GENETIC CHARACTERISATION OF RABIES VIRUS ISOLATES IN BOSNIA AND HERZEGOVINA

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Abstract

Serotyping of five rabies virus isolates with monoclonal anti-nucleoprotein antibodies for classical rabies virus and rabies-related viruses and phylogenetic relationships among sequences indicate that viruses circulating in population of animals in Bosnia and Herzegovina belong to the sero-genotype 1 of classical rabies virus. Phylogenetic relationships among sequences of our viruses have shown the presence of two phylogenetic lines, one which is present in the northwestern part and other which is present in the northeastern part of the country. Our viruses are closely related to Westeuropean isolates of rabies virus.

KEY WORDS: acetylsalicylic acid (aspirin), lymphocyte cell culture

Introduction

Rabies is an acute fatal zoonotic viral infection of the central nervous system that is transmitted by the bite of rabbit animals and is capable of infecting all warm-blooded species (1). The etiological agent of rabies- the rabies virus (order *Mononegavirales*, genus *Lyssavirus*, family *Rhabdoviridae*) is an RNA virus with a negatively polarized single-stranded RNA. According to the valid taxonomy the genus *Lyssavirus* is classified into four serotypes and seven genotypes. *Classical rabies virus* (sero-genotype 1), *Lagos Bat virus* (sero-genotype 2), *Mokola virus* (sero-genotype 3) and *Duvenhage virus* (sero-genotype 4), *European bat lyssavirus* 1 (EBL1) genotypes 5, *European bat lyssavirus* 2 (EBL2) genotype 6 and *Australian bat lyssavirus* (ABL) genotype 7. All genotypes except genotype 2 have caused human and/or animal deaths in nature (2). Despite continued attempts of prevention rabies is still present in many parts of the world. In endemic countries, the virus is maintained in two interrelated ecological niches: an urban one largely limited to feral dogs and cats and sylvatic limited to wildlife. The most important reservoir of rabies in Europe is red fox, rabid animals having progressively moved from Eastern Europe after the Second World War and spread throughout Western Europe by the middle of the 1980s (3). After October 1982, when the first fox rabies cases were found on the territory of the town Livno, wildlife (sylvatic) rabies has become of increasing health and economic importance in Bosnia and Herzegovina (4).

Materials and Methods

We studied five brain samples that were positive by the fluorescent antibody test used for routine diagnosis (5) which come from the following animals: 3 foxes, one cat and one dog collected during 2004. form five (Tuzla, Vareš, Tešanj, Livno and Mostar) different part of Bosnia and Herzegovina. Murine neuroblastoma cell cultures (Na42/13) at passages 30 to 50 were used for isolation of virus from brain tissue (6), for serotyping of viral isolates we used panel of monoclonal anti-nucleoprotein antibodies for classical rabies virus (W239.17, W187.5, W187.11.2 and MW187.6.1) and rabies related viruses (MSA6.3, LBV7.36, DUV6.15.19, 862.1.2, P 41 and Z144.88) performed as indirect imunofluorescence test with an FITC-conjugated antimause immunoglobulin (7). The RT-PCR was used for the detection of RNA sequences of the rabies virus genome encoding for the nucleoprotein and phosoprotein (N-P) with primers N1161P (5'-AAGAACTTCAAGAATACGAGGC-3'-3') and N1579M (5'-TTCAGCCATCTCAAGATCGG-3') for classical rabies virus (8). The purified 400 pb PCR products from (N-P) gene segment were labelled for sequencing. In total, 381 nucleotides were sequenced, nucleotides 1199-1579 on the SAD B19 genome (3). The sequences were compared between each other and with published sequences from SR Yugoslavia (Serbia and Montenegro) and France. Phylogenetic relationships among sequences obtained were estimated by using BioEdit Sequence Alignment Editor, Version 5.0.9 and Tree View programs. Isolation of virus, indirect imunofluorescence test and sequencing was performed on the Freidrich-Loeffler-Institute, Federal Research Institute for Animal Health, Wusterhausen, Germany.

Results

From all positive FAT (Figure1 1) and PCR samples (Figure 2) we isolated viruses in murine neuroblastoma cell culture (Figure 3) for further examination.

