

1 Title: Ebola virus epidemiology and evolution in Nigeria

2 Running title: Nigeria EBOV epidemiology and evolution

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4 Onikepe A. Folarin^{1,2*}, Deborah Ehichioya^{1,2*}, Stephen F. Schaffner^{3,4*}, Sarah M. Winnicki^{3,4*},

5 Shirlee Wohl^{3,4*}, Philomena Eromon^{1,2}, Kendra L. West³, Adrienne Gladden-Young³, Nicholas

6 E. Oyejide¹, Christian B. Matranga³, Awa Bineta Deme⁵, Ayorinde James⁶, Christopher

7 Tomkins-Tinch³, Kenneth Onyewurunwa^{1,2}, Jason T. Ladner⁷, Gustavo Palacios⁷, Dolo

8 Nosamiefan³, Kristian G. Andersen⁸, Sunday Omilabu⁹, Daniel J. Park^{2,3}, Nathan L. Yozwiak^{2,3,}

9 ⁴, Abdusallam Nasidi¹⁰, Robert F. Garry^{2,11}, Oyewale Tomori^{1,12}, Pardis C. Sabeti^{2,3,4,13}, Christian

10 T. Happi^{1,2}

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1 **Footnotes**

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3 *Contributed equally to this work.

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5 ¹ Department of Biological Sciences, Redeemer's University, Ede, Osun State, Nigeria

6 ² African Center of Excellence for Genomics of Infectious Diseases, Redeemer's University,

7 Ede, Osun State, Nigeria

8 ³ Broad Institute of Harvard and MIT, Cambridge, MA 02142 USA

9 ⁴ FAS Center for Systems Biology, Department of Organismic and Evolutionary Biology,

10 Harvard University, Cambridge, MA 02138 USA

11 ⁵ Department de Parasitologie et Mycologie, Université Cheikh Anta Diop de Dakar, Fann,

12 Dakar, Senegal

13 ⁶ Department of Biochemistry, Lagos University Teaching Hospital, Lagos, Nigeria

14 ⁷ Center for Genome Sciences, US Army Medical Research Institute of Infectious Diseases,

15 Frederick, MD 21702 USA

16 ⁸ The Scripps Research Institute, Scripps Translational Science Institute, La Jolla, CA 92037

17 USA

18 ⁹ Department of Medical Microbiology and Parasitology, College of Medicine, University of

19 Lagos, Lagos, Nigeria

20 ¹⁰ Nigeria Centre for Disease Control, Abuja, Nigeria

21 ¹¹ Department of Microbiology and Immunology, Tulane University, New Orleans, LA 70118

22 USA

23 ¹² Nigerian Academy of Science, Akoka-Yaba, Lagos, Nigeria

24 ¹³ Howard Hughes Medical Institute, Chevy Chase, MD 20815 USA

25

26 Correspondence should be addressed to:

27

28 Christian Happi

29 Redeemer's University

30 Ede, Osun State, Nigeria

31 Tel: +234-802-338-3684

32 E-mail: happic@run.edu.ng

33

34 Stephen F. Schaffner

35 Broad Institute of Harvard and MIT

36 Cambridge, MA 02142

37 Tel: 617-714-7634

38 E-mail: sfs@broadinstitute.org

39

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2 Abstract

3 Containment limited the 2014 Nigerian Ebola virus disease outbreak to 20 reported cases and 8
4 fatalities. We present here clinical data and contact information for at least 19 cases, and full-
5 length Ebola virus (EBOV) genome sequences for 12 of the 20. The detailed contact data permits
6 nearly complete reconstruction of the transmission tree for the outbreak. The EBOV genomic
7 data is consistent with that tree. It confirms that there was a single source for the Nigerian
8 infections, shows that the Nigerian EBOV lineage nests within a lineage previously seen in
9 Liberia but is genetically distinct from it, and supports the conclusion that transmission from
10 Nigeria to elsewhere did not occur.

11 *Key words: Ebola, genomic, phylogeny, epidemiology, Nigeria, sequencing, outbreak*

12 Introduction

13 The 2014 outbreak of Ebola virus disease (EVD) in Nigeria was one branch of the major West
14 African epidemic that spanned 2013-2016. As of 13 March 2016, 28,639 EVD cases and 11,316
15 deaths have been reported in 10 countries. The majority of EVD burden has occurred in Liberia,
16 Sierra Leone, and Guinea, with exported cases responsible for additional transmissions in the
17 United States, Mali, and Nigeria, and diagnosed cases with no transmissions in the United
18 Kingdom, Italy, Senegal, and Spain (1).

