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Epidemiology and Management of the 2013–16 West African Ebola Outbreak

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Abstract

The 2013–16 West African Ebola outbreak is the largest, most geographically dispersed, and deadliest on record, with 28,616 suspected cases and 11,310 deaths recorded to date in Guinea, Liberia, and Sierra Leone. We provide a review of the epidemiology and management of the 2013–16 Ebola outbreak in West Africa aimed at stimulating reflection on lessons learned that may improve the response to the next international health crisis caused by a pathogen that emerges in a region of the world with a severely limited health care infrastructure. Surveillance efforts employing rapid and effective point-of-care diagnostics designed for environments that lack advanced laboratory infrastructure will greatly aid in early detection and containment efforts during future outbreaks. Introduction of effective therapeutics and vaccines against Ebola into the public health system and the biodefense armamentarium is of the highest priority if future outbreaks are to be adequately managed and contained in a timely manner.

Keywords

Ebola virus; filoviruses; epidemiology; outbreak management; viral genetics; viral therapeutics; viral vaccines; viral diagnostics; point-of-care testing

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DISCLOSURE STATEMENT

The authors are members of the Viral Hemorrhagic Fever Consortium (http://www.vhfc.org), a public-private partnership of academic and industry scientists who are developing diagnostic tests, therapeutic agents, and vaccines for Ebola, Lassa fever, 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. If commercial products are developed, consortium members may receive royalties or profits.

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INTRODUCTION

Ebola virus (EBOV) has periodically caused outbreaks of Ebola, otherwise known as Ebola virus disease (EVD), within Central African countries including Gabon, Republic of the Congo, Democratic Republic of the Congo (DRC; formerly Zaire), Sudan, Uganda, and Kenya over the past 40 years (1–7). The 2013–16 West African Ebola outbreak is the largest, most geographically dispersed, and deadliest on record, with 28,616 suspected cases and 11,310 deaths documented as of January 20, 2016 (8). The outbreak was caused by the EBOV Makona variant (9), which diverged from Central African lineages approximately a decade ago (10). It appears likely that undetected limited spillover of EBOV Makona from its as-yet-unconfirmed animal reservoir to humans occurred during this timeframe (11, 12). The current outbreak, declared in March 2014 in Guinea, was traced back to the end of 2013 (13), and subsequently expanded to involve the neighboring countries of Sierra Leone and Liberia. Additional cases caused by transmission from humans who had traveled from one of these countries occurred in Nigeria (14–16), Senegal (17), Mali (18), Spain (19), and the United States (20, 21). Here, we discuss the epidemiology and management of the 2013–16 Ebola outbreak in West Africa and reflect on lessons learned that may improve the response to the next international health crisis caused by an emerging viral pathogen.

EBOLA VIRUS STRUCTURE AND PATHOGENESIS

EBOV is a member of the *Filoviridae* family of enveloped, nonsegmented, negative-strand RNA viruses. The viral particles are characteristically filamentous with a diameter of 80 nm. Infectious particles are approximately 970 nm in length, but particles can reach up to 14,000 nm in length due to variations in assembly or budding. The RNA genome has seven genes, encoding nucleoprotein, viral protein (VP)24, VP30, VP35, VP40, glycoprotein (GP), and an RNA-dependent RNA polymerase (L polymerase) (22). All the genes are monocistronic except for the GP gene, which encodes GP1 and GP2, forming the trimeric surface glycoprotein spikes (23). VP30 is a viral transcription factor (24), and VP35 is the cofactor for L polymerase (25). The VP40 matrix protein is a peripheral membrane protein and mediates budding and viral particle release (26). The VP24 matrix protein is involved in virion assembly but also has important roles in both viral RNA transcription and replication (27).

After it enters the body via mucosal surfaces or breaks in the skin, the principal cellular targets of EBOV are resident monocytes, dendritic cells (28), and macrophages. The virus, while establishing systemic infection, can also infect adrenal cortical cells, hepatocytes, fibroblasts, and endothelial cells (23). The initial targeting of peripheral monocytes, dendritic cells, and macrophages followed by release of free virions in the lymph and blood quickly disseminates EBOV to various tissues. Its ability to disseminate to multiple tissues combined with its affinity for several surface receptor targets accounts for EBOV's pantropism (29).

EBOV is internalized through macropinocytosis into early endosomes (30, 31). Once the viral particles are internalized into endosomes, they are trafficked to acidified late endosomes, where cleavage of GP1 allows conformational changes and insertion of the GP2

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fusion loop into the endosome membrane, triggering partial fusion of the viral and cellular membranes (32–34). This insertion into the cell membrane also leads to the masking and downregulation of adhesion molecules, MHC class I proteins, and EGF receptors (35–39). Eventually, full fusion of the viral and cellular membranes releases the viral genome and associated nucleocapsid and matrix proteins into the host cytoplasm (40).

EBOV VPs and GPs are responsible for inhibiting early immune responses, contributing to viral pathogenesis (41). In the absence of an early innate immune response, unimpeded viral translation and replication lead to exponential release of progeny virions and cytopathic depletion of target host cells such as dendritic cells and T cells, further retarding effective innate and adaptive responses (23). The EBOV GPs also contribute to both the cytopathic effect and immune evasion by altering the expression of adhesion molecules and causing display of surface receptors, destabilization of membrane integrity, and release of soluble GP (sGP) to neutralize any humoral immune response. All these viral defense mechanisms contribute to the pathogenesis and the characteristic high morbidity and mortality of Ebola.

EBOLA CLINICAL PRESENTATION

The incubation period of EVD can be as long as 21 days; however, cases typically become symptomatic 6–12 days after infection. EVD typically presents with high fever (40°C), chills, malaise, myalgia, and arthralgia at onset and often progresses to gastrointestinal signs including anorexia, nausea, vomiting, abdominal pain, and diarrhea. These symptoms are characteristic of viral hemorrhagic fevers (VHFs). Lassa fever and EVD share similarities in early clinical manifestations with malaria and typhoid fever, which can lead to misdiagnosis of both diseases, often with fatal consequences (42–45). Respiratory signs include chest pain, cough, and shortness of breath. Vascular signs of dizziness, conjunctival injection, and edema may occur. Neurological signs include headache, confusion, delirium, and coma (46). Hemorrhage typically occurs in fewer than 30% of cases but may include maculopapular rash, petechiae, bleeding at the site of injection, mucosal hemorrhage, hematemesis, hematuria, and hematochezia (46). In acute EVD, hepatic and renal involvements are indicated by elevated levels of serum transaminases [aspartate transaminase (AST) and alanine transaminase (ALT)], blood urea nitrogen (BUN), and creatinine as well as proteinuria (47). Thrombocytopenia, longer prothrombin times, and high D dimer levels are indicators of disseminated intravascular coagulation and can contribute to multiple organ failure and shock, which can be fatal in >50% of cases (38, 48).

Schieffelin et al. (45) examined clinical data from the first 106 EVD patients admitted to Kenema Government Hospital (KGH) in Kenema, Sierra Leone. Mean time from onset to admission was 5.7 ± 0.5 days, and mean time from onset to death was 9.8 ± 0.7 days. An elevated case fatality rate (CFR) was observed in cases that presented with a viral load $\geq 10^5$ copies/mL. Persons >45 years old had a CFR of 94%, compared with 57% for persons <21 years old (overall CFR = 74%). There was no significant difference in overall CFR for males and females. Typical signs and symptoms described above were observed in the Kenema EVD patients (45). Fever and headache were the most common for both fatal and nonfatal EVD. Weakness, dizziness, and diarrhea were significantly ($p < 0.05$) more prevalent in fatal than in nonfatal EVD. Hemorrhage was observed in only one patient, and other studies have

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reported that less than 5% of West African cases developed hemorrhage, suggesting that infection with the Makona variant may cause fewer hemorrhagic symptoms than infections with Central African EBOV (49). Metabolic testing indicated hepatocellular damage and impaired kidney function (45). Levels of AST and ALT, which measure liver function, were elevated, with a significant increase ($p = 0.009$) in fatal versus nonfatal EVD. Similarly, BUN and creatinine levels, tests for kidney function, were significantly elevated in fatal versus nonfatal EVD. The higher ratio of AST to ALT indicates that damage to tissues apart from the liver is occurring, and rhabdomyolysis (muscle breakdown) may be contributing to morbidity and renal manifestations. Although excessive fluid loss due to diarrhea contributes to the elevated BUN and creatinine levels, renal insufficiency is seen in patients without vomiting and diarrhea as well (47).

