Evidence of coinfection of African swine fever virus genotype X and Porcine parvovirus type 3 at pig farms in the North Kivu province, Eastern Democratic Republic of Congo

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Abstract

Background

African swine fever virus (ASFV) and Porcine parvovirus (PPV) are highly contagious viral pathogens that can cause devastating diseases in the pig industry. The absence of studies on the coinfection of ASFV and PPV in North Kivu raises concerns, especially given the uncontrolled movement of pigs and pig products between North and South Kivu in neighbouring countries where both diseases have been reported in domestic and wild pigs. A multifaceted approach was carried out to identify putative pathogens and risk factors associated with the transmission and spread of African Swine Fever (ASF) and PPV coinfections at pig farms in North Kivu province.

Materials and methods

A structured questionnaire was administered to collect risk factors associated with ASFV and PPV transmission dynamics, alongside 117 blood samples collected from domestic pigs and tested for the presence of ASFV and PPV using Polymerase Chain Reaction (PCR). The resulting PCR amplification products were sequenced using the standard Sanger sequencing method.

Results and Discussion

The results of Multivariable logistic regression analysis indicated that free-ranging system of pigs (OR = 3.64 95% CI: 1.076–12.92) is a major risk factor positively associated with ASFV and PPV3 infections (OR = 4.82, 95% CI: 1.06–20.86) at pig farms in North Kivu Province, eastern DRC. Free-ranging systems, where pigs roam outdoors, may increase the risk of exposure to infected vectors, wildlife reservoirs, or contaminated environments, thereby facilitating the spread of ASFV and PPV3. From thirteen PCR-positive pigs for ASFV (23.5%), four were co-infected with PPV. Phylogenetic analysis of ASFV p72 and p54 protein genes revealed that ASFV field isolates belong to genotype X whilst PPV clustered with porcine reproductive virus (PPV type 3). Owing to the transboundary nature of ASFV and PPV3 infections, viruses can easily spread across borders. This highlights the need for enhanced surveillance, early detection, and rapid response mechanisms to prevent and control the spread of these diseases regionally and internationally.

INTRODUCTION

The piggery industry is emerging as a lucrative enterprise in the Democratic Republic of Congo (DRC), significantly addressing critical issues like poverty reduction, food security, and economic livelihoods. The DRC occupies the second position in central Africa, after Cameroon, with a standing pig population of 1 million [1]. One of the major factors affecting efficient pig production in DRC is the presence of high-impact transboundary animal disease, the African swine fever (ASF). Various pathogenic agents can co-

infect pigs in natural settings, impacting reproductive outcomes. Porcine Parvovirus (PPV), a significant contributor to global reproductive failure in pigs [2], underscores the importance of considering multiple pathogens in research and control efforts. Coinfection with both viruses can exacerbate the disease burden on pig farms, leading to increased mortality rates and reproductive failure among the swine population.

ASF can cause up to 100% mortality in a susceptible pig population and represents the most economically devastating disease in the pig industry [3]. African swine fever virus (ASFV) has a considerably large genome of around 170–194 kb in length, is a member of the *Asfarviridae* family and is the only known linear double-stranded DNA arbovirus [4]. The ASFV has been reported in several African and European countries, where it is maintained in a sylvatic cycle involving Ornithodoros soft ticks and asymptomatically infected warthogs and bush pigs. It threatens food security and livelihoods, as infected pigs may need to be culled to control the spread of the virus. Effective control measures, biosecurity practices, and public awareness campaigns are crucial in managing and preventing the spread of ASF in the pig population in the absence of vaccines [5, 6].

Porcine parvovirus (PPV) stands as a primary culprit behind embryonic and fetal death in pigs, leading to adverse outcomes such as mummification, stillbirths, and delayed return to the estrus cycle [2]. With a single-stranded linear DNA genome approximately 5 kb long, PPV belongs to the Parvoviridae family, specifically the Parvovirinae subfamily that infects vertebrates. This subfamily is divided into eight genera, with four affecting swine: Bocaparvovirus, Copiparvovirus, Protoparvovirus, and Tetraparvovirus [7]. Notable genera infecting swine include classical porcine parvovirus 1 (PPV1), novel porcine parvovirus type 2 (PPV2), PPV3, PPV4, PPV5, PPV6, PPV7, porcine bocavirus 1 (PBoV1), and several others [7]. Control measures encompass stringent hygiene protocols, vaccination of sows for immune protection, and vigilant monitoring for early PPV detection. Collaborative efforts among stakeholders are vital for effective PPV control in DRC.

The agro-pastoral province of North Kivu in the eastern part of the DRC faces significant challenges in its livestock sector. With approximately 200,000 head of livestock, including smallholder pig farms, the region grapples with insecurity, inadequate road infrastructure, and insufficient government support. The absence of studies on the coinfection of ASFV and PPV in North Kivu raises concerns, especially given the uncontrolled movement of animals and pig products between North and South Kivu, as well as neighbouring countries where both diseases have been reported in domestic and wild pigs [8] [9]. Conducting a comprehensive investigation of ASFV and PPV coinfections in pig farms in North Kivu province will provide valuable insights into the epidemiology, transmission dynamics, and risk factors associated with these diseases, ultimately contributing to the development of targeted control strategies to safeguard pig health and mitigate the economic impact on local pig farming communities. Thus, this study aimed to determine the presence of ASFV and PPV coinfections in apparently healthy domestic pigs from North Kivu province. This knowledge is crucial for implementing targeted control measures, enhancing biosecurity, and developing effective vaccination strategies to mitigate the impact of these diseases on the swine industry in the affected areas and prevent further spread of ASFV and PPV in DRC.

