Isolation of Irkut Virus from a *Murina leucogaster* Bat in China

Ye Liu, Shoufeng Zhang, Jinghui Zhao, Fei Zhang, Rongliang Hu*

Laboratory of Epidemiology and Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Military Veterinary Research Institute, Academy of Military Medical Sciences, Jingyue Economy Development Zone, Changchun, China

Abstract

**Background and objectives:** Bats are recognized as a major reservoir of lyssaviruses; however, no bat lyssavirus has been isolated in Asia except for Aravan and Khujand virus in Central Asia. All Chinese lyssavirus isolates in previous reports have been of species rabies virus, mainly from dogs. Following at least two recent bat-associated human rabies-like cases in northeast China, we have initiated a study of the prevalence of lyssaviruses in bats in Jilin province and their public health implications. A bat lyssavirus has been isolated and its pathogenicity in mice and genomic alignment have been determined.

**Results:** We report the first isolation of a bat lyssavirus in China, from the brain of a northeastern bat, *Murina leucogaster*. Its nucleoprotein gene shared 92.4%/98.9% (nucleotide) and 92.2%/98.8% (amino acid) identity with the two known Irkut virus isolates from Russia, and was designated IRKV-THChina12. Following intracranial and intramuscular injection, IRKV-THChina12 produced rabies-like symptoms in adult mice with a short inoculation period and high mortality. Nucleotide sequence analysis showed that IRKV-THChina12 has the same genomic organization as other lyssaviruses and its isolation provides an independent origin for the species IRKV.

**Conclusions:** We have identified the existence of a bat lyssavirus in a common Chinese bat species. Its high pathogenicity in adult mice suggests that public warnings and medical education regarding bat bites in China should be increased, and that surveillance be extended to provide a better understanding of iruk virus ecology and its significance for public health.

Introduction

**Lyssavirus** genus presently comprises 12 species: *rabies virus* (RABV), *Lagos bat virus* (LBV), *Mokola virus* (MOKV), *Duvenhage virus* (DUVV), *European bat lyssavirus types 1 and 2* (EBLV-1 & 2), *Australian bat lyssavirus* (ABLV), *Aravan virus* (ARAV), *Khujand virus* (KHUV), *Irkut virus* (IRKV), *West Caucasian bat virus* (WCBV), and *Shimoni bat virus* (SHIBV) [1]. Additionally, two recent isolates, *Ikoma lyssavirus* (IKOV) and *Bokeloh bat lyssavirus* (BBLV), have not yet been assessed taxonomically [2,3].

Bats are the most important reservoirs of lyssaviruses [1]; however, little is known about bat lyssaviruses in Asia. ARAV and KHUV were isolated in Central Asia [1], and IRKV in eastern Siberia and the Russian Far East [1]. In 2009, a human rabies case was reported in Primorsky Kray, Russian Far East, in a patient bitten by an insectivorous bat. The isolated virus, Ozernoe, was closely related to IRKV [1,4].

A 1998 survey in Philippines found that 22/231 (9.52%) serum samples from insectivorous bats contained neutralizing antibody (VNA) to ABLV [5]. In Cambodia, 2000-1, 30/187 (16.04%) bat serum samples were found to have VNA against different lyssaviruses [6]. In Thailand, 2002-3, 16/394 (4.06%) serum samples from fruit bats had detectable VNA against ARAV, KHUV, IRKV, or ABLV [7]. Three of 288 (1.04%) bat serum samples collected in Bangladesh during 2003-4 cross-reacted with ARAV and KHUV [8], and in China, 2010, low levels of rabies VNA were demonstrated in 15/685 (2.19%) bat serum samples [9]. However, there has been no report of bat lyssavirus isolation in any of these countries. Although isolation of “rabies” viruses has been reported from Thailand [10] and India [11], these isolates were not characterized or stored for further studies.

