

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



LSHTM Research Online

Sow, Abdourahmane; Nikolay, Birgit; Faye, Oumar; Cauchemez, Simon; Cano, Jorge; Diallo, Mawlouth; Faye, Ousmane; Sadio, Bakary; Ndiaye, Oumar; Weaver, Scott C; +3 more... Dia, Anta T; Sall, Amadou Alpha; Malvy, Denis; (2020) Changes in the Transmission Dynamic of Chikungunya Virus in Southeastern Senegal. *Viruses*, 12 (2). p. 196. ISSN 1999-4915 DOI: <https://doi.org/10.3390/v12020196>

Downloaded from: <http://researchonline.lshtm.ac.uk/id/eprint/4656193/>

DOI: <https://doi.org/10.3390/v12020196>

Usage Guidelines:

Please refer to usage guidelines at <https://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: <http://creativecommons.org/licenses/by/2.5/>

<https://researchonline.lshtm.ac.uk>

1 Article

2 Changes in the transmission dynamic of 3 Chikungunya virus in South Eastern, Senegal

4 Abdourahmane Sow^{*1,8,9}, Birgit Nikolay^{2,3,4}, Oumar Faye¹, Simon Cauchemez^{2,3,4}, Jorge Cano⁵,
5 Mawlouth Diallo⁶, Ousmane Faye¹, Bakary Sadio¹, Oumar Ndiaye¹, Scott C. Weaver⁷, Anta T
6 Dia⁸, Amadou Alpha Sall¹ and Denis Malvy⁹

7 ¹ Institut Pasteur Dakar, Arbovirus and viral Hemorrhagic Fevers Unit, 36, Avenue Pasteur, BP 220, Dakar,
8 Senegal.; West African Health Organization (WAHO)175 Avenue Ouezzin Coulibaly, BP 153 Bobo-
9 Dioulasso, Burkina Faso

10 ² Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France.

11 ³ CNRS, URA3012, Paris, France.

12 ⁴ Center of Bioinformatics, Biostatistics and Integrative Biology, Institut Pasteur, Paris, France.

13 ⁵ Faculty of Infectious Diseases and Tropical Medicine, London School of Hygiene & Tropical Medicine,
14 London, United Kingdom.

15 ⁶ Institut Pasteur Dakar, Medical entomology Unit, 36, Avenue Pasteur, BP 220, Dakar, Senegal.

16 ⁷ Institute for Human Infections and Immunity, Center for Tropical Diseases and Department of Pathology,
17 University of Texas Medical Branch, Galveston, Texas, USA,

18 ⁸ Institut Santé et développement (ISED), Université Cheikh Anta Diop, Dakar, Senegal,

19 ⁹ INSERM U1219 University of Bordeaux, Bordeaux, France.

20 * Correspondence: asow20@gmail.com; Tel: +226 66894712

21 Received: date; Accepted: date; Published: date

22 **Abstract:** In Senegal, chikungunya virus (CHIKV) is maintained in a sylvatic cycle and causes
23 sporadic cases or small outbreaks in rural areas. However, little is known about the influence of the
24 environment on its transmission. To address the question, One hundred twenty villages were
25 randomly selected in the region. Samples were tested for anti CHIKV IgG antibodies by ELISA. We
26 investigated the association of CHIKV seroprevalence with environmental variables using logistic
27 regression analysis and the spatial correlation of village seroprevalence based on semivariogram
28 analysis. 54% [51-57] of individuals were tested positive for CHIKV-specific IgG. CHIKV
29 seroprevalence was significantly higher in population living close to forest (NDVI, OR = 1.90 [1.42-
30 2.57] and was negatively associated with population density, (OR= 0.76 [0.69-0.84]. In contrary in
31 gold mining sites where population density was >400 people per km², seroprevalence peaked
32 significantly among adults (46%, [27-67]) compared to all other individuals (20% [12-31]; p=0.013).
33 Higher exposure to CHIKV in areas with lower population density and close to the forest is
34 consistent with transmission through sylvatic mosquitoes such *Aedes furcifer*. However traditional
35 gold mining activities changes significantly the transmission dynamic of CHIKV leading a potential
36 increase in the risk of human exposition in the region.

37 **Keywords:** Chikungunya – Spatial autocorrelation - Environmental risk- Gold mining - Senegal

38

39 1. Introduction

40 Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that belongs to the *Togaviridae*
41 family [1]. It was first isolated in 1953 from the serum of a febrile patient during an epidemic in
42 Newala district, Tanzania [2-3]. Acute CHIKV infection in humans can cause a flu-like syndrome
43 associated with severe arthralgia and rash [4-7]. CHIKV is maintained in a sylvatic cycle involving
44 non-human primates as reservoir hosts [8-9] and forest dwelling mosquitoes [10-11]. Sylvatic vectors
45 can be responsible for sporadic cases or small outbreaks among humans living in rural areas [8, 12-

46 13]. In urban areas, CHIKV is transmitted between humans by *Ae. aegypti* and *Ae. albopictus*
47 mosquitoes [14].

48 Since the outbreak in Tanzania in 1952, CHIKV outbreaks have been reported in Africa, Asia
49 and southern-America between the 1960s and 2000s[15]. More recently, CHIKV was recognized as
50 emerging arbovirus with important public health impact after major epidemics occurred in 2004 in
51 numerous countries (Kenya, Comores and islands in the Indian Ocean). The largest outbreak
52 occurred in La Reunion with 300,000 infected cases and an attack rate of about 35% [16]. In addition
53 a significant outbreak occurred in Italy in 2007 and imported cases have been detected ever since
54 elsewhere in Europe and the USA [17-20] due to the spread of the anthropophilic mosquito *Ae.*
55 *albopictus* outside Africa and the global movement of viremic individuals [21], emphasizing CHIKV
56 as a re-emerging threat to global public health.

