



1. African swine fever (ASF), the pig health challenge of the century

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Abstract

More than one hundred years ago African swine fever (ASF) was first diagnosed in Kenya. Since then, diverse approaches have been applied to the study of the causative virus, the sole member of the family *Asfarviridae*, aimed at characterising its properties, genome organisation and replication, its antigenic and biological properties as well as to develop treatment and a vaccine. The disease evolved and has persisted in Africa in a sylvatic cycle involving wild suids and soft ticks for a long time, but was introduced, usually through contaminated waste food, into other regions on multiple occasions since 1957. The most recent introduction, into Georgia in 2007, resulted in the spread of the disease to the European Union in 2014 and to the establishment of an international and multidisciplinary network of scientists funded by the European Cooperation in Science and Technology (COST) two years later. The network included a broad variety of scientific fields, animal health and food safety authorities, hunting associations, wildlife managers and food and livestock industries with the goal of increasing preparedness and attempting to stop ASF spread. This book represents the summary of the collective and integrated work of almost 300 dedicated participants in tackling the complex challenge posed by ASF. Here we summarise the state-of-the-art knowledge on this lethal disease, with a focus on the European situation, and identify areas that still need to be explored.

Keywords: African swine fever, history, multidisciplinary, Europe

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1.1 Where ASF-STOP comes from

More than one hundred years ago, R. Eustace Montgomery diagnosed for the first time, in Kenya, the occurrence of a swine disease characterised by clinical and pathological features similar but not related to classical swine fever (CSF) or hog cholera. His observations and studies, developed from 1910 up to 1917, showed relevant aspects of the 'East African swine fever' now known as African swine fever (ASF) (Montgomery, 1921). The author's findings are still considered to be pillars for the knowledge attained since then on this disease, that currently inflicts alarming outbreaks with devastating economic and social consequences in numerous countries in Africa, Europe, Asia and Oceania. Hundreds of researchers and specialists in different fields of knowledge, of private and public National and International institutions, have produced, along the years, countless documents and scientific papers dedicated to explore and deepen key aspects on intricate details of the virus biology, disease maintenance and dissemination, complex viral-host interactions, strategies to prevent and control disease outbreaks and dissemination, and efforts to develop safe and successful vaccines. Recent reviews, including the chapters of this book, fully cover different aspects of ASF and it would be superfluous to describe facts already available for the likely already informed readers. Thus, the main ground-breaking contributions that have provided new insights into the current knowledge on ASF are presented here, to reinforce strategies towards prevention and control of ASF.

Since Montgomery's findings, African swine fever virus (ASFV) has been studied through diverse approaches aiming at characterising its structural and physicochemical properties, genome organisation and replication, as well as its antigenic and biological properties. This complex virus is a large double stranded DNA virus (170-190 kb) that encodes more than 150 open reading frames (ORFs), depending on the virus isolate. It is the only known DNA arbovirus and the sole member of the family *Asfarviridae*, classified into the Nucleocytoplasmic Large DNA Viruses superfamily (Takamatsu *et al.*, 2011). The virus infects all members of the *Suidae* family and it is maintained in different epidemiological cycles related to distinct ecosystems. It was diagnosed in Africa for the first time in 1910, when occurrence of ASF outbreaks was related to the presence of warthogs (*Phacochoerus* sp.) and bush pigs (*Potamochoerus* sp.), both shown to survive experimental inoculations with the causative virus without manifesting clinical signs. However, their blood collected up to five to seven days post inoculation was infectious and caused ASF experimentally in domestic pigs. The infected wild suids did not, however, appear capable of disseminating the virus by contact, urine, and faeces although he considered possible the involvement of an arthropod vector, and was able to exclude fleas and lice (Montgomery, 1921).

Many years later, in the 1960s, when ASF was spreading in Europe, Sanchez Botija found that ASFV infected soft ticks (*Ornithodoros erraticus*), collected in pig sties, were acting as the biological vectors of ASFV in Spain (Sanchez-Botija, 1963). This finding opened new insights regarding the study of ASF epidemiology in Africa. Plowright and colleagues (1969) described thereafter that soft ticks of the *Ornithodoros moubata* complex were vectors and reservoirs of ASFV in Africa. This new knowledge was further pursued by the identification of the main role of *O. moubata* ticks in the ASF sylvatic epidemiological cycle. This cycle was shown to be connected to common warthogs (*Phacochoerus africanus*) in Eastern and Southern Africa (Penrith *et al.*, 2004), the niche of the disease whose aetiological agent has been described as having evolved over

300 years with a time to the most recent common ancestor in the early 18th century (Michaud *et al.*, 2013).

