viruses

MDPI

Article

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

Abdourahmane Sow*[1,8,9], Birgit Nikolay[2,3,4], Oumar Faye[1], Simon Cauchemez[2,3,4], Jorge Cano[5], Mawlouth Diallo[6], Ousmane Faye[1], Bakary Sadio[1], Oumar Ndiaye[1], Scott C. Weaver[7], Anta T Dia[8], Amadou Alpha Sall[1] and Denis Malvy[9]

[1] Institut Pasteur Dakar, Arbovirus and viral Hemorrhagic Fevers Unit, 36, Avenue Pasteur, BP 220, Dakar, Senegal.; West African Health Organization (WAHO)175 Avenue Ouezzin Coulibaly, BP 153 Bobo-Dioulasso, Burkina Faso
[2] Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France.
[3] CNRS, URA3012, Paris, France.
[4] Center of Bioinformatics, Biostatistics and Integrative Biology, Institut Pasteur, Paris, France.
[5] Faculty of Infectious Diseases and Tropical Medicine, London School of Hygiene & Tropical Medicine, London, United Kingdom.
[6] Institut Pasteur Dakar, Medical entomology Unit, 36, Avenue Pasteur, BP 220, Dakar, Senegal.
[7] Institute for Human Infections and Immunity, Center for Tropical Diseases and Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA,
[8] Institut Santé et développement (ISED), Université Cheikh Anta Diop, Dakar, Senegal,
[9] INSERM U1219 University of Bordeaux, Bordeaux, France.
* Correspondence: asow20@gmail.com; Tel: +226 66894712

Received: date; Accepted: date; Published: date

Abstract: In Senegal, chikungunya virus (CHIKV) is maintained in a sylvatic cycle and causes sporadic cases or small outbreaks in rural areas. However, little is known about the influence of the environment on its transmission. To address the question, One hundred twenty villages were randomly selected in the region. Samples were tested for anti CHIKV IgG antibodies by ELISA. We investigated the association of CHIKV seroprevalence with environmental variables using logistic regression analysis and the spatial correlation of village seroprevalence based on semivariogram analysis. 54% [51-57] of individuals were tested positive for CHIKV-specific IgG. CHIKV seroprevalence was significantly higher in population living close to forest (NDVI, OR = 1.90 [1.42-2.57] and was negatively associated with population density, (OR= 0.76 [0.69-0.84]. In contrary in gold mining sites where population density was >400 people per km^2, seroprevalence peaked significantly among adults (46%, [27-67]) compared to all other individuals (20% [12-31]; p=0.013). Higher exposure to CHIKV in areas with lower population density and close to the forest is consistent with transmission through sylvatic mosquitoes such *Aedes furcifer*. However traditional gold mining activities changes significantly the transmission dynamic of CHIKV leading a potential increase in the risk of human exposition in the region.

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

1. Introduction

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

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

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

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

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

2. Materials and Methods

Serological study:

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

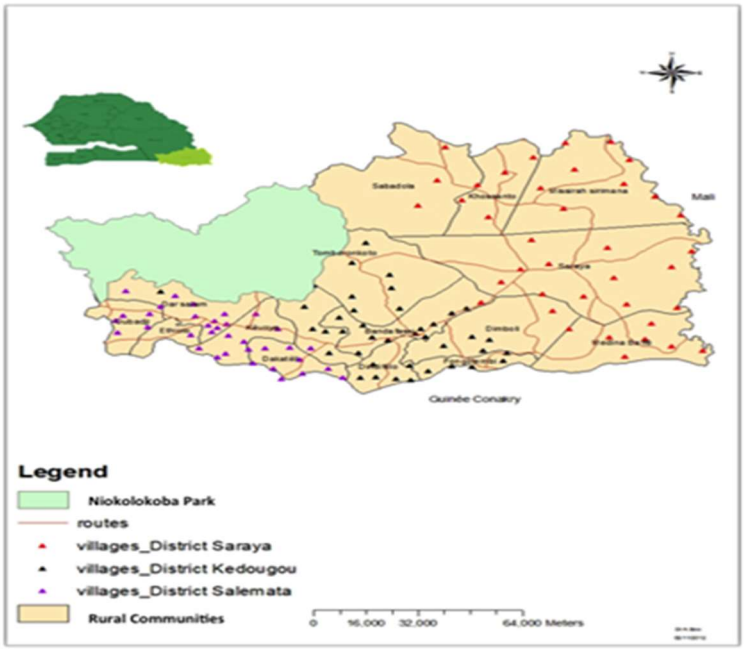


Figure 1. Investigated villages in Kedougou region.

