Forward genomic surveillance advances DoD biomedical research toward combating high-consequence emerging infectious diseases

Innovations from a Global Health Engagement and Rapid Response during the 2013-2015 Western African Ebola virus outbreak

USAMRIID
United States Army
Medical Research Institute
of Infectious Diseases

Biodefense solutions to protect our nation

Center for Genomic Sciences
USAMRIID

USAMRIID increases Liberia's capacity to combat the Ebola Virus

Liberian Institute of Biomedical Research (LIBR)

PURPOSE: To build country capacity by establishing a permanent and sustained Ebola diagnostic laboratory and a high-throughput sequencing capability at LIBR

We were postured to respond:

- 8 filovirus diagnostic assays with pre-emergency use authorizations (EUA) approved by FDA since 2013
- Depth of expertise in full genome sequencing and analysis methodologies for filoviruses

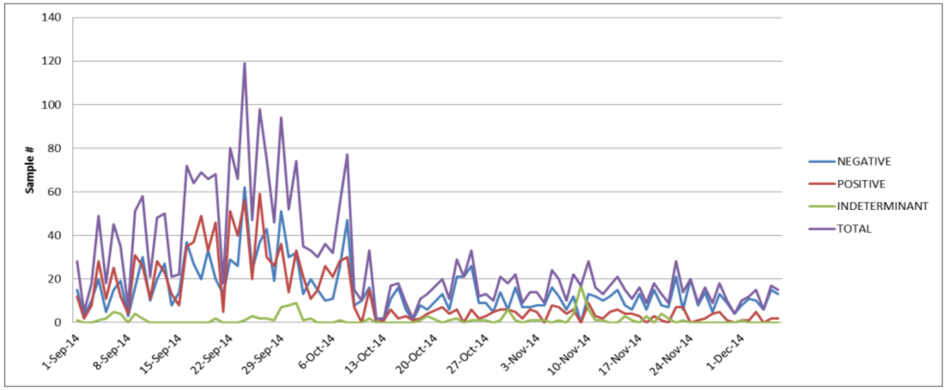
We responded:

1. Diagnostic Capacity built beginning in April 2014 and continues today
    - To assist patient triage and isolation guidelines for ETU
    - 1 of 10 labs in country; < 3 exist today
    - First EUA in U.S. for Ebola with CLIP certification for testing of American Citizens and U.S. Military personnel supporting Operation United Assistance .
    - Capacity today transitioned to EID diagnostics –Lassa fever (CDC-ACCEL)

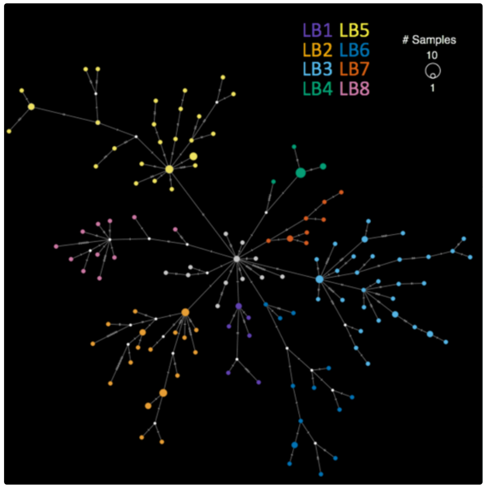
2. Sequencing Capacity built December 2014 and continues today
    - Risk of target erosion of diagnostics and sequence-based therapeutics
    - Only Sequencer in Liberia

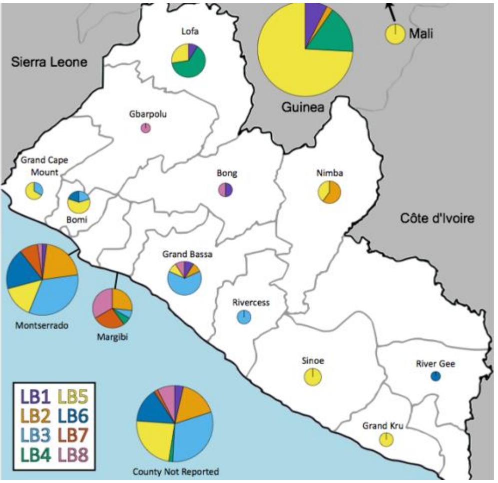
High Throughput Sample Processing to Robust Data Analysis

01 SEP – 6 DEC 2014:
~3000 samples tested by diagnostics



~200 complete sequenced genomes





IMPACT:

- Largest collection of samples and genetic data for variants that circulated in Liberia for this outbreak
- 8 lineages circulated in Liberia,
  - 7 of which in Monrovia;
  - lineages are geographically associated
- Calculated rate of evolution consistent with neighboring countries
  - 9.17 × 10^{-4} substitutions/site/year
- Target Erosion Confirmed but low risk to efficacy:
  - 26 mutations to epitopes recognized by immunotherapies
  - 5 mutations in siRNA therapy binding sites
  - 2 mutations in diagnostic primer binding sites (binding not impacted)

