Seroepidemiology of Saffold Cardiovirus Type 2

To the Editor: Saffold virus (SAFV) is a new human virus belonging to the genus Cardiovirus of the family Picornaviridae (1–6). The virus has also been named human Theiler’s-like cardiovirus (4). To date, 8 SAFV genotypes are known, based on molecular variation in the P1 region, which codes for the viral capsid (5). SAFVs are detected in respiratory and fecal samples from infants and children <6 years of age. The virus is generally detected at low frequency (0.5%–3%) in the general population, but in Afghanistan and Pakistan higher incidences have been reported (10%–12%) (5). Detection is mainly based on molecular techniques because virus isolation is cumbersome. Other than gastroenteritis, no clear disease manifestations are known (1–8), although recently, researchers in Japan have suggested that for a select group of children, SAFV infection may cause pharyngitis and tonsillitis (7). In rodents, however, cardioviruses are serious pathogens that can cause myocarditis, meningoencephalitis, and pancreatitis.

Previously, we have shown that SAFV-3 infections are ubiquitous and are transmitted early in life (6). This conclusion was based on antibody testing by virus neutralization, a highly specific test that can discriminate serotypes within a single species, but formal proof for existence of serotypes was lacking. Extrapolated to other genotypes, the finding would indicate a high infection rate for SAFVs, which is at odds with the low detection frequency reported in most studies. Most SAFV-2 isolates, from our laboratory and others, grow poorly in cell culture, which hampers reading of neutralization. Recently, however, a SAFV-2 strain was isolated in Finland that grows well in HeLa cells and shows clear cytopathic effects (SAFV-2-FIN2008, GenBank accession no. FR682076; S. Blomqvist et al., unpub. data). This strain enabled us to set up a virus neutralization test similar to that described for SAFV-3 (6). For comparison, we used strain SAFV-3(NL2007) (GenBank accession no. FM207487), which was isolated in Nijmegen (6). The virus neutralization test was performed on HeLa cells with 100 TCID<sub>50</sub> of virus per serum dilution. Virus–serum mixtures were incubated for 1 hour at 37°C and overnight at 7°C to stabilize virus–antibody complexes (6). Human serum samples submitted previously were tested by using 3-fold dilution steps and duplicate testing (6). The results are presented in the Table. A low seroprevalence was found for SAFV-2 and SAFV-3 at 9 months of age, which is at the nadir of immunoglobulin G levels in infants. At the age of 24 months, a high seroprevalence of antibodies was found in the Netherlands for SAFV-2 and -3, pointing to early acquisition of infection with both strains. In Finland, the seroprevalence for SAFV-2 and -3 was lower in young children, which suggests somewhat lower infection rate similar to what has been reported for enteroviruses (9). A high seroprevalence of 97%–100% was found in persons >4 years of age in Cameroon, Indonesia, and the Netherlands (Table). A high prevalence of SAFV-2 antibodies was recently also reported in blood donors from the United States (8). Thus, the seroepidemiology of SAFV-2 is similar to that of SAFV-3. As depicted in the last 3 columns of the Table, on several occasions serum samples independently neutralized either SAFV-2 or SAFV-3, which suggests that the viruses behave as different serotypes.

In conclusion, SAFV-2 and SAFV-3 show an almost identical epidemiologic pattern with infection acquired early in life and with a high seroprevalence in different continents. The outcome is concordant with universal occurrence of infection by both genotypes. Because several times there was a clear discrepancy between antibody titers against one or the other of the 2 genotypes, we conclude that they behave as separate serotypes, although weak cross-reactivity (below the detection limit of 1:15) cannot be excluded. Accepting that SAFV genotypes correspond with existence of different serotypes, the infection rate in the first years of life must be quite high, similar to that for human parechoviruses and enteroviruses, which are found in 15%–18% of stool samples from children <5 years of age (10). The

Table. SAFV-neutralizing antibodies in blood samples from humans of different ages and from different geographic regions*

