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Diagnosis and molecular characterization of rabies virus from a buffalo in China: a case report

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Abstract

Background: Rabies virus (RABV) can infect many different species of warm-blooded animals. Glycoprotein G plays a key role in viral pathogenicity and neurotropism, and includes antigenic domains that are responsible for membrane fusion and host cell receptor recognition.

Case presentation: A case of buffalo rabies in China was diagnosed by direct fluorescent antibody test, G gene reverse-transcriptase polymerase chain reaction, and RABV mouse inoculation test. Molecular characterization of the RABV was performed using DNA sequencing, phylogenetic analysis and amino acid sequence comparison based on the G gene from different species of animals.

Conclusion: The results confirmed that the buffalo with suspected rabies was infected by RABV, which was genetically closely related to HNC (FJ602451) that was isolated from cattle in China in 2007. Comparison of the G gene among different species of animal showed that there were almost no amino acid changes among RABVs isolated from the same species of animals that distributed in a near region. However, there were many changes among RABVs that were isolated from different species of animal, or the same species from different geographic regions. This is believed to be the first case report of buffalo rabies in China, and the results may provide further information to understand the mechanism by which RABV breaks through the species barrier.

Background

Rabies virus (RABV) is one of the seven species in the genus Lyssavirus in the Rhabdoviridae family [1]. All warm-blooded animals, including raccoons, skunks, bats and foxes, are susceptible to RABV, and domestic dogs act as the main reservoir and transmitter [2]. The annual number of human deaths caused by rabies is estimated to be 55,000 worldwide [3], with about 32,000 in Asia [4]. The total number of human deaths was 108,412 between 1950 and 2004 in China [5]. The average number was 1,524 from 1996 to 2008, and 50% of cases were reported in Guangxi, Hunan and Guizhou provinces [6]. Therefore, the disease continues to be a serious public and animal health problem in China.

RABV has a non-segmented, negative-sense, single-stranded RNA genome about 12 kb in length [7], with five genes: L (RNA polymerase), G (glycoprotein), M (matrix protein), P (phosphoprotein), and N (nucleoprotein) [8]. The order of relative conservation of these five genes from high to low could be either N>L>M>P>G, or N>L>M>G>P[9]. Glycoprotein G of RABV plays an important role in pathogenicity [10,11] and viral neurotropism [12] because it contains membrane fusion sites [13] and host cell receptor domains [14,15]. In recent years, molecular epidemiology [16-18] and diagnosis [19,20] of rabies have been based on the G gene.

The aims of the present study were to diagnose a case of buffalo rabies that occurred in Wuhan City, Hubei Province, using three different methods, and to compare the sequences with different RABVs that were isolated from different species, based on the G gene. This is believed to be the first report of the phylogenetic analysis of buffalo RABV in China compared with other isolates from different animals.

Case presentation

Specimens were collected from the gyrus hippocampi of the buffalo with suspected rabies in Wuhan City (114.3°E, 30.8°N), Hubei Province, China. Direct fluorescent

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antibody test (dFAT) of the specimen was performed as previously described [21-23]. Normal buffalo brain samples were used as a negative control. RABV isolation by mouse inoculation test was performed as described previously [24,25].

The total RNA from buffalo brain was extracted with Trizol reagent (Invitrogen) according to the manufacturer's instructions. Primer design and reverse-transcriptase polymerase chain reaction (RT-PCR) of the G gene were performed as described previously [17]. RT-PCR products were visualized under UV light after electrophoresis on 1% agarose gels containing ethidium bromide. The amplified products were purified with a QIAquick PCR gel extraction kit (QIAGEN) according to the manufacturer's protocol. The sequencing was carried out in an Applied Biosystems 3730 DNA automated sequencer. After the raw sequences were edited by ClustalX Version 1.82 [26], 1575 nt sequences of the G gene were obtained and submitted to GenBank.

The phylogenetic tree based on the deduced amino acid sequences was constructed by using the neighbor-joining method with 1,000 bootstrap replicates using MEGA version 4.0 software [27], based on the complete sequence of the RABV G gene from 10 different species of animal (Table 1). Bootstrap values >70% were considered significant [28]. Genetic distance analysis for the G gene was conducted with PHYLIP version 3.63 software [29]. Glycoprotein nucleotide sequences from different animals were identified, translated into amino acid sequences, edited, and pair aligned using BioEdit software [27]. Multiple alignments were performed by ClustalX software [26].

