Technical Appendix

Technical Appendix Table 1. Primers used for CHUV detection and full-length genome amplification of segments 1–10*

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>Length, bp</th>
<th>Function†</th>
<th>Reference‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHUV-D-F</td>
<td>5'-GTGTTCGACTGATAACATCATCG-3’</td>
<td>364</td>
<td>CHUV detection</td>
<td>KT887181,</td>
</tr>
<tr>
<td>CHUV-D-R</td>
<td>5'-GCTGGACTGTTGATTATCCCTC-3’</td>
<td></td>
<td></td>
<td>AB014725</td>
</tr>
<tr>
<td>CHUV-Seg-1-F</td>
<td>5'-GGTCAATCATGCAAGACGCATC-3’</td>
<td>3903</td>
<td>VP1 amplification</td>
<td>KT887180</td>
</tr>
<tr>
<td>CHUV-Seg-1-R</td>
<td>5'-GGTGACGCTAAAACAAATTTAGATTCTG-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-2-F</td>
<td>5'-TCGGCAATGGGATGTTTTCG-3’</td>
<td>3034</td>
<td>VP2 amplification</td>
<td>KT002589</td>
</tr>
<tr>
<td>CHUV-Seg-2-R</td>
<td>5'-GCAACACGTGATTGCACCTACAG-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-3-F</td>
<td>5'-TGGAGCCTTGTGCAGTGTTAC-3’</td>
<td>2736</td>
<td>VP3 amplification</td>
<td>KT887182</td>
</tr>
<tr>
<td>CHUV-Seg-3-R</td>
<td>5'-GCAGGTTGGTCAGTCTATACAG-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-4-F</td>
<td>5'-CTTTCGAGTTTCACCTACTCTTT-3’</td>
<td>1938</td>
<td>VP4 amplification</td>
<td>KT887183</td>
</tr>
<tr>
<td>CHUV-Seg-4-R</td>
<td>5'-CTTTCGAGTTTCACCTACTCTTT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-5-F</td>
<td>5'-CGTTTCAGAATGGAACGATTCC-3’</td>
<td>1719</td>
<td>NS1 amplification</td>
<td>KT887184</td>
</tr>
<tr>
<td>CHUV-Seg-5-R</td>
<td>5'-CACCGAACGATCCTAACCTAAAC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-6-F</td>
<td>5'-GAACACGGATGGTGTTTCTG-3’</td>
<td>1593</td>
<td>VP5 amplification</td>
<td>KT002593</td>
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<tr>
<td>CHUV-Seg-6-R</td>
<td>5'-GCTGGACTGTTGATTATCCCTC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-7-F</td>
<td>5'-GCTGGACTGTTGATTATCCCTC-3’</td>
<td>1102</td>
<td>VP5 amplification</td>
<td>KT887186</td>
</tr>
<tr>
<td>CHUV-Seg-7-R</td>
<td>5'-TGTAGGATGGATGCTCAACGTA-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-8-F</td>
<td>5'-CCTTTCGAGTTTCACCTACTCTTT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-8-R</td>
<td>5'-GGTGTCGTTGGTGCTACATGAC-3’</td>
<td>1024</td>
<td>NS2 amplification</td>
<td>KT887187</td>
</tr>
<tr>
<td>CHUV-Seg-9-F</td>
<td>5'-GGTGTCGTTGGTGCTACATGAC-3’</td>
<td>839</td>
<td>VP6 amplification</td>
<td>KT887188</td>
</tr>
<tr>
<td>CHUV-Seg-9-R</td>
<td>5'-GTGTGGTGGTGGTGCTACATGAC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHUV-Seg-10-F</td>
<td>5'-GAAATGTTGGCGCGGTCGTA-3’</td>
<td>662</td>
<td>NS3 amplification</td>
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</tr>
<tr>
<td>CHUV-Seg-10-R</td>
<td>5'-CGTTTCGAGTTTCACCTACTCTTT-3’</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*CHUV, Chuzan virus; D detection; NS, nonstructural protein; F, forward; R, reverse; seg, segment; VP, viral protein.
†The aim of the primers used in this study.
‡The reference used for specific primers designation.
### Technical Appendix Table 2. Results of Chuzan virus detection in yaks by reverse transcription PCR for 3 provinces surrounding Qinghai Tibetan Plateau, China, 2016–2017