19

20 The Nigeria EVD outbreak began on 20 July 2014, when a traveller from Liberia (the index
21 case) infected with Ebola virus (EBOV), arrived by commercial aircraft to Murtala Muhammed
22 International Airport in Lagos. The movement of this traveller was quickly restricted, patient
23 samples were confirmed EBOV positive by independent PCR tests within days, and intensive

1 contact tracing was conducted. The Nigeria EVD outbreak ended on 20 October 2014, when the
2 country was declared Ebola-free by the World Health Organization. During that period, 20
3 individuals are reported to have been infected, of whom 8 died.

4
5 Despite emerging in the megacity of Lagos, the Nigeria EVD outbreak was well documented and
6 well contained because of rapid detection of the index case and thorough contact tracing
7 throughout the outbreak. Contact tracing provides a detailed understanding of viral spread, which
8 is key to controlling any viral outbreak. Sequencing of patient samples can also be used to
9 understand transmission routes, and is especially important in cases where contact tracing is not
10 available, or when contact tracing cannot completely resolve a transmission chain.

11 The EVD outbreak in Nigeria is unique because both genetic and contact tracing data are
12 available. The complete transmission chain could be reconstructed with considerable confidence,
13 and detailed clinical records were available for most patients. Viral sequencing data and
14 sampling dates can be used to estimate general transmission patterns between patients and
15 regions, and are used in this case to confirm and inform the transmission chain suggested by
16 contact tracing. Comparing the two methods highlights the strengths of each, and the importance
17 of both contact tracing and genomic sequencing during an outbreak.

18
19 We present here an account of the Nigeria 2014 EVD outbreak that includes clinical,
20 epidemiological and viral sequence data for most of the affected patients. We also describe
21 sequencing results generated in Nigeria and in duplicate in the U.S. for the purposes of both
22 outbreak investigation and validation of viral sequencing capabilities in new laboratories.

23

1 **Materials and Methods**

2

3 **Management of Contacts and Cases of EVD**

4 The index case presented to a private hospital in Lagos on 20 July 2014 with fever and body
5 weakness, denied contact with known EVD cases or funeral attendance, and was treated with
6 anti-malarial drugs and analgesics. Over the next 3 days the patient's condition worsened (fever
7 escalated, vomiting and diarrhea persisted), and EVD was suspected. Filovirus PCR testing was
8 conducted at Lagos University Teaching Hospital (LUTH), and on 23 July the index case was
9 reported as filovirus positive. Samples were then shared with Redeemer's University (RUN) for
10 EBOV-specific PCR testing, which was confirmed on 25 July 2014. The index patient died on 25
11 July 2014 (see Case Supplement and Supplementary Data 1).

12

13 All persons who were exposed to the index case and their contacts were traced, placed under
14 surveillance and monitored for clinical features of EVD. If contacts exhibited fever or other
15 symptoms, they were admitted into the Ebola Treatment Centre (ETC) as suspected cases; blood
16 samples were then collected and tested by RT-PCR for presence of EBOV at both LUTH and
17 RUN. Those positive by RT-PCR were moved to the confirmed ward of the ETC. This
18 combination — history of contact with an EVD case, presentation with symptoms, and RT-PCR
19 evidence of EBOV infection — defined a confirmed case. Each patient was counselled on their
20 need for at least 4 litres of oral rehydration solution (ORS) daily. They were also placed on
21 antibiotics because of their immunosuppression and antimalarials due to the endemicity of
22 malaria in Nigeria. They were placed on nutritional supplements and vitamins. The only

1 analgesic administered was paracetamol. Injectables and invasive procedures were avoided
2 unless patients were too ill or weak to take ORS.

3

4 Infection prevention and control procedures and protocols were strictly adhered to in patient
5 management. Prior to discharge, patients were confirmed negative for EVD by RT-PCR. When
6 discharged, they were decontaminated before being allowed to leave the ETC and were not
7 allowed to take clothing or other personal items. Replacement clothes, footwear and basic
8 personal effects were provided by family or the ETC depending on each individual's
9 circumstances.

10

11 **Data Collection and Review**

12 ETC-case management, clinical data, and laboratory data of all confirmed EVD cases identified
13 during 20 July–30 September were reviewed by qualified medical professionals in the case
14 management team. The following case data were compiled: socio-demographic (age, sex,
15 occupation, city of residence), clinical (respiratory rate, pulse rate, blood pressure, presenting
16 symptoms, signs, syndromes, outcome), laboratory (RT-PCR) and administrative data (date of
17 symptoms onset, duration of symptoms, length of stay (LOS)).

18

19 Each patient's exposure history, presenting symptoms, history of presenting symptoms, course of
20 illness, excerpts of clinical management and illness outcome were abstracted from their medical
21 records or contact tracing interview notes (including Suspect Evacuation Forms, Case
22 Investigation Forms, Laboratory Request and Report Forms, Clinical Notes and Charts, and
23 Contact Tracing Interview Notes) and summarised as case histories.

