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Short communication

Vaccine-associated rabies in red fox, Hungary

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ABSTRACT

Rabies vaccine strain was isolated from a red fox (*Vulpes vulpes*) with signs of neurological disorder during an oral vaccination campaign in 2015, Hungary. The whole genome sequence of the isolated strain shared >99.9% nucleotide sequence identity to the whole genomes of vaccines strains recently used in Hungarian oral vaccination campaigns. The neuroinvasive potential of rabies vaccines that leads to development of clinical manifestations is rarely seen among wild animals; however, the observed residual pathogenicity needs awareness of field experts and requires close monitoring of rabies cases in areas where elimination programs are implemented.

Since the 1980's oral immunization of wildlife by vaccine baits has helped numerous European countries to control and eliminate sylvatic rabies [1]. In most of the countries, live attenuated vaccine strains have been used [2–6]. These vaccines have originated from the Evelyn Rokitnicki Abelseth (ERA) virus strain derived from the original Street Alabama Dufferin (SAD) virus that was isolated from a rabid dog [7]. Oral vaccination campaigns (ORV) in Hungary starting from 1992 were performed using different SAD-based vaccine baits (SAD B19 in Fuchsoral, IDT Biologika GmbH; SAD P5/88 in Rabifox, IDT Biologika GmbH; SAG 1 in SAG1 in Oral Fox Vaccine, Virbac) [8]. The SAD Bern (Lysvulpen, Bioveta) vaccines have been in use since 2008 [1,9].

Residual pathogenicity of live rabies vaccines currently used in wildlife has been observed for SAD P5/88 [2], SAD B19 [2,10], SAD Bern [11,12], and ERA [13], therefore, rabies viruses isolated from animals in a vaccination area needs to be characterized to identify possible vaccine-induced rabies cases [13–15].

In a region of south-eastern Hungary (Csabaszabadi, Békés county) where vaccination campaigns are routinely carried out, a one and a half years old red fox (*Vulpes vulpes*) with altered behaviour was observed in November 2015. The fox showed unsteady behaviour and moved wobblingly. After shooting the apparently ill animal, the carcass was transferred to the Veterinary Diagnostic Department of the National Food Chain Safety Office. Recommended as well as traditional techniques were used for differential diagnosis of rabies as detailed elsewhere [14,16]. In brief, the methods we utilized included immunofluorescence antibody test (FAT) performed on smears of spinal bulb and cortex and mouse inoculation test (MIT); FAT gave positive results whereas in MIT assay 7 days post infection dyspnoea, ataxia and exsiccosis were observed in specific-pathogen-free NMRI mice. A total of 6 mice were inoculated via intracranial route and were observed up to 28 days post infection. All 6 mice showed clinical signs between 9 and 12 days post infection and were humanely euthanized at the end of observation. Histopathological examination of brain slices showed cellular aggregations consisting of glial cells around medulla oblongata (i.e. Babes nodules; data not shown). Rabies virus (RABV) isolation was performed on N2a cells. The presence of RABV RNA in the homogenized brain specimens of fox and mice was demonstrated by reverse-transcription PCR and direct sequencing of short genomic sequences encoding the nucleoprotein (N gene) and the glycoprotein (G gene) [17–20]. A newly developed DIVA-TaqMan RT-PCR assay to differentiate between vaccine strain and wild-type RABV strains circulating in Hungary was also utilized (see Supplementary file). ELISA test (Platelia™ RabiesII Kit, Bio-Rad) showed greater than 0.5 equivalent units per ml serum IgG titer of the shot red fox specimen [21]. The routine method for age determination in foxes was counting the layers of cementum in sections of canine teeth under 40× magnification [22,23]. In addition, tetracycline marker (TCM) examination of the canine teeth indicated that the animal consumed bait during a preceding immunization campaign (data not shown) [24].

