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LETTER TO THE EDITOR

The phylogenetic analysis of a novel genetic subtype of caprine arthritis encephalitis virus (CAEV) in the Czech Republic

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Lentiviruses of sheep and goats, referred to as small ruminant lentiviruses (SRLV), cause severe diseases that affect health, productivity and international animal trade, causing significant economic losses. Infections with maedi visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are characterized by long incubation period and life-long persistent infection of sheep and goats (*1,2*). Both infections are subclinical, and viruses can be spread and propagated without any clinical symptoms for months or years and can lead to death of infected animals (*3*).

The SRLV screening and detection in flocks of sheep and goat were done over the last three years in the Czech Republic (*4*). Considering the current economic impact and animal health status of the SRLV infection, both serological and molecular-based methods have been used to detect infected animals and to evaluate the distribution and prevalence of MVV and CAEV infections among the herds in the Czech Republic. In this study we analyze genotypes of small ruminant lentiviruses in an imported goat flock using the molecular approach.

All blood samples were collected from adult Alpine goat flock imported from France (dept. Alpes-Maritimes, France) and located on a small family farm in North Bohemia (Jablonné v Podještědí, N50° 45' 55'', E14° 45'38'', Czech Republic). A volume of 5 ml of blood was drawn by venepuncture and collected into tubes with ethylenediaminetetraacetic acid (K_3EDTA). A buffy coat preparation and a white blood cell isolation was performed according to the previously described protocol (*4*). Genomic DNA was extracted from the white blood cells using MagCore Genomic DNA Whole Blood Kit (RBC Bioscience) following the manufacturer's instructions. The extracted DNA was stored at -20°C until processed.

The proviral DNA was amplified by PCR according to the procedure described previously using two sets of *gag* and *pol* PCR primers (*5*). The resulting 833 bp fragments were gel-purified using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and cloned into pCR2.1-TOPO vector (Invitrogene). Recombinant plasmid DNA was sequenced by Macrogen Co. Ltd. (South Korea) using universal M13 sequencing primers.

The output sequence data were processed in the Geneious 8.1.8 (https://www.geneious.com, *6*) and processed sequences were compared via the BLAST algorithm with sequences in the NCBI database. Multiple alignment of analyzed sequences together with reference SRLV sequences was accomplished with MUSCLE (*7*). Phylogenetic analyses were performed using the Neighbour Joining (NJ) method. The statistical confidence of the topologies was assessed using 1000 bootstrap replicates.

Altogether 35 animals from one goat flock were analyzed. Based on the analysis of the *TMEM154* gene, all goats belong to the E35 haplotype group with a higher risk of SRLV infection (*8*). All analyzed animals were serologically SRLV-positive and using PCR reaction we successfully

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Abbreviations: SRLV = small ruminant lentiviruses; CAEV = caprine arthritis encephalitis virus; MVV = maedi visna virus