In 2002 and 2010, two bat-associated human rabies cases were reported in northeast China, in Tonghua county and Longjing city respectively [12,13]. One (2002) was reported to the local Jilin Center for Disease Control and Prevention; the other (2010) was not. The victims suffered bat bites on ear and hand respectively. Both cases developed an acute progressive encephalomyelitis which consistent with rabies infection, 30 and 20 days after the bites, and both died 10 days following the first manifestation of disease symptoms. However, both cases were diagnosed only clinically, without laboratory confirmation. Additionally, according to the information provided by local residents, there was another human death from rabies following a bat bite in Tonghua county in the 1990s.
Author Summary

The Lyssavirus genus presently comprises 12 species and two unapproved species with different antigenic characteristics. Rabies virus is detectable worldwide; Lagos bat virus, Mokola virus, Duvenhage virus, Shimon bat virus, and Ikoma lyssavirus circulate in Africa; European bat lyssavirus types 1 and 2, Irkt virus, West Caucasian bat virus, and Bokeloh bat lyssavirus are found in Europe; and Australian bat lyssavirus has been isolated in Australia. Only Aravan and Khujand viruses have been identified in central Asia. Bats are recognized as the most important reservoirs of lyssaviruses. In China, all lyssavirus isolates in previous reports have been rabies viruses, mainly from dogs; none has been from bats. Recently, however, at least two bat-associated human rabies or rabies-like cases have been reported in northeast China. Therefore, we conducted a search for bat lyssaviruses in Jilin province, close to where the first bat-associated human rabies case was recorded. We isolated a bat lyssavirus, identified as an Irkt virus isolate with high pathogenicity in experimental mice. Our findings suggest that public warnings and medical education regarding bat bites in China should be increased, and that surveillance should be extended to provide a better understanding of Irkt virus ecology and its significance for public health.

Since 2002, efforts have been made to obtain human specimens in hospitals and bat specimens in the field. Human specimens have not been obtained, however, since bat-associated human rabies is rare, even though there have been several reports of bat bites [13–15]. To investigate more extensively the prevalence of lyssaviruses in bats in Jilin province and their public health implications, therefore, we conducted the field survey described below.

Materials and Methods

Ethics statement

All animals experiments described in this paper have been conducted according to the Guideline on the Humane Treatment of Laboratory Animals stipulated by the Ministry of Science and Technology of the People’s Republic of China (MOST) [16] and approved by the Animal Welfare Committee of the Military Veterinary Research Institute, Changchun, China. All animals were housed in a climate-controlled laboratory with a 12 h day/12 h night cycle. No human patient derived clinical materials and their brains were collected and removed for testing by the Veterinary Research Institute, Changchun, China. All animals approved by the Animal Welfare Committee of the Military Technology of the People’s Republic of China (MOST) [16] and Ethics statement

Materials and Methods

All animals experiments described in this paper have been conducted according to the Guideline on the Humane Treatment of Laboratory Animals stipulated by the Ministry of Science and Technology of the People’s Republic of China (MOST) [16] and approved by the Animal Welfare Committee of the Military Veterinary Research Institute, Changchun, China. All animals were housed in a climate-controlled laboratory with a 12 h day/12 h night cycle. No human patient derived clinical materials and their brains were collected and removed for testing by the Veterinary Research Institute, Changchun, China. All animals approved by the Animal Welfare Committee of the Military Technology of the People’s Republic of China (MOST) [16] and Ethics statement.

Materials and Methods

Animal Summary

The Lyssavirus genus presently comprises 12 species and two unapproved species with different antigenic characteristics. Rabies virus is detectable worldwide; Lagos bat virus, Mokola virus, Duvenhage virus, Shimon bat virus, and Ikoma lyssavirus circulate in Africa; European bat lyssavirus types 1 and 2, Irkt virus, West Caucasian bat virus, and Bokeloh bat lyssavirus are found in Europe; and Australian bat lyssavirus has been isolated in Australia. Only Aravan and Khujand viruses have been identified in central Asia. Bats are recognized as the most important reservoirs of lyssaviruses. In China, all lyssavirus isolates in previous reports have been rabies viruses, mainly from dogs; none has been from bats. Recently, however, at least two bat-associated human rabies or rabies-like cases have been reported in northeast China. Therefore, we conducted a search for bat lyssaviruses in Jilin province, close to where the first bat-associated human rabies case was recorded. We isolated a bat lyssavirus, identified as an Irkt virus isolate with high pathogenicity in experimental mice. Our findings suggest that public warnings and medical education regarding bat bites in China should be increased, and that surveillance should be extended to provide a better understanding of Irkt virus ecology and its significance for public health.

