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SUPPLEMENT ARTICLE

An Outbreak of Ebola Virus Disease in the Lassa Fever Zone

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Background. Kenema Government Hospital (KGH) has developed an advanced clinical and laboratory research capacity to manage the threat of Lassa fever, a viral hemorrhagic fever (VHF). The 2013–2016 Ebola virus (EBOV) disease (EVD) outbreak is the first to have occurred in an area close to a facility with established clinical and laboratory capacity for study of VHFs.

Methods. Because of its proximity to the epicenter of the EVD outbreak, which began in Guinea in March 2014, the KGH Lassa fever Team mobilized to establish EBOV surveillance and diagnostic capabilities.

Results. Augustine Goba, director of the KGH Lassa laboratory, diagnosed the first documented case of EVD in Sierra Leone, on 25 May 2014. Thereafter, KGH received and cared for numbers of patients with EVD that quickly overwhelmed the capacity for safe management. Numerous healthcare workers contracted and lost their lives to EVD. The vast majority of subsequent EVD cases in West Africa can be traced back to a single transmission chain that includes this first diagnosed case.

Conclusions. Responding to the challenges of confronting 2 hemorrhagic fever viruses will require continued investments in the development of countermeasures (vaccines, therapeutic agents, and diagnostic assays), infrastructure, and human resources.

Keywords. West African Ebola outbreak 2013–16; Ebola virus; Lassa virus; research capacity building; viral hemorrhagic fever diagnostics; epidemiology; genomic sequencing.

Kenema Government Hospital (KGH) is the largest public health facility in the Eastern province of Sierra Leone. An advanced clinical and laboratory research capacity has been developed at KGH to manage the threat of Lassa fever, a viral hemorrhagic fever (VHF) that is endemic in parts of West Africa. The Sierra Leone Ministry of Health and Sanitation (MoHS) and the Viral Hemorrhagic Fever Consortium (VHFC), a public-private partnership of academic and biotechnology researchers, have worked in concert for over a decade on Lassa fever, providing diagnosis, nationwide surveillance, investigation of cases, contact tracing, rodent control, and community education and sensitization [1].

The West African Ebola outbreak originated only about a 4-hour drive from KGH, in an area of Guinea that is close to the borders of Sierra Leone and Liberia. The 2013–2016 Ebola virus (EBOV) disease (EVD) outbreak is the first to have occurred in an area close to a facility with both clinical and laboratory capacity for study of VHFs (Supplementary Figure 1). Because of its proximity to the epicenter of the EVD outbreak in Guinea, the Lassa fever team mobilized to establish EBOV diagnostic capabilities for the first time at KGH (Supplementary Figure 2). Months after the outbreak had begun in neighboring Guinea and was thought to be subsiding, Augustine Goba, head

This article is dedicated to all healthcare workers who cared for patients with Ebola virus disease during the outbreak and to the people of Sierra Leone.

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of the MoHS Lassa fever laboratory at KGH, provided the first EVD diagnosis in Sierra Leone, on 25 May 2014. Here, we review the response to the outbreak by KGH staff as they received and cared for patients with EVD. We also discuss the ongoing challenges for a region of the world confronted with 2 highly pathogenic hemorrhagic fever viruses.

METHODS

Human Subjects

Research involving human subjects at the KGH was approved by the Sierra Leone Ethics and Scientific Review Committee and the Tulane University Institutional Review Board (IRB) in accordance with National Institutes of Health guidelines. All patients enrolled through 25 May 2014 provided informed consent and were enrolled as having suspected Lassa fever prior to the first diagnosis of an Ebola case in Sierra Leone. Because of the Ebola outbreak, a waiver of written consent was granted for testing samples obtained from patients who enrolled after 25 May. Approval from the Sierra Leone Ethics and Scientific Review Committee, the MoHS, and VHFC IRBs was obtained to use excess samples in further testing, including EBOV genomic sequencing. Permission to use personal photographs was obtained from all individuals or from legal relatives of deceased persons.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Diagnostic Assays

In late March 2014, VHFC scientists Kristian Andersen and Stephen Gire, with support from the MoHS, assisted KGH/MOHS Laboratory Director Augustine Goba and technicians Mambu Momoh and Mohamed Fullah in implementation of RT-PCR diagnostic testing for EBOV (Supplementary Figure 2*A*). RNA was extracted using QIAamp viral RNA mini kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The FiloA/B primer set targeting the L gene of EBOV was based on primers designed by Panning et al [2], as modified by Baize et al [3] in the article describing the initial West African EBOVs from Guinea. The expected size of the PCR product is 285 base pairs. Modified FiloA/B primers (L target) were as follows: forward, 5′-GTCAAAGCATTTCCTAGCAACATGATGG-3′; reverse, 5′-ATAATAATCACTCACATGCATATAACA-3′.

Another assay designed by the VHFC used all available EBOV sequence data from previous outbreaks, to optimize the primers. The KGH primers targeting the VP40 gene were homologous with nearly all EBOV Makona variant sequences. The expected size of the PCR product is 122 base pairs. KGH primers (VP24 target) were as follows: forward, 5′-GTC GTTCCAACAATCGAGCG-3′; reverse, 5′-CGTCCCGTAG CTTTRGCCAT-3′.

The RT-PCR assay for detection of Lassa virus (LASV) infection was previously established in the KGH/MOHS Laboratory. The modification by Ölschläger et al [4] of the Demby primer set [5] targets the GPC gene. The expected size of the PCR product is 318 base pairs. Modified Demby primers (GPC target) were as follows: forward, 5′-ACCGGGGATCCTAGGCA TTT-3′; reverse, 5′-GTTCTTTGTGCAGGAMAGGGGCAT KGTCAT-3′.

RESULTS

The Lassa Fever Program at KGH

The Lassa fever zone stretches from Guinea (and perhaps Senegal) in the west to Mali in the north and Nigeria in the east [6]. The world's highest incidence of the disease occurs in the Eastern Province of Sierra Leone [7]. The MoHS, with support from the Centers for Disease Control and Prevention (CDC), established Lassa fever treatment wards and research facilities in the Eastern Province (Figure 1*A*). Three centers, at Panguma, Segbwema, and KGH, became major sites for Lassa fever research throughout the 1970s and 1980s (Figure 1*B*) [9]. The violent civil conflict from 1991 to 2002, sometimes referred to as the Blood Diamond War, forced suspension of CDC involvement. However, admission to the KGH Lassa ward, led by Dr Aniru Conteh, a Sierra Leonean physician who transferred in 1991 from Segbwema, continued throughout the civil conflict. Following the cessation of hostilities, a consortium of Lassa fever researchers, in close collaboration with the MoHS, began rebuilding the clinical and laboratory infrastructure at KGH. Tragically, Dr Conteh contracted Lassa fever and died of the disease in 2004 [10]. A then-recent graduate from the medical school in Freetown, Dr S. Humarr Khan became the new director of the KGH Lassa fever program.