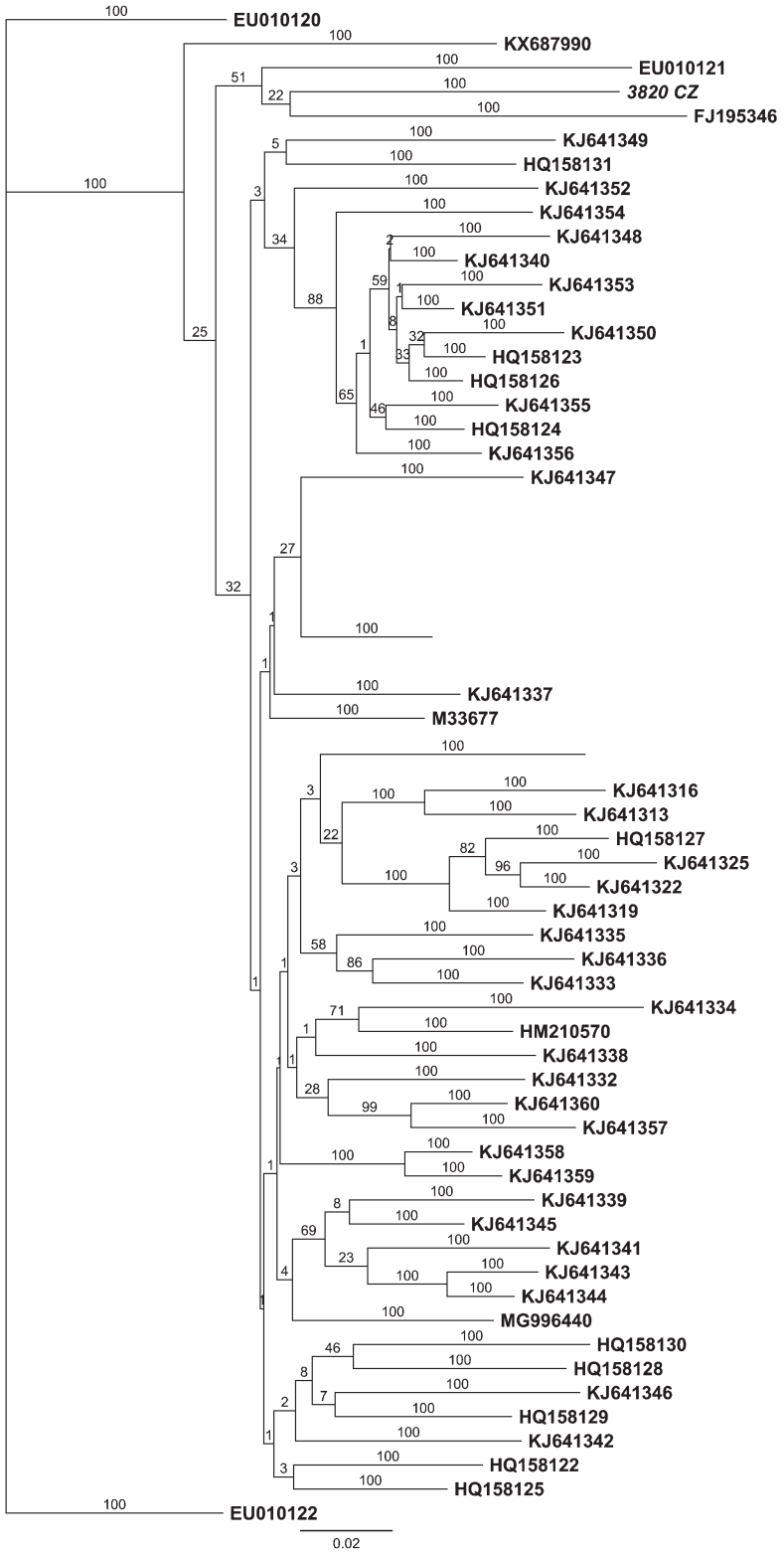

Fig. 1

Phylogenetic relationships inferred from 3820 CZ strain, comparison in 0.8 kb *gag-pol* amplicon

3820 CZ strain from goats were analyzed together with GenBank sequences denoted with their accession numbers. Phylogenetic tree was constructed from the nucleic acid alignments using the F84 Neighbor Joining method of the program Geneious 8.1.8 software using 1000 bootstrap replicates.

amplified 833 bp long fragment of the *gag-pol* CAEV gene. The sequence analysis gave the same results, so all animals are infected by the same viral genotype. The edited sequence is marked as 3820 CZ and was compared to sequences in NCBI GenBank. Isolates from the Czech Republic differ from type CAEV isolate (GenBank M33677) but are still clustered into CAEV group.

The topology of unrooted phylogenetic tree based on *gag-pol* sequences from the type samples (A1, A2, A3, B1 and B2 genotypes) and constructed using the distance-based NJ model indicated that 3820 CZ belongs to the genetic subtype B1 (figure is not shown). The sequences corresponding to different SRLV strains were retrieved from NCBI GenBank database according to the possible origin of infection and the viral strain-determined typology (*7,9*).

Our SRLV isolates from the goat flock compared to BLAST search results share a nucleotide sequence identity ranging from 85 % up to 92.5 % and are clustered with genotype B1. Phylogenetic analysis of the sequenced 833 bp fragment of proviral DNA with available NCBI sequences showed clustering of 3820 CZ sequence into the European B1 groups of SRLV (Fig. 1). This group belongs to the Italian genotype from goats and Spanish genotypes isolated from sheep, both clustered among viruses related to the CAEV isolates (*7, 10*). Although sequence 3820 CZ is clustered into B1 group, this particular genotype differs from the sequenced genotypes, thus we suggest that a new genotype of goat retrovirus was isolated in the Czech Republic for the first time. This is the first finding of B1 genotype of SRLV in the small ruminant flocks in the Czech Republic (see SRLV genotypes distribution described in *4*). Since there is often a cross infection of lentivirus genotypes between sheep and goats, it can be assumed that a new strain will be spread among flocks of small ruminants in the Czech Republic in the future (*11, 12, 13*).

Although we described a new genotype of SLRV in goats in the Czech Republic, a further characterization of long sequences and studies of recombination would be useful to better understand the position of CR isolates in small ruminant populations. Our finding sheds the light on the distribution of genotypes in the Czech Republic. Further experiments are required to accomplish more extensive surveys of the distribution of SRLV genotypes in the Czech Republic. More studies could also clarify recombinant variants of SRLV in goat and sheep flocks in the Czech Republic.

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