Research Paper

Ebola Virus Persistence in Ocular Tissues and Fluids (EVICT) Study: Reverse Transcription-Polymerase Chain Reaction and Cataract Surgery Outcomes of Ebola Survivors in Sierra Leone☆


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Abstract

Background: Ebola virus disease (EVD) survivors are at risk for uveitis during convalescence. Vision loss has been observed following uveitis due to cataracts. Since Ebola virus (EBOV) may persist in the ocular fluid of EVD survivors for an unknown duration, there are questions about the safety and feasibility of vision restorative cataract surgery in EVD survivors.

Methods: We conducted a cross-sectional study of EVD survivors anticipating cataract surgery and patients with active uveitis to evaluate EBOV RNA persistence in ocular fluid, as well as vision outcomes post cataract surgery.

Keywords:
Ebola virus disease
Ebola virus

ARTICLE INFO

Article history:
Received 15 October 2017
Received in revised form 16 March 2018
Accepted 17 March 2018
Available online 23 March 2018

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Ebola virus disease
Ebola virus

☆ This work has been presented, in part, at the American Uveitis Society Winter Symposium (Dr. Shantha; All Co-Authors; Park City, Utah, January 14–16, 2017) and as a presentation at Association for Research in Vision and Ophthalmology Annual Meeting (Dr. Yeh; All Co-Authors; Baltimore, Maryland, May 7–11, 2017).

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ABSTRACT

Background: Ebola virus disease (EVD) survivors are at risk for uveitis during convalescence. Vision loss has been observed following uveitis due to cataracts. Since Ebola virus (EBOV) may persist in the ocular fluid of EVD survivors for an unknown duration, there are questions about the safety and feasibility of vision restorative cataract surgery in EVD survivors.

Methods: We conducted a cross-sectional study of EVD survivors anticipating cataract surgery and patients with active uveitis to evaluate EBOV RNA persistence in ocular fluid, as well as vision outcomes post cataract surgery. Patients with aqueous humor that tested negative for EBOV RNA were eligible to proceed with manual small incision cataract surgery (MSICS).

https://doi.org/10.1016/j.ebiom.2018.03.020
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1. Introduction

Uveitis syndromes due to ocular viral infection can lead to significant visual morbidity and blindness (Connors et al., 2015). In addition to commonly recognized pathogens (e.g., herpes simplex virus, cytomegalovirus), emerging viruses (e.g. chikungunya, zika) are increasingly implicated as causes of uveitis (Connors et al., 2015). The West African Ebola virus disease (EVD) outbreak in 2013–2016 brought attention to the range of uveitis findings ranging from anterior uveitis to sight-threatening panuveitis as a sequelae of Ebola virus infection diagnosed in 13% to 34% of EVD survivors (Varkey et al., 2015; Tiffany et al., 2016; Shantha et al., 2017; Hereth-Hebert et al., 2017). A complex disease spectrum was noted, leading to severe vision impairment or blindness in nearly 40% of affected eyes (Shantha et al., 2017). Vision loss due to uveitis impacts overall quality-of-life amidst a number of other clinical sequelae of EVD, including arthralgias, myalgias, headache, and abdominal pain (Epstein et al., 2015; Vetter et al., 2016).

Ebola virus (EBOV) has been noted to persist in immune privileged sites including the aqueous humor (Varkey et al., 2015) and cerebrospinal fluid (Jacobs et al., 2016), leading to severe uveitis and meningoencephalitis, respectively, during EVD convalescence. Long-term EBOV RNA detection in semen (Deen et al., 2017; Soka et al., 2016), breast milk (Sissoko et al., 2017), and placenta (Bower et al., 2016), with rare transmission events reported (Sissoko et al., 2017; Bower et al., 2016; Mate et al., 2015; Diallo et al., 2016), highlight the individual and public health consequences of EBOV persistence and emphasize the urgent need to investigate EBOV RNA clearance from immune-privileged sites.

