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MAJOR ARTICLE

Chikungunya Outbreak in Kedougou, Southeastern Senegal in 2009–2010

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Background. In Senegal, Chikungunya virus (CHIKV), which is an emerging mosquito-borne alphavirus, circulates in a sylvatic and urban/domestic cycle and has caused sporadic human cases and epidemics since 1960s. However, the real impact of the CHIKV sylvatic cycle in humans and mechanisms underlying its emergence still remains unknown.

Methodology. One thousand four hundred nine suspect cases of CHIKV infection, recruited from 5 health facilities located in Kedougou region, south-eastern Senegal, between May 2009 to March 2010, together with 866 serum samples collected from schoolchildren from 4 elementary schools in May and November 2009 from Kedougou were screened for anti-CHIKV immunoglobulin (Ig)M antibodies and, when appropriate, for viral nucleic acid by real-time polymerase chain reaction (rPCR) and virus isolation. In addition, mosquitoes collected in the same area from May 2009 to January 2010 were tested for CHIKV by rPCR and by virus isolation, and 116 monkeys sera collected from March 2010 to May 2010 were tested for anti-CHIKV IgM and neutralizing antibodies.

Results. The main clinical manifestations of the CHIKV suspect cases were headache, myalgia, and arthralgia. Evidence for CHIKV infection was observed in 1.4% (20 of 1409) of patients among suspect cases. No significant difference was observed among age or sex groups. In addition, 25 (2.9%) students had evidence of CHIKV infection in November 2009. Chikungunya virus was detected in 42 pools of mosquitoes, mainly from *Aedes furcifer*, and 83% of monkeys sampled were seropositive.

Conclusions. Our findings further documented that CHIKV is maintained in a sylvatic transmission cycle among monkeys and *Aedes* mosquitoes in Kedougou, and humans become infected by exposure to the virus in the forest.

Keywords. Chikungunya virus; Kedougou; outbreak; Senegal; sylvatic circulation.

Chikungunya virus (CHIKV) is a member of the Semliki Forest virus antigenic group of the genus *Alphavirus* (family *Togaviridae*) and was first isolated in 1953 in Tanzania [1, 2]. This arthtrogenic virus is composed of 2 lineages: African and Asiatic. When symptomatic, cute CHIKV infection in human can cause a flu-like syndrome with rash, but joint pains are the dominant complaint and might evolve to persistent and incapacitating manifestations. In Africa, CHIKV is maintained in nature among nonhuman primates and forest-dwelling *Aedes* sp. In rural areas, these sylvatic vectors can be responsible for sporadic cases or small outbreaks [3, 4]. In urban areas, CHIKV is transmitted to humans by *Aedes aegypti* and *Aedes albopictus* [5]. Several studies have reported CHIKV circulation in West Africa, especially in Nigeria between 1963 and 1977 [6, 7]. Major CHIKV epidemics were recently reported in Africa and Asia [8]. A significant outbreak occurred in Italy in 2007, and numerous imported cases have been detected elsewhere in Europe and the United States of America [9–13], emphasizing CHIKV as a re-emerging public health threat worldwide.

In Senegal, CHIKV was first isolated in 1962 from bats [14, 15], and CHIKV outbreaks and sporadic cases were subsequently reported in 1966, 1982, 1996, 1997, and 2004–2006 [4, 16, 17] (Figure 1). In addition, CHIKV has been repeatedly isolated from wild caught *Aedes furcifer*, *Aedes luteocephalus*, and *Aedes taylori* in a sylvatic focus near Kedougou in southeastern Senegal [5, 18–20] as part of an entomological surveillance programme that began in 1972 [21]. Various vertebrates, including monkeys, bats, gophers, and galagos, have been proven for implication as hosts of CHIKV by serological evidence or viral isolation [5]. Amplifications of CHIKV in the Kedougou area have occurred at approximately 5-year intervals, which is hypothesized to be the time necessary for the turnover of susceptible vertebrate hosts [5]. Despite active circulation of CHIKV in the sylvatic cycle in Senegal, limited information is available on its transmission on human and wildlife

Received 14 July 2017; editorial decision 18 November 2017; accepted 27 November 2017.

