in China. Although reassortment was not detected in this co-infection, a potential risk for emergence of a new pandemic strain by reassortment between these 2 viruses (with humans as mixing vessels) should not be ignored. To reduce the risk for emergence of new viral subtypes, the public health and scientific communities should enhance surveillance for co-infection with influenza (H7N9) virus and other influenza virus subtypes.

This research was supported by the National Megaprojects of China for Infectious Disease (nos. 2012ZX10004211 and 2014ZX10004002-003-004), National Natural Science Foundation of China (nos. 81341004, 81102283, and 81370131), Outstanding Academic Leader of Health System in Shanghai (no. XBR2013078), Ministry of Science and Technology (no. KJYJ-2013-01-01), Shanghai Municipal Health and Family Planning Commission (no. 2013QLG002), Key Discipline Construction Project of Pudong Health Bureau of Shanghai (no. PWZx2014-10), Academic Leader Training Project in Health System of Pudong Health Bureau of Shanghai (no. PWRd2010-01), and Key Medical Specialties of Shanghai (no. ZK2012A28).

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# Nairobi Sheep Disease Virus RNA in Ixodid Ticks, China, 2013

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DOI: http://dx.doi.org/10.3201/eid2104.141602

**To the Editor:** Nairobi sheep disease virus (NSDV; genus *Nairovirus*, family *Bunyaviridae*) causes acute hemorrhagic gastroenteritis in sheep and goats (*1*,*2*). First identified in Nairobi, Kenya, in 1910, it is considered endemic in East Africa (*1*,*3*). Ganjam virus, a variant of NSDV, is found in India and Sri Lanka (*2*). NSDV has a limited effect on animals bred in areas to which the virus is endemic but can cause large losses of animals during introduction of new livestock or transport of animals through these areas (*4*). In humans, NSDV infection can cause febrile illness, headache, nausea, and vomiting (*5*).

Ticks are the main transmission vectors of NSDV and many other viral pathogens and therefore pose a major threat to public health (*6*,*7*). Here, we describe a newly discovered NSDV, named NSDV (China), identified by viral metagenomic analysis of ticks collected from the northeast region of the People's Republic of China (Liaoning, Jilin, and Heilongjiang provinces) during May–July, 2013, and divided into 9 groups according to tick species and sampling sites. Four tick species were morphologically identified: *Haemaphysalis longicornis* (84.8%); *Dermacentor silvarum* (7.2%); *D. nuttalli* (5.5%); and *Ixodes persulcatus* (2.5%) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/4/14-1602-Techapp1.pdf).

Of the 6,427 ticks collected, 3,410 were divided into 9 groups (average 379 ticks/group, range 163–512); each group was homogenized in SM buffer (50 mmol/L Tris, 10 mmol/L $MgSO_4$, 0.1 mol/L NaCl, pH 7.5). Viral RNA extraction, Solexa sequencing, and analysis are described in the online Technical Appendix. Among the sequences annotated to mammalian viruses, 65 contigs were found to target the small (n = 15), medium (n = 27), and large (n = 23) segments of the NSDV genome (online Technical Appendix Tables 2–4).

[1]These authors contributed equally to this article.

To confirm the Solexa results, a 376-nt fragment of the NSDV small gene segment was amplified by reverse transcription PCR (RT-PCR) by using primers P1 (5′-AGCAAAGAGCACATTGACTGGGC-3′) and P2 (5′-CTGTCACACCTGCCTTCCAA-3′). Ticks in 3 *H. longicornis* groups were positive for NSDV: group 1 from sheep in Jian, Jilin Province (125°34′E, 40°52′N); group 2 from cattle in Jinxing, Jilin Province (130°38′E, 42°25′N); and group 5 from sheep in Dandong, Liaoning Province (124°23′E, 40°07′N). Ticks in the other groups were negative. The obtained sequences shared 92% identity with NSDV from *H. intermedia* in India.

The full-length sequence of NSDV was then obtained from group 2 by RT-PCR by using primers based on the Solexa sequences or the conserved sequences of nairoviruses (online Technical Appendix Table 5). The complete sequences of the small, medium, and large segments of NSDV (China) (GenBank accession nos. KM464724–KM464726) contained 1,590, 5,077, and 12,081 nt, respectively; that is, they were similar to other NSDVs. Sequence comparisons showed 75.1%–89.6% identity with other NSDVs at the nucleotide level and 81.3%–96.7% at the deduced amino acid level (online Technical Appendix Table 6). Compared with other member species within the genus *Nairovirus* (Dugbe, Kupe, Hazara, and Crimean Congo hemorrhagic fever viruses), low identities (37.5%–68.6%) were observed at both nucleotide and amino acid levels (online Technical Appendix Table 6). Phylogenetic analysis based on the amino acid sequences grouped the virus together with NSDVs from Africa and South Asia (Figure).

The remaining tick samples of the NSDV-positive groups were used to determine the infection frequency by using RT-PCR to analyze primers P1 and P2. We assayed 104 tick pools (average 15 ticks/pool, range 8–40), 13 pools of 416 ticks in Jian Province and 91 pools of 1,095 ticks in Jinxing Province; 12.5% (13/104) tested positive, 38.5% (5/13) in Jian and 8.8% (8/91) in Jinxing. The higher prevalence in Jian Province may result from more ticks in the pools. Attempts to isolate virus from the positive samples in cell lines (Vero and BHK-21) and suckling mice were unsuccessful; thus, its pathogenicity could not be determined.

