Seroprevalence of Ebola virus infection in Bombali District, Sierra Leone

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Abstract

A serosurvey of anti-Ebola Zaire virus nucleoprotein IgG prevalence was carried out among Ebola virus disease survivors and their Community Contacts in Bombali District, Sierra Leone. Our data suggest that the specie of Ebola virus (*Zaire*) responsible of the 2013-2016 epidemic in West Africa may cause mild or asymptomatic infection in a proportion of cases, possibly due to an efficient immune response.

Introduction

Ebola Virus Disease (EVD) spreads in communities through person-to-person transmission, with infection resulting from direct contact with blood, secretions, other body fluids or organs, and indirect contact with contaminated environmental materials or infected animals. In Sierra Leone, a total of 8704 confirmed cases and 3589 deaths were reported by the end of December 2015,[1] of which 307 (221 deaths) were among health care workers (HCWs).[2] In Bombali District, located in the northern region of the country, a total of 1050 confirmed EBV cases have been cumulatively reported since May 2013 to the end of December 2015. Retrospective serologic data[3] suggest that EBOV might have been in circulation in Sierra Leone since 2006.

The role of antibody responses in viral clearance and protection against Ebola Virus (EBOV) in humans is not fully understood. Among EVD survivors, antibodies appear as early as day-5 after symptom onset, peak 2 weeks after recovery, then decline slowly over several years.[4-7] In patients with fatal outcome, antibody titers are low or absent.[8] The presence of detectable anti-EBOV antibodies in asymptomatic individuals suggests exposure and a putative role of antibody response in the control of EVD and provides information on EBOV seroprevalence in at risk populations.[9] Monitoring anti-EBOV antibodies in EVD-community contacts (CCs) of index cases is crucial for assessing immune protection against EBOV. Due to the large number of infected HCWs, evaluation of anti-Ebola antibodies in this group is a useful tool for measuring the risk of occupational exposure to EBOV.

The aim of the study was to assess the extent of asymptomatic or mild cases of EBOV infection among the CCs of laboratory confirmed EVD cases (survivors or deceased) and in HCWs from health facilities not involved in the management of EVD cases.

Materials and Methods

Study populations

The study was conducted between February and March 2015 in the rural area of Sanda Loko Chiefdom, in the villages of Kamalo, Maron and Makasa (Figure 1) in the context of Ebola survivor Mobile Health Clinic program aimed to provide integrated primary healthcare services to address the medical and psychosocial needs of Ebola survivors living in areas with low medical coverage.[10]

A total of 6 EVD-deaths and 10 EVD-survivors were reported (Table 1). EVD cases were reported from Kamalo (4 deaths and 10 survivors) and Maron (2 deaths) villages, whereas no cases were reported from Makasa village (Table 2). EVD survivors were defined as individuals who showed EVD clinical signs or symptoms with EBOV infection confirmed by RT-PCR assay. Considering the organization of the local rural society, CCs were individuals from communities placed under quarantine.

In addition, seventy-nine HCWs were included in this study from Holy Spirit Hospital (n=59) and Loreto Clinic (n=20), two religious health care facilities in Makeni municipality (headquarter of Bombali District) that were not involved in the management of EVD cases (Table 1). The definition of HCWs included physicians, nurses, laboratory technicians, administrative personnell and cleaners (Table 1).

The Ethical Review Board of the Ministry of Health and Sanitation of Sierra Leone approved this study, and participants gave their approval by signing or fingerprinting a written consent form. Demographic data and information about clinical history were collected using pre-

tested questionnaires before obtaining 4 ml of peripheral blood by venipuncture.

Anti Ebola virus antibody level

Anti-Zaire Ebola Nucleoprotein (ZEBOV-NP) IgG antibodies were quantified using Enzyme-Linked Immunosorbent assay (ELISA) by a commercial kit (AE320620-1, Alpha Diagnostic International [ADI], Texas, USA), according to the manufacturer's instructions. Positive control and calibrators provided by the kit were used in each test run. Optimal sample dilution was previously determined at 1:500 by testing 68 positive control plasma samples from EVD-survivors and 10 negative control plasma samples from expatriates not exposed to Ebola infection.

Threshold index to discriminate positive and negative antibody reactivity to ZEBOV NP was calculated following manufacturer's instructions.

Statistical analysis

Data were collected and entered in a customized template in EpiData software version 3.1. The prevalence was calculated by dividing the number of seropositive cases by the number of examined individuals. Comparison among frequency of seropositive CCs from different villages was performed by one-tail Fisher's exact test assuming the hypothesis of seroprevalence association.

Results and Discussion

Among Sanda Loko EVD survivors, all those enrolled from Kamalo were ZEBOV-NP-IgG positive (n=4/4). Anti-ZEBOV-NP-IgG were detected in 12 out of the 105 tested CCs (11.4% seroprevalence rate) (Table 1). As described in Table 2, the seroprevalence for ZEBOV-NP-IgG in CCs from different villages was 5.9% (n=3/51) in Kamalo, 20.0% (n=7/35) in Maron, and 10.5% (n=2/19) in Makasa.