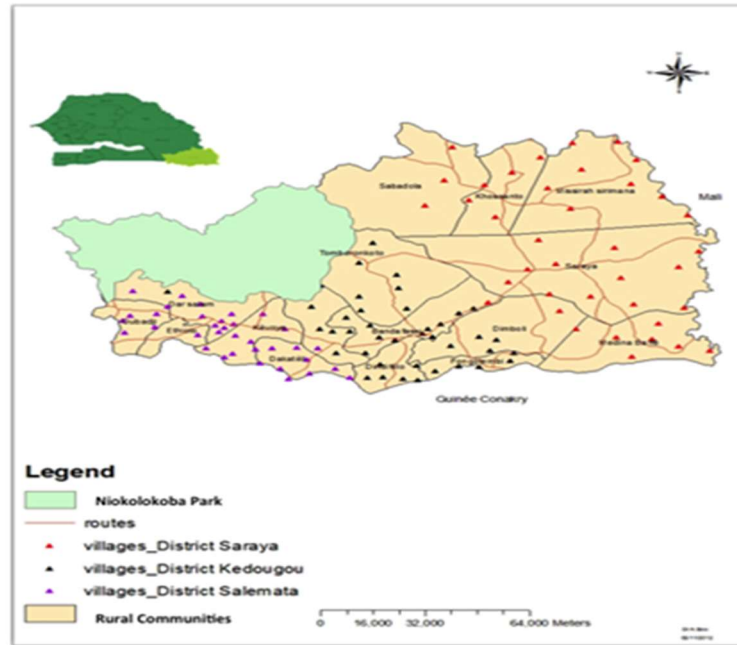
57 In Senegal, CHIKV was first isolated from a bat in 1962 [22-23], and since then, sporadic human
58 cases and outbreaks of CHIKV were regularly reported [13, 18, 24-26]. Since 1972 the Pasteur Institute
59 has implemented an entomological surveillance in the Kedougou area, located on the border of
60 Guinea, South-Eastern Senegal, where CHIKV has been repeatedly isolated from *Ae. furcifer*, *Ae.*
61 *luteocephalus* and *Ae. taylori* [8, 26-29]. Amplifications of CHIKV have been detected at approximately
62 5-year intervals. This interval is hypothesized to be the time necessary for the turnover of susceptible
63 vertebrate hosts [6]. Following amplification, the virus is likely to go locally extinct and must be
64 reintroduced to initiate a new amplification cycle (Althouse et al., manuscript submitted). In 2009 a
65 CHIKV zoonotic amplification occurred in Kedougou region both among humans and mosquitoes.
66 In deed 20 confirmed human cases were reported in Kedougou and Saraya districts mainly in gold
67 mining sites. In parallel 42 CHIKV infected pools were obtained by rPCR from September to
68 December 2009 mainly from *Ae. furcifer* (16 pools), *Ae. taylori* (5 pools), and *Ae. luteocephalus* (5 pools).
69 [30]

70 Despite active sylvatic circulation of CHIKV in Kedougou region, limited information is
71 available about its impact on human health and its interaction with environmental conditions. To
72 address these questions, we conducted a serosurvey in 2012 following the last detected virus
73 amplification in 2009. Here we report the results of the serosurvey implemented in Kedougou place,
74 South-Eastern Senegal.

75 **2. Materials and Methods**

76 **Serological study:**

77 The study was carried out in Kedougou region located in the extreme south-east of Senegal
78 between 12 ° 33 ' north latitude and 12 ° 11' west longitude (Figure 1). It extends over an area of 16,896
79 km² with an estimated population of 153,476 inhabitants among which 55% are under 20 years and
80 an average density of 8 persons per km² [31]. The population is predominantly rural (84%), and
81 ethnically diverse. On average, annual rainfall in the area is estimated between 1,200 mm and 1,300
82 mm. Agriculture remains the principal economic activity but traditional gold mining has increased
83 considerably leading to massive human migration and important eco-environmental changes.



84

85

Figure 1. Investigated villages in Kedougou region.

86

87

88

89

90

91

92

93

The sampling method was based on a two-level cross sectional randomized cluster sampling adapted from WHO. The sampling frame was the list of villages drawn up for the 2002 national census. The Kedougou region was first divided into 3 districts. For each district, 40 villages were randomly selected using the cumulative total method. In each of selected villages, unless 10 persons by randomly selected household were sampled. From each consented individual, 5 ml of intravenous blood were taken. Samples were centrifuged and serum aliquoted and sent in liquid nitrogen at Dakar Pasteur Institute where sera were tested for anti CHIKV IgG antibodies by ELISA assay as described by Traore-Lamizana and *al.* [32].

94

Environmental data

95

96

97

98

99

100

101

102

103

104

105

106

107

A suite of environmental, topographical and demographic datasets was used to explore potential drivers of CHIKV outbreaks in the study area. From the Moderate Resolution Imaging Spectroradiometer (MODIS) [33] products, we downloaded global MOD13Q1 data, which includes vegetation indices such as Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI) and mid-infrared band (MIR) which has been found to be useful to discriminate water surfaces [34]. Forest cover for the study area was obtained from the Global Forest Change project (University of Maryland) [35]. The elevation dataset at 250m resolution was derived from a gridded digital elevation model produced by the Shuttle Radar Topography Mission (SRTM) [36]. Finally, gridded maps at 100m resolution of estimated population density for Senegal in 2010 and 2015 were obtained from the World Pop project [36]. Environmental, topographic and demographic data were extracted for village point locations as average values over a buffer zone of 1km radius. We further assessed sensitivity of estimates to buffer size by repeating the analysis with a buffer zone of 3km radius to account for movement of individuals around village locations.

