2	0	18
3,979	1 BBK	609	1 ArDp,BAG	646	0	11,248
24	0	10	0	18	0	60
10	0	24	0	29	0	63
19	0	9	0	10	0	40
115	0	34	0	50	0	278
1,169	3 RVF	1,069	0	302	0	3,578
4,174	2 WN, 10 RVF	6,971	0	14,953	0	40,991
0	0	0	0	0	0	1
139	0	129	0	82	0	502
184	0	66	0	307	0	1,167
21	0	58	1 NRI	78	0	706
9	0	1	0	3	0	33
1	0	10	0	6	0	17
59	0	93	0	22	0	292
64	0	43	0	16	0	296
482	1 BBK	298	1 ArDp,BAG, 1 ArDp,BAG-BBK	1,085	0	3,544
343	0	44	0	246	0	1,361
2	0	0	0	0	0	22
1,669	1 WN, 1 WN-BAG	2,037	0	1,478	0	6,689
0	0	0	0	0	0	46
3,602	9 BBK, 5 BAG, 49 ArD65238, 3 WN, 4 WN-BBK	950	8 ArDp, BAG, 1 WN, 1 WN-ArDp,BAG	1,409	0	9,226
0	0	0	0	0	0	37
330	2 ArD65239 1 WN, WN-BAG	59	4 ArD66707	140	0	904
28,63	1 BBK, 17 BAG, 60 ArD65239, 10 WN	7,014	1 ArDp,BAG, 4 WN	6,737	082,563	
291	0	0	0	0	0	630
182	0	360	0	1,572	0	3,591
602	4 WN, WN-BBK	3	0	0	0	1,02
492	3 BBK	0	0	2	0	790
5	0	2	0	1	0	23
6	0	0	0	0	0	6
4,634	2 ArD65239, 1 WN	5,65	0	474	0	12,293
925	1 ArD65239, 1 WN	140	0	3,026	0	5,473
219	0	0	0	0	0	1,216
0	0	0	0	0	0	1
18	0	0	0	6	0	1,834
0	0	0	0	119	0	1,227
72	0	17	0	618	0	824
63	0	0	0	6	0	176
9,716	0	724	0	4,336	0	34,336
0	0	0	0	0	0	56
0	0	0	0	0	0	176
111	0	7	0	25	0	51
0	0	0	0	0	0	
0	0	1	0	0	0	1
72,014	41 SAB, 4 ArD95737	42,736	21 SAB, 1GF, 2 CHP	66,145	8 SAB, 4 GF, 4 CHP, 6 ArD9573	214,812
0	0	0	0	155		155
1,105	0	1,171	0	66		2,527
60,153	15 BBK, 2 BAG, 114 ArD65239 23 WN, 41 SAB, 2 WN-BAG 7 WN-BBK, 4 ArD95737, 13 RVF	21,951	11 ArDp,BAG, 5 WN, 21 SAB 2 NRI, 1 GF, 4 ArD66707, 2 CHP, 1 WN-ArDp,BAG 1 ArDp,BAG-BBK	39,117	28 SAB, 4 GF 4 CHP, 6 ArD95737	454,585

Table 2. Viruses isolated from Diptera collected in Barkedji area, 1990–1995

Genera	Virus	1990	1991	1992	1993	1994	1995	Total
Alphavirus	Babanki	1	2	0	15	0	0	18
Flavivirus	Bagaza	8	0	9	22	0	0	39
	Ar D 65239 (Bagaza-like)	0	0	0	114	11	0	125
	Wesselsbron	0	0	1	0	0	0	1
	West Nile	44	4	3	23	5	0	79
	Koutango	0	0	1	0	0	0	1
	Saboya	0	0	4	41	21	28	94
Bunyavirus	Buniamwera	0	0	5	0	0	0	5
	Ngari	0	0	1	0	2	0	3
Phlebovirus	Gabek Forest	0	0	0	0	1	4	5
	RVF-Zinga	0	0	0	13	0	0	13
Orbivirus	Ar D 66707 (Sanar)	0	0	0	0	4	0	4
Rabdovirus	Chandipura	0	0	1	0	2	4	7
Unclassified	Ar D 95737	0	0	0	4	1	6	11
Mixed infections	Ar D 63239 (BAG-like)-BBK	0	0	0	0	1	0	1
	West Nile-Ar D 65239 (BAG-like)	0	0	0	0	1	0	1
	West Nile-Bagaza	17	0	0	2	0	0	19
	West Nile-Babanki	0	0	0	7	0	0	7
	Total	70	6	25	241	49	42	433

Ar D p. BAG = Ar D 54239 (Bagaza-like).

determining sampling strategies for mosquitoes during RVF virus investigations.