Lesson Learned: Mapping diagnostic and sequencing data reveals probable viral escape event missed during mission

LEGEND

Diagnostic EBOV Indeterminant

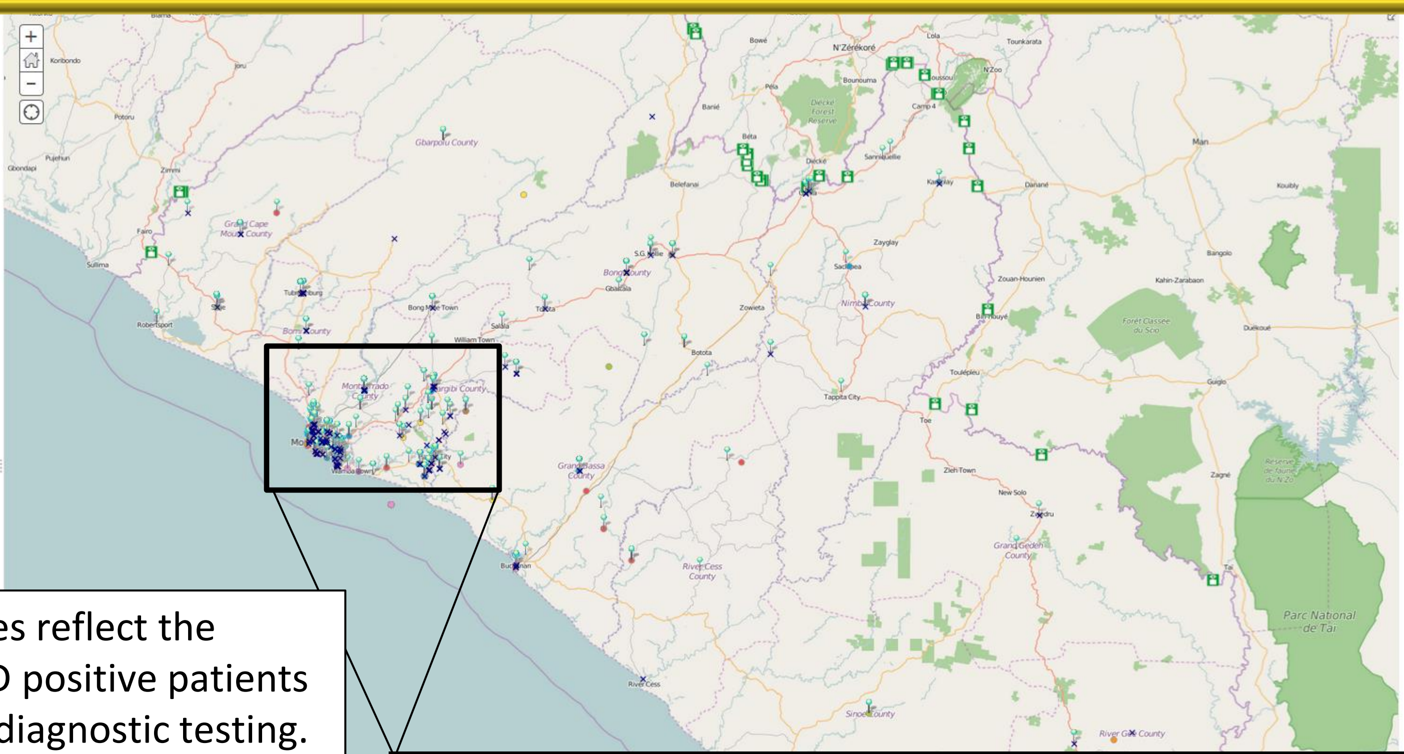
×

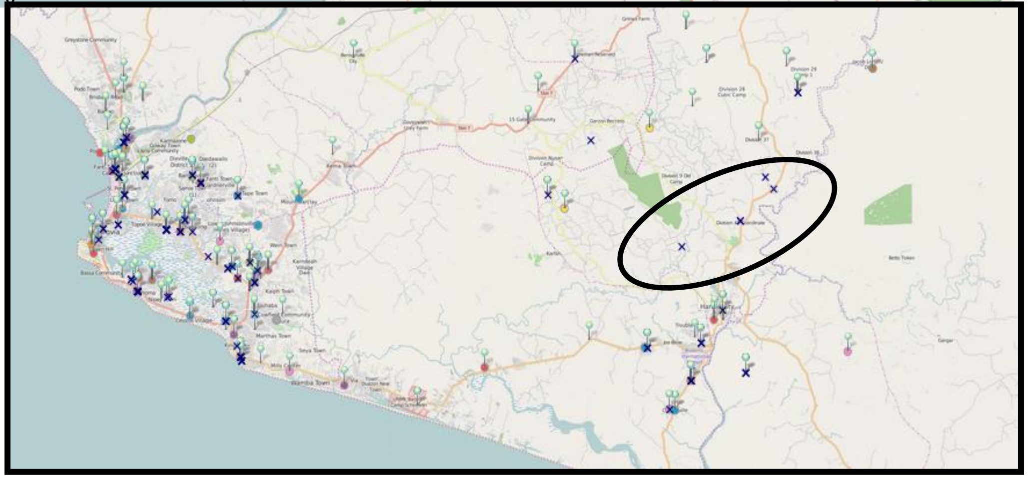
Diagnostic EBOV Positive

EBOV Lineages – sequenced genomes

● LB3
● LB2
● LB5
● LB7
● LB6
● LB8
● NA
● LB1
● SL2
● LB4
● Other

