



## LETTER

# Fox- and raccoon-dog-associated rabies outbreaks in northern China

Dear Editor,

Rabies is a generally fatal disease caused by the rabies virus (RABV), and is transmitted mainly by Carnivora and Chiroptera (Fooks A R, et al., 2014; Tao X, et al., 2013). In China, stray dogs and some wild animals (e.g., Chinese ferret badgers, foxes, and raccoon dogs) are the principal reservoirs for RABV (Hu R L, et al., 2009). Historically, rabies in wild foxes and raccoon dogs (*Nyctereutes procyonoides*) was recorded in the early 1980s in China, but has only been sporadically reported in the Xinjiang Autonomous Region and the Inner Mongolia Autonomous Region (IMAR) in the past years (Wang L H, et al., 2014). Here, we report an outbreak of wild fox and raccoon-dog rabies and its spread to domestic animals in the IMAR and in Heilongjiang Province, China.

China has implemented projects and policies relating to the conversion of degraded farmland into forests and grasslands, and trees and grasses have been extensively planted in deserted regions since 2000. However, the rat population has exploded with these improvements in the green ecology of the IMAR and Heilongjiang, bringing foxes, raccoon dogs, and other carnivores. In the spring of 2013, local farmers reported dead foxes and raccoon dogs in pastures, and some cattle and goats died of suspected rabies. This year, several hundred foxes and raccoon dogs have been found dead in Bayannur, IMAR, and Tongjiang City, Heilongjiang, and herdsmen have reported dogs, cattle, goats, horses, and camels falling ill with rabies.

We performed an epidemiologic investigation of some of the affected regions and phylogenetically analyzed virus isolates. The brain specimens of one rabid red fox (*Vulpes vulpes*) and one cow bitten by the fox in the IMAR, and one rabid raccoon dog and one goat bitten by the raccoon dog in Heilongjiang, were collected for direct fluorescence assay and nested reverse transcriptase-PCR analysis (Liu Y, et al., 2013). The four samples were found to be positive for RABV by direct fluorescence assay, and the nucleotide sequences of the nucleoproteins were determined and submitted to GenBank (accession nos. KM016901, KM016899, KM016900, and KM016898). A phylogenetic tree (Figure 1) was constructed using the maximum

likelihood method in MEGA 5.0 (Tamura K, et al., 2011), in which the reliability of the phylogeny groupings was evaluated using bootstrapping with 1000 replicates. The general time-reversible model incorporating invariant sites and a GTR+I+ $\gamma_4$  model was favored for all datasets.

Using BLAST, the nucleoprotein gene nucleotide sequences from the rabid fox and cow were found to be highly similar (99%) to isolates from red foxes in Mongolia, especially isolate Mongolia 6 (GenBank no. EF614254), and the nucleoprotein gene nucleotide sequences from the rabid raccoon dog and goat were highly similar (99%) to raccoon-dog isolate 857r (GenBank no. AY352458) from Russia. However, nucleotide identity between the red-fox- and raccoon-dog-associated RA-BVs was only 88%, indicating two separate phylogenetic groups (Figure 1). The phylogenetic tree showed that the raccoon-dog-associated RABV isolates were closely related to raccoon-dog and dog-related iso-lates from South Korea (Kuzmin I V, et al., 2004; Shao X Q, et al., 2011), but distinct from the ferret-badger and dog RABVs that are widely distributed in China (Hu R L, et al., 2009; Liu Y, et al., 2010).

The identification of four RABV isolates suggests the introduction of fox- and raccoon-dog-associated rabies into north China from elsewhere. The outbreaks have caused the deaths of wild and domestic animals, and pose a threat to human health. Phylogenetic analysis indicates that they have resulted from cross-border transmission between China and Mongolia or China and Russia. Therefore, regular surveillance of cross-border wild animals is required for rabies control.

Rabies is a preventable disease. Providing pre-exposure prophylaxis (i.e., preventive vaccination) to the herds in these regions would avoid all losses. For the control of rabies in wild animals, more research should be devoted to the development of an oral vaccine.

## FOOTNOTES

This project was funded by the National “973” project (no. 2011CB500705) and the Special Fund for Agro-Scientific Research in the Public Interest (no. 201203056).

All the authors declare that they have no competing interests.

