Genotyping of porcine teschoviruses isolated from 1960 to 1980 in the former Czechoslovakia and new *Porcine teschovirus* isolates obtained from piglets with diarrhoea

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ABSTRACT: The porcine enteroviruses (family Picornaviridae) were described as causative agents of neurological disorders known as Teschen/Talfan disease, reproductive failure, dermal lesions, enteric disease and pneumonia of swine. Recently, porcine enteroviruses were reclassified on the basis of genome sequencing data. A new *Picornavirus* genus named *Teschovirus* (formerly PEV CPE group I.) was established. The aim of the study was retyping and reclassification of the 27 strains of porcine enteroviruses deposited in the Collection of Animal Pathogenic Microorganisms (CAPM). Viral strains were isolated over the period 1960–1980 predominantly from pigs with encephalomyelitis. Twenty-four of 27 isolates were classified as porcine enterovirus serotype 1 (PEV-1) based on physicochemicals properties of their virions and growth characteristics. The viral strains were reclassified using RT-PCR protocol that allowed detection of the genus *Teschovirus* and the *Porcine teschovirus* serotype 1 (PTV-1). Two PCR amplifications with cDNA were performed for detection of the genus *Teschovirus* and PTV-1. Amplification of fragment characteristic for the genus *Teschovirus* was succesful for all tested viral strains. The fragment characteristic for PTV-1 was detected with the exception of three strains (Kr69TK, 95 and 172). Used RT-PCR method was subsequently applied to the detection of *Porcine teschovirus* in pig fecal samples. Ten of 22 faecal samples were found to be *Porcine teschovirus* positive, however none of 22 samples generated amplicon specific for the PTV-1.

Keywords: *Teschovirus*; PTV-1; fecal samples; reverse transcription; polymerase chain reaction

The first evidence of porcine teschovirus/enterovirus infection to be reported was the occurence of Teschen disease with high mortality, in Czechoslovakia over 75 years ago. On the basis of findings during the Teschen disease outbreak, the article titled "The Massive Ilness of Swine in Teschen Area" was published and a serious disease of piglets and saws with fever and neurological disorders was described. However, the cause of the disease was diagnosed incorrectly as a solanine intoxication (Trefny, 1930). In the 1950s, the disease spread throughout Europe and caused huge losses to the pig breeding industry. A milder form of the disease with almost no mortality was identified in Wales as Talfan disease (Knowles, 2008).

Nonenveloped porcine teschoviruses and enteroviruses shown high resistance to chemical inactivation (Dvorakova et al., 2008) and environment. The only known natural host for porcine teschoviruses and enteroviruses is the pig (Knowles et al., 2006). Therefore, their presence in water should indicate contamination with pig fecal residues (Jimenez-Clavero et al., 2003).

Although *Porcine teschovirus* (PTV) infections are most frequently asymptomatic and remain

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unapparent (Knowles, 2006), strains of *Porcine teschovirus* serotype 1 (PTV-1) were described as causative agents of polioencephalomyelitis (Trefny, 1930; Harding et al., 1957). *Teschovirus encephalomyelitis* (previously Teschen/Talfan diseases, and later *Enterovirus encephalomyelitis*) is an acute condition of pigs characterised by central nervous system disorders (Knowles, 2008). On the other hand, serotypes other than PTV-1 were described as causative agents of enteric disease (Izawa et al., 1962), pneumonia (Meyer et al., 1966), aphtae-like dermal lesions of swine (Nardelli et al., 1968; Knowles, 1988) and reproductive failure (Dunne et al., 1965).

Based on the cytopathic effect (CPE), replication properties in different host cell lines and serological assays, the porcine enteroviruses were divided into three group: CPE group I comprises serotypes

Table 1. List of analysed viral strains with the results of RT-PCR assays and molecular identification

