12. Conclusions

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Abstract

The COST Action ASF-STOP brought together an extensive network of scientists of remarkable excellence on African swine fever (ASF) vaccinology, virology, immunology, diagnostics and pathology. The network also includes global leaders in wild boar ecology and management, renowned epidemiologists specialised in ASF and disease control and scientists with vast expertise in the pig sector in Europe, biosecurity, cleaning and disinfection in pig holdings. This book collects updated knowledge in these fields, with a focus on the European situation. ASF-STOP coordinated and integrated research on ASF. Despite the new knowledge generated on ASF by scientists in Europe and worldwide, many unknowns still remain. For example, many expressed ASF virus (ASFV) genes remain uncharacterised and the information is needed for efficient antiviral drug and vaccine development. Crucial interactions between ASFV and cells like macrophages or dendritic cells are not yet completely understood. Research is needed on the mechanisms of protective immunity and identifying further viral proteins for inclusion in subunit vaccines. An in-depth analysis of the impact of ASF on the structure of the pig farming

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system in the EU has not yet been conducted, and transmission risks from the wild boar-habitat epidemiological cycle to the domestic pig cycle are still not fully understood. The pig sector is one of the most economically significant farming sectors in the EU and pork is the most consumed meat. The EU is the world's second biggest producer of pork. In both the wild boar-habitat and the domestic pig epidemiological cycle fully implemented biosecurity can hinder or eliminate virus transmission. National legislation and EU regulations set out clear regulations for controlling ASF. However, across Europe, wild boar populations are growing in size and range and the control of ASF continues to be a major challenge. The great socio-economic impact of ASF calls for further collaborative efforts to tackle this disease.

Keywords: wild boar, pig, African swine fever, ASF-STOP, COST

12.1 Summary and conclusions

The COST Action ASF-STOP brought together an extensive network of more than 270 scientists from 42 countries covering a wide range of disciplines related to African swine fever (ASF). The excellence of the ASF-STOP network is remarkable, including scientists with outstanding international records in the fields of ASF vaccinology, virology, immunology, diagnostics and pathology. The network also includes global leaders in wild boar ecology and management, renowned epidemiologists specialised in ASF and disease control and scientists with vast expertise in the pig sector in Europe, biosecurity, cleaning and disinfection in pig holdings. This book collects updated knowledge in these fields, with a focus on the European situation. Some of the main conclusions are summarised below.

The African swine fever virus (ASFV) is a large double-stranded DNA virus and the only member of the *Asfaviridae* family of viruses. ASFV encodes ~150-170 open reading frames with a very low mutation rate, expressed in a precise and regulated fashion, with most of the genes being actively transcribed. Recent next-generation sequencing approaches enable mapping of ASFV gene transcripts from start to finish, elucidating molecular mechanisms of ASFV transcription, including promoter motifs, factors involved, and temporal gene expression patterns. However, many expressed ASFV genes remain uncharacterised, highlighting genome-wide studies as a useful tool for efficient antiviral drug and vaccine development.

Recent studies on the architecture and composition of the infectious ASFV particle have provided a comprehensive model of the ASFV architecture that integrates compositional, structural and functional information, shedding light on the huge complexity of ASFV structure and biology. ASFV infects predominantly macrophages by a very rapid dynamin and clathrin-mediated process of endocytosis, engaging unknown cell receptor(s), with movement of viral particles and endosomes along microtubules. Inside the cells, ASFV inhibits different innate immune responses.

The complex work leading to the development of an effective drug against ASFV can be greatly accelerated by using modern approaches like genome-wide CRISPR/Cas screens or computational methods. Indeed, more *in silico* approaches should be used in order to find new

antiviral agents targeting ASFV proteins or host factors involved in the virus lifecycle, which alone or in combination with vaccines will stop the spread of ASFV.

The immune system is designed to detect and subsequently eliminate harmful pathogens while maintaining a homeostatic relationship with beneficial microbes in the body. Upon infection with ASFV a variety of immunological mechanisms are triggered, but these are not sufficiently broad or powerful to eliminate the virus. The first line of defence against pathogens is the innate immune system. Crucial interactions between ASFV and cells like macrophages (Mφ) or dendritic cells (DC) are not yet completely understood. Despite the central role of Mφ in ASFV infection biology, little is known about the responses of different subsets of Mφ to ASFV. The same applies to DC subsets. Remarkably, it has been shown that ASFV uses several mechanisms to counteract type I interferon (IFN) action, also subverting interferon stimulated genes, showing the key role of this family of cytokines in ASFV infection. In fact, it has been suggested that ASFV has evolved mechanisms to become 'tolerant' to the action of type I IFN.

