The Journal of Infectious Diseases

EDITORIAL COMMENTARY

Ebola Mysteries and Conundrums

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(See the Major Article by Hoff et al on pages 517–25.)

Keywords. Ebola; Ebola virus seroprevalence; Ebola immunoassays; healthcare workers.

In this issue of *The Journal of Infectious Diseases,* Hoff et al report results of a survey of serological markers for Ebola virus (EBOV) among healthcare workers in the Boende Health Zone of the Democratic Republic of the Congo (DRC) [1]. The study was conducted in the aftermath of the seventh outbreak of Ebola in the DRC since the discovery of EBOV there in 1976. This outbreak occurred between 24 August 2014 and 20 November 2014 and included 38 laboratory-confirmed cases and 28 probable cases with 49 deaths (nominal case-fatality rate, 74%) in the villages of Watsi Kengo, Lokolia, Boende, and Boende Muke of Equateur Province. While this outbreak in the DRC occurred simultaneously with the massive 2013–2016 West African outbreak, EBOV genomic sequencing revealed no epidemiological link between the outbreaks [2]. Inexplicably, 2014 Boende EBOV is phylogenetically similar to EBOVs isolated during the 1995–1996 outbreaks in Middle Africa. Such sequence similarity is much greater than expected, given the rate of EBOV evolution determined previously [3], indicating that the EBOV causing the Boende outbreak evolved at a lower rate than EBOVs involved in subsequent outbreaks. The now uncertain evolutionary trajectory of EBOV is only one of many mysteries and conundrums surrounding Ebola.

One of the major unknowns in Ebola is the provenance of serological markers to EBOV in persons with no known disease or infection. Some people with no known history of Ebola or who live in areas where no Ebola outbreak has occurred produce antibodies that react, often strongly, with EBOV proteins. Proven explanations for the presence of these markers are lacking, but it is not for lack of trying. The serosurvey by Hoff et al is not the first to find EBOV markers among a substantial portion of persons not known to have ever been infected with EBOV. Indeed, in a meta-analysis published in January 2017, Bower and Glynn [4] identified 51 prior studies covering 84 distinct populations and >44 000 subjects from areas with no known outbreaks. Seroprevalences in regions of nonendemicity as high as 32% by immunofluorescence assay (IFA) [5] and 17% by enzyme-linked immunosorbent assay (ELISA) [6] has been noted and is rarely 0% [4]. Complicating interpretations of these many studies are wide variances in sampling strategies and immunoassay methods. For example, the current study is one of the few to assay immune responses to >1 EBOV protein. The researchers measured responses to EBOV glycoprotein (GP), nucleoprotein, and viral protein 40. Additionally, the researchers assessed the potential of serum from the subjects in their cohort to neutralize pseudoparticles that were dependent on EBOV GP for cell entry. It is revealing that most subjects (168 of 303) whose sera were reactive in any ELISA were reactive to only a single EBOV protein. Sera from only 6 subjects reacted to all 3 proteins. This suggests (but does not prove) that most subjects were not exposed to intact replication-competent EBOV. Rather, it is likely that, for most subjects in this cohort, their exposure(s) was to disrupted noninfectious particles or to non-EBOV proteins that share a limited number of cross-reactive epitopes with a subset of EBOV proteins.

One of the major Ebola mysteries is the identity of EBOV's reservoir species. Fruit bats have been implicated as reservoirs and vectors for transmission of filoviruses in Africa. EBOV sequences and antibodies have been found in fruit bats (*Hypsignathus monstrosus, Epomops franqueti*, and *Myonycteris torquata*) [7]. However, the inability to date to culture infectious EBOV from bats precludes definitively assigning any bat species as the EBOV reservoir. One hypothesis to explain the presence of serological responses to EBOV in humans is the exposure to EBOV on fruit such as mangoes. Filoviruses that are present in bat saliva would be expected to be transferred to fruit and then may be inactivated by sunlight, heat, drying, or the enzymes present in the saliva or fruits themselves. EBOV proteins subjected to these conditions may still retain some antigenicity, and oral exposure to the proteins could induce a humoral immune response, and repeated exposures could boost this response. Whether this explains the seroprevalence rates observed in serosurveys by Hoff et al and others must await identification of the EBOV reservoir.

Received 30 July 2018; editorial decision 30 July 2018; accepted 30 July 2018; published online August 4, 2018.

