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2017 Outbreak of Ebola Virus Disease in Northern Democratic Republic of Congo

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Background. In 2017, the Democratic Republic of the Congo (DRC) recorded its eighth Ebola virus disease (EVD) outbreak, approximately 3 years after the previous outbreak.

Methods. Suspect cases of EVD were identified on the basis of clinical and epidemiological information. Reverse transcription–polymerase chain reaction (RT-PCR) analysis or serological testing was used to confirm Ebola virus infection in suspected cases. The causative virus was later sequenced from a RT-PCR–positive individual and assessed using phylogenetic analysis.

Results. Three probable and 5 laboratory-confirmed cases of EVD were recorded between 27 March and 1 July 2017 in the DRC. Fifty percent of cases died from the infection. EVD cases were detected in 4 separate areas, resulting in > 270 contacts monitored. The complete genome of the causative agent, a variant from the *Zaire ebolavirus* species, denoted Ebola virus Muyembe, was obtained using next-generation sequencing. This variant is genetically closest, with 98.73% homology, to the Ebola virus Mayinga variant isolated from the first DRC outbreaks in 1976–1977.

Conclusion. A single spillover event into the human population is responsible for this DRC outbreak. Human-to-human transmission resulted in limited dissemination of the causative agent, a novel Ebola virus variant closely related to the initial Mayinga variant isolated in 1976–1977 in the DRC.

Keywords. Ebola virus; outbreak; virus sequence.

On 12 May 2017, roughly 1 year after the end of the largest Ebola virus (EBOV) disease (EVD) outbreak ever recorded, the Ministry of Health of the Democratic Republic of the Congo (DRC) declared that EVD cases were detected in Likati District, Bas Uele Province, located in northern DRC (Figure 1A). The close proximity of Likati District to the Central African Republic raised concerns that EBOV could again spread across borders. Likati District, which is approximately 1700 km from the capital of the DRC, Kinshasa, is very secluded, and access is limited. To reach Likati, the first 1235 km, from Kinshasa to Kisangani, can be easily covered via a 30-minute plane ride using commercial airlines. In contrast, the remaining 470 km, from Kisangani to Likati, require > 11 hours of road travel. The initial 330 km of this journey takes approximately 5 hours, using standard road vehicles. The remaining 144 km, however, requires the use of motorbikes on dirt roads and can take upwards of 6 hours.

The index case of the eighth-recorded EBOV epidemic in the DRC was a 45-year-old man who started showing clinical symptoms reminiscent of viral hemorrhagic fever disease on 27 March 2017 and died on 6 April. Direct human-to-human transmission led to a single documented chain of EBOV transmission during this short outbreak. Two family members (caregivers) of the index case contracted the disease (1 died on 25 April), probably while transporting him on a motorbike to and from a traditional healer and the local healthcare facilities. The secondary cases transmitted the disease to an additional 5 individuals.

In this article, we report the clinical and epidemiological information related to the 2017 EBOV outbreak in the DRC, as well as the characterization of the causative agent, a novel *Ebolavirus* variant from the *Zaire ebolavirus* species, denoted Ebola virus/H. sapiens-wt/COD/2017/Muyembe.1.

METHODS

Case Definitions

The same case definitions for suspected, probable, and confirmed case of EVD used during the 2014 EBOV outbreak in the DRC were used for this outbreak [1]. Whole-blood specimens, nasal swab specimens, or oral swab specimens were collected from anyone fitting the definition of a suspected case.

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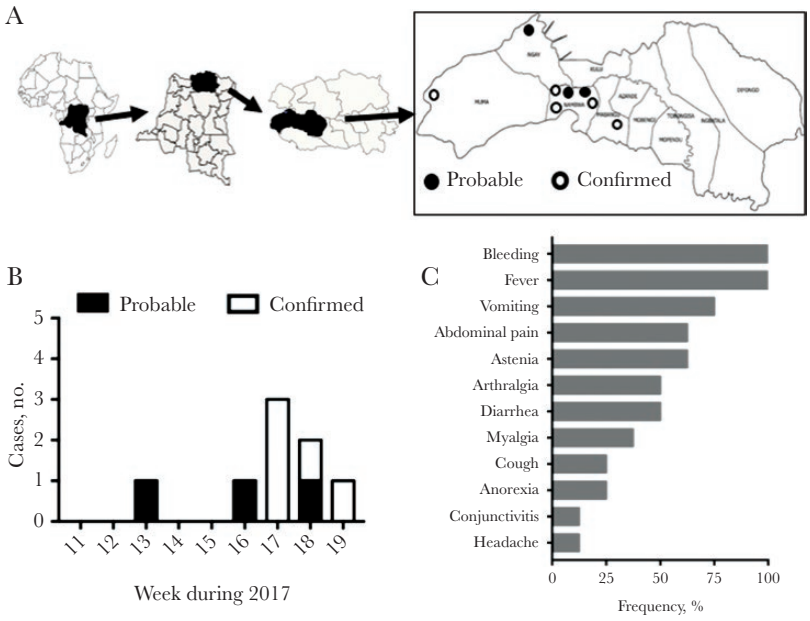

