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First investigation on the presence of porcine parvovirus type 3 in domestic pig farms without reproductive failure in the Democratic Republic of Congo

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ABSTRACT

Porcine Parvovirus (PPV) is one of the major pathogens responsible for reproductive failure in sows. However, the information on its frequency in the Democratic Republic of Congo (DRC) is largely unknown. Thus, the present study was carried out to detect and genetically characterize some of known Parvovirus namely porcine parvovirus 1, 2, 3, 4, porcine bocavirus (PBoV) 1, and porcine bocavirus-like virus (PBolikeV) in 80 randomly selected archive pig farm samples during an African swine fever (ASF) survey in South Kivu, eastern DRC by polymerase chain reaction (PCR). The majority of animals analyzed (82.5%) were local breeds, and most of them (87.5%) were adults (above one year old). The majority of the animals (65%) were from the free range farms. The PCR result indicated that only PPV3 was detected in 14/80 pigs. Seven swine herds (8.7%) were co-infected with PPV3 and ASFV. Morever, a significantly high PPV3 infection rate was observed in the spleen (66.7%, $P<0.0001$) compared to the others type of samples. Further, the phylogenetic analysis of partial PPV3 sequences revealed one clade of PPV3 clustered with PPV3 isolates reported in a previous study in Cameroun, China, Slovakia, Germany, and China. This study is the first to report the detection of PPV in DRC. Further studies are needed to assess the levels of PPV3 viremia and the impact in co-infections with other endemic pig viruses, including ASFV.

1. Introduction

Pig farming has become a very attractive business venture across the Democratic Republic of Congo (DRC) to increase food, income, and employment. Pigs are preferred for their unique features such as prolificacy and their relatively short generation time that results in high production turnover for sale and consumption (Afolabi et al., 2019). Despite the economic importance of pig farming, this sector is hampered by various constraints, including diseases such as swine infertility infectious pathogens(Dione et al., 2014). Several studies on pig diseases in DR Congo have focused mainly on African swine fever and gastrointestinal parasites (Praet et al., 2014; Mulumba-Mfumu et al., 2017; Bisimwa et al., 2020), thereby neglecting the exploration of other diseases patchy. In most instances, farmers often mistake other diseases for "swine fever" due to inadequate or lack of accurate diagnosis. There is a wide variety of pathogenic viral agents involved in the development of diseases that affect pigs' reproductivity. This includes the porcine parvovirus, the most important cause of porcine reproductive failure worldwide (Mengeling et al., 2000). Parvoviruses infect a wide range of species ranging from non-vertebrate arthropods to higher mammals, including human beings. Their high diversity further influences the clinical manifestation on their hosts, varying from non-pathogenic

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infections to severely fatal diseases' manifestations even though most of them cause only mild or subclinical infections in infected hosts (Kailasan et al., 2015).

Porcine parvovirus is the main causative agent of embryonic and fetal death in swine, which causes mummification, stillbirths, and delayed return to estrus (Mengeling, 2006). Porcine Parvovirus belongs to the *Parvoviridae* family comprising two subfamilies, namely *Densovirinae* (which infect arthropods) and *Parvovirinae* (whose members infect vertebrates). The *Parvovirinae* subfamily is further classified into eight Genera with the addition of several new species and viruses as members of the family. These include *Dependoparvovirus, Erythroparvovirus, Amdoparvovirus, Averparvovirus, Bocaparvovirus, Copiparvovirus, Protoparvovirus,* and *Tetraparvovirus* (Cotmore et al., 2014). The last four genera presently contain the viruses that infect swine, and the most representatives include the classical porcine parvovirus 1 (PPV1), the novel porcine parvovirus type 2 (PPV2), PPV3, PPV4, PPV5, PPV6, PPV7, porcine bocavirus 1 (PBoV1), PBoV2, PBoV3A, PBoV3B, PBoV3C, PBoV3D, and PBoV3D (Cotmore et al., 2019; Gava et al., 2015). The classical porcine parvovirus 1 (PPV1) belongs to the *Parvovirus* genus in the subfamily of *Parvovirinae.* The PPV2 and PPV3 belong to the genus of *Tetraparvovirus,* while PPV4 is currently classified together with bovine parvovirus type 2 within the genus of *Copiparvovirus.* More recently, through metagenomics assays, a novel (but unclassified) porcine parvoviruses have been identified and provisionally designed as PPV5 in the USA (Xiao et al., 2013), PPV6 in Poland (Cui et al., 2017), and more recently PPV7 in China (Ouh et *al.*, 2018; Xing et *al.*, 2018).

