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Review

African swine fever – A review of current knowledge

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ABSTRACT

African swine fever (ASF) is a viral hemorrhagic disease with exceptionally high lethality in domestic pigs and Eurasian wild boar. Over the last decade, ASF has emerged in several European and Asian countries and has now an unprecedented distribution. Against this background, the presented review focuses on current knowledge and advances in ASF virology, clinical disease upon infection with recent strains, epidemiology, diagnosis, and control. This review highlights knowledge gaps and controversial opinions related to ASF.

1. Introduction

African swine fever (ASF) is a viral hemorrhagic disease with exceptionally high lethality in domestic pigs and Eurasian wild boar. Despite its limited host range and absent zoonotic potential, its socio-economic impact is very high and many stakeholders are involved. For this reason, the disease is notifiable to the World Organisation for Animal Health (OIE). In its worst-case scenario, the disease involves domestic pigs, reservoir hosts in wildlife, i.e. wild boar or other feral swine, inanimate fomites (e.g. carcasses, contaminated habitats, tools, other mechanical vectors), and competent arthropod vectors (soft ticks). Control measures rely on strict sanitary measures as neither a licensed vaccine nor any treatments are currently available.

In 2007, ASF, that has its roots in a sylvatic cycle in Sub-Saharan Africa, was introduced into Georgia. Subsequently, the virus spread to the Trans-Caucasian region and reached the Russian Federation. From Russia, the virus moved further and invaded the European Union in 2014. In August 2018, the disease also reached the world's largest pig producer, China, and is now spreading in several Asian countries. The latest affected countries were Papua New Guinea at the very doorstep of Australia, and India in 2020. Thus, over the last 13 years, ASF has gained unprecedented spread and importance and the current pandemic has impacted even distant industries. Especially the epidemic situation in Asia has revealed weaknesses in the veterinary and agricultural sectors, but also various direct and indirect links between the pig industry and the recycling and use of by-products. Not only is the supply of heparin and the availability of gelatin for food and confectionery affected, but also the utilisation of animal fats, skins and bristles. Furthermore, the careless use of porcine materials as a protein source for pigs works like a kind of "fire accelerator" for the epidemic.

Against this background, this review tries to summarize the available data, especially the knowledge gained over the last five years, and to draw conclusions on major knowledge gaps. For the latter, the recent gap analysis report of the Global African Swine Fever Research Alliance has been taken into account (the 2018 document is available online at: https://www.ars.usda.gov/GARA/reports.htm).

2. Virus properties

2.1. Virus characteristics and replication

The causative agent of ASF is African swine fever virus (ASFV), a large double-stranded DNA virus of the genus *Asfivirus* within the *Asfarviridae* family (Alonso et al., 2018). According to the 2019 taxonomy release of the International Committee on Taxonomy of Viruses (EC 51, Berlin, Germany, July 2019), the family *Asfarviridae* has been included into the order of *Asfuvirales* and the class of *Pokkesviricetes*. Apart from this official nomenclature, it has been controversially discussed to include ASFV into the tentative order of *Megavirales* containing the monophyletic but heterogeneous clade of nucleo-cytoplasmatic large DNA viruses (NCLDV) (Andres et al., 2020). This tentative group would also comprise *Poxviridae* (now in the new order of *Chitovirales*), *Iridoviridae* (now in the order *Pimascovirales*), *Asfarviridae, Phycodnaviridae* (now in the order of *Algavirales*), *Mimiviridae* (now *Imitervirales*), *Ascoviridae* (now *Pimascovirales*) and *Marseilleviridae* (now *Pimascovirales*) (Iyer et al., 2006). Due to the continuous discovery of new giant viruses like pandoraviruses (Abergel et al., 2015; Legendre et al., 2014; Philippe et al., 2013; Scheid, 2015), faustoviruses

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(Benamar et al., 2016; Reteno et al., 2015), molliviruses (Abergel et al., 2015; Christo-Foroux et al., 2020), kaumaoebavirus (Bajrai et al., 2016), cedratviruses (Rodrigues et al., 2018; Silva et al., 2018), and pacmanvirus (Andreani et al., 2017), this group will probably grow in the near future (Andres et al., 2020) and nomenclature will remain under discussion.

The virion has a very complex structure and an overall diameter of 175–215 nm. So far, it has been acknowledged that the virion consists of a nucleoprotein core (70 − 100 nm in diameter), a core shell surrounded by an internal lipid layer, an icosahedral capsid with 1892–2172 capsomers, and a dispensable lipid envelope (Alonso et al., 2018; Salas and Andrés, 2012). However, detailed knowledge on structure and architecture has still been missing.