<table>
<thead>
<tr>
<th>Country</th>
<th>No. samples</th>
<th>Patient age†</th>
<th>Years collected</th>
<th>SAFV-2 pos‡</th>
<th>SAFV-3 pos‡</th>
<th>SAFV-3 pos§</th>
<th>SAFV-2 pos + SAFV-3 neg</th>
<th>SAFV-2 neg + SAFV-3 pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>29</td>
<td>9 mo</td>
<td>2006–2007</td>
<td>15 (52)</td>
<td>4 (14)</td>
<td>15 (52)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Netherlands</td>
<td>26</td>
<td>24 mo</td>
<td>2006–2007</td>
<td>21 (81)</td>
<td>20 (77)</td>
<td>25 (96)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Netherlands</td>
<td>30</td>
<td>18–39 y</td>
<td>2004</td>
<td>30 (100)</td>
<td>29 (97)</td>
<td>30 (100)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Finland</td>
<td>30</td>
<td>2–2.5 y</td>
<td>1997–1998</td>
<td>10 (33)</td>
<td>21 (70)</td>
<td>24 (80)</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Cameroon</td>
<td>29</td>
<td>5–15 y</td>
<td>1997</td>
<td>28 (97)</td>
<td>28 (97)</td>
<td>29 (100)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Indonesia</td>
<td>30</td>
<td>4–40 y</td>
<td>1997–1998</td>
<td>30 (100)</td>
<td>30 (100)</td>
<td>30 (100)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*SAFV, Saffold virus; pos, positive; neg, negative.
†Samples were collected from patients at this age or within this age range.
‡Titer >15.
§Cumulative positive for antibodies against SAFV-2 and/or SAFV-3.
low SAFV detection rate remains thereby difficult to explain.

The outcome can be explained by a short duration of virus excretion in stool, which, however, is unlikely for an infection spreading by the fecal–oral route. Alternatively, it may be that the virus is unstable in stool and rapidly degrades, such that fecal samples are inadequate for diagnosis of the infection. Other specimens, however, such as respiratory samples, yielded also low numbers of positive findings (4). Remarkably, the study with a high prevalence of positive stool samples made use of primers selected in a conserved region of 2C helicase (5), whereas other studies used primers in the 5′ noncoding region (3, 4). Hence, a difference in sensitivity between the different PCRs may be responsible for the discrepancy between seroepidemiology and the low diagnostic yield by PCR. This discrepancy, however, awaits further investigation.

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Alkhoruma Hemorrhagic Fever in Travelers Returning from Egypt, 2010

To the Editor: The report of 2 visitors from Italy being infected by Alkhoruma hemorrhagic fever virus (AHFV) in southeastern Egypt near the border with Sudan (1) provides useful data to help clarify the evolutionary origin of these tick-borne flaviviruses. AHFV was first isolated in Saudi Arabia and is associated with camel ticks (2). It is a genetically close relative of Kyanusan Forest disease virus, which was first isolated in India in 1957. Following the original isolation of Kyanusan Forest disease virus, there was no clear explanation for its apparent isolation in the Indian forests. Indeed, its subsequent discovery in southern China (3) suggested that migratory birds might carry the infected ticks to or from that region.

The most likely explanation for these outbreaks of hemorrhagic disease now begins to fit a pattern that can be interpreted in terms of the diseases’ evolutionary origin in Africa. Thousands of animals are annually transported from Africa and other countries to Mecca, Saudi Arabia, to meet the human demand for food and transport during the Hajj. Many of these animals, including camels, are infested with ticks that may carry AHFV and thus provide the source of this human infectious agent. Phylogenetic evidence had previously suggested that the tick-borne encephalitic flavivirus serocomplex originated in Africa and gradually evolved and dispersed across the Northern Hemisphere of the Old World (4,5). This concept is totally consistent with the discoveries of AHFV in Saudi Arabia and now in southeastern Egypt. Thus, Africa is a likely source of infected ticks that are regularly moved between Africa.