

Chuzan Virus in Yaks, Qinghai-Tibetan Plateau, China

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We detected Chuzan virus (CHUV) in domestic yaks from the Qinghai-Tibetan Plateau, western China, indicating CHUV probably has been transmitted to yaks in recent years. Awareness for CHUV surveillance and transmission and livestock health management in these special regions should be raised to avoid outbreaks and animal loss.

Chuzan virus (CHUV) belongs to the Palyam serogroup of genus *Orbivirus*, family *Reoviridae*. A CHUV outbreak, first reported in Japan in 1985, was the causative agent of disease that resulted in many reproductive disorders in cattle, including abortion, stillbirth, and congenital malformation (1) and in considerable economic loss in the cattle industry.

Like other orbiviruses, CHUV consists of 10 double-stranded RNA segments (Seg-1 to Seg-10), which encode 7 structural viral proteins (VP1–VP7) and 4 nonstructural proteins (NS1–NS4) (2–4). Seg-2 (VP2) and Seg-6 (VP5) of the Palyam serogroup show the highest levels of variation in genome sequence, which correlates with virus serotype specificity (2). These viruses usually are transmitted by arthropod vectors (5,6). Therefore, CHUV has been widespread in many countries of Asia, such as South Korea (7) and mainland China (8,9), which have reported CHUV infection in cattle. However, no information was available about CHUV in yaks (*Bos grunniens*) on China's Qinghai-Tibetan Plateau.

Yaks are an important livestock in the Qinghai-Tibetan Plateau. They have been farmed with other livestock, such as Tibetan sheep and Tibetan pigs. The high prevalence of bluetongue virus (BTV) infection, also belonging to genus *Orbivirus*, has been reported in yaks and Tibetan sheep (10). A study in 2016 found an abortion rate in yaks of 21.39% in part of Qinghai Province, presumably because of the high prevalence of BTV and other related pathogens (10). All these data and CHUV

infection in cattle in China motivated us to study whether CHUV infects yaks.

During August 2016–April 2017, we randomly collected 208 blood samples from apparently healthy domestic yaks, 71 yaks from Gansu Province (46 <1 year of age), 64 yaks from Qinghai Province (23 <1 year of age), and 73 yaks from Sichuan Province (29 <1 year of age). Soon after sampling, total RNAs were extracted and used as templates to amplify full-length cDNA by reverse transcription PCR (RT-PCR; SuperScript III Synthesis Kit, Invitrogen, Carlsbad, CA, USA). One pair of specific primers was designed based on VP2 genome sequence of CHUV (online Technical Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/24/12/17-1414-Techapp1.pdf>) and used to detect CHUV in yaks. We also performed serologic assay by using the CHUV 2nd detection kit (iNtRON, IPC11028, Gyeonggi-do, South Korea), and the results of the assay were then authenticated by RT-PCR.

For phylogenetic and identity analysis of genome sequence of 10 segments from CHUV, we designed 10 pairs of primer based on known sequences deposited in GenBank (online Technical Appendix Table 1) to obtain the open reading frame genome of these proteins. Phylogenetic and identity analyses were performed based on these genome sequences and the corresponding sequences available in GenBank.

Five (7%) of the 71 samples were positive for CHUV in Gansu Province, 4 of which were <1 year of age (Figure; online Technical Appendix Table 2). CHUV infection in yaks was not found in Qinghai and Sichuan provinces. Relatively low prevalence of CHUV infection in yaks is consistent with the report of CHUV infection in cattle in South Korea (7), which was also significantly lower than BTV infection in yaks (17.34%) (10). We also obtained similar results in serologic investigation. We observed neither illness nor disease in these yaks; our results are consistent with CHUV infection in sentinel cattle (8) and thus suggest that subclinical infection of CHUV occurs in cattle. We used heparinized blood samples to inoculate baby hamster kidney 21 cells for 5 blind passages, as described previously (6,8,9). Infected cells that exhibited a wrinkled morphology and were detached from the bottom of culture flasks within 5 days (online Technical Appendix Figure 1), and 19 segments of CHUV could also be detected by RT-PCR.