CAPM No.	Strain	Source	Year and place (region, country) of isolation	Amplification of the 316 bp fragment characteristic for genus *Teschovirus*	Amplification of the 683 bp fragment characteristic for PTV-1
V-20	Krupina	pig/brain	1963, Krupina (SK)	+	+
V-49	Kr69TK	pig/lung	1967, Krepice (CZ)	+	–
V-76	Konratice	pig/brain	unknown, Konratice (CZ)	+	+
V-84	Cadca	pig/brain	1964, Cierne (Cadca, SK)	+	+
V-85	Lipovany	pig/brain	1966, Lipovany (Lucenec, SK)	+	+
V-86	Roznava	pig/brain	1965, Roznava (SK)	+	+
V-109	95	pig/lung	1962, Chlumec (CZ)	+	–
V-110	172	pig/lung	unknown	+	–
V-111	Praha	pig/brain	1962, Praha (CZ)	+	+
V-180	10873/V75	pig/fetus	1976, Zdislavice (Havlickuv Brod, CZ)	+	+
V-182	Z8566/V76	sow/brain	1976, Zaborna (Jihlava, CZ)	+	+
V-372	Celadna	pig/brain	unknown, Celadna (CZ)	+	+
V-373	J. Balco	pig/brain	unknown	+	+
V-374	Ciprus	pig/brain	1966, unknown	+	+
V-375	Guty	pig/brain	1965, Guty-Trinec (CZ)	+	+
V-376	Jesenske	pig/brain	1966, Jesenske (Rimavska Sobota, SK)	+	+
V-378	Kysel	pig/brain	unknown, Kyselka (Rimavska Sobota, SK)	+	+
V-379	Kysucke Nove Mesto	pig/brain	1964, Kysucke Nove Mesto (SK)	+	+
V-380	Lest	pig/brain	1966, Zvolen (SK)	+	+
V-381	Lucenec	pig/brain	1965, Lucenec (SK)	+	+
V-382	Medzianky	pig/brain	1965, Medzianky (Vranov nad Toplou, SK)	+	+
V-383	Mosovice	pig/brain	1965, unknown	+	+
V-384	Oroslani	pig/brain	unknown	+	+
V-386	Rejdova	pig/brain	1965, Rejdova (Roznava, SK)	+	+
V-388	Rimavska Sobota 1	pig/brain	1965, Rimavska Sobota (SK)	+	+
V-389	Stec	pig/brain	1965, Ropice (CZ)	+	+
V-392	Zabreh	pig/brain	unknown	+	+
V-37	Talfan, control strain	pig/brain and spinal cord	1956, United Kingdom	+	+

CZ = Czech Republic, SK = Slovak Republic

1–7 and 11–13, CPE group II comprises serotype 8 and CPE group III comprises serotypes 9 and 10. Recently, porcine picornaviruses, previously classified in the genus *Enterovirus,* have been reclassified on the basis of genome sequencing data. Two species in the genus *Enterovirus*, *Porcine enterovirus A* (PEV-8) and *Porcine enterovirus B* (PEV-9 and PEV-10) and new genus *Teschovirus* comprising a single species *Porcine teschovirus* (PTV), with 11 serotypes, were established (Zell et al., 2001; Krumbholz et al., 2002; Stanway et al., 2005).

Here we described genotyping of 27 viral strains of the porcine teschoviruses/enteroviruses, isolated from 1960 to 1980 in former Czechoslovakia and held in the Collection of Animal Pathogenic Microorganisms (CAPM). Used RT-PCR method was subsequently applied to the detection of *Porcine teschovirus* in pig fecal samples.

MATERIAL AND METHODS

Source and propagation of viruses

All 27 used porcine teschovirus/enterovirus strains and one reference strain are held in the CAPM. Viral strains with CAPM number, year and place of isolation and molecular identification are listed in Table 1. Viral strains were propagated in porcine kidney cell line (PK-15), which was maintained in Minimal Essential Modified Eagle Medium (PAA) supplemented with 10% fetal bovine serum. Time of harvest for the propagated virus strains was dependent on the extent of CPE. Incubation was ended when 90% of the monolayers were destroyed, generally from 18 hours to three days postinfection.

The porcine fecal or gut content samples were collected from piglets with diarrhoea from 2005 to 2007 in different regions of the Czech Republic. The teschovirus positive fecal and gut content samples, year and region of sampling and molecular identification are listed in Table 2. All samples were examined by negative contrast electron microscopy. The sample P1380/1 was rotavirus positive, P1358 astrovirus positive and samples P1558/2 and P1559 were rotavirus and coronavirus positive.

RNA extraction and cDNA synthesis

RNA from infected cell culture supernatant and faeces or gut content supernatant was purified by QiaAmp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. Approximatelly 5 µg of total RNA was reverse transcribed with 2.5µM random nonamers (Sigma) and 4 IU Omniscript Reverse Transcriptase (Qiagen) in a total volume of 20 µl. The reaction was incubated at 37°C for 1 h and synthetized cDNA was stored at –20°C.

Table 2. List of *Porcine teschovirus* positive fecal samples with the result of RT-PCR assay analysed in this work

Sample No.	Source	Year/region of isolation	Amplification of fragments characteristic for genus *Teschovirus*		Amplification of the 683 bp fragment characteristic for PTV-1
			1st PCR 316 bp	2nd PCR 158 bp	
P1353/3	faeces/piglet	2005/Benesov	+	+	–
P1380/1	faeces/piglet	2005/Pribram	+	+	–
P1379/2	faeces/piglet	2005/Hradec Kralove	+	+	–
P1358	faeces/piglet	2005/Ceske Budejovice	+	+	–
P1367	faeces/piglet	2005/Novy Jicin	+	+	–
P1541/1	faeces/piglet	2007/Rychnov nad Kneznou	+	+	–
P1558/2	faeces/piglet	2007/Louny	+	+	–
P1559	faeces/piglet	2007/unknown	+	+	–
P1563/1	gut content of piglet	2007/Znojmo	+	+	–
P1564	faeces/piglet	2007/Svitavy	+	+	–

