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Role of wild boars and domestic pigs in the spread of African swine fever in the Russian Federation (2007-2013)

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African swine fever (ASF) is an infectious and notifiable swine disease which has devastating consequences for swine sector. In the last years (2007-2013) the high spread of the disease in The Russian Federation has caused significant economic losses in the swine industry and current status is endemic country (OIE). The role of wild boar in the spread of ASF is well defined in the first stages in Southern areas of the Russian Federation, but is inconclusive within the five years subsequent of spread of ASF to Northern areas. The recent introduction of ASF in wild boars into Eastern European Union (EU) increases the risk for other EU-member countries. This introduction was related to wild boar movement from neighboring third countries where that disease is present (CD 2014/178/EU). Therefore a better knowledge about the interaction of ASF between wild boar and domestic pigs is required to a better understand in quantifiable terms the potential mechanisms of virus spread in the Russian Federation. It is a prerequisite to develop and focus programs to control or prevent the spread of ASF in this region and its fatal economic consequences. A spatiotemporal analysis using kriging methodology was proposed here in order to identify the role of wild boar in the spread of ASF. To do it the source of infection (domestic pigs or wild boars) of each ASF outbreak notified during 2007-2013 in The Russian Federation was evaluated. Results show that the source of infection could be attributed to wild boars in 32,23% cases (126 outbreaks) in domestic pigs and in 28.77% cases (84 outbreaks) in wild boars, which could be interpreted as the role of wild boar is lower than the role of domestic pigs in the spread of ASF. Results presented allow understand better the role of wild boar as ASF spreader in the current Russian epidemic and could help to formulate and parameterize the necessary ASF spread models for the disease in wild boars and consequently contribute to the improved of control plans against for ASF.

Environmental factors related with the presence of African swine fever in wild boars in the Russian Federation (2007-2013)

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Abstract text: African swine fever (ASF) is a highly contagious and fatal disease of domestic pigs, feral pigs and wild boar. In the last years (2007-2013) 683 outbreaks of ASF were reported in the Russian Federation (RF) (292 in wild boars and 391 in domestic pigs) causing significant economic losses in the swine industry. The recent introduction of ASF in wild boars in EU increases the risk for other EU-member countries. The role of wild boar in the epidemiology of the disease in RF is not yet completely understood. Therefore a better understanding about related risk factors associated with the presence of ASF in wild boar in RF is still required in order to develop and focus programs to control or prevent the spread of ASF. The objective of this study was to identify the environmental risk factors associated with outbreaks of ASF in wild boars in RF (2007-2013). To do it, a case-control study was conducted using a generalized linear model (GLM). Cases were defined as those areas where significative spatiotemporal clusters of ASF outbreaks in wild boars had been identified using a scan statistics permutation analysis (nine case areas) (Iglesias et al., 2014*). Eighteen randomly selected areas were defined as ”control areas”. Ten environmental variables (climatic and biological) selected because their importance in the biology of ASF virus, were explored comparing their mean values between cases and control areas.

Those variables associated (Kruskall-Wallis test) with sanitary status of the areas and without significant correlation (Pearson test) among them were included in a GLM analysis

The model that best fitted the data (AIC=30.99) included “wild boar presence” as predictor variable ($P < 0.05$) (b=9.2). ”Maximum isolation” could be included but showed a low effect on the probability of occurrence of the event (b=0.004) and showed a high correlation with wild boar presence. A subsequent analysis indicated that the number of ASF domestic pigs cases showed a high association with cases/control areas (KWH=11.16; p=0.0008). Results could be interpreted as environmental factors might not be as relevant as ASF domestic pig cases in the ASF transmission in wild boar. For a deeper analysis of this finding, a spatiotemporal analysis of interactions between domestic and wild ASF cases was developed (results showed in Iglesias et al., 2014**).

* Iglesias et al., 2014. Título. Identification of the Reproductive ratio for the local spread of African swine fever in wild boars in the Russian Federation. EPIZONE 2014 comunication.

** Iglesias et al., 2014. Título. Role of wild boars and domestic pigs in the spread of African swine fever in the Russian Federation (2007-2013)EPIZONE.