108

Statistical analysis

109

Descriptive analysis

110

111

Age was classified as <5, 5 to 9, 10 to 19, 20 to 39, 40 to 59 and ≥60 years. Associations of seroprevalence with age and sex of individuals were investigated by logistic regression analysis and

112 statistical significance was assessed with a likelihood ratio test. The models included random
113 intercepts for villages and rural communities to adjust for clustering of surveyed individuals.
114 Confidence intervals of seroprevalence by sex or age-group were obtained based on the exact
115 binomial method. We included a sex-age interaction term to explore potential greater exposure to
116 infections on certain groups (i.e. male adults be more exposed during their activities outside their
117 resident villages) considering age groups that reflect different occupational activities (<20, 20 to 59,
118 and ≥60 years) and stratifying by population density (locations with ≤400 and >400 people per km²).

119 *Spatial patterns of seroprevalence*

120 We aggregated individuals by villages and rural communities to assess the spatial variation in
121 seroprevalence levels. We investigated the spatial correlation of village seroprevalence based on
122 semivariogram analysis using the geoR package.

123 *Environmental risk factors*

124 We first investigated the association of CHIKV seroprevalence with environmental variables by
125 univariable logistic regression analysis including random intercepts for villages and rural
126 communities. We classified environmental variables into quintiles to assess departure from linearity
127 in associations and included these as categorical terms in the models. For each variable, we compared
128 the model fit to a model including the variable as continuous term. The decision to include variables
129 as categorical or continuous terms was based on the lowest Akaike information criterion (AIC). For
130 variables associated with seroprevalence that were highly correlated (Pearson's $r > 0.7$), we performed
131 a preliminary variable selection based on lowest likelihood ratio test p-values and lowest AIC. Due
132 to convergence problems when including all variables simultaneously, we chose a forward model
133 selection approach starting with the variable with lowest p-value and lowest AIC, adding additional
134 variables in order of increasing p-values and AIC. Variables were retained in the model if
135 significantly associated ($p \leq 0.05$). Random intercepts were retained in the final model if these were
136 significantly associated ($p \leq 0.05$) and improved the model fit.

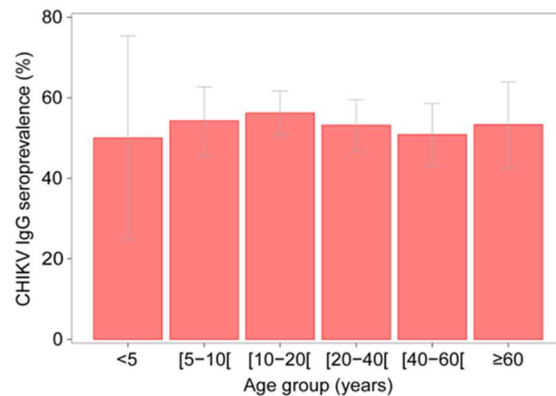
137 To assess spatial patterns in the unexplained variation of seroprevalence by villages, we
138 investigated spatial correlation of village random-effects by semivariogram analysis as described
139 above. Additionally, we compared model fit of the selected logistic regression model to a
140 geostatistical model that additionally accounted for spatial correlation between village random
141 effects.

142 Basic statistical analysis was performed using the R computing environment and parameters of
143 geostatistical models were estimated using Bayesian methods implemented in Winbugs [38].

144 **3. Results**

145 *Serological investigation for CHIKV IgG in 2012*

146 In total, 998 individuals living in 101 villages and 15 rural communities in the Kedougou region
147 were tested for CHIKV IgG. The age of tested individuals ranged from 1-99 years (median 21, IQR
148 12-41) and 56% of tested individuals were male. Fifty-four percent of individuals [51-57] were found
149 positive for CHIKV IgG. Seroprevalence did not vary significantly by sex (males 53% [49-57]; females
150 55% 95% [50-60]; $p = 0.522$) or age group ($p = 0.485$) (Figure 2).



151

152

Figure 2. Seroprevalence by age group and exact binomial 95% confidence intervals.

153

Spatial variation in CHIKV IgG seroprevalence

154

Seroprevalence against CHIKV varied between villages and rural communities in the study area (Figure 3A); village seroprevalence levels were however not spatially correlated (Supplementary Figure 1). Table 1 shows that in univariate analysis CHIKV Seroprevalence was significantly 2 times higher in population living close to forest with great vegetation (NDVI, OR = 1.90 [1.42-2.57].

158

Table 1. Univariable analysis of the association between CHIKV seroprevalence and environmental variables. The models were adjusted for clustering of individuals in villages and rural communities (random intercept).

159

160

	OR (95%CI)	LRT p-value	AIC
Environmental variables:			
EVI_max (per 0.1 increase)	1.54 (1.16; 2.02)	0.002	1354
EVI_mean (per 0.1 increase)	2.23 (1.44; 3.47)	<0.001	1351
EVI_sd (per 0.01 increase)	1.14 (1.03; 1.27)	0.010	1357
NDVI_max (per 0.1 increase)	1.90 (1.42; 2.57)	<0.001	1348
NDVI_mean (per 0.1 increase)	1.85 (1.35; 2.52)	<0.001	1350
NDVI_sd (per 0.01 increase)	1.16 (1.03; 1.30)	0.012	1357
MIR_max (per 0.1 increase)	0.68 (0.35; 1.35)	0.258	1362
MIR_mean (per 0.1 increase)	0.22 (0.10; 0.51)	<0.001	1352
MIR_sd (per 0.01 increase)	1.02 (0.82; 1.28)	0.829	1363
Distance to water bodies (km)	1.01 (1.00; 1.03)	0.048	1360
Distance to rivers (km)	1.02 (0.99; 1.05)	0.202	1361
Population density per km² (log-transformed)	0.76 (0.69; 0.84)	<0.001	1340
Slope (degree)	1.12 (0.98; 1.29)	0.089	1360
Altitude (meters)	1.00 (1.00; 1.00)	0.937	1363
Forest area (proportion, per 0.1 increase)	1.06 (0.99; 1.13)	0.081	1360
Distance to forest (km)	0.86 (0.76; 0.98)	0.023	1358
Accessibility (travel time to city per hour increase)	1.00 (1.00; 1.00)	0.985	1363
Random intercepts:			
Village only	NA	0.003	1362
Rural community only	NA	0.094	1368
Village and rural	NA	<0.001	1361

