Novel Lyssaviruses Isolated from Bats in Russia

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Two new rabies-related viruses were discovered in Russia during 2002. Viruses were isolated from bats in Eastern Siberia near Baikal Lake and in the western Caucasus Mountains. After preliminary antigenic and genetic characterization, we found that both viruses should be considered as new putative lyssavirus genotypes.

Rabies is an acute, fatal encephalitis caused by lyssaviruses that are perpetuated in reservoir mammals, principally certain carnivores and bats. Although the disease has been known among carnivores, such as dogs, for centuries, the paradigm of rabies in bats has been appreciated fully only over the past 50 years (1–3). Recent findings of bat lyssaviruses throughout the world have prompted a taxonomic reconsideration of the Lyssavirus genus, family Rhabdoviridae. To date, besides their occurrence in the Americas, Africa, and Australia, at least four additional bat lyssaviruses have been identified in Eurasia (4–7). One of these has been reported from Russia, a “Duvenhage-like” virus isolated from a patient who died in 1985 after being bitten by a bat at a site near the Ukrainian border (8). We describe the isolation and preliminary identification of two new bat lyssaviruses discovered in Russia.

The Study

During preliminary infectious disease surveys, bats were obtained randomly at different locations by hand at roosts and from mist netting at cave entrances and at routes of nocturnal foraging. From 1979 to 2002, a total of 210 bats were collected in the Baikal Lake region, including 98 Vespertilio murinus, 3 Myotis brandtii, 55 M. daubentonii, 2 M. ikhnikovii, 29 Eptesicus nilssonii, 22 Plecotus auritus, and 1 Murina leucogaster. In the Caucasus Mountains, 129 bats were collected during a field expedition in July 2002, including 6 Rhinolophus ferrumequinum, 10 Myotis blythii, 43 M. daubentonii, 4 M. emarginatus, 9 Pipistrellus kuhlii, 2 P. pipistrellus, 3 Barbastella bar-...
A second isolate was obtained from the Caucasus (about 100 km southeast of the town of Krasnodar). All bats collected in this survey appeared healthy. No sick bats or carcasses were found in caves or other roosts. ELISAs of 129 bat brain samples gave negative results. The MIT produced one positive result, from the brain of a male Common Bent-winged Bat (Miniopterus schreibersi), captured during departure for nocturnal foraging at a cave entrance, together with 23 other males of the same species. Inoculated mice became paralyzed and died 9–13 days after intracerebral inoculation. Mouse brain impressions were DFAT-positive with at least log 5.7 MIC 50/0.05 mL. Based on the location, the virus was named West Caucasian bat virus (WCBV).

In antigenic typing, both viruses reacted with Wistar N-MAB 502-2, but only the African nonrabies lyssaviruses reacted with N-MAB 422-5 (Table). With CDC N-MAbs, the patterns obtained for Irkut virus were similar to those of Duvenhage and European bat lyssavirus, type 1 (EBLV-1), but distinguishable from both of them, whereas WCBV demonstrated unique patterns.

When phylogenetic analysis was performed, Irkut virus was recognized as a member of a cluster joining lyssavirus genotypes 4 and 5 (76% bootstrap support). However, the degree of diversity did not allow us to consider it a representative of one of these genotypes (Figure). WCBV was connected to the cluster of genotypes 2 and 3, but bootstrap support of this joining was insignificant (68%), illustrating that this virus is the most divergent member of the Lyssavirus genus examined to date. Further analysis of the

| Table. Antigenic patterns of new bat virus isolates compared to other lyssaviruses by a panel of N-MAbsa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Virus | 3-1 | 8-2 | 11-1 | 15-2 | 22-3 | 23-4 | 24-5 | 24-10 | 52-1 | 52-2 | 61-1 | 62-4 | 71-2 | 97-3 | 97-5 | 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 b | - | - | + | - | + | - | - | + | + | - | - | - | - | + | - | - | + | + | + | - |
| Duvenhage virusb | - | - | + | - | + | + | + | - | - | - | - | - | - | + | - | - | + | + | + | - |
| EBLV-1b | + | - | + | - | + | + | + | - | - | - | - | - | - | + | - | - | + | + | + | - |
| EBLV-2b | + | - | + | - | + | + | + | - | - | - | - | - | - | + | - | - | + | + | + | - |
| Aravan virus | - | - | + | - | - | - | - | + | + | - | - | - | - | + | - | - | + | + | + | - |
| Khujand virus | o | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | - |
| Rabies, Red fox (West Europe)b | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Rabies, Red fox (Caucasus) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Rabies, CVS | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |

aN-MAbs, antinucleocapsid monoclonal antibodies; - , absence of reaction; zero, reduced reaction with 10-fold less diluted antibody; +, positive reaction; WCBV, West Caucasian bat virus; EBLV, European bat lyssavirus; CVS, challenge virus standard.