In Africa, NSDV is primarily transmitted by *R. appendiculatus* ticks (*5*). In South Asia (India and Sri Lanka), NSDV has been isolated from ticks (*H. intermedia*, *H. wellingtoni*, and *R. haemaphysaloides*), mosquitoes, sheep and humans; *H. intermedia* ticks are considered the main vector for the virus (*5*,*8*,*9*). NSDV had not previously been reported from East Asia. The isolate we identified, NSDV (China), is genetically divergent from the NSDVs of South Asia and Africa and is therefore a novel strain, with *H. longicornis* likely the main vector. Nairobi sheep disease has not been reported in China and East Asia, but our results

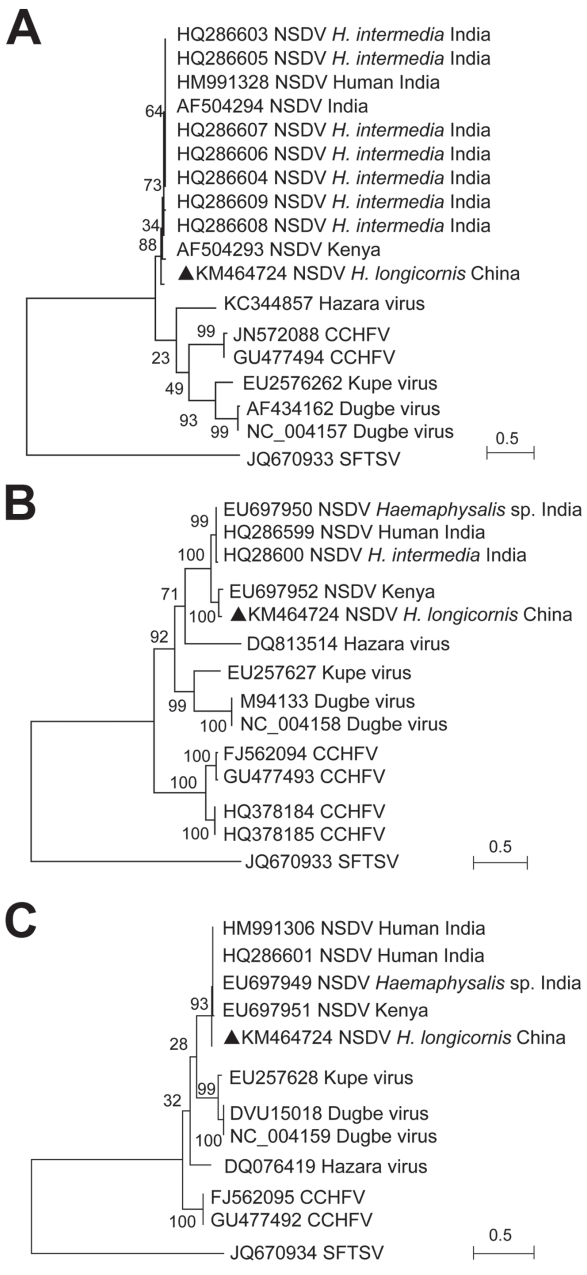


**Figure.** Phylogenetic analysis of Nairobi sheep disease virus (China) and other nairoviruses. The phylogenetic trees were generated in MEGA5.2 software (http://www.megasoftware.net). The complete coding regions for nucleocapsid protein in the small segment (A), glycoprotein precursor in the medium segment (B), and RNA dependent RNA polymerase in the large segment (C) were analyzed by the maximum-likelihood method. An emergent severe fever thrombocytopenia syndrome virus (SFTSV; family *Bunyaviridae,* genus *Phlebovirus*) was used as the outgroup. Bootstrap testing (1,000 replicates) was performed, and the bootstrap values are indicated. Sequences are identified by their GenBank accession numbers, followed by the virus name, host, and country. Black triangles indicate novel strain NSDV (China). Scale bars indicate substitutions per site. CCHFV, Crimean-Congo hemorrhagic fever virus.

indicate the risk of its occurrence in these regions, where *H. longicornis* is widely distributed (*10*). More extensive investigation to clarify the natural circulation of NSDV among ticks should be conducted and surveillance of sheep improved to prevent outbreaks of Nairobi sheep disease in China and East Asia.

This work was supported by the Science and Technology Basic Work Program from the Ministry of Science and Technology of China (2013FY113600), and the Military Medical Innovation Program of Academy of Military Medical Sciences (2012CXJJ019).

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# Avian Influenza A(H10N7) Virus–Associated Mass Deaths among Harbor Seals

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DOI: http://dx.doi.org/10.3201/eid2104.141675

**To the Editor:** Avian influenza A viruses occasionally cross the species barrier; influenza A(H5N1) virus and the recently emerged influenza A(H7N9) virus are prime examples of bird-to-human transmission (*1*,*2*). In addition, avian influenza A viruses can cross to various other mammalian species, including pinnipeds (e.g., seals) (*3*,*4*).

Recently, mass deaths have occurred among harbor seals (*Phoca vitulina*); hundreds of carcasses washed up the shores of Sweden (March 2014), Denmark (July 2014), and Germany (October 2014). Approximately 1,400 dead harbor seals were seen in the coastal waters of Schleswig-Holstein in Germany alone, where the population is ≈12,000 animals.

We report the investigation of the deaths of 17 seals from different age groups that were found dead on the islands of Helgoland and Sylt, Germany, during the second week of October 2014. Complete necropsies were performed on the carcasses, which were in variable nutritional conditions, ranging from very poor to good. Necropsy results showed consistently poorly retracted lungs with severe congestion, occasional diffuse consolidation, and multifocal firm nodular areas of gray-yellow discoloration with varying numbers of metazoic parasites. Histologic examinations showed acute necrotizing bronchitis and adenitis of bronchial glands with sloughing of epithelial cells (Figure, panel A). Occasionally, mild interstitial pneumonia was found. Multifocal pyogranulomatous to necrotizing pneumonia was associated with parasite infestation. A few animals had suppurative to necrotizing or nonsuppurative rhinitis and tracheitis.

Because mass deaths among seals were caused by phocine distemper virus in the same area in 1988 and 2002,

Article DOI: http://dx.doi.org/10.3201/eid2104.141602

# Nairobi Sheep Disease Virus RNA in Ixodid Ticks, China, 2013

## Technical Appendix

### Tick Collection, RNA extraction and Processing, Solexa Sequencing, and Analysis of Data

In the present study, ticks were collected respectively from sheep, cattle and by netting over the vegetation (Technical Appendix Table 1). Of the ticks collected from animals, some were fed, but the ticks collected from grass were unfed. All the ticks were adult with most of them being female. The ticks were pooled and examined according to their species and sampling site.

Tick samples were prepared for megagenomic analysis as described by He et al. (2013). The tick number of each pool was shown in Techncial Appendix Table 1. A total of 9 groups were pooled and homogenized in SM buffer [1:10 (w/v); 50 mM Tris, 10 mM MgSO4, 0.1 M NaCl, pH7.5], respectively. The homogenized samples were centrifuged at 8000×g at 4°C for 30 min to remove cell debris and foreign materials, and the supernatants were immediately filtered through 0.45-μm and 0.22-μm filters (Millipore, Billerica, MA). Host genome and other free nucleic acids were eliminated by digestion of DNase (Ambion, Austin, TX), Benzonase Nuclease (Novagen, San Diego, CA) and RNase I (Fermentas, Ontario, Canada).

