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7 **Genetic characterization of maedi-visna virus (MVV) detected in Finland**

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## 1 Abstract

2 The aim of the study was to characterize the small-ruminant lentiviruses (SRLVs) detected in Finland by  
3 defining their phylogenetic relationships and by studying the evolution of the virus based on a well-known  
4 epidemiology. The study material comprised lung tissue samples of 20 sheep from five different farms, a cell-  
5 cultured virus from one of the original sheep lung samples, and a blood sample of a goat. The sheep were  
6 identified as positive during seroepidemiologic screenings in 1994-1996 and the goat in 2001. Initial  
7 classification of a 251 nt nucleotide sequence within *gag* gene amplified from the uncultured samples as well  
8 as from the cell-cultured virus showed that the SRLVs were genetically close and that they were more closely  
9 related to the prototype ovine maedi-visna viruses (MVVs) than to the caprine arthritis-encephalitis virus  
10 (CAEV). The lentivirus detected from the goat aligned within the cluster of the Finnish ovine viruses,  
11 demonstrating a natural sheep-to-goat transmission. Further phylogenetic analysis of the proviral *gag*, *pol* and  
12 *env* sequences confirmed the initial classification and showed that they constituted a new subtype within the  
13 diverse MVV group. The sequence analyses also showed that the virus had remained genetically relatively  
14 stable, in spite of the time given for virus evolution, an estimated 20 years, and in spite of the virus crossing  
15 the host species barrier.

16 *Keywords:* Small-ruminant lentivirus; MVV; Sheep; Goat; Phylogenetic relationships

17

## 18 1. Introduction

19 Maedi-visna virus (MVV) and caprine arthritis-encephalitis virus (CAEV) are small-ruminant lentiviruses  
20 (SRLVs) that cause persistent inflammatory infections in sheep and goats (reviewed by Pépin et al., 1998). The  
21 phylogenetic relationships of the SRLVs were first defined with full-length genome sequences of the MVV  
22 prototype strains (Sonigo et al., 1985; Querat et al., 1990; Sargan et al., 1991), and the CAEV type strain  
23 (Saltarelli et al., 1990). These sequences indicated, along with the first partial genomic data that MVV of sheep  
24 and CAEV of goat are related but distinct viruses. Subsequent studies based on partial regions within *gag*, *pol*  
25 and *env* genes showed, however, that viruses of ovine and caprine origin are interspersed in the phylogenetic  
26 tree irrespective of the host, suggesting that interspecies infections had occurred between the host animals  
27 (Leroux et al., 1997; Zanoni, 1998; Rolland et al., 2002). According to a recently proposed nomenclature based

1 on 1.8 kb *gag-pol* and 1.2 kb *pol* sequences, the SRLVs are classified into four equidistantly related groups,  
2 A-D (Shah et al., 2004a). Group A can be further divided into at least seven subtypes, A1 to A7, where the  
3 subtype A1 is identified by the genetically and geographically heterogeneous MVVs and group B refers to the  
4 CAEV type and comprises only two distinct subtypes, B1 and B2. Groups C and D are represented by few  
5 isolates or recognized only by *pol* sequence (Shah et al., 2004a). Recently, direct evidence for natural sheep-  
6 to-goat and goat-to-sheep transmissions of particular subtypes (A4 and B1) of the virus has been shown (Shah  
7 et al., 2004b; Pisoni et al., 2005).

8 In Finland, the presence of a SRLV infection was first revealed among sheep during a serological survey  
9 conducted in 1994, and it had spread into thirteen sheep farms until detected in the survey (Sihvonen et al.,  
10 1999, 2000). Maedi-visna has a notifiable disease status in Finland which provides for official restrictive  
11 measures to which all infected herds are subject. In 1995, a voluntary MVV/CAEV control programme for both  
12 sheep and goats was initiated to extend the official control efforts, and since 2001 the programme has been  
13 compulsory for all flocks of more than 20 ewes and/or she-goats. In 2001, a goat from a newly established goat  
14 farm was detected as seropositive.

15 This study was started with an aim to genetically characterize the small-ruminant lentiviruses of sheep and goat  
16 screened seropositive and thereby to develop a molecular based method for direct virus identification. A  
17 further aim of this study was to determine the position of our viruses in the current taxonomic classification  
18 (Shah et al., 2004a) and to study the evolution of the virus. The genetic analysis was performed on the basis of  
19 different PCR amplifications of *gag*, *pol* and *env* gene regions of the Finnish viruses.

