Research article

Prevalence and genomic characterization of rotavirus group A genotypes in piglets from southern highlands and eastern Tanzania

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ABSTRACT

Animals have been identified as the potential reservoirs of rotavirus group A (RVA) for human infection. However, very little is known regarding the genotype and genomic profiles of circulating RVA in Tanzanian piglets. The rotavirus genetic diversity and genome analysis was assessed among piglets from Southern highlands and Eastern Tanzania. A total of 241 faecal samples were collected from piglets in the regions of Mbeya, Iringa, and Morogoro. RVA was detected and genotyped using reverse transcription polymerase chain reaction (RT-PCR). Sanger dideoxynucleotide cycle sequencing of the viral protein (VP) 4 and VP7 genes was afterwards performed to confirm the RT-PCR results. Selected genotypes were subjected to whole genome sequencing. The overall prevalence of RVA was 35.26% (85/241) in piglets (30.58% in Mbeya, 43.75% in Iringa and 31.16% in Morogoro). Upon genotyping, the G genotypes were G4 (26), G9 (10), G3 (6), G5 (3) and the remaining 40 were untypeable, while the P genotype, were P[6] (35), P[13] (3) and the remaining 47 were untypeable. The G4P[6] were the predominant genotype followed by G3P[6], G3P[13], G4P[13] and G5P[13] were most common genotypes combinations. On phylogenetic analysis, G4 was grouped to lineage V, sublineages VIIa and VIIc, G9 to lineage I, G5 to lineage II, G3 to lineage IV, P[6] to lineage V and sublineage Ic and the P[13] to lineage IV. We revealed amino acid differences between the circulating G4 and the G4 in the ProSystems RCE vaccine used in pigs. The whole genome reveals genomic constellation of G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1, G5-P[x]-I5-R1-C1-M1-A8-N1-Tx-E1-H1, G3/G4-P[13]/P[6]-Ix-R1-C1-M1-A8-N1-T1-E1-H1, G3-P[6]-Ix-R1-C1-M1-A8-N1-Tx-E1-H1 and G9-P[x]-Ix-R1-C1-M1-Ax-N1-Tx-E1-H1. The VP7 gene of G9, the VP4 gene of P[6] and NSP4 (E1) gene of some genotypes clustered together and closely related to humans origin or porcine-human reassortant strains with nucleotide similarities ranging from 97.90% to 99.74% from neighboring countries, implying possibility intragenogroup reassortment and interspecies transmission. The higher strain diversity observed within the gene segments highlight the importance of genomic analysis and continuous monitoring of RVA genotypes. Further research is needed to determine the risk factors associated with RVA infection in Tanzanian pigs in order to properly design a control program.

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1. Introduction

Rotavirus is a major cause of diarrhea in children under the age of five and pigs worldwide (Komoto et al., 2016; Liu et al., 2012; Vidal et al., 2018; Xue et al., 2018). Rotavirus causes severe diarrhea in piglets, which leads to dehydration, stunted growth, and death. RVA has been shown to cause massive economic losses in domestic pigs due to its negative effects on growth and productivity (Martella et al., 2006a,b; 2010; Midgley et al., 2012). However, animals are also regarded as rotavirus reservoirs of genetic and antigenic human rotavirus (Gómara et al., 2004; Martella et al., 2006a,b; Nakagomi and Nakagomi, 2002). There is evidence of direct RVA interspecies transmission from domestic pigs to humans (Yodmeeklin et al., 2017; Tacharoenmuang et al., 2021). As a result, studying animal rotaviruses is a critical step towards better understanding rotavirus epidemiology, evolution, and ecology. There is no data on the genetic diversity of rotavirus in piglets in Tanzania, and only one study reported the prevalence of RVA in Arusha, Tanzania (Gachanja et al., 2016). Data on animal origin genotypes such as G8P[4], G8P[6], G8P[8], and G3P[6] in under-five children in Tanzania are seldom available (Malakalinga et al., 2019). A large population of Humans in Tanzania live in close proximity to animals, putting them at risk of zoonotic pathogens (Assenga et al., 2015; Kamaghe et al., 2014; Lupindu et al., 2015; Zhang et al., 2016). Interspecies transmission is known to drive rotavirus diversity (Dóró et al., 2016; Vlasova et al., 2017), which could affect vaccine effectiveness in humans and animals. Consequently, data on the burden and diversity of RVA in pigs is critical for developing strategic RVA control strategies to protect human and pig health in the Country. The virus genome consists of 11 segments of double stranded RNA that code for six structural proteins (VP1-VP4, VP6 and VP7) and five non-structural proteins (NSP1-NSP5/NSP6) (Estes and Kapikian, 2007). The VP7 and VP4 proteins form a dual classification system of rotavirus, play a very important role in inducing genotype-specific neutralizing antibodies and are used for vaccine development (Gentsch et al., 2005; Matthijnssens et al., 2008a). The VP7 protein is glycoprotein referred to as the G; G1, G2, G3,......G(nth) (Greenberg and Estes, 2009). Because of the discrepancy between neutralization assay and sequencing for the VP4 (protease-sensitive protein or P type antigen), a dual system for P typing was proposed. Whereas P serotypes are identified by serotype numbers (e.g., P1, P2,…P nth) and P genotypes are identified by numbers in brackets (e.g., P[1], P [2],…P[nth]) (Greenberg and Estes, 2009). To date The Rotavirus Classification Working Group (RCWG) has identified approximately 41 G genotypes and 57 P genotypes in humans and animals worldwide (RCWG, 2020). Rotavirus is classified into eight groups (RVA-RVI) based on immunoreactivity with specific monoclonal antibodies (Matthijnssens et al., 2012), with rotavirus group A (RVA) being the most important group that affects both humans and animals (Matthijnssens et al., 2011). The RVA G and P genotypes that are commonly found include G3–5, G9, and G11, as well as P[6], P[7], P[13], and P[19] (Amimo et al., 2015; Dhama et al., 2009; Martella et al., 2010).