EBOLA EPIDEMIOLOGY

EBOV can be transmitted to humans through contact with tissue or fluids (saliva, blood) of infected animals (Figure 1). Large-droplet aerosol transmission has also been observed in animal model studies (50). Transmission can occur through consumption of infected bushmeat. Chimpanzees, gorillas, and duikers (a small- to medium-sized antelope) have all been suggested to serve as intermediate hosts for transmission to humans (Figure 1*a*) (51, 52). Bats are potential reservoirs, although definitive evidence for this has not been shown. Ebola outbreaks are sustained by secondary human-to-human transmission (Figure 1*b*) that occurs through exposure to the fluids described above as well as semen, sweat, and breast milk. In the hospital setting, nosocomial transmission is a significant concern. Full personal protective equipment, including an impermeable gown or coveralls, respiratory protection (such as an N95 mask), face shield, double gloves, boot covers, and an apron, and barrier nursing protocols need to be strictly observed and human waste and contaminated bedding disposed of correctly. Safe burial practices must be followed to eliminate transmission that would otherwise occur during ritual burial rites that include washing of the corpse.

Evidence of virus infection and persistence in immunologically privileged tissues such as the testes has existed for decades from studies of primate (53–55) and human infection (Figure 1*c*). The well-documented case of laboratory-acquired EVD stemming from the investigation of the 1976 Sudan and Zaire EVD outbreaks (56) established that EBOV could be isolated from semen up to 61 days postonset. Following the 1995 Kikwit Ebola outbreak, prospective studies of EVD convalescent patients identified four of five semen donors having positive quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) samples ranging from 47 to 91 days postonset. These survivors tested negative for antigen in their semen, and virus isolation was not successful. Screening of household contacts for this study produced indirect evidence of EBOV transmission from one of the survivors to a household contact through sexual contact via semen (57, 58).

PRIOR FILOVIRUS OUTBREAKS

Hemorrhagic fever caused by a filovirus was first characterized in 1967 during an outbreak in Marburg and Frankfurt (Germany) and Belgrade (former Yugoslavia) among laboratory workers who were exposed to blood and tissue of African green monkeys (*Cercopithecus*

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aethiops) imported from Uganda (59). After the outbreak of Marburg virus, two other filoviruses emerged in 1976 during nearly simultaneous outbreaks in southern Sudan and northern Zaire (now DRC) (7). The Sudan outbreak had 150 fatalities out of 284 cases (53% CFR) (60). The second outbreak in Zaire (DRC) resulted in 284 deaths out of 318 cases (89% CFR) (61). It was subsequently determined that these outbreaks were caused by representatives of two related species of filoviruses, Sudan virus (SUDV; formerly Sudan ebolavirus) and EBOV (formerly Zaire ebolavirus). The 1976 outbreak mortality rates remain characteristic for these two filoviruses.

There was an absence of EVD cases after 1979 until the 1994 reemergence of EBOV in Gabon and Kikwit, DRC. The size of the Kikwit outbreak (318 cases and 284 deaths) grew due to multiple nosocomial transmission events and the occurrence of two superspreaders that were suspected to account for approximately 20% of the cases (6, 62). During a subsequent SUDV outbreak in the early 2000s, a dispersed outbreak surrounding Gulu, Uganda, resulted in 173 deaths from 425 cases (63). One of the last large EVD outbreaks prior to the 2013–16 West African outbreak occurred in the town of Luebo, DRC, between May and November 2007. This outbreak, which involved 260 cases and 186 deaths, was significant because the investigation into the index case produced the strongest evidence to date of the direct transmission of EBOV from migrating fruit bats to a human (Figure 2) (3).

FOUR PHASES OF THE WEST AFRICAN EBOLA OUTBREAK

The 2013–16 West African EVD outbreak appears to have originated near the village of Meliandou in the Guéckédou District of forested Guinea, which is close to the borders of Sierra Leone and Liberia (13). The Kissi people of Sierra Leone, Guinea, and Liberia populate this tricountry region and move freely across the borders for commercial, family, and social interactions. The West African outbreak progressed in four phases (Figure 3). Phase 1, from the emergence of EVD in late 2013 to May 25, 2014, included 258 diagnosed or probable cases. On May 25 Augustine Goba, director of the VHF laboratory at KGH, diagnosed the first EVD case in Sierra Leone. This case (Case B) was referred by the community health officer at the Koindu Health Center; Koindu is a Kissi village in Kailahun District, Sierra Leone, located a few kilometers from the Guinea border. It is noteworthy that another person from Koindu with EVD had already presented to KGH days prior to this diagnosis. KGH is about a four-hour drive from Koindu and is the largest health facility in the Eastern province. Case A, a pregnant woman, was initially believed to have Lassa fever, which has severe manifestations in pregnancy (44, 64). When Case A tested negative for Lassa fever, the doctor in charge, the late Sheik Humarr Khan, strongly suspected that she had EVD, which was confirmed by Goba. Case A lost her baby but survived.

Phase 1 of the outbreak was not contained by the interventions of the multiagency response; rather, it is evident that EBOV continued to spread in a limited fashion. Moreover, Phase 1 was not confined to Guinea and Liberia, where the international community had established diagnostic laboratories and treatment centers, but was also circulating in the entire tricountry border region, including Sierra Leone. The failure to recognize and respond to suspected cases in the border region allowed EBOV to disseminate unabated in early 2014. In mid-March 2014 there was a World Health Organization (WHO) report of a probable EVD case

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in Kailahun District, Sierra Leone, that was not further investigated. This probable case was epidemiologically linked to a subsequent seminal event of Phase 1 (65), the funeral of a Sierra Leonean healer (herbalist) from Kpondu, a village located a short distance from Koindu. The funeral of the healer, reportedly attended by hundreds, heralded the second, much deadlier phase of the outbreak, Phase 2, during which case numbers grew exponentially in all three countries (Figure 3). The first 14 cases diagnosed in Sierra Leone were all women, including Cases A and B (10). All were either relatives of the healer or members of her social circle. All 14 had attended the funeral of the healer on April 8, an event that has been previously reported as occurring on April 28 (66). Factors that contributed to the massive size of the subsequent epidemic include the higher population density and the greater mobility of the population in West Africa compared to Middle Africa, where prior outbreaks had occurred.

For reasons that are not clear, the number of EVD cases plummeted in West Africa at the end of 2014, a trend that continued into early 2015. A combination of factors, including interventions by the international community, forced quarantines and lockdowns, enhanced community awareness, and environmental factors, may have contributed to the abrupt decrease in case numbers. The dramatic drop-off in caseload marked the beginning of Phase 3, during which limited transmission chains occurred that were blunted by community quarantines and aggressive case-finding (Figure 3). Phase 3 in Sierra Leone was characterized by approximately 3 months during which 30–40 EVD cases were diagnosed per week, followed by approximately 4 months of 10 or fewer cases per week. Phase 3 also was marked by maximal engagement by the international community. More efficient diagnosis, improved clinical case management, and enhanced engagement limiting exposures are factors that blunted transmission chains in Phase 3. The WHO declared the outbreak over in Liberia on May 9, 2015; in Guinea on December 29, 2015; and in Sierra Leone on November 7, 2015. These dates marked the end of Phase 3 in these respective countries (Figure 3).

The West African outbreak was not yet over. The large number of EVD survivors exposed an underappreciated aspect of EBOV biology, the ability of the virus to be maintained in immunologically privileged sites such as the testes. Phase 4 has been characterized by the continuing sporadic reemergence of Ebola, triggered in at least some cases by sexual transmission from male survivors (Figure 3; see sidebar, Evidence of Sexual Transmission). Each of the three West African countries most affected by the outbreak has seen new Ebola cases in Phase 4. As in Phase 3, transmission chains in Phase 4 are limited in scope due to quarantines and aggressive case-finding and management. The major tool used to identify Ebola cases in Phase 4 has been the testing of oral fluids of corpses by qRT-PCR or on a limited basis by immunodiagnostics. Ring vaccination with experimental EBOV vaccines has also been deployed in Phase 4, and this appears to have had an impact on limiting transmission (67–70).