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DOI: 10.1093/ofid/ofx259

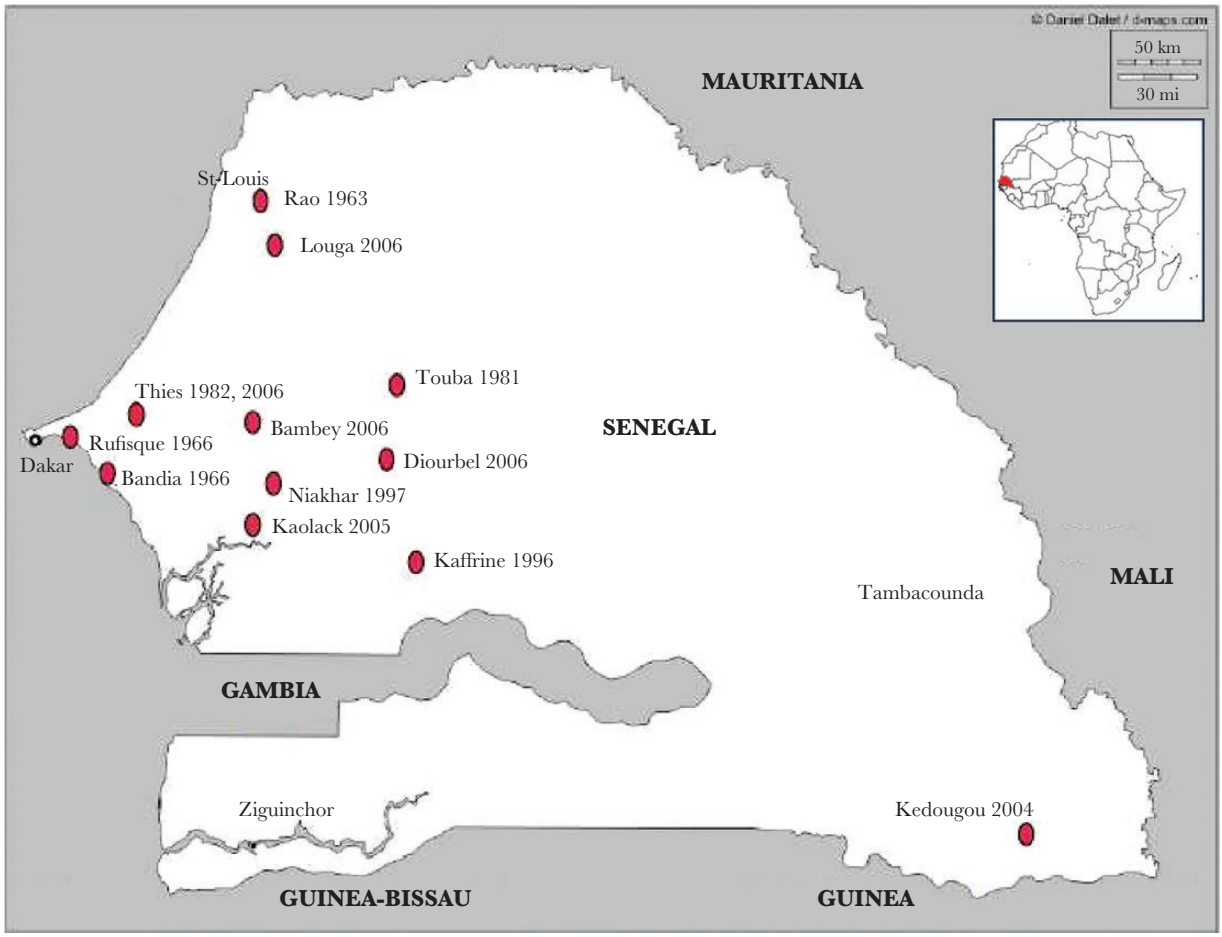

Figure 1. Map of Senegal showing locations where Chikungunya outbreaks have been reported. Red circle indicates the localities and the years of Chikungunya virus outbreaks.

populations. To address this question, we conducted passive surveillance for CHIKV infection among patients attending 5 health clinics with febrile illness, together with active surveillance of students recruited from 4 schools in Kedougou. In a parallel effort, we also investigated monkey serosampling in the region and surveyed mosquitoes in the area for CHIKV. In this study, we report the investigation of the CHIKV zoonotic amplification that occurred among monkeys, mosquitoes, and humans in the Kedougou region by 2009–2010.

MATERIALS AND METHODS

Ethics Statement

The protocols for human and monkey studies were approved by the National Ethics Committee of Senegal and the University of Texas Medical Branch Institutional Review Board and Animal Care and Use Committees, respectively. The protocol used for animals adheres to the Senegal national guideline and approved by the Institutional Review Board of the Interstate School for science and veterinary medicine. Written informed consent for adults and children was obtained. Concerning children, sera were collected with the consent of the parents. An audit of the protocol implementation was regularly conducted by the authorities of the National Ethics Committee of Senegal.

Study Areas

The study was conducted in the Kedougou region of southeastern Senegal (12°32'N, 12°11'W) (Figure 2). The human population is 133 487 inhabitants with an average density of 8 persons per km^2, 55% of whom are under 20 years of age. The climate is Sudano-Guinean with a single rainy season extending from May to November. The landscape consists of wooded grassland or woodland and dense gallery forest. The average annual temperature is 28.2°C. The main economic activity in the region is agriculture and livestock, but hunting and logging are a source of human contact with the forest. Currently, gold mining is expanding as one of the most important economic activities in the area.

Human Surveillance

Passive human surveillance focused on patients who attended local health facilities for acute febrile illness, and active surveillance involved the periodic seromonitoring of a prospective cohort of schoolchildren living in the Kedougou region. All patients were interviewed by experienced public health

