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Letter

Investigation of the Evolutionary History of the Lyssaviruses

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Dear Editor,

We report the results of evolutionary history estimation of the lyssaviruses based on an analysis of the Glycoprotein (G) sequences gene using the BEAST software package. The most recent common ancestor (TMRCA) of all the lyssavirus strains was estimated to be approximately 5030 years (95% HPD 3988-6069 years), and there was a significant spread of the rabies virus throughout the world range in the last 200 years, consistent with significant time points in development and migration of human civilizations. We speculate that increased and expansion of human migration during this time period may have promote the increase in lyssavirus diversity. In addition, evidence of host switching in lyssavirus history from the Chiroptera to the Carnivora orders was also identified.

Rabies is one of the oldest infectious diseases known to man, ancient Mesopotamian literature from more than 4000 years ago contains reports consistent with this fatal infection (Nel L H, et al., 2007). Rabies is caused by lyssaviruses. The genome of lyssavirus is approximately 12kb, comprising five genes (3'-5'), encoding nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (Nel L H, et al., 2007).

Previous investigations of the evolutionary history of the lyssaviruses have estimated the emergence of carnivore rabies from chiropteran lyssaviruses was determined to have occurred between 888 to 1,459 years ago (Badrane H, et al., 2001). Rabies virus (RABV), the best known of the lyssavirus, is estimated to have begun to diverge about 500 years ago (Badrane H, et al., 2001; Bourhy H, et al., 2008; Holmes E C, et al., 2002). However, because of the emergence of new species and collection of greater numbers of isolates in more divergent regions in recent years, a reinvestigation of the characteristics of lyssaviruses evolution is timely. In this study we combined the newly completed G sequences of Chinese isolates identified in our lab with almost all lyssavirus G sequences available in Genbank to explore the evolutionary history and characteristics of the lyssavirus genera.

Specimens were collected as part of the trial Chinese national surveillance program (Lang S L, et al., 2012; Tao X Y, et al., 2009; Yu J, et al., 2012). All complete G sequences with full background information of rabies street strains (as of Jan 6, 2011) were downloaded from GenBank and combined with the newly sequenced samples in our lab to generate an initial set of 533 sequences (Tao X, et al., 2013). To avoid redundancy and reduce computational requirements, a smaller dataset was selected that only contained sequences with less than 98% homology in RABV as detected by the T-coffee software package (Notredame C, et al., 2000), to form a final set of 266 sequences.

The GTR+I+G model was determined by MODELTEST to be the best nucleotide substitution model for the lyssavirus G gene, consistent with RABV analysis results (Kuzmin I V, et al., 2012). Both strict and relaxed (uncorrelated $\log_{\text{normal}}$) molecular clocks were considered under the Bayesian skyline model. The former was the best supported according to the estimated Bayes Factors. The mean rate of nucleotide substitution estimated for the lyssavirus G gene was 3.0×10^{-4} substitutions (subs) per site per year (95% HPD values=$2.5\text{-}3.5\times10^{-4}$ subs per site per year), which are in agreement with previous

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studies of lyssavirus evolution (Badrane H, et al., 2001; Bourhy H, et al., 2008).

Using the BEAST software, the TMRCA of all the lyssavirus strains was estimated to be approximately 5030 years (95% HPD 3988-6069 years) (Fig. 1), Which appears to be consistent with evidence of the earliest human civilizations (ancient Mesopotamia, ancient Egypt, ancient India and ancient China). A previous estimate, based on 55 G sequences, estimated the TMRCA of lyssaviruses was approximately 7000 years ago (Badrane H, et al., 2001). In recent years, more and more lyssavirus sequences have become available, therefore, our current analysis may be closer to the real situation. In addition, we estimated the emergence of carnivore rabies from chiropteran lyssaviruses to have occurred 550 to 783 years ago, which also is less than the corresponding estimate by Badrance et.al estimated (888 to 1,459 years ago), and also has a narrower 95HPD interval.

