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Chapter

Molecular Diagnostics of Ebola Patient Samples by Institut Pasteur de Dakar Mobile Laboratory in Guinea 2014–2016

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Abstract

As part of the laboratory response to the Ebola virus outbreak in Guinea, the Institut Pasteur de Dakar mobile laboratory (IPD-ML) was set up in Donka hospital from 2014 to 2016. EBOV suspected samples collected at Ebola Treatment Centers (ETC) and from community deaths were sent daily to IPD-ML. Analysis was performed using dried oligonucleotide mixes for real-time RT-PCR designed for field diagnostic. From March 2014 to May 2015, a total of 6055 patient samples suspected for EBOV collected from seven regions of Guinea were tested by real-time RT-PCR. These patients' clinical included serum samples ($n = 2537$ samples) and swabs ($n = 3518$ samples) with positivity rates of 36.74 and 6.88% respectively. Females were significantly more affected than males with positivity rates of 22.39 and 17.22% respectively (p -value = $5.721e-7$). All age groups were exposed to the virus with significant difference (p -value $\leq 2.2e-16$). The IPD-ML contributed significantly to the surveillance and patient management during the EBOV outbreak in Guinea. Furthermore, dried reagents adapted for field diagnostic of EVD suspect cases could be useful for future outbreak preparedness and response.

Keywords: Ebola disease virus, West-Africa epidemic, mobile laboratory deployment, social benefits

1. Introduction

Ebola virus (EBOV) belongs to the family *Filoviridae*, genus *Filovirus*. *Filoviridae* are non-segmented negative RNA viruses belonging to the order of *Mononegavirales* together with the families *Paramyxoviridae* and *Rhabdoviridae*. Ebola virus disease (EVD) is caused by five species of the genus *Ebolavirus* represented by Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV), Bundibugyo virus (BDBV),

Tai Forest virus (TAFV). TAFV has been associated with only one human case [1]. RESTV has only been found in non-human primates (NHP) and was found in swine suffering from porcine reproductive and respiratory disease syndrome [2].

The natural hosts of the EBOV remain unknown. However frugivorous bats of the family *Pteropodidae* are the most likely reservoir. Humans are infected with EBOV through direct contact with blood, secretions, organs or body fluids of diseased or dead animals found in the rainforest or most likely during slaughter of infected animals such as chimpanzees, gorillas, frugivorous bats, monkeys, duikers or porcupines. Human-to-human transmission is then achieved through direct contact with the secretions or biological fluids of infected persons. In hospitals and communities' cases of infections through contact with the patients and during funerals were described [3, 4]. Sexual transmission has also been documented and the virus can be isolated in the seminal fluid after several weeks of convalescence [5].

Since the discovery of EVD in 1976 in Nzara (now South Sudan) and Yambuku (Democratic Republic of the Congo (DRC)) close to the Ebola River [6], EBOV outbreaks were sporadically reported in Eastern and Central Africa from 1976 to 2014 in five countries: DRC, Sudan, Gabon, Uganda and Congo. More than 2000 cases have been reported with over 1500 deaths. EVD therefore appeared mostly geographically limited to remote villages in Central Africa until the 2014–2016 outbreak in West Africa [7–9].

In February 2014, Guinea recorded numerous cases of fatal fever in the south of the country. In March 2014, a local team conducted a first investigation in the Guinea Forest region, particularly in the prefectures of Macenta, Gueckedou, Kissidougou and Nzerékoré to collect blood samples from suspect cases of hemorrhagic fever patients. Thirty-three blood samples were sent to the Institut Pasteur of Lyon, France for laboratory investigation.

Since biosecurity concerns by airlines, obstructed air transport of more samples from Guinea to Senegal a request was issued by the Guinean Minister of Health to the WHO for the deployment of a team of the Institut Pasteur of Dakar (IPD) to Guinea to support diagnostics of the suspected hemorrhagic fever cases on March 20, 2014. On 22 March 2014 first laboratory results by the Institut Pasteur de Lyon, in France confirmed EVD cases in Guinea. The Institut Pasteur Dakar Mobile Laboratory (IPD-ML) was deployed on 23 March 2014, to the Donka hospital in Conakry and tested a first positive sample on the same day. Here, we present a summary of the laboratory's activities between March 23, 2014 and May 31, 2015 using field-based sensitive and/or rapid molecular diagnostics tools.