In EVD survivors, invasive ophthalmic procedures (e.g. cataract surgery, open globe repair, retinal detachment surgery) currently pose an uncertain risk of EBOV transmission via ocular fluid to health care workers and close contacts of EVD survivors. We conducted the Ebola Virus Persistence in Ocular Tissues and Fluids (EVICT) study to establish an evidence base for a safe, effective approach to invasive ophthalmic procedures in EVD survivors. Prior chamber paracentesis was performed in patients with active uveitis or in patients who require ophthalmic surgery to test for EBOV viral persistence before intraocular surgery. Herein, we report the clinical ophthalmic phenotypes, prevalence of EBOV RT-PCR in ocular fluid of a cohort of Sierra Leonian EVD survivors anticipating ocular surgery or with active uveitis. We also describe the vision restorative outcomes of patients meeting criteria for cataract surgery.

2. Methods

We designed a cross-sectional study to evaluate EBOV RNA persistence in ocular fluids and tissues of EVD survivors. Institutional Review Board approval was obtained from Emory University and the Office of Ethics and Scientific Review Committee, Sierra Leone Ministry of Health and Sanitation (MOHS). Human research was conducted according to the Tenets of the Declaration of Helsinki, and informed consent was obtained with the assistance of Sierra Leonean interpreters in the native dialect of enrolled patients. During the ocular fluid sampling portion of the EVICT Study, patients underwent ocular fluid testing for EBOV RNA by RT-PCR. Patients who tested negative for EBOV RNA were then eligible for the surgical portion of the EVICT Study, which included manual small-incision cataract surgery (MSCS) with intraocular lens (IOL) implantation when medically indicated.

2.1. EVICT Facility, Study Site Preparation, and Personal Protective Equipment

Patient ophthalmologic evaluations were conducted at the Lowell and Ruth Gess Eye Hospital in Freetown, Sierra Leone. An ophthalmic procedure room was designed adhering to World Health Organization (WHO) guidelines (World Health Organisation, 2016), and guidance from the Emory University Serious Communicable Disease Unit (SCDU), with high-level safety precautions for potential EBOV exposure (Fig. 1). Eye care providers performed the ocular fluid sampling procedure in full personal protective equipment (PPE) with monitoring from an infectious disease physician trained in the care of EVD in the acute Ebola treatment unit (ETU) setting.

2.2. Patient Recruitment

EVD survivors anticipating ophthalmic surgery (cataract and/or retinal surgery) were identified via an ophthalmic screening program conducted by the MOHS National Eye Care Program from March 2015 through March 2016. In addition, EVD survivors were referred from local eye clinics for vision loss and cataract evaluation. These centers included Connaught Government Hospital (Freetown), Lunsar Baptist Eye Hospital (Port Loko) and Kenema Government Hospital (Kenema), as well as direct referral from the Sierra Leone Association of Ebola Survivors (SLAES).

2.3. Patient Screening, Ophthalmic Exam, and Follow-Up

Ophthalmic exams for EVD survivors included corrected visual acuity (VA), pupil examination, confrontational visual fields, ocular motility and intraocular pressure (tonopen, Avia, Reichert Technologies). Anterior chamber (AC) cell grade was measured per Standardization of Uveitis Nomenclature guidelines via slit lamp examination (Jabs et al., 2005). Cataract was classified as nuclear sclerotic, posterior subcapsular, anterior subcapsular, uveitic white cataract, uveitic white cataract with anterior capsular fibrosis, and graded from one to four. Funduscopic evaluation was performed with a 90- and 28-diopter...
lens. When media opacity (e.g. dense cataract) precluded adequate view of the retina, a B-scan ultrasound was performed. Patients who were anticipating intraocular surgery or with active uveitis were offered EVICT enrollment for ocular fluid sampling. Exclusion criteria included clinical findings suggesting minimal to no benefit from intraocular surgery (e.g. chronic tractional retinal detachment by B-scan would preclude cataract surgery) and was determined by the clinical investigator. Following their ocular fluid (aqueous humor or vitreous) sampling procedure, patients were seen at one-day, one-week, and one-month post procedure. Patients were evaluated sooner when clinically indicated. Patients who underwent MSICS with IOL implantation were evaluated at postoperative day one (POD1), at week one (POW1), at month one (POM1), and at three to four months (POM 3/4).

