Supplementary Materials for

Emerging Disease or Diagnosis?


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This PDF file includes:

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References S1
Figs. S1 and S2
Tables S1 to S3
Note S1
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References S1

Introducing emerging diseases


2. [http://www.niaid.nih.gov/topics/emerging/Pages/list.aspx](http://www.niaid.nih.gov/topics/emerging/Pages/list.aspx)

Recognizing possibility of overlooked pathogens


Factors in disease emergence


Symptoms of Lassa fever and Ebola


**Malaria overdiagnosis**


**Lassa fever outbreak due to misdiagnosis**


**Discovery of Lassa, Ebola, and Marburg viruses**


**Example of an emerging disease**


**General background on use of IFA and ELISA in Lassa fever and Ebola**


doi:10.1016/S0140-6736(10)60667-8 Medline

*Seroprevalence of Lassa virus*


doi:10.1007/s004300100061 Medline


doi:10.1093/infdis/155.3.437 Medline


**Epidemiology of Lassa fever**


**Seroprevalence of Ebola virus**


   doi:10.1016/S0140-6736(82)91871-2 Medline

   doi:10.1016/0035-9203(82)90089-X Medline


   doi:10.1016/0035-9203(82)90113-4 Medline


   doi:10.1016/S0923-2516(89)80112-8 Medline

   doi:10.1016/0035-9203(89)90519-1 Medline


Also references 24, 25, and 31

Lassa virus reservoir: Mastomys natalensis


**Proposed Ebola reservoirs**

   doi:10.1038/438575a Medline


   doi:10.1089/vbz.2008.0167 Medline
**Data on Animal Habitats**

   <http://www.iucnredlist.org>. Downloaded on 10 January 2012

**Enduring Transmission in Animal Outbreak**

   doi:10.1126/science.1133105 Medline

85. E. M. Leroy et al., Multiple Ebola virus transmission events and rapid decline of central

    doi:10.1371/journal.pbio.0030371 Medline

87. E. M. Leroy et al., A serological survey of Ebola virus infection in central African nonhuman

**Worldwide distribution of filoviruses and arenaviruses**

88. T. Briese et al., Genetic detection and characterization of Lujo virus, a new hemorrhagic
    doi:10.1371/journal.ppat.1000455 Medline

89. A. T. B. Peterson, J. T. Bauer, J. N. Mills, Ecologic and geographic distribution of filovirus

90. A. Negredo et al., Discovery of an ebolavirus-like filovirus in europe. PLoS Pathog. **7**, 
    e1002304 (2011). doi:10.1371/journal.ppat.1002304 Medline

91. F. D. Mathiot CC, Georges AJ, Coulanges P, Antibodies to haemorrhagic fever viruses in
Filovirus integration into mammalian genomes


Lassa and Ebola virus genome evolution


Natural selection on malaria resistance

Evidence for natural selection at the gene LARGE


Role of LARGE in Lassa fever pathogenesis


Evidence of natural selection for other genes linked to viral infection


   
   doi:10.1371/journal.pbio.0020275 Medline

   
   doi:10.1007/978-3-642-02175-6_3 Medline


   
   doi:10.1016/j.tim.2010.06.010 Medline

   
   doi:10.1371/journal.ppat.1000443 Medline


   
   doi:10.1371/journal.ppat.1000300 Medline

**Evidence for natural resistance to Lassa virus**


**Evidence for natural resistance to Ebola virus**


**Examples of surveillance and vaccine efforts**


**Current drug treatment for Lassa fever**


**Evidence for utility of early diagnosis to changing patient outcomes**


   doi:10.1016/S0163-4453(95)90670-3 Medline


**Lassa fever efforts in Sierra Leone and Nigeria**


Lassa Fever in Nigeria 2012


Figure S1. Current list of emerging viruses (1, 2).