The newly established classification of rotavirus by whole genome sequencing (WGS) (Matthijnssens et al., 2008a,b) pave understand of reassortment events and interspecies transmission towards better knowledge on the epidemiology and evolution (Matthijnssens et al., 2008a,b). The methods involve sequencing all segments and the genotypes are assigned based on nucleotide percentage identity for each segment established by the RCWG (Matthijnssens et al., 2008a,b). The nomenclature of the complete genome is annotated by Gx-P [x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx are used for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/NSP6 (Matthijnssens et al., 2008a,b). The WGS also provides information about genomic constellation and assign the strain in their appropriate genogroup. The example of genomic constellation includes, WA-like (Gx-P[x]-I1-R1-C1-M1-A1-N1--T1-E1-H1), DS-1 (Gx-P[x]-I2-R2-C2-M2-A2-N2-T2-E2-H2) and AU-1(Gx -P[x]-I3-R3-C3-M3-A3-N3-T3-E3-H3) and other several genogroup (Matthijnssens et al., 2008b). Some strain have been reported to have mixed genome constellations due to reassortment and therefore hindering its characterization and tracking by conventional method such as nucleic acids hybridization techniques (Banerjee et al., 2007). Therefore, genome classification by sequencing critical to countries where the genomic data are limited for better understanding the rotavirus epidemiology. The whole genome has been reported to reveal the common origin of human Wa-like rotavirus and porcine rotaviruses, DS-1-like and bovine rotavirus in Belgium (Matthijnssens et al., 2008a,b). The reassortment and interspecies transmission were also reported between human, porcine and bovine rotaviruses in South Korea, Uganda, Cameroon, Ethiopia, South Africa, Togo, Uganda, Zambia, Zimbabwe Nigeria, Thailand and Italy (Bwogi et al., 2017; Komoto et al., 2016; Medici et al., 2015; Mokoena et al., 2021; Tacharoenmuang et al., 2018). The aim of this study was to investigate genetic diversity of pigs RVA in southern highland and eastern Tanzania to comprehend the rotavirus epidemiology, improve biosecurity and to document the zoonotic threats and the phylogenetic relationship to global RVA strains. In addition, RVA strains were compared to the RVA vaccine ProSystems RCE strains used in pigs to investigate antigenic disparities.

2. Material and methods

2.1. Description of the study area and design

This cross-sectional study was conducted in three districts of Tanzania, namely Mbarali, Kilolo and Mvomero which were purposively picked from three regions such as Mbeya, Iringa (Southern highlands) and Morogoro (eastern Tanzania), respectively. Random sampling of faecal samples was done between April, 2019 and May, 2020. Mbarali is located between latitudes S07°41′ and S09°25′ and longitudes E33°40′ and E35°40′ at an altitude ranging from 1000 to 1800 m above sea level (masl). The average rainfall range in the region is between 300 mm and 940 mm per annum. The dry season starts in May and November and the rain season December to April. The human population in the region has been estimated at 300,215 (NBS, 2012) whereas pigs and cattle numbers stand at 70,000 and 198,316 respectively (HWMB, 2020). Kilolo district is located between latitudes 35°54′E and 35°57′E and longitudes 8°01′S and 8°06′S in the Iringa region. The human population is 218,130 (NBS, 2012), pig is 75,000 and cattle is 79,500 (HWKI, 2020). Mvomero is located between 6°07′ and 7°05′ South and 37°17′ and 37°65′ East. The district altitude varies between 300 and 400 masl in Turiani, Mvomero and Mzumbe division up to a range of 1000 and 1700 masl in Mgeta division. The temperature ranges from 19 °C to 31 °C whereas the average rainfall is 1100 mm per annum. In this district the rainfall is bimodal, heavy rains falling between March and May, while the short rains fall between October and December. Human, cattle and pig populations were estimated at 312,109, 125,988 and 50,254 respectively (HWMV, 2020; NBS, 2012).

2.2. Ethical considerations

The permit to research on animal subject was obtained from Sokoine University of Agriculture through the College Research Innovations and Publication Committee of CVMBS (SUA/CVMBS//R.1/2019/1). Permission was also granted by the respective Regional Administrative Secretary (RAS) and District Administrative Secretary (DAS).

2.3. Sample collection

Faecal samples were aseptically collected from piglets (less than 3 months of age). Approximately a pea-size faecal sample was picked from the piglet and placed in duplicate into sterile cryogenic vials (Corning, Lowell, USA). The vials contained 1 mL Trizol reagent (Life Technologies Corporation, Carlsbad, USA) and 1 mL of viral transporting media (Remel Micro test M6, Lenexa, USA). A total of 241 stool samples (84 samples from Mbeya, 80 from Iringa and 77 from Morogoro) were collected from

symptomatic (piglets with diarrhea; a loose or watery stool without blood) and asymptomatic (piglets without diarrhea) piglets. Among 241 samples, 45 samples were collected from piglets with diarrhea and 196 from piglets without diarrhea. Feacal samples were obtained and transported in liquid nitrogen to Sokoine University of Agriculture and deep freezed at −80 °C in ultra-low temperature freezer (NUAIRE, Plymouth, USA) until time of RNA extraction.

2.4. Sample preparation and RNA extraction

Before RNA extraction the samples were allowed to thaw in class II biological safety cabinet (SterilGard, Sanford, USA) and centrifuged at 5000 *g* for 8 min in a 5717R centrifuge (Eppendorf, Hamburg, Germany). Then, 250 μL of supernatant was collected and used for RNA extraction using the direct zol-RNA MiniPrep Kit (Direct zol RNA MiniPrep, Tustin, United States of America) according to the manufacturer's instructions. The final volume of dsRNA after extraction was 50 μL which was deep freezed at −80 °C until time of analysis.

2.5. cDNA synthesis

The cDNA was synthesized using SuperScript® III First-Strand Synthesis System Kit (Invitrogen, Carlsbad, USA) according to instructions of the manufacturer. Before synthesis, 25 μL of the dsRNA was first denatured at 97 °C for 5 min and chilled on ice for 2 min to make single stranded RNA of which 8 μL was needed. The final volume of the cDNA was 20 μL and was stored at -80 °C until molecular analysis.

2.6. Detection of rotavirus group A

Rotavirus group A (RVA) was identified using RT-PCR with primer sets (Macrogen, Seoul, Republic of South Korea) targeting the non-structural protein 3 (NSP3) gene (NSP3-F 5′-ACCATCTACA-CATGACCCTC-3′ and NSP3-R 5′-GGTCACATAACGCCCC-3′) (Amimo et al., 2015; Pang et al., 2004). The Invitrogen Platinum *Taq* kit (Invitrogen, Carlsbad, USA) was used along with these primers following the manufacturer's instructions. The PCR master mix contained 2.5 μL 10× PCR Buffer, 0.75 μL 50 mM MgCl$_2$, 0.5 μL 10 mM dNTP, 0.1 μL Platinum *Taq* DNA polymerase, 1 μL (10 μm) NSP3 forward primer, 1 μL (10 μm) NSP3 reverse primer and 18.15 μL molecular grade water. Then, 1μL of cDNA template, RNA extraction control, positive RVA cDNA control (Rotarix Vaccine and Bovine rotavirus Indiana strain) and PCR control (molecular grade water) as negative control were added to the respective PCR master mix. The PCR conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C of 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The electrophoresis was performed using 3% agarose gel (AMRESCO, Solon, USA) stained with GelRed nucleic acid stain (Biotium, Fremont, USA) at 110 voltage for 1.30 h. The DNA marker (Bionexus, Oakland, USA) of 15 μL was loaded along with the PCR product. The gel visualization of amplicon size was performed using Dual UV Trans-illuminator (Analytic Jena US, Upland, USA).