As shown in Fig. 1, the West Caucasian bat virus (WCBV) isolated (suggested to belong to phylogroup III) (Nel, 2007) divided first, then phylogroup I and II divided about 4000 years ago. Approximately 2000 years ago, different species began to appear. For RABV, with the widest distribution and largest number, the TMRCA was estimated to be around 663 years ago, which is consistent with the estimation by Bourhy et al. based on the N gene (about 749 years ago) and the G gene (about 583 years ago) of dog strains in RABV species (Bourhy H, et al., 2008). The Carnivora and Chiroptera host group of RABV species divided about 500 years ago, and the

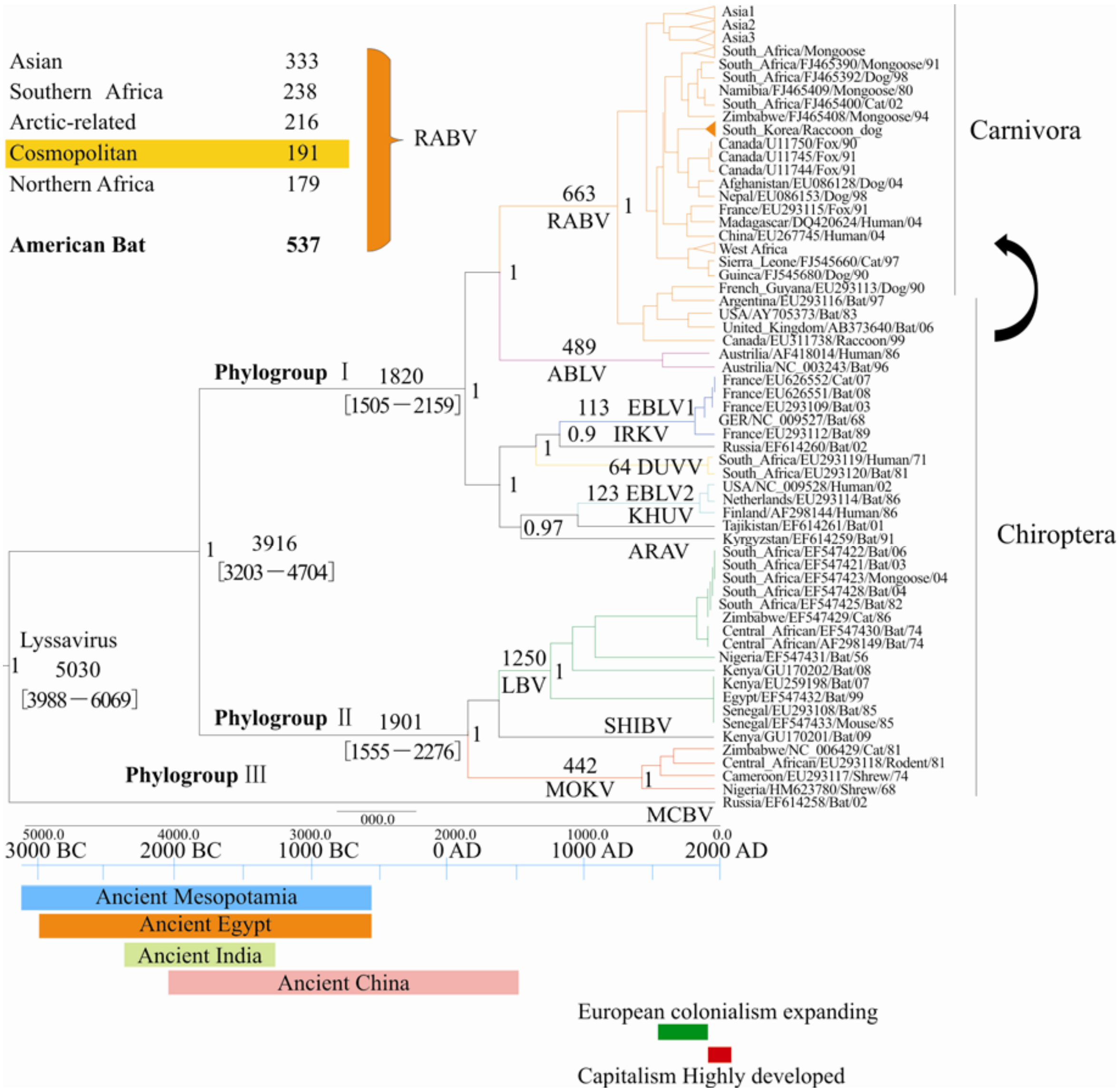

Fig. 1. MCC tree of lyssavirus G sequences (top) and human civilizations timeline (bottom), showing the correspondence between historical events in human civilization and major phylogenetic events in the estimated tree. Taxa names in the tree are in the format (Country/GenBank number/ Host/Year).

Table 1. Geographical distribution and hosts of different species of lyssavirus used in this study.