Liberia_Border_Crossings



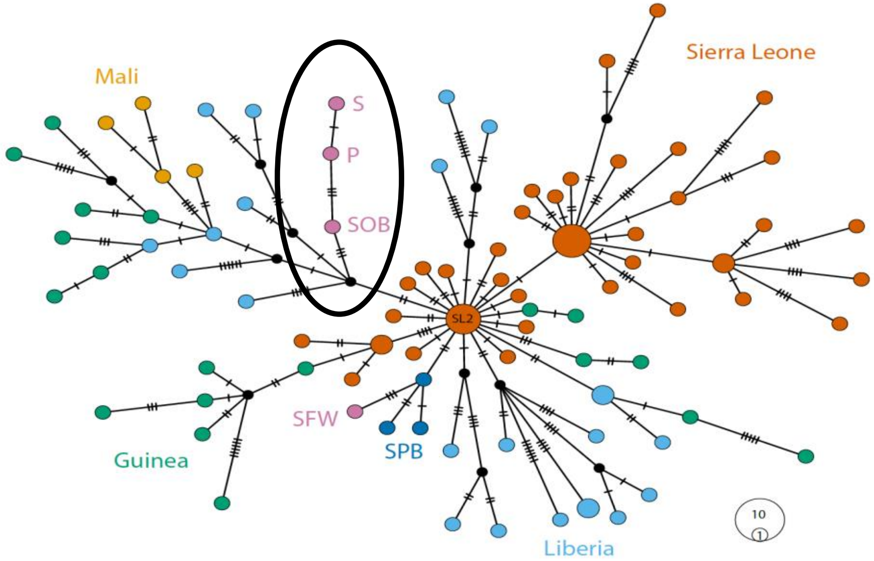


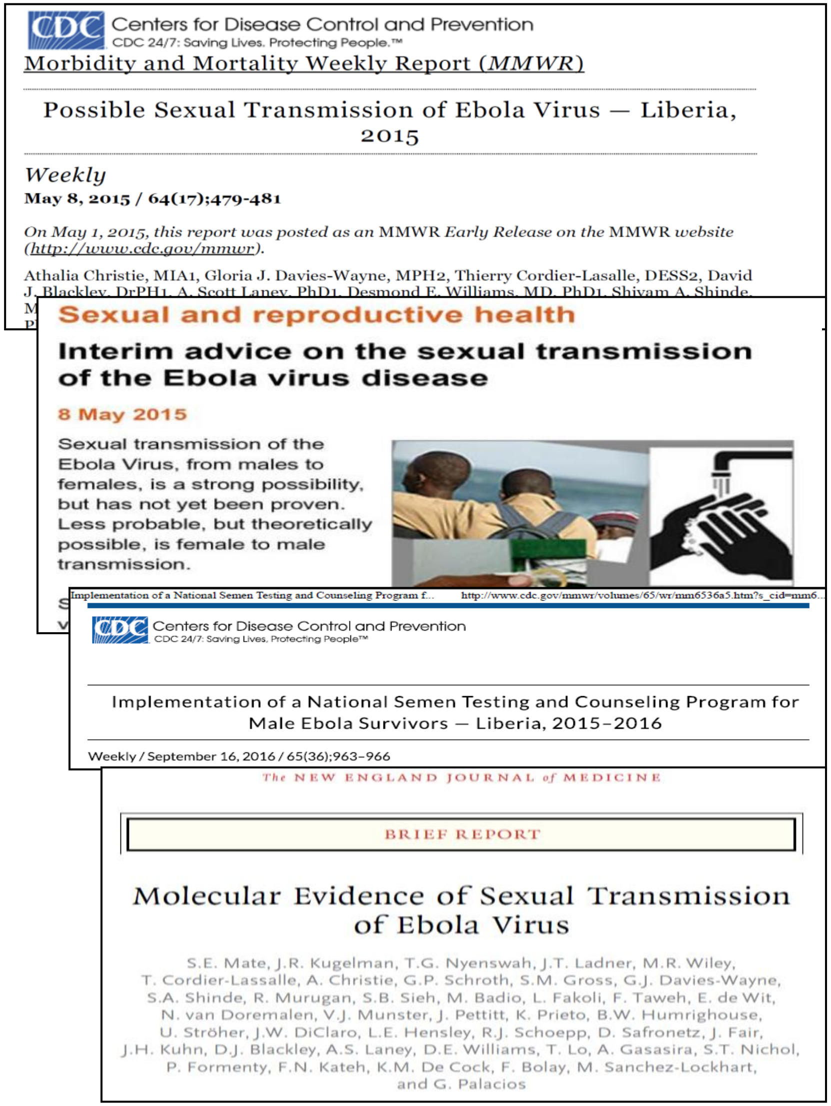
- Sequenced samples reflect the distribution of EVD positive patients confirmed during diagnostic testing.
- Sequencing covered regions with indeterminate results except for one region (circled) which could indicate mutation of virus that escapes diagnostic testing assays.

