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Epidemiological analysis of porcine rotavirus A genotypes in Germany

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ABSTRACT

Group A porcine rotaviruses are a global threat to animal health in stock breeding. While certain genotypes have shown predominance in other countries, data from Europe's second largest swine population is still scarce. Therefore, porcine rotaviruses taken from different areas of Germany were genotyped to create a basis for comparison with data from neighboring countries. In addition, the potential predominance and regionality based on regions (federal states) have been investigated by examining 101 samples.

The study revealed the dominance of the VP7 genotypes G9, G4, G5 as well as VP4 genotypes P[23], P[6], P[32]. The most common genotype combinations were G9P[23], G4P[6], and G9P[32]. Analysis focusing on the regionality aspect revealed that areas with high pig populations promote the emergence of dominant genotype combinations. However, pig populations in Germany cannot be considered individually and therefore results were put into international context, taken from already published genotyping data. In consequence, our data contributes to the fundamental understanding of regional and supraregional rotavirus epidemiology. The detected genotypes provide a basis for prospective porcine rotavirus surveillance, that first of all helps to identify interspecies transmission. Furthermore it may provide supporting data for the selection of particular genotypes, suitable for the production of porcine rotavirus A vaccine candidates.

1. Introduction

Rotaviruses (RVs) can affect the health of mammalia and birds causing severe diarrhea and subsequent dehydration. RVs are responsible for globally 215.000 child deaths per year (Tate et al., 2016). Although the incidence of rotavirus (RV) infections is correlated with the gross national product, it is a global threat (Patel et al., 2013). In consequence, human RV vaccine programs have been established all over the world. In piglets, diarrhea and subsequent clinical symptoms are most frequently observed from 7 to 60 days (Zimmerman, 2012), but also younger piglets are affected. Germany has the second largest pig livestock in the European Union (Agriculture, forestry and fishery statistics op., 2016 p.103). Recent data seize 27 million pigs, including 7.9 million piglets (Federal Statistical Office 2017, p.19). RVs not only cause massive consequences for animal welfare, but also for economic efficiency with regard to routine production schedules within farms. Differences in breeding procedure, herd size, and the multifactorial character of diarrhea complicate the definition of specific economic losses caused by RV diarrhea. However, they can be substantial, taking into account an average mortality rate of 5% and 1 kg lower average weight at weaning. Even though porcine RV diarrhea is a widely spread infectious disease no licensed vaccine for pigs is available in Europe. Hence, Germany's high swine population, situated in Central Europe, can be seen as an indicator for relevant circulating RV genotypes. RVs are classified into ten groups (A-J) which are based on the antigenic characteristics of VP6, one of the structural proteins. Group I and J can be defined preliminary (Banyai et al., 2017; Mihalov-Kovács et al., 2015). Of clinical and economical relevance for pigs are groups A, B, C, E and H, whereas diagnostic in livestock breeding mainly is focused on rotavirus A (RVA), which is considered to be the primary cause of diarrhea in pigs (Marthaler et al., 2014). RV genus belongs to the family of *Reoviridae* and is a nonenveloped virus, featuring a double-stranded and segmented RNA genome. The particles are triple-layered, in which the structural proteins VP4 and VP7 form the outer layer. Sequence analysis of the glycoprotein VP7 (G-type) and the protease sensitive protein VP4 (P-type) genes is a widely accepted method to determine RV genotypes. In 2008, the Rotavirus Classification Working Group proposed the extended full-genome sequence classification system, which is based on the sequence data of all 11 gene segments (Matthijnssens et al., 2008). Based on the dual typing system (G-/P-types) many genotype studies for porcine RVA have been published as recently reviewed by Vlasova et al. (2017) and Papp et al. (2013).

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Nonetheless, data on present RV genotypes in pigs bred in Germany is not available and thus comparison to data taken from studies worldwide, especially to neighboring EU-countries is difficult. Since German pigs and piglets are exported alive, it is crucial to see RV epidemiology in an international context and porcine RVA genotyping data is required for comparison with international epidemiologic studies. For this reason we designed this study to evaluate whether RVA with specific genotypes in German pigs show a regional distribution and if the possible pattern fits in the European context. In addition, we investigated whether particular G- and P-genotypes and their combinations are emerging thus providing a basis for comparison with already available data from other countries.

2. Materials and methods

2.1. Origin of samples

The study was performed using 101 fecal swabs, feces, and small intestine samples that had been sent to Vaxxinova GmbH diagnostic facility between March 2016 and March 2017. The samples which were proven RVA or RVA and rotavirus C (RVC) positive were organized by the federal states and administrative districts they came from. Two different samples from every available administrative district were chosen randomly for RVA genotyping.

2.2. RNA preparation

The nucleic acid extraction was performed using Qiagen MagAttract 96 cador Pathogen Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with 10% fecal solutions in Phosphate-Buffered Saline (PBS) respectively fecal swabs mounted in PBS. Tissue samples were pretreated with a ball mill before performing the nucleic acid extraction.