METHODS

Study area description

This study was conducted in the North Kivu province, which is one of the 26 provinces of the Democratic Republic of Congo (DRC), with an area of 59,631 Km^2 and an estimated population of 65 million. It is located in the east of DRC and is bordered by Rwanda and Uganda to the east, Oriental Province to the north, Maniema Province to the west, and South Kivu Province to the south. The North Kivu province is among the main pig production regions in the DRC, which suggests that the region plays a crucial role in contributing to the country's overall pig farming industry. Additionally, most pigs produced in this region are imported from neighbouring countries and provinces within DRC, which indicates a dynamic and interconnected pig market, both domestically and internationally. Pig samples were collected from only three districts, namely Masisi, Nyiragongo, and the city of Goma in the North Kivu province. The focus on these districts was influenced by accessibility and insecurity concerns in other territories. The distribution of samples across tree districts was designed to capture the diversity of pig farming practices, conditions, and potential health issues within the North Kivu province. The study area showing sampling sites is presented in Fig. 1.

Study design and sample selection

A cross-sectional survey was conducted to collect epidemiological data and pig blood samples for 3 months from February 2021 to April 2021. A Kobocollect form was administered to gather information on risk factors associated with the transmission and spread of ASF and PPV infections. The following risk factors were incorporated into the Kobocollect form: sex, age, breed, farming systems, origin of animals, management of dead animals, contact with infected pigs, mixing of pigs with other animals and vaccination status. On the other hand, blood samples were collected from pig farms and slaughter slabs using EDTA vacutainer tubes. The blood samples were selectively collected from local and exotic breeds older than 5 months with no clinical signs specific to ASFV or Parvovirus infections.

Blood sample collection

A qualified veterinary personnel collected 1- 3mL blood from the jugular vein of apparently healthy or sick pigs in EDTA-treated vacutainer tubes. A total of 117 blood samples were collected, amongst which 47 were from pig farms in Masisi, 34 from Nyiragongo, and 37 were from pig slaughter slabs in Goma town. All blood samples were transported to the laboratory under a cold chain and were stored at − 20ºC until DNA extraction.

DNA extraction

Total DNA was extracted directly from 200µL of blood using a DNeasy Blood and Tissue extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Briefly, 200µL of blood was poured into a 1.5ml Eppendorf tube and mixed with 20µL of proteinase K and 200µL of lysis buffers (AL). The volume was then mixed and incubated for 10 minutes at 56°C. Then, 200µL of Ethanol (99%) was added

to the mixture to allow precipitation of the DNA. The entire mixture was then poured into the columns provided in the kit, and two successive washes were performed by adding 500µL of wash buffer (AW1 and AW2). The DNA was eluted in 50µL of elution buffer and stored at -20°C until nucleotide amplification by polymerase chain reaction (PCR).

African swine fever virus and Porcine parvovirus detection by PCR

The presence of ASFV and PPV in collected samples was confirmed by PCR (ProFlexM, Applied Biosystems). The ASFV DNA was amplified using ASF diagnostic primers PPA1 and PPA2, targeting a 257 bp region of the B646L gene encoding the p72 major capsid protein, as previously described by Aguero *et al.* [10]. The variable 3'-end of the *B646L* gene encoding the major capsid protein p72 was amplified using the following primers: p72-D/p72-U, with a targeted amplicon of 478 bp as previously described by Bastos *et al.*. [11]. The amplification profile was as follows: initial denaturation step of 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 62°C for 30 s and extension at 72°C for 30 s. These cycles were followed by an extension step of 72°C for 7 min and terminated by a holding step at 4°C. Similarly, different porcine parvoviruses were detected by PCR as previously described by Csagola *et al.* [12]. The primer sequences used for the detection and characterisation of PPV were adopted from Bisimwa *et al.*. [9]. All PCR products were analysed by gel electrophoresis on a 1.5% agarose gel stained with GelRed nucleic acid stain and visualised with a UV transluminator (Bio-Rad, Hercules, CA, USA). PCR-positive amplicons of ASFV and PPV were purified using a QIAquick PCR purification Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The purified amplicons were sent to Macrogen Europe BV (Amsterdam, The Netherlands) for sequencing.

Nucleotide sequencing and phylogenetic analysis

The nucleotide sequences of ASFV and PPV were obtained by automated dideoxynucleotide cycle sequencing of PCR products using BigDye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA). The quality of sequences was analysed using CLC Main WorkBench version 8 software. The chromatograms were inspected and cleaned to remove noisy sequences for each amplified region. Both 5' and 3' ends of the nucleotide sequences of forward and reverse sequences with poor quality sequences were trimmed using the trimming option on CLC Main WorkBench software. Clean sequences were assembled to obtain consensus sequences. The consensus sequences were exported in fasta format to search for similarity of nucleotide sequences obtained in this study to other nucleotide sequences available at GenBank (BLASTn: https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed after multiple sequences aligned using Clustal W. The maximum Likelihood method and Kimura 2-parameter model with a bootstrap frequency of 1000 replicates implemented by MEGA X [13] infer the evolutionary history of ASFV and PPV [14].