NDVI: Normalized Difference Vegetation Index, EVI: Enhanced Vegetation Index
MIR: Mid-Infra-Red band, OR: Odds Ratio, CI: confident Interval

LRT : Likelihood Ratio Test, AIC : Akaike Information Criteria

161 The model that best explained the observed spatial variation in seroprevalence was based on
 162 population density (Figure 3B).and accounted for clustering of individuals in villages (village
 163 random effects). Indeed, Seroprevalence was negatively associated with population density, so that
 164 for each one-unit increase in population density at the log-scale, the seroprevalence decreased by an
 165 Odds Ratio (OR) of 0.76 [0.69-0.84] (Table 2).

166 **Table 2. Multivariable analysis of the association between CHIK seroprevalence and**
 167 **environmental variables.** The models were adjusted for population density (log-scale) and clustering
 168 of individuals in villages (random intercept).

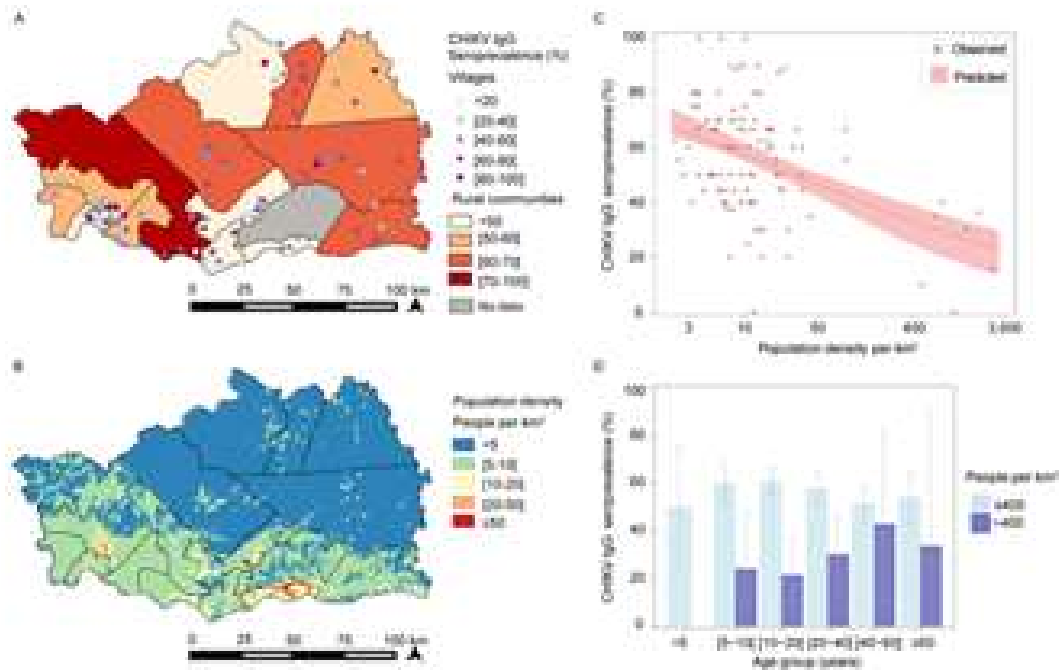
Environmental variables	OR (95%CI)	LRT p-value	AIC
Population density per km ² (log-transformed)	0.76 (0.69; 0.84)	0.008	1338
NDVI_max (per 0.1 increase)	1.17 (0.76; 1.81)	0.485	1340
Distance to forest (km)	0.97 (0.86; 1.10)	0.614	1340
Distance to water bodies (km)	1.00 (0.99; 1.01)	0.735	1340

NDVI: Normalized Difference Vegetation Index, OR: Odds Ratio, CI: confident Interval, LRT : Likelihood Ratio Test, AIC : Akaike Information Criteria

169 This translates for example into a predicted seroprevalence of 57% [55-62] at a population
 170 density of 10 persons per km², compared to 32% [23-38] at a population density of 500 persons per
 171 km² (Figure 3C). Village random effects were not spatially correlated and including spatial
 172 dependency did not improve model fit (Supplementary Figure 1).

173 In contrary, among individuals living in villages where population density was >400 people per
 174 km² (i.e., seven villages in the rural community of Bandafassi which are the main sites of traditional
 175 gold mining), seroprevalence against CHIKV peaked among adults (Figure 3D); in particular it was
 176 significantly higher among male adults 20-59 years old 46%, [27-67] compared to all other individuals
 177 (20% [12-31]; p=0.013). There was suggestive evidence for an interaction between sex and age
 178 (p_{interaction}=0.098).

179 Among individuals living at population densities ≤400 people per km², seroprevalence among
 180 male adults did not differ significantly from other individuals (p=0.091) and no interaction between
 181 age and sex was detected (p_{interaction}=0.766).



182

183 **Figure 3. Spatial variation in CHIKV IgG Seroprevalence.** (A) Seroprevalence by village and rural
 184 community. (B) Spatial variation in population density. (C) Observed and predicted seroprevalence
 185 by population density. The 95% CI of the prediction was obtained by bootstrap (2,000 iterations) (D)
 186 Age patterns by population density (≤ 400 vs. >400 people per km^2).

