

## Xi River virus, a new bat reovirus isolated in southern China

Linfeng Du · Zongji Lu · Yu Fan · Keyin Meng ·  
Yu Jiang · Yan Zhu · Shumin Wang · Wanjun Gu ·  
Xiaohuan Zou · Changchun Tu

Received: 30 January 2010 / Accepted: 29 April 2010 / Published online: 22 May 2010  
© Springer-Verlag 2010

**Abstract** Nelson Bay orthoreovirus (NBV) is a species in the genus *Orthoreovirus*, family *Reoviridae*, containing 4, possibly 5, members. Here, we report a putative sixth member, Xi River virus (XRV), isolated from fruit bats collected in a location near the Xi River, Guangdong Province, China. This virus showed the same electron microscopic morphology as NBV, fusogenic CPE, and a 10-segmented double-strand RNA genome, as well as high sequence identity to NBV members. It is the first bat reovirus isolated in China.

The family *Reoviridae* consists of a large number of diverse viruses containing segmented, double-stranded RNA (dsRNA) genomes. Its members are classified taxonomically into 12 genera and have a wide host range, from mammals, birds, fish, and insects to reptiles and plants. The

virus particles are non-enveloped and icosahedral, with a diameter of 60–80 nm [13]. Within the family *Reoviridae*, members of the genus *Orthoreovirus* were originally divided into fusogenic and nonfusogenic groups based on their ability to cause cell fusion and syncytia [7], but they are now classified into five species based on their host range: *Mammalian orthoreovirus* (MRV), *Avian orthoreovirus* (ARV), *Nelson Bay orthoreovirus* (NBV), *Baboon orthoreovirus* (BRV) and *Reptilian orthoreovirus* (RRV), with MRV being non-fusogenic and the other four fusogenic. The dsRNA genomes of orthoreoviruses contain approximately 23,500 base pairs in 10 segments divided into three size classes based on their mobility in SDS-polyacrylamide gel electrophoresis (SDS-PAGE): three large (L1, L2 and L3), three medium (M1, M2 and M3) and four small segments (S1, S2, S3 and S4) [3].

In 1968, Nelson Bay virus was isolated from a fruit bat in Nelson Bay, New South Wales, Australia, giving rise to the first report of a bat reovirus [8]. Later, it was classified as belonging to a separate species within the genus *Orthoreovirus* [7]. In 2006, a second bat reovirus, Pulau virus (PuV), was reported, isolated from fruit bats during a search between 1998 and 1999 for Nipah virus in this species on Tioman Island, Malaysia. It was then characterized as a new member of the NBV species, since it was serologically and genetically closely related to NBV [14]. In 2007 and 2008, two new members of the NBV species, Melaka virus (MeV) and Kampar virus (KaV) were discovered in Malaysia [5, 6]. Although MeV and KaV were not directly isolated from bats, their close relationship to NBV and PuV and preliminary epidemiological data suggested that both are most likely bat-associated orthoreoviruses.

The mammalian orthoreoviruses have been considered to have limited human pathogenicity, causing at most only

---

Linfeng Du, Zongji Lu contributed equally to the study.

---

**Electronic supplementary material** The online version of this article (doi:10.1007/s00705-010-0690-4) contains supplementary material, which is available to authorized users.

---

L. Du · Y. Fan · K. Meng · Y. Jiang · X. Zou · C. Tu (✉)  
Institute of Veterinary Sciences, Academy of Military Medical  
Sciences, 1068 Qinglong Road, Changchun 130062,  
People's Republic of China  
e-mail: changchun\_tu@hotmail.com

Z. Lu · S. Wang · W. Gu  
Veterinary College of Foshan University, Foshan,  
People's Republic of China

Y. Zhu  
Veterinary College of Northeast Agricultural University,  
Harbin, People's Republic of China

mild upper respiratory tract illness. NBV and PuV have not been found associated with human infection, but both MeV and KamV have been isolated from humans with acute respiratory syndrome and with possible human-to-human transmission [5, 6]. This discovery has given rise to increasing concern about bat-transmitted reovirus infections in humans. Recently, another fusogenic reovirus was isolated in Hong Kong from an ill tourist who had a history of travel in Bali, Indonesia, and who might have acquired the infection from an unknown animal vector in Bali [4]. This virus (strain HK23629/07) has a high sequence identity with current bat reoviruses, suggesting not only its membership in the NBV species but also the possibility of its being bat-borne.