Statistical analysis

The epidemiological data collected during the survey was encoded in Microsoft Excel version 2010 (Microsoft, California, USA). The Chi-square test was used to determine the association between ASF and

porcine parvovirus infections along with other risk factors such as sex, age, breed, farming systems, the origin of animals, management of dead animals, contact with infected pigs, mixing of pigs with other animals and vaccination. We used logistic regression analysis to model the odds of being a positive case based on RT-PCR results as a function of the dichotomous risk factors measures. The significance level was set at $p < 0.05$. R software (Version 4.2.1) was used to perform the analysis.

RESULTS

African swine fever virus and Porcine parvovirus type 3 infections at pig farms

Out of 117 blood samples collected from pigs and screened for ASFV and PPV by PCR during this field surveillance, 11.1% (95% CI, 0.06–0.18) were ASFV-positive, while 13.7% (95%CI, 0.08–0.21) were infected *Porcine parvovirus type 3* (PPV3). The highest proportion of ASFV-positive samples were obtained from Masisi district with [14.3% (95% CI, 0.06–0.27)] followed by Nyiragongo district with [12.5% (95% CI, 0.76–6.15)]. Equally, the highest proportional of PPV3 samples were collected from the Nyiragongo territory [18.7% (95%CI, 0.88–17.55)] followed by the Goma territory [16.7% (95% CI, 0.67–14.06)] (Table 1).

Table 1
PCR-positive cases of African swine fever and Porcine parvovirus type 3 infections at pig farms in the North Kivu province

Territories	Number tested	ASFV + N(%)	95% CI	PPV3 + N(%)	95% CI
Masisi	49	7 (14.3)	0.06–0.27	4 (8.1)	0.02–0.19
Goma	36	2 (5.6)	0.44–3.81	6 (16.7)	0.67–14.06
Nyiragongo	32	4 (12.5)	0.76–6.15	6 (18.7)	0.88–17.55
Total	**117**	**13 (11.1)**	**0.06–0.18**	**16 (13.7)**	**0.08–0.21**

African swine fever and Porcine parvovirus type 3 coinfections at pig farms

Of the 117 examined pigs, 13 (11.1%) were ASFV positive and 16 (13.7%) PPV positive. Out of the 13 animals that were ASFV PCR positive,4 (30.7%) were PPV PCR positive. Additionally, 9 pigs which were PPV3 negative were found to be ASFV positive by PCR assay. In addition, 12 pigs which were PPV positive were ASFV negative after PCR (Table 2).

Table 2
The number of coinfections between African swine fever virus and Porcine parvovirus type 3 at pig farms in North Kivu province

Viruses	ASFV			
PPV3		Positive (¨%)	Negative (%)	Total (%)
	Positive	4 (25)	12	16
	Negative	9	92	101
	Total (%)	13	104	117 (100)

Univariable Logistic regression analysis of risk factors associated with African swine fever virus and Porcine parvovirus type 3 positivity

The univariable logistic regression analysis did not reveal any statistical significance for all the risk factors analysed at $p > 0.05$. The free-range pig production system, consumption of dead pigs within farms, contact with infected pigs and non-vaccination status were the major factors associated with ASFV infection at OR > 2 (Table 3). In contrast, only the free-range pig production system contributes to PPV3 spread at OR > 3.

Table 3
Univariable logistic regression analysis of risk factors associated with African swine fever and Porcine parvovirus type 3 positive cases at pig farms in the North Kivu province

Variables	Category	PPA			PPV3		
		Positive %	OR	P-value	Positive%	OR	P-value
Sex of animals	Female	4,27		0.917	5,13	0.98	0.978
	Male	6,84	1.05		8,55		
Raising system	In housing	4,27		0.105	10,26	3.033	0.096
	Free raising	6,84	2.55		3,42		
Management of dead animals	Sell	8,55			8,55		
	Seizure	0,00	1.09	0.99	0,85	0.58	0.677
	Consumption	2,56	2.163	0.315	4,27	1.69	0.504
Origin of animals	Neighbouring villages	6,84			6,84		
	Within the farm	4,27	1.329	0.4785	6,84	0.577	0.455
Selling place	Market	0,85		**0.077**	0,85	0.89	0.928
	Slaughterhouse	10,26	1.43		12,82		
Contact with infected pigs	Yes	4,27	2.07	0.21	2,56	0.36	0.223
	No	6,84			11,11		
Assistance from the Vet of a neighbouring farm	Yes	7,69	1.07	0.916	6,84	0.37	0.256
	No	3,42			6,84		
Testing of animals before buying	Yes	7,69	0.59	0.201	7,69	0.63	0.506
	No	3,42			5,98		
Mixing of pigs of different ages	Yes	8,55	1.045	919	0,00	0.89	0.902
	No	2,56			13,68		
Sharing of farm equipment/materials with neighbours	Yes	5,13	1.02	0.961	5,98	0.83	0.806
	No	5,98			7,69		
Access to sanitary treatment	Yes	5,13	0.8	0.693	8,55	0.52	0.329
	No	5,98	-	-	5,13		