<table>
<thead>
<tr>
<th>Province</th>
<th>Age, mo.</th>
<th>2016</th>
<th>2017</th>
<th>No. positive (%)†</th>
<th>Total positive (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gansu</td>
<td>≤1</td>
<td>35</td>
<td>11</td>
<td>4/46 (8.7)</td>
<td>5/71 (7.04)</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>13</td>
<td>12</td>
<td>1/25 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Qinghai</td>
<td>≤1</td>
<td>8</td>
<td>15</td>
<td>0/23 (0)</td>
<td>0/64 (0)</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>27</td>
<td>14</td>
<td>0/41 (0)</td>
<td></td>
</tr>
<tr>
<td>Sichuan</td>
<td>≤1</td>
<td>14</td>
<td>15</td>
<td>0/29 (0)</td>
<td>0/73 (0)</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>21</td>
<td>23</td>
<td>0/44 (0)</td>
<td></td>
</tr>
</tbody>
</table>

*The number of yak samples collected from each province with 2 age groups at various times (2016–2017) in the study.

†Number and percentage of positive samples in each age group of different provinces.

‡Number and percentage of positive samples in all age groups of different provinces.

### Technical Appendix Table 3. Nucleotide and amino acid identities of segments 1–10 of CHUV discovered in this study with the strain GX871/China deposited in GenBank*

<table>
<thead>
<tr>
<th>CHUV segment</th>
<th>CHUV discovered in this study</th>
<th>CHUV/GX871/China</th>
<th>Nucleotide/amino acid, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg-1</td>
<td>MH090056/CHN-GS-16</td>
<td>KT887180/GX871/China</td>
<td>99.59/99.07</td>
</tr>
<tr>
<td></td>
<td>MH090057/CHN-GS-26</td>
<td></td>
<td>99.48/98.83</td>
</tr>
<tr>
<td></td>
<td>MH090058/CHN-GS-70</td>
<td></td>
<td>99.43/98.88</td>
</tr>
<tr>
<td>Seg-2</td>
<td>MF642326/CHN-GS-16</td>
<td>KT887181/GX871/China</td>
<td>99.03/98.76</td>
</tr>
<tr>
<td></td>
<td>MF642327/CHN-GS-26</td>
<td></td>
<td>98.83/98.29</td>
</tr>
<tr>
<td></td>
<td>MF642328/CHN-GS-70</td>
<td></td>
<td>98.69/98.09</td>
</tr>
<tr>
<td>Seg-3</td>
<td>MH090059/CHN-GS-16</td>
<td>KT887182/GX871/China</td>
<td>99.33/98.65</td>
</tr>
<tr>
<td></td>
<td>MH090060/CHN-GS-26</td>
<td></td>
<td>99.41/98.65</td>
</tr>
<tr>
<td></td>
<td>MH090061/CHN-GS-70</td>
<td></td>
<td>99.56/99.02</td>
</tr>
<tr>
<td>Seg-4</td>
<td>MH090062/CHN-GS-16</td>
<td>KT887183/GX871/China</td>
<td>99.43/98.90</td>
</tr>
<tr>
<td></td>
<td>MH090063/CHN-GS-26</td>
<td></td>
<td>99.27/98.43</td>
</tr>
<tr>
<td></td>
<td>MH090064/CHN-GS-70</td>
<td></td>
<td>99.32/98.43</td>
</tr>
<tr>
<td>Seg-5</td>
<td>MH090065/CHN-GS-16</td>
<td>KT887184/GX871/China</td>
<td>99.96/98.90</td>
</tr>
<tr>
<td></td>
<td>MH090066/CHN-GS-26</td>
<td></td>
<td>99.51/98.43</td>
</tr>
<tr>
<td></td>
<td>MH090067/CHN-GS-70</td>
<td></td>
<td>99.51/98.43</td>
</tr>
<tr>
<td>Seg-6</td>
<td>MF642329/CHN-GS-16</td>
<td>KT887185/GX871/China</td>
<td>98.91/99.08</td>
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<tr>
<td></td>
<td>MF642330/CHN-GS-26</td>
<td></td>
<td>98.65/98.71</td>
</tr>
<tr>
<td></td>
<td>MF642331/CHN-GS-70</td>
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<td>98.38/98.71</td>
</tr>
<tr>
<td>Seg-7</td>
<td>MH090068/CHN-GS-16</td>
<td>KT887186/GX871/China</td>
<td>99.71/99.71</td>
</tr>
<tr>
<td></td>
<td>MH090069/CHN-GS-26</td>
<td></td>
<td>99.81/100.00</td>
</tr>
<tr>
<td></td>
<td>MH090070/CHN-GS-70</td>
<td></td>
<td>99.81/99.71</td>
</tr>
<tr>
<td>CHUV segment</td>
<td>CHUV discovered in this study</td>
<td>CHUV/GX871/China</td>
<td>Nucleotide/amino acid, %</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Seg-8</td>
<td>MH090071/CHN-GS-16</td>
<td>KT887187/GX871/China</td>
<td>99.50/99.40</td>
</tr>
<tr>
<td></td>
<td>MH090072/CHN-GS-26</td>
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<td>99.70/99.10</td>
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<tr>
<td></td>
<td>MH090073/CHN-GS-70</td>
<td></td>
<td>99.70/99.10</td>
</tr>
<tr>
<td>Seg-9</td>
<td>MH090074/CHN-GS-16</td>
<td>KT887188/GX871/China</td>
<td>99.88/99.63</td>
</tr>
<tr>
<td></td>
<td>MH090075/CHN-GS-26</td>
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<td>99.51/98.89</td>
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<td></td>
<td>MH090076/CHN-GS-70</td>
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<td>99.51/98.89</td>
</tr>
<tr>
<td>Seg-10</td>
<td>MH090077/CHN-GS-16</td>
<td>KT887189/GX871/China</td>
<td>99.68/99.52</td>
</tr>
<tr>
<td></td>
<td>MH090078/CHN-GS-26</td>
<td></td>
<td>99.68/100.00</td>
</tr>
<tr>
<td></td>
<td>MH090079/CHN-GS-70</td>
<td></td>
<td>99.53/99.05</td>
</tr>
</tbody>
</table>

