Letter to the Editor



Detection and Analysis of Ebola Virus in Sierra Leone-China Friendship Biosafety Laboratory from March 11 to April 20, 2015^{*}

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Ebola virus disease reemerged in Western Africa in 2014. Chinese Center for Disease Control and Prevention dispatched the first Ebola virus (EBOV) detection team to run newly established Sierra Leone-China Friendship Biological Safety Laboratory. The aims of study were to understand epidemiology, clinical manifestations and survival time of EBOV in patient's blood. A total of 913 specimens were tested between March 11 and April 20, 2015. EBOV positivity occurred in 7.37% of the blood and 0.53% in throat swabs. Most commonly reported symptoms of laboratory confirmed patients were intense fatigue, anorexia, and fever. EBOV RNAs persisted in blood for almost 4 weeks and the real-time RT-PCR Ct values showed close correlation with the sampling time after onset.

Ebola virus disease (EVD) is caused by Ebola virus (EBOV), a highly contagious RNA virus of the *Filoviridae* family, and it has be considered a most virulent and deadly pathogen since its discovery^[1]. In early 2014, EVD emerged in Western Africa, purportedly originating in Guinea^[2]. The epidemic quickly spread and become the largest known EVD outbreak to date^[3]. As of 21 April 2015, a total of 26,109 confirmed, probable, and suspected cases of EVD have been reported in a handful of countries in Africa (http://www.who.int/csr/disease/ebola/en/). A total of 10,835 deaths have been reported. Sierra Leone, Guinea, and Liberia are the countries with widespread and intense transmission^[3-4]. The most important control strategies included isolation

patients, implementation of infection control, and taking public health preventive measures, such as safe funeral, restrictions on travel, public gatherings, and market, restaurant, and business hours, etc.

The Chinese government began to offer large-scale medical aid to patients in the three most affected countries shortly after the EVD outbreak^[5]. Sierra Leone-China Friendship Biological Safety Laboratory (hereafter 'SLE-CHN Bio-safety Lab') was built since November 20, 2014 and put into use for EBOV detection on March 11, 2015. It contains a negative-pressure BSL-3 laboratory, а BSL-2 laboratory, and other two auxiliary laboratories. Fourteen virologists from the National Institute for Viral Disease Control and Prevention (IVDC), Chinese Center for Disease Control and Prevention (China CDC) formed the first team working in this laboratory.

The aims of the present study were to get more knowledge of the EBOV epidemiology, clinical manifestations, and the survival time of EBOV in patient's blood. During the study period (March 11-April 20, 2015), a total of 913 blood and throat swab samples were tested for EBOV and malaria at the SLE-CHN Bio-safety Lab. Of them, 44 were positive for EBOV and 82 were positive for malaria. Our data revealed a close correlation between the real-time reverse transcription-polymerase chain reaction (RT-PCR) Ct value of the blood sample and sampling timing after onset. Assay of the serially collected blood samples of several confirmed EVD

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cases illustrated that EBOV RNA persisted in the blood for almost 4 weeks after onset. The epidemiological and clinical features of the EVD patients were recorded.

The human specimens tested in the SLE-CHN Bio-safety Lab were collected by the sample center of the Ministry of Health and Sanitation, Sierra Leone. The blood specimens were collected from patients with suspected or lab confirmed EVD patients, while the throat swab specimens were collected from cadavers (one specimen per body). The Sierra Leone-China Friendship Biological Safety Laboratory had authorization from government of Sierra Leone to detect the human specimens collected by the sample center of the Ministry of Health and Sanitation, Sierra Leone. Blood samples were simultaneously tested for EBOV (Real-time RT-PCR method) and malaria (colloidal gold method), while throat swabs were tested for EBOV only. A commercial double-channel real-time RT-PCR detection kit (China Registration No.: 20143402058) that targets the GP and NP genes of EBOV (Zaire strain) was used for detecting EBOV^[6], which was originally designed and developed by IVDC, China CDC. The clinical and epidemiological data of each case were obtained from the clinical information form (CIF) that was submitted with each individual sample. Specimens were temporarily stored in 4 °C refrigerator before they were transported to the core area of the BSL-3 for EBOV detection. All the test results were reported within 24 h after collection of samples according to the requirements of the Ministry of Health and Sanitation of Sierra Leone.