Virus Isolation. Overall, 433 isolates composed of 14 viruses were made from 9,490 pools of female Diptera (Table 2). The viruses isolated included the following: one Alphavirus, Babanki (BBK); 6 Flaviviruses, Bagaza (BAG), Ar D 65239, Wesselsbron (WSL), West Nile (WN), Koutango (KOU), Saboya (SAB); two Bunyaviruses, Bunyamwera (BUN) and Ngari (NRI); two Phleboviruses, Rift Valley Fever (RVF) and Gabek Forest (GF); one Orbivirus, Ar D 66707 (Sanar); one Rhabdovirus, Chandipura (CHP) and one unclassified virus, Ar D 95537. Of these, 261 were obtained from *Culex* (60.3% of the total number of isolates), 117 from Phlebotomines (27%), 21 from *Mimimyia* (4.8%), 17 from *Aedes* (3.9%), 10 from *Mansonia* (2.3%), four from *Aedeomyia* (0.9%), two from *Anopheles* (0.5%), and one from *Uranotaenia* (0.2%). WN virus also was isolated from the serum of one of two febrile staff tested, and antibodies to WN virus were detected in the serum of the second.

Overall, 160 (37% of all virus isolates) were made from mosquitoes collected around Niakha pond, 136 (31%) in Beli Boda, 121 (28%) in Mogre, and 16 (4%) in Barkedji. When tested by chi-square, there were no significant differences between Niakha and Beli Boda, Beli Boda and Maugré; but there were significant differences between Niakha and Maugré, Niakha and Barkedji, Beli Boda and Barkedji, and Maugré and Barkedji.

Most virus isolates (169) were made during October (39% of total isolates), followed by 118 in November (27.2%), 49 in March (11.3%), 28 in February (6.4%), 25 in April (5.7%), 16 in January (3.7%), 13 in September (3.0%), 12 in December (2.8%), three in July, and one in August (>1%) (Table 3).

Discussion

Our main objective was to extend our knowledge of the presence of Rift Valley fever virus in this possible natural focus in Senegal and to identify factors that trigger epidemics or epizootics. We also revealed the exposure of humans and animals to additional Alphavirus, Flavivirus, Bunyavirus, Phlebovirus, Orbivirus, Rhabdovirus, and an unidentified virus.

Mosquito Collection. From October 1990 to December 1995, a combination of different trapping techniques was used to survey the mosquito fauna. Mosquito prevalence was influenced by the proximity of suitable larval habitats, because practically all species collected develop in temporary surface pools.

Mosquito populations of all species were most abundant during 1993, followed by 1992, 1995, and then 1991, 1990, and 1994. *Aedes* were the first species collected after flooding, followed successively by *Anopheles, Culex, Mansonia, Mimomyia, Aedeomyia,* and *Uranotaenia. Aedes (Aedimorphus)* emerged after a single flooding of the eggs in the soil near ponds. There was no indication that water fluctuation significantly contributed to population levels in any of the *Aedes* ssp., and there was no recurrence of *Aedes* following their rapid population increase and decline, indicating the emergence of one generation.

The abundance of *Culex, Mansonia, Mimomyia, Aedeomyia,* and *Uranotaenia* peaked about 1 mo after the *Aedes.* The gradual increase in population levels, indicated that these species may have produced multiple generations. In 1995, for example, *Ae. vexans* appeared 4 d after the first rain, whereas the first *Culex, Mansonia, Mimomyia,* and *Aedeomyia* were collected 10 d thereafter, and the first *Uranotaenia* after 3 mo. These observations indicated rapid recolonization by dispersing females. Phlebotomines were collected 4 mo after the first *Aedes* mosquitoes.

