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RE-EMERGENCE OF YELLOW FEVER IN KEDOUGOU, SOUTHEASTERN SENEGAL IN 2010-2011

Medical Science

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ABSTRACT

Yellow Fever (YF) is an infectious viral disease transmitted by Aedes mosquitoes that causes 200,000 human cases and 30,000 deaths annually in tropical Africa and South America. YF has emerged from its sylvatic cycle in the Kedougou region in Senegal regularly over the past five decades without any major urban outbreaks, but changes have occurred since 2007, including gold mining, increased urbanization and massive immigration. Such changes have the potential to disrupt on the epidemiological pattern of YF in this region. We report results of surveillance and YFV outbreaks among humans, non-human primates and mosquitoes in Kedougou, Senegal during 2010 and 2011. Serum samples were collected from, 9,213 patients recruited from 7 clinics in Kedougou region and 13 confirmed and 10 probable YF cases were diagnosed. We also detected YF virus in 67 pools of mosquitoes, mainly Aedes furcifer, from September to December 2010, and evidence of recent infection was found in monkeys at the same period. Entomological investigations during the YF outbreak showed that epidemic vectors were found in the domestic context and entomologic risk indexes were well above the epidemic threshold. These data emphasize the concern that gold mining and associated changes occurred in Kedougou region could lead to emergence and propagation of YF human outbreaks.

KEYWORDS:

Introduction

Yellow Fever (YF) is an acute infectious viral disease transmitted by *Aedes* mosquitoes that causes 200,000 human cases and 30,000 deaths annually in tropical Africa and South America [1]. YF virus (YFV) circulation is based on a temporal and spatial dynamics. The virus circulates continuously in sylvatic, endemic foci in forests and wet savannahs between non-human primates and sylvatic *Aedes* and can be introduced into regions of dry savannahs through movement of NHPs or mosquitoes to seed human epidemics [2]. In West Africa, low vaccine coverage and urbanization led to YF resurgence in urban outbreaks [3-5] in Abidjan (Côte d'ivoire, 2001), Conakry (Guinea, 2002) Dakar and Touba (Senegal, 2002) Bobo Dioulasso (Burkina Faso, 2004) [6].

In Senegal, since 1972 entomological surveillance has been carried out in Kedougou region [7] which is considered to be as an emergence zone [8] where YF sylvatic amplifications in mosquitoes have been reported at 4 to 6 year intervals [7,9-11]. Such amplifications have always coincided or been followed by human outbreaks in central Senegal (1965 Diourbel, Sine Saloum, 1978, 1995 koungueul, Kaffrine 1996, Bambey 2001, Touba and Dakar in 2002) [12-16] or in West Africa [11] but never in Kedougou region itself [17]. In 1993, a serosurvey conducted in Kedougou following a sylvatic YF amplification showed that no recent human infection could be detected and immunity against YFV was as high as 77.1% among the local population [11] as previously described by Cordellier [2]. In endemic areas the level of immunity was mostly acquired via natural infection rather than vaccination. Since 2007, gold mining has become the main economic activity in Kedougou, leading to increased urbanization, more activity in the forest and massive migration of non-immune populations. A YF outbreak occurred in Kedougou during 2010 and 2011 at the same time as these disruptive social changes. In this paper we report epidemiological, virological and entomological information about this outbreak.

Materials and methods

Study area

The study was conducted in the Kedougou region located in South East Senegal (12°32'N, 12°11'W) (**Figure 1**), with 133 487 inhabitants of which 55% are under 20 years and an average density of 8 persons per km^2. Kedougou region borders Guinea, Mali and Gambia. The Gambia river and its tributaries run through the region, some of these tributaries dry up during the dry season, creating temporary ponds with dense vegetation around the edges. The region is located between isohyets 1200 mm and 1300 mm. The climate is Sudano-Guinean with a single rainy season from May to November. Temperatures are generally high with an annual average of 28.4 ° C. The landscape consists of wooded savannah or woodland and dense gallery forest and the fauna is diverse, including several known NHP hosts of YFV, such as *Chlorocebus Sabaeus,Papio papio, and Erythrocebus patas.* Agriculture is the main economic activity in the region but hunting and logging are a source of human contact with the forest. Recently, gold mining has become one of the most important economic activities in the area.