2. Material and methods

2.1 Organization of the laboratory

The IPD-ML was set up at Donka hospital in the Infectious Disease Department. It was organized in pre-analytical, analytical and post-analytical phases as described previously [10].

2.2 Sample collection

Clinical samples were collected at the MSF isolation ward by personnel wearing personal protective equipment (PPE) including a surgical mask, cap, shield or goggles, gown, apron, gloves (two pairs) and boots. Swab samples (nasal and

oral) were collected using cotton tipped applicators (Deltalab, Spain). Whole blood samples were collected using EDTA and serum vacutainer tubes. For transport, tubes were disinfected with a 0.5% hypochlorite solution and put in triples packages bags. An Ebola investigation request form was also filled out and sent with sample to the laboratory.

2.3 Sample handling and RNA extraction

Collected samples were manipulated at IPD-ML by personnel wearing Tyvek suits, goggles and gloves. Samples were inactivated in a class II biosafety cabinet (BDK Luft, Genkingen, Germany) using QIAamp viral mini kit as recommended by the supplier. Then, RNA extraction was performed in a class II biosafety cabinet (Nuaire, Minnesota, USA) and eluted in 50 µl of buffer. All waste material was treated with 1% Incidin (Ecolab, Germany) solution and incinerated on the same day.

2.4 RT-PCR diagnostic assays

RNA was detected using the quantitect RT-PCR Master Mix Probe kit (Qiagen). Briefly, 5 µl of RNA was added to 20 µl of master mix containing 2x QuantiTect Probe RT-Master Mix buffer and dried oligonucleotide mixes (Roboscreen, Jena, Germany) prepared in 4x and 10x reactions as described [11]. The real time PCR assay was performed using a Smartcycler thermocycler (Cepheid, Sunnyvale, CA). The thermal profile used was a reverse transcription for 10 min at 50°C, 15 min at 95°C for reverse transcriptase inactivation and DNA polymerase activation followed by 45 amplification cycles of 5 sec at 95°C and 50 sec 60°C (annealing-extension step).

The samples were considered positive if there was an apparent logarithmic phase in the amplification curve with a Ct value of <38. For Ct values between 38 and 42, the samples were considered suspect and undetermined and negative if there was no apparent logarithmic amplification.

2.5 Data management and statistical analysis

Patient results were recorded in a database including patient ID, date of sample collection and laboratory results. Daily, the database was sent to the ETC, the WHO and the Guinean MoH for rapid care of the patients and an update of the EVD response.

The statistical analyses were performed using the R statistical software (version 3.3.2) [12]. All calculated p-values were results of Fisher's Exact Test with a two-sided alternative hypothesis (testing for inequality).

3. Results

The algorithm for laboratory testing is presented in **Figure 1**. The IPD-ML was operational within 1 hour after the arrival in Guinea and gave results of suspected EVD cases in less than 3 hours from the time of sample receipt. From March 2014 to May 2015, a total of 6055 patient samples suspected for EBOV (53% of the total of samples tested in Guinea) were tested by the IPD-ML setup in Donka hospital, Guinea, of which 1157 tested positive by real time RT-PCR. Among the 6055 samples, 7 were EVD suspect cases from Liberia received at the IPD-ML on March 29, 2014, of which two tested positive for EBOV, confirming the first circulation of

the virus in this country. The clinical specimens included mainly serum samples (n = 2537 samples) and swabs (n = 3518 samples) with positivity rates of 36.74 and 6.88% respectively (Table 1).

Figure 2 shows the geographical distribution of patient samples tested at IPD-ML. Overall, most of the samples tested were from Lower Guinea regions: Conakry (70%), Coyah (6.42%), Dubreka (5.38%), Forecariah (5.07%), Kindia (3.12%), and Boffa (2.59%). Furthermore, we also tested patient samples collected from the remaining regions of the country: Middle Guinea, Upper Guinea and Forest Guinea.