** Produced by the National Geospatial Intelligence Agency – Experience Directorate

USAMRIID provides molecular evidence for sexual transmission of EBOV

- **March 20, 2015 – 2 New EVD Case** Identified 30 Days into "the count"
- **April** - USAMRIID confirmed source was a male survivor who had a persistent infection for 179 Days
- **May** –Change in health policy and infection control measures
- **July** – Men's Health Program opened to permit




Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™
Morbidity and Mortality Weekly Report (*MMWR*)

Possible Sexual Transmission of Ebola Virus — Liberia, 2015

Weekly
May 8, 2015 / 64(17);479-481

On May 1, 2015, this report was posted as an MMWR *Early Release on the* MMWR *website (http://www.cdc.gov/mmwr).*

Athalia Christie, MIA1, Gloria J. Davies-Wayne, MPH2, Thierry Cordier-Lasalle, DESS2, David J. Blackley, DrPH1, A. Scott Laney, PhD1, Desmond E. Williams, MD, PhD1, Shivam A. Shinde,

Sexual and reproductive health

Interim advice on the sexual transmission of the Ebola virus disease

8 May 2015

Sexual transmission of the Ebola Virus, from males to females, is a strong possibility, but has not yet been proven. Less probable, but theoretically possible, is female to male transmission.

Implementation of a National Semen Testing and Counseling Program f... http://www.cdc.gov/mmwr/volumes/65/wr/mm6536a5.htm?s_cid=mm6...

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Implementation of a National Semen Testing and Counseling Program for Male Ebola Survivors — Liberia, 2015–2016

Weekly / September 16, 2016 / 65(36);963–966

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Molecular Evidence of Sexual Transmission of Ebola Virus

S.E. Mate, J.R. Kugelman, T.G. Nyenswah, J.T. Ladner, M.R. Wiley, T. Cordier-Lassalle, A. Christie, G.P. Schroth, S.M. Gross, G.J. Davies-Wayne, S.A. Shinde, R. Murugan, S.B. Sieh, M. Badio, L. Fakoli, F. Taweh, E. de Wit, N. van Doremalen, V.J. Munster, J. Pettitt, K. Prieto, B.W. Humrighouse, U. Ströher, J.W. DiClaro, L.E. Hensley, R.J. Schoepp, D. Safronetz, J. Fair, J.H. Kuhn, D.J. Blackley, A.S. Laney, D.E. Williams, T. Lo, A. Gasasira, S.T. Nichol, P. Formenty, F.N. Kateh, K.M. De Cock, F. Bolay, M. Sanchez-Lockhart, and G. Palacios