After sequencing of the partial N and G genes we observed that the Csabaszabadi RABV sample (Csabaszabadi denotes the nearest settlement to the location were the rabid red fox was identified) clustered with the Lysvulpen vaccine strain and not wild type RABV se-

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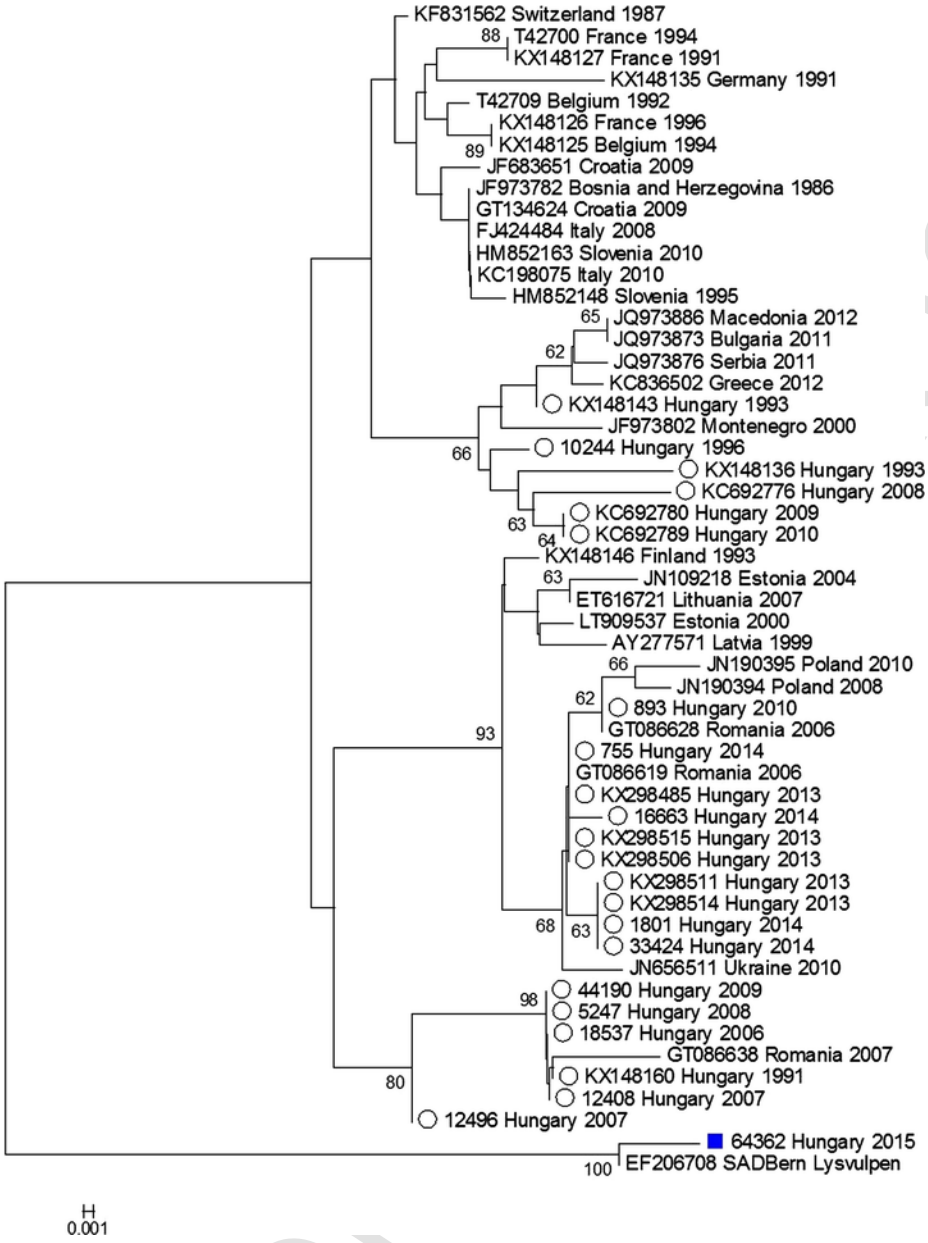

