Article

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Bacary Djilocalisse Sadio [1,*,†], Martin Faye [1,†], Aboubacry Gaye [2], Cheikh Tidiane Diagne [3], Oumar Ndiaye [1], Bocar Sow [2] Gamou Fall [1], Oumar Faye [1], Alassane Mbengue [4], Ousmane Faye [1], Ndongo Dia [1], Amadou Alpha Sall [1] and Abdourahmane Sow [1,5]

[1] Virology Department, Institut Pasteur de Dakar, 220 Dakar, Senegal.

[2] Epidemiology, Clinical Research and Data Science Unit, Institut Pasteur de Dakar, Dakar 220, Senegal.

[3] MIVEGEC (Infectious Diseases and Vector: Ecology, Genetics, Evolution and Control), Uni. Montpellier, IRD, CNRS, 34394 Montpellier, France.

[4] G4 Malaria Experimental Genetic Approaches & vaccines, IMI, Institut Pasteur de Dakar, 220, Senegal.

[5] West African Health Organization, 175, Avenue Ouezzin Coulibaly, 01BP: 153 Bobo Dioulasso 01, Burkina Faso.

* Correspondence: Bacary Djilocalisse SADIO, PhD student, 36, avenue Pasteur, Institut Pasteur of Dakar, Dakar, Senegal, bacary.sadio@pasteur.sn; Phone: +221.77.748.00.96.

† These authors contributed equally to this work.

Abstract: Rapid proliferation of traditional gold mining sites in Kédougou a Southeast region in Senegal, led to mass population migration from the neighboring West African countries and rapid expansion of small mining villages with poor hygiene and sanitation conditions. An outbreak of hepatitis E was reported in 2014 with several cases of febrile jaundice among traditional mine workers. In this study, we analyzed both HEV IgM and IgG seroprevalence and the associated risk factors of infection by testing any suspected case and contacts collected from February 2012 to November 2014. RNA-negative sera from suspected cases and contacts were tested for anti-HEV IgM and anti-HEV IgG. A total of 799 sera were collected from 290 suspected cases, 470 contacts and 39 individuals with missing information. The median age of the cohort study was 19 years (1-88 years) with a male/female sex-ratio of 1.9. We found an overall prevalence of 43.68% (332/760) of anti-HEV IgM and 38.15% (290/760) of anti-HEV IgG sera. Our data provide new insights into the HEV epidemiology and point to the crucial need to estimate the disease's burden in Kédougou and assess the viral mechanisms driving the disease's severity in pregnant women.

Keywords: Hepatitis E Virus; seroprevalence; risk factors; mining gold villages; Southeastern Senegal

1. Introduction

Hepatitis E is a widespread disease worldwide and is a serious public health concern. Acute hepatitis E can cause febrile jaundice, asthenia, anorexia, and nausea-vomiting but regresses spontaneously within 4-6 weeks[1]. The disease can progress to fulminant and acute hepatitis infection therefore causing death. Every year there are an estimated 20 million HEV infections worldwide, leading to an estimated 3.3 million symptomatic cases of hepatitis E. The mortality rate of hepatitis E in humans ranges from 0.5 to 4%[2]. Pregnant women are more susceptible to complicated forms of the disease resulting in a 20% mortality rate in the last trimester[3]. Indeed, there is strong evidence that HEV is a major contributor to maternal morbidity and mortality in Southeast Asia, particularly in pregnant women in the third trimester. This makes HEV infection the most serious of all viral hepatitis in pregnancy[4-7].

The causative agent, Hepatitis E virus (HEV), is a non-enveloped, single-stranded, positive RNA virus with a 7.5 kilobases (kb) genome size of, belonging to the Hepeviridae family, *Hepevirus* genus[8]. HEV presents a large diversity with four distinct HEV genotypes (1-4) circulating worldwide with

specific geographical distribution of each genotypes[9–11]. Both HEV genotypes 3 and 4 infect several infect several mammalian animals, with occasional transmission to humans [12].