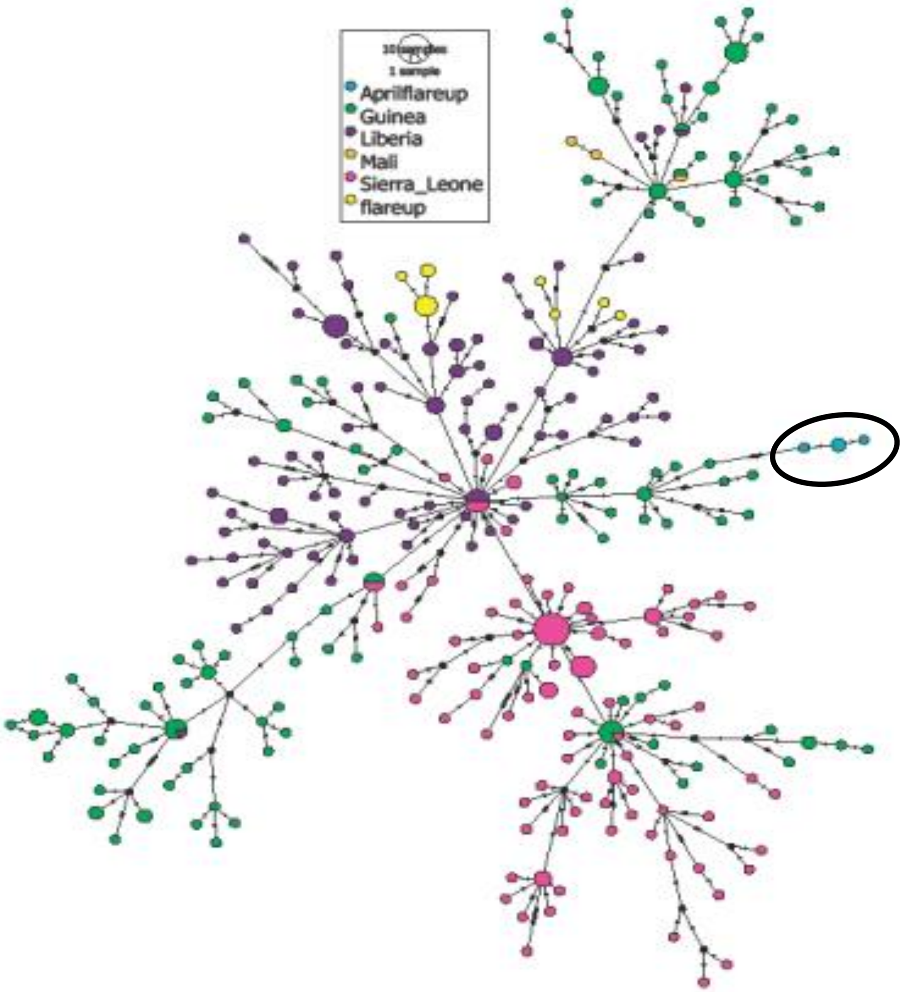
Sequencing confirmed:

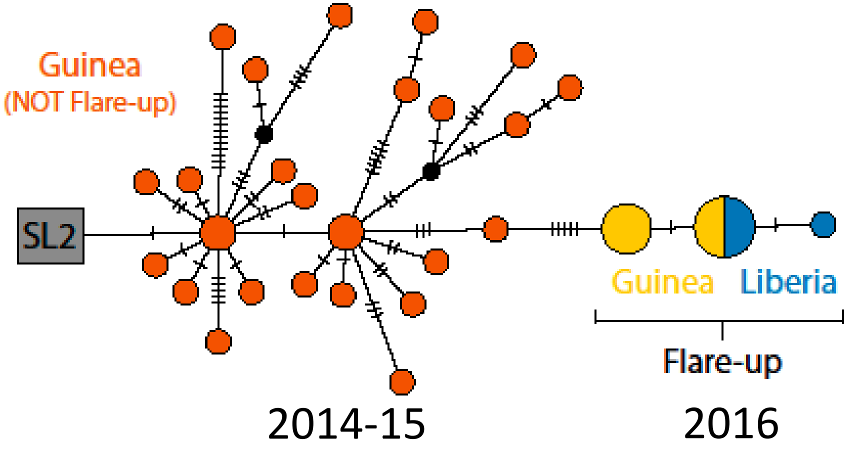
- Variant had circulated in Liberia
- Variant from semen was nearly identical to the new infection
- Both variants exhibited unique substitutions never seen before
- Variants exhibited a reduced rate of evolution = persistent infection & reservoir

USMRIID:CDC unite to apply molecular sequencing to epidemiologic case tracing
April 2016 Flare-up – 3 EVD cases in Liberia

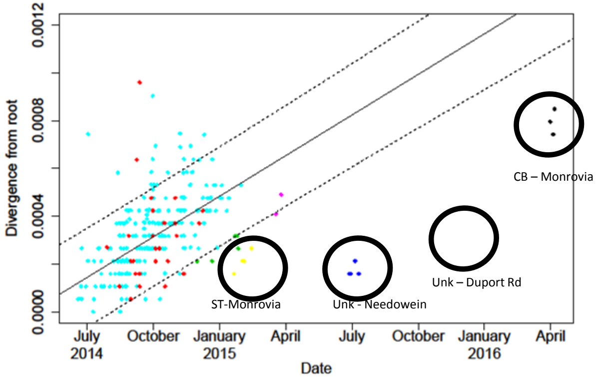
Evolutionary analysis revealed:

- Patients were not infected by EBOV that circulated n Liberia
- Closest to variants from Guinea
- Cases were nearly identical to variants from flare-up in N'Zérékoré, Guinea



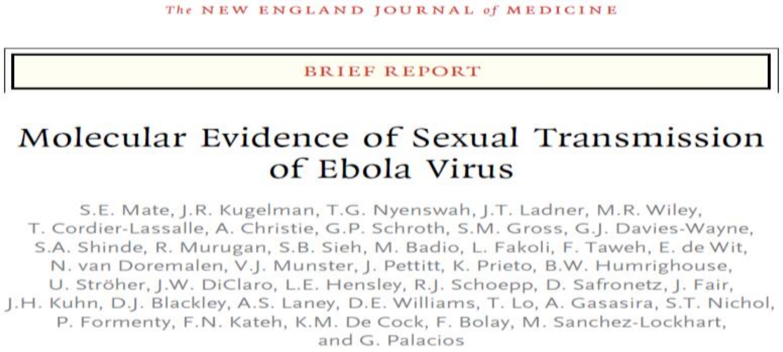


- Reduced evolutionary rate was consistent with the source for the flare-up in Guinea, a persistently infected Survivor.



