THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Jacobs M, Rodger A, Bell DJ, et al. Late Ebola virus relapse causing meningoencephalitis: a case report. *Lancet* 2016; published online May 18.
http://dx.doi.org/10.1016/S0140-6736(16)30386-5.

APPENDIX

SEQUENCING METHODS

RNA extraction was carried out in containment level 3 as follows: 100ul of RNA was eluted from 200ul of plasma and 200ul CSF on the easyMag platform according to manufacturer's instructions.

cDNA synthesis and NGS library preparation: A volume of 25 ul of CSF eluate was treated with RNase-free DNase I (Ambion), purified with RNAClean XP magnetic beads (Beckman Coulter) and eluted into 11 ul of water. Plasma eluate was concentrated from 50 to 11 ul using magnetic beads as indicated above, in the absence of DNase I treatment. Both samples were reverse-transcribed using Superscript III (Invitrogen) followed by dsDNA synthesis with NEB Next(r) mRNA Second Strand Synthesis Module (NEB). Libraries were prepared using a KAPA DNA Library Preparation Kit (KAPA Biosystems), following a modified protocol that includes ligation of the NEBnext adapter for Illumina (NEB), followed by indexing with TruGrade oligonucleotides (Integrated DNA Technologies) to eliminate tag crossover. Resulting libraries were quantified using a Qubit 3.0 fluorometer (Invitrogen) and their size determined using a 2200 TapeStation (Agilent). Libraries were pooled in equimolar concentrations.

Sequencing: CSF and plasma samples were sequenced on the Illumina MiSeq platform. 26,566,048 sequence read pairs were obtained (2x150bp - 8 Gbp) and 92% of reads had a quality score of >Q30. Samples were sequenced in parallel on the NextSeq platform. 454,033,194 sequence read pairs were obtained (2x150bp - 136 Gbp) and 82% of reads had a quality score of >Q30. An independent repeat of the plasma sample sequencing was carried out on the NextSeq platform. 455,650,487 read pairs (2x150bp - 137 Gbp) were obtained and 82% of reads had a quality score of >Q30.

Bioinformatic analysis: Sequence reconstruction was carried out using 3 different assemblers (Tanoti, BWA and Bowtie2) and by *de novo* assembly. In all cases, the consensus sequence from CSF and plasma were identical with 2 variations compared with the original sample obtained in December 2014 (Appendix Figure 1 and accession numbers KP658432, KU052669 and KU052670).

Appendix figure 1

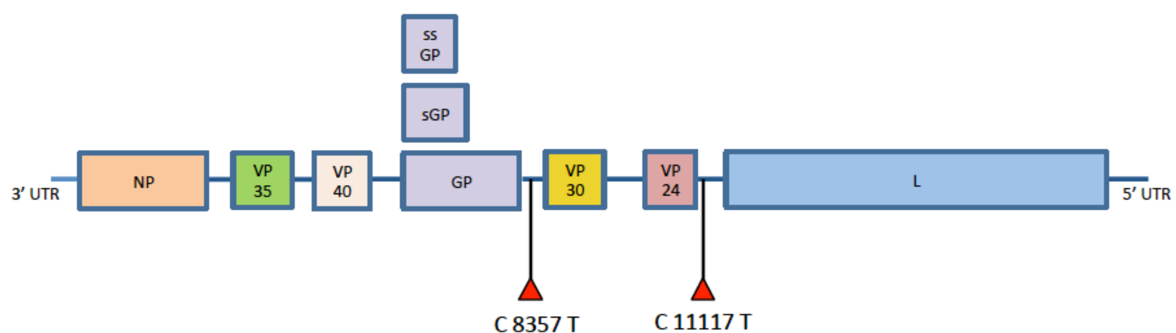


Diagram showing the organisation of the Ebola virus (EBOV) genome and sequence variation between initial admission with EVD in December 2014 and EBOV relapse in October 2015. Two non-coding C to T mutations at positions 8357 and 11117 were evident in both plasma and CSF on readmission when compared with the baseline genome. NP - nucleoprotein, vp35 - polymerase cofactor, vp40 - matrix protein, GP - glycoprotein, sGP - small secreted glycoprotein, ssGP - super small secreted glycoprotein, vp30 - transcription activator, vp24 - matrix 2 protein, L - RNA polymerase.

VIRUS ISOLATION

CSF, Serum and Plasma samples were inoculated into Vero E6 cells under serum free conditions. Cultures (P0) were tested for virus growth by RT-PCR after 7-10 days and then at 14-21 days post inoculation. Confirmation of isolation was by passage (P1) into new Vero E6 with (a) RT-PCR evidence of virus replication at 7 and 14 days and (b) indirect cell ELISA at 72 and 96 hours, post infection.