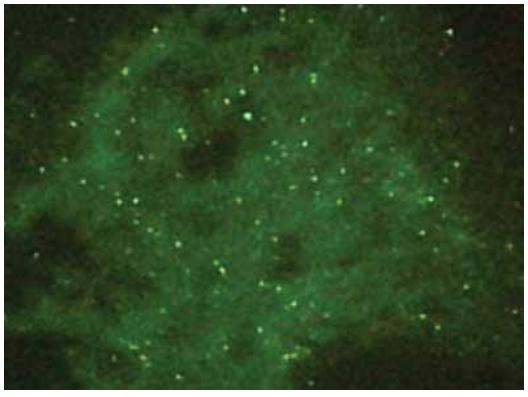

FIGURE 1. Positive fluorescent antibody test, magnification X 400

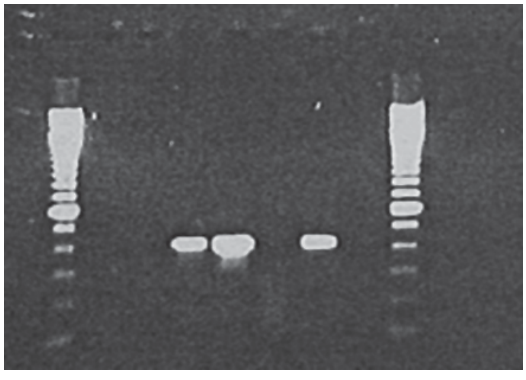

FIGURE 2. Two positive PCR reactions

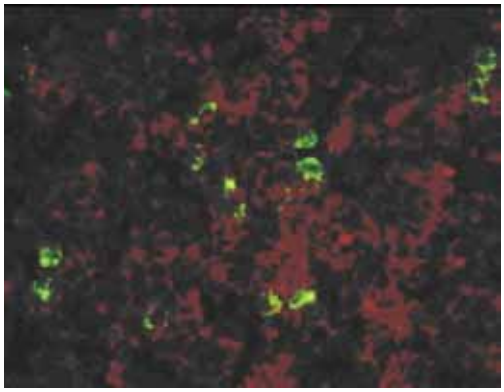

FIGURE 3. Murine neuroblastoma cell cultures infected with rabies virus, magnification X 300

The results of serotyping of our five virus isolates called 8192 Tz, 8193 Va, 8194 Tš, 8195 Li and 8196 Mo (Table 1) with monoclonal anti-nucleoprotein antibodies for classical rabies virus (W239.17, W187.5, W187.11.2 and MW187.6.1) and rabies related viruses (MSA6.3, LBV7.36, DUV6.15.19, 862.1.2, P 41 and Z144.88) and result of phylogenetic relationships among sequences (Figures 4 and 5) indicate that viruses circulating in population of animals in our country belongs to the sero-genotype 1 of classical rabies virus.

Comparison of three our isolates sequences from northeastern part of country has shown that they have very similar arrangement of nucleotides of the N-P gene segment (only one mutation), but sequences of two isolates from northwestern part have 9 mutations of nucleotides of the N-P gene segment. The rate of genetic similarity between isolates from northeastern part and isolates from northwestern was 97,4%.

Comparison of sequences and phylogenetic relationships among sequences of our isolates with published sequences from Serbia and Montenegro and France showed that isolates from Bosnia and Herzegovina are closely related to Westeuropean (France) isolates of rabies (Figure 5). Phylogenetic relationships among sequences of our viruses has shown the presence of two phylogenetic groups (lines); one WE (western European) which is present in the northwestern part of the country and other EE (eastern European) which is present in the northeastern part of the country (Figure 6).

Discussion

By applying indirect immunofluorescence technique on all the five isolates we received a positive reaction only with antibodies for a classic rabies virus (Table 1), and consequently, we have managed to prove that the virus circulating among the animal population in our country is a classic rabies virus belonging to the serotype 1 which is in conformity with earlier investigations of rabies virus in our area (10), as well as investigations conducted worldwide which have proved that the serotype 1 has been circulating in the populations of domestic and wild animals, mostly carnivores, all over the world, but also among the bat populations on the American continent (11).

