This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2009.009a,bV***	(to be completed by ICTV officers)

Short title: Create 4 species named *Aravan virus, Khujand virus, Irkut virus and West Caucasian bat virus* in the genus *Lyssavirus* in the family *Rhabdoviridae* in the order *Mononegavirales*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Ivan V. Kuzmin (ibk3@cdc.gov);
Noel Tordo (ntordo@pasteur.fr)

Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

ICTV-EC or Study Group comments and response of the proposer:

Approved by the EC at EC41, checked by SGS

Date first submitted to ICTV:	26.05.09
Date of this revision (if different to above):	22.06.09

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.009aV***	(assigned by ICTV officers)

To create 4 species with the name(s):

Aravan virus
Khujand virus
Irkut virus
West Caucasian bat virus

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.009bV***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Lyssavirus***
Subfamily:	
Family:	***Rhabdoviridae***
Order:	***Mononegavirales***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

The species within the *Lyssavirus* genus are demarcated based on several criteria. These include: (1) genetic distance which allows the operational classification into genotypes, with threshold of 80-82% nucleotide identity for complete nucleoprotein (N) gene, which provides the same phylogenetic topology of the tree that other gene sequences, but with a better quantitative resolution for the threshold; (2) antigenic patterns in reactions with panels of antinucleocapsid monoclonal antibodies (which preceeded with serologic cross-reactivity and definition of lyssavirus serotypes, using polyclonal sera). Interestingly, phylogenetic and serologic relationship correlate, which contributed into delineation of two major phylogroups within *Lyssavirus* genus. Phylogroup 1 includes *Rabies virus* (RABV), *Duvenhage virus* (DUVV), *European bat lyssaviruses, type 1* and *2* (EBLV-1 and 2, respectively), and *Australian bat lyssavirus* (ABLV). Phylogroup 2 includes *Lagos bat virus* (LBV) and *Mokola virus* (MOKV). There is a significant serological neutralization within phylogroups, but very limited cross-neutralization has been detected between phylogroups. In addition, while RABV circulates worldwide among carnivores and bats, other species within the genus have more limited distribution and host species ranges. Bats are primary or sole reservoir hosts for all lyssaviruses except MOKV (for which the reservoir species has not been clearly identified as of yet).

Based on these demarcation criteria, each of the four viruses named in this proposal: *Aravan virus* (ARAV), *Khujand virus* (KHUV), *Irkut virus* (IRKV) and *West Caucasian bat virus* (WCBV) can be considered as new independed species within the *Lyssavirus genus*. ARAV, KHUV and IRKV cross-react serologically with members of phylogroup 1, whereas WCBV does not cross-react serologically with any of the two phylogroups.

Complete genome sequences of the 4 new viruses indicated they all include 5 structural genes, typical to lyssaviruses: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) genes. Phylogenetic studies confirmed that they belong to the *Lyssavirus* genus (Annex, figure 1). Using different phylogenetic methods applied on different genome regions, more subtle relationships can be seen between the species. KHUV, ARAV and IRKV appear phylogenitically more related to EBLV1, EBLV2 and DUVV. However, comparaison of their N protein coding region indicates that all viruses are almost equidistant while comparison of the G ectodomain coding sequence suggests that KHUV and EBLV-2 on the one hand, IRKV and EBLV-1 on the other hand, would be more related, ARAV occupying a somehow intermediate position between them (Annex, figure 2). The WCBV appears to be the most phylogenetically distinct member described to date within the genus, equally distant from other lyssavirus species. For each ARAV, KHUV, IRKV and WCBV, the nucleotide identity levels to the most closely related established species were less than the identities within the species. These values, along with the topology of phylogenetic trees, support that neither of these 4 viruses could be included into any established lyssavirus species (Annex, figure 2).