The viral RNAs and DNAs were then extracted immediately using TRIzol (TaKaRa, Dalian, China) according to the manufacturer's protocol. Total viral nucleic acids were dissolved in RNase-free $H_2O$ (TaKaRa) and used immediately for the following reverse transcription with SuperScript III reverse transcription (Invitrogen, Carlsbad, CA) using random primers according to the manufacturer's protocol. To synthesize dscDNA, a Klenow fragment (New England

Biolabs, Beijing, China) was added to the cDNA mixture, and incubated at 37°C for 60 min. After inactivation of the enzyme, phosphates and free single-stranded bases in the dscDNA reaction was removed using shrimp alkaline phosphatase and exonuclease I (TaKaRa).

To obtain sufficient viral nucleic acid, SISPA was employed to amplify the dscDNA with the Accuprime Taq DNA Polymerase System (Invitrogen) according to the manufacturer's protocol. Briefly, a 50 µl reaction system containing 10 µl of the above dscDNA mixture, random primers (20 mM), 10×Accuprime buffer I, and Taq DNA Polymerase (1 U) was denatured at 94°C for 2 min, followed by 40 cycles of 94°C denaturing for 30 s, 55°C annealing for 30 s, 68°C extending for 1 min with final 68°C extension for 8 min. The PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and dissolved in 50 µl TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH8.0). The purified PCR products of the 9 groups were pooled together and then subjected to Solexa sequencing in one lane by the Beijing Genome Institute (BGI, Shenzhen, China).

## Virus Isolation Procedure

The homogenates were prepared in cooled medium, and virus isolation was conducted according to the standard procedures. Briefly, NSDV-positive samples were ground with cold serum-free minimum essential medium (MEM; Sigma-Aldrich) and centrifuged at 12,000 × g for 10 min at 4°C. Supernatants were passed through 0.22-µm syringe filters (Sartorius), and the filtrates were added to Vero and BHK cell lines with MEM containing 2% fatal bovine serum (FBS; GIBCO). The cell cultures were observed daily during incubation with 5% $CO_2$ at 37°C and passaged for 5 times. The cell cultures of each passage were subjected to RT-PCR screening of NSDV.

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**Technical Appendix Table 1**. Tick species, origin, and number screened and analyzed for presence of Nairobi sheep disease, China, 2013

| Group* | Tick species† | Sampling site | Animal host‡ | No. collected ticks (%) | No. ticks for metagenomic analysis | No. remaining ticks§ |
|---|---|---|---|---|---|---|
| 1 | *Haemaphysalis longicornis* | Jian, Jilin (125°34′ E, 40°52′ N) | Sheep | 920 (14.3) | 504 | 416 |
| 2 | *H. longicornis* | Jinxing, Jilin (130°38′ E, 42°25′ N) | Cattle | 1,525 (23.7) | 430 | 1,095 |
| 3 | *H. longicornis* | Chunhua, Jilin (131°04′ E, 43°11′ N) | Cattle | 679 (10.6) | 512 | 167 |
| 4 | *H. longicornis* | Nanshan, Jilin (130°47′ E, 42°84′ N) | Cattle | 1,826 (28.4) | 487 | 1,339 |
| 5 | *H. longicornis* | Dandong, Liaoning (124°23′ E, 40° 07′ N) | Sheep | 326 (5.1) | 326 | 0 |
| 6 | *H. longicornis* | Huma, Heilongjiang (125°03′ E, 50°49′ N) | No | 174 (2.7) | 174 | 0 |
| Subtotal | | | | 5,450 (84.8) | 2,433 | 3,017 |
| 7 | *Dermacentor nuttalli* | Harbin, Heilongjiang (125°42′ E, 44°04′ N) | No | 143 (2.2) | 143 | 0 |
| | | Songlin, Jilin (130°54′ E, 42°84′ N) | Cattle | 78 (1.2) | 78 | 0 |
| | | Huma, Heilongjiang (125°03′20″ E, 50°49′ N) | No | 130 (2.0) | 130 | 0 |
| Subtotal | | | | 351 (5.5) | 351 | 0 |
| 8 | *D. silvarum* | Harbin, Heilongjiang (125°42′ E, 44°04′ N) | No | 56 (0.9) | 56 | 0 |
| | | Songlin, Jilin | Cattle | 182 (2.8) | 182 | 0 |
| | | Huma, Heilongjiang (125°03′ E, 50°49′ N) | No | 225 (3.5) | 225 | 0 |
| Subtotal | | | | 463 (7.2) | 463 | 0 |
| 9 | *Ixodes persulcatus* | Harbin, Heilongjiang (125°42′-130°10′ E, 44°04′-46°40′ N) | No | 31 (0.5) | 31 | 0 |
| | | Nanshan, Jilin (130°28′ E, 42°28′ N) | Cattle | 62 (1.0) | 62 | 0 |
| | | Chunhua, Jilin (131°04′ E, 43°11′ N) | Cattle | 23 (0.3) | 23 | 0 |
| | | Huma, Heilongjiang (125°03′ E, 50°49′ N) | No | 47 (0.7) | 47 | 0 |
| Subtotal | | | | 163 (2.5) | 163 | 0 |
| Total | | | | 6,427 | 3,410 | 3,017 |

* Group 1–6: each group comprises ticks collected from a single site. Group 7–9: each group comprises pooled ticks collected from multiple sites.

† Species was identified according to tick morphology described by Cheng and Pang (1992).

| Group* | Tick species† | Sampling site | Animal host‡ | No. collected ticks (%) | No. ticks for metagenomic analysis | No. remaining ticks§ |
|---|---|---|---|---|---|---|

‡ No indicates that the ticks were collected by sweep netting the vegetation.

§This column shows the numbers of remaining ticks not subjected to metagenomic analysis, in which the ticks only in groups 1 and 2 were analyzed for prevalence testing since these 2 groups were NSDV RT-PCR positive.