Lassa fever causes initial symptoms such as fever or headache that are common in other diseases, such as malaria. Therefore, the focus of Lassa fever research in postconflict Sierra Leone was development of improved laboratory diagnostic assays. A major step was renovation of a preexisting building and installation of laboratory infrastructure that was completed in 2005 (Figure 1*A*). Driven by the terrorist attacks of 2001, the National Institutes of Health (NIH) increased funding for emerging diseases, including Lassa fever. A grant application to the NIH, written in 2003–2004, was funded in August 2005, just days before Hurricane Katrina made landfall. Despite the challenges of this natural disaster for the New Orleans–based investigators, recombinant antigen–based enzyme-linked immunosorbent assays were developed and implemented and have been used consistently at the KGH Lassa ward since 2008 [1]. Luis Branco and the Biofactura team (Rockville, Maryland) were wholly responsible for cloning LASV proteins and deriving anti-LASV monoclonal antibodies. Autoimmune Technologies provided expertise in antibody production. Vybion joined the consortium to express LASV proteins to scale. Corgenix (Broomfield, Colorado) assembled and validated the ReLASV diagnostic assays, which are capable of identifying 95% of active Lassa fever cases, as confirmed by PCR. A lateral flow immunoassay (a rapid diagnostic test [RDT]) requiring only a drop of blood

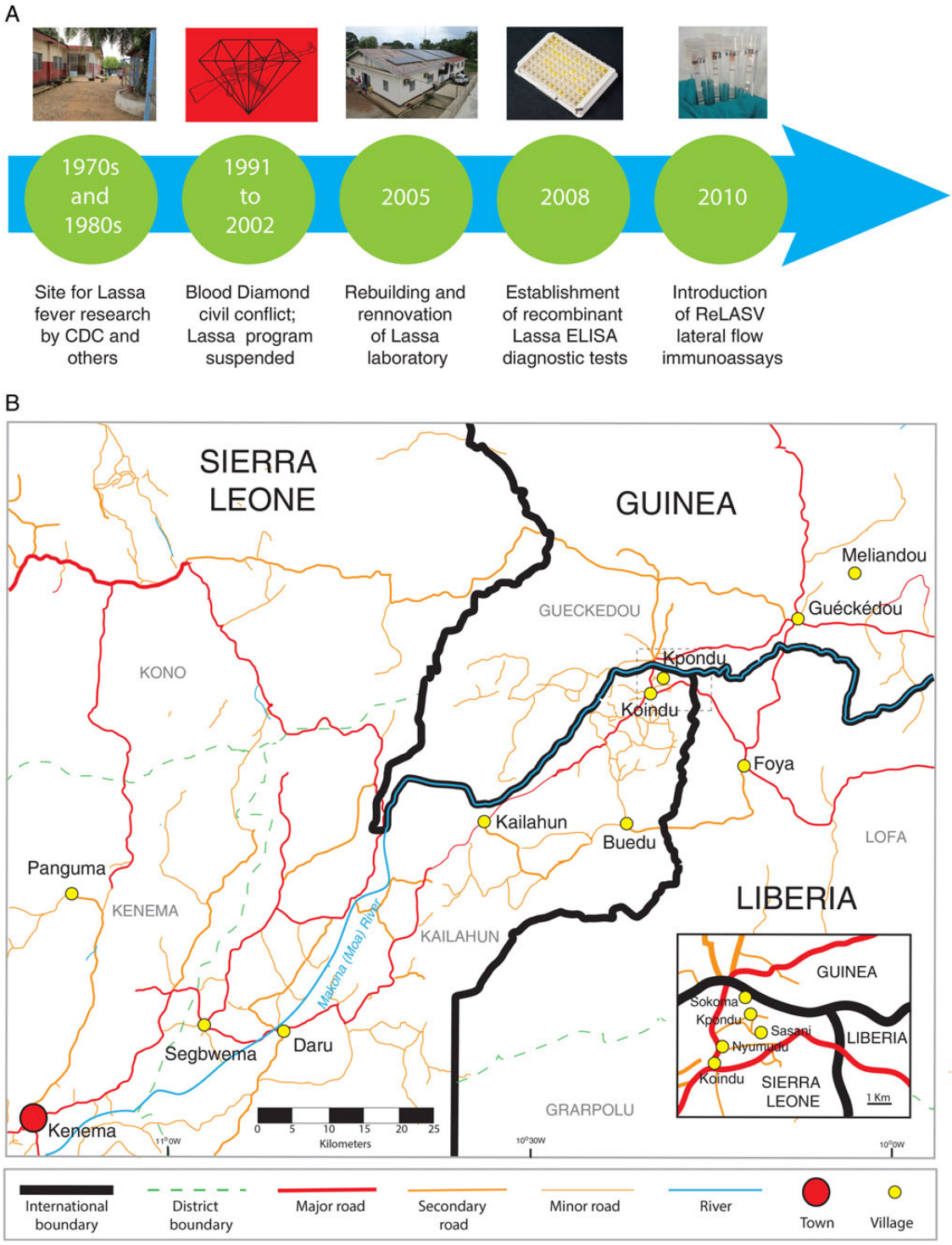

Figure 1. Key events during the development of the Lassa fever program at Kenema Government Hospital and important locations of the 2013–2016 West African Ebola outbreak. *A*, Time line of major Lassa fever research programs and initiatives at Kenema Government Hospital prior to the emergence of Ebola virus (EBOV) disease in West Africa. *B*, Map of area where Sierra Leone, Guinea, and Liberia converge. The 2013–2016 EBOV variant is named after the Makona (Moa) river, which forms parts of the Guinea–Sierra Leone and Guinea-Liberia borders [8]. Abbreviations: CDC, Centers for Disease Control and Prevention; ELISA, enzyme-linked immunosorbent assay.