Species	Geographical Distribution	Host species
Rabies virus (RABV)		
Asian	Asia	Dog,Human
Southern Africa	Southern Africa	Dog,Mongoose,Cat
Arctic-related	North America,Asia	Dog,Human,Red fox,Raccoon dog,Sheep
Cosmopolitan	America,Africa,Europe,Asia	Dog,Human,Skunk,Fox,Raccoon dog,Cat,Horse,Cattle,Sheep,Pig
Northern Africa	Northern Africa	Dog,Cat,Human,Camel
Indian subcontinent	South Asia	Dog,Human
American bat	America	*Insectivorous Bat and Vampire Bat*,Horse,Cattle,Sheep,Dog
Australian bat lyssavirus (ABLV)	Australia	*Insectivorous Bat and Frugivorous Bat*, Human
European bat lyssavirus 1 (EBLV1)	Europe	*Insectivorous Bat*,Cat
Irkut virus (IRKV)	Russia	*Insectivorous Bat*
Duvenhage virus (DUVV)	Africa	*Insectivorous Bat*,Human
European bat lyssavirus 2 (EBLV2)	Europe	*Insectivorous Bat*,Human
Khujand virus (KHUV)	Tajikistan	*Insectivorous Bat*
Aravan virus (ARAV)	Kyrgyzstan	*Insectivorous Bat*
Lagos bat virus (LBV)	Africa	*Frugivorous Bat*,Mouse,Cat,Mongoose
Shimoni bat virus (SHIBV)	Kenya	*Insectivorous Bat*
Mokola virus (MOKV)	Africa	Rodent、Cat
West Caucasian bat virus (WCBV)	Russia	*Insectivorous Bat*

Cosmopolitan group distributed throughout the whole world appears to have expanded within the last 200 years. These important lyssavirus evolution time points appear to coincide with some important stages in the development of human civilization (Fig. 1).

According to the background of the sequences analyzed in this study (Supplementary Table S1), we next considered the geographical and host distribution of each species according to the sequence background as shown in Table 1 (these statistics are based on the 533 strains analyzed in this study). By comparison with other species, RABV appears to be the most widely distributed, in particular the Cosmopolitan group, followed by the Arctic related group; other species are located in a specific continent, region or country. The host distribution of RABV also varied with other species, and both the various carnivores and bats (Chiroptera) were included in RABV species. Moreover, the Chiroptera group became divided earlier than the Carnivora group (Fig. 1). In the remaining 11 species (except RABV), only the MOKV strains have not been isolated from bats, however, the reservoir hosts in other species are bats, especially the insectivorous bats, and that these species became divided earlier than RABV. So, the most comprehensive data analysis in our study support the inference that host in lyssavirus switched from the Chiroptera to the Carnivora orders (Badrane H, et al., 2001).

The natural evolution history of lyssavirus appears to be associated with the history human civilizations. The TMRCA of lyssavirus can be traced back to about 5000 years (95% HPD 3988-6069 years) ago, when ancient Mesopotamian civilizations began to rise; as early as 4000 years ago, there were records of rabies in Mesopotamia (Badrane H, et al., 2001). About 2,000 years ago, ancient civilizations reached unprecedented levels of prosperity and the commercial trade, for example, the ancient Silk Road, was established between distant countries. At this same time, the diversity of lyssavirus became more pronounced evidenced by the appearance of different phylogroups (Fig. 1). After the opening of new sea routes in the late 15th century, human activities were extended on a more global level, and in this period the RABV, especially the Carnivora order, the biggest threat to human, began to be disseminated world-wide (Badrane H, et al., 2001; Holmes E C, et al., 2002). In the last 400 years (especially recent 200 years), capitalism developed further and colonialism continued to expand globally, and in this period, the Cosmopolitan and other groups of the RABV species completed their worldwide expansion (Fig. 1). Therefore, we can speculate that the increasing and expansion of human migrations aided the development of lyssavirus diversity. The infectious spectrum of lyssaviruses is broad, and almost all the warm-blooded animals are susceptible (Rupprecht C E, et al., 2002). Dogs and some wildlife (e.g. foxes, raccoons and bats) can transmit the virus more easily and are effective hosts for keeping the virus in circulation; On the other hand, herbivores such as horses, cattle, sheep, camels and human are dead end hosts of rabies infections.

Until now, only MOKV strains have not been found in bats (Markotter W, et al., 2006; Paweska J T, et al., 2006). Lyssaviruses belong to the Rhabdoviridae family which infect a wide range of hosts comprising mammals, plants, and arthropods (Nel L H, et al., 2007). However, the bat host species of lyssavirus are almost exclusively insectivorous bats (Table 1) leading to speculation that lyssaviruses originated from an insect rhabdovirus, which insectivorous bats contracted from insects; host switching then occurred at some point during the history of lyssaviruses (Badrane H, et al., 2001). This is consistent with the host switching event from the Chiroptera to the Carnivora orders observed in our estimated tree.

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