Identification of the Reproductive ratio for the local spread of African swine fever in wild boars in the Russian Federation

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African swine fever (ASF) is a notifiable viral disease of swine that produces devastating consequences for the swine industry of affected regions. Over the last 7 years has caused substantial economic losses to the swine industry of the Russian Federation from where disease has spread to a number of neighboring countries. Wild boars have traditionally played an important role on ASF spread. Quantitative knowledge of the dynamics of ASF transmission in wild boar is required to understand the mechanisms of virus spread in the region. The disease reproductive ratio (R_0) is a key parameter for understanding disease dynamics, its quantification helps to design effective control, preventive and surveillance strategies at local and regional levels. Here, R_0 of ASF was estimated in spatiotemporal clusters of ASF outbreaks in wild boar in the Russian Federation (2007–2013). Clusters were defined using a permutation scan statistics model to detect clusters in time and space (Using SatScan software). R_0 was identified applying an algorithm that does not make use of population data. The median range value of R_0=1.58 (1.13-3.77) was lower compared to values previously estimated for ASF transmission in domestic pigs, which may associated with the lower animal density of wild boars compared to industrial systems. This result is consistent with active disease propagation in the Russian Federation. Estimates of R_0 presented here are useful for a better parameterization of ASF spread models in wild boars that will contribute to enhance the effectiveness of national and regional surveillance and control disease programs.

Experimental infection of pregnant sows with African swine fever virus (ASFV Georgia 2007): Clinical outcome, pathogenesis and vertical transmission

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African swine fever virus (ASFV) causes a severe haemorrhagic disease in domestic pigs. The infection can lead to very high levels of mortality. In recent years (since 2007), the virus has become established in Eastern Europe. It was initially introduced into Georgia and then the virus spread throughout the Caucasus and the Russian Federation. Multiple outbreaks have occurred in domestic pigs and wild boar in these countries. Recently (in 2014) the disease has spread as far west as Poland. Therefore there is an increased risk of further transmission across Europe. In order to determine the properties of the recently circulating ASF virus strain, particularly relating to the effect on pregnant sows, and to obtain samples for diagnostic investigations, 3 pregnant sows (late, ca. 100 days, in gestation; about 2 weeks prior to expected farrowing) were inoculated, intramuscularly, with a high dose ($10^{4.3}$ TCID) of ASFV Georgia (2007). The sows were examined on a daily basis and samples were taken for analysis of circulating blood cells and virus. All 3 sows developed fever, lost their appetite, became disorientated and then moribund. Two of the animals died and one was euthanized (due to animal welfare reasons) at 4-5 days post inoculation. At necropsy, tissue samples were collected from the sows and from the fetuses (ca. 20 fetuses per sow). The blood counts indicated a severe fall in the level of circulating B- and T-cells within the infected sows on the 4^{th} day post infection. Analyses of virus distribution within these infected animals and the transmission of virus to the fetuses are in progress. Initial results show that ASFV DNA can be detected within lymphoid tissue of the fetuses from two sows, thus showing that transplacental transmission of ASFV to the fetuses has occurred.

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Detection of genetic heterogeneity in African swine fever virus populations in Russia.

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Since 2007 there were periodic outbreaks of African swine fever (ASF) reported in the territory of Russian Federation (RF). Within the next 5 years no changes were detected in biological or genetic virus traits of the African swine fever virus (ASFV) or in development of the disease. However, the results of recent studies suggest possible heterogenicity within circulating viral isolates.

Our goal was to analyze a collection of the viral isolates obtained on the territory of Russian Federation through the years 2009-2013 for detection of possible changes in the most variable sites of the viral genome.

We used the methods of genetic typing and AFLP of the viral genome as recommended by World Organisation for Animal Health. We also used nucleotide sequence analysis of the B602L gene site.

There were 50 viral isolates obtained from the following RF territories: Rostov, Volgograd, Tver, Smolensk, Voronezh, Tula, Tambov, Pskov, Saratov, Vladimir, Yaroslav, Krasnodar, Stavropol districts and from the Republic of Belarus.