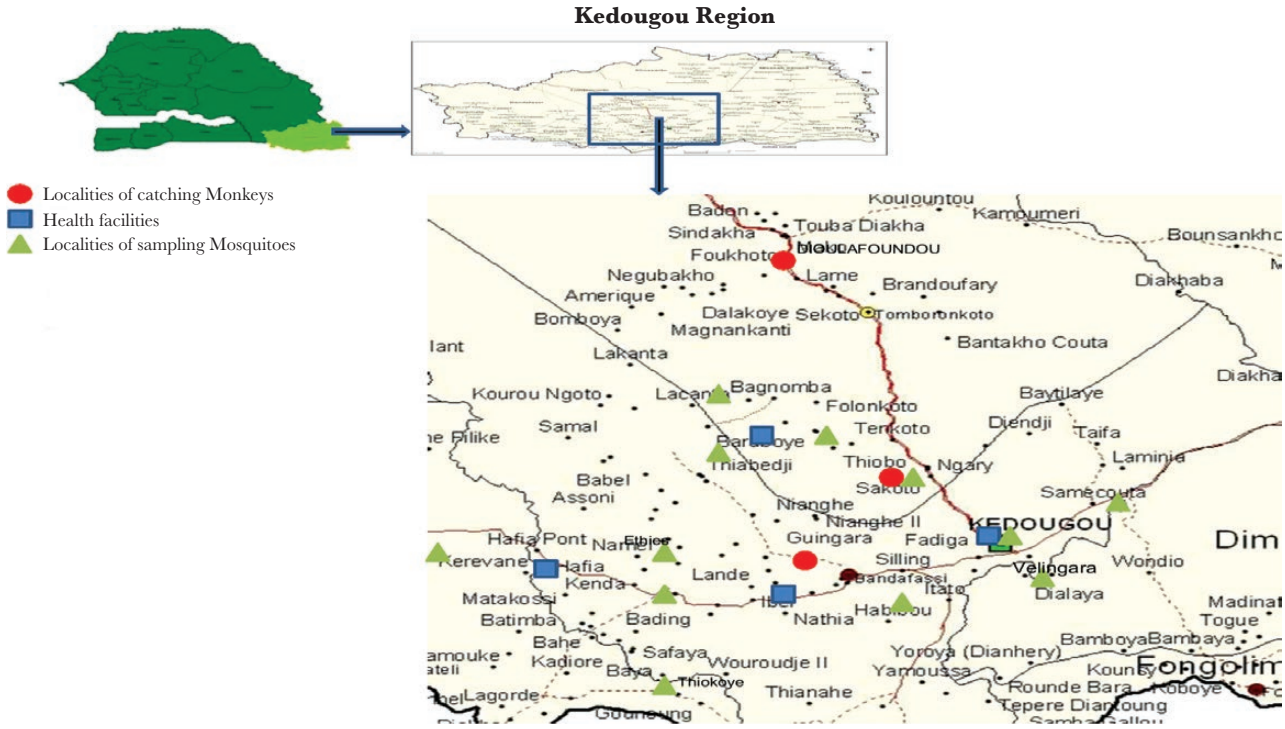

Figure 2. Map of Kedougou showing the sites sampled for Chikungunya surveillance during this study. Red circles indicates locations of monkey sampling, blue squares indicate health facilities, and green triangles show sites of mosquito sampling.

workers. The clinical manifestations and demographic data were recorded for each patient on a standardized interview form. In addition, similar data were obtained from both the healthy nonsymptomatic and febrile students.

Passive Surveillance of Chikungunya in Health Facilities

Five healthcare facilities located in Kedougou region were selected for human surveillance, including the Ninefesha Hospital, the Kedougou Health Center, the Bandafassi Clinic, the Military Health Centre, and the Catholic Mission, which has a mobile team that provides healthcare to indigenous populations in remote areas (Figure 2). Suspected CHIKV cases were defined as patients over 1 year of age with a fever over 38.5°C and at least 2 of the following clinical symptoms: headache, maculopapular cutaneous exanthema, eye pain, joint pain or injury, myalgia, fatigue, vomiting, dyspnea, diarrhea, jaundice, disorientation, and hemorrhagic manifestations.

Active Surveillance of Chikungunya Among Students' Cohorts

Four primary schools in Kedougou Department were selected for active surveillance including the Ninefesha, Bandafassi, and Ngari primary schools and the Catholic Mission School with some boarding residents as well as daily attendees (students from villages around Kedougou city). Serum samples were obtained from students each year before and after the rainy season (May and November) and tested for arboviruses of interest in the region including CHIKV antibody testing. In addition, schools were visited twice a week to identify students reaching criteria for the "suspect case" definition. Students not attending during the weekly visit at school were visited in their household to check whether they even met the "suspect case" definition. Suspect students cases were sent to the health center involved in the study to perform a blood sample for CHIKV virological diagnostic testing.

Surveillance of Chikungunya Among Primates

The study focused on 3 species of monkeys in the Kedougou area that are considered potential reservoirs of arboviruses: African green monkeys ([AGMs] *Chlorocebus sabaeus*), Patas monkeys (*Erythrocebus patas* [EP]), and Guinea baboons (*Papio papio* [PP]). Monkey blood sampling was conducted during the dry season (January–May 2010) around temporary ponds around the villages of Silling, Bafoundou, and Ngari-Sekoto (Figure 2). The monkeys were trapped in large cages baited with peanuts and anaesthetized with ketamine. Five milliliters of blood was collected and centrifuged, and the serum fraction was collected and stored at −20°C or −80°C until tested for antibody as described below. The capture date, location, age, sex, and weight for each captured monkey were systematically recorded.

Laboratory Analysis

Malaria Test

Human blood samples were tested by Giemsa-stained blood smears and rapid malaria tests kits for plasmodia [22].