Variables	Category	PPA			PPV3		
		Positive %	OR	P-value	Positive%	OR	P-value
Vaccination	Yes	3,42	2.2	0.083	3,42	1.13	0.84
	No	7,69			10,26		

In addition, the generalised linear model (GLM) was used to perform multivariate logistic regression analysis on risk factors, which showed p-values < 0.1 in the univariate logistic regression. The results revealed that pigs under free-ranging systems had significantly higher risks for African swine fever virus infection (OR, 3.645; p = 0.038) and Porcine Parvovirus (OR, 4.826;p = 0.033) than in housing. In addition, pigs from Nyiragongo territory were at higher risk for Porcine parvovirus infection (OR, 1.949; p = 0.061) (Table 4).

Table 4
Multivariate logistic regression analysis of risk factors associated with pig infection

	ASFV				PPV3			
Variables	OR(95% CI)	ß	Z-value	P-value	OR (95% CI)	ß	Z-value	P-value
Free-ranging	3.6 (1.07–12.9)	1.2	2.07	0.038 *	4.82 (1.06–20.86)	1.293	2.07	0.033 *
Selling at slaughterhouse	0.38 (0.05–1.7)	-0.9	-1.09	0.275	0.42 (0.01–3.04)	-0.95	-1.09	0.472
Nyiragongo territory	1.41(0.84–2.3)	0.3	1.32	0.186	1.94(0.98–4.04)	0.346	1.32	0.061
Access to sanitary treatment	0.47 (0.18–1.1)	-0.7	-1.59	0.11	0.42 (0.12–1.45)	-0.746	-1.59	0.162

Legend: PPV, parvovirus; ASFV, African swine fever virus; β, standardized regression coefficient;; OR, odds ratio; CI, confidence interval

Nucleotides sequences and phylogenetic analyses of African swine fever virus and porcine parvovirus type 3

Only six samples out of 13 PCR-positive pigs for ASFV were successfully sequenced based on the p72 gene (Fig. 2). Only four samples were sequenced when the p54 gene was targeted (Fig. 3). At the nucleotides level, p72 and p54 indicate a high degree of identity between 99 and 100% with other nucleotide sequences at GenBank (Fig. 3). A phylogenetic analysis of p72 and p54 sequences showed that ASFV isolates collected from North Kivu clustered with genotype X of ASFV. The comparison of nucleotide sequences with those of other ASFV strains revealed the highest nucleotide identity (99 to 100%) with the ASFV isolate reported in the South Kivu province of DRC, Burundi, Kenya, Tanzania and

Uganda. Equally, 8 samples out of 17 PCR positive for PPV3 were successfully sequenced (Fig. 4). The comparison of the second open reading frame (ORF2) for PPV3 sequences with those of other PPV type 1–7 strains revealed the highest nucleotide identity (99 to 100%) with the PPV3 isolate reported in South Kivu province of DRC, Cameroon, China, Slovakia and Germany (Fig. 4).

DISCUSSION

ASFV and PPV3 are highly contagious viral pathogens that can cause devastating diseases in domestic and wild pigs. ASFV, in particular, is associated with high mortality rates in affected pigs, while PPV3 can lead to reproductive disorders and decreased productivity in breeding sows. Coinfection with both viruses can exacerbate the disease burden on pig farms, increasing the swine population's morbidity and mortality rates. The detection of ASFV and PPV3 infections at pig farms in the North Kivu province suggests a complex epidemiological situation with concurrent infections with two important veterinary viral pathogens.

The coinfection of ASFV genotype X and PPV3 could probably be due to the immunosuppressive potential of the PPV3 that exposes pigs to the risk of multiple infections [15]. For this reason, the infection with PPV3 most probably occurred before ASFV infection. This may lead to severe socio-economic losses due to increased mortality, reduced reproductive performance, and decreased growth rates in surviving pigs. Additionally, outbreaks of ASFV can lead to trade restrictions on pork products, further impacting the economic viability of pig farming operations in DRC.

The proportion of coinfections indicates that 25% of the pigs that tested positive for PPV3 were also positive for ASFV. These results corroborate with a previous study in South Kivu province, DRC [9] and China [16] where ASFV was found in pigs infected with PPV .This is further supported by reports of a survey on the Namibian warthog ecosystem showing a concurrent infection between ASFV and PPV type 1 (PPV1) [17]. High infection rates of ASFV and PPV3 were detected from Misisi and Goma territories, and this was mainly due to the increased displacement of pig breeders and their belongings from rural areas to towns as people fled heightened rebel insurgency in rural villages. The viruses could quickly move from rural areas and beyond due to the transboundary nature of ASFV and PPV3 infections. This scenario may necessitate stringent biosecurity measures, quarantine protocols, and targeted control strategies to contain the spread of these viruses and prevent further transmission within the swine population.

