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Genetic diversity of porcine group A rotavirus strains in the UK

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ABSTRACT

Rotavirus is endemic in pig farms where it causes a loss in production. This study is the first to characterise porcine rotavirus circulating in UK pigs. Samples from diarrheic pigs with rotavirus enteritis obtained between 2010 and 2012 were genotyped in order to determine the diversity of group A rotavirus (GARV) in UK pigs. A wide range of rotavirus genotypes were identified in UK pigs: six G types (VP7); G2, G3, G4, G5, G9 and G11 and six P types (VP4); P[6], P[7], P[8], P[13], P[23], and P[32]. With the exception of a single P[8] isolate, there was less than 95% nucleotide identity between sequences from this study and any available rotavirus sequences.

The G9 and P[6] genotypes are capable of infecting both humans and pigs, but showed no species cross-over within the UK as they were shown to be genetically distinct, which suggested zoonotic transmission is rare within the UK. We identified the P[8] genotype in one isolate, this genotype is almost exclusively found in humans. The P[8] was linked to a human Irish rotavirus isolate in the same year. The discovery of human genotype P[8] rotavirus in a UK pig confirms this common human genotype can infect pigs and also highlights the necessity of surveillance of porcine rotavirus genotypes to safeguard human as well as porcine health.

1. Introduction

Rotaviruses have a broad host range that includes mammalian and avian species. In children, group A rotavirus (GARV) is the leading cause of severe gastroenteritis worldwide, and is associated with significant morbidity and mortality, with most children having been exposed by the time they are 5 years old (Tate et al., 2012). In pigs, rotavirus has a significant economic impact through loss in production and is most prevalent in neonatal pigs (<7 days) and piglets at the time of weaning (21–28 days) (Katsuda et al., 2006; Svensmark et al., 1989). Rotavirus can be transmitted zoonotically between pigs and humans. To date there are no reported studies of rotavirus genotypes in symptomatic UK pigs.

Rotaviruses belong to the *Reoviridae* family. They are non-enveloped, double stranded RNA viruses with a segmented genome. The 11 genome segments code for six structural proteins (VP1–4, 6–7) and six non-structural proteins (NSP1–6). There are eight different serogroups of rotavirus (Group A–H), all of which are found in animals or birds (Kindler et al., 2013; Molinari et al., 2014), but only A–C are found in humans (Estes and Cohen, 1989). Pigs are affected by rotavirus serogroups A, B, C, E and H (Molinari et al., 2014; Pedley et al., 1986). The outer capsid of the virus particle is constituted of VP7 (a glycoprotein) and VP4 (a protease sensitive protein), both elicit neutralising

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antibodies and form the basis of the dual classification of rotaviruses into G and P types, respectively (Estes and Cohen, 1989; Estes and Kapikian, 2007). To date, 27 G-types and 37 P-types of GARV have been identified (Matthijnssens et al., 2011; Trojnar et al., 2013).

Genotype diversity among rotavirus strains is generated by genetic drift, through the accumulation of point mutations, leading to genetic lineages within genotypes and monotypes within serotypes that possess altered epitopes and specific antibody recognition patterns (Coulson and Kirkwood, 1991). In addition, due to the segmented nature of the rotavirus genome, gene reassortment which can take place during co-infection with more than one strain can lead to further rotavirus strain diversity of co-circulating strains. The widespread presence of rotaviruses throughout the animal kingdom constitutes a large reservoir of rotavirus strains, and interspecies transmission combined with reassortment can lead to the emergence of novel or unusual strains that may spread globally. Numerous reports have described interspecies transmission leading to sporadic cases of human disease with rotaviruses from different animal species origin (Ben Hadj Fredj et al., 2013; Doan et al., 2013; Luchs et al., 2012; Mukherjee et al., 2013; Papp et al., 2013). The emergence of epidemiologically important strains such as G9P[8] globally, G10P[11] in India and G8P[4] in Africa, Europe and the USA, in the human population is postulated to have resulted from reassortment with animal strains leading to host adaptation and spread (De Donno et al., 2009; Iturriza-Gomara et al., 2000a; Jere et al., 2011; Leite et al., 2008; Nyaga et al., 2013; Pietsch et al., 2009; Ramani et al., 2009; Than et al., 2013; Weinberg et al., 2012). Worldwide, common porcine rotavirus genotypes are G3, G4, G5, G11 and P[6], P[7], P[13], P[19], P[23], P[26], P[27] (Martella et al., 2010). In Europe, genotypes G1–6, 9–12 and P[6]–P[10], P[13], P[22], P[23], P[27] and P[32] have been identified in pigs (Collins et al., 2010a,b; Midgley et al., 2012).