TRACKING EBOLA VIRUS EPIDEMIOLOGY BY GENOMIC SEQUENCING

Insight into the dynamic nature of the epidemic has been obtained by tracking molecular changes in the EBOV genome, including the emergence of intrahost variants, throughout the

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course of the outbreak. An important conclusion of the phylogenetic and epidemiological data is that the West African outbreak likely involved only one introduction of EBOV from its unknown animal reservoir to a human (10). This observation permitted a focus on breaking human transmission chains, rather than on restricting bushmeat consumption. The tracking of genetic changes in real time during the outbreak was also essential to ensure that diagnosis of the disease, which relied almost exclusively on qRT-PCR-based molecular assays, was not compromised by mutations in the EBOV genome during the unprecedented numbers of human-to-human transmission events. Data in nonhuman primate challenge studies suggest that the Makona variant may have somewhat slower replication kinetics (71), and a trend to lower virus loads as the outbreak progressed has been observed (72). As noted above, the West African disease was associated with more gastrointestinal symptoms and less overt bleeding than in prior Central African outbreaks (45, 51). However, the effects of the accumulated mutations in the EBOV genome on viral replication, entry, or pathogenesis cannot be determined from the current data.

Phase 1 of the outbreak included the initial EBOV variants sequenced from early Guinean cases in March 2014, referred to variously as GN-1, GUI-1, or lineage A variants (Figure 4) (9, 73, 74). GN-1 variants were subsequently dispersed throughout Guinea, with more limited incursions into Liberia and Sierra Leone. The progenitor of the GN-1 variant was likely to have been associated with the original animal reservoir-to-human transmission event, which occurred in or near Guéckédou District. As GN-1 viruses spread, their evolution was characterized by the accumulation of multiple nonsynonymous mutations in the genes encoding nucleoprotein, VP35, and GP that are not found in other EBOV Makona variants.

With the exception of GN-1 viruses that cocirculated with other variants in the greater Conakry region later in the outbreak, Phase 2 of the outbreak involved predominantly EBOV variants that were phylogenetically linked to those that emerged in Sierra Leone at the transition from Phase 1 to Phase 2, as described by Gire et al. (10) from samples collected at KGH. The first reported Ebola cases in Sierra Leone stemmed from two genetic clusters. SL1 was closely related to the three early GN-1 isolates from Guéckédou District, differing by two to five mutations. This close genetic relationship of SL1 to early GN-1 viruses is consistent with a single animal reservoir-to-human transmission event. The second cluster, SL2, was characterized by four additional mutations (Figure 4). SL2 appears to have evolved from SL1 months before it was observed among the first cases in Sierra Leone. A third lineage, SL3, derived from SL2, emerged widely in mid-June 2014 (Figure 4) (10, 45). The base haplotype of SL3 differs from that of SL2 by a single mutation (nucleotide 10218 of the EBOV Makona genome) and was first found as an intrahost variant (polymorphism) within a single individual.

SL1 and SL2 were both associated with the funeral of the healer (10), so it is possible that this event may have reignited the epidemic. SL3 became the most prevalent cluster in Sierra Leone, with SL1 disappearing in Sierra Leone soon after the appearance of SL3. Likewise, the SL2 variant was largely replaced by SL3 in Sierra Leone. Of the genomes from Sierra Leone viruses collected from August 20, 2014, through January 10, 2015, 97% carried the SL3 mutation at nucleotide 10218, and only 3% belonged to SL2. Tong et al. (75) sequenced

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175 EBOV genomes from July to November 2014 in Sierra Leone and found a similar result, although SL3 was the only cluster present in their samples. These results indicated that there were no additional imported EBOV lineages, link all Sierra Leonean EVD cases to the initial introduction of EBOV into Kailahun District, Sierra Leone, and confirm that SL3 became more genetically diverse as the virus spread from eastern to western Sierra Leone (Figure 4).

All four phases of the outbreak were evident in Liberia. Genomic sequencing is consistent with a Guinean source for the Phase 1 EVD cases detected in Liberia in March through May 2014 (74, 76). In contrast to the situation in Guinea, where GN-1 variants persisted at low levels, GN-1 viruses died out in Liberia (Figure 4). Most Liberian EBOV sequences group with those of cluster SL2, although a minor fraction of SL1-like variants were detected. Importantly, some sequences of Liberian EBOV have the basal haplotype of cluster SL2, suggesting that the introduction of SL2 variants into Liberia may have occurred contemporaneously with introduction of EBOV in Sierra Leone that lead to infection of the healer. Following the introduction of SL2 EBOV into Liberia, the viral population rapidly diversified. Phase 2 of the Liberian outbreak was dominated by eight sublineages of SL2 referred to as LB1–LB8. Four sequences from Mali from October and November 2014 also fell within the Liberia-derived SL2/GN-2 cluster. These four sequences include representatives from two independent introductions of EBOV to Mali, both of which have been traced to the movement of infected individuals from Guinea within transmission chains that can be traced back to Liberia (18).

The EBOV transmission patterns in Phase 2 (May 2014-January 2015) for Liberia and Sierra Leone were driven primarily by within-country spread (Figure 4) (77). Following the initial introduction of the SL2 lineage into Liberia, there was no evidence of additional introductions. In contrast, Phase 2 of the Guinean outbreak appears to involve several reintroductions of EBOV from both Liberia and Sierra Leone, although continuous spread and diversification also contributed to EBOV diversity in Guinea (Figure 4) (73). This interpretation is consistent with single, large peaks of cases in Sierra Leone and Liberia, but multiple, smaller peaks in Guinea (Figure 3). The predominant Phase 2 clusters of EBOV in Guinea are referred to as GN-2 and GN-3. GN-2 is most closely related to SL2 and could represent either a reintroduction from Sierra Leone or the continued spread in Guinea of variants related to those initially introduced to Sierra Leone. There is epidemiological evidence for reintroductions of SL2-derived EBOV from Sierra Leone into Guinea (10, 73). Examples of such cross-border virus traffic include cases that initiated a transmission chain in Conakry and Dalaba, each of which is directly linked to different travelers from Sierra Leone. GN-3 was found in Conakry, Forécariah, Dalaba, and to a limited extent Coyah. GN-3 is genetically similar to SL3 and likely arose by introduction of this variant by a traveler from Sierra Leone.

MANAGEMENT OF THE WEST AFRICAN EBOLA VIRUS DISEASE OUTBREAK WITH DIAGNOSTICS

The international response to outbreaks of EVD has evolved over the past several decades from small investigative teams and limited equipment to more coordinated and rapid

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responses capable of establishing field hospitals, laboratories, and surveillance teams. When there are isolated and limited cases, this approach can efficiently contain an emerging outbreak. However, even prior to the 2013–16 West African EVD outbreak, the locations of outbreaks were so remote that they grew to involve dozens to hundreds of cases and contacts before outbreak management teams could arrive to establish diagnostic and treatment capacity, and in some cases, laboratory diagnosis was still conducted elsewhere (3, 78, 79).

What has been missing from VHF outbreak surveillance and management is the capacity to deploy simple and reliable rapid tests in these remote communities. Even in a small country such as Sierra Leone, where Lassa fever is endemic and a regional VHF lab at KGH is available, the time from onset of symptoms to medical interventions can be 5 to 10 days and often includes misdiagnosis and multiple referrals (Figure 5*a*) (64, 80). For any VHF, which is difficult to manage clinically even with early diagnosis, delays this long can increase fatalities. Whereas the gold standard for EBOV diagnosis is viral genome detection by qRT-PCR, rapid diagnostic tests (RDTs) are typically based on detection of EBOV antigens and are visually read tests that do not require specialized equipment with consistent power supply. They can easily be performed with basic disposable laboratory supplies, and training requirements are minimal (Figure 5*b*). RDT reagents and kits are also very stable, and most do not require long-term refrigeration for storage. Furthermore, they can be produced at much lower costs than more complex tests such as qRT-PCR and can be less prone to contamination and false-positive results.