SEROLOGY

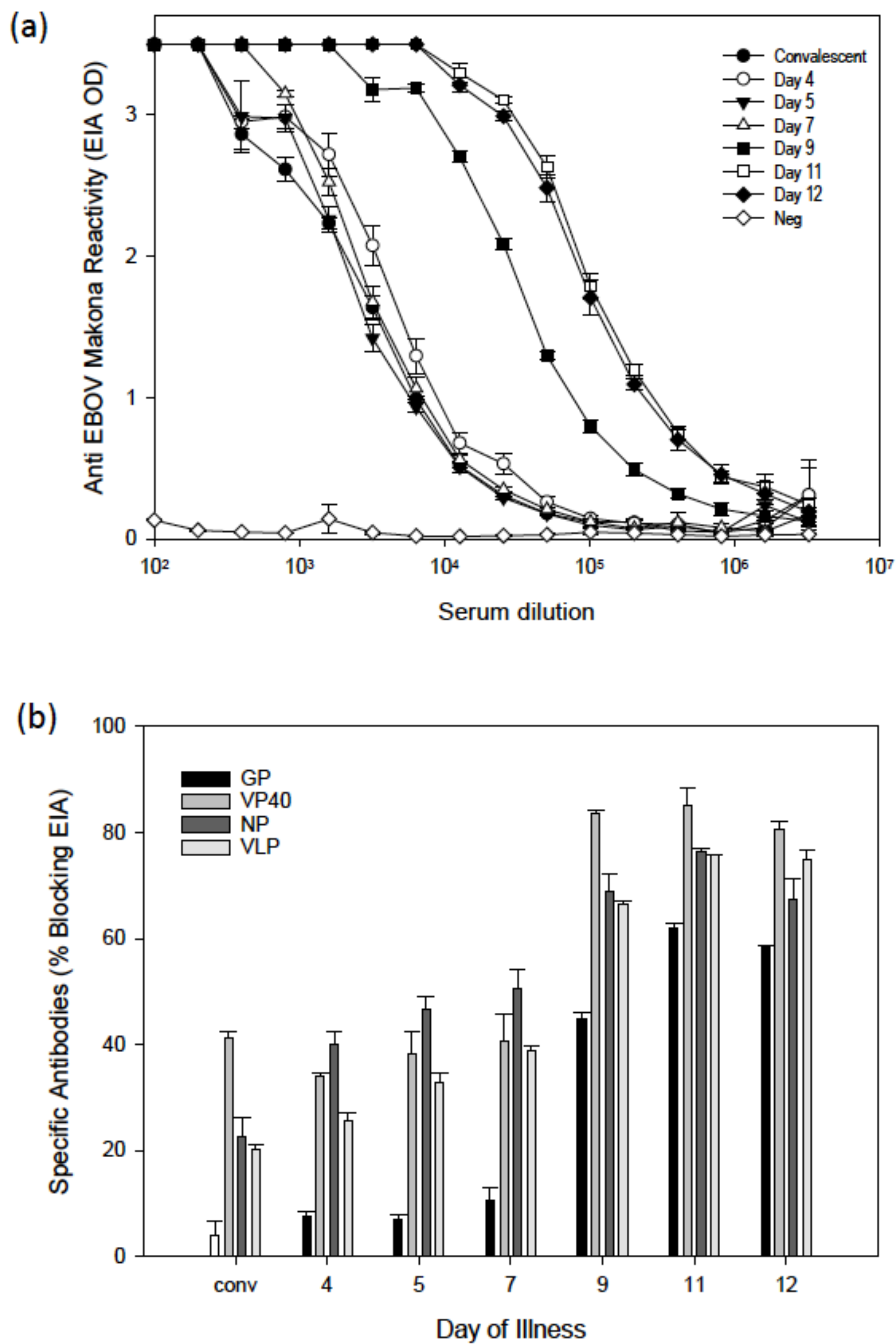
Virus: Total anti-EBOV and protein specific IgG reactivity were determined by Enzyme Immunoassay (EIA) against partially purified Ebola Makona (2014) virions (virus provided by Stephan Gunther, Bernard-Nocht Inst.).

EIA Antigen: Briefly, virus was grown in VeroE6 cells and virions pelleted from supernatant by ultracentrifugation through a 20% glycerol cushion. Microplates for both Indirect and blocking EIA were coated with a 10,000-fold dilution of a preparation with an infectious virus titre of 10E9 focus forming units per ml.

Indirect EIA: Total anti-EBOV IgG was determined by reactivity of serial serum dilutions against EBOV on 384 well microplates. Bound human IgG was detected with a specific anti-human horseradish peroxidase conjugate (DAKO).

Blocking EIA: Human serum antibodies to EBOV GP (glycoprotein), VP40, NP (nucleoprotein) and VLP (virus-like particles) were determined by the ability to block the binding of specific rabbit anti-peptide (GP, VP40, NP) and anti-VLP (Zaire EBOV) antibodies (IBT Bioservices) to EBOV Makona virion coated microplates . Briefly, EBOV patient serum and negative control serum dilution (1/100) were reacted on virion coated microplates for 4-6 hours. Serum was removed and plates were then reacted with EBOV specific rabbit antibodies. Bound rabbit antibodies were detected by specific anti-rabbit horseradish peroxidase conjugate (DAKO). Evidence of EBOV protein specific human antibodies was determined by the reduction in the binding of the corresponding rabbit antibody compared to the reactivity with the negative control. Results were expressed as a percentage reduction of the negative control binding.

Appendix figure 2



Figures show presence of antibodies to EBOV Makona virions in serum samples taken during the convalescent period (February 2015) and between day of illness 4 and 12 of EBOV relapse. (a) Indirect EIA. (b) Blocking EIA. For each value n=3 with standard error bars shown.