Epizootic of vesicular disease in pigs caused by coxsackievirus B4 in the Soviet Union in 1975

Natalia F. Lomakina,1 Elena Yu Shustova,1 Olga M. Strizhakova,2 Jan Felix Drexler3,4 and Alexander N. Lukashev1

1Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia
2National Research Institute for Veterinary Virology and Microbiology of Russia, Pokrov, Vladimir Region, Russia
3Institute of Virology, University of Bonn Medical Centre, Bonn, Germany
4German Centre for Infection Research (DZIF), Bonn-Cologne, Germany

Swine vesicular disease virus (SVDV) is an epizootic pathogen causing significant morbidity among pigs in Europe and Asia (Escribano-Romero et al., 2000). Serological analysis identified SVDV as a human enterovirus, i.e. coxsackievirus B5 (CVB5) (Brown et al., 1973). Phylogenetic analysis revealed that the transfer of CVB5 into swine occurred only a short time before the epizootic (Zhang et al., 1999). The first outbreak of SVDV was registered in Italy in 1966 (Nardelli et al., 1968) and several epizootics occurred worldwide in the following years. The most recent outbreak was reported in Portugal in 2007 (Knowles et al., 2007). After the emergence of SVDV as a swine pathogen from an ancestral human virus, there have been no reports of enterovirus host switches between humans and non-primate hosts.

Many European countries, Hong Kong and Japan were affected by swine vesicular disease outbreaks in 1970–1981 (Brocchi et al., 1997). Knowledge on swine vesicular disease in the former Soviet Union is limited because most of the investigation results were either not made publicly available at all or published only in the internal proceedings of the Soviet veterinary institutions (e.g. Fedorischeva et al., 1980).

The first reports of swine vesicular disease in the Soviet Union date to 1972. The epizooty originated in the Odessa region, one of the major ports of the Soviet Union (Karpov, 1979). The isolated strain O72 (Odessa-1972) was typed serologically as an SVDV with morphological, physical and chemical properties similar to strains that were circulating globally at that time (Karpov, 1979). In 1975, another outbreak of 24 488 cases of swine vesicular disease was registered at 22 farms in the Tambov region (Central Russia). Virus spread was linked to contaminated transport and bone meal. The infection was introduced to three farms in closely situated Voronezh and Saratov regions, infecting another several thousand pigs. According to the existing guidelines for investigation of vesicular disease outbreaks in pigs, the isolate was tested for the presence of SVDV, foot-and-mouth disease virus and vesicular exanthema of swine virus. None of these agents were detected. The virus was identified as an enterovirus by physical properties and by electron microscopy. However, the virus could not be neutralized by serum to the classical SVDV and was therefore termed SVDV serotype 2, whilst the classical SVDV was termed SVDV serotype 1 (Fedorischeva, 1980). The isolate was not tested with antisera to human enteroviruses because of standard practices of that time in veterinary institutions and was not transferred to other institutions, presumably because of the government’s intention to maintain secrecy.

The virus was isolated from pigs at several farms in 1975; however, only one isolate termed T75 (Tambov-1975) was preserved as a reference isolate and was available for this study. High titres of neutralizing antibodies to T75 were found in most of the diseased pigs from affected farms, although seroconversion has never been demonstrated in a natural setting because pre-morbid serum samples were not available (Fedorischeva, 1980). Inoculation of pigs with strain T75 resulted in clinical manifestations that did not differ significantly from SVDV infection (Fedorischeva et al., 1980). Infectious virus could be isolated from saliva, faeces, urine and other body fluids of experimentally infected pigs. Experimentally infected pigs transmitted the virus to contact animals. When symptomatic and healthy animals were kept together, 10 of 12 contact pigs developed
symptomatic disease and two were asymptomatic, although they excreted virus and seroconverted. In another experiment, convalescent animals that secreted virus 1 and 3 months after infection (at about the same titres as in the acute phase) also transmitted virus to contact pigs. The latter also excreted virus and seroconverted, but did not develop a symptomatic disease (Fedorischeva, 1980). Therefore, the virulence of the virus was likely waning over the course of infection. Interestingly, recent outbreaks in Italy were noted for a significant proportion of asymptomatic cases and decreasing virulence of the virus was discussed as one of the explanations (Bellini et al., 2007).