ASF has persisted in Africa in a sylvatic cycle involving wild suids and soft ticks, spreading through infected *O. moubata* ticks to domestic pig populations in which, afterwards, it readily spreads through direct and indirect transmission from infected to healthy pigs (Penrith *et al.*, 2004). The disease was introduced, probably from Angola, to Portugal in 1957 (Manso Ribeiro *et al.*, 1958) in ASFV contaminated waste food from planes that was fed as swill to pigs.

The disease was considered eradicated the following year but was again introduced in 1960 (Manso Ribeiro and Rosa Azevedo, 1961) and it remained endemic in Portugal and Spain up to the early 1990s. Besides being maintained in the domestic pig populations through direct and indirect transmission, it persisted in populations of free ranging Iberian pigs, kept in premises, built mostly with stones and adobe, during the night periods, allowing the establishment of the epidemiological cycle including the soft ticks *Ornithodoros erraticus* as biological reservoirs (Caiaado *et al.*, 1988; Sanchez-Botija, 1963). In Europe, the distribution of this soft tick is limited to the Iberian pig extensive production areas, in Central Spain and Southwestern quarter of Iberia (Montado/Portugal and Dehesa/Spain), the last locations from which the disease was eradicated. The occurrence of a sporadic outbreak, in 1999 in the South of Portugal, has been epidemiologically connected with the presence of *O. erraticus* in the pig premises, as these arthropods are very resistant to fasting and may harbour active ASFV for up to 5 years (Boinas *et al.*, 2011). As a consequence, the repopulation of the infested premises in the area became limited since it was only allowed based on the result of a compulsory risk evaluation. Most of them were depopulated, which led to a significant decrease in the prevalence of *O. erraticus* in more recent years (Wilson *et al.*, 2013).

Although the epidemiological role of the Eurasian wild boar (*Sus scrofa*) (WB) during the endemic period in Iberia was considered negligible, epidemiological surveys done at the time showed that WB caused between 5-6% of the ASF outbreaks (Ordas *et al.*, 1983; Perestrelo-Vieira, 1993). Later on, towards the end of the previously mentioned period, serological surveys in Portugal showed only 2.3% seroprevalence in the hunted WB population in 1990 with no positives in 1992 (Commission of the European Communities-Directorate General for Agriculture, 1994). The significant increase of WB in recent years in the whole of Iberian Peninsula, and the increase of free-ranging pig farms, mainly in the Southern areas, certainly would play a relevant role in case of ASF reintroduction.

The WB is known to be as susceptible as the domestic pig to ASF (Blome *et al.*, 2013; Polo Jover and Sanchez-Botija, 1961), and is currently considered to be the main driver of a new epidemiological cycle in disease maintenance and spread since it emerged in the Caucasus, Russia, Eastern Europe and Baltic States (Guberti *et al.*, 2020). This is further described in detail later in this book (Chapters 8 and 9). In the Italian island of Sardinia, ASF has been endemic since 1978, although eradication is likely to be achieved soon. Its persistence for more than 30 years has frequently been associated with the large populations of free-range 'brado' pigs that live in the mountainous area in close contact with WB, although WB is considered of minor importance in this epidemiological scenario (Laddomada *et al.*, 2019).

Studies developed on the role of several species of blood-sucking invertebrates found in pig or warthog habitats have shown that they do not transmit the virus after having an infected blood meal. Although not acting as biological vectors in nature like the argasid ticks, it was experimentally shown that stable flies (*Stomoxys calcitrans*) may act as mechanical vectors of ASFV (Mellor *et al.*, 1987; Olesen *et al.*, 2018).