The 2013–16 West African EVD outbreak proved to be a very challenging environment to quickly establish sufficient laboratory capacity in the affected countries. In Sierra Leone, the disease emerged in the Kailahun and Kenema Districts of the Eastern Province. At the time, the only hospital laboratory with the capacity to routinely run both molecular diagnostics (qRT-PCR) and immunoassays (ELISA, Western blot analysis, and RDTs) for VHFs was the VHF laboratory at KGH. As the EVD outbreak spread, international health organizations set up additional temporary laboratories or added testing capacity to existing laboratories throughout the three affected countries. Based on WHO guidance, these new testing sites all used qRT-PCR as their EVD confirmatory test method (Figure 5*a*). The centralized nature of qRT-PCR test methods and the implementation of different target amplicons, instrument platforms, and data interpretation (including determination of cutoffs) created difficult logistical problems in transporting samples from the surveillance teams and Ebola treatment units (ETUs). Biosecurity protocols and data management issues created further delays in the reporting of laboratory results. When the outbreak had reached national emergency status, the volume of samples exceeded laboratory capacity, resulting in delays up to 5 days (and sometimes more) for reported results.

Against this backdrop, several groups addressed these hurdles to EVD testing in austere laboratory environments by developing rapid, point-of-care immunoassays that incorporate the use of recombinant protein technology (44, 81–87). Corgenix Inc. (Broomfield, CO) and the Viral Hemorrhagic Fever Consortium (VHFC) partners worked with the US Food and Drug Administration (FDA) and the WHO to expedite the validation of the ReEBOV® Antigen Rapid Test (ReEBOV RDT) at KGH to provide a point-of-care method for EVD screening in the field and at ETUs (Figure 5*b*). By November 2014, the EVD outbreak in

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Kenema District had subsided. A holding center at KGH was still accepting suspected EVD cases for transport to the International Federation of Red Cross and Red Crescent Societies (IFRC) ETU located outside the city of Kenema. The VHF laboratory at KGH maintained the Ebola sample bank and continued to receive samples from the IFRC ETU and others for Lassa fever and EVD diagnostic screening. The dynamic course of the EVD outbreak in the Eastern District compromised the full implementation of a prospective clinical study; therefore, clinical effectiveness was established using surplus banked clinical samples collected and maintained at KGH (88–90). The WHO also provided an independent performance evaluation working with two sites in the Western Area and Freetown, the European Mobile Laboratory at Hastings and the African Union/European Mobile Laboratory at Prince of Wales.

The WHO study compared RDT results to the RealStar® Filovirus Screen qRT-PCR Kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany); the ReEBOV RDT had a sensitivity of 91.8% and specificity of 84.2% compared with qRT-PCR. The samples were obtained at presentation to an ETU, when EBOV loads are likely higher than at earlier points in the disease course. The ReEBOV RDT received emergency use authorization from the US FDA and the WHO in February 2015. Two additional EVD RDTs have since received emergency use approval. The OraQuick Ebola Rapid Antigen Test (OraSure Technologies Inc., Bethlehem, PA) uses the same Ebola-specific antibodies (Autoimmune Technologies LLC, New Orleans, LA) as the ReEBOV RDT and received US FDA emergency use authorization in August 2015 (sensitivity 84.0%, specificity 98.0%). The SD Q Line Ebola Zaire Antigen (SD Biosensor, Inc., Seoul, South Korea) received WHO emergency use approval in September 2015 (sensitivity 84.9%, specificity 99.7%). Further studies are required to determine when RDTs are reliably positive during the course of illness and their utility in various phases of an Ebola outbreak.

Vogt et al. (91) evaluated the performance of the triage system based on EVD case definition while awaiting qRT-PCR test results at the Médecins Sans Frontières (MSF) Case Management Centre (CMC) site in Kailahun, Sierra Leone. Suspect EVD cases (433) were separated by symptoms at triage into Suspected (41%) and Highly Suspected (59%) isolation wards while they awaited qRT-PCR results. Based on qRT-PCR, the positive predictive value of symptom classification at triage was only 46% for Suspected and 76% for Highly Suspected cases, resulting in a combined false-positive rate of 39% of patients potentially exposed to nosocomial transmission of EVD. Another study, by Fitzpatrick et al. (92), analyzed the Ebola viral load, based on qRT-PCR cycle time, on admission to the MSF CMC in Kailahun during Phase 2 (June–October 2014). The qRT-PCR used a cutoff comparable to that used in the three ReEBOV RDT validation studies and determined that a cycle threshold (Ct) of 25 was equivalent to 1.3×10^7 copies/mL, which is nearly 2 log above the ReEBOV RDT limit of detection. The Ct values at admission for both survivors (interquantile ranges: 25–34) and fatal cases (20–25) were below the recommended cutoff ($Ct < 37$) of the KGH validation study. This suggests that use of the ReEBOV RDT during triage would be of benefit, because acceptable time to result for the qRT-PCR was 5 days for inclusion in this study. Suspected and Highly Suspected cases had similar virus loads (Ct = 25–32 and 21–32, respectively), which suggests that the clinical diagnosis was not capable of identifying the most infectious cases. These studies suggest that implementation of RDT

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screening during Phase 3 would have contributed to case triage management efforts in areas where EVD transmission was still active. The potential utility of EVD RDTs in triage scenarios and surveillance programs was modeled by Nouvellet et al. (93), who suggested that combined use of RDTs at the point of care followed by qRT-PCR confirmation could have reduced the size of the epidemic.

EBOLA VIRUS DISEASE TREATMENT, THERAPEUTICS, AND VACCINE DEVELOPMENT

Currently there are no regulatory approved therapies for EVD, though there are several promising therapeutics and vaccines in clinical trials that have been accelerated in response to the 2013–16 West African EVD outbreak (Tables 1 and 2) (94–96). Since the discovery of filoviruses, survivor's blood or plasma has been used as a therapeutic, with benefits, if any, presumed to be due to the presence of protective antibodies. Although studies have shown this to be an ineffective intervention for Lassa fever (97–100), convalescent serum has continued to be used in the treatment of EVD, particularly in the current outbreak. During this outbreak, several subjects were enrolled in independent studies evaluating the effectiveness of survivor's blood or convalescent plasma alone or in combination with drug therapies (Table 1). A study in Guinea treated 84 EVD patients with plasma from individual convalescent donors at the time of or up to 2 days after diagnosis (101). Compared with the control group (patients treated at the same center during the previous 5 months), and matched for age group and qRT-PCR Ct value, the treatment group received no benefit from plasma therapy. Individual case reports from 2015 described the survival of subjects treated with convalescent plasma and brincidofovir or TKM-Ebola (102, 103). Authors were unable to draw conclusions about the effectiveness of these therapies, though, because of the small sample size and the use of concurrent therapies. Convalescent plasma efficacy studies are ongoing, but the mixed results obtained so far necessitate the development of modern, highly scalable, recombinant antibody-based therapies, which could introduce a superior therapeutic option in the armamentarium of countermeasures for EVD.

ZMapp, a cocktail of chimerized murine monoclonal antibodies (Table 1), has shown protective efficacy against EBOV infection (104). The ZMapp immunotherapeutic was granted emergency use authorization during the current EVD outbreak and has been used in combination with other antivirals and convalescent serum. Due to its generation in transgenic tobacco plants, the ZMapp production process is too long and its throughput too low to support widespread use. It also has not completed full clinical effectiveness studies to support FDA approval.

