Final Report Summary - ASFORCE (Targeted research effort on African swine fever)

Executive Summary:
African swine fever (ASF) is a devastating disease affecting domestic pigs (DP) and wild boars (WB), caused by a complex virus of the Asfarviridae family, the African swine fever virus (ASFV), able to cause significant socioeconomic losses. The disease is enzootic in the great majority of Sub-Saharan countries and up to 2007 it was confined in EU, to the island of Sardinia, after its eradication in early 90’s in Portugal and Spain. In 2007 it was declared in Georgia where from it rapidly spread to other Eastern European countries (Armenia, Azerbaijan, the Russian Federation, Ukraine, and Belarus) and since 2014 to the EU countries Lithuania, Poland, Latvia and Estonia.

Due to the fact that no vaccine has been obtained so far, and to the complexity of the different epidemiological scenarios involving DP, WB and vectors (argasids, Ornithodoros spp), the prevention, control, and eradication of ASF is mainly based on its early detection and implementation of strict (and challenging) sanitary measures.

The ASFORCE project aimed at researching and transferring knowledge on prevention and control of ASF, by: i) providing help in the design of more cost-effective surveillance and control strategies in different risk scenarios; ii) identifying risk factors for disease spread, including wildlife considerations namely the role of WB and argasids; iii) contributing to the development of safer vaccines through different strategies, namely by rational deletion of genes to produce attenuated ASFV vaccine strains, the development of Defective Infectious Single Cycle (DISC) viruses and the identification of protective antigens to be incorporated into vectored virus vaccines, iv) developing diagnostic tests for early detection of ASFV infection; v) improving preparedness for ASF in pig farmers, hunters, veterinarians and governmental agencies in EU and ASF European affected countries.

The contribution of highly qualified expertise from EU, Russian Federation and Switzerland, allowed the achievement of successful outcomes on critical aspects of ASF and the production of useful tools to prevent and control ASF, namely through:

• Evaluation of driving forces in pig trade to identify risk factors for disease spread in EU and the identification of new genetic markers allowing the differentiation of viral isolates and increased traceability of virus dissemination. A cost-benefit analysis of existing surveillance and control measures and the identification of parameters driving ASF transmission, allowed the development of a disease spread model to estimate cost-effectiveness of risk-based surveillance strategies. Finally, guidelines for the cost-effective prevention and control of ASF were produced and published in downloadable paper format in the project’s webpage.

• Wildlife investigations providing essential data on the role of ticks and WB on ASF dynamics. The studies developed, could not find Ornithodoros spp ticks in Georgia, Bulgaria and Russian Federation suggesting these argasids are not relevant for the epidemiology of the disease in these specific areas. The evaluation of DP - WB interactions (via noninvasive methods) provided essential information on location of interface areas (via mapping activities), seasonality (via questionnaires), evidence of contacts (via bio-markers), patterns of movement and ecology of hosts (via telemetry radio tracking), showing that the WB habitat is the most significant factor for disease maintenance and spread. Altogether these investigations offer a relevant
contribution to assist veterinary services, wildlife management authorities, and decision makers when designing ASF surveillance strategies.

- Characterization of virus deletion mutants representing an important step in the production of rationally attenuated candidate vaccines. Further genome manipulations will be required to achieve safety and efficacy requirements for registration. Identification of T cell epitopes conserved in different ASFV isolates, open new insights to identify key protective antigens for inclusion in viral-vectored vaccines. Studies on the development of DISC viruses may provide a safer alternative to the use of live attenuated virus.
- Development of a novel lateral flow device (LFD) for detection of virus antigen and an ELISA test to detect IgM, provide improved methods for early diagnosis of infection.
- Training and knowledge transfer on relevant aspects of ASF was conducted through: i) the organization of workshops attended by around 400 veterinarians including CVOs from EU and non-EU countries; ii) the dissemination of a flyer on ASF in different occasions and in the ASFORCE website; and iii) production of an interactive course on ASF and videos on “ASF awareness” and “Spread of African swine fever in Eastern Europe and EU countries since 2007,” available on the website for wide dissemination.

Overall ASFORCE contributed to a better preparedness against ASF, therefore decreasing the present and future socioeconomic consequences of the disease in Europe.

Project Context and Objectives:
In response to the threat of African swine fever spread in the EU, posed by its presence in Eastern Europe, the ASFORCE project aimed to provide pig farmers, hunters, veterinarians and policy makers with practical answers and prevention tools to help control the disease.

Coordination and management activities were carried out under Theme 1 (Coordination and management), the scientific and technological work plan of ASFORCE was further divided in four Themes, each with particular objectives to achieve the overall goals of the project, as described hereafter:
Theme 2 - Prevention, control and eradication models for ASF
Research work developed under this theme aimed to provide essential information to design more cost-effective surveillance and control strategies for ASF. This would minimize the economic losses in the different risk scenarios, by providing valuable tools for policy makers, administrations, veterinarians and pig producers.

The specific objectives of the work developed aimed to:
- Collect information on pig production systems in EU and neighbouring countries, including the presence of risk factors that could facilitate ASF introduction and/or spread
- Develop models that reproduce observed pig trade networks to understand the mechanisms underlining the organisation of pig trade networks, and to highlight the key differences between European pig production systems.
- Analyse the genetic properties of ASFV isolates currently circulating and the geographical and molecular spatial-temporal patterns, to trace the dynamics of ASF infection.
- Review and perform cost-benefit analysis of the surveillance and control strategies for ASF in Sardinia, Corsica and the Russian Federation. To evaluate existing surveillance strategies and contingency plans in several countries namely in Sardinia (Italy), Georgia and the Russian Federation as endemic areas, Corsica (France) and Bulgaria as free regions, and Poland and Lithuania as regions at risk (ASF cases were detected in dead wild boars at the time studies were performed).

Theme 3 - Pig - wild boar-argasidae interactions relevant for ASF epidemiology
Research aimed to provide essential data to identify key points for designing new control strategies including potential spread involving wildlife. This involved study of the epidemiology of ASF in wild boar in its natural habitat, their interactions with domestic pig and the potential role of soft ticks in the disease epidemiology. This information is important to improve the knowledge on ASF spread in different epidemiological situations and a basis for the development of more reliable measures to control ASF.
The specific objectives of the work carried out aimed to:

- Develop cartographic and experimental models to predict the distribution of ticks in Eurasia, to investigate their presence in different geographic areas in EU and Russian Federation, and assess the vector competence of European Ornithodoros spp.
- Adapt non-invasive methods to assess the pig-wild boar interactions in affected areas from Eastern Europe and Sardinia.
- Analyze information on ASF transmission dynamics in wild boars in Eastern Europe and Sardinia and develop models to predict wild boar distribution and ASF spread in wild boar and from wild boar to domestic pigs.

Theme 4 - Development of protection tools against ASF

Vaccines are not available for ASF and this severely limits strategies for disease control. The overall objective of Theme 4 was to advance work leading to vaccine development, through different strategies. In addition diagnostic tests were developed to detect earlier infection in pigs.

The specific objectives of the work developed aimed to:

- Construct virus gene deletion mutants and test these by immunization and challenge experiments in pigs. Assess the persistence and transmission of attenuated strains to contact pigs.

NHV/P68 and OURT88/3, isolates are naturally attenuated genotype I virus. These isolates can induce good levels of protection against virulent virus challenge but can result in adverse reactions in pigs, specifically swelling of joints and necrotic skin lesions. One strategy was to delete additional genes from these already attenuated isolates to try and reduce adverse post-immunization reactions, but maintaining good levels of protection against challenge. A second strategy was to delete genes from virulent isolates that inhibit innate immune responses to produce novel attenuated viruses.

- A modification of the above described approaches aimed to develop replication defective infectious single cycle (DISC) ASFV vaccine candidates. These viruses enter cells and express most of the encoded proteins, thus inducing immune responses against many targets. However, infectious progeny virions are not produced and therefore the risk of the vaccine spreading during field use is reduced. To develop ASFV DISC viruses, helper cell lines expressing the essential target genes were constructed and work was progressed to use these to isolate ASFV deletion mutants lacking those genes.

- Further studies aimed to identify antigens which can induce protection against ASFV infection in pigs, in particular regarding those important in inducing protective CD8+ cytolytic T cell responses in the context of MHC Class I antigens. These cells were previously identified as playing a major role in protection against infection. For this purpose, a selection of ASFV encoded antigens was tested by an in silico screen to predict those with conserved T cell epitopes which are expressed early in infection. These were expressed in virus vectors, (host-restricted parapox virus) and virus-like particles derived from parvovirus (Parvo-VLPs) which display either B or T cell epitopes, for preliminary testing in pigs.

- Diagnostic tests for early detection of ASFV infection were developed. These included a lateral flow device for rapid penside virus detection and an ELISA test to detect early antibody responses in ASF infected pigs.

Theme 5 - Training and knowledge transfer

The work plan of this theme aimed to improve preparedness for ASF at different levels and involved activities targeting pig farmers, hunters, veterinarians, and governmental agencies. Thorough knowledge on ASFV, including understanding the pathology and clinical presentation of the disease and understanding its epidemiology is crucial for stakeholders of EU countries at risk and of countries recently affected by ASF. They all need to be aware of their responsibilities and their role in preventing and combating the disease as well as their options for effective prevention and control.

The specific objectives of the work developed aimed:

- To increase the preparedness for ASF through investigation of the attitude of pig farmers, butchers/middlemen and hunters towards disease reporting. To prepare and distribute material for disease awareness campaigns directed at farmers and hunters in EU countries at risk and non-EU countries recently affected.

- To increase preparedness for ASF among veterinarians, in particular in regions at risk in the EU and in recently affected countries in Caucasus and in Russia. The objectives were to organize workshops and provide training for veterinarians to increase risk awareness and capacity of incident response.
• To provide representatives of different governmental agencies in the EU and in countries at risk, through their participation in different workshops, a platform to discuss and update their prevention strategy and to adjust contingency plans.

• Further disseminate learning materials on different aspects of ASF (flyers, a training course and videos in electronic formats).

Project Results:
I - Theme 2 - Prevention, control and eradication models for ASF

Research was developed under eight work-packages subdivided in several Tasks aiming at the attainment of the Theme objectives as described hereafter:

WP 2 - Characterization of the pig industry in the EU Member States and ASF affected areas in Europe

1) Summary Objectives of the work package
The aim of WP2 was the description, evaluation and mapping of existing pig production within several EU states and neighbouring countries, especially in the South-Eastern Europe.

2) Summary of objectives and results of Tasks
Task 2.1. Collection and evaluation of data on pig farming in densely populated areas
Task 2.2. Collection and evaluation of data on the pig populations in areas with traditional backyard farming, especially in South-Eastern Europe
Task 2.3. Assessment of management systems, productivity, price differentials as driving force for trade and evaluation of ASF-potential risk factors for each production types

Tasks 2.1 and 2.3 aimed at describing pig production in Europe and neighbouring countries. In order to achieve it, Germany, Spain, Georgia, Romania, Bulgaria and Russian Federation (RF), were selected as representative examples of European pig producers. Task 2.1 was focused on densely populated areas while Task 2.2 was focused on traditional backyard farming. Lately, Task 2.3 aimed to evaluate the driving forces in pig trade by conducting a systematic evaluation of the management systems, productivity, price differentials and other related factors. The main objective was the better understanding of the factors that influence patterns and changes in pig trade under diverse environments.