Since early studies, the protective role of antibody responses against ASFV has been a controversial topic and their neutralisation ability has received little attention. Further studies on antibodies generated after infection with different isolates of ASFV are required to elucidate their role in ASFV infection. Conversely, new data on cellular responses show the importance of αβ T cell receptors +T cells, like CD8+ T cells, CD4+T cells or invariant natural killer T cells, or cell subsets, such as natural killer or γ-T cells within ASFV infection. The ASFV epitopes recognised by them is an area of recent discoveries that will be useful in future vaccine design studies. Recent results on regulatory T cells show that the interplay and plasticity of the immune system is subverted by ASFV infection. Unfortunately, many knowledge gaps still exist regarding immune responses to ASFV in natural infection compared to experimental infection. Moreover, identification of the correlates of immune protection is still one of the main unknowns in ASFV infection.

Pathology plays a key role in studying the pathogenesis of ASF and complements other knowledge fields to provide a broad understanding of host-virus interactions. Development of lesions over time, from early stage to terminal or late disease can be correlated with clinical signs, virus presence and immunological response. Pathology techniques, such as immunohistochemistry to label ASFV, allow characterising the co-localisation of virus with lesions, and virus loads. In this way tissue and cell targets of the virus can be identified and associated to lesions. Pathology scoring of severity and extent of lesions allows systematic semi-quantitative comparisons of outcomes of experimental infections, for example for evaluation of ASF vaccine candidates. Several host and virus-related factors, such as the virulence of the ASFV isolate, affect the clinical presentation, presence and severity of lesions and the final outcome of the infection. Highly virulent isolates are usually involved in peracute or acute forms of disease while moderately virulent isolates can induce acute, subacute and even chronic forms of ASF. Natural infection of pigs in immunologically naïve herds by highly virulent ASFV has a case fatality of up to 90-100% four to ten days post infection. The pathology presentation of ASF is variable. Fluid exudation into body cavities, such as hydrothorax and hydropericardium, and macroscopic haemorrhagic lesions are considered hallmarks of acute and subacute forms of ASF. Significant macroscopic lesions affect the lymphoid system. The spleen displays an increase in size (hyperaemic splenomegaly) and lymph nodes are often swollen, oedematous and haemorrhagic. The lungs show diffuse congestion and oedema. The kidneys characteristically show petechiae. Macroscopic lesions associated with subacute

ASF forms are similar to those in acute forms but characterised by more severe and extensive haemorrhages and oedema as a consequence of longer clinical courses induced by less virulent isolates, severe and prolonged thrombocytopenia and consumption of coagulation factors. Animals presenting chronic forms of ASF are characteristically immunocompromised and susceptible to infection with opportunistic pathogens, mainly bacteria, which ultimately cause non-specific macroscopic lesions such as fibrinous and fibrous pericarditis, pleural adhesions, lung consolidation or necrotic and caseous pneumonia with mineralisation or necrotic areas on tonsils and tongue. Joint swelling and necrotic skin lesions are also characteristic macroscopic lesions observed not only in natural chronic forms of ASF but also in experimental infections with naturally attenuated low virulent isolates used as potential vaccine candidates; the pathogenic mechanisms remain unclear.

For the diagnosis of ASF there are currently very good tools in terms of sensitivity, specificity and robustness. Several commercial kits for the detection of either antibodies (ELISA) or viral genome (PCR) have been fully validated. Inconsistent results are most often due to poor quality of the sample or to the environment (laboratory contamination) rather than to the performance of the diagnostic tools. It is highly recommended to perform PCR with an internal control to obtain reliable results particularly with poor quality samples. Either for serological or virological methods, confirmatory methods should be applied to verify positive results, especially before the declaration of the first case of ASF in a new area in the absence of an obvious epidemiological link. The overall diagnostic results depend on several factors, such as the sampling design and the population targeted. For example, in a farm with a suspicion of ASF, it is more efficacious to target sick pigs than applying random sampling of all animals. The demand for penside tests is increasing following the expansion of the disease through the Eurasian continent. Currently, a penside test for antibody detection is commercially available, while the validation of a penside test for antigen detection is still ongoing. However, the use of penside tests for ASF surveillance is still being discussed, especially considering that ASF is a notifiable disease.

Classical methods of generating vaccines for African swine fever virus have not been successful. Inactivated vaccines induce antibody responses in pigs, but do not prevent animals from developing disease after being infected. Repeated passage of ASFV through tissue culture leads to genetic changes that attenuate the virus, and these attenuated viruses can protect pigs from virulent virus. However, these tissue culture adapted viruses cause a chronic form of disease and are therefore not suitable for deployment in the field. An alternative to attenuating ASFV by passage through tissue culture is targeted gene deletion. This approach has led to the development of a number of vaccine candidates with good efficacy and promising safety profiles. A large-scale trial of one of these viruses is currently under way in China with promising initial results. A subunit vaccine would avoid the safety issues associated with live ASFV vaccines, but the complexity of the virus makes this approach challenging. Combinations of viral proteins have been tested in pigs using different vaccine platforms including viral vectors, whole proteins, and DNA plasmids with varying degrees of success. Research on the mechanisms of protective immunity and identifying further viral proteins for inclusion in subunit vaccines is required for success.