187 4. Discussion

188 Two years after a Chikungunya outbreak in Kedougou region [39], our survey showed that over
 189 50% of studied individuals had a history of CHIKV infection. Seroprevalence was homogenously
 190 distributed over all age ranges, including very young children, suggesting a simultaneous and recent
 191 exposure of the population to CHIKV circulation. Continuous circulation of CHIKV within this
 192 population, on the contrary, would have led to a significant age pattern with increasing
 193 seroprevalence by age.

194 Seroprevalence against CHIKV was highest in remote areas with low population density.
 195 Individuals living in those areas were indeed 1.24 times more likely exposed to CHIKV than those
 196 living in areas with high population density. This can be explained by CHIKV transmission through
 197 sylvatic mosquitoes such as *Ae. fuscifer*, which is more frequent in rural areas close to the forest
 198 galleries and was identified as the main vector in the 2009 epidemic [39]. The univariate analysis also
 199 showed that populations living close to the forest and the rivers (forest galleries) were significantly
 200 more exposed than the others (Table 1).

201 Although overall seroprevalence was low in Bandafassi rural community, CHIKV
 202 seroprevalence were significantly higher in gold mining sites where the population density was
 203 relatively high especially among male adults. In addition, during the outbreak in 2009-2010, those
 204 villages harbor such gold mining sites were most affected by CHIKV [40]. A similar pattern was
 205 observed also during the CHIKV outbreak in 2015, where confirmed cases in Saraya district clustered
 206 in villages where the main gold mining sites were located in 2015 (unpublished data). This suggests
 207 that traditional gold mining by attracting thousands of indigenous and foreign populations to remote
 208 rural areas, particularly close to the forest galleries, may increase exposure of humans to CHIKV
 209 through the enzootic cycle. Moreover, environmental changes linked to human activity in sites with

210 a high human concentration favor the development of domestic larval breeding sites [40]. Although
211 no CHIKV cases have been previously reported in the Salemata district, CHIKV seroprevalence was
212 found to be high (>50% were seropositive). This suggests either CHIKV circulation with low clinical
213 expression, which however has been observed in only around 15% of infected individuals [12], or
214 more likely a limited capacity of the surveillance system to detect cases. Indeed the weakness of the
215 surveillance in this area is potentially due to difficult access to the health facility of the district which
216 is the remotest area of the region and the absence of sentinel sites in contrary than Kedougou and
217 Saraya districts.

218 The elevated exposure to CHIKV among human populations living in rural Kedougou area
219 suggests a high spill over risk into rural or domestic transmission cycles during amplification years.
220 Particularly gold mining sites that attract a large number of highly mobile individuals may act as
221 hotspots for the emergence and dissemination of new CHIKV strains. Given the abundance of CHIKV
222 vectors in the Kedougou region, the weakness of surveillance system and the massive human
223 migrations, it is urgently necessary to strengthen the CHIKV surveillance system in Kedougou region
224 in order to prevent the establishment of a domestic CHIKV transmission cycle and the potential
225 global spread of newly introduced virus strains.

226 **Supplementary Materials:**

227 **Environmental data**

228 A suite of environmental, topographical and demographic datasets was used to explore
229 potential drivers of CHIKV outbreaks in the study area. Due to the relatively small study area, we
230 resorted to high resolution satellite images provided by the Moderate Resolution Imaging
231 Spectroradiometer (MODIS) instrument operating in the Terra spacecraft (NASA) [33], which
232 measure 36 spectral bands and it acquires data at lowest spatial resolution of 250m. From the family
233 of MODIS products, we downloaded global MOD13Q1 data, which are provided every 16 days at
234 250m spatial resolution. The MOD13Q1 product includes vegetation indices such as Normalized
235 Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI). The latter minimized
236 canopy background variations and maintains sensitivity over dense vegetation conditions. It also
237 includes mid-infrared band (MIR) which has been found to be useful to discriminate water surfaces;
238 water highly reflects wavelength in the range of MIR band (2.1 μm) [34]. Fortnightly continuous
239 gridded maps of NDVI, EVI and MIR for the study area were produced for 2009 and aggregated by
240 calculating the mean, maximum and standard deviation of the rainy season (May to December).

241 Forest cover for the study area was obtained from the Global Forest Change project (University
242 of Maryland) [35]. This project, which has been conceived to monitoring global forest extent, provides
243 gridded maps of forest and non-forest areas based on high-resolution satellite images obtained by
244 Landsat mission between 2000-2014. We later calculated the Euclidean distance (straight line
245 distance) in kilometers from the communities to the nearest forest area. Likewise, we produced a
246 continuous surface of distances in km to the nearest water body based on the Global Database of
247 Lakes, Reservoirs and Wetlands.