20

## 21 **2. Materials and methods**

### 22 *2.1. Virological samples*

23 Lung tissue samples from 20 sheep identified as seropositive for a SRLV were available for this study. The  
24 sheep originated from five different farms known to be epidemiologically linked (coded B, C6, D4, D5 and E,  
25 according to Sihvonen et al., 1999, 2000) (Fig. 1). A cell-cultured virus (coded B S604 substrain 1) originally  
26 isolated from one of the sheep lung samples (farm B sheep 604) was passaged once in sheep choroid plexus  
27 (SCP) cells and included in the study. In addition, a blood sample from a seropositive goat (named Lotta)

1 obtained during the MVV/CAEV control programme in 2001 was included in the study. From the goat, 10 ml of  
2 heparin-anticoagulated blood was drawn by venipuncture. PBMCs were isolated by the standard protocol of  
3 NH<sub>4</sub>Cl treatment followed by centrifugation. SRLV strain M88 (a gift from Dr. G. Pétursson, Institute for  
4 Experimental Pathology, Iceland), originally isolated from a maedi-affected sheep lung in Iceland in 1961  
5 (Gudnadóttir and Pálsson, 1967) and with a passage history including nine rounds in SCP cells in our  
6 laboratory, was included as a positive control in the study.

## 7 2.2. DNA extraction, PCR and sequencing

8 Proviral DNA was extracted from 25 mg of sheep lung tissue, 50 µl aliquot of the goat PBMC pellet or from  
9 200 µl of the virus infected cell culture by DNeasy Tissue Kit (QIAGEN GmbH, Hilden, Germany), according  
10 to the manufacturer's instructions. Oligonucleotide primers were selected from the conserved sites found when  
11 prototype MVVs (strains K1514, SAOMVV and EV1) and CAEV prototype strain Cork were compared. A  
12 251 nt *gag* region (nt 1026-1276; numbering according to the MVV K1514 strain; Sonigo et al., 1985) coding  
13 for the highly conserved N-terminal part of the capsid (CA) protein was obtained using primers *gag5* (sense;  
14 5'AGCATGGACTTGTGTCCGAGGA) and *gag4* (antisense; 5'ACGCTGTTATTACCCACTGCA). An  
15 extended 853 nt *gag* region (nt 612-1464) covering 307 nt of the C-terminal part of the matrix (MA) protein  
16 followed by 546 nt of the N-terminal part of the CA protein was constructed from two overlapping amplicons  
17 flanked by primer pairs *gag1* (sense; 5'CTTGACAGAAGGGAATTGTCTATG [nt 588-611]) / *gag2*  
18 (antisense; 5'TGTGCTCTATTCCCAGGCATCAT [nt 1115-1093]) and *gag5* / *gag6* (antisense;  
19 5'CTTTTGACAGTCTGTGCTAGCATT [nt 1488-1465]). A 514-517 *env* region (nt 7450-7951) of the viral  
20 genome covering 487-490 nt of the C-terminal region of the surface glycoprotein (SU) and the adjacent 27 nt of  
21 the N-terminus of the transmembrane glycoprotein (TM) was amplified using primers *env1* (sense;  
22 5'ACAAATTGGGATGGATGTAA [nt 7409-7428]) and *env4* (antisense;  
23 5'GCAGCAGTTGCGTTAGCAAG [nt 8058-8039]) for primary PCR and *env7* (sense;  
24 5'CTGTACAAGGTCAGGAAATCA [nt 7429-7449]) and *env2* (antisense;  
25 5'GCAGCGATTATTGCCATGAT [nt 7971-7952]) for nested PCR. Oligonucleotide primers for a nested  
26 PCR, specific for the *gag-pol* and the *pol* gene region and designed by Shah et al. (2004a), were used to  
27 amplify a 1.8 kb and a 1.2 kb sequence, respectively. Uniform PCR conditions were used; 5 µl of the sample