Virus Isolation from Mosquitoes. RVF virus (Daubney et al. 1931) was isolated from mosquitoes collected in October and November 1993 and detected by the seroconversion of a sentinel sheep (Z.H.G., unpublished data). In Senegal, RVF virus previously was isolated from *Ae. dalzieli* (Theobald) and was described as Zinga virus (Ar B 1276) by Digoutte et al.

Table 3. Number of virus strains isolated monthly in Barkedji area

Year	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1990										8 BAG, 33 WN 12 WN-BAG, 1 BBK	11 WN, 5 WN-BAG	
1991		2 BBK									4 WN	
1992				4 SAB					1 WSL	1 KOU, 1NRI	9 BAG, 3 WN	5 BUN, 1 CHP
1993	4 Ar D 95737, 3 SAB		35 SAB						3 BAG, 6 WN 1 Ar D p. BAG	19 BAG, 9 WN, 10 RVF 72 AR D p. BAG	41 Ar D p. BAG, 15 BBK, 3 RVF 8 WN, 2 WN-BGA, 7 WN-BBK	3 SAB
1994	2 Ar D p. BGA, 1 WN2 SAB, 1 Ar D 95737	4 SAB	12 SAB 2 CHP	3 DAB 1 GF			3 Ar D 66707	1 NRI	2 WN	1 WN, 1 NRI 1 BBK-Ar D 54239	8 Ar D p. BAG, 1 Ar D 66707 1 WN-Ar D p. BAG	1 WN 1 Ar D p. BAG
1995	2 SAB, 1 CHP	15 SAB 4 GF 3 Ar D 95737		11 SAB, 3 CHP 2 Ar D 95737								
No. strains	16	28	49	24			3	1	13	168	118	12

(1974). Meegan et al. (1983) later showed that Zinga and RVF virus were the same virus. Before 1993, four strains of RVF virus were isolated from *Ae. dalzieli* from the Kedougou area, one in 1974 and three in 1983, indicating that RVF virus was present in Senegal (Digoutte et al. 1992).

RVF vectors in the Barkedji area were *Ae. vexans* and *Ae. ochraceus* (Theobald) (Fontenille et al. 1995, 1998). In the current study, seven isolates were made from *Ae. vexans;* minimal field infection rates (per 1,000 mosquitoes tested) were 5.89 for *Ae. vexans* and 3.0 from *Ae. ochraceus* (minimal field infection rates, 3.52) in October 1993 at Mogre pond. In November, RVF virus was isolated from *Ae. vexans* at Mogre, Beli Boda, and Niakha ponds and from a sheep near Barkedji ponds, indicating local dispersal at the end of the 1993 rainy season. *Ae. mcintoshi* (Huang), one of the main vectors of RVF virus in East Africa, has not been found infected in Senegal.

Many mosquitoes (*Aedes, Culex,* and *Eretmapodites*) transmit the RVF virus and are infected naturally (Turell and Bailey 1987, Turell and Perkins 1990, Turell et al. 1990, Turell and Cynthia 1991). *Aedes* (*Aedimorphus* and *Neomelaniconion*) are considered the main vectors. Isolations of the virus from *Ae. mcintoshi* males and females reared from larvae and never blood fed as adults provided strong evidence for vertical transmission (Logan et al. 1991). A possible annual emergence vertically of infected *Aedes* mosquitoes may maintain RVF virus at enzootic foci (Swanepoel 1981). Sand flies were not involved in RVF virus maintenance, yet they allowed the replication virus experimentally (Fontenille et al. 1994, Trouillet et al. 1995). *Phlebotomus dubosqi,* one of the species identified in the Barkedji area, has been shown experimentally to transmit RVF virus (Turell and Perkins 1990). *Culicoides* were tested for virus with negative results.

Serologic surveys indicated that RVF virus continuously circulates in Senegal (Saluzzo et al. 1987; Guillaud et al. 1988; Thiongane et al. 1994, 1995; Digoutte 1995; Zeller et al. 1995). *Ae. vexans* and *Ae. ochraceus* feed on mammals; and the preferred host was sheep, followed by bovine, horse, chicken, and human (Findlay et al. 1936, Traoré-Lamizana 1997). The possible transmission cycles of RVF virus in Sahelian western Africa including enzootic virus maintenance around temporary surface ponds by *Ae. vexans* and *Ae. ochraceus,* movement of infected animals, and direct transmission (aerosol, contact with abortion products) have been analyzed by Zeller et al. (1997). Between epizootic periods, RVF virus possibly circulates in an enzootic maintenance cycle near these areas or was reintroduced (Saluzzo et al. 1987, Guillaud et al. 1988, Provost 1989, Zeller et al. 1992, Thiongane et al. 1994, Digoutte 1995). RVF virus also may persist in the eggs of vertically infected flood-water *Aedes,* where it may remain dormant in eggs for several dry seasons. Therefore, reintroduction may not be necessary to produce a focal enzootic transmission. When enzootic transmission of RVF virus was detected in the Barkedji area in 1993, RVF virus was epizootic among ruminants in