Other drugs previously being investigated for utility in treating EVD were advanced to clinical trials during the outbreak with limited success. Brincidofovir, developed to interfere with viral DNA replication (Table 1), was experimentally used in combination with convalescent plasma to treat one EVD patient (102). Though the patient survived, the positive outcome could not be attributed with any certainty to the drug. At the beginning of 2015, MSF agreed to oversee a clinical trial of brincidofovir on behalf of Chimerix in Liberia, but Chimerix abruptly ended the study after enrolling only four patients and did not

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report its findings (105). Another clinical trial evaluating TKM-Ebola began in March 2015. This small interfering RNA drug targets L polymerase, VP24, and VP35 (Table 1) and demonstrated promising results in monkeys and safety in phase I clinical trials (106–110). However, the study was terminated after enrolling 14 patients, citing that the results indicated the drug was unlikely to show significant benefit (105). Favipiravir, an influenza drug developed and approved in Japan (Table 1), inhibits EBOV in cell culture studies and limits EVD in small animal models (111, 112). France's National Institute for Health and Medical Research oversaw the largest EVD clinical drug trial in Guinea to test favipiravir. The researchers described the results they collected from more than 200 subjects as encouraging; however, the study has been criticized because it was not randomized and controlled (105). Unlike convalescent plasma, ZMapp, brincidofovir, TKM-Ebola, and favipiravir, the interferons were not considered a priority treatment option by the WHO due to their unfavorable side effects, such as fever and muscle pain, despite being successful in monkeys and guinea pigs (Table 1) (113–115). However, a study was pushed through at a time when patient numbers were declining, and although the WHO does not recognize its results because of the small sample size, the research claims there is statistical significance (105).

Vaccine development has had limited success to date (Table 2). Attenuated virus and γ-inactivated virus vaccines have not proved effective for EBOV. New DNA-based vaccines are still in early development stages, and clinical effectiveness has not been demonstrated. Recombinant viral vaccines have shown mixed success. These vaccines incorporate EBOV protein expression in vaccinia virus (rVV) or vesicular stomatitis virus (rVSV) (116). The rVSV platform has demonstrated better effectiveness for EBOV infections in animal models than other approaches. An rVSV-based EBOV vaccine is progressing through clinical trials (69). Another potentially useful vaccine platform is based on virus-like particle technology. These nonreplicative enveloped particles display native viral immunogens and can elicit fully protective responses in vaccinated animal models (117–124).

Although progress in the testing of Ebola countermeasures has been realized, it is imperative that development of improved Ebola diagnostics, therapeutics, and vaccines continue. Future outbreaks of the disease, as well as outbreaks of disease caused by other filoviruses and other VHF agents, will benefit from having these improved management tools, which must be deployed with greater urgency than was evident in the 2013–16 West African Ebola outbreak.

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EBOV: Ebola virus

EVD: Ebola virus disease

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Viral proteins (VPs): Ebola VP24, VP30, VP35, and VP40 aid in viral replication and budding; they are also capable of disrupting early immune responses

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Glycoproteins (GPs): Ebola GP1 and GP2 bind cellular receptors and allow viral genomic entry; GP2 is implicated in cellular pore formation, causing the severe diarrhea seen in this outbreak

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VHF: viral hemorrhagic fever

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Kenema Government Hospital (KGH): hospital located in Kenema District in eastern Sierra Leone; responsible for diagnosing the first EVD case in the country

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Phase 1: the first phase of the outbreak, from the initial case in late 2013 to the cross-border spread in April 2014

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Phase 2: the longest phase of the outbreak, from May 2014 until early 2015, during which case numbers increased exponentially

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Phase 3: a phase characterized by slowed growth of the outbreak; transmission chains were blunted due to community quarantines and aggressive case-finding

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Phase 4: the final outbreak phase, during which sporadic cases have occurred in Liberia, Sierra Leone, and Guinea

RDT: rapid diagnostic test

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ETU: Ebola treatment unit

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ReEBOV® Antigen Rapid Test (ReEBOV RDT): this test received FDA emergency use authorization and is listed as an approved RDT by the WHO

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EVIDENCE OF SEXUAL TRANSMISSION

Horizontal transmission of EBOV through sexual contact has become a particular area of concern and has complicated containment of the West African outbreak (Figure 1*c*). One case of possible sexual transmission occurred in Monrovia during March 2015 (125). A 44-year-old woman was triaged with suspected EVD, which was confirmed by qRT-PCR 7 days postonset. Prior to the patient's death on day 13 postonset, she reported having unprotected sex with a known EVD survivor 7 days prior to her onset of symptoms. The patient had no travel history to other affected countries or contact with people with EVD symptoms. Genomic sequencing of EBOV from her blood specimen identified six mutations consistent with mutations identified in the EBOV isolated from the survivor. This set of mutations was not found in any of 132 EBOV genomes from Liberia, Guinea, Mali, and Sierra Leone.

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SUMMARY POINTS

1. EBOV is a member of the *Filoviridae* family of enveloped, nonsegmented, negative-strand RNA viruses. The viral particles are filamentous, with a uniform diameter but variable length. EBOV can infect a variety of cell types, including monocytes, dendritic cells (28), macrophages, adrenal cortical cells, hepatocytes, fibroblasts, and endothelial cells.
2. Spillover events from natural and/or intermediate hosts such as bats and apes followed by human-to-human transmission chains have been responsible for several EVD outbreaks since 1976; the current 2013–16 West African EVD outbreak is the largest in history.
3. Symptoms of the West African EVD outbreak begin as a nonspecific febrile illness and commonly progress to dizziness and severe diarrhea in fatal cases, with very little to no signs of hemorrhage.
4. The outbreak has progressed in four phases: Phase 1 represented the initial spillover event in Guinea and spread across borders into Liberia and Sierra Leone. In Phase 2, this spread quickly transitioned into exponential growth. Phase 3 eventually followed after mediation of the outbreak by local intervention, an international response (delayed by the belief that the outbreak would be self-limited), and greater community awareness. Finally, Phase 4 is characterized by sporadic, isolated cases.
5. Viral genomic sequencing revealed that after initial introduction of the virus into Liberia and Sierra Leone in Phase 1, Phase 2 in these two countries was dominated by intra-country diversification; in contrast, Guinea appears to have experienced multiple reintroductions from both Liberia and Sierra Leone.
6. The WHO established qRT-PCR as the standard for diagnosing EVD during the outbreak, but due to the time it took to establish laboratories capable of running qRT-PCR, the number of cases being seen in Phase 2, and the time from sample draw to diagnosis, testing could not be used to triage patients and EVD continued to aggressively spread through communities and holding centers at ETUs.
7. The WHO has issued guidance on field-deployable rapid diagnostic tests for EBOV antigens that present opportunities for better management of EVD outbreaks because they can easily be used in remote villages for screening, can provide an initial diagnosis at ETUs within minutes, and are much more cost effective, making them ideal for surveillance.
8. The West African EVD outbreak accelerated trials of potential therapeutics and vaccines in human subjects, most of which ended with no significant results due to limited manufacturing capacity, a decline in the number of

patients, and/or the simultaneous administration of treatments with no randomized controls.

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FUTURE ISSUES

1. Vaccine strategies including rVV, rVSV, and virus-like particles have shown success in animal models, and some are advancing to human trials in West Africa. Results from these will potentiate important progress toward preventing large future outbreaks.
2. The limited clinical trials that took place during the outbreak on preexisting drugs and treatment strategies proved to have modest impact. Monoclonal antibodies are an emerging section of therapeutics that may provide the most success and should be explored further.
3. Diagnostics that provide sensitivity and specificity comparable to qRT-PCR but take less time will greatly aid in containment. Improvements in the performance of RDTs, which require very little infrastructure to store and run, would greatly improve triaging practices.
4. The unanticipated occurrence of EVD in West Africa proves that this virus may be more prevalent than previously thought, necessitating surveillance and reliable RDTs for austere environments. Evaluation of enhanced surveillance methods is ongoing by the WHO and others.
5. The turning point in the outbreak was achieved in large part due to community education; therefore, continual education and community outreach about VHFs will assist in containment of future outbreaks.