1 DNA and 50 pmol of the primers were added to the reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50  
2 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.5 mM of each dNTP and 2.5 units of AmpliTaq Gold DNA polymerase (Applied  
3 Biosystems, Foster City, CA, USA) in a final volume of 100 µl. The PCR amplification was started with an  
4 initial denaturation for 10 min at 95°C. The tubes were subjected to 40 amplification cycles, each consisting of  
5 1 min denaturation at 95°C, 1 min annealing at 55°C and 1 min extension at 72°C. Within *env* region, three  
6 specimens (B S23, C6 S1 and D4 S1) that could not be amplified in the first round of PCR were subjected to  
7 nested PCR, using cycling conditions identical to the primary PCR. The PCR products were purified using  
8 either the MicroSpin S-400 Columns (Amersham Pharmacia Biotech, Piscataway, NJ, USA) or, if non-specific  
9 products were seen in the gel electrophoresis, the QIAquick Gel Extraction Kit (QIAGEN) for the excised gel  
10 fragments. The DNA was sequenced with the PCR primers using dye-terminator chemistry (Applied  
11 Biosystems). Capillary electrophoresis was carried out on an ABI PRISM 3100 Avant Genetic Analyzer  
12 (Applied Biosystems). The final sequence data was generated by repeatedly sequencing the amplification  
13 products of several independent PCR assays. Ambiguous positions resulting from a mixture of lentiviral  
14 sequence forms were entered using the IUPAC-IUB codes. The GenBank/EMBL accession numbers of the  
15 sequences obtained in this study are AM084187-AM084209, AM084215-AM084220 and xxxxxx-xxxxxx.

### 16 2.3. Analysis of the sequence data

17 Sequence editing was performed using the program EditSeq of the LASERGENE software package  
18 (DNASTAR Inc., Madison, WI, USA), and the pairwise sequence divergences were calculated using the  
19 MegAlign program of LASERGENE, with default settings. Alignments of nucleotide sequences were  
20 performed with CLUSTAL X (Thompson et al., 1997), and later edited manually. All positions with ambiguous  
21 codes or alignment gaps were excluded from the analyses. Phylogenetic analyses were conducted using MEGA  
22 version 3.1 (Kumar et al., 2004). Pairwise nucleotide distances were estimated with Kimura's two-parameter  
23 model with a transition/transversion ratio 2, and phylogenetic trees were constructed from the distance matrices  
24 with the neighbor-joining algorithm; the robustness of the trees was evaluated by bootstrap analysis on 1,000  
25 repeats.

26 All the 20 sheep lung samples, the cell-cultured isolate (B S604 substrain 1), the goat blood sample (Lotta) and  
27 the Icelandic SRLV strain M88 were used in sequence analysis of the 251 nt *gag* CA region, whereas six

1 samples (B S23, B S604, C6 S1, D4 S1, D5 S4 and E S1) representing five different sheep farms and Lotta  
2 from the goat farm were selected for a further phylogenetic analysis of the 853 nt region of *gag* MA/CA and  
3 the 514-517 nt region of *env* SU/TM. In constructing the phylogenetic tree within the *env* SU region a 411-414  
4 nt (nt position 7450-7842 in MVV K1514) was used to match the new sequences to the published sequences in  
5 the databases. For phylogenetic comparison with the recently published Swiss sequences (Shah et al., 2004a),  
6 PCR amplifications of the 1.8 kb *gag-pol* and 1.2 kb *pol* gene regions were carried out with B S604 substrain 1  
7 representing the Finnish virus type.

8

### 9 **3. Results**

10 A 251 nt *gag* CA sequence was successfully amplified directly from the 20 sheep lung samples and from the  
11 goat PBMCs as well as from the cell-cultured B S604 substrain 1 and the Icelandic strain M88. The *gag*  
12 MA/CA sequence was obtained from all of the seven samples tested (B S23, B S604, C6 S1, D4 S1, D5 S4, E  
13 S1 and Lotta). Within the *env* SU/TM region, viral sequences were obtained after the first round of  
14 amplification from four (B S604, D5 S4, E S1 and Lotta) out of the seven samples tested. Two specimens (C6  
15 S1 and D4 S1) which failed to react with the first combination of *env* primers were amplified using the nested  
16 primers, whereas no amplification product was obtained from one sample (B S23).