**Technical Appendix Table 2**. Sequences of S gene contigs and their identities to NSDV strain 708, China, 2013

| Contig | Location | Sequence (5′→3′) | Sequence identity (%) |
|---|---|---|---|
| es.1 | 678–877 | CAAGTTCCTTGTTGTCTTCAATCCACCATGGGGTGATATCAACAAGGCAGGAAAGTCAGGGATTGCCTTAGCAGCGAC AGGTATGGCAAAATTGATAGAGCTGGATGGTCCCAAAATTGCAGAGGACCTGAGGGAATCTCTGAAGGGTCTTGTGG CATGGATCAATGCCCACAAGGATGAAGTGGAGAACGGTAAAGAGG | 87 |
| 2 | 865–1109 | AACGGTAAAGAGGTTGTTGATGGTTTGACCAAGCACCTGCAGAAAGCCCTTGAATTAGCCAAGCAATCAAGTGCCATG AGAGCCCAAGGGGCTCAGATTGACACTGTCTTTAGCAGCTACTACTGGCTTTGGAAGGCAGGTGTGACAGCGGAGAT GTTTCCGACAGTCTCACAGTTTCTTTTTGAGCTCGGCAAGGTGCCCAGGGGAAATAAAAAAATGAAGAAAGCACTATC AAGTATGCCTCT | 92 |
| 3 | 1096–1259 | TCAAGTATGCCTCTGAAGTGGGGAAAGAAGTTGCTAGCACTCTTTGCTGATGATAGCTTCACTGCTAATCGGATTTACA TGCACCCTGGTGTCCTAACAGCTGGGAGAATGTCTGAGCTCGGTGTTTGCTTCGGAGCAATCCCAGTTGCCAATCCT GACGATGC | 92 |
| 4 | 1368–1478 | AGGCTTCGACATTGAGAGCATGGACATAGTTGCCTCGGAGCATCTGCTACACCAGTCACTCGTTGGAAAGAGATCTCC TTTTCAGAATGCCTACAACATCCGGGGAAATGC | 90 |
| 5 | 394–572 | ACGGAAGTACCCAGCCCTGAACAACTGCTGGGTTACCAGAGAGCAGCTCTTAAGTGGAGAAAGGATACCAAGTACGG GATCAACAGAAATACAGCTGCACTGGCCGCTGCAATTGCAACCGAGTATCGGAGCTCTGCAGATATCGTGGTGAATGT CAAGGACATGCTGTCGGACATGATCA | 78 |
| 6 | 538–680 | TCGTGGTGAATGTCAAGGACATGCTGTCGGACATGATCAGGAGAAGGAACAAGATCCTGAACAGAGACGGCAGTGAA GATGTTCCGAAAAGGGGACCCGTCAGCAAAGAGCACATTGACTGGGCTAGGGACCTTGCTCAAGGTAA | 88 |
| 7 | 457–626 | GATACCAAGTACGGGATCAACAGAAATACAGCTGCACTGGCCGCTGCAATCGCAACCGAGTATAGAGTCCCTGGATC AATTGTGGTGAATGTCAAGGACATGCTGTCGGACATGATCAGGAGAAGGAACAAGATCCTGAACAGAGACGGCAGTG | 88 |

| Contig | Location | Sequence (5′→3′) | Sequence identity (%) |
|---|---|---|---|
| | | AAGATGTTCCGAAAAG | |
| 8 | 406–578 | AGCCCTGAACAACTGCTAGGTTACCAGAGAGCAGCTCTTAAGTGGAGAAAGGATACCAAGTACGGGATCAACAGAAA<br>TACAGCTGCACTGGCCGCTGCAATTGCAACCGAGTATAGAGTCCCTGGATCAATTGTGGTGAATGTCAAGGACATGCT<br>GTCGGACATGATCCGGAG | 85 |
| 9 | 1365–1500 | GGCCGGCTTCGACATTGAGAGCATGGACATAGTTGCCTTGGAGCATCTGCTACACCAGTCACTCGTTGGAAAGAGAT<br>CTCCTTTTCAGAATGCCTACAACATCCGGGGAAATGCCACCAG | 89 |
| 10 | 204–349 | GCTTGTTTCATCGGAGAGTGAGAAGGACTCAGTGTATGTATCTGCCCTGGTGGCCGCGACTAAATTCTGTGCTCCTAT<br>ACTGGAGTGTGCCTGGACCAGCTGCACGGGGATGGTCGAGCGTGGTCTGGACTGGTTTGAAAACAACA | 86 |
| 11 | 531–657 | ATCAATTGTGGTGAATGTCAAGGACATGCTGTCGGACATGATCAGGAGAAGGAACAAGATCCTGAACAGAGACGGCA<br>GTGAAGATGTTCCGAAAAGGGGACCCGTCAGCAAAGAGCACATTGACTGG | 89 |
| 12 | 264–437 | CAACTTCTGTGCTCCTATACTGGAGTGTGCCTGGACCAGCTGCACGGGGATGGTCGAGCGTGGTCTGGACTGGTTTG<br>AAAACAACAAGGAAACAGTGAAGATTTGGGATGCTGAGTACGGGAAGCTAAGAACGGAAGTACCCAGCCCTGAACAA<br>CTGCTGGGTTACCAGAGAGC | 81 |
| 13 | 562–681 | TCGGACATGATCAGGGGAAGGAACAAGATCCTGAACAGAGACGGCAGTGAAGATGTTCCGAAAAGGGGACCCGTCA<br>GCAAAGAGCACATTGACTGGGCTAGGGACCTTGCTCAAGGTAAG | 88 |
| 14 | 1345–1459 | GCCCTATTCAACATTCAGAAAGCCGGCTTCGACATTGAGAGCATGGACATAGTTGCCTCGGAGCATCTGCTACACCAG<br>TCACTCGTTGGAAAGAGATCTCCTTTTCAGAATGCTT | 89 |
| 15 | 1366–1457 | GCCGGCTTCGACATTGAGAGCATGGACATAGTTGCCTCGGAGCATCTGCTACACCAGTCACTCGTTGGAAAGAGATC<br>TCCTTTTCAGAATGC | 90 |

