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# Risk factors of African swine fever virus in suspected infected pigs in smallholder farming systems in South-Kivu province, Democratic Republic of Congo

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## ABSTRACT

**Background:** African swine fever (ASF) is an infectious viral disease of domestic pigs that presents as a hemorrhagic fever, and for which no effective vaccine is available. The disease has a serious negative social and economic impact on pig keepers. There is limited information on the potential risk factors responsible for the spread of ASF in South Kivu.




**Objective:** The aim of this study was to determine the potential risk factors associated with ASF infection in suspected ASF virus (ASFV)-infected pigs.

**Methods:** We sampled whole blood from 391 pigs. Additionally, 300 pig farmers were interviewed using a structured questionnaire. Viral DNA was detected by using the real-time polymerase chain reaction technique.

**Results:** The majority of pigs sampled, 78% (95% confidence interval [CI], 74.4–82.6), were of local breeds. Over half, 60.4% (95% CI, 55.5–65.2), were female, and most of them, 90.5% (95% CI, 87.6–93.4), were adult pigs (> 1 year old). Viral DNA was detected in 72 of the 391 sampled pigs, indicating an overall infection rate of 18.4% (95% CI, 14.5–22.4). Multivariable logistic regression analysis revealed several risk factors positively associated with ASFV infection: feeding with swill in pen (odds ratio [OR], 3.8; 95% CI, 2.12–6.77); mixed ages of pigs in the same pen (OR, 3.3; 95% CI, 1.99–5.57); introduction of new animals to the farm (OR, 5.4; 95% CI, 1.91–15.28). The risk factors that were negatively (protective) correlated with ASFV positivity were the presence of male animals and the use of an in-pen breeding system.

**Conclusion:** Local pig farmers should be encouraged to adopt proper husbandry and feeding practices in order to increase the number of ASF-free farms.

**Keywords:** African swine fever; diagnosis; domestic pigs; epidemiology; transmission

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#### Conflict of Interest

The authors declare that they have no competing interests.

#### Author Contributions

Conceptualization: Bisimwa PN, Bisimwa B, Steinaa L, Ongus J; Data curation: Bisimwa PN, Dione M, Mushagalusa AC; Formal analysis: Bisimwa PN, Bisimwa B, Mushagalusa AC, Ongus J; Methodology: Bisimwa PN, Dione M, Mushagalusa AC, Steinaa L; Supervision: Ongus J; Validation: Dione M, Mushagalusa AC, Steinaa L; Writing- original draft: Bisimwa PN; Writing-review & editing: Dione M, Bisimwa B, Steinaa L, Ongus J.

## INTRODUCTION

Pig farming has become very popular across Africa continent as a means of increasing food supply, income, and employment. The economic potentials of this farming sector are related to some of its characteristics that are distinct from farming of other domestic animals, including prolificacy (up to 20 piglets per litter), relatively short generation time, and high feed to flesh conversion rate, resulting in high production turnover for sale and consumption [1]. However, this sector faces several constraints, with the associated disease burden being a major problem [2]. One of the most common swine diseases is African swine fever (ASF), a complex, contagious, lethal, and economically important viral disease of domestic pigs that causes hemorrhagic fever and leads to considerable economic loss due to morbidity and death of animals, and for which no effective vaccine is available [3,4]. In addition, the disease threatens food supply security by limiting the availability of animal protein, particularly in regions where alternative livestock production is a challenge.

ASF is endemic in Sub-Saharan Africa and has been reported in more than 26 countries [5]. Outbreaks of ASF have serious social and economic implications, both in areas where it is endemic and where it has been introduced, due to its high mortality rate, which can approach 100 percent. In several endemic African countries, ASF affects smallholder pig producers primarily due to a lack of biosecurity infrastructure and a lack of effective prevention and biosecurity measures [6].

The clinical presentation of ASF in domestic pigs depends mainly on the viral isolate's virulence, the pig breed, the infective dose, and the route of infection [7,8]. Typical signs in infected animals include severe depression, high fever (40–42°C), cyanotic skin on ears, bleeding from bodily orifices, breathing difficulty, nasal discharge, abortion in pregnant sows, and coma and death [9].

