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Molecular characterization of African swine fever virus in apparently healthy domestic pigs in Uganda

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African swine fever (ASF) is a highly lethal and economically significant disease of domestic pigs in Uganda where outbreaks regularly occur. There is neither a vaccine nor treatment available for ASF control. Twenty two African swine fever virus (ASFV) genotypes (I - XXII) have been identified based on partial sequencing of the C-terminus of the major capsid protein p72 encoded by the *B646L* gene. The majority of previously characterized Ugandan ASFV strains belong to genotype IX. The major aim of the current study was to determine the ASFV genotypes among asymptomatic slaughter pigs at Wambizi slaughterhouse and in some parts of the country where surveillance was done. Three discrete regions of the ASFV were analysed in the genomes of viruses detected in asymptomatic domestic pigs. The analysis was conducted by genotyping based on sequence data from three single copy ASFV genes. The E183L gene encoding the structural protein P54 and part of the gene encoding the p72 protein were used to delineate genotypes, before intra-genotypic resolution of viral relationships by analysis of tetramer amino acid repeats within the hypervariable central variable region (CVR) of the B602L gene. All the ASF viruses obtained from this study clustered with previous viruses in genotype IX based on analysis of the *p72* and *P54* genes. Analysis of the *CVR* gene grouped the viruses in three different subgroups; 13, 23 and 25. Only one genotype is circulating in Uganda among asymptomatic domestic pigs and it is the same virus causing outbreaks in the country and parts of neighbouring Kenya.

Key words: African swine fever virus, asymptomatic, slaughterhouse, P54, p72, *CVR* gene, genotypes.

INTRODUCTION

African swine fever (ASF) is an important, highly contagious and lethal disease of domestic pigs caused by

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Abbreviations: ASF, African swine fever; **CVR,** central variable region; **ASFV,** African swine fever virus.

an icosahedral double stranded DNA virus that is presently the sole member of the *Asfivirus* genus within the family *Asfarviridae* (Dixon et al., 2000). Outbreaks of ASF have been sporadic in the different regions of Uganda (Atuhaire et al., 2013).

Epidemiology of ASF confirms that presence of the disease in one area has a potential risk for introduction and further spreading in any direction despite the natural and artificial borders and distance (Wieland et al., 2011). The viral genome comprises around 170 to 195 kb (depending on the isolate), which encode more than 150 different proteins.

Sequence analyses of virus genomes (Chapman et al., 2008; Chapman et al., 2011; de Villiers et al., 2010; Yanez et al., 1995) have established that the central region is relatively conserved but large length variations occur at the termini, particularly within 40 kbp of the left end of the genome, but also within 15 kbp from the right end of the genome.

Molecular epidemiology has been used to describe the heterogeneity and epidemiological links of ASFV (Bastos et al., 2003; Boshoff et al., 2007; Gallardo et al., 2011). Twenty two ASFV genotypes have been identified based on partial sequencing of the C-terminus of the major capsid protein p72 encoded by the *B646L* gene (Bastos et al., 2003; Boshoff et al., 2007; Lubisi et al., 2007). Previously characterized Ugandan ASF viruses have been placed in genotype IX (Atuhaire et al., 2013; Gallardo et al., 2011) and genotype X (Nix et al., 2006).

Previous studies have demonstrated the value of full P54 gene sequencing for providing additional, intermediate resolution when typing of ASFV viruses (Gallardo et al., 2009.

The ASFV P54 is an externally located viral structural protein of 25-27 kDa, encoded by the virus gene - the open reading frame (ORF) E183L (Rodriguez et al., 1996).

The ASFV protein P54 is involved in the adsorption of the virion on susceptible cells and the early steps of viral infection (Rodriguez et al., 2004).

In addition to p72 and P54 genotyping, higher resolution for viral discrimination has been achieved by use of the B602L central variable genome region (CVR) which contains 12-bp repeats which encode 4 amino acids that vary in number and sequence when genomes of different isolates are compared (Irusta et al., 1996; Nix et al., 2006). Therefore by combining p72, P54 and B602L, a high level resolution approach is achieved for viral discrimination (Gallardo et al., 2011; Lubisi et al., 2007).

The aims of the present study were to genotype ASFV in asymptomatic domestic pigs by p72, P54 and CVR sequencing and determine the relationship of these viruses from the abattoir and field surveillance, and viruses causing ASF outbreaks in Uganda (2010 to 2013) was obtained from GenBank.