The results of the conducted investigations (Figure 4), arrangement sequences of nucleotides of N-P gene segments of PCR products (367 pb) of our five isolates show that three isolates from the "northeast" (8192 Tz, 8193 Va and 8194 Tš) have almost the identical, examined genome region (99,8% homologenity), while isolates from the "southwest" (195 Li and 8196 Mo) show 97,4% of homologenity in relation to the previous three isolates. The obtained nucleotide sequences of our isolates and a phylogenetic analysis (Figures 4 and 5) suggest their belonging to the genotype 1 of the classic rabies virus. Classification of the isolates under genotype 1 of the classic rabies virus has been asserted both in Serbia and Montenegro (12), but the same holds true for the isolates from the eighties deriving from Bosnia and Herzegovina (13). By mutually assessing the phylogenetic relations of the sequences of N-P gene of our isolates of the rabies virus, and then with the sequences of the

	Monoclonal anti-nucleoprotein antibodies for classical rabies virus				Monoclonal anti-nucleoprotein antibodies for rabies related viruses					
Virus Isolate	MAb1 W239. 17	MAb2 W187. 5	mAb3 W187.11.2	mAb4 MW187. 6.1	mAb5 MSA 6.3	mAb6 LBV7. 36	mAb7 DUV6. 15.19	MAb8 862.1.2	mAb9 P 41.	mAb10 Z 144. 88
8192 Tz	+	+	+	+	-	-	-	-	-	-
8193 Va	+	+	+	+	-	-	-	-	-	-
8194 Tš	+	+	+	+	-	-	-	-	-	-
8195 Li	+	+	+	+	-	-	-	-	-	-
8196 Mo	+	+	+	+	-	-	-	-	-	-

TABLE 1. Serotyping of viral isolates with of monoclonal antibodies for classical rabies virus and rabies related viruses in indirect imunofluorescence test