Susceptible animals are infected through either the sylvatic or domestic cycles or between interaction between the sylvatic cycle and domestic pigs [10]. In the sylvatic cycle, the ASF virus circulates between wild pigs, the natural reservoir, and soft ticks of the *Ornithodoros* spp. [11]. Ticks transmit the virus to common warthogs (*Phacochoerus* spp.) while the ticks feed on the wild pig's blood. The domestic cycle occurs when the virus is transmitted from one infected domestic pig to another, either by direct contact or through ingestion of an infected pork product, and without the involvement of sylvatic cycle hosts or arthropod vectors [11,12]. Transmission between the sylvatic cycle and domestic pigs occurs when domestic pigs are infected through tick bites, which occurs in those areas of Africa where warthogs are present. Furthermore, an infection can occur when domestic pigs are fed ASF virus (ASFV)-contaminated warthog carcasses or come in contact with warthog feces [13]. Prevention and control methods rely mainly on early diagnosis, restriction of the movement of pigs and pig products, strict application of biosecurity measures, and culling of infected swine herds [14]. Some of the mitigative measures against ASF spread in domestic pig farms are the adoption of biosecurity practices, proper disposal of carcasses, and efficient reporting of suspected outbreaks.

The Democratic Republic of Congo (DRC) has the second-largest pig population in central Africa, after Cameroon, with a standing pig population of 1 million [15]. These are distributed unequally among the different provinces of the country. Although pig farming is an important livestock activity among farmers in several rural areas of South Kivu province, this region is one of the provinces with a small pig population. The relatively low population

is because of the frequent occurrence of confirmed and suspected ASF outbreaks and the resulting high mortality. To date, most of the ASF outbreak reports have been based on clinical diagnosis without the use of any confirmatory testing; moreover, there is a reluctance among farmers to report suspected outbreaks [15].

The South Kivu provinces of the DRC have a pig population of approximately 66,636 [15]. Smallholder pig farmers have faced a serious challenge due to the regular occurrence of hemorrhagic fever outbreaks, leading to high domestic pig mortality and a notable negative effect on productivity. Pigs are raised mostly by smallholders in various farming environments ranging from municipal to rural areas [16], and such farmers can easily sell their pigs to cover school fees, medical bills, and other family requirements [17]. In this region, 3 pig-farm management systems, intensive, tethering, and extensive, are used [16,17]. Intensive pig farming involves housing pigs in pens to restrict their movement and provide them with feed and water. In the tethering system, pigs are tied by a rope to a post to restrict their movement within a specified radius. In the extensive system, pigs are left to scavenge in the open to obtain food and water, which places them at a very high risk of infections.

A previous study conducted in South Kivu province focused mainly on genetic characterization of isolated ASFV strains, based on sequence analysis of the major capsid protein (p72) [18]. That study reported the circulation of ASFV genotype IX, which was identical to the isolates responsible for outbreaks in other DRC provinces with ASF outbreaks [19]. The same genotype has been reported in Tanzania, which borders the South Kivu province of DRC. This indicates that ASFV can plausibly move between DRC provinces, and movements between Tanzania and South Kivu might have occurred. Despite the devastating effects of swine diseases, the lack of routine diagnostic and the poor husbandry practices observed in smallholder pig farms, limited studies have been conducted in South Kivu to determine the infection rate and prevalence of ASF in suspected clinically infected pig farms by using a sensitive diagnostic method (such as real-time polymerase chain reaction [RT-PCR]). Such information is important when differentiating ASFV infection from other pig diseases with similar clinical manifestations and when investigating epidemiology in South Kivu. Therefore, this study aimed to identify the risk factors associated with the occurrence of ASF and its spread among smallholder pig farmers. The results will contribute to the elucidation of ASF transmission, which is necessary for the implementation of proper control measures.