17 Nucleotide sequences were first obtained from the 251 nt *gag* CA region from all the viruses included in this  
18 study and compared with the SRLV sequences previously identified elsewhere (Fig. 2). In the phylogenetic  
19 tree, a group identified by the prototype MVVs was clearly distinguished from the CAEVs with a high  
20 bootstrap value (87%). The Finnish viruses formed a cluster of identical or highly related sequences within the  
21 genetically and geographically heterogeneous MVV group. The virus found in a goat flock (Lotta) aligned with  
22 the ovine viruses detected several years earlier. The analysis also showed a close relationship between the two  
23 Icelandic MVVs, the prototype strain K1514 and the strain M88 (Fig. 2).

24 The pairwise nucleotide divergences among the Finnish viruses varied from 0.2 to 3.9% in the *gag* MA/CA  
25 region and from 3.3 to 8.0% in the *env* SU/TM region, and the variation between the Finnish viruses and the  
26 prototype MVVs (strains K1514, SAOMVV and EV1) was 17% and 28%, respectively. At the amino acid  
27 level, the divergences among the Finnish viruses varied from 1.1 to 4.4% (284 aa) in the MA/CA region and in

1 the SU/TM region from 6.7 to 16.3% (171-172 aa), and the variation between the Finnish viruses and the  
2 MVVs was 11% and 25%, respectively. The deduced amino acid sequences of MA/CA and SU/TM proteins  
3 of the Finnish MVVs and the prototype MVV/CAEVs were aligned (the most variable site of SU/TM alignment  
4 is shown in Fig. 3). In the MA/CA region, the alignment revealed a difference in the sequence variability when  
5 comparing the MA and CA regions. In the MA region (102 aa) the Finnish viruses varied in 15 positions, eight  
6 of which were biochemically conservative, whereas the variation in CA region (182 aa) was limited to five  
7 conservative substitutions. When comparing the Finnish sheep and goat viruses with each other and with the  
8 ovine MVV prototypes, no insertions or deletions were present. The comparison with the CAEV prototype,  
9 however, showed an insertion of the size of 7 amino acids (aa 181-187 in MVV strain K1514) at the C-terminus  
10 of MA and a deletion of 2 amino acids (between aa 283 and 284) at the adjacent N-terminal part of CA. In the  
11 SU/TM region, the amino acid differences between the Finnish viruses were concentrated in a variable region  
12 identified as V4 by Valas et al. (2000), whereas only a few substitutions were seen at or near the variable  
13 region identified as V5 (Fig. 3). The RKKR sequence, which has been hypothesized to be the proteolytic site  
14 between SU and TM, was fully conserved among the viruses. Amino acid changes were especially seen in a  
15 short stretch (deleted in strain K1514) positioned within a region previously defined in MVV as the major  
16 neutralization epitope (aa 566-598 in strain K1514) by Skraban et al. (1999). Within this peptide site, the goat  
17 virus (Lotta) had an additional amino acid in comparison with the sheep viruses (B S604, C6 S1, D4 S1, D5 S4  
18 and E S1).

19 To analyse further the genetic relationships between Finnish MVVs, prototype MVV/CAEVs and other small-  
20 ruminant lentiviruses, phylogenetic trees were constructed from alignments of the *gag* MA/CA and *env* SU  
21 regions (Fig. 4a and b). The results show that the Finnish viruses formed a highly supported cluster (100%) and  
22 grouped with the prototype MVVs, which was consistent with the results obtained in the analysis of the  
23 relatively short (251 nt) *gag* fragment. However, as could be expected from the highly variable nature of the C-  
24 terminus of the SU protein, the viruses seemed more diverged in the *env* analysis than in the *gag* analyses. In  
25 the phylogenetic trees constructed according to the classification proposed by Shah et al. (2004a) (Fig. 4c and  
26 d), the closest relatives of the Finnish virus type was found either in the A1 subtype (19% nucleotide  
27 divergence in *gag-pol*) or with a virus recently isolated in Switzerland (subtype A5, 19.5% nucleotide

1 divergence in *pol*). Since these associations were not supported by high bootstrap values, the Finnish virus type  
2 can be best described as forming a new subtype in the diverse group A.