Serology

Blood samples collected from patients, students, and primates were centrifuged and stored at −20°C until tested for antibody. Samples were tested for CHIKV by immunoglobulin (Ig)M antigen-capture enzyme-linked immunosorbant assay (ELISA). A differential diagnosis of CHIKV infection among other arboviruses such as yellow fever virus, dengue virus (DENV), West Nile virus, Rift Valley fever virus, and Crimean-Congo hemorrhagic fever virus, which are endemic in the study area, was also performed by IgM antibody-capture ELISA. Monkey serum samples were analyzed for specific CHIKV-neutralizing antibodies by plaque reduction neutralization tests (PRNTs) as described by De Madrid and Porterfield [23].

Molecular Detection

Real-time polymerase chain reaction (rPCR) was used to test human sera and mosquito samples for CHIKV. The mosquito sampling and testing protocols were extensively describes by Diallo et al 2012 [24].

Sequencing of E1 Coding Region

The whole genome of CHIKV strains was initially amplified using the Titan One Tube RT-PCR system (Roche, Mannheim, Germany), following the strategy reported by Volk et al [25]. Due to the extremely low level of variation in initially assessed genome sequence (data not shown), only the region that corresponded to the E1 envelope glycoprotein gene was subjected to further sequencing and analyses [2]. Primer sequences and specific PCR and sequencing protocols are available from the authors.

Phylogenetic Analysis

Genome sequences representing the spatiotemporal distribution of CHIKV were downloaded from GenBank and aligned using MUSCLE [26] and manually adjusted in Se-Al (available at http://tree.bio.ed.ac.uk/software/seal/) according to amino acid sequence homology. The E1 region was then excised and combined with the 34 newly generated CHIKV E1 sequences, leading to a final data set containing 103 sequences and 1317 nucleotides. A maximum likelihood tree was then inferred using the PAUP v4.0b package [27], based on the best-fit nucleotide substitution model determined by MODELTEST [28]. The database and operation script is available upon request from the authors.

Statistical Analysis

Data were analyzed using R software. The χ^2 test was used to compare the difference between 2 proportions, with a statistical significance level set at $P < .05$.

RESULTS

Patient Samples

One thousand four hundred nine suspect cases of CHIKV were evaluated from 5 healthcare facilities in Kedougou between May 2009 and March 2010 and tested for evidence of acute CHIKV infection using virus isolation, viral genome, or IgM antibody detection. Table 1 summarizes the distribution of findings relating to human sera collected, 50.4% of which were negative for active malaria parasitemia. Overall, evidence for CHIKV infection was observed in 1.4% (20 of 1409) of patients including 6 patients who tested positive for CHIKV-specific IgM antibody (Table 1). Among the 1409 human sera sampled, 144 were referred to acute stage injury (<5 days of illness) and subsequently tested for CHIKV detection or for viral genetic material evidence. Hence, CHIKV ribonucleic acid (RNA) was detected by rPCR in 9.7% (14 of 144) of patients. Of note, the majority of CHIKV-confirmed cases based on the detection of virus or viral RNA from acute sera (9 of 14; 64 %) were recruited from the Kedougou healthcare center (Table 1).

In terms of the spatial distribution and among the 20 confirmed humans cases, 90% were reported in the Kedougou district, suggesting an incidence rate ranging from 0.55 to 9.38/1000 inhabitants, and 10% (2 of 20) of cases were reported in the Saraya district (Table 1), with an incidence rate at 1.34/1000 inhabitants.

The median age of infected patients was 24 years (7–55), the sex ratio male/female was 1. Adult individuals, especially those between 31 and 45 years old, were significantly more affected than others. However, there was no significant difference between sexes ($P > .05$). The most common clinical symptoms among patients were headache (70%), myalgia (70%), and arthralgia (60%) followed by vomiting (30%), cough (25%), diarrhea (10%), and cutaneous rash (5%) (Figure 3A). Among the CHIKV-infected confirmed cases, 20% (4 of 20) were diagnosed with malaria coinfection and presented with all the previously described symptoms except rash. Diarrhea and vomiting were more common in malaria coinfection (33% and 50%,

Table 1. Enrolled Patients and CHIKV-Positive (+) Cases in Kedougou in Five Health Facilities From July 2009 to March 2010

Health Facilities	No. Sera Collected	No. CHIKV ELISA IgM+	No. Acute Sera Tested	No. CHIKV rPCR+	Total Positive
Kedougou	319	0	47	9	9
Military Camp	658	5	40	0	5
Bandafassi	205	0	28	3	3
Ninefesha	199	1	29	2	3
Catholic Mission	28	0	0	0	0
Total	1409	6	144	14	20

Abbreviations: CHIKV, Chikungunya virus; ELISA, enzyme-linked immunosorbant assay; IgM, immunoglobulin M; rPCR, real-time polymearse chain reaction.