## MATERIALS AND METHODS

### Study areas

The study was conducted in 6 different districts in South Kivu province in the eastern part of the DRC, including Kabare, Kalehe, Fizi, Mwenga, Uvira, and Walungu districts (**Fig. 1**). The South Kivu province is a large region (surface area of 66,814 km<sup>2</sup>) located between longitudes 26° 10' 30" and 29° 58' east, and latitudes 00° 58" North and 4° 51' 21" South. The rainy season in this region lasts for 9 months (September to May) and the dry season for 3 months (June to August). The annual average rainfall is approximately 1,300 mm. The 6 study districts were selected following consultation with and obtaining permission from the Provincial Ministry of Agriculture Livestock and Fishery; moreover, the districts included purposive sampling locations where ASF was previously reported. The selected districts were chosen as they have had major and/or suspected ASF outbreaks in the recent past.

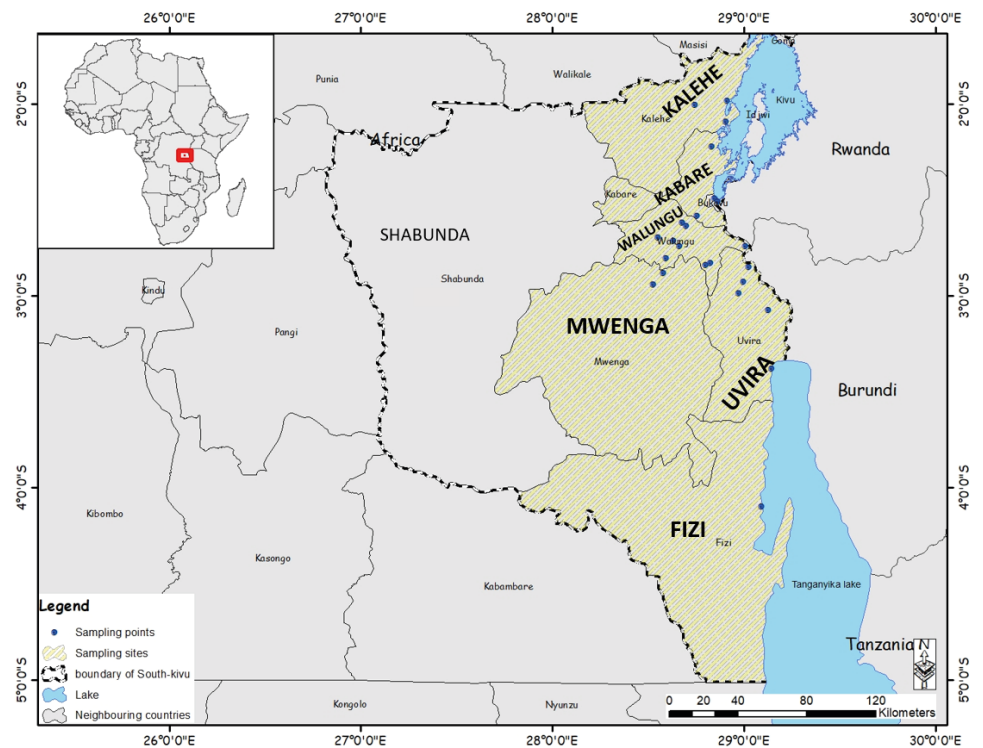


Fig. 1. Map of South Kivu province showing sampling districts and sites (blue dots) where African swine fever was identified (Drawn with ArcMap).

### Study design, sample size determination and sample collection

The study was carried out between November 2018 to January 2019.

#### Sample size for farms and pigs

The target population comprised all domestic pigs in the 6 districts of South Kivu that were identified and recorded by the Ministry of Agriculture Livestock and Fishery as the main pig-producing, marketing, and consuming areas of South Kivu. The sample size for blood sampling was established to achieve a 5% level of precision at a 95% confidence level and was determined using the formula described by Dohoo et al. [20]:  $n = \frac{Z^2 * P * (1 - P)}{d^2}$ , where  $n$  = sample size,  $Z = 1.96$  at 95% confidence level,  $P$  = probable prevalence of ASFV in pig herds (set at 23%) [16], and  $d$  = Standard error (set at 4). Based on that formula, 404 pigs were sampled. However, blood samples from only 391 pigs were included because 13 samples were discarded due to hemolysis.

Overall, between 61 and 69 pigs were sampled in each of the 6 districts. The average herd size in the study area was 4 pigs, and 1–3 pigs were sampled per farm.