3

#### 4 **4. Discussion**

5 The aim of the study was to characterize the small-ruminant lentiviruses detected in Finland by defining their  
6 phylogenetic relationships and by studying the evolution of the virus based on a well-known epidemiology.  
7 The suitability of the highly conserved *gag* region for direct virus identification is also considered. The  
8 nucleotide sequences were first obtained and the phylogenetic relationships defined using a relatively short 251  
9 nt *gag* gene region coding for the highly conserved N-terminal part of the CA protein. Despite the high  
10 conservation at the protein level, the nucleotide sequence analysis allowed the initial classification of the  
11 Finnish SRLVs into the group of the prototype MVVs (Fig. 2). We analysed an extended 853 nt *gag* MA/CA  
12 region as well as the *env* SU region from samples selected from each flock in order to confirm the phylogenetic  
13 result obtained from the 251 nt *gag* CA alignment. Similar results were obtained from all the studied regions:  
14 the small-ruminant lentiviruses found in Finland formed a cluster of highly related viruses positioning closer to  
15 the prototypic ovine MVVs than to the CAEVs (Fig. 4a and b). The clustering obtained among the predicted  
16 amino acid sequences of the MA/CA and SU regions further confirmed the phylogenetic result (data not  
17 shown). According to the taxonomic classification proposed by Shah et al. (2004a) the Finnish virus type  
18 identified in this study formed a new subtype in the already diverse group A. The phylogenetic analyses also  
19 showed that the subtype A1 is genetically too heterogeneous to form a well supported subtype. The high  
20 genetic heterogeneity seen among the SRLVs, as well as the possibility of cross-infections between the host  
21 animals, which can cause host-specific changes, show that more extensive analyses of different isolates are  
22 needed to understand the genetic relationships between and within the genetic clusters.

23 In this study, the phylogenetic analyses showed that the similarity between the Finnish MVVs and the prototype  
24 MVVs and, on the other hand, the difference between the MVVs and the CAEVs could most clearly be  
25 demonstrated in the analyses based on *gag* region (Figs. 2 and 4a). This observation was supported by the  
26 result obtained from the paired comparisons as well as by the presence of the two highly conserved  
27 insertion/deletion sites in the *gag* alignment (an insertion of the size of 7 amino acids at the C-terminus of MA

1 and a deletion of 2 amino acids at the adjacent N-terminal part of CA). These results suggest that the *gag*  
2 region could reflect a long-term evolution of the MVV/CAEV-specific division of the virus. Especially in the  
3 *gag* MA region, the differences between the MVVs and the CAEVs were remarkable, with pairwise sequence  
4 divergences varying from 51 to 53% at the nucleotide level and from 63 to 70% at the amino acid level.  
5 Previous studies have indicated that the variable V4 and V5 regions of SU glycoprotein are important  
6 determinants for SRLV infection (Skraban et al., 1999; Valas et al., 2000). A type-specific, conformational  
7 neutralization domain has been mapped within the V4 region, and moreover, it has been suggested that this  
8 region defines a binding site for a cellular receptor or a coreceptor for MVV (Skraban et al., 1999). In our  
9 study, the analysis showed that the amino acid changes of the Finnish viruses were concentrated within the V4  
10 region, whereas only a few changes were seen at or near the variable region, V5, located at the C-terminus of  
11 SU (Fig. 3). Moreover, within V4, the changes could especially be seen in a short 3 amino acid stretch  
12 positioned at the proposed lower linear epitope of the conformational domain. Within this peptide site the virus  
13 detected from the Finnish goat had an additional amino acid in comparison with the ovine viruses. The same  
14 insertion could be seen in viral sequences of all the studied organs of the goat (data not shown) suggesting that  
15 the insertion could be a consequence of the cross-species transmission of the virus.

16 The virus found in a newly established goat flock in 2001 aligned with the Finnish ovine viruses detected  
17 several years earlier demonstrating a natural sheep-to-goat transmission of a new SRLV subtype. Our further  
18 study indeed showed an epidemiological link between the goat and a sheep flock (D1) found infected in 1996,  
19 and thus, the infection could be traced back to one importation of sheep from Sweden in 1981 (Fig. 1). The  
20 results obtained from *gag* and *env* regions showed that the virus had remained genetically relatively stable, in  
21 spite of the time given for virus evolution, an estimated 20 years, and in spite of the virus crossing the host  
22 species barrier. The highest degree of nucleotide and especially amino acid variation was seen in the *env*  
23 region and could be due to the location of important antigenic determinants at the C-terminus of *env* SU. As a  
24 result of the variability, the studied *env* region provided more detailed information for the epidemiological  
25 study than the other genomic regions. The maximum nucleotide divergences of 8% and amino acid divergences  
26 of 16% were seen between the most distant sequences of the infection-chain, whereas the direct transmission of  
27 the virus between two farms showed the lowest divergences (data not shown).