Table 3. Oligonucleotide primers used for nested PCR and PCR

Primer	Sequence	Fragment size	Reference
Porcine teschovirus (PTV) specific primers			
pev1a	5'-AGTTTTGGATTATCTTGTGCCC-3'	316 bp	Krumbholz et al., 2003
pev1e	5'-CGCGACCCTGTCAGGCAGCAC-3'		
pev1c	5'-TGAAAGACCTGCTCTGGCGCGAG-3'	158 bp	
pev1d	5'-GCTGGTGGGCCCCAGAGAAATCTC-3'		
PTV-1 specific primers			
TTD1	5'-ATGCCTTTGAGACCTGTTAATGA-3'	683 bp	Zell et al., 2000
TTD2	5'-CAACATTAGTCATCTTTGTAATTGT-3'		

PCR assay

Porcine teschoviruses were detected by nested PCR using primer pairs pev1a/pev1e for first PCR designed to bind to the teschoviral highly conserved 5´-non-translated region and pev1c/pev1d for second PCR according Krumbholz et al. (2003). *Porcine teschovirus* serotype 1 was detected by PCR using primer pair TTD1/TTD2 designed to bind VP3/VP1 genome region according Zell et al. (2000). For sequences of primers refer to Table 3. PCR was done in total volume of 25 µl. PCR detection of PTV's in cell culture supernatant was performed according following conditions: 1 × PCR Buffer (Qiagen), 200µM of dNTP's, 1.5mM $MgCl_2$, 0.5µM of each primer, 2.5 IU of HotStar *Taq* DNA polymerase (Qiagen) and 1 µl of the DNA. For the PCR detection of PTV's in faeces, 1 × PCR Buffer (Qiagen), 200µM of dNTP's, 3mM $MgCl_2$, 0.8µM of each primer, 1 × Q-Solution (Qiagen), 2.5 IU of HotStar *Taq* DNA polymerase and 2.5 µl of the DNA was used. Each amplification mixture was subjected to initial denaturation 95°C for 15 min, 35 cycles of 94°C for 50 s, 55°C for 50 s and 72°C for 1 min and final extension at 72°C for 5 min. Presence of PCR products was visually confirmed after agarose gel (1.5% w/v) electrophoresis and staining with ethidium bromide.

RESULTS AND DISCUSSION

In the present study, molecular typing of PTV strains isolated from pigs in former Czechoslovakia from 1960 to 1980 was performed. PCR design allowed classification of all viral strains and field samples into genus *Teschovirus*. All tested teschovirus/enterovirus strains were confirmed to belong to the genus *Teschovirus* since they showed positive amplification of 316 bp fragment specific for the genus *Teschovirus*. Furthermore, the PCR with primers specific for serotype 1 was performed and specific product was detected in all except for three lung isolates Kr69TK, 95 and 172 (Table 1). Besides the PTV serotype 1, serotypes 2 and 3 were recognized as causative agents of pneumonia. However, it is probable that alone they rarely cause clinical signs of respiratory disease (Knowles, 2006). The remaining 23 strains isolated from brain, one strain isolated from fetus and control strain Talfan were characterized as PTV-1. Currently, PTV-1 is still frequently isolated from the feces, tonsils, and other nonneural organs of apparently unaffected pigs. On the other hand, other serotypes different from PTV-1 are increasingly identified to be the cause of neurologic disorders of swine (LaRosa et al., 2006). It is not known whether this observation reflects changes of virus prevalence or is the result of improved methods of virus detection (Zell et al., 2001). There have been recent incidents of neurological disease in piglets in The United States, Germany, Japan and China caused by PTV (Roost et al., 2002; Pogranichnyi et al., 2003; Yamada et al., 2004; Feng et al., 2007).

In relation to genotyping of formerly isolated teschoviral strains, the detection of *Porcine teschovirus* in fecal samples obtained from piglets with diarrhea was performed. Using nested RT-PCR, 10 of 22 samples were found to be *Porcine teschovirus* positive. None of 22 fecal isolates generated amplicon (683 bp) by RT-PCR with the *Porcine teschovirus* serotype 1 specific primers pair. The

role of *Porcine teschovirus* as enteric pathogens is uncertain, because they have frequently been isolated from the feces of piglets with diarrhoea as well from normal piglets (Knowles, 2006). Since diarrhoea can be caused by a variety of other viral and bacterial agents, the presence of porcine teschoviruses may be coincidental or disease can be causally associated with multiple agents.

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