To elucidate the origins of viruses causing swine vesicular disease in the former Soviet Union, we obtained both prototype strains T75 and O72 from the pathogen collection of the National Research Institute for Veterinary Virology and Microbiology. Viruses were propagated in RD (human rhabdomyosarcoma) cell culture. A typical enterovirus cytopathic effect was observed after 48–72 h. Viral RNA was isolated directly from the storage tube and used for

Fig. 1. Phylogenetic tree of CVB4, including strain T75 (in bold). The dataset contained 65 full VP1 sequences (924 nt). Phylogenetic analysis was performed using a Bayesian likelihood-based algorithm implemented in BEAST 1.7.5 (Drummond & Rambaut, 2007). The SRD06 substitution model was used with a relaxed log-normal clock. Analysis was run over 50 million generations and trees were sampled every 10,000 generations, resulting in 50,000 trees. Trees were annotated with TreeAnnotator 1.4.8 using a burn-in of 1000 trees and visualized with FigTree 1.3.1. Bar, time in years. Ages of nodes indicated with circles are provided in the text. Nodes marked with dots had posterior probability values >0.95.
sequencing. Partial genomic sequence obtained from the passaged virus was identical to that in the original tube. The complete genomic sequence of strain T75 was obtained by Sanger sequencing using PCR with degenerate primers to 5’ NTR, VP1 and 3D genome regions of enterovirus B (Lukashev et al., 2004), and then specific primers to strain T75 were used for long-range PCR and sequencing. Genome termini were sequenced using a Roche RACE kit (Roche Diagnostics). For comparison of the Soviet SVDV isolate O72 with available strains, the complete VP1 genome region was identified for strain O72 using a similar approach. The GenBank accession numbers of sequences are KT006373 and KT0063734.

Viruses belonging to the same enterovirus type share >75 % nucleotide sequence identity in the VP1 genome region (Knowles et al., 2012). Strain T75 shared 80.0–90.4 % identity with coxsackievirus B4 (CVB4) sequences available in GenBank and therefore belongs to the CVB4 type. SVDV was shown previously to be CVB5. The Soviet strain O72 was 87.3–97 % identical to other SVDV isolates, confirming its assignment to this type. All CVB4 and CVB5 sequences for which the full VP1 genome region, isolation place and year were available were extracted from GenBank and used for a phylogenetic analysis.

Molecular clock analysis was performed to identify time of SVDV and strain T75 emergence. Date of the most recent common ancestor (tMRCA) of strain T75 and other CVB4 strains was 1950 (Fig. 1, node A), and the 95 % highest probability density (HPD) interval of this node’s date was 1945–1954. Therefore, T75 virus most likely diverged from human CVB4 after 1945, but before its isolation in 1975. This date of strain T75 emergence was compared with the predicted time of emergence of the classical SVDV. The tMRCA of all SVDV isolates dated back to 1962 (95 % HPD 1959–1964; posterior probability 1), and the tMRCA of SVDV and the most closely related CVB5 was 1954 (95 % HPD 1948–1960; posterior probability 0.99) (Fig. 2, nodes B and C, respectively). Therefore, SVDV most likely emerged between 1948 and 1964 (extreme boundaries of the 95 % HPD intervals). The predicted time intervals of SVDV and strain T75 emergence are thus very close. Strain O72 was most similar and grouped with SVDV strains isolated in Hong Kong in 1970–1971. However, the common ancestor of O72 and these SVDV strains dated 3–5 years (95 % HPD) before isolation of O72, and it is not possible to conclude if the virus was introduced to the Soviet Union directly from Hong Kong or via some intermediate location.

Cross-species transfer of human enteroviruses to animals is common only for non-human primates (Harvala et al., 2011; Oberste et al., 2013). Apart from SVDV, there have been no records of human enteroviruses successfully switching into genetically less related animal hosts. However, there is multiple evidence of enterovirus transfer between other

Fig. 2. Phylogenetic tree of CVB5 and SVDV (in bold). The dataset contained 126 non-redundant sequences (including 21 SVDV sequences) spanning positions 25–834 of the VP1 genome region. Only the tree fragment that included SVDV is shown. Analysis parameters were the same as in Fig. 1. Bar, time in years. Ages of nodes indicated with circles are provided in the text. Nodes marked with dots had posterior probability values >0.95.
distantly related host species. For example, enterovirus F (formerly bovine enterovirus 2) was detected in possums (Zheng, 2007), and an enterovirus E (formerly bovine enterovirus 1) isolate was found in a dolphin (Nollens et al., 2009) and in non-human primates (Oberste et al., 2013). Moreover, such cross-species transfer may produce viruses that are recombinants of distinct enterovirus species (Boros et al., 2012). Further sampling of animal enteroviruses is required to see if they are indeed more prone to host switch than enterovirus A–D that are found in primates.

Interestingly, a cross-species transfer of CVB4 occurred at about the same time as a transfer of SVDV from CVB5. Hypothetically, agricultural practices, such as use of untreated food waste and bone meal to feed swine, may have facilitated cross-species transfer of two human enteroviruses to pigs at about the same time. Experimental evidence of limited transmissibility of strain T75 compared with SVDV (Fedorischeva, 1980) may explain why CVB4 did not become established as a swine pathogen. It is possible that dead-end transfers of human enteroviruses to other species are more common than currently known and the two types of coxsackievirus B that switched to swine may be a model to study factors limiting efficient cross-species transfer of enteroviruses.

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References