When using standard analysis protocols, we did not find genetical alterations in genes p72, p54, p30 and CD2v. In contrast sequence analysis of the B602L gene site revealed a non synonimous substitution of adenine to thymine in the ASFV genome. The change was verified by running independent PCR tests and by repeating the sequence analysis. As the result of the analysis of SNP mutation among the ASF viral isolates the following was discovered: originally the change in the B602L gene was found in a specimen of 2010 isolated at Southern Federal District (Rostov Divisiont and Krasnodar Division). Later outbreaks of the disease (years of 2011-2013) revealed increase in number of mutant variations of the ASF virus. Interestingly, the mutant variation of the ASF virus dominated in the regions where the original virus was endemic (Tula and Tver territories).

The performed research revealed heterogenicity among the isolates of ASF virus that circulate on the territories of Russian Federation. The mutation in B602L gene could be used as SNP marker for express-test of viral ASF isolates thus serving as an additional method of molecular epidemiology.

Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar

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In view of the fact that African swine fever (ASF) was recently introduced into the wild boar population of the European Union and that classical swine fever (CSF) keeps reoccurring, targeted surveillance is of utmost importance for early detection. Introduction of both diseases is usually accompanied by an increased occurrence of animals found dead. Thus, fallen wild boar are the main target for passive surveillance. However, encouraging reporting by hunters and sampling of these animals is difficult. Partly, these problems could be solved by providing a pragmatic sampling approach. For this reason, we assessed the applicability of three different dry/semi-dry blood swabs, namely a cotton swab, a flocked swab, and a forensic livestock swab, for molecular swine fever diagnosis. After nucleic acid extraction using manual and automated systems, routine quantitative real-time polymerase chain reactions (qPCR) were carried out. Results obtained from swabs or their fragments were compared to results generated from EDTA blood.
It was shown that reliable detection of both diseases was possible by qPCR. Shifts in genome copy numbers were observed, but they did not change the qualitative results. In general, all swabs were suitable, but the forensic swab showed slight advantages, especially in terms of cutting and further storage. Taken together, swab samples could be recommended as a pragmatic approach to sample fallen wild boar.

Spatiotemporal exploration of African swine fever outbreaks in wild boar in Sardinia (2012-2014)

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African swine fever (ASF) is a notifiable viral disease which affects swine and wild boars. It even is endemic in Sardinia from 1978. Surveillance in wild boars has increased in the last 3 years. Here we present a spatiotemporal analysis of ASF cases in Sardinia during 2012-2014, in order to explore the pattern of ASF in wild boars and the comparison with domestic ASF occurrence. A better knowledge about the dynamic of ASF in wild boar is required to improve programs to control or prevent the spread of ASF in this region.

A total of 295 outbreaks notified in Sardinia during 2012-2014 were included in the analysis (198 in domestic pigs and 97 in wild boars). Exploration of ASF cases included the following analysis: 1) Seasonality correlation between wild and domestic cases 2) Association (Anova test) between topographic variables and wild boar and domestic pig cases 3) A spatial point pattern analysis using K-Ripley function 4) A spatiotemporal analysis using a permutation model of scan statistic.

Results show a significant seasonality correlation (Pearson test=0.89; p=0.000) of 6 months between wild and domestic cases. Peaks of domestic pig outbreaks occur in summer and peaks of wild boars outbreaks in winter (hunting season). High altitude shows a significant association with wild boar outbreaks. The K(d) function analysis indicated a maximum distance of significant spatial association between ASF cases of 15 km and 25 km for domestic pigs and wild boars respectively. Five significant ($p<0.005$) time-space clusters of ASF domestic outbreaks were detected in the Northern (4) and Central (1) area of Sardinia respectively, within a total of 45 cases. One significant ($p<0.1$) time-space cluster of ASF wild boar outbreaks was detected between Sassari and Nuoro provinces, which included 3 cases.

These results describe the pattern of notified ASF cases in Sardinia during 2012-2014, highlighting the identification of the maximum distance of related cases in wild boars (25km) and domestic pigs (15 km) which could help to define the zone used to control and surveillance of ASF in Sardinia.

Targeted research effort on African swine fever

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In response to the threat of African swine fever entry in EU, posed by its spreading in Eastern Europe, the ASFORCE project aims at providing veterinarians, pig farmers, hunters and policy makers with practical answers and prevention tools against the disease. Under coordination and management activities planned under Theme 1 (Coordination and management), the scientific and technological work plan of ASFORCE is divided in four Themes, each aiming at particular objectives to reinforce the overall goals of the project:

Prevention, control and eradication models for ASF (Theme 2)

Ongoing research aims at providing essential information to design more cost-effective surveillance and control strategies for ASF into different risk scenarios, providing valuable tools for veterinarians, pig producers and policy makers. The ultimate goal is to identify key points for a better prevention and control of ASF minimizing the economical loses on endemic or on potential new infected areas.