The SLE-CHN Bio-safety Lab received 913 clinical samples (350 blood from 244 cases and 563 throat swabs from 563 cadavers) between March 11-April 20, 2015. Among the 350 blood samples, 244 samples were submitted from 244 suspected EVD cases (patients who had symptoms such as fever, headache, chills, and weakness). And among the 244 patients, 59 patients were collected twice if the first sampling time is within 3 days of onset, and 7 patients with confirmed EVD (patients whose samples were detected positive for EBOV) were sampled repeatedly in order to monitor the viral excretion status of the patient, and confirmed EVD patients can only be discharged from the hospital if their blood samples detected continuously negative at least twice. There were 21 EBOV-positive patients including 18 detected from blood samples (7.37%, 18/244) and 3 from swab samples (0.53%, 3/563). Screening for EBOV among the cadavers who were not considered as suspected EVD definitely benefits to cut off the potential transmission *via* traditional funeral^[7]. Additionally, a total of 59 malaria-positive cases (24.18%, 59/244) were detected. Obviously, more malaria cases were preliminarily suspected as EVD and needed to be distinguished during that period. Only two tested samples from two cases were positive for both EBOV and malaria, implying that co-infection of those two pathogens is uncommon in this period.

Among the 21 confirmed EVD cases, 17 were from Western Area Urban, three were from Western Area Rural, and only one was from Moyamba (Figure 1A). The cases of confirmed EVD at the SLE-CHN Bio-safety Lab showed obviously time-dependent decline during the six weeks study period. Among the 18 EVD patients with EBOV-positive in blood, six each were identified in the first 2 weeks, three in the third week, two in the fourth week, and one in the fifth week. No EVD case was found in the last week of the study (Figure 1B). The decline in positive EBOV specimens agreed well with the data of the entire country of Sierra Leone (Figure 1C), which may also reflect the achievement of success via the implementation of a strict strategy for EVD control and prevention in Sierra Leone^[8].

The onset ages of the 21 laboratory-confirmed EVD patients varied from 1 to 65 year-old (median, 29 years). The time from symptom onset to clinic visit of the 18 EVD patients with EBOV-positive blood samples were 1-9 days (median, 4 days). The reported clinical manifestations of those 18 EVD patients were diverse, and intense fatigue (16/18; 88.87%), anorexia (15/18; 83.33%) and fever (12/18, 66.67%) were most frequently noted. Various positions of pain (abdominal pain, joint pain, muscle pain, etc) were also frequently reported. In line with the other observations in this EVD outbreak in Western Africa^[9], hemorrhage-related manifestations appear in a relatively small proportion of the patients (seven presents with conjunctivitis). Those unspecific clinical manifestations of EVD cases in this outbreak may complicate the clinical diagnosis, highlighting again the importance in EBOV pathogenic detection for EVD diagnosis^[7].

The real-time RT-PCR Ct values of the first tested blood samples from the 18 EVD patients ranged from 16.94 to 38.31. There are strict quality control in the different experiments, and the difference of Ct value of the positive controls from different experiments were less than one, which guaranteed



---- Ebola positive specimens detected in whole contory in Sierra Leone

Figure 1. Epidemiological data of Ebola virus disease (EVD) patients detected in Sierra Leone-China Friendship Biosafety Laboratory (SLE-CHN Bio-Safety Lab), March 11-April 20, 2015. (A) Geographic distribution of 21 patients with laboratory-confirmed EVD. (B) Time-dependent decline in the number and percentage of patients with laboratory-confirmed EVD. (C) Ebola virus-positive cases in the SLE-CHN Bio-safety Lab and all of Sierra Leone.

the Ct value comparable obtained from different experiment performed in different time. То address the potential correlation between Ct values and sampling time after onset, all 18 EVD cases were divided into three groups: sampling within 5 days after onset (nine cases), 6-10 days (five cases), and >10 days (four cases). Our findings revealed a notable increase in Ct value with increasing sampling time after onset. The average Ct value in the cases within 5 days after onset was 22.08, whereas those of the groups of 6-10 days and >10 days were 30.61 and 34.40, respectively (Figure 2A). A statistical analysis illustrated a significant difference (P=0.0074) in Ct value among those three groups, indicating a close correlation between Ct value and sampling time after onset. This phenomenon is reasonable since severe viremia is usually present in the early phase of an acute viral infection^[10].