southeastern Mauritania, 250 km east of Barkedji (Zeller et al. 1995).

McIntosh and Jupp (1981) and Davies et al. (1985, 1992) associated Rift Valley fever epizootics with heavy rainfall. They suggested that RVF virus probably is maintained during interepizootic periods by vertical transmission in *Aedes* that breed in temporary surface ponds (dambos). Periods of persistent, heavy rainfall raise the ground-water table to a level where the eggs of *Aedes* become flooded (Linthicum et al. 1983, 1984b). Flooding of the "dambo" formation induces the hatching of *Aedes* eggs and subsequent emergence of very large numbers of adult mosquitoes, which frequently feed on cattle (Linthicum et al. 1985). If these mosquitoes are infected vertically with RVF virus, vertebrate bloodmeal hosts may become infected and, in turn, develop a viremia. These vertebrates may infect other mosquitoes that may be competent to transmit this virus (McIntosh 1972). The humid conditions and cloud cover present during the prolonged rainy periods allow a large proportion of the adult *Aedes* population to survive through more feeding-oviposition cycles than during the hot, dry conditions usually prevailing in these areas (Davies et al. 1985). In the Barkedji area, virus isolations were made only during 1993, a year without heavy rainfall, questioning the predictive relationship between increased rainfall and RVF epizootics in Senegal.

Overall, 79 isolates of WN virus were made from mosquitoes and one from humans, indicating a high level of enzootic activity. WN virus was isolated previously in 1988 from *Cx. poicilipes* Theobald (4 strains), *Cx. tritaeniorhynchus* Giles (3), *Cx. antennatus* Becker (5), and *Ma. uniformis* Theobald (2) from the lower basin of the Senegal river, and from *Cx. antennatus* in October 1989 and from *Ae. vexans* in October 1990 in Yonofere village (Traoré-Lamizana et al. 1994). West Nile specific antibody was detected in 80% of 90 samples of human sera collected at Lagbar village (80 km N of Barkedji) in December 1989 (H.G.Z., unpublished data). Previously, *Culex* (*univittatus, neavei, antennatus, modestus* Ficalbi, *vishnui* Theobald, *molestus* Forskal), *Anopheles* (*subpictus* Grassi, *maculipennis* Meigen, *coustani* Laveran), *Mansonia* (*metallica* Theobald, *aurites* Theobald), and *Aedes* (*circumluteolus* Theobald, *cantans* Meigen) mosquitoes were found to be infected naturally with WN virus. The high prevalence of infected pools of *Cx. poicilipes, Mi. hispida, Mi. lacustris, Mi. splendens, Mimomyia* ssp., and *Cx. neavei* observed in 1990 (Traoré-Lamizana et al. 1994), and the consistent isolations made from 1991 to 1994, led us to consider these species as vectors, even though their vector competence has never been studied.

Ornithophagic *Culex* of the *pipiens* group (Boreham and Snow 1973, Snow and Boreham 1973, Rickenbach et al. 1974), especially *Cx. neavei* (McIntosh et al. 1972, 1978) and *Cx. antennatus* (Taylor et al. 1953, 1956), most frequently have been found infected with WN virus. Ecological investigations in Africa (McIntosh et al. 1976, 1980, Fontenille et al. 1989, Tsai et al. 1998) suggest an enzootic maintenance cycle of WN virus involving birds and ornithophagic mosquitoes, with occasional tangential transmission to mammals including humans (Briese et al. 1999). WN strains from Pakistan, France, Israel, African countries, and the former USSR are closely related antigenically (Hammann et al. 1965, Gaidamovich and Sokhey 1973, Briese et al. 1999). A phylogenic analysis of the complete amino acid sequence of the viral envelope glycoprotein demonstrated a close relationship between 1996 mosquito isolates from Romania and from Rift Valley Province, Kenya. This observation supports the possible role of migratory birds in disseminating WN virus between Africa and Europe (Miller et al. 2000).

