VETERINARSKI ARHIV 86 (1), 23-34, 2016

Phylogenetic investigation of partial G gene sequences in Lithuanian rabies virus field isolates

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ZIENIUS, D., G. PRIDOTKAS, M. MORKŪNAS, M. RUŽAUSKAS: Phylogenetic investigation of partial G gene sequences in Lithuanian rabies virus field isolates. Vet. arhiv 86, 23-34, 2016.

ABSTRACT

The objective of this study was to evaluate the molecular epidemiology of rabies virus G-gene partial sequences in rabies positive field samples, isolated in different regions of Lithuania, and to compare the sequences phylogenetically with various rabies virus isolates of wild carnivores from the Baltic Sea region, Europe and Russia. Twenty brain samples of rabid animals, collected during the period 2006-2011, were investigated. Multiple alignments of 358 nt G gene (nt 3324-3682) sequences were performed using ClustalW with default settings. Phylogenetic and molecular evolutionary analyses were conducted using the Neighbor-Joining method (NJ) in MEGA version 4. The phylogenetic investigations of rabies virus glycoprotein indicate that the Lithuanian rabies virus isolates' G-sequences were closely related and showed 89.8-98.9 % of nt identity. The G-sequences between Lithuanian raccoon dog and red fox rabies virus isolates were more conservative (97.2 % nt identity) than the rabies virus isolates inside the raccoon dog group (95.1 % nt identity). Two Lithuanian raccoon dog rabies virus isolate G-sequences were rather divergent (90.8 and 91.2 % nt identity) and were closely associated with rabies virus isolate G-sequences from Estonia (92.5 % nt identity), Russia (91.0 %) and Poland (89.3 %) respectively. The comparative investigation of G-sequences between the Lithuanian rabies virus isolates and different isolates from Germany, Slovenia and France indicated 84.2 % nt identity, whereas the G-sequences of Lithuanian rabies virus isolates and rabies virus isolate G-sequences from Russia were 88.1 % nt identical.

Key words: rabies, raccoon dog, red fox, Baltic, molecular epidemiology

Introduction

Over the past 15 year period, rabies in raccoon (*Nyctereutes procyonoides*) dogs had increased more than six-fold, especially in North-East Europe and the European part of

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ISSN 0372-5480
Printed in Croatia

the Baltic Sea region. Red fox and raccoon dog rabies together accounted for a constant level of 90-94 % of wildlife rabies in the last decade. Taking into consideration the fact that both species directly share the same territory of habitation and hunting areas, the possibilities of interspecific spread of rabies viruses are quite realistic. The speculation that raccoon dogs in Eastern Europe may form a separate, independent infectious cycle of rabies viruses (POTZSCH et al., 2006) should be analysed using the data from phylogenetic investigations of racoon dog and red fox rabies virus isolates.

Rabies virus (RV) investigation using the reverse transcription - polymerase chain reaction test (RT-PCR), subsequent nucleotide sequencing and phylogenetic analysis is an important tool for rabies molecular epidemiology. Several genome regions are used for these purposes, but the molecular analysis of field RV isolates has concentrated on the N, G gene and G-L non-coding regions. Although the G gene and G-L intergenic region (pseudo-gene, *c*-region) are far more variable than the N gene (JOHNSON et al., 2002), both of these regions have been used for genetic characterization of rabies viruses (BOURHY et al., 1999; KUZMIN et al., 2004; HYUN et al., 2005). The G gene of RV is an important target for genetic analyses because it encodes the glycoprotein containing determinants of the rabies virus pathogenicity, and potentially host specificity. External viral glycoprotein was previously used to study genetic diversity or host switching in the *Lyssavirus* genus (BADRANE and TORDO, 2001). Glycoprotein (G) is a type 1 transmembrane protein, 505 amino acids (aa) in length. The first 19 aa are the signal sequence. Upon DNA polymorphism analysis, the signal sequence and cytoplasmic domain of the G-gene were more divergent, while the ectodomain and transmembrane domain were relatively conserved. The overall nucleotide diversity of the G gene is 0.18520 (WU et al., 2007). Lyssavirus transcription processes are sequentially interconnected. The freedom of one gene from the others is therefore rather restricted. Additional evidence supports the concept that RV pathogenicity is not due to a single gene or protein, but rather is a multigene event (YAMADA et al., 2006). In addition, recent studies, employing reverse genetic techniques, have demonstrated the importance of a number of specific glycoprotein residues in conferring viral pathogenicity (TAKAYAMA-ITO et al., 2006). Several phylogenetic and molecular-epidemiological studies on the RV G-gene conducted over the past 15 years. BOURHY et al. (1999) classified rabies viruses of European origin into four main groups: the NEE group (North-East Europe), the EE-group (East Europe), the WE-group (Western Europe) and the CE-group (Central Europe). KUZMIN (2004) studied a wide range of rabies viruses isolated in the territory of the former Soviet Union, and classified them into five groups (A, B, C, D and E). Recently, rabies virus nucleoprotein (N) partial sequence analysis in Lithuanian RV isolates (ZIENIUS et al., 2008) indicated that all raccoon dogs and red foxes RV N-sequences belonged to RV genotype 1 and showed significant bootstrap support inside the North-Eastern Europe group (NEE). The phylogenetic relationship between Lithuanian red fox and raccoon dog RV isolate