**Technical Appendix Table 3**. Sequences of M gene contigs and their identities to NSDV strain 708, China, 2013

| Contig | Location | Sequence (5′→3′) | Sequence identity, % |
|---|---|---|---|
| 1 | 3162–3337 | CTTTCTGGAAGGTCAACAGGAATCATAAAACTCCAGGAAAGAACTGGTCTGATGTGGAAGATGTCATCAGAAAAGGCATC<br>AGAAAGCAAAAACCTTCTTGTTTCAGTGATGGATTTTTCCAGCTCTACAATTCCATATTTCAGTATATAACGGGTGACAG<br>GAGTCTTTCAGAATG | 89 |
| 2 | 1743–1901 | GAATGCCAGGGAAGCTGCCTCTTTGCCATCCCACAGGACACAGGAGACATCACAATTGACTGCTCTGGAGGAAGGCAGCA | 90 |

| Contig | Location | Sequence (5′→3′) | Sequence identity, % |
|---|---|---|---|
| | | TTACCTGGAAATTAATATAGTTGACATACACTGTCCAGGGAAAGACAAATGGAAAGGGTTTATGCTCTACATATGCAGG | |
| 3 | 4512–4678 | ATTGAGCACAAAGGGACCATCATACAGCACTACAACAAAAGTTGTGATGAGGGATATAATTGCTGGGTTGGATCTGTTTC<br>TGGATTCTTTGTTGGAGTGAAAGACTTTTTTGAAAAGAACCTTGGAGGTGTACTTATTGGTCTTGTAAGCACAATACTTC<br>CTTTAGT | 88 |
| 4 | 4396–4542 | GGGGTGTAAAGAAGTTGTGCCTGGAAGTGGTTGAGAAGGACTACTGTCCTTCCTGCACCGTAGAAGATTTGAAGATATGT<br>GTTGATGTGTCTCTTGAGCCCCCTAAAGACATACTCATTGAGCACAAAGGGACCATCATACAGCACT | 91 |
| 5 | 4071–4211 | TGTAAAGAAGTTGTGCCTGGAAGTGGTTGAGAAGGACTACTGTCCTTCCTGCACCGTAGAAGATTTGAAGATATGTGTTG<br>ATGTGTCTCTTGAGCCCCCTAAAGACATACTCATTGAGCACAAAGGGACCATCATACAGCA | 79 |
| 6 | 313–468 | AGAATTTTATCAAAAATGGGCATGGCCAGAGACTTCGCAGATGAGGAGTTAGACGTCTGGTGTCACGAGCGGTTTGACAA<br>CTGTAGCTCTAATGACATTGAAAATAGAATCAGGGATTTCTTTCTCATCAGTGATCAGACTGAGTGCTTTGATAAA | 76 |
| 7 | 1573–1720 | GTGTCAAGCAGGCCTTTAATTGCCTTGACATTTGGATTGTGGCTTGCAGCAGGCTACCTAATAACATGCCTTGTTTCCTT<br>TATCATCTATAATGCTGTTTTGCTTCTCAGCATTGCCATCAAGAAAGTGAAACAAGGGAGAGAAAA | 74 |
| 8 | 1580–1748 | TAACAAGGTGATAACAGGAAAGGCATGTAAAGTGGGAAACTCCACAGGATCCTGTGAGGTGCAAACAGAGCTACAGAAGT<br>GCGAGACTGGTAAATGCATACTGGTGAAACAGAAAAGCAAAGGAGTGGTGAAACTGAAAAGAGGAAAGACTGTAATCAT | 88 |
| 9 | 3477–3629 | TGCTGTGGGATGGATGGTGAGAGGTACTTTAATAAATACTTCGGTGTGAAATGGGCCTTAGAATATGTTAGAACTGATGT<br>AGTAGTGTGTGTGGAACTCACAAATGAGGAAAGACATTGTGACCTTGTGCAGGCCGGAAGCCGCTTTTCCATA | 88 |
| 10 | 618–763 | AAAATGAAAAAACTGCTTAGCCAAAGAATTTTATCAAAAATGGGCATGGCCAGAGACTTCACAGATGAGGAGTTAGACGT<br>CTGGTGTCACGAGCGGTTTGACAACTGTAGCTCTAATGACATTGAAAATAGAATCAGGGATTTTTT | 88 |
| 11 | 4000–4154 | TCACCAATCTCATAAACACAGAGACCGATTACACAAGCAAGTTCCACTTCCACTCAAAAAGGATTTCAGCTAGAGATGAC<br>ACTTTGCAGATGGACCTGAAAGCAAGACCTAACTCCGGAGGCGGTGAGATGACAGTTTTGGTAGAGGTTAATGGG | 86 |
| 12 | 4602–4764 | GTTGGAGTGAAAGACTTTTTTGAAAAGAACCTTGGAGGTGTACTTATTGGTCTTGTAAGCACAATACTTCCTTTAGTGCT<br>GACAGTTCTGTTCTTTATTTATGGCAAGAAGCTGTTCTGCCTGTGCCGACTGTGCCACAAGAAATGTTGTAGAGGCTCAG<br>GAA | 85 |
| 13 | 1458–1571 | ATGACAACGGCTGTGTCTGTTCATAGTGACAAAAAAGGAGGACCTGGAAAGAAGCTCACCATATGCAATGGTACCACAGT<br>CTCAGATTCAACACTCAATGAAGGGCTCGGATGT | 91 |
| 14 | 4830–4937 | AGCAGGAACGGCGAACTACTAGGGAAGGGTGAAAATGACAAAAGAACTGTGGCAAGGATGTTCATGGATGGGCAAAGTAC<br>AAAGAAGGCAATCAAGGAAGTAGCTTAA | 91 |