Human sampling

Human Surveillance: Health facilities involved in the human surveillance cover a population of 109,034 inhabitants including 70,005 inhabitants in the Kedougou health district (Kedougou health care center, Bandafassi outpatient clinic, Ninefesha hospital and Kedougou catholic mobile team working in the area of responsibility of the health center of Tomboronkoto) and 39,029 in Saraya health District (Saraya health center and Khossanto outpatient clinic). Any patient older than 1 year with an acute (less than 2 weeks from the onset of disease) febrile (temperature ≥ 38 °C) syndrome and exhibiting 2 or more of the following symptoms: headache, myalgia, eye pain, arthralgia, cough, nausea / vomiting, diarrhea, jaundice, bleeding and neurological signs, was enrolled once a written informed consent was signed.

Cases definition during outbreak investigation: YF case investigation was undertaken using case definition for suspect, probable and confirmed cases. A "suspect YF case" was defined as any patient in Kedougou and Saraya between May 1st 2011 and August 31th 2011 and presenting fever associated with jaundice or bleeding within 2 weeks after the onset of symptoms. A "probable YF case" was defined as any suspect case that died before being tested for YF infection markers. A confirmed case was defined "any suspect case" whose sample was positive for anti-yellow fever IgM or YFV viral genome in absence of any documented vaccination. Family members and neighbors of YF confirmed cases were defined as contact cases and were also investigated to identify asymptomatic and/or mild cases.

Mosquito sampling

Entomological surveillance: a portion of the Kedougou region was divided into 10 blocks of equal size. In each block five biotopes were chosen: forest, savannah, bare soil, agriculture and villages. Mosquitoes collection was made by teams of three catchers using human landing collection method simultaneously in forest canopy, forest ground, savanna, bare soil, agriculture, village indoor and village outdoor from 6 pm to 9 pm. The captures were performed monthly for 2 consecutive nights in each site. In each village, 5 households were selected on a virtual transect from the center to the periphery of the village and mosquitoes were collected indoor and outdoor alternatively in 3 of these houses every night. In order to avoid bias in mosquito collection, position of collectors were rotated within and among households.

After each night of capture, the mosquitoes were frozen and sorted in monospecific pools on chill table using identification keys established by Edwards (1941) , Ferrara *et al.* (1983) , Huang (1986) , and Jupp (1997) for Culicines by Diagne *et al.* (1994) – for anophelines and stored in liquid nitrogen until their use for virus isolation and identification.

Entomological outbreak investigation method: Host-seeking mosquitoes were collected indoors and outdoors in each and surrounding households where confirmed and probable human YF cases were identified. All water-holding containers were surveyed for pre-imaginal mosquito stages in the domestic and peridomestic areas where the outbreak occurred. Pre-imaginal stages were reared in insectary and emerging adult stages were collected and identified. Emerging and wild caught adult mosquitoes were frozen, sorted in monospecific pools for YFV detection.

Monkey sampling

Monkey capture and sampling: Three monkeys species, African green (*Chlorocebus Sabaeus*), the patas (*Erythrocebus patas*) and Guinea baboons (*Papio papio*), were captured in the dry season (January to May) around water points next to Silling, Bafoundou and Ngari-Sekoto villages (Figure 1) in 2010 and 2011 using trapping methods previously described [24]. Monkeys were anesthetized with ketamine; morphometric parameters were measured, dental casts prepared for use in age determination, and 3 to 5 ml of blood sampled for YFV and antibody detection. Blood samples were centrifuged for 15 min at 2000 rpm and the plasma was removed and stored in liquid nitrogen. The monkeys were then marked with a microchip to detect re-sampling and released in nature.

Laboratory tests

Serological test: Human blood samples collected through the surveillance were systematically tested for malaria and for IgM against YFV, dengue 2, Chikungunya fever, Rift Valley fever, Crimean-Congo and West Nile viruses as previously described . During the YFV outbreak investigation, human and monkey samples were also tested for anti-YFV IgG [11].

Virus isolation: Virus isolation was attempted by intracerebral inoculation of suckling mice with human sera collected at the early phase of infection (up to 5 days after the disease onset) followed by 21 days of monitoring of mice behavior to detect viral replication . Furthermore, mosquito pools were ground in Leibovitz 15 medium supplemented with 20% fetal calf serum and inoculated into continuous AP61cell lines. Virus identification was performed by indirect immunofluorescence as previously described.

Molecular detection: YFV was detected using RT-PCR of human (on acute malaria negative sera and on 10% of acute malaria positive sera) and mosquito samples. RNA was extracted from homogenates of mosquito batches using the QIAamp® Viral RNA kit (Qiagen GmbH,

Heiden, Germany) as recommended by the supplier. The extracts were taken up in 60 l of buffer AVE and then used for detection of YFV by real time RT-PCR on an ABI Prism 7500 SDS Real-Time apparatus (Applied Biosystems, Foster City, USA) as previously described.