2.4. Laboratory Workup and Serologic Testing for Causes of Uveitis

Serologic evidence of prior EBOV infection was evaluated on sera collected from enrolled patients using ReEBOV® IgG ELISA Test Kits (Zalgen Labs, Germantown, MD) as described in the Supplementary Appendix by the Kenema Government Hospital Lassa Hemorrhagic Fever Laboratory. Patients also underwent laboratory evaluation for other causes of uveitis, including serum testing for HIV 1/2- Antigen-Antibody (Alere Determine™, San Diego, CA), and syphilis testing by rapid plasma reagin (RPR). Specific syphilis antibody testing with FTA-ABS or syphilis IgG was not available. Serology for Lassa fever (LASV) IgG using ReLASV™ Pan-Lassa IgG/IgM ELISA Test Kit (Zalgen Labs) was also performed.
2.5. Ocular Fluid Sampling Procedure

We performed ocular fluid sampling in full PPE in a facility with infection prevention measures following guidelines from the WHO and Emory SCDU (Fig. 1). Patients underwent extra- and intraocular fluid sampling by Retina Fellowship-trained ophthalmologists. Anterior chamber paracentesis or vitreous tap was performed as detailed in the Supplemental Appendix. Approximately 100–200 µl were aspirated from the aqueous humor and 500 µl were obtained from the vitreous humor. We obtained conjunctival swabs from the inferior conjunctival fornix pre-procedure and immediately post procedure with a Dacron swab, and placed them in viral transport media (Hardy Diagnostics, Springboro, OH). Ocular fluid sampling proceeded in two phases with Phase 1 occurring in June 2016 and Phase 2 occurring in July and August 2017. The patients in Phase 1 were largely from Freetown and included Port Loko and Western Area Districts, whereas the patients in Phase 2 included individuals from more distant locations including Bo, Kenema, Moyamba, Tonkolili.

2.6. EBOV Reverse Transcription Polymerase Chain Reaction (RT-PCR) Testing of Ocular Fluid

EBOV RT-PCR was performed on conjunctival swab specimens and ocular fluid following RNA extraction using the QiAamp Viral RNA Mini kit (Qiagen, Germantown, MD) at the Kenema Government Hospital (KGH). Patient samples then underwent EBOV RT-qPCR analysis using the KGH primer set (Gire et al., 2014) and Power SYBR Green RNA-to-Ct 1-Step qRT-PCR assay (Life Technologies, Carlsbad, CA). The details of the EBOV RT-PCR procedure are outlined in the Supplemental Appendix.

2.7. Manual Small-Incision Cataract Surgery Procedure

Patients with ocular fluid specimens that tested negative for EBOV RNA by RT-PCR had the opportunity to undergo MSICS with IOL implantation to achieve a postoperative refractive error between -0.50 and -1.00 diopters. PPE was modified to sterile surgical attire (fluid impermeable gown, gloves, mask and shoe covers). The details of the MSICS are summarized in the Supplemental Appendix.

2.8. Statistical Analysis

Data were presented as percentage frequencies as appropriate or medians with interquartile range (IQR) for continuous variables. VA was converted to logarithm of the minimal angle of resolution (logMAR) for statistical analysis. Counting fingers and hand motions were converted to logMAR as previously described (Holladay, 2004). Patients with only light perception or no light perception were excluded from statistical analyses. We used a paired t-test to compare preoperative to postoperative VA at follow-up. The Jeffrey’s interval approach was used to calculate a confidence interval estimating the true proportion of ocular specimens with EBOV presence. Descriptive and univariate analyses were carried out using Microsoft Excel (v2013, Redmond, WA) and SAS (v9.3, Cary, NC). All statistical tests were two-sided and a p value < 0.05 was considered statistically significant for all comparisons.

3. Results

3.1. Characteristics of Screened and Enrolled Patients

We examined 137 EVD survivors from Sierra Leone in from June 2016 – August 2017 (Fig. 2, Consort diagram), of whom 32 (23%) EVD-survivors showed evidence of cataract but did not fulfill EVICT eligibility criteria due to non-visually significant (i.e. immature) cataract (16 patients), retinal or optic nerve disease precluding VA improvement (11 patients) and hypopyon/phthisis bulbi (5 patients). Fifty-one patients met eligibility criteria and one patient deferred enrollment. Fifty (35%) EVD survivors were enrolled into the EVICT study for ocular fluid sampling.