Analysis of all samples tested by sex, suggests a statistically significant difference between the females (n = 2608 samples) and males (n = 3397 samples) with positivity rates of 22.39 and 17.22% respectively (p-value = 5.721e-7). Analysis by sex of samples collected in Conakry, showed statistically significant difference between the females (n = 1550 cases) and males (n = 2169 cases) with positivity rates of 16.1 and 12.7% respectively (p-value = 0.01231). Furthermore, the distribution of EVD cases by age of samples collected in the Conakry region showed that all age groups were exposed to the virus with significant difference (p-value <= 2.2e-16). Patients aged 50 years and over seemed to be less affected by the outbreak.

Analysis of alive suspect EVD patients by sex show a total of 1111 females versus 1415 males tested (serum patients); for these patients 41.3% (459/1111) of females and 33.4% (472/1415) of males were tested positive with significant difference of

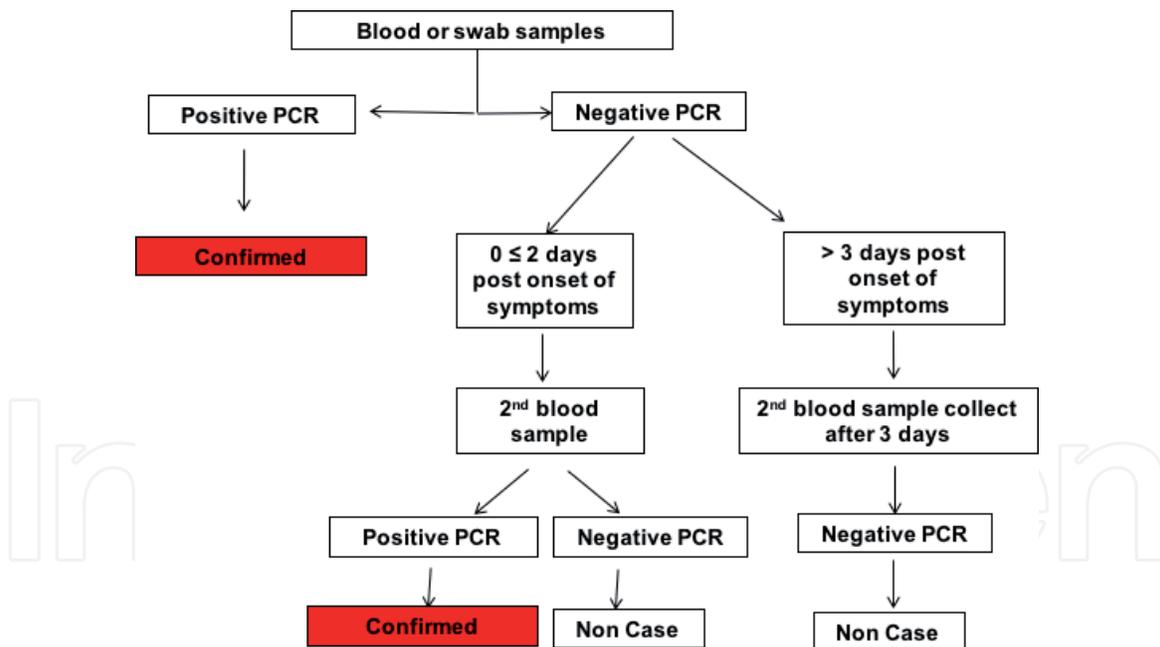


Figure 1. Diagnostic algorithm for IPD - ML testing.

Samples source	Tested	Positives/negatives
Blood samples	2537	932/1605
Swab samples	3518	242/3276
Total	6055	1174/4881

Table 1. Samples tested and lab results from March, 23, 2014, to May 31, 2015.

infection between the two genders ($p = 4.615e-5$). Females exhibited a higher ratio of infection than males during the period of study.

Analysis of alive patients by age show significant difference of infection in terms of age group with a higher rate of infection for people aged between 35 and 49 years (p -value = 0.01704) (Table 2).