2.7. Genotyping of rotavirus group A

The G and P genotyping was performed by multiplex reverse transcription nested PCR (RT-PCR) using primer sets and amplification conditions as previously described (Ansari et al., 2013; Gouvea et al., 1990; Gentsch et al., 1992; Gómara et al., 2004). The Invitrogen Platinum *Taq* kits were used for PCR amplification, following manufacturer's instructions. The primer set (VP7-F and VP7-R) (Gómara et al., 2001) for round 1 PCR target the VP7 gene for all RVA, resulting in a band size of 881 bp on gel electrophoresis. The primer sets for round 2, multiplex PCR for G typing targeted G1, G2, G3, G4, G8, G9, G10 and G12 yielding PCR products with band sizes of 618, 521, 682, 452, 754, 179, 266 and 387 bp, respectively (Gouvea et al., 1990; Ansari et al., 2013). The PCR was performed at following conditions; preheating at 94 °C for 4 min for

initial denaturation, followed by 35 PCR cycles at 94 °C for 1 min, 42 °C for 2 min and 72 °C for 1 min and a final extension at 72 °C for 7 min. Whereas for the P typing, the primer set (Con3 and Con2) (Gentsch et al., 1992; Gouvea et al., 1994) for round 1 targeted the VP4 gene for RVA which produces a band size of 876 bp on gel electrophoresis. The round 2 targeted P[4], P[6], P[8], P[9], P[10] and P[11] producing PCR products with a band sizes of 483, 267, 345, 391, 583 and 312 bp, respectively (Ansari et al., 2013; Gouvea et al., 1990; Gómara et al., 2004). The PCR was performed at following conditions; preheating at 94 °C for 2 min for initial denaturation, followed by 35 PCR cycles at 94 °C for 1 min, 45 °C for 2 min and 72 °C for 1 min and a final extension at 72 °C for 7 min. Gel electrophoresis was performed using a 2% agarose gel stained with GelRed at 110 V for 1.30 h. A gel documentation system was used to visualize the amplicon size on the agarose gel.

2.8. Sequencing of structural (VP1–VP3 and VP6) and non-structural (NSP1–NSP5/NSP6) gene

All genes segments were sequenced either partial or full using the Sanger dideoxy sequencing method in the ABI 3130XL using a Big-dye Terminator kit (Applied Biosystems, Carlsbad, USA). For the segment with large base size such as segment 1, 2, 3, 4 and 5 primer walking method was used to cover the entire sequence as previously described (Yodmeeklin et al., 2017).

2.9. Sequence processing

The ABI sequence files of the forward and the reverse sequences were edited, aligned and consensus sequences were generated with the BioEdit sequence alignment Editor 7.2.5. The rotavirus genotypes were assigned based on the nucleotide percentage identities using the ViPR (ViPR, 2021) based on the RotaC algorithm developed by Maes et al. (2009) and Basic Local Alignment Search Tool (BLAST).

2.10. Phylogenetic analysis and amino acid alignments

The phylogenetic tree of VP genes and NSP genes nucleotide sequences was constructed using Mega X software by maximum likelihood method and Tamura-Nei model for nucleotides analysis with 1000 bootstraps replicates (Kumar et al., 2018; Tamura and Nei, 1993).

3. Results and discussion

3.1. Prevalence and genotypes distribution of RVA in piglets

The overall RVA prevalence was 35.26% (85/241) (Table 1), among the RVA positive samples (85), 53 were from piglets without diarrhea and 32 were from piglets with diarrhea. The RVA was found in 30.58% of pigs in Mbarali, 43.75% in Kilolo, Iringa, and 31.16% in Mvomero, Morogoro (Table 1). The infection rate in the villages is shown in Table 2. The overall prevalence of RVA in piglets was comparable to a previous report in peri-urban Arusha, northern Tanzania (Gachanja et al., 2016), as well as other countries such as Kenya, the Republic of Korea, Vietnam, and India (Amimo et al., 2015; Anh et al., 2014; Das and Handique, 2016; Kim et al., 2010; Xue et al., 2018). The prevalence in Mbeya, Iringa, and

Table 1. Rotavirus group A prevalence in piglets in Mbarali, Kilolo, and Mvomero districts of Mbeya, Iringa, and Morogoro regions, respectively.

Region	District	Piglets	
		n	Rotavirus positive (%)
Overall		241	85 (35.26%)
Mbeya	Mbarali	84	26 (30.58%)
Iringa	Kilolo	80	35 (43.75%)
Morogoro	Mvomero	77	24 (31.16%)

Table 2. Rotavirus group A infection rate in piglets in villages discovered in respective districts.

Region	District	Ward	Village	n	Rotavirus positivity rate (%)
Mbeya	Mbarali	Ubaruku	Mkombwe	16	5 (31.25%)
			Mpakani	12	3 (25%)
			Ubaruku	8	4 (50%)
		Chimala	Mwale	12	5 (41.66%)
			Lyambogo	10	4 (40%)
		Luhanga	Luhanga	11	4 (36.36%)
		Kongolo	Kongolo Mkola	6	0 (0%)
			Kongolo Mswisi	9	1 (11.11%)
Iringa	Kilolo	Ihimbo	Utengule	9	1 (11.11%)
			Itarula	11	7 (63.62%)
			Ihimbo	13	0 (0%)
		Ng'uruhe	Lukani	12	6 (50%)
			Ng'uruhe	11	8 (72.72%)
		Image	Image 8	11	5 (45.45%)
			Uhominyi	6	4 (66.66%)
			Ilawa	7	4 (57.14%)
Morogoro	Mvomero	Mhondo	Kichangani	45	10 (22.22%)
		Manyinga	Diongoya	9	3 (33.33%)
		Ndugutu	Nyandira	23	11 (47.82%)

Morogoro were comparable, indicating that rotavirus severity was similar across regions. Despite the fact that RVA has a negative economic impact on the pig industry and the risk to humans (Martella et al., 2006a, b), little effort is being made to control rotavirus livestock in Tanzania. Other countries, including Mexico, North America, the United Kingdom, New Zealand, and Brazil, have used RVA vaccines to combat rotavirus infection (Rodríguez-Limas et al., 2009). RVA vaccines available include ProSystems RCE, Scourguard 3, Scourguard 4, and others. However, proper rotavirus vaccine selection based on circulating genotypes and their antigenic relationship is more important for the successful intervention of infection in piglets.