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].

Environmental data

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.

Statistical analysis

Descriptive analysis

Age was classified as <5, 5 to 9, 10 to 19, 20 to 39, 40 to59 and ≥60 years. Associations of seroprevalence with age and sex of individuals were investigated by logistic regression analysis and

statistical significance was assessed with a likelihood ratio test. The models included random intercepts for villages and rural communities to adjust for clustering of surveyed individuals. Confidence intervals of seroprevalence by sex or age-group were obtained based on the exact binomial method. We included a sex-age interaction term to explore potential greater exposure to infections on certain groups (i.e. male adults be more exposed during their activities outside their resident villages) considering age groups that reflect different occupational activities (<20, 20 to 59, and ≥60 years) and stratifying by population density (locations with ≤400 and >400 people per km^2).

Spatial patterns of seroprevalence

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

Environmental risk factors

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

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

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

3. Results

Serological investigation for CHIKV IgG in 2012

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

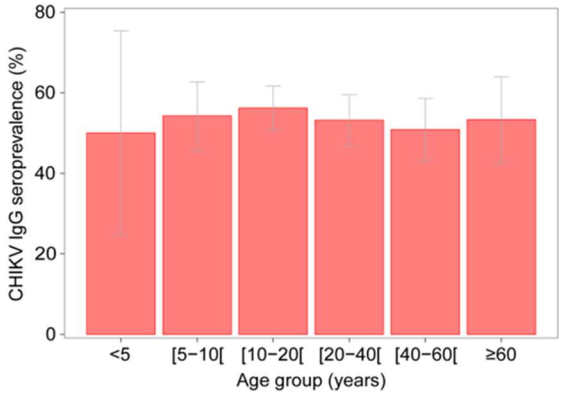


Figure 2. Seroprevalence by age group and exact binomial 95% confidence intervals.

Spatial variation in CHIKV IgG seroprevalence

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].

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).

	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 km^2 (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

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

Table 2. Multivariable analysis of the association between CHIK seroprevalence and environmental variables. The models were adjusted for 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)	0.76 (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

This translates for example into a predicted seroprevalence of 57% [55-62] at a population density of 10 persons per km^2, compared to 32% [23-38] at a population density of 500 persons per km^2 (Figure 3C). Village random effects were not spatially correlated and including spatial dependency did not improve model fit (Supplementary Figure 1).

In contrary, among individuals living in villages where population density was >400 people per km^2 (i.e., seven villages in the rural community of Bandafassi which are the main sites of traditional gold mining), seroprevalence against CHIKV peaked among adults (Figure 3D); in particular it was significantly higher among male adults 20-59 years old 46%, [27-67] compared to all other individuals (20% [12-31]; p=0.013). There was suggestive evidence for an interaction between sex and age ($p_{interaction}$=0.098).

Among individuals living at population densities ≤400 people per km^2, seroprevalence among male adults did not differ significantly from other individuals (p=0.091) and no interaction between age and sex was detected ($p_{interaction}$=0.766).