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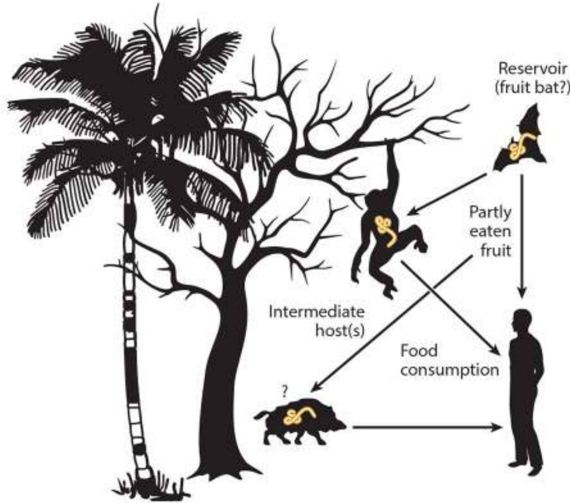

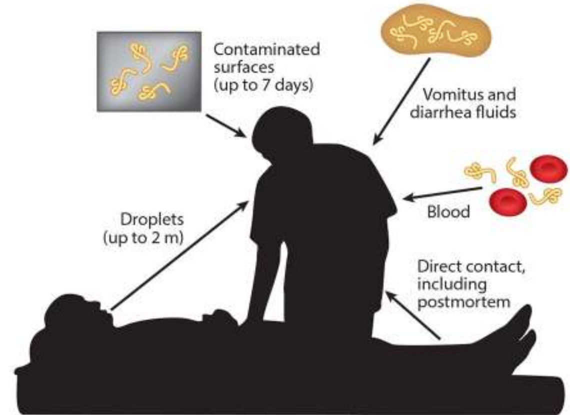

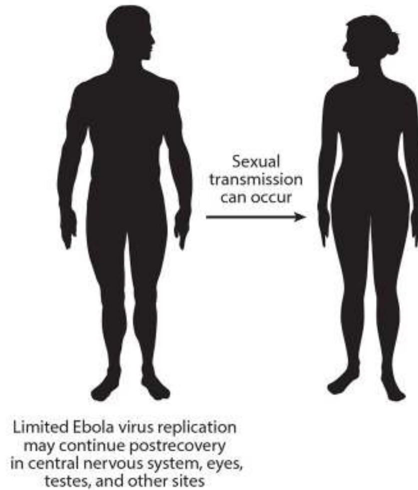

Figure 1.

Transmission of Ebola virus (EBOV). (*a*) The natural reservoir(s) of EBOV is unknown. There is evidence that fruit bats may serve this role. A number of animals, including apes, may be infected with EBOV and serve as intermediate hosts by transmitting the virus to humans. Humans may be infected by exposure to EBOV-contaminated surfaces or food, such as fruit, or by consuming an EBOV-infected animal. Sequencing studies have suggested that the West African Ebola virus disease outbreak was likely initiated by a single animal-to-human transmission event. (*b*) Human-to-human transmission of EBOV sustained the outbreak. Humans can be infected by direct contact with cases, because the virus can be found in sweat and saliva. Bodily fluids, such as blood, or diarrhea fluids can also serve as a source of infection and may be stable on surfaces for up to one week. EBOV on surfaces is inactivated by exposure to sunlight or disinfectants (such as bleach) or by drying. Transmission of EBOV from humans via suspended small aerosol droplets (<5 μm) is not

known to occur, but large droplets (>5 μm) potentially transmit EBOV at a distance of up to 2 m (126). (*c*) EBOV can persist in immunologically privileged sites such as the central nervous system, eyes, or testes. Reactivation in or from these sites coupled with evasion of immune responses can lead to inflammation and other sequelae. EBOV in semen can result in sexual transmission from males. It is unclear whether sexual transmission from female Ebola virus disease survivors occurs.

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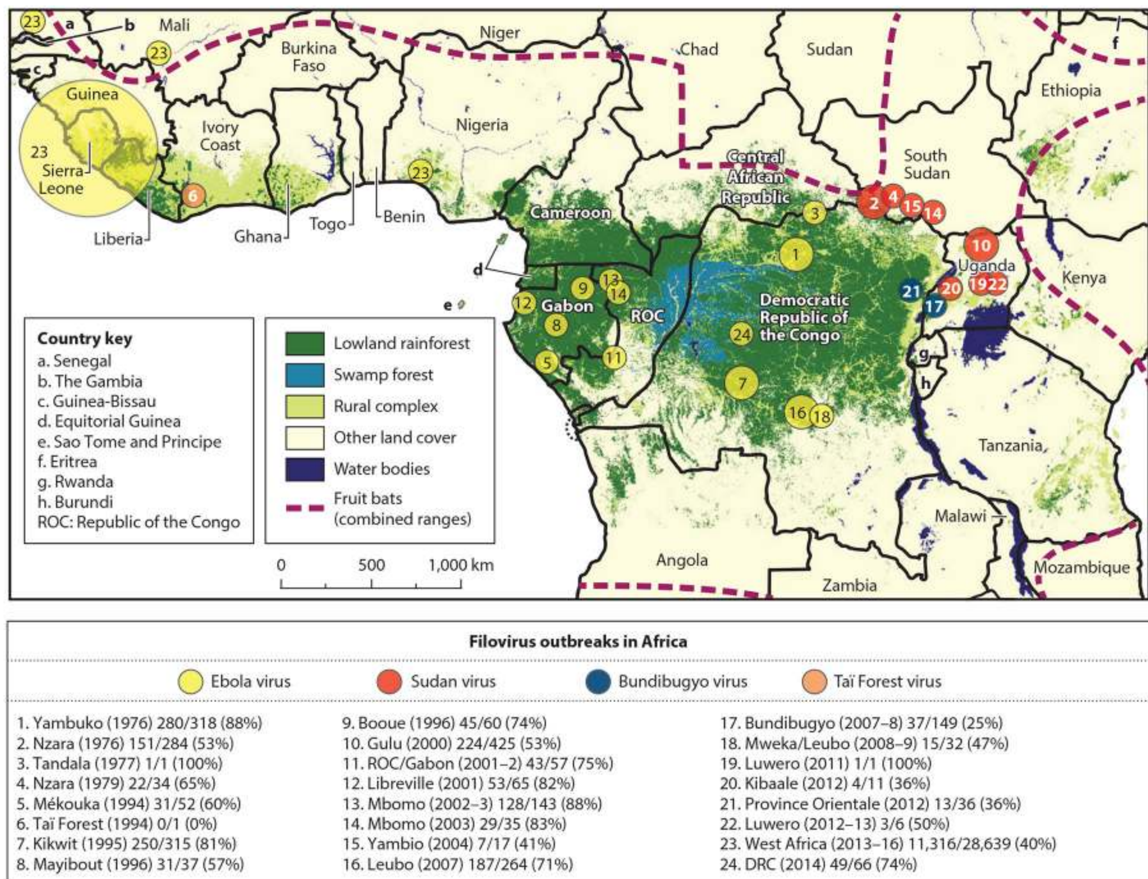

Figure 2.

Past outbreaks of Ebola virus and related filoviruses. The 2013–16 outbreak is the first filovirus outbreak to have occurred in West Africa, except for the lone human case of Taï Forest virus infection in 1994. Outbreaks of filovirus disease in Africa have involved representatives of four species: Ebola virus (*yellow*), Sudan virus (*red*), Bundibugyo virus (*blue*), and Taï Forest virus (*orange*). The size of each outbreak is approximated by the size of the corresponding circle. Some outbreaks involving single cases or limited importation from endemic regions are not depicted. Also delineated (*dashed line*) is the approximate combined range of several prominent species of fruit bats that may serve as reservoirs for Ebola virus. Each virus outbreak is listed with its year(s), the number of deaths over the total number of cases, and the case fatality rate. The map showing African rainforests is reproduced from Mayaux et al. (127), with permission.