Vertical transmission of WN virus was demonstrated for *Cx. tritaeniorhynchus, Ae. albopictus,* and *Ae. aegypti,* allowing them to be considered as virus reservoirs as well as horizontal vectors (Baqar et al. 1993). Miller et al. (2000) published the first field evidence for vertical transmission of WN virus by the isolation of WN virus from males in *Cx. univittatus* complex from Rift Valley province, Kenya.

Bagaza (BGA, Flaviviridae, genus Flavivirus) was first isolated in 1966 in the Central African Republic from *Culex* ssp. (J. P. Digoutte in Karabatsos 1985) and then recovered in Cameroon and Senegal from *Cx. perfuscus* Edwards, *Cx. guiarti* Blanchard, *Cx. thalassius* Theobald, and *Cx. grahami* Theobald (Karatbatsos 1985). Other isolations from *Cx. thalassius, Ae. dalzieli, Cx. ethiopicus, An. nili* Theobald, and *Cx. univittatus* were reported in southern Senegal (Digoutte 1991). In northern Senegal, BAG virus was recovered from *Cx. neavei* in October 1989 (Digoutte 1991). This virus has a wide distribution and was recovered from eastern Senegal to the Senegal estuary, and from Senegal to the Central African Republic (Digoutte 1991). These mosquitoes are ornithophagic, and BAG virus presumably circulates between mosquitoes and birds. Serological surveys have not been done to determine human infection. In the current study, numerous isolations were made from several *Culex* species, incriminating these species as probable vectors of BAG virus. BAG virus was associated for the first time with *Mimomyia* species that feed on birds.

A new virus identified as Ar D 65239 (Bagaza-like) was related closely to Ntaya and BAG virus, but with some differences. Identified initially by immune ascitic preparation, it was difficult to separate from BAG virus using indirect immunofluorescent antibody assays or complement fixation test, but these viruses clearly were different by endpoint seroneutralization test.

The isolation of Bagaza and Ar D 65239 (Bagaza-like) from mosquitoes incriminated these species as potential vectors. BAG virus has never been isolated from human or other vertebrate hosts, and pathogenicity in vertebrates is still unknown. Ar D 95537 antigen was compared in complement fixation reactions, with immune ascitics made from reference virus strains in the WHO collaborative Center for Research on Arboviruses. Reactions were negative except for

the homologous immune ascitic fluid. The reaction also was negative with seven mixed groups of polyvalent immune ascitic acid fluid. Homologous ascitic fluid was compared with all antigens found in the WHO Center with negative findings. This new virus does not grow on Vero cells, but grew readily on *Aedes pseudoscutellaris* cells. Therefore, Ar D 95535 was considered as new to science. The new virus prototype has been sent to the Yale Arbovirus Research Unit (Yaru), International Center for reference on arboviruses for further study.

Babanki (BBK, genus Alphavirus) was first isolated in Cameroon by Milan and Germain in 1969 from *Mansonia africana* (Robin et al. 1970). BBK virus was recovered from *Culex* ssp., *Ae. africanus* (Theobald), *Ae. simpsoni* (Theobald), *Anopheles* ssp., *An. squamosus* Theobald, *Eretmapodites oedipodius* Graham, *Amblyoma* ssp., and *Boophilus* ssp. in Cameroon; from *Cx. decens* group Theobald and human sera in Madagascar; from *Culex* ssp., *Ae. vittatus* (Bigot), and *An. brohieri* Edwards in Senegal; from *Cx. telesilla* (De Meillon & Lavoipierre), *Cx. tigripes* De Granpre & De Charmoy, *Cx. weschei* Edwards, and *Cx. cinereus* Theobald, and human sera in Central African Republic; and from *Cx. decens* group and *Cx. perfuscus* in Ivory Coast (Robin 1970). This virus has been isolated from human sera and mosquito pools in Madagascar and from rodents in Nigeria and *Cricetus critecus* (hamster) in Europe (Digoutte 1995). BBK virus is near Sindbis virus, and birds are the presumed natural host. BBK virus has been known for 60 yr, and was isolated in Sweden during a 1982 outbreak (named "Edsbyn," Espmark). BBK virus has been identified previously as Ockelbo disease, and has been found in the Central Sweden, Finland (Pogosta disease), and Russia (Karelie fever). Isolates from Sweden and Russia were related closely to mosquito and human isolates from Africa. Symptoms in humans include rash, arthralgia, and fever (Lundstrom et al. 1991). In Africa, BBK virus does not seem to be responsible for human morbidity.