(30 μL) obtained with a safety lancet and capable of detecting LASV antigenemia within 15 minutes was implemented in 2010 [11]. Since 2007, every diagnosis of Lassa fever at KGH has been performed by MoHS personnel by using recombinant immunoassays provided by the VHFC. In addition to the laboratory, the Lassa fever program also supports the activities of a

clinical staff experienced in the care of patients with Lassa fever, as well as those of an ecology team, which provides rodent-control measures to villages with Lassa fever cases (Supplementary Figure 1). The outreach/surveillance team performs case investigations, follow-up, and community sensitization.

Emergence of EBOV in West Africa and Its Spread to Sierra Leone

The 2013–2016 West Africa Ebola outbreak originated in an area that is inhabited by the Kissi people, where the borders of Guinea, Sierra Leone, and Liberia converge (Figure 1*B*). There are no restrictions to movement across the international borders for commercial, family, and social interactions. Epidemiological studies suggest that the initial EVD cases occurred in Meliandou in 2013 [12] (Figure 1*B*). With the exception of Taï Forest virus, which has not been seen since 1994 [13], this is the first time that any active filovirus infection had been detected in the Lassa fever zone of West Africa. From the emergence of EBOV in 2013 to 23 May 2014, there were officially 258 diagnosed or probable cases of EVD, all in either Guinea or Liberia (Figure 2*A*). The World Health Organization (WHO) was only days away from announcing that the outbreak had ended when, on 25 May, Augustine Goba, director of the VHF laboratory at KGH, diagnosed the first known EVD case in Sierra Leone (Figure 2*B*). Mr Goba was awarded a Presidential Citation from His Excellency Ernest Bai Koroma on 18 December 2015 (Supplementary Figure 2*B*). Subsequent investigations revealed that the outbreak had not been confined to Guinea and Liberia, where the international community had established diagnostic laboratories and treatment centers [17, 18]. Instead, the official data underestimated the true number of EBOV cases and failed to account for circulation before May 2014 of EBOV among the Kissi people, including members of the chiefdom who were living in Sierra Leone.

The West African Ebola outbreak was officially declared on 23 March 2014 (Figure 2*A*). Prior to the declaration, a WHO memorandum on 18 March documented 35 cases (22 deaths) of a disease considered likely to be Lassa fever in Guinea. Samples from these cases were sent to Institute Pasteur facilities in Senegal and Lyon, where they tested positive for EBOV. Another WHO memorandum, dated 19 March, described additional cases, including case A (Figure 3). Case A lived in Kailahun District of Sierra Leone, across the border from Guinea, in or near Kpondu, and had recently traveled to visit a son in Guinea in a vehicle with a sick individual, who subsequently died. Case A died on March 3. Although the 19 March memorandum was translated from French, which is spoken in Guinea, to English, which is spoken in Sierra Leone, the information in the memo was not communicated effectively or was ignored. Subsequent investigations revealed that case A was epidemiologically linked to each of the first 17 PCR-confirmed EVD cases in Sierra Leone. Moreover, genetic analyses of EBOV suggest that, with the exception of a limited number of cases linked to the original animal reservoir-to-human transmission event in Guéckédou District, all subsequent cases of EVD West Africa, including those involving foreign healthcare workers, can be traced back to this single transmission chain involving case A [14, 16]. The transmission chain from case A leads plausibly to case F, a revered traditional healer (herbalist) and a prominent leader of Kpondu (Figure 3 and Table 1). Although her death was previously reported as occurring on 30 April 2014 [19], the funeral of case F, the healer, occurred on 8 April 2014. The funeral was reportedly attended by hundreds [20] and heralded the prolonged, much deadlier phase of the outbreak that followed.

The first 14 EVD cases diagnosed in Sierra Leone occurred in women [14, 16]. All were either relatives of case F, the healer, members of her social circle, or otherwise epidemiologically linked to her (Figure 3 and Table 1). Case 1, a pregnant woman from Koindu, presented to KGH on 23 May 2014 and was initially believed to have Lassa fever, which has severe manifestations in pregnancy [1, 21]. When case 1 tested negative for Lassa fever, the doctor in charge, Dr Khan, strongly suspected that she had EVD, which was confirmed on 25 May 2014. Case 1 lost her baby but became the first known survivor of EVD infection in Sierra Leone, likely because of treatment by the KGH Lassa fever clinical team, which included a dilation and curettage procedure. Case 1 had earlier shared a room at the Koindu public health unit (PHU) with case L, another pregnant woman, who died on 5 May 2015 of probable EVD. Case I, who died of suspected EVD, is linked to the earlier cases in Koindu though case L, the granddaughter of case I and daughter of case K, who died of suspected EVD. Case I was the younger sister of case F, the healer. Case I infected her mother, case J, who died of EVD. Case J was the mother-in-law of case 2, whose sample was sent to KGH by the director of the Koindu PHU on 24 May 2014. On 25 May, case 2 became the first Sierra Leonean to receive a diagnosis of EVD, by Augustine Goba (Figure 2*B*). A sample from case 3, who attended the funeral of the healer and died of EVD (Buedu village), was also diagnosed as EVD positive on 25 May 2014 (Figure 3 and Table 1).

With the knowledge that EVD cases were occurring in upper Kailahun District, attempts were made to identify additional persons from this area that may have come to KGH to seek medical attention. Case 4, a midwife from Koindu PHU who had attended to case L during her pregnancy, was identified in the Annex Ward and received a diagnosis on 26 May 2014 (Figure 3, Table 1). Dr Khan sent the KGH outreach team to Koindu and nearby villages to collect samples from individuals with suspected EVD. Nine additional EBOV-positive individuals (cases 5–13) were identified on 27 May, all of whom had attended the funeral of the healer. Further attempts by the KGH team to identify additional patients in and around Koindu were resisted by the communities for several weeks. The first individuals from outside the Kpondu/Koindu regions with EVD