| Contig | Location | Sequence (5′→3′) | Sequence identity, % |
|---|---|---|---|
| 15 | 3992–4159 | AGACAGCTCCAATCTCATAAACACAGAGACCGATTACACAGGCAAGTTCCACTTTCACTCCAAAAGGATTTCAGCTAGAG ATGACACTTTGCAAATGGATCTGAAAGCAAGACCTAACTCCGGAGGCGGTGAAATGACAGTTTTGGTAGAGGTTAATGGG TTGGA | 84 |
| 16 | 4017–4178 | ACAGAGACCGATTACACAGGCAAGTTCCACTTTCACTCCAAAAGGATTTCAGCTAGAGATGACACTTTGCAAATGGATCT GAAAGCAAGACCTAACTCCGGAGGCGGTGAAATGACAGTTTTGGTAGAGGTTAATGGGTTGGAGCTGCATTCAAAAAGGA TA | 84 |
| 17 | 4462–4590 | ATTTTAAGATATGTGTTGATGTGTCTCTTGAGCCCCCTAAAGACATACTCATTGAGCACAAAGGGACCATCATACAGCAC TACAACAAAAGTTGTGATGAGGGATATAATTGCTGGGTTGGATCTGTTT | 90 |
| 18 | 4568–4711 | TAATTGCTGGGTTGGATCTGTTTCCGGATTCTTTGTTGGAGTGAAAGACTTTTTTGAAAAGAACCTTGGAGGTGTACTTA TTGGTCTTGTAAGCACAATACTTCCTTTAGTGCTGACAGTTCTGTTCTTTATTTATGGCAAGAA | 86 |
| 19 | 1037–1182 | CATGCACTGCTTAAACATAGAGAATGGGTTAGCTAAACCATCCAAAATGGTTGTTCTTAATGTATTAATGACAACAGTAG AGGTTAGACTGGAATCCTGCCGTGCGTTCATAAATGCACATCAATGCATTTACACACAACATGCAG | 86 |
| 20 | 3693–3785 | ATGACAATTCAAGAAATTGCTGATAATGGCATCTTGGACCTGATGCATGTGAGCAAAGTCATCTCAGCAGAGAATGCCTG CAAACTCCAGAGC | 90 |
| 21 | 4309–4427 | GCAGATATCGTGGTGGTTGCTGAGACCAGTGTAACAGCCAGGAAGGTAGAGACAGGAGCTAGGAGCGGCTTCAGAGCCTT TGCAGTAAGGGATGTAAAGAAGTTGTGCCTGGAAGTGGTT | 88 |
| 22 | 1248–1408 | ACGATGAGCTTCAACTTGACTGATGAAAGAAATAGAGCATGCATCATCAGAACAACTTGTGAGGTCAAAGGAAAAGAGGT AAAGAAGGGACAGAGTCAATTGAGAGGTTTCCCAACAAGTATCAGACTCTTCAAGTCTGTGACAGGAAAAAGAAGGCTCA | 91 |
| 23 | 1293–1435 | ATCAGAACAACTTGTGTGGTCAAAGGAAAAGAGGTAAAGAAGGGACAGAGTCAATTGAGAGGTTTCCCAACAAGTATCAG ACTCTTCAAGTCTGTGACAGGCAAAAGAAGGCTCATGTCAGAGGAGGGAGATTGGACTGACTG | 92 |
| 24 | 3620–3764 | TTTTTCCATAGGTCCTGTGTCTGTAACTCTGTCTGACCCCCAGAATGTGGTGAACCGTCTCTCGAGCAACATCATGACAA TTCAAGAAATTGCTGATAACGGCATCTTGGACTTGATGCATGTGAGCAAAGTGATATCTGCAGAG | 91 |
| 25 | 1897–2049 | GCAGGGAGGCAAGCAGGCCTTTAATTGCCTTGACATTTGGATTGTGGCTTGCAGCAGGCTACCTAATAACATGCCTTGTT TCCTTCATCATCTATAATGCTGTTTTGCTTCTCAGCATTGCCATCAAGAAAGTGAAACAAGGGAGAGAATGGA | 88 |
| 26 | 994–1151 | TTATGAACGAACTTCAGGCCCCACTATCCAAAACTATGATAGCATGCACTGCTTAAACATAGAGAATGGGTTAGCTAAAC CATCCAAAATGGTTGTTCTTAATGTATTAATGACAACAGTAGAGGTTAGACTGGAATCCTGCCGTGCGTTCATAAAT | 86 |

| Contig | Location | Sequence (5′→3′) | Sequence identity, % |
|---|---|---|---|
| 27 | 2243–2494 | TTTATTCTGACAATTTCACCGGTGCAGGGATTCCCATTGGAGAGTCCACCTATTGGCGCAGCAACTGACAGTAGAATCAT<br>GTATGGGGTAGTGGGGTTCATGTTTGCACTAATAATGATGCTTAATCTTAAAAAATCACACTACGGTTCCAT | 86 |

**Technical Appendix Table 4**. Sequences of L gene contigs and their identities to NSDV strain 708, China, 2013

| Contig | Location | Sequence (5′→3′) | Sequence identity (%) |
|---|---|---|---|
| 1 | 971–1121 | TAGATGCTGGCGGCCTCCTACGTTCAGCATTTCCAGGAATGGGATTAGAGAGGAACCTGCAATTCTTGCACTCAGAGGTTCTG<br>CTTGATGTGTGCACAGTGGTTGTTGCTGTCCTACTGTCTTCCTTCCTGTACGGGTCAAACAACAGAAA | 89 |
| 2 | 1122–1291 | TAAGAAGACATTTATAACAAACTGCTTGCTGAACACAAGCCTTTCAGGGAAGAGAGTCTTCAAGGCACTAGGAAAGCTAACAGG<br>CACCACTCTTTACAAAAGTCCTAGGAATGCTTTGTCCCATGTGTGTCAAACACTCTATGGCAAGATGACAGGAGATATCTGCAG<br>AGCT | 86 |
| 3 | 2632–2812 | AGACAGATACTACTAGTTGAGGTTGGTTATCAGACCGATGTGGACGGCAAAATAACAACAGACTTTAAGAAGTGGAAAGACATC<br>TTGAGGCTCCTAGAGATGTTGGAAATCAAGTGTTCATTCATAGCCTGTGCAGACTGCACATCAACACCTGCGGACAACTGGTGG<br>ATATCTGTCGACA | 92 |
| 4 | 9940–10105 | GACAAGACATCTCTTCTCATAAGCTTCCTCAACTGGAAAGTTTCACCTGACTCAATGGCACAAGATTGTCCGCTTTACAAACAGG<br>AACATGCTGTCATCAGTGAATTTGCAGGACAGGTTGTTGTGAACACACTTGCCAGTGAGCTTAGCTCTGTTAGGAGAGATG | 92 |
| 5 | 6811–6964 | TGCAAGCGTCTGACTGGCAGAACAACTGGTGAAAGGCTTCCAAGGAGTGTGAGAAGCAAAGTAATTTATGAAATGGTAAAGCTT<br>GTTGGGGAAACTGGAATGGCAATACTACAACAACTGGCCTTTGCCCAAGCTCTTAATTACGATCATAGAT | 94 |
| 6 | 5253–5423 | AAGCTTAAGTGGGAGACGTTCTTACTGTTCTAGTAGAGGGAGGATTCAGAGCATCTTTGGCAGGGACAATTCAAACAAAAAACC<br>TTTTGAGTTTAGACCAGGCTTAGAAGTTAGAGCAGATCCAATGAATGATTTCGAGCAAGCAGTCACACACACAACGATATCTGC<br>AGA | 90 |
| 7 | 6853–7015 | AGGAGCGTGAGAAGCAAAGTAATTTATGAAATGGTAAAGCTTGTTGGGGAAACTGGAATGGCAATACTACAACAACTGGCCTTT<br>GCCCAAGCTCTTAATTACGATCATAGATTTTATGCAGTGTTGGCACCAAAAGCCCAACTTGGAGGGACTAGATATCTGT | 90 |
| 8 | 1061–1227 | TGTGCACAGTGGTTGTTGCTGTCCTACTGTCTTCCTTCCTGTACGGGTCAAACAACAGAAACAAGAAGACATTTATAACAAACTG<br>CTTGCTGAACACAAGCCTTTCAGGGAAGAGAGTCTTCAAGGCACTAGGAAAGCTAACAGGCACCACTCTTTACAAAAGTCCT | 89 |
| 9 | 6262–6418 | AGAATGAAGAAGGATAATCCCAGCGTAAGCTTCACCAAGGAAGAAGTTCTTGTGAAAAGACTTGAGAAGTCATTCCTGAAGAAA | 91 |