N-sequences showed strong nucleotide identity, were related to other NEE lineages, and there was no reason to consider them as species - specific to the raccoon dogs.

The objective of the present study was to evaluate and compare the molecular epidemiology of rabies virus G-gene partial sequences in Lithuanian raccoon dog and red fox RV field samples. In addition, the predicted glycoprotein sequences were compared phylogenetically with various RV field strains of wild carnivores from Europe, the Baltic Sea region and Russia.

Materials and methods

Sample collection and RNA extraction. In total, 20 Lithuanian racoon dog and red fox RV isolates, diagnosed as rabies-positive by both the referent fluorescent-antibody test (FAT) and the rabies tissue-culture infection test (RTCIT) (OIE, 2008) received from the Lithuanian National Food and Veterinary Risk Assessment Institute (NFVRAI), were used in this study. Brain samples of rabid animals were collected from 30 of the 44 administrative regions of Lithuania. The isolates were stored at -70 °C until RNA extraction, and investigated in the 2006-2011 period, during oral rabies vaccination campaigns in Lithuania. Total RNA was extracted from infected brain samples using the TRIzol method (Invitrogen, Life Technologies, MD, USA) following the manufacturer's recommendations. After RNA extraction, all the RNA samples were dissolved in RNase-free distilled water (50 µL) and stored at -70 °C until used in RT-PCR.

Primer design and RT-PCR. The primer set for RT-PCR was designed using the Perl Primer (MARSHALL, 2004) primer design program, with positions relative to the PV (strain Pasteur vaccines) strain (GenBank Acc. No M13215, (TORDO et al., 1988) RVG3324F/M (for) 5'- CTCAGGCTCTCCTGTTTGTACC -3'; RVG3682R/M (rev) 5'- GGCCATCTTCCAGTTGTACG -3' for amplification of the expected size of the 358 nt (nucleotide) amplicon from the G gene (nt 3324-3682). RT-PCR was performed in principle as described earlier (SZANTO et al., 2008) with some modifications. 2 µL of RNA sample was mixed with 2.5 µL (50 pmol) each of primers with 13 µL water (nuclease free) in a PCR tube (20 µL), heated at 65 °C/5 min and rapidly cooled in ice water. 30 µL of master mix was added to each tube: water (nuclease free) 19.4 µL; 10×PCR buffer 5 µL; $MgCl_2$ 3 µL (1.5 mmol/L); each of dNTP's (10 mM) 1 µL; Ribolock (40 U) 0.5 µL (20 U); M-MLV (100U) 0.5 µL (50U) and Taq DNA polymerase (3U) 0.6 µL. The thermocycling format used one cycle of RT at 42 °C/5 min, followed by denaturation at 94 °C/3 min; 35 amplification cycles with denaturation at 94 °C/60s, annealing 55 °C/60s and extension at 72 °C/60 s; and final incubation at 72 °C 5 min. The modified attenuated SAD Bern strain (biological activity 1.8×10^6-1.8×10^8 TCID50/1.8 mL (Tissue Culture Infective Dose) RV samples was used for the RT-PCR positive and DEPC (Diethyl pyrocarbonate) water for the negative controls.