Since 2002, efforts have been made to obtain human specimens in hospitals and bat specimens in the field. Human specimens have not been obtained, however, since bat-associated human rabies is rare, even though there have been several reports of bat bites [13–15]. To investigate more extensively the prevalence of lyssaviruses in bats in Jilin province and their public health implications, therefore, we conducted the field survey described below.

Materials and Methods

Ethics statement

All animals experiments described in this paper have been conducted according to the Guideline on the Humane Treatment of Laboratory Animals stipulated by the Ministry of Science and Technology of the People’s Republic of China (MOST) [16] and approved by the Animal Welfare Committee of the Military Veterinary Research Institute, Changchun, China. All animals were housed in a climate-controlled laboratory with a 12 h day/12 h night cycle. No human patient derived clinical materials and non-human primates were used in the completion of these studies.

Sample collection and assay

In April 2012, 261 bats of five species, Plecotus auritus (n = 12), Myotis daubentonii (n = 66), Rhinolophus ferrumequum (n = 59), and Myotis skomini (n = 20), were collected in Tonghua county, Jilin province (i.e., where the first bat-associated human rabies case was recorded in 2002) and identified by both morphological traits and amplification and sequencing of the cytochrome B (cyB) gene as described previously [17]. This area is also close to Longjing city where the other human case was reported in 2006 from a rabid dog in China, was maintained in unvaccinated dogs in previous experiments [19]. Mouse neuronal-2a (N2a) cell line was maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, and 100 μg/mL streptomycin sulfate at 37°C in a 5% CO2 humidified incubator.

To compare the pathogenicities of lyssavirus isolate and BD06 at the same dose in mice, both kinds of these viruses were cultivated in N2a cells as described previously [19]. Only two passages in the infected N2a cells were performed to minimize cell adaptation and possible reduction of pathogenicity. The titers of lyssavirus isolate and BD06 in N2a cells were assayed as described previously [19] and were from 10^5.5–10^5.7 TCID50/mL.

Animals and virus inoculation

One-day-old suckling and 8-week-old adult Kunming mice were purchased from the Changchun Institute of Biological Products, and respectively used for lyssavirus isolation from the DFA-positive samples and in vivo pathogenicity assay. Virus isolation was conducted using the mouse inoculation test (MIT) method as described previously [20]. For pathogenicity, serial 10-fold dilutions of the viruses (second passage in N2a cells) were prepared in PBS/2% horse serum and injected i.m. (the hindlimb skeletal muscle) into 4 groups of 10 adult mice (0.1 mL containing 10^7–10^4 TCID50, 1 dilution per group).

All the inoculated mice were observed for ~28 days and the sick and the final survived mice were euthanized by cervical dislocation respectively before their death and at the end of the experimental period. The infected mice were re-checked for the presence of lyssavirus by DFA.

Viral RNA isolation, primer designs, amplification and sequencing

Preparation of total RNA from bat brain tissue was conducted with TRIzol (Invitrogen Life Technologies, USA) according to the supplier’s instructions.

For amplification and analysis of the viral genomes, a total of 9 pairs of primers of overlapping fragments covering the full length of the viral cDNA were designed based on the similar sequences identified using BLAST. Fragments containing the 3' and 5' termini of the viral genome were obtained using a 3' and 5' Full RACE Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The PCR products were purified, and nucleotide sequencing was performed on both forward and reverse strands of each fragment by Genscript Nanjing Co., China.
Sequence alignment and phylogenetic analysis

Phylogenetic tree was constructed using the Maximum Likelihood method in MEGA 5.10 [21], 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+Γ4 model was favored for all datasets. The percentage identities and similarity scores of the viral protein sequences were calculated using DNastar Lasergene software, (DNASTAR, Inc., Madison, USA).

Complete genome sequences of each lyssavirus species were used as follows: RABV (16 sequences), LBV (4 sequences), MOKV (2 sequences), DUVV (4 sequences), EBLV-1 (5 sequences), IRKV (3 sequences), EBLV-2 (2 sequences), ABLV, ARAV, KHUV, WCBV, BBLV and SHIBV (1 sequence each).