1 In conclusion, the virus responsible for the small-ruminant lentivirus infection in Finland could uniformly be  
2 classified as MVV in phylogenetic analyses based on distinct regions (*gag*, *pol* and *env*) of the viral genome.  
3 Analysis of the Finnish MVVs showed a correlation between molecular and epidemiological data confirming a  
4 close linkage between the infected farms and supporting the idea of one importation as a source of infection.  
5 Moreover, this study provides an overview of molecular evolution of the MVV in Finland.

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Fig. 1. Schematic representation of transmission of the MVV infection in Finland. Only the farms included in the present study are shown. The infection transmitted from the primary infection farm A to the secondary infection farms B and E, and further from farm B to all the rest of the infected farms (C6, D1, D4, D5). The epidemiological linkage to the goat farm (goat named Lotta) is demonstrated. The year of the serological detection is shown. The year of introduction of the virus to a farm, if known, is shown in parenthesis.

Fig. 2. Phylogenetic tree based on analysis of a 251 nt fragment at the CA protein coding region of *gag* (nt 1026-1276; numbering according to MVV K1514 strain; Sonigo et al., 1985) of the studied SRLVs. The Finnish ovine lentiviruses are indicated by flock code (B, C6, D4, D5 or E) followed by sheep (S) number and the Finnish caprine lentivirus by the name Lotta. The cell culture isolate representing the Finnish virus type is indicated B S604 substrain 1 by the specimen of origin. The prototype MVV strains K1514, SAOMVV and EV1, and the prototype CAEV strain Cork (bold) together with selected representatives of SRLVs were included in the analysis, and indicated by GenBank accession numbers. Bootstrap probabilities for the major clusters are indicated (only values exceeding 60% of the replicates are shown). The scale for the genetic distances is indicated. Country abbreviations: BRA, Brazil; FIN, Finland; FRA, France; GBR, Great Britain; ISL, Iceland; NED, The Netherlands; NOR, Norway; POR, Portugal; RSA, South Africa; SUI, Switzerland; USA, United States of America.

Fig. 3. Alignment of partial *env* SU/TM precursor amino acid sequences of the studied Finnish viruses (B S604, C6 S1, D4 S1, D5 S4, E S1 and Lotta) and the SRLV prototypes (MVV strains K1514, SAOMVV and EV1, and CAEV Cork). Dots represent amino acid residues identical to those of the consensus sequence (Cons) while dashes indicate gaps. Previously identified (Valas et al., 2000) variable regions, V4 (a) and V5 (b), are delineated by overlines. The positions of the two spatially important cysteine residues at the neutralization domain (Skraban et al., 1999) are underscored and the sites of the proposed linear epitopes of the neutralization domain are shaded. The highly variable peptide site (3 aa) found in this study is boxed and the additional amino acid (N, Asn) in the goat sequence (Lotta) is indicated by an arrow. X indicates ambiguous amino acids in the consensus sequence and amino acids K, E, N or D in the sequence of B S604.

\*, conserved cysteine residue; - - -, conserved potential N-linked glycosylation site.

Fig. 4. Neighbor-joining trees constructed from parts of the MA and CA coding regions of *gag* (nt 612-1464; numbering according to MVV K1514 strain; Sonigo et al., 1985) (a) and part of the SU coding region of *env* (nt 7450-7842) (b) of the studied SRLVs. The Finnish ovine lentiviruses are indicated by flock code (B, C6, D4, D5 or E) followed by sheep (S) number and the Finnish caprine lentivirus by the name Lotta. No amplicon was obtained from sample B S23 in the analysis of the *env* region. Neighbor-joining trees constructed from the 1.8 kb *gag-pol* region (almost-complete, nt 1114-2669) (c) and the 1.2 kb *pol* region (nt 3724-4853) (d). The cell culture isolate representing the Finnish virus type is indicated B S604 substrain 1. The prototype MVV strains K1514, SAOMVV and EV1 and the prototype CAEV strain Cork (bold) together with selected representatives of SRLVs were included in the analysis, and indicated by GenBank accession numbers. Bootstrap probabilities for the major clusters are indicated (only values exceeding 60% of the replicates are shown). The scale for the genetic distances is indicated. Previously published full-length sequences of MVV K1514 (M10608), SAOMVV (M31646) and EV1 (S51392) strains, CAEV Cork strain (M33677), PIOLV (AF479638) and G4668 (AY445885) were used in the comparisons. Country abbreviations: BRA, Brazil; FIN, Finland; FRA, France; GBR, Great Britain; ISL, Iceland; NOR, Norway; POR, Portugal; RSA, South Africa; SUI, Switzerland; USA, United States of America.