Conclusion

dFAT indicated the presence of RABV antigen in the brain specimens from the buffalo with suspected rabies (Figure 1A), whereas normal buffalo brain did not (Figure 1B). The expected size of the G gene fragment was obtained from the suspected buffalo brain by RT-PCR (data not shown). RABV from buffalo was isolated successfully in suckling mice (data not shown), and the RABV was named Hubei070308 strain. The positive result was supported by G gene sequencing, and the sequences were submitted to GenBank under the accession number EF643518.

The G gene sequence, together with reference sequences from seven countries and 10 species of animals were aligned (Table 1). We showed that the G gene of RABV had relative territorial specificity but not species specificity (Figure 2). The genetic relationship of the RABV in this study was differed greatly from PG(AY009097) that was used as a vaccine strain in China in 1931. Compared with 18 other RABV strains, Hubei070308 shared 85.0-99.8% sequence identity at the amino acid level (data not shown). We demonstrated that Hubei070308 strain was close to the cattle strain FJ602451 and the human strain DQ849063, which belong to Chinese group I [17], but it was far from coyote strain U52946, which was isolated in 1996 in the United States (Figure 2).

Table 1 Detailed information of the G gene used in the present study

No.	Virus strains	Country and year of isolation	Accession Number	Host species
1	**NNV-RAB-H**	**India 2007**	EF437215	homo sapiens
2	**ZAMRAV51/00**	**Zambia 2000**	AB285215	dog
3	**HN06**	**China 2005**	DQ849062	dog
4	**FY3**	**China 2004**	DQ849046	dog
5	**THA1-HM**	**Thailand 1983**	AF325488	human
6	**MAL1-HM**	**Malaysia 1985**	AF325487	human
7	**CHI1-BK**	**China 1986**	AF325471	deer
8	**COSRV**	**USA 1996**	U52947	coyote
9	**SHBRV**	**USA 1996**	U52946	bat
10	**90RABN5850**	**Canada 2001**	U11754	vulpes
11	**PA R89**	**Canada 2001**	U27217	Procyon
12	**92RBG1741**	**Canada 2001**	AF344305	skunk
13	**Hubei070308**	**China 2007**	EF643518	buffalo
14	**PG**	**China 1931**	AY009097	dog(Vaccine Strain)
15	**QC**	**China 2006**	DQ849063	human
16	**HNC**	**China 2007**	FJ602451	cattle
17	**LuoH**	**China 2007**	FJ602453	Homo sapiens
18	**92RBGL0867**	**Canada 1992**	AF344307	Striped skunk
19	NY516	USA 1995	U27214	raccoon

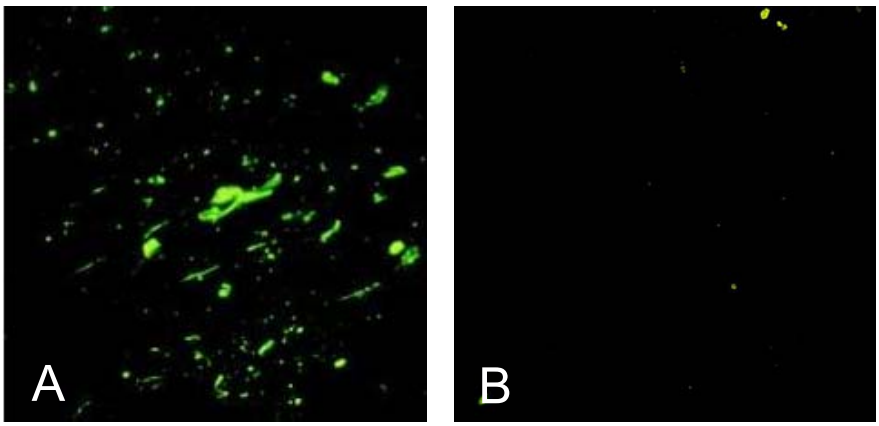

Figure 1 Results of dFAT of specimens from brain of buffalo with suspected rabies. (A) suspected buffalo brain sample; (B) normal buffalo brain sample.

