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Short communication

Molecular characterization of African swine fever virus isolates originating from outbreaks in the Russian Federation between 2007 and 2011

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ABSTRACT

African swine fever is one of the most important viral diseases of pigs and which caused significant economic damage on the pig production worldwide. Nowadays, it is still present on the African continent, in Transcaucasus countries (TCC), on Island of Sardinia and in Russia. Outbreaks of the disease have been reported in Russia for the last four years, affected especially the Southern Federal District of the country. Since 2010, a new outbreak area has been observed in the Northwestern Federal District.

In order to study the evolution of African swine fever virus (ASFV) isolates, strains were collected in the Russian Federation from 2007 to 2011 and investigated by means of partial sequencing and fragment length polymorphism. In detail, 7 variable regions, namely B646L, E183L, I196L, B602L, I73R/I329R, I78R/I215L and KP86R were investigated. Phylogenetic analyses revealed 100% nucleotide identity of B646L and E183L gene sequences of all examined isolates. All isolates formed one genetic cluster within genotype II. Moreover, no amplified fragment length polymorphism (AFLP) was observed for B602L, I196L, I73R/I329R, and I78R/I215L genes. The flanking primers used to amplify the KP86R gene failed to amplify a product in all the isolates. The obtained data strongly suggests that only one ASFV virus variant caused the outbreaks from 2007 to 2011 in the territory of the Russian Federation.

1. Introduction

African swine fever (ASF) is among the most dangerous diseases impairing pig production worldwide. ASF is highly contagious and acute forms of the disease have a high rate of mortality that may result in substantial economic losses, due to compulsory pig slaughtering and the international ban on trade of live animals and pig products (Penrith, 2009; Tulman et al., 2009). Epidemiology of ASF confirms that presence of the disease in one area has a potential risk for introduction and further spreading to any directions despite the natural and artificial borders and distance (Wieland et al., 2011). Clinical symptoms of ASF vary considerably, but in general, ASF is well known as a disease with high morbidity and mortality. The disease affects domestic and wild pigs, and can be transmitted through contact as well as via soft ticks of the *Ornithodorus* complex (Jori and Bastos, 2009; Parker et al., 1969; Plowright et al., 1970). The causative agent is a large enveloped double-stranded DNA virus, sole member of the genus *Asfivirus* within the *Asfarviridae* family (Kleiboeker and Scoles, 2001). The viral genome comprises around 170–195 kb, which encode more than 125 different proteins. According to the nucleotide sequence analysis of the variable region of the B646L gene (p72), 22 genotypes can be distinguished (Bastos et al., 2003; Lubisi et al., 2005).

Better phylogenetic resolution between closely related ASFV isolates was reached using other virus genes (E183L

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(p54), B602L, KP86R, I196L) and intergenic regions (J286L, BtSj, I73R/I329R, I78R/I215L) as the markers for genetic variations (Gallardo et al., 2009; Nix et al., 2006; Rodriguez et al., 1994).

The repeat region next to J268L contains a set of internal repeated sequences composed of 5 types of 200-bp-long tandemly repeated units. These units contain a G-rich core of 10–14 nucleotides surrounded by regions with a high A and T content. The ORF KP86R is cysteine-rich and contains tandem repeats identical to those in the ORF DP86L at the right-hand end of the genome.

Two intergenic region I73R/I329R, I78R/I215L and open reading frame I196L placed 10.6 kbp from the right end of ASFV genome (EcoRI-I fragment) and characterized by presence of terminal repeat sequences (TRS).

The ORF B602L encodes a central region containing twelve-base-pair repeats. The variable region of ORF B602L consists of repeated amino acid tetramers that vary in number and type. The reasons for the variability of the B602L protein are still not clear. The protein has been reported to act as a chaperone involved in assembly of the p72 capsid protein into virions, although B602L protein itself was reported not to be incorporated into virions (Nix et al., 2006).