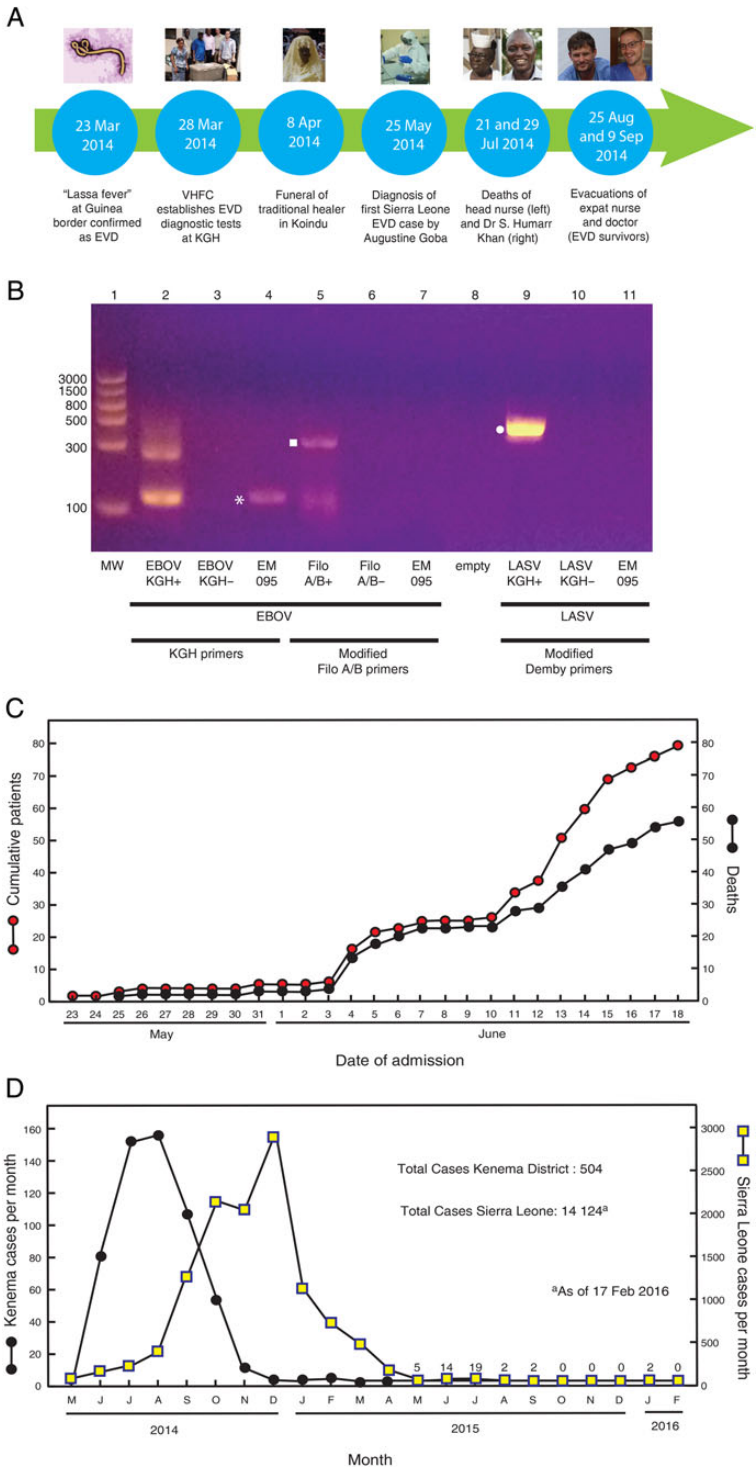

Figure 2. Diagnosis of the first case of Ebola virus (EBOV) disease (EVD) and changes in caseload and case numbers over time. *A*, Time line of events at Kenema Government Hospital (KGH) following the introduction of EBOV into Sierra Leone. Images from left to right are a colorized version of a electron micrograph of EBOV, taken by F. Murphy (Centers for Disease Control and Prevention); (2) a photograph of a team from the Viral Hemorrhagic Fever Consortium (VHFC; from left to right, Simbirie Jalloh [program manager], Mambu Momoh [laboratory technician], Dr S. Humarr Khan [director, Lassa fever program], Dr Kristian Andersen [then in the laboratory of Pardis Sabeti at Harvard/Broad and currently at the Scripps Research Institute], and Stephen Gire [then in the laboratory of Pardis Sabeti and currently at NextGen Jane) assists in establishing diagnostic capability for EBOV at KGH following the declaration of an EVD outbreak in nearby Guinea; cropped photograph of healer [17]. *B*, Diagnosis of first EVD case in Sierra Leone by Augustine Goba on 25 March 2014. An asterisk indicates the location of the 122–base pair positive band, using a VHFC-designed polymerase chain reaction assay (performed by K. Andersen, using KGH primers) [14]. Other primers include the Baize et al [3] modification of the Filo A/B [15] primer set for EBOV detection (285–base pair control band; white square, negative for sample) and the Ölschläger et al [4] modification of the Demby et al primer set [5] for Lassa virus (LASV) detection (318–base pair control band; white circle, negative for sample). There is 1 mismatch in the forward primer and 1 mismatch in the reverse primer, compared with the EM095 sequence (accession number KM034550) [14], which may explain why the modified FiloA/B primer set failed to amplify this sequence. EM095 is also referred to as case 2. Case 1 presented