In the vertebrate host, the aetiological agent replicates preferentially in monocyte and macrophage cells of the mononuclear phagocytic system (van Furth and Cohn, 1968) and causes a range of syndromes and lesions, from peracute to chronic and unapparent forms of disease (Gallardo *et al.*, 2015; Gómez-Villamandos *et al.*, 2013). The clinical evolution of ASF is often related to the diverse virulence of the aetiological agent, which still remains to date one of the most intriguing details of ASFV biology. When ASF was confined to Africa and Iberian countries up to the early '60s, viral isolates in natural occurring outbreaks were shown to be highly virulent, inducing haemorrhagic disease with mortality approaching 100% of infected animals (Manso Ribeiro *et al.*, 1958; Montgomery, 1921). However, even though several authors claimed that ASFV virulence would attenuate in time after the initial outbreaks, possibly through viral adaptation to the host (Plowright *et al.*, 1994), this was not the case. For example, in Portugal highly virulent haemadsorbing isolates (see Chapter 2) were identified up to the end of the endemic period, or even when an isolated and controlled outbreak of disease occurred in 1999 (Wilson *et al.*, 2013).

However, while outbreaks caused by the highly virulent haemadsorbing isolate ASFV Lisbon 60 (L60) prevailed, infections with low virulent non-haemadsorbing isolates were also identified in apparently healthy pigs showing chronic pulmonary lesions (Vigário *et al.*, 1974). This parallel occurrence of both low and highly virulent ASFV isolates in nature is not fully understood. However, it is unlikely that genetic changes have naturally occurred in the highly virulent L60 to give origin to the non-haemadsorbing, low virulent virus. The latter was recently shown to have significant genetic differences when compared to the virulent isolate (Portugal *et al.*, 2015). The haemadsorption effect caused by some virus isolates, and demonstrated by Malmquist and Hay (1960), was thought for a while to be a virulence marker. However, findings in Africa during natural occurring outbreaks have shown that non-haemadsorbing ASFV isolates can be both virulent and non-virulent (Pini and Wagenaar, 1974; Thomson *et al.*, 1979). The origin of non-haemadsorbing isolates, showing different virulence, is not clearly understood. It is accepted that they may occur in natural conditions, originating from parental virus composed of a heterogeneous mix of different viruses, as demonstrated *in vitro* by Pan and Hess (1984, 1985).

The development of molecular methods has been a major advance to study different aspects of ASFV biology. A landmark for ASFV genome characterisation was achieved through the analysis of the complete viral genome sequence of BA71V, an avirulent ASFV isolate, adapted to grow in Vero cells (Yáñez *et al.*, 1995). This work has prompted further research towards the functional characterisation of viral genes that has been pursued by different authors using other viral isolates. As recently reviewed, ASFV encodes for at least 150 proteins and, so far, 38 of them are associated with known or predicted functions in nucleotide metabolism, transcription, replication and repair; more than 24 proteins are involved in virion structure and morphogenesis, and at least 8 are likely involved in host cell interactions, although the functions of a large number of ASFV encoded proteins still remain unknown (Gaudreault *et al.*, 2020). Genome sequencing has allowed molecular characterisation of small conserved DNA regions enabling

the detection of origin and traceability of ASFV during outbreaks. The current approach is based on the analysis of the C-terminal end of gene B646L, encoding the major protein p72 (Bastos *et al.*, 2003). More recently, this procedure has been enhanced through the sequencing of the Central Variable Region (CVR) within the B602L gene, or other regions (e.g. E183L encoding p54 protein, CP204L encoding p30 protein), to distinguish between geographically and temporally constrained p72 genotype viruses (Gallardo *et al.*, 2015). This approach has allowed 24 different p72 genotypes to be identified among the currently known virus isolates. Out of these, genotype I is predominant in West Africa and was the only one found outside Africa (Europe, America, and the Caribbean) until the introduction of genotype II from East Africa into Georgia in 2007. This latter genotype is the one currently present in parts of the Caucasus, Europe, the Russian Federation, Southeast Asia and Oceania (Gallardo *et al.*, 2015; Gaudreault *et al.*, 2020). Although important for determining epidemiological details of ASFV, the above-mentioned genotyping does not correlate to the virulence of ASFV isolates as often wrongly stated. The quest for the identification of ASFV virulence factors or markers has been pursued for a long time.