Results

Virus isolation

Of all 261 bat specimens tested, only a single brain sample from an adult male Murina leucogaster bat was found positive by DFA. From this sample, a 255-bp sequence was obtained using nested RT-PCR. Using BLAST, its sequence was found to be highly similar (92%/98%) to that of both IRKVs, especially isolate Ozernoe. All the eight inoculated suckling mice with lyssavirus isolate developed neurological signs between days 7–9 post-inoculation, at which point they were euthanized. Each mouse brain sample was DFA positive. During the next intracranial passage, the incubation period was reduced to 3–4 days in suckling mice. All the other bat brain samples were DFA negative.

As originally proposed by Kuzmin et al., N gene nucleotide identities provided unequivocal separation of all lyssavirus species with an identity threshold of 82% [22]. Alignment analysis based on the full nucleoprotein (N) sequences clearly demonstrated that the isolate clustered with IRKV (Figure 1), sharing 92.4%/98.9% (nucleotide) and 92.2%/98.8% (amino acid) identity (Table 1) with the two available isolates from eastern Siberia and the Russian Far East. The identity within the N sequences for other lyssaviruses (Table 1) ranged from 70.0%–79.2% (nucleotide) and 70.2%–79.1% (amino acid), indicating that the virus is an isolate of the species IRKV [22,23] and therefore was designated IRKV-THChina12 (''TH'' representing Tonghua county and ''12'' 2012). The complete genome sequence of IRKV-THChina12 was submitted to GenBank (Accession no. JX442979).

Pathogenicity of IRKV-THChina12

Following i.m. injection of adult mice at the same doses, rabies-like symptoms were observed 6–10 d post-injection of IRKV-THChina12, whereas the incubation period of BD06 was 8–11 d. All injected adult mice died 24–48 h following appearance of rabies-associated symptoms such as lethargy, ruffled hair, muscle weakness, loss of body weight and progressive paralysis of one or both hind limbs. Table 2 shows that 10/10 adult mice died of IRKV-THChina12 injection at dose 10^5 TCID_{50}, with 7/10 dying from BD06 injection at the same dose. At lower doses (10^3–10^5 TCID_{50}), some animals survived in every group; however, given 10^2 TCID_{50} or more, the mortalities in each IRKV-THChina12 group were higher than that in BD06 groups at the same dose.

Genomic organization of IRKV-THChina12

The genome of IRKV-THChina12 was determined to be 11980 nucleotides (nt) in length with a G+C content of 44.72%, similar to the G+C content of 44.49% of Irkut virus and 44.72% of Ozernoe virus (Table 1). The genomic organization was as follows:

Phylogenetic analysis

As shown in Figure 1, all lyssaviruses studied can be separated into three major branches previously defined as different “phylogroups” [25]. Although IRKV, ARAV, BBLV, KHUV, DUVV, WCBV, EBLV-1 and EBLV-2 within phylogroup 1 have all been found in insectivorous bats widely distributed in Eurasia, IRKV, ARAV, KHUV and WCBV have also been isolated in countries neighbouring China. IRKV-THChina12 was closely related to EBLV-1 and DUVV as well as the other IRKVs, somewhat divergent from ARAV and KHUV, and most divergent from WCBV and IKOV within phylogroup 3. These data are consistent with that of a previous study [22]. The tree summarizing an analysis of the whole N gene (Figure 1) showed that IRKV-THChina12 clustered with isolate Ozernoe from the China-Russia border and with original isolate Irkut virus.

IRKV-THChina12 and the two other IRKV isolates all provide independent origins for the species IRKV. However, according to the branch length, IRKV-THChina12 has undergone more changes since the three isolates diverged from their common ancestor; i.e., the evolution rate in the lineage leading up to IRKV-THChina12 is greater than that leading up to the isolate Ozernoe. It has been previously suggested that complete genomes provide the best tools for genotyping [26]. In this study, similar conclusions were drawn from a full genome analysis (data not shown).