Figure 3 shows EVD cases admitted to the ETC analyzed by IPD-ML between March 2014 and May 2015. The number of confirmed EBOV cases was almost the same per month between March and July 2014 with a total of 34.22% (128/374) confirmed cases with an average of 26 positives cases by month, and peaked in December 2014 with 253 confirmed cases.

Figure 4 and Table 3 represent swab samples collected from deceased analyzed by month and age. Out of the 3518 swab tests performed, 242 were tested EBOV

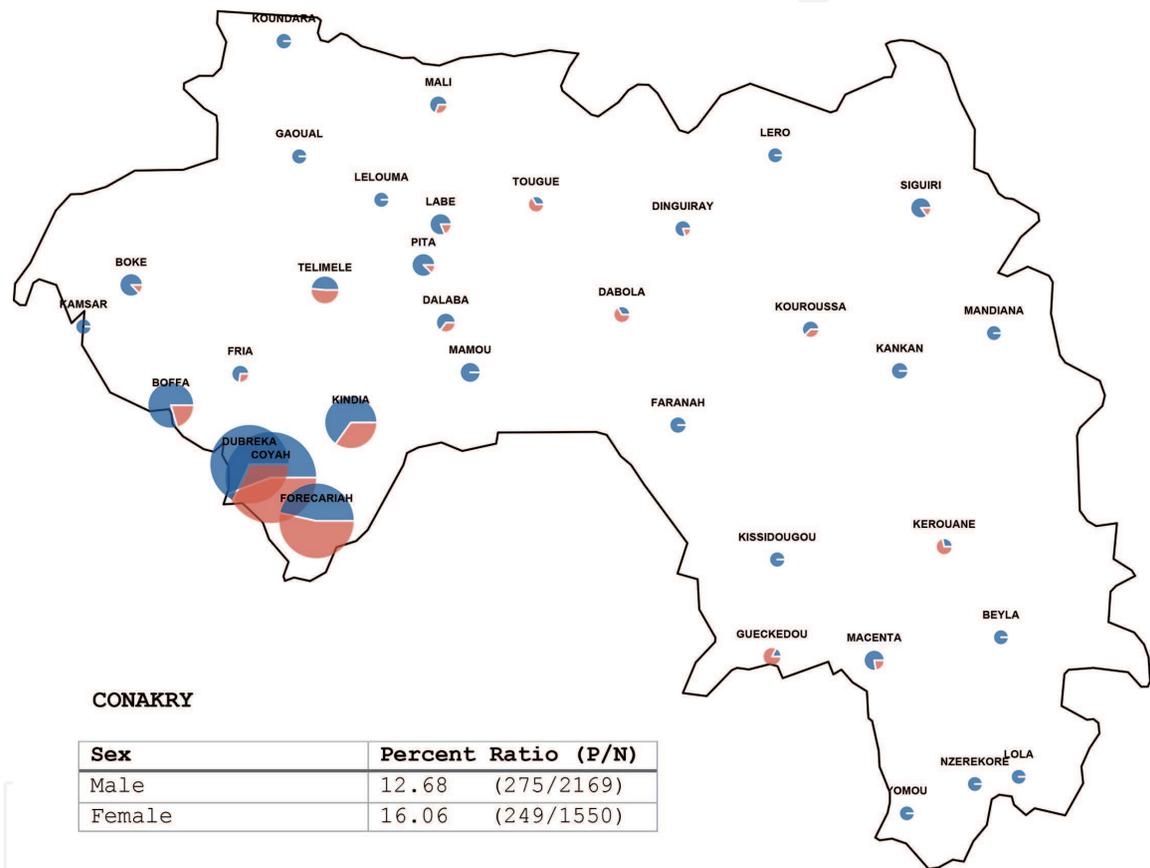


Figure 2. Outbreak distribution map. Geographic mapping of the distribution of the epidemic throughout the country of Guinea with the exception of its capital, Conakry. Red and blue colors represent positives and negatives cases respectively. Because about two thirds (4285/6055) of all tested patients resided in Conakry, an extra table represents numbers tested and confirmed cases in the Conakry region. Inclusion of these data throws the proportion of the remaining pie charts off balance.