MATERIALS AND METHODS

Ethical consideration

Full ethical clearance was obtained from the Uganda National Council for Science and Technology (UNCST) and the College of Veterinary Medicine, Animal Resources and Biosecurity, of Makerere University under reference number VAB/REC/11/110. Permission was obtained from the Wambizi slaughter house administrative authority. For collection of field samples, permission was obtained from area Veterinary Officers and farmers. All animals were handled humanely during sample collection.

Study design

The study design, study sites and sampling strategy were as described previously by Atuhaire et al. (2013).

DNA extraction

Viral DNA was extracted directly from 200 µl aliquots of blood collected in EDTA tubes using the DNeasy Blood and tissue kit (QIAGEN®, USA).

ASFV detection

A 278 bp region corresponding to the central portion of the p72 gene was amplified using the diagnostic primers, primer 1 (5'-ATGGATACCGAGGGAATAGC-3') and primer 2 (5'-CTTACCGATGAAAATGATAC-3') to confirm the presence of ASFV DNA (Wilkinson, 2000).

ASFV molecular characterization

Epidemiological primers which amplify the C-terminal region of the p72 gene (478 bp), p72-U (5'-GGCACAAGTTCGGACATGT-3') and p72-D (5'-GTACTGTAACGCAGCACAG-3') as described previously were used for p72 genotyping (Bastos et al., 2003). The complete gene encoding the P54 protein was amplified using the primers PPA722 (5'-CGAAGTGCATGTAATAAACGTC-3') and PPA89 (5'-TGTAATTTCATTGCGCCACAAC-3') flanking a 676 bp DNA fragment (Gallardo et al., 2009). The CVR located in the B602L gene was amplified using the primer pairs CVR-FL1 (5'-TCGGCCTGAAGCTCATTAG-3') and CVR-FL2 (5'-CAGGAAACTAATGATGTTCC-3') flanking a variable in size DNA fragment (Bastos et al., 2004). Conditions for PCR assays were as previously described (Gallardo et al., 2009) with slight modifications in the annealing temperature which was reduced from 55 to 50°C.

Sequencing and sequence analysis

Amplification products of the expected size were identified against a molecular weight marker, following electrophoresis on a 2% agarose gel. Bands of correct size were excised and purified by means of a Ron's Gel Extraction Kit (BIORON®, Germany) according to manufacturer specifications and sent to Macrogen Europe for sequencing. Analysis of sequence data was performed with Chromas (www.technelysium.com.au), BioEdit (www.mbio.ncsu.edu/ BioEdit/BioEdit.html) and ClustalX version 1.83 (www.clustal.org). For the tetrameric repeat sequences (TRS), analyses including that of the CVR sequences and deduced amino acid sequences were manually aligned with gaps being inserted to optimize the alignment. Two datasets were generated for phylogenetic analyses conducted using MEGA version 5.0 (Kumar et al., 2001), the p72 and P54 gene data sets. Sequences generated in this study from the Ugandan domestic pig viruses were analysed together with homologous sequences of viruses that were representative of genotype X, IX, VII and II identified in previous studies. Neighbour joining (NJ) trees were constructed employing the p-distance nucleotide substitution model as implemented in the MEGA 5.0 program. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. To determine the degree of statistical support for each node in the resulting p72 and P54 trees, data were re-sampled 1000 times using the bootstrap method. Out of the 14 virus isolates analysed, 12 p72 and 11 P54 generated consensus sequences on alignment.

RESULTS

The origins of ASF viruses

Fourteen (14) ASF viruses obtained from the Wambizi slaughterhouse were selected for use in this study. The samples that tested positive by PCR were from Busoga sub region (Eastern region), Lango sub region (Northern region), Kalungu district, Nakasongola district, Sembabule district, and Nakaseke district all from the central region. These origins depended entirely on the information provided by the traders at the slaughterhouse. One ASF virus was obtained during field surveillance in Kibaale district in Western Uganda.