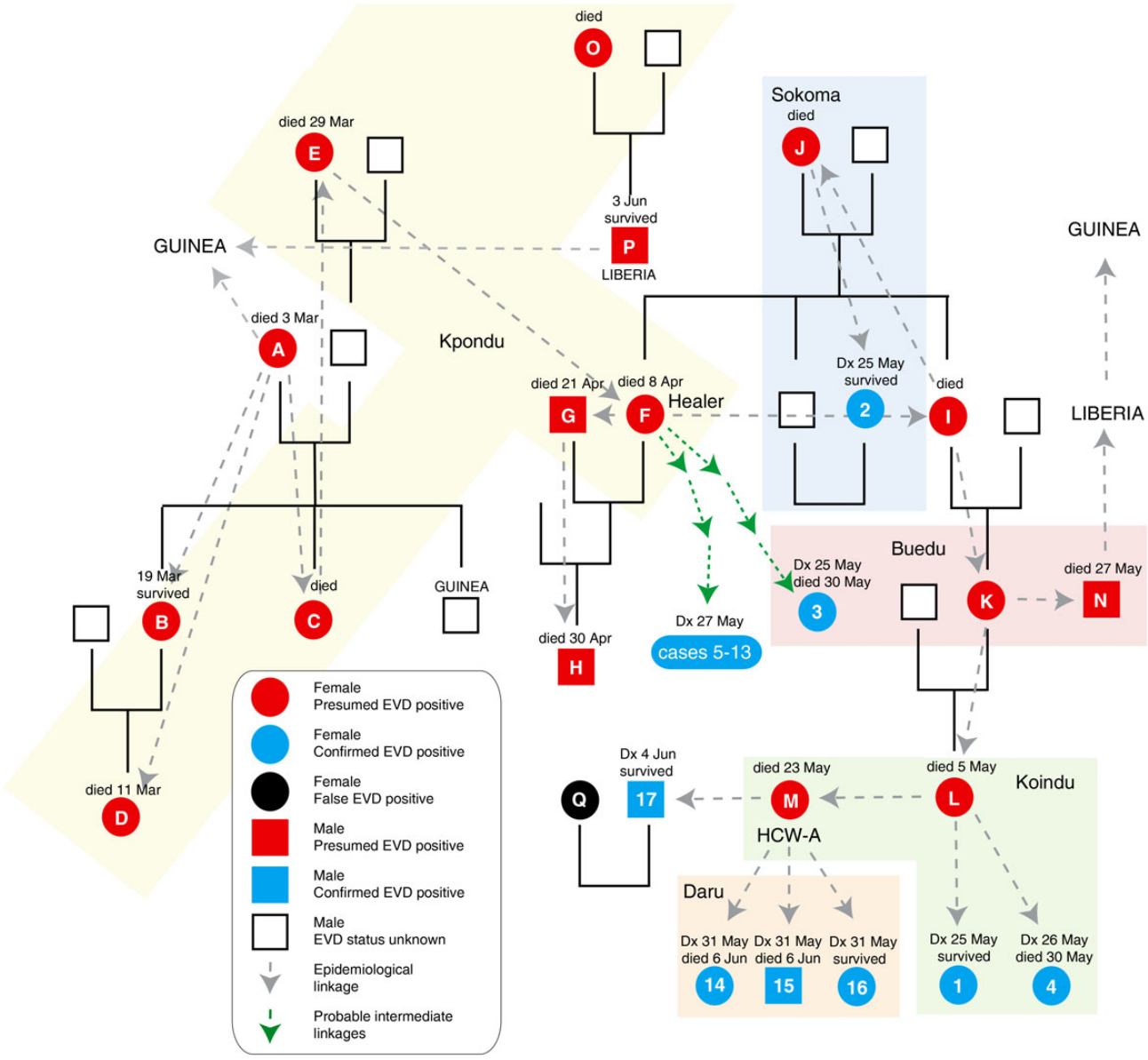

Figure 3. Transmission chains involving the early cases of Ebola virus disease (EVD) as the disease spread into Sierra Leone. Details of the transmission chains are provided in Table 1 and the text. Abbreviation: Dx, diagnosed.

diagnosed involved staff of the Daru PHU (cases 14–16, who had EVD diagnosed on 31 May), including the first male (case 15), and case 17 (who had EVD diagnosed on 4 June), from Kambia District in northeastern Sierra Leone. Each of these cases were also linked to the transmission chain described above.

By the second week of June 2014, numerous cases were being admitted to KGH from villages throughout Kailahun District, many from villages in and around Daru (Figure 1*B*, Figure 3, and Table 1). By the third week of June, 2 cases had been detected in Kenema District. The actual magnitude of the outbreak in Sierra Leone at this time was underreported amid fears that the outbreak would have a deleterious influence on mining and other commerce. Despite the growing numbers, it was felt by some that the outbreak would soon burn out in weeks to months [20]. An interview on National Public Radio in the

Figure 2 continued. to KGH on 23 March 2014 but received a diagnosed of EVD after case 2 tested positive. *C*, Cumulative numbers of EVD cases admitted to KGH from 25 May to 18 June 2014. These cases were the basis of the genomic sequencing article by Gire et al [14] and the clinical study by Schieffelin et al [16]. *D*, Numbers of EVD cases diagnosed per month in Kenema District and in Sierra Leone between 25 May 2014 and 17 February 2016.

Table 1. Epidemiological Linkages of First Known Ebola Virus Disease (EVD) Cases in Sierra Leone

Case	Sex; Age, y	Residence	Date of Diagnosis or Known Illness	Date of Death or Survival Status	Epidemiological Linkage(s)
A	F; unknown	Kpondu or vicinity	Presumed EVD	3 Mar 2014	Mother of case D, grandmother of case B, traveled to Guinea in vehicle with sick individual to visit son, 5 sisters reported to have washed body and died in Guinea, reported in WHO/Guinea MoH 19 Mar 2014 memorandum (village was referred to as Peluon)
B	F; 21	Kpondu	19 Mar 2014	Survived	Daughter of case A, reported in WHO/Guinea MoH 19 Mar 2014 memorandum
C	F; unknown	Kpondu	Presumed EVD	Died (date unknown)	Daughter of case A
D	F; 3	Kpondu	Presumed EVD	11 Mar 2014	Granddaughter of case A, daughter of case D, mother of case C, reported in WHO/Guinea MoH 19 Mar 2014 memorandum
E	F; unknown	Kpondu	Presumed EVD	29 Mar 2014	"Mother-in-law" or other relative of case A, close friend or assistant of case F
F	F; unknown	Kpondu	Presumed EVD	8 Apr 2014	Traditional healer (herbalist), visited case E while case E was sick
G	M; unknown	Kpondu	Presumed EVD	21 Apr 2014	Husband of case F (healer)
H	M; unknown	Kpondu	Presumed EVD	30 Apr 2014	Grandson of cases G and F
I	F; unknown	Nyumudu	Presumed EVD	Died (date unknown)	Sister of case F
J	F; unknown	Sokoma	Presumed EVD	Died (date unknown)	Mother of cases F and I, mother-in-law of case 1
K	F; unknown	Buedu	Presumed EVD	Unknown	Daughter of case I
L	F; unknown	Koindu	Presumed EVD	5 May 2014	Daughter of case K, pregnant, shared room with case 1, died at Koindu PHU
M	F; unknown	Koindu	Presumed EVD	23 May 2014	HCW at Koindu PHU, contact with case L and case 1, attempts to go the KGH in vehicle driven by case 17, because of severe illness stops at Daru Health Center
1	F; 20	Koindu	23 May 2014	Survived	Pregnant, shared room at Koindu PHU with case L, first documented EVD case to present to KGH (on 23 May 2014), where she was treated by the Lassa fever clinical team
2	F; 39	Sokoma	25 May 2014	Survived	Daughter-in-law of case J, sister-in-law of cases F and I, reported to have attended funeral of healer; first case diagnosed in Sierra Leone by Augustine Goba of KGH Lassa laboratory
3	F; 45	Buedu	25 May 2014	30 May 2014	Reported to have attended funeral of healer, died in Buedu PHU
4	F; 50	Koindu	26 May 2014	30 May 2014	Midwife at Koindu PHU, reported contact with case L and case 1, died in annex of KGH
N	M; unknown	Buedu	Presumed EVD	27 May 2014	Possible link to case K; died in Foya, Liberia; many family members in Foya and Monrovia reportedly became sick and died
5	F; 15	Nyumudu	27 May 2014	Unknown	Reported to have attended funeral of healer
6	F; 8	Nyumudu	27 May 2014	Unknown	Reported to have attended funeral of healer
7	F; 55	Kolosu	27 May 2014	Unknown	Reported to have attended funeral of healer
8	F; 54	Kolosu	27 May 2014	Unknown	Reported to have attended funeral of healer
9	F; 57	Fokoma	27 May 2014	Unknown	Reported to have attended funeral of healer
10	F; 19	Sasani	27 May 2014	Unknown	Reported to have attended funeral of healer
11	F; 50	Kpondu	27 May 2014	Unknown	Reported to have attended funeral of healer
12	F; 27	Buedu	27 May 2014	Unknown	Reported to have attended funeral of healer
13	F; 38	Buedu	27 May 2014	Unknown	Reported to have attended funeral of healer
14	F; 38	Daru	31 May 2014	6 Jun 2014	HCW at Daru PHU, contact with case M
15	M; 43	Daru	31 May 2014	6 Jun 2014	HCW at Daru PHU, contact with case M
16	F; 45	Daru	31 May 2014	Survived	HCW at Daru PHU, contact with case M
0	F; unknown	Kpondu	Late May	Died (late May)	Mother of case P
P	M; unknown	Liberia	3 Jun 2014	Survived	Son of case O, reportedly treated case O with intravenous fluids in Koindu and returned to Liberia, admitted to Foya ETU, had relatives who reportedly died in Guinea
17	M; 42	Kambia	4 Jun 2014	Survived	Driver of vehicle transporting case M (HCW-A) to Daru PHU, returned from Kambia to KGH with outreach team and case Q
Q	F; unknown	Kambia	4 Jun 2014 (false-positive diagnosis by Metabiota)	NA	Wife of case 17