<table>
<thead>
<tr>
<th>Arenavirus</th>
<th>Herpesvirus</th>
</tr>
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<tbody>
<tr>
<td>Lassa Virus</td>
<td>Human herpesvirus-6/7-7/8</td>
</tr>
<tr>
<td>Lujo Virus (Zambia)</td>
<td>Herpes Simplex- 1/2</td>
</tr>
<tr>
<td>Junin, Machupo, Guanarito and Sabia</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Bunyavirus</th>
<th>Orthomyxovirus</th>
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<tbody>
<tr>
<td>California Serogroup Viruses</td>
<td>Influenza</td>
</tr>
<tr>
<td>Crimean-Congo (CCHF) Virus</td>
<td></td>
</tr>
<tr>
<td>Hanta Virus</td>
<td>Papillomavirus (HPV)</td>
</tr>
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<td>Rift Valley Fever</td>
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<table>
<thead>
<tr>
<th>Calicivirus</th>
<th>Paramyxovirus</th>
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</thead>
<tbody>
<tr>
<td>Noroviruses</td>
<td>Hendra or equine morbilli virus</td>
</tr>
<tr>
<td>SARS</td>
<td>Measles</td>
</tr>
<tr>
<td></td>
<td>Mumps</td>
</tr>
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<td>Nipah Virus</td>
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<tr>
<th>Coronavirus</th>
<th>Parvovirus</th>
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<td>Enterovirus</td>
<td>Human Parvovirus B19</td>
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<table>
<thead>
<tr>
<th>Filovirus</th>
<th>Reovirus</th>
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<tr>
<td>Ebola Virus</td>
<td>Rotaviruses</td>
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<tr>
<td>Marburg Virus</td>
<td></td>
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<td>Lluvio Virus (Spain)</td>
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<table>
<thead>
<tr>
<th>Flavivirus</th>
<th>Retrovirus</th>
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<tbody>
<tr>
<td>Dengue Virus</td>
<td>Human T-Cell Leukemia Virus (HTLV)</td>
</tr>
<tr>
<td>Hepatitis C Virus</td>
<td>HIV-1/2</td>
</tr>
<tr>
<td>Japanese Equine Encephalitis (JEE)</td>
<td></td>
</tr>
<tr>
<td>Omsk, Kyasanur (tick-borne)</td>
<td></td>
</tr>
<tr>
<td>Yellow Fever Virus</td>
<td></td>
</tr>
<tr>
<td>West Nile Virus</td>
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</tr>
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<table>
<thead>
<tr>
<th>Hepadnavirus</th>
<th>Rhabdovirus</th>
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</thead>
<tbody>
<tr>
<td>Hepatitis B Virus</td>
<td>Australian bat lyssavirus</td>
</tr>
<tr>
<td></td>
<td>Irkut Virus</td>
</tr>
<tr>
<td></td>
<td>Khujand Virus</td>
</tr>
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<td></td>
<td>Rabies Virus</td>
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<table>
<thead>
<tr>
<th>Hepevirus</th>
<th>Togavirus</th>
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</thead>
<tbody>
<tr>
<td>Hepatitis E Virus</td>
<td>Chikungunya Virus</td>
</tr>
<tr>
<td></td>
<td>Eastern Equine Encephalitis (EEE)</td>
</tr>
<tr>
<td></td>
<td>Ross River Virus</td>
</tr>
<tr>
<td></td>
<td>Venezuelan Equine Encephalitis (YEE)</td>
</tr>
<tr>
<td></td>
<td>Western Equine Encephalitis (WEE)</td>
</tr>
</tbody>
</table>
**Figure S2.** Ebola and Lassa seroprevalence and distribution of animal habitats.