Porcine parvoviruses are small, non-enveloped, single-stranded linear DNA viruses with a genome size ranging from approximately 4–6.3 kb. They have a palindromic hairpin terminus and contains two major open reading frames (ORFs), including the ORF1, which encode non-structural protein (NS1) located at the 59-end and ORF2 encoding at least two capsid proteins (VP1 and VP2) located at the 39-end (Bergeron et al., 1993; Allander et al., 2005). The capsid protein VP2 contains major antigenic domains of PPV, which induce the production of neutralizing antibodies (Kong et al., 2014). Thus, VP2 is the main target for PPV diagnosis and immune prophylaxis.

Additionally, emerging parvoviruses grouped under the genus *Bocaparvovirus* have been reported for the first time in some parts of Africa, such as Uganda and Cameroon (Blomström et al., 2013 Ndeze et al., 2013). In DRC, only human parvoviruses B19 in children under five years old have been reported, but no study is available on the prevalence of porcine parvoviruses in this country. Recently, a cross-sectional study was carried out in South Kivu province in the Eastern part of DR Congo to detect the presence of African swine fever virus (ASFV) in suspected infected pigs. Of the 391 blood samples tested, 6.7% were ASFV positive. This low frequency of ASFV in symptomatic pigs was attributed to the possibility that most pigs sampled may not have been infected by ASFV. This suggests that pigs may have been affected by other swine diseases with similar symptoms to ASF, including porcine parvoviruses or classical swine fever, which are not routinely diagnosed. In addition, a previous study conducted in this region by Akilimali et al. (2017) showed that a large number of pig farmers had reported occurrence of several cases of abortion, sows farrowing a low number of piglet (less than eight a time) or dead fetuses, all of which are reproductive disorder associated with PPVs.

Despite the growing concerns about porcine parvoviruses (PPVs), information about their frequency in Eastern D.R. is lacking, and the circulating viruses have never been characterized. This study aimed, therefore, to provide the first detection and genetic characterization of porcine parvoviruses in domestic pig farms in DR Congo with ASFV infection-related clinical signs.

2. Materials and methods

2.1. Study area

The study was conducted in the South Kivu province, located in the Eastern part of the Democratic Republic of Congo (Fig. 1). It is a 66.814 Km^2 region, located between longitudes 26° 10′ 30" and 29° 58′ east, and latitudes 00′ 58" North and 4° 51′ 21" South.

Based on the importance of pig farming, six out of eight districts were selected for the purpose of this study. These include Fizi, Kabare, Kalehe, Mwenga, Uvira, and Walungu. A key factor in selecting the sample sites was the presence of pig-farming and, pig-product trade. Equally, high pig-product consumption areas, especially where swine disease outbreaks had been reported by the Provincial Ministry of Agriculture Livestock and Fishery (South Kivu, DRC), were considered.

2.2. Sample collection and DNA extraction

A total of 80 samples were randomly selected from archived samples collected from both sick and apparently healthy female pigs (sows) during an African swine fever investigation study in South Kivu province between December 2018 and January 2020. Previously, a molecular survey was conducted for detecting and characterizing ASFV in this region using these samples. The suspected sick pigs presented a variety of clinical signs, including hemorrhagic diarrhea, high fever, reddening of the ears and snout, coughing and difficulties in breathing, vomiting, inability to stand, and loss of appetite. No signs of reproductive disorder were recorded on the animals. The age of the pigs tested ranged from 4 months and above. The viral DNA was extracted directly from 300 µl of spleen and liver tissues homogenates and 200 µl of whole blood using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's guidelines. The quality and integrity of the DNA were assessed by 0.8% agarose gel electrophoresis after staining with 0.5 µg/ml Nucleic Acid Gelred (Biotium) (Fisher Biotech, Australia), while the content and purity were estimated by Nanodrop (PCRmax Lambda) spectrophotometer. The extracted DNA was then stored at -20°C in the molecular biology laboratory at Université Evangélique en Afrique (UEA) before being shipped to Kenya. After extracting DNA, all samples were shipped in a cold scheme to the molecular biology laboratory at Pan African University Institute of Basic Sciences Technology and Innovation in Nairobi, Kenya, for downstream analysis. Once in Nairobi, all DNA samples were stored at -20°C before being used.