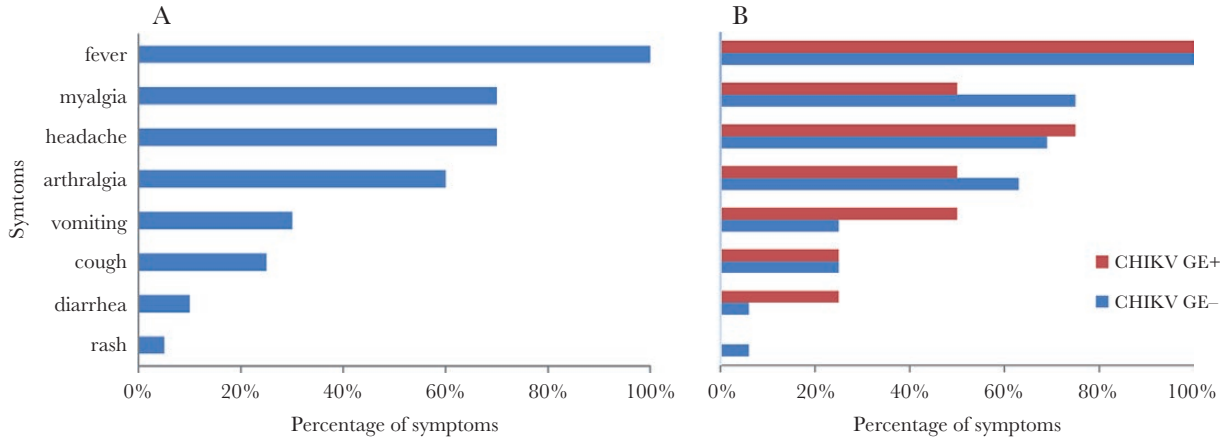

Figure 3. Clinical features of all Chikungunya (CHIKV) cases (A) and cases with and without malaria coinfection (3B). CHIKV GE^{-} were negative for malaria, whereas GE^{+} were positive for malaria.

respectively) than in the sole CHIKV infection with no evidence of active malaria (6% and 25%) (Figure 3B). However, no significant difference was observed in the frequency of signs and symptoms between the malaria and CHIKV-only groups ($P > .50$).

Analysis of temporal distribution of CHIKV-positive mosquito pools and CHIKV-infected cases showed an overlap between the 2, with a lag of approximately 1 month between the first detection of CHIKV-infected mosquitoes and first detection of a human case (Figure 4). The epidemic curve showed intermittent peaks that began in October 2009 with the first peak in week 2 (6 cases). In November 2009, the number of CHIKV-infected confirmed cases peaked in the first week 1 (6 cases), and then declined gradually by half (3 cases per week) at weeks 2 and 3, and finally dropped to 1 case at week 4 of November 2009. No case was detected between December 2009 and February 2010. In March 2010, 1 more confirmed CHIKV case was detected during the relating second week (Figure 4).

Entomological Findings

From June 2009 to January 2010, a total of 39 799 mosquitoes, grouped in 4211 pools, were collected and analyzed. The most abundant species among the potential CHIKV vectors were *Aedes vittatus* (23.0% of the host-seeking females), *A furcifer* (18.7%), *Aedes dalzieli* (15.6%), and *A luteocephalus* (13.1%). A total of 42 CHIKV-infected pools were obtained by rPCR from September to December 2009 mainly from *A furcifer* (16

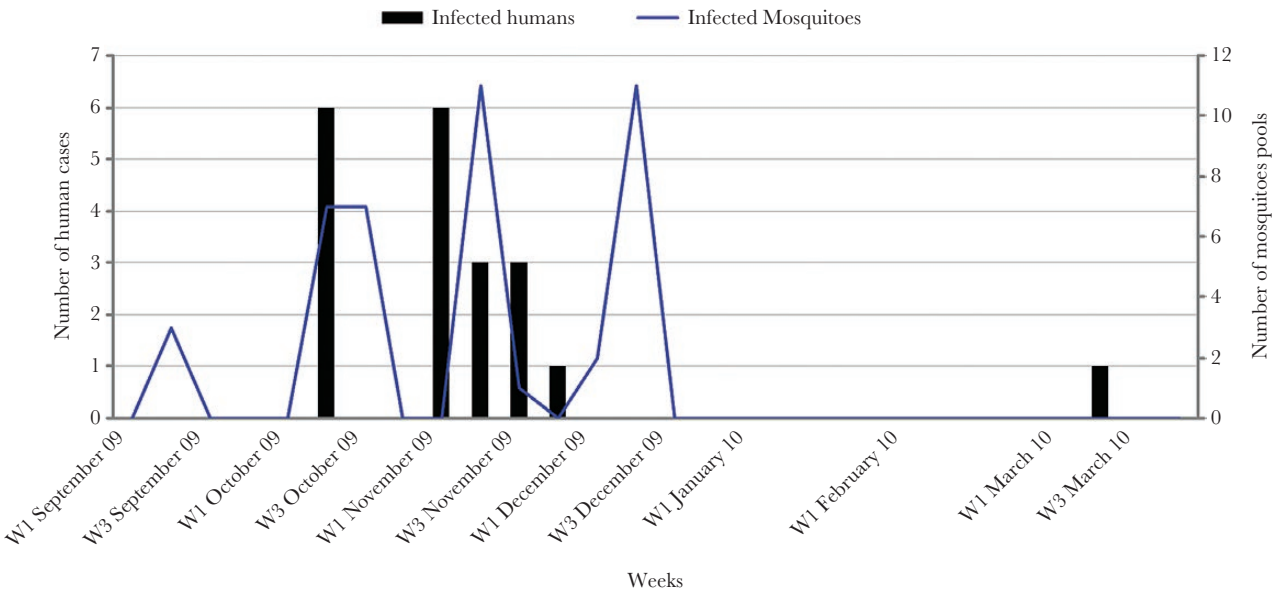

Figure 4. Temporal distribution of Chikungunya cases and detection of Chikungunya-infected mosquito pools from May 2009 to March 2010 in Kedougou. W, week; W1, the first week of the month.

pools), *A taylori* (5 pools*), and A luteocephalus* (5 pools), which represented 66.9% of the CHIKV-positive pools.