3.2. Baseline Enrolled Patient Characteristics

The median age of the 50 EVD survivor participants was 24.0 years (IQR 17–35). Thirty-five patients (70%) were female. The median time spent in an ETU was 21 days (IQR 14–45). The median time from EVD diagnosis to AC paracentesis in Phase 1 of ocular fluid sampling was 19 months (IQR 18–20); in Phase 2 of ocular fluid sampling, the median time from EVD diagnosis to AC paracentesis was 34 months (IQR 32–36 months). The most commonly observed ophthalmic complaints at the time of EVICT enrollment included vision loss (49%), eye pain (33%), and tearing (27%) (Supplemental Table 1). Systemic complaints at study enrollment were summarized in Supplemental Table 2, with headache, joint pain, and weight loss being most frequently observed.

The median logMAR visual acuity of the affected eyes was 3 (IQR 1.2 – 3.0; Snellen visual acuity equivalent hand motions; IQR 20/320 – Hand Motions). Thirty eyes (60%) involved the left eye. Indications for enrollment included visually significant cataract (46 eyes, 92%), active uveitis (2 eyes, 4%), subluxed lens (1 eyes, 2%), and blind painful eye due to chronic uveitis (1 patient, 2%). Cataracts were identified in 46 (92%) patients. Uveitic white cataract (25, 54.3%) and posterior subcapsular cataract alone (8, 17.4%) were most frequently seen. Other cataract subtypes included uveitic white cataract with anterior capsular fibrosis (4, 8.7%), combination nuclear sclerotic/posterior subcapsular (4, 8.7%), combination anterior subcapsular/posterior subcapsular (3, 6.5%), and age-related nuclear sclerotic (2, 4.3%) (Fig. 3).

Two enrollees (4%) had active uveitis, one of whom also had a non-visually significant cataract. Forty-six survivors (92%) had a history of uveitis. Structural complications in affected eyes included posterior synechiae (40, 80%), chorioretinal scars (7, 14%), inactive, pigmented keratic precipitates (9, 18%), active keratic precipitates (2, 4%) and band keratopathy (3, 6%). B-scan ultrasonography was required due to inadequate view of the posterior segment in 36 (72%) patients (Fig. 4). Ocular treatments prior to study enrollment included oral prednisone (4, 8%), sulfamethoxazole/trimethoprim 800 mg/160 mg and oral prednisone (1, 2%), topical prednisolone acetate 1% (2, 4%) and a combination of topical prednisolone acetate 1% and oral prednisone (15, 30%).

3.3. Procedures, Safety Monitoring, and Follow-Up

Forty-nine (98%) patients underwent an AC paracentesis and 1 patient had a vitreous tap. The median volume of the AC paracentesis was 140 (IQR 110–220) µl. The vitreous sample was 500 µl. We obtained pre-procedure and immediate post procedure conjunctival swabs in all patients. One adverse event was observed following ocular fluid sampling. Specifically, the patient who underwent the vitreous tap developed a 1.0 mm hyphema with a transient elevation of intraocular pressure that resolved to a normal intraocular pressure with topical prednisolone acetate, atropine, and ocular hypotensives. One month after the procedure, all patients were stable with no new adverse events. Visual acuities and intraocular pressures remained stable at 2-months follow-up.

3.4. Laboratory Investigations

Sera collected from enrolled EVD survivors yielded seropositive results for EBOV IgG in 49 (98%) patients. LASV IgG, HIV, and RPR were positive in 8 (16%), 1 (2%), and 1 (2%) patients respectively.
### 3.5. Ocular Fluid Analysis

Forty-nine aqueous humor samples and one vitreous aspirate tested EBOV RNA-negative by RT-PCR. Pre-procedure and immediate post procedure conjunctival swab specimens tested negative by EBOV RT-PCR in all patients. Following negative testing results for EBOV RNA of ocular fluid specimens, the 46 patients with visually significant cataract were deemed eligible for MSICS. With all 50 ocular fluid specimens testing negative for EBOV RNA by RT-PCR, it is estimated with 95% confidence that the true EBOV presence in ocular fluid specimens would be no greater than 5% in our cohort of patients assessed.

