Phylogenetic reconstruction of two large HAV outbreaks occurring simultaneously in different regions in Bulgaria

Anton Andonov
Public Health Agency of Canada
National Microbiology Laboratory

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HAV genome organization

HAV genotypes: 1, 2, 3 of human origin and 4, 5 and 6 of simian origin
(based on sequence variation; 15% for genotypes and 7% for subtypes)
<table>
<thead>
<tr>
<th>Patient #</th>
<th>ALT-1</th>
<th>ALT-2</th>
<th>Jaundice</th>
<th>Anti-HAV IgM</th>
<th>Anti-HAV IgM</th>
<th>Anti-HAV IgG</th>
<th>Anti-HAV IgM</th>
<th>Anti-HAV IgM</th>
<th>Anti-HAV IgM</th>
<th>HAV RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>420</td>
<td>237</td>
<td>wk</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>90</td>
<td>161</td>
<td>348</td>
<td>no</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>91</td>
<td>87</td>
<td>122</td>
<td>no</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>( )</td>
<td>( )</td>
<td>(+)</td>
</tr>
<tr>
<td>92</td>
<td>32</td>
<td>115</td>
<td>wk</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>( )</td>
<td>( )</td>
<td>( )</td>
<td>ND</td>
</tr>
<tr>
<td>93</td>
<td>126</td>
<td>208</td>
<td>wk</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>ND</td>
</tr>
<tr>
<td>114</td>
<td>1639</td>
<td>1991</td>
<td>Yes</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
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</table>
Stolipinovo (Столипиново) is a district of the Bulgarian town of Plovdiv and the most populous predominantly Roma-inhabited district on the Balkans with a population of about 45,000 people. It is located on the outskirts of the city, in its northeastern part on the right bank of the Maritsa.

The study included 216 children, 118 Roma and 98 non-Roma of 9 years median age (range 5-15 years). Among Roma children 98.3% had detectable antibodies to HAV, compared with 32.7% among non-Romas (P < 0.0001).
HAV VP1 = 300 aa

HAV VP3 = 246 aa
Number of HAV cases in the regions of Plovdiv and Svoge,
M Kojouharova; Eurosurveillance, v. 11, No. 40, 2006
Fig. 1 Phylogenetic analysis of HAV strains from outbreaks in Plovdiv and Svoge, 2006.

N-J tree based on sequencing of the VP1/2a junction.

Legend: HAV strains from Plovdiv in blue colour; HAV strains from Svoge in red colour.
Number of HAV cases in the regions of Plovdiv and Svoge,
M Kojouharova; Eurosurveillance, v. 11, No. 40, 2006
HAV strains with identical RNA “fingerprint” based on 373 bp VP1/2A amplicon; comparison of VP1 complete sequences.
1. B38, B12, B9, B36
2. B114, B90
3. B1
The aftermath:
• 150kg of washing powder donated
• 8500 packs of soap and wet wipes urgently delivered
• 40 tons of refuse removed
• Illegal wood and tin housing
• 1 million leva ($650,000 US) to sanitize the district
• The area between housing blocs drained and asphalted
• All children immunized
“Unless living conditions in the Roma quarters changed, the epidemic would repeat itself in five years”, Kunchev told BTA.
Conclusions:

• Parallell transmission chains of at least two genetically closely related, albeit different HAV strains belonging to subgenotype 1A were identified phylogenetically in both outbreaks (Stolipinovo and Svoge).
• The mutant spectrum of HAV quasispecies varied allowing for rapid evolution of viral sublineages to be established among individual hosts.
• Presence of polymorphic sites within VP1/2A junction region may indicate emergence of phylogenetic closely related viral variants due to high number of rounds of replication in a prolonged HAV outbreak.
• The significance of the well established phylogenetic comparison within the VP1/2A junction as a sole criterion for RNA “fingerprinting” needs careful re-evaluation.
• Our data did not support the hypothesis that the HAV outbreak in Stolipinovo could have been caused by immune escape HAV.