Here, we report the first isolation and characterization of a bat reovirus in China to support the claim that this virus is a new member of the NBV species. In this study, lung specimens of 150 fruit bats *Rousettus leschenaultia* were collected from a location near the Xi River of Guangdong province, China (Fig. 1). Equal amounts of lung tissue from five bats were pooled, and each pooled sample was homogenized in minimum essential medium (MEM) supplemented with antibiotics and clarified by centrifugation. Supernatants were inoculated onto conventional monolayer cultures of Vero-E6 cells in MEM supplemented with 5% newborn calf serum (Hyclone, Logan, UT) and antibiotics and incubated at 37°C in 5% CO<sub>2</sub>. The cultures were



**Fig. 1** Global distribution of bat-origin reoviruses. The locations of viral sample collection are indicated. The magnified map on the right shows the location of the XRV sample collection near the Xi river of Guangdong province, China

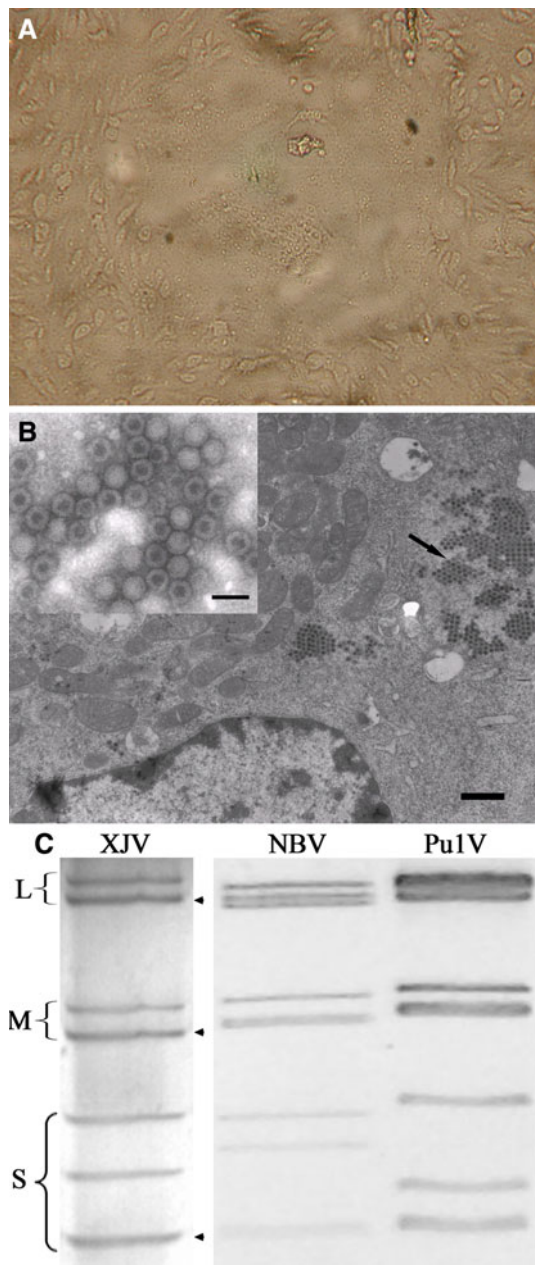
examined daily for development of a cytopathogenic effect (CPE) and blind passaged three times before being discarded if no CPE was observed.

The clarified supernatant of a cell culture showing CPE was examined by electron microscopy after negative staining with 2% (w/v) phosphotungstic acid, pH 6.8. Ultrathin sections of infected cells were also made, using a published method [14]. All samples were examined with a JEM-1200E II electron microscope (JEOL, Tokyo, Japan).

Virus pellets were prepared by ultracentrifugation of the 6% PEG8000-treated, infected BHK-21 cell culture [9]. Double-stranded viral RNA was extracted, separated on 10% SDS-PAGE and visualized by silver staining using a published method [14]. Individual viral RNA segments were separated on a 1% agarose gel, then excised and purified using a QIAquick Gel Extraction Kit (Qiagen, Germany). The single-primer amplification technique (SPAT) was used to generate S segments of the isolated virus using published methods [1, 2]. The PCR products were ligated to pMD-20 vector, and at least three clones of each PCR product were sequenced using an ABI 3730 Genetic Analyzer with an ABI-PRISM Dye Termination Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). Sequence comparisons using DNASTar software (DNASTAR, Madison, WI) and phylogenetic analysis based on the S1 and S3 genes were conducted using the neighbour-joining algorithm of MEGA software version 3.1 (<http://www.megasoftware.net/>) and the maximum parsimony method of PHYLIP software version 3.63 (Seattle, WA, USA). The tree was evaluated statistically using 1000 bootstrap replicates and visualized using the TREEVIEW program (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>).

