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Molecular investigations on the prevalence and viral load of enteric viruses in pigs from five European countries

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ABSTRACT

Enteric viral infections in pigs may cause diarrhea resulting in ill-thrift and substantial economic losses. This study reports the enteric infections with porcine astrovirus type 4 (PAstV4), porcine group A rotavirus (GARV), porcine group C rotavirus (GCRV), porcine circovirus type 2 (PCV2) and porcine kobuvirus (PKoV) in 419 pigs, comprising both healthy and diarrheic animals, from 49 farms in five European countries (Austria, Germany, Hungary, Spain and Sweden). Real-time RT-PCR assays were developed to test fecal samples and to compare the prevalence and viral load in relation to health status, farms of origin and age groups. The results showed that PAstV4 (70.4%) was the dominant virus species, followed by PKoV (56.7%), PCV2 (42.2%), GCRV (3%) and GARV (0.9%). Diarrheic pigs had a higher viral load of PAstV4 in the nursery and growing-finishing groups. Rotaviruses were mainly detected in diarrheic pigs, whereas PCV2 was more often detected in clinically healthy than in diarrheic pigs, suggesting that most PCV2 infections were subclinical. PAstV4, PCV2 and PKoV were considered ubiquitous in the European pig livestock and co-infections among them were frequent, independently of the disease status, in contrast to a low prevalence of classical rotavirus infections.

1. Introduction

Pigs are an important source for food supply worldwide. Maintaining pig health and improving feed conversion rate are crucial to meet the increasing demand of food for a fast growing human population in the world. Certain highly contagious, transboundary diseases such as classical swine fever have been controlled and even eradicated from most European countries by different strategies including strict stamping-out policy, vaccination, and improved diagnosis and biosafety, resulting in a better health status of pigs in these countries. By contrast, endemic diseases are still present in spite of being managed by veterinarians and farmers. Among these pathological conditions, diarrhea in young pigs causes substantial economic losses due to increased mortality or morbidity with decreased growth and prolonged time for reaching market weight.

Pig diarrhea is a multi-factorial problem, also interpreted as a disease complex. Besides bacteria such as enterotoxigenic *Escherichia coli* (ETEC) and *Clostridium perfringens* types A and C, a number of viruses have been reported either causing or triggering the disease. Rotaviruses are one of the major classical causes of

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diarrhea in piglets (neonatal, nursery and post-weaning) as well as in many other species including humans. Rotaviruses are non-enveloped, double-stranded RNA viruses with a genome comprising 11 segments, and are divided into seven (A–G) antigenically distinct serogroups based on the serological characteristics of the membrane protein VP6. Serogroups A and C are the most frequent ones causing diarrhea in piglets. Group A rotavirus (GARV) is detected most frequently in pigs younger than 60 days of age (Chang et al., 2012), with the highest prevalence at 3–5 weeks of age (Bohl, 1979). By contrast, group C rotavirus (GCRV) is becoming dominant in diarrheic suckling piglets (less than 20 days of age) in the US (Marthaler et al., 2014). Rotaviruses are transmitted primarily via fecal-oral route and viral infection is widespread in swine herds. The widely accepted mechanism of rotavirus-induced diarrhea in pigs and humans is related to villous atrophy with loss of intestinal absorptive cells (Chang et al., 2012), which leads to malabsorption (Kapikian, 2001).

Several "emerging" viruses such as astroviruses and kobuvirus have been detected more often in diarrheic pigs as well as in healthy pigs (Khamrin et al., 2010; Park et al., 2010; Dufkova et al., 2013). Porcine astrovirus (PAstV) belongs to the genus *Mamastrovirus* of the family *Astroviridae*. Astroviruses are non-enveloped and contain a single-stranded RNA genome. The viruses infect a broad range of hosts such as humans, cattle, sheep/goats, pigs, mink and bats, and are phylogenetically grouped into different lineages. Porcine astroviruses can be divided into five types 1–5 (PAstV1–PAstV5) (Indik et al., 2006; Laurin et al., 2011; Luo et al., 2011; Shan et al., 2011). All five types of astroviruses have been reported in Europe (Brnić et al., 2013, 2014; Machnowska et al., 2014; Monini et al., 2015; Reuter et al., 2011). As astroviruses are found both in diarrheic and in healthy pigs, their role as a causative agent of pig diarrhea has not been unequivocally established. Similarly, a causal role of porcine kobuvirus (PKoV), a small picornavirus with a single-stranded RNA genome, in disease has yet to be defined despite a high prevalence of infection in diarrheic as well as in healthy pigs (Khamrin et al., 2009; Dufkova et al., 2013).