Data are based in part on investigations by Drs Sylvia Olayinka Blyden and Sheri Fink. Relationships indicated may not represent biological relatedness.

Abbreviations: ETU, Ebola treatment unit; HCW, healthcare worker; KGH, Kenema Government Hospital; MoH, ministry of health; NA, not applicable; PHU, public health unit; WHO, World Health Organization.

United States by one of us (R. F. G.) on 18 June that recounted the deaths in the vicinity of Daru was depicted as needlessly alarmist by factions in the United States and Sierra Leone governments and elsewhere. Médecins Sans Frontières (MSF) was another group that appealed for more international assistance at this time but was likewise labeled alarmist [22].

EBOV Infections Among Healthcare Workers at KGH

The caseload at KGH continued to increase beyond its capacity (Figure 2*C*). Nurses from other KGH wards were recruited to assist with patients who had EVD. In part because of the delayed engagement by the international community, personal protective equipment was in short supply at KGH, particularly in the general wards. By the middle of July 2014, several members of the nursing staff had been infected with EBOV [23]. One of the nurses who became infected was pregnant. Four nurses, including the head nurse of the Lassa ward, who had worked with the Lassa fever program for >25 years, attempted to save the life of their colleague by inducing a stillbirth delivery, a procedure that offers a chance of survival during Lassa fever and likely contributed to the survival of case 1. Despite this procedure, the nurse died of EVD, and each of the 4 nurses were infected with EBOV. All 4 died, including, on 21 July, the head nurse (Figure 2*A* and Supplementary Figure 3). On 22 July, Dr Khan tested positive for EVD, as did one of the Lassa laboratory staff. It is likely that the laboratory staff member acquired EBOV infection in the community. Dr Khan made the decision to transport himself to the Ebola treatment unit (ETU) in Kailahun, to reduce stress on the KGH staff. In a teleconference organized by the VHFC and including representatives from the NIH, the WHO, the CDC, and MSF, the use of ZMapp, an experimental therapy, consisting of a cocktail of humanized mouse monoclonal antibodies to EBOV [24], for Dr Khan was discussed. It was stated that Dr Khan was to receive the experimental drug, but this decision was reversed [25]. Dr Khan died on 29 July 2014 (Supplementary Figure 3).

While the West African EVD outbreak was not declared a public health emergency of international concern until 8 August 2014, representatives of the WHO Clinical Response Team and other volunteers had already come to KGH (Supplementary Figure 4). On 22 August 2014, a nurse became the first British citizen to be infected with EBOV during the West African outbreak (Figure 2*A*) [26]. The nurse was a resident of the VHFC project house in Kenema at the time and was evacuated to London on 25 August 2014, where he made a quick recovery and returned to Sierra Leone to treat patients with EVD. Another KGH laboratory technician received a diagnosis of EVD, on 3 September. This diagnosis turned out to be based on a false-positive PCR result. The technician spent 2 days in the KGH Annex Ward with an EBOV-infected nurse who later died of EVD. This case highlights the deficiencies of triage procedures that rely on a single diagnostic assay. Use of a rapid immunoassay at the point of care, followed by confirmation with quantitative RT-PCR targeting at least 2 distinct nonoverlapping gene regions, would be a superior triage approach, compared with screening based on a single technology. A short time later, a physician, a WHO volunteer, contracted EVD and, on 9 September, was evacuated to Emory Hospital (Atlanta, Georgia). The physician had a severe EVD course and experienced recrudescence involving EBOV replication in his left eye and other complications [27].

The CDC assumed responsibility for EBOV diagnostic testing at the KGH laboratory in late August 2014 [28]. The VHF laboratory at KGH continued to receive samples from the International Federation of Red Cross and Red Crescent Societies and other ETUs for Lassa fever and EVD diagnostic screening. By November 2014, the Ebola outbreak in Kenema District had largely subsided, which can be attributed to aggressive case finding and contract tracing (Figure 3*B*). KGH was no longer treating EVD cases. A holding center at KGH was still accepting suspected EVD cases for transport to the IFRC ETU located between the cities of Kenema and Bo. The CDC moved its diagnostic laboratory in late September to Bo, within the MSF ETU, because of the shifting burden of EVD cases to the west. EVD cases continued in Sierra Leone throughout 2015, with 2 cases occurring in January 2016 (Figure 2*D*).