Of 30 pooled samples, sample 824-828L showed syncytial CPE in Vero-E6 cells at the second passage 12 h post-inoculation (Fig. 2a), but virus particles were not seen by electron microscopy until the fourth passage. Morphological observation revealed that the isolated virus had a typical non-enveloped reovirus morphology, with a diameter of approximate 80 nm. The virus particles possessed a double-capsid icosahedral structure with conspicuous “spikes” situated on the inner core (inset of Fig. 2b). Ultrathin sections showed paracrystalline arrays of viral particles in semi-electron-dense inclusion bodies in the cytoplasm of infected cells (Fig. 2b). The observed syncytial CPE and morphogenesis suggest classification of the isolated virus among the orthoreoviruses, and it was therefore named Xi River virus (XRV) after the sample collection location, in accord with the current way of naming NBV members.

Genomic pattern analysis on SDS-PAGE showed that XRV had 10 dsRNA segments that migrated in a pattern highly consistent with those of members of the NBV species,



**Fig. 2** Characterizaion of XRV. **a** XRV causes large syncytia CPE in infected Vero-E6 cells; **b** An ultrathin section of XRV-infected BHK-21 cells (bar 200 nm) shows the paracrystalline arrays of XRV particles (arrow). The inset shows negative staining of XRV (bar 100 nm); **c** SDS-PAGE of XRV genomic segments in comparison with those of NBV and Pu1V [14]. *L*, *M* and *S* represent large, medium and small segments, respectively. The arrows indicate co-migration of two segment bands

dividing XRV RNAs into three size classes: three large (L1, L2 and L3), three medium (M1, M2 and M3) and four small (S1, S2, S3 and S4) segments with L2 and L3, M2 and M3, S3 and S4 co-migrating in the gel (Fig. 2c). For further characterization, SPAT was used to generate the four S segments, and complete S1 and S3 segments were

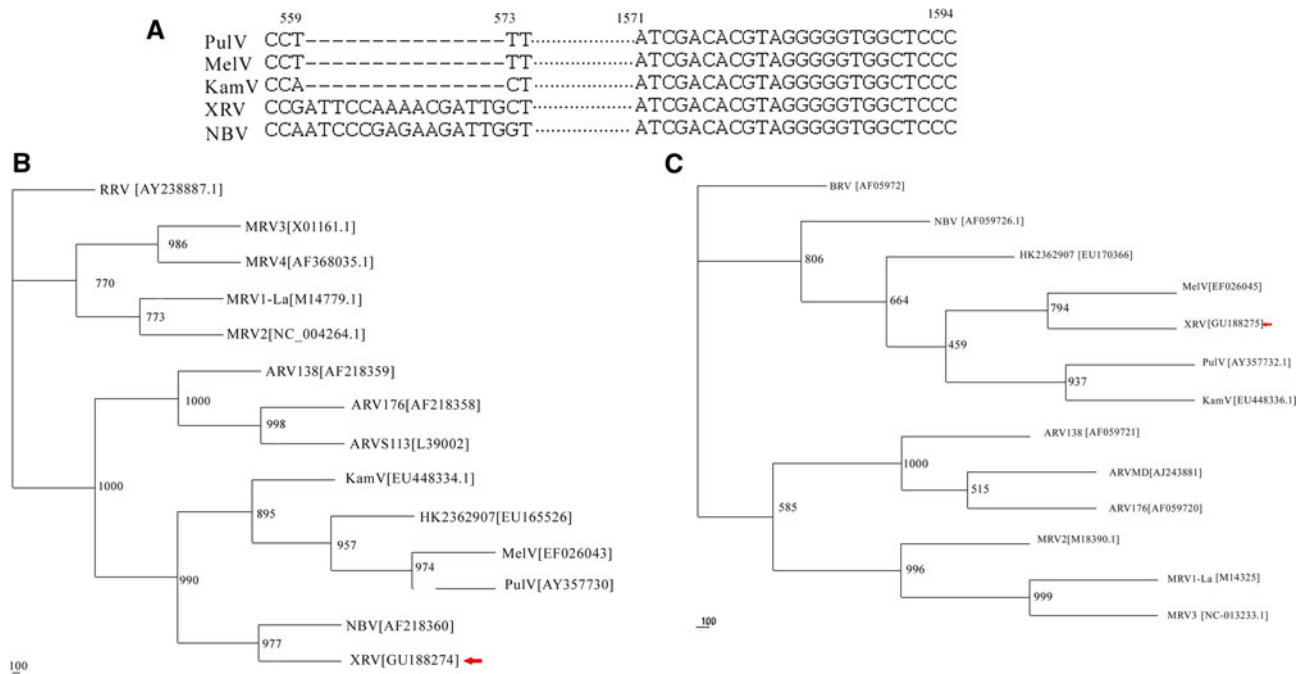
eventually cloned and sequenced. Sequence analysis showed that S1 and S3 of XRV were 1,617 and 1,192 bp in length, respectively, with 5' and 3' terminal sequences typical of members of the NBV species: 5'-GCUUAA.....UCAUC-3' [6]. The complete sequences of XRV S1 and S3 have been deposited in GenBank with accession numbers *GU188274* and *GU188275*, respectively.