2.3. PCR amplification of viral DNA

The genomic DNA of different porcine parvoviruses were detected by conventional Polymerase Chain Reaction (PCR) techniques using primers and PCR protocols as previously reported (Cságola et al., 2012; Afolabi et al., 2019). The primer sequences used for both diagnosis and characterization, their nucleotide sequences, and sources are presented in Table 1. A 20µl PCR setup containing 1.25 µM of each primer, 5x FIREPol Master Mix with blue dye (Solis BioDyne, Estonia) which contains (12.5 mM $MgCl_2$, 1 mM dNTPs, 25 units/ml FIREPol DNA polymerase (500 U/100 µl), 5x reaction Buffer B), 10 µg of DNA template and nuclease-free water added to make-up a 20 µl reaction mixture was prepared. PCR amplification of the viral DNA was done in a thermocycler machine (ProFlex^M, Applied Biosystems) using optimized annealing temperatures ranging between 51 to 58°C. All PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel stained with GelRed nucleic acid stain in the presence of molecular weight markers and visualized using U.V. light. For sequencing, PCR products were cleaned up using Quick PCR purification Kit (QIAGEN, USA) following the manufacturer's instructions and sent to Macrogen Europe B.V. (Amsterdam, The Netherlands) for Sanger sequencing.

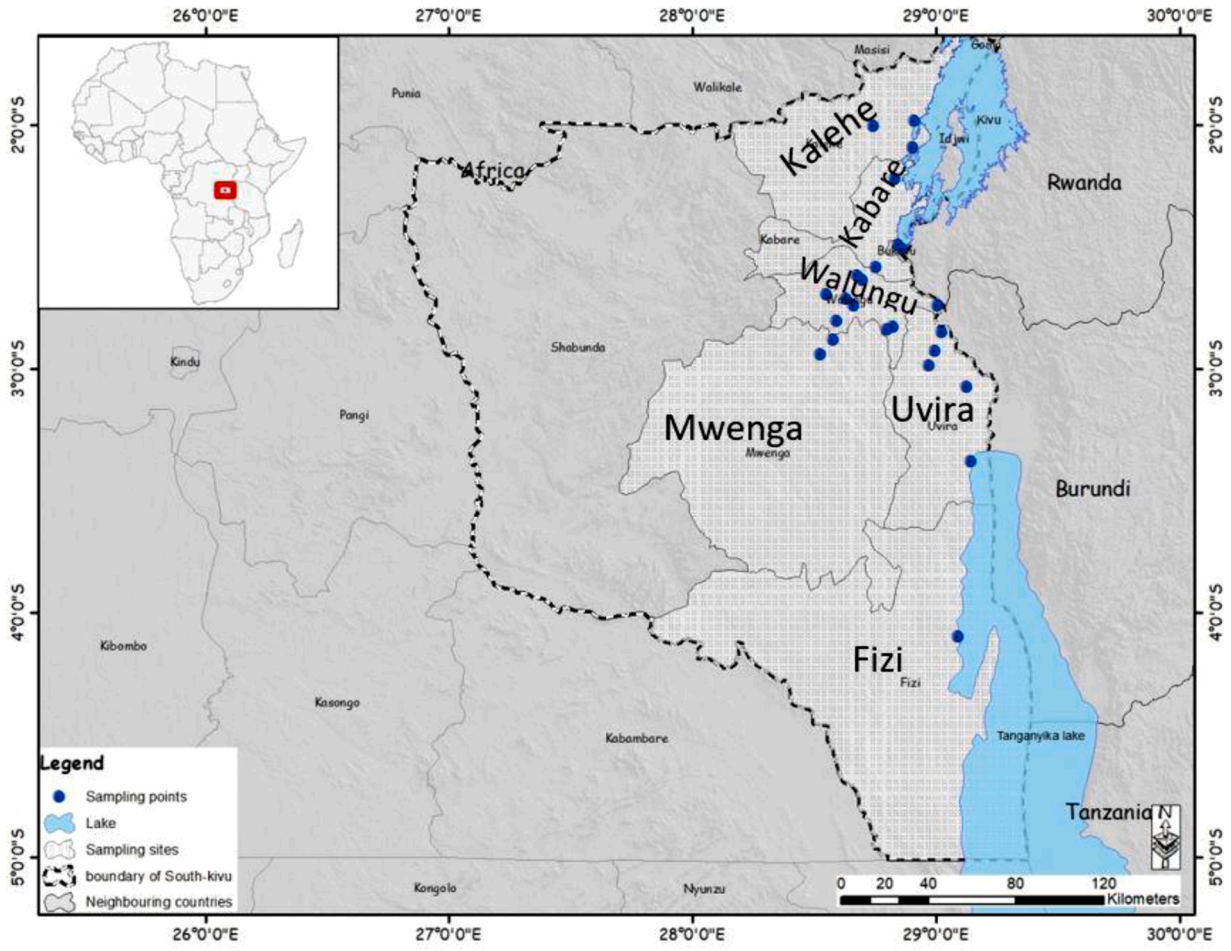

Fig. 1. Map of South Kivu province in the Democratic Republic of Congo highlighting the sampling sites and dots show the sampling points (Arc GIS, UEA).

Table 1
PPVs' primer sequences previously reported and used for detection and molecular characterization.