Pig-wild boar-argasidae interactions relevant for ASF epidemiology (Theme 3)

Research work is providing data essential to identify key points for designing new control strategies including wildlife considerations namely through understanding the epidemiology of ASF in wild boar in its natural habitat, their interactions with domestic pigs and the potential role of soft ticks in the disease epidemiology. Data collected and analyzes of information on the transmission dynamics will provide a basis for the development of more reliable control measures and biosecurity practices with the goal of mitigating the risk of ASF impact on swine production.

Development of protection tools against ASF (Theme 4)

The overall ongoing work under this theme aims at advancing research leading to vaccine development through two approaches: 1) the rational deletion of genes to produce attenuated and non-replicating candidate ASFV vaccine strains; 2) the identification of protective antigens and their incorporation into vectored virus vaccine, both further complemented by the assessment of pig-carrier state induced in experimentally “vaccinated animals” , to be applied in field conditions through the development of improved diagnostic tests for viral and antibody detection.

Training and knowledge transfer (Theme 5)

Work under this theme aims at improving preparedness for ASF at different levels and activities targeting veterinarians, pig farmers, hunters and policy makers, through dissemination of knowledge on ASF, including among others the recognition and understanding the clinical and pathological features of the disease and its epidemiology, relevant for the improvement of prevention and control strategies in EU countries at risk and in countries recently affected by ASF.

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Development of the Research on the African swine fever Vaccines

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African swine fever is a fatal hemorrhagic disease of domestic pigs caused by African swine fever. Since ASF was first described in Kenya in 1921, the disease has spread to many countries so far. In recent years the popular of ASF is particularly frequent in Caucasus where is near to our country. Although lots of research on African swine fever vaccines were reported, there is no vaccine available for ASFV. The role of cellular immunity and humoral immunity played is still uncertain.

Revisiting the use of Live-Atenuated Viruses as models to study the pathogenesis and the mechanisms involved in protection against African swine fever

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African Swine Fever (ASF) is a highly infectious viral disease that provokes dramatic losses in the affected countries. The causative agent of ASF, ASF virus (ASFV), is a large, double-stranded DNA virus that encodes more than 150 different proteins and against which there is no vaccine available. Despite their biosafety-derived problems, naturally and /or classically - attenuated viruses have demonstrated to confer solid protection against ASF. With this work we pretend to confirm live attenuated viruses as ideal models to unravel the immunological mechanisms involved in protection against ASF. To achieve this main objective, we planned the following specific objectives:

1. Establishing an attenuated-infection model in the laboratory, based on the E75CV1 *(Ruiz Gonzalvo et al; 1986)*
2. Characterizing its protective potential against lethal ASFV challenge
3. Comparing the early and late immune response induced by these two viruses using a multidisciplinary approach (following virological, immunological and proteomic/genomic approaches).

Animals immunized with the optimal dose of E75CV1 barely showed visible signs of ASF. Surprisingly enough, immunization with a 10-times higher dose or with a 100-times lower dose of E75CV1 became lethal for some animals. Similarly, specific pathogen free (SPF) pigs proved to be much more sensitive to infection with E75CV1 than conventional animals, showing the fine balance between protection and pathogenesis and confirming the risks related to the potential use of classically attenuated strains in the field.

E75CV1-immunized pigs were able to resist the infection with a lethal dose of the homologous E75L virulent virus but did not confer solid protection against the heterologous BA71 virulent strain..

Both the attenuated E75CV1 and the virulent E75L homologous viruses were capable to modulate the host-immune response from very early after infection, albeit they did it in almost opposite ways:

A) The virulent E75L virus was able to suppress the activation of immune system as early as at 1 day post-infection (1dpi), allowing the replication and spread of the virus. Coversely, a massive activation of pro-inflammatory mediators was detected at day 7pi, both by real-time PCR in lymph-nodes and by ELISA in serum, coinciding with the ocurrence of a severe leucopenia and with the death of the animals
B) In clear contrast, E75CV1 was efficiently recognized by the innate immune system as early as at 1dpi, priming for a late Th1-like response, detectable both by RT-PCR and ELISPOT and coinciding with the late development of adaptive immune responses (specific humoral and cellular responses)

Preliminary proteomic approaches corroborated these findings allowing the characterization of many host mediators as key players during the infection for each one of the viral strains used.