Sequence analysis revealed 100% identity of genomes for Seg-1 to Seg-10 of 3 CHUV sequences in yaks (CHN-GS-70). Identity analysis showed that genome sequences for Seg-1 to Seg-10 of CHUV shared >98.38% nt identities and >98.09% aa identities with CHUV strain KT887181/GX871/China in previous studies (8,9) (online Technical Appendix Table 3). We constructed 2 phylogenetic trees based on VP2 and VP5 genome sequences

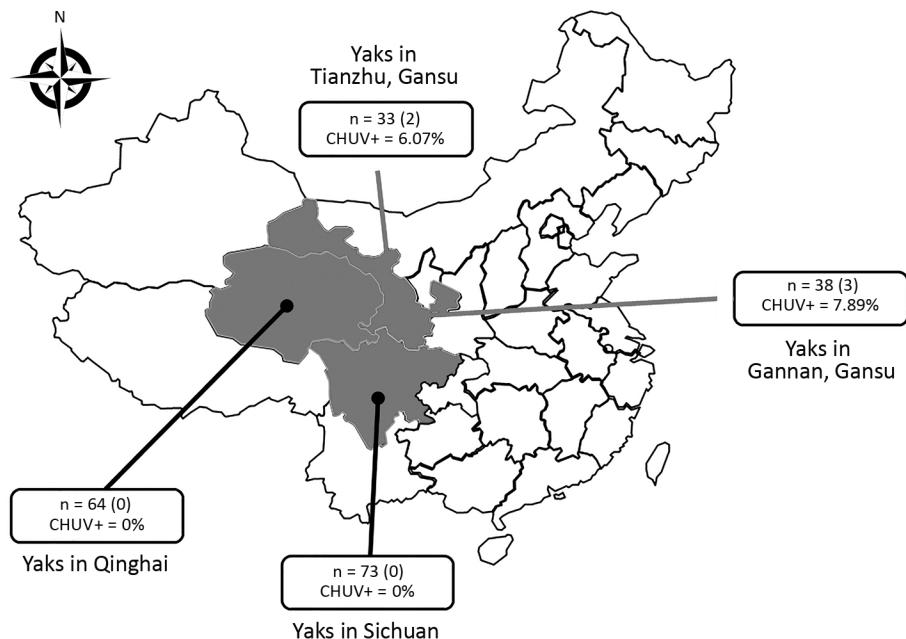


Figure. Number and species of yaks from provinces around the Qinghai Tibetan Plateau, China, 2016–2017. The 3 provinces where sampling was performed, yak species, and occurrence of CHUV are indicated. n values indicate the total number of samples in each province; numbers in parentheses indicate the numbers of positive samples in each province; CHUV+ percentages indicate the CHUV prevalence rate. CHUV, Chuzan virus.

of CHUV and other members in genus *Orbivirus* (online Technical Appendix Figures 2, 3). All strains from our study were grouped in a new separate cluster and shared an ancestor with the strains KT002589/SZ187/China and KT887181/GX871/China. Furthermore, CHN-GS-26 and CHN-GS-70 were located in the same separate sub-cluster (online Technical Appendix Figures 2, 3), which demonstrated a complicated and transregional transmission cycle for CHUV in China.

The yaks that were positive for CHUV were located in 2 cities of Gansu Province \approx 600 km apart, which indicates that transmission of CHUV has spread rapidly around the Qinghai-Tibetan Plateau. Further studies are needed to determine the epidemiology and evolution of CHUV in livestock with concomitant virus isolation and phylogenetic analysis. The awareness of livestock health management in these special regions should also be raised.

Acknowledgment

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Conservation of White Rhinoceroses Threatened by Bovine Tuberculosis, South Africa, 2016–2017

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During 2016–2017, when Kruger National Park, South Africa, was under quarantine to limit bovine tuberculosis spread, we examined 35 white and 5 black rhinoceroses for infection. We found 6 infected white rhinoceroses during times of nutritional stress. Further research on *Mycobacterium bovis* pathogenesis in white rhinoceroses is needed.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* or *M. bovis* has been reported in captive rhinoceroses since the early 1800s (1–3). Bovine TB is endemic in many wildlife populations worldwide, including among those in Kruger National Park (KNP), South Africa (4). KNP contains the largest free-living population of white rhinoceroses in the world (estimated at 6,649–7,830). However, prolonged drought in South Africa (2015–2017) raised concerns that starvation and disease could increase the mortality rate and affect conservation efforts for this species (5).