The *p72* gene phylogeny

The analysis of the p72 partial gene sequences from each of the 12 ASF viruses showed that they were almost identical at the nucleotide level with minor differences resulting from manual trimming of the aligned sequences. The phylogenetic analysis established that all the Ugandan viruses obtained in this study were placed in the p72 genotype IX together with some viruses isolated in previous studies in Uganda, Kenya, and Congo as shown in Figure 1. The 12 ASF viruses (marked with (●) in Figure 1) submitted to the GenBank and their accession numbers include; Uga12.Nakasongola-KF303310, Uga12.Kalungu1-KF303311, Uga12.Kalungu2-KF303312, Uga12.Kalungu3-KF303313, Uga12.Sembabule-KF303314, Uga12.Kibaale-KF303315, Uga12.Nakaseke-KF303316, Uga12.Busoga1-KF303317, Uga12.Busoga2-KF303318, Uga12.Lango1-KF303319, Uga12.Lango2-KF303320 and Uga12.Lango3-KF303321.

The *p54* gene phylogeny

Previous studies have confirmed P54 sequencing as a valuable additional genotyping method for molecular epidemiological studies of genotype IX ASF viruses (Gallardo et al., 2009; Nix et al., 2006). PCR amplification of the fragment containing the complete P54 gene from all of the Ugandan viruses in this study produced products of approximately 670 to 680 bp. The nucleotide sequence analysis of the P54 gene showed that all the isolates were identical. The sequences of the 11 Ugandan viruses were compared with 50 P54 ASFV sequences retrieved from GenBank. The phylogeny revealed that the Ugandan viruses obtained in this study cluster with the majority of the viruses from previous outbreaks in Uganda, Kenya and Congo (Figure 2). The P54 sequences of ASF viruses (marked with (●) in Figure 2) submitted to the GenBank and their accession numbers include; Uga12.Nakaseke-KF303302, Uga12.Busoga1-KF303303, Uga12.Lango1-KF303304, Uga12.Lango3-KF303305, Uga12.Nakasongola-KF303306, Uga12.Kalungu2-KF303307, Uga12.Kalungu3-KF303308, Uga12.Sembabule-KF303309.

Intra-genotypic resolution (CVR) of homogenous p72 genotype IX Ugandan viruses from asymptomatic pigs

In order to delineate the p72 genotype IX obtained in this study at a higher resolution, the CVR of the B602L gene was analysed. Amplification of the CVR gave products of varying sizes (400 to 600bp). The Ugandan viruses characterized in this study clustered with isolates from previous studies in Uganda and some from Kenya. However, differences were mainly observed in the number of tetrameric amino acid repeats of the viruses obtained in this study. The viruses obtained in this study clustered in three different subgroups; 13, 23 and 25 based on the analysis of the tetrameric amino acid sequences (TRS) (Figure 3). Viruses Uga12.Kibaale and Uga12.Kalungu1 had the same repeat sequences with an additional single internally located tetrameric repeat (CAST). Viruses Uga12.Busoga1, Uga12.Lango4, Uga12.Busoga3 and Uga12.Nakaseke clustered together and were different from the others in this study due to the absence of a single CAST repeat and presence of a CADI sequence instead of a CADT sequence. Virus Uga12.Nakasongola was unique due to the absence of 11 tetrameric repeat sequences. The CVR sequences of the ASF viruses (highlighted in Figure 3) submitted to the GenBank and their accession numbers include; Uga12.Nakasongola-KF303295, Uga12.Busoga1-KF303296, Uga12.Lango4-KF303297, Uga12.Busoga3-KF303298, Uga12.Kibaale-KF303299, Uga12.Kalungu1-KF303300, and Uga12.Nakaseke-KF303301.

When compared with sequences of viruses causing outbreaks in Uganda (2010 to 2013) obtained from the GenBank (Atuhaire et al., 2013), viruses Uga12.Busoga1, Uga12.Lango4, Uga12.Busoga3 and Uga12.Nakaseke