1

2 Sample collection and processing

3 Suspected EVD patient samples were shipped both to the Virology laboratory at LUTH for
4 diagnostics and to the African Center of Excellence for Genomics of Infectious Diseases
5 (ACEGID) at RUN for diagnostics and sequencing. Whole blood samples shipped to RUN were
6 inactivated using Buffer AVL (Qiagen) or TRIzol LS (Life Technologies) in a 4:1 ratio, both
7 following the manufacturer's protocol. Inactivated samples were stored in a -20°C freezer.
8 Buffer AVL and TRIzol LS have been used extensively in virus inactivation including EBOV (2-
9 7).. Samples inactivated in Buffer AVL were extracted using the QIAamp Viral RNA Mini Kit
10 extraction protocol (Qiagen) according to manufacturer's protocol. Samples inactivated in
11 TRIzol were extracted using chloroform modified with an AVL inactivation and QIAamp Viral
12 RNA Mini Kit extraction protocol. Following this modified protocol, 140 µL of chloroform was
13 added to 1 mL of TRIzol inactivated sample. After vortex and centrifugation, 200 µL of the
14 aqueous phase was transferred to a tube with 700 µL of AVL without carrier RNA added. The
15 sample was then processed following the manufacturer's protocol for extraction using the
16 QIAamp Viral RNA Mini Kit. Extracted RNA samples were divided into aliquots for sequencing
17 at both RUN and the Broad Institute of MIT and Harvard (Broad). Samples destined for the
18 Broad were shipped on dry ice and subsequently stored at -80°C.

19

20 Diagnostics performed at RUN

21 EBOV-specific diagnostic tests were performed on the suspected EBOV samples at RUN with
22 RT-PCR using the SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity
23 DNA Polymerase (Life Technologies). The 25 µL assay mix included 5 µL RNA, KGH primer

1 set (2) at 250 nM final concentration (fwd: GTC GTT CCA ACA ATC GAG CG, rvs: CGT CCC
2 GTA GCT TTR GCC AT), 12.5 μ L 2x Reaction Mix and 0.5 μ L 0.5 μ L SuperScript™ III RT/
3 Platinum® Taq High Fidelity Enzyme Mix. The cycling conditions were 60° C for 20 min and
4 94° C for 5 min, followed by 35 cycles of 94° C for 15 sec, 58° C for 15 sec and 68° C for 15 sec
5 with a final extension at 68° C for 2 min. RT-PCR was performed on an Eppendorf Mastercycler
6 thermocycler. The samples were run on a 1.5% agarose gel and visual results recorded.

7

8 **qRT-PCRs performed at RUN and the Broad**

9 To assess sample quality, extracted RNA was quantified using qRT-PCR for both EBOV and
10 human rRNA (18S). RNA selected for sequencing was quantified using the Power SYBR Green
11 RNA-to-Ct 1-Step qRT-PCR assay (Life Technologies). The *Kulesh* assay protocol was adapted
12 from a probe-based qPCR assay to a SYBR qPCR assay by omitting the probe (8). The 10 μ L
13 assay mix included 3 μ L RNA, 0.3 μ M primer *Kulesh fwd* (TCT GAC ATG GAT TAC CAC
14 AAG ATC), 0.3 μ M *Kulesh rv* (GGA TGA CTC TTT GCC GAA CAA TC), 5 μ L 2x Power
15 SYBR Green RT-PCR Mix and 0.08 μ L RT Enzyme Mix. The cycling conditions were 48° C for
16 30 min and 95° C for 10 min, followed by 45 cycles of 95° C for 15 sec and 60° C for 30 sec
17 with a melt curve of 95° C for 15 sec, 55° C for 15 sec and 95° C for 15 sec. qRT-PCR was
18 performed on the LightCycler 96 (Roche) instrument at both RUN and the Broad. Synthetic
19 oligonucleotide amplicons were prepared as a standard to quantify the viral copy number in the
20 qRT-PCR assays. These amplicons represent a portion of the EBOV segment within the L gene
21 as a template for PCR. The amplicons were cleaned using AMPure XP beads (Beckman Coulter
22 Genomics) and quantified by TapeStation (Ambion). Amplicon concentrations were converted to
23 EBOV copies per microliter for quantification.

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RNA processing and library preparation

DNA was depleted from the RNA samples using TURBO DNase (Ambion), then host rRNA was depleted from the samples using an RNase H selective depletion method described previously (2,9-10). cDNA was then synthesized from the resulting depleted RNA, Nextera XT libraries were constructed and Illumina sequencing was carried out according to methods described previously (2,11), with the following modification: Nextera libraries were generated using 16-18 cycles of PCR. At RUN samples were sequenced on the MiSeq, while at the Broad samples were sequenced on both the MiSeq and HiSeq2500 platforms (Illumina).