Fig. 1. Nucleoprotein gene based phylogenetic tree generated with Kimura 2-parameter substitution model with gamma distribution and maximum likelihood statistical method using 1000 bootstraps. The Hungarian Csabaszabadi sample (blue square) cluster with the SADBern Lysvulpen sequence and not with wild-type RABV strains detected over the past decade in Hungary (empty circles) or neighboring countries. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quences identified over the past two decades in Hungary (Fig. 1). This preliminary analysis also demonstrated sequence divergence between the reference Lysvulpen vaccine sequence and the Csabaszabadi RABV. Therefore, we decided to perform full genome sequencing of the isolated RABV strain and five batches of the Lysvulpen vaccine (0519, 1819, 6321, 0922, 0622). The sequenced vaccine virus strains were manufactured between April 2012 and July 2015. Among the batches produced during 2015, #0622 and #0922 were used in the autumn campaign of ORV in Hungary. Regarding the Csabaszabadi RABV strain we determined the genome derived from cell culture isolates (after passage #2) as well as the genome of the re-isolated strain from mouse brain. For each RABV isolate we amplified partially overlapping genomic regions using six primer pairs (Table S1). Then, amplicons belonging to the same isolate were mixed and subjected to whole genome sequencing by using published protocols of semiconductor sequencing on an Ion Torrent PGM [25,26]. As a result, 11,874 base pair long genomic sequences were obtained (GenBank accession numbers, MK111075–MK111080). When analyzing the genomic sequences, >99.9% nucleotide sequence identities were found among the Csabaszabadi RABV isolate and the Lysvulpen vaccine strains whose sequences were determined in this study. Whole-genome sequence based phylogenetic tree was generated to compare the Hungarian origin sequences to reference vaccine sequences (Fig. 2). Phylogenetic analysis of whole genome sequences showed that Lysvulpen vaccine batches used during most recent Hungarian ORV campaigns and the fox origin RABV vaccine