Primer name	Primer sequence	Amplicon size (bp)	Virus type	Region	Reference
PPV1F	CACAGAAGCAACAGCAATTAGG	203	PPV1	ORF2	Ogawa et al., (2009)
PPV1R	CTAGCTCTTGTGAAGATGTGG				
PPV2AF	ACACGATGAGCGGTACGA	279	PPV2	ORF2	Cságola et al., (2012)
PPV2AR	TCCTCACGAGGTCTCTTCTG				
PPV3DF	GCAGTCTGCGCTTAACTT	392	PPV3	ORF2	Cságola et al., (2012)
PPV3DR	CTGCTTCATCCACTGGTC				
PPV4DF	TCATAGCACTATGGCGAGC	284	PPV4	ORF2	Cságola et al., (2012)
PPV4DR	AGC ATT CTG CGT TGG ACA				
SbocaF	GGGCGAGAACATTGAAGAGGT	495	PBo-likeV	ORF2	Zhai et al., (2010)
SbocaR	TTGTGAGTATGGGTATTGGTG				
PBoVF	TGGTGGAACGTCTCTCTGACA	466	PBoV1/V2	NS1/NP1	Cságola et al., (2012)
PBoVR	GAGTCATTCGGTCTCCTCCAT				

2.4. Sequence and phylogenetic analysis

The sequences were trimmed and assembled using CLC Genomics Workbench 8 (QIAGEN, USA) software. Homology search was carried out with the BLASTN program on the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequence alignment of the partial sequences of ORF2 was created using Clustal W implemented into MEGA X, and the output alignments were then subjected to phylogenetic analysis with MEGA X (Kumar *et al.,* 2016). Sequences obtained from PCR products and publicly available porcine parvovirus sequence data in GenBank were used to generate a comparable topology tree. The evolutionary trees were built using the Maximum Likelihood method with the Kimura 2-parameter model and bootstrap test of 1000 replicates.

The phylogenetic dataset included nine(9) sequences obtained in this study and 23 PPVs geographically related strains retrieved from GenBank. The DR Congo PPV3 nucleotide sequences were submitted to GenBank, and the assigned accession numbers are presented in Table 2 alongside the sequences retrieved from GenBank used as references for comparison.

2.5. Statistical analysis

A descriptive statistical and univariate analysis was carried out using CDC Epi-info ™ version 7 (Centers for Disease Control and Prevention, 2016) software. Each factor was coded as a dichotomous independent

Table 2
PPV3 sequences obtained in this study and other PPV's sequences previously reported and used in phylogenetic analysis.

Virus	Strain	Location	Host	Accession number	Source
PPV1	PPV1-SA10	South Africa	pig	MG846614	GenBank
	PPV1-DJH24	China	Wild boar	MK092384	GenBank
	PPV1-SA5	South Africa	pig	MG846609	GenBank
PPV2	F2-1NB	Romania	pig	JQ860239	GenBank
	PPV2-DSH1	China	Wild boar	MK092389	GenBank
PPV3	PPV3-GX-2012	China	pig	KJ842609	GenBank
	Cam79	Cameroun	pig	KF225548	GenBank
	Cam82	Cameroun	pig	KF225549	GenBank
	PPV3 VIRES NX02	China	pig	MK378240	GenBank
	PPV3 VIRES GZ03	China	pig	MK378232	GenBank
	2376KA	Slovakia	Wild boar	KP768509	GenBank
	PPV3-JS-2012	China	pig	KJ842611	GenBank
	PPV3-ZJ-2012	China	pig	KJ842613	GenBank
	70T	Germany	-	KC296751	GenBank
	PPV3-SK-DRC-2	DRC	Pig	MW355754	This study
	PPV3-SK-DRC-9	DRC	Pig	MW355753	This study
	PPV3-SK-DRC-10	DRC	Pig	MW355748	This study
	PPV3-SK-DRC-18	DRC	Pig	MW355751	This study
	PPV3-SK-DRC-45	DRC	Pig	MW355750	This study
	PPV3-SK-DRC-49	DRC	Pig	MW355749	This study
	PPV3-SK-DRC-237	DRC	Pig	MW355747	This study
	PPV3-SK-DRC-240	DRC	Pig	MW355755	This study
	PPV3-SK-DRC-244	DRC	Pig	MW355752	This study
PPV4	JS0918a	China	Pig	HM031134	GenBank
	F3-7BN	Romania	pig	JQ868707	GenBank
	PPV4-21PL	Poland	pig	KC701352	GenBank
PPV5	ND564	USA	pig	JX896322	GenBank
	PPV5-DSH33	China	Wild boar	MK092443	GenBank
PPV6	KSU4-NE-2014	USA	pig	KR709265	GenBank
	K17-10	Poland	pig	KX384820	GenBank
PPV7	PPV7/COL	Colombia	pig	MT747168	GenBank
	GD-2014-3	China	pig	KY996758	GenBank

PPV3 nucleotide sequences generated from this study are highlighted.

