African swine fewer virus fragments Sequence Analysis in wild boars and domestic pigs

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Introduction

The ASFV genome is a linear double-stranded DNA molecule that ranges in length between isolates from about 170 to 193 kbp. ASFV encodes for between 151 and 167 open reading frames (ORFs) and the differences in genome length and gene number are largely due to gain or loss of ORFs from the multigene families (MGF) encoded by the virus. Sequence analysis of virus genomes have established that central region is conserved but large length variations occur at the terminal ends, particularly within 40kbp of the left end of the genome, bur also within 15kbp from right end of genome. ASFV genotyping is based on the analysis of four regions located at the conserved central or right area of the ASFV genome : sequencing of C-terminal end of the gene B646L encoding protein p72; sequencing of the central variable region within B602L-gene (CVR); sequencing of intergenic region between I73R and I329L genes in African swine fever virus and characterized by the presence of TRS; sequencing of multigene family member MGF505-10R this region are present in regions close to the genome termini.

For ASFV-positive clinical samples (spleen, kidney, lung, bone marrow, blood) collected in 2014-2016 years from infected wild boars and domestic pigs initial genetic characterization was performed by using standardized genotyping procedures on virus DNA extracted directly from homogenized tissues bone marrow and from blood samples. We compared the nucleotide sequences obtained from PCRs with those of Caucasus 2007 previously described representative isolates. We used Clustal Omega (http://www.clustal.org/) to perform multiple sequence alignments.

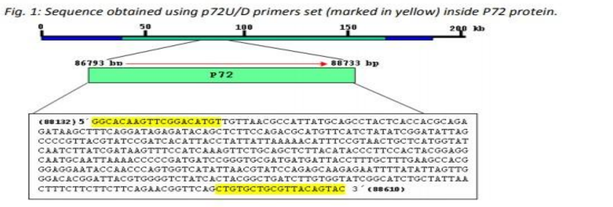
Material and methods

For experiments were used positive samples collected from different affected regions in Lithuania in 2014-2016.

Total DNA was extracted from 140-500µL wide range of sample: tissue, bone, blood-EDTA, serum using “Viral RNA Extraction Kit“(Qiagen) for blood and “RNeasy Mini Kit” (Qiagen) for tissue following the manufacturer’s procedure. Final elution was done with 80-125 µL elution buffer. Wild boars and domestic pigs samples were tested by ASFV genotyping. Analysis is based of four regions located at the conserved central area and termini of the ASFV genome : sequencing of C-terminal end of the gene B646L encoding protein p72; sequencing of the central variable region within B602L-gene (CVR); sequencing of intergenic region between I73R and I329L genes; sequencing of multigene family member MGF505-10R in African swine fever virus and characterized by the presence of TRS using primers sequences. Four different set of primers using to amplify four independent regions of the ASFV genome. Samples were amplified by PCR analysis and after that were performed sequencing analysis.

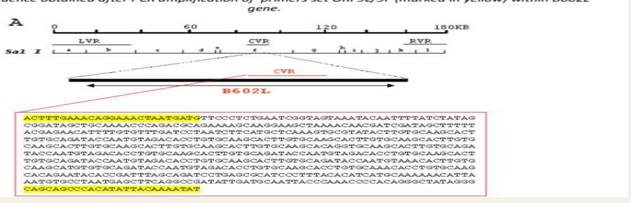
PCR amplification of the C-terminal region of p72 protein using primers p72- U and p72-D. These primers amplify 478 bp from the protein p72 of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 1) and have been previously described by Bastos et al., 2003.

Fig. 1: Sequence obtained using p72U/D primers set (marked in yellow) inside P72 protein.

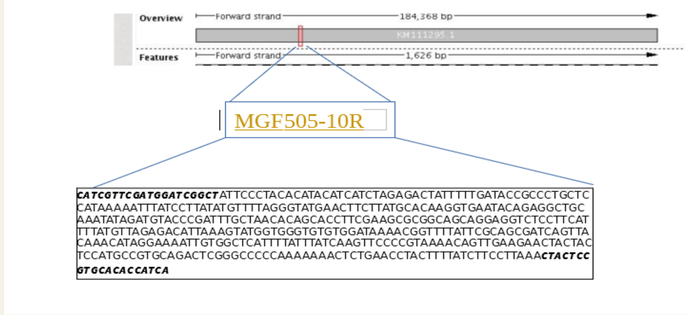


PCR amplification of the CVR within the *B602L* gen using the primer set CVR1 and CVR2. These primers amplify 665 bp of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 3) containing the amino acid tandem repeats and have been previously described by Gallardo *et* al., 2011.