Sequencing and phylogenetic analysis: All available YFV sequences containing the envelope gene (E), excluding vaccine strains and engineered clone sequences, were downloaded from Genbank and aligned using MUSCLE [28] and manually adjusted in Se-Al [29] according to amino acid sequence homology. The E regions were then excised and combined with the newly generated YFV E gene sequences, leading to a final data set containing 82 sequences of 1479 nt in length. Due to the high sequence identity of newly sampled sequences, only 4 sequences containing at least one mutation from each other were included in analyses. A maximum likelihood (ML) tree was then inferred using the PAUP v4.0b package [30], based on the best-fit nucleotide substitution model determined by MODELTEST [31]. The reliability of all phylogenetic groupings was evaluated through a bootstrap resampling analysis (1000 pseudo-replicates of neighbour-joining trees estimated under the ML substitution model). The database and operation script is available upon request from the authors.

Statistical analysis Statistical analysis was performed with Epi Info version 6 and R software version 2.14.2 [32]. Chi2 (^{2}) tests were used for proportions, with statistical significant set at $p<0.05$.

Ethical considerations:
The protocols for the enrollment of humans and use of monkeys in this study 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.

Results

YF human surveillance in Kedougou

From January 2010 to December 2011, 9,213 patients including 6763 (73.4%) acute cases (*i.e.* 5 days after the date of onset) were enrolled from 7 clinics. During that period, 13 YF confirmed cases (0.14%) including 12 IgM positives and 2 PCR positives and 10 probable cases were identified (Table 1). Geographical distribution of confirmed YF cases (Figure 1) showed that 84.6% (11/13) and 15.4% of them were respectively located in Kedougou and Saraya districts. Although the YF incidence rate in Kedougou district (0.45‰ inhabitants) was higher than in Saraya district (0.21‰ inhabitants), incidencies were not significantly different in these districts ($p>0.05$).

Ten probable cases were retrospectively identified during the investigation. The first probable cases occurred in May 2011 in the village of Saraya followed by 5 other cases between June and August 2011 in Saraya district (Wassangran, Ilimalo, Bondala and Diakhaba) (figure1). The 4 remaining cases (40%) were notified in Kedougou districts including 3 in Kedougou town and 1 in the village of Bantaco August 2011 (Figure 1 and Table 1). In parallel, the 2 first confirmed cases occurred in June 2010 followed by a peak of activity in November 2010, subsidence and resurgence of cases from June 2011 to October 2011. Among confirmed YF cases, children under 5 (30.8% of cases) were significantly less affected than young [15-29 years] adults 53.84% ($P < 0.0001$). The number of YF cases (69.2%) were significantly higher among men ($p <0.0001$) (Table1).

In addition, 53.8% (7/13) of YF confirmed cases were co-infected with malaria. The mild form of malaria was diagnosed among confirmed cases with headache and fever (100%), vomiting (46%), arthralgia (38%), myalgia and cough (30%), jaundice and diarrhea (15 %), but probable cases presented more severe signs of the disease like jaundice (80%), gastrointestinal bleeding (20%), neurological signs (10%). Miners were the most affected among professionals representing 23% (3/13) of the cases ($p <0.0001$).

During the YF epidemic investigation, 134 contacts of confirmed and probable cases were enrolled and sampled. IgM antibodies were not found among the contacts but the overall seroprevalence of YFV IgG antibodies as confirmed by seroneutralization was 54%, with the lowest seroprevalence of 36% in Bantaco (Table 2). Seroprevalence of neutralizing antibodies was higher in adults 61% (43/71) than in children 46% (29/63) ($p=0.131$).

Entomological findings

Entomological surveillance: Three thousand four hundred and seventy seven (3,477) and 1,793 mosquito pools were collected and tested in 2010 and 2011, respectively. From september to december 2010, YFV was detected in 67 pools (1.9 %) by RT-PCR (35, 57,3%), isolation (11, 16,4%) or both methods (21, 31,3%). *Aedes furcifer* (55.55%), *Aedes luteocephalus* (33.33%), *Aedes taylori* (6.35%), *Aedes africanus* (3.17%) and *Aedes vittatus* (1.58%) were the mosquito species found infected. No mosquito pools collected in 2011 were tested positive for YFV.