Cre-recombinase expressing WSL cell lines efficiently remove LoxP-flanked reporter genes from the genome of recombinant African swine fever virus

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Isolation of African swine fever virus (ASFV) recombinants generated by homologous recombination in permissive cells is a laborious and time consuming task. After transfection of the cells with transfer plasmids and infection with the target ASFV strain, recombinants have to be separated from a huge excess of parental virus. Isolation of the recombinants can be supported by insertion of reporter genes encoding e.g. green or red fluorescing proteins. However, presence of foreign genes is undesirable with regard to live vaccine development. Furthermore, overexpression of the reporter proteins may interfere with virus replication and distort functional characterization of the introduced gene deletions. Recently, it was demonstrated that a marker gene flanked by LoxP sequences can be removed from recombinant ASFV after transient transfection of infected cells with a plasmid encoding Cre recombinase (Abrams & Dixon 2012, Virology 433, 142–148). To further facilitate the removal of reporter genes, we attempted to generate stably Cre-expressing wild boar lung (WSL) cell lines, which were chosen because they enable propagation of both laboratory and field strains of ASFV. The open reading frame encoding modified Cre without a nuclear localization signal was inserted into plasmid pIRES1neo (Clontech), which permitted expression of the transgene under control of the human cytomegalovirus immediate early promoter together with neomycin phosphotransferase from a bicistronic mRNA containing an internal ribosomal entry site (IRES). Therefore, a considerable proportion of the stably neomycin (G418) resistant WSL cell clones obtained after plasmid transfection also possessed an intact Cre gene, as shown by PCR amplification and sequencing of chromosomal DNA. Infection of these cell lines with an ASFV recombinant containing a LoxP-site flanked, p72-promoter regulated DsRed gene at the nonessential I8L gene locus (see also presentation by Keil et al.), reproducibly resulted in rapid disappearance of red fluorescence from virus plaques coinciding with the expected gene deletion. In conclusion, WSL-Cre cells are a valuable tool for genetic engineering of ASFV.

Comparison of genotype and serogroup classification of African swine fever virus

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African swine fever (ASF) is a viral disease of domestic pigs caused by ASF virus (ASFV). The disease ranges from acute to chronic form and apparently asymptomatic animals that are carriers of the virus. There is no doubt that the extreme antigenic diversity of African swine fever virus is still one of the main obstacles for developing a safe and efficacious vaccine against ASFV.

Genotyping ASFV based on partial nucleotide sequencing of the B646L gene provides essential data to identify the origin of the virus, but unfortunately it is not capable of discriminating strains of different virulence and other biological properties.

Researchers at the VNIIVViM developed a classification of ASFV isolates based on a hemadsorption inhibition assay (HAI) with ASFV reference immune antisera. ASFV serogrouping is closely linked with the cross-protection. Here, we present the results of an analysis of ASFV isolates in terms of genotypes and serogroup clustering.

The main aim of our study was to combine the results of serological ASFV classification and well-known genotype distributions to reveal new relationships between distant ASFV variants in regards to disease epidemiology.

Our experiments were based on the results of HAI of ASFV strains maintained at the VNIIVViM. These include isolates from disease outbreaks in Africa, Europe, the Caribbean, and more recently from the Russian/Trans-Caucasian epizootic and attenuated variants. The ASFV isolates were clustered in one serogroup (SG) if the hemadsorption was inhibited by serum belonging to a certain group (SG I - SGVIII).

The ASFV isolates from the depository at Pokrov's Institute that were well-characterized into serogroups were additionally classified using a partial B646L nucleotide sequencing protocol.

The results of the current study support previous reports that areas with high genotype and serogroup ASFV diversity are located in countries where multiple mechanisms of ASF infection (mixed sylvatic and domestic cycle) are established. The data demonstrate the high level of heterogeneity of ASFV isolates within one genotype. We have shown that strains that are closely related genetically may have different phenotypes and therefore form homology serogroups.