Figure 1. Geographic distribution (*A*), weekly distribution (*B*), and associated symptoms (*C*) of all probable and confirmed cases of Ebola virus disease during the 2017 outbreak in the Democratic Republic of the Congo.

Diagnostic Assays

Viral infection was confirmed in suspected or probable cases by reverse transcription–polymerase chain reaction (RT-PCR) analysis. For diagnostic RT-PCR, total RNA was extracted from 140 µL of whole-blood specimens, oral swab specimens, or nasal swab specimens, using the QIAmp Viral RNA Mini Kit (Qiagen, Toronto, Canada). Levels of EBOV RNA polymerase (L) and nucleoprotein (NP) were detected by RT-PCR analysis, using LightCycler 480 RNA Master Hydrolysis Probes (Roche, Laval, Canada). The primers and probes were ZebovLF, ZebovLR, and ZebovLP2, for the L gene, and GACGASGAGGACACTAAGCC (forward primer), TGGCCCTTTTGACTGTTSTT (reverse primer), and TGCCTAATAGATCR ACCAAGGGTGG (probe), in the 5′ to 3′ orientation, for the NP gene, all of which were previously described [2–4]. When possible, repeat testing of symptomatic patients was performed approximately 3 days after a negative RT-PCR result, for confirmation.

RT-PCR–negative samples, collected from suspected or probable cases >10 days from the reported onset of disease, were also screened using a serological assay. Briefly, each well was coated with 0.1 µg of EBOV antigen (IBT Biosciences, Gaithersburg, MD). Milk diluent/blocking solution (Mandel Scientific, Guelph, Canada) was used for blocking, as well as for sample and secondary antibody dilution. Samples were revealed using ABTS Substrate (Mandel Scientific). Wells were washed extensively between steps. Any RT-PCR–negative individual was considered confirmed as recovering from EBOV infection (ie, a survivor) if (1) both immunoglobulin M (IgM) and immunoglobulin G (IgG) responses against EBOV were detectable; (2) IgG but no IgM antibodies against EBOV were detected in samples collected > 2–3 months after onset of disease, and symptoms and epidemiological information matched those of EBOV infection; or (3) EBOV-specific IgM antibodies could not be tested but a strong and/or rising IgG response was detected shortly after symptom onset, and the clinical presentation and epidemiological information were compatible with EBOV infection.

Viral Sequencing

RNA extracted from individuals who tested positive for EBOV by quantitative RT-PCR was sequenced. Ten microliters of RNA extracted by a Qiagen kit was treated with the Turbo DNA-free kit (Thermo Scientific, Waltham, MA). DNAse treatment was followed by an RNAeasy MinElute Cleanup kit (Qiagen), and complementary DNA (cDNA) synthesis was completed using the Maxima H Minus Double-Stranded cDNA synthesis kit (Thermo Scientific). Remaining RNA was removed by RNAse-I. cDNA purification was performed using Agencourt Ampure XP Beads (Beckman coulter, Mississauga, Ontario) and the resulting cDNA was resuspended in 40 µL of sterile water. Subsequently, cDNA was quantified by a Qubit fluorometer (Thermo Fisher) and the 2200 TapeStation device (Agilent, Santa Clara, CA). The cDNA library was prepared for deep-sequencing analysis, using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) according to the manufacturer's instructions. The library was quantified as mentioned above to determine double-stranded DNA quality and the distribution of fragment lengths. Standard normalization was made to 2 nM to obtain a final concentration of 20 pM. DNA was sequenced using a MiSeq Sequencer (Illumina). De

novo assembly of the EBOV genome was performed using Ray 2.3.1, and a k-mer length of 31 [5]. The sequence provided was obtained as a single contig.