Entomological investigation during YF outbreak: Entomological investigations were performed in 16 sites to collect adult mosquitoes populations for virus detection and assess epidemic risk by zone using larvae risk indexes. Larvae investigation in 914 habitations units and 3,795 potential breeding sites showed that container and Breteau indexes were well above the epidemic threshold in all areas especially in Kedougou town and Bantaco (Table 3, figure 1). Abandoned containers and building bricks were the main sources of infestation. Also, 2,744 adult mosquitoes from 17 species including 4 YF vectors (*Aedes aegypti, Aedes luteocephalus, Aedes vittatus* and *Eretmapodites chrysogaster)* emerged from larvae collection and showed that *Ae aegypti and Aedes vittatus* was the most abundant vector.

Three hundred and sixteen adult mosquitoes belonging to 16 species including 4 YFV vectors (*Aedes aegypti, Aedes furcifer, Aedes vittatus and Eretmapodites chrysogaster*) were collected in investigated households. Although, *Aedes aegypti* was collected in all localities, *Aedes furcifer* was the predominant species (Table 5).

Overall, 555 mosquitoes pools were prepared from mosquitoes emerging from larvae or caught as adults during the field investigation but YFV was not detected in any of them.

Monkey serosurvey

From January to May 2010, 158 monkeys were captured (Figure 1), sampled and released. Anti-YFV IgM was detected in one monkey (0.63 %, Table 3); 55 (34.8%) exhibited anti-YFV IgG by seroneutralization. During the same period in 2011, 88% (193/220) of monkey sera were positive for seroneutralization comparing to 2010 ($p < 0.05$). Seroprevalence among juvenile monkeys increased from 4% in 2010 to 42% in 2011 ($p< 0.05$) (Figure 3).

Phylogenetic analysis of YFV E gene

Maximum Likelihood tree was inferred from E protein coding region nucleic sequences. As shown in figure 3, the sequences of YFV isolates from mosquitoes in 2010 were closely related to each other and belong to the lineage 4 of YFV and clustered with isolates collected from Senegal, Guinea and Gambia in 2000 - 2001.

Discussion

Starting in 2010, a sylvatic YFV circulation intensified among monkeys and mosquitoes in Kedougou region, as confirmed by the increase of seroprevalence of YFV neutralizing antibodies among juvenile monkeys from 4 to 42% between 2010 and 2011. Using surveillance of acute febrile illnesses, the first human YF confirmed cases were detected in June 2010 in Kedougou town. No further cases were reported throughout the following wet and dry seasons up to June 2011 when new YF confirmed cases were detected, suggesting that YFV may have been maintained through vertical transmission among mosquitoes. Two hypotheses could explain the early human infections in 2011: i) hatching of eggs infected from a previous amplification, ii) a long-lasting IgM of humans infected from a undetectable circulation of the virus either during the previous rainy season or the dry season. Phylogenetic analysis of the YFV isolated in 2010 clustered in clade 4 with YFV isolates from 2000 sylvatic amplification and suggests that the YFV strain causing the 2010-2011 outbreaks re-emerged from a local sylvatic focus and is consistent with previous data underlying vertical transmission of YFV as an important mechanism for YFV maintenance in nature [34]. However, the detection of IgM from a monkey collected between January to May (a period preceding the

amplification, during which few mosquitoes were active) supports the second hypothesis.

Among the 13 confirmed YF cases detected, only 2 (15%) met the "YF suspect case" definition of acute febrile jaundice proposed by WHO [35] and would have been identified through the national YF surveillance system. Such an observation not only emphasizes that YF incidence is probably underestimated [36] but also the importance of laboratory facilities capable of investigating etiologies of acute febrile illness in malaria endemic regions of Africa. Indeed, all YF confirmed cases detected during this outbreak visited a healthcare center for an acute febrile illness, which could have represented a wide array of tropical diseases including malaria and others flaviviruses and patients recovered a few days after a symptomatic treatment. Such a situation suggests that surveillance should be based more on a syndromic approach of case definition with investigation of agents causing such syndromes rather than vertical approaches focusing on one specific disease.

The high incidence of YF among children under 5 during this outbreak is consistent with similar patterns observed in several previous outbreaks in west Africa, including Senegal (1965), the Gambia (1978, 1979) and northern Ghana (1977, 1979) and could be explained by low natural immunity as children are less exposed to wild infected vectors, or by low vaccine coverage through the expanded program of immunization. Indeed, (30%) of YF infected children have never been vaccinated against YF because they live in remote areas or difficult to access particularly during rainy season due to flooding or mountains.

The young adult [15-29 years] age group was the most affected population during the outbreak (Table 1), probably because they are exposed to infected vectors in the forest in Kedougou in relation with their professional activities especially traditional gold mining. The latter activity attracts many of indigenous and foreign populations in villages to mines, which are located at edge of forests gallery (Figure 1), favoring close contact with the sylvatic amplification of YFV. In this regard, it is noteworthy that 4 confirmed and 2 probable YF cases occurred in villages with gold extracting activity (Bantaco, Diyabougou, Bondalla and Tenkoto).