Our results also indicate the degree of heterogeneity in terms of HAI assays between ASFV variants isolated from one parental wild type ASF field isolate with a conservative genotype. The diversity of ASFV viral populations have been shown previously, but the ability to identify ASF virus variants using a serological approach has not been demonstrated before.

Thus, serogroup classification could possibly help the understanding of the heterologous cross-protection of ASFV isolates among many genotypes.

Centrifugal enhancement of plasmid delivery enhances transgene expression and generation of African Swine fever virus recombinants.

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Nucleic acid delivery into cells which are difficult to transfect is frequently challenging, especially in cases where generation of recombinant viruses depends on a distinct permanent cell line. Wild boar lung cells (WSL), which proved to be suitable for propagation of both African swine fever virus field isolates and laboratory strains, are cells which are hard to transfect, irrespective whether chemical, physical or biological methods were used so far. Targeted generation of ASFV recombinants in WSL cells, however, is reliant on the transfection efficacy so a novel transfection method was tested for the abitily to increase target gene expression efficacy in these cells. The K2® Transfection System consisting of cationic lipids and a coreagent that decreases the cells' ability to detect nucleic acids, increased - in comparison to e.g. transfection using polyethylene immine, Superfect (QIAGEN) or Lipofectamin (life technologies®) - significantly the number of transgene expressing WSL cells. This augmentation was irrespective of whether the promoter driving expression was constitutively active or dependent on transactivation by ASFV superinfection. Surprisingly, K2® transfection efficacy could be further increased about tenfold by centrifugation of the transfected cultures at 600 x g for 1 h. Accordingly, generation of ASFV recombinants in WSL and WSL-Bu cells as recently described (Keil et al., Arch Virol, DOI 10.1007/s00705-014-2095-2), increased within the same magnitude. In addition, monitoring the effect of K2® mediated transfection followed by centrifugal enhancement revealed that the increase in transgene expression is not due to specifics of WSL cells but applies also, to differing extents, to other cell lines. Thus, the centrifugation enhanced plasmid delivery into cells is beneficial not only for transient expression of proteins but also for generation of ASFV in WSL cultures and probably other virus recombinants in respective cells.

Deletion of multiple genes from a virulent African swine fever virus strain results in attenuation and induction of a protective immune response

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Gene deletion mutants were constructed of the virulent, genotype I African swine fever virus isolate Benin 97/1. The effect of the deletions on virus replication in macrophages and pathogenesis and induction of a protective immune response in pigs was compared with the parental virus and a natural attenuated ASFV isolate OURT88/3. Deletion or interruption of 5 genes from multigene family (MGF) 360 and 4 of MGF 505 did not significantly reduce replication of the virus in macrophages compared to parental virus. Groups of pigs were inoculated with this deletion mutant, BeninΔMGF, or with the attenuated OURT88/3 strain and boosted with the same strain 3 weeks later. Of the 5 pigs immunized with BeninΔMGF , 3 developed a transient fever for one day at day 5 or 6 but no other clinical signs. After a further 3 weeks all pigs were challenged with the virulent Benin 97/1 isolate in parallel with 3 non-immune pigs. All of the pigs immunized with the BeninΔMGF virus were protected against challenge and did not show clinical signs. Of the 4 pigs immunized with OURT88/3 two were protected and the 4th was terminated at the humane endpoint of the experiment. A low level of transient viraemia was detected in blood from some of the pigs by qPCR. All of the control non-immune group developed clinical signs and high levels of virus genome in blood typical of acute ASf. Antibody and cellular responses were compared. No neutralizing antibodies were detected. Numbers of IFN-gamma producing T cells detected following stimulation of PBMCs with virus were lower in pigs immunized with BeninΔMGF compared to OURT88/3, indicating differences in the magnitude and possibly function of the T cell response. The results suggest this is a possible route to rational development of live attenuated ASFV candidate vaccines.

In vitro characterization of African swine fever virus promoters – implications for recombinant virus construction.