Serological diagnosis of HEV infection rely on both IgG and IgM antibodies detection by ELISA. IgM antibodies, which indicate a recent disease, are detectable from the early symptomatic stages with a maximum peak after a month following the primary infection. This is followed by substantial IgM decrease of IgM from 2 to 6 months at most following the infections[13]. However, IgG antibodies known to indicate an o infection, persist from 18 months to more than 10 years in the body, (depending on the sensitivity of the used assay[14]. However, serological cross-reactivity has been described between the four HEV genotypes[15–17].

Rapid proliferation of traditional gold mining sites in the Kédougou region, Southeastern Senegal, led to an increasing demography in the area with many people originating from the neighbouring West African countries. The elevated number of newly established mining villages is unfortunately associated with poor hygiene, unsafe water usage and thus bad sanitation conditions triggering disease's occurrence. In 2014, a hepatitis E outbreak has been reported in the Kédougou region including several cases of febrile jaundice. Investigations exhibited the epidemic's beginning from March 2012[18]. Herein, we investigated the HEV seroprevalence in population of the Kédougou region using serum samples collected from patients with jaundice from 2012 to 2014.

2. Methods

Ethical Statement

The Senegalese National Ethical Committee at the Ministry of Health and Social Action (MoHSA) has approved our current surveillance protocol. Consent forms were obtained from all adults or parents for minors included in this study. Field investigations were regularly supervised by the authorities of the MoHSA in Senegal. Additional samples used in this study were collected in the frame of the national integrated surveillance program of fevers in Senegal and were available from the collection of the WHO Collaborating Centre for Arboviruses and Hemorrhagic Fevers at Institut Pasteur de Dakar. All methods including the use of human samples, were performed in accordance with the Declaration of Helsinki**.**

