MOLECULAR CHARACTERIZATION OF AFRICAN SWINE FEVER IN A BACKAYRD SWINE FARM IN BULACAN PHILIPPINES

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Introduction

African swine fever is an infectious transboundary disease of domestic pigs and wild swine causing high fever, hemorrhages, severe depression with mortality rates approaching 100%. The disease is caused by a large, complex and multi-enveloped DNA virus, from the genus *Asfivirus*, family *Asfarviridae* (Ge *et al*. 2018; Mazur-Panasiuk *et al*. 2019).
The Philippines has reported its first cases of African swine fever last September 2019 in Rizal and Bulacan provinces.

In this study, we aim to identify the African swine fever virus genotypes from an outbreak in a backyard farm in Bulacan to contribute sequence data for molecular epidemiology studies of the disease.

Materials and Methods

A total of fifteen FTA samples were collected from Plaridel, Bulacan last September 20, 2019. Samples comprise of 4 blood samples from apparently healthy pigs, 2 blood samples from sick pigs, tissues from dead pigs, feeds from warehouse, feed sample from 2 pens with sick pigs feeds, water from irrigation, water from the entrance of the gate, water from the nearby building, canal water and mud from the connecting pathway. PCR test was done on site and was performed as recommended by the Office International Des Epizooties; Manual of standards for diagnostic test and vaccines.

Samples were subjected to conventional PCR targeting the central portion of the p72 gene generating a 278 bp gel band product with forward primer 5- ATG-GAT-ACC-GAG-GGAATA-GC -3 and reverse primer 5- CTT-ACCGAT-GAA-AAT-GAT-AC-3. Thermocycling conditions were as follows: pre-warming at 95°C for 3 minutes, 40 cycles of amplification such as denaturation at 95°C for 30 seconds; annealing at 50°C for 30 seconds; and extension at 72°C for 30 seconds (Atuhaire *et al.* 2013). Final elongation was done at 72°C for 10 minutes. Amplicons were analyzed in 1.2% agarose in TAE buffer gel containing gel red by electrophoresis. PCR products were sent to the Philippine Genome Center for purification and bidirectional nucleotide sequencing. Sequence assembly and editing were performed using CodonCode Aligner® (version 3.7.1, CodonCode Corporation, MA) and ClustalX® (version 2.1, Conway Institute UCD Dublin, Ireland). Deduced amino acid sequences were determined using Bioedit® software package version 7.1.3.0. Confirmation of identity and homology was performed using BLAST http://www.ncbi.nlm.nih.gov. Phylogenetic analysis was performed with the neighbor-joining method using MEGA version 4.0 with the maximum composite likelihood substitution model at 1000 bootstrap replicates. The nucleotide sequences of field strains were compared with the nucleotide sequences of reference strains reported in GenBank.

Discussion

Out of the fifteen samples collected, direct nucleotide sequencing confirmed that 3 blood samples from apparently healthy pigs, 2 blood samples from sick pigs and tissue sample from dead pigs (n=6) contained nucleotide sequences of the p72 gene of African Swine Fever Virus. This indicates that exposed pigs can harbor ASFV in the blood before showing any

observable clinical signs. The negative PCR test results of feeds and water from different sources as well as dirt, mud and other environmental samples may indicate the presence of inhibitors in the samples affecting the results, minimal viral shedding and environmental contamination in the early stages of infection or the effect of disinfectants on the level of ASFV contamination in the environmental samples.

In this study, the genetic analysis of samples that tested positive showed that the p72 gene of the ASFV were 100% similar to each other indicating that the virus maybe identical. The comparison of the partial sequence of the p72 gene also showed that the ASFV strain from the samples were 97100% closely related to Georgia ASFV (2007), Krasnodar, Russia ASFV (2012), Estonia ASFV (2014), Poland ASFV (2015), Etalle, Belgium ASFV (2018), China ASFV (2018) and Vietnam ASFV (2018).

The published information in the Genbank and phylogenetic analysis of the p72 gene confirmed that ASFV genotype II, a highly virulent and predominant strain, was responsible for the reported disease occurrence in Bulacan.

References

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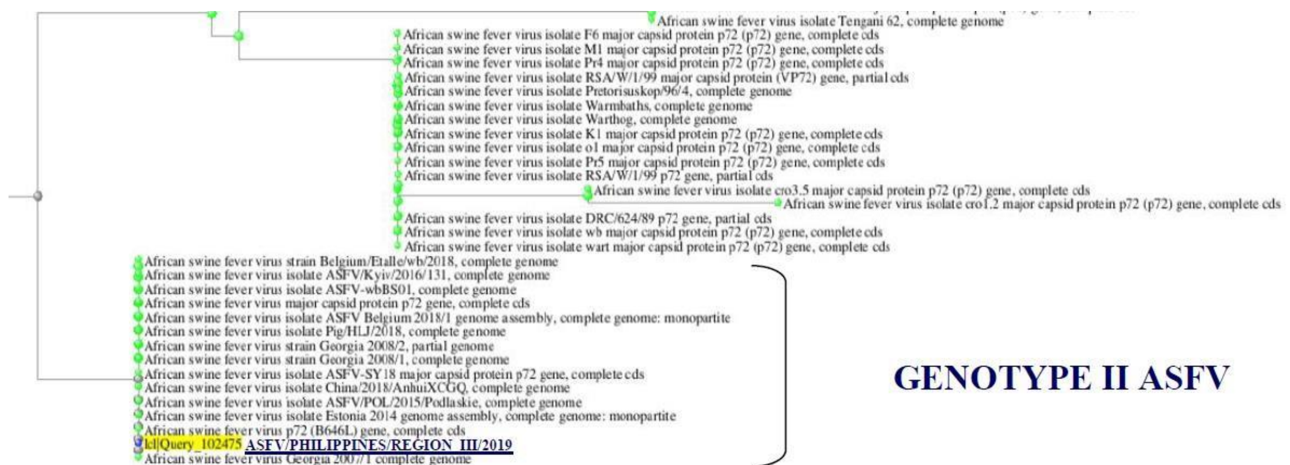

Figure 1. Phylogenetic analysis of the detected ASFV strain