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Commentary

First Detection of African Swine Fever (ASF) Virus Genotype X and Serogroup 7 in Symptomatic Pigs in the Democratic Republic of Congo: A Short Commentary

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BACKGROUND AND JUSTIFICATION

The biggest threat to global animal-source food security is infectious disease. African swine fever (ASF) is a contagious, notifiable and highly infectious fatal hemorrhagic viral disease of domestic pigs with significant economic effects due to massive losses in pig population in affected regions [1,2]. It is caused by African swine fever virus (ASFV), a large, linear double-stranded DNA virus. It is the sole member of the genus Asfivirus within

the Asfarviridae family, with a genome of 170-193 kbp in length. Warthogs, bush pigs and the soft tick of the genus Ornithodoros are the major reservoirs [3-5]. ASFV infection is almost always fatal in domestic pigs and the lack of effective vaccines and treatments is a big challenge for its prevention and control. The disease is endemic in more than 25 sub-Saharan African countries. Frequent epidemics of ASF in Russian Federation (2007-2020) and large parts of Eastern Europe (2014-2020) [6,7], as well as disease spread in China in 2018, the producer of approximately half of the world's one billion pigs-highlight the significant threat of this disease to the global pig industry, which is worth more than USD150 billion a year [8].

A solid knowledge of the molecular epidemiology of ASFV is important for efficient prevention, control and eradication of ASF, and prevention of the spread of the virus. To date, 24 ASFV genotypes have been reported, all of them known to circulate in Africa [9,10]. Recent studies have shown the circulation of genotypes I, IX and XIV in some regions of the Democratic Republic of Congo (DRC) [11]. Despite reports from the Provincial Ministry of Agriculture Livestock and Fishery (PMALF) and the local veterinary authority indicating occurrence of recurrent ASF outbreaks in South Kivu province, no study was conducted in that region, which is bigger than the size of Burundi and Rwanda put together. Taking this gap into consideration, this study was conducted in South Kivu province to determine the ASFV strains causing disease outbreaks in order to enhance our understanding of ASFV evolution and spread throughout the South Kivu province, and design improved disease control strategies.

METHODOLOGY AND FINDINGS

We conducted a cross-sectional study in six of the eight districts of South Kivu province by collecting blood samples from indigenous pigs with symptoms of ASF during the December 2018-January 2019 outbreaks. First, we used the ASFV diagnostic PCR primers PPA1/PPA2 to ascertain the presence of ASFV in suspected pigs sampled and determine the prevalence of the infection. Of the 391 blood samples tested, 6.7% were positive for the presence of ASFV. This low prevalence of ASFV in symptomatic pigs may be due to the low sensitivity of the conventional PCR used (in comparison to real-time PCR), suggesting that most pigs tested may have low virus load not detectable by the PCR technique used. In addition, most pigs sampled may not have been infected by ASFV and therefore, may have been affected by other diseases and conditions with similar symptoms to ASF such as porcine reproductive and respiratory syndrome, porcine dermatitis, nephropathy syndrome, salmonellosis, as well as classical swine fever mainly found in Latin America, Europe, Asia and parts of Africa.

During the period from May-August 2020 in Kenya, we observed a similar low PCR-positivity from suspected pigs in ASF outbreaks. Since the PPA-primers will detect all known ASFV genotypes, the presence of different genotypes should not account for the observed low prevalence. Since infected pigs can produce ASFV specific antibody detectable using indirect Enzyme Linked Immunosorbent Assay (ELISA), and because some pigs could be affected by other diseases with similar symptoms to ASF, we will include differential diagnosis as well as ELISA in future epidemiological studies of ASF to avoid confusion of ASF with other pig diseases and complement molecular diagnostics with antibody-based assay.

For a better insight into the South Kivu ASFV evolution and spread,

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as well as their relationship with other known virus strains/isolates, particularly those in circulation in the region, we genotyped the South Kivu strains and compared them with reported ones. In that respect, we carried out partial sequence comparison at four loci—the C-terminus of B646L gene encoding the p72 protein, the E183L gene encoding the p54 protein, the central hyper variable region (CVR) of the B602L gene encoding the J9L protein, and the intergenic region between the I73R and I329L genes. We found that p72 sequences of the South Kivu ASFV shared 99%-100% identity due to some few synonymous mutations while the p54 sequences were 100% identical. Both p72 and p54 phylogenetic analyses clustered ASFV strains in South Kivu province with ASFV genotype X retrieved from the GenBank database. ASFV genotype X is the major genotype associated with ASF outbreaks in Burundi (AF449463) and in some parts of Tanzania (JX403648, AF301546, MF437291), Kenya (AY261360) and Uganda (KJ526359). In addition, partial sequence alignments of p72 gene showed 100% identity between the South Kivu strains and ASFV Burundi 1984. This genetic similarity suggests that South Kivu ASFV may have originated from or could have expanded to Burundi. South Kivu province and Burundi share a common border through the river Ruzizi and Lake Tanganyika and frequent, uncontrolled cross-border movement of pigs and pork products are observed in the region.

We then used the Tetrameric Repeat Sequence (TRS) of the CVR amino acid sequence and the intergenic region between the I73R and I329L genes to ensure a high-level resolution for the South Kivu ASFV and discriminate between closely related strains. We found that the ASFV p72/p54 genotype X population in South Kivu is composed of two subgroups, the Uvira subgroup with 10 TRS repeats (AAAABNAABA) and another subgroup with 8 TRS repeats (AABNAABA) formed by strains from the five other districts studied. Based on CVR, the latter subgroup was very similar to ASFV Uganda 95/3 (AABNBABA). However, the two CVR variants found in our study were different from previously reported variants, including DR Congo ASFV strains [11]. Our study also showed that South Kivu ASFV strains had identical but unique I73R and I329L intergenic region that differentiated them from other known ASFV, including the virus Kenya 1950 (AY261360) which showed a high sequence identity but had an insertion of 33 bp. Both the CVR and intergenic region analyses suggested that the ASFV genotype X in circulation in South Kivu province of DR Congo identified in this study may be a new ASFV strain.

Finally, we determined the virus serological specificity. Haemadsorption inhibition (HAI)-based serogroup classification using the ASFV proteins CD2v (EP402R) and/or C-type lectin (EP153R) was suggested as a better correlate for *in vivo* cross-protection among strains compared to the p72 genotyping [12]. This has significant importance for vaccine development against ASF. CD2v is a transmembrane glycoprotein located in the viroplasm and in the plasma membrane of infected cells. We found that all the South Kivu ASFV strains analyzed had identical CD2v which clustered with a homolog (at 99.2% identity) from the only member of the serogroup 7 reported to date, the Uganda genotype X and serogroup 7 (KM609361.1) suggesting that the ASFV strains characterized in this study may belong to serogroup 7.

CONCLUSION

Combining molecular characterization methods in this study provided significant insights into genetic variations and serotype specificity among ASFV for discriminating between closely related ASFV isolates and strains in South Kivu and the region. It was also helpful in understanding the evolution and spread of ASFV better, tracking the potential origin of ASF disease outbreaks and formulating vaccine development strategies. This study identified for the first time ASFV p72 genotype X and CD2v serogroup 7 in DRC, molecularly and serologically different from other strains reported so far in Congo. Our findings emphasize the need for improved coordination of efforts to control ASF, and further studies to conduct in-depth comparative sequence analyses including whole genome sequencing of ASFV strains circulating in the area.

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