Study area

The study was conducted in Kédougou, a region located in Southeastern Senegal (12°32′ N,12°11′ W). Insets are obtained from ArcGIS Desktop V10.8.1 (ESRI 2021, Redlands, CA: Environmental Systems Research Institute; https:// desktop. arcgis. com/ en/) and sampling sites are placed on base map using their respective XY coordinates and ArcGIS Desktop (Figure 1a). The population of Kédougou is of 184,276, 5.1% of whom are under 18 years old, and the average population density is of 8 persons per km2[19]. Kédougou shares borders with the Republic of Guinea and Mali. The climate is Sudano-Guinean with a single rainy season from May to October. Temperatures are generally high with an annual average of 28.4 °C. Agriculture is the main economic activity in the region, but hunting and logging are also source of contact between humans and wildlife [19]. The Gambia River flows through the region and creates during the dry season temporary pools with dense vegetation. Recently, gold mining has become one of the most important economic activities in the region.
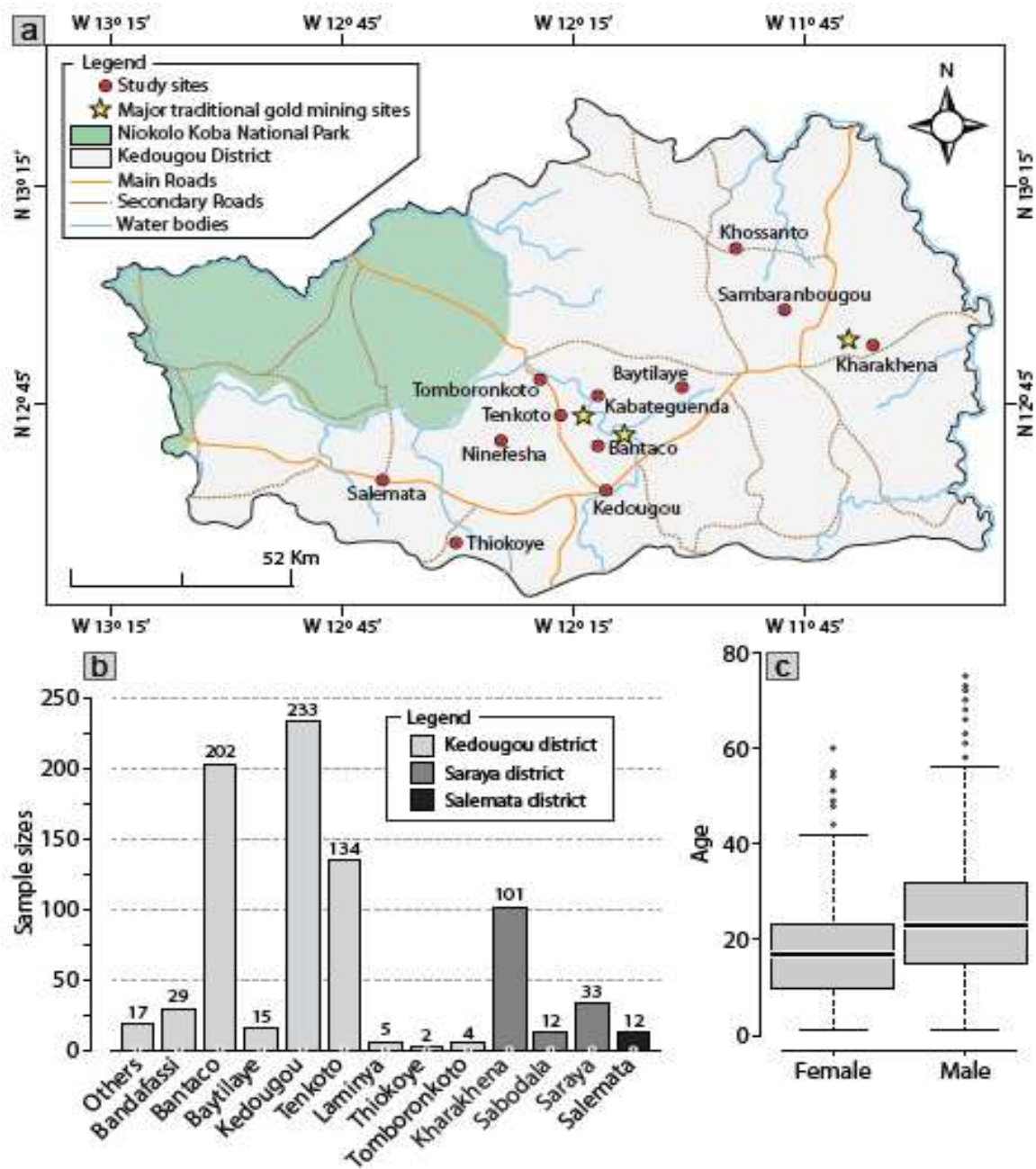
Figure 1. Geographical distribution of included patients. **a)** Kédougou's map is represented. The mining gold villages are highlighted (stars). This work was done in collaboration with the three health districts of Saraya, Kédougou (town) and Salemata. Kharakhena. **b)** 233 individuals residing in the city of Kédougou, 437 gold miners from the three traditional mining sites: Bantaco (n=202), Tenkoto (n=134), and Kharakhena (n=101) were included, while the other patients came from the surrounding villages. **c)** The median age based on the sex ratio (1.9) shows a population with a median age of 19.4 for women versus 24.4 for men.

Data collection

Following 2014 HEV outbreak in Kédougou, a retrospective sero-surveillance study was carried out from February 2012 to November 2014. All patients included in this study were working in the three main traditional mining gold villages (Kharakhena, Bantaco, Tenkoto) which are supervised by the health districts of Kédougou and Saraya, respectively. Twelve samples from suspected individuals from the health district of Salemata, a non-gold mining area were included in our study.

Clinical and epidemiological data retrieved from health care registration books and the database of the virology department of the Institut Pasteur de Dakar in Senegal, were analyzed in addition to both anti-HEV IgM and anti-HEV IgG serological data.