Age group	Positive	Negative	Ratio (P/N)	P-value
[−19]	186	323	0.57585	0.01704
[20–34]	330	612	0.53922	
[35–49]	240	325	0.73846	
[50+]	139	268	0.51866	

Table 2. Distribution of patients (alive) by age group.

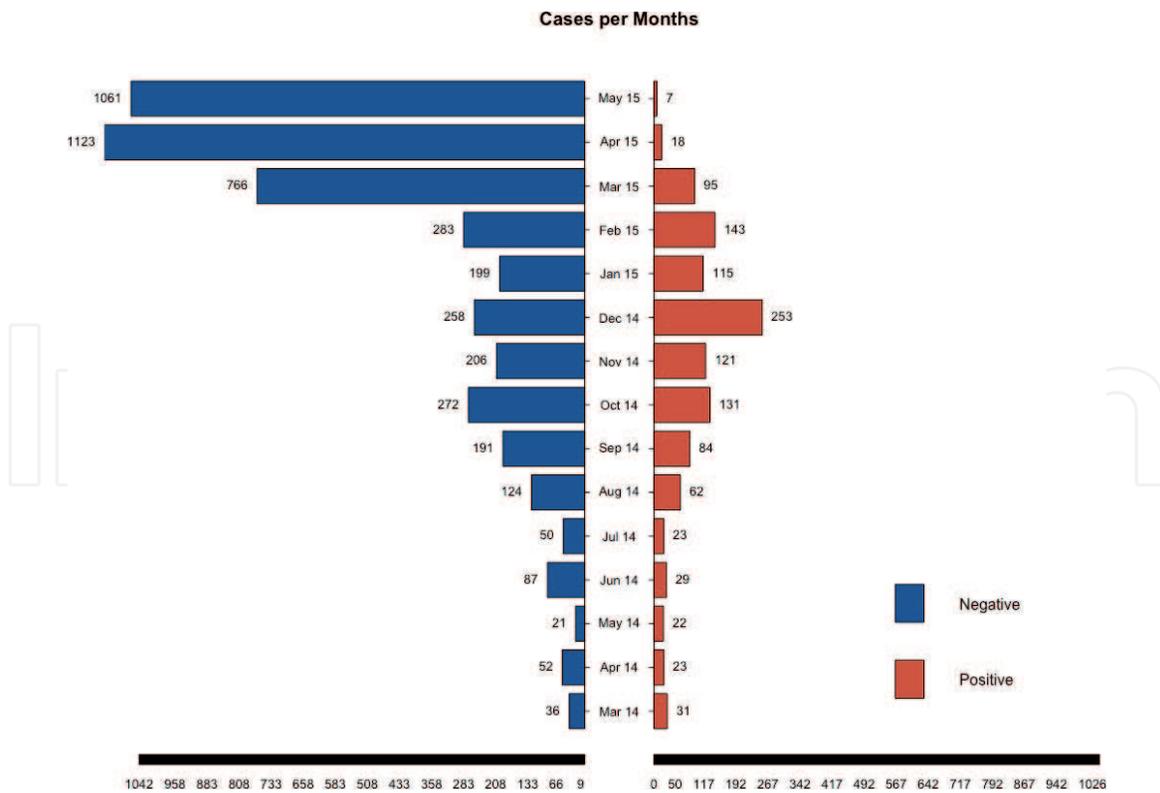


Figure 3. Number of cases per months. The horizontal bar plot shows the number of people that were tested every month from January 2014 to May 2015. Red and blue bars represent positive and negative cases respectively.