The proportion of ASFV-PCR positivity rates obtained from this study is lower than the previous study in the South Kivu province of DRC, where 22.8% of apparently healthy pigs were positive [8]. This result agreed with previous findings [18], where the ASFV genome was detected in asymptomatic Nigerian indigenous pigs. In contrast, the study conducted in Uganda did not detect ASFV DNA despite the high apparent disease incidence [19]. However, only 13.6% of PPV3 (originally named porcine hokovirus) was detected, confirming the observation by a recent study conducted in South Kivu province, where 17.5% of PPV3 strain was detected in pigs [8]. Equally, previous studies have shown variations of PPV3 positivity in different countries and regions. This could be attributed to different sampling criteria, health status, and sample size, directly influencing research outcomes [20] [21]. For instance, this study involved analysis of

pig farms without symptoms related to reproductive disorders, while the previous study conducted in South Africa by Afolabi *et al.*. [21] targeted only confirmed porcine circovirus type 2 (PCV2) infected pigs.

Logistic regression analysis revealed that meat consumption from dead pigs, breeding pigs from the same farm, and not testing pigs before buying and mixing pigs of different ages in the same pen were the most prominent risk factors associated with ASFV infection in North Kivu province. Mixing pigs of different ages within the same pen as a factor associated with ASFV transmission may be likely attributed to direct pig-to-pig contact, especially on confined farms with poor biosecurity measures. Similar results were reported in South Kivu province (DRC)[8] in Sardina [22] and in Madagascar [23], where pens with mixed pigs of different species and ages were associated with ASFV positivity. Moreover, feeding pigs with kitchen leftovers is common in most pig-keeping households. This feed may be mixed with meat from ASFV-infected pigs. This increases the risk of consuming ASFV-contaminated products. These results corroborate with studies conducted in DRC [8], Uganda and Kenya [24], which reported swill feeding as a potential risk factor for ASF transmission. However, our finding contrasts with a study conducted in Malawi, where feeding kitchen leftovers was not a possible risk factor in ASFV transmission [25]. Additionally, free range system was a potential risk factor for both ASFV and PPV3 transmission. A free-range system, practised in several parts of North Kivu, allows pigs to move and scavenge around the farms. Hence, pigs in this system are more likely to be in contact with other infected domestic and wild pigs and possible pig products that may facilitate the spread of ASFV and PPV3.

Genomic comparison of North Kivu field isolates, together with other available partial sequences of ASFV genotype X and PPV3 available on GenBank, showed a high level of sequence conformity, and this is in agreement with previous study by Sliz *et al.*. [26]. Similarly, sequence analysis of PPV3 revealed overall high nucleotide homology between North Kivu PPV3 sequences and other PPV3 isolates previously reported in DRC, in particular South Kivu province [8], Cameroon, China and Germany, suggesting that the viruses might have also evolved from a common ancestor [9]. On the other hand, phylogenetic analysis revealed a high nucleotide identity (99–100%) with ASFV strains currently circulating in East Africa, indicating a common origin. The North Kivu ASFV isolates are closely related to isolates from South Kivu Eastern DRC, Kenya, Burundi, Tanzania and Uganda. This strongly suggests the historical and geographical connections between the ASF viruses and East African countries that mainly border DRC. However, genomic surveillance for ASF is poor and a lack of complete-genome sequencing from other regions likely biases this result. For instance, samples that were analysed in the current study were collected from local (indigenous) or cross-bred pigs, suggesting ASFV could probably spread from within Africa, specifically from the neighbouring province of South Kivu or from Uganda, which borders North Kivu province through regular pig trade.

Conclusion

Pig free-ranging system was a major risk factor associated with ASFV and PPV infections in domestic pigs at pig farms in North Kivu province, Eastern DRC. A proper husbandry system, such as housing system is needed since the transmission of ASFV and PPV3 was mainly associated with scavenging pigs.

This study confirmed the presence of ASFV genotype X and PPV3 in domestic pigs in North Kivu province that clustered with other viruses in DRC and neighbouring Eastern African countries such as Burundi, Tanzania, Uganda, and Kenya. Thus, a large field study and phylogeographic analysis of complete genomes are needed to clarify the transmission dynamic of ASFV and PPV infections in the East African episystem. Understanding the genetic diversity and evolution of ASFV and PPV strains can provide insights into their transmission dynamics and pathogenicity.

Declarations

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Disclosure Statement

The authors report that there are no competing interests to declare.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Consent and Approval

Ethical approval and authorisation for the collection of pig 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 133/2023). A consent form which described the purpose of the study was signed by pig farm managers willing to participate in the study after translation into their local languages.

Data Availability

Nucleotide sequence datasets generated in this study have been submitted to NCBI GenBank and will be publicly available under accession numbers (PP432674-PP432683 for ASFV and PP386327-PP386334 for PPV3)

Author Contribution

Patrick Bisimwa Ntagereka: design the experiment, data collection, analysed, and interpreted the data, wrote the draft and Methods; **Edson Kinimi:** conceived and designed the experiments, analysed, interpreted the data, review and editing the draft; **Dieudonnée Wasso Shukuru**: conceived and designed the experiments, analysed, and interpreted the data; **Elie Ntale Ya Mushagalusa:** Performed the experiments, data collection, hypothesis development, review the draft; **Jean-Paul Mugisho Basedeke**: conceived and designed study review, data collection, and editing the draft, **Fabrice Bantuzeko**: Data analysis, review & editing the draft; **Justin Aksanti Bashimbe:** design the experiment, data collection,

analysed **Ronald Tonui**: Data analysis, review & editing the draft; **Simon Patrick Baenyi**: conceived and designed study review and editing the draft; **Ahadi Bwihangane Birindwa:** performed the experiments, data collection, hypothesis development, review the draft and supervision.