Porcine circovirus type 2 (PCV2) is a single-stranded DNA virus belonging to the genus *Circovirus* in the family *Circoviridae* (Allan and Ellis, 2000; Segalés et al., 2005). There are two major genotypes (2a, 2b): PCV2b has replaced PCV2a and became predominant in swine farms (Segalés et al., 2013). In addition, a third genotype, PCV2c, is found in Danish archived samples (Dupont et al., 2008; Segalés et al., 2008). PCV2 infection is associated with several diseases or disease clusters with diarrhea as one of the clinical signs. Moreover, PCV2 modulates the immune response (Kekarainen et al., 2008); therefore, co-infection with PCV2 may contribute to severe clinical diseases.

The prevalence of rotaviral and emerging viral enteric infections in most European pig herds is poorly documented. Therefore, the objective of this study was to provide data on the prevalence of PAstV4, GARV and GCRV, PCV2 and PKoV infections in both clinically healthy and diarrheic pigs from farms in Austria, Germany, Hungary, Spain and Sweden.

2. Materials and methods

2.1. Sample collection

A total of 419 fecal samples from 49 pig farms were collected by veterinarians in five European countries during 2010–2013. The number of samples from each country was 136 (Austria), 44 (Germany), 50 (Hungary), 83 (Spain), and 106 (Sweden). These samples originated from pigs at different ages: suckling (0–5 weeks), nursery (6–10 weeks), growing-finishing (11–18 weeks) and unknown aged pigs. With the exception of the German samples, which included only diarrheic feces, both healthy and diarrheic pigs were sampled in each country. The samples were transported in cool containers to the laboratory and stored in a −20 °C freezer until analyzed. Specificity of the assays was evaluated by testing ten swine virus isolates, namely, EU and NA strains of porcine reproductive and respiratory syndrome virus (PRRSV), two strains of transmissible gastroenteritis coronavirus (TGEV), porcine epidemic diarrhea virus (PEDV), porcine parvovirus (PPV), GARV CN86 strain, PCV2, border disease virus 137/4 and classical swine fever virus (CSFV).

2.2. Nucleic acid extraction

Approximately 0.2 g of feces were resuspended in 500 μl of water containing 10^6 pfu of bacteriophage MS2 as extrinsic control. The mixture was centrifuged at 4000 × *g* for 20 min and 350 μl of

Table 1
Primers and probes designed for real-time PCR assays in this study.

Target virus and region	Primer or probe name	Sequence (5′–3′)[a]	5′end position (accession no.)	Reference
GRAV (VP6)	T207-F	GGAGGTTCTGTAYTCATTGTCAAAA	26 (X94617)	Logan et al., 2006
	T234-R	CCTATTCCTCCTGTTTGAAAATCAT	178 (U10031)	This study
	T223-ROX	AAT(+C)AAA(+T)GATAG(+T)CAC(+T)ATGA	120 (U10031)	This study
GCRV (VP6)	T148-F	CCGTGAAGAGAATGGTGATGTAGA	1187 (M94157)	Chun et al., 2010
	T243-R	CATAGTTCACATTTCATCCTCCTG	1348 (M94157)	This study
	T150-Quasar670	AACCAATCTCTATGTGGACTACATACCA	1225 (M94157)	Chun et al., 2010
PAstV1 (capsid)	T217-F	CCAAAACCAGCAATCCGTCAA	260 (Y15938)	This study
	T218-R	GCCCCTAAAGCAACGATCGG	420 (Y15938)	This study
	T219-Quasar705	TTCTTGTCAAGGATAATACGGGG	363 (Y15938)	This study
PAstV4 (RdRp)	T220-F	ACAGCGCTGCATGGGAAACTC	863 (GU562296)	This study
	T221-R	AGGCTTATGCTTTGGTCCGC	1045 (GU562296)	This study
	T222-FAM	AGGCAGATGGACAGGCTTTGGAG	1001 (GU562296)	This study
PKoV (5′UTR)	T248-F	TCTCTGACCTCTGAAGTGCACT	462 (JX401523.1)	This study
	T249-R	TGAAGAAGCCATGTGTCTTGTC	589 (JX401523.1)	This study
	T250-FAM	GGTTGCGTGGCTGGGAATCCAC	486 (JX401523.1)	This study
PCV2 (Rep)	T176-F	GGCCACCTGGGTGTGGTAAA	571 (JQ002672)	Gagnon et al., 2010
	T177-R	CCCACCACTTGTTTCTAGGTGGTT	660 (JQ002672)	Gagnon et al., 2010
	T178–FAM	TTTGCAGACCCGGAAACCACATACTGGA	609 (JQ002672)	Gagnon et al., 2010
MS2	T210-F	TGGCACTACCCCTCTCCGTATTCAC	289 (NC.001417)	Liu et al., 2011a
	T211-R	GTACGGGCGACCCCACGATGAC	387 (NC.001417)	Liu et al., 2011a
	T212-TET	CACATCGATAGATCAAGGTGCC	330 (NC.001417)	Liu et al., 2011a

Note: (+C) or (+T) indicates a locked nucleic acid (LNA).