Ngari (NRI, Bunyamvera, genus Bunyavirus) was isolated originally from *Ae. simpsoni* (Theobald) males that were reared to adults from eggs collected near Ngari village in 1979. It was isolated in the same year from *Ae. neoafricanus* Cornet, Valade & Dieng, and in 1980 from *Ae. vittatus* (Bigot) (Karabatsos 1985). Other isolations were made in 1988 from *Ae. hirsutus* (1), *An. gambiae* (1), *An. pharoensis* Theobald (8), *Cx. antennatus* (2), *Cx. tritaeniorhynchus* (2), and *Cx. poicilipes* (2) (Gordon et al. 1992). NRI virus was isolated from Kedougou area (Zeller et al. 1996). This virus also has been isolated from cattle in Mauritania and antibody prevalence was as high as 50%, indicating possible transmission by contact among ruminants (Gonzalez 1988).

Generally, specificity exists between mosquito hosts and arboviruses; but this was not indicated for NRI virus, which has been isolated from Culicidae in three genera and 17 species (H.G.Z.), Phlebotomines, and *Culicoides*. It was isolated most frequently from *Anopheles* (Gordon et al. 1992), but one or two isolates were obtained from eight *Aedes* species. NRI virus was isolated during the RVF epizootic in Mauritania in 1988 from an aborted sheep. A potential pathogenicity of NRI virus in humans was suspected when the virus was isolated from febrile patients in Dakar hospital in October and November 1993 (Zeller et al. 1996). The wide geographical distribution of NRI virus over different bioclimatic regions indicated the broad adaptibility of this virus. It was isolated in Ouest, Central Africa, and in Madagascar Karabatsos 1985, Mathiot et al. 1986). Further investigation is required to determine the public health significance of this emerging virus (Morse 1995).

Bunyamwera (BUN, Bunyavirus, Bunymwera group) previously was isolated in Senegal from *Ae. argenteocephalus* (1), *Ae. dalzieli* (6), *Ae. vittatus* (1), *Ae. furcifer-taylori* (Edwards) (1), and from humans (1) in the Kedougou area (Digoutte in Karabatsos 1985). *Ma. africana* and *Ma. uniformis* were new mosquito hosts for this virus group in Senegal. Although isolated from humans (Digoutte in Karabatsos 1985), this virus has never been implicated in disease. Bunyamwera virus epidemiology will need further investigation to determine it's potential pathogeny.

Wesselsbron (WSL, Flaviviridae, genus Flavivirus) was first isolated from a Merino lamb in South Africa in 1956 (Weiss et al. in Karabatsos 1985). Subsequently, it was isolated from humans, mosquitoes, and many vertebrates. It has a wide geographical distribution in Africa and Madagascar. Before our Barkedji isolations, WSL virus had been isolated in Senegal from *Anopheles: domicola* Edwards (1 isolate), *freetownensis* Evans (1), *funestus* Giles (1), *pharoensis* (1), *rufipes* (Gough) (2); *Aedes: dalzieli* (10), *minutus* (7), *ochraceus* (2), *vittatus* (1), *luteocephalus* (1); *Culex: antennatus* (1), *perfuscus* (1), *tritaeniorhynchus* (1); and *Uranotaenia mashonaensis* Theobald (1). WSL virus was not rare in the forested zone of Senegal (Institut Pasteur, Dakar, Senegal, unpublished data), but it was isolated infrequently in the Barkedji area.