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variable, and the odds of being a PPV case based on PCR results were then modeled as a function of the dichotomous determinant measures, using conditional logistic regression models. Group differences were tested using chi-square statistics for categorical variables, while Fischer's exact test was used for discrete variables using the 95% confidence level.

Table 3
Characteristics of pigs screened and husbandry practices in each study district.

Variables	Characteristics	Fizin	Kabaren	Kalehen	Mwengan	Uviran	Walungun	Total N=80(%)
Breed	Local	10	9	6	9	16	16	66(82.5)
	Crossed	0	3	5	0	0	6	14(17.5)
Age	Adult	9	10	9	8	15	19	70(87.5)
	Young adult	1	2	2	1	1	3	10(12.5)
Husbandry system	Free-range	9	5	3	1	16	9	43(53.8)
	Housed	1	7	8	8	0	13	37(46.2)
Source of pig	Own farm	5	7	6	8	12	14	52(65)
	Bought/market	3	3	3	0	1	6	16(20)
	NGOs	2	2	2	1	3	2	12(15)
Swill feeding	Yes	1	4	3	3	6	8	25(31.3)
	No	9	10	10	7	12	16	64(80)
Administration of vitamins	Yes	0	0	2	1	2	2	7(8.7)
	No	10	12	9	8	14	20	73(91.3)
Sharing pen	Yes	6	10	7	7	10	14	54(67.5)
	Non	4	2	4	2	6	8	26(32.5)

3. Results

3.1. Characteristics of domestic pigs and husbandry practices in the South Kivu province

During this study, a total of 80 samples were randomly selected from 391 ASF suspected domestic pigs with clinical signs related to African swine fever ASF from 300 farms in the South Kivu province in the years 2018 and 2019. Previously, a molecular detection survey of ASFV was conducted to evaluate the ASFV infection rate. Out of the 391 samples tested, ASFV was detected in only 26 samples (6.6%) (Bisimwa et al., 2020). The majority of animals analyzed in this study (82.5%) were local breeds, and most of them (87.5%) were adults (above 1-year-old). The majority of the animals (65%) were from free-range farms, while over half (53.8%) were housed (semi-intensive) breeding systems (Table 3). In addition, the pig feeding patterns were similar in all the districts, with crop residues being the major feeding source and a low proportion of the pigs inspected (31.3%) were fed with unprocessed pigswill. Moreover, a high number of pigs of different ages (67.5%) share pens.

3.2. Detection of porcine parvoviruses viral DNA in domestic pigs in South Kivu province, east of Democratic Republic of Congo

A total of 80 pig samples were tested for the presence of PPV genomes. Resolved PCR product in 1.5% gel produced an expected band of 400 bp in some domestic pig samples for porcine parvovirus 3 (PPV3) (Fig. 2). PPV3 genomes were detected in 14 swine samples representing an overall frequency of 17.5% (Table 4). However, PPV1, PPV2, PPV4, porcine bocavirus type 1 and 2 (PBoV1/2) were not detected in any of the samples analyzed. The highest infection rate was observed in Walungu (31.8%), followed by Uvira (12.5%), while the lowest was in Kabare (8.3%). Seven swine samples (8.7%) were co-infected with PPV3 and ASFV, with the highest co-infection (18.7%) reported in the Uvira district. The sequences of the ASFV positive samples were reported in our previous study (Bisimwa et al., 2020), and they can be found in GenBank under accession number MN689307 MN689309, MN689310, MN689311, MN689312, MN689313, and MN689317.

3.3. Regression analysis of factors associated with PPV3 positivity

The statistical analysis revealed that PPV3 infection in swine varied significantly according to sample type ($p<0.0001$), where a high infection rate was detected in the spleen (66.7%) followed by liver (40%) (Table 5). Based on geographical location, no significant difference was detected, although the frequency seemed to be highest in Walungu district (31.8%) while the lowest was reported in Kabare (8.3%). No statistical difference was detected between PPV3 infection and the husbandry system.

Table 4
Proportion and distribution of porcine parvoviruses type 3 genomes and co-infections with ASFV in domestic pigs from South Kivu province, East of DR Congo.