Antigenic patterns of ARAV, KHUV, IRKV and WCBV, studied with a selected panel of antinucleocapsid monoclonal antibodies, were distinct from each other and from any other lyssavirus species (Annex, table 1). Serologic cross-reactivity was detected between all phylogroup I lyssaviruses, and from this standpoint ARAV, KHUV and IRKV must be considered as members of this phylogroup. In contrast, WCBV did not demonstrate significant serologic cross-reactivity to any other lyssavirus, either from phylogroups I and II, and therefore must be considered as a representative of a new phylogroup III (Annex, table 2). Immunization of animals with the commercially available rabies biologics provided incomplete protection against ARAV, KHUV and IRKV, whereas no protection was demonstrated for WCBV (Annex, table 3).

Geographically ARAV, KHUV, IRKV and WCBV viruses were isolated in Eurasia, but in areas distanced from those where the most phylogenetically similar lyssaviruses (EBLV-1, EBLV-2, DUVV) have been identified. Moreover, they were isolated from the host species from which the mentioned above viruses were never isolated as well: the only exception is bat *Miniopterus schreibersi*, from which the DUVV was isolated in Africa and the WCBV was isolated in south-eastern Europe.

***Aravan virus* (ARAV)**

- Isolated from the lesser mouse-eared bat (*Myotis blythi*) in Kyrghizstan in 1991. Further, serologic evidences of circulation of viruses related to ARAV and KHUV were demonstrated in bats in Bangladesh.
- Pathogenic to laboratory mice, hamsters, and bats via intracranial and intramuscular inoculation routes, causing acute progressive fatal encephalitis (rabies).
- During infection, forms typical to lyssaviruses intracytoplasmic inclusions, detected by staining with polyclonal or monoclonal anti-rabies antibodies.
- Antigenic patterns in reactions with selected panels of antinucleocapsid monoclonal antibodies are different from those of other lyssaviruses.
- Serologically cross-react with phylogroup I lyssaviruses. Use of commercially available rabies biologicals provide significant protection against ARAV, although less efficient than against RABV.
- The genome (GenBank Accession No. EF614259) consists of 11918 nucleotides and includes 5 structural genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) genes.
- Phylogenetically belongs to the *Lyssavirus* genus. Comparing the entire nucleoprotein (N) gene sequence, ARAV is closer to KHUV (78.8% identity), then to DUVV (78.1%) and EBLV-1 (77.8-78.0%).

***Khujand virus* (KHUV)**

- Isolated from the whiskered bat (*Myotis mystacinus*) in Tajikistan in 2001. Further, serologic evidences of circulation of viruses related to ARAV and KHUV, were demonstrated in bats in Bangladesh.
- Pathogenic to laboratory mice, hamsters, and bats via intracranial and intramuscular inoculation routes, causing acute progressive fatal encephalitis (rabies).
- During infection, forms typical to lyssaviruses intracytoplasmic inclusions, detected by staining with polyclonal or monoclonal anti-rabies antibodies.
- Antigenic patterns in reactions with selected panels of antinucleocapsid monoclonal antibodies are different from those of other lyssaviruses.
- Serologically cross-react with phylogroup I lyssaviruses. Use of commercially available rabies biologicals provide significant protection against KHUV, although less efficient than against RABV.
- The genome (GenBank Accession No. EF614261) consists of 11903 nucleotides and includes 5 structural genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) genes.
- Phylogenetically belongs to the *Lyssavirus* genus. Comparing the entire nucleoprotein (N) gene sequence, KHUV is closer to EBLV-2 (79.0% identity), then to ARAV (78.8%).

Irkut virus (IRKV)

- Was isolated from the greater tube-nosed bat (*Murina leucogaster*) in Russia (East Siberia) in 2002.
- Pathogenic to laboratory mice, hamsters, and bats via intracranial and intramuscular inoculation routes, causing acute progressive fatal encephalitis (rabies).
- During infection, forms typical to lyssaviruses intracytoplasmic inclusions, detected by staining with polyclonal or monoclonal anti-rabies antibodies.
- Antigenic patterns in reactions with selected panels of antinucleocapsid monoclonal antibodies are different from those of other lyssaviruses.