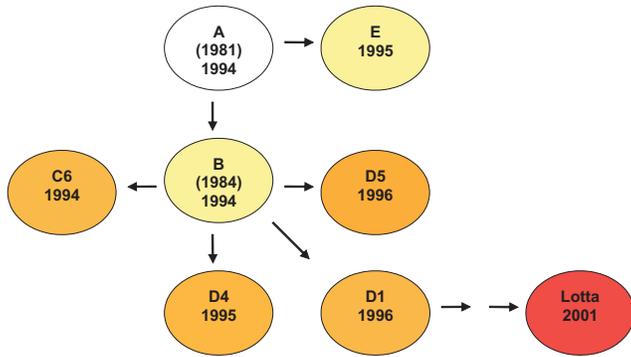


Fig. 1.

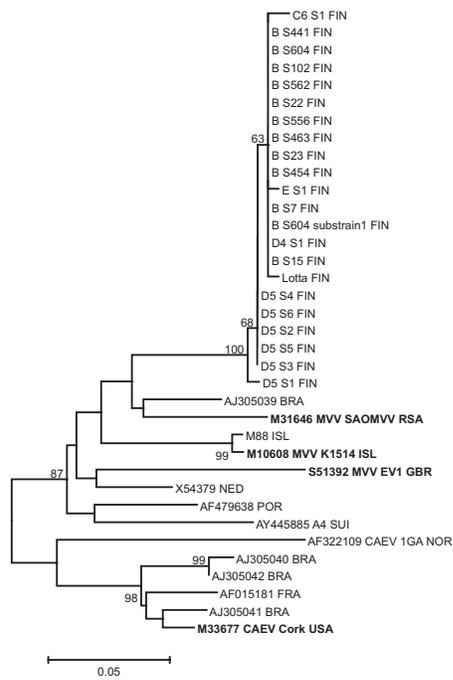


Fig. 2.

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E S1      . . . . . T T . . . . . T G S E . . . . . Q P . . . . . T . . . . . R K H
          . . . . . A K . . . . . I E . . . . . L L . . . . . L L . . . . . Q E F
B S604    . . . . . E . . . . . T . . . . . E R . . . . . Q . . . . . X X N
          . . . . . G S . . . . . G S . . . . . . . . . . . . . . . . T
C6 S1     S . . . . . E . . . . . T . . . . . T D . . . . . Q . . . . . K . . . . . R D G
D4 S1     K . . . . . I . . . . . G . . . . . G . . . . . K . . . . . E . . . . . S G F
D5 S4     . . . . . E . . . . . I . . . . . T . . . . . R . . . . . T K . . . . . S . . . . . R G G Q
          . . . . . A N . . . . . A N . . . . . . . . . . . . . . . . K E D
Lotta     S M . . . . . I . . . . . N . . . . . K . . . . . I . . . . . R . . . . . R E . . . . . R E G Q
          . . . . . D . . . . . Q . . . . . . . . . . . . . . . . G
M V V K1514 . . . . . N I . . . . . Q . . . . . R . . . . . N . . . . . S S . . . . . D . . . . . S . . . . . N . . . . . L K . . . . . R . . . . . S N . . . . . K S . . . . . Q
M V V S A O M V V . . . . . G I . . . . . E . . . . . K . . . . . S . . . . . D . . . . . D . . . . . N . . . . . I . . . . . Y . . . . . S N . . . . . N S K K
M V V E V - 1 . . . . . E I . . . . . S . . . . . S . . . . . D . . . . . I . . . . . I . . . . . I . . . . . R . . . . . N T . . . . . A A S R G N
C A E V C o r k . . . . . G I . . . . . A . . . . . D N . . . . . E . . . . . G . . . . . A . . . . . K . . . . . S N . . . . . P . . . . . Q . . . . . R D G . . . . . T