Serological testing

A total of 799 archived sera collected from PCR-negative suspected cases and jaundiced contacts in the Kédougou region were tested using the IgM ELISA 3.0 (ref: 2P35-01(23160-096) and MP Diagnostics IgG ELISA (ref: 2F09-02(21150-096T) kits according to the manufacturer's procedures (MP DIAGNOSTICS), for the detection of IgM HEV and IgG HEV antibodies. All samples were processed in duplicates.

Statistical analysis

Statistical analyses were performed using R version 4.0.3 (R Corporation). The age distribution by sex was represented by a boxplot showing the age distribution in the two groups, as well as the geographical distribution of the patients. The 95% confidence intervals (CI) were calculated using analysis of variance. Univariate comparison of proportions between groups (age, sex, status, and geographic location) was performed using analysis of variance. To estimate the strength of association, odds ratios (ORs) and associated 95% CIs were used. Variables with significant univariate associations at a P value < 0.05 were entered into a multivariate binomial regression model. P<0.05 or a 95% CI not spanning 1.0 was considered statistically significant for all analyses.

3. Results

Study population

Senegal is a West African country where mining activities have emerged over the last years particularly in Kédougou. The large number of undeclared migrants working in the traditional gold mining villages continue to increase (Figure 1-a). The majority of the included patients lived in the Kédougou district (n= 641), followed by the Saraya district (n= 146) and the non-mining gold sanitary district of Salemata (n= 12) (Figure 1-b). Out of the 799 patients included in our study, we identified 470 contacts and 290 suspected cases. The median age was 19 years (1-88 years) with a male/female sex ratio of 1.9 (Figure 1-c). Of the 799 sera analyzed, laboratory tests showed a total of 404 samples positive for anti-HEV, including 332 IgM, 251 IgG and 179 IgM+IgG.

Sero-epidemiological analysis

Statistical analysis showed that in Kédougou district, the villages of Thiokoye (100%, n= 1), Laminya (60%, n= 3), Baytillaye (53.3%, n= 8) and Tenkoto (29.8%, n= 40) had the highest seroprevalences for IgG (p-value= 1.88x10⁻⁶) (Table 1). In Saraya district, the highest seroprevalences were observed in Sabadola village (81.8%), Saraya town (60.6%) and Kharakhena village (47.5%) (p-value= 0.043). In Saraya district and Kédougou district, there was a positivity rate of 20/145 (53.1%) and 163/619 (26.3%), respectively. However, in Salemata district, we found that 8 out of 12 suspected patients (66.6%) tested positive for anti-HEV IgG. For IgM seroprevalence, the statistical analysis showed a highest seroprevalence in Kédougou district (220/620). This overall 35,5% recent HEV infections were distributed in Baytilaye 11/15, Thiokoye 1/2, Tenkoto 60/134, Laminya 2/5 and the city of Kédougou 86/233 (36.9%) (p-value= 1.88x10⁻⁶). In Saraya district, the highest seroprevalence rate were observed in the villages of Sabadola 11/12 (91.7%), Saraya 25/33 (75.8%) and Kharakhena 53/101 (52.5%) (p-value= 0.043). As for IgG, we found a high rate of IgM seroprevalence in the Salemata district 10/12 (83.3%). Overall, Saraya was associated with 70% old HEV infections while Kédougou showed 35% of anti-HEV IgM. Of the 8 deaths recorded among the included patients with positive IgM serology, 3 were from the Kédougou district and 5 from the Saraya district while 50% of these patients included pregnant women (n= 4). These results indicate that both old and recent HEV infections were circulating since 2012 two years before the declared 2014 outbreak. More importantly,

our data showed that HEV infections spread from a traditional mining gold village also reported to be the epicenter of HEV outbreak (Kharakhena) to the surrounding villages and city. This impact of human movement on virus circulation was also observed in Salemata district.

Table 1. IgM Seroprevalence and HEV death rate in the different districts of Kédougou, Saraya and Salemata.