The observed high prevalence of rotavirus infection in domestic pigs suggests that RVA infection is endemic in the study areas. However, a higher number of positives were observed in asymptomatic piglets, implying that asymptomatic piglets may act as a source of pathogen persistence in farms as well as a port for RVA diversity and rotavirus disease attribution may also be complicated during pig diarrhea outbreaks. Consequently, further research may be necessary to understand the role of asymptomatic piglets in RVA infection, diversity, and evolution. RVA was found in asymptomatic piglets, which was comparable to previous reports in Arusha, Tanzania, Kenya, Uganda, Vietnam, and Slovenia (Steyer et al., 2008; Anh et al., 2014; Amimo et al., 2015; Gachanja et al., 2016). Higher infection rates were observed in piglets with diarrhea, indicating that RVA may be a major contributor to diarrhea in piglets, though other diarrhea-causing pathogens were not identified. Poor husbandry and hygiene in piggery farms may contribute to the higher RVA infection rate in pigs, allowing pathogens to spread quickly. Pig farmers must therefore be educated on biosecurity measures such as good sanitation and hygiene, routine disinfection of the room, segregation of pigs based on their ages, separation of piglets with diarrhea from the rest, testing new incoming pigs in the farm, and limiting birds' easy access to the farm, in addition to vaccination of young pigs. These practices will aid in reducing the spread and introduction of rotavirus infection on the farm and in neighboring pig farms.

The G4 (26/45) was the most common G genotypes followed by G9 (10/45), G3 (6/45) and G5 (3/45) on multiplex RT-PCR genotyping while remaining 40 RVA positive samples were untypeable. P[6] (35/38) was the most common P genotype, followed by P[13] (3/38) and 47 were untypeable P genotypes out of 85 RVA positive samples. This is the first report on RVA genotypes circulating in piglets in Tanzania. Rotavirus group A was genetically diverse in piglets. These genotypes have also been reported in pigs in neighboring country Kenya (Amimo et al., 2015) and elsewhrere (Anh et al., 2014; Chandler-Bostock et al., 2014; Parra et al., 2008; Wu et al., 2022; Xue et al., 2018). The G4 and P[6] genotypes are widely distributed in all three regions (Mbeya, Iringa, and Morogoro), whereas the G3 genotype occurred only in Morogoro, the G9 genotype in Mbeya, the G5 genotype in Iringa, and the P[13] genotype in Iringa and Morogoro. As a result, additional research is required to truly understand the regional genotype specific across the country, which is required for vaccine development and selection. There were 34 genotype combinations identified, with G4P[6] (25/34) being the most common, followed by G3P[6] (5/34), G5P[13](1/34), G3P[13](1/34), G4P[13](1/34), and G9P[6](1/34) (Table 3). This does not imply that this genotype will always be prevalent; for example, in the United Kingdom, G5P[32] was the most prevalent in 2009, but G4P[32] was the most prevalent in 2010 (Chandler-Bostock et al., 2014). Similar phenomena were observed in Taiwan, where G9P[19] was prevalent in 2015 and G9P13] was prevalent in 2016 (Wu et al., 2022). The prevalent of G4P[6] has also been reported in other countries such as Canada and Vietnam (Anh et al., 2014; Lamhoujeb et al., 2010).

3.2. Genome constellation

The ViPR was used to determine the genotype of all 11 segments of the selected 9 strains based on VP7 sequence clustering on phylogeny. We discovered that the fully characterised strain belonged to the genogroup constellation G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 (Table 4) with evidence of intra-genogroup reassortment between pig and human rotavirus but other non-completely characterized genomes displayed G5-P[x]-I5-R1-C1-M1-A8-N1-Tx-E1-H1, G3/G4-P[13]/P[6]-Ix-R1-C1-M1-A8-N1-T1-E1-H1, G3-P[6]-Ix-R1-C1-M1-A8-N1-Tx-E1-H1 and G9-P[x]-Ix-R1-C1-M1-Ax-N1-Tx-E1-H1 genome constellation. However, due to insufficient nucleic acid or primer failure due to mutation, segments 6 (I genotype) of six strains, segment 7 (T genotype) of three strains, and segment 5 (A genotype) of one A strain did not produce detectable sequences. Genomic analysis reveals higher diversity in genome segments among the isolates. We detected two kinds of I gene (VP6 i.e. segment 6) which include I1 and I5. The RP072 and RP074 strains had similar Gottfried genome constellations (G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1), but phylogenic analysis revealed nucleotide differences by clustering to different lineages and clusters. The genomes constellations discovered were consistent with previous research in pigs from neighbouring countries such as Uganda (Bwogi et al., 2017), Kenya (Amimo et al., 2015) and in human in Zambia (Maringa et al., 2020) and elsewhere in Thailand (Tuanthap et al., 2019) and Switzerland (Baumann et al., 2022). The I5-R1-C1-M1-A8-N1-Tx-E1-H1 and I1-R1-C1-M1-A8-N1-T1-E1-H1 genotype constellation are commonly found in pigs worldwide (Hull et al., 2020; Guo et al., 2021; Nyaga et al., 2015). The segment were successfully assigned accession numbers by gene bank which are OP082194-OP082202 (segment 1, VP1), OP082207–OP082215 (segment 2, VP2), OP082220–OP082228 (segment 3, VP3), OP082182 (segment 5, NSP1), ON058281–ON058283 (segment 6, VP6), ON092385–ON092390 (segment 7, NSP3), ON092395–ON092403 (segment 8, NSP2), ON092408–ON092416 (segment 10, NSP4) and ON109526–ON109534 (segment 11, NSP5/6).

3.3. Phylogenetic analysis of structural protein (VP7, VP4, VP1, VP2, VP3 and VP6) and amino acid analysis of VP7

The phylogenetic analysis of VP7 revealed that Tanzanian G4 genotypes are diverse since were located in two different lineages (lineage V and V11) and different clusters within the lineage. The G4 strains in lineage V form three distinct clusters, two clusters had strains from Iringa

Table 3. Rotavirus group A individual genotypes for VP4 and VP7 genotyping circulating in piglets in Mbeya, Iringa and Morogoro, along with gene bank accession numbers.

