Molecular characterization of P[8] human rotaviruses isolated from pigs from Disaneng Village, South Africa

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ABSTRACT

Rotavirus infection threatens pig farming because it serves to be the main income for rural poor farmers who belong to less developed areas and rely solely on the profits of this venture. In addition, it plays an important role in the swine industry especially in cases that can remain without viruses being identified. An exploratory study was undertaken; to report on the presence of P[8] rotavirus found in pigs from Mahikeng and the surrounding areas of Mahikeng, North West Province, South Africa. Diarrhea stool samples of pigs were collected around Mafikeng using aseptic techniques. Isolates that tested positive for rotavirus were P6 and P11, from which RNA was isolated using the Trizol method. cDNA was synthesized and characterized according to VP4 types using VP4 primers (Con2, Con3) and products were sequenced and analysed. Using the RotaC software, the P type detected from samples P6 and P11 was P[8]. Future studies from the current one, requires VP7 characterization for binary characterization of the G and P rotavirus strains and to expand the study to other areas in the North West Province. Public awareness about rotavirus infections thus needs to be encouraged; target group being, pet owners, parents to young children and people in rural areas that might be in need of this information.

Key words: Swine, zoonotic, diarrhea, gastroenteritis.

Abbreviations: VP, Viral proteins; G, glycoprotein; P, protease sensitive; NSP, Non-structural proteins.

INTRODUCTION

Pig farming serves as the main source of income for rural poor farmers who reside in to less developed areas (Malik et al., 2014). This activity may be threatened by swine diarrhea which plays an important role in the swine industry especially in cases where the virus is unidentified (Shan et al., 2011). Currently, viral gastroenteritis has been identified as the most significant public health problem throughout the world, predominantly in developing countries (Chan et al., 2011). Furthermore, rotavirus has been identified among the agents responsible for a significant proportion of neonatal diarrhoea in mammals and poultry (Miyazaki et al., 2013; Malik et al., 2014; Katsuda et al., 2006). Rotavirus infections are mostly encountered during two critical points of a piglet’s life: the suckling period and after weaning (Theuns et al., 2014b).

The morphological appearance of rotavirus resembles a wheel with short spokes and a well-defined rim (Estes and Cohen, 1989). The mature virus particles possess a triple-layered icosahedral protein capsid which is approximately 75 nm in diameter. The core contains 120 copies of VP2, transcription enzyme complex of VP1 (RNA dependent RNA polymerase, VP3 (guanylyl and methyl transferase) and a genome that consists of 11 segments of RNA which encode 12 viral proteins; 6 structural proteins (VP1, VP2,
VP3, VP4, VP6 and VP7) and 5 or 6 (depending on the strain) non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6) (Farkas et al., 2009; Ruiz et al., 2009). All genomic segments are monocistronic except for the shortest segment, which encodes NSP5 and NSP6 (Zeller et al., 2012). Complete genomic sequencing of all the 11 ds-RNA segments has revealed heterogeneity and this has provided evidence for frequent similarities between the evolution of human and swine rotaviruses (Martella et al., 2010). Recently, the 11 genomic segments have been assigned letter codes for each gene and have been classified into 6R, 6C, 7M, 35P, 13I, 27G, 16A, 6N, 8T, 12E and 8H genotypes based on specific nucleotide sequence identity (Rajendran and Kang, 2014).

It has been suggested that pig and human rotavirus strains possess a common evolutionary ancestor (Theuns et al., 2014b) and swine are natural reservoirs of a large variety of viruses capable of causing human diseases (Shan et al., 2011), this includes swine group A rotaviruses. This phenomenon plays a great role in generating newly adapted emerging strains (Parra et al., 2008), thus playing an important role in the diversification of group A rotaviruses in human and other animal species (Collins et al., 2010). In addition, swine rotavirus serves as a serious threat to the human population due to the possibility of genetic reassortment; that is, exchange of gene segments between animals and human to produce animal-human reassortants. As such, swine rotaviruses are considered to have significant zoonotic impact (Ramani and Kang, 2007).

Rotavirus infection is commonly observed in 1-4 weeks old or weaned piglets but in mature herd or piglets aged 1 month and older, the infection may not cause clinical disease unless it is complicated by other pathogens (Dhama et al., 2009). This is because as the pigs mature, susceptibility to the disease decreases due to acquired immunity from previous infection (Estes et al., 2007). The rotavirus associated with viral gastroenteritis is of great concern not only for the economic impact but also as a potential source of heterologous infection in humans and other animal species (Dhama et al., 2009). This may lead to the generation of reassortants with a wide variety of other mammalian rotaviruses including the human rotaviruses thus leading to the fast evolution of rotaviruses because of point mutations and reassortants generation especially in the case of multiple infections (Malik et al., 2014).