The sequences of the XRV S1 and S3 genes were compared with those of NBV, Pu1V, Me1V and KamV (S1 and S3 of HK23629/07 were not used, since the GenBank sequences lack termini). The result showed that S1 was highly variable, while S3 was highly conserved among the five reoviruses. Similarities of XRV with NBV, Pu1V, Me1V and KamV ranged from 54.8–65.4% for S1 and 87.3–96.2% for S3. XRV had the highest S1 identity with NBV and the highest S3 identity with Me1V. The S1 sequences of XRV and NBV were the same length, while those of the other three viruses were 12–17 bp shorter. Alignment of S1 sequences revealed a deletion of a 15-bp sequence in Pu1V, Me1V and KamV from nt 559 to 573, and the presence of an identical 24-bp sequence (nt 1571–1594) in all five reoviruses (see Fig. 3a). Phylogenetic analysis using S1 and S3 sequences placed XRV among the NBV rather than the other orthoreovirus species (Fig. 3b, c).

The above genomic pattern, sequence comparison and phylogenetic analysis strongly suggest that XRV should be grouped within the NBV species as a new member.

Bats are the only true flying mammals and have the broadest distribution of any mammalian taxon in the world. It has been shown that bats represent the largest natural reservoir for zoonotic viruses belonging to more than 60 species, with most being highly pathogenic RNA viruses [16]. Isolation of bat-associated viruses such as Chikungunya virus, Ross River virus, Japanese encephalitis virus, Russian spring summer encephalitis virus, SARS-like viruses and other coronaviruses have been reported in China, indicating that bats in this country present a risk of transmission of zoonotic viruses [11, 12, 15].

Our recent study showed that some Chinese *Rousettus leschenaultia* bats were naturally positive for rabies antibody [10]. The lung tissues from this study were later used to investigate the possibility of their carrying other viruses, which is how XRV was isolated and identified. Following its isolation in Vero-E6 cells, XRV was tested for its syncytium-forming ability in other cell lines available in our laboratory, since viruses of the NBV species can induce extensive syncytial CPE in mammalian cell lines. The results showed that XRV could also induce fusogenic CPE in Vero, BHK-21, PK-15 and MDCK cell lines, with CPE developing more rapidly than during the primary passage (data not shown). The bat origin and syncytial CPE, along with the morphology and genomic pattern, are highly



**Fig. 3** **a** Partial S1 sequence comparison. **b, c** phylogenetic analysis using S1 and S3 sequences respectively, in which all bat reovirus sequences were compared with those of mammalian orthoreoviruses

consistent with the characteristics of the NBV species, but the definitive classification has to be based on nucleotide sequencing. Currently, only S segments of bat reoviruses have been sequenced and used for this purpose [4–6, 14], and the sequences of their L and M segments have not been determined. Therefore, SPAT was used to clone XRV S segments, and only S1 and S3 sequences were obtained and then used for phylogenetic analysis, which clearly characterized XRV as a new bat reovirus and a potential sixth member of the NBV species.

In the ecosystem of bat reoviruses, Nelson Bay virus was isolated from *Pteropus poliocephalus* [8], PuV from *Pteropus hypomelanus* [14] and, in the present study, XRV from the fruit bat *Rousettus leschenaulti*, indicating that NBVs have a wide range of reservoir bats. Although the natural hosts of MelV, KamV and HK23629/07 have not been identified, fruit bats are the likely host species. In addition, the discovery of XRV has significantly expanded the geographical distribution of bat reoviruses northward (Fig. 1). All of these studies emphasize the likelihood that additional reoviruses will be isolated from further bat species and that bat reoviruses have a much wider geographical distribution than currently identified.

Bat reoviruses were not associated with pathogenicity until 2006, when a human case presenting with high fever and acute respiratory disease was reported to be associated with a reovirus of bat origin, Melaka virus [5]. Since then, two more bat reoviruses have been isolated from human cases with acute respiratory disease [4, 6]. These findings

(MRV), avian orthoreoviruses (ARV), a baboon orthoreovirus (BRV) and a reptile orthoreovirus (RRV). GenBank accession numbers are shown in brackets

have challenged the concept that bat reoviruses are not human pathogens. Given that bats are very important reservoir animals for a number of zoonotic viruses and play a significant role in the transmission of emerging zoonotic diseases to humans [16], the pathogenicity of XRV merits further study. The increasing number of viruses isolated in bats further highlights the urgent need for a systematic survey of the ecology of bat-borne viruses in their natural habitats in proximity to human communities. This will enable effective risk assessments of viral spillover in order to permit development of better strategies for control and to prevent potential outbreaks of emerging infectious diseases.