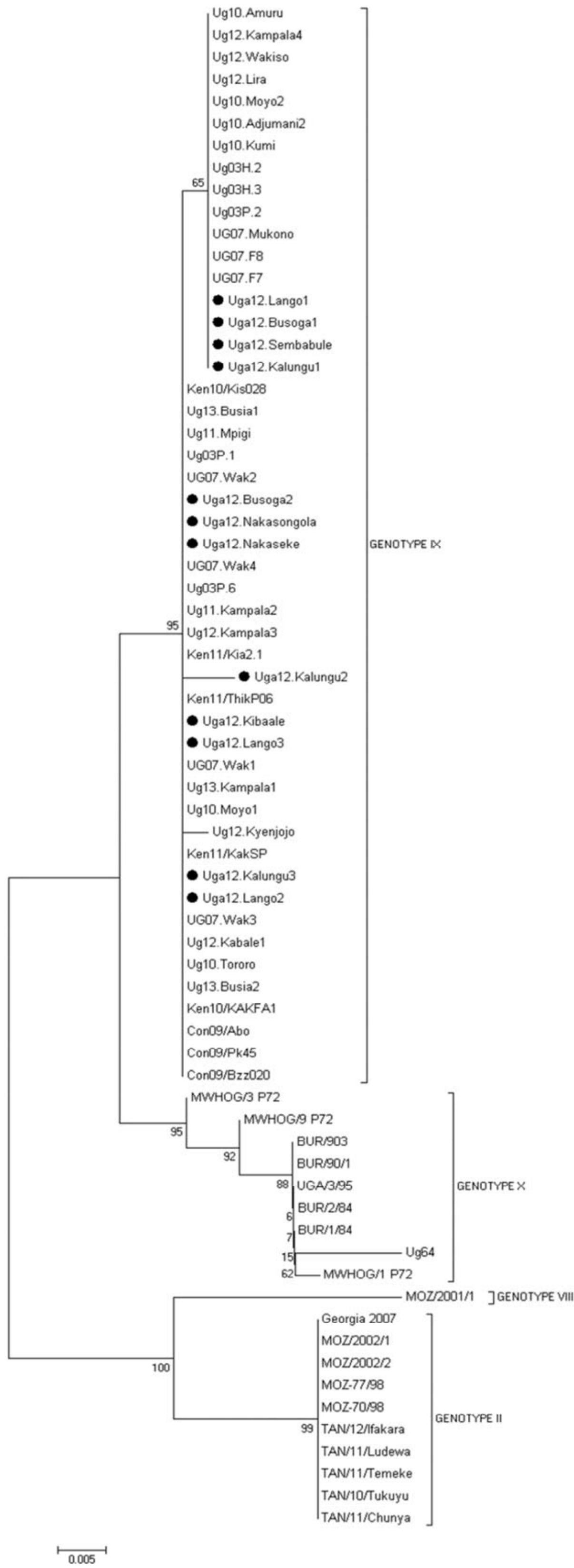

Figure 1. Evolutionary relationships of p72 genotypes: Neighbor-Joining tree of the p72 gene. The analysis involved 69 nucleotide sequences. The p72 sequences from this study are marked with ●. There were a total of 376 positions in the final dataset.