Analysis of the E1 Sequences

As shown in Figure 5, all of our samples from 2009, including those from both mosquitoes and patients, were closely related to each other. All were within the West-Africa lineage, with a closest neighbor from Senegal in 2005. All of the CHIKV strains had an alanine residue at E1 position 226, consistent with enzootic strains rather than the recent strains responsible of Asian or South American epidemics.

Active Surveillance Among School Children

A total of 866 students were investigated for CHIKV circulation in May and November 2009 in 4 schools. Enzyme-linked immunosorbent assay (ELISA) IgM was performed on all sera collected. Table 3 shows that no evidence of recent CHIKV infection was detected in May. However, ELISA IgM performed on sera collected from the same children in November 2009 showed that 25 were positive for CHIKV in Bandafassi, Ninefesha, and Catholic Mission schools with infection rates of 4% (7 of 171), 8% (15 of 180), and 0.6% (3 of 464), respectively. The infection rates were significantly different among the schools ($P < .000003$; Fisher's exact test). However, in Bandafassi and Ninefesha schools, the infection rate was statistically similar ($P = .12$; Fisher's exact test). Between November 2009 and May 2010, a total of 65 suspected students were followed. However, none was positive for CHIKV test.

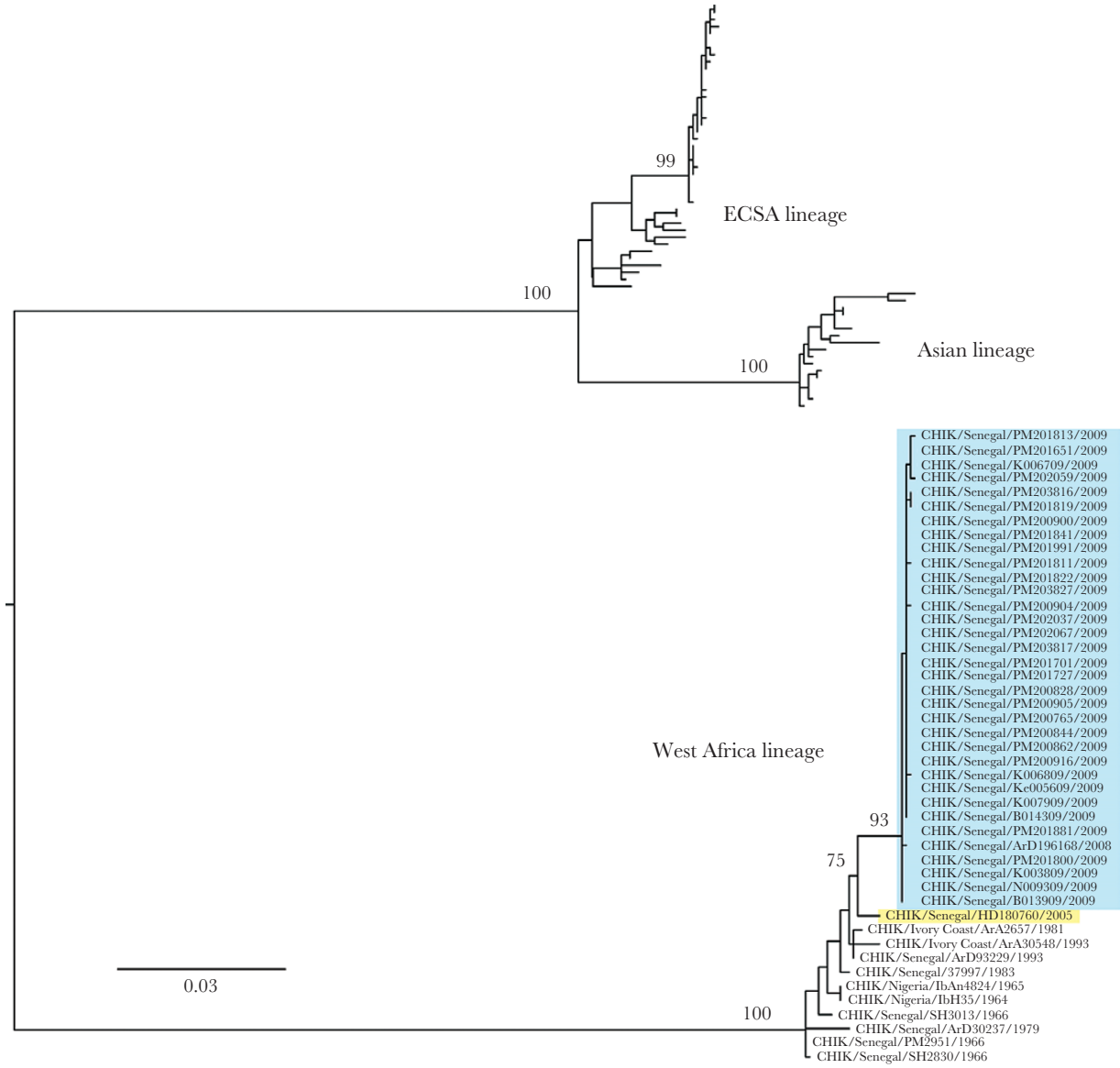

Figure 5. Maximun likelihood tree of the E1 gene of Chikungunya virus. Isolates from humans and mosquitoes obtained between May 2009 and March 2010 in Kedougou are highlighted in blue, and the most recent, previous Senegal strain is highlighted in yellow. Bootstrap value higher than 70 are labeled along the major branches.