#### Farm data collection

The farm survey involved 300 pig owners who were selected based on the following criteria: farmer experience of more than 2 years in pig farming; at least 2 pigs on the farm; the farm had reported at least one suspected ASF outbreak in 2017–2018. Moreover, pig keepers who had at least one pig suspected to be sick during the sampling period were interviewed. Between 40 and 61 pig farmers were surveyed in the 6 different districts based on the above selection criteria.

### Inclusion criteria for pig sampling

Sampled study animals included pigs older than 3 months to avoid interference from maternal antibodies. Sows with a litter more than 3 months old and non-pregnant sows were sampled. Clinical signs observed on suspected sick pigs at the time of sampling included high fever (> 40°C), redness of the skin, ears, or extremities, growth retardation, difficulty standing, emaciation, and respiratory distress.

### Blood collection

At all sampled farms, any pig suspected to be sick or presenting the symptoms described above was retained for blood collection. Vacutainer EDTA tubes (Thomas Scientific, USA) were used to collect 3–5 mL of whole blood samples aseptically from the jugular vein. After collection, all samples were transported unrefrigerated for not more than 6 h to the molecular biology laboratory at Université Evangélique en Afrique, where they were stored at –20°C before being shipped to the Pan African University Institute of Basic Sciences Technology and Innovation in Nairobi, Kenya, for analysis.

### Survey administration

Each farmer signed a consent form after a short briefing on the background of the study. Thereafter, a personal interview was conducted by a team composed of assistant researchers and veterinarians from the Department of Animal Sciences and Production at the Université Evangélique en Afrique. The interview included the completion of a structured questionnaire that was translated to the local language (French) to allow a clear understanding of the questions presented to the farmers. The questionnaire was designed to gather information about farm demographics, pig production systems, ASF awareness, and major potential risk factors (i.e., person-reported farm-level biosecurity practices). In addition, respondents were retained to prevent non-response bias due to people unwilling or unable to take the survey. To avoid desirability bias, all survey responses were anonymous, and the questionnaire was designed so that some of the questions were repeated in different ways. These potential limitations were considered during the interpretation of the data.

### DNA extraction and ASFV detection by RT-PCR

Viral DNA was extracted directly from 200 µL aliquots of blood using DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's recommendations. RT-PCR amplification assay was carried out using TaqMan (Applied Biosystems, USA) probe FAM-5'-CCACGGGAGGAATACCAACCCAGTG3'-TAMRA and primers, with the sequences: ASFV-F5'-CTG CTCATGGTATCAATCTTATCGA-3' (Forward) and ASFV-R-5'-GATACCACAAGATCRGCCGT-3' (Reverse). They were also used to amplify a conserved target region of the 3'-end of the VP72 gene as described previously [21].

The PCR mix was prepared using the Luna Universal qPCR Master Mix (New England BioLabs Inc., USA) by following the manufacturer's protocol. A threshold cycle (CT) value was attributed to each PCR reaction [21] after PCR amplification, and a CT value > 40 corresponded to a negative test sample or blank control. Samples with a high viral load were further amplified by conventional PCR using the ASF diagnostic primers PPA1/PPA2 (PPA1, 5-AGTTATGGGAAACCCGACCC-3; PPA2, 5-CCCTGAATCGGAGCATCCT-3), which generated an amplicon of 257 bp from the *B646L* gene that encodes the major capsid protein p72 [22]. The amplification products were loaded on a 1.5% agarose gel and visualized by ultraviolet irradiation after staining with Gel Red. The gel was photographed in order to provide a soft copy result for the diagnostic products.

### Statistical analysis

The information on farm management factors included in the questionnaire was used to define explanatory variables for testing in a logistic regression model to assess the association between the infection status (PCR-positive) of pigs (outcome variable) and the hypothesized risk factors. Each potential risk factor was coded as a dichotomous independent variable. The odds of being an ASF case based on PCR results was then modeled as a function of the dichotomous risk factors measures, using conditional logistic regression models. All variables with a  $p$  value  $< 0.1$  were selected as potential candidates for multivariate analysis. Correlations among variables were analyzed using the generalized variance-inflation factor to check for multicollinearity [23].