Ebola virus genome assembly and analysis

Raw sequencing reads from all sequencing runs were processed together and assembled using the viral-ngs pipeline (12,13) with mostly default parameters. Reads from 2 flowcells were not included due to suspected contamination. Two parameters were varied from defaults: the minimum length of assembly (expressed as a fraction of the reference genome length) and minimum fraction of unambiguous bases were both decreased to allow assembly of lower quality samples (assembly_min_length_fraction_of_reference=0.8; assembly_min_unambig=0.7).

Consensus variants were called using a custom pipeline and annotated using SnpEff (14). Multiple alignments were done using MAFFT v7.017 (15,16) with default parameters. Within-host variants were identified as part of the viral-ngs pipeline with default minimum read and strand bias filters.

1 The maximum likelihood tree was made using IQ-TREE v1.3.13 (17), a TIM+I substitution
 2 model selected by ModelFinder (implemented in IQ-TREE), and 1000 bootstrap replicates.
 3 Liberian EBOV sequences included all genomes publicly available on GenBank as of 17
 4 February 2016 (Supplementary Data 3).

5

6 **Data analysis**

7 As noted in the Discussion, new SNPs were observed to be clustered, with 6 SNPs appearing in
 8 one sample, 2 in another and zero in the remaining 9 samples. To determine whether this was
 9 unlikely given a uniform mutation rate per transmission, a p-value was calculated as follows.
 10 From the transmission tree, the sequenced cases represent a minimum of 11 transmissions from
 11 the index case. Assume that new SNPs in a transmission occur in a Poisson process at an
 12 unknown rate μ_s . For a given μ_s , calculate the probability of seeing 4 new SNPs in at least 1
 13 case, and then integrate over all values of μ_s , weighting by the probability of observing 6 SNPs in
 14 11 transmissions. That is,

15

$$p = \frac{\int p(S_t = 6|\mu_s)(1 - p(S_s < 4|\mu_s)^{N_t})d\mu_s}{\int p(S_t = 6|\mu_s) d\mu_s}$$

16

17 where S_t is the total number of new SNPs, S_s is the number of new SNPs seen in a single case,
 18 and N_t is the number of transmissions. The first probability is the Poisson pdf, $p(S_t|\mu_s) =$

19 $\frac{(\mu_s N_t)^{S_t} e^{-\mu_s N_t}}{S_t!}$, and the second is the cdf: $e^{-\mu_s} \sum_{i=0}^{N_t-1} \left(\frac{\mu_s^i}{i!}\right)$

20

21 **Results**

1

2 Clinical data

3 Available metadata on the Nigerian EVD patients is summarized in Supplementary Data 1, while
4 symptoms and outcome for all 20 are summarized in Supplementary Tables 1 and 2. Among the
5 20 EVD cases, the median age was 33 years (range: 26-62 years); 55% were female. Most (65%)
6 were less than 40 years of age, and most were health workers (65%). At presentation, the most
7 common symptoms were fever (85%), fatigue (70%) and diarrhea (65%). The pulse rate and
8 blood pressure were within normal range in 50% of the patients; however, the respiratory rate
9 was elevated in 90% of the cases with available data. The common clinical syndromes
10 documented were gastroenteritis (45%), haemorrhage (30%) and encephalopathy (15%). Of 20
11 cases, 12 (60%) survived, with 1 having post illness mental health complication requiring
12 follow-up. The average duration from onset of symptoms to presentation at the ETC was 3 ± 2
13 days among survivors, compared to 5 ± 2 days for non-survivors. The mean duration from
14 symptom onset to death or discharge from the ETC was 15 ± 5 days for survivors and 11 ± 2 days
15 for non-survivors.

16

17 Sequencing data

18 We prepared 16 samples from 13 of the 20 confirmed cases and discharge samples for 3 of these
19 cases. This includes case #9, which could not be confidently matched to a sample (suspected
20 match to E030). We prepared an additional 16 samples from suspected cases where the sample
21 could not be clearly associated with a particular case because of incomplete records. Dates and
22 RT-qPCR results for each of these samples are reported in Supplementary Data 1. Because this
23 table includes re-tested and discharge samples, as well as incomplete information collated many

1 months after the outbreak, we were not able to confirm that there were exactly 20 EVD cases in
2 Nigeria. Following inactivation and extraction at RUN, we divided RNA from each sample into
3 2 aliquots for independent library preparation and sequencing at RUN and the Broad. Extracted
4 RNA samples contained an average of 3.97×10^6 18S copies/mL (range: 3.28×10^4 – 2.31×10^7
5 copies/mL) as determined by qRT-PCR.