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The Journal of Infectious Diseases® 2019;219:511–3
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DOI: 10.1093/infdis/jiy476

It is possible that some subjects studied by Hoff et al and in prior serosurveys were exposed to filoviruses that are only distantly related to EBOV. Like EBOV, the filoviruses Sudan virus and Bundibugyo virus have been associated with hemorrhagic fever outbreaks in Africa. Filoviruses that appear nonpathogenic for humans, such as Tai Forest virus and Reston virus, may also circulate in Africa, Southeast Asia, and other locations. Known filoviruses likely represent only a fraction of the diversity of the *Filoviridae*. In 2002, colonies of Schreiber's bats (*Miniopterus schreibersii*) sustained massive die-offs in caves in France, Spain, and Portugal [8]. A genetically distinct filovirus named Lloviu virus was detected during the investigation of these bat die-offs. Filoviruses also appear to be present in wide variety of Philippine bats, but prevalence rates and virus loads are low [9]. A better understanding of the species distribution of filoviruses could inform studies to explain EBOV seroprevalence rates in humans. Furthermore, filoviruses are ancient viruses whose sequences entered the germ lines of diverse animals tens of millions of years ago [10]. The recent discovery of a filovirus in a ray-finned fish confirms that filoviruses have ancient evolutionary histories [11]. It is possible that the EBOV GP responses that dominated in the cohort of Hoff et al represent exposures to nonfiloviruses, as well. Boid snakes (boas and pythons) carry arenaviruses with GPs that are more closely related to the GP of EBOV than to GPs of mammalian arenaviruses [12].

Hoff et al are correct that there are no gold standards for EBOV serological testing. Better standards for EBOV serological assays would be valuable, particularly for comparing results of serological surveys. In this regard, the authors are to be commended for paying more attention than most to immunoassay cutoffs. All immunoassays must have a cutoff, and where the cutoff is set can dramatically affect the interpretation of any serosurvey. Underappreciated is the fact that nucleic acid–based tests also must have cutoffs—cycle thresholds over a certain level represent background noise. Thus, polymerase chain reaction tests (even quantitative polymerase chain reaction analyses) can fail as gold standards [13]. Moreover, simply having better standards and stricter cutoffs will not answer all of the conundrums regarding the presence of serological responses to EBOV in nonoutbreak populations. Answers to the questions of whether the serological responses are due to a subclinical infection, exposure to inactivated or disrupted virus, cross-reactivity to a nonpathogenic filovirus, or other spurious cross-reactivities will not be answered by more-precisely calibrated tests. To answer these questions, new techniques will need to be applied to the problem. Serological profiling of the epitopes recognized by subjects of serosurveys could shed light on these questions. Persons exposed to replicating EBOV would be expected to make polyclonal responses to all or most proteins; several epitopes on multiple proteins will be recognized. Persons exposed to a related nonpathgenic filovirus would be expected to recognize fewer EBOV epitopes, only epitopes on conserved structures, or, in the case of linear epitopes, conserved sequences. If the serological responses are spurious cross-reactions to nonfilovirus proteins (or other macromolecules, including glycans), then the number of epitopes recognized should be few. Scanning mutagenesis has been applied to EBOV proteins [14], and techniques using a panel of mutated proteins could be used to compare the breath of responses to EBOV proteins in confirmed Ebola survivors and persons with serological responses but no documented infection. Likewise, single-particle electron microscopy is increasing being used to map epitopes [15] and could be adapted to profile the breath of epitopes recognized. It may also prove possible to compare the immune repertoire to EBOV in known survivors and those without known disease, using high-throughput sequencing techniques [16].

There is still much to learn about EBOV and its interactions with animals and humans. EBOV is good at evading the immune system [17] and encodes at least 1 toxic protein [18], but the mechanism(s) for its high mortality rate is (are) not established. We do not yet know whether any drug or drug combination can meaningfully influence survival in an outbreak situation. Although the recombinant vesicular stomatitis virus—based Zaire EBOV vaccine is promising [19], we also do not know whether it is possible to provide durable protection against Ebola with any vaccine. Important studies such as those reported here by Hoff et al will continue to chip away at Ebola's many mysteries and conundrums.

Notes

Financial support. This work was supported by the National Institutes of Health (grants AI067188, AI082778, AI088843, AI104621, and AI115754, and contracts HSN272200900049C and HHSN272201400048C).

Potential conflicts of interest. R. F. G. is the program manager of the Viral Hemorrhagic Fever Consortium (available at: http://www.vhfc.org), a public-private partnership of academic and industry scientists who are developing diagnostic tests, therapeutic agents, and vaccines for Lassa fever, Ebola, and other severe diseases. Tulane University and its various academic and industry partners have filed US and foreign patent applications on behalf of the consortium for several of these technologies. Technical information may also be kept as trade secrets. If commercial products are developed, consortium members may receive royalties or profits. This does not alter his adherence to all policies of the National Institutes of Health and *The Journal of Infectious Diseases* on sharing data and materials. The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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