### 3.6. Manual Small Incision Cataract Surgery Outcomes

Thirty-four of 46 (74%) patients with visually significant cataract underwent MSICS with IOL implantation (Fig. 2). Twenty of the patients (53%) were female. Eighteen patients (53%) had a cataract in the right eye and thirty patients (88%) required B-scan ultrasound for evaluation of the posterior segment. Preoperative median logMAR VA (IQR; Median Snellen VA) improved from 3 (Snellen VA equivalent Hand motions; IQR 1.0–3.0; IQR 20/200 – Hand motions) to 0.54 (IQR 0.18–0.78; Median Snellen VA 20/70, IQR 20/30 – 20/120) at POM1 (p < 0.001) and 0.18 (IQR 0.0-0.69; Median Snellen VA 20/30, IQR 20/20–20/100) at POM3/4 (Fig. 5).

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**Fig. 2.** CONSORT Diagram depicting Ebola virus disease survivors screened, excluded, and enrolled for EVICT Study. Following negative Ebola virus RT-PCR testing of ocular fluid, survivors with visually significant cataract underwent surgery.
Twenty-four of 34 eyes (70%) in this cohort met the International Classification of Disease-10 (ICD-10) definition of blindness in the affected eye with a preoperative VA < 20/400. Of these 24 patients with poorer than 20/400 visual acuity, 20 patients (83%) improved by 3 or more lines of visual acuity during follow-up. Of the 34 patients undergoing MSICS, 27 patients experienced VA improvement of ≥3 Snellen eye chart lines by their final visit with 20 patients (60%) demonstrating VA of 20/40 or better at their last visit. Five patients remained poorer than 2’/200E (i.e. counting fingers vision) due to vitreoretinal pathology. Nine patients underwent yttrium aluminum garnet (YAG) capsulotomy during follow-up. Monitoring for uveitis recurrences is ongoing.

4. Discussion

This study is the first to systematically evaluate EBOV persistence in the eyes of EVD survivors with cataract or active inflammation. Because uveitis has been estimated in 13 to 34% of EVD survivors, and cataract blindness is a disabling, but potentially treatable complication of uveitis, the study findings provide initial evidence to directly impact clinical care and timing of vision rehabilitation via cataract surgery. Amidst public health uncertainty around the persistence of EBOV in immune privileged sites (Varkey et al., 2015), interim WHO guidance includes the avoidance of elective surgery (i.e. cataract surgery) until further data is obtained (World Health Organization, n.d.). The step-wise approach involving ocular screening, ocular fluid sampling, and subsequent cataract surgery was safe, feasible, and vision restorative in this cohort of EVD survivors.

Among the 50 EVD survivors we enrolled, the majority presented with severe vision impairment or blindness in the affected eye. All 50 tested negative for EBOV RNA in their ocular fluid and conjunctiva at a median of 19 months after EVD diagnosis in Phase I of ocular fluid sampling and at a median of 34 months after EVD in Phase 2 of ocular fluid sampling. Survivors with a cataract could then proceed with eye surgery with preliminary reassurance to eye care providers that aqueous humor was free of EBOV by RT-PCR. The 34 EVD survivors who underwent cataract surgery experienced significant VA improvement over three to four months of follow-up. It is particularly notable that the preoperative vision of over 70% of eyes was poorer than Snellen visual acuity 20/400 or big “E” optotype. The vision loss was reversible with treatment despite signs of previous inflammation and the need for a complex MSICS procedure.
The mechanism by which the dense cataracts leading to blindness rapidly developed in association with uveitis in EVD survivors is unknown. Contributing factors may include chronic, untreated inflammation, acute severe episodic inflammation, corticosteroid-related factors, or EBOV persistence. While EBOV persistence in seminal fluid has been evaluated in several studies (Deen et al., 2017; Mate et al., 2015; Christie et al., 2015), the clearance dynamics of EBOV from other immune privileged sites (i.e. eye, central nervous system) require further investigation. Recent studies in non-human primates have demonstrated that EBOV may persist in monocytes and macrophages within the vitreous humor in association with uveitis (Zeng et al., 2017). Moreover, in vitro studies have shown that human retinal pigment epithelial cells are permissive to EBOV infection (Smith et al., 2017).