As mentioned above, ASFV virulence was initially correlated to the haemadsorption phenomenon (Malmquist and Hay, 1960). Progressively, studies have been devoted to compare genome sequencing of different virus, for example through the use of isolates of different virulence from the same origin (Portugal *et al.*, 2015). Such approaches are likely relevant to identify genes that may be of importance to be used in vaccine designing. Development of efficient and safe vaccine(s) against ASF is the ultimate goal pursued for more than one hundred years (see Chapter 6) with the first attempts done by Montgomery, who unsuccessfully tried to vaccinate domestic pigs with inactivated ASFV and who also found that anti-ASF serum did not neutralise the virus (Montgomery, 1921). Research on vaccine development against ASF has been extensively reported by many authors covering, among others, details on the complexity of the ASFV particle, the large number of proteins encoded by its genome, the ASFV-host interactions, the mechanisms of protective immunity, and approaches for vaccine development (Arias *et al.*, 2017). Herein, a few aspects will be emphasised regarding viral-host interactions that may be considered as a cautionary route when evaluating vaccine design against ASF. First of all, the preferential replication of ASFV in pig macrophages is important. Numerous studies have been conducted using pig macrophages to characterise aspects of viral modulation of innate immune responses potentially relevant *in vivo*, thus ideally to be used as markers to test efficacy of different vaccine models. For instance, *in vitro* studies have shown that the viral infection did not alter the expression of Complement Fixation (Fc) receptors or the ability to mediate antibody cellular cytotoxicity; in contrast, phagocytosis, antibody mediated phagocytosis and chemotaxis were abrogated independently of the virulence of ASFV isolate (Martins *et al.*, 1987). However, a low virulent ASFV (ASFV/NH/P68 – NHV) was shown to induce an enhanced expression and production of relevant regulatory cytokines interferon (IFN) (IFN α), tumour necrosis factor (TNF) (TNF α) and interleukin (IL) (IL-12p40) on porcine macrophages *in vitro*, when compared to the effect of the virulent ASFV Lisbon 60 (L60) (Gil *et al.*, 2008). More recently, other studies have suggested that both L60 and NHV were able to inhibit interferon I (IFN I) production in macrophages, through a mechanism not dependent on IRF3 modulation (Portugal *et al.*, 2018), while macrophage responses to NHV have been associated with enhanced sensitivity to type I IFN and cytokine responses from classically activated macrophages (Franzoni *et al.*, 2020). Modulation of cellular IFN responses to infection with attenuated virus and induction of

different protective immunity has been demonstrated through deletion of genes from multigene families (MGF) on a virulent ASF isolate (Reis *et al.*, 2016).

ASF evolves frequently in domestic pigs as a contagious and usually acute haemorrhagic and lethal disease, although some pigs may survive, which is common when they are infected with low virulent ASFV isolates although, in such cases, chronic type infections may develop (Gallardo *et al.*, 2015; Leitão *et al.*, 2001). In general, pigs surviving ASF are resistant to challenge with homologous or, in some cases, with closely related virus isolates, suggesting the presence of a not yet fully understood efficient protective immunity against infection. On one hand, anti-ASF antibodies may interfere with disease development, as seen by reduced mortality, reduced virulence and a delayed onset of infection in pigs treated with those antibodies (Onisk *et al.*, 1994; Wardley *et al.*, 1985). However, conversely to what occurs in many different animal viral infections, antibodies do not neutralise the pathogenic capacity of ASFV so that vaccines can stimulate the development of the host humoral immune responses are not efficient. On the other hand, cellular immune mechanisms as ASF-specific cytotoxic T (cluster of differentiation) CD8⁺ lymphocyte and natural killer (NK) cell activities were shown to occur in pigs surviving experimental infection with low virulent NHV isolate (Leitão *et al.*, 2001; Martins *et al.*, 1988, 1993). The cytotoxic T CD8⁺ lymphocyte activity was seen to play an important role in ASF protective immunity in animals exposed to the low virulent OUR/T88/3 strain that were no longer protected from challenge with the virulent OUR/T88/1 isolate when depleted of CD8⁺ lymphocytes (Oura *et al.*, 2005). The above mentioned immune responses suggest that ASFV specific antibody alone is not sufficient for protection against ASFV infection, and that there is an important role for the CD8⁺ lymphocyte subset in ASFV protective immunity (Takamatsu *et al.*, 2013).