○ 4 Flare-ups in Liberia & Source
ST = Sexual Transmission
CB= Cross-Border transmission

Persistence in immune privileged tissues:
Ebola outbreak largest impact to public health knowledge and continues to be a public health concern



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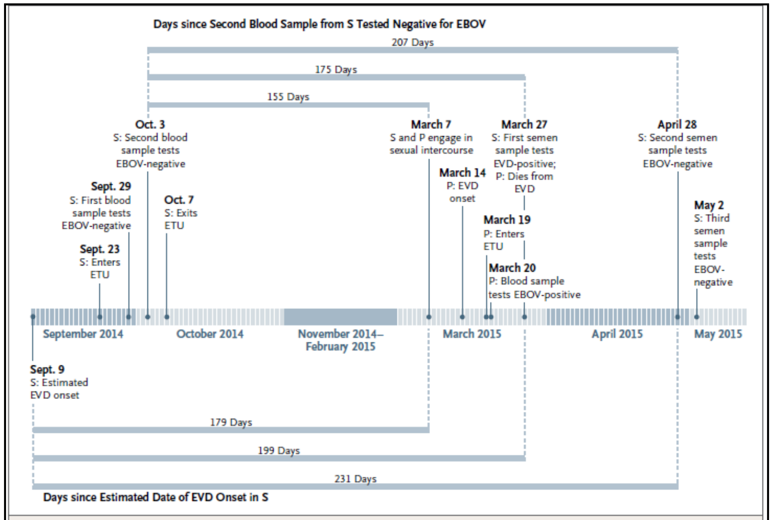
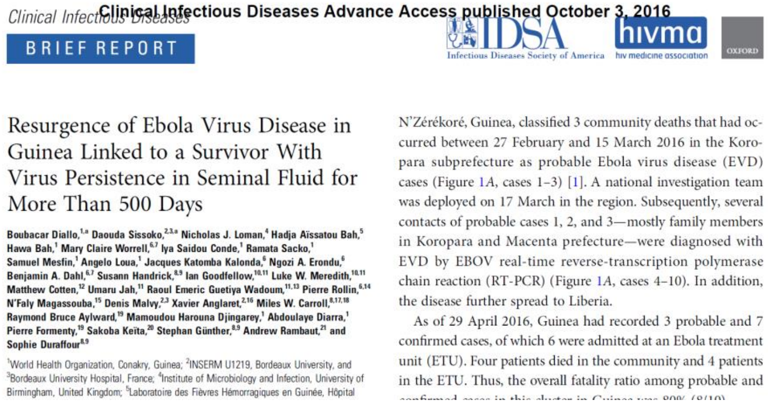


Figure 1. **Clinical Timelines for the Patient and the Survivor, from September 2014 through May 2015.**

Shown are key dates regarding the Ebola virus disease (EVD) presentation, diagnostic tests, and outcomes for the survivor (S) and the patient (P). Horizontal bars estimate the number of days of persistence of the Ebola virus (EBOV) since the date of disease onset and since the date of clearance from blood. ETU denotes Ebola treatment unit.



Clinical Infectious Diseases

Clinical Infectious Diseases Advance Access published October 3, 2016

BRIEF REPORT

IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

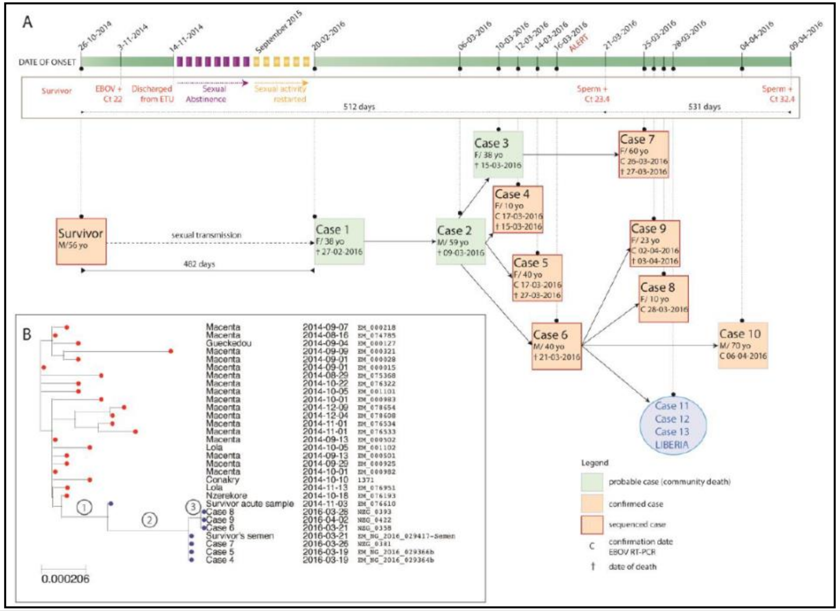
Resurgence of Ebola Virus Disease in Guinea Linked to a Survivor With Virus Persistence in Seminal Fluid for More Than 500 Days