In June 2016, a black rhinoceros (*Diceros bicornis minor*) with an *M. bovis* infection was discovered (6). Thereafter, a surveillance program was initiated to screen rhinoceros carcasses in KNP, leading to 35 white and 5 black rhinoceros carcasses being examined during June 2016–October 2017. To determine which animals were infected, we conducted macroscopic examinations and collected samples for histopathologic studies and mycobacterial culture, as previously described (7). Research protocols were approved by the South African National Park Animal Use and Care Committee and ethics committee of Stellenbosch University.

No additional cases of *M. bovis* infection were found in black rhinoceroses. However, we confirmed *M. bovis* infection in 6 white rhinoceroses (Table). Grossly visible lesions, mostly found in the retropharyngeal or tracheobronchial lymph nodes or lung, were typically small and localized and could easily be missed or mistaken for granulomas caused by other pathogens if careful dissections of tissues were not performed (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/12/18-0293-Techapp1.pdf>). On histologic examination, we found granulomatous inflammation in lung or lymph node sections and rare acid-fast organisms in some granulomas (Table). We typed these *M. bovis* isolates as strain SB0121, the most common strain found in KNP (8).

Four of the infected animals were found during September–November 2016, near the end of the drought, and the remaining 2 animals were found in September and October 2017, at the end of the next winter. The timing of infections suggests that animals under nutritional stress might be more susceptible to infection, similar to observations in other species (9). The low number of positive cases and localized paucibacillary lesions support the hypothesis that white rhinoceroses, although susceptible to infection,

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Technical Appendix

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

Primers	Sequences	Length, bp	Function†	Reference‡
CHUV-D-F	5'-GTGTTCACTGATAACATCATCG-3'	364	CHUV detection	KT887181,
CHUV-D-R	5'-GCTGGACTGTTGATTATCCCTC-3'			AB014725
CHUV-Seg-1-F	5'-GGTCAATCATGCAAGACGCATC-3'	3903	VP1 amplification	KT887180
CHUV-Seg-1-R	5'-GGTGACGCTAAACAAATTGATTCTG-3'			
CHUV-Seg-2-F	5'-TTCCGCAATGGATGAATTTTCG-3'	3034	VP2 amplification	KT002589
CHUV-Seg-2-R	5'-GCAACACGTAGTTGTACACTACG-3'			
CHUV-Seg-3-F	5'-TGTAGGATGGATGCTCAACGTA-3'	2736	VP3 amplification	KT887182
CHUV-Seg-3-R	5'-ACAGGTTGGTCAGTCCTATACAG-3'			
CHUV-Seg-4-F	5'-CATGGAGCCTTGTGCAGTGTAC-3'	1938	VP4 amplification	KT887183
CHUV-Seg-4-R	5'-CCTTCGAGTTTCACCTACTCTT-3'			
CHUV-Seg-5-F	5'-CGTTTCAGAATGGAACGATTCC-3'	1719	NS1 amplification	KT887184
CHUV-Seg-5-R	5'-CACCGAACGATCCTAACCTAAC-3'			
CHUV-Seg-6-F	5'-GAACACGATGGGTCCGTTTCGTGA-3'	1593	VP5 amplification	KT002593
CHUV-Seg-6-R	5'-GCTGATTTGCTCCTGACCGACT-3'			
CHUV-Seg-7-F	5'-TCTCCTCGAGATGGATGCGATTG-3'	1102	VP5 amplification	KT887186
CHUV-Seg-7-R	5'-ATCTAGTGTGACTGATGCATTGTG-3'			
CHUV-Seg-8-F	5'-CCTTGACATCATGGGTGACAG-3'	1024	NS2 amplification	KT887187
CHUV-Seg-8-R	5'-CCTATCAGTCAACTAGTGGACAG-3'			
CHUV-Seg-9-F	5'-GTTGTGGTTGATGACGACGTCTC-3'	839	VP6 amplification	KT887188
CHUV-Seg-9-R	5'-CGTGCCAATCCTACATATGATC-3'			
CHUV-Seg-10-F	5'-GAAATGTTGGCGGGTCGCTA-3'	662	NS3 amplification	KT887189
CHUV-Seg-10-R	5'-CGTTCGGAATCCAATACTCG-3'			

*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

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

*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.