The aims of this study were to genotype rotavirus in symptomatic UK pigs, to determine the likelihood of zoonotic transmission between pigs and humans within the UK and to compare porcine rotavirus in the UK to genotypes prevalent in Europe and the rest of the world. The findings from the study will not, in themselves, improve biosecurity but will contribute to a better understanding of the potential threat of zoonosis.

2. Methods

2.1. Sample collection

Porcine faecal and intestinal content samples were collected from UK pigs; 66% were obtained from the Animal Health Veterinary Laboratories Agency (AHVLA), 34% samples were referred directly to our lab from veterinarians. The samples obtained from the AHVLA had previously tested positive for rotavirus using gel electrophoresis. Other samples were suspected rotavirus infection and were confirmed using RT-PCR (described below). In total, there were 63 samples from 54 different locations between autumn 2010 and spring 2012. The distribution of these samples in the UK is shown in Fig. 1. All samples were obtained and analysed in accordance with the University of Nottingham ethical guidelines.

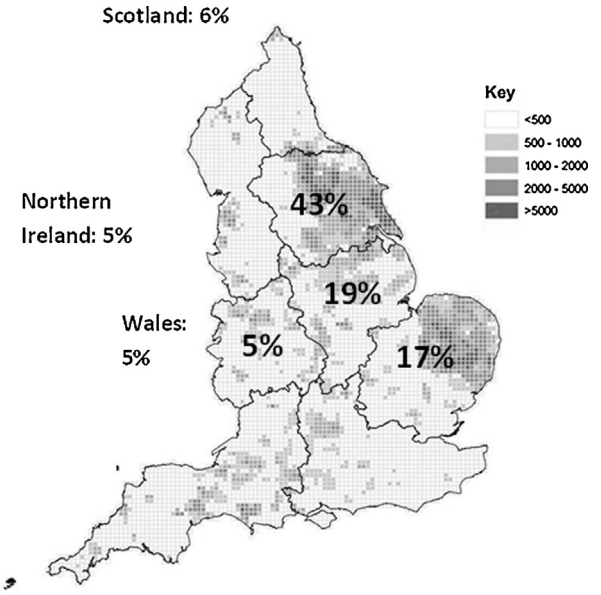

Fig. 1. Map of England showing the distribution of pigs per 5 km^2 in 2010 separated by region, adapted from DEFRA (2010). Percentages on map represent the percentage of samples from each region of England and the percentage taken from Scotland, Wales and Northern Ireland.

2.2. RNA preparation

Nucleic acid extraction was carried out with QiaXtractor platform (Qiagen) using the specified plastics and the VX reagent kit, as per manufacturers' instructions, from 10% faecal solutions in Dulbecco's modified Eagle's Medium (DMEM).

2.3. RT-PCR amplification of VP7 and VP4

VP7 and VP4 rotavirus genes were amplified from extracted nucleic acids by RT-PCR using methods and primers previously described by Gomara et al. (2001), Gray and Iturriza-Gomara (2011) and Gentsch et al. (1992). Samples producing a band for either VP7 or VP4 were considered positive. Samples that did not amplify in the VP7 and VP4 assays were considered negatives as they were also negative in a VP6-specific qPCR (Gomara et al., 2002). PCR products were purified using QIAQuick PCR Purification Kit (Qiagen) and the forward and reverse strands of VP7 and the VP8* portion of VP4 genes were sequenced using Sanger sequencing (MWG Eurofins) and the same primers as for amplification. Sequences have been added to Genbank database VP7 accession numbers KJ135124–KJ135172 and VP4 accession numbers KJ135173–KJ135220.

2.4. Sequence analysis

Genotypes were determined using the RotaC genotyping tool (Maes et al., 2009) and compared to similar