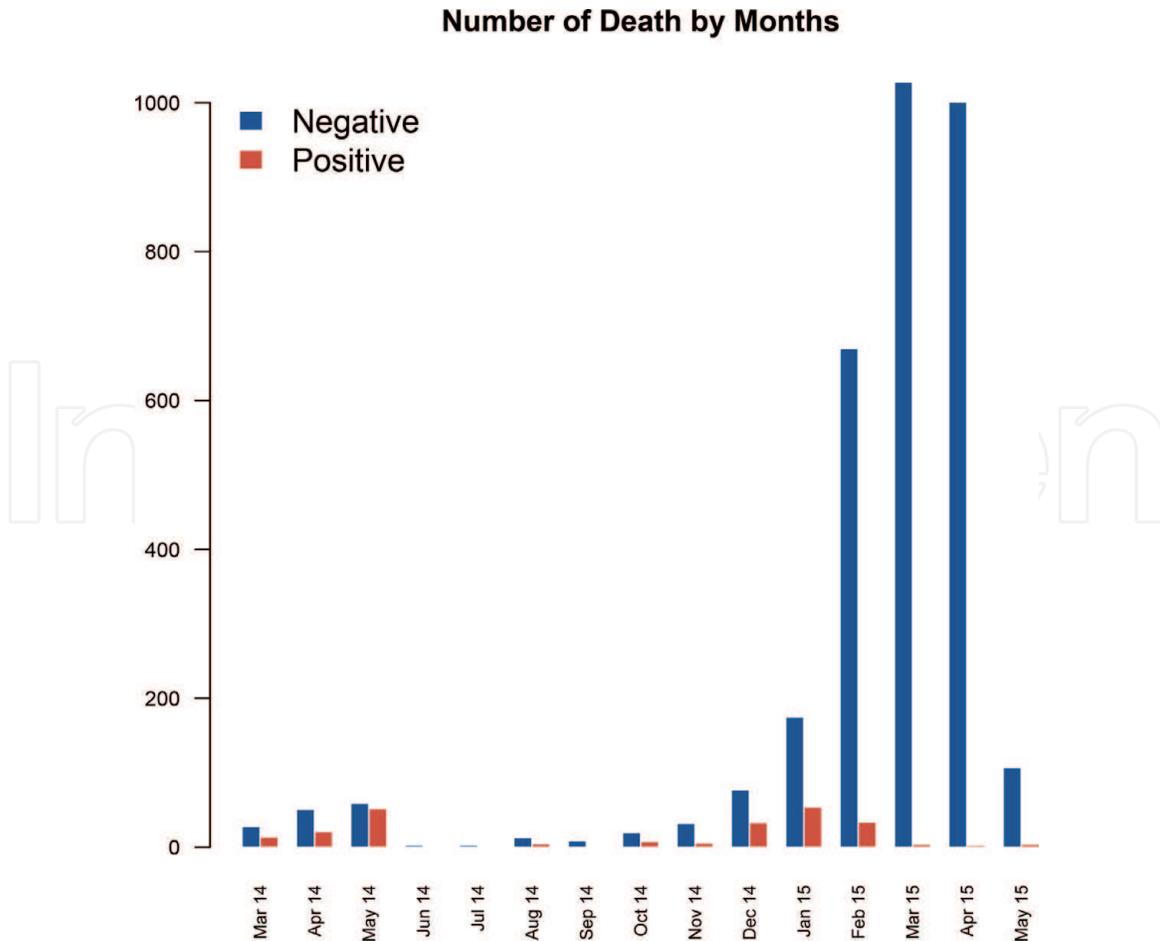


Figure 4. Number of deaths per months. The bar plot classifies all the defunct tested patients through the months and segregates the patients by the result of their tests.

Age group	Female	Male	Ratio (M/F)	P-value
[-19]	21	19	0.90476	0.9952
[20-34]	33	28	0.84848	
[35-49]	35	32	0.91429	
[50+]	32	27	0.84375	

Table 3.
Distribution of death by Ebola per age group.

positive i.e. the cause of death had been EVD against 3276 patients that died of other causes. However, analysis of the 242 aforementioned patients grouped by age shows that there is not enough evidence to correlate deaths caused by EBOV to gender (p-value = 0.9952).

3.1 Need for rapid and portable assay for Ebola diagnostics

As in previous epidemics contacts of Ebola patients or deaths in West-Africa were found to feel helpless while fearing to die a premature death, to mourn the loss of relatives and friends and to lose the trust in others. Especially during the waiting time for the test result, they feel helpless and hopeless [13].