Agarose gel electrophoresis and PCR products purification. A 25 µL aliquot of amplified PCR product from each sample was separated onto 2 % agarose gel in 0.56 TBE buffer (0.045 mol/L Tris borate, 0.001 mol/L EDTA), stained with ethidium bromide (10 µL) and visualized under UV light. PCR products were purified from the agarose gel slice, using a Gene JET Gel Extraction Kit (Fermentas, Lithuania), according to the manufacturer's instruction. The purified DNA was stored at -20 °C until the DNA sequencing procedures.

DNA sequencing and phylogenetic analysis. Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, USA) according to the manufacturer's protocol. The following reaction mixtures were used: terminator ready reaction mix 8.0 µL, template 10 ng, primer 5.0 pmol and deionised water up to 20 µL. The following cycling conditions were used: initial denaturation 96 °C/1 min, followed by 25 cycles: 96 °C/10 sec, 50 °C/5 sec and 60 °C/4 min. Ethanol/EDTA precipitation method was used for extension product purification. An ABI Prism 310 Genetic Analyzer was used for sample electrophoresis and data analysis. The sequencing results were prepared in ABI format (chromatogram files) and analyzed by Chromas Lite (version 2.01, Technelysium, Australia), saved in the FASTA format and used for the next investigation. Multiple alignments were performed using ClustalW with default settings (LARKIN et al., 2007). Phylogenetic and molecular evolutionary analyses were conducted using MEGA v. 4 (TAMURA et al., 2007). The Neighbor-Joining method (NJ) tree was obtained using the Maximum Composite Likelihood algorithm (TAMURA et al., 2004), the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches. Bootstrap values above 70 % were considered as significant (HILLIS and BULL, 1993).

Results

The RT-PCR with the RVG3324F/M and RVG3682R/M primer set obtained positive results in 20 of tested brains samples. The samples were sequenced, using the partial region from the amino acid terminus of the G gene (358 nt). According to the objective of the present study, 20 Lithuanian RV G-sequences of raccoon dogs (n = 12) and red foxes (n = 8) were included in the phylogenetic study. 25 varieties of RV isolate G-sequences, published in the GenBank database (http://www.ncbi.nlm.nih.gov), were used for internal comparative sequence analysis (Table 1). Sequencing with the amplification primers yielded 380 bases of sequence on average, trimmed to a consensus 358 nt for comparative analysis. The sequence evolutionary relationships of the 20 Lithuanian rabies virus isolates (Fig. 1) and 25 rabies field viruses from various parts of Europe and Russia were identified. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

Table1. Rabies virus (RV) isolates used in glycoprotein partial phylogenetic analysis of Lithuanian raccoon dog and red fox rabies virus isolates. Lithuanian RV isolates presented in Bold (RF - red fox; RD - raccoon dog).