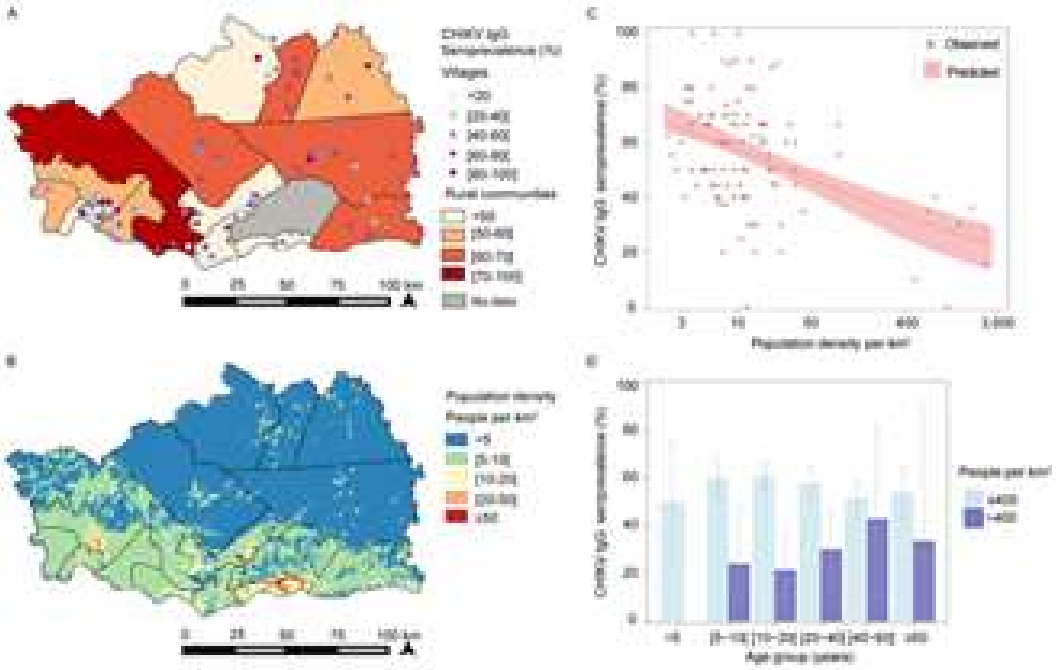


Figure 3. Spatial variation in CHIKV IgG Seroprevalence. (A) Seroprevalence by village and rural community. (B) Spatial variation in population density. (C) Observed and predicted seroprevalence by population density. The 95% CI of the prediction was obtained by boostrap (2,000 iterations) (D) Age patterns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

4. Discussion

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

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

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

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

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

Supplementary Materials:

Environmental data

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

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

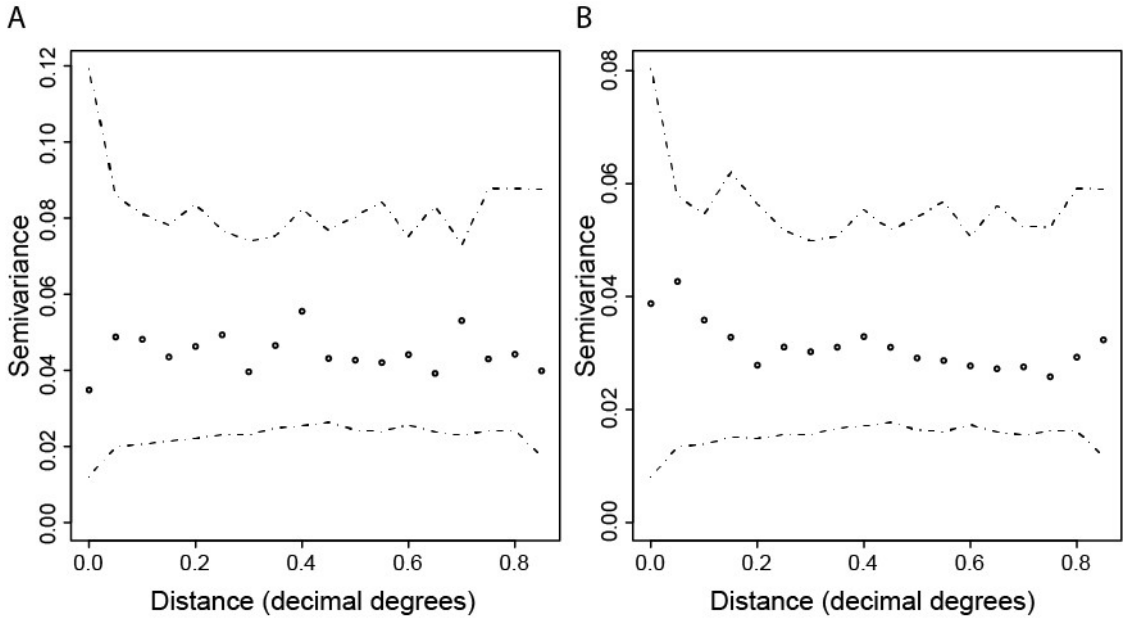
The elevation dataset at 250m resolution was derived from a gridded digital elevation model produced by the Shuttle Radar Topography Mission (SRTM) [36]. This elevation surface was then processed to obtain slope in degrees. In addition, a gridded map of urban accessibility at 1 km resolution was obtained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

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

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

Spatial correlation of village seroprevalence levels

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



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

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

Supplementary Table 1: Univariable analysis of the association between CHIKV seroprevalence and environmental variables using 3km buffers around villages. The models were adjusted for 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

Supplementary Table 2: Multivariable analysis of the association between CHIK seroprevalence and environmental variables using 3km buffers around villages. The models were adjusted for 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

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "conceptualization, A.A.S. and S.C.W. 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 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—original draft preparation, A.S.; J.C.; and B.N.; writing—review and editing, A.A.S.; D.M.; A.T.D.; visualization, B.N.; funding acquisition, A.A.S; and S.C.W."

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

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

Competing interests: The authors declare no competing interests.

Appendix

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).

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

Figure 1: Investigated villages in Kedougou region

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

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

References

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

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

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