Very recently, single-particle cryo-EM analyses of the three-dimensional structure of the ASFV particle have revealed that the nucleoid (viral genome and associated proteins, i.e. DNA-binding protein p10, pA104R, and parts of the transcriptional machinery) is in fact surrounded by two distinct icosahedral protein capsids and two lipoprotein membranes, one following the icosahedral symmetry surrounding the inner capsid, one the outer and originating from the budding process (Andres et al., 2020). The inner protein layer, organized as a T = 19 capsid, confines the core shell. It comprises proteins derived from the viral polyproteins pp220 (p5, p14, p34, p37, and p150) and pp62 (p35, p15, and p8). Due to the content of beta-strands, p15 could be the major capsid protein of this layer. The outer capsid (with a triangulation number of T = 277) forms a hexagonal lattice composed of 8280 copies of major capsid protein p72, arranged in trimers, and 60 copies of a penton protein at the vertices. In a nutshell, the extracellular virion consists of an outer envelope that is acquired by budding, the outer capsid, an icosahedral membrane, an icosahedral inner capsid, and the enclosed core shell and nucleoid (Andres et al., 2020). A schematic presentation of the structure is depicted in Fig. 1.

Recently, the complex capsid structure has been solved further showing that the major capsid protein (p72) and four stabilizing minor proteins (H240R, M1249L, p17, p49) are interacting as penta- and trisymmetrons (Wang et al., 2019). In the process of correct p72 formation (Liu et al., 2019a), B602L is needed as chaperone (Epifano et al., 2006a; Liu et al., 2019a).

The ASFV genome consists of a double-stranded DNA molecule of 170–190 kbp that contains between 151 and 167 open reading frames (ORFs), depending on the virus strain (Dixon, 2013). The ends of the genome show terminal inverted repeats and are closed by hairpin loops (Salas and Andrés, 2012). The genome encodes many proteins that are involved in virus assembly, DNA replication and repair. Moreover, proteins involved in immune modulation, e.g. interfering with type I interferon and cell death pathways, are encoded (Reis et al., 2017). About half of the ASFV genes still lack any known or predictable function (Alejo et al., 2018).

Up to very recently, 54 structural proteins were known based on two-dimensional analyses (reviewed by Salas and Andrés, 2012). As mentioned above, the outer envelope is acquired from the host cell through budding, and virus attachment protein p12 seems to localize there (Carrascosa et al., 1993). Another outer envelope protein that deserves attention is the EP402R gene product, CD2v. It shares sequence homology with the T-lymphocyte surface adhesion receptor CD2. This protein is required for the hemadsorption phenomenon and is probably important for pathogenesis but also for replication in the arthropod vector (Rowlands et al., 2009). It also interacts among others with adaptor protein 1 (AP-1) and could be involved in cellular traffic remodeling (Perez-Nunez et al., 2015). Attenuated strains often have truncated CD2v proteins (Karger et al., 2019). Another protein in the outer envelope is a cellular protein named p24. The main capsid component is p72, others are pB438L (p49), pE120R (p17), H240R, and M1249L (Andrés et al., 2001; Andres et al., 2020; Epifano et al., 2006b; Liu et al., 2019b; Wang et al., 2019). The inner envelope seems to be derived from the endoplasmic reticulum (Rouiller et al., 1998) and contains the membrane proteins p54, p17, p12 (is also found in the outer envelope), and pE248R. The so-called core shell, now defined as inner capsid (Andres et al., 2020) consists of pp220 and pp62 products as well as pS273R. The latter is the enzyme that processes viral polyproteins. Sequential cleavage results in p150, p37, p34, p14, p35, and p15. As mentioned above, p15 could be the main capsid protein of this inner capsid. The nucleoproteins in the core are p10 and pA104R (histone-like protein). The core is also supposed to contain the transcriptional machinery (reviewed by Salas and Andrés, 2012).

The use of proteomic tools has recently given rise to a better understanding of the viral proteins and virus host interactions (Karger et al., 2019). Along these lines, Alejo et al. (2018) could establish an updated atlas of the ASFV particle with the vero-adapted “BA71V” using mass spectrometry and immunoelectron microscopy to localize the detected proteins. In total, 68 viral proteins were identified, among them all previously known structural proteins (see above) and 44 so far unrecognized proteins. Interestingly, twenty-three discovered proteins could not be assigned to a function but present significant parts of the virion.

Another study used a recombinant derivative of OURT88/3 to study gene expression in mammalian cell lines of susceptible (wild boar) and non-susceptible hosts (human, monkey) (Kessler et al., 2018). Proteins could be identified for 94 out of 157 ORFs of OURT88/3. Interestingly, some of the most abundant proteins were also uncharacterized. Among them are pK145R and pI73R. These proteins are candidates for future analyses. Karger et al. (2019) summarized and compared both above-mentioned studies. It can be shown that for most predicted ORFs, the existence of the protein could be demonstrated. However, evidence of expression is still lacking for others, e.g. members of the multigene families. These might play a role only in the infected host or its primary target cells. This could also explain why these genes are quite often deleted in cell culture adapted ASFV strains (Krug et al., 2015).

As can be seen, the above-mentioned studies were done on permanent cell lines rather than the main target cells of ASFV. This could have

