

SHORT REPORT

Disease outbreaks caused by steppe-type rabies viruses in China

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Received 5 May 2014; Final revision 29 June 2014; Accepted 9 July 2014;
first published online 31 July 2014

SUMMARY

While rabies is a significant public health concern in China, the epidemiology of animal rabies in the north and northwest border provinces remains unknown. From February 2013 to March 2014, seven outbreaks of domestic animal rabies caused by wild carnivores in Xinjiang (XJ) and Inner Mongolia (IM) Autonomous Regions, China were reported and diagnosed in brain samples of infected animals by the fluorescent antibody test (FAT) and RT-PCR. Ten field rabies viruses were obtained. Sequence comparison and phylogenetic analysis based on the complete *N* gene (1353 bp) amplified directly from the original brain tissues showed that these ten strains were steppe-type viruses, closely related to strains reported in Russia and Mongolia. None had been identified previously in China. The viruses from XJ and IM clustered separately into two lineages showing their different geographical distribution. This study emphasizes the importance of wildlife surveillance and of cross-departmental cooperation in the control of transboundary rabies transmission.

Key words: China, rabies virus, steppe-type.

On the night of 24 February 2013, a wild animal attacked a sheep flock of about 115 head in Tuoli county (TC), Tacheng city, Xinjiang Uygur Autonomous Region (XJ) (Fig. 1) and then ran off. Darkness prevented the sheep owner from identifying the attacker,

although it had a large tail and therefore was suspected to be a wild fox. Bitten sheep began to show clinical signs of rabies including anxiety, salivation and paralysis after 2 weeks, and 26 had died by 17 March. Brain tissue samples collected from three dead sheep were submitted by the local Centre for Animal Diseases Control and Prevention to our OIE Reference Laboratory for Rabies for confirmation.

On 3 April 2013, a cow and a sheep were bitten by wild foxes in different locations within Sonid Youqi County (SYC), Inner Mongolia Autonomous

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Fig. 1. Map of north and northwest China with bordering countries. District designation: 1, Altai; 2, Tacheng; 3, Ili; 4, Kizilsu. Black dots indicate the location of steppe-type viruses isolated in border countries during the recent outbreaks.

Region (IM) (Fig. 1). A week later, the injured animals developed clinical signs of rabies, including restlessness, hyperexcitation, running, furious butting and sensitivity to noise, followed by paralysis and death. On 24 February 2014, a rabid camel died in Alxa Youqi County (AYC), IM; however, the attacker was not identified. On 2 March, another camel and a bovine raised by the same herdsman in AYC showed clinical signs such as irritability, restlessness and anxiety with loss of appetite and eventual death. The camel was seen bitten by a steppe fox (*Vulpes corsac*) 8 days before development of clinical signs. The bovine was also likely bitten by rabid wild carnivores since four wild foxes were found dead near the herdsman's yard. Between 12 and 23 March 2014, three head of cattle from two different herds in Sonid Zuoqi County (SZC), IM, showed signs of excitement (anxiety, salivation and wariness), followed by paralysis and death. Brain tissues of the eight domestic animals and one of the four wild foxes were collected and submitted to the rabies laboratory.

The tissues were examined by the fluorescent antibody test (FAT) [1] and acetone-fixed brain smears were stained with FITC-conjugated anti-rabies monoclonal antibody (Fujirebio Diagnostics Inc., USA). All brain tissues were positive for Lyssavirus antigen. Viral RNA was extracted from each sample and subjected to RT-PCR using the SuperScript III First-Strand Synthesis System and the Platinum *Taq* DNA Polymerase High Fidelity kit (Invitrogen, USA) to obtain the complete coding sequence of

each nucleoprotein (*N*) gene. Methods and primers have been described previously [2].

Sequence comparison using Clustal W of MEGA 5.1 [3] and DNASTar v. 7.2 (DNASTAR Inc., USA) showed that the *N* genes of the viruses from the three XJ sheep were 100% identical, of which one sequence, designated XJTCS01, was used for further analysis. The viral sequences of the sheep and cow from SYC were 99.9% identical, and designated NMSH01 and NMC01, respectively. The viral sequences of the two camels, one cow and one fox from AYC, designated NMCAM01, NMCAM02, NMAYC01 and NMFOX01, were 98.9–99.9% identical. Identity of NMCAM02, NMAYC01 and NMFOX01 was 99.9%, indicating that the rabid cattle and camel were probably infected by rabid foxes. The viral sequences of three cattle samples from the two SZC herds were 99.5–99.9% identical and designated NMC02, NMC03 and NMC04. Sequence identity between XJ and IM strains was 96.6–97.2%. All ten sequences have been submitted to GenBank with accession numbers KJ152772–KJ152774, KJ748631–KJ748636 and KJ737435.