- Serologically cross-react with phylogroup I lyssaviruses. Use of commercially available rabies biologicals provide significant protection against IRKV, although less efficient than against RABV.
- The genome (GenBank Accession No. EF614260) consists of 11980 nucleotides and includes 5 structural genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) genes.
- Phylogenetically belongs to the *Lyssavirus* genus. Comparing the entire nucleoprotein (N) gene sequence, IRKV is closer to EBLV-1 (78.2-78.6% identity), then to DUVV (78.0%).

West Caucasian bat virus (WCBV)

- Was isolated from the Schreiber's bent-winged bat (*Miniopterus schreibersi*) in Russia (western Caucasus) in 2002. Seroprevalence to WCBV was detected in several species of *Miniopterus* spp. Bats from Kenya.
- Pathogenic to laboratory mice via intracranial route, and to hamsters, non-human primates and bats via intracranial and intramuscular inoculation routes, causing acute progressive fatal encephalitis (rabies).
- During infection, forms typical to lyssaviruses intracytoplasmic inclusions, detected by staining with polyclonal or monoclonal anti-rabies antibodies.
- Antigenic patterns in reactions with selected panels of antinucleocapsid monoclonal antibodies are different from those of other lyssaviruses.
- Serologically does not demonstrate any detectable cross-reactivity with other lyssaviruses. Use of commercially available rabies biologicals does not provide protection against WCBV.
- The genome (GenBank Accession No. EF614258) consists of 12278 nucleotides and includes 5 structural genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) genes. There is an exceptionally large non coding region between G and L coding regions (about 700 nucleotides) wich contains a potential open reading frame, the corresponding protein having not been evidenced in vitro.
- Phylogenetically belongs to the *Lyssavirus* genus. Within the genus, is placed ancestrally to all other lyssaviruses, and is the most divergent lyssavirus described to date. Can not be included in either phylogroup I or II, and should be considered as a representative of independent phylogroup III.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

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Kuzmin, I.V., Niezgoda, M., Carroll, D. S., Keeler, N., Hossain, M.J., Breiman, R.F., Ksiazek, T.G. and Rupprecht, C.E. (2006). Lyssavirus surveillance in bats, Bangladesh. Emerging Infectious Diseases 12, 486-488.

Kuzmin I.V., Niezgoda M., Franka R., Agwanda B., Markotter W., Beagley J.C., Urazova O.Y., Breiman R.F., Rupprecht C.E. (2008). Possible emergence of West Caucasian bat virus in Africa. Emerg. Infect. Dis. 14, 1887-1889.

Kuzmin I.V., Wu X., Tordo N., Rupprecht C.E. (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.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders.
The use of Figures and Tables is strongly recommended.

Figure 1. Phylogenetic tree of *Rhabdoviridae* based on the alignment of partial nucleoprotein (N) gene sequences (1027 nucleotides).