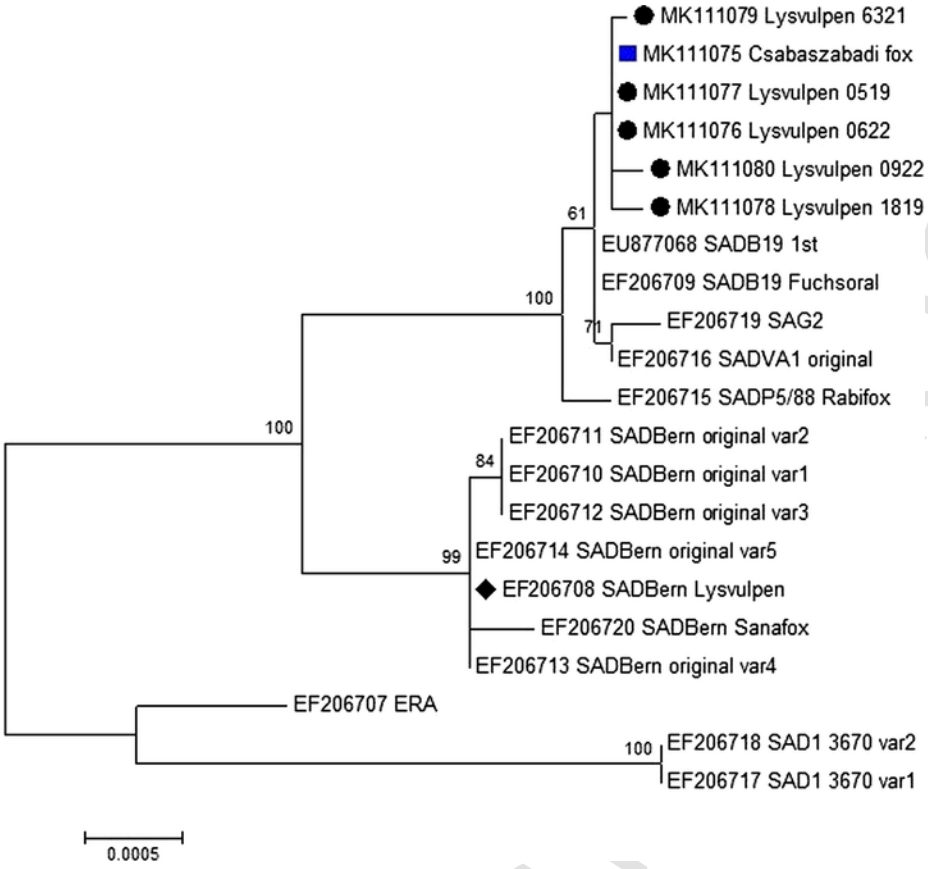

Fig. 2. Whole genome based phylogenetic tree generated with Tamura-3 substitution model and maximum likelihood statistical method using 1000 bootstraps. The Hungarian Csabaszabadi sample (blue square) and the vaccine strains used in Hungary (black circles) cluster with SAD B19, SAG2, SAD VA1, and SAD P5/88 vaccines. In contrast, the original Lysvulpen vaccine (black diamond) clusters with SAD Bern vaccines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specimen are more closely related to the SAD Bern based vaccine viruses (≥99.9% nt similarity, ≤6 nt substitutions) than to the original Lysvulpen vaccine strain (GenBank accession number, EF206708; ∼99.7% nt similarity, 27–30 nt substitutions). In previous studies similar findings were reported about the genetic relationships among SAD Bern strain and vaccine derived field isolates and deeper analyses of available sequence data suggested that some of the commercial vaccines exist as a mixture of variants [27–29]. This observation may explain why a SAD B19-like vaccine strain was found in several batches of Lysvulpen. Moreover, sequencing of different batches of Lysvulpen vaccines may help fully resolve the history and population structure of currently used rabies vaccines across Europe.

In summary, oral rabies vaccines containing live replication-competent attenuated virus strains may cause vaccine-induced rabies [14]. Examples include the attenuated SAD Bern, SAD B19, and SAD P5/88 vaccines which can be pathogenic for some rodent species during cerebral, muscular and oral inoculation route [2,30–33]. In addition, sporadic SAD Bern or SAD B19 vaccine associated field cases in wildlife were reported from Austria, Germany, Latvia, Romania, Slovenia, and Switzerland following oral rabies vaccination campaigns [2,11,12,15,29]. Thus, despite the good safety profile, authorities and field experts need to be aware that oral rabies vaccines may possess some residual pathogenicity and represent potential risk of vaccine-associated rabies. In this paper we report a vaccine-induced rabies case in Hungary, the first case identified since the implementation of ORV based rabies elimination program launched in our country. The high genome sequence similarity among the Csabaszabadi red fox RABV isolate and the Lysvulpen vaccine strains used in the ORV programs together with the positive TCM test suggested that this isolated Hungarian case was caused by infection from the vaccine strain most likely during the campaign in autumn 2015. The affected animal was a bit older than expected given that vaccine-associated rabies cases are detected predominantly in juvenile animals [2,10,29]. Unfortunately, we did not have the opportunity to examine the salivary gland tissues of the shot red fox; in this regard speculations remain whether red foxes could acquire vaccine virus from the bite of an animal which consumed vaccine baits and subsequently continue to transmit this virus to other hosts [10]. Nonetheless, literature data are not conclusive concerning the vaccine-strain transmission potential of red foxes as it has been demonstrated that salivary glands may test negative by PCR in experimentally infected animals [2]. Apparently further investigations are required to understand virus and host genetic factors that contribute to the development of neurovirulence and transmissibility of vaccine-strains via salivary glands. Collectively, even though published reports on residual pathogenicity of SAD Bern vaccines associated with ORV in wildlife are scarce, this additional case from Hungary emphasizes the need for extended screening to detect such cases in the field.

1. Contribution

BF, ÁH, and KB designed the study. BF, SM, SK, and ÁH collected and validated the data. BF, SM, SK, ÁH, and KB analyzed the data. All authors participated in writing the manuscript and have ap-

proved the final article. All authors attest they meet the ICMJE criteria for authorship.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.014.