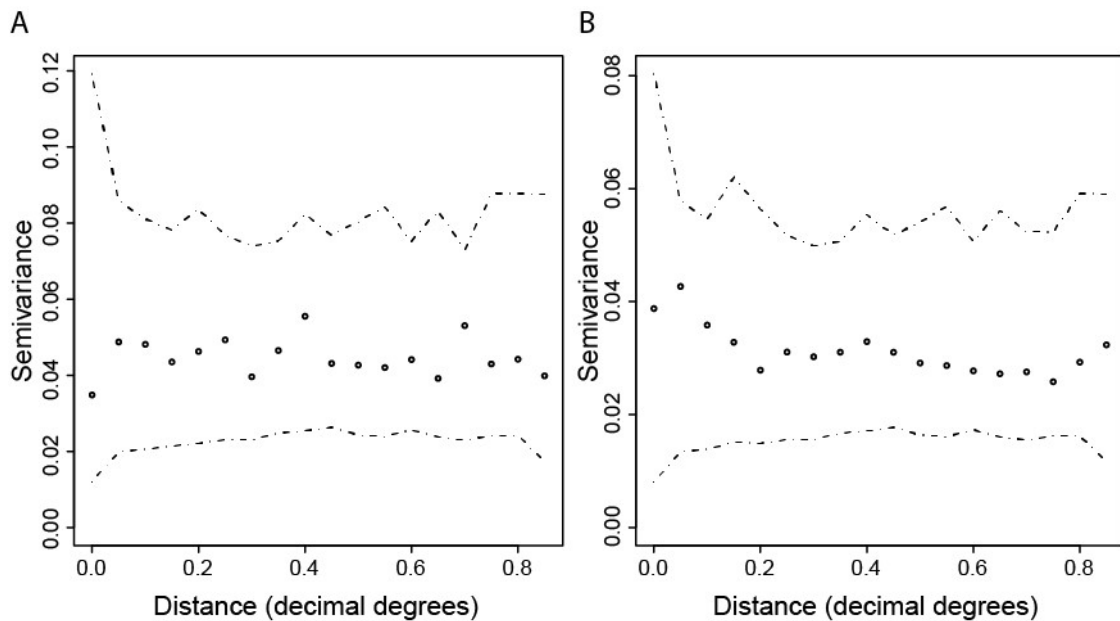
248 The elevation dataset at 250m resolution was derived from a gridded digital elevation model
249 produced by the Shuttle Radar Topography Mission (SRTM) [36]. This elevation surface was then
250 processed to obtain slope in degrees. In addition, a gridded map of urban accessibility at 1 km
251 resolution was obtained from the European Commission Joint Research Centre Global Environment
252 Monitoring Unit (JRC) [41]. This dataset defined urban accessibility as the predicted time taken to
253 travel from that grid cell to a city of $\geq 50,000$ persons in the year 2000 using land- or water-based travel.
254 Finally, gridded maps at 100m resolution of estimated population density for Senegal in 2010 were
255 obtained from the WorldPop project [37].

256 Survey and environmental data were linked in ArcGIS 10.3. (ESRI Inc., Redlands CA, USA)
257 based on the WGS-1984 Web Mercator projection at 250m x 250m resolution. Nearest neighbour was

258 applied to resample raster data sets Input grids were either extended or clipped to match the
259 geographic extent of a map of the study area, and eventually aligned to it.

260 Spatial correlation of village seroprevalence levels

261 Semivariogram analysis of village seroprevalence and village random-effects adjusting for
262 population density did not show any spatial dependency in village infection levels (Supplementary
263 Figure 1). The absence of spatial correlation was further confirmed by Bayesian geostatistical
264 modelling using Winbugs software [38]. Including a spatial exponential decay function for village-
265 level random effects did not improve model fit (DIC non-spatial 1333, DIC spatial 1339).



266

267 **Supplementary Figure 1.** (A) Semivariogram of CHIKV village prevalence and (B) village random
268 effects adjusting for log-transformed population density. Envelopes to assess significance of spatial
269 dependency were computed by simulating 1000 permutations.

270

Sensitivity analysis of environmental risk factors using a 3 km buffer zone.

271 **Supplementary Table 1: Univariable analysis of the association between CHIKV seroprevalence**
272 **and environmental variables using 3km buffers around villages.** The models were adjusted for
273 clustering of individuals in villages and rural communities (random intercept).

	OR (95%CI)	LRT p-value	AIC
Environmental variables:			
EVI_max (per 0.1 increase)	1.79 (1.22; 2.56)	0.002	1355
EVI_mean (per 0.1 increase)	2.84 (1.55; 5.22)	0.001	1352
EVI_sd (per 0.01 increase)	1.17 (1.02; 1.31)	0.018	1358
NDVI_max (per 0.1 increase)	2.58 (1.71; 3.97)	<0.001	1347
NDVI_mean (per 0.1 increase)	2.14 (1.54; 3.82)	<0.001	1350
NDVI_sd (per 0.01 increase)	1.16 (1.01; 1.30)	0.028	1359
MIR_max (per 0.1 increase)	0.44 (0.18; 1.08)	0.067	1360
MIR_mean (per 0.1 increase)	0.09 (0.03; 0.25)	<0.001	1347
MIR_sd (per 0.01 increase)	0.94 (0.72; 1.22)	0.638	1363
Distance to water bodies (km)	1.01 (1.00; 1.03)	0.044	1360
Distance to rivers (km)	1.02 (0.99; 1.05)	0.196	1361
Population density per km ² (log-transformed)	0.73 (0.64; 0.81)	<0.001	1339
Slope (degree)	1.03 (0.90; 1.18)	0.674	1363
Altitude (meters)	1.00 (1.00; 1.00)	0.825	1363
Forest area (proportion, per 0.1 increase)	1.07 (0.99; 1.16)	0.071	1360
Distance to forest (km)	0.87 (0.75; 1.01)	0.062	1360
Accessibility (travel time to city per hour increase)	1.00 (1.00; 1.00)	0.966	1363

Random intercepts:			
Village only	NA	0.003	1362
Rural community only	NA	0.094	1368
Village and rural	NA	<0.001	1361

274 **Supplementary Table 2: Multivariable analysis of the association between CHIK seroprevalence**
 275 **and environmental variables using 3km buffers around villages.** The models were adjusted for
 276 population density (log-scale) and clustering of individuals in villages (random intercept).

Environmental variables	OR (95%CI)	LRT p-value	AIC
Population density per km ² (log-transformed)	0.73 (0.64; 0.81)	<0.001	1337

277 **Author Contributions:** For research articles with several authors, a short paragraph specifying their individual
 278 contributions must be provided. The following statements should be used “conceptualization, A.A.S. and S.C.W.
 279 and M.D.; methodology, O.F; B.N.; J.C.; software, B.N and J.C.; validation, A.A.S; D.M; A.T.D. and S.C.; formal
 280 analysis, A.S.; B.N.; and J.C.; investigation, A.S.; O.N; and B.S.; data curation, A.S.; O.N.; and B.S.; writing—
 281 original draft preparation, A.S.; J.C.; and B.N.; writing—review and editing, A.A.S.; D.M.; A.T.D.; visualization,
 282 B.N.; funding acquisition, A.A.S; and S.C.W.”