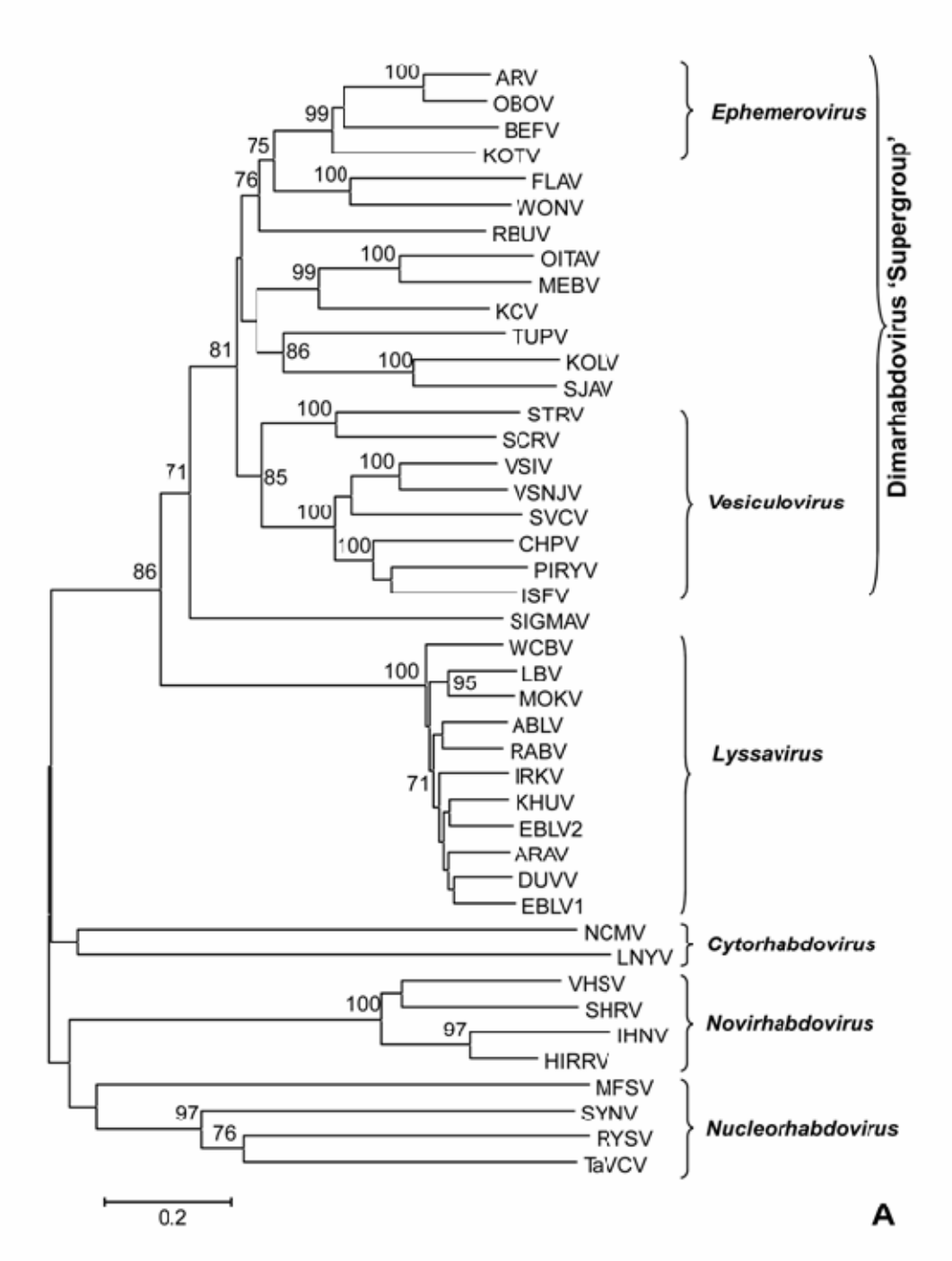

Figure 2. Unrooted phylogenetic trees based on the entire lyssavirus N gene nucleotide sequences (*a*) and glycoprotein ectodomain amino acid sequences (*b*) (Kuzmin et al., 2005).