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African swine fever virus (ASFV) causes ASF, a highly contagious disease of domestic swine and wild boar and a substantial threat for worldwide pig husbandry. It still lacks a vaccine for prevention. ASFV is a double stranded DNA virus with a 170-190 kb genome predicted to code for 150 to 167 genes. Viral gene expression, DNA replication and morphogenesis take place in the cytoplasm. Transcription of viral genes is temporally regulated. DNA replication, beginning around 6h after infection, defines the transition from early to late regulated gene expression, though some genes are expressed in both phases of the infection. Little is known about the performance of ASFV promoters with regard to strength and thus efficacy of transcription. The major capsid protein p72 promoter has been - and still is - extensively used to direct e.g. reporter gene expression in recombinant viruses. Current approaches for development of vaccines against ASFV include generation of recombinants expressing duplicates of immunogenic authentic proteins or immunomodulatory proteins. To identify strong promoters, we inserted the 5' sequences of different viral genes upstream from the Firefly luciferase open reading frame (ORF). These genes included: p30/32 (CP204L) coding for a phosphoprotein abundantly expressed at early infection; alpha-like DNA polymerase (G1211R), expressed at both early and late phases of infection; p72 (B646L) for comparison and the CD2 homologue (EP402R) responsible for haemadsorption, both expressed at late infection. Since the essential promoter sequence motifs of ASFV genes, except for p72, are not known, we also varied the upstream length of the 5' sequences and included the start codons, since previous studies on the p72 promoter have shown that it may be necessary for full promoter activity. These were placed in frame with the start codon of the Firefly luciferase reporter ORF. In the case of the DNA polymerase, for which two ATG codons exist in frame in the beginning of its ORF, a frameshift was also inserted in between, to confirm which one is used for translation initiation. To determine promoter activities, wild boar cell line WSL was used for transfection with the different reporter constructs, followed by infection with ASFV. A plasmid expressing Renilla luciferase under control of MCMVie1 promoter was co-transfected as a reference, and luciferase expression levels were recorded 22h p.i.. The results showed that DNA polymerase promoter driven expression was the highest, followed by the p30 promoter. p72 and CD2 promoters revealed comparable activities. No clear difference was observed between DNA polymerase promoters with or without frameshift, confirming that the second ATG codon is used for translation initiation. These results may reflect the viral genes temporal expression, since DNA polymerase is expressed throughout infection, whereas p30, p72 or CD2 expression is limited to early or late phases of infection. Nonetheless, these results are useful for expression studies in the context of ASFV infection and for the design of expression cassettes for generation of mutant viruses expressing selectable markers or other sequences of interest.

ID Screen® African Swine Fever Indirect ELISA: improved performance thanks to new interpretation criteria

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INTRODUCTION

ASF control and eradication programs require accurate and reliable diagnostic tests. The ID Screen® African Swine Fever Indirect ELISA detects anti-AFSV antibodies in both domestic and wild pigs. Unique features of the ELISA include the coating of three recombinant ASFV antigens (P32, P62, and P72), and the ability to use the test with blood filter paper and meat juice as well as serum and plasma.
Thanks to a new protocol and lowered cut-off, IDvet has improved test performance. This study presents validation data obtained for this new cut-off (30-40%)

METHODS AND RESULTS

Specificity

- 763 disease-free sera from domestic pigs, wild boars, and Iberian pigs were tested. Measured specificity = 99.61% (CI $_{95\%}$: 98.96% - 99.90%).
- 100 samples from disease-free animals in France were tested.
Measured specificity = 100% (CI $_{95\%}$: 96.30% - 100%), n=100.
- 90 negative animals were tested by both the serum and filter paper protocols. All sera were found negative by both protocols.

Sensitivity

- 3 sera from vaccinated and challenged pigs were tested. After challenge, all three animals gave strong positive results with the ID Screen® ELISA.
- 8 reference sera from the ASF EURL were correctly identified as positive.
- 3 positive sera were titrated and tested by both the serum and filter paper protocols. The measured analytical sensitivity was similar regardless of the sample type tested.
- Test sensitivity for meat juice was evaluated through the analysis of spiked samples. All spiked samples were correctly identified as positive.

CONCLUSION

The ID Screen® African Swine Fever Indirect ELISA shows excellent test performance.
It is an efficient and reliable tool for the diagnosis of ASF in both domestic pigs and wild boars.
The serum application has been validated by the ASF European Reference Laboratory, and the test can also be used for filter paper and meat juice samples.