Discussion

The identification of IRKV-THChina12 represents the first isolation of a bat lyssavirus in China. Although it was found in only a single sample (1/104) from Murina leucogaster bats, other considerations suggest that this bat species should receive special attention concerning its association with IRKV. The greater tube-nosed bat (Murina leucogaster) is a non-migratory species, distributed throughout India, Mongolia, Siberia and the Far East of Russia, China, Korea, and Japan [26]. The original IRKV isolate was obtained from this species [1], as was our isolate. Potentially, while the circulation patterns of IRKV in all bat populations require extensive surveillance since only a single isolate was obtained in this study, it is likely that the greater tube-nosed bat (Murina leucogaster) is the principal vector of the virus. IRKV may circulate in bats of this species across all this vast territory.

The overall genomic organization of the isolate IRKV-THChina12 is similar to those of IRKVs and other lyssaviruses [27], with minor variations. Although complete sequences of both IRKV genomes have been available for several years, neither has been used to estimate the evolutionary relationship among lyssaviruses. Differences in rates of evolution are common and
Figure 1. Maximum likelihood of the lyssavirus-rooted phylogenetic tree based on the complete N sequences. The isolate, IRKV-THChina12, is marked using black triangle. Bootstrap values are presented as percentages for key nodes on the tree, and branch lengths are indicated using a scale bar. EBLV-1: European bat lyssavirus-1; DUVV: Duvenhage virus; IRKV: Irkut virus; ARAV: Aravan virus; BBLV: Bokeloh bat lyssavirus; KHUV: Khujand virus; EBLV-2: European bat lyssavirus-2; ABLV: Australian bat lyssavirus; RABV: rabies virus; WCBV: West Caucasian bat virus; SHIBV: Shimoni bat virus; MOKV: Mokola virus; LBV: Lagos bat virus; and IKOV: Ikoma lyssavirus.

doi:10.1371/journal.pntd.0002097.g001
References

1. Kuzmin IV, Tordo N. (2012) Genus Lyssavirus. In: Dietzgen RD, Kuzmin IV, editors. Rhabdoviruses: Molecular Taxonomy, Evolution, Genomics, Ecology, Host–Vector Interactions, Cytopathology and Control. London: Caister Academic Press. 37–57 p.

2. Marston DA, Horton DL, Ngeleja C, Hampson K, McElhinney LM, et al. (2011) Novel lyssavirus in Natterer’s bat, Germany. Emerg Infect Dis 17: 1519–1522.

3. Freuling CM, Beer M, Courathis CD, Finke S, Hoffmann B, et al. (2012) Ikoma lyssavirus, highly divergent novel lyssavirus in an African civet. Emerg Infect Dis 18: 664–667.

4. Leonova GN, Belikov SI, Kondratov IG, Krylova NV, Pavlenko EV, et al. (2002) Lyssavirus surveillance in bats, Bangladesh. Emerg Infect Dis 12: 486–488.

5. Kuzmin IV, Niezgoda M, Carroll DS, Keeler N, Hossain MJ, et al. (2006) Lyssavirus surveillance in bats, Bangladesh. Emerg Infect Dis 12: 486–488.

6. Yang XJ, Wang JN (2010) A 18-year-old male from Longjing city was bitten by a dog and then developed rabies. Ybnews. Available: http://www.ybnews.cn/news/newsyb/201007/113456.html. Accessed 21 Jul 2010.

7. Lamliertdacha B, Boongird K, Wanghongsa S, Wacharapasudee S, Chanhoom L, et al. (2005) Survey for bat lyssaviruses, Thailand. Emerg Infect Dis 11: 232–236.

8. Kuzmin IV, Niezgoda M, Carroll DS, Keeler N, Hossain MJ, et al. (2006) Lyssavirus surveillance in bats, Bangladesh. Emerg Infect Dis 12: 486–488.

9. Jiang Y, Wang L, Lu Z, Xuan H, Han X, et al. (2010) Seroprevalence of rabies virus antibodies in bats from southern China. Vector Borne Zoonotic Dis 10: 117–118.

10. Smith PC, Lachowaowski K, Vick WE, Stanton JS (1968) Isolation of rabies virus from fruit bats in Thailand. Nature 216: 384.

11. Pal SR, Arora BM, Chuttani PN, Broer S, Choudhury S, et al. (1980) Rabies virus infection of a flying fox bat, Pteropus poliocephalus, in Chandigarh, northern India. Trop Geogr Med 32: 265–267.