Fig. 3: Sequence obtained after PCR amplification of primers set ORF9L/9F (marked in yellow) within B602L gene.



PCR amplification region of MGF505-10R protein using MGF4F and MGF4R primers. These primers amplify 422bp from protein MGF505-10R (1600bp) of the Pig/Kenya/KEN-50/1950 and have been previuosly described by Bishop et.al. 2015



Results

We compared the nucleotide sequences obtained from the p72-, CVR intergenic region between I73R I329L genes and multigene family member MGF505-10R PCRs from Lithuania different region infected by ASF with those of previously described representative isolates (Table)

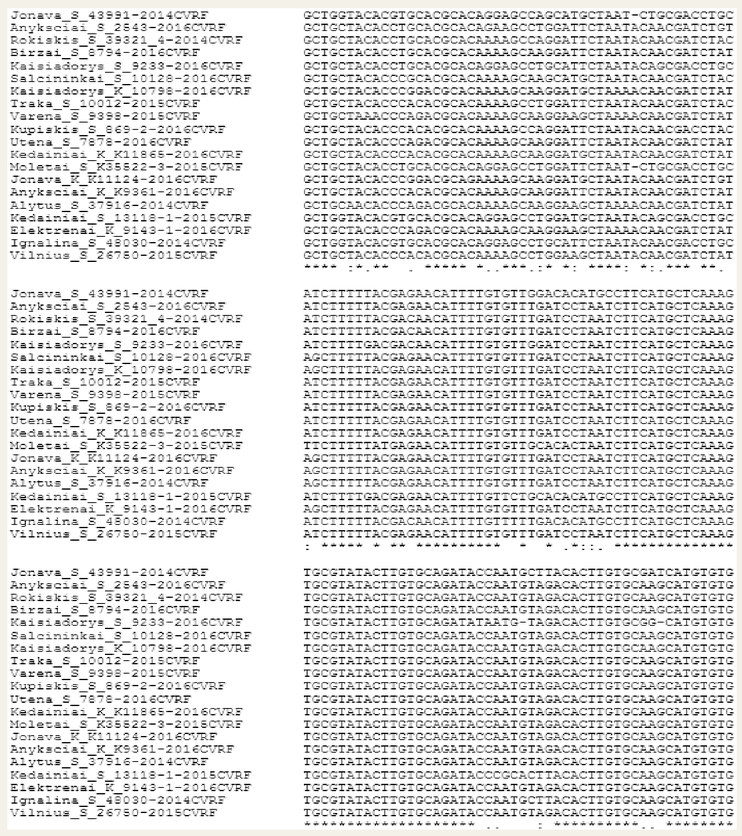
Table 1. African swine fever virus samples from different Lithuania region selected for a study of the genetic variation among genotype II viruses in Lithuania, 2014–2016

Sample	Source	Area	Onset of outbreak
39321_4-2014	Wild boar	Rokiškis	2014
37916-2014	Wild boar	Alytus	2014
43991-2014	Wild boar	Jonava	2014
48030-2014	Wild boar	Ignalina	2014
9398-2015	Wild boar	Varėna	2015
10012-2015	Wild boar	Trakai	2015
13118-1-2015	Wild boar	Kėdainiai	2015
26750-2015C	Wild boar	Vilnius	2015
K35522-3-2015	Wild boar	Molėtai	2015
869-2-2016	Wild boar	Kupiškis	2016
2543-2016	Wild boar	Anykščiai	2016
7878-2016	Wild boar	Utena	2016
8794-2016	Wild boar	Biržai	2016
9233-2016	Wild boar	Kaišiadorys	2016
10128-2016	Wild boar	Šalčininkai	2016
10798-2016	Domestic pig	Kaišiadorys	2016
K11865-2016	Domestic pig	Kėdainiai	2016
9143-1-2016	Domestic pig	Elektėnai	2016
K9361-2016	Domestic pig	Anykščiai	2016
K11124-2016	Domestic pig	Jonava	2016
9233-2016	Domestic pig	Kaišiadorys	2016
10128-2016	Domestic pig	Šalčininkai	2016
10798-2016	Domestic pig	Kaišiadorys	2016

Presented results confirm, that primers for p72 amplify a fragment of 478 bp within the C-terminal end of the p72 protein, all sequenced samples belongs to II ASF genotype.