6
7 We prepared Nextera libraries for all 32 of the samples. Using the Kulesh qRT-PCR assay, we
8 detected EBOV RNA in 18 of the 32 samples, including 2 discharge samples and 3 samples
9 unassociated with a particular case. After library construction, we used Kulesh qPCR to detect
10 the presence of any EBOV copies in the libraries. Based on the results, we sequenced 23 samples
11 using a combination of the MiSeq and HiSeq 2500 platforms (Illumina). We were able to
12 generate assembled EBOV genomes from 12 of these samples, all from confirmed EVD cases
13 with associated case histories. We combined the MiSeq and HiSeq sequencing data from RUN
14 and the Broad for analysis. The median sequencing coverage was 225.5x (range: 6 – 4864x)
15 (Table 1). Although we recorded combined sequencing data, the MiSeq data from RUN
16 separately confirmed EBOV reads in 6 of the 12 samples with assembled EBOV genomes.

17

18 **Consensus and within-host variants**

19 We identified 17 consensus-level variants (9 synonymous, 5 non-synonymous, 3 non-coding, all
20 relative to the earliest EBOV sequence from the West African outbreak (accession number
21 KJ660346.2)) in EBOV genomes from the 12 sequencing-positive Nigerian samples (Table 2).
22 Variants characteristic of the LB5 (Liberia sublineage 5) (18) were shared by all Nigeria EBOV
23 genomes.

1

2 The Nigerian EBOV genomes also shared 3 variants not common in Liberia, at positions 4037,
3 17016, and 18754 (Table 2). These variants were present in all Nigerian samples sequenced,
4 including the index case (we note that 2 samples did not have coverage at position 18574). Two
5 of these variants were unique to Nigeria, and 1, the variant at position 18754, was also seen in 2
6 EBOV genomes from Liberia (accession numbers: KT725314, KT725261), suggesting a close
7 relationship of the Nigeria clade to those samples. Two Nigerian samples had unique additional
8 consensus variants.

9

10 We also identified 31 intrahost variants (iSNVs) in 5 of the 12 EBOV genomes from Nigeria (5
11 synonymous, 5 non-synonymous, 5 non-coding SNPs, and 16 insertions/deletions)
12 (Supplementary Data 2). We sequenced each of the 5 samples with iSNVs at least twice from
13 replicate libraries, and iSNV calls were concordant between libraries. Eight of these iSNVs were
14 shared by 2 or more samples, and 2 iSNVs (positions 7551 and 10503), both found in sample
15 E027, were also consensus variants in sample E030. Presence and number of iSNVs found
16 correlated roughly with sample coverage; only samples with >100x coverage had more than 1
17 iSNV call that passed our basic filters.

18

19 **Phylogenetic tree**

20 To better understand the evolutionary relationship between the EVD outbreak in Nigeria and the
21 West African outbreak as a whole, we created a maximum likelihood tree (Figure 1). The tree
22 confirms that the EVD outbreak in Nigeria was due to a single introduction from Liberia, as
23 suggested by contact tracing. More specifically, the EBOV genomes from Nigeria are

1 descendants of the LB5 clade in Liberia (18). No EBOV sequences yet sampled outside Nigeria
2 descend from the Nigerian EBOV isolates (2,19-23), indicating containment of EVD cases in
3 Nigeria within the larger outbreak, as also suggested by contact tracing.

4

5 **Reconstructed transmission tree**

6 Given the phylogenetic tree of the sampled viruses, along with their dates, it is possible to infer
7 at least the outlines of the chain of transmission from one patient to another (Figure 2a). Ten
8 Nigerian EBOV have identical consensus sequences, suggesting that these sequences are closely
9 connected by direct transmissions. Date information identifies sample E001, the index case, as
10 the earliest-sampled case in Nigeria (collection date: 22 July 2014). Of the other 9 identical
11 genomes, 7 have collection dates from 4 August 2014 – 8 August 2014.

12

13 The close proximity of the sample collection dates to each other suggests that each of the
14 corresponding cases was infected by the index case (i.e. it is unlikely that an individual
15 presenting symptoms on 8 August 2014 would have been infected <4 days previously) (24). The
16 remaining 2 cases with viral genomes identical to the index case are dated 15 August and 1
17 September, and therefore may have been infected by one of the earlier cases. The presence of
18 additional SNPs in the viral genomes corresponding to cases #2 and #9 make it difficult to place
19 these samples within the transmission chain. However, case #6 has an iSNV at each of the 2 case
20 #9 SNP positions (position 7551, 21% minor allele frequency; position 10503, 16% minor allele
21 frequency) (Supplementary Data 2), suggesting these 2 cases are closely linked.