No	Country	Isolates No. and abbreviation	Reference / source	Species	Gen Bank accession No.
1	Estonia	9339EST	BOURHY et al., 1999	Raccoon dog	AF134335
2	Estonia	9142EST	BOURHY et al., 1999	Raccoon dog	AF134339
3	Estonia	RV437	HORTON et al., 2010	Raccoon dog	GU936879
4	Germany	9212ALL	BOURHY et al., 1999	Red fox	AF134337
5	Germany	RV313	HORTON et al., 2010	Red fox	GU936875
6	Germany	9202ALL	BOURHY et al., 1999	Red fox	AF134338
7	Hungary	9384HON	BOURHY et al.,, 1999	Red fox	AF134340
8	France	9445FRA	BOURHY et al., 1999	Red fox	AF134332
9	France	9223FRA	BOURHY et al., 1999	Red fox	AF134326
10	France	FRA1-FX	BADRANE and TORDO, 2001	Red fox	AF325461
11	Yugoslavia	86107YOU	BOURHY et al., 1999	Red fox	AF134345
12	Yugoslavia	8653YOU	BOURHY et al., 1999	Wolf	AF134341
13	Slovenia	664-02SVN	RIHTARIC et al., 2011	Red fox	HM852168
14	Slovenia	587-94SVN	RIHTARIC et al., 2011	Marten	HM852175
15	Slovenia	1209-08SVN	RIHTARIC et al., 2011	Badger	HM852183
16	Slovenia	1647-07SVN	RIHTARIC et al., 2011	Red fox	HM852184
17	Russia	rv262	KUZMIN et al., 2004	Red fox	AY353859
18	Russia	rv245	KUZMIN et al., 2004	Human	AY353877
19	Russia	rv299	KUZMIN et al., 2004	Red fox	AY353880
20	Russia	rv1596	KUZMIN et al., 2004	Red fox	AY353876
21	Russia	rv298	KUZMIN et al., 2004	Cow	AY353879
22	Kazakhstan	rv259	KUZMIN et al., 2004	Red fox	AY353892
23	Poland	POL1RD	BADRANE and TORDO, 2001	Raccoon dog	AF325464
24	Poland	POL2HM	BADRANE and TORDO, 2001	Human	AF325465
25	South Africa	DUVVSA81		Chiroptera	EU623443
					Isolation
26	Lithuania	**12143PV1RF**	This study	Red fox	2011
27	Lithuania	**5121PV1RF**	This study	Red fox	2011
28	Lithuania	**14639PV1RF**	This study	Red fox	2011
29	Lithuania	**13446PV1RD**	This study	Raccoon dog	2010
30	Lithuania	**15189PV1RD**	This study	Raccoon dog	2010
31	Lithuania	**26388V1RD**	This study	Raccoon dog	2010

No	Country	Isolates No. and abbreviation	Reference / source	Species	Gen Bank accession No.
32	Lithuania	**4063V1RD**	This study	Raccoon dog	2010
33	Lithuania	**15188PV1RD**	This study	Raccoon dog	2010
34	Lithuania	**13687PV1RD**	This study	Raccoon dog	2010
35	Lithuania	**1689PV1RF**	This study	Red fox	2010
36	Lithuania	**9930LTRD**	This study	Raccoon dog	2010
37	Lithuania	**5240LTRD**	This study	Raccoon dog	2010
38	Lithuania	**5437LTRF**	This study	Red fox	2010
39	Lithuania	**6155LTRF**	This study	Red fox	2010
40	Lithuania	**4655V1RD**	This study	Raccoon dog	2009
41	Lithuania	**3968PV1RD**	This study	Raccoon dog	2009
42	Lithuania	**18217PV1RD**	This study	Raccoon dog	2006
43	Lithuania	**12143V1RF**	This study	Red fox	2008
44	Lithuania	**5382PV1RD**	This study	Raccoon dog	2009
45	Lithuania	**9683PV1RF**	This study	Red fox	2009

The sequence analysis in the NJ tree was subdivided into two main genetic groups (I and II) with bootstrap support of 75 % (Fig. 1). The first group (bootstrap support 80 %) joined all 20 RV isolates from Lithuania and the RV isolates G-sequences from Estonia, Poland and Russia. The 18 RV isolates, including ten raccoon dog and eight red fox G-sequences originating from Lithuania were grouped together in a rather conservative subgroup 1 with bootstrap support of 75-93 %. All the Lithuanian G-sequences in subgroup 1 were associated (bootstrap support of 75 %) with sequences from Estonia (GU936879 437EST raccoon dog) and Russia (AY353876 rv1596RU red fox). The G-sequence of the raccoon dog RV isolate from Poland (POL1RD AF325464) was related (bootstrap support of 77 %) to the Lithuanian subgroup 1 and with the same RV isolates from Estonia and Russia. Two G-sequences of raccoon dog RV isolates from Lithuania (4655V1RD and 4063V1RD) were relatively divergent (subgroup 2) and directly associated with the sequence of raccoon dog RV isolate from Estonia (AF134339 9142EST). They were grouped together (bootstrap support of 79 %) with the isolates from Estonia (AF134335 9339EST), Russia (AY353877 rv245RU) and Poland (AF325465 POL2HM). The G-sequences of the red fox RV isolate from Slovenia (HM852184 1647-07SVN) was associated with the first and the second subgroups (bootstrap support of 70 %). The four G-sequences of RV isolates from Russia clustered together (bootstrap support of 90-96 %) in the subgroup 3. The conservative genetic group II contained the G-sequences of RV isolates from Slovenia, France and Germany (bootstrap support of 72-98 %). The Lithuanian partial G sequences of raccoon dog/red fox RV isolates were similar and their