*The newly identified CHUVs sequence surrounding Qinghai-Tibet Plateau of China, and have been deposited in GenBank. CHUV, Chuzan virus; seg, segment.

**Technical Appendix Figure 1.** Cytopathic effects of baby hamster kidney (BHK-21) cells that were inoculated with Chuzan virus (CHUV). (A) BHK-21 cells at fifth passage without CHUV infection. Original magnification ×200. (B) BHK-21 cells at fifth passage infected with CHUV at first passage. The infected cells that exhibited a wrinkled morphology and were detached from the bottom of culture flasks. Original magnification ×200.
Technical Appendix Figure 2 Phylogenetic analysis of CHUV based on viral protein 2 genome sequences of CHUV and other members in Palyam serogroup of genus Orbivirus available in August 2017. The phylogenetic tree was constructed by using the neighbor-joining method with MEGA 7.01 software (http://www.megasoftware.net). Bootstrap values were calculated with 1,000 replicates. The number on each branch indicates bootstrap values. Black circles indicate the newly identified CHUVs sequence surrounding Qinghai-Tibet Plateau of China, and have been deposited in GenBank (GenBank accession no. MF642326/CHN-GS-16, MF642327/CHN-GS-26 and MF642328/CHN-GS-70). The reference sequences obtained from GenBank are indicated by GenBank accession numbers, strain abbreviations and countries. Scale bar indicates nucleotide substitutions per site. BCV, Bunyip Creek virus; CHUV, Chuzan virus; DAV, D’Agullar virus.
Technical Appendix Figure 3  Phylogenetic analysis of CHUV based on viral protein 5 genome sequences of CHUV and other members in genus *Orbivirus* available in August 2017. The phylogenetic tree was constructed by using the neighbor-joining method with MEGA 7.01 software (http://www.megasoftware.net). Bootstrap values were calculated with 1,000 replicates. The number on each branch indicates bootstrap values. Black circles indicate the newly identified CHUV sequence surrounding Qinghai-Tibet Plateau of China, which have been deposited in GenBank (GenBank accession no. MF642329/CHN-GS-16, MF642330/CHN-GS-26 and MF642331/CHN-GS-70). The reference sequences obtained from GenBank are indicated by GenBank accession numbers, strain abbreviations and countries. Scale bar indicates nucleotide substitutions per site. AHSV, African horse sickness virus; BTV, bluetongue virus; CHUV, Chuzan virus.