Furthermore, the absence of YF confirmed cases among contacts and family members during the epidemiological investigation conducted immediately after detection of confirmed and probable cases suggests limited or no domestic transmission. Adult YF confirmed cases had been probably infected with the forest cycle of the virus during their professional activities. Children rarely enter the forest alone but instead may have been infected either during a stay in the forest with their parents or by mosquitoes which moved from forests into households. Notably, infected *Aedes furcifer* were detected in villages (Baraboye and Velingara).

During the entomological investigation, *Ae aegypti* was collected in domestic context as adults or larvae in all localities. The presence of this species, which has never been reported for Kedougou in the past [36] may be the consequence of the rapid and increased urbanization and expansion of new settlements; certainly by using building bricks as breeding sites as they were identified as the primary source of *Aedes aegypti* larvae (Table 4). This change may have a major impact on arbovirus transmission in Kedougou town, as the virus circulation in domestic context may be favored and create conditions for domestic outbreak. Containers and Breteau indices were well above the threshold of epidemic risk.

Our active and integrated surveillance system for YFV did allow early detection of this YFV amplification virus in 2011 and facilitated the mobilization of effective preventive measures for protection of the population. This effort demonstrates the importance of a multidisciplinary collaboration between clinicians, epidemiologists, virologists, entomologists and primatologists for an effective fight against YF in Africa. This study showed that despite the absence of major outbreak, YFV transmission in Kedougou may be changing in the future in concert with urbanization and migration of susceptible populations. In particular, social changes may favor adaptation of *Ae. Aegypti* to domestic contexts and created breeding sites for *Ae. furcifer*, which could lead to intermediate or urban transmission of YF and a return to the deadly human outbreaks of the past [4-5]. Therefore the strengthening of EPI (Expanded Program on Immunization) should be performed to protect children against YFV especially in miner's villages and in areas with difficult access. Also the epidemiological surveillance system should be reinforced mostly in bordering between Senegal, Mali and Guinea where immigration may be diluting herd immunity to YFV.

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Figures:

Figure 1. Map of Kedougou showing the sites sampled for yellow fever surveillance during this study. Red human symbols indicate sites where confirmed human cases were found, black human symbols indicates sites where probable cases were found, Red Trees indicate locations of monkey sampling, H symbol indicate health center, and green triangles show sites of mosquito sampling.

Figure 2. Temporal distribution of yellow fever cases and detection of mosquito pools containing yellow fever virus from May January 2010 to December 2011 in Kedougou.

Figure 3. Seropositive monkey sera according to species and age during the dry season in 2010 and 2011.PRNT=Plaque reduction Neutralization Test. AGM=African green monkey (Chlorocebus Sabaeus), PP= Papio papio, EP= *Erythrocebus patas.*

Figure 4. Maximum Likelihood tree of the E1 gene of yellow fever virus. Isolates from mosquitoes obtained between January 2010 to December 2011 in Kedougou are highlighted in red and the most recent, previous Senegal strain is highlighted in blue. Bootstrap value higher than 70 are labeled along the major branches.

Table 1: Yellow fever Incidence Rate (IR) and probable cases according to age and sex in Kedougou region between June 2010 et October 2011.

	Affected population		Confirmed cases			Probable cases	
District		Number	Number	%	IR(/1000)	Number	%
Kedougou	**Sex**						
	male	11666	7	53.84%	0.6	2	50%
	female	12639	4	46.16%	0.32	2	50%
	age group						
	0-4 years	4132	4	30.76%	**0.96**	0	0%
	5-14 years	6805	0	0%	0	0	0%
	15-29 years	6805	6	46.15%	**0.88**	3	30%
	30-44 years	3646	1	7.70%	0.27	1	10%
	>45 years	2917	0	0%	0	0	0%
	Subtotal	**24305**	**11**	**84.60%**	**0.45**	**4**	40%
Saraya	**Sex**						
	male	4471	2	15.40%	0.45	4	67%

	female	4844	0	0%	0	2	33%
	age group						
	0-4 years	1584	0	0%	0	3	30%
	5-14 years	2608	0	0%	0	0	0%
	15-29 years	2608	1	7.70%	0.38	0	0%
	30-44 years	1397	0	0%	0	1	10%
	>45 years	1118	1	7.70%	0.89	2	20%
	Subtotal	**9315**	**2**	**15.40%**	**0.21**	**6**	60%
Total		**33620**	**13**	**100%**	**0.39**	**10**	**100%**

Table 2: Yellow Fever Seroprevalence in designated localities in Kedougou region during August 2011.