1.2 Where ASF-STOP stands

Soon after ASF emerged in Georgia in 2007 and carefully following the development of the situation in Russia, the international scientific community became aware of not only the approaching threat to the European pig industry but also the increased importance of wild boar in this epidemic. With the aim to understand the role of wild boar in ASF epidemiology, improve diagnosis and surveillance in this species and develop management practices that would decrease the role of wild boar in ASF epidemiology, a network of wildlife scientists, under the umbrella of the European Wildlife Disease Association (EWDA), came together to tackle the challenge of ASF expansion into new territories. A core group met at a workshop in Uppsala, Sweden, on 6-7 March 2014 to plan a proposal to the European Cooperation in Science and Technology (COST), a funding organisation under the Horizon 2020 programme of the European Union. The COST Action (Action is the term used by COST and it means 'networking project') ASF-STOP was launched on 1 May 2016. The main objective of the Action was 'to stop African swine fever from spreading further in Europe and protecting the European pig industry by combating ASF through a comprehensive, multi- and interdisciplinary approach'. The network included a broad variety of fields comprising, among others, virology, wild boar management, pathology, biosecurity, vaccinology and communication (Figure 1.1). The inclusiveness of the network led to the involvement of research institutions like universities and veterinary institutes side by side with animal health and food safety authorities, hunters' associations, wildlife managers and food

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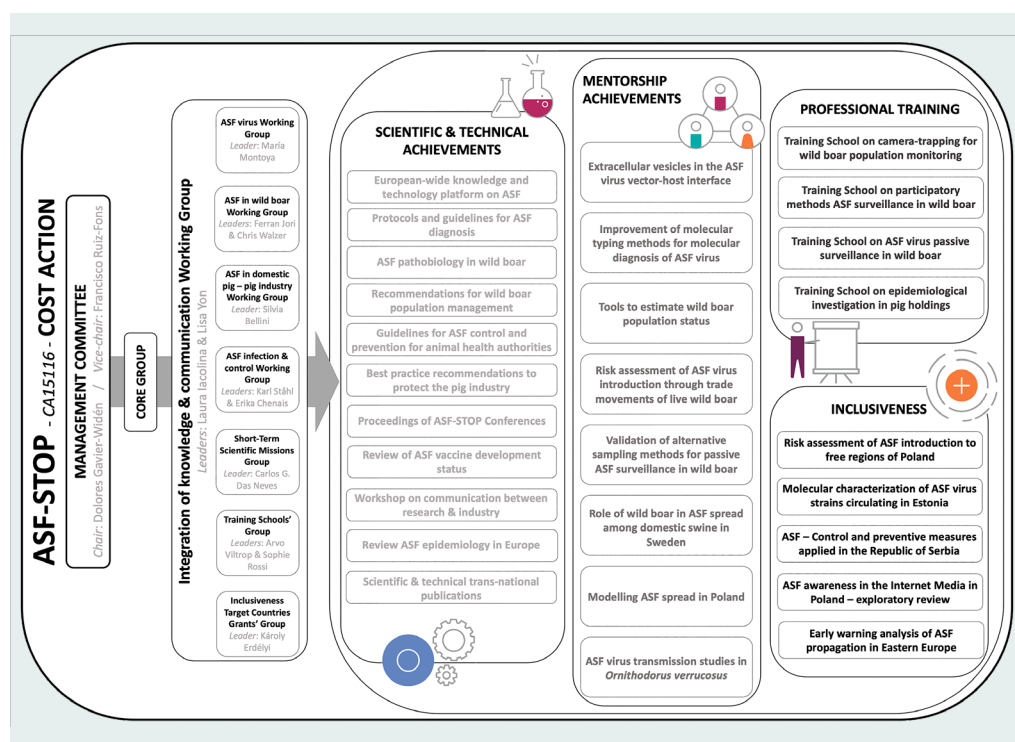


Figure 1.1. Structure of ASF-STOP and overview of the main outputs.

and livestock industries. Non-European partners also joined the network, shared their experience (from work in African and previously ASF infected countries) and contributed to the exchange of knowledge. Most European institutions conducting research on ASF participated in ASF-STOP, as well as many ASF national and international reference laboratories. Overall, the network included 37 countries; 17 of them were inclusiveness target countries in Europe, and three international Agencies, with a total of almost 300 participants (Figure 1.2). All worked together in a coordinated and integrated effort to collectively address the multiple challenges posed by ASF, moving towards the common goal of increasing preparedness and attempting to stop its spread. ASF-STOP worked in close collaboration with the Global African Swine Fever Research Alliance (GARA) and participated in the creation of a global research agenda (GARA <https://www.ars.usda.gov/GARA/>). In collaboration with GARA, to facilitate the flow of information and, at the same time, avoid unnecessary overlap or duplication of efforts, ASF-STOP created an inclusive environment where experts from different fields came together to complement each other's knowledge and build solutions.