Until recently, only countries of Sub-Saharan Africa and Sardinia were endemically infected with ASFV. In June 2007, Institute of Animal Health (Pirbright, UK) notified of a new outbreak of ASF in the Republic of Georgia, coherently the outbreaks were registered in Armenia, Azerbaijan and the Russian Federation (Rowlands et al., 2008). Since the first introduction in 2007 and until 2011, ASF outbreaks were regularly detected in different regions of the Russian Federation (Balyshev et al., 2010; Gulenkin et al., 2011; Kurinnov, 2009). From 2008 to 2011, the disease was continuously present in the Southern Federal District. Since 2010, a new outbreak area has been observed in the Northwestern Federal District.

Considering the endemic situation in some territories of Russia over the last four years, there is a clear possibility for genetic changes in ASFV isolates which are obtained from different times, places, and animals.

In this regard, the main goal of our study was to carry out a comparative analysis of ASFV isolates, from different outbreaks of the disease on the territory of Russia since 2007 based on phylogenetic relationship and molecular characteristics.

2. Materials and methods

2.1. Virus isolates

16 ASFV positive samples of organs (mainly spleens) obtained from naturally infected wild boars and domestic pigs and stored in the virus collection at the SRI NRIVVaM RAAS were used to study genetic differences between them. The samples for investigation from different ASF outbreaks were provided by the Russian State Veterinary Service and Federal Service for Veterinary and Phytosanitary Surveillance from North Caucasian Federal District, Southern Federal District, Volga Federal District, Central Federal District and Northwestern Federal District.

2.2. Viral DNA

Viral DNA was extracted from the tissue samples of naturally ASF infected animals using the DNA easy Tissue Kit (Qiagen) according to the manufacturer's instructions, and was stored at −70 °C until testing by polymerase chain reaction (PCR).

2.3. PCR assay

The following genome regions were chosen to investigate strain variability: B646L and E183L were used for sequencing and phylogenetic analysis: B602L, I196L, I73R/I329R, I78R/I215L and KP86R were applied for AFLP (Bastos et al., 2003; Nix et al., 2006). To this means, gene specific primer pairs were used as previously described. All reactions were carried out according to protocols which are designed by the European Community ASF reference laboratory (Valdeolmos, CISA-INIA). PCR reactions were carried out using a PalmCycler machine (Corbette Research).

The results of variable regions amplification were visualized in 1.5% agarose gel under standard conditions. Comparative analysis of specific patterns of amplified fragment length was carry out using Quantity One System 4.6.9. (BioRad Laboratories, USA) with accompanied software. For following sequencing of B646L and E183 genes the same PCR primers described previously were used (Bastos et al., 2003; Gallardo et al., 2009).

2.4. Nucleotide sequencing

DNA fragments obtained by conventional PCR were purified from agarose gel using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). Sequencing was carried out using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the protocol recommended by the manufacturer. Nucleotide sequences were obtained with a 3130 Genetic Analyzer (Applied Biosystems, USA).

2.5. Phylogenetic analysis

Nucleotide sequences were edited and analysed using the BioEdit Sequence Alignment Editor 7.0.5.3. with a ClustalW alignment package. Subsequently, aligned nucleotide sequences were used to calculate phylogenetic trees with the Neighbor-Joining method and p-distance model with 1000 bootstrap as implemented in MEGA 5.0. Publicly available sequence data of reference strains and isolates were used to generate a comparable topology of the tree.

3. Results

To estimate strain variability of ASFV isolates which were obtained from naturally infected wild boars and domestic pigs from different regions of the Russian Federation from 2007 to 2011, seven variable genome regions (B646L, E183L, I196L, B602L, I73R/I329R, I78R/I215L and KP86R) were analysed.