2.3. RT-qPCR assay rotavirus A, C

The samples were tested for the presence of RVA and RVC RNA using the modified method previously described by Marthaler et al. (2014).

2.4. One-step amplification of gene segments 4 (VP4) and 9 (VP7)

The extracted RNA was used for VP7- and VP4-gene amplification using primers previously described by Fujii et al. (2012), Gentsch et al. (1992), Isegawa et al. (1993), Iturriza Gómara et al. (2004) and Simmonds et al. (2008). The amplification of the gene segments was carried out in a one-step polymerase chain reaction (PCR) utilizing the qScript® XLT 1-Step RT-PCR Kit (Quantabio, Beverly, MA) following the manufacturer's instructions using the forward and reverse primer (10 µmol) and underwent 5 min at 95 °C followed by immediate chilling in ice cold water. Next, mastermix was added and the reaction was carried out under the following conditions: reverse transcription for 20 min at 48 °C, 3 min initial denaturation at 94 °C, 30 cycles of 15 s denaturation at 94 °C, 40 s at the primer-dependent annealing temperature described below, 50 s elongation at 68 °C, followed by a final extension for 10 min at 72 °C.

Annealing temperatures for primers were 50 °C for Bov4Com5/Bov4Com3 (Isegawa et al., 1993) and VP4F/VP4R (Simmonds et al., 2008), 55 °C for VP7 F/VP7 R (Fujii et al., 2012) and VP7-F/VP7-R (Iturriza Gómara et al., 2004), and 47 °C for con 2/con 3 (Gentsch et al., 1992).

Every scheduled sample (see 2.1) underwent amplification with one primer pair for the VP7 and one for VP4 gene segment. The PCR products were analyzed on 1,5% agarose gels and visualized under UV light. If the result was negative, i.e. no amplicon was present at the expected base pair length, the amplification was repeated with the other previously described primer pairs until the amplification was successful. In case none of the primers for VP7 or VP4 or even both lead to a band the sample was defined as nontypeable.

PCR products from positive samples were purified and sequenced at Seqlab Sequence laboratories (Göttingen, Germany) via Sanger sequencing with the same primers used for the PCR.

2.5. Sequence analysis

Sequence data was analyzed using the software Chromas (version 2.6.4; Technelysium Pty Ltd) and for the determination of genotypes the RotaC genotyping tool was applied (Maes et al., 2009). In addition, all sequences were compared with sequences available from NCBI blast genbank database (NCBI Resource Coordinators, 2016) under the default settings.

3. Results

3.1. Rotavirus genotypes in german pig population

The samples used for determining RV genotypes in the German pig population originated from ten different federal states. Overall, we found 27 different genotype combinations in the 79 typeable samples. Among these, 8 different G-types (depicted in Fig. 1) and 7 different P-types (depicted in Fig. 2) could be detected. 22 samples were untypeable. The most frequent VP7 genotypes were G9 (38%), G4 (31%), G5 (14%) and G11 (6%). Prevalent VP4 genotypes were P[23] (37%), P[6] (29%), P[32] (18%), and P[13] (9%). The outstanding dominant genotype combinations were G9P[23] (23%), G4P[6] (19%), and G9P[32] (8%). Only two samples could be defined by either G- or P-type since their sequence results laid below the cut-off value of 80% as proposed for VP4 and VP7 by Maes et al. (2009). Therefore, they are considered to be new genotypes (suggested GX and P[X]), which were not yet described.

3.2. Genotype prevalence in federal state-centered view

Analyzing the genotype distribution on a narrow federal state based level, we found a high occurrence of genotype G9 in specific combinations. Hereby, G9P[23] represents the most common genotype combinations in the district of Baden-Württemberg, Saxony-Anhalt as well as Thuringia and belongs to the dominant combinations in Lower Saxony, North Rhine-Westphalia, and Bavaria. The most frequent RV combination found in North Rhine-Westphalia and Lower Saxony is G4P[6]. The third noticeable genotype combination is G9P[32], one of the dominant types in Lower Saxony and North Rhine-Westphalia.

Getting focused on the absolute distribution of RV genotypes, five noticeable combinations are emerging. In addition to the regional dominance as described in the above-mentioned paragraph, G9P[23] is collaterally present in seven different federal countries. The same conclusion can be made for G4P[6] which is in fact dominant in some regions, but is additionally widely spread over five different federal countries. The third dominant genotype G9P[32], was only detected in two different federal states. In contrast to this, two genotypes (G4P[23] and G5P[23]) weren't noticeable under the aspect of dominance within single federal states, but are widely spread throughout five different federal states each. Additionally, these states are located in different directions of Germany. The occurrence of all genotype combinations found within the federal states is listed in Table 1.

4. Discussion

4.1. Regional emerging genotypes and genotype combinations

The collected data shows the predominance of certain RVA genotype combinations as well as specific VP7 and VP4 genotypes. It is