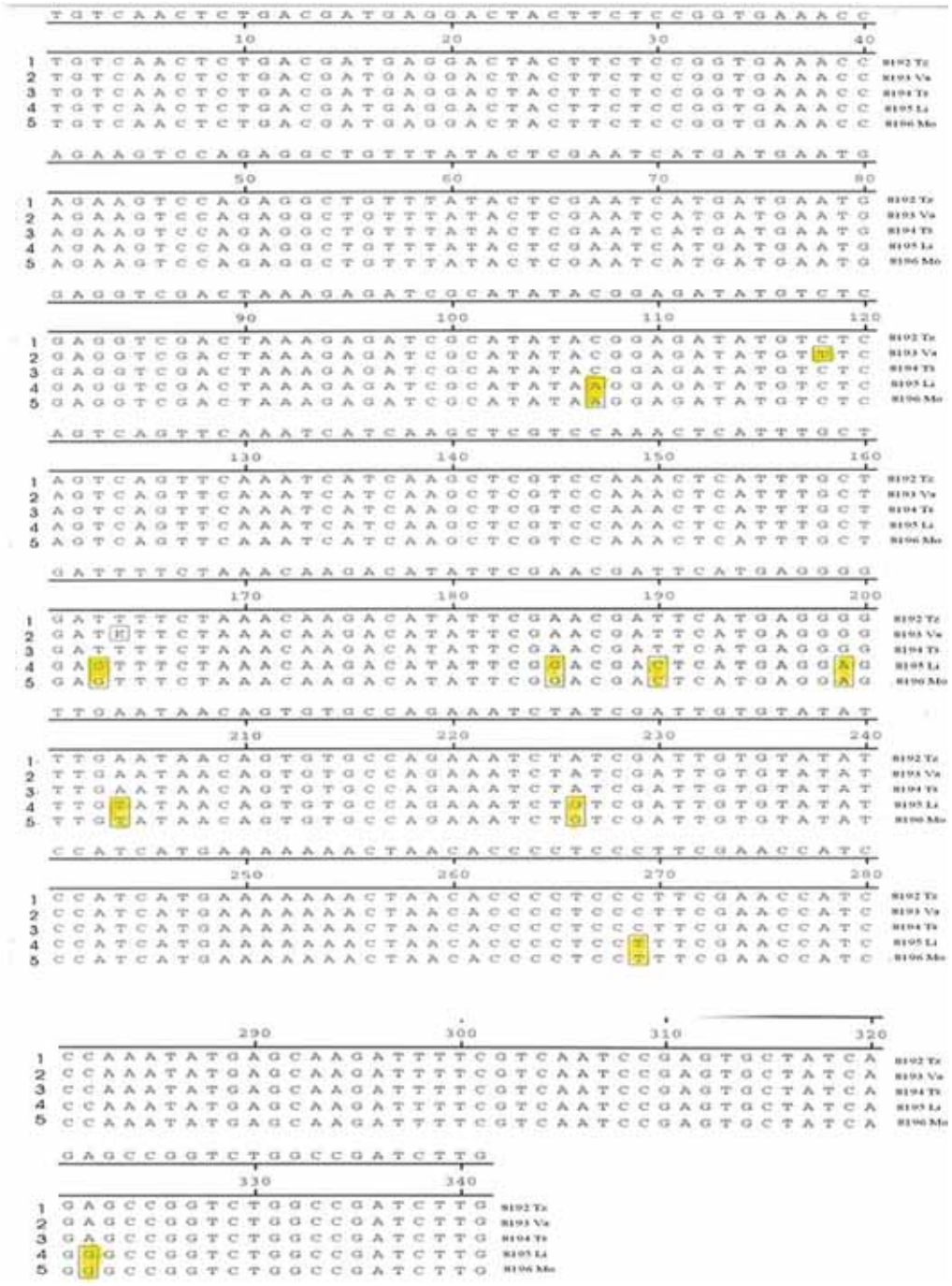

FIGURE 4. Nucleotide sequence of five rabies samples from Bosnia and Herzegovina

rabies virus isolates from France and those from Paster's Institute in Novi Sad (Figure 5), we can observe that our isolates constitute two phylogenetic groups. The isolates from the „northeast" belong to the first group, while the isolates from the „southwest" belong to the second group, and this has confirmed our earlier assumption (10) whereby in the area of Bosnia and Herzegovina there exist viruses which differ in the genome structure. This has also confirmed the allegations that in this area there are two antigene groups (lines), namely the West-European (WE) which has spread from southern Poland as far as France and Belgium, also affecting Switzerland, Austria, Slovenia, Croatia and Bosnia and Herzegovina, and the second East European (EE) antigene line which reached the northern parts of our country from Poland via the Chezh Republic and Hungary (13). The illustration of entry and spreading directions of the sylvatic form of rabies in our country (Figure 6),

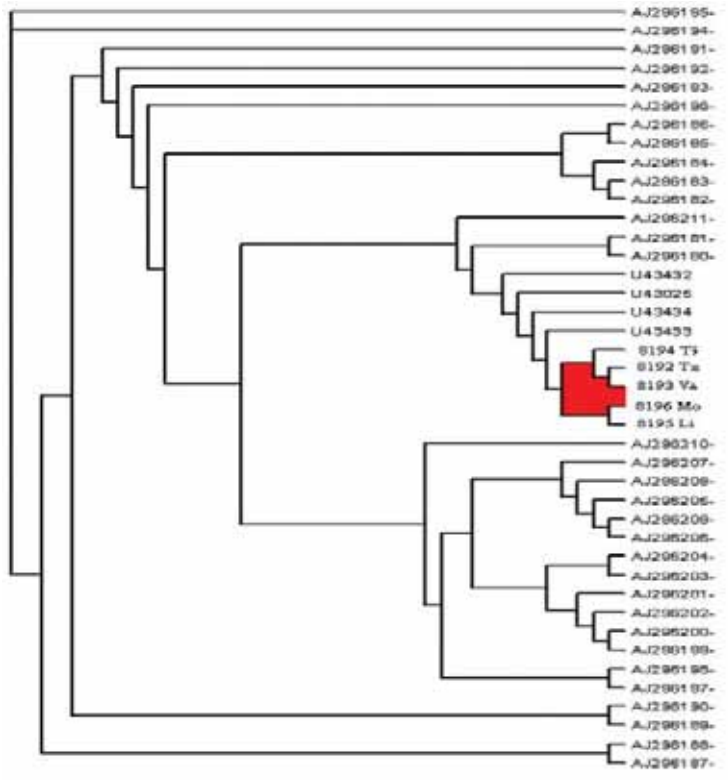

FIGURE 5. Phylogenetic relationships among sequences of our isolates with published sequences from Serbia and Montenegro and France