Ethics approval and consent to participate

The study was carried out in compliance with the IPEL (Inspection Provincial de l'Elevage) guidelines. All protocols including live animals were approved by the National Comitee Ethic Of Health (CNS 001/DPSK/415/PM/2024.

Consent for publication

Not applicable

Consent to Participate declaration

Not applicable

Competing interests

The authors declare no competing interest

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Figures

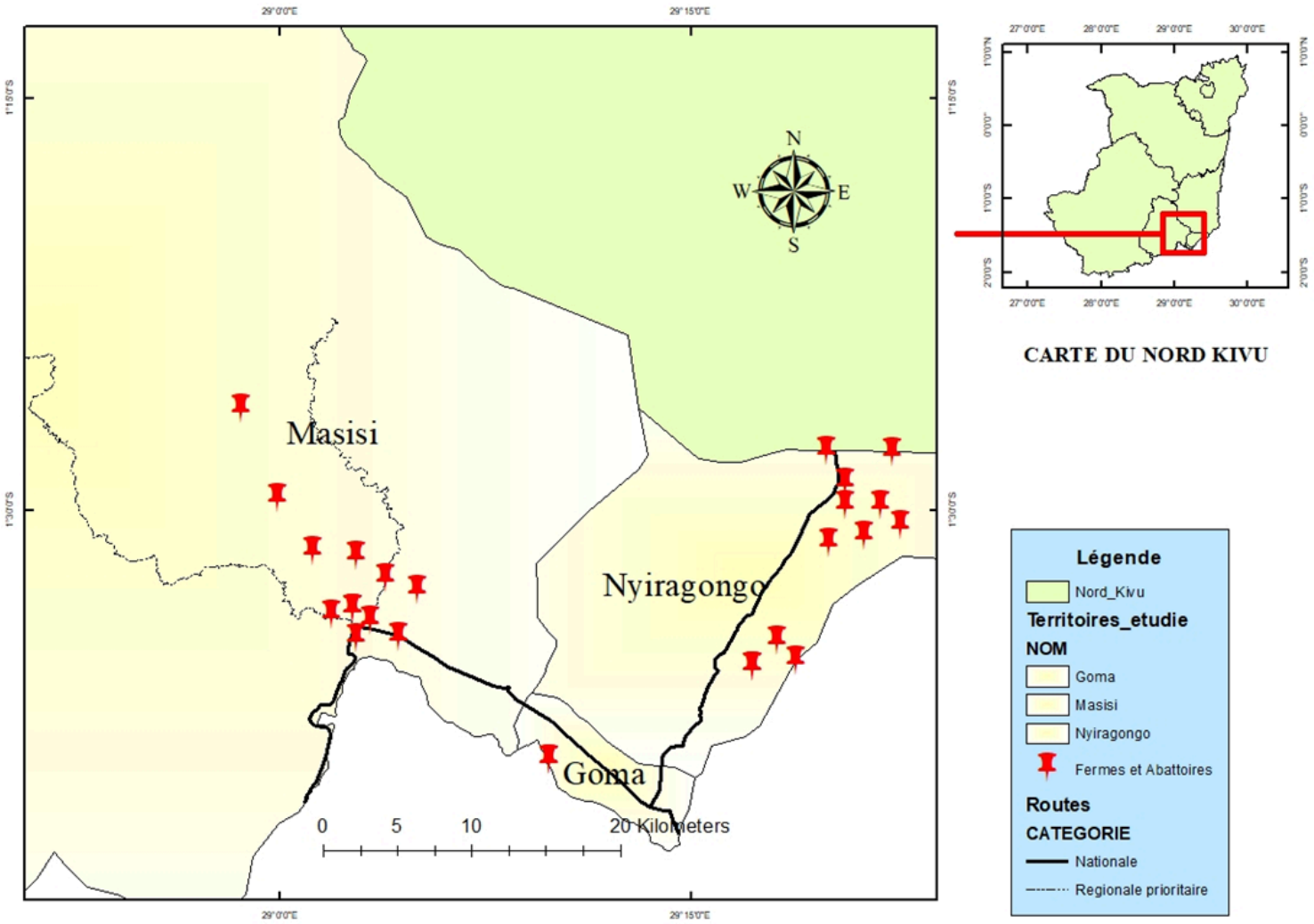

Figure 1

A map of North Kivu province showing sampling sites of pig samples used in this study. The pig samples analysed were collected from pigs in three districts, namely Masisi, Nyiragongo, and Goma, marked in red pins.