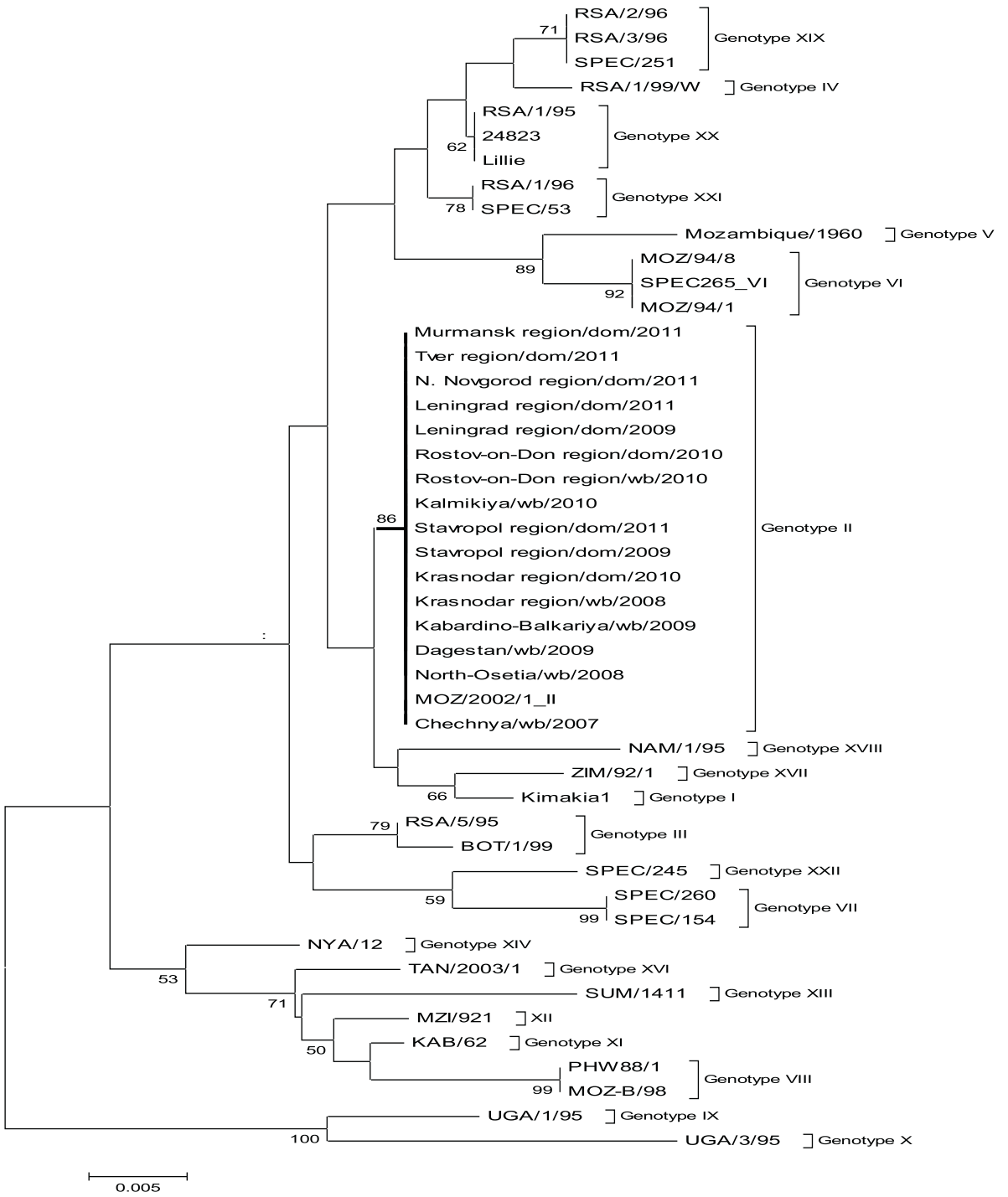

Fig. 1. Phylogenetic relationship between Russian isolates of ASFV (2007–2011) and previously identified ASFV genotypes according to nucleotide sequencing of the p72 gene fragment represented by the neighbor-joining tree. Numbers at nodes represent the percentage of 1000 bootstrap replicates (values <50 are not shown).