Long periods of waiting for the test result, enhanced a high social burden for the affected communities because of stigmatization: “When the burial team lead told [the relatives] that it could take between 2-21 days before they would know the test results, some relatives complained about the delay, noting: “the whole time, the stigma on you” [5].

In Liberia, there was a big fear among the communities to be erroneously quarantined for a non-Ebola illness so people became reluctant to search health-service when they experienced symptoms. Additionally, there was a fear of an entire households being stigmatized with Ebola and quarantined whenever deaths occurred which were most likely to be due to other diseases. Similar responses were reported from the EVD outbreak in Kikwit in 1995 and in Gulu 2001 [14, 15].

Lacking definite EVD confirmation or exclusion, the community started to hide deaths and performed secret burials in order to avoid inappropriate stigmatization and quarantine. Receiving a negative test result freed families from stigmatization due to an Ebola case.

Communities began demanding a timelier return of test results and specifically demanded a rapid diagnostic test: “I suggest that the government improve the system. People will die at home. That will always happen. But let us say, if in 2- or 3-hours’ time, if government able to tell you that this is Ebola body or not Ebola body that will be very fine.” (Community member FGD, Site B) [5].

Rapid point of care assays like the highly sensitive and specific RPA used in Guinea shorten the burden of emotional distress by significantly decreasing the amount of waiting time until the Ebola status is assessed.

A quicker return of results also shortens the time of inappropriate quarantine and facilitates contact tracing efforts. It counters low acceptance of testing for EVD which risks underdiagnosing EVD patients thereby facilitating spread of disease by continuous contact with other community members [5]. Rapid diagnostic tests are a key tool for providing the appropriate care for patients, according to the Ebola Response Anthropology Platform [16].

3.2 Use of recombinase polymerase amplification

The EBOV RT-RPA was successfully used in a campaign to roll back EVD cases in western parts of Conakry following an upsurge of EVD cases connected to non-reporting, reticence, and transmission chains connected to funeral rites toward the end of [17]. Mobile suitcase labs using RT-RPA tested oral swabs from all deceased at the morgue in Matoto in March and April 2015. Results were provided 30–40 min after receipt of samples to the safe and dignified burial (SDB) teams.

Altogether the suitcase based RT-RPA assay for the detection of EBOV in saliva samples proved to be very efficient and as sensitive and specific as real time PCR. The combination of SPEED Extract extraction in a hard plastic glovebox and RT-RPA in a suitcase using RPA pellets which already contained primers and probe allowed biosafe handling of samples, reduced time to result and allowed a large turnover in very simple settings [18].

There was no concomitant study to measure the impact of the RPA deployment. However the joint IPD-guinea team noticed this strategy of lowering the time to results successfully contributed to the reduction of the population reluctances to be tested. When knowledge spread in Matoto about the fast time to result for the obligatory screening of deceased, SDB teams were suddenly actively addressed and directed to deceased to take samples in order to clear bodies for traditional burials, essentially changing the behavior of the local population.

The RPA assay was deployed not only in Conakry but also during campaigns organized by the coordination committee for active search of Ebola suspected cases in the Forécariah, Coyah, Dubréka and Boké prefectures. During these campaigns, 2509 samples were tested.

The end of the Ebola epidemic was declared by the WHO on December 29, 2015. However due to the persistence of the virus in survivors, WHO recommended to strengthen the surveillance in the whole country for three additional months. Hence, a lab deployment strategy was set up in the Guinea to screen all Ebola suspected cases samples collected in the health care and community death. Then mobile laboratory based on the RPA assay was recommended by the “Coordination Nationale de Riposte au virus Ebola” to continue the real time monitoring of Ebola virus due to the rapidity and user-friendly of the assay. On 17 March 2016, the RPA system allowed the detection of the two last Ebola cases reported in Guinea, in the Nzérékoré forest area of the country [19].