Additional File 1, Figure S1 shows that the amino acid changes were mainly focused on the transmembrane areas (aa 440-461), inner-membrane zone (aa 462-505) and signal peptide range of mature RABV glycoprotein. Glycoprotein sequences of Hubei070308 were identical to HNC (FJ602451) that was isolated from cattle in 2007. However, there were many amino acid substitutions when it was compared with RABVs from other animals such as skunk, dog, human, bat and deer. The linear epitope (aa 14-19) at antigenic site II and the minor site between aa 342 and 343 were highly conserved, which was consistent with the findings of Meng *et al*. [17]. Among all the RABVs, SHBRV strain that was isolated from bats was the most variable at the amino acid level. Many different animals can be infected by RABV [2], and cases of transmission from bats to humans [30], dogs to humans [17] and even dogs to pigs [31] have been reported. For a virus shed by one host to infect another, it must break through entry barriers (e.g., epithelium, mucus, and alveolar macrophages) and find its way to tissues in which it can replicate [32].

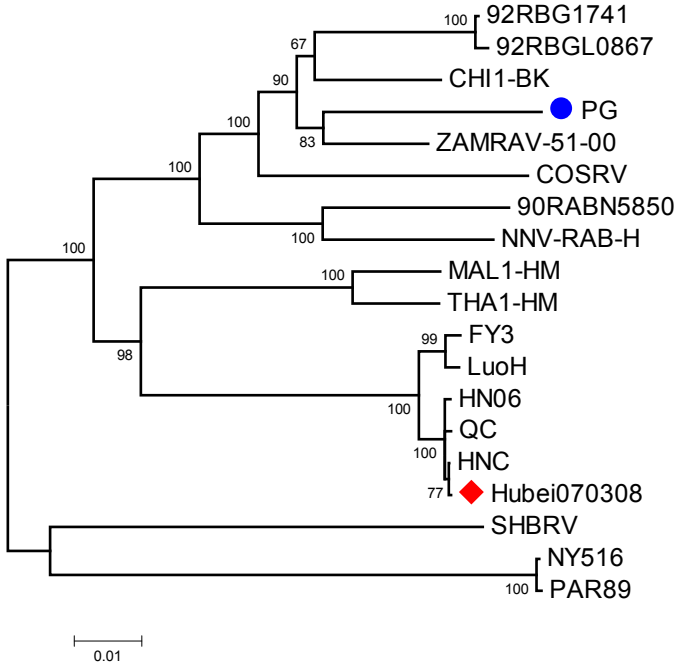

Figure 2 Phylogenetic analysis based on deduced amino acid sequence of complete G gene. The phylogenetic tree was constructed by the neighbor-joining algorithm using MEGA version 4.0, and bootstrap analysis was performed with 1,000 trials. All sequences were collected from GenBank. The red spot indicates Hubei070308 that was isolated in the present study. The blue spot indicates the 1931 RABV vaccine strain.

It has been reported that several amino acids in the RABV glycoprotein are responsible for pathogenicity [33,34]. Therefore, RABV glycoprotein is the best target protein to study virus-host interaction, or it may be the main protein that is responsible for breakthrough of the species barrier. In the present study, many amino acid substitutions in G protein were found among RABVs that were isolated from different animal species, or from the same species distributed in different geographic regions. These substituted amino acids may explain why RABV can break through the host barrier to infect one species of animal from another. This hypothesis needs to be confirmed by further experiments.

Additional material

Additional file 1: Figure S1. Comparative analysis of G gene amino acid with other RABVs isolated from different animals. Dots represent identity among all sequences. Arrows mark the range of the signal peptide, antigenic site, linear epitope and endo-domain. Transmembrane (TM) domain was framed. SP: signal peptides; ENDO: endo-domain; AS2: antigenic site II; AS3: antigenic site III; LE: linear epitope.

List of abbreviations

dFAT: direct fluorescent antibody test (dFAT); G: Glycoprotein; RABV: rabies virus; RT-RCR: reverse-transcriptase polymerase chain reaction.

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Authors' contributions

BW was the leader of the project. KZ carried out most of the studies and drafted the manuscript. ZX and MX amplified the complete G gene. HC and JG provided consultation and preparation of the final report. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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