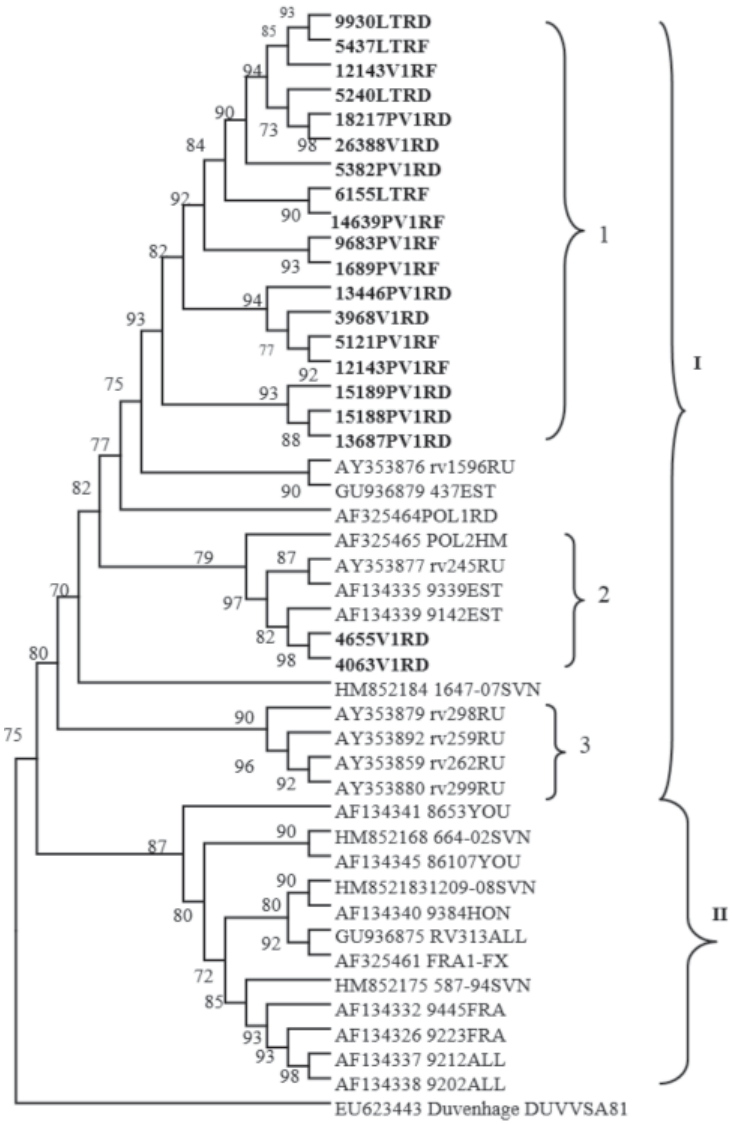

Fig. 1. The Neighbor-Joining (NJ) phylogenetic tree of rabies virus G-coding (358 bp) region showing the relationships among 45 isolates of lyssavirus. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) shown next to the branches. Duvenhage DUVVSA81 (EU623443) used as out-group. Lithuanian RV isolates G-sequences presented in Bold.

nt pair identity was 89.8-98.9 %. The G-sequences between Lithuanian raccoon dog and red fox RV isolates were more conservative (97.2 % of nt identity) than the sequences of RV isolates inside the raccoon dog group (95.1 %). Statistical pair identity between G sequences of Lithuanian RV isolates amounted to 95.3 %, however, two of the 20 analysed rabies virus G sequences (raccoon dogs) were relatively divergent: 4655V1RD (90.8 % nt identity) and 4063V1LTRD (91.2 % nt identity). The statistical average (nt homology) of G partial sequence (358 bp) pair comparison of Lithuania racoon dog/ red fox RV isolates and other 25 field isolates amounted to 86.8 %. G-sequences of RV field isolates from Russia (AY353876 rv1596RU) and Estonia (GU936879 437EST) were closely associated with most of the Lithuanian RV G-sequences (91.2 % nt identity). At the same time, the G-sequences of Lithuanian raccoon dog RV isolates (4655PV1LTRD and 4063PV1RD) were directly associated with the G-sequence of RV isolates from Estonia (AF134339 9142EST 94.5 % nt identity).