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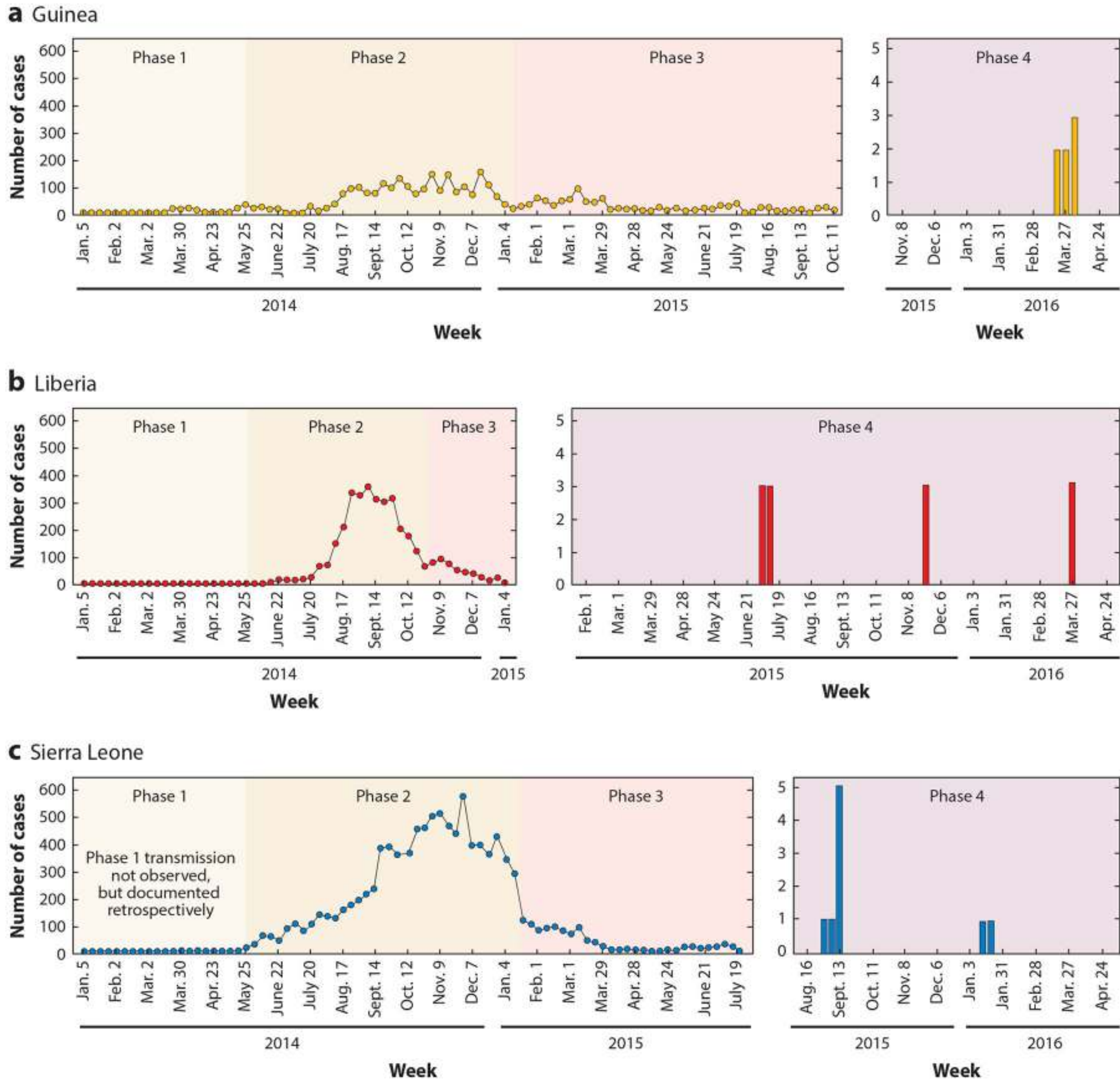

Figure 3.

Four phases of the 2013–16 West African Ebola virus disease outbreak. Weekly numbers of confirmed Ebola virus disease cases in Guinea, Liberia, and Sierra Leone are depicted. Although no cases were confirmed in 2013, investigations suggest that Phase 1 of outbreak began with limited local transmission in November or December of that year. Phase 2 of the outbreak, marked by widespread transmission, began May 25, 2014, when the first case of Ebola virus disease was diagnosed by Augustine Goba, director of the Kenema Government Hospital viral hemorrhagic fever laboratory. Phase 3 involved sporadic transmission chains that were blunted by the international and community responses begun in January 2015. Phase 4 of the outbreak is driven at least in part by sexual transmissions from survivors and began in August 2015.

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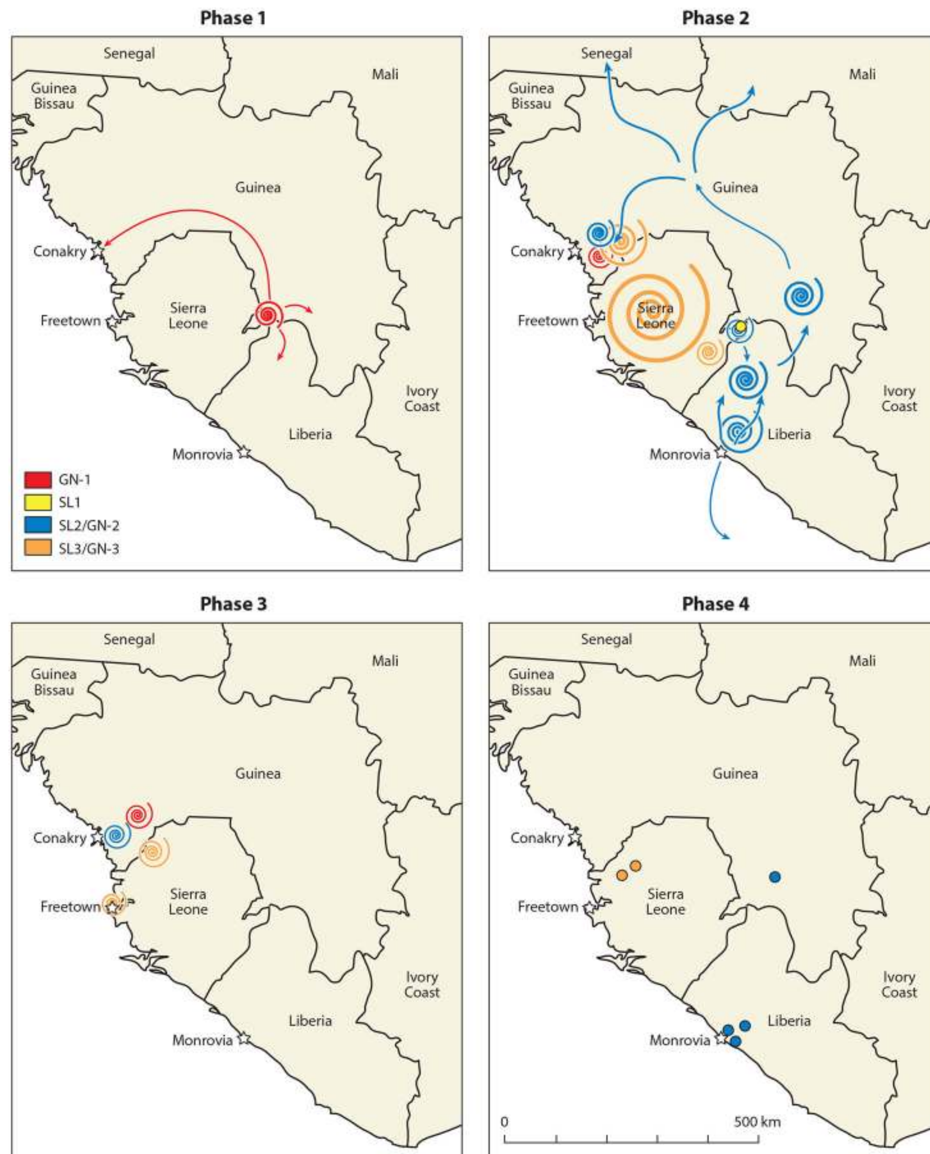

Figure 4.

Spread of major Ebola virus Makona variants during the four phases of the West African outbreak. Phase 1 of the outbreak involved limited local transmission of the Guinea 1 variant (GN-1) in the tricountry area. There were limited incursions of GN-1 to Conakry, Guinea, and to southern Liberia. Phase 2 began with Sierra Leone 1 (SL1) and Sierra Leone 2 (SL2) variants, which appear to have arisen from GN-1 in the tricountry area. SL2 was largely replaced by the SL3 variant, which spread via major roads in Sierra Leone from east to west. SL2 and the genetically related sublineages GN-2 and Liberia variants LB1–LB8 were dominant in Phase 2. Sporadic transmission chains involving a mixture of GN-1 and SL3 variants occurred in western Sierra Leone and the Conakry area of Guinea during Phase 3. Viruses related to those that were prominent during Phases 2 and 3 have been isolated from a limited number of cases in Phase 4. Because these Phase 4 viruses show limited genetic

change, they appear to have come from sites of limited replication in Ebola virus disease survivors.

