Chikungunya Outbreak in Kedougou, Southeastern Senegal in 2009–2010

Abdourahmane Sow,1,10,11 Oumar Faye,1 Mawloukh Diallo,1 Diawo Diallo,2 Rubing Chen,1 Ousmane Faye,1 Cheikh T. Diagne,1 Mathilde Guerbois,3 Manfred Weidmann,4 Youssoupha Ndiaye,4 Cheikh Sadibou Senghor,1 Abdourahmane Faye,4 Ousmane M. Diop,1 Bakary Sadio,1 Oumar Ndiaye,1 Douglas Watts,3 Kathryn A. Hanley,1 Ana T. Dia,10 Denis Malvy,10,a Scott C. Weaver,3,a and Amadou Alpha Sall1

1Institut Pasteur Dakar, Arbovirus and Viral Hemorrhagic Fevers Unit, Senegal; 2Institut Pasteur Dakar, Medical Entomology Unit, Senegal; 3Institute for Human Infections and Immunity, Center for Tropical Diseases and Department of Pathology, University of Texas Medical Branch, Galveston; 4Department of Virology, University Medical Center Göttingen, Germany; 5District Sanitaire Mathilde Guerbois,3 Manfred Weidmann,4 Youssoupha Ndiaye,4 Cheikh Sadibou Senghor,1 Abdourahmane Faye,4 Ousmane M. Diop,1 Bakary Sadio,1 Oumar Ndiaye,1 Douglas Watts,3 Kathryn A. Hanley,1 Ana T. Dia,10 Denis Malvy,10,a Scott C. Weaver,3,a and Amadou Alpha Sall1

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Correspondence: A. Sow, MD, MPH, Professional Officer/Epidemics Control-Public Health Laboratories, West African Health Organisation (WAHO), 175 Avenue Daessiz Coulibaly, BP 153, Bobo-Dioulasso, Burkina Faso (asow20@gmail.com).

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 arthropod 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...
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 km², 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...
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.

Results

Statistical Analysis
Data were analyzed using R software. The χ² 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%, respectively).

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

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

Abbreviations: CHIKV, Chikungunya virus; ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; rPCR, real-time polymerase chain reaction.
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...
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.** Maximum 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.
CHIKV-neutralizing antibodies were found by PRNT in 87% (25 of 33) of AGM, and 71% (5 of 7) of EP were sampled from Chlorocebus sabaeus in March to May 2010 in Silly, Ngari, and Bafoundou (Figure 1).

One hundred seventeen monkeys including 77 PP, 33 AGM, and 7 EP were sampled from 3 primates species (Fisher’s exact test).

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

<table>
<thead>
<tr>
<th>Locality</th>
<th>Recruited Patients</th>
<th>Population</th>
<th>CHIKV+</th>
<th>CHIKV+ (% in 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bandafassi</td>
<td>94</td>
<td>1134</td>
<td>1</td>
<td>1.028</td>
</tr>
<tr>
<td>Boundoucondi</td>
<td>16</td>
<td>137</td>
<td>1</td>
<td>5.64</td>
</tr>
<tr>
<td>Ibel</td>
<td>17</td>
<td>1000</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>Thoketian</td>
<td>17</td>
<td>512</td>
<td>1</td>
<td>1.953</td>
</tr>
<tr>
<td>Niemeneke</td>
<td>1</td>
<td>800</td>
<td>1</td>
<td>1.25</td>
</tr>
<tr>
<td>Ninefesha</td>
<td>34</td>
<td>213</td>
<td>2</td>
<td>9.38</td>
</tr>
<tr>
<td>Kedougou</td>
<td>834</td>
<td>19731</td>
<td>11</td>
<td>0.55</td>
</tr>
<tr>
<td>Others</td>
<td>394</td>
<td>23405</td>
<td>18</td>
<td>0.769</td>
</tr>
<tr>
<td>Subtotal</td>
<td>1407</td>
<td>26376</td>
<td>33</td>
<td>0.56</td>
</tr>
<tr>
<td>Sabadola</td>
<td>2</td>
<td>1454</td>
<td>2</td>
<td>1.375</td>
</tr>
<tr>
<td>Total</td>
<td>1409</td>
<td>26859</td>
<td>20</td>
<td>0.804</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>School</th>
<th>Number Students</th>
<th>May 2009</th>
<th>November 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bandafassi</td>
<td>171</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Ninefesha</td>
<td>180</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Ngari</td>
<td>51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Catholic Mission</td>
<td>464</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>866</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

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

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 (alga-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 coinfect patients presented the same symptoms and signs described above for CHIKV-only infection. Diarrhea and vomiting were more frequently reported among confirmed malaria cases coinfect 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 vil-
lages 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 Maldives islands (65.2/1000) [36]. This observation
may be explained by the low density of the human population in
Kedougou, which is 8 people/km² and probably not sufficient for
human-mosquito-human CHIKV transmission. Previous stud-
ies showed that human population densities of approximately
3000–7000/km² 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 Ninefescha and Bandafassi
schools, which are located in the forest area. In addition, the con-
firmed 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 prox-
imity to the forest environment, which could facilitate con-
tact 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 evi-
denced with a history of recent travel to forested areas.

Chikungunya virus isolates obtained from humans and mos-
quitoses [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, agri-
culture, 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 transmis-
sion 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 main-
tained 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 proba-
bly 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 pre-
vious 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 resi-
due 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 ampli-
fication 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 diag-
nosis, 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 circu-
lation 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.

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