| Contig | Location | Sequence (5′→3′) | Sequence identity (%) |
|---|---|---|---|
| | | TTTAACAAGGAAGCAATGAAGTTTGTCAATCTAGTGTTCTTTTGTTCACTCTCTGCCCCTTGGTGTGTTCATT | |
| 10 | 6289–6443 | AGCTTCACCAAGGAAGAAGTTCTTGTGAAAAGACTTGAGAAGTCATTCCTGAAGAAATTTAACAAGGAAGCAATGAAGTTTGTCA ATCTAGTGTTCTTTTGTTCACTCTCTGCCCCTTGGTGTGTTCATTACAAGTCTTTAGAGTCTTACTTGGT | 92 |
| 11 | 8921–9083 | CTGCAGATATCTCCAGACTAAAACAGACCTTGACAGCCAGAAACGTCCTGCATGGCCTTGCTGGAGGTATCAAGGAGCTGTCA CTTCCTATCTATACCATCTTCCTAAAGTCGTACTTTTTTAAAGACAATGTCTTCTTAGACCTTGAGGACAGATGGTCAAC | 90 |
| 12 | 6900–7051 | GGAAACTGGAATGGCAATACTACAACAACTGGCCTTTGCCCAAGCTCTTAATTACGATCATAGATTTTATGCAGTGTTGGCACCA AAAGCCCAACTTGGAGGGGGTAGGGATCTGCTAGTCCAAGAGACCGGCACAAAAGTTATCCATGCCA | 91 |
| 13 | 4969–5112 | AACAGTGACCGACAGTTAATCTTTGACATTTACAATGTACACATCTACAACAAGGAGATGGACAATTTTGATGAAGGATGCATAA GTGTGTTAGAAGAGACTGCTGAAAGACACATGCTGTGGGAAATGGATCTTCTTAGGTCA | 91 |
| 14 | 8692–8859 | GATGGGACAGATAAGCAAGTTAAGGCATCTCTCAATAGGGACGACAATAGAGTTATCGAAGACCCCATGATTCAACTAGTACCT GAAAAGCTGAGAAGAGAGCTAGAGAGGCTGGGAGTCTCTAGGATGGAGATCGATGAACTAATGCCTTCTATAAAGCATGACGA A | 86 |
| 15 | 4909–5055 | TTGAAAGACTTGTGCCCTGAAGTCACAATTCCATGCTTCTCCGTCTATGGTGTATTTGTGAACAGTGACCGACAGTTAATCTTTG ACACTTACAATGTACACATCTACAACAAGGAGATGGACAATTTTGATGAAGGATGCATAAGT | 86 |
| 16 | 6786–6906 | GATATCAAACAGTAACTTCAATGTCTGCAAGCGTCTGACTGGCAGAACAACTGGTGAAAGGCTTCCAAGGAGTGTGAGAAGCA AAGTAATTTATGAAATGGTAAAGCTTGTTGGGGAAACT | 91 |
| 17 | 3477–3582 | TGCAGCTCTGCAGATATCGATAGGGTGGTTCGGTTGACTTTACCTGGCAAAACTGAAAAGGAGAGAAGGATTAAGAGAAATGTT GAAACACTAATCCTGTTGATG | 89 |
| 18 | 5638–5741 | CCTAGAACACACATAATGCTCAAAGACTGTTTCAAGATTCTTCTCGGCACTGAGAACAAGAAAATCGTCAAGATGCTAAGAGGC AAGCTAAAGAAGCTGGGTGC | 88 |
| 19 | 5026–5099 | ATGGACAATTTTGATGAAGGATGCATAAGTGTGTTAGAAGAGACTGCTGAAAGACACATGCTATGGGAAATGGA | 95 |
| 20 | 8680–8766 | TCATTTGGTAGCGACGGGACAGATAAGCAAGTTAAGGCATCTCTCAATAGGGACGACAATAGAGTTATCGAAGACCCCAT GATTCAA | 90 |
| 21 | 8968–9092 | CTTCATGGCTTGGCTGGAGGTATAAAAGAACTGTCCCTGCCTCTTTACACCATCTTCCTCAAATCATACTTCTTCATAGA TAAGGTATTTTTATCCCATGCAGATAGGTGGAACACCAAGCACAG | 74 |
| 22 | 7836–7969 | AAGAGGCTGTCAGATGAAGGACTCTGCAAAAACGCTCATAGGAGATGTCATGTGTGAGTTTTACAGTGAGTTTATGCTAT | 73 |

| Contig | Location | Sequence (5′→3′) | Sequence identity (%) |
|---|---|---|---|
| | | ACCATAGGGTGACACCTGCAGTCATTAAGTTCATTATCGC | |
| 23 | 7537–7639 | TATATATCCAAAGGAAAGCTGGCCCTAGACTGCTACAACCACATGGGACAGGGCATACACCATGCCACCTCATCAGTAAT GACCTCTTGCATGGCTGAACTGT | 77 |