The statistical comparative evaluation of nucleotides and amino acids (nt/aa) of all the identified RV G-sequences showed that the average of nt/aa differences within the Lithuanian isolates was 17.2/ 5.8 respectively. The identical investigation between Lithuanian and the G-sequences of the other 25 RV isolates indicates 48.8/ 6.5 nt/aa differences.

Discussion

The European continent represents an ideal opportunity to undertake a RV molecular epidemiology study because several strains of rabies virus co-circulate within this region and infect a range of mammalian species. Analyses of these data revealed two distinct, but clearly related patterns: viruses from the same geographical area tend to group together, as do isolates taken from the same host species, although less strongly. The rabies virus NEE strain can be identified over a wide geographical area, including Poland, Estonia, Lithuania and Finland. The NEE group includes viruses isolated from both red foxes and raccoon dogs, showing that both species are effective reservoirs for this variant of rabies virus. The NEE group is found in the region where the population of raccoon dogs is large. The density of susceptible hosts, as well the close proximity of a donor species, are major ecological factors in the establishment of rabies virus in a new host species. Perhaps the NEE strain is preferentially adapted to this species (BOURHY et al., 1999). The investigation of the RV G-region of Lithuanian field isolates showed that two Lithuanian raccoon dog rabies virus isolate G-sequences were rather divergent and were related to isolates from Estonia (92.5 % nt identity), Russia (91.0 % nt identity) and Poland (89.3 % nt identity). The G-sequence of the RV isolate from Slovenia (HM852184 1647-07SVN) was independently associated (88.9 % mean of nt identity) with two subgroups of RV isolates including all of the Lithuania RV G-sequences. Recently investigated Slovenian RV field isolate (RIHTARIC et al., 2011) partial G gene sequences (672 nt) showed 94.6-

100 % nucleotide homology to each other and formed two genetic groups of closely related strains, belonging to the Western Europe (WE) and Eastern Europe (EE) groups according to BOURHY et al. (1999). No isolates within the Balkan panel were found to be part of the Northeast European (NEE) sublineage (McELHINNEY et al., 2011). A recent phylogenetic study of Romanian RV (TURCITU et al., 2010) reported the presence of a number of viruses widely dispersed throughout Romania, which aligned closely with the NEE sublineage. The occurrence of the NEE variant so far south may have resulted from a Westward incursion from Ukraine or Moldova. However, a NEE RV isolate (94250SLK, U43007) was previously identified in Slovakia (BOURHY et al., 1999) and therefore it is plausible that the variant moved into Romania from the North. The rabies virus NEE variant has been particularly associated with raccoon dogs (*Nyctereutes procyonoides*) in Northwest Russia and North-East Europe (KUZMIN et al., 2004). Raccoon dogs were first recorded in Serbia in 1978 (CIROVIC and MILENKOVIC, 1999) and have been associated with a single case of rabies in 2002 (Rabies Bulletin Europe); however, the virus isolate was not available for study. Insufficient data are available for Hungary and Ukraine to make any firm conclusions about the direction in which the NEE variant dispersed (McELHINNEY et al., 2011).

The five RV isolates from Russia (KUZMIN et al., 2004) included in this study were genetically more related (88.1 % average nt identity) to the G-sequences of raccoon dog and red fox RV isolates from Lithuania, than to the "G1 classical member" RV field sample G-sequences (84.2 % of average nt identity) from Western or Central Europe. The G-sequences of red fox RV field isolates from the Russian Pskov region (AY353876 rv1596RU) and of raccoon dogs from Estonia (GU936879 437EST) were closely associated (92.8 % mean of nt identity) with 18 of 20 Lithuanian raccoon dog and red fox RV G-sequences respectively. The earlier identified Russian group E (KUZMIN et al., 2004) consists of RV isolates from the Baltic region (Estonia and Finland) and the North-Western part of Russia. Raccoon dogs were frequently involved in RV circulation in this region. The raccoon dog was suggested as the possible intermediary host in the switch of rabies virus from dogs and wolves to foxes. The raccoon dog population density in the region is estimated at 3-20 times lower than that of the red fox population. It appears that the red fox is more affected in the Russian part of this area. However, raccoon dog rabies cases predominated during the rabies outbreak in Finland in 1988-89. Phylogenetically, this group is related to other European lineages, and there was no reason to consider it as specific to the raccoon dog. Previous genetic and antigenic studies have confirmed that the isolates responsible for the outbreak in Finland (1988-89) were related but not identical to the viruses isolated in the Baltic region. This suggests that rabies had perhaps gradually moved from the Russian-Estonian territories after prolonged circulation among local hosts before being detected (JOHNSON and FOOKS, 2005). The RV North-European group includes viruses from Finland and Estonia, plus the Pskov, Bryansk and Leningrad