References

[1] J. Vitasek, A review of rabies elimination in Europe, Vet Med (Czech) 49 (2004) 171–185.
[2] T. Müller, H.J. Bätza, A. Beckert, C. Bunzenthal, J.H. Cox, C.M. Freuling, et al., Analysis of vaccine-virus-associated rabies cases in red foxes (Vulpes vulpes) after oral rabies vaccination campaigns in Germany and Austria, Arch Virol 154 (2009) 1081–1091, https://doi.org/10.1007/s00705-009-0408-7.
[3] T. Müller, H.J. Bätza, C. Freuling, A. Kliemt, J. Kliemt, R. Heuser, et al., Elimination of terrestrial rabies in Germany using oral vaccination of foxes, Berl Munch Tierarztl Wochenschr 125 (2012) 178–190, https://doi.org/10.2316/0005-9366-125-117.
[4] C.M. Freuling, K. Hampson, T. Selhorst, R. Schröder, F.X. Meslin, T.C. Mettenleiter, et al., The elimination of fox rabies from Europe: determinants of success and lessons for the future, Philos Trans R Soc Lond B Biol Sci 368 (2013) 20120142, https://doi.org/10.1098/rstb.2012.0142.
[5] P. Mähl, F. Cliquet, A.L. Guiot, E. Niin, E. Fournials, N. Saint-Jean, et al., Twenty year experience of the oral rabies vaccine SAG2 in wildlife: a global review, Vet Res 45 (2014) 77, https://doi.org/10.1186/s13567-014-0077-8.
[6] T.F. Müller, R. Schröder, P. Wysocki, T.C. Mettenleiter, C.M. Freuling, Spatio-temporal use of oral rabies vaccines in fox rabies elimination programmes in Europe, PLoS Negl Trop Dis 9 (2015) e0003953, https://doi.org/10.1371/journal.pntd.0003953.
[7] P. Fenje, Propagation of rabies virus in cultures of hamster kidney cells, Can J Microbiol 6 (1960) 479–484.
[8] King AA, Fooks AR, Aubert M, Wandeler AI. Historical perspective of rabies in Europe and the Mediterranean Basin, France, OIE; 2004.
[9] European Commission. Survey Program for Rabies Hungary. Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses; 2012. https://ec.europa.eu/food/sites/food/files/safety/docs/cff_animal_vet-progs_2012_dec-2011-807-ec_rabies_hun.pdf.
[10] P. Hostnik, E. Picard-Meyer, D. Rihtarič, I. Toplak, F. Cliquet, Vaccine-induced rabies in a red fox (Vulpes vulpes): isolation of vaccine virus in brain tissue and salivary glands, J Wildl Dis 50 (2014) 397–401, https://doi.org/10.7589/2013-07-183.
[11] E. Robardet, E. Picard-Meyer, M. Dobroštana, I. Jaceviciene, K. Mähar, Z. Muižniece, et al., Rabies in the Baltic States: decoding a process of control and elimination, PLoS Negl Trop Dis 10 (2016)e0004432https://doi.org/10.1371/journal.pntd.0004432.
[12] V. Vuta, E. Picard-Meyer, E. Robardet, G. Barboi, R. Motiu, F. Barbuceanu, et al., Vaccine-induced rabies case in a cow (Bos taurus): Molecular characterisation of vaccine strain in brain tissue, Vaccine 34 (2016) 5021–5025, https://doi.org/10.1016/j.vaccine.2016.08.013.
[13] C. Fehlner-Gardiner, S. Nadin-Davis, J. Armstrong, F. Muldoon, P. Bachmann, A. Wandeler, ERA vaccine-derived cases of rabies in wildlife and domestic animals in Ontario, Canada, 1989–2004, J Wildl Dis 44 (2008) 71–85, https://doi.org/10.7589/0090-3558-44.1.71.
[14] WHO. Expert Consultation on Rabies, second report; 2013. http://apps.who.int/iris/bitstream/handle/10665/85346/9789240690943_eng.pdf?sequence=1&isAllowed=y.
[15] A.I. Wandeler, S. Capt, A. Kappeler, R. Hauser, Oral immunization of wildlife against rabies: concept and first field experiments, Rev Infect Dis 10 (Suppl 4) (1988) S649–S653.
[16] Á. Hornyák, T. Juhász, B. Forró, S. Kecskeméti, K. Bányai, Resurgence of rabies in Hungary during 2013–2014: An attempt to track the origin of identified strains, Transbound Emerg Dis 65 (2018) e14–e24, https://doi.org/10.1111/tbed.12658.
[17] G. Sato, T. Itou, Y. Shoji, Y. Miura, T. Mikami, M. Ito, et al., Genetic and phylogenetic analysis of glycoprotein of rabies virus isolated from several species in Brazil, J Vet Med Sci 66 (2004) 747–753.