Based on data collection, pig farming was characterized with regard to district proximity, proximity to water bodies or forest, distance to other pig holdings, information on pig owner, on holding and on its management. Furthermore, information on breeding management, wild boar (WB) contact, trade issues and participation in exhibitions and auctions was also collected.

Regarding driving forces in pig trade, outcomes indicated that driving forces significantly differed, depending on the pig production system. In intensive pig production systems, animal movements oscillated during studied years but without clear seasonal patterns while a clear seasonal pattern was identified in extensive and small-scale pig production systems.

WP 3 - Characterization of contact patterns between pig populations through social network analysis

1) Summary Objectives of the work package
The objective of WP3 was to evaluate the spatial and temporal patterns of pig movements, and the structure of pig trade networks, in densely populated areas and areas with traditional backyard farming.

2) Summary of objectives and results of Tasks
Task 3.1 Commodity chain analysis to evaluate the spatial and temporal patterns of pig movements within and between pig farming in densely populated areas and areas with traditional backyard farming

Task 3.2 Understand the pattern of pig movements in areas with traditional backyard farming related to trade with the view to set hypothesis on potential factors that explain the observed choices of trading points

The first objective of Tasks 3.1 and 3.2 was to analyse the commodity chain in order to evaluate spatial-temporal patterns of pig shipments between pig farming in densely populated areas (France, Italy, Germany and Spain) and areas where traditional and or/backyard pig production is predominant (Bulgaria, Georgia, Romania, RF, Corsica and Sardinia islands), with the view to set hypothesis on potential factors that explain the observed choices of trading points. The final goal was to generate random networks using exponential random graph models, constructed to resemble those previously obtained.
High detail of information from France, Spain, Italy and Bulgaria allowed to intensively explore pig trade patterns at high level of spatial and temporal resolution, and to characterize in detail trade patterns among different production systems. Three important findings were obtained regarding trade networks most premises had few connections while few premises had many connections. Secondly, pig trade communities were identified which tended to cover large areas and to overlap when they include industrial farms. Finally, important trading points were identified and characterized in areas where industrial or backyard/extensive productions are predominant. Exponential random graph models were used to reproduce, understand and predict pig trade networks in different European pig production systems (small-scale in Bulgaria, extensive in Extremadura [Spain] and intensive in Côtes d’Armor [France]).

**WP 4 - Update of the epidemiological disease status in Europe, and the genetic properties of ASFV**

1) **Summary Objectives of the work package**

The aim of WP4 was to update the epidemiological situation of ASF in Europe, the genetic properties of ASFV and the geographical and molecular spatial-temporal patterns.

2) **Summary of objectives and results of Tasks**

**Task 4.1 Description of the epidemiological disease status in EU (Sardinia), Armenia and Russian Federation based on sampling collection and identification of the geographical spatial-temporal patterns**

In order to increase the knowledge about the epidemiological disease status, Task 4.1 was focused on epidemiological findings obtained from official sources, as well as molecular epidemiological on ASFV isolates currently circulating in Europe, RF and in Trans-Caucasian countries (TCC).

Results for Eastern European countries showed that ASF is spreading from RF towards west affecting Ukraine and Belarus. Regarding temporal patterns, the highest incidence of cases in RF occurred from June to August with a second peak in autumn. The lowest incidence was observed from December to May. A high level of correlation between WB and domestic pig (DP) cases was found, which could be interpreted as interactions between WB and DP in this scenario; however, WB-to-DP transmission played only a secondary role in ASF spread. WB has been involved in ASF spread both at local and trans-boundary levels, being 133 km the maximum distance of significant spatial association for the relation between cases in WB.

In addition, WBs are responsible of only 28 % of outbreaks whereas DP are the source of 72 % of the outbreaks. Spatial patterns allowed to identify hotspot areas located in Southern West (2009-2010) and in Southern Central (2008-2009). Finally, developed studies revealed acute clinical forms associated to virulent ASFV isolates, which induce 94 % - 100 % mortality. Despite this fact, some animals survive for over a month and are able to recover from the infection or even remain sub-clinically infected.

In Sardinia, field studies revealed acute forms of the disease in DP whereas WB showed low mortality (important difference compared with the European scenario), furthermore a 5 % of the WB population presents antibodies against the virus. Regarding temporal patterns, outbreaks have presented periods of hyper- and hypo-endemicity, that are likely associated with sociocultural and economic fluctuation on the island. Seasonality (2013-2014) was mainly identified in May and early summer in DP and from October to February in WB. Taking into consideration spatial patterns, endemic areas have been historically located in the Central-East part of Sardinia where almost 75 % of the total outbreaks were concentrated. Unfortunately, outbreaks have conquered naïve areas in the South-Western (2004) and Northern regions (2012) of the island. In addition, hot spot areas where the virus is persistent especially in DP, have been identified. The maximum spatial association between notifications (15 km in DP and 25 km in WB) was within the current surveillance radii supporting the adequacy of the applied measures. The secondary role of WB in ASF transmission could be explained as result of certain socio-economic factors, mainly illegal free-range pig breeding or the mingling of herds.

**Task 4.2 Evaluation of ASF viral genotypes circulating in Europe (Sardinia) and Russian Federation using standardized procedures.**

**Task 4.3 Characterization of additional ASFV genome markers as supplemental tool for molecular and biological characterization**

Tasks 4.2 and 4.3 aimed to determine virus sequences of ASFV isolates from Europe by using international standardized
procedures and by the analysis of the additional ASFV genome marker regions

After carrying out molecular studies, all Eastern European ASFV isolates clustered within p72 genotype II pointing out a single introduction in 2007 from East Africa. The intergenic region (IGR) between I73R and I329L genes (IGR73R-I329L) revealed the presence of two genetic variants [GII-IGR-1 and GII-IGR-2] co-circulating in RF since 2012. GII-IGR-2 was characterized by a TRS insertion that was absent in previous Eastern European ASFV isolates. The results showed that the isolate responsible of the outbreak occurred three months later in Ukraine in DP had this TRS insertion. Further subtyping analysis of Russian ASFV isolates has allowed determining that GII-IGR-2 variant prevail among current outbreaks in both WB and DP, as well as it is the one circulating in the EU. Furthermore, CVR amplification allowed to find a new genetic variant (named Gil-CVR-2) that is recently circulating within WB population from Estonia. Sardinian ASFV isolates were placed in p72 genotype I indicating a single introduction in 1978. TRS studies within CVR region revealed the presence of 12 repeats identical to those included into the previously defined CVR sub-group X which is represented by isolates collected from 1990 up to 2010. Deeper subtyping analyses of IGR73R-I329L region showed 100% of homology among all ASFV isolates. These results combined with the ones obtained by the classical genotyped indicated a low-rate evolution in Sardinian isolates having a field presence of 25 years (1990–2015).

Task 4.4 Generation of full-length sequences of ASFV isolates circulating in the Russian Federation and Trans-Caucasian countries

Finally, Task 4.4 aimed to generate full-length sequences of ASFV isolates from RF and TCC. Comparing four nearly full-length genomes of recent Russian ASFV isolates, only minor changes were observed over the whole genome. All sequences had the highest sequence identity of 99.9 % to strain ASFV Georgia2007/1. Then, phylogenetic analyses with the four nearly full-length genomes revealed two clusters whereby ASFV Georgia 2007/1 and ASFV from Tulskaya (Tula dp 06/2012) formed one cluster. The other three strains investigated formed other cluster.

WP 5 - Evaluation and cost benefit analysis of existing surveillance, contingency plans and control strategies

1) Summary Objectives of the work package

The aim of WPS is to review and evaluate the historical and current surveillance and control programs in EU member states and in Eastern European countries.

2) Summary of objectives and results of Tasks

Task 5.1. Review and cost-benefit analysis of the surveillance and control strategies for ASF in Sardinia, Corsica

Task 5.2. Review and cost-benefit analysis of the surveillance and control strategies for ASF in the Russian Federation

Tasks 5.1 and 5.2 reviewed and cost-benefit analysed the surveillance and control strategies for ASF in Sardinia and RF. In Sardinia, the comparison of the surveillance and control programs before and after the modification of the area considered at risk (in November 2011), and the modification of the applied control measures gave a detailed information about the situation in Sardinia. This analysis clearly reflects that European, Italian and Sardinian authorities have invested many efforts and resources for fighting against ASF, especially during the last years. Time is still too close to fully evaluate the actions of these measures. However, the results were not satisfactory revealing a spread of the disease in the territory. Some measures implied great costs without revealing important success, whereas other less important measures easily effects on the disease spread.

On the other hand, ASF legislation in RF is stricter in relation with the slaughtering of animals in case of outbreaks and the extension area for movement restrictions, although the effective implementation of these measures is not always adequate. Similarly, repopulation is permitted one year or exceptionally 6 months after one outbreak is notified; this excessive time could lead to breaking laws with worse consequences. The need of well-equipped veterinary services, formed by reliable and trained personnel was detected, as well as the necessity of giving compensations to farmers as incentive to enhance their reports. Some advantages, such as different approaches for different regions (affected, unaffected, at-risk zones), large control and protective zones were also identified. Cost benefit analysis was performed at regional level, concretely in Krasnodar region. Furthermore, very detailed information on costs was obtained from another four affected regions: Belgograd, Tver, Bryanskaya and Volgograd.
Task 5.3. Evaluation of existing surveillance strategies and contingency plans in Eastern European countries

The goal of the Task 5.3 was to evaluate the existing surveillance strategies and contingency plans in Eastern European countries: Bulgaria, Georgia, Poland and Lithuania. In Bulgaria, uniform and standardized disease control requirements are difficult to be followed since Bulgarian pig sector is heterogeneous. From a legal point of view all pig holdings (professional or non-professional) are equal and have to fulfil same requirements related to ASF control and eradication. However, non-professional producers are often not fully under the control of authorities. A great identified strength is the close cooperation with hunters which allows investigating WB carcasses for visible lesions of ASF. In Georgia, the State Veterinary Service does not perform any active or passive surveillance; furthermore staff cuts have reduced the number of veterinarians from 2,000 to less than one tenth. Disease prevention and control is challenging due to the low levels of ASF awareness, low biosecurity, poor compliance of animal regulations and lack of animal identification and traceability systems. There are no funds to investigate suspected outbreaks neither any compensation. Finally, Poland and Lithuania followed a similar early detection program, mainly focused on sampling DP located in high risk areas and WB distributed among the whole country. Differences were found in terms of program coverage and techniques employed. Lithuanian program covered a higher proportion of the population, and was based on the parallel detection of virus and antibodies. However, in Poland, PCR was the most important technique used, especially for WB.

WP 6 - Dynamic disease modelling to identify key spread mechanisms and quantify potential local spread

1) Summary Objectives of the work package

The aim of the WP6 was to develop a dynamic spread disease model of ASF in the network of pig trade (from WP3). Other goal was to better know relevant parameters related to ASF epidemiology, including transmission rates and ASFV survival.