283 **Funding:** The research was supported by the National Institutes of Health (NIH), Grant Number AI1069145.

284 **Acknowledgments:** The authors thank the population, healthcare workers and medical authorities in Kedougou
 285 region for their support and cooperation in conducting this study.

286 **Competing interests:** The authors declare no competing interests.

287 Appendix

288 Table 1: Univariable analysis of the association between CHIKV seroprevalence and
 289 environmental variables. The models were adjusted for clustering of individuals in villages and rural
 290 communities (random intercept).

291 Table 2: Multivariable analysis of the association between CHIK seroprevalence and
 292 environmental variables. The models were adjusted for population density (log-scale) and clustering
 293 of individuals in villages (random intercept).

294 Figure 1: Investigated villages in Kedougou region

295 Figure 2: Seroprevalence by age group and exact binomial 95% confidence intervals

296 Figure 3: Spatial variation in CHIKV IgG Seroprevalence. (A) Seroprevalence by village and
 297 rural community. (B) Spatial variation in population density. (C) Observed and predicted
 298 seroprevalence by population density. The 95% CI of the prediction was obtained by bootstrap (2,000
 299 iterations) (D) Age patterns by population density (≤ 400 vs. >400 people per km²).

300 References

- 301 1. Schuffenecker I.; Iteman I.; Michault A.; Murri S.; Frangeul L.; Vaney MC.; et al. Genome microevolution of
 302 chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* **2006**; 3(7): e263.
- 303 2. Ross R.W. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the
 304 epidemic. *J Hyg* 1956; 54: 177-91.
- 305 3. Powers AM.; Brault A.C.; Shirako Y.; Strauss EG.; Kang W.; et al. Evolutionary relationships and
 306 systematics of the Alphaviruses. *J Virol* **2001**; 75: 10118 -31.
- 307 4. Deller J.J. Jr.; Russell PK. An analysis of fevers of unknown origin in American soldiers in Vietnam. *Ann*
 308 *Intern Med* **1967**; 66: 1129-43.