Koutango (KOU, Flaviviridae, genus Flavivirus) was first isolated from *Tatera kempi* trapped in 1968 near Saboya village in Senegal (Robin 1972). KOU virus was later isolated in Senegal from *Taterillus* sp. (3), *Mastomys* sp. (7), and *Mastomys erythroleucus* (2), and in Central African Republic from *Lemniscomys striatus* (2) and *Mastomys* sp. (2). KOU virus is a rodent virus, but it also has been isolated from a human in Senegal (Karabatsos 1985). Clinical manifestations include fever, headache, articular pains, ocular pains, and rash. KOU virus was isolated from *Cx. neavei* in Senegal. Rodents are the principal hosts in the zoonotic maintenance cycle, and humans are tangentially infected.

Ar D 66707 (Sanar) is an Orbivirus (Corriparta group). A prototype strain has not been registred in the *International Catalog of Arboviruses*. It was isolated for the first time in 1990 from *Cx. poicilipes* in the Saint Louis area and then from *Cx. perfuscus* (2) and *Cx. quiquefasciatus* Say (1) in Kedougou area, and *Cx. perfuscus* (4) in Barkedji area. Its vertebrate hosts and disease association are unknown.

Virus Isolation from Sand Flies. Saboya (SAB, Flaviviridae, genus Flavivirus) first was isolated from *Tatera kempi* (gerbil) in 1968 near Saboya village in Senegal (Karabatsos 1985), and since then has been recovered from several rodent species: *Tatera kempi* (4), *Arvicanthis niloticus* (6), *Mastomys* (2), *Mastomys* sp. (2), *Mastomys erythroleucus* (1), and *Mus musculus* (1). SAB virus also has been isolated in the Central African Republic in 1981 from *Ae. vittatus* (2) and *Ae. africanus* group (1) (Institut Pasteur, Dakar, Senegal, unpublished data). SAB virus was isolated for the first time from Phlebotomine sand flies in Senegal, West Africa (Fontenille et al. 1994). Isolation of SAB virus from Phlebotomines strongly indicates a transmission cycle involving rodentophagic sand flies and rodents of which there are five species in the Barkedji area (Duplantier, Fontenille et al. 1994). SAB has never been isolated from humans and animals other than rodents.

Chandipura (CHP, Rhabdoviridae, genus Rhabdovirus, of the vesicular stomatitis virus group) was described by Bhatt and Rhodrigues (1967). It was first isolated in India from patients with febrile illness and from a fatal case in a child with encephalitis. It also was isolated from Phlebotomine species caught in human dwellings and cowsheds in the Maharashtra State (Dhanda et al. 1970). Transovarial transmission of CHP virus was demonstarted in *P. papatasi*: 80% of the F_1 offspring of intrathoracically infected females were infected (Tesh and Modi 1983). Experimental transmission was obtained from mouse to mouse with *Ae. aegypti, Ae. albopictus, Ae. stephensi* Liston, and *Cx. tritaeniorhynchus* (Rao et al. 1967). CHP virus has been isolated in Africa from *Atelerix spiculus* (hedgehogs) and from humans in Nigeria (University of Ibadan 1971–1972). The Senegal strains reported here are the first isolates from arthopods in Africa (Fontenille et al. 1994).

Gabek Forest (GF, Phleboviridae, genus Phlebovirus) first was isolated by Heymann (1961) from a spiny mouse (*Acomys cahirinus*) in Sudan (in Karabatsos 1985). It also was recovered from *Atelerix albiventrix* (hedgehog), *Tatera kempi, T. gracilis, Tatellirus nigeriae,* and *Tatera* sp. (gerbil), *Arvicanthis niloticus* (rat), *Mastomys natalensis* (mouse) in Nigeria, and from *Tatera kempi, Tatellirus nigeriae* and *Mastomys natalensis* in Dahomey (Kemp et al. 1974), and from *Tatera* sp. in Central African Republic (Digoutte 1982). GF virus was isolated in Senegal from *Tatera kempi* in 1981, followed by other isolations from *Taterillus* sp. (7), *Arvicanthis niloticus* (4), *Mastomys* sp. (2), and *Mastomys erythroleucus* (1). GF virus was isolated from Phlebotomines in 1994 (1 isolate) and 1995 (5). Although frequently isolated from rodents, it was isolated for the first time from Phlebotomines during the current study, indicating that they were the potential vectors of this Phlebovirus. GB was not transmitted transovarilly by experimentally infected *P. papatasi* (Tesh and Modi 1984). Isolations have not been made from humans, but antibody was detected in Sudan, Nigeria, Egypt, Greece, Somalia, and Iran.

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