To investigate the genetic relationship of the *N* gene sequences of the 10 causative viruses with previously reported rabies virus strains from the Far East, China and Europe, phylogenetic analysis was conducted. Results (Fig. 2) showed that the 10 sequences grouped with steppe-type rabies viruses, and were only distantly related to all previously reported Chinese strains. The *N* gene sequence of XJTCS01

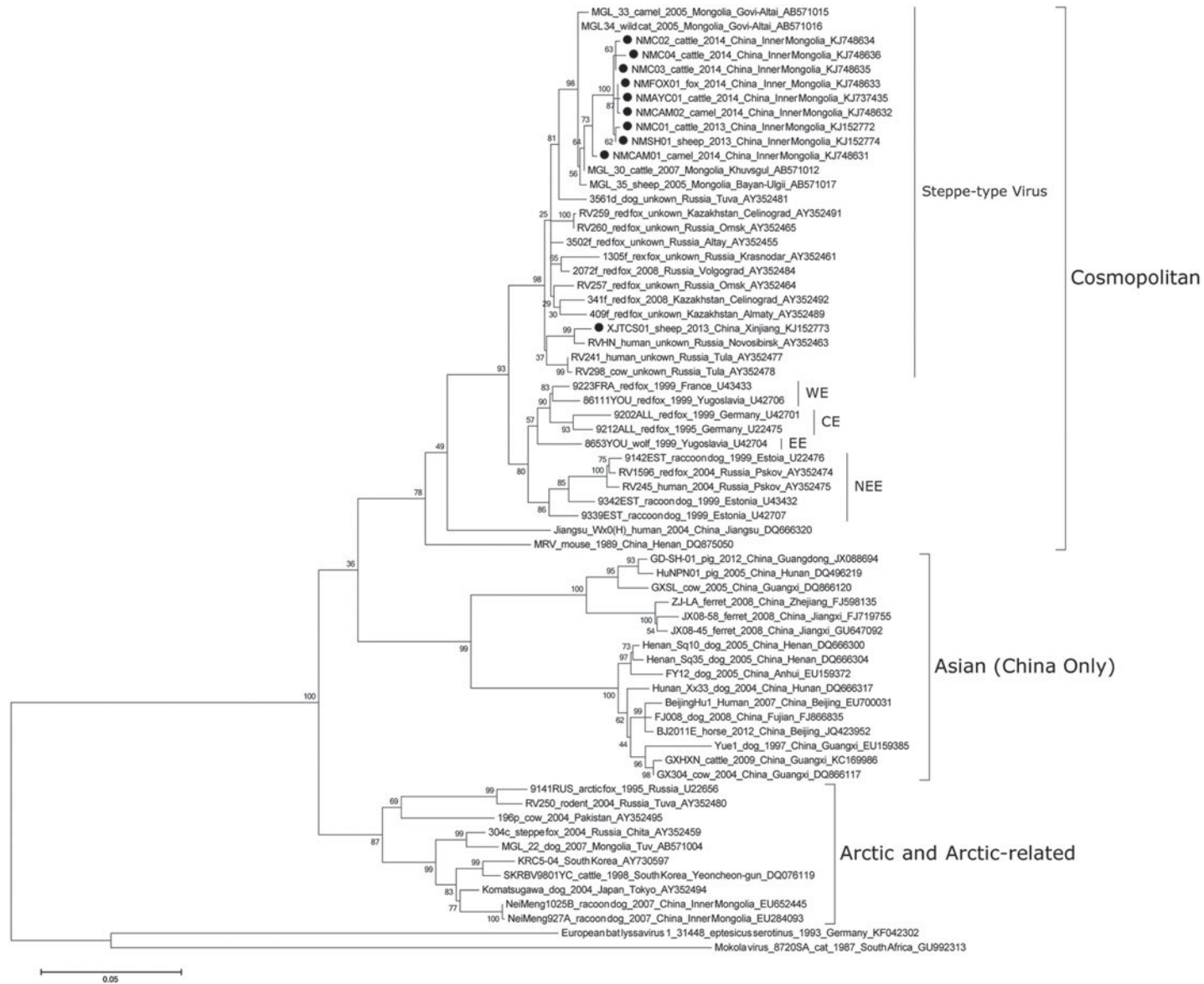


Fig. 2. Phylogenetic tree based on the *N* gene of representative rabies virus strains, as established by the MEGA 5.1 program [3] using the maximum likelihood method with 1000 bootstrap replicates. Background information and GenBank accession numbers of some representative strains or isolates from clades of cosmopolitan, Asian (China only), Arctic and Arctic-related viruses are shown in the tree. EE, NEE, CE and WE represent the isolates from East, Northeast, Central and Western Europe, respectively. The ten strains identified in the present study are marked by black dots. EBLV1 and MOKV were used as outgroup sequences.