Contributions of KGH to the Scientific Understanding of the West African Outbreak

After receiving approvals from the Sierra Leone Ethics and Scientific Review Committee and MoHS to ship sample excess no longer needed for diagnosis, VHFC scientists led by Pardis Sabeti of Harvard University and the Broad Institute analyzed the genomes of EBOV from the initial patients with EVD diagnosed at KGH (Figure 2*C*) [14]. The study demonstrated that human-to-human spread, rather than repeated introductions of EBOV from the animal reservoir, drove the outbreak and documented the expected accumulation of mutations in the viral genome. Sequence analyses indicated that the West African variant of EBOV (Makona) diverged from Middle African lineages approximately a decade ago. This was consistent with serological evidence for possible exposure to filoviruses in Sierra Leoneans dating back to 2009 [29]. These genetic studies were subsequently expanded by a number of groups, including our collaborative study with the CDC [30].

An in-depth clinical study of EVD in the early cases admitted to KGH (Figure 2*C*) was also performed [16]. Clinical and laboratory factors at presentation that were associated with a fatal outcome included fever, weakness, dizziness, diarrhea, and elevated levels of blood urea nitrogen, aspartate aminotransferase, and creatinine. Age of >45 years and a plasma EBOV load of $>10^8$ genomes/mL were also associated with fatal outcomes, findings that were subsequently confirmed in other cohorts [31–33]. Our clinical study showed that the West African variant of EBOV causes predominately a gastrointestinal illness and focused attention on this aspect for control of disease spread, rather than on bleeding manifestations that predominated in prior outbreaks.

KGH and the VHFC also worked with the Food and Drug Association and the WHO to validate the ReEBOV Antigen Rapid Test (ReEBOV RDT). The ReEBOV RDT received WHO Emergency Use Authorization and Listing on 20 February 2015 and Food and Drug Administration Emergency Use Authorization

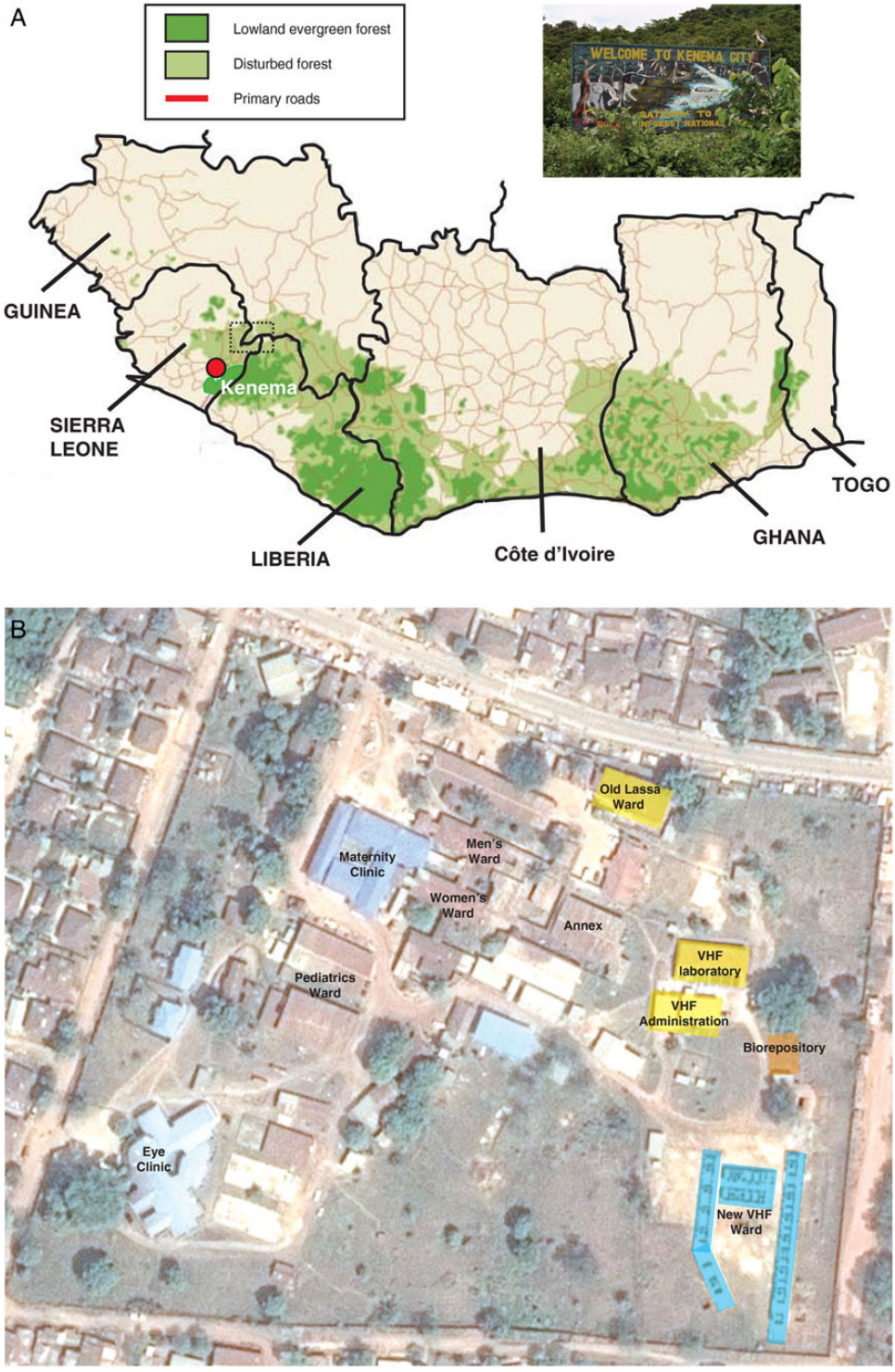

Figure 4. Possible reemergence of Ebola virus (EBOV) disease in eastern Sierra Leone and fortification of Kenema Government Hospital (KGH) for managing viral hemorrhagic fevers (VHFs). *A*, Distribution of the Gola Forest across West Africa. Location of Kenema town on the edge of the forested area suggests that the area is at risk for reemergence of EBOV. The box with dotted lines is the area in which EBOV emerged in the 2013–2016 outbreak. The inset shows a billboard posted on the main road into Kenema reading, "Welcome to Kenema: Gateway to the Gola Rainforest National Park." *B*, Existing and planned infrastructure to manage VHFs at KGH. The satellite image of the KGH complex is from Google Maps. The figure was inspired by a presentation by Jason Moses (IGLU, London, United Kingdom), who designed the new VHF ward. A 44-bed VHF clinical ward to replace the historic 14-bed Lassa ward will be completed in 2016 through funding by the Naval Engineering Facilities Command. The facility will feature high-efficiency solar power generation, LED lighting, ensured water supply, enhanced infection control, and improved decontamination facilities and waste disposal. The KGH laboratory is equipped for diagnosis of VHFs (via rapid diagnostic tests, enzyme-linked immunosorbent assays, and quantitative polymerase chain reaction analyses) and for performing clinical chemistry analyses, hematological assays, flow cytometry, and cell culture. Establishment of genomic sequencing capacity is expected during the first quarter of 2017. A ruggedized liquid nitrogen generator and cryostorage tanks were delivered in January 2016, A biorepository is expected to be operational in the second quarter of 2016.