Boubacar Diallo,[1,a] Daouda Sissoko,[2,3,a] Nicholas J. Loman,[4] Hadja Aïssatou Bah,[5] Hawa Bah,[1] Mary Claire Worrell,[6,7] Iya Saidou Conde,[1] Ramata Sacko,[1] Samuel Mesfin,[1] Angelo Loua,[1] Jacques Katomba Kalonda,[6] Ngozi A. Erondu,[6] Benjamin A. Dahl,[6,7] Susann Handrick,[8,9] Ian Goodfellow,[10,11] Luke W. Meredith,[10,11] Matthew Cotten,[12] Umaru Jah,[11] Raoul Emeric Guetiya Wadoum,[11,13] Pierre Rollin,[6,14] N'Faly Magassouba,[15] Denis Malvy,[2,3] Xavier Anglaret,[2,16] Miles W. Carroll,[8,17,18] Raymond Bruce Aylward,[19] Mamoudou Harouna Djingarey,[1] Abdoulaye Diarra,[1] Pierre Formenty,[19] Sakoba Keïta,[20] Stephan Günther,[8,9] Andrew Rambaut,[21] and Sophie Duraffour[8,9]

[1]World Health Organization, Conakry, Guinea; [2]INSERM U1219, Bordeaux University, and [3]Bordeaux University Hospital, France; [4]Institute of Microbiology and Infection, University of Birmingham, United Kingdom; [5]Laboratoire des Fièvres Hémorragiques en Guinée, Hôpital

N'Zérékoré, Guinea, classified 3 community deaths that had occurred between 27 February and 15 March 2016 in the Koropara subprefecture as probable Ebola virus disease (EVD) cases (Figure 1A, cases 1–3) [1]. A national investigation team was deployed on 17 March in the region. Subsequently, several contacts of probable cases 1, 2, and 3—mostly family members in Koropara and Macenta prefecture—were diagnosed with EVD by EBOV real-time reverse-transcription polymerase chain reaction (RT-PCR) (Figure 1A, cases 4–10). In addition, the disease further spread to Liberia.

As of 29 April 2016, Guinea had recorded 3 probable and 7 confirmed cases, of which 6 were admitted at an Ebola treatment unit (ETU). Four patients died in the community and 4 patients in the ETU. Thus, the overall fatality ratio among probable and



Infection from Persistence > 179 Days post onset

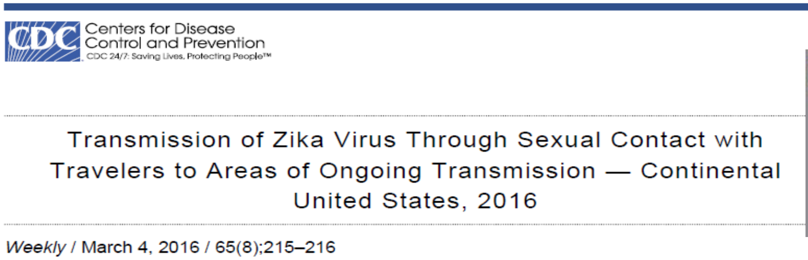
April 2015

Infection from Persistence > 471 Days post onset

April 2016

USAMRIID is now applying the same **Genome Surveillance Strategy** to combat Zika on the Homeland

Reports demonstrate sexual transmission and persistence of Zika virus.



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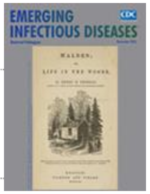
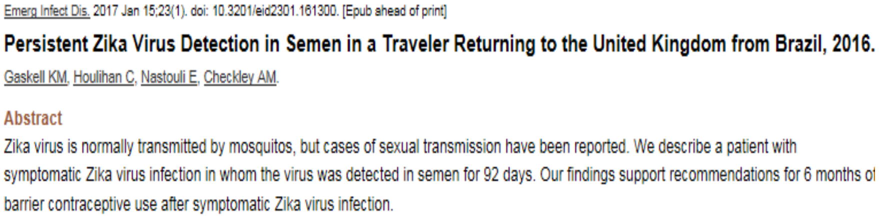
Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission — Continental United States, 2016

Weekly / March 4, 2016 / 65(8);215–216





Emerg Infect Dis. 2017 Jan 15;23(1). doi: 10.3201/eid2301.161300. [Epub ahead of print]

Persistent Zika Virus Detection in Semen in a Traveler Returning to the United Kingdom from Brazil, 2016.