The pig sector is one of the most economically significant farming sectors in the European Union (EU) and pork is the most consumed meat. The EU is the world's second biggest producer

of pork, after China, and the biggest exporter of pork and pork products. The main producer countries, Germany, Spain and France, represent about half of the EU's total production. The major production basin extends from Germany to Belgium and accounts for 30% of the sows in the EU. Other important pig producing regions are Catalonia, Murcia, Lombardy, Bretagne and some areas of central Poland and northern Croatia. Small-scale pig producers are mostly found in eastern Europe where small units rearing 3.8% of pigs account for 73.3% of the pig farms. Following the current ASF epidemic in the eastern EU, which started in 2014, the pig sector has greatly changed. The number of mainly small-sized pig holdings has decreased. An in-depth analysis of the impact of ASF on the structure of the pig farming system in the EU has, however, not yet been conducted.

Many ASF outbreak investigations have reported biosecurity shortcomings as a critical element for virus introduction and spread. The structure of the European swine industry makes it necessary to put in place differentiated biosecurity measures, in order to meet the different risk levels for introduction and spread of ASF among the diversified farming systems. The risk of exposure to ASF for the individual farm depends on the country, area and farm location in relation to the infectious status in the surroundings. This implies that biosecurity measures should take into consideration the virus persistence in the environment, routes of transmission and excretion as well as the characteristics of the farming systems and the health situation of the territory. Biosecurity programmes are normally applied to commercial holdings. However, all holdings that have access to markets, i.e. sell pigs and products, should be included in such biosecurity programmes. Backyard holdings that sell animals have an important role in the spread of ASF.

Cleaning and disinfection (C&D) procedures are fundamental for pathogen inactivation, to prevent the spread of the disease and to facilitate the repopulation after an outbreak. The completion of the C&D procedure is also one of the requirements foreseen by the OIE and by the EU legislation for the recovery of the free status after the occurrence of ASF.The choice of disinfectants and of procedures for disinfection shall take into consideration the nature of the premises, vehicles and objects to be treated. Disinfectants should further be officially authorised by the veterinary service and the conditions for their use strictly followed.

The introduction of ASFV genotype II to Georgia in 2007 and further to the EU in 2014 sparked a new era of global ASF research. This has led to the identification of specific characteristics of the epidemiology of ASF relating to wild boar, i.e the wild boar-habitat epidemiological cycle. In this epidemiological cycle wild boar carcasses and the resulting contamination of the environment play key roles for virus persistence. Transmission risks from this cycle to the domestic pig cycle are still not fully understood. Several field studies, experimental infection trials and literature reviews have confirmed that previous knowledge concerning important factors in ASF epidemiology are valid for both wild boar and domestic pigs infected with ASFV genotype II: high case fatality rate, low contagiousness, and no evidence of asymptomatic virus carriers. In both the wild boar-habitat and the domestic pig epidemiological cycle of ASF, fully implemented biosecurity can hinder or eliminate virus transmission. National legislation and EU regulations set out clear regulations for controlling ASF in this regard. However, if these are not entirely complied with, and adequate measures thus not implemented where the disease is transmitted during the daily activities of people, they are of no value for disease control and eradication. The global pattern of the current epidemic confirms the role of humans in transmitting ASF to domestic pigs and

wild boar. Therefore, achieving control requires adapting biosecurity measures to local conditions to increase their implementation, as well as to improve understanding of the sociocultural, economic, and political dimensions of domestic pig production and wild boar value chains.

The positive examples from the Czech Republic and Belgium show that control and eradication of ASF in wild boar can be achieved. Early detection and swift actions combining several different efforts to first restrain wild boar from leaving the infected area, and secondly, eliminate affected populations, seem to be keys to success in this regard. Such endeavours require multi-stakeholder cooperation and communication on international, national, and local levels.

Across Europe, wild boar populations are growing in size and range, in parallel with increased damage to agricultural crops, forests and biodiversity, higher number of traffic accidents and higher levels of detected infectious pathogens maintained and disseminated by wild boar, including ASF. Currently, a high priority goal in wild boar management practice is to reduce wild boar population densities and avoid overpopulation in order to mitigate the negative impacts.

Since wild boar is one of the most popular and widespread game species in Europe, considerable expertise has been built and lessons have been learned regarding management of wild boar populations in order to curb their associated economic and environmental impact. This expertise encompasses lethal and non-lethal methods to reduce wild boar populations and methods and tools to contain and influence their movements and behaviour. During the current ASF epidemic in the EU, many of these wild boar management methods have been combined with other disease control strategies such as regionalisation, fencing and enhanced passive surveillance to attempt control or eradication of ASF from wild boar populations. Field experience shows that successful control requires collaboration of different stakeholders including animal health authorities, local authorities, hunting associations, wildlife managers, farmers, landowners, the general public using the forests, and occasionally army or police forces.

The COST Action ASF-STOP coordinated and integrated research on ASF. It also contributed to knowledge dissemination and capacity building. Despite the new knowledge generated on ASF by scientists in Europe and worldwide many unknowns still remain. The control of ASF continues to be a major challenge, which in recent years has acquired a global dimension. The great socio-economic impact of ASF calls for further collaborative efforts to tackle the disease.

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