12. Yang XJ, Wang JN (2010) A 10-year-old male from Longjing city was bitten by a bat of unknown species, developed rabies and died. Ybnews. Available: http://www.ynbnews.cn/news/newsyb/201007/113456.html. Accessed 21 Jul 2010.
14. Chen J (2006) An old woman was bitten by a bat. Eastday. Available: http://why.eastday.com/eastday/node/33/node178/node14417/u1a172787.html. Accessed 31 Aug 2006.

15. Gao CC (2012) A male from Tieling city was bitten on the lip by a bat. Ifeng. Available: http://news.ifeng.com/society/4/detail_2012_07/05/15793296_0.shtml. Accessed 5 Jul 2012.

16. The Ministry of Science and Technology of the People’s Republic of China (MOST) (2006) Guideline on humane treatment of laboratory animals. Available: http://www.most.gov.cn/fggw/zfwj/zfwj2006/zf06wj/zf06hfw/200609/t20060930_54196.htm. Accessed 30 Sep 2006.

17. Linacre A, Lee JC (2005) Species determination: the role and use of the cytochrome B gene. Methods Mol Biol 297: 45–52.

18. Meslin FX, Kaplan MM, Koprowski H (1996) Laboratory Techniques in Rabies. 4th edition. Geneva: World Health Organization. 80–168 p.

19. Liu Y, Zhang SF, Zhang F, Hu RL (2012) Adaptation of a Chinese ferret badger strain of rabies virus to high-titered growth in BHK-21 cells for canine vaccine development. Arch Virol 157: 2397–402.

20. Bourhy H, Rollin PE, Vincent J, Sureau P (1989) Comparative field evaluation of the fluorescent-antibody test, virus isolation from tissue culture, and enzyme immunodiagnosis for rapid laboratory diagnosis of rabies. J Clin Microbiol 27: 519–523.

21. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739.

22. Kuzmin IV, Hughes GJ, Botvinkin AD, Orciari LA, Rupprecht CE (2005) Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. Virus Res 111: 28–43.

23. Kissi B, Tordo N, Bourhy H (1995) Genetic polymorphism in the rabies virus nucleoprotein gene. Virology 209: 526–537.

24. Marston DA, McElhinney LM, Johnson N, Muller T, Conzelmann KK, et al. (2007) Comparative analysis of the full genome sequence of European bat lyssavirus type 1 and type 2 with other lyssaviruses and evidence for a conserved transcription termination and poly adenylation motif in the G-L 3’ non-translated region. J Gen Virol 88: 3102–1314.

25. Delmas O, Holmes EC, Talbi C, Larrous F, Dacheux L, et al. (2008) Genomic diversity and evolution of the lyssaviruses. PLoS One 3: e2037–2062.

26. Smith AT, Xie Y (2008) A guide to the mammals of China. Princeton: Princeton University Press. 385 p.

27. Wunner WH (2007) Rabies virus. In Jackson AC, Wunner WH, editors. Rabies. 2nd edition. San Diego: Academic Press. pp. 23–60.

28. Bourhy H, Kissi B, Tordo N (1993) Molecular diversity of Lyssavirus genus. Virology 194: 70–81.

29. Johnson N, McElhinney LM, Smith J, Lowings P, Fooks AR (2002) Phylogenetic comparison of the genus Lyssavirus using distal coding sequences of the glycoprotein and nucleoprotein genes. Arch Virol 147: 2111–2123.

30. Szanto AG, Nadín-Davis SA, White BN (2008) Complete genome sequence of a raccoon rabies virus isolate. Virus Res 136: 130–139.

31. Kuzmin IV, Wu XF, Tordo N, Rupprecht CE (2008) Complete genomes of Aravan, Khujand, Irkut and West Caucasian bat viruses, with special attention to the polymerase gene and non-coding regions. Virus Res 136: 81–90.

32. Hanlon CA, Kuzmin IV, Blanton JD, Weldon WC, Manangan JS, et al. (2005) Efficacy of rabies biologics against new lyssaviruses from Eurasia. Virus Res 111: 44–54.