bPatterns obtained from Smith (11).
entire N and other genes should be conducted to refine the phylogenetic relationships of both these viruses.

Conclusions

Estimating the potential public health significance of these two newly recognized lyssaviruses is critical. Other bat lyssaviruses cause fatal human encephalitis, even in so-called “rabies-free” countries (2,3,13). Given bat mobility and the opportunity for infecting new areas quickly, no major geographic area can be considered truly free from lyssaviruses. For example, the Irkutsk Province was considered free of rabies for 35 years before the Irkut virus was isolated. Additionally, although the Caucasus had been considered as a rabies-endemic area, virus reservoirs were identified only among the canids. Public health authorities need to be aware of the potential of bats to transmit lyssaviruses and increase surveillance and public education. Attention should focus on the protective efficacy of commercially available rabies virus vaccines and immune globulins against these novel nonrabies lyssaviruses, before human infection occurs.

Acknowledgments

We thank staff in the Rabies Section, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, for their kind assistance and expertise; and T. Mebatsion and J.H.Cox, National Research Institute of Health, Addis-Ababa, Ethiopia, for providing Mokola and Lagos bat virus sequences from Ethiopia.

This study was supported in part by the Russian Foundation of Basic Research (grant 00-04-48004) and by the Association of Public Health Laboratories, international fellowship program of 2002-2003.

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References

1. Schneider LG, Cox JN. Bat lyssaviruses in Europe. In: Rupprecht CE, Dietzschold B, Koprowski H, editors. Lyssaviruses. Berlin: Springer-Verlag 1994. p. 207–18.
2. Hooper PT, Luni RA, Gould AR, Samarutunga H, Hyatt AD, Gleson LG, et al. A new lyssavirus—the first endemic rabies-related virus recognized in Australia. Bulletin de l’Institut Pasteur 1997;95:209–18.
3. Fooks AR, Finningar C, Johnson K, Mc Elhinney L, Manser P. Human case of EBL type 2 following exposure to bats in Angus, Scotland. Vet Rec 2002;151:679.
4. King A, Davies P, Lawrie A. The rabies viruses of bats. Vet Microbiol 1990; 23:165–74.
5. Amengual B, Whitby JE, King A, Serra Cobo J, Bourhy H. Evolution of European bat lyssaviruses. J GenVirol 1997;78:2319–28.
6. Arai YT, Kuzmin IV, Kameoka Y, Botvinkin AD. New Lyssavirus Genotype from the lesser mouse-eared bat (Myotis blythii), Kyrgyzstan. Emerg Infect Dis 2003;9:333–7.
7. Kuzmin IV, Orciari LA, Arai YT, Smith JS, Hanlon CA, Kameika Y, Rupprecht CE. Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. Virus Res 2003;97:65–79.
8. Selimov MA, Tatarov AG, Botvinkin AD, Klueva EV, Kulikova EV, Khismatullina NA. Rabies-related Yuli virus: identification with a panel of monoclonal antibodies. Acta Virol 1989;33:542–5.
9. Meslin, F.-X, Kaplan MM, Koprowski H, editors. Laboratory techniques in rabies. Fourth edition. Geneva: World Health Organization; 1996. p. 476
10. Wiktor TJ, Koprowski H. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. Proc Natl Acad Sci U S A 1978;75:3938–42.
11. Smith JS. Rabies virus epistopic variation: use in ecologic studies. Adv Virus Res 1989;36:215–53.
12. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 2001;17:1244–5.
13. Lumio J, Hillbom M, Roine R, Ketonen L, Haltia M, Valle M., et al. Human rabies of bat origin in Europe. Lancet 1986;1:378.

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