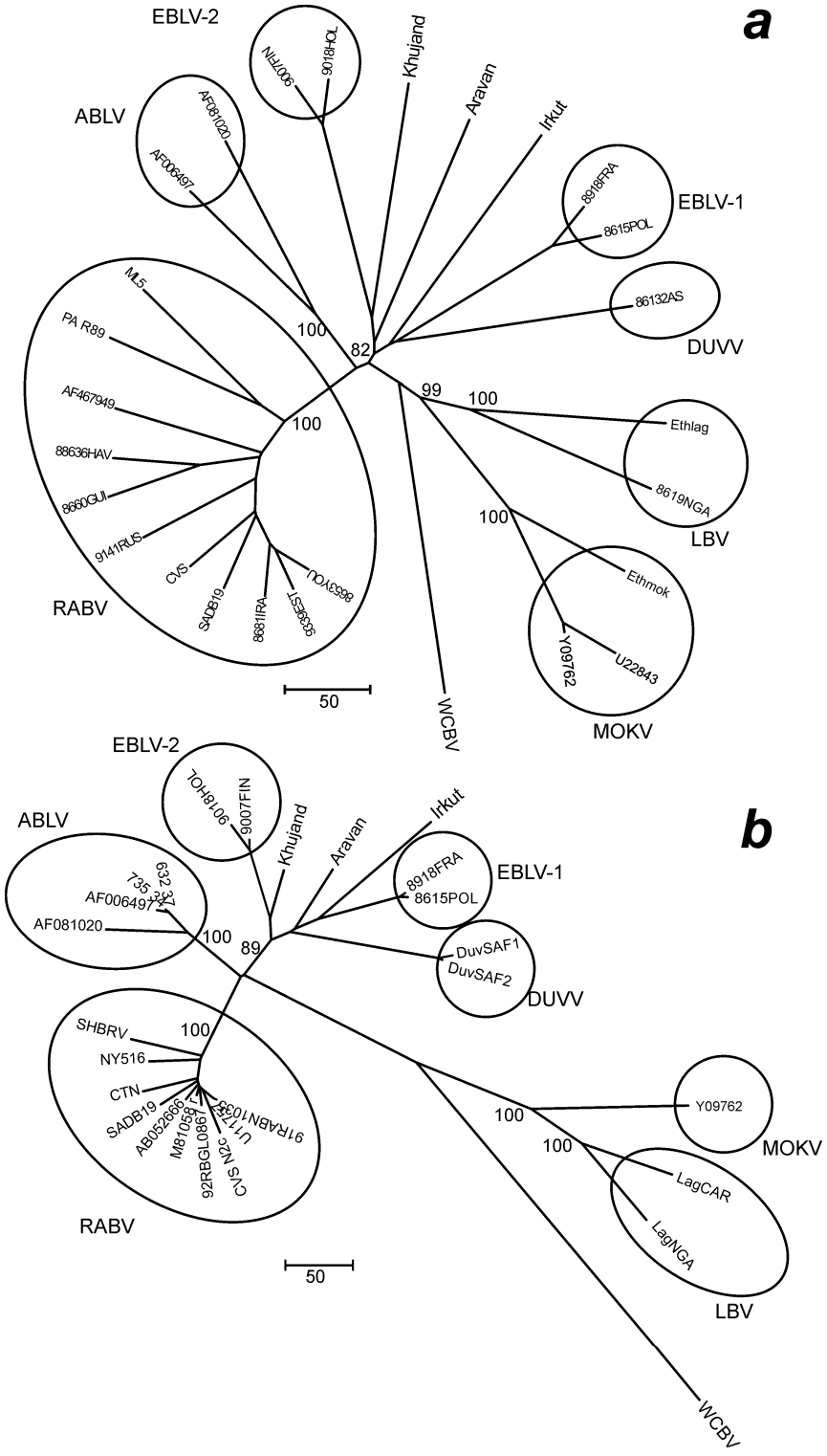

Table 1. Antigenic patterns lyssavirus representatives with a panel of antinucleopcapsid monoclonal antibodies of CDC (Botvinkin et al., 2003).