Sample ID	Location	G GenBank accession no.	P GenBank accession no	Symptoms
RVA/Pig-wt/TZA/Mbeya-MP001/2019/G9P[x]	Mbeya	MW718925	ND	Diarrhea
RVA/Pig-wt/TZA/Mbeya-MP003/2019/G9P[x]	Mbeya	MW718926	ND	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP006/2019/G9	Mbeya	MW718927	ND	Diarrhea
RVA/Pig-wt/TZA/Mbeya-MP025/2019/G9	Mbeya	MW718928	ND	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP051/2019/G4P[X]	Mbeya	MW718895	ND	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP056/2019/G4P[6]	Mbeya	MW718896	OM471829	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP058/2019/G4P[X]	Mbeya	MW718897	ND	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP060/2019/G4P[X]	Mbeya	MW718898	ND	No diarrhea
RVA/Pig-wt/TZA/Mbeya-MP063/2019/G4P[X]	Mbeya	MW718899	ND	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP013/2019/G4P[6]	Iringa	MW718900	OM471830	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP014/2019/G4P[X]	Iringa	MW718901	ND	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP037/2019/G4P[6]	Iringa	MW718902	MW718936	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP038/2019/G4P[6]	Iringa	MW718903	OM471831	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP043/2019/G4P[6]	Iringa	MW718904	MW718937	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP044/2019/G4P[6]	Iringa	MW718905	MW718938	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP045/2019/G4P[6	Iringa	MW718906	OM471832	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP046/2019/G4P[6]	Iringa	MW718907	OM471833	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP047/2019/GxP[6]	Iringa	ND	OM471834	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP054/2019/G5P[13]	Iringa	MW718921	MW718949	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP055/2019/G5P[x]	Iringa	MW718922	ND	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP056/2019/G4P[6]	Iringa	MW718908	OM471835	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP057/2019/G5P[x]	Iringa	MW718923	ND	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP058/2019/G4P[6]	Iringa	MW718909	MW718939	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP059/2019/GxP[6]	Iringa	ND	MW718940	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP061/2019/G4 P[X]	Iringa	MW718910	ND	Diarrhea
RVA/Pig-wt/TZA/Iringa-IP062/2019/G4P[6]	Iringa	MW718911	OM471836	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP070/2019/G4P[x]	Iringa	MW718912	NA	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP071/2019/G4P[6]	Iringa	MW718913	OM471837	No diarrhea
RVA/Pig-wt/TZA/Iringa-IP072/2019/G4P[6]	Iringa	MW718914	OM471838	No diarrhea
RVA/Pig-wt/TZA/Morogoro-RP011/2019/G3P[x]	Morogoro	MW718894	ND	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP012/2019/G3P[13]	Morogoro	MW718890	MW718950	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP013/2019/G3P[x]	Morogoro	MW718891	ND	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP015/2019/G3P[6]	Morogoro	MW718892	MW718941	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP016/2019/G3P[x]	Morogoro	MW718893	ND	Diarrhea
RVA/Pig-wt/TZA/Morogoro- RP018/2019/G4P[6]	Morogoro	MW718920	MW718942	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP019/2019/G4P[6]	Morogoro	MW718915	MW718943	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP020/2019/G4P[6]	Morogoro	MW718916	MW718944	Diarrhea
RVA/Pig-wt/TZA/Morogoro-RP072/2019/G4P[6]	Morogoro	MW718917	MW718945	No diarrhea
RVA/Pig-wt/TZA/Morogoro-RP073/2019/G4P[6]	Morogoro	MW718918	MW718946	No diarrhea
RVA/Pig-wt/TZA/Morogoro-RP074/2019/G4P[13]	Morogoro	MW718919	MW718951	No diarrhea

ND = Not determined.

and the other cluster contained G4 strain from Morogoro. For the first time in Tanzania, we were able to establish RVA lineages of genotypes circulating in pig. Different lineages are known to express different antigenic properties, each lineage may necessitate its own vaccine. The G4 isolates in lineage VII were classified into sublineage VII-a and VII-c (Figure 1A). The G4 strains of sublineage VII-a closely related to known human and pig isolates from Kenya and South Africa with higher nucleotide identity to human ranging from 97.23% to 98.8% and lower to pig ranging from 90.01% to 91.4%. Similar observation of clustering was observed in strains of Sublineage VIIc. The sublineage VII-c form two clusters, one contained G4 from Iringa and the other from Mbeya which clustered with human G4 strains from Dominica republic (KX778620) and Sri Lanka (LC389890), however the strain shown to be of porcine origin. Lineage V strains are divided into three distinct clusters, with each cluster containing strains from a specific region. All identified G4 strains clustered distantly to G4 strain found in the ProSystems RCE vaccine used in pig vaccination, which belongs to lineage III (X06759.1). Because the

VP7 gene plays an important role in inducing strain specific immunity, which is important for vaccine development, additional studies in other regions are required to provide a clear picture on relationship between wild type and the vaccine strain across the country (Gentsch et al., 2005; Matthijnssens et al., 2008a,b).

On amino acid alignment between circulating G4 and G4 in the vaccine, we discovered significant differences in amino acid sequences between circulating these strains. The amino acid variation included key amino acids found at antigenic epitopes 7-1a, 7-2b, and 7-2c (Table 5) suggesting the circulating G4 strains might be antigenically different to G4 strain present in the vaccine. This could be due to an accumulation of point mutations that resulted in these amino acid variations. The amino acid variation at the antigenic epitopes were associated with escape from neutralization antibodies induced by vaccine (Aoki et al., 2009; Caust et al., 1987; Dyall-Smith et al., 1986). Similar finding were also observed in the study conducted in Thailand (Tuanthap et al., 2019). This is critical information on the performance of this vaccine during its introduction

Table 4. The whole genome constellation of characterized piglet RVA strains circulating in Southern highland and Eastern Tanzania was compared to corresponding pig and human reference strains. This study's strains are highlighted.

Strain	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
	G	P	I	R	C	M	A	N	T	E	H
RVA/Pig-wt/TZA/Mbeya-MP003/2019/G9P[x]	G9	P[x]	Ix	R1	C1	M1	Ax	N1	Tx	E1	H1
RVA/Pig-wt/TZA/Iringa-IP044/2019/G4P[6]	G4	P[6]	Ix	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Iringa-IP058/2019/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Morogoro-RP012/2019/G3P[13]	G3	P[13]	Ix	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Morogoro-RP015/2019/G3P[6]	G3	P[6]	Ix	R1	C1	M1	A8	N1	Tx	E1	H1
RVA/Pig-wt/TZA/Morogoro-RP019/2019/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Iringa-IP072/2019/G4P[6]	G4	P[6]	Ix	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Morogoro-RP074/2019/G4P[13]	G4	P[13]	Ix	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/TZA/Iringa-IP057/2019/G5P[x]	G5	P[x]	I5	R1	C1	M1	A8	N1	Tx	E1	H1
RVA/Pig-wt/UGA/KYE-14-A047/2014/G3P[13]	G3	P[13]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-tc/USA/Gottfried/1983	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-tc/USA/WI61/1983	G9	P[8]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	G9	P[8]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/UGA/Ug-453/G5P[13]	G5	P[13]	I5	R1	C1	M1	A8	N1	T1	E1	H1

against the wildtype G4 strain. However, these amino acid variation cannot confirm the impact on antigenicity, therefore further studies on antigenicity using immunoassay techniques are necessary to understand the impact of these amino acid changes.