In order to determine the genetic relationship between the obtained isolates and the 22 already identified ASFV genotypes according to nucleotide sequencing of the p72 gene fragment, phylogenetic trees were constructed utilizing the neighbour-joining method.

Phylogenetic analysis of the obtained sequences (B646L) showed that all Russian isolates used in this study were identical and form one cluster within genotype II (Fig. 1). In order to represent sequence of p72 gene fragment of analysed ASFV isolates one of them

(Rostov-on-Don/Dom/2010) was submitted in GenBank as JF260952.1.

Working on the reasonable hypothesis to find out more close relationship between the investigated isolates E183L nucleotide sequencing and subsequently phylogenetic analysis were applied. The E183L (p54) sequences from all used isolates were absolutely identical.

To reveal any possibility of occurred genetic changes in ASF isolates analysis of length amplification products for five variable regions (I196L, B602L, I73R/I329R, I78R/I215L and KP86R) was performed. These regions contain different size short nucleotide repeats in central (B602L) and right (I196L) part of ASF genome and also include intergenic regions (I73R/I329R, I78R/I215L). The number of repeats is specific for closely related isolates or strains which have similar source of origin or evolution history.

Amplification of the B602L variable region produced fragments in size 190 bp, of the I196L – 490 bp, of the intergenic region I73R/I329R – 385 bp and of the I78R/I215L – 593 bp for all ASFV isolates used in this study. The flanking primers used to amplify the KP86R gene failed to amplify a product in all the isolates. As a positive control of amplification of KP86 gene fragment were used strains of ASFV in SRI NRIVVaM RAAS collection such as L57 and Angola 72 (250 bp fragment), France 64 (320 bp fragment). Absence of amplified gene fragment can be explained by presence a multiple mismatches in specific primer site or partial/complete deletion of this gene in all Russian isolates.

4. Discussion

Genetic typing, nucleotide sequencing, and further phylogenetic analyses are worldwide applied the techniques to confirm epidemiological data on a qualitatively different level. It also allows us to estimate virus evolution over time.

Comparison analysis of ASFV genome variable regions containing array of the tandem repeats enables to provide more information about molecular patterns of isolates and allow to separate them in multiple sub-groups even if they were clustered together basis of on sequencing of more conserve regions. This method can clarify questions regarding ASFV isolates origin and their heterogeneity (Nix et al., 2006; Rodriguez et al., 1994).

In this study comparison analysis of PCR size fragments of 7 variable regions enables to conclude absence of detectible changes in ASFV isolates caused a disease outbreaks 2007–2011 in Russia. Previously described results of our collaboration with CISA-INIA (Valdeolmos, Spain) of nucleotide sequencing and phylogenetic analysis of variable regions (p72, p54, CVR) of isolates collected in Russia and TCC (2007–2009) neither revealed any genetic modifications between isolates (Gallardo, C., 2010 Oral presentation on ASF/CSF NRL meeting, Pulawy, 2010). All isolates have the same patterns for specific variable regions and can not be separate into subgroups. These data suggest that the same variant is causing the outbreaks in Russia but it also indicates that no significant genetic changes have occurred in the regions evaluated over the 4 year period.

5. Conclusion

Identical molecular characteristics and genetic patterns of analysed ASF isolates seem to be very likely the result of disease spreading from one common source without an appearance and long-term existing of cylvatic or domestic cycles. Finally, our data obtained based on investigation of variable regions in right (RVR) and central (CVR) part of ASF virus genome allow to conclude single source of origin for all used isolates and absence of genetic changes have occurred in the regions evaluated over the 4 year period that can also suggest a close relation of biological characteristic for these isolates.

Conflict of interest

The authors declare no conflict of interest. None of the authors of this publication has any financial or personal relationships with other people or organizations that could inappropriately influence this work.

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