Districts	Number animal	PPV3 positive	Positive (%)	Co-infection with ASFV n (%)
Fizi	10	2	20	0 (0)
Kabare	12	1	8.3	0 (0)
Kalehe	11	1	9	1 (9)
Mwenga	9	1	11.1	0 (0)
Uvira	16	2	12.5	3(18.7)
Walungu	22	7	31.8	3 (13.6)
Total	**80**	**14**	**17.5**	**7 (8.7)**

3.4. Nucleotide sequences and phylogenetic analysis

Of the 14 PPV3 positive samples, we successfully amplified and sequenced 9(64.3%) samples for ORF2. Cross comparison among the ORF2 nucleotide sequences obtained from this study was done alongside other PPV3 reference sequences retrieved from GenBank. Nucleotide sequence analysis showed identity ranging from 99.4 to 100% between all the PPV3 from this study and from 98.1 to 100% with other PPV3 sequences. The evolutionary relationship tree was constructed based on the PPV3 sequences identified in the current study, and 23 representatives reported porcine parvoviruses' sequences type 1 to 7. The phylogenetic analysis revealed that PPV3 sequences originating from DRC were genetically very close related to sequences from studies in Cameroun, China, Slovakia, Germany, and China (Fig. 3), but the DRC PPV3 strains appeared to be most related to a China PPV3 strain (KJ842609) and Slovakia PPV3 strain (KP768509) with a 98.8% and 98.5% nucleotide identity respectively (Data not shown).

4. Discussion

Porcine parvoviruses are among the most important swine infertility infectious pathogens responsible for infertility and pathogenesis in the reproductive and respiratory system. They constitute a major threat to the development of the pig industry and are commonly present in most pig-rearing countries (Saekhow and Ikeda, 2015). Cases of abortions or farrowing of dead or mummified fetuses are common in DRC, but information on the causative agents remains scarce. This study explored

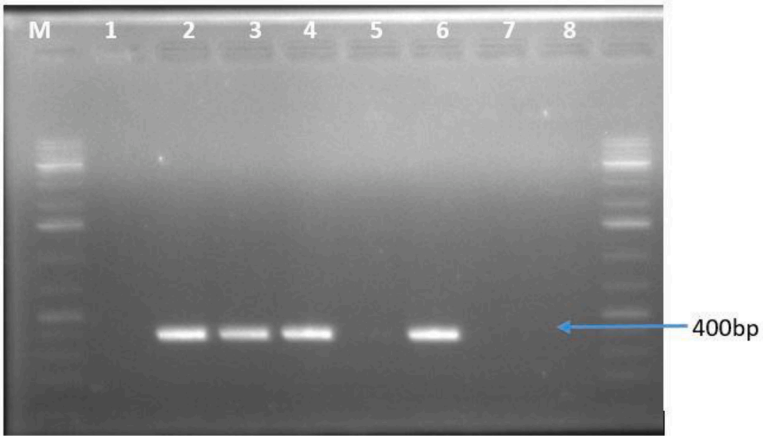

Fig. 2. PCR amplification of the PPV3. A 1.5% agarose gel showing the bands obtained with the diagnostic primers targeting the ORF2 of PPV3. Lane M: 1Kb plus (Thermo Fisher Scientific) Molecular weight DNA marker; Lanes 2, 3, 4, and 6 are PPV3 positive samples with an approximately 400 bp band size. Lane 1, 5, and 7 are PPV3 negative samples, while lane 8 is a negative control.

Table 5
Linear regression analysis of PPV3 in the tested pigs according to the breeding system, sample type, and geographical region.

Variable	Category(n)	Positive n (%)	Coefficient	Std Error	F-test	P-value
Age	Adult (70)	10 (14.3)				0.472
	Young adult (10)	3 (30)	0.080	0.111	0.522	
Breed	Exotic (14)	1 (7.1)				0.269
	Local (66)	12 (18.9)	0.117	0.105	1.238	
Husbandry system	Free-range (43)	7 (16.3)				
	Housed (37)	6 (16.2)	0.093	0.093	0.989	0.323
Districts	Fizi (10)	2 (20)				
	Kabare (12)	1 (8.3)	-0.165	0.148	1.2430	0.268874
	Kalehe (11)	1 (9)	-0.122	0.165	0.550	0.460
	Mwenga (9)	1 (11.1)	-0.225	0.167	1.801	0.184
	Uvira (16)	2 (12.5)	-0.249	0.141	3.112	0.082
	Walungu (22)	7 (31.8)	-0.111	0.143	0.600	0.441
Sample type	Blood (59)	3 (5.08)				
	Liver (5)	2 (40)	0.381	0.160	5.694	0.019*
	Spleen (12)	8 (66.7)	0.621	0.110	31.878	<0.0001***
CONSTANT			0.019	0.144	0.016	0.898