Gaskell KM, Houlihan C, Nastouli E, Checkley AM.

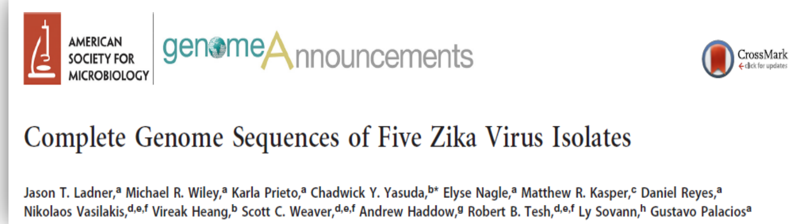
Abstract

Zika virus is normally transmitted by mosquitos, but cases of sexual transmission have been reported. We describe a patient with symptomatic Zika virus infection in whom the virus was detected in semen for 92 days. Our findings support recommendations for 6 months of barrier contraceptive use after symptomatic Zika virus infection.

Different Virus- Same questions for molecular epidemiology

- When did local transmission actually begin?
- How many introductions have contributed?
- Which cases are epidemiologically linked?
- Reduced rates of evolution signify persistence?

We are already assisting public health officials and increasing public knowledge



AMERICAN SOCIETY FOR MICROBIOLOGY genomeAnnouncements

Complete Genome Sequences of Five Zika Virus Isolates

Jason T. Ladner,[a] Michael R. Wiley,[a] Karla Prieto,[a] Chadwick Y. Yasuda,[b*] Elyse Nagle,[a] Matthew R. Kasper,[c] Daniel Reyes,[a] Nikolaos Vasilakis,[d,e,f] Vireak Heang,[b] Scott C. Weaver,[d,e,f] Andrew Haddow,[g] Robert B. Tesh,[d,e,f] Ly Sovann,[h] Gustavo Palacios[a]

AMERICAN SOCIETY FOR MICROBIOLOGY genomeAnnouncements

Complete Genome Sequences of Zika Virus Strains Isolated from the Blood of Patients in Thailand in 2014 and the Philippines in 2012

D. W. Ellison,[a] J. T. Ladner,[b] R. Buathong,[c] M. T. Alera,[a] M. R. Wiley,[b] L. Hermann,[a] W. Rutvisuttinunt,[a] C. Klungthong,[a] P. Chinnawirotpisan,[a] W. Manasatienkij,[a] M. C. Melendrez,[d] I. Maljkovic Berry,[d] B. Thaisomboonsuk,[a] P. Ong-ajchaowlerd,[a] W. Kaneechit,[a] J. M. Velasco,[a] I. A. Tac-An,[e] D. Villa,[e] C. B. Lago,[a] V. G. Roque, Jr.,[f] T. Pilpat,[c] A. Nisalak,[a] A. Srikiatkhachorn,[g] S. Fernandez,[h] I. K. Yoon,[i] A. D. Haddow,[b] G. F. Palacios,[b] R. G. Jarman,[d] L. R. Macareo[a]

References

Publications by USAMRIID Investigators:

Mate, S.E., Kugelman, J.R., Nyenswah et al. Molecular Evidence of Sexual Transmission of Ebola Virus. (2015) N Engl J Med. 373(25):2448-54.

Christie, A., Davies-Wayne, G.J., Cordier-Lasalle, et al. Possible sexual transmission of Ebola virus - Liberia, 2015. MMWR Morb. Mortal. Wkly. Rep. 64: 479–481.

Kugelman, J.R., Wiley, M.R., Mate, S., et. al. Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia. (2015) Emerg. Infect. Dis. 21: 1135–1143.

J. T. Ladner, M. R. Wiley, S. Mate, et al. Evolution and spread of Ebola virus in Liberia, 2014–2015. (2015) Cell Host Microbe 18, 659–669.

David J. Blackley, Michael R. Wiley, Jason T. Ladner et al. Evolution and Spread of Ebola Virus in Liberia, 2014–2015. (2015) Cell Host & Microbe 18:659–669.

Gytis Dudas , Luiz Carvalho , Trevor Bedford et al. Virus genomes reveal the factors that spread and sustained the West African Ebola epidemic. Nature. Submitted August 31, 2016. Manuscript Under Consideration.

Ladner JT, Beitzel B, Chain PS, et al. Standards for sequencing viral genomes in the era of high-throughput sequencing. (2014) MBio. 5(3):e01360-14

Other Publications cited :

Diallo B, Sissoko D, Loman NJ, et al. Resurgence of Ebola virus disease in Guinea linked to a survivor with virus persistence in seminal fluid for more than 500 days. (2016) Clinical Infectious Diseases (Pre-Pub).