Summary of objectives and results of Tasks

Task 6.1 Development of disease spread model

Task 6.2 Evaluation of control measures

The works undertaken as part of Task 6.1 aimed at increasing understanding of transmission process of ASF in order to inform simulation models that can be used to calibrate intervention measures. A second goal of this task was to investigate the major routes of transmission at local levels in outbreaks from 2007 to 2014 located in Krasnodar and Tver regions. Finally, Task 6.1 aimed to estimate ASFV survival in contaminated materials stored under different conditions. The objectives of the task 6.2 are threefold: to summarise the epidemiological outputs for different transmission simulation scenarios of ASF in order to be able to conduct an economic analysis (to inform the task 7.1); to estimate and compare the effectiveness of different mitigation strategies for ASF for each type of index case; to provide recommendations as to how to modify the current mitigation strategy to maximise its effectiveness.

Results show that within a pen the within-farm basic reproduction number (R0) is 3.9 (95 % confidence interval [CI]: 1.9-6.4) and between pens, it is 1.9 (95 % CI: 0.9-3.2) for the Georgia 2007/1 ASFV strain (by using a stochastic susceptible-exposed-infectious-recovered [SEIR] model). Regarding viral survival, results shown that ASFV survived in faeces from 8.48 days at 4 °C to 3.71 days at 37 °C and in urine from 15.33 days at 4 °C to 2.88 days at 37 °C. Moreover, statistical analyses of the spatial and spatio-temporal distributions of ASF cases in the RF from 2007 to 2014 were conducted. Results suggest that spatial proximity to an infectious farm was a strong risk factor for infection of a susceptible farm. Then, the joint spatial distribution of cases in WB and DS in the Tver region may be explained by regular spill-overs from the domestic pig to the wild boar population. Furthermore, occurrence of ASF outbreaks in the South was found to be positively associated with human population density. Finally, through disease spread model, a risk-based surveillance strategy targeting farms that are highly connected to highly connected farms could be considered as the most effective mitigation strategy.

WP 7 - Economic evaluation of different alternative disease spread and control scenarios

1) Summary Objectives of the work package

Work package 8 aimed to develop a decision support tool for the EU member states to identify appropriate and cost-effective ASF prevention and control strategies under different scenarios at risk.
2) Summary of objectives and results of Tasks

Task 7.1 Economic model for the evaluation of different disease spread and control scenarios

The main objective of Task 7.1 was to develop an economic module to be combined with the different economic outputs obtained as part of WP6. By combining this work and all the knowledge that was generated as part of ASFORCE project, task 7.1 also aimed at defining a decision support tool that will allow identifying appropriate control strategies (including both surveillance and intervention strategies) optimised for specific ASF risk scenarios. In WP 6, simulations of ASF spread were run for Spain under different control strategies (including a baseline strategy) and different virus introduction scenarios. Under the baseline strategy, for all virus introduction scenarios considered in this study, the direct consequential costs associated with movement restrictions in surveillance and protection zones were shown to account for around 80% of the direct economic impact of the disease. It was also shown that implementing the alternative control strategies would be beneficial. However, this benefit is not expected to be substantial so that the benefit-cost ratio was smaller than in a large majority of the simulations, whatever the alternative mitigation strategy. In addition, decision support tool showed a list of 21 surveillance and 22 intervention strategies for ASF control. Finally, surveillance and intervention strategies presenting the best compromise between effectiveness and practicality were identified for each epidemiological context.

WP 8 - Guidelines for a cost-effective control and prevention including communication

1) Summary Objectives of the work package

The goal of WP8 was to produce a set of guidelines for cost-effective prevention and control of ASF by incorporating the results obtained in the previous work packages (from 2 to 7).

2) Summary of objectives and results of Tasks

Task 8.1 Guidelines for cost-effective prevention and control of ASF, including communication

The main goal of Task 8.1 was to establish a guidelines to prevent and control ASF, considering two risk scenarios (free area and area at risk) in order to be cost-effective. Cost-effective guidelines collected a brief introduction about ASF, as well as a description of the current situation in Europe. Furthermore, main relevant results obtained from Theme 2, which was focused on epidemiology, were compiled in these guidelines. Outcomes as pig production in Europe, pig trade forces in several European countries, molecular characterization of current European isolates, new genome markers and preventing and control strategies identified as most effective and practical for two risk scenarios (ASF-free scenario and ASF-infected scenario) were summarized in these guidelines. In addition, other interesting ASFORCE information as videos or courses were also included. From all these results, a concise and easy to read guidelines are available in English at ASFORCE website.

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

Research was developed under three work-packages subdivided in several Tasks aiming at the attainment of the Theme objectives as described hereafter:

WP 9 - Ornithodoros ticks’ distribution, competence and interactions with wild boars and domestic pigs

1) Summary Objectives of the work package

The objective of this WP was to identify the role of ticks in the currently ASF affected areas in Europe, covering:

• The development of a predictive map of the tick’s distribution in some EU and neighboring countries (Russian Federation and Trans-Caucasian Countries) based in the suitable habitats to predict the presence of Ornithodoros ticks that may transmit and maintain ASFV in the EU and neighboring countries “at risk”.
• The development of a ticks manual capture protocol and adaptation of anti-ticks serology ELISA assay to assess the interaction between domestic pigs, wild boar and ticks in order to elucidate the tick presence in Eastern Europe and Sardinia.
• The development of experimental models to assess the vector competence of European Ornithodoros spp.

2) Summary of objectives and results of Tasks
Task 9.1 Ornithodoros tick distribution in the EU (Sardinia, Bulgaria) and neighboring countries (Russian Federation and Trans-Caucasian Countries i.e. Ukraine and Armenia)

The predictive map on Ornithodoros ticks distribution (1 km resolution) has been developed in the palearctic region based on historical data, climatic and ecological conditions. Tick presence (argasids) has been investigated (Georgia, Russia, Sardinia and Bulgaria) in locations selected based on the two predictive tools previously developed (tick’s map and anti-ticks serology ELISA test). Collection of Ornithodoros ticks was done according to a developed sampling protocol. A practical training course on tick collection was performed in Georgia in June 2013, organized by CIRAD and FAO. Missions were performed in Georgia (Two mission: Gardabani district and Lagodekhi region (June 2013) and Kvermo Kartli and Kakheti region (May 2014)); Bulgaria (Four missions (May, June and October 2014 and February 2015) in 36 animal holdings, 21 pig farms and 15 sheep and cattle holdings); The Russian Federation (Three mission: Krasnodar/Daghestan mission (September 2013), Astrakhan region (June 2014) and Volgograd region and Stavropol region (July 2015)); and Sardinia (Two missions). In summary, according to the obtained results, it is possible to conclude the absence of Ornithodoros soft ticks in Bulgaria and Sardinia. Regarding Russian Federation, due to the high extent of the country it is not possible to clearly conclude either the absence or presence of Ornithodoros ticks. In Georgia, due to the insufficient sampling efforts, we cannot conclude the absence of Ornithodoros ticks and we highly suspect that the ticks are present in south-eastern regions.

Task 9.2. Development, assessment and use of a serological test efficient for the detection of tick-wild boar and tick-pig interactions

The available ELISA test protocol against O. erraticus antibodies have been improved and adapted for its use with wild boar samples. Unfortunately, this ELISA test could not be adapted to other Ornithodoros tick species, due to the lack of progress in tick collection missions. It has been employed to assess potential interactions between ticks and hosts (domestic and wild boar) in each ASF scenario and to identify places where ticks could be present. In summary, the ELISA test has shown to be a good screening tool. According to previous published results no cross reaction with other known ectoparasities occurs. For WB, no indication of interaction with ticks has been recorded. For DP, Russian Federation is the country where there is a probability of existing contact between Ornithodoros and domestic pigs, but it is very low in Germany, Sardinia and Bulgaria.

Task 9.3. Quantitative characterization of vector competence of the European O. erraticus and the Caucasian O. asperus and assessment of environmental stresses and gut microbiota as factors impacting on vector competence

A methodology has been developed to test the tick competence methodology including the method for rearing ticks, the protocol for detection of virus in ticks, the PCR using UPL vp72, adapted to all states of tick’ life. Studies have been conducted with O. erraticus collected in Portugal in autumn 2013 due to the failure to obtain O. asperus ticks.

Vector competence: The different trials conducted indicated that O. erraticus is able to be infected by ASFV Georgia2007/1 and to amplify the virus. Results suggested a dose effect with a positive correlation between the infective dose and the infection success. However O. erraticus were not able to transmit the virus 5 month later to healthy pigs through biting.

Effect of intrinsic factors on vector competence: Only O. erraticus were able to be infected among the different tick strains assayed (O. erraticus from field, O. moubata maintained in laboratory for more than 50 years, and O. porcinus imported three years ago from Madagascar), with clear virus amplification following the infectious bloodmeal. Apart from possible tick species effect, it is likely supposed that the status of the tick colony plays a major role on the success of tick infection by selecting refractory specimens through laboratory rearing. Regarding the tick saliva and ASFV horizontal transmission, experiments showed that O. porcinus tick saliva significantly modulates the local pig immune response through the decrease of Langherans cell density in the epidermis and the increase of macrophage density in the derma. Such reactions create a suitable environment for local ASFV amplification and may play a role in the success of pig infection through tick bite. Similar patterns should be expected with O. erraticus.

Effect of extrinsic factors on vector competence: experiments on External temperature and Tick microbiome were still running by the end of the second period of the project and final results are expected to be published in the near future.

WP 10 - Ecology of contacts between domestic and wild pigs in different epidemiological settings

1) Summary Objectives of the work package
The objective of this WP was to assess and quantify the contacts between wild and domestic swine that could potentially favor the transmission of ASFV by comparing different noninvasive methods in affected areas from Eastern Europe and Sardinia, through:

- Development and use of questionnaires among farmers and hunters in Spain, Corsica, Sardinia, Bulgaria, Germany, Russia and Georgia.
- Adaptation and use of GPS radiotracking in domestic and wild pigs in Corsica, Sardinia, Spain and Russia.
- Test and assess the use of biomarkers (fecal E. coli and hepatitis) in the different regions of the Russian Federation, Bulgaria and some Mediterranean countries (France, Spain and Italy).
- Development of domestic pigs low biosecurity holdings and wild boar population density maps based on their modeled, spatial distributions and, interaction maps between both populations in Europe.

Methodologies were compared to assess their strength and weakness.

2) Summary of objectives and results of Tasks

**Task 10.1 Assessment of interaction through the use of questionnaires**

Questionnaires have been developed, translated in the different languages and implemented in Western Spain (Extremadura), France (Corsica), Eastern Bulgaria, Russian Federation and Germany. They appear to be an efficient tool, easy and cheap, which can provide abundant and unique information. In consequence, they represent a good selection to be applied at large scale (country level). Interactions between domestic pigs and wild boars were reported in all the countries/regions investigated. Although they cannot confirm direct contacts, the existence of these interface areas represent scenarios where indirect contacts may occur such as disposal of carcasses, hunting infected wild boar... establishing possibilities of infection cross-over from one population to another. However, despite efforts of harmonization in the methodologies of data collection and questionnaire implementation, it has proven challenging to implement the same methods in all settings. Moreover, questionnaires validity appears to be strongly associated to the nature of the participants (conflicts with confidentiality and sensitivity) for which they should be selected with extremely care.