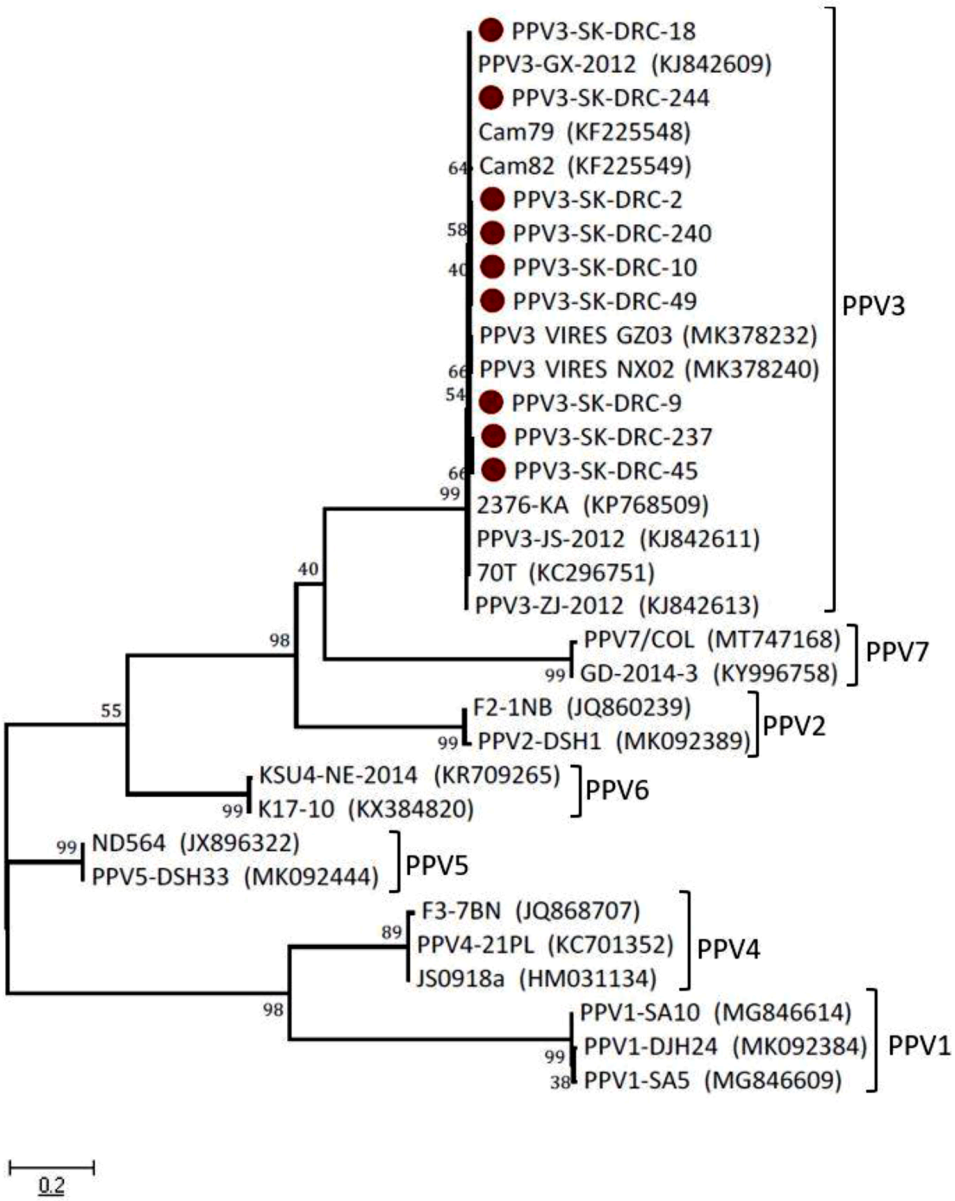

Fig. 3. Phylogenetic tree from the alignment of VP2 gene of the seven types of PPVs. The evolutionary history was inferred using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model. This tree included 23 porcine parvoviruses' sequences grouped from PPV type 1 to type 7. The phylogeny was inferred following 1,000 bootstrap replications, and the node values show percentage bootstrap support. Scale bar indicates nucleotide substitutions per site. The GenBank accession numbers for the different PPVs sequences are indicated in parenthesis. Sequences from this study are presented by a red circle (•).

the presence, and molecular characteristics of Porcine Parvovirus (PPV) strains in sows without reproductive failure in South-Kivu province, Eastern DRC, where pig farming is common. The study was carried out on 25 farms from which 80 samples were collected comprising blood and tissues from pigs with clinical signs related to ASF. They were analyzed by conventional PCR for the presence of PPV1–PPV4 as well as PBoV1 genomes. Information on the circulating PPVs in different pig farms will provide more relevant data and contribute to better understanding the frequency and distribution of these viruses.