Lassa (A) and Ebola (B) seroprevalence levels as identified by IgG in ELISA-based studies are represented by countries highlighted in gradated orange - darker being higher seroprevalence. (See Table S1-2 and References. We show only estimates from studies based on ELISA, as studies performed with IFAT may not as accurately reflect prevalence rates), as well as distribution of animal habitats. In A, while it is not fully known what regions or subspecies of *Mastomys natalensis* may carry Lassa Fever, the striped area represents the Lassa risk map developed by Fichet-Calvet *et al.* based on climate and annual rainfall (74). In B, great ape habitats include *Gorilla gorilla*, *Pan paniscus*, and *Pan troglodyte* species. Bat habitats include *Epomops franqueti*, *Hypsognathus monstrosus*, and *Myonycteris torquata* species. Animal habitat data is from the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (83).
Table S1. Lassa virus seroprevalence in African countries.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cohort Size</th>
<th>Test</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>Guinea &amp; Nigeria</td>
<td>75</td>
<td>IFA</td>
<td>23%</td>
<td>27</td>
</tr>
<tr>
<td>N/A</td>
<td>Uganda</td>
<td>50</td>
<td>IFA</td>
<td>0%</td>
<td>27</td>
</tr>
<tr>
<td>1978</td>
<td>Sierra Leone</td>
<td>953</td>
<td>IFA</td>
<td>26%</td>
<td>28</td>
</tr>
<tr>
<td>1980-1982</td>
<td>Liberia</td>
<td>1491</td>
<td>IFA</td>
<td>6.5%</td>
<td>29</td>
</tr>
<tr>
<td>1987</td>
<td>Sierra Leone</td>
<td>3456</td>
<td>IFA</td>
<td>8-52%</td>
<td>30</td>
</tr>
<tr>
<td>1988</td>
<td>Nigeria</td>
<td>1677</td>
<td>IFA</td>
<td>21.3%</td>
<td>31</td>
</tr>
<tr>
<td>1990-92</td>
<td>Guinea</td>
<td>3126</td>
<td>ELISA</td>
<td>3.4-54.9%</td>
<td>32</td>
</tr>
<tr>
<td>1993</td>
<td>Guinea (Southeast)</td>
<td>232</td>
<td>IFA</td>
<td>2.6%</td>
<td>33</td>
</tr>
<tr>
<td>1993</td>
<td>Guinea (Northwest)</td>
<td>751</td>
<td>IFA</td>
<td>14%</td>
<td>33</td>
</tr>
<tr>
<td>2000</td>
<td>Guinea</td>
<td>977</td>
<td>IFA</td>
<td>12%</td>
<td>34</td>
</tr>
<tr>
<td>2007</td>
<td>Guinea</td>
<td>213</td>
<td>ELISA</td>
<td>12-20%</td>
<td>35</td>
</tr>
<tr>
<td>2007</td>
<td>Ivory Coast</td>
<td>50</td>
<td>ELISA</td>
<td>20%</td>
<td>35</td>
</tr>
<tr>
<td>2007</td>
<td>Ghana</td>
<td>480</td>
<td>ELISA</td>
<td>3.8%</td>
<td>35</td>
</tr>
<tr>
<td>2007</td>
<td>Benin</td>
<td>101</td>
<td>ELISA</td>
<td>9.9%</td>
<td>35</td>
</tr>
<tr>
<td>2007</td>
<td>Nigeria</td>
<td>116</td>
<td>ELISA</td>
<td>10.3%</td>
<td>35</td>
</tr>
</tbody>
</table>

*Year samples were collected
Table S2. Ebola virus seroprevalence in Africa, Asia and Central America since 1961