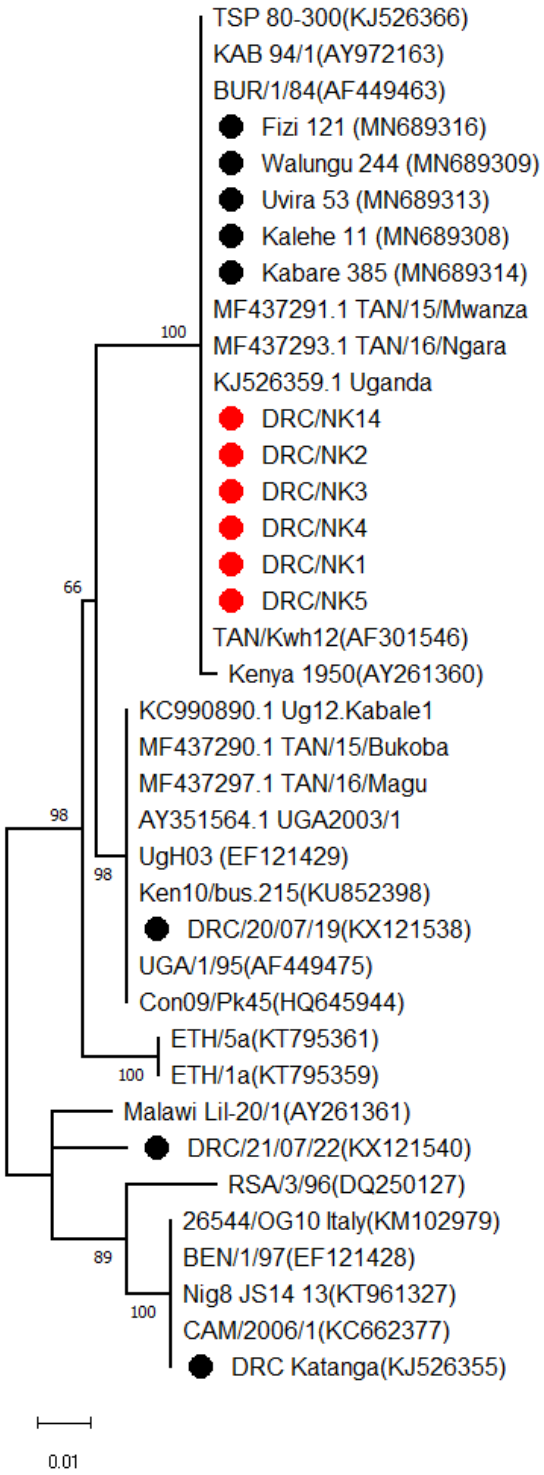

Figure 2

Phylogenetic relationship between the 6 ASFV strains isolated in this study and 32 previously identified ASFV genotypes based on partial sequencing of the B646L gene. The Maximum likelihood method and Kimura 2-parameter model were used to construct the phylogenetic tree in MEGA X. The phylogeny was inferred following 1,000 bootstrap replications, and the node values show percentage bootstrap support. The scale bar indicates nucleotide substitutions per site. The p72 sequences isolated in this study are

marked by a red circle (•). ASFV p72 isolates reported in some parts of DRC are marked by a black circle (•).