Phylogenetic Analysis

The sequence of the 2017 *Ebolavirus* variant and 30 complete genomes from the 5 *Ebolavirus* species, representing variants from a wide variety of geographical locations and isolated between 1976 and 2017 were included in the analysis. Multiple genome alignment was performed using the MUSCLE algorithm within the MEGA7 software package [6]. Phylogenetic analysis was done using maximum likelihood and a bootstrap of 100. All positions containing gaps and missing data were excluded from the analysis. As a result, 16 780 nucleotides were analyzed.

RESULTS

Clinical and Epidemiological Data

Between 27 March and 1 July 2017, 8 EVD cases, of which 5 were confirmed and 3 were probable, were reported as part of the eighth EVD outbreak in the DRC (Figure 1). The index case started showing symptoms on 27 March and died of infection of 6 April. An alert was received on 7 April by the DRC minister of health from a local nurse in Likati regarding a possible case (case 1) of EBOV infection. The causative agent of the outbreak was confirmed on 11 May by the DRC national reference laboratory (Institut National de Recherche Biomedicale). A team of experts and a mobile laboratory arrived at the epicenter of the epidemic on 15 and 17 May, respectively, roughly 5 weeks after the death of the index case. A total of 62.5% of cases (5 of 8) originated from the Nambwa area within Likati District. The remaining 3 cases occurred in 3 separate districts, namely Mabango, Muma, and Ngay. Several logistical challenges were associated with the management of EBOV cases in 4 separate locations. Initially, a rapid assessment of the geolocation of cases and contacts, response capacities, and risks of disease spread informed the deployment and coordination of an initial rapid response team. An Ebola treatment center, a portable laboratory, and a larger multidisciplinary team were positioned in Likati within the largest populated area of the region. Subsequently, subcoordination focal points were located in 3 additional locations, Nambwa, Muma, and Ngay, within Likati. Subcoordination teams were responsible for implementing the outbreak response, including close monitoring and reporting on the activities previously adopted by the central coordination team, based in Likati. Every sample collected throughout the outbreak was transported to Likati, where the diagnostic laboratory processed them within 3 hours of reception. In the absence of other communication means (including a cellular network), communication between subcoordination teams and the central coordination in Likati could only done by satellite device between the subcoordination teams and, whenever possible, occurred 3 times daily, at 7:30 AM, 12:30 PM, and 5:30 PM.

A total of 277 contacts linked to confirmed or probable cases were identified and followed for 21 days. Of these, 97 developed nonspecific clinical symptoms that could have been associated with EVD. However, only 4 received a diagnosis of EVD, with the remaining 93 individuals testing negative by RT-PCR and serological analyses (see Methods for the diagnostic laboratory algorithm). Of note, a novel serological assay protocol was used during this outbreak. The assay demonstrated robust performance in the field, with a low background (<0.3 OD at the lowest sera dilution of 1:100) and a sensitivity capable of detecting EBOV-specific IgG in serum samples diluted to up to 1:25 600 in challenging field conditions. Three cases of EVD, in individuals 2, 4, and 5, were confirmed on the basis of this novel serological assay and epidemiological findings (Figure 2). Of 16 tested suspected cases selected out of 97 with a strong epidemiological link to confirmed or probable cases, EBOV-specific antibodies (IgG) were present in only 3 (18.7%). The low prevalence of a humoral response to EBOV also suggests that this population may have been largely immunologically naive to EBOV. Indeed, 12 local individuals with no known epidemiological link to this outbreak who were used as baseline controls tested negative for EBOV, with ODs <0.3 at the lowest serum dilution tested of 1:100.

The overall case-fatality rate (50% [4 of 8 patients]) documented from this outbreak was higher than in the 2014–2016 EVD in West Africa (approximately 39.5%), but lower than in the 2014 DRC outbreak in Lokolia Parish, Boende District (49/69; 71%) [1, 7]. The median time (±SD) between reported symptom onset and death was 7.5 ± 2.4 days. Fever and hemorrhages (include at least 1 of the following: epistaxis, gingival bleeding, subconjunctival hemorrhage, hematochezia, hematemesis, petechiae, and large hematomas) were detected in all cases, while vomiting (detected in 75% of cases), abdominal pain (in 62.5%), and asthenia (in 62.5%) were prevalent in many but not all infected individuals. Arthralgia, diarrhea, and myalgia were also reported in ≤50% of cases (Figure 1C).