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(b)

V5

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636.      SU >< TM 672.
Cons      G V V D M P K S Y A E N --- K R K T T A L T Q K R K K R G I G L V I V L A
E S1      . . . . . N . . . . . S . . . . . G . . . . . D . . . . .
B S604    . . . . . N . . . . . R . . . . .
C6 S1     . . . . . E . . . . .
D4 S1     . . . . . N . . . . . R . . . . . L . . . . .
D5 S4     . . . . . T . . . . .
Lotta     . . . . . D . . . . . R . . . . .
M V V K1514 . . . . . E . . . . . Q . . . . . M . . . . . E R G E N . . . . . R S R R N L . . . . .
M V V S A O M V V . . . . . A . . . . . R . . . . . K N M R N K R S . . . . . E I S R P . . . . .
M V V E V - 1 . . . . . E . . . . . Q . . . . . L . . . . . K . . . . . N . . . . . R N A F K K E . . . . .
C A E V C o r k . . . . . I E . . . . . E N . . . . . K T R I I N . . . . . K R E . . . . . S H . . . . . V . . . . . M . . . . . V

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Fig. 3.

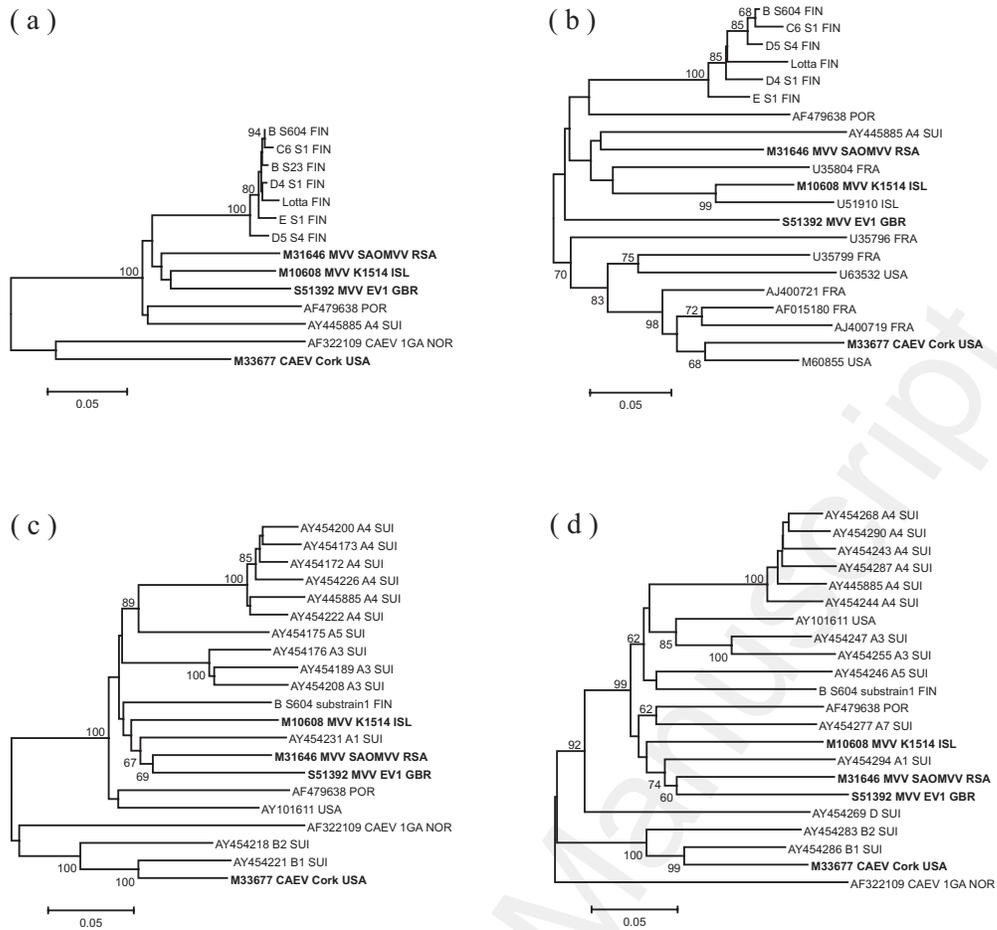


Fig. 4.