District	Village	Total number of analysed samples	IgG Seroprevalence n (%)	Total number of analysed sample	IgM Seroprevalence n (%)	Death (n = 8)	Pregnant women (n= 4)	p--value
Kédougou	Bandafassi	29	8 (27,6)	29	08 (27,6)	0	0	1.88x 10^{-6}
	Bantaco	202	54 (26,7)	202	52 (25,7)	0	0	
	Baytilaye	15	8 (53,3)	15	11 (73,3)	0	0	
	Kédougou	233	49 (21,0)	233	86 (36.9)	3	2	
	Laminya	5	3 (60,0)	5	02 (40,0)	0	0	
	Tenkoto	134	40 (29,8)	134	60 (44.8)	0	0	
	Thiokoye	1	1 (100,0)	2	01 (50,0)	0	0	
Total		619	163(26,3)	620	220(35,5)	3	2	
Saraya	Kharakhena	101	48 (47,5)	101	53 (52.5)	2	1	0.043
	Sabodala	11	9 (81,8)	12	11 (91.7)	1	0	
	Saraya	33	20 (60,6)	33	25 (75.8)	2	1	
Total		145	77(53,1)	146	89(70,0)	5	2	
Salemata	Salemata	12	8(66,7)	12	10 (83.3)	0	0	
Total		12	8(66,7)	12	10 (83.3)	0	0	