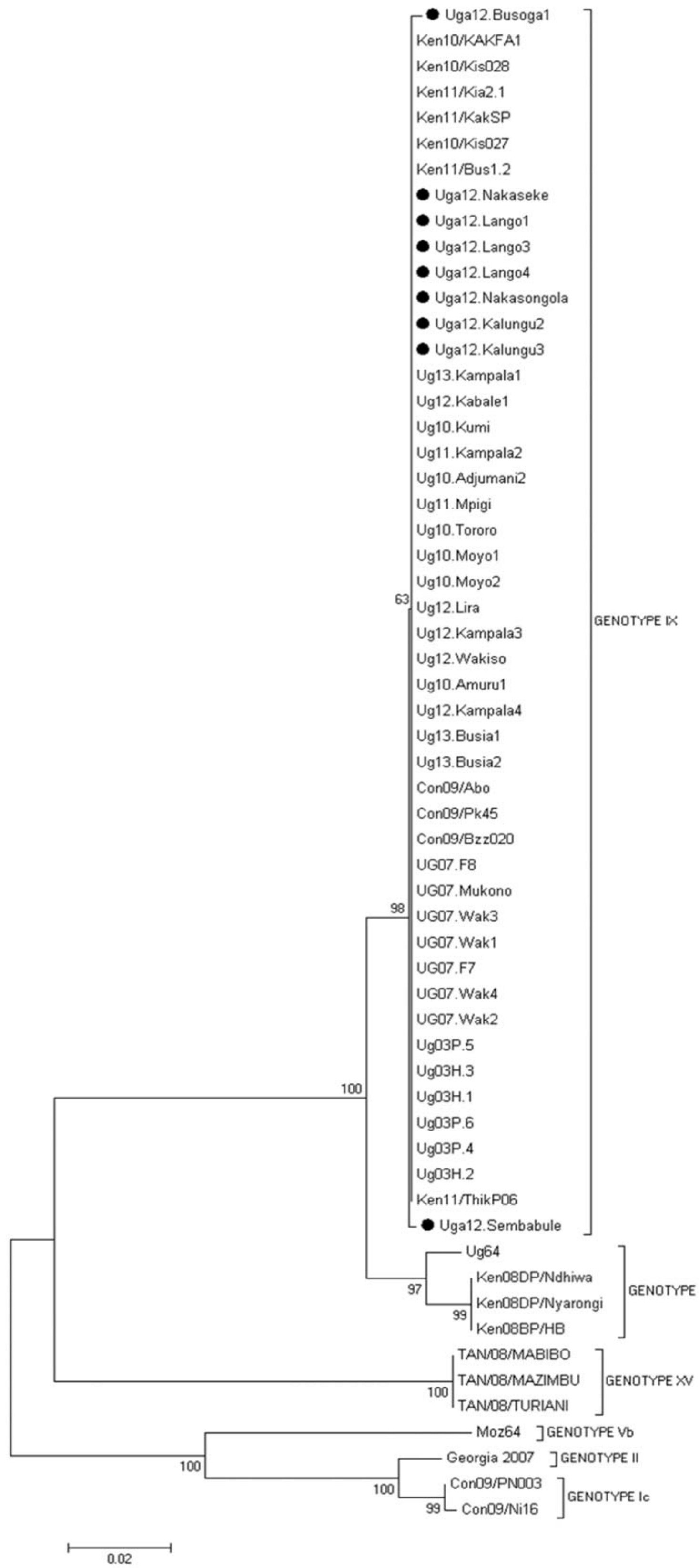

Figure 2. Evolutionary relationships of P54 genotypes: The Neighbor-Joining tree of the P54 gene. The analysis involved 61 nucleotide sequences. The P54 sequences from this study are marked with ●. There were a total of 535 positions in the final dataset.

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Uga12.Busoga1       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga12.Lango4        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga12.Busoga3       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga12.Nakaseke      LHAQSAYTCASTCASTCAST----CADTNVDTCASTCVDI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Kumi           LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug11.Kampala2       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADI----------------CADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga12.Kibaale       LHAQSAYTCASTCASTCASTCAST--------CAST----CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga12.Kalungu1      LHAQSAYTCASTCASTCASTCAST--------CAST----CADTNVDTCASTCADTCADTNVDTWASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVYTCVSTCADTCASTE
Uga12.Nakasongola   LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADT--------------------------------------------NVDTCVSTCADTCASTE
Ug13.Kampala1       LHAQSAYTCASTCASTCASTCAST--------CAST----CADTNVDTCASTCADT------------------------------------------------NVDTCVSTCADTCASTE
Ug12.Kabale1        LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Adjumani2      LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug11.Mpigi          LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Moyo1          LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug12.Lira           LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Amuru1         LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug12.Wakiso         LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug12.Kampala4       LHAQSAYTCASTCASTCASTCAST----------------CADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Tororo         LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Moyo2          LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug12.Kampala3       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug12.Kyenjojo       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug13.Busia1         LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug13.Busia2         LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ug10.Namasuba       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken11/KakSP         LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken11/Kia2.1        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken10/Kis028        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken10/KAKFA1        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken11/ThikP06       LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken11/Bus1.2        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
Ken10/Kis027        LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.Wak2           LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.Wak4           LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.F7             LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.Wak1           LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.Mukono         LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
UG07.F8             LHAQSAYTCASTCASTCAST----CADTNVDTCASTCADICADTNVDTCASTCADTCADT----------------CVSTCVST----CADTNVDTCASTCADTNVDTCVSTCADTCASTE
Uga_95/1            -HAQSAYTCASTCASTCAST----CADTNVDTCASTCADTCADTNVDTCASTCADTCADTNVDTCASTCADTCADTCVSTCVSTCADTCADTNVDTCASTCADTNVDTCVSTCADTCASTE
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Figure 3. Amino acid sequence alignment of the tetrameric tandem repeats identified within the central variable region (CVR) of gene B602L from Ugandan viruses obtained during abattoir and field surveillance during 2012 (Uga12). The sequences obtained were compared with CVR sequences from isolates associated with outbreaks in Uganda between 2010 and 2013, viruses from an outbreak in Uganda in 2007 as well as UGA95/1 and sequences causing outbreaks in Kenya in 2010 and 2011.