Table 2. Comparisons of Lassa Fever and Ebola Virus Disease

Variable	Lassa Fever	Ebola Virus Disease (Makona Variant)	Reference(s)
Epidemiologic feature			
Estimated introduction in West Africa	150 y ago	10 y ago	[14, 39]
Reservoir host	*Mastomys natalensis*	Unknown/inconclusive	[40, 41]
Primary mode of spread	Rodent to human	Human to human	[39]
Viral feature			
Genetic variability	High	Moderate	[14, 39]
Evolutionary rate	Moderate	Moderate	[39]
Ability to spread by aerosol	Experimental evidence suggests possibility	Hypothetical	[42, 43]
Virion stability	Unknown	7 d (in corpses)	[44]
Countermeasure			
Therapeutics	Ribavirin; experimental	Experimental only	[45, 46]
Vaccines	Experimental only (VSV-GPC, VLP)	Experimental only (VSV-GP, ChAd3 recombinant, VLP)	[47–49]
Diagnostic assays	RDT, ELISAs, PCR in use	PCR widely used, RDTs approved for emergency use but not widely deployed	[1, 38]
Clinical feature			
Overall case-fatality rate	Unknown	Unknown	
Case-fatality rate in persons presenting while viremic, %	50–80	Variable by site (approximately 32–75)	[1]
Fever	Variable	Frequent	[16, 50]
Bleeding	Common	Infrequent	[16, 51, 52]
Vomiting, diarrhea	Common	Common	[16, 51]

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RDT, rapid diagnostic test; VLP, virus-like particle.

on 24 February 2015. The combined WHO clinical performance for the ReEBOV RDT showed a positive percentage agreement of 91.8% and a negative percentage agreement of 84.6% with quantitative PCR and was a distinct improvement to clinical diagnostic likelihood based solely on case definition without laboratory testing [16, 34–36]. An informal deployment of prototype RDTs by the MoHS following a reemergence of EVD in Kono District in December 2014 found 100% correspondence with PCR results and anecdotally found utility in the use of RDTs for effective triage, particularly in the case of several infants, for whom a clinical evaluation was challenging. While this limited deployment tested the point-of-contact concept by using blood specimens obtained by finger stick, rather than stored blood specimens, it was not sanctioned by the VHFC and was halted by a WHO field team. An independent study that used finger stick or fresh venous whole-blood specimens started in February 2015 and was conducted by Partners In Health (Boston) and Public Health England (Porton Down, United Kingdom) [37]. The clinical performances for both finger stick and whole-blood specimens were identical, with a positive percentage agreement of 100% and a negative percentage agreement of 92.2%. A modeling study suggested that combined use of RDTs and PCR confirmation may have reduced the size of the epidemic [38].

DISCUSSION

Kenema District is a major gateway into the Gola Rainforest, which extends from Sierra Leone into Guinea, Liberia, Côte d'Ivoire, Ghana, and Togo (Figure 4*A*). While it is unknown whether EBOV is present in reservoir species throughout this forested area of West Africa, it is prudent to prepare for the possibility that Kenema and surrounding districts may experience future reemergence of EBOV. KGH must fortify its emergency infrastructure and logistical capabilities to respond to a future Ebola outbreak or other health emergency. Work is being completed on a new VHF ward that will anchor the Khan Center of Excellence at KGH (Figure 4*B*). In the event of a new Ebola outbreak in eastern Sierra Leone, the facility will serve as a triage and treatment facility until, if needed, ETUs outside of KGH are established. KGH and VHFC team members are active sponsors and in some cases members of the Kenema Ebola Survivors Association. A similar association for Lassa fever survivors is being established. Training opportunities for West African scientists are being expanded through support by the NIH International Center for Infectious Disease Research program, the Human Health and Heredity Program, Wellcome Trust, and the African Center of Excellence for the Genomics of Infectious Diseases.

LASV, a member of the *Arenaviridae*, and EBOV, a member of the *Filoviridae*, represent 2 of 4 families of enveloped RNA viruses that include members that induce VHF. There are differences in the virus particles and disease characteristics that influence the management of disease due to these 2 pathogens (summarized in Table 2). Lassa fever is primarily spread from its rodent reservoir to humans, while human-to-human transmission occurs with low efficiency [39]. In contrast, the reservoir-to-human spread of EBOV is rare. Once this transmission

occurs, outbreaks are typically sustained by human-to-human transmission, which is moderately effective. In view of these differences, a surveillance system for Kenema District has been developed. This so-called hub-and-spoke system will ultimately use Lassa and Ebola RDTs and appropriate confirmatory diagnostic assays to provide advanced detection of these public health threats in locations that have minimal laboratory infrastructure.

The lessons learned at KGH may provide guidance for improving the responses to international health crises in regions of the world where healthcare infrastructure is severely limited. It is essential that resources from the international community are deployed with greater urgency than was evident at the beginning of the 2013–2016 Ebola outbreak. Completely open data, including sharing pathogen genomic sequences and anonymized patient clinical records, must become a practice embraced by the scientific community. International teams already on the ground must cooperate closely with local ministries of health and the WHO, which was not always done. While control of the outbreak must remain the first priority, testing of potential diagnostic assays, therapeutic agents, and vaccines, which was needlessly delayed in the West African Ebola outbreak, must be given higher prioritization. If future outbreaks are to be adequately managed and contained in a timely manner, there must be greatly increased investments in human resources and public health infrastructure in underdeveloped countries.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. The Viral Hemorrhagic Fever Consortium (available at: http://www.vhfc.org) is a partnership of academic and industry scientists who are developing diagnostic tests, therapeutic agents, and vaccines for Lassa fever, Ebola, and other severe diseases. Tulane University and its various academic and industry partners have filed US and foreign patent applications on behalf of the consortium for several of these technologies. Technical information may also be kept as trade secrets. If commercial products are developed, consortium members may receive royalties or profits. This does not alter our adherence to all policies of the NIH and *The Journal of Infectious Diseases* on sharing data and materials. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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