Gender and age distribution of IgM and IgG HEV positive cases

Out of the of 332 IgM-positive cases, the active population aged from 16-30 years-old group show the (47.9%) highest recent HEV infections rate, followed by the 15 years old population, the 31-45 years-old group and the age group above 45 years with 25%, 19.6% and 7.5%, respectively (p-value = 0.05592). Table 2 displays the same pattern for old HEV infections rates. Indeed, out of the of 251 IgG-positive cases, the highest rate was recorded in the 16-30 years-old group with 48.2%, followed by the 31-45 years-old group, the age group under 15 years and the and the age group above 45 years with 22.7%, 19.5% and 9.6%, respectively (p-value = 0.05592) (Table 2). Moreover, if we consider gender balance, we found that HEV infections were affecting most likely men than women also see that men with 68.6% vs. 31.4% of IgM-positive and 75.3% vs. 24.7% of IgG positive cases p-value = 4.496x10^{-6} (Table 2). Next, we sought to map out the overall distribution of HEV infections. Figures 2a and 2-b showed the risk factors of HEV infection including both suspected and confirmed cases. For both IgM and IgG seroprevalence, we found a similar pattern with patients over 45 years-old, showing a higher risk of developing the disease of 88.8% (n= 24) and 96.15% (n= 25), respectively. However, the 31-45 years-old, 16-30 years-old and under 15 years groups exhibited prevalence of (n= 57) 74.02%, (n= 121) (51.05%) and (n= 49) (24.13%), respectively (Figure 2-a, b). In terms of geographical distribution, the villages of Kharkhena, the 2014 HEV epidemic epicentre, Bantaco and Tenkoto, which are all villages hosting the largest traditional gold panning sites, had more cases developing both IgG and IgM antibodies indicating that the virus was present in the population from 2012 to 2014. This is also the case for Kédougou, which hosts the reference center for the entire Kédougou region (Figure 2-c). Table 3 indicates that of the 290 suspected cases included in our study, 35.7% (n= 123) tested positive for IgG antibodies. However, the rate of IgM-positive cases was significantly higher (63.8% (n= 185)), (p-value = 2.2x10^{-16}). The overall prevalence of suspected cases developing both IgM and IgG antibodies at the same time was 37.2% (n= 108). In addition, the same
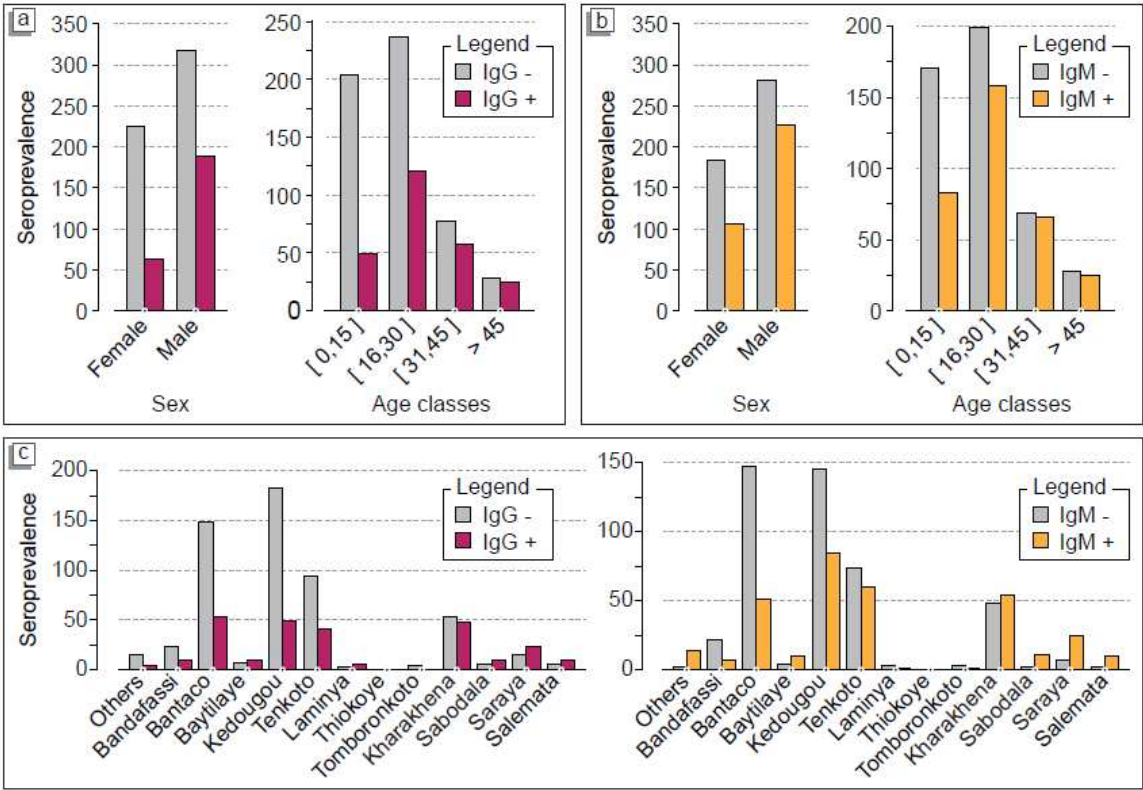
trend was observed among contacts. Among the 470 contacts, 25.3% (n=119) were positive for IgG antibodies (p-value = 2.2×10^{-16}) while 30% (n= 141) of them were positive for IgM (p-value = 2.2×10^{-16}) (Table 3). The overall prevalence of contacts developing both IgM and IgG antibodies at the same time had an antibody rate of 14.25% (n= 67).

Table 2. Seroprevalence of IgM and IgG antibodies according to age and sex.

	IgM		IgG	
	n (%)	**p-value**	**n (%)**	**p-value**
Age group (years old)				
<15	83(25)		49(19,5)	
16-30	159(47,9)	0.05976	121(48,2)	0.05592
31-45	65(19,6)		57(22,7)	
>45	25(7,5)		24(9,6)	
Total	**332 (100)**		**251(100)**	
Sex distribution				
M	227(68,6)	0.01907	189 (75,3)	4.496x10⁻⁶
F	104(31,4)		62 (24,7)	
Total	**331(100)**		**251(100)**	

Table 3. recapitulative of the seroprevalence of suspected cases and contacts according to IgM and IgG antibodies and cases positive at the same time for IgM and IgM.

| Suspected cases (n= 290)	Number of positive individuals n (%)	p-value
IgG+	123 (35.7)	
IgM+	185 (63.8)	2.2×10^{-16}
IgM+/ IgG+	108 (37.2)	
Contacts (n= 470)		
IgG+	119 (25.3)	
IgM+	141(30)	2.2×10^{-16}
IgM+/ IgG+	67 (14.25)	

Figure 2. seroprevalence by sex and age and the geographic distribution of IgM and IgG antibodies in the general population (n=799).