had the same number of tetrameric repeats as Ug11.Kampala2, Ug12.Kampala3, Ug10.Namasuba, Ug10.Tororo, Ug13.Busia1, Ug13.Busia2, Ug10.Kumi, Ug10.Moyo2 and Ug12.Kyenjojo. These viruses were identical to viruses causing outbreaks in Kenya in 2010 and 2011 suggesting that the same virus is circulating between the two countries. Viruses Uga12.Kibaale and Uga12.Kalungu1 were similar to Ug12.Kampala4, Ug11.Mpigi, Ug12.Wakiso, Ug10.Adjumani2, Ug10.Moyo1, Ug12.Lira, Ug10.Amuru and Ug12.Kabale1 in that they had an extra CAST tetrameric repeat, however, they were different in the total number of amino acid tetrameric repeats (Table 1). Uga12.Nakasongola and Ug13.Kampala1 had 13 amino acid tetrameric repeats each (Table 1), with a difference in only one repeat sequence. Uga12.Nakasongola had a CADT sequence while Ug13.Kampala1 had a CAST sequence (Figure 3).

DISCUSSION

African swine fever continues to hamper the development of the pig industry in Uganda with outbreaks occurring sporadically throughout the year. The disease is endemic in the country (Atuhaire et al., 2013; OIE, 2010). The inability of ASFV to induce neutralizing antibodies has hampered the prevention and control of the disease by vaccination and to date there is no vaccine for ASF. In the absence of effective vaccines, control is based on rapid laboratory diagnosis and the enforcement of strict sanitary measures (Sánchez-Vizcaíno et al., 2009). The formulation of appropriate disease control strategies requires intensive molecular epidemiological investigations not only during disease outbreaks but also field and abattoir surveillance. This would be of value in determining the nature of the viruses circulating in asymptomatic domestic pigs and comparing them with

Table 1. Amino acid sequence of the tetrameric repeats that constitute the CVR of the B602L gene identified in viruses belonging to p72 genotype IX.

Virus name	Country	p72 genotype	CVR amino acid sequence	No. of repeats	CVR GenBank accession no.	Reference
Uga12.Busoga1	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KF303296	This study
Uga12.Lango4	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KF303297	This study
Uga12.Busoga3	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KF303298	This study
Uga12.Nakaseke	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KF303301	This study
Ug10.Kumi	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990858	Atuhaire et al., 2013
Ug11.Kampala2	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990859	Atuhaire et al., 2013
Uga12.Kibaale	Uganda	IX	AAAAABNABBNABBaaBBNABNaBA	25	KF303299	This study
Uga12.Kalungu1	Uganda	IX	AAAAABNABBNABBaaBBNABNaBA	25	KF303300	This study
Uga12.Nakasongola	Uganda	IX	AAAABNABBNaBA	13	KF303295	This study
Ug13.Kampala1	Uganda	IX	AAAAABNABNaBA	13	KC990856	Atuhaire et al., 2013
Ug12.Kabale1	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990857	Atuhaire et al., 2013
Ug10.Adjumani2	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990860	Atuhaire et al., 2013
Ug11.Mpigi	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990861	Atuhaire et al., 2013
Ug10.Moyo1	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990863	Atuhaire et al., 2013
Ug12.Lira	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990865	Atuhaire et al., 2013
Ug10.Amuru	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990868	Atuhaire et al., 2013
Ug12.Wakiso	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990867	Atuhaire et al., 2013
Ug12.Kampala4	Uganda	IX	AAAABNABBNABBaaBBNABNaBA	24	KC990870	Atuhaire et al., 2013
Ug10.Tororo	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990862	Atuhaire et al., 2013
Ug10.Moyo2	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990864	Atuhaire et al., 2013
Ug12.Kampala3	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990866	Atuhaire et al., 2013
Ug12.Kyenjojo	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990869	Atuhaire et al., 2013
Ug13.Busia1	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990871	Atuhaire et al., 2013
Ug13.Busia2	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990872	Atuhaire et al., 2013
Ug10.Namasuba	Uganda	IX	AAABNABBNABBaaBBNABNaBA	23	KC990873	Atuhaire et al., 2013
Ken11/KakSP	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93414.1	Gallardo, 2012
Ken11/Kia2.1	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93412.1	Gallardo, 2012
Ken10/Kis028	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93410.1	Gallardo, 2012
Ken10/KAKFA1	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93408.1	Gallardo, 2012
Ken11/ThikP06	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93413.1	Gallardo, 2012
Ken11/Bus1.2	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93411.1	Gallardo, 2012
Ken10/Kis027	Kenya	IX	AAABNABBNABBaaBBNABNaBA	23	AGC93409.1	Gallardo, 2012
UG07.Wak2	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18202.1	Gallardo et al., 2011
UG07.Wak4	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18204.1	Gallardo et al., 2011
UG07.F7	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18206.1	Gallardo et al., 2011
UG07.Wak1	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18201.1	Gallardo et al., 2011
UG07.Mukono	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18205.1	Gallardo et al., 2011
UG07.F8	Uganda	IX	AAABNABBNABBaaBNABNaBA	22	ACZ18207.1	Gallardo et al., 2011
Uga_95/1	Uganda	IX	ABNABBNABBNABBaaBBNABNaBA	25	CAJ90783.1	Nix et al., 2006