(Saint Petersburg) regions. It is obvious that viruses circulating in Estonia and the Pskov region of Russia were very similar. However, surveillance data suggested that some RV from the North-European group were positive for the P-41mAb indicating the Arctic antigenic group. The P-41-reactive viruses were identified in raccoon dogs (*Nyctereutes procyonoides*) and red foxes (*Vulpes vulpes*) in the Baltic region (METLIN et al., 2004). Despite this, the NEE virus variants isolated from raccoon dogs in Baltic countries and North-Western Russia were not closely related to the Arctic-like variant found in the natural territories of raccoon dogs (McELHINNEY et al., 2008). Nucleotide sequencing has facilitated the more precise differentiation and subsequent phylogenetic placement of viruses isolated during the last few decades.

Conclusions

Rabies virus glycoprotein partial 358 nt G-sequence comparative and phylogenetic investigations in 20 Lithuanian raccoon dog and red fox rabies virus field isolates indicate that 18 of 20 the samples were closely related and showed high identity with rabies virus isolate G-sequences from Estonia, Russia, and Poland. Two Lithuanian raccoon dog rabies virus isolates were comparatively divergent. They were more closely associated with the G-sequences of rabies virus isolates from Estonia and Russia, rather than with sequences of RV isolates from Western and Central Europe.

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Received: 5 November 2014
Accepted: 9 June 2015

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SAŽETAK

Svrha ovog rada bila je odrediti molekularnu epidemiologiju djelomičnih sekvencija gena G virusa bjesnoće u terenskim uzorcima pozitivnima na bjesnoću iz različitih područja Litve te ih filogenetski usporediti s različitim izolatima virusa bjesnoće iz divljih mesojeda na području Baltičkog jezera, Europe i Rusije. Pretraženo je 20 uzoraka tkiva mozga bijesnih životinja prikupljenih u razdoblju od 2006. do 2011. Višestruka poravnanja sekvencija od 358 nukleotida gena G (nt 3324-3682) učinjena su pomoću programa ClustalW. Filogenetska i molekularno evolucijska analiza provedena je metodom združivanja genetski najsličnijih sojeva (engl. Neighbour-joining method) u MEGA verziji 4. Filogenetska istraživanja glikoproteina virusa naznačuju da su G-sekvencije izolata litavskoga virusa bjesnoće usko srodne i pokazuju 89,8-98,9 % nt identičnosti. G-sekvencije izolata virusa bjesnoće iz litavskog rakunskog psa i crvenih lisica bile su sačuvanije (97,2 % nt identičnosti) nego izolati virusa bjesnoće izdvojeni iz rakunskog psa (95,1 % nt identičnosti). G-sekvencije dvaju izolata iz litavskih rakunskih pasa bile su prilično divergentne (90,8 i 91,2 % nt identičnosti) i usko srodne s G-sekvencijama izolata virusa bjesnoće iz Estonije (92,5 % nt identičnosti), Rusije (91,0 %) i Poljske (89,3 %). Komparativno istraživanje G-sekvencije litavskih izolata virusa bjesnoće i različitih izolata iz Njemačke, Slovenije i Francuske pokazalo je 84,2 % nt identičnosti, dok je identičnost između G-sekvencije između litavskih izolata virusa bjesnoće i ruskih izolata bila na razini 88,1 % nt identičnosti.

Ključne riječi: bjesnoća, rakunski pas, crvena lisica, Baltik, molekularna epidemiologija