The network worked together on coordinated objectives, which included all aspects connected to ASF (summarised in this book). High scientific excellence, active collaboration and integration of disciplines led to the achievement of the objectives. Special focus areas were the development of standardised methods, the identification of best practices applicable to different field conditions and the development of preventive measures, including advancing in vaccine development. Multidisciplinary teams worked in close collaboration to understand the role of wild boar in

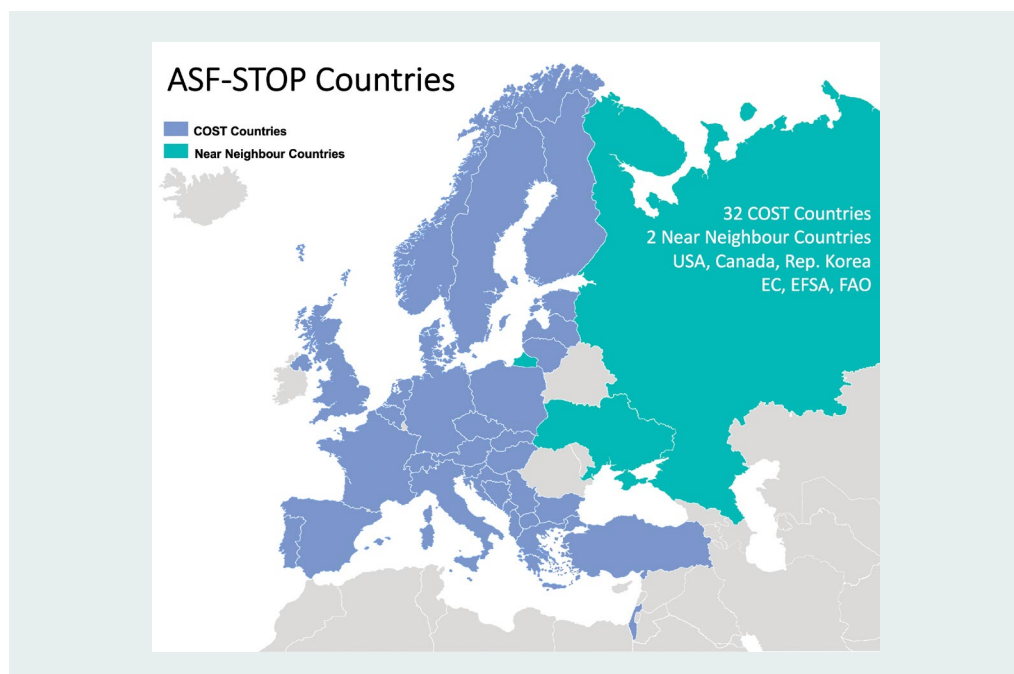


Figure 1.2. Map of the ASF-STOP participating countries.

virus transmission in different conditions and management practices. The teams designed control measures adapted to the different European habitats and wild boar densities. Leaders in virology, vaccinology, pathology, diagnostics and immunology came together to address aspects of the virus biology, its effects on the host, potential therapies and the best ways to detect infection in animals with varying levels of immunological status as well as in samples with different preservation conditions. This latter aspect is particularly relevant in the current epidemiological context where wild boar, as proven by the work of epidemiologists and wildlife biologists in the network, constitute a new epidemiological cycle for the virus in Europe and passive monitoring of wild boar carcasses has proven crucial for early detection of outbreaks. Close monitoring of the health status of wild boar is important for the prevention of introduction of ASFV into the pig production system. The pig industry and food production system are extremely variegated in terms of size, practices, needs and cultural importance. To protect this economically important sector and the welfare of the animals, experts in biosecurity, pig production systems and cleaning and disinfection collaborated with the pig and food industry and animal health and food safety authorities to identify potential risks for ASFV introduction and define guidelines specific for different branches of pig production, from industrial to backyard.

ASF-STOP also worked towards the following capacity building objectives: (1) creation of a Europe-wide scientific knowledge and technology exchange platform to prevent, control and mitigate ASF; (2) enabling informed decision-making based on current scientific data; (3) building capacity in Europe and strengthening trans-national collaboration; (4) achieving inclusive collaboration; (5) achieving wide dissemination of the outcomes and results of ASF-STOP; and (6) establishing a Pan-European research agenda.