A, CAST, WAST; a, CVST; B, CADT, CVDI, CADI; N, NVDT, NVYT.

viruses causing disease outbreaks. The major aim of the current study was to detect and characterize ASFV obtained during an abattoir surveillance and field surveillance in selected parts of the country. We used the combined p72, full length P54 and CVR approach to achieve optimal levels of discrimination of even the closely related viruses as previously described (Gallardo et al., 2011). The ability to delineate ASF viruses using the p72, P54 and CVR genes without the need to first isolate the viruses was explored in this study.

We used the OIE recommended diagnostic PCR with primers (Wilkinson, 2000) to confirm the presence of ASFV

DNA in blood samples collected from asymptomatic domestic pigs during abattoir and field surveillance. It could be that the pigs brought for slaughter are subclinical or chronic carriers of ASF. More so, our findings agree with a study carried out in Rakai district in Uganda where ASFV was detected in asymptomatic domestic pigs (Björnheden, 2011). A recent study in Uganda has also detected ASFV in apparently healthy domestic pigs in the same slaughter house (Atuhaire et al., 2013). A seroprevalence study in abattoirs in Mubende has also detected ASFV in domestic pigs (Muwonge et al., 2012). This emphasizes the role of sub-clinical and/or chronically infected carrier domestic pigs in the epidemiology of ASF and factors that lead to resurgence of the virus to cause active infection need to be investigated further.

In this study, phylogenetic analysis based on the p72 and P54 genes grouped all the Ugandan viruses into genotype IX. The results of this study agree with other previous studies in Uganda that grouped viruses causing outbreaks into the same genotype (Atuhaire et al., 2013; Gallardo et al., 2011). Our viruses are also similar to viruses causing outbreaks in neighbouring Kenya in 2010 and 2011 (Gallardo, 2012) emphasizing the role of neighbouring countries in the epidemiology of the disease.

Our findings suggest that there is no significant variation in the ASF viruses circulating in Uganda based on their p72 and P54 genome regions characterized at nucleotide level, confirming a remarkable genetic stability of these regions.

Although p72 and P54 genes are useful for identifying the major ASFV genotypes, higher discrimination of viruses enables more detailed dissection of the genotypes for epidemiological analysis and classification. The analysis of the B602L gene of the CVR revealed the presence of minor differences in the number of TRS placing the viruses into three clusters (subgroups). Uga12.Busoga1, Uga12.Lango4, Uga12.Busoga3 and Uga12.Nakaseke clustered together with 23 TRS. Viruses Uga12.Kibaale and Uga12.Kalungu1 clustered together and had 25 TRS. Uga12.Nakasongola had only 13 TRS which compared with an isolate Ug13.Kampala1 (Accession number GenBank: KC990856) from a previous study in Uganda (Atuhaire et al., 2013). Our findings confirm the value of the *CVR* gene as an additional marker for delineating ASFV in addition to p72 and P54 genotyping.

In conclusion, only one genotype is circulating in Uganda among asymptomatic domestic pigs and it is the same virus genotype causing outbreaks in the country and parts of neighbouring Kenya based on molecular characteristics and genetic patterns of the analysed ASF viruses. The fact that ASFV was detected in asymptomatic domestic pigs emphasizes their role in the epidemiology of the virus.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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