				Seroprevalence			
	Investigated patients			IgG+			
Sites	**Suspected cases**	**Contacts**	**Total**	**Vaccinated**	**Non Vaccinated**	**Unknown**	**Total**
Kedougou	11	18	29	0	1	12	13 (45%)
Tenkoto 2	0	6	6	3	2	0	05 (83%)
Angoussaka	0	24	24	0	1	8	09 (38%)
Wassangran	0	23	23	8	0	7	15 (65%)
Diyabougou	1	4	5	0	2	0	02 (40%)
Bantaco	7	15	22	0	3	5	08 (36%)
Diakhaba	0	2	2	0	1	1	02 (100%)
Commune de Saraya	0	2	2	0	1	2	03 (100%)
Kanomery	1	3	4	0	1	1	02 (67%)
Ngary	1	16	17	1	8	4	13 (77%)
Total	**21**	**113**	**134**	**12(16%)**	**20(28%)**	**40(56%)**	**72 (54%)**

Table 3 : Breeding sites found with water and epidemic risk indices in designated localities in Kedougou region

Localities	**VH**	**HU**	**SC**		**AC**		**tires**		**bricks**		**others**		**Total**		**CI**	**BI**
			Po	Pr.	P0	Pr	Po	Pr	Po	Pr	Po	Pr	Po	Pr.		
Bantaco	10	62	10	30	5	11	4	6	47	58	7	10	73	115	63	117
Bondala	10	56	1	5	36	73	4	7	11	33	0	2	52	120	43	92
Diakhaba	10	115	2	63	11	50	3	11	87	116	2	10	105	250	42	91
Kedougou	44	268	16	116	63	155	28	56	918	1097	22	62	1047	1486	70	390
Ndebou	10	49	12	70	4	9	0	0	0	0	2	6	18	85	21	36
Saraya	10	33	2	14	25	92	2	5	0	0	1	1	30	112	26	90
Wassangran	10	63	4	40	27	70	1	5	0	0	12	26	44	141	31	69
Total	148	914	63	454	234	615	70	146	1981	2401	68	179	2416	3795	63	264

Table 5: Adult mosquitoes collected in the field or obtained from emergence of collected larvae

Species	**Human landing catch**	**Emergence**
Aedes aegypti	39	1742
Aedes centropunctatus	1	0
Aedes dalzieli	1	0
Aedes furcifer	112	0
Aedes fowleri	1	0
Aedes. hirsutus	0	5
Aedes. luteocephalus	0	37
Aedes minutes	28	0
Aedes. vexans	0	3
Aedes vittatus	35	127
Total Aedes	**217**	**1914**
Total Anopheles	**15**	**10**
Total Culex	**81**	**815**
Eretmapodites chrysogaster	1	5
Mansonia uniformis	2	0
Total	**316**	**2744**