Group differences were tested using  $\chi^2$  statistics for categorical variables. Whenever the chi-squared result was not appropriate, Fischer's exact test was used. Each variable's results were expressed as a  $p$  value and as an odds ratio (OR) with 95% confidence interval (95% CI). For the multivariate analysis, a binary logistic regression (R software Logit Link Function) model was used to identify the features associated with ASF infection. All analyses were computed using R software (R core team, 2016).

### Ethics approval and consent to participate

A consent form translated into the local language and which described the aim of the study was signed by all farmers willing to participate in the study. Ethical approval for this study and permission to collect samples was provided by the Interdisciplinary Centre for Ethical Research (CIRE) at the Université Evangélique en Afrique, Bukavu, DR Congo (UEA/SGAC/KM 132/2016).

## RESULTS

### Descriptive analysis of the characteristics of the respondents in South Kivu province

Descriptive analysis was performed to determine the proportions of the different variables in each district (**Supplementary Table 1**). In total, 300 pig farmers were interviewed, including but not limited to 221 (73.7%) females with ages between 18 and 30 years. The majority of respondents ( $n = 156$ , 52%) had a formal education, and most ( $n = 158$ , 52.7%) had experienced 1–3 suspected ASF outbreaks. Most households ( $n = 207$ , 69%) had a pig herd size between 1 and 5 pigs. The number of farms visited in each district ranged from 40 to 63, and the highest number of pigs tested was 69 (17.6%) in the Uvira district.

### Characteristics of domestic pigs during field surveys in the South Kivu province

During this study, 391 suspected ASF-infected domestic pigs from the 6 districts were sampled and tested for the presence of ASFV antibodies and viral genomes. About 307 (78.5%) of the pigs were a local breed, and the rest were exotic (European); over half ( $n = 236$ , 60.4%) were female animals and most ( $n = 354$ , 90.5%) were adults ( $> 1$  year old). In addition, many of the pigs ( $n = 216$ , 54.2%) were kept in pen (semi-intensive) breeding systems (**Supplementary Table 2**).

**Table 1.** Pigs tested and found to be ASFV PCR-positive during farm surveillance in the selected districts of South Kivu province, DRC

Pig origin district	PCR-positive		
	Number tested	%	95% CI
Fizi	7	11.5	4.06–20.8
Kabare	11	17	9.1–28.7
Kalehe	7	11	4.9–21.9
Mwenga	5	7.7	2.9–17.8
Uvira	22	31.9	21.5–44.3
Walungu	20	29.9	19.6–42.4
Total	72	18.4	14.5–22.4

ASFV, African swine fever virus; PCR, polymerase chain reaction; DRC, Democratic Republic of Congo; CI, confidence interval.

### ASFV PCR-positive pig farms in South Kivu province

Of the 391 blood samples analyzed during field surveillance, the average percentage of ASFV-positive pigs was 18.4 (95% CI, 14.5–22.4%). A higher percentage of ASFV PCR-positive samples was detected in the Uvira district (31.9%; 95% CI, 21.5–44.3%) with Walungu district having the second-highest percentage (29.9%; 95% CI, 19.6–42.4%) (**Table 1**).

### Univariable and multivariable logistic regression analysis for risk factors associated with ASFV positivity

The univariable logistic regression analysis revealed that pigs from Uvira (OR, 4.2; 95% CI, 1.61–11.47;  $p = 0.004$ ) and Walungu (OR, 3.9; 95% CI, 1.45–10.52;  $p = 0.007$ ) had significantly higher risks of ASFV infection than that of the Fizi district. In addition, risk factors such as feeding with swill in pens (OR, 3.8; 95% CI, 2.12–6.77;  $p < 0.001$ ), sharing of work utensils among pig farmers (OR, 3; 95% CI, 1.32–6.81;  $p = 0.009$ ), mixed ages of pigs in a pen (OR, 3.3; 95% CI, 1.99–5.57;  $p < 0.001$ ), and introduction of new pig(s) to a herd (OR, 5.4; 95% CI, 1.91–15.28;  $p = 0.001$ ) were the major factors significantly associated with ASFV infection (**Table 2**). However, the use of an in-pen breeding system (OR, 0.4; 95% CI, 0.25–0.72;  $p = 0.002$ ) and the presence of male pigs (OR, 0.4; 95% CI, 0.28–0.87;  $p = 0.016$ ) were considered protective factors as they were associated with a low likelihood of infection.