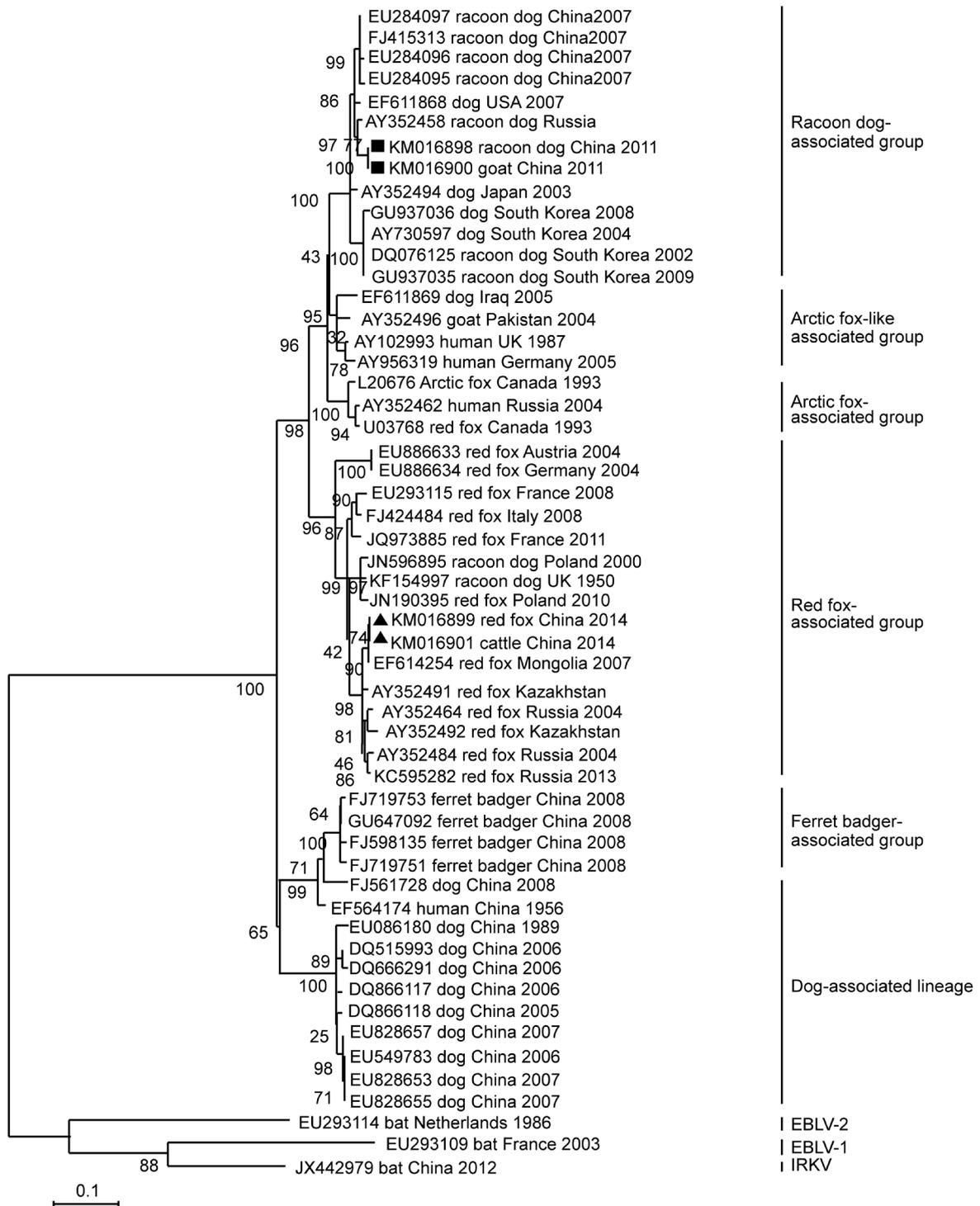


Figure 1. Maximum likelihood phylogenetic tree based on 525 nucleotide fragment of the nucleoprotein gene. The tree is rooted with Irkut virus isolate JX442979, European bat lyssavirus type 1 (EBLV-1) isolate EU293109, and EBLV-2 isolate EU293114. The red-fox- and raccoon-dog-associated RABV isolates are marked using black triangles and black squares, respectively.

The samples were collected under a research protocol approved by the Animal Welfare Committee of the Military Veterinary Research Institute, Changchun, China.

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Published online: 28 September 2014

## REFERENCES

- Fooks A R, Banyard A C, Horton D L, et al. 2014. *Lancet*, Epub ahead of print.
- Hu R L, Tang Q, Tang J R, et al. 2009. *Vector Borne Zoonotic Dis*, 9:1–12.
- Kuzmin I V, Botvinkin A D, McElhinney L M, et al. 2004. *J Wild Dis*, 40:617–631.
- Liu Y, Zhang S F, Wu X F, et al. 2010. *BMC Infect Dis*, 10:234.
- Liu Y, Zhang S F, Zhao J, et al. 2013. *PLoS Neg Trop Dis*, 7:e2097.
- Shao X Q, Yan X J, Luo G L, et al. 2011. *Epidemiol Infect*, 139:629–635.
- Tamura K, Peterson D, Peterson N, et al. 2011. *Mol Biol Evolut*, 28:2731–2739.
- Tao X, Han N, Guo Z, Tang Q, et al. 2013. *Virolog Sin*, 28(2):116–123.
- Wang L H, Tang Q, Liang G D. 2014. *Int J Infect Dis*, Epub ahead of print.