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Irkutsk may possess somewhat higher virulence to mice as compared with TBE viruses distributed in far-eastern region.

To establish the preventive vaccination for TBE in east-Siberian and far-eastern regions of Russia, the efficacy of the European TBE vaccine against recent Irkutsk, Vladivostok and Khabarovsk isolates was evaluated. The sera of vaccinated humans showed efficient neutralizing antibody titers against Irkutsk, Vladivostok and Khabarovsk isolates.

In addition, all vaccinated mice were asymptomatic and survived against lethal challenge of each virus strain. These results demonstrated that the European vaccine could induce efficient neutralizing antibodies in humans and protective immune responses in animal model against TBE viruses in east-Siberian and far-eastern regions. Therefore, it was expected that the European vaccine can prevent the TBE virus infection in human in east-Siberian and far-eastern regions.

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## Genetic and antigenic analyses of porcine enteroviruses

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Genetic and antigenic characters of porcine enterovirus serotype 1 (PEV-1) reference strain, Talfan, were analyzed. Furthermore, genetic reclassification of other members of PEVs was performed.

First, the majority of the genomic sequence of Talfan was determined and it was compared with other picornaviruses. Genome structure of Talfan was characteristic in possessing some features; (1) a leader protein coding region at 5' end of the open reading frame, (2) 2A region coding an foot-and-mouth disease-like protease, and (3) putative poly (C) tract in the 5' non-translated region. Moreover, the amino acid identities (a. a. i.) among them was significantly low throughout the genome except 2A protein. Even in 3D region coding RNA-dependent RNA polymerase (RdRp) which is considered to be the

most conserved protein among picornaviruses, the a. a. i. were less than 40%, although it possessed picornavirus characteristics. Phylogenetic analysis of RdRp revealed that Talfan fell into a distinct cluster within picornaviruses. Based on these observation, I proposed that PEV-1 Talfan be regarded as the prototype of a new genus for the family *Picornaviridae*. Consequently, the new genus *Teschovirus* was approved in the eleventh International Congress of Virology in 1999.

Second, antigenic structures of "porcine teschovirus (PTV; renamed from PEV-1)" Talfan strain was analyzed. Neutralizing antigenic sites on Talfan were identified through epitope mapping of neutralizing monoclonal antibodies (MAbs) by using synthetic peptides spanning the capsid proteins. All of the 11 MAbs obtained recognized peptides in the EF

loop("puff") of VP 2 (site A) ; in addition, 1 MAb concurrently reacted to a peptide in the GH loop of VP 1 (site B) , and another reacted to a peptide in the C terminus of VP 1 (site C) . A computer-simulated 3 -dimensional model of Talfan confirmed that all of those antigenic sites were exposed on the virion surface. Moreover, the distances between "puff" and the other two sites seemed to be short enough for recognition by a single antibody, indicating that site A/B and site A/C may function as discontinuous epitopes. These observations suggested that "puff" is the most immunodominant neutralizing antigenic site in this virus.

Finally, genetic diversity of PTVs and most serotypes of PEVs was studied. The nucleotide and derived amino acid sequences in 3 genomic regions involving different biological functions, the RdRp region, capsid VP 2 region and the 3' non-translated region (3' NTR), were compared among PTVs/PEVs and with other picornaviruses. Phylogenetic analyses of RdRp/VP 2 and the predicted RNA secondary structure analysis of 3' NTR indicated that PEVs should be reclassified genetically into at least 3 groups, the Talfan group, PEV- 8 V 13 group and PEV- 9 UKG 410/73 group. The 14 strains represented by Talfan, in particular, were completely distinct

from other picornaviruses, indicating that these strains should be reclassified as PTVs. In addition, since the genetic difference between the PEV- 9 UKG 410/73 group and PEV- 8 V 13 group was not negligible, these 2 groups should be distinguished from each other, e.g., as PEV-A and PEV-B. Interestingly, these 3 genotypes were consistent with biological phenotypes such as CPE types, thermoresistance and pH stability, supporting the validity of this reclassification. Moreover, among PTVs, the phylogenetic analysis of 28-29 amino acids in VP 2 "puff" , which is considered to be a dominant neutralizing antigenic site, was performed. In which, all the strains including prototype strains and field isolates were found to be classified in completely consistent with the serological classification. On the other hand, the strains isolated from CNS or brain could not be genetically differentiated from others in this analysis. Further directions of the study in PTVs/PEVs and enterovirus encephalomyelitis would be clarification of host-and-virus interaction and the mechanism of developing clinical symptoms. The observations described in this study would contribute to develop new methods for diagnosis and epidemiological survey of PTV infection.

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