22

1 In the limited Nigeria EVD outbreak, it was also possible to reconstruct a nearly complete
2 transmission chain based on contact tracing alone (Figure 2b). Such a reconstruction is feasible
3 in this case because (i) EBOV spreads primarily through direct contact, (ii) there were few cases
4 (multiple exposures were uncommon), and (iii) intensive efforts were made to trace and monitor
5 all suspected contacts. The contact tracing information resulted in a transmission tree similar to
6 that suggested by genetic data, with the index case responsible for a majority of transmissions.
7 This data also revealed that one individual (case #18) traveled from Lagos to Port Harcourt while
8 infected with EBOV, where he acted as the index patient in a small secondary outbreak
9 containing 4 additional EVD cases.

10

11 **Discussion**

12 The 2014 Nigeria outbreak is unusual for an EVD outbreak in the detailed information available
13 about its development: we have both a good reconstruction of the transmission chain of 20
14 patients, and viral genomic data from most of the cases in the chain. The completeness of the
15 record reflects the public health situation: Nigeria was prepared for the arrival of EBOV, and was
16 able to implement thorough contact tracing promptly after the index case was diagnosed, while
17 the number of cases was still small. That effort was critical in containing the outbreak, but it is
18 also very helpful in reconstructing its details afterward. Combined with sequence data, the
19 transmission chain helps us interpret the changes occurring in the virus, since it generally lets us
20 pinpoint where in the chain each new mutation actually occurred.

21

22 Viewed by itself, sequence data can serve to provide a broad picture of an outbreak, and that is
23 true of this EVD outbreak. This capability is obviously useful when contact tracing is absent or

1 incomplete, as is usually the case with epidemics. In the 2014 Nigeria outbreak, sequencing
2 alone makes it clear that the entire outbreak stemmed from a single introduction of EBOV into
3 the country. It also places the Nigerian outbreak in its larger context, identifying a particular
4 branch of the Liberian LB5 lineage of EBOV as the source, and showing that the Nigerian
5 lineage did not spread into other countries.

6
7 Identifying individual links in the transmission chain is usually beyond the resolution of
8 sequence data, however, and requires contact tracing in the field. The resolution of genomic data
9 is limited because new variants arise less often than new cases, meaning that many cases will be
10 genetically indistinguishable. This can be seen in our data in Figure 2a, in which multiple
11 successive links in the chain share identical genomes. In addition, when mutations do occur,
12 more than one can arise in a single patient, making genetic distance an imperfect guide to the
13 number of transmission links that have occurred. Thus, most of the cases infected directly by the
14 index patient in Nigeria had identical genomes, but one case (#4) differed by 4 mutations, even
15 though it too resulted from a single transmission. Contact tracing (Figure 2b) — when it is
16 available — does not suffer from such limitations.

17
18 Within-host variants (iSNVs) that are shared between patients can provide a more detailed
19 picture of transmission routes, but our data point up some important caveats about their
20 usefulness. First, detection of iSNVs requires deep sequencing of good quality samples, and that
21 is not always possible: deep enough sequencing could only be achieved for two-thirds of our
22 sequenced samples. Second, even when iSNV data is available, it may not all be meaningful.
23 Some of the iSNVs we observed have previously been documented in unrelated datasets from

1 Sierra Leone and Liberia (2,13,18); these included all 8 of the shared iSNVs. Most of our iSNVs,
2 including most shared iSNVs, were low-frequency frameshift insertions or deletions. Because
3 they can disrupt protein structure, they are unlikely to be transmitted. More likely, these
4 recurrent iSNVs represent either recurring mutations in highly mutable regions of the EBOV
5 genome, or sequencing errors, especially since many of them occur in homopolymer regions. In
6 either case, their value for determining transmission chains is uncertain. More research is
7 necessary to fully utilize within-host genomic data in understanding transmission, including
8 better sequencing coverage for all samples and improved methods to identify false positives.

9
10 One aspect of our genomic data that is slightly surprising is the distribution of new variants,
11 which is not at all uniform. Our sequenced samples include the results of 11 transmissions from
12 the index case. Nine of these produced no new consensus SNPS, while one produced 4 new
13 SNPs and another produced 2 (Figure 2a). This clustering of mutations in certain samples
14 suggests the possibility that the mutation rate was not uniform across all of the cases. This is no
15 more than a possibility, though, since the clustering is not statistically significant ($p = 0.07$).