This work was supported by the following grants to CT: Chinese National Commonwealth Funding Project on Agriculture (No.200803014), National Key Technology R&D Program (No.2010BAD04B01), and Key Project of Department of Science and Technology of Guangdong Province (No. 2004A20403001). YJ was funded by National Nature Science Foundation of China (No. 30600445).

## References

- Attoui H, Biagini P, Stirling J, Mertens PP, Cantaloube JF, Meyer A, de Micco P, de Lamballerie X (2001) Sequence characterization of Ndelle virus genome segments 1, 5, 7, 8, and 10: evidence for reassignment to the genus *Orthoreovirus*, family *Reoviridae*. *Biochem Biophys Res Commun* 287:583–588



2. Attoui H, Billoir F, Cantaloube JF, Biagini P, de Micco P, de Lamballerie X (2000) Strategies for the sequence determination of viral dsRNA genomes. *J Virol Methods* 89:147–158
3. Chappell JD, Duncan R, Mertens PPC, Dermody TS (2005) Genus Orthoreovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) *Virus taxonomy*. Eighth report of the international committee on taxonomy of viruses. Elsevier Academic Press, San Diego, pp 455–465
4. Cheng P, Lau CS, Lai A, Ho E, Leung P, Chan F, Wong A, Lim W (2009) A novel reovirus isolated from a patient with acute respiratory disease. *J Clin Virol* 45:79–80
5. Chua KB, Crameri G, Hyatt A, Yu M, Tompang M, Rosli J, McEachern J, Crameri S, Kumarasamy V, Eaton BT, Wang L-F (2007) A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. *Proc Natl Acad Sci USA* 104:11424–11429
6. Chua KB, Voon K, Crameri G, Tan HS, Rosli J, McEachern JA, Suluraju S, Yu M, Wang L-F (2008) Identification and characterization of a new orthoreovirus from patients with acute respiratory infections. *PLOS One* 3 (11):e3803. doi:[10.1371/journal.pone.0003803](https://doi.org/10.1371/journal.pone.0003803)
7. Duncan R (1999) Extensive sequence divergence and phylogenetic relationships between the fusogenic and nonfusogenic orthoreoviruses: a species proposal. *Virology* 260:316–328
8. Gard G, Compans R (1970) Structure and cytopathic effects of Nelson Bay virus. *J Virol* 6:100–106
9. Jiang S, Ji S, Tang Q, Yang H, Cui X, Bi Y, Kan B, Gao S (2006) Cloning of the complete genome and sequence analysis of the VP4, VP6, and VP7 genes of a novel adult diarrhea rotavirus strain J19. *Chin J Virol* 22:413–420
10. Jiang Y, Wang L, Lu Z, Xuan H, Han X, Xia X, Zhao F, Tu C (2010) Seroprevalence of rabies virus antibodies in bats from southern China. *Vector Borne Zoonotic Dis* 10:177–181
11. Li W, Liang G, Liang B, Hu Z, Shi Z, Zhang S (2004) Progress of research on the viruses in bats. *Virol Sin* 19:418–425
12. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang L-F (2005) Bats are natural reservoirs of SARS-like coronaviruses. *Science* 10:1126–1136
13. Mertens PPC, Duncan R, Attoui H, Dermody TS (2005) Family Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) *Virus taxonomy*. Eighth report of the international committee on taxonomy of viruses. Elsevier Academic Press, San Diego, pp 447–454
14. Pritchard LI, Chua KB, Cummins D, Hyatt A, Crameri G, Eaton BT, Wang L-F (2006) Pulau virus; a new member of the Nelson Bay orthoreovirus species isolated from fruit bats in Malaysia. *Arch Virol* 151:229–239
15. Tang XC, Zhang JX, Zhang SY, Wang P, Fan XH, Li LF, Li G, Dong BQ, Liu W, Cheung CL, Xu KM, Song WJ, Vijaykrishna D, Poon LL, Peiris JS, Smith GJ, Chen H, Guan Y (2006) Prevalence and genetic diversity of coronaviruses in bats from China. *J Virol* 80:7481–7490
16. Wong S, Lau S, Woo P, Yuen KK (2007) Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 17:67–91