Limitations of this cross-sectional study include the length of time after acute EVD when ocular fluid sampling occurred. Specifically, our study cannot determine if survivors with uveitis may have harbored EBOV in their eye at earlier time points when ocular inflammation was active. Although the RT-qPCR is very sensitive, the low amount of sample extracted could affect the ability to capture EBOV RNA, especially when using conjunctival swabs, which have very little total RNA. Moreover, only approximately 50–150 μl of aqueous humor can be safely obtained from the anterior chamber of the eye to avoid complications, which include ocular hypotony, hyphema, damage to the lens capsule, and vision loss. Nonetheless, it currently remains unknown whether EBOV may persist at low levels after infection, which remain beneath the threshold of detection of RT-PCR, or in other locations within the eye, which were not specifically assessed in this study. Another limitation in the evaluation of cataract outcomes was the variable follow-up. The majority of patients, however, returned for their 3-month follow-up appointments and their visual acuity outcomes showed statistically significant and clinically meaningful improvement.

Future study considerations include assessment of survivors at time points earlier than 19 months, patients with active uveitis, and a larger sample size. While our study findings in 50 EVD survivors support that the expected true proportion of EBOV RNA-positive patients would be no greater than 5% in a comparable patient population, a larger sample size could provide greater reassurance to eye care providers performing invasive surgical procedures to restore vision. Specifically, the minimum number of subjects testing negative for EBOV RNA by RT-PCR to achieve a 95% confidence interval of 0.99–1.00 (i.e. expected proportion of EBOV RNA positive samples ≤1%) is 250.

In summary, the EVICT Study provides the first systematically collected evidence evaluating EBOV persistence in ocular fluid of EVD survivors. We also demonstrate that cataract surgery can be performed safely with vision restorative outcomes. Given the magnitude of the West African EVD outbreak and the thousands of survivors at risk of complications of uveitis, including cataract, efforts to address the ongoing ophthalmic medical and surgical needs of survivors are urgently needed.

Funding Sources

Supported by an unrestricted grant from Research to Prevent Blindness to the Emory Eye Center and National Eye Institute, National Institutes of Health Core Grant to Emory University (P30EY006360), Supported by the Building Interdisciplinary Careers in Women's Health Awards Program (JGS) and the Heed Ophthalmic Foundation (JGS).

Authors’ Contributions

Literature search - JGS, JM, KGB, FI, CK, SM, RF, JS, LAG, IC, TMU, DGB, RFG, MV, SY.

Figures - JGS, JM, AG, KGB, BRH, LAG, RR, MR, MT, IC, TMU, RFG, MV, SY.
Study design - JGS (Shantha), JGM, AG, KGB, FL, CK, BRH, JH, JGS (Shaffer), SM, AKC, RF, TOD, EK, TH, RR, MR, LAG, KO, SK, AW, MM, NA, WL, SB, GP, MT, KD, JC, IC, PEF, TMU, DGB, RFC, MVJ, SY.


Data analysis and interpretation - All authors contributed.

Manuscript drafting - JGS (Shantha), JM, AG, KGB, JH, JGS (Shaffer), TMU, DGB, IC, MV, SY.

Manuscript revision and approval of the final manuscript - All authors contributed.

Acknowledgments

We gratefully thank the patients in the EVICT Study and their families for accompanying them on their journey to the eye clinic and through the ophthalmic procedures. We are grateful to the Sierra Leone Association of Ebola Survivors organization and leadership. We also thank the Kissy United Methodist Church Hospital, Kissy UMC Medical Board, and Bishop John Yambasu; Christian Blind Mission International; Lowell and Ruth Gess Eye Hospital staff and eye care providers, Lead Outreach Administrator Mr. Ibrahim Conteh; Central Global Vision Fund; Ministry of Health and Sanitation, Sierra Leone; Chief Medical Officer Dr. Brima Kargbo for guidance in the EVICT Study; Program Implementation Unit, Comprehensive Program for Ebola Survivors, Ministry of Health and Sanitation; Partners in Health; Emory Eye Center, Emory Global Health Institute, Emory University School of Medicine, Dr. Allen Beck, Dr. Timothy Olsen, Dr. Alcides Fernandes, Mr. Andy Garrard, Mrs. Kimberly Monroe, Ms. Anita Hester; World Hope International; Kenema Government Hospital Lassa Fever Laboratory; Alcon Foundation; and Helen Keller International.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.03.020.

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