Table 2. Spatial Distribution, Gender, and Age Distribution of CHIKV-Positive (+) Cases in Kedougou From July 2009 to March 2010

Localities		Recruited Patients			CHIKV+	
District		Number	%	Population	Number	IR[a] (1/1000)
	Bandafassi	94	6.67	972	1	1.028
	Boundoucondi	16	1.13	177	1	5.64
	Ibel	17	1.20	1000	1	1
	Thioketian	17	1.20	512	1	1.953
	Niemeneke	1	0.07	800	1	1.25
	Ninefesha	34	2.41	213	2	9.38
	Kedougou	834	59.19	19 731	11	0.55
	Others	394	27.96		0	0
	Subtotal	1407	99.85	23 405	18	0.769
	Sabadola	2	0.15	1454	2	1.375
	Total	1409	100	25 859	20	0.804
Sex						
	Male	747	53	11933	10	0.838
	Female	661	47	12926	10	0.773
Age Group						
	0–4 years	165	11.71	4227	0	0
	5–14 years	494	35.06	6960	6	0.862
	15–29 years	427	30.30	6960	5	0.718
	30–44 years	194	13.76	3729	6	1.609
	>45 years	106	7.54	2983	3	1.005
	Missing	23	1.63	—	—	—
	Total	1409	100.00	24859	20	0.804

Abbreviations: CHIKV, Chikungunya virus; IR, incidence rate.

Primates Samples Analysis

One hundred seventeen monkeys including 77 PP, 33 *Chlorocebus sabaeus* (AGM), and 7 EP were sampled from March to May 2010 in Silly, Ngari, and Bafoundou (Figure 1). No IgM antibody-positive animals were detected, but CHIKV-neutralizing antibodies were found by PRNT in 87% (66 of 76) of PP, 75% (25 of 33) of AGM, and 71% (5 of 7) of EP; 34% of infant and juvenile primates had neutralizing antibodies. Chikungunya virus seroprevalences did not differ significantly among the 3 primates species ($P > .22$; Fisher's exact test).

Table 3. Number of Student Samples Tested and Number of Students Positive (+) by ELISA IgM Against CHIKV in May and November 2009

		CHIKV+	
School	Number Students	May 2009	November 2009
Bandafassi	171	0	7
Ninefesha	180	0	15
Ngari	51	0	0
Catholic Mission	464	0	3
Total	866	0	25

Abbreviations: CHIKV, Chikungunya virus; ELISA, enzyme-linked immunosorbant assay; IgM, immunoglobulin M.

DISCUSSION

Chikungunya virus is a re-emerging mosquito-borne viral infection. In the Kedougou region, Southeastern Senegal, several epizootics of CHIKV have been reported at intervals of approximately 5 years since 1972, but little information is available on the impact of CHIKV among human populations and mechanisms involved in its emergence. In the present study, we reported a sylvatic outbreak of CHIKV in the Kedougou region that was evidenced from patients recruited in 5 health facilities and among a prospective cohort of living locally students.

Overall, a combination of serological IgM, rPCR testing, and virus isolation performed on 1409 patients enrolled revealed 20 that were infected by CHIKV in the survey area. Seventy percent of CHIKV-positive patients had symptoms matching the case CHIKV disease definition. This result is similar to that reported during CHIKV epidemics that occurred in Thailand [29] and in the Indian Ocean [30]. Overall, the clinical manifestations observed (algo-febrile eruptive illness, with vomiting and diarrhea) were similar to those described previously for acute CHIKV infection [11]. Our study also revealed coinfections of CHIKV with malaria in 20% of CHIKV-confirmed cases and supporting previous observations on CHIKV-malaria coinfections reported in the Democratic Republic of the Congo and in Senegal [31, 32]. Except for the absence of cutaneous rash, the coinfected patients presented the same symptoms and signs described above for CHIKV-only infection. Diarrhea and vomiting were more frequently reported among confirmed malaria cases coinfected with CHIKV, but no significant difference was observed when comparing with patients infected with CHIKV only. These results (1) underscore the difficulties and challenges in clinically differentiating these conditions in areas where malaria is endemic [3] and (2) reinforce malaria as an "umbrella" infection potentially masking arboviral disorders in the context of sub-Saharan Africa. Therefore, in suspected cases of malaria, CHIKV infection should be included as an alternative diagnosis, as well as other arboviral infection options. As previously described, fever and joint involvement were the most frequent manifestations, affecting most of Chikungunya patients [11, 30]. Also our study showed that 60% of infected patients presented arthralgia for which 10% experienced persistent arthralgia.

Our finding of equal prevalence of CHIKV infection between the genders and the age groups is also consistent with a rural outbreak of CHIKV that occurred in Cameroon [33]. In contrast to studies carried out in Nigeria in the 1960s–1970s, where children from 1 to 4 years of age were significantly more infected [34, 35], all age groups were found to be to be susceptible for infection with CHIKV. Distinctly, an exception for children under 4 years was noteworthy, suggesting that little or no domestic transmission of CHIKV occurred during this outbreak. Indeed, children younger than 4 years of age tend to remain in the household, unlike adults and schoolchildren

(7 years old and older) whose activities (agriculture, traditional gold extraction) and daily school attendance from remote villages expose them to forests and forest-living mosquitoes.