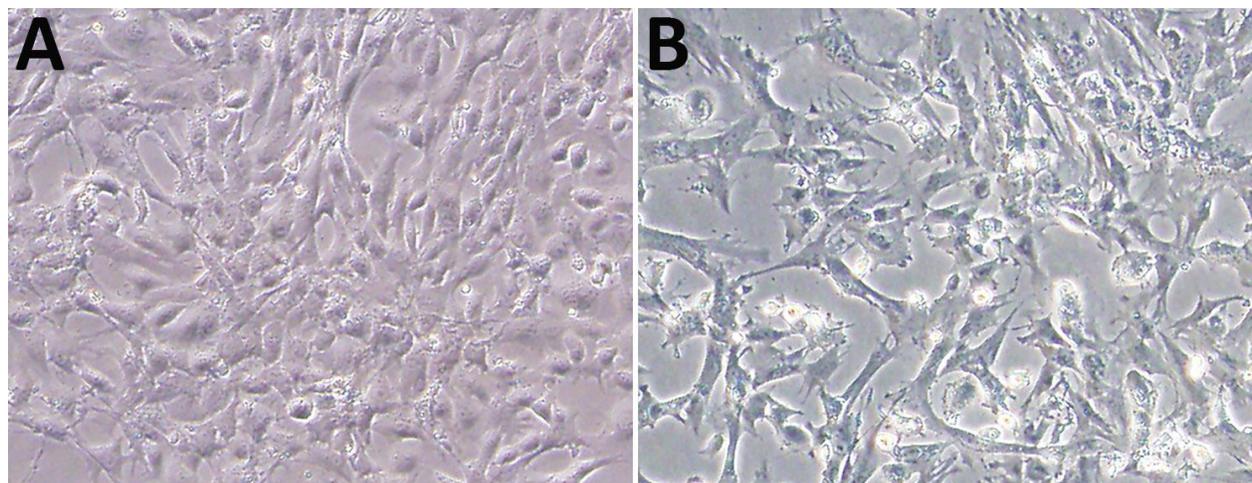
‡Number and percentage of positive samples in all age groups of different provinces.

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*

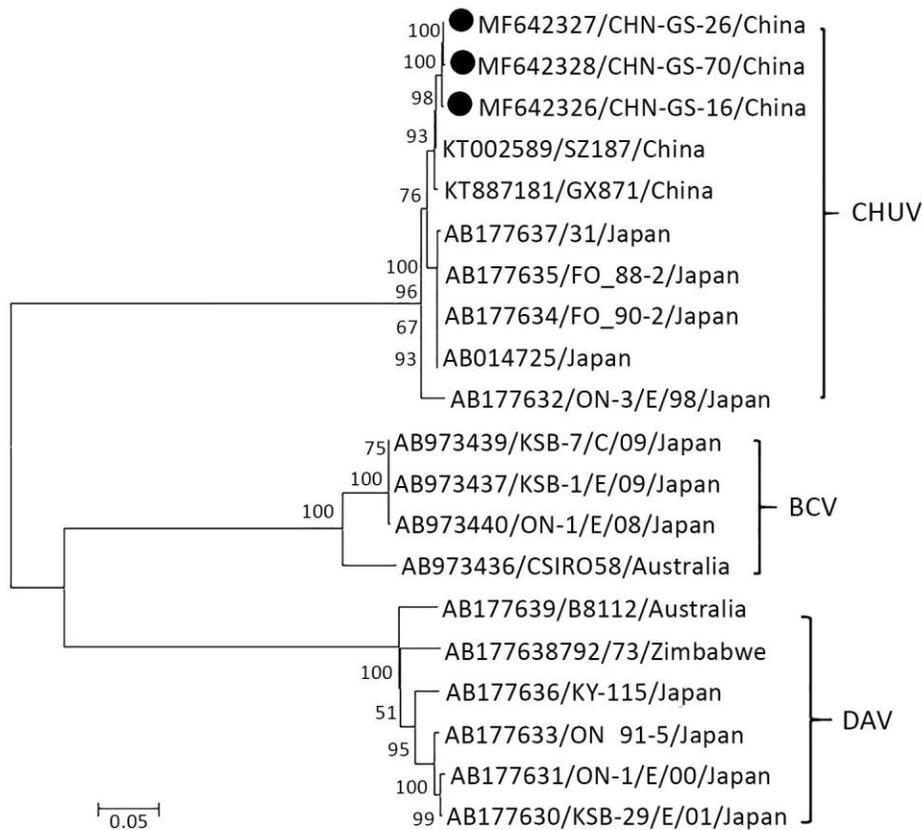
CHUV segment	CHUV discovered in this study	CHUV/GX871/China	Nucleotide/amino acid, %
Seg-1	MH090056/CHN-GS-16	KT887180/GX871/China	99.59/99.07
	MH090057/CHN-GS-26		99.48/98.83
	MH090058/CHN-GS-70		99.43/98.88
Seg-2	MF642326/CHN-GS-16	KT887181/GX871/China	99.03/98.76
	MF642327/CHN-GS-26		98.83/98.29
	MF642328/CHN-GS-70		98.69/98.09
Seg-3	MH090059/CHN-GS-16	KT887182/GX871/China	99.33/98.65
	MH090060/CHN-GS-26		99.41/98.65
	MH090061/CHN-GS-70		99.56/99.02
Seg-4	MH090062/CHN-GS-16	KT887183/GX871/China	99.43/98.90
	MH090063/CHN-GS-26		99.27/98.43
	MH090064/CHN-GS-70		99.32/98.43
Seg-5	MH090065/CHN-GS-16	KT887184/GX871/China	99.96/98.90
	MH090066/CHN-GS-26		99.51/98.43
	MH090067/CHN-GS-70		99.51/98.43
Seg-6	MF642329/CHN-GS-16	KT887185/GX871/China	98.91/99.08
	MF642330/CHN-GS-26		98.65/98.71
	MF642331/CHN-GS-70		98.38/98.71
Seg-7	MH090068/CHN-GS-16	KT887186/GX871/China	99.71/99.71
	MH090069/CHN-GS-26		99.81/100.00
	MH090070/CHN-GS-70		99.81/99.71