at XJ was 99.0% identical to steppe-type virus strain RVHN isolated from a rabid human in Novosibirsk, Russia [4]. The other nine sequences from IM showed high sequence identities (98.6–99.6%) to those steppe-type viruses isolated in Khuvsgul and Govi-Altai, Mongolia (Fig. 2) [5].

Rabies is a transboundary zoonotic disease and endemic in the countries adjacent to China. However, information about the rabies situation in border areas of China is limited. In Mongolia rabies is endemic with nearly 6000 animal cases and 34 human deaths reported between 1972 and 2006. Endemic Mongolian rabies viruses are divided mainly into steppe-type and Arctic-like strains with wild carnivores as the main reservoirs. They constitute main transmission sources of human and domestic animal rabies with cattle being principally affected, followed by camels, sheep, goats and horses [5, 6]. Steppe-type rabies viruses circulate in the huge steppe and forest-steppe lands of Russia (from its European part to Tuva), East Kazakhstan (Almaty and Celinograd) and West Mongolia (Fig. 1), with steppe foxes and wolves as the main reservoirs [6], but have never been reported previously in China. In the present study, seven rabies outbreaks in domestic animals were laboratory-confirmed with four being associated with fox attack and other three having unknown exposure history. Of the seven outbreaks only one contained a sample collected from a fox which was probably the transmission source. Further sequence analysis showed that all ten viruses causing these outbreaks were closely related steppe-type rabies viruses indicating that these outbreaks were most likely transmitted by foxes.

Rabies is a serious problem in China, with about 1000 human cases annually of which domestic dogs account for more than 95%. XJ and IM are large provinces, comprising the major part of China in the north and northwest and sharing a long border with Kazakhstan, Russia and Mongolia (Fig. 1). While animal husbandry is an important economic resource, local rabies is still a neglected disease with limited information available. In Altai district of XJ, rabies was first officially reported in 1941, with outbreaks in humans, dogs, horses, sheep and cattle recorded between 1941 and 1986 being mainly caused by wolves [7]. From 1987 to 1992, 13 people and >600 animals died of rabies in Ili district alone [8]. In 2006 three domestic animal rabies outbreaks were reported in Kizilsu district, caused by foxes and wolves [9]. Unfortunately, all these outbreaks in XJ were

clinically diagnosed without laboratory confirmation, and therefore the isolation, identification and sequence information of the rabies viruses is lacking. In IM, rabies virus was first identified only in 2011 when an Arctic rabies-like strain was isolated from an outbreak in raccoon dogs [10]. The identification of steppe-type isolates in the present study has contributed to our understanding of the natural circulation and transmission of rabies viruses in IM.

Phylogenetic analysis showed that the virus strains in XJ and IM identified in the present study formed two lineages (Fig. 2). The XJ lineage is closer to the virus strains reported in Russia and Kazakhstan, while the IM lineage is closer to the virus strains reported in Mongolia, suggesting the existence of their transboundary circulation in Russia/Kazakhstan/XJ and Mongolia/IM, respectively. As the border mountain between Russia, Mongolia and China (Fig. 1), Altay Mountain provides a natural barrier and probably constrains the spread of rabies. It may therefore be a factor contributing to the geographical distribution of the two virus lineages.

Sheep, bovines and camels in XJ and IM are raised mainly in a traditional nomadic way, being permitted to roam freely on the grasslands. The field investigation conducted in AYC of IM after the rabies occurrence revealed that the fox population had been increasing in recent years due to the more plentiful supply of grassland gerbils resulting from weakened anti-rodent campaigns. The expansion of this wild food chain has led an increasing exposure of domestic animals to rabid foxes, resulting in a high risk of animal rabies outbreaks. Since no measures have been established in China to control fox rabies, mass vaccination of domestic animals has been proposed. In the meantime, however, our observations identify the need for increased wildlife surveillance and dog vaccination in the rabies-affected areas to prevent further transmission of steppe rabies to humans and domestic animals

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China (grant no. 31302043), National '973' Programme (grant no. 2012CB722501), National Key Technology R&D Programme (grant no. 2010BAD04B01) and Special Fund for Agro-scientific Research in the Public Interest (grant no. 201103032).

DECLARATION OF INTEREST

None.

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