4. Discussion

The use of mobile laboratories is crucial for rapid management and control of epidemics in infrastructure poor settings. During the EVD outbreak in West Africa, several mobile laboratories were deployed in the countries most affected by the deadly virus, i.e. Sierra Leone, Liberia and Guinea. In this context, the IPD deployed a mobile laboratory in Conakry, Guinea, at the Donka Hospital. Overall, a total of 4881 samples of EVD-suspect patients including live and community deaths were analyzed between March 2014 and May 2015 with 1174 confirmed positives for EBOV.

Epidemics prior to 2014 had been reported in rural areas only [9, 20]. The IPD-ML tested samples collected from all the four natural regions of Guinea. The majority of the confirmed cases tested were collected toward the end of 2014 in the Conakry region. This was due to movements of EVD cases inside a densely populated area combined with ignorance toward the disease by the population in general and in particular by medical staff although EVD had been ongoing in the country for months.

The results of the IPD-ML were systematically sent out 3 hours after samples receipt at the hospital Donka Ebola treatment center managed by Médecins Sans Frontières. Patients which tested positive were placed in the isolation zone for better care and to avoid contamination of others.

Depending on the development of the disease, the viral genome can be detected at day 2 or 3 after the onset of the disease until about day 10, and IgM and IgG can be detected at about the same time after the onset of the disease (8–10 days) [21]. Therefore, in the first 3 days of illness, and beyond 10 days after the beginning of the disease, molecular testing cannot detect the virus genome, which can lead to false negatives. Therefore, RT-PCR should be repeated for early samples taken up to 72 hours after onset of disease [22, 23]. Patients negative in the initial test therefore were observed for 48 hours in a separate zone and a second control test had to score negative before they were released.

Furthermore, for Ct values ranging between 38 and 42, the patient was also considered as a suspected case and a control test was recommended to the ETC. The real time RT-PCR method used by IPD-ML was very sensitive with a detection limit of 10 copies of RNA [18, 24, 25].

In addition, we carried out studies to improve our diagnostic platform deployed by the IPD-ML. In the face of an upsurge of EBOV cases in Conakry and transmission of the virus via funeral rites in 2014, we developed a rapid isothermal test for rapid detection of the virus in 10–15 min [18]. This method allowed rapid rendering of results and secure burial of deaths, thus contributing to a significant reduction of the transmissions of the virus during funeral rites and a reduction of social reluctance to submit samples often reported due to the expectation of the results and the consequent impact on burial rites.

It is important to note that in Conakry, where most tested patients resided, women had a significant higher infection rate which can be attributed to gender-related risk factors. The Guinean societal and organizational culture holds women responsible for nursing and caring for the patients. In an attempt to fulfill their gender roles, women were left more exposed to the virus [26].

The major challenge encountered during this deployment was the electricity supply, which can have an impact on the storage of reagents and the time to results. During the IPD-ML deployment in Guinea, these obstacles were overcome. A generator of 10 KVA was installed which supported the PFHG laboratory building. In the event of a breakdown of our generator, our diagnostic platform composed of a class III cabinet glovebox, a centrifuge, a Smart Cycler and other small bench-top equipment was powered by a system of batteries coupled with solar panels. Moreover, the use of freeze-dried primers and probes with good performances at temperatures of 40°C relieved us of energy problems.

In addition, it is important to have a differential diagnosis approach in the event of an outbreak for rapid management of non-EBOV patients. Indeed, the signs of EBOV are often similar to certain diseases such as malaria, typhoid fever, dengue that co-circulate in the same geographical areas [27].

During the 2014–2015 EVD outbreak in Guinea, the mobile laboratory running the Ebola RPA assay was run by local laboratory technicians, a sustainable option which provides local capacity for potential future outbreaks. The redeployment and strategic placement of this RPA instrument to complement EVD isolation facilities throughout the country has strengthened preparedness and response capabilities for future EVD outbreaks in Africa, particularly in West Africa [26].

The development of portable mobile platform is crucial for rapid epidemic management in resource-poor regions. The lessons learned during the Ebola epidemic,

in particular the development of new equipment and reagents, will allow better management of future epidemics.

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Conflict of interest

No conflict of interest for any of the authors declared.

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