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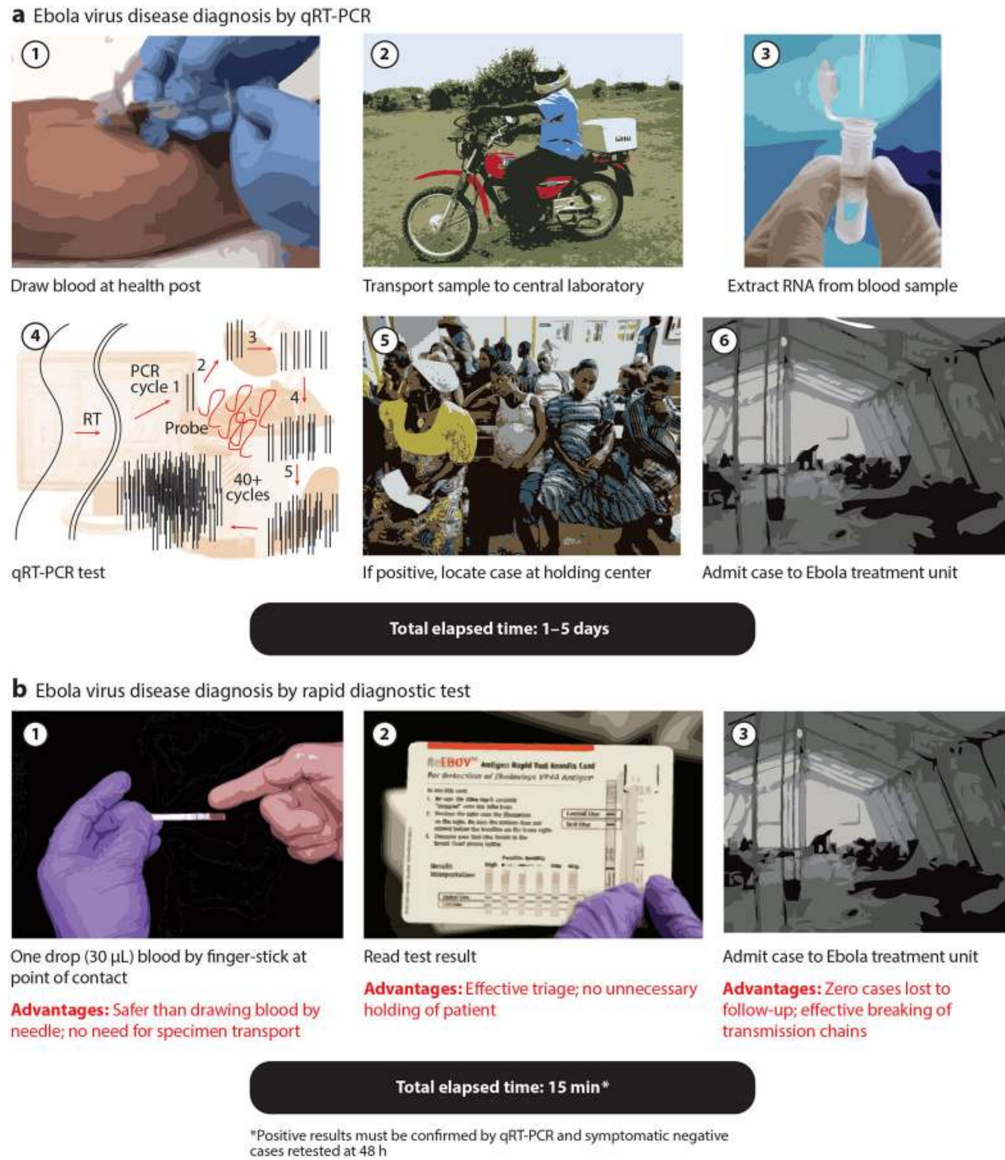

Figure 5.

Comparison of Ebola virus diagnosis using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) and rapid immunodiagnostics. (*a*) Management of the Ebola virus disease outbreak in West Africa relied on diagnosis of the disease in central laboratories by qRT-PCR. (*b*) An alternative strategy involving the use of antigen capture lateral flow immunodiagnostic assays followed by qRT-PCR confirmation could improve case identification and triage of Ebola virus disease patients. Some images have been digitally modified from unattributed photographs.

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Table 1

Ebola therapeutics administered during the outbreak

Therapeutic candidate	Format	Sponsor(s)	Profile
Convalescent blood product	Whole blood or plasma	Not applicable	Delivers matured adaptive immunity
ZMapp	Humanized monoclonal antibody cocktail	Mapp Biopharmaceutical (San Diego, CA)	Produced in tobacco plants and in CHO cells
Brincidofovir (CMX001)	Antiviral prodrug	Chimerix (Durham, NC)	Prodrug of cidofovir
TKM-Ebola	Small interfering RNA	Tekmira (now Arbutus Biopharma; Canada)	Lipid-encapsulated small interfering RNA delivery
Favipiravir (T705, Avigan)	Antiviral drug	Toyama Chemical (Japan)	Pyrazinecarboxamide derivative
Interferon-β	Recombinant protein	Biogen (Cambridge, MA) Bayer HealthCare (Pittsburgh, PA) Merck KGaA (Germany)	Activates host immune response

The WHO prioritized and recommended therapeutic treatment options to undergo clinical trials during the outbreak. Convalescent plasma, brincidofovir, TKM-Ebola, favipiravir, and interferon therapy (not recommended by the WHO) were administered to EVD patients in different countries with varying success. The largest barrier to these studies was the inability to perform randomized controlled trials.

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Table 2

Ebola virus vaccine candidates

Vaccine candidate	Format	Sponsor	Stage
ChAd3-ZEBOV	Recombinant vector derived from ChAd	GlaxoSmithKline (Coraopolis, PA)	Clinical
rVSV-ZEBOV	Recombinant vector based on VSV	NewLink Genetics (Ames, IA) and Merck (Kenilworth, NJ)	Clinical
Ad26-EBOV and MVA-EBOV	Two-dose vaccine, heterologous prime-boost	Crucell (Johnson & Johnson subsidiary; Leiden, Netherlands), Bavarian Nordic (Kvistgaard, Denmark)	Clinical
EBOVGP nanoparticle	Adjuvant vaccine, baculovirus-derived EBOV GP nanoparticles + Matrix M adjuvant	Novavax (Gaithersburg, MD)	Clinical
rVSVN4CT1 EBOV	Recombinant Ad5 viral vector vaccine; replicates incompetent live virus oral vaccine monovalent (Zaire) + VSV vector backbone used in influenza vaccine candidates	Profectus (Baltimore, MD); manufactured by Novasep	Preclinical
VXA ZEBOV-GP	Recombinant VSV vector vaccine; replicates competent live virus oral vaccine monovalent (Zaire) + VSV vector backbone used in HIV-1 vaccine candidates	VaxArt (South San Francisco, CA); manufactured by Lonza	Preclinical
Rabies-EBOV GP	Recombinant rabies virus vector vaccine; replicates a competent live virus vaccine trivalent (Zaire, Sudan, Marburg) + rabies virus vector backbone used in HIV-1 vaccine candidate	Thomas Jefferson University (Philadelphia, PA); manufactured by IDT Biologika	Preclinical

Recombinant vector vaccines (Ad, rVV, and rVSV) are the most prevalent candidates and have had varying degrees of success in preclinical and clinical trials. Of the vaccines undergoing clinical trials, the WHO decided to support a clinical trial in Guinea of rVSV-ZEBOV. Abbreviations: Ad, adenovirus; ChAd, chimpanzee adenovirus; EBOV, Ebola virus; GP, glycoprotein; HIV-1, human immunodeficiency virus type 1; MVA, modified vaccinia virus Ankara; VSV, vesicular stomatitis virus; VV, vaccinia virus.