<table>
<thead>
<tr>
<th>Year*</th>
<th>Country</th>
<th>Cohort Size</th>
<th>Test</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961-62</td>
<td>Ethiopia</td>
<td>277</td>
<td>IFA</td>
<td>19.8%</td>
<td>40</td>
</tr>
<tr>
<td>1977</td>
<td>DRC</td>
<td>984</td>
<td>IFA</td>
<td>4%</td>
<td>41</td>
</tr>
<tr>
<td>1977</td>
<td>Sudan (Maridi)</td>
<td>214</td>
<td>IFA</td>
<td>33%</td>
<td>41</td>
</tr>
<tr>
<td>1977</td>
<td>Sudan (Nzara)</td>
<td>218</td>
<td>IFA</td>
<td>6.4%</td>
<td>41</td>
</tr>
<tr>
<td>1977</td>
<td>Zimbabwe</td>
<td>243</td>
<td>IFA</td>
<td>3%</td>
<td>41</td>
</tr>
<tr>
<td>1977</td>
<td>Panama</td>
<td>200</td>
<td>IFA</td>
<td>2%</td>
<td>41</td>
</tr>
<tr>
<td>1978</td>
<td>DRC</td>
<td>1096</td>
<td>IFA</td>
<td>7.2%</td>
<td>42</td>
</tr>
<tr>
<td>1978</td>
<td>Liberia</td>
<td>481</td>
<td>IFA</td>
<td>6%</td>
<td>24</td>
</tr>
<tr>
<td>1979</td>
<td>CAR</td>
<td>1344</td>
<td>IFA</td>
<td>3%</td>
<td>25</td>
</tr>
<tr>
<td>1979</td>
<td>Sudan</td>
<td>23</td>
<td>IFA</td>
<td>25%</td>
<td>43</td>
</tr>
<tr>
<td>1979-82</td>
<td>CAR</td>
<td>1909</td>
<td>IFA</td>
<td>4.5%</td>
<td>44</td>
</tr>
<tr>
<td>1980</td>
<td>Kenya</td>
<td>84</td>
<td>IFA</td>
<td>5%</td>
<td>45</td>
</tr>
<tr>
<td>1980</td>
<td>Gabon</td>
<td>253</td>
<td>IFA</td>
<td>6.3%</td>
<td>46</td>
</tr>
<tr>
<td>1980</td>
<td>Cameroon</td>
<td>1517</td>
<td>IFA</td>
<td>9.7%</td>
<td>47</td>
</tr>
<tr>
<td>1980</td>
<td>CAR</td>
<td>499</td>
<td>IFA</td>
<td>3.4%</td>
<td>48</td>
</tr>
<tr>
<td>1980</td>
<td>Zimbabwe</td>
<td>486</td>
<td>IFA</td>
<td>1.8%</td>
<td>49</td>
</tr>
<tr>
<td>1981</td>
<td>Kenya</td>
<td>1899</td>
<td>IFA</td>
<td>1.4%</td>
<td>50</td>
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<tr>
<td>1981-82</td>
<td>Liberia</td>
<td>225</td>
<td>IFA</td>
<td>13%</td>
<td>51</td>
</tr>
<tr>
<td>1981-85</td>
<td>DRC</td>
<td>137</td>
<td>IFA</td>
<td>1%</td>
<td>52</td>
</tr>
<tr>
<td>1982-83</td>
<td>Guinea</td>
<td>138</td>
<td>IFA</td>
<td>8%</td>
<td>53</td>
</tr>
<tr>
<td>1983</td>
<td>Ethiopia</td>
<td>250</td>
<td>IFA</td>
<td>0%</td>
<td>40</td>
</tr>
<tr>
<td>1984</td>
<td>CAR</td>
<td>296</td>
<td>IFA</td>
<td>2.6%</td>
<td>54</td>
</tr>
<tr>
<td>1984</td>
<td>Kenya</td>
<td>471</td>
<td>IFA</td>
<td>10%</td>
<td>55</td>
</tr>
<tr>
<td>1984</td>
<td>Uganda</td>
<td>132</td>
<td>IFA</td>
<td>4.5%</td>
<td>56</td>
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<tr>
<td>1984-86</td>
<td>Botswana</td>
<td>154</td>
<td>IFA</td>
<td>0%</td>
<td>57</td>
</tr>
<tr>
<td>1985</td>
<td>Gabon</td>
<td>213</td>
<td>IFA</td>
<td>9.4%</td>
<td>54</td>
</tr>
<tr>
<td>1985</td>
<td>CAR</td>
<td>659</td>
<td>IFA</td>
<td>22%</td>
<td>54</td>
</tr>
<tr>
<td>1985</td>
<td>Cameroon</td>
<td>375</td>
<td>IFA</td>
<td>2%</td>
<td>58</td>
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<td>1987</td>
<td>CAR</td>
<td>4295</td>
<td>IFA</td>
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<td>59</td>
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<tr>
<td>1987</td>
<td>CAR</td>
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<td>IFA</td>
<td>17.6%</td>
<td>59</td>
</tr>
<tr>
<td>1986-87</td>
<td>Nigeria</td>
<td>1677</td>
<td>IFA</td>
<td>1.7%</td>
<td>31</td>
</tr>
<tr>
<td>1987</td>
<td>Chad</td>
<td>334</td>
<td>IFA</td>
<td>3.6%</td>
<td>60</td>
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</table>
**Table S2 (Continued).** Ebola virus seroprevalence in Africa, Asia and Central America since 1961

<table>
<thead>
<tr>
<th>Year*</th>
<th>Country</th>
<th>Cohort Size</th>
<th>Test</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Cameroon</td>
<td>1152</td>
<td>IFA</td>
<td>7.7%</td>
<td>60</td>
</tr>
<tr>
<td>1987</td>
<td>DRC</td>
<td>728</td>
<td>IFA</td>
<td>10%</td>
<td>60</td>
</tr>
<tr>
<td>1987</td>
<td>Equatorial Guinea</td>
<td>688</td>
<td>IFA</td>
<td>16.1%</td>
<td>60</td>
</tr>
<tr>
<td>1988</td>
<td>Madagascar</td>
<td>381</td>
<td>IFA</td>
<td>4%</td>
<td>61</td>
</tr>
<tr>
<td>1989-90</td>
<td>Phillipines</td>
<td>186</td>
<td>IFA</td>
<td>6%</td>
<td>62</td>
</tr>
<tr>
<td>1995</td>
<td>CAR</td>
<td>1331</td>
<td>ELISA</td>
<td>5.3%</td>
<td>63</td>
</tr>
<tr>
<td>1995</td>
<td>DRC</td>
<td>414 City</td>
<td>ELISA</td>
<td>2.2%</td>
<td>64</td>
</tr>
<tr>
<td>1995</td>
<td>DRC</td>
<td>161 Village</td>
<td>ELISA</td>
<td>9.3%</td>
<td>64</td>
</tr>
<tr>
<td>1996</td>
<td>Phillipines</td>
<td>231</td>
<td>ELISA</td>
<td>0.4%</td>
<td>65</td>
</tr>
<tr>
<td>1996</td>
<td>Gabon</td>
<td>236 (Jan)</td>
<td>ELISA</td>
<td>10%</td>
<td>66</td>
</tr>
<tr>
<td>1996</td>
<td>Gabon</td>
<td>205 (Spring)</td>
<td>ELISA</td>
<td>17%</td>
<td>66</td>
</tr>
<tr>
<td>1997</td>
<td>Gabon</td>
<td>979</td>
<td>ELISA</td>
<td>1.4%</td>
<td>67</td>
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<tr>
<td>2005-08</td>
<td>Gabon</td>
<td>4349</td>
<td>ELISA</td>
<td>15.3%</td>
<td>68</td>
</tr>
</tbody>
</table>

*Year samples were collected; CAR (Central African Republic), DRC (Democratic Republic of Congo).