- 309 5. McGill PE. Viral infections: alpha-viral arthropathy. *Baillieres Clin Rheumatol* **1995**; 9: 145-50.
- 310 6. Adebajo AO. Rheumatic manifestations of tropical diseases. *Curr Opin Rheumatol* **1996**; 8: 85-9.
- 311 7. Ligon BL. Reemergence of an unusual disease: the chikungunya epidemic. *Semin Pediatr Infect Dis* **2006**;
- 312 17: 99-104
- 313 8. Diallo M.; Thonnon J.; Traore L.M.; Fontenille D. Vectors of Chikungunya virus in Senegal: current data
- 314 and transmission cycles. *Am J Trop Med Hyg* **1999**; 60: 281-86.
- 315 9. Volk SM.; Chen R.; Tsetsarkin KA.; Adams AP.; Garcia TI.; Sall AA.; et al. Genome-scale phylogenetic
- 316 analyses of chikungunya virus reveal independent emergences of recent epidemics and various
- 317 evolutionary rates. *J Virol* **2010**; 84: 6497- 6504.
- 318 10. Barrett ADT.; Weaver SC. Arboviruses: alphaviruses, flaviviruses and bunyaviruses. In: Greenwood D,
- 319 Slack RCB, Peutherer JF., *Medical microbiology*. 16 edn. London: Churchill Livingstone, **2002**: 484-501.
- 320 11. Brooks G.F.; Butel J.S.; Morse SA. Human arboviral infections. In: Jawetz. Melnick and Adelberg's *Medical*
- 321 *microbiology*. 23rd edn. Singapore: Mc Graw Hill, **2004**: 514-24.
- 322 12. Alessandra LP.; Alessia L.; Eleonora C.; Gianguglielmo Z.; Massimo C. Chikungunya virus, epidemiology,
- 323 clinics and phylogenesis: A review. *Asian Pac J Trop Med* **2014**; 7: 925-32.
- 324 13. Thonnon J.; Spiegel A.; Diallo M.; Diallo A.; Fontenille D. Chikungunya virus outbreak in Senegal in 1996
- 325 and 1997. *Bull Soc Pathol Exot* **1999**; 92: 79 - 82
- 326 14. Pfeiffer M.; Dobler G. Emergence of zoonotic arboviruses by animal trade and migration. *ParasitVectors*
- 327 **2010**;8 : 3:35
- 328 15. Devaux CA. Emerging and re-emerging viruses: A global challenge illustrated by Chikungunya virus
- 329 outbreaks. *World J Virol* **2012**; 1: 11-22
- 330 16. Weaver S.C.; and Reisen W.K. Present and Future Arboviral Threats. *Antiviral Res.* **2010**; 85: 328-45.
- 331 17. Lanciotti RS.; Kosoy O.L.; Laven J.J.; Panella A.J.; Velez J.O.; et al. Chikungunya virus in US travelers
- 332 returning from India, 2006. *Emerg Infect Dis* **2007**; 13: 764-67.
- 333 18. Pistone T.; Ezzedine K.; Boisvert M.; Receveur M.C.; Schuffenecker I.; Zeller H.; Lafon M.E.; Fleury H.;
- 334 Malvy D. Cluster of chikungunya virus infection in travelers returning from Senegal, 2006. *J Travel Med.*
- 335 **2009**; 16:286-88.
- 336 19. Moro M.L.; Gagliotti C.; Silvi G.; Angelimni R.; Sambri V.; et al. Chikungunya virus in north-eastern Italy:
- 337 a serosurvey. *Am J Trop Med Hyg* **2010**; 82: 508 -11.
- 338 20. Grandadam M.; Caro V.; Plumet S.; Thiberge J.M.; Souarès Y.; et al. Chikungunya Virus, Southeastern
- 339 France. *Emerg Infect Dis* **2011**;17: 910 – 13.
- 340 21. Powell J. New contender for most lethal animal. *Nature* **2016**; 540:525.
- 341 22. Bres P.; Chambon L.; Pape Y.; Michel R. Isolement de plusieurs souches d'arbovirus à partir des glandes
- 342 salivaires de chauves-souris. *Bul Soc Med Afr Noire Langue Fr* **1963**; 8 : 710-12.
- 343 23. Robin Y.; Bres P. Arboviruses in Senegal. Current status. *Bull Soc Med Afr Noire Lang Fr* **1969**; 14: 722-28.
- 344 24. Roche S.; Robin Y. Human infections by CHIK virus in Rufisque (Senegal). *Bull Soc Med Afr Noire Lang*
- 345 *Fr* **1966**; 12: 490-96.
- 346 25. Saluzzo J.F.; Cornet M.; Digoutte JP. Outbreak of a CHIK virus epidemic in western Senegal in 1982. *Dakar*
- 347 *Med* **1983**; 28: 497-00.
- 348 26. Monlun E.; Zeller H.; Le Guenno B.; Traoré L.M.; Hervy J.P. Surveillance de la circulation des arbovirus
- 349 d'intérêt médical dans la région du Sénégal Oriental (1988 – 1991). [Bull Soc Pathol Exot.](#) **1993**; 86:21-8.
- 350 27. Cornet M.; Robin Y.; Chateau R.; Hème G.; Adam C.; et al. Isolement d'arbovirus au Sénégal oriental à
- 351 partir de moustiques (1972-1977) et notes sur l'épidémiologie des virus transmis par les *Aedes*, en particulier
- 352 du virus Amaril. *Cah. O.R.S.T.O.M. Sér Ent Méd Parasitol* **1979** ; 17:149-63.
- 353 28. Diallo M.; Sall AA.; Moncayo AC.; Ba Y.; Fernandez Z.; et al. Potential role of sylvatic and domestic African
- 354 mosquito species in dengue emergence. *Am J Trop Med Hyg* **2005**; 73: 445- 49.
- 355 29. CRORA (Centre collaborateur OMS de référence et de recherche pour les arbovirus et virus de fièvres
- 356 hémorragiques). <http://www.pasteur.fr/recherche/banques/CRORA/virus>. Accessed 2017 October 01.
- 357 30. Sow A.; Faye O.; Diallo M.; Diallo D.; Cheng R.; Faye O.; et al. Chikungunya Outbreak in Kedougou,
- 358 Southeastern Senegal in 2009 – 2010. *OFID* **2017**; Doi: 10.1093/ofid/ofx259.
- 359 31. Agence nationale de la démographie et de la Statistique (2015). Service Régional de la Statistique et de la
- 360 Démographie de Kédougou. Rapport annuel de la Situation économique et régionale. Kedougou, Sénégal.
- 361 Aboubacar Sédikh BEYE. 138p. <http://www.ansd.sn/ressources/ses/SES-Kedougou-2013.pdf> Accessed
- 362 2017 July 17.

- 363 32. Traore L.M.; Fontenille D.; Zeller H.G.; Mondo M.; Diallo M.; et al. Surveillance for yellow fever virus in
364 eastern Senegal during 1993. *J Med Entomol* **1996**; *33*: 760-65.
- 365 33. NASA EOSDIS Land Processes DAAC, USGS Earth Resources Observation and Science (EROS) Center,
366 Sioux Falls (<https://lpdaac.usgs.gov>), accessed September15, 2017
- 367 34. Hay S.I.; Tatem A.J.; Graham A.J.; Goetz S.J.; Rogers D.J. Global environmental data for mapping
368 infectious disease distribution. *Adv Parasitol* **2006**; *62*:37-77.
- 369 35. Hansen M.C.; Potapov P.V.; Moore R.; Hancher M.; Turubanova S.A.; Tyukavina A.; et al. High-Resolution
370 Global Maps of 21st-Century Forest Cover Change. *Science* **2013**; *342*:850-853
- 371 36. Farr T.G.; Kobrick M. Shuttle Radar Topography Mission produces a wealth of data. *Amer Geophys Union*
372 *Eos* **2000**; *81*(48): 583-5.
- 373 37. The WorldPop demography project .<http://www.worldpop.org.uk/>. accessed September15, 2016
- 374 38. Lunn D.J.; Thomas A.; Best N.; Spiegelhalter D. WinBUGS - a Bayesian modelling framework: concepts,
375 structure, and extensibility. *Statistics and Computing* **2000**; *10*:325-337.
- 376 39. Diallo D.; Sall AA.; Buenemann M.; Chen R.; Faye O.; Diagne C.T.T.; et al. Landscape Ecology of Sylvatic
377 Chikungunya Virus and mosquito Vectors in Southeastern Senegal; *PLoS Negl Trop Dis.* **2012**; *6*: e1649.
- 378 40. Sow A.; Faye Oum.; Diallo D.; Chen R.; Faye Ous.; Diagne C.T.T.; et al. Re-emergence of Yellow fever in
379 Kedougou, Southeastern Senegal in 2010-2011. *Int J Sc Research.* **2017**; doi 10.1016/j.ijid.2014.03.878.
- 380 41. Travel time to major cities: A global map of Accessibility.
381 <http://bioval.jrc.ec.europa.eu/products/gam/index.htm>., accessed September15, 2018
382



© 2019 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

383