The presence of viral DNA was confirmed by conventional PCR amplification of DNA from selected RT-PCR products that presented higher viral load after using the PPA1 and PPA2 diagnosis primers [22], which amplify the *B646L* gene encoding the p72 capsid protein. An expected 257 bp band was observed in ASFV-infected pigs, while no band was observed in the negative control pigs (**Supplementary Fig. 1**). Similarly, a binary logistic regression (Logit Link Function) model was used to perform multivariable analysis. Eleven variables were included in the final model. The statistical results revealed that districts such as Kabare (OR, 4.7; 95% CI, 1.26–18.25;  $p = 0.022$ ), Uvira (OR, 6.1; 95% CI, 1.92–19.8;  $p = 0.002$ ), and Walungu (OR, 6.6; 95% CI, 1.91–22.89;  $p = 0.003$ ) were most at risk of ASFV infection. However, the main risk factors associated with ASFV positivity included in-pen feeding with swill (OR, 4.4; 95% CI, 2.58–8.67;  $p < 0.001$ ), presence of mixed-aged pigs in the pen (OR, 5.1; 95% CI, 2.67–10;  $p < 0.001$ ), and the introduction of a new animal to the farm (OR, 4.8; 95% CI, 1.53–15.47;  $p = 0.007$ ). The analysis also revealed that the presence of male animals (OR, 0.43; 95% CI, 0.22–0.83;  $p = 0.013$ ) was a protective factor (**Table 3**).

**Table 2.** Univariable logistic regression analysis of potential risk factors associated with ASFV PCR positivity in sampled pig farms

Variables	Category	OR (95% CI)	$\beta$	SE	Z-value	p value
District	Fizi					
	Kabare	1.87 (0.64–5.41)	0.62	0.54	1.15	0.25
	Kalehe	1.13 (0.36–3.65)	0.12	0.59	0.2	0.84
	Mwenga	0.7 (0.22–2.64)	-0.27	0.63	-0.43	0.671
	Uvira	4.2 (1.61–11.47)	1.46	0.5	2.9	0.004 <sup>b</sup>
Age	Walungu	3.9 (1.45–10.52)	1.36	0.51	2.69	0.007 <sup>b</sup>
	Adult					
Breed	Young	0.5 (0.18–1.52)	-0.66	0.55	-1.12	0.23
	Exotic (European)					
Sex	Local	0.8 (0.46–1.55)	-0.17	0.31	-0.56	0.57
	Female					
Breeding system	Male	0.4 (0.28–0.87)	-0.70	0.29	-2.42	0.016 <sup>c</sup>
	Free-range					
Origin of feed	In pen	0.4 (0.25–0.72)	-0.85	0.27	-3.17	0.002 <sup>b</sup>
	Forages					
	Market	0.7 (0.21–2.64)	-0.30	0.65	-0.46	0.64
Farm near pig slaughter slabs	Swill in pen	3.8 (2.12–6.77)	1.33	0.30	4.5	< 0.001 <sup>a</sup>
	No					
Shared working utensils with other pig farmers	Yes	1.3 (0.78–2.23)	0.28	0.27	1.04	0.3
	No					
Restricted access for visitors	Yes	3 (1.32–6.81)	1.1	0.42	2.62	0.009 <sup>b</sup>
	No					
Foot bath in farm	Yes	0.6 (0.23–2.00)	-0.39	0.55	-0.71	0.47
	No					
Daily cleaning of working utensils	Yes	0.7 (0.16–3.40)	-0.30	0.78	-0.38	0.70
	No					
Mixed pigs of different ages	Yes	0.3 (0.04–2.41)	-1.16	1.04	-1.12	0.26
	No					
Daily cleaning of pen floor	Yes	3.3 (1.99–5.57)	1.22	0.27	4.48	< 0.001 <sup>a</sup>
	No					
Introduction of new animal to herd	Yes	1.03 (0.33–3.06)	-0.00	0.57	-0.00	0.99
	No					
Farm near park	Yes	5.4 (1.91–15.28)	1.69	0.53	3.18	0.001 <sup>b</sup>
	No					
Farm near park	Yes	0.4 (0.22–1.13)	-0.71	0.42	-1.66	0.09
	No					

ASFV, African swine fever virus; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval;  $\beta$ , standardized regression coefficient; SE, standard error; OR, odds ratio.