Overlapping IgM and IgG distribution and evolution pattern from 2012 to 2014.

The comparative analysis of individuals developing IgM type antibodies as well as those developing IgG type antibodies came from Kharahena (considered the epicenter of the epidemic), Tenkoto and Bantako which are villages that host the largest traditional gold panning sites in the region (Figure 3-a). The commune of Kédougou recorded a significant number of patients with both HEV IgM and IgG antibodies (Figure 3-a). The comparative study of individuals developing both IgM and IgG antibodies over the years showed there was a discrete/silent HEV evolution loci from 2012 to 2013. However, in 2013 there was a rapid expansion of HEV infections in the Kégoudou region leading to the outbreak previously declared from January 2014[20]. Figure 3-b showed that both old and recent HEV infections increased from 2013 with two peaks of infection recorded in February and May 2014, respectively.
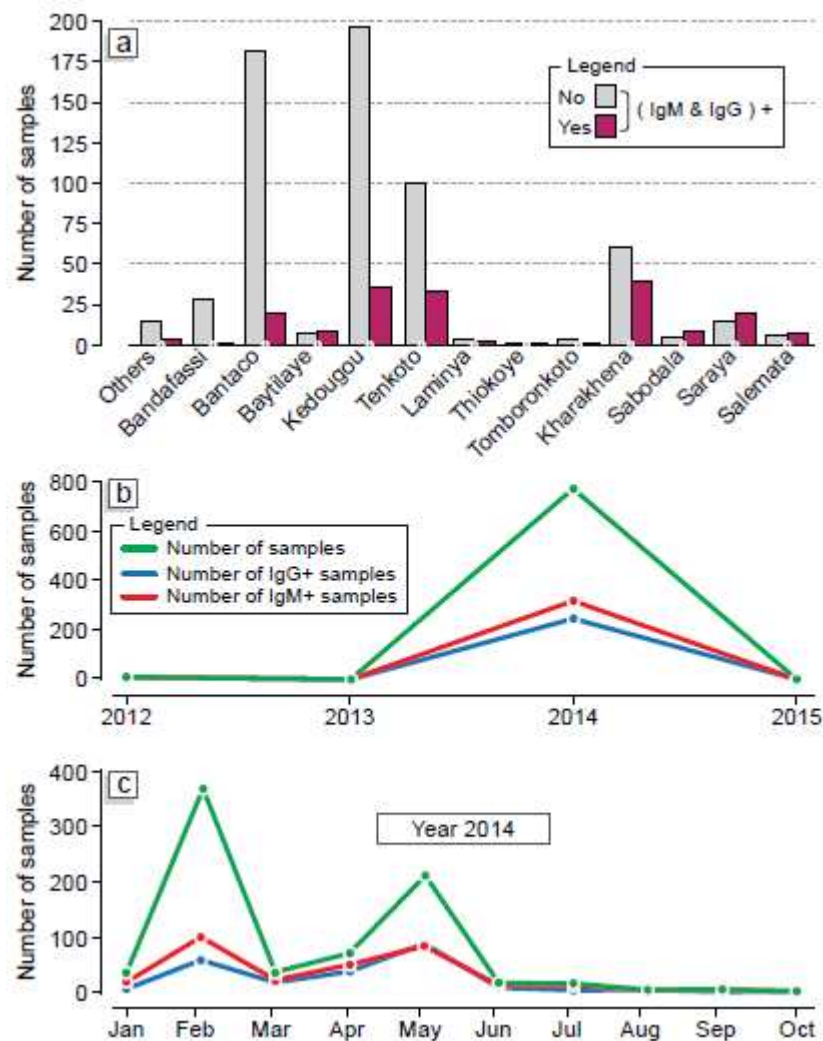
Figure 3. Geographical (a) and Temporal (b and c) distributions of IgM/IgG positive cases from 2012 to 2014 and monthly distribution of IgM/IgG positive cases in 2014.