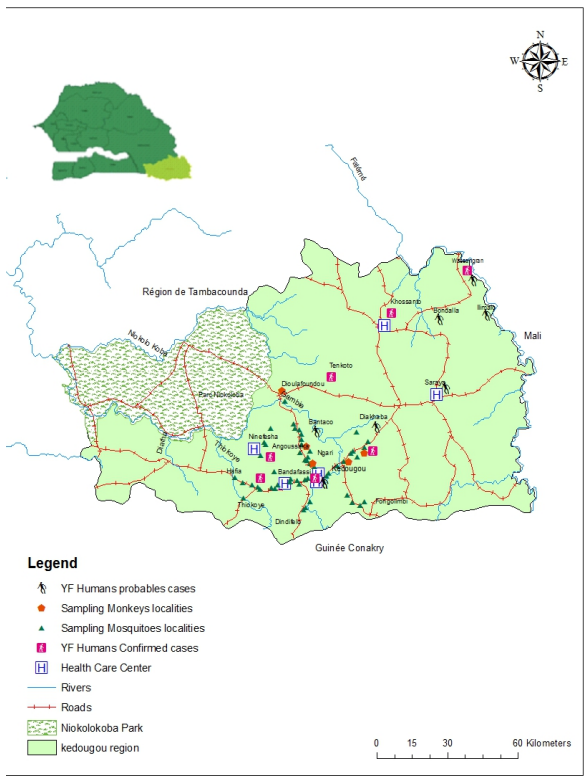
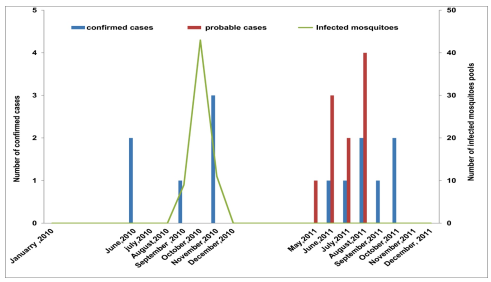
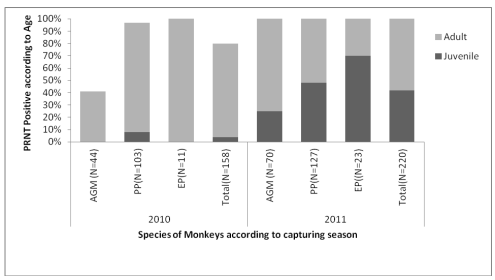
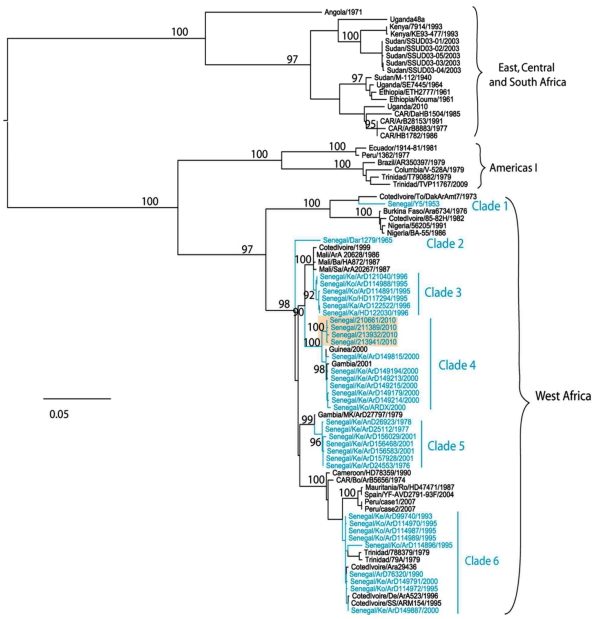


Figure 1: Study area, sampling sites and geographical distribution of YF cases in Kedougou region between January 2010 and December 2011.







References:

1. OMS (2000) Surveillance de la fievre jaune: lignes directrices à l'échelon du district. Organisation mondiale de la Santé, Département Vaccins et produits biologiques CH-1211 Genève 27 (Suisse).64p.
2. Cordellier R (1991) [The epidemiology of yellow fever in Western Africa]. Bull World Health Organ 69: 73-84.
3. WHO (2011) Le point sur les épidémies. Wkly Epidemiol Rec 86: 37-44.
4. WHO (2011) Yellow fever in the WHO African and American Regions, 2010. Wkly Epidemiol Rec 86: 370-376.
5. WHO (2011) Yellow fever in Africa and Central and South America, 2008-2009. Wkly Epidemiol Rec 86: 25-36.
6. WHO (2005) Progress in the control of yellow fever in Africa. Wkly Epidemiol Rec 80: 50-55.
7. Cornet M, Robin Y, Château R, Héme G,Adam C et al.(1979) 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. et Parasitol.,vol XVII,n°3,1979: 149-163.
8. Taufflieb R, Cornet M, Le Gondec G, Robin Y (1973) foyer selvatique de Fièvre jaune au Sénégal Oriental. Cah. O.R.S.T.O.M.,Sér.Ent.méd. et Parasitol.,vol XI,n°3,1973: 211-220.
9. Faye O, Diallo M, Dia I, Ba Y, Faye O, et al. (2007) [Integrated approach to yellow fever surveillance: pilot study in Senegal in 2003-2004]. Bull Soc Pathol Exot 100: 187-192.
10. Monlun E, Zeller H, Le Guenno B, Traore-Lamizana M, Hervy JP, et al. (1993) [Surveillance of the circulation of arbovirus of medical interest in the region of eastern Senegal]. Bull Soc Pathol Exot 86: 21-28.
11. Traore-Lamizana M, Fontenille D, Zeller HG, Mondo M, Diallo M, et al. (1996) Surveillance for yellow fever virus in eastern Senegal during 1993. J Med Entomol 33: 760-765.
12. WHO African Region (2003) Yellow fever epidemic in senegal in 2002: lessons learnt. Communicable Diseases Epidemiological Report. October 2003:1-3.
13. Cornet M, Robin Y, Hannoun C, Corniou B, Bres P, et al. (1968) [An epidemic of yellow fever in Senegal in 1965. Epidemiological studies]. Bull World Health Organ 39: 845-858.
14. Salaun JJ, Germain M, Robert V, Robin Y, Monath TP, et al. (1981) [Yellow fever in Senegal from 1976 to 1980 (author's transl)]. Med Trop (Mars) 41: 45-51.
15. Thonnon J, Fontenille D, Tall A, Diallo M, Renaudineau Y, et al. (1998) Re-emergence of yellow fever in Senegal in 1995. Am J Trop Med Hyg 59: 108-114.
16. Thonnon J, Spiegel A, Diallo M, Sylla R, Fall A, et al. (1998) Yellow fever outbreak in Kaffrine, Senegal 1996: epidemiological and entomological findings. Trop Med Int Health 3: 872-877.
17. ReliefWeb report (2005) Yellow fever in Senegal. Available: http://reliefweb.int/node/414466. Accessed 13 February 2013.
18. Diallo D, Sall AA, Buenemann M, Chen R, Faye O, et al. (2012) Landscape ecology of sylvatic chikungunya virus and mosquito vectors in southeastern Senegal. PLoS Negl Trop Dis 6: e1649.
19. Edwards FW (1941) Mosquitoes of the Ethiopian Region. III. Culicine Adultsand Pupae. London: British Museum of Natural History.Faye O, Diallo M, Dia I, Ba Y, Faye O, et al. (2007) [Integrated approach to yellow fever surveillance: pilot study in Senegal in 2003-2004]. Bull Soc Pathol Exot 100: 187-192.
20. Ferrara L, Germain M, Hervy JP (1984) Aedes (Diceromyia) furcifer (Edwards, 1913) et Aedes (Diceromyia) taylori (Edwards, 1936): le point sur la diffe´rentiation des adultes. Cah ORSTOM, Entomol Med Parasitol 22: 95–98.
21. Huang YM, Ward RA (1986) A pictorial key to the identification of the mosquitoes associated with Yellow Fever in Africa. Mosq Syst 13: 138–149.
22. Jupp PG (1996) Mosquitoes of Southern Africa: Culicinae and Toxorhynchitinae. Hartebeespoort: Ekogilde Publishers.
23. Diagne ND, Fontenille D, Konate L, Faye O, Lamizana MT, et al. (1994) Les Anopheles du Senegal: liste comment& et illustree. Bull Soc Path, Exp 87: 1–9.
24. Belchior EM. Implication des singes en tant qu'hote- reservoir dans le cycle selvatique de la dengue dans la region de Kedougou (Senegal oriental). These de doctorat veterinaire, Ecole nationale veterinaire d'alfort ,99p.2004, Creteil.
25. Draper CC (1971) Malaria. Laboratory diagnosis. Br Med J 2: 93-95.
26. Digoutte JP, Calvo-Wilson MA, Mondo M, Traore-Lamizana M, Adam F (1992) Continuous cell lines and immune ascitic fluid pools in arbovirus detection. Res Virol 143: 417-422.
27. Weidmann M, Faye O, Faye O, Kranaster R, Marx A, et al. (2010) Improved LNA probe-based assay for the detection of African and South American yellow fever virus strains. J Clin Virol 48: 187-192.
28. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797.
29. Molecular Evolution, Phylogenetics and Epidemiology. Available: http://www.tree.bio.ed.ac.uk. Accessed 13 February 2013.
30. Rogers JS, Swofford DL (1998) A fast method for approximating maximum likelihoods of phylogenetic trees from nucleotide sequences. Syst Biol 47: 77-89.
31. Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.
32. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
33. Galat G. Socio-écologie du Singe vert (Cercopithecus aethiops sabaeus), en référence de quatre Cercopithécinés forestiers sympatriques (Cercocebus atys, Cercopithecus campbelli, C. diana, C. petaurista) d'Afrique de l'Ouest. Thèse de Doctorat ès Sciences,Université Pierre et Marie Curie,500p.1983,Paris.
34. Sall AA, Faye O, Diallo M, Firth C, Kitchen A, et al. (2010) Yellow fever virus exhibits sloweevolutionary dynamics than dengue virus. J Virol 84: 765-772.
35. World Health Organization (Department of Immunization, Vaccines and Biologicals) (2004). Manual for the monitoring of yellow fever virus infection , Geneva, Switzerland, 68 p.
36. OMS (1987) Lutte contre la FJ en Afrique.Organisation Mondiale de la santé, Genêve. 104p.
37. OMS (1993) Les bases immunilogiques de la vaccination. Organisation mondiale de la santé Genève, 1993.23p.