<sup>a</sup>Highly significant; <sup>b</sup>Very significant; <sup>c</sup>Significant.

**Table 3.** Multivariable logistic regression of variables associated with ASFV infection on pig farms

Variables	OR (95% CI)	$\beta$	SE	Z-value	Pr (> z )
District Kabare	4.7 (1.26–18.25)	1.57	0.68	2.3	0.022 <sup>a</sup>
District Kalehe	3.3 (0.79–14.38)	1.21	0.74	1.64	0.101
District Mwenga	2.9 (0.63–14)	1.08	0.79	1.37	0.171
District Uvira	6.1 (1.92–19.8)	1.82	0.59	3.06	0.002 <sup>b</sup>
District Walungu	6.6 (1.91–22.89)	1.89	0.63	2.98	0.003 <sup>b</sup>
Sex male	0.4 (0.22–0.83)	-0.85	0.34	-2.5	0.013 <sup>a</sup>
In-pen breeding system	0.4 (0.2–1.07)	-0.77	0.43	-1.8	0.071
Food from market	0.9 (0.23–3.88)	-0.05	0.72	-0.07	0.946
Food from swill in pen	4.4 (2.58–8.67)	1.49	0.34	4.38	< 0.001 <sup>a</sup>
Shared working utensils with other pigs	2.1 (0.88–5.39)	0.78	0.46	1.69	0.092
Mixed pigs of different ages	5.1 (2.67–10)	1.64	0.34	4.87	< 0.001 <sup>a</sup>
Introduction of new animal	4.8 (1.53–15.47)	1.58	0.59	2.68	0.007 <sup>b</sup>
(Intercept)		-5.82	0.96	-6.06	< 0.001 <sup>a</sup>

ASFV, African swine fever virus;  $\beta$ , standardized regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

<sup>a</sup>Highly significant; <sup>b</sup>Very significant; <sup>c</sup>Significant.



## DISCUSSION

This study was conducted from November 2018 to January 2019, a period in which there was no official report of an ASF outbreak in the sampled districts. The purpose of the study was to determine the proportion of ASFV-positive animals among the symptomatic domestic pigs sampled and determine the associated potential risk factors for ASFV spread among domestic pigs.

The limited access to formal education for the majority of farmers interviewed during this investigation and the lack of good husbandry practices might negatively affect pig-farm management and may favor disease occurrence. This finding agrees with previous studies conducted in Uganda [2,14], which showed that insufficient knowledge of husbandry practices and pig management were major constraints on pig farmers.

An average ASFV PCR-positive rate of 18.4% was detected in this study, close to that in a study conducted in Kenya, where 28% were positive [24] but much lower than the 42.8% reported in another study in symptomatic pigs in Cameroon [25]. Identification of symptomatic pigs that were ASFV PCR-positive in South Kivu indicates current or previous infections in the province, suggesting that outbreaks might be occurring in the region; however, a proper reporting system is lacking, so outbreaks may not be officially recorded. Similar results were obtained in previous studies conducted in the same region and in some other parts of the DRC where ASFV viral DNA was detected in domestic pigs with ASF-specific clinical signs [17,26].

The highest level of PCR-positive pigs was observed in the Uvira district, followed by that in the Walungu district. This may be related to the use of a free-range system, which is practiced in Uvira and some parts of Walungu and allows pigs to move around the farm area. Pigs in a free-range system are far more likely to have more frequent contact with infected animals or materials that may facilitate infection. Logistic regression analysis revealed that the feeding of pigs with swill in pen, the presence of pigs of mixed ages in the same pen, and the introduction of a new animal to a farm were the most prominent risk factors associated with ASFV positivity in South Kivu province. The swill feeding practice, common in most pig-keeping households, increases the risk of consuming ASFV-contaminated products. This linkage has been suggested in previous studies conducted in Uganda and Kenya [14], which showed that swill feeding is a potential risk factor for ASF transmission, particularly where many farms in peri-urban areas practice the same system, probably because of their proximity to many restaurants and hotels. However, a study conducted by Allaway et al. [27] found that feeding kitchen leftovers was not a potential risk factor in the spread of ASF in Malawi. Regardless, treating swill before feeding can be an efficient way of preventing the introduction of ASFV in pig farms.