Swine rotaviruses are distributed worldwide (Teodoroff et al., 2005) and they belong to groups A (which is the most prevalent), B, C and E (Martella et al., 2007). Furthermore, group A rotaviruses are the most important due to their high prevalence and pathogenicity in humans as well as in pigs (Papp et al., 2013). Due to sequence analysis of VP4 or VP7 genes, P or G serotypes have been identified within group A rotaviruses. These are the genes encoding the outer capsid proteins and they are of utmost importance because they induce neutralizing antibodies (Matthijnssens et al., 2008b) and are the basis of binary classification system defining G types (glycoprotein) and P types (protease sensitive), respectively (Matthijnssens et al., 2008b). Partial or complete sequence of all 11 gene segments are analyzed and compared with other sequences and this approach allows direct determination of genetic relationship (Matthijnssens et al., 2008b).

Using the binary classification system, 12 different G genotypes G1 to G6, G8 to G12, and G26 and 13 different P genotypes [P1], [P5] to [P8], [P11], [P13], [P19], [P23], [P26], [P27], [P32], and [P34] have been reported in pigs worldwide (Martella et al., 2010). While genotypes G2, G3, G4, G5, G9, and G11 in combination with [P6], [P7], [P13], [P23], or [P27] were demonstrated in Belgium (Theuns et al., 2014a) and in Japan serotypes G9 with P serotypes [P13] and [P23] have also been reported (Teodoroff et al., 2005). According to Martella et al. (2010), sporadic G genotypes are G1, G6, G8, G10, G12 and those occurring epidemically are G2, G3, G4, G5, G9, G11.

The hybridization technique has been very useful to investigate possible reassortment events between human strains belonging to different genogroups (Nakagomi et al., 1990; Matthijnssens et al., 2008a) or between human and animal strains (Nakagomi and Nakagomi, 2002; Matthijnssens et al., 2008a). A surveillance study may reveal the presence of uncommon serotypes in a human population that may be commonly found in domestic animals (Ramani and Kang, 2007; Malik et al., 2005). Therefore, it is of high importance to conduct a surveillance study and accumulate information on the genetic heterogeneity of swine viruses to identify unusual strains (Collins et al., 2010).

To date, only few studies have provided detailed information on the prevalence of swine rotavirus strains in the North West Province or in South Africa. This study has identified a gap to be filled in the current body of knowledge and will be among the first to report on the findings of the presence of swine rotavirus in the North West Province, South Africa.

**MATERIALS AND METHODS**

**Sample collection and preparation**

An exploratory study was under taken, where diarrheal stool samples of pigs were brought into the NWU laboratories from various farms around Mahikeng. Aseptic techniques were used for collection of samples, as previously described by Speich et al. (2010). From these samples, stool suspensions were made using distilled water by keeping them in water overnight and then the suspensions were treated with Vertrell for cleaning. RNA was isolated using the Trizol method described by Simms et al. (1993). RNA that was recovered was suspended in TE buffer and stored at -80°C.
Genotyping of VP4 gene

cDNA was synthesized using the SuperScript III One Step RT-PCR System with Platinum Taq (Invitrogen, Thermo Fisher Scientific, USA) following the manufacturer’s instructions. Gene-specific primers used for PCR amplification were VP4F (5’-ACG ACA CCT GGC GTA ATT GT-3’) and VP4R (5’-GCT GTC CTA GCG TTA GGT GT-3’) and the conditions set on the thermal cycler (BIO RAD-TM100, USA) were as follows: 1 cycle of cDNA synthesis at 60°C for 30 min, 40 cycles of denaturing at 94°C for 2 min, annealing at 60°C for 30 s, extension at 68°C for 60 s and 1 cycle of final extension of 68°C for 5 min.

Nucleotide sequence analysis

Rotavirus strains from the NCBI database were included in the study for genomic analysis. The aim was to compare VP4 genetic composition of obtained isolates (from the study) with the strains from the online database. The nucleotide sequences obtained were aligned and edited using the Bio-Edit Sequence Alignment Editor (version 7.2.6) and then compared with the corresponding sequences of selected rotavirus strains available in the GenBank database. The phylogenetic analysis was performed using Mega (version 7.0.26) (Figure 3).

RESULTS AND DISCUSSION

Two stool samples that tested positive for rotavirus were watery, dark colored with a very foul smell, especially sample P11. Samples were first tested using the immunochromatography strip test specific for rotavirus called VIKKIA Rota-Adeno kit. When samples were viewed through the TEM, an image shown in Figure 2A and B was generated, which resembled an icosahedral shape as described by Farkas and Jiang (2009). The virus measured at approximately 75 nm was found in all samples. The cDNA synthesized using the VP4 primers was constructed and used for PCR amplification. About 8 µL of the cDNA together with 5 µL of the loading dye were loaded onto the gel bands separated and measured at 551 bp.