Our data also showed low CHIKV incidence rates (0.55–9.38/1000), in comparison with those reported from Kaffrine (with an incidence rate of 35.3%), Senegal in 1996–1997 [3] and on the Maldive islands (65.2/1000) [36]. This observation may be explained by the low density of the human population in Kedougou, which is 8 people/km^2 and probably not sufficient for human-mosquito-human CHIKV transmission. Previous studies showed that human population densities of approximately 3000–7000/km^2 are needed to produce CHIKV outbreaks [37]. It is also likely that only severe cases attend healthcare centers and clinics, and the asymptomatic forms of the disease—which is believed to be a vast majority of cases—were missed by the recruitment process used in this investigation.

Among the cohort of schoolchildren, 25 seroconverted for CHIKV IgM antibody in Bandafassi, Ninefesha, and Catholic Mission schools between May and November 2009, suggesting that CHIKV circulated during the rainy season. The infection rate was observed in students of Ninefecha and Bandafassi schools, which are located in the forest area. In addition, the confirmed CHIKV cases belonging to the Catholic Mission School (located in Kedougou city) were students who returned to their villages during week ends and holidays. Overall, the exposure of these students to CHIKV could be explained by their proximity to the forest environment, which could facilitate contact with CHIKV-infected mosquito vectors. This explanation is further strengthened by the level of difference for infection rates between the student cohort (2.88%) and the framework of the local healthcare facilities (1.42%). Furthermore, up to 36% of the CHIKV patients found in the Kedougou area were evidenced with a history of recent travel to forested areas.

Chikungunya virus isolates obtained from humans and mosquitoes [24] were found between October and December 2009, confirming previous studies showing that in the Kedougou area arboviruses are mostly isolated from October to December, which corresponds to the end of the rainy season [18, 19]. Chikungunya virus was mostly isolated from *A furcifer*, which is probably the primary vector of this outbreak. In a companion study [24], we showed that *A furcifer* occurred in all 5 major land cover classes in the Kedougou region (forest, village, agriculture, savannah, and barren). On this behalf, there were no significant differences in the abundance of this species among these land cover classes, and the *A furcifer* species was rarely found inside households in villages and was distinctly more abundant on the periphery than inside the villages. In addition, CHIKV was also detected at equal rates in all 5 lands cover classes. Given that, humans are rarely staying in forests during the moment when sylvatic CHIKV vectors are the more active. Because *A furcifer* shows high biting rates in villages [24], we suggest that humans are likely exposed to sylvatic CHIKV while outside of their homes but within their villages. Such an hypothesis is reinforced by the fact that no domestic transmission of CHIKV occurred given the absence of infection among children under 4 years, who are more likely to spend time within the household. Our results also suggest that the CHIKV epizootic began in September 2009. Hence, sylvatic circulation must precede epidemic amplification in the emergence area [38, 39]. The CHIKV outbreak episode that emerged in March 2010 raises a question about the transmission of the virus during the dry season in southeastern Senegal. Based in our data, the *Anopheles* genus found infected with CHIKV could be partly involved in transmission, consistent with a previous study that demonstrated experimental CHIKV transmission by *Anopheles* spp [40].

The sylvatic CHIKV cycle in Senegal is believed to be maintained through the nonhuman primates. We found evidence for past CHIKV infection in 3 monkey species collected between March and May 2010. Although sampling of primates occurred after our studies of humans and mosquitoes (May 2009 to March 2010), the detection of CHIKV-neutralizing antibodies among infant primates strongly suggest that they were probably infected recently during the sylvatic amplification between May and November 2009. Previous studies have shown that the turnover of monkey populations is necessary for the emergence of arboviruses [41].

As shown in our phylogenetic tree (Figure 5), the CHIKV strains isolated from patients in Kedougou in 2009 clustered in the West African genotype and were closely related to previous isolates from Senegal, suggesting continuous circulation of CHIKV in rural or sporadic local transmission of the virus. The isolates sequenced exhibited an alanine at amino acid residue 226 of the E1 envelope glycoprotein. Previous studies have demonstrated that a substitution of valine (A226V) provides a selective advantage for the replication and transmission of CHIKV by *A albopictus* [42, 43]. However, no *A albopictus* was detected so far in the study area, in Senegal and in turn in West Africa. Our phylogenetic data supported by the sylvatic amplification of CHIKV described by Diallo et al [24], are similar to previously studies based on DENV, which suggest that sylvatic cycle is a natural source of emergence [20, 44].

CONCLUSIONS

In conclusion, the present study highlights the importance of surveillance including clinical observations, laboratory diagnosis, and cohort serosurveillance to detect outbreaks caused by CHIKV and other arboviruses in the Kedougou region. The combination of serological and molecular tests identified an epidemic of CHIKV in Kedougou after an amplification from the sylvatic cycle that would otherwise have been overlooked. Our results support the hypothesis that sylvatic enzootic circulation can be a source of emergence of CHIKV into domestic or urban transmission cycles and underscore the value of effective,

real-time surveillance to improve the detection and prevention of arboviral diseases.

Acknowledgments

We thank the population, healthcare workers, and professional of health facilities in Kedougou for their support and cooperation in conducting this study.

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. The research was funded by the National Institutes of Health (Grant Number 5R01A 1069145).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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