**Technical Appendix Table 5**. Primer sequences used to amplify the complete genome of NSDV, China, 2013

| Genome segment, interest fragment | Forward (location) (5′→3′) | Reverse (location) (5′→3′) |
|---|---|---|
| Small | | |
| 1–1012 | S-F1: TCTCAAAGACAAACGTGCCGCTTTCGCC (1–28) | S-R1: CTGTCACACCTGCCTTCCAA (1012–993) |
| 637–1012 | S-F2: AGCAAAGAGCACATTGACTGGGC (637–659) | S-R2: CTGTCACACCTGCCTTCCAA (1012–993) |
| 826–1335 | S-F3: CTTGTGGCATGGATCAATGC (826–845) | S-R3: CTGTGCGCAGGGGTTGCCAG (1335–1316) |
| 1165–1590 | S-F-4: CGGATTTACATGCACCCTGGTGTC (1165–1188) | S-R4: TCTCAAAGAGATCGTTGCCGCACAGCC (1590–1564) |
| Medium | | |
| 1–1451 | M-F1: TCTCAAAAGAGATAGTTGCGGCACTAGCAGG (1–30) | M-R1: CTGTGTGCCAGATCCGCAGTCAGT (1451–1428) |
| 1428–1979 | M-F2: ACTGACTGCGGATCTGGCACACAG (1428–1451) | M-R2: GGAAACAAGGCATGTTATTAGATAGCC (1979–1953) |
| 1953–3187 | M-F3: GGCTATCTAATAACATGCCTTGTTTCC (1953–1979) | M-R3: ATGATTCCTGTTGACCTTCCAGAAAG (3187–3162) |
| 3162–4431 | M-F4: CTTTCTGGAAGGTCAACAGGAATCAT (3162–3187) | M-R4: TCTCAACCACTTCCAGGCACAACTT (4431–4407) |
| 4056–5075 | M-F5: TACACAGGCAAGTTCCACTTTCAC (4056–4079) | M-R5: TCTCAAAGAGATAGTGGCGGCACAGCA (5077–5051) |
| Large | | |
| 1–1098 | L-F1: TCTCAAAGATATCAATCCCCCCGTTACCCCAGAGTTGC (1–38) | L-R1: ACAGGAAGGAAGACAGTAGGACAGC (1098–1078) |
| 995–2246 | L-F2: CAGCATTTCCAGGAATGGGATTAG (995–1018) | L-R2: TCAGGCAGAGGAAACATCTTCTTCT (2246–2222) |
| 1775–2875 | L-F3: GCATTGAGCCAATTGCGATAATATG (1775–1799) | L-R3: CAGGAGAGTTCTTTGTGAGGCTGCT (2875–2851) |
| 2850–3833 | L-F4: TAGCAGCCTCACAAAGAACTCTCCT (2850–2874) | L-R4: GCTCTAGGCACTGAACACTTGGA (3833–3811) |
| 3811–5046 | L-F5: TCCAAGTGTTCAGTGCCTAGAGCATG (3811–3836) | L-R5: TCCTTCGTCAAAATTGTCCATCTC (5046–5023) |

| Genome segment, interest fragment | Forward (location) (5′→3′) | Reverse (location) (5′→3′) |
|---|---|---|
| 5023–6847 | L-F6: GAGATGGACAATTTTGACGAAGGA (5023–5046) | L-R6: GCCTTTCACCAGTTGTTCTGCCAGTC (6847–6822) |
| 6696–7767 | L-F7: AGACGAGAAGCTGTTGCATCAGAC (6696–6719) | L-R7: GAGTTCTTTAGGCAGTAGCCCAGT (7767–7744) |
| 7744–8737 | L-F8: ACTGGGCTACTGCCTAAAGAACTC (7744–7767) | L-R8: TGTCGTCCCTATTGAGAGATGCCTT (8737–8713) |
| 8713–9807 | L-F9: AAGGCATCTCTCAATAGGGACGACA (8713–8737) | L-R9: GACTAGCTCAGACACCGTGGGC (9807–9786) |
| 9786–11367 | L-F10: GCCCACGGTGTCTGAGCTAGTC (9786–9807) | L-R10: GGTGGCAACTGCTTCAATTTCTT (11367–11345) |
| 11345–12081 | L-F11: AAGAAATTGAAGCAGTTGCCACC (11345–11367) | L-R11: TCTCAAAGAAATCGTTCCCCCCCCACCCC (12081–12053) |

**Technical Appendix Table 6.** Gene lengths and identity between NSDV from specimens collected in China and other nairoviruses*

| | Small segment | | | | | Medium segment | | | | | Large segment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GenBank accession | | % | | % | GenBank accession | | % | | % | GenBank accession | | % | | |
| Virus | No. | nt | ID | aa | ID | no. | nt | ID | aa | ID | no. | nt | ID | aa | % ID |
| NSDV (China) | KM464724 | 1590 | NA | 482 | NA | KM464725 | 5077 | NA | 1628 | NA | KM464726 | 12081 | NA | 3992 | NA |
| NSDV (India) | AF504294 | 1590 | 87.9 | 482 | 96.7 | EU697950 | 5094 | 75.1 | 1624 | 81.3 | EU697949 | 12081 | 88.7 | 3991 | 96.6 |
| NSDV(Kenya) | AF504293 | 1590 | 88.4 | 482 | 95.6 | EU697952 | 5077 | 87.6 | 1627 | 93.2 | EU697951 | 12081 | 89.6 | 3991 | 96.1 |
| Dugbe virus | NC_004157 | 1716 | 59.8 | 483 | 59.6 | NC_004158 | 4888 | 51.1 | 1551 | 44.8 | NC_004159 | 12255 | 66.9 | 4036 | 67.6 |
| Kupe virus | EU257626 | 1694 | 61.8 | 483 | 61.9 | EU257627 | 4818 | 52.5 | 1549 | 48.1 | EU257628 | 12330 | 64.8 | 4050 | 67.5 |
| Hazara viru | KC344857 | 1677 | 63.3 | 485 | 62.1 | DQ813514 | 4576 | 54.2 | 1421 | 48.8 | DQ076419 | 11980 | 64.0 | 3923 | 68.6. |
| CCHFV | GU477494 | 1673 | 59.9 | 482 | 62.0 | GU477493 | 5377 | 39.5 | 1697 | 37.5 | GU477492 | 12158 | 64.9 | 3945 | 64.7 |

*nt, nucleotide length; % ID, percentage identities of nt and aa (amino acid) between NSDV (China) and other nairoviruses; NA, not applicable; CCHFV, Crimean Congo hemorrhagic fever virus.