The G9 were located in lineage II containing only G9 strains from Mbeya which form their own Tanzanian cluster that shared nucleotide identity ranging from 99.19% to 100%. The G9 clustered together in the same lineage II with human G9 strains from Uganda, Zambia, and Kenya which shared maximum nucleotide identities ranging from 97.90% to 98.60% although these strains clustered distantly with porcine strain (Figure 1B). These results suggest interspecies transmission or reassortment event between porcine and human rotavirus. The G3 located in lineage IV covered by only G3 strains from Morogoro and clustered together to pig isolates from China and Uganda, with nucleotide similarities of ranging from 95.04 % to 96.9% (Figure 1C). The G5 strains were located in lineage III which clustered together with mostly pig isolates (Figure 1D), however, our G5 strains located in the same lineage with the OSU strain (X04613 RVA/pig-tw/USA/OSU) which is one of the component of ProSystems RCE vaccine and also clustered with the equine (AF242393) and bovine (DQ683523) isolates.

The phylogenetic analysis of the VP4 gene revealed that P[6] is genetically diverse and located lineages, sublineages and clusters including lineage I sublineage I-c and lineage V (Figure 1E). The results are in agreement that east African porcine P[6] are more divergent at VP4 from global strains (Amimo et al., 2015). The sublineage I-c contained strains from Mbeya, Morogoro, and Iringa however Iringa strain form two distinct cluster (Figure 1e) with known pig isolates from Italy, Sri lank, Thailand and China. The Lineage V composed P[6] strains from Morogoro shared nucleotide similarities ranging from 99.88% to 100%. These strain were previous identified as porcine-like human strains from neighbouring country Kenya, Democratic republic of Congo (DRC) (KJ870903-RVA/Human-wt/COD/KisB332/2008/G4P[6]) (Heylen et al., 2014) and Zambia (MT271026-RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]) (Maringa et al., 2020) which shared nucleotide percentage identities of 98.74 %, 98.17 %, and 98.24 %, respectively. On the other, P[6] isolates in lineage V, on clustered distantly to piglet P[6] from Italy which shared a lower nucleotide identity of 92.47 % (Martella et al., 2006a,b). The VP4 gene results suggest Tanzanian P[6] in lineage V might be resulted from reassortment event between porcine and human strains. On ViPR genotyping tool, these P[6] were similar to human strain isolated in Dhaka (RVA/Human-wt/BGD/Dhaka12/2003/G12P6) which shared

nucleotide identities ranging from 86.35% to 86.38%. The diversity of P[6] have been also reported in Italy (Martella et al., 2006a,b). The P[13] belonged to lineage IV and was closely related to pig P[13] isolates from Vietnam (KX363348.1), China (MG066587.1) P[13] with nucleotide similarities of 96.12%, 96.07 %, and 88.46%, respectively (Figure 1F). The P[13] common genotypes circulating found in pigs. The P[13] have also been reported in the neighbouring country Kenya and Uganda (Amimo et al., 2015).

The VP1 genes belong to R1 and appeared divergent because they are divided into three clusters (Figure 1G). One cluster contains strains from Morogoro and Iringa which clustered with known human and porcine isolates from China and Vietnam (Dong et al., 2013; Kaneko et al., 2018) with nucleotide identities ranging from 95.23% to 96.56%. The strain were distantly related to African strains such as porcine strain from Uganda (KY077640 and KX988275) (Bwogi et al., 2017) which shared nucleotide identities of 93.39% and 93.27%. Few Africans were related to the study strains and this may be due to little studies on rotavirus genotypes in pig, thus lack of data for comparison and ascertains strain origin. Another cluster contains strains from Iringa and the final cluster contains strains from Mbeya. All strains clustered with known pig and human strains from china, with nucleotide identities ranging from 95.74% to 97.05% and shown to have porcine origin.

VP2 genes of all nine strains belong to C1, which is divided into three different clusters. One cluster included three Morogoro strains (RP012, RP015, and RP019) and one Iringa strain (IPO58) (Figure 1H). The strains shared nucleotide identities ranging from 96.44% to 96.97% with piglets and a human strain from China. Another cluster contained only Morogoro strains (RP072 and RP074), which were closely related to human strains from the Republic of Congo (KJ870901; RVA/Human-wt/COD/KisB332/2008/G4P[6]) and pig strains from Uganda (KY055417; RVA/Pig-wt/UGA/BUW-14-A008/2014/G12P8) and USA strain (KR052758; RVA/Pig-tc/USA/LS00006_OSU/1975/G5P[X]) (Bwogi et al., 2017; Heylen et al., 2014) with nucleotide identities ranging from 98.35% percent to 98.57% percent and 95.60%–95.70% percent, respectively. However all strain originated from porcine strains. The strain from DRC was reported as porcine-human reassortant (Bwogi et al., 2017). The final cluster included two Iringa strains (IP044 and IP057) and one Mbeya strain (MP003), as well as human and pig strains from Sri Lanka (LC389886) and China (MK410287) with nucleotide identities ranging from 95.99% to 96.94% and 95.44%–98.26%, respectively.

Figure 1. The nucleotide sequence phylogeny tree depicting the genetic relationship and lineages of circulating RVA genotypes for VP7; G4 (A), G9 (B), G3 (C) and G5 (D), VP4; P[6] (E) and P[13] (F), VP1 (G), VP2 (H), VP3 (I) and VP6 gene (J). This study's isolates are labelled with a black filled square. Blasting was used to obtain reference strains from the gene bank.

Table 5. Amino acid differences (green and pink colored) at the antigenic epitopes of the VP7 gene of circulating G4 compared to G4 (Gottfried) present in the ProSystems RCE vaccine.