The field epidemiological investigation concluded that the index case was infected through close contact with bush meat. A hunter brought back carcasses of a wild boar and a nonhuman primate to the village of the index case. The index case butchered the wild boar, while the nonhuman primate was brought to and sold at the local market. It was not possible to recover samples from either animal to confirm the presence of EBOV RNA. Following the original single zoonotic transmission, limited human-to-human transmission occurred, mainly through relatives caring for sick individuals (Figure 2), most of whom (6 of 8) in this outbreak were male (Table 1).

Characterization of the *Ebolavirus* Variant

To better situate and characterize the variant responsible for this EVD outbreak, blood samples from case 7 (Figure 2), which demonstrated the highest level of viral genomic RNA by RT-PCR

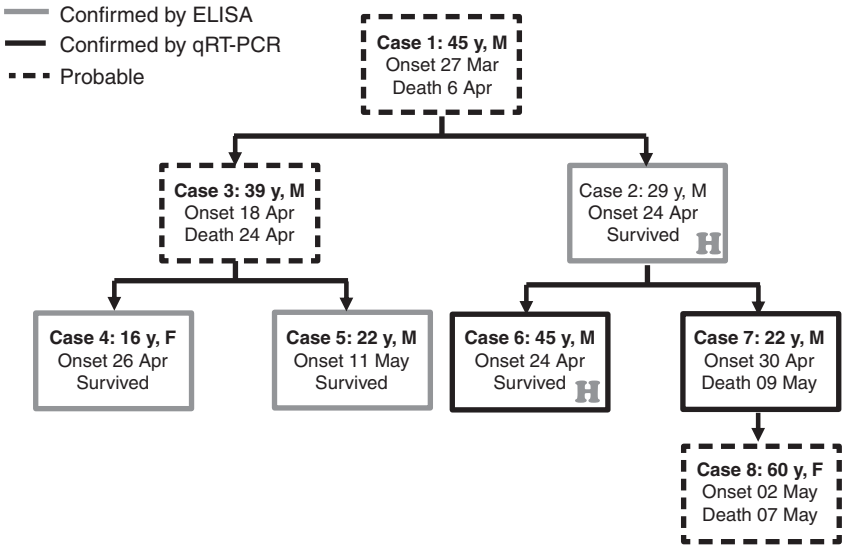

Figure 2. Chain of Ebola virus disease transmission during the 2017 outbreak. Probable cases are represented by dotted lines, whereas cases confirmed by quantitative reverse transcription–polymerase chain reaction (qRT-PCR) analysis or enzyme-linked immunosorbent assay (ELISA) are in black and gray solid lines, respectively. Case number, age, sex, date of symptom onset, and time of death (when relevant) are indicated. F, female; M, male; H, nosocomial transmission.

(cycle thresholds, 24.86 and 27.75 for EBOV L and NP, respectively), were analyzed by next-generation sequencing. A complete genome of 18 899 nucleotides was obtained and named Ebola virus/H.sapiens-wt/COD/2017/Muyembe.1 according to the standard filovirus nomenclature (GenBank accession number MH613311) [8], where "COD" denotes the DRC. Phylogenetic comparative analyses with *Zaire ebolavirus* variants from previous outbreaks, as well as variants from the 4 other *Ebolavirus* species, were performed. The analyses confirmed that the strain responsible for this outbreak belonged to *Zaire ebolavirus* (Figure 3). Interestingly, the 2017 variant was most closely related (98.73% homology) to the first EBOV isolated from the 1976 outbreak in Yambuku, DRC (Mayinga) and from a single EBOV case from the 1977 outbreak in Bonduni, DRC (GenBank accession numbers NC_002549.1 and KC242791.1). A total of 213 mutations were noted between EBOV/Muyembe.1 and its 1977 counterpart. Most occurred within the L, glycoprotein, and NP genes, with 68, 37, and 29 mutations, respectively. Whether any of these mutations affect the virulence or transmission efficiency of EBOV/Muyembe.1 will require further investigation.

Of note, high sequence homology of 98.47 was also observed between the EBOV/Muyembe.1 variant and additional complete genome sequences from the 1995 (Kikwit) and 2014 (Lomela) EBOV outbreaks in the DRC (GenBank accession numbers KP271020.1, KP271018.1, KC242796.1, and AY354458.1). Overall, the phylogenetic analysis suggests that the EBOV/Muyembe.1 isolate is a local variant that has been circulating in the DRC for at least the past 30 years.