FIGURE 6. Two phylogenetic lines of rabies virus which are present in Bosnia and Herzegovina.

made in accordance with the above indicated data has confirmed our research findings that the virus isolates circulating in the population of our animals in phylogenetic respect are closer to those originating from France than the virus lines from Serbia and Montenegro. The obtained genetic differences of our isolates in respect of geographical origin can be accounted for by historic spreading of sylvatic rabies in our country and the influence of natural barriers. The obtained results of the molecular epidemiological examinations (Figures 4, 5 and 6) have shown that rabies epizootics in our country is more considerably influenced by the epizootical situation in Croatia than that in Serbia and Montenegro because of the geographical barriers with the latter, namely the river courses of the Drina, the Sava and the Danube.

Conclusion

1. Serotyping of isolates indicate that viruses circulating in population of animals in our country belong to the serogenotype 1 of classical rabies virus.

2. Phylogenetic relationships among sequences of our viruses have shown the presence of two phylogenetic groups (lines): one WE (western European) which is present in the northwestern part of the country, and other EE (eastern European) which is present in the northeastern part of the country.

3. Our isolates are closely related to isolates from France than to isolates from Serbia and Montenegro.

References

(1) Fu, Z.F. Rabies and rabies research: past, present and future. Vaccine 1997; 15: 20-24.

(2) Murphy F.A., Gibbs, P.J.E., Horzinek, M.C., Studdert, M.J. Rhabdoviridae. In: Veterinary Virology 3rd ed. Academic Press. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto,1999; pp.: 435 –439.

(3) Poetsch C.J., Mueller T., Kramp M. Summarizing the rabies situation in Europe 1990-2002. Rabies Bulletin Europe. 2002;26, 11-16

(4) Velić R., Santrač V. Rabies in Bosnia and Herzegovina 2004 –2006. Rabies Bulletin Europe. 2007; 1: 11-12.

(5) Dean D.J., Abelseth M.K. The fluorescent antibody test. In: Laboratory techniques in Rabies. (Kaplan M.M., Koprowski H., eds.), 3rd ed. World Health Organization. Geneva. 1973; 73- 84.

(6) Rudd R.J., Trimarchi C.V., Abelseth M.K. Tissue culture technique for routine isolation of street strain Rabies virus. J.Clin. Microbiol. 2002; 4: 590-593.

(7) Smith J.S., King A.AMonoclonal antibodies for the identification of rabies and non-rabies lyssaviruses. In: Laboratory techniques in rabies. (Meslin F.X., Kaplan M.M., Koprowski H., Eds), Geneva. World Health organization, 1996; pp. 145-156.

(8) East M.L., Hofer H., Cox J.H., Wulle U., Wilk H., Pitra C. Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas. PNAS. 2001; 98, 15026-15031.

(9) Conzelman K.K. Genetic manipulation of non-segmented negative-stranded RNA viruses. J. Gen. Virol. 1996;77: 381-389.

(10) Kubelka D., Tabaković B., Nevjestić A., Rukavina LJ., Hodžić E., Golubović S., Kovačević S., Šarić M., Bajrović T., Sabirović M. Neke neobičnosti u pojavi, širenju i karakteru bjesnila u Bosni i Hercegovini. Simpozijum o besnilu sa međunarodnim učešćem i 70 godina Pasterovog Zavoda u Novom Sadu,1991.

(11) Bedrane H., Tordo N. Host switching in Lyssavirus history from Chiroptera to the Carnivora orders. J. Virology. 2001;17: 8096-8104.

(12) Stankov, S. Typing of field rabies virus strains in FR Yugoslavia by limited sequence analysis and monoclonal antibodies. Med. Pregl. 2001; 9-10: 446-452.

(13) Bourhy H., Kissi B., Audry L., Smreczak M., Sadowska-Todys M., Kullonen K., Tordo N., Zmudzinski J.F., Holmes E.C. Ecology and evulution of rabies virus in Europe. J. Gen. Virol. 1999;80, 2545-2557.