16
17 Also puzzling is a pair of variants that were seen twice, once as consensus SNPs (in case #9) and
18 once as iSNVs (in case #6). Based on sample dates and contact data, both of these cases were
19 infected by the index patient, so presumably they inherited these variants from that patient. We
20 do not, however, find them in the sample from the index case, either as consensus SNPs or as
21 iSNVs, despite high sequencing depth. Nor do they appear as consensus SNPs in the other cases
22 derived from the index, or as iSNVs in the one other case that was deeply sequenced and that
23 was sampled around the same time as samples #6 and #9. The explanation may simply be that

1 the variants were present in the index but at too low a frequency for us to detect. It is also
2 possible that their frequency changed in the index patient between the time he was sampled and
3 transmission to the other cases, or that they differed across tissues within the patient. Better
4 understanding of the dynamics of within-host evolution and transmission, and of our power to
5 detect iSNVs, would help to clarify this issue.

6
7 The genomic data was invaluable in revealing what was happening to the virus during the
8 outbreak, but it would have been even more informative had samples been of uniformly high
9 quality. Many samples did not produce whole-genome assemblies because of poor sample
10 quality, and a third of those that did could not be used to detect iSNVs. This highlights the
11 importance of rapid sequencing in clinical settings during outbreaks, with well-established
12 sample collection and processing protocols. Although at the time of the outbreak, sequencing
13 was not yet ready on site, sequencing capability is now becoming increasingly available
14 throughout many regions. With high-throughput deep sequencing now being routinely performed
15 by ACEGID at RUN, high resolution pathogen information can now be generated to better
16 understand outbreak dynamics and response, both in Nigeria and throughout West Africa.

17
18 Data handling could similarly benefit from good protocols established in advance. In the case of
19 the data presented here, clinical and contact data were separated from sequence data, and the
20 correspondence between the two had to be established post hoc, a process that was both
21 laborious and uncertain. In an outbreak setting, keeping track of different kinds of data is not the
22 highest priority, but valuable information can be lost as a result. Having a system for collecting
23 and maintaining both clinical and laboratory data established in advance would be very helpful.

1 Sequence assemblies are available from GenBank and reads available from SRA, accessible
2 under BioProject PRJNA316870.

3

4 The content of this publication does not necessarily reflect the views, policies, or official
5 positions of the US Army.

6

7 **Figure and Table Legends**

8

9 **Figure 1.** Maximum likelihood tree. Phylogenetic analysis confirms a single introduction of
10 EBOV into Nigeria from Liberia, and places all Nigerian sequences as descendents of Liberia
11 sublineage 5 (LB5). Two LB5 genomes (accession numbers: KT725314 and KT725261) cluster
12 closely with Nigerian samples due to a shared variant at position 18754.

13

14 **Figure 2.** Transmission tree. (a) Transmission reconstructed from EBOV genome sequence and
15 sample dates only. Arrows indicate likely transmission; cases not connected to arrows cannot be
16 placed within the transmission tree given the available data. (b) Transmission reconstructed from
17 contact tracing only. Contact tracing provides more precise information, but is not always
18 available. Samples were collected in Lagos, Nigeria unless otherwise identified. Each case is
19 labeled with its sample collection date; cases not connected to sequenced samples are labeled
20 with date of hospitalization. LB5: Liberia sublineage 5 reference. Samples are colored by
21 consensus sequence; i.e. samples with identical viral genomes are similarly colored. Cases
22 colored in grey are those for which genetic data is not available.

23

1 **Table 1.** Sample coverage. Percent coverage: percent of bases with $\geq 1x$ coverage. x Coverage:
2 median depth of coverage.

3

4 **Table 2.** Consensus SNPs seen in Nigeria. All variants and positions are relative to the
5 KJ660346.2 Guinea genome from early in the outbreak. The “Lineage” column indicates
6 previously published clade-defining SNPs ancestral to the Nigeria lineage. The 3 highlighted
7 SNVs are novel to Nigeria (with the exception of 18754, which is shared by 2 EBOV genomes
8 from Liberia) and are shared by all Nigerian samples.

9

10 **Supplementary Table 1.** Clinical records for 20 Nigerian EBOV patients.

11

12 **Supplementary Table 2.** Additional clinical information for Nigerian EBOV patients.

13

14 **Supplementary Data 1.** Sample metadata.

15

16 **Supplementary Data 2.** Annotated intrahost variants (iSNVs).

17

18 **Supplementary Data 3.** GenBank accession numbers for Liberian EBOV genomes used in
19 phylogenetic analysis.

20

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14

15 **References**

16

17 1. WHO. Ebola Situation Report. 16 March 2016. [http://apps.who.int/ebola/current-](http://apps.who.int/ebola/current-situation/ebola-situation-report-16-march-2016)
18 [situation/ebola-situation-report-16-march-2016](http://apps.who.int/ebola/current-situation/ebola-situation-report-16-march-2016)

19 2. Gire SK, Goba A, Andersen KG, et al. Genomic surveillance elucidates Ebola
20 virus origin and transmission during the 2014 outbreak. *Science* **2014**; 345(6202):1369–
21 1372.