DISCUSSION

In April 2017, the DRC recorded is eighth EVD outbreak. This outbreak was limited, with only 8 cases detected. These cases were confirmed on the basis of virus detection by RT-PCR analysis or EBOV-specific antibody detection by serological analysis during an epidemiological investigation of person, place, and time data. To our knowledge, it is the first EBOV outbreak in which cases have been identified in real time at the site of the outbreak, based on serological data in concordance with epidemiological findings.

No large viral amplification was detected in humans, with infected individuals transmitting the disease to ≤2 individuals. Whether differences in human genetic factors, infectious dose and mode of infection, virulence, and transmission efficiency between this variant and other *Ebolavirus* variants from the

Table 1. Characteristics of 8 Patients With Ebola Virus Disease (EVD), by Location

Location	Confirmed EVD, No. (%)	Probable EVD, No. (%)	Male Sex, No. (%)	Died, No. (%)
Mabango	1 (12.5)	0	0	0
Muma	1 (12.5)	0	1 (12.5)	1 (12.5)
Nambwa	3 (27.5)	2 (25)	5 (62.5)	2 (25)
Ngay	0	1 (12.5)	0	1 (12.5)
Overall	5 (62.5)	3 (37.5)	6 (75)	4 (50)

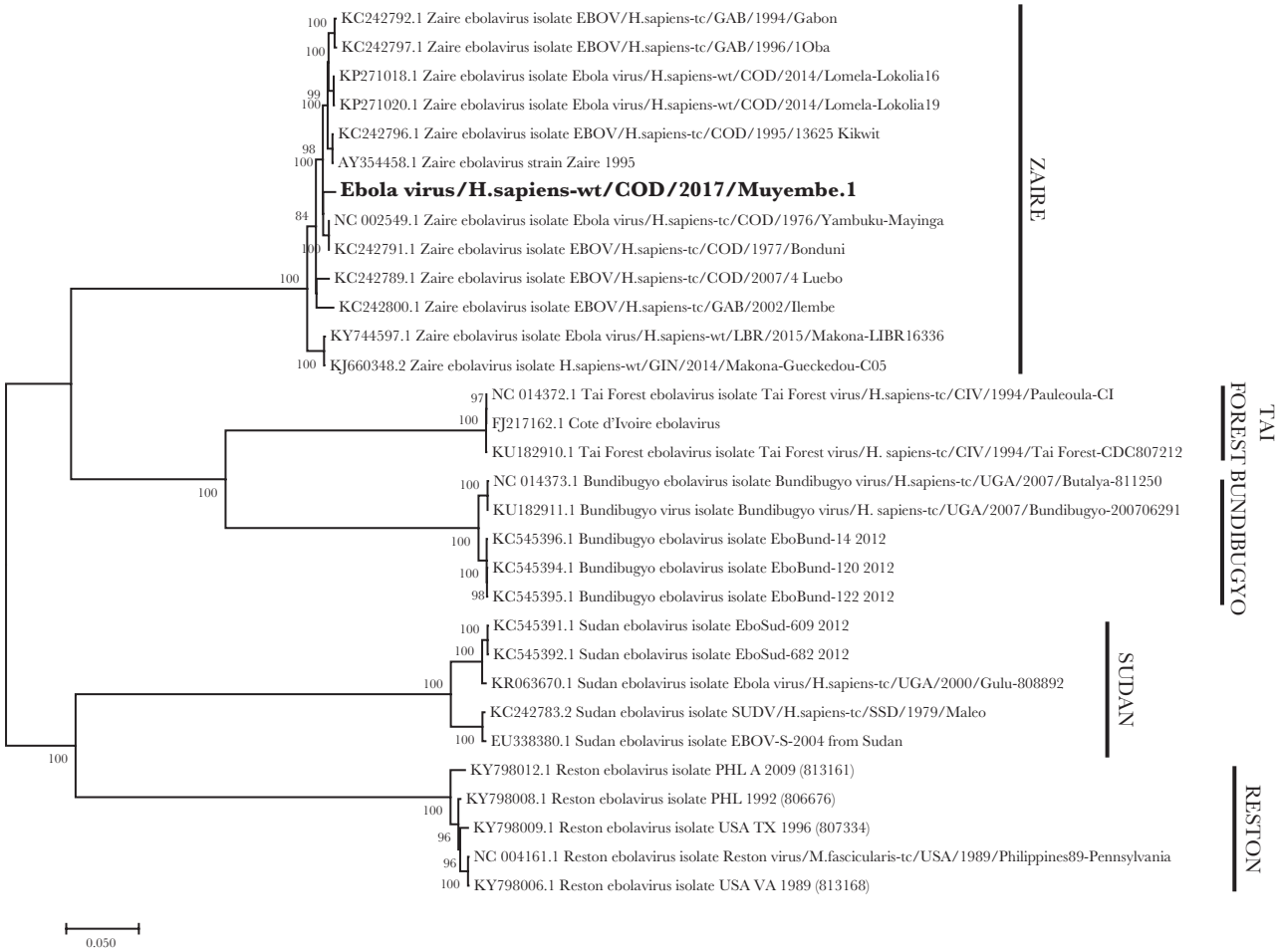

Figure 3. Phylogenetic analysis of the 2017 *Ebolavirus* variant. Phylogenetic analysis of the obtained sequence (Ebola virus/H.sapiens-wt/COD/2017/Muyembe-1, highlighted in bold) and 30 additional sequences from the 5 different *Ebolavirus* species, denoted by vertical bars, was generated on the basis of the Tamura-Nei model. The tree with the highest log likelihood is shown.

Zaire ebolavirus species are responsible for the difference in outbreak size and case-fatality rate will require further investigation. The 98.73% homology between this variant and the closest EBOV variant (Mayinga) surpasses previous variations between other *Ebolavirus* variants (eg, Mayinga and Kikwit) and therefore supports the recognition of this novel variant with the name proposed here: "EBOV/Muyembe.1." This genetic variant was named after Prof Jean-Jacques Muyembe-Tamfum (with his permission), who was part of the team that investigated the first documented EVD outbreak in 1976 and has since been instrumental in the fight against this virus in Central Africa. Owing to the limited number of cases detected during this outbreak, no conclusion on EBOV/Muyembe.1's viral properties can be drawn. However, phylogenetic analysis indicates that the EBOV/Muyembe.1 isolate is an ancient strain that has been present in the DRC since at least 1976, suggesting that the efficacy of the local outbreak response, rather than differences in viral fitness, may have been responsible for the limited outbreak observed. Past outbreaks have mostly been detected and confirmed >3 months after the death of the index case, thus allowing the virus to spread undetected for a longer period and, consequently, making outbreak control more challenging. In contrast, during this outbreak, the confirmation of the etiological agent was obtained on 11 May, roughly 6 weeks after the index cases developed clinical symptoms.