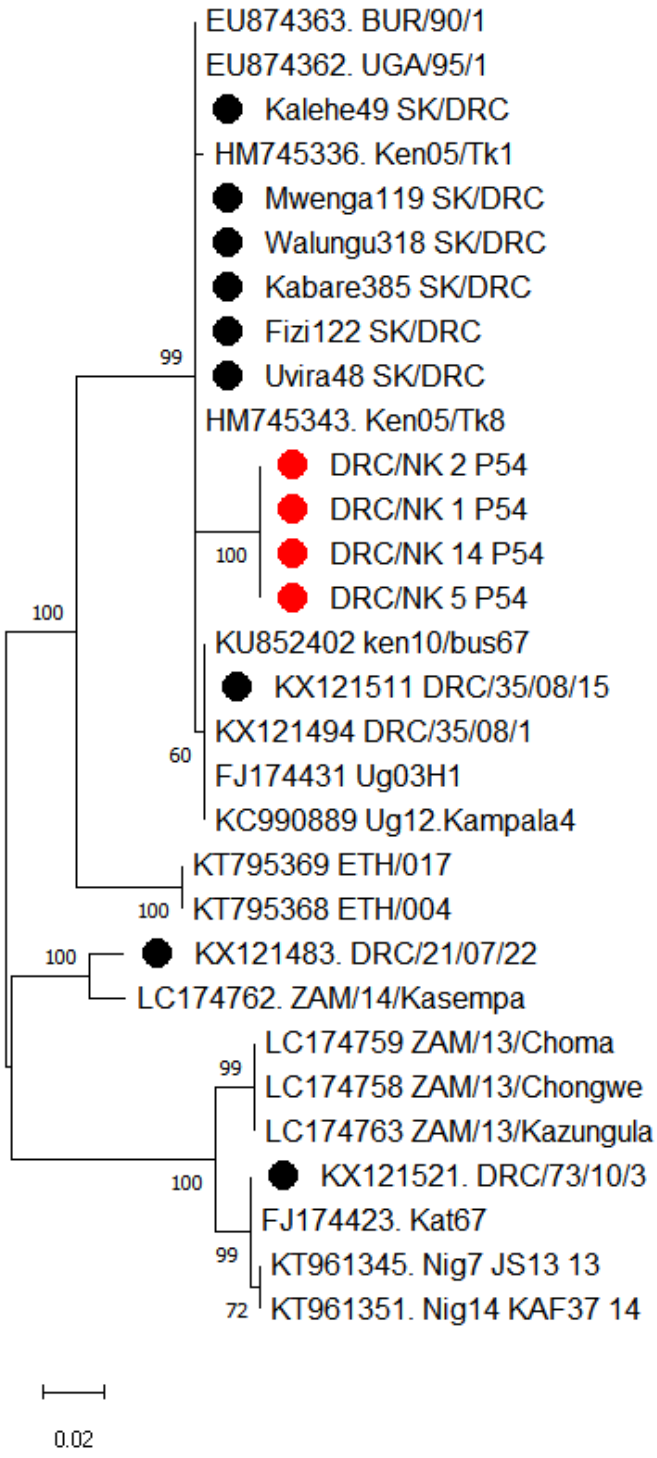

Figure 3

Phylogenetic relationship between four ASFV strains isolated in this study and 26 previously identified ASFV genotypes based on partial sequencing of the E183L gene (p54). The Maximum likelihood method and Kimura 2-parameter model were used to construct the phylogenetic tree in MEGA X. The phylogeny

was inferred following 1,000 bootstrap replications, and the node values show percentage bootstrap support. The scale bar indicates nucleotide substitutions per site. The p54 sequences isolated in this study are marked by a red circle (•), and ASFV p54 isolates reported in some parts of DRC are marked by a black circle (•).

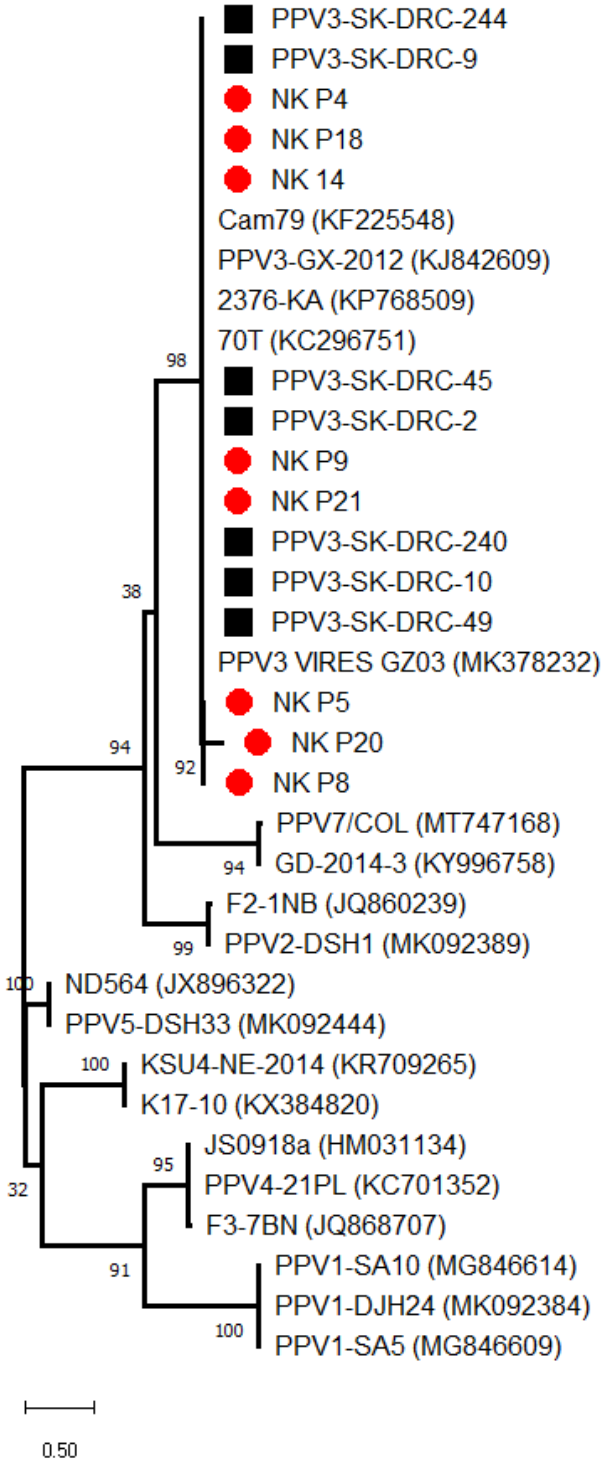

Figure 4

Phylogenetic relationship between eight PPV3 strains isolated in this study and 26 previously identified PPV3 genotypes. The Maximum likelihood method and Kimura 2-parameter model were used to construct the phylogenetic tree in MEGA X. The phylogeny was inferred following 1,000 bootstrap replications, and the node values show percentage bootstrap support. The scale bar indicates nucleotide substitutions per site. The PPV3 sequences isolated in this study are marked by a red circle (•), and the other PPV isolates reported in different areas of DRC are marked by a black circle (•). The GenBank accession numbers for the different PPV sequences are indicated in parentheses.