Serial blood specimens from seven laboratory-confirmed EVD patients were well studied, including three patients with four serial blood samples, one with five, one with seven, and two with ten. The times of the first samples of those seven cases received in the laboratory were 2-20 days after onset (Figure 2B). All cases showed obviously declining tendencies along with the prolonging clinical courses. Five of seven cases were EBOV RNA negative in the latest samples. The clearance times of EBOV RNA in the blood of these five patients were 21-30 days after onset (median, 27), highlighting a long clearance time. Although it remains impossible for us to definitely state the value of real-time RT-PCR Ct for judging the infectivity of the tested sample. Nevertheless, for such a virulent and fatal infectious disease, our results proved again that it is crucial to use real-time RT-PCR negativity of a blood sample as the discharge criterion for EVD patients.

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Competing Interests The authors declare that no competing interests exist.

Ethical Approval This study did not involve human participants or experimentation. The only human material used was blood specimens that

collected from EVD patients and the throat swab specimens that collected from cadavers at the instigation of the Ministry of Health and Sanitation of Sierra Leone for public health purposes. This study was approved by the second session of the Ethics Review Committee of the National Institute for Viral Disease Control and Prevention, China CDC, and the methods were carried out in accordance with the approved guidelines.



Figure 2. EBOV RNAs persisted in blood and the correlation between the real-time RT-PCR Ct values and the sampling time after onset. (A), Ct values of the first tested blood samples of 18 patients with Ebola virus disease (EVD). The patients with EVD were grouped according to sampling times after onset: 0-5 days, 6-10 days, and >10 days. The mean±SEM is shown in each group. (B), Clearance time of Ebola virus (EBOV) RNA from the blood samples of seven patients with laboratory-confirmed Ebola virus disease. The time (in days) after onset is shown on the x-axis and the Ct value is shown on the y-axis. The points in the curve of every case represent the EBOV sampling and testing points.

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REFERENCES

 Weyer J, Grobbelaar A, Blumberg L. Ebola virus disease: history, epidemiology and outbreaks. Curr Infect Dis Rep, 2015; 17, 1-8. 447

- Simon-Loriere E, Faye O, Faye O, et al. Distinct lineages of Ebola virus in Guinea during the 2014 West African epidemic. Nature, 2015; 524, 102-4.
- Shaman J, Yang W, Kandula S. Inference and forecast of the current west african ebola outbreak in Guinea, sierra leone and liberia. PLoS Curr, 2014; 6.
- Carroll MW, Matthews DA, Hiscox JA, et al. Temporal and spatial analysis of the 2014-2015 Ebola virus outbreak in West Africa. Nature, 2015; 524, 97-101.
- Li Y, Tang SL, Sato K, et al. Chinese People's Liberation Army on Action of Fighting against Ebola in Africa: Implications and Challenges. Chin Med J (Engl), 2015; 128, 1420.
- Li A, Li W, Li J, et al. Development of Real-time RT-PCR assay for detection of Zaire Ebolavirus. Chinese Journal of Experimental and Clinical Virology, 2014; 28, 321-3. (In Chinese)
- Siedner MJ, Gostin LO, Cranmer HH, et al. Strengthening the detection of and early response to public health emergencies: lessons from the West African Ebola epidemic. PLoS Med, 2015; 12, e1001804.
- Koroma M, Lv S. Ebola wreaks havoc in Sierra Leone. Infect Dis Poverty, 2015; 4, 10.
- Schieffelin JS, Shaffer JG, Goba A, et al. Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med, 2014; 371, 2092-100.
- Wang Y, Menne S, Baldwin BH, et al. Kinetics of viremia and acute liver injury in relation to outcome of neonatal woodchuck hepatitis virus infection. J Med Virol, 2004; 72, 406-15.