CHUV segment	CHUV discovered in this study	CHUV/GX871/China	Nucleotide/amino acid, %
Seg-8	MH090071/CHN-GS-16	KT887187/GX871/China	99.50/99.40
	MH090072/CHN-GS-26		99.70/99.10
	MH090073/CHN-GS-70		99.70/99.10
Seg-9	MH090074/CHN-GS-16	KT887188/GX871/China	99.88/99.63
	MH090075/CHN-GS-26		99.51/98.89
	MH090076/CHN-GS-70		99.51/98.89
Seg-10	MH090077/CHN-GS-16	KT887189/GX871/China	99.68/99.52
	MH090078/CHN-GS-26		99.68/100.00
	MH090079/CHN-GS-70		99.53/99.05

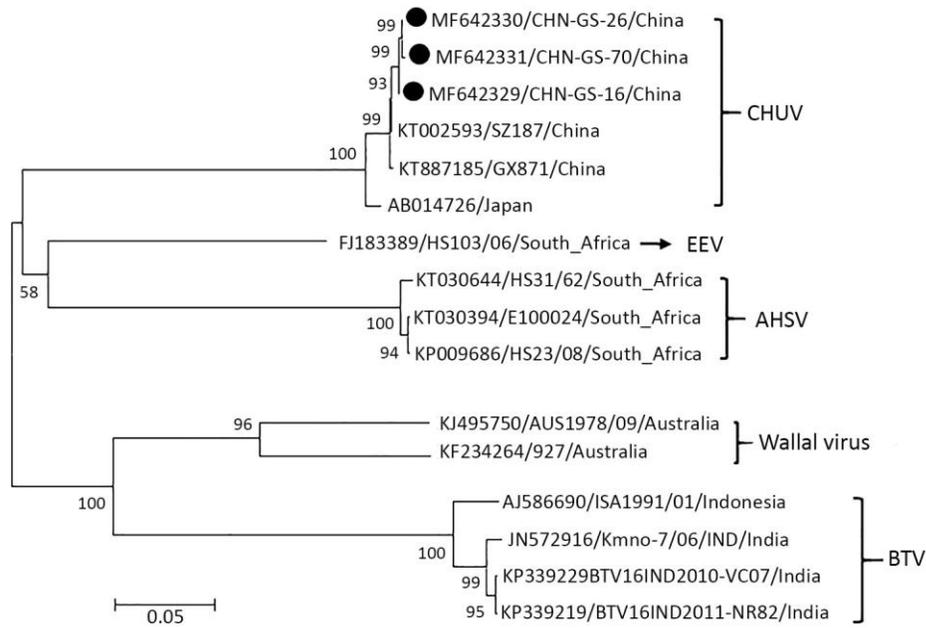
*The newly identified CHUVs sequence surrounding Qinghai-Tibet Plateau of China, and have been deposited in GenBank. CHUV, Chuzan virus; seg, segment.



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 $\times 200$. (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 $\times 200$.



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’Aguiar 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.