This is one of the studies that addresses the identification of rotavirus strains (Figure 1) in Mahikeng, North West Province, in particular to swine rotavirus. Surveillance studies such as these, are crucial because they help in identifying prevalent strains in a given area which could shed light into their antigenic and genome properties (Trojnar et al., 2013). In addition, determining the G and P-types of the rotavirus strains will eventually assist in the development of efficient vaccines which have the ability to cover the whole spectrum of rotaviruses.
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Figure 2 A and B: Transmission Electron Microscope images of isolates P6 and P11, respectively. Stool samples were taken to the Laboratory for Electron Microscopy Chemical Resource Beneficiation Potchefstroom Campus North-West University, and were investigated by Transmission Electron Microscopy (TEM) using negative staining.

Figure 3: Phylogenetic analysis performed using the maximum likelihood method provided by MEGA 7.0. Nucleotide accession numbers collected from GenBank: MG181593.1, KT921094.1, KJS60526.1, AB905370.1, KJ753730.1, MF407507.1, KX315700.1, KX646589.1, KP222878.1, JF790352.1, KP793016.1, MG676189.1, MF186788.1, KX774441.1, KX498078.1, KX011952.1, KP017295.1, MF469269.1.

which could also reduce the dramatic effects of the disease (Trojnar et al., 2013).

Knowledge of the prevalence of swine rotavirus in Mahikeng, North West Province is not known; considering that pigs are known to be a source of zoonotic pathogens which could be transmitted to humans indirectly or directly. Some of these pathogens (rotavirus included) may not necessarily cause disease in the animal itself (asymptomatic reservoir pigs) but may cause serious illness in humans (Machnowska et al., 2014).

The objective of the study was to screen stool samples for rotavirus infections, in the Mahikeng area. Stool samples that tested positive for rotavirus were collected from a private farm from Disaneng Village, which is about
34 km outside Mahikeng. Disaneng village, is one of the regions where people live in close proximity with their livestock, which could pose as a potentially serious public health threat exposing humans to infectious animal pathogens such as rotavirus (Amimo et al., 2015). The prevalence of rotavirus in this region is not known, thus surveillance studies such as this one are crucial with the hope of shedding light about swine rotavirus to the public, thus reducing the transmission to humans as pigs play an important role as reservoirs for human rotavirus strains (Palombo, 2002).

The P11 and P6 (isolates from this study) clustered with human P[8] isolates from the database showed a 99% nucleotide similarity to them on the first hit. The isolates from this study were classified as P[8] genotypes and were highly genotypically similar to human rotaviruses based on nucleotide comparison using the NCBI database, except ours were isolated from pig stool samples. All the strains from the database were isolated from humans from different parts of the world and were classified as P[8] genotype. The P11 isolate for instance showed 99% nucleotide similarity with isolates from children from the Chokwe District, Southern Mozambique (Langa et al., 2016). In addition, the P11 isolate also showed 99% similarity to a strain isolated from Memphis, USA from a 5 year old female in March 2013.

The genotype P[8] in combination with the G12 was also determined in the study. The rotavirus strain was isolated in the year 2011 and showed a genotype of the G12 P[8] combination. The genotype P[8] in combination of G12-type are the most frequent combinations of rotaviruses found throughout the world (Matthijnssens et al., 2011) and P[8] genotypes are commonly associated with human infections (Santos and Hoshino, 2005). This information therefore corresponds with Langa et al. (2016) where the majority (57%) of their samples was of the P[8] genotype in combination with G12. However, in a study conducted in India in different districts of Odisha, the most common strain detected (at 62%) from children experiencing diarrhoea, had the G/P type combination of G1P[8] (Mohanty et al., 2017). Similar results was seen in a study done in Africa, where the predominant G/P combination was G1P[8] isolated from children experiencing diarrhea (Seheri et al., 2014). Variation of genotypes can be seen from region to region and country to country, because each year strains cannot be entirely predicted due to the complex epidemiology of rotavirus; they could fluctuate over time (Seheri et al., 2014).

The pig's anatomy is very similar to that of humans (Meuren et al., 2012) and also based on their genetics, they are comparable to each other (Hart et al., 2007). In addition to this, transmission between human and pigs is possible through direct and indirect contact particularly in rural areas like Disaneng. There is close proximity of animals and humans and possible contamination of food and water, thus increasing the risk of zoonotic transmission (Steyer et al., 2008). Zoonotic transmission of swine rotavirus occurs and based on the segmented nature of rotavirus; they are able to re assort during co-infection therefore, whole-genome sequencing is a useful tool as it has provided new and important information on the complex origin of strains and reassortment of human and animal strains (Ghosh and Kobayashi, 2011).

Limitations of this study include: how stool samples were transported and stored considering that the place of collection was far from the Virology Laboratories, and lack of VP7 (G) gene identification. But analysis of VP4 and VP7 genes is not sufficient information on the overall genetic diversity (Ghosh and Kobayashi, 2014), so whole genome sequencing still needs to be done.

**Conclusion**

This is the first study to report on the presence of rotavirus infections in Mahikeng. We were able to identify rotavirus from pigs which were predominantly identified to be human strains. It is likely that interspecies transmission might have occurred and this could pose serious threat to the community. Sources of rotavirus infections of humans to pigs and vice versa could have arisen from contaminated water sources, raw food considering close proximity of both humans and pigs.

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