	N-MAbs [a]																			
Virus	3-1	8-2	11-1	15-2	22-3	23-4	24-1	24-10	52-1	52-2	61-1	62-4	71-2	97-3	97-11	141-1	143-1	146-3	164-2	502-2	422-5
Irkut virus	+	-	+	-	+	o	-	+	+	+	-	-	-	-	-	+	-	-	-	+	-
WCBV	-	+	-	-	+	-	+	-	+	-	+	+	+	-	-	-	-	-	-	+	-
Lagos bat virus (variant 1)[b]	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	+
Lagos bat virus (variant 2)[b]	-	-	+	-	+	-	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+
Mokola	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+	-	+	+
Duvenhage virus[b]	-	-	+	-	+	+	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+
EBLV-1	+	-	+	-	+	+	-	+	+	+	-	-	-	-	-	+	-	-	+	+	-
EBLV-2	+	-	+	-	+	-	-	+	+	+	-	-	+	-	-	+	+	+	-	+	-
Aravan virus	-	-	+	-	+	+	-	+	+	+	-	-	-	-	-	+	-	+	-	+	-
Khujand virus	o	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-
Rabies, Red fox (West Europe)	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-
Rabies, Red fox (Caucasus)	+	+	+	+	+	+	-	+	+	+	+	o	-	+	+	+	-	+	+	+	-
Rabies, CVS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

[a]N-MAbs, antinucleocapsid monoclonal antibodies; –, absence of reaction; zero, reduced reaction with 10^{x} less diluted antibody; +, positive reaction; WCBV, West Caucasian bat virus; .EBLV, European bat lyssavirus; CVS, challenge virus standard.

Table 2. Comparative neutralization activity against lyssaviruses (Hanlon et al., 2005).

Antibody	Virus[a]						
	CVS	DUVV	EBLV:H	EBLV:/	ARAV	KHUV	IRKV
Rabies	**0.0**	0.7	0.6	0.6	0.7	0.4	0.7
DUVV	1.3	**0.0**	0.3	0.6	0.8	0.6	1.1
EBLV-1	2.2	1.2	**0.0**	0.5	0.6	0.3	0.7
EBLV-2	1.1	0.7	0.3	**0.0**	0.9	0.3	0.8
ARAV	1.1	0.8	0.5	0.1	**0.0**	0.0	0.6
KHUV	0.9	1.1	0.2	0.7	0.5	**0.0**	0.8

Antibody	Virus[b]			
	CVS	LBV	MOKV	WCBV
Rabies	250	<5	<5	<5
LBV	95	3125	17	≤11
MOKV	≤11	431	989	≤11
WCBV	≤11	≤11	≤11	6390

The bold values indicate anti-sera against the inducing virus.

[a] Mouse anti-sera (antibody) were prepared against rabies virus (challenge virus standard, CVS-11), Duvenhage (DUVV), European Bat *Lyssavirus*H UEBLV: HSw European Bat*lyssavirus*/ UEBLV:/Sw Aravan UARAVSw and Khujand UKHUVS viruses using standard techniques and evaluated using the rapid buorescent focus inhibition test with CVS:HHw DUVVw EBLV:Hw EBLV:/w ARAVw KHUVw and IRKVL Results represent the log titer difference in the amount required for equivalent neutralizationL

[b] Mouse anti:sera UantibodyS were prepared against rabies virusw Lagos Bat virus ULBVSw Mokola virus UMOKVS and West Caucasian Bat virus UWCBVS using standard techniques and evaluated using the rapid buorescent focus inhibition test with CVS:HHw LBVw MOKVw and WCBVL Results are reported in reciprocal titers

Table 3. Protection of Syrian hamster against various lyssaviruses via pre- and post-exposure prophylaxis (Hanlon et al., 2005).