Antigenic epitopes		7-1a														7-1b						7–2								
	LN	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
G4 of ProSystems RCE vaccine (Gottfried)	III	S	T	N	N	E	W	K	D	Q	N	L	I	D	K	Q	N	T	A	D	T	R	A	S	G	E	S	T	S	G
MP051	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	I	A	G	E	S	M	N	G
MP056	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	I	A	G	E	S	M	N	G
MP058	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	I	A	G	E	S	M	N	G
MP060	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	I	A	G	E	S	M	N	G
MP063	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	I	A	G	E	S	M	N	G
IP046	VIIc	T	T	G	N	E	W	K	D	Q	N	L	I	D		Q	N	A	G	D	T	R	I	A	G	E	S	M	N	G
IP056	VIIc	T	T	G	N	E	R	K	D	Q	N	L	I	D		Q	N	A	G	D	T	R	I	A	G	E	S	M	N	G
IP058	VIIc	T	T	G	K	E	R	R	D	Q	N	L	I	D		Q	N	A	G	D	T	R	I	A	G	E	S	M	N	G
IP062	VIIc	T	T	G	N	E	R	K	D	Q	N	L	I	D		Q	N	A	G	D	T	R	I	A	G	E	S	M	N	G
RP018	VIIa	T	T	G	T	E	R	R	D	Q	N	L	I	D		Q	N	T	G	D	T	R	V	A	G	E	L	M	N	G
RP019	VIIa	T	T	G	T	E	R	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	V	A	G	E	L	M	N	G
RP020	VIIa	T	T	G	T	E	W	K	D	Q	N	L	I	D		Q	N	T	G	D	T	R	V	A	G	E	L	M	N	G
IP013	V	T	T	N	N	E	W	K	D	Q	N	L	V	D		Q	N	T	N	N	T	R	V	S	G	E	S	T	S	G
IP014	V	T	T	N	N	E	W	K	D	Q	N	L	V	D		Q	N	T	N	N	T	R	V	S	G	E	S	T	S	G
IP037	V	T	T	S	N	E	W	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP038	V	T	T	N	N	E	W	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP043	V	T	T	S	N	E	W	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP044	V	T	T	S	N	E	W	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP045	V	T	T	N	N	E	W	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP070	V	T	T	N	N	E	W	K	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP071	V	T	T	N	N	E	W	K	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
IP072	V	T	T	N	N	E	W	K	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
RP072	V	T	T	N	N	E	R	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
RP073	V	T	T	N	N	E	R	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G
RP074	V	T	T	N	N	E	R	R	D	Q	N	L	V	D		Q	N	T	N	D	T	R	A	S	G	E	S	T	S	G

The VP3 gene of all nine strains have the M1 VP3 gene, which is divided into two clusters (Figure 1I). One cluster includes strains from Iringa and Morogoro that were closely related to rotavirus strain isolates from piglets (MK227390), humans (KF041443), and giant panda (HQ641295) in China, with maximum nucleotide identities of 96.73%, 96.73%, and 97.47%, respectively.

Only three samples were classified for the VP6 gene, and the rest were untypeable using the used primers. The IP058 and RP019 strains were assigned to I1 genotype on ViPR classification, which form two clusters on phylogeny whereas the IP057 was assigned to I5 genotype (Figure 1J). The I5 genotype is most common in pigs worldwide and have reported in pigs in neighbouring country Kenya and Uganda (Amimo et al., 2015). In human, I5 genotype have been reported in china (Wang et al., 2010). The study I5 genotyped clustered with pig isolates and with I5 genotype isolated in Murine (MK606443) in china. The IP058 strain clustered together and was closely related to several known human and piglet strains, including human (LC389889) from Sri Lanka and pig (KX363319) from Vietnam, which shared nucleotide similarities of 96.8 % and 96.5 %, respectively. The RP019 strain clustered together with

human from Democratic republic of Congo (KJ870904) and Zambia (MT271027) and piglet strains from African countries only, including Uganda (KX988268), suggesting common origin with other African strain.

3.4. Phylogenetic analysis of non-structural protein genes: NSP1, NSP2, NSP3, NSP4 and NSP5/NSP6

The NSP1 gene from eight strains belongs to the A8 genotype and is divided into two clusters (Figure 2A). Strains from Iringa and Morogoro clustered together on phylogeny whereas the Mbeya strain was unable to be genotyped. Both strains in the respective cluster shared nucleotide identities ranging from 93.96% to 96.66% and 94.64%–95.67% with human (KF726072, JN104616) and pig (MH137274, LC095905, MN102369) isolates respectively. These humans strains from China and Vietnam were identified as porcine origin strains (Ghosh et al., 2012; Zhou et al., 2015). The NSP2 gene in all nine isolates belongs to the N1 genotype and is clustered in four different clusters (Figure 2B). One cluster includes strains from Morogoro and Iringa that are closely related
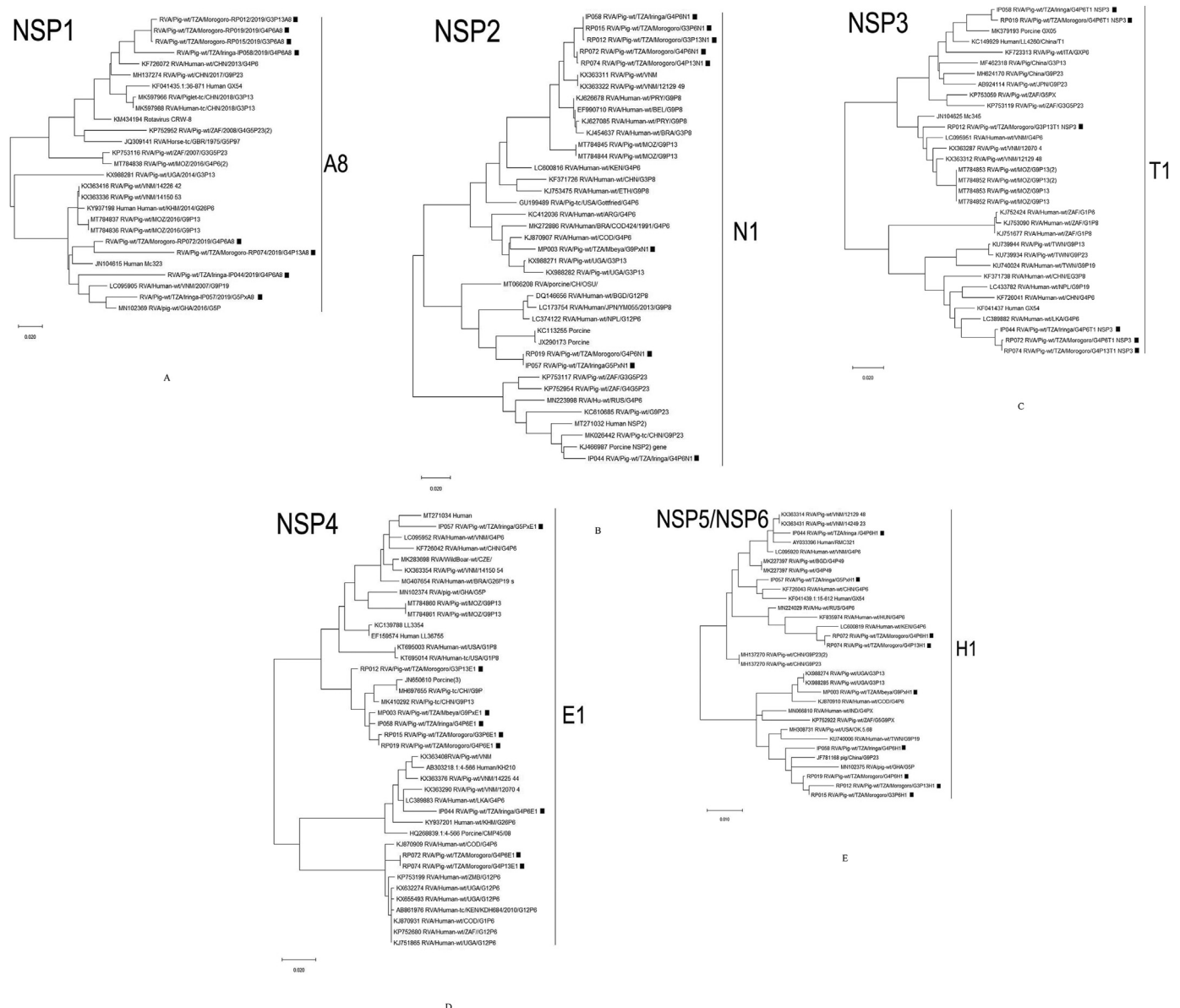