Of all the PPVs analyzed, only the PPV3 (originally named porcine hokovirus) genomes were detected in 17.5% (14/80) of the samples. To the best of our knowledge, this is the first study reporting PPV3 circulating in pig farms in DRC. This finding is in contrast to a recent study carried out in South Africa, where all these PPV strains were detected (Afolabi et al., 2019). This observation may be due to the sampling procedures used that might influence the results. Our study involved analysis of pig farms with no clinical signs related to reproductive disorders, while the previous study conducted by Afolabi et al. (2019) in South Africa was based only on confirmed porcine circovirus type 2 (PCV2) infected pigs.

The overall high frequency of PPV3 suggests a persistent infection, but no evidence could be identified to explain the pathogenicity of the virus. The frequency obtained from this study is higher than 5.5% obtained in pigs in South Africa (Afolabi et al., 2019), but it is relatively lower than 20%, 69%, and 73% reported in previous studies in Germany (Streck et al., 2013), Cameroun (Adlhoch et al., 2013) and in Thailand (Saekhow and Ikeda, 2015), respectively. These differences in the frequency of PPV3 between the current and previous studies may be associated with the type of samples analyzed. Indeed, this study analyzed blood, spleen, and liver samples in which the virus load could be lower compared to tonsil, kidney, heart, and fetus samples in previous studies. This hypothesis is in agreement: with a previous report showing that differences in frequency in PPV2, PPV3, and PPV4 are attributable to the organs where samples are drawn. Genomes were detected better from tonsils and lymphoid compared to other organs (Streck et al., 2013; Li et al., 2013; Saekhow and Ikeda, 2015). The detection of PPV3s in blood samples may be an indicator of an ongoing infection at a population level.

The higher infection rate detected in spleen samples is in accordance with a previous study carried out in China in 2011, indicating a high frequency of PPV3 in spleen samples (Pan et al., 2012). PPV3 was suggested to have universal distribution after being detected in various parts of the world (Cságola et al., 2012)., There was no significant association between geographical location and the frequency of PPV3, although a relatively high number of cases was recorded in the Walungu district (31.8%). These findings corroborate a study conducted in Hungary where geography was not associated with the distribution of PPV3 among domestic pig herds (Cságola et al., 2012). In addition, detection of PPV3 in adult pigs beyond one-year-old is suggestive that pigs may have been infected early in their lives. It also indicates persistent infections (Adlhoch et al., 2010; Cadar et al., 2011). Seven out of the 80 swine samples analyzed were co-infected with PPV3 and ASFV. This finding is in agreement with a previous report in China where PPVs were detected in serum samples of pigs presenting high fever (Wang et al., 2010). This was one of the clinical signs related to ASF in wild boar samples collected during classical swine fever surveillance in Slovakia (Sliz et al., 2015). The reason behind this is not known; however, probably be due to the immunosuppressive potential of the PPV that puts swine at risk of multiple infections (Saekhow and Ikeda, 2015).

Sequence analysis revealed very high homology between South Kivu PPV3 isolates, indicating that they may have evolved from a common ancestor with a low mutation frequency (Sliz et al., 2015). The phylogenetic tree generated after analysis of the ORF2 region displayed overall high similarity between South Kivu PPV3 sequences and others reported in different countries, including Cameroun, China, Slovakia, and Germany. Since samples analyzed in the current study were taken from local (indigenous) or cross-bred pigs, the PPV3 could probably spread from within Africa, specifically from Cameroun through pig trade in the last century. Further investigations are necessary to confirm this hypothesis with more samples from African countries.

5. Conclusion

The present study reported the first occurrence, frequency and genetic characteristics of porcine parvovirus type 3 (PPV3) in pigs from DRC. It provides preliminary insights into the presence of PPV in domestic swine herds of the country. The infection rate within the South Kivu region was significantly different according to the sample types. Our findings will ultimately be useful in contributing to an improved PPV control and surveillance strategy in the country. Despite the lack of routine diagnosis in smallholder farmers, lack of data on the geographical distribution of PPVs in DRC, and considering the global trading network, it can be assumed that these viruses could be circulating in pig-rearing regions in the country as they can spread easily due to inadequate control of the movement of live animals and pork products. However, more studies that span different parts of the country are recommended to elucidate the true frequency of PPVs and their distribution in the DRC.

Ethics approval and consent to participate

A consent form that described the aim of the study was signed by farmers willing to participate in the study after translation into local languages. Ethical approval for the study reported here, and the permission for the collection of samples was provided by the Interdisciplinary Centre for Ethical Research (CIRE) established by the Evangelical University in Africa, Bukavu, DR Congo, with reference (UEA/SGAC/KM 132/2016).

Supplementary materials

The datasets analysed during the present study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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