4. Discussion

In our study, 799 HEV RNA-negative samples were tested for IgM and IgG antibodies which are indicating recent and old infections. With 332 IgM-positive cases versus 251 IgG-positive cases we found despite negative PCR detection a significant IgM/IgG ratio in Kédougou from 2012 to December 2014 as previously reported[18]. Our IgG serological data shows that HEV was circulating in the region since 2012. This shows that the classical PCR detection method alone is not sufficient to assess the HEV surveillance. ELISA must be coupled with routine PCR detection methods to prevent HEV outbreaks. Our work has shown the impact of human movements from traditional mining gold villages where sanitation, precarious hygienic conditions and access to drinking water are often challenges on HEV expansion. In fact, the highest seroprevalence recorded in the Salemata district could be related to the return of patients from gold mining sites such as Kharakhena, Tenkoto and Bantaco. Seroprevalence and HEV risk associated were found to directly impact males than females with 68.6% versus 31.4% IgM+ and 75.3% versus 24.7% IgG+. These data are similar to those previously reported from Asia[21]. However, we found that pregnant women are highly susceptible to HEV infection as previously reported[4] hus, training of clinicians and healthcare workers in the Kédougou region in the appropriate management of this disease whose signs in terms of jaundice are reminiscent of yellow fever, could be needed to avoid high mortality rate in this vulnerable group in the future. In addition, clinical monitoring of Senegalese women for HEV during pregnancy and postpartum, especially in high-risk settings as Kédougou, is critical during the last trimester of

pregnancy[22,23]. The fact that the 16-30 years old group was significantly affected by the disease could be due to the routine activities as workers in the traditional gold panning mines as previously reported in Nepal[24]. In addition, the risk of contracting HEV in people over the age of 45 years may be related to their fragile immunity, which can rapidly deteriorate over the years[20]. Given the seriousness of hepatitis E, particularly in pregnant women, in Senegal, and more particularly in Kédougou, the hepatitis E virus must be suspected in the presence of any unexplained acute hepatitis, even if it does not involve travel to an endemic zone and especially if it occurs in a patient over 40 years of age. Although there is no etiological treatment for this disease, vulnerable populations such as pregnant women and the elderly should be particularly vigilant regarding detection and early management in order to avoid high mortality. Although a low seroprevalence was found in children under 15 years in our study (25% IgM+ and 19% IgG+), more seroprevalence studies could be promoted in children across Sub-saharan Africa, particularly those under 2 years as an unusual high mortality (13%) has been previously reported in Uganda[25]. However, their possible involvement as disease vector to pregnant women could be also assessed. The non-negligible rate of IgM antibodies in contacts could be explained by the asymptomatic nature of the majority of hepatitis E cases in an outbreak[26,27], symptomatic cases could have also participated to the progressive spread of the disease in Kédougou from 2012 to 2014[20], in contrast to the brutal and massive circulation generally reported in HEV epidemics[28,29]. To evaluate the real burden of future hepatitis E epidemics, dual testing of suspected cases using both serology testing and real-time PCR could be considered.

Given the similarity of HEV to yellow fever in terms of signs, HEV testing should be used as differential diagnosis. In addition, it is necessary to sensitize the population on the modes of HEV transmission and the usual hygiene measures such as systematic hands washing after leaving the toilets, before preparing the meal, after contact with live animals or products of animal origin, using clean latrines, covering wells[23] Next, it is necessary to supply the population with drinking water in the gold mining sites by installing new boreholes and water purification systems at the household level. Finally, improvement of sanitation and wastewater management systems in gold mining sites could be prioritized.

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