**Table S3.** Positive selection in humans associated with emerging viral infection.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Analysis</th>
<th>Cellular function</th>
<th>Reported viral associations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARGE</td>
<td>CMS*</td>
<td>Glycosyltransferase that modifies α-dystroglycan</td>
<td>Lassa virus</td>
<td>99-102</td>
</tr>
<tr>
<td>APOBEC3</td>
<td>dN/dS</td>
<td>Cytidine deaminase and antiviral restriction factor</td>
<td>HIV, SIV, MLV, Hepatitis B virus</td>
<td>105-106</td>
</tr>
<tr>
<td>TRIM5</td>
<td>dN/dS</td>
<td>E3 ubiquitin ligase and antiviral restriction factor</td>
<td>HIV, SIV, EIAV, MLV</td>
<td>107-108</td>
</tr>
<tr>
<td>BST-2/ Tetherin**</td>
<td>dN/dS</td>
<td>Prevents viral budding</td>
<td>Ebola, Marburg, Lassa, HIV, SIV, MLV, HTLV-1, KSHV, EIAV, spumaviruses, XMRV</td>
<td>109-112</td>
</tr>
</tbody>
</table>

*Composite of multiple signals; **certain codons; HTLV-1 (human T-cell leukemia virus), HIV (human immunodeficiency virus), SIV (simian immunodeficiency virus), MLV (murine leukemia
virus), EIAV (Equine infectious anemia virus), KSHV (Kaposi’s sarcoma herpes virus) and XMRV (Xenotropic murine leukemia virus-related virus)
Note S1. Background on Lassa and Ebola Virus Seroprevalence Studies

Seroprevalence studies attempt to estimate the number of individuals in a given population that have been exposed to a specific microbe. They generally test for the presence of specific IgM or IgG antibodies produced in response to previous infections. Two types of biochemical assays have been used to determine the prevalence of Lassa and Ebola virus immunoglobulin prevalence in various populations: the indirect fluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA).

The Indirect fluorescent antibody (IFA) test was a widely used technique for viral hemorrhagic fever serology prior to 1990. However—for at least Ebola virus—the test lacks specificity in populations that have never been exposed to African filoviruses. For example, in tests of 449 randomly individuals from primary care facilities in the United States—who had no exposure to primates or travel in Africa—12 were found to test positive for Ebola IgG (22). Because of IFATs low specificity, results from seroprevalence studies using this test should viewed with caution.

The enzyme-linked immunosorbent assay (ELISA), has improved specificity and sensitivity compared to IFA tests (19). This test is now considered the gold standard for Lassa and Ebola seroprevalence studies and several ELISAs for the detection of arena virus and filo-virus specific antibodies have been developed. However, because viruses within the same family can be closely related, the ability to distinguish between specific species can be difficult; sera and plasma from some patients shows significant cross-reactivity with antigens from multiple arenavirus and filovirus species (19, 23). Thus, it is likely that current ELISA assays will detect closely related species or even distantly related species. The limited ability to specifically distinguish between related strains suggests that these tests might detect related, as well as divergent, species circulating in the observed population.
The sensitivity of the ELISA appears to be robust and antibodies can be detected long after infection. In one study Ebola-specific IgG persisted in non-human primates for at least 400 days; in two human samples it persisted for more than 10 years (23). It should also be noted that a positive result does not necessarily mean the individual was exposed to a pathogenic virus capable of replication in its host. A positive test result could result from contact with a noninfectious viral particle or exposure to very low levels of antigen.

Unlike chronic viral infections in which the host becomes persistently infected, detection of acute viral infections can be challenging. Therefore, assessing the incidence of viral hemorrhagic fevers, like Ebola and Lassa, can be particularly difficult. During a two-year period (2009 through 2010) 1,650 suspected cases of viral hemorrhagic fever were tested at the Irrua Specialist Teaching Hospital using PCR. 198 tested positive indicating the incidence of Lassa among suspected cases was 12% (128).