Mixing of pigs of different ages in the same pen as a factor influencing ASFV transmission is likely due to direct pig-to-pig contact or spreading through fomites, especially in confined farms with poor or no biosecurity measures. Similar results were reported in Sardinia and Madagascar [28,29]. The introduction of new pigs from another farm to an existing herd (without a quarantine area) is an obvious risk factor, as ASFV can be introduced through such a route. The surveys conducted in this study revealed that pig keepers usually buy pigs from sellers or neighboring farms without knowing the animals' health status, and such transactions are followed by the introduction of the newly purchased pig into their herd. This practice is also conducted for breeding purposes. The farmer may borrow a boar, or a sow in

heat may be sent to a boar for mating, leading to the spread of ASF. This approach was also reported in studies in Uganda [30] and Tanzania [31]. Logistic regression analysis revealed that the presence of male pigs and the used of an in-pen breeding system are less likely to be infected by ASF; thus, these were considered protective factors. These findings support those in a previous study in Uganda where farm perimeter fencing was one of the protective factors against ASF infection [32].

However, the epidemiology and risk factor information from this study are different from findings in a similar study carried out in Uganda [32], which revealed that prompt disposal of dead pigs on farms, wild animals present in the village, and farmers obtaining drugs from stockists to be the major risk factors associated with ASF outbreaks in Uganda. These differences could be attributed to diversity among the 6 geographical districts included in the present study compared to the previous study in Uganda, in which samples were from only 3 districts. Furthermore, this study was conducted over a shorter period (3 months) than that of the study in Uganda (5 months).

Our findings are also different from those reported in Nigeria [33], where the sharing of tools was negatively associated with the spread of infection, whereas it was positively associated with ASF infection in the current study. This could be due to differences in the implementation of biosecurity measures between the 2 countries. For example, it is possible that tools shared between pig farms in Nigeria are regularly washed and disinfected; hence, the observation of a negative association (ASF protection).

Despite the informal approach to most livestock farming, particularly for small-scale pig farming in rural areas, the security issues in several villages in the study region, the lack of transport to reach some areas due to road damage, and the lack of up to date animal registration lists, the data for the 6 districts included in this study may not be representative of all pig farms in the study region. Therefore, the results obtained may not be conclusive. Further study covering all of the districts of South Kivu is recommended.

In conclusion, most ASF studies carried out in South Kivu province (DRC) have focused on the clinical diagnosis and molecular characterization of ASFV. This study, however, highlights molecular-based ASFV diagnosis and describes important potential risk factors for the spread of ASF. Such factors may contribute to the regular occurrence of ASF outbreaks and most importantly may result in maintenance of an ASFV population in South Kivu. The observed ASFV infectious rate in clinically infected pigs is evidence of the active circulation of ASFV in a domestic pig population. Improved biosecurity, including fencing, strict control of feed sources, foot baths, and remote quarantine areas for new pigs (with foot baths), are important recommended practices for the control of ASFV transmission in South Kivu province, DRC.

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## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

Demographic characteristics during the field survey and the number of farms and animals in each district

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### Supplementary Table 2

Characteristics of pigs screened according to sex, age, breed, breeding system and geographical region

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### Supplementary Fig. 1

PCR amplification of the *B646L* gene encoding the p72 protein. A 1.5% agarose gel showing the bands obtained using diagnostic primers targeting the central portion of the p72 gene of ASFV. Lane M is 1 Kb plus (Thermo Fisher Scientific) molecular weight DNA marker; Lanes 1, 2, 3, 4, 5, 6, 7, 8, and 9 are selected positive samples showing an approximately 257 bp band size, Lane NC is the negative control; and lane PC is a positive control.

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