The testing of 158 samples (blood specimens, oral and nasal swab specimens, or sperm specimens) from the 97 potential contacts who developed nonspecific clinical symptoms within the span of a little more than 2 months demonstrates the overall immediate and robust mobilization deployed to control and stop this outbreak. One of the most critical lessons learned from this outbreak was the importance of the education of frontline healthcare workers and the local population regarding hemorrhagic fever disease presentation. During this outbreak, the alert was first raised by a nurse from the Nambwa health facility, located > 1700 km from the capital, Kinshasa. This nurse participated in a local training program on the recognition of EVD cases in June 2016, which helped her rapidly identify an initial

EVD case. In addition, on 9 May, because of an unusual death and similar cases in a close community, relatives implemented ad hoc the protected burial, which included decontamination of some personal belongings.

These examples highlight the need and the usefulness of training healthcare professional and sensitizing local communities in remote rural areas where outbreaks are likely to occur.

Retrospectively, whether the scale of the outbreak response was disproportionate for the size of the 2017 DRC outbreak is a point of discussion. However, multiple international agencies in charge of infectious disease outbreak response have adopted a no-regret policy to prevent additional public health events of international concern, as witnessed during the 2014–2016 Ebola outbreak in West Africa.

This is the second EVD epidemic, including the 2014 outbreak, that the DRC successfully managed and confined to limited local transmission. These successes suggest that a quick international mobilization in combination with a strong local leadership and outbreak management system are critical for swift containment of emerging-pathogen outbreaks in low- and middle-income countries that could face similar events. It is worth pointing out that, since the 2017 outbreak, 2 EBOV outbreaks have occurred in the DRC. The first took place between May and July 2018 in Equateur Province and claimed the lives of 33 of 54 infected individuals [9]. The second outbreak, which, at the time of writing, is ongoing in North Kivu, has been raging since July 2018. To date, > 620 individuals have been infected, with 377 dying from infection, making the North Kivu outbreak the largest ever recorded in the DRC [10, 11].

Finally, the findings described in this article have important implications with regard to the genetic stability of EBOV in the environment over decades, and they highlight the importance of understanding the natural evolution of filoviruses and the selection pressure at play that promote the jump of specific variants into humans.

Notes

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