Without the collective and integrated work of ASF-STOP the impact of all these efforts in research and development would have been mostly confined to specific fields. The continuous commitment to share this information within and beyond the network, communicating and engaging policy makers, breeders and farmers, hunters, wildlife managers and society as a whole largely contributed to the implementation of the knowledge and the impact of ASF-STOP. As a whole, ASF-STOP made important contributions towards the control and eradication of ASF in Europe, developed and communicated new knowledge, built an extended international network of scientists working on ASF and contributed to capacity building in Europe.

1.3 What ASF-STOP paved the way for

Within the four years of its life span, ASF-STOP has faced many challenges and resulted in even more success stories. A lot has been learnt but several gaps of knowledge have been identified in the process. This book aims to present the overview of the currently available knowledge on ASF, with special focus on the European situation, including but not limited to the results of the Action. This book is also a call for action for the scientific community to investigate those areas that still need to be explored, for policy makers and funding bodies to support those efforts and for society to actively engage in the global challenge of stopping ASF.

Recent research on ASFV (Chapter 2) has shown how the virus inhibits different immune responses and how multiple factors can lead to different lethality rates and that, although knowledge on the architecture of ASFV has progressed over time, more light needs to be shed on this aspect. At the same time, Chapter 2 shows how genome and computational methods could prove fundamental for the development of antiviral drugs and a vaccine.

The immune system is an organism barrier against harmful pathogens and while the host immune system reacts to ASFV infection, the virus interactions with macrophages or dendritic cells remain largely unknown, as is the immune response to ASFV in natural infections compared to experimental settings (Chapter 3).

Knowledge on pathology provides the foundation for understanding pathogenesis and host response to the pathogen. Disease caused by ASFV may range from peracute to subclinical or inapparent depending on different factors, among which the virulence of the isolates is particularly important. The presence and severity of lesions are highly variable and may affect multiple organs (Chapter 4).

Building on this body of knowledge on the lesions caused by ASF, the immunological processes and the characteristics of the virus several diagnostic tools have been developed with good sensitivity and specificity (Chapter 5). However, it remains of paramount importance to carefully follow guidelines for the choice of the most appropriate tool and sampling procedure based on the situation at hand, the field conditions and the requirements.

An aspect that has gained renewed attention over the past few decades is the development of a vaccine. Over time multiple approaches have been tested but most of them were either not

successful or led to undesired side effects; however, a number of promising candidates with good efficacy are currently being tested (Chapter 6), with the whole pig sector awaiting the results of large-scale trials. This industry is not only economically important but is also highly relevant for food security, which may be threatened by the ability of ASF to significantly reduce both the availability and affordability of adequate supplies of pork. To prevent the ravages of ASF, the industry has already undergone major rearrangements and changes, particularly in small size pig holdings that, together with backyard farms, are those most at risk (Chapter 7). For this reason, specific guidelines have been developed for the different farming premises with biosecurity information (Chapter 10) and best practices focusing on cleaning and disinfection procedures (Chapter 11) to prevent the introduction of ASFV into the pig holding. Both biosecurity and cleaning and disinfection procedures, if carefully and routinely implemented, have proven to be effective preventive measures even when the virus is circulating in the wild boar population around the farm.

The density and widespread distribution of wild boar in Europe has led to the identification of a new ASF epidemiological cycle between the wild boar and its habitat (Chapter 9). Several studies have reported the same epidemiological principles in both wild boar and domestic pigs and highlighted the important role played by humans in transmitting ASF to both wild and domestic pigs, thus confirming the paramount importance of correct implementation of biosecurity practices. At the same time, the identification of the wild boar-habitat cycle brought renewed attention to the need to manage the increasing European wild boar population (Chapter 8). Expertise developed in wildlife biology and management has been complemented with all aspects of ASFV transmission, survival and pathology to develop integrated approaches that combine management methods and pathogen transmission prevention practices. Positive field implementations in countries, such as Czech Republic and Belgium showed early detection and swift collaborative action involving all stakeholders can lead to control and, ultimately, eradication of ASF from wild boar populations.

Overall, the present summary of different relevant aspects of ASFV biology and viral-host interactions, acquired by different authors up to date, reinforces that to overcome the main challenges highlighted, certain major efforts should be pursued to prevent and control ASF. In Europe the following aspects are particularly important: improvement of sanitary and biosecurity measures in all operations regarding production of domestic pigs; control of WB populations responsible for maintaining and spreading the disease and above all, the development of an efficient vaccine.

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Figure 1.3. ASF-STOP final conference group picture (by G. Spalenza).

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