Pre-exposure vaccination[a]

Treatment group	Virus				
	WCBV	ARAV	IRKV	KHUV	Rabies
(A) Commercial human vaccine	4/9[b]	5/9	6/9*	8/9*	9/9*
(B) Commercial veterinary vaccine	2/9	9/9*	8/9*	9/9*	9/9*
(C) Vaccinia-rabies glycoprotein	1/9	4/9	5/9	9/9*	9/9*
(D) Unvaccinated controls	2/9	0/9	0/9	1/9	0/9
Statistical *p*-value	–	$p<0.01$	$p<0.01$	$p<0.001$	$p<0.001$

[a] Hamsters were vaccinated with 0.05 ml intramuscularly in the left gastrocnemius muscle. Vaccines consisted of a commercial human vaccine (Human Diploid Cell Vaccine, Imovax IM, Lot no. W0182), a commercial veterinary vaccine (Rabdomun, Serial no. A240929B) and a live vaccinia-rabies glycoprotein recombinant virus vaccine (log 10^5 pfu/ml). Five weeks later, the animals were inoculated intramuscularly in the left gastrocnemius muscle with Aravan (ARAV) (log $10^{3.9}$ $MICLD_{50}$/0.05 ml), Khujand (KHUV) (log $10^{4.3}$ $MICLD_{50}$/0.05 ml), Irkut (IRKV) (log $10^{4.7}$ $MICLD_{50}$/0.05 ml) or West Caucasian bat virus (WCBV) (log $10^{5.7}$ $MICLD_{50}$/0.05 ml) (0.05 ml of mouse-brain-passaged homogenate) or $10^{3.4}$ $MICLD_{50}$ salivary gland homogenate from a naturally infected dog (#323) from Texas (dog/coyote rabies virus variant).

[b] Number survived/number challenged.

* Statistically different from controls.

Post-exposure prophylaxis after lyssavirus infection[a]

Treatment group	Virus					
	WCBV	IRKV	ARAV	KHUV	Rabies	Rabies
(A) HRIG + vaccine	1/9	1/9	4/9	7/9	9/9**	9/9**
(B) HRIG-HT + vaccine	0/9	0/9	3/9	9/9*	9/9**	9/9**
(C) ERIG + vaccine	0/9	0/9	3/9	7/9	9/9**	8/9**
(D) Vaccine only	0/9	0/9	1/9	3/9	0/9	0/9
(E) Mab 62-71-3 + vaccine	–	–	9/9*	9/9*	9/9**	9/9**
(F) Controls	0/9	0/9	0/9	2/9	0/9	0/9

[a] Virus inoculation consisted of 0.05 ml administered intramuscularly in the left gastrocnemius muscle with Aravan (ARAV) (log $10^{3.9}$ $MICLD_{50}$/0.05 ml), Khujand (KHUV) (log $10^{4.3}$ $MICLD_{50}$/0.05 ml), Irkut (IRKV) (log $10^{4.7}$ $MICLD_{50}$/0.05 ml) or West Caucasian Bat virus (WCBV) (log $10^{5.7}$ $MICLD_{50}$/0.05 ml) (mouse-brain-passaged homogenate) or $10^{3.4}$ $MICLD_{50}$ salivary gland homogenate from a naturally infected dog (#323) (dog/coyote rabies virus variant) or $10^{3.1}$ $MICLD_{50}$ salivary gland homogenate from a naturally infected gray fox (#393) (gray fox rabies virus variant), both from Texas. Four hours after virus inoculation, post-exposure prophylaxis was initiated consisting of 50 l of a commercial human rabies vaccine (human diploid cell vaccine; Imovax IM®, Lot no. W0182) administered in the right gastrocnemius muscle on days 0, 3, 7, 14 and 28, and, in groups (A–C), a single administration of commercial human rabies immunoglobulin (human rabies immunoglobulin-BayRab; human rabies immunoglobulin, heat-treated – Imogam-HT) or equine rabies immune globulin (Behring Equine Rabies immunoserum) at the site of virus inoculation on day 0. Group D received only vaccine. Group E received an experimental murine monoclonal antibody (62-71-3) and the series of vaccinations. The control group (F) received no treatment. Fractions represent number survived/number challenged.

* Statistically signicant difference from controls, $p<0.01$.

** Statistically signicant difference from controls, $p<0.001$.