Figure 2. The nucleotide sequence phylogeny tree illustrating the genetic relationship of non-structural proteins (a) NSP1 (b) NSP2 (c) NSP3, (d) NSP4 and (e) NSP5/6 of circulating the RVA genotypes. This study's isolates are labelled with a black filled square, and the reference strains were obtained from the gene bank via blasting.

to human and pig isolates from Vietnam (KX3363311), Belgium (EF990710), and Paraguay (KJ626678) which have shown to originate from ancestral Gottfried (GU199489) porcine rotavirus. Another cluster contained only MP003 strain, which was closely related to human strains from the Democratic Republic of the Congo (KJ870907; RVA/Human-wt/COD/KisB332/2008/G4P[6]) and pig strains from Uganda (KX988271; RVA/Pig-wt/UGA/KYE-14-A047/2014/G3P[13]), with nucleotide identities of 97.4% and 97.2%, respectively. The remaining two clusters grouped with human and pig isolates. Regardless of clustering with human and pig isolate, strain shown to originate from porcine strains.

Only six strains successfully genotyped for the NSP3 gene, with the remaining three strains failing to produce sequence results. NSP3 was discovered to belong to the T1 genotype, forming three distinct phylogenetic clusters (Figure 2C). One cluster includes Iringa and Morogoro strains (IP044, RP072 and RP074) that are close related and clustered with human origin isolates from Sri Lanka (LC389882) (Yahiro et al., 2018), China (KF041437) (Dong et al., 2013), and Nepal (LC433782) (Takatsuki et al., 2019) with nucleotide identities ranging from 95.32% to 96.56%, suggesting our strains are porcine-human reassortant. Another cluster contained two Iringa (IP058) and Morogoro (RP019) strains that were closely related to pig (MK379193) and human (KC149929) strains. The final cluster contained only one strain from Morogoro which clustered together porcine and human strain.

The NSP4 gene in all nine strains belongs to the E1 genotype and is divided into four clusters (Figure 2D). The Morogoro RP072 and RP074 strains were grouped together in one cluster and were closely related to only human strains from neighbouring countries such as the Democratic Republic of the Congo (KJ87093), Kenya (AB861976), Uganda (KX655493), and Zambia (KP753199), which shared the highest nucleotide identities ranging from 98.51 percent to 98.76 percent. On ViPR the strain were related to RVA/Pig-tc/USA/OSU/1977/G5P7 by nucleotide identity of 91.51%. The human strain which clustered with our strain were previous reported as porcine-human reassortant strains (Bwogi et al., 2017; Heylen et al., 2014). The results indicate possible of rotavirus transmission to human in the study areas. Another cluster collected five of the nine strains (RP012, RP015, RP019, MP003 and IP058) from all three study regions (Morogoro, Iringa, and Mbeya). In this cluster, the Tanzania strain clustered together with only Pig strains from China, with nucleotide identities ranging from 97.08% to 98.48%. Another cluster contained only IP057 from Iringa, which clustered with pig, human, and one wild boar strain. The IP044 strain formed its own cluster with pig and human strains that shared 96.98 %–98 % nucleotide identities. Similar observation of close relationship to human isolate was observed in NSP5/NSP6 gene, were the RP072 and RP074 strains clustered together with human strains and shared 99% and 98% nucleotide identities with human strains from Kenya and Russia, respectively (Figure 2E). The other strains in the study, which were divided into four groups, were closely related to both pig and human strains.

4. Conclusions

RVA infections are endemic and widespread in both symptomatic and asymptomatic piglets, according to our findings, to prevent the spread and introduction of RVA in their farm, we recommend educating pig farmers biosecurity measures such as good hygiene, sanitation, and pig vaccination against RVA. RVA genotypes are genetically diverse, with different lineages, sublineages, and clusters, which is important information for the strategic design and development of vaccines for effective RVA infection control. Higher nucleotide similarities and clustering of some genome segments between the identified piglet strain and the reference human strain suggest possibility interspecies transmissions and reassortment, emphasizing the importance of whole genome analysis surveillance systems of animal rotavirus strains to understand genetic diversity. The study provided preliminary epidemiology data that will aid in understanding rotavirus infection in pigs and pave the way for

future research in Tanzania on risk factors associated with RVA infection in pigs.

Declarations

Author contribution statement

Joseph Malakalinga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gerald Misinzo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

George Msalya: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mariana Shayo: Analyzed and interpreted the data; Performed the experiments; Wrote the paper.

Rudovick Kazwala: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

4.1. Data availability statement

Data associated with this study has been deposited at Gene Bank under the accession number [MW718925, MW718926, MW718927, MW718928, MW718895, MW718896, MW718897, MW718898, MW718899, MW718900, MW718901, MW718902, MW718903, MW718904, MW718905, MW718906, MW718907, MW718921, MW718922, MW718908, MW718923, MW718909, MW718910, MW718911, MW718912, MW718913, MW718914, MW718894, MW718890, MW718891, MW718892, MW718893, MW718920, MW718915, MW718916, MW718917, MW718918,MW718919, OM471829, OM471830, MW718936, OM471831, MW718937, MW718938, OM471832, OM471833, OM471834, MW718949, OM471835, MW718939, MW718940, OM471836, OM471837,OM471838, MW718950, MW718941, MW718942, MW718943, MW718944, MW718945, MW718946, MW718951, OP082194–OP082202, OP082-207–OP082215, OP082220–OP082228, OP082182, ON058281–ON05-8283, ON092385–ON092390, ON092395–ON092403, ON092408–ON09 2416, ON109526–ON109534].

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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