Among HCWs (Table 1), no ZEBOV-seropositive cases were found in the Loreto Clinic group (n=0/20), whereas 3 seropositive cases (1 nurse and 2 cleaners) out of 59 (5.8%) were found at the Holy Spirit Hospital. Overall, the seroprevalence among HCWs was 3.8% (n=3/79).

ZEBOV-seropositive CCs did not report fever or other symptoms/signs suggestive of EVD during the previous 8 months. Three of them reported mild, non-specific symptoms, including headache (n=1), joint pain (n=1), or both (n=1). Risk behaviours, such as hunting activity in the forest, or participating in a burial service during the past 8 months, were reported by one and two per-

sons respectively.

Although the knowledge of Ebola dynamics, pathogenesis and clinical course is improving, some aspects remain unclear, including the role for naturally-acquired humoral immunity.

The influence of humoral immunity on EVD outcome was observed in Gabon[8] and confirmed by a serosurvey in Uganda.[7] A larger EBOV serosurvey in Gabon[9] showed significant seroprevalence across the country even in areas considered Ebola-free, and raised important questions about EBOV spread, virulence and the existence of natural protective immunization.

The overall seroprevalence rate among asymptomatic CCs in the area of Sanda Loko Chiefdom was 11.4% with significant differences observed among the three villages. Anti-EBOV-positive subjects were found in all villages with or without confirmed EVD cases. Interestingly, the highest seroprevalence rates was observed in villages with the lowest incidence of EVD

cases (P<0.05, Table 2) suggesting that EBOV circulated in those populations without causing disease.

In this paper we report a high seroprevalence in asymptomatic CCs of Sanda Loko Chiefdom although we have recently shown that only 2.6% (10 of 388) of asymptomatic members of Ebola-affected households had evidence of Ebola virus infection in Sierra Leone.[11] The extend of asymptomatic EBOV infection is still unclear and several studies reported a wide variability of seroprevalence (from 1% to 46%),[9,12-14] among the household and CCs. This high variability can be ascribed to the different antigens and assays used and also the definition of contact varied in the studies.

The seroprevalence rate among all HCWs was 3.8% even if the health facilities where they worked were not included in the network for managing EVD cases. We cannot rule out that they may have been exposed to EVD outside the work place. The observed moderate grade of EBOV

Table 1. Prevalence of ZEBOV-seropositivity in EVD survivors, community contacts and health care workers.

	N	Tested (n)	Seropositives (n)	Seroprevalence (%)
Sanda Loko EVD-survivors	10*	4	4	100
Sanda Loko EVD deaths	6	-	-	-
Sanda Loko CCs	NA	105	12	11.4
Total HCWs	79	79	3	3.8
Holy Spirit Hospital	59	59	3	5.8
Loreto Clinic	20	20	0	0.0

*4 survivors were enrolled and tested in this study. NA, not available.

Table 2. EVD deaths and survivors, community contacts and seroprevalence in Sanda Loko Chiefdom villages.

Village	Deaths (n)	Survivors (n)	CCs (n*)	Anti-ZEBOV positive CCs (n)	Seroprevalence (%) among CCs
Kamalo	4	10°	51	3	5.9#
Maron	2	0	35	7	20.0
Makasa	0	0	19	2	10.5

*CC (n) refers to the number of Community Contacts included in this study; °four survivors were enrolled and tested in this study; #P<0.05.

Figure 1. Maps of Sierra Leone, Bombali District and Sanda Loko Chiefdom. Kamalo and Makasa villages are reported in local language as Kamalu and Makassa respectively.

transmission in HCWs needs further analysis of larger groups, along with assessing the adherence to use of personal protection (gowns, gloves, masks). This data is consistent with previous serosurvey performed in Gabon[9] and led to hypothesize that the host-EBOV interaction might determine the development of specific antibodies capable to control the infection, leading to mild or asymptomatic EVD.

Our findings that anti-ZEBOV-IgG was detected in both EVD-survivors and CCs, although at different prevalence rates, suggests that the immunity, previously reported as persistent among survivors,[7] may be important for protection that allowed subclinical disease among CCs.

Conclusions

Our study provides significant new insight into population exposure to EBOV and outcomes of virus infection. This causes us to question the assumption that EBOV is highly fatal. Instead, EBOV, similar to Lassa Fever virus[15,16] and many other viral pathogens, may have high fatality as a proportion of clinical cases but relatively high seroprevalence among persons without evident disease arguing the fatality rate is actually lower than has been predicted. These findings raise important questions about EBOV pathogenicity and argue that some individuals have achieved long lasting protection following attenuated infection[7] that should be taken in account for vaccine programs.

There is a strong rationale for expanding the serological screening of exposed populations and HCWs in countries affected by EVD to understand their risk of infection and to guide the implementation of quarantine when needed, and vaccine programs when available.

Ebola Study Group

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