[18] E. Picard-Meyer, V. Bruyere, J. Barrat, E. Tissot, M.J. Barrat, F. Cliquet, Development of a hemi-nested RT-PCR method for the specific determination of European Bat Lyssavirus 1: comparison with other rabies diagnostic methods, Vaccine 22 (2004) 1921–1929, https://doi.org/10.1016/j.vaccine.2003.11.015.
[19] P.R. Wakeley, N. Johnson, L.M. McElhinney, D. Marston, J. Sawyer, A.R. Fooks, Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of lyssavirus genotypes 1, 5, and 6, J Clin Microbiol 43 (2005) 2786–2792, https://doi.org/10.1128/JCM.43.6.2786-2792.2005.
[20] A.R. Fooks, N. Johnson, C.M. Freuling, P.R. Wakeley, A.C. Banyard, L.M. McElhinney, et al., Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century, PLoS Negl Trop Dis 3 (2009) e530, https://doi.org/10.1371/journal.pntd.0000530.
[21] M. Feyssaguet, L. Dacheux, L. Audry, A. Compoint, J.L. Morize, I. Blanchard, et al., Multicenter comparative study of a new ELISA, PLATELIA RABIES II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people, Vaccine 25 (2007) 2244–2251, https://doi.org/10.1016/j.vaccine.2006.12.012.
[22] S. Harris, Age determination in the red fox (Vulpes vulpes)–an evaluation of technique efficiency as applied to a sample of suburban foxes, J Zool 184 (1978) 91–117.
[23] J. Roulichova, M. Andera, Simple method of age determination in red fox, Vulpes vulpes, Folia Zool 2007 (56) (2007) 440–444.
[24] D.H. Johnston, D.R. Voigt, A baiting system for the oral rabies vaccination of wild foxes and skunks, Comp Immunol Microbiol Infect Dis 5 (1982) 185–186.
[25] Z.G. Homonnay, E.W. Kovács, K. Bányai, M. Albert, E. Fehér, T. Mató, et al., Tembusu-like flavivirus (Perak virus) as the cause of neurological disease outbreaks in young Pekin ducks, Avian Pathol 43 (2014) 552–560, https://doi.org/10.1080/03079457.2014.973832.
[26] E. Fehér, S. Marton, Á.G. Tóth, K. Ursu, K. Wernike, M. Beer, et al., Sequence analysis of Schmallenberg virus genomes detected in Hungary, Acta Microbiol Immunol Hung 64 (2017) 373–384, https://doi.org/10.1556/030.64.2017.038.
[27] F. Cliquet, E. Robardet, E. Picard-Meyer, Genetic strain modification of a live rabies virus vaccine widely used in Europe for wildlife oral vaccination, Antiviral Res 100 (2013) 84–89, https://doi.org/10.1016/j.antiviral.2013.07.012.
[28] D. Höper, C.M. Freuling, T. Müller, D. Hanke, V. von Messling, K. Duchow, et al., High definition viral vaccine strain identity and stability testing using full-genome population data–The next generation of vaccine quality control, Vaccine 33 (2015) 5829–5837, https://doi.org/10.1016/j.vaccine.2015.08.091.
[29] Pfaff F, Müller T, Freuling CM, Fehlner-Gardiner C, Nadin-Davis S, Robardet E, et al. In-depth genome analyses of viruses from vaccine-derived rabies cases and corresponding live-attenuated oral rabies vaccines. Vaccine [Epub ahead of print] doi: https://doi.org/10.1016/j.vaccine.2018.01.083.
[30] M. Artois, C. Guittré, I. Thomas, H. Leblois, B. Brochier, J. Barrat, Potential pathogenicity for rodents of vaccines intended for oral vaccination against rabies: a comparison, Vaccine 10 (1992) 524–528.
[31] J. Bingham, C.M. Foggin, H. Gerber, F.W. Hill, A. Kappeler, A.A. King, et al., Pathogenicity of SAD rabies vaccine given orally in chacma baboons (Papio ursinus), Vet Rec 131 (1992) 55–56.
[32] European Commission. The oral vaccination of foxes against rabies. Report of the Scientific Committeeon Animal Health and Animal Welfare; 2002. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out80_en.pdf.
[33] A. Vos, A. Neubert, O. Aylan, P. Schuster, E. Pommerening, T. Müller, et al., An update on safety studies of SAD B19 rabies virus vaccine in target and non-target species, Epidemiol Infect 123 (1999) 165–175.