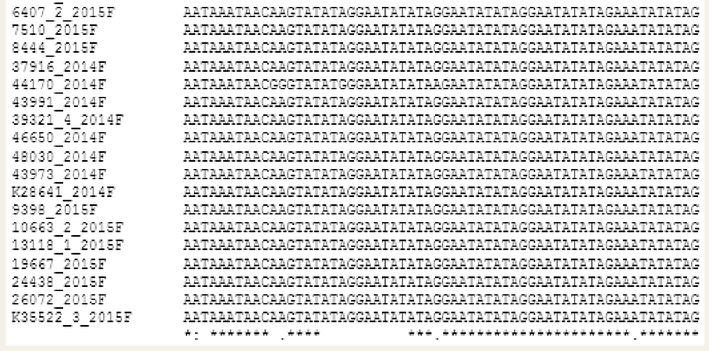
Comparison samples amplified for CVR region, primers allows to establish size variation, and single nucleodite differences are observed in the sequence . (Figure 1)

Figure 1. African swine fewer samples sequence for CVR fragments



Sequencing samples for intergenic region between I73R and I329L genes in African swine fever virus, nucleotide sequence analysis of the PCR products revealed that the size difference was caused by the insertion of an additional TRS (GGAATATATA) at nt 136, also and single nucleodite differences are observed in the sequence (Figure 2)

Figure 2. Nucleotide sequence alignment of the intergenic region between I73R and I329L in African swine fever virus (ASFV) samples

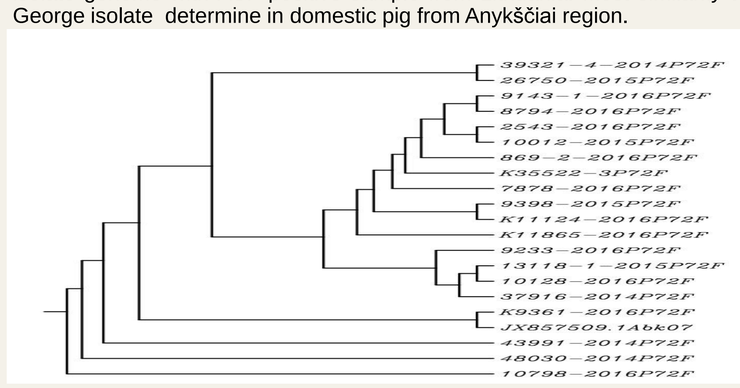


Sequencing samples for region MGF505-10R (422bp) in African swine fever virus, nucleotide sequence analysis of the PCR products revealed that the size difference primers allows to establish single nucleodite differences in the sequence, but no significant result were observed.

Conclusion

ASF has emerged in several European countries. Since it is complicated to control wild boar population and movements, the ASF spread among animal in this area is getting progressive. While ASF is spreading in territory of Lithuania, PCR positive prevalence is increasing, analyzing ASF virus spreading 2014-2016 were selected wild boars and domestic pigs samples from different Lithuania region for further sequencing experiments. For experiments were selected of three regions located at the conserved central area of the ASFV genome : sequencing of C-terminal end of the gene B646L encoding protein p72; sequencing of the central variable region within B602L-gene (CVR); sequencing of intergenic region between I73R and I329L genes in African swine fever virus and characterized by the presence of TRS. Experiments for p72 sequencing confirm that sequenced samples from Lithuania belongs to II ASF genotype, which has been circulating in eastern European countries since the introduction of ASFV into Georgia in 2007. To determine the phylogenetic relationship between the Georgia 2007/1 isolate and analyzed positive ASF samples from Lithuania (Table), the concatenated nucleotide sequences of p72 were compared (Figure 3).

Figure 3. Comparison of the Georgia 2007 African swine fever virus (ASFV) isolate genome with ASF positive sample from Lithuania. Most similarity to George isolate determine in domestic pig from Anykščiai region.



Analysing CVR fragment sequencing results were observed single nucleotide differences in sequence in different region of Lithuania, it is difficult to noticeable the trend, so it required more detailed study.

Sequencing samples for intergenic region between I73R and I329L genes in African swine fever virus, results showed that viruses had TRS insertion as was presented in previous experiments by Spain researcher, as was mentioned previous ASFVs detected in Lithuania most probably originated from Belarus. Also and single nucleotide differences are observed some regions of Lithuania.

Analysing results of MGF505-10R (422bp)sequencing in African swine fever virus, nucleotide sequence analysis of the PCR products revealed that the size difference primers allows to establish single nucleodite differences in the sequence, but no significant result were observed, for further works 902bp amplified product of MGF505-10R fragment will be sequenced.

References

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