22 3. Günther S, Asper M, Röser C, et al. Application of real-time PCR for testing

- 1 antiviral compounds against Lassa virus, SARS coronavirus and Ebola virus in vitro.
2 Antiviral Res **2004**; 63(3):209–215.
- 3 4. Grard G, Biek R, Tamfum J-JM, et al. Emergence of divergent Zaire ebola virus
4 strains in Democratic Republic of the Congo in 2007 and 2008. J Infect Dis **2011**; 204
5 Suppl 3:S776–84.
- 6 5. Kobinger GP, Leung A, Neufeld J, et al. Replication, pathogenicity, shedding, and
7 transmission of Zaire ebolavirus in pigs. J Infect Dis **2011**; 204(2):200–208.
- 8 6. Hoenen T, Jung S, Herwig A, Groseth A, Becker S. Both matrix proteins of Ebola
9 virus contribute to the regulation of viral genome replication and transcription. Virology
10 **2010**; 403(1):56–66.
- 11 7. Blow JA, Mores CN, Dyer J, Dohm DJ. Viral nucleic acid stabilization by RNA
12 extraction reagent. J Virol Methods **2008**; 150(1-2):41–44.
- 13 8. Trombly AR, Wachter L, Garrison J, et al. Comprehensive panel of real-time
14 TaqMan polymerase chain reaction assays for detection and absolute quantification of
15 filoviruses, arenaviruses, and New World hantaviruses. Am J Trop Med Hyg **2010**;
16 82(5):954–960.
- 17 9. Matranga CB, Andersen KG, Winnicki S, et al. Enhanced methods for unbiased
18 deep sequencing of Lassa and Ebola RNA viruses from clinical and biological samples.
19 Genome Biol **2014**; 15(519).
- 20 10. Morlan JD, Qu K, Sinicropy DV. Selective Depletion of rRNA Enables Whole

- 1 Transcriptome Profiling of Archival Fixed Tissue. PLoS One **2012**
- 2 11. Adiconis X, Borges-Rivera D, Satija R, et al. Comparative analysis of RNA
3 sequencing methods for degraded or low-input samples. Nat methods **2013**; 10(7):623–
4 629.
- 5 12. Park DJ, Jungreis I, Tomkins-Tinch C, Lin M. viral-ngs. Available at:
6 <http://dx.doi.org/10.5281/zenodo.17560>
- 7 13. Park DJ, Dudas G, Wohl S, et al. Ebola Virus Epidemiology, Transmission, and
8 Evolution during Seven Months in Sierra Leone. Cell **2015**; 161(7):1516–1526.
- 9 14. Cingolani P, Platts A, Wang LL, et al. A program for annotating and predicting
10 the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of
11 *Drosophila melanogaster* strain w1118; iso-2; iso-3. Fly **2012**; 6(2):80.
- 12 15. Katoh K, Misawa K, Kuma K-I, Miyata T. MAFFT: a novel method for rapid
13 multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. **2002**;
14 30(14):3059–3066.
- 15 16. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7:
16 improvements in performance and usability. Mol Biol Evol. **2013**; 30(4):772–780.
- 17 17. Nguyen L-T, Schmidt HA, Haeseler von A, Minh BQ. IQ-TREE: a fast and
18 effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol
19 and Evol **2015**; 32(1):268–274.
- 20 18. Ladner JT, Wiley MR, Mate S, et al. Evolution and Spread of Ebola Virus in

- 1 Liberia, 2014-2015. *Cell Host and Microbe* **2015**; 18(6):659–669.
- 2 19. Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola Virus
3 Disease in Guinea — Preliminary Report. *N Engl J Med* **2014**.
- 4 20. Tong Y-G, Shi W-F, Di Liu, et al. Genetic diversity and evolutionary dynamics of
5 Ebola virus in Sierra Leone. *Nature* **2015**; 524(7563):93–96.
- 6 21. Carroll MW, Matthews DA, Hiscox JA, et al. Temporal and spatial analysis of the
7 2014-2015 Ebola virus outbreak in West Africa. *Nature* **2015**; 524(7563):97–U201.
- 8 22. Simon-Loriere E, Faye O, Faye O, et al. Distinct lineages of Ebola virus in
9 Guinea during the 2014 West African epidemic. *Nature* **2015**; 524(7563):102–U210.
- 10 23. Kugelman JR, Wiley MR, Mate S, et al. Monitoring of Ebola Virus Makona
11 Evolution through Establishment of Advanced Genomic Capability in Liberia. *Emerg*
12 *Infect Dis* **2015**; 21(7):1135–1143.
- 13 24. Chowell G, Nishiura H. Transmission dynamics and control of Ebola virus
14 disease (EVD): a review. *BMC Med* **2014**; 12:196.

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