**Task 10.2 Use of GPS telemetry to assess and monitor interactions between wild and domestic pigs**

Field studies were performed in Corsica, Sardinia, Spain and RF. This methodology has shown to be the most power tool able to confirm, quantify and characterize the direct contact between both hosts. They have demonstrated to be very useful to gather field and real-time information on contact patterns. However, it is the most-time consuming tool with the highest economical and personnel cost for which its use should be limited to well predefined objectives. In this case, field data have been obtained to feed the spreading model of ASF transmission: the wild boar home range (4.4-9 km), population structure, frequency and type of resources used (the use of extensive farm resources to livestock, supplementary feeding, is frequent and widespread by WB). Some inconveniences have been identified: the price of the technology, organization of collaring needs important time and human involvement and also animal’s capture (not easy to organize).

**Task 10.3 Assessment of contacts through the use of E. coli as biological markers**

Biomarkers have shown promising results. Under experimental conditions E. coli has shown potential as biomarker whereas HEV molecular studies on full-genome basis did not reveal markers that would allow tracing of transmission direction. Conversely, field studies performed in Spain with HEV clearly shows the interaction in the same hunting area of wild boar and Iberian pig, indicating that transmission of the virus among domestic animals and wildlife is bidirectional. Therefore, HEV has been proposed as a useful marker of contact between wildlife and Iberian pigs although the direction of transmission cannot be defined.

**Task 10.4 Mapping activities to assess the interactions between wild boar and domestic pigs**

Three maps were developed under this task: A low biosecurity pig density (PD) map in Eurasia based on demographic and environmental data; A wild boar (WB) population density map in Europe based on demographic and environmental data (5 km resolution) that was highly accurate both against the original input data as well as the independent data (Melis et al. 2006) and; A wild boar/backyard pig interface map in Europe. The predicted WB densities, BP densities as well as WB/BP interface maps represent useful tools for decision makers, dealing with WB and BP epidemiology, surveillance, prevention and control
although they should be carefully employed to assess the real-life epidemiological situations. It needs to be understood that backyard pig numbers vary strongly on the local scale and also temporarily (seasonally).

Task 10.5 Comparative evaluation of the different methods used to assess wild and domestic pigs interactions

The evaluation of the DP-WB interactions (noninvasive methods) provided essential information on location of interface areas (mapping activities), seasonality (questionnaires) and evidence of contacts (biomarkers), patterns of movement and ecology of hosts (radio tracking) offering a highly contribution to assist surveillance system. Their strength and weakness were compared

WP 11 - Modelling of ASF transmission in wildlife and between wild boars and domestic pigs

1) Summary Objectives of the work package

The objective of this WP was to identify the role of the wild boar in the ASF transmission (2007-2013; mainly Sardinia and the Russian Federation) through the identification of key parameters and development of models to fit the wild boar distribution, specifically:

- To estimate the rate of transmission of ASF among wild boar populations in the affected areas (the Russian Federation and Sardinia)
- To develop a cartographic tool about wild boar distribution based on available suitable habitat.
- To identify the main risk factors for ASF transmission in wild boar in the Russian Federation.
- To model the potential ASF transmission from wild boars to domestic pigs in Eastern Europe based on previous improvements.

2) Summary of objectives and results of Tasks

Task 11.1 Identification of reproductive ratio for the local spread of ASF in the areas with wild boar population affected from 2007 to 2011

The transmission rate (R0) of the wild boar ASF cases has been investigated. The R0 in the Russian Federation (2007-2013) was 1.58 (95% CI: 1.13-3.77) demonstrating an effective transmission between WB populations. However, it could not be identified in Sardinia (2012-2014) due to no significant time-space cluster were detected.

Task 11.2. Mapping wild boar free ranging distribution based on habitat suitability and hunting data (when available)

Different approaches have been conducted to finally develop a Eurasian map of the wild boar distribution based on habitat suitability (QAH) and Expert opinion. The map showed a high agreement with wild boar presence data (>12,000) and ASF cases (80% of them fall in areas with the highest category value).

Potential uses of the density (Task 10.4) and QAH (Task 11.2) WB maps were identified:

- QAH map can be useful to focus surveillance of infectious diseases on domestic pigs shared with WB: to identify areas where the highest population of should be expected, to identify potential WB corridors between countries borders and regions, etc. Additionally, the distribution and types of agricultural areas should be taken into account to evaluate risk of the disease spreading in share areas between WB and DP.
- WB predicted densities map (Task 10.4) was highly accurate both against the original input data as well as the data by Melis et al. (2006). The map represents a useful tool for decision makers, dealing with wildlife, the epidemiology of WB diseases and related disease interventions (surveillance, prevention and control).

Task 11.3 Identification of main risk factors for ASF transmission in wild boar in the affected areas from 2007 to 2011

Main risk factors identified to explain the occurrence of ASF transmission in wild boars in the Russian Federation. The risk of ASF introduction into the EU by wild boar movements was assessed based on main risk factors (wild boar presence) and ASF cases and the available habitat map for WB. The highest risk values were found in Romania, Slovakia, Czech Republic and Finland stressing the importance of two drivers, the progress of the disease through the EU and the occurrence of new entrances from endemic areas outside the EU. This methodology can be used successfully for evaluating the risk of ASF introduction allowing carrying out cross-country comparisons with limited input data and supporting sustainable decision-making to health authorities in at-risk countries.
Task 11.4. Spatial modeling of the potential ASF transmission from wild boars to domestic pigs

A spatial risk model (large scale – the Russian Federation) and a dynamics risk model (small scale – Tver region) have been developed to identify risk areas and simulate ASF spreading from WB to DP. These models could be used as a tool for mapping at-risk areas in time and space, at a regional scale.

WP 12 - Biological characterization of the Caucasian ASFV isolates in wild boar

1) Summary Objectives of the work package

The objective of this WP was the further characterization of the Caucasian ASFV isolates in European wild boar, especially upon low dose infection in order to supplement and complete existing data on the behavior of the Caucasian ASFV isolates in wild boar that could help to understand the epidemiology and transmission modes in affected areas.

2) Summary of objectives and results of Tasks

Task 12.1 Animal trials will be conducted to assess the risk of chronic forms and carriers after high, moderate, and low dose infections with Caucasian ASFV strains

Animal trials have conducted with low dose oro-nasal infection of European wild boar and domestic pigs. It was observed that only a few weak animals got directly infected. However, after virus amplification by these animals, all other trial mates got infected. While course and outcome of infection (acute lethal disease on individual level) were comparable among all animals, the clinical presentation was less pronounced in the initial cases with the direct low dose infection. On group level, the disease was present up to 36 days confirming a rather moderate contagiousness. This prolonged course on the “herd-level” together with an exceptionally low dose that proved to be sufficient to infect a running wild boar, could be most important for disease dynamics in wild boar populations and under backyard settings as well as for the initial introduction into ASFV free regions. Finally, no indications were observed for prolonged or chronic individual courses upon low dose infection in either species. The additionally tested Sardinian isolate showed high virulence in European wild boar with 100 % mortality within a week.

III - Theme 4 - Development of protection tools against ASF

Research was developed under three work-packages subdivided in several Tasks aiming at the attainment of the Theme objectives as described hereafter:

WP 13 - Development of deletion mutants as attenuated and DISC vaccine strains

1) Summary Objectives of the work package

NHV/P68 and OURT88/3 are naturally attenuated genotype I isolates from a pig or tick respectively. These isolates can induce good levels of protection against virulent virus challenge but can result in adverse reactions in pigs, specifically swelling of joints and necrotic skin lesions. One strategy for vaccine development was to delete additional genes from these already attenuated isolates to try and reduce adverse reactions post-immunization but maintain good levels of protection against challenge. The sequence conservation between the OURT88/3 strain and virulent genotype I strain Benin 97/1 mean that the same plasmid transfer vectors can be used to delete genes from the Benin 97/1 genome as from the OURT88/3 strain. For this reason we deviated from the original plan and deleted genes from the Benin 97/1 isolate rather than from strains circulating in Caucasus/Russia Defective Infectious Single Cycle (DISC) strains it is first necessary to construct helper cell lines expressing genes that are essential for virus replication. These cell lines can then be used to delete the essential gene from the virus genome to produce a virus that replicates productively in the helper cell line but undergoes a single cycle of replication in other cells. The predicted essential genes of interest were selected based on studies from WP 14. Different strategies were followed to produce helper cell lines in this WP.

2) Summary of objectives and results of Tasks

Task 13.1 Further attenuation of existing attenuated strains (NHV/P68, OURT88/3) through deletion of genes involved in virus/host interactions, replication and virulence

1. Deletions of A276R and A528R from NHV/P68 virus (CSIC). Primary porcine macrophages are the main target cell for ASFV replication in vivo but these cells would not be suitable for commercial production of vaccines and therefore COS cells were
also tested as an alternative cell line for producing deletion mutants, since this cell line is already licensed for vaccine production. The viruses obtained from COS cells were purified by Percoll gradients. The genes deleted from NHV/P68 included A276R and A528R. The deletion of A276R was used in immunisation and challenge experiments in pigs (WP 15.1).

2. Attempts to delete D250R gene from NHV/P68 genome (CSIC). Another gene selected for deletion from NHV/P68 was the D250R gene, a Nudix hydrolase with mRNA de-capping activity. Attempts to generate this deletion mutant failed suggesting that this gene may be essential for virus replication. Instead this gene may be a target for construction of DISC viruses (see task 13.3).

3. Attempts to generate recombinant ASFV over expressing A238L protein (CSIC). It was planned to generate a NHV/P68 recombinant virus with an “extra” copy of A238L gene (NHV-A238LPLUS) since it might suppress the inflammatory response in immunised pigs. It was predicted this may reduce adverse reactions caused by immunization with NHP68. Attempts to introduce this extra copy of A238L into the virus thymidine kinase (TK) gene locus failed using porcine macrophages. On the contrary, virus NHVDA238L mutant, showed to protect pigs over 65% after challenge with virulent Armenia strain, showing no clinical signs.

4. Additional gene deletions from the OURT88/3 strain (PIR). Three deletion mutants were constructed from the attenuated OURT88/3 strain, these lacked genes MGF 360-9L, DP148R or I329L genes. Attempts to obtain deletions of the K205R gene were not successful indicating that this gene may have an essential role in the virus replication. All of these genes have been shown to inhibit type I IFN responses (WP 14.1). These were constructed and purified using primary porcine macrophage cultures. The deletion mutants OURT88/3ΔDP148R and OURT88/3ΔI329L were tested in vivo in immunization and challenge experiments.

Task 13.2. Attenuation of strains circulating in Caucasus/Russia through gene deletions

The sequence conservation between the OURT88/3 strain and virulent genotype I strain Benin 97/1 mean that the same plasmid transfer vectors can be used. Three deletion mutants were constructed in the Benin 97/1 isolate: BeninΔMGF (lacked genes from the multigene families MGF360 and MGF 505), BeninΔDP148R gene and BeninΔDP71L and ΔDP96R genes. Deletion of the MGF 360 and 505 genes resulted in increased production of IFN-β mRNA in infected macrophages indicating that these genes encode proteins that suppress type I IFN. The DP148R gene was studied in WP 14.1 and shown to inhibit type I IFN induction and IFN- stimulated pathways. The DP71L gene prevents shut-off of host protein synthesis and DP96R has also been described as a virulence factor. These were tested in pigs by immunization and challenge (WP15.1). The BeninΔMGF and BeninΔΔDP148R mutants were shown to be attenuated and to induce good levels of protection in pigs. The BeninΔDP71LΔP96R deletion mutant was not attenuated in pigs (WP 15.1).

Task 13.3. Development of potential DISC ASFV vaccine strains

1. Development of helper cell lines to obtain gene essential delta ASFV as DISC vaccines Eight cell lines expressing the ASFV ORF’s A104R, I215L, E301R, QP509L, Q706L, C962R, F1055L and P1192R were generated both in Vero E6 cells and COS-1 wild type cells. Results of immunofluorescence allowed the confirmation of cell lines for A104R and I215L. Initial attempts developed by FMV-ULisboa aiming at the generation of cell lines (either COS- or VERO-based) stably expressing GFP-P1192R failed. Therefore, a new strategy was adopted based on the pIRESnneo, in which the gene of interest and the G418-resistance gene are expressed in the same mRNA, which contains an IRES from ECMV and allows for translation of two open reading frames from a single mRNA. Vero and COS-1 cells were transfected with each of these constructs. In Vero cells we obtained lines for GFP (as a control), GFP-E301R and GFP-F1055L, while in COS-1 we obtained lines for GFP, GFP-E301R, GFP-C962R and GFP-P1192R. We also obtained for P1192R a stable cell line in Vero and COS-1.

2. Development of helper cell lines expressing D250R protein (CISC). We attempted to generate a third helper cell line using COS Flip in cells. We used this system since these cells possess a FLP recombination site integrated at their genome. However, we discontinue it due to the fact that prior results from FMV-ULisboa demonstrated that COS Flip in cells lines cannot be infected by NHV strains. Thus, we aimed to generate the deletion virus using transient transfection of the D250R gene as the extra copy required for an effective virus production. We were able to obtain small positive clones, however, were lost after successive rounds of infection.

3. Generation of ASFV DISC mutants. To generate ASFV DISC mutants, constructs containing the left and right homology arms
of each gene intended to delete, fused with a selection marker were inserted in a vector plasmid and ready to transfect infected cells. So far, we obtained the plasmids with the constructs for deletion of ASFV ORF’s A104R, I215L, E301R, QP509L, Q706L, C962R and P1192R. Since the left and right homology arms (500-600 bp) flanking each of the genes under study are conserved among sequenced ASFV isolates (with the exception of A104R) these constructs may be used for the deletion of these genes in any viral isolate. These constructs were transfected into Vero cells previously infected with Ba71V. For all the genes, it was found that the titre of recombinant virus was much lower when compared with the wild type. Overall, our studies strongly suggest that the development of DISC viral particles is feasible and they may consist on a relevant approach towards the design of an effective ASFV vaccine.

WP 14 - ASFV-cell interactions aimed at the identification of new gene candidates for construction of attenuated strains

1) Summary Objectives of the work package

The objective of this task is to identify and determine the mechanism of action of selected ASFV inhibitors of the interferon response. Our initial experiments revealed seven ASFV genes modulating Interferon responses using luciferase reporter assays. From these, we have selected a total of 4 genes for determination of their targets in the IFN system signal transduction pathway. During this period the main goal of this task was to further characterize the molecular mechanisms used by these genes to manipulate the IFN signaling pathway. Moreover, the ASFV entry pathways and the characterization of porcine macrophage cells lines in order to produce vaccines are important goals in this WP.

2) Summary of objectives and results of Tasks

Task 14.1. Study of ASFV genes modulating the IFN and other immune responses

The ASFV A276R gene from MGF360 inhibited the induction of IFN-β the cytosolic pathways. The DP148R gene from pathogenic ASFV virus strains inhibits the IFN response by targeting both MAVS and IRF3, whereas the gene from non-pathogenic virus only inhibits MAVS. Finally, the I329L gene inhibits both TLR3 and TLR4 activation. The genes, A276R, DP148R and I329L were deleted from virus genomes (WP 13.1 13.2) and tested in vivo in immunization and challenge experiments (Task15.1 and Task15.2). Our results confirmed that IFNbeta was clearly reduced in the presence of either A276R or A528R ASFV genes. Cells infected with the parental NHV isolate showed an increased IFNbeta expression as compared with mock or virulent L60-infected macrophages, and those cultures infected with either NHV-ΔA276R or NHVΔA528R deletion mutants displayed an even higher IFNbeta mRNA expression, again indicating that these ASFV genes are involved in the inhibition of IFN expression. Similar results were obtained with the Benin ΔMGF deletion mutant in comparison with Benin 97/1 strain. ELISA analyses also showed that the NHV-ΔA276R induced reduced levels of IFNgamma, and similar levels of IL-6, IL-10 and TNF-alpha, as compared with NHV infection, but the profile of cytokine production changed dramatically depending on the type of (Diomune)-TLR ligand added to the virus infection.

Task 14.2. Study of ASFV genes involved in replication, transcription and cellular regulation to identify targets for attenuation and construction of DISC viruses

Functional studies on A104R activity revealed its binding to both ss and ds DNA, in a large range of temperatures, pH and salt concentrations. No differences in binding activity of A104R were detected in the presence/absence of ATP. To demonstrate that pP1192R is a functional type II topoisomerase, ORF P1192R was expressing in JCW26 cells. Total extracts of JCW26 cells expressing pP1192R, a pP1192R catalytic mutant, the pP1192R-Top2pCterm fusion or the wild-type Top2p, and observed that pP1192R was able to decatenate kDNA as efficiently as wild-type Top2p. Also, mutation of the predicted catalytic residue fully abrogated the activity of pP1192R. Recombinant pP1192R was purified and determined as possessing decatenation and relaxation activities. Using the decatenation assay, some type II topoisomerase poisons and inhibitors were tested for their effect on pP1192R. Preliminary results indicate that pP1192R is likely to be present in the viral particle as decatenation activity is observed and increases with an increase of the amount of particles used in the assay. Through qPCR analysis we observed that mRNAs from ORFs E301R, C962R and F1055L are detected around 6 hours post-infection and accumulate at late times and are present both in the nucleus and in the cytoplasm of transfected cells, and upon infection of these cells the fusion proteins are seen co-localizing with viral factories at 8 hours post-infection, while GFP-C962R is observed only in the cytoplasm of transfected cells and its localization does not change upon infection of these cells. We found that pC962R is
detected from 8 hours post-infection onwards and accumulates over time. Further studies focused in the gene D250R (g5R), an early-late gene that could probably control either the temporal ASF viral gene expression and the cellular shut off (or both). Interaction of the viral protein with cellular and viral mRNAs was analyzed, and we observed that the protein binds cellular and viral poly (A) RNAs during the infection. Thus, the deletion of this gene would induce an accumulation of early ASFV mRNA, unbalancing transcription and stopping the late mRNA expression. This will probably produce ASFV-pseudo infecting cells expressing early viral proteins, without further progression of the infection, which will hopefully be enough to protect.

Task 14.3. Characterization of cellular mechanisms and molecules involved in ASFV entry, internalization traffic and replication

Focusing on the process of viral uptake of several ASFV strains (NHV, E70 and Georgia), we found that ASFV exploits macropinocytosis as the main endocytic mechanisms for viral entry being the function of Na+/H+ channels, Pak1, EGFR and PI3K important for the process. The results showed that ASFV-NHV induces Akt phosphorylation at 8hpi and this activation is mediated by an early process during viral entry and/or internalization.

The generation of a porcine macrophage cell line able to efficiently support ASFV infection is necessary to develop models for viral entry mechanisms in target cells and vaccine studies which are linked to the development of this project. Four different porcine cell lines (IPAM WT, IPAM-CD163, CD2+ and WSL) were tested to support ASFV production. The results indicate that neither of them are fully matured macrophages Regarding ASFV susceptibility, the percentage of p72 positive cells was lower than PAM p72 positive cells in all cell lines analyzed. Importantly, a slightly increase of p72 expression in IPAM-CD163 was obtained compared to the non-transfected IPAM WT, suggesting an important role for this receptor during ASFV infection. Finally, we found that neither of the cells lines are as productive for ASFV as PAM, although WSL and IPAM cells were able to produce new infective viral particles.

Task 14.4. Evaluation of virus deletion mutants through in vitro studies on host responses and virus replication in cells infected with deletion mutants. Cytokine virulence profile, MHC

Diomune characterized K84, CP1 and CP2 adjuvants in in vitro assays in cell culture to confirm their predicted effects on cytokine production, showing that- K84 promotes Th17 response with a low inflammatory response, CP1 promotes a major Th2 response compared to a lower Th1 response and did not induce an inflammatory response. CP2 promoted a Th2 response and did not induce an inflammatory response. These adjuvants could be used in future in vivo since they do not show toxicity in vitro and bind to TLR receptors resulting in differential effects described above. Samples obtained from WSL cells mock or infected by L60, NHV, Ba71V, HindeAtt or UgaAtt isolates were tested for mRNA of IFNalpha and beta, IL1beta, IL6L, IL12p40, IL15, TNFalpha and TGFbeta. Similar levels of expression were observed in L60 and NHV/P68 infections, except for IFNbeta, that was clearly overexpressed in NHV/P68 infections. This increase was also observed in WSL cells infected with other attenuated ASFV strains), supporting the correlation of IFNbeta mRNA induction as an indicator associated with virus attenuation. Studies developed in Sw Mac confirmed the results obtained in WSL.

WP 15 - In vivo testing of selected mutant ASFV and assessment of the carrier state. Development of penside/front line tests

1) Summary Objectives of the work package

Data from in vitro assays will be used to select deletion mutants to test in vivo in vaccination and challenge experiments in pigs. Clinical scores, virus replication and development of antibody and cell-mediated immune response will be measured. Assessment of carrier status will be approached. An IgM ELISA will be developed and a LFA for antigen detection based on the use of MAb against VP72 protein of ASFV will be developed.

2) Summary of objectives and results of Tasks

Task 15.1 Phase I: In vivo testing of selected deletion mutants in vaccination/challenge models in pigs.

One group of 6 pigs was inoculated with BeninΔMGF and a group of 5 pigs with BeninΔDP148R and challenged with virulent Benin97/1 104 TCID50 IM in parallel with a group of 3 non-immune control pigs. After challenge all pigs inoculated with BeninΔDP148R survived without showing clinical signs. In the group inoculated with BeninΔMGF, 2 pigs did not survive challenge although the onset of clinical signs was delayed compared to the control pigs. The results show that deletion of a single gene that inhibits IFN induction attenuates virulent virus. A second experiment was carried out to test
OURT88/3ΔDP148R and OURT88/3ΔI329L. After challenge four of the 6 pigs inoculated with OURT88/3ΔI329L were terminated at day 3 or 4 post-challenge with signs typical of acute ASF. Three of the 6 pigs inoculated with OURT88/3ΔDP148R were terminated on day 4 or 5 post-challenge with signs typical of chronic ASF. The results showed that deletion of genes that inhibit IFN responses from attenuated OURT88/3 strain significantly reduced levels of protection.

**Task 15.2 Phase II: In vivo testing of selected deletion mutants in vaccination/challenge models in pigs**

A second phase of testing was completed for deletion mutant BeninΔMGF. After challenge with Benin ΔMGF, 3 of 6 inoculated with 102 did not survive challenge, 2 of those inoculated with 103 IM and IN did not survive and 1 of those immunized with 104 IM did not survive. The results showed a good safety profile for the BeninΔMGF across the range of doses and routes tested. In the previous experiment 100% protection was achieved with the BeninΔMGF strain. In other exp developed by CSIC-CISA,, total of 3 pigs survived the ASFV infection with the high virulent virus comprising: 2 out of 5 (40%) immunized with NHV/P68-DΔA238L-COS percoll purified and 1 out of 3 (33.3%) in the group receiving the parental virus (NHV/P68 -COS) Percoll purified. However, while the two immunized animals with the deletion mutant died between 8 to 14 dpc, a delay on the onset of the disease was observed in the two pigs NHV/P68 -parental-COS immunized pigs dying between 15 to 22 dpc. By the end of the experiment, at day 65 p.i. survivor pigs were totally recovered, not showing clinical signs of ASF, although viremia was intermittently detected across the experiment in the immunized pigs. The five animals immunized with the NHV/P68ΔA276R-PAM virus died between 7 (2 pigs) to 9-10 (3 pigs) dpc. In clear contrast with that observed in the group immunized with the deletion mutant, the two immunized animals with the parental virus survived to the infection without exhibiting significant clinical signs related to acute disease.

**Task 15.3 In vivo testing of selected deletion mutants in oral vaccination/challenge models in wild boar**

The respective study comprised five European wild boars with an age of six to seven months. Upon oral vaccination, clinical, virological, and serological investigations were carried out according to standard procedures, and a full necropsy was performed at the end of the trial. Upon immunization, all animals developed ASF-specific antibodies by day 21 post vaccination. Low amounts of viral DNA were still detected in three out of five animals at the end of the trial (28 dpi). No adverse effects were observed. The respective deliverable (15.3) was submitted.

**Task 15.4 Assessment of the carrier state induced in experimentally infected pigs**

Experimental studies have shown transmission of the low virulent NHV/P68 virus to pigs in direct contact was possible after 3 months of the primary infection. After 3 months, the donor pigs inoculated with NHV/P68 viruses had virus in 2 out 15 tissues. Another study of virus transmission from recovered animals was carried out using ASF control pigs inoculated with NHV/P68 virus. These two pigs had been inoculated with NHV/Mac virus and then challenged with Armenia virus. At the time the contact pigs were exposed to the two inoculated and recovered pigs (65 dpi), the carriers didn´t show clinical signs, showed weak viremia (NHV) and a high antibody response. The transmission of this NH/ P68 attenuated strain to the contact pigs after 3, 5 months of the primary infection, and its persistence in tissues confirms the potential role of infected-surviving pigs in the maintenance and dissemination of the disease.

**Task 15.5 New test for viral and antibodies detection**

The IgM seroconversion occurred as early as day 10 pi. The first antibodies were produced against the VP72 protein, despite been produced later during infection of cells and the majority of the pigs that showed an increase in the IgM response, were protected and survived the challenge with ASFV. A LFA for antigen detection based on the use of MAbs against VP72 protein of ASFV has been developed. However, when compared with PCR, the sensitivity of the penside-test was considerably lower.

**WP 16. Identification of immunogenic ASFV antigens and testing in vaccination challenge in pigs**

1) **Summary Objectives of the work package**

Research efforts by different authors have shown that mechanism of protection against ASFV mainly involve cell mediated immunity, as suggested by the fact that ASFV specific CD8 + CTL activity is activated in pigs surviving infection with low virulent isolates. A subset of ASFV encoded antigens was analyzed by an in silico screen to identify those with conserved T cell
epitopes which are expressed early. Within the consortium several licensed virus vectors are available and these have been shown to be effective in delivery of antigens to pigs. The vectors of particular interest are a host-restricted parapox virus vector, and virus-like particles derived from parovirus (Parvo-VLPs) which display either B or T cell epitopes.

2) Summary of objectives and results of Tasks

Task 16.1 Selection of ASFV antigens for screening

The genome of ASFV strain Georgia 2007 (GenBank accession number FR682468.) which was selected for the in-silico analysis contains 188 open reading frames. All 188 putative proteins were screened for presence of 9mer peptides which might bind to 45 different pig haplotypes. The datasets obtained were inspected for presence of peptides which are predicted to be strong binders for at least the majority of MHC Class I swine haplotypes. From the proteins remaining after exclusion the following were included into the prioritized list (corresponding to deliverable 16.1) of ASFV proteins to be tested in pigs (the proteins underlined are examined and resulted in vectored vaccine candidates): MGF_360-1L, MGF_360-4L, MGF_505-1R, C475L, C962R, D1133L, I329L, B646L, I8L, B602L,G1340L, D345L, S273R, P1192R, H339R, H233R, R298L, E423R, E301R, E248R, EP296R, EP402R, E111R, E66L, I267L, DP238L, MGF360-16R, MGF505-11L, MGF100-1L, L11L.

Task 16.2 Testing antigens for induction of protective immune responses in pigs using BacMams for an initial high throughput screen

ORFs for p72, MGF505-1R, C475L, C962R and B602 have been integrated into BacMam viruses. ORFs for MGF360-4L-1R, I329L and I8L have been integrated into BacMam transfer vectors. To verify protein expression via transcription in the nucleus, ORFs coding for amino- and carboxyterminal Strep-tagged proteins were also constructed. Monospecific rabbit sera against bacterial expressed fusion proteins specific for p72, pB602L, pC962R, pC475L, and pMGF_505-1R were raised in rabbits. Sera against MGF_360-4L, I329L and I8L were tested after the first booster immunization. Surprisingly, only anti-p72 and anti-p602 reacted with their target proteins after transfection or BacMam transduction. Also negative was the examination of more than 40 transgenic RK13 and WSL cell lines in whose genomes presence of the target protein ORFs were detected by PCR. These results indicated that the enhancer promoter elements from the human and murine cytomegalovirus were not capable to direct detectable expression of the abovementioned ASFV proteins. It was therefore decided to test the transcription regulating element contained in expression vector pCAGGS. Using this expression vector for pC962R, pC475L and pMGF_505-1R we succeeded to detect synthesis of the respective proteins. However, the latter results were only obtained during the last few months of the project and were thus much too late for in vivo screening. For this reason the related work was replaced by performance of a proof-of-concept animal trial using heterologous ASFV epitopes (the in-silico predicted antigens, see above) incorporated into recombinant parovirus VLPs and recombinant Parapox (Orf) viruses). Diomune complemented the studies through provision of a suitable adjuvant (DIO1). The studies, included different vaccination schemes with PPV-VLPs alone, Parapox alone, and a prime-boost combination of approaches.

Task 16.3 Cloning of genes for selected antigens identified in Task 16.2 in a parapox vector and testing in immunization and challenge experiments in pigs

From the identified genes, the following genes were selected as candidates to be expressed in the Parapox Orf virus (ORFV) vector system: I329L, MGF_360-4L, MGF_505-1R, C475L, C962-R and I8L. Due to the difficulties described for task 16.2 it was decided to prioritize constructs containing His-tag sequences for the proof-of-concept of the recombinant ASF parapox viruses. Subsequently, transfer vectors containing I329L, MGF_360-4L, MGF_505-1R, C475L, C962-R and I8L genes with the His-tag sequence were generated. Recombinant ORFV were generated by homologous recombination and recombinant viruses were rescued and cloned by dilution in susceptible cells. For the rORFV-ASF vaccination, small-scale virus productions were performed. Recombinant ASF parapox viruses production methods were set-up in cell cultures and the five antigen candidates were produced from Vero cells cultures infected at low MOI. Parapox recombinants were sent to FLI for the vaccination and challenge trial.

Task 16.4 Cloning of selected antigens identified in Task 16.2 in parovirus VLPs to display either B cell or T cell epitopes

The main goal has been the production, purification and characterization of the PPV-VP2-ASFV VLPs generated. Several
constructs of the VP2 gene containing ASFV epitopes (with flanking sequences or 6 His tail) were prepared (Table 1). The genes were cloned in the baculovirus expression vector pBacPack8-PPV VP2. Then, recombinant viruses were obtained by co-transfection. All mutants were expressed at high levels except PPV VP2-I329L. This result correlated with the high predicted secondary structure of the epitope, as it was described in P. Rueda et al. 2004. Moreover, soluble fractions of infected cells were analyzed for their capability to band in CsCl and in sucrose gradients. In the case of VLPs carrying epitopes 2, 2-His, 3 and 4, an opalescent band was readily visualized in the gradient, indicating the presence of VLPs that band to the same density of wild-type empty virions (1.3gr/cm3). In parallel, VLPs were also purified by sucrose gradient, with similar results to the CsCl gradient. The final and more definitive confirmation of the presence of particles was obtained by electron microscopy (EM). The VLPs have been produced at high scale in order to perform vaccination experiments in pigs. To test the antigens expressed by parvovirus VLPs and parapox vectors, respectively, a proof-of-concept trial was conducted. In brief, the study comprised a total of 19 cross-bred domestic weaner pigs between six to eight weeks of age. Upon vaccination, no local or systemic adverse effects were observed. However, after challenge infection, all animals developed an acute-lethal course of ASF and showed pathomorphological signs indicative for an ASFV infection. Laboratory investigations revealed high and comparable viral loads in EDTA-blood samples from days 3 and 7 post challenge and in tissue samples. In antibody ELISAs, values rose upon infection but did not reach the positive range of the test system. Thus, no protection was conferred by the constructs used in this study and no differences were observed among groups with regard to viral loads or clinical signs.

Task 16.5 Construction of a BacMam virus, pseudotyped with immunodominant ASFV proteins in the envelope and capsid and which expresses up to three antigens identified in Task 16.2 after gene transfer into host cells

Due to the fact that immune-dominant proteins could not be identified within Task 16.2 pseudotyped BacMam viruses could not be developed.

IV- Theme 5 - Training and knowledge transfer

The work plan was developed under three work-packages subdivided in several Tasks aiming at the attainment of the Theme objectives as described hereafter:

WP 17 - Disease awareness in pig producers and hunters
1) Summary Objectives of the work package

The aim of this work package was to improve preparedness for ASF among pig producers in the EU, in particular in areas at risk, and in non-EU countries recently affected.

2) Summary of objectives and results of Tasks

Task 17.1 Investigate the attitude of pig farmers, butchers/middlemen and hunters towards disease reporting in EU countries at risk and non-EU countries recently affected

An online survey was developed by RVC to investigate the attitude and beliefs of pig farmers and hunters in Germany, Bulgaria and the Russian Federation towards reporting suspected cases of ASF. The results showed that farmers who would not immediately report suspected cases are more likely to believe that their reputation would be adversely affected if they were to report it, that they can control the outbreak without the involvement of veterinary services and that laboratory confirmation would take too long.

Task 17.2 Material for disease awareness campaigns will be developed and distributed in countries at risk in the EU and outside

A leaflet was prepared, in the format of “Frequently Asked Questions” and the following sub-topics were included: the etiological agent, geographical distribution, clinical signs and disease progress, virus transmission: dynamics and cycle, what to do when facing a potential case, why and how. The leaflet was translated into additional languages (English, Portuguese, Spanish, French, Italian, Russian, German, Bulgarian and Chinese) and is available for download electronically (http://asforce.org/african-swine-fever-and-asforce-project). It has been downloaded more 500 times. This number is very likely to be an under-estimate as it is being distributed by secondary organizations that get it off the internet. It was distributed in all the countries represented in the consortium as well as Belarus, Estonia, Hungary, Latvia, Lithuania, Moldova, Poland, Romania,
Slovakia, Ukraine, USA and Vietnam. Furthermore, a banner was designed and produced with key information on the ASFORCE project, and it was present in the consortium meetings, as well as all the training workshops, and different scientific events, across Europe, that happened in the past three years.

WP 18 - Training and disease awareness of veterinarians

1) Summary Objectives of the work package

The aim of this work package was to increase preparedness for ASF among veterinarians, in particular in regions at risk in the EU and in recently affected countries in Caucasus and in Russia. The objectives were to organize workshops and provide training for veterinarians to increase risk awareness and capacity of incident response.

2) Summary of objectives and results of Tasks

Task 18.1 At least 3 training workshops on ASF will be organized for pig veterinarians in EU countries and in non-EU countries recently affected by ASF

The initially planned three workshops were organized as follows:

- Workshop in Madrid, Spain, held on April 15-16, 2013.
  24 participants attended the workshop: 7 participants from Portugal, 4 participants from France, 8 participants from Spain and 5 participants from Italy.

- Workshop in Pokrov, Russia, held on June 17-19, 2013.
  58 Russian veterinarians and 2 participants from Belarus attended the workshop.

- Workshop in Sofia, Bulgaria held on September 9-11, 2013. 44 participants attended the workshop: 38 participants from Bulgaria, 3 from Romania and 3 from Macedonia.

In total, >100 veterinarians were trained during these workshops. Each workshop consisted of a similar programme with modifications to address local needs.

By the end of the workshop, invited veterinarians were expected to:

a) Be able to recognize a possible ASF case;
b) Understand transmission routes and pathogenesis of ASF;
c) Be familiar with ASF spread risk factors;
d) Be aware of the global epidemiological current situation, with particular focus on the European one, and on the recent outbreaks in Eastern Europe;
e) Have a general understanding of how the laboratory diagnosis is performed and to be able to interpret laboratory results;
f) Have a clear idea of the risk of failing to control an ASF outbreak and its economic impact.

Speakers included ASFORCE partners as well as local experts. All PowerPoint slides/presentations were translated into the local relevant language, and are available per request. Translation was available during the workshops when needed. Feedback from participants indicated successful dissemination to veterinarians in the targeted regions including practitioners as well as official veterinarians from different administrative levels. Participants received a certificate of completion of the training.

Organization of these three workshops is available at the ASFORCE webpage at: http://asforce.org/blog/88

Two extra workshops were also organized:

“Eradication of African Swine Fever in Sardinia: Is it a feasible goal?”, was held in Cagliari, Sardinia in Italy on October 23th, 2013. The workshop aimed at gathering veterinarians from the Local Health Units of Sardinia, from the regional technical agencies and in general all the veterinarians involved with African swine fever issues in Sardinia, to review and discuss fundamental and practical aspects of the disease namely those related to its prevention, control and eradication. 150 participants attended this workshop (http://asforce.org/blog/94).

A fifth workshop was organized in Pokrov, Russian Federation on May 21, and taking advantage of the presence of several
members of the consortium at this date in Russia. It followed the same structure as the previous workshops and was attended by more than 100 participants, from Russia (vast majority) and Belarus.

Task 18.2 Distance learning material on ASF will be prepared and made available through the project website UCM developed a set of online training modules related to ASF which is available from the web site (http://asforce.org/course/). Three modules are available: 1) Suspicion; 2) Confirmation, control and eradication; 3) Prevention & Biosecurity. The course was disseminated in the countries represented in the consortium as well as South Africa (AfriVIPPortal) and the network of the European College of Porcine Health Management (ECPHM). To date, > 2500 users have accessed the training material online. A webinar for high-school students was given on December 10, 14:00 (CET) as a spin-out from this WP. After a unanimous vote from the consortium in favor of the making of a new/up-to date ASF awareness video, and official support from the European Commission, the consortium got engaged in this “extra” activity. The video was officially presented both to the consortium on during the last full consortium meeting and the ASFORCE Symposium (22-24 September 2015 in Lisbon). It is available on the project website under: http://asforce.org/blog/111. The feedback received so far is extremely positive.

WP 19 - Regional Workshops for governmental agencies
1) Summary Objectives of the work package
The aim of this work package was to provide representatives of different governmental agencies in the EU and in countries at risk a platform to discuss and update their prevention strategy and to adjust contingency plans based on the results of Themes 2-4.

2) Summary of objectives and results of Tasks
Task 19.1 At least 3 regional workshops for representatives of governmental agencies of EU Member States and countries recently affected by ASF will be organized
The first workshop held in Berlin on February 24th 2015, targeting representatives from veterinary services from Central Europe and neighboring countries. Representatives from Austria, Denmark, Finland, Germany, Holland Latvia, Norway, Poland, Slovakia, Switzerland and United Kingdom, attended the workshop. Following the welcome and introductory note by Prof. Hans-Joachim the representative from the Ministry of Agriculture of Germany and the presentation of a brief background on ASFORCE and an introduction to ASF, theme leaders of ASFORCE presented the main achievements in the different areas of work under the project. After this, a practical exercise was completed with the contribution of all participants, under the topic “Is it possible to prepare an ASF recipe for preventing the introduction of ASF?”
All experts agreed that the main ingredients in ASF “recipe” should be a mixture of early detection, contingency plan and training.

The second workshop, held in Rome, was hosted by FAO on the 10th of March 2015. It was attended by representatives from veterinary services in Southern Europe, namely Bulgaria, Hungary, Italy, Slovenia and Spain. Following the welcome and introductory note by Dr. Juan Lubroth (CVO, FAO) the structure was as above described for the workshop in Berlin. After this, Dr. Sofie Dhollander, guest to the workshop, presented an overall view on EFSA’s risk assessments on ASF. During the afternoon, each representative at the workshop, presented and discussed with the other participants, relevant aspects on the measures organized for prevention and control of ASF in their countries.

The final workshop was organized as the ASFORCE Symposium, in Lisbon (Portugal), on September 24, 2015. Program included key note presentations by Luis-Vivas Alegre (EC Project Officer), Carlos Martins (FMV-UL, PT), Alberto Laddomada (IZS, Sardinia, Italy) and Juan Lubroth (CVO, FAO). The key results of ASFORCE themes 2 – 5 were presented by the related key consortium members. Participants included representatives from several Portuguese institutions, as well as from international organizations (exs. COPA-COGECA, OIE) and from the veterinary services of Portugal, Serbia, Latvia, Macedonia, Spain, Armenia, Poland, Finland, Romania, Belgium, Georgia, Lithuania and Poland.

Finally, the ASFORCE project and its results were also presented during the GF-TADS 6th meeting of the Regional Steering Committee, in Brussels, September 30-October 1, 2015. The presentation was done by Jorge Pinto Ferreira (SAFOSO, CH) after feedback received by the project coordinator and theme leaders. This meeting was attended by high representatives of FAO,
OIE and EC, and it was an excellent dissemination opportunity, that allowed us to satisfy the suggestions received on the consolidated review report (strengthening of the links with these international organizations)

Potential Impact:
1. Strategic impact

1.1 Expected impacts listed in the work programme

The ASFORCE project addressed the expected impacts listed under the area 2.1.3 Optimised animal health, production and welfare across agriculture, fisheries and aquaculture, of the Work Program Cooperation 2012 Call KBBE.2012.1.3-02: “Targeted research effort on African swine fever”, through the implementation and development of research efforts targeting prevention and control of the ASF threat to the EU. According to this, the research effort was focused on:

1. The design of prevention, control and eradication models for ASF (ASFORCE, Theme 2);
2. The analysis of the domestic pig-wild boar-argasidae interactions relevant for ASF epidemiology (ASFORCE, Theme 3);
3. The development of protection tools against ASF by attenuation of ASFV vaccine strains and identification of protective antigens and the development of diagnostic tests for earlier detection of the disease (ASFORCE, Theme 4);
4. The implementation of Training and Knowledge Transfer activities (ASFORCE, Theme 5).

The importance of a devastating disease like ASF in domestic pigs has been recognized since its first description on 1921 in Africa. Although a reasonable situation regarding the control and prevention of ASF was finally achieved after the dissemination of the disease in Europe, Caribbean Islands and Brazil, with only Sardinia and Africa being the sole places where ASF was enzootic, the circumstances have changed dramatically since the declaration of the disease in Armenia, Georgia, Azerbaijan and Russia in 2007. In this new scenario, in which the EU was at high risk of introduction of the disease by legal or illegal movements of animals and animal products, particularly through its Eastern borders, the research efforts developed by the ASFORCE project contributed to provide knowledge and tools for preparedness programmes in this evolving situation.

1.2 Generation of tools and strategies for the prevention and control of African Swine Fever.

As above indicated, these impacts were fulfilled by the development of Themes 2, 3 and 4.

Theme 2: Prevention, control and eradication models for ASF

In order to design a rational eradication model that takes into account different risk scenarios, knowledge about the pig production and contact pattern was mandatory. In this Theme, the pig industry within the EU and in ASF affected areas in Europe was characterized by collecting data on pig farming in densely populated and traditional backyard farming areas. Management systems and their impact on productivity and price differentials were assessed. The spatial and temporal patterns of pig and pork movements within member states and other participant countries were evaluated. These were integrated and used to deduce the corresponding risk factors and to generate models to realistically simulate the potential spread of ASF within and between those countries. This delivered impact in “validating tools for risk analysis”.

There is an increased need for knowledge about the epidemiological and molecular features of currently circulating ASFV isolates in Europe, with special regards to those present in the EU, Trans-Caucasian countries and the Russian Federation. To address this need, epidemiological surveillance and molecular typing of isolates was integrated, to trace the dynamic of infection spread. The results obtained with additional ASFV genome markers provided strong evidence of the genetic variability among genotype II ASFV circulating in Europe, and described a useful methodology to distinguish closely related ASFV isolates. Results revealed the presence of two genetic variants co-circulating in the Russian Federation since 2012. The evaluation of the cost-benefit of existing ASF surveillance and control strategies were the basis for the development of an economic module that was incorporated in the disease spread model. Estimated parameters of ASF transmission were implemented in disease models to simulate the spread of ASF and estimate the cost-benefit of different risk-based surveillance strategies. Finally, thanks to an expert elicitation, the surveillance and intervention strategies perceived to have the best compromise between effectiveness and practicality were identified for different epidemiological context.

In conclusion, Theme 2 aimed to provide essential information to design more cost-effective surveillance and control strategies for ASF in different risk scenarios. This provided valuable tools for policy makers, administrations, pig producers and veterinarians. The ultimate goal would be to better prevent and control ASF and minimize the economical losses of endemic or potential new infected areas. All outputs produced under Theme 2 were summarized in clear and concise guidelines with the...
most recommended actions to be taken in each considered risk scenario (Guidelines for the cost-effective prevention and control of African swine fever), disseminated through the ASFORCE webpage.

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

Theme 3 proposed to provide essential data to identify key points for designing new control strategies including wildlife considerations. This was achieved by gaining further understanding and knowledge of the interaction of ASF virus with its hosts in field conditions in Eastern Europe and Sardinia.

Based on the results obtained the role of Ornithodoros soft ticks in the disease epidemiology was not relevant as it is absent. Although it has been demonstrated that O. erraticus will be able to be infected by ASFV Georgia2007/1 and to amplify the virus, an improving knowledge on the vector competence is still needed. Wild boar (WB) played a secondary role in disease spreading, compared to domestic pigs (DP) in low biosecurity farms, but an ASF significant transmission rate between wild boar populations could only be confirmed in the case of Russia, indicating that an effective transmission was taking place. High virulence of the recent Sardinian isolate and moderate/low contagiosity of Caucasian ASFV isolate at low oral dose were proved in wild boar. Main risk factor for ASF presence in wild boar appeared to be the presence of illegal pig breeding in the Sardinian scenario and the presence of areas suitable for wild boar and ASF cases in backyard in the Russian scenario. The evaluation of the DP-WB interactions (noninvasive methods) provided essential information on location of interface areas (mapping activities), seasonality (questionnaires) and evidence of contacts (biomarkers), patterns of movement and ecology of hosts (radio tracking) offering a highly contribution to assist surveillance system. ASF transmission models on WB identified risk countries of ASF introduction (Romania and Slovakia as countries at highest risk) stressing the importance of two drivers, the progress of the disease through the EU and the occurrence of new entrances from endemic areas outside the EU, e.g. as consequence of the current progressing of the disease in Ukraine.

The development of cartographic tools to predict the tick and the wild boar distribution in Eurasia and, the improvement of laboratory tests (e.g. ELISA test and biomarkers) to assess the tick-pig/WB and the DP-WB interactions, as well as the different ASF transmission models developed, are of major relevance to veterinary services, wildlife management authorities, and decision makers, enabling the use with geospatial products and screening tools for ASF surveillance and the identification of at-risk areas.

All achievements above mentioned constitute important aspects to improve the knowledge on ASF scenarios and a basis for the development of more reliable control measures to fight against ASF.

Theme 4: Development of protection tools against ASF

Vaccines are not available for ASF and this severely limits strategies for disease control. The overall objective was to advance work leading to vaccine development through three different strategies i) the construction of virus deletion mutants and testing by immunization and challenge experiments in pigs. Persistence and transmission of candidate attenuated vaccine strains to in contact pigs was evaluated; ii) the development of Defective Infectious Single Cycle (DISC) viruses, and iii) the prediction of potential protective antigens by computer analysis and their expression in virus vectors for preliminary testing in pigs. Within this theme, improved diagnostic tests developed included a lateral flow device for rapid penside virus detection and an ELISA test to detect early antibody responses in infected pigs, thus improving earlier detection the disease.

The virus deletion mutants were produced by deleting genes that inhibit innate immune responses that were characterized in this project. These were deleted from a virulent strain or an already attenuated strain. The deletion mutants characterized represent a first step in the production of rationally attenuated candidate vaccines. Further genome manipulations will be required to achieve safety and efficacy requirements for registration. Helper cell lines expressing target essential genes for construction of DISC viruses were constructed. These are being used to produce DISC viruses lacking these essential genes. This approach may provide a safer alternative to the use of live attenuated virus. T cell epitopes conserved in different ASFV isolates and predicted to be presented with a diverse range of swine leucocyte antigen I (SLA-I) haplotypes were predicted. Five of these were expressed in virus vectors and used to immunization and challenge experiments in pigs. Although these did not induce protection, future testing may identify key protective antigens for inclusion in viral-vectored vaccines. The two novel diagnostic tests developed (Lateral Flow Device (LFD) for virus antigen detection and ELISA to detect IgM will provide improved methods for early diagnosis of infection. The use of the LFD device will be particularly useful for rapid diagnosis in
1.3 Training of EU and third countries researchers

The impact in this section was fulfilled in the ASFORCE project through the development of Theme 5.

**Theme 5: Training and Knowledge Transfer**

Work developed under this Theme aimed at improving preparedness for ASF at different levels and activities targeting pig farmers, hunters, veterinarians and governmental agencies.

These objectives have been fulfilled by the development of different activities briefly described below aiming at:

- Increasing the preparedness for ASF among pig producers in the EU, in particular in areas at risk, and in non-EU countries;
- Increasing preparedness for ASF among veterinarians, in particular in regions at risk in the EU and in recently affected countries in Caucasus and in Russia, through the organization of workshops and providing training for veterinarians to increase risk awareness and capacity of incident response;
- Providing representatives of different governmental agencies in the EU and in countries at risk a platform to discuss and update their prevention strategy and to adjust contingency plans based on the results of Themes 2-4.

To fulfill these objectives, a number of training workshops, practical courses and on-line training modules via the ASFORCE webpage, have been prepared to increase risk awareness and capacity of incident response of pig farmers, hunters, veterinarians and representatives of the governmental EU agencies, coordinated by different Participants in the project including FAO. The materials for disease awareness were developed and distributed in countries at risk in the EU and outside, in collaboration with national pig organizations, veterinary associations, hunter associations and representatives of the pig industry and governmental agencies. Materials as downloadable print media or videos are also offered via the webpage site (www.asforce.org).

1.4 Advancement of knowledge with contribution to improving the quality of EU research.

Contributions for this impact are mainly expected from the research developed in Themes 2 and 3 (identification of risk factors – transmission model development, guidelines for evaluation of ASF control measures including wild life considerations), and on Theme 4 (development of vaccine candidates and diagnostic tests for earlier detection of the disease), which have been disseminated in several publications in high impact scientific journals, the development of PhD and master’s thesis as further described, and through the presentation of research results in scientific meetings.

1.5 Contribute to the competiveness of European pig production and international trade.

This impact is fulfilled by the development of tools and strategies for the prevention and control of African Swine Fever (Theme 2), by assessing the role of wild boars in the maintenance and ASF transmission to domestic pigs (Theme 3), and by developing knowledge and tools for the development of vaccines and improved diagnostic tests for earlier detection of the disease. (Theme 4). Further contribution to this end is reinforced by training and knowledge activities developed under Theme 5.

Overall and through the results obtained in the above mentioned areas, ASFORCE contributes on a relevant manner to the sustainability and competiveness of the agri-food sector.

1.6 European added value in different aspects e.g. critical mass of researchers and activities internationally recognized and addressing a pan-European challenge.

The control of transboundary animal diseases such as ASF cannot adequately be addressed by local or national approaches. Expertise in individual countries and groups is limited. A European consortium such as ASFORCE, which brings together partners with the best expertise, skills, experience and potential for product development available, is the most effective way to advance knowledge and achieve objectives. An intense and productive collaboration was developed from the Consortium assembled in the project with the participation of 18 Groups from seven EU countries, Russian Federation and Switzerland, including most of the major, internationally recognized institutions involved in ASF research all of them performing collaborative work addressing a pan-European challenge, posed by the continuing ASF dissemination during recent years.
2. Dissemination activities
2.1 Publications in scientific journals

So far, work developed under the project allowed the following publications:


2.2 PhD and Master's thesis
PhD Thesis
Beneficiary #1- FMV - ULisboa
Beneficiary #3 - UCM
Beneficiary #5 - CSIC
Ana Quintas Gorozarri, ‘ASFV-mediated regulation of RNA metabolism: g5Rp, a putative viral decapping enzyme’. PhD approved on April 2015. Universidad Autónoma de Madrid. Classification: Cum Laude
Beneficiary #11 - ANSES
Beneficiary #15 - BFSA

Masters’ Thesis
Beneficiary #1 - .FMV-ULisboa
João Miguel Carvalho da Costa, “Construction of helper cell lines expressing African swine fever virus helicases toward the development of DISC-particles”. Master’s degree in Microbiology. Approved, 24th November 2015. ULisboa
Beneficiary #2 - FCG-IGC


Beneficiary#6 - CIRAD

Sebastien Trabucco, ‘Characterization of the contacts between wild and domestic pigs: assessment of human practices having an impact on transmission of diseases’. Masters approved, Institute AGROPARITECH.

Morgane Laval, ‘Dynamics of circulation and excretion of hepatitis E virus in domestic and wild suids in Corsica’. Masters approved (ISARA), University of Lyon.

Jaimee Hung, ‘Generation of Ornithodoros tick-derived cell lines and development of a real time RT-PCR to follow their infection by African swine fever virus’. Masters, approved, University Montpellier II.

Caroline Fano, ‘Development of methods to detect and quantify African swine fever virus in Ornithodoros erraticus ticks: Application on experimentally infected ticks’. DUT (Technical University Diploma) approved, Université d'Avignon et des Pays du Vaucluse.

2.3 Other activities

Improvement of preparedness for ASF at different levels was strongly achieved through dissemination activities, reported elsewhere in this report, namely through the ASFORCE webpage (www.asforce.org), partners’ webpages, establishment of dissemination connections with international projects (GARA, LINKTADs) and through other activities as posters and oral communications at different scientific meetings, organizations of workshops and conferences, participation on exhibitions, media briefings, production and dissemination of videos and press releases, dissemination through TV clips.

3. Patents submitted

Patent summary:
Application reference : PCT/GB2015/051798
Intellectual Property Organization: UK
Title of application: “ASFV Deletion Mutant”
Confidential
Applicants: Charles Abrams, Anna-Luisa Reis, Chris Netherton, Linda Dixon, Pedro Sanchez-Cordon
URL of application: https://www.ipo.gov.uk/p-ipsum/Case/ApplicationNumber/GB1410971.4

4. Commercial exploitation of R&D results

1- Method for the diagnosis of infection by African Swine Fever Virus (ASFV). The method is based on the determination of the presence of the virus in a blood or serum sample by immunochromatographic method. The assay is especially useful at field level.

Lateral Flow Assay for ASFV detection
Sector(s) of application: A1.4.6- Raising of swine/pigs
Owner & Other Beneficiary(s) involved: INGENASA (Beneficiary #13)

2- An ELISA for detection of IgM antibodies as an indirect indicator of ASFV infection. The assay was based on an early antigen of the virus

Capture ELISA for ASFV IgM detection
Sector(s) of application: A1.4.6- Raising of swine/pigs
Owner & Other Beneficiary(s) involved: INGENASA (Beneficiary #13)/CSIC (Beneficiary#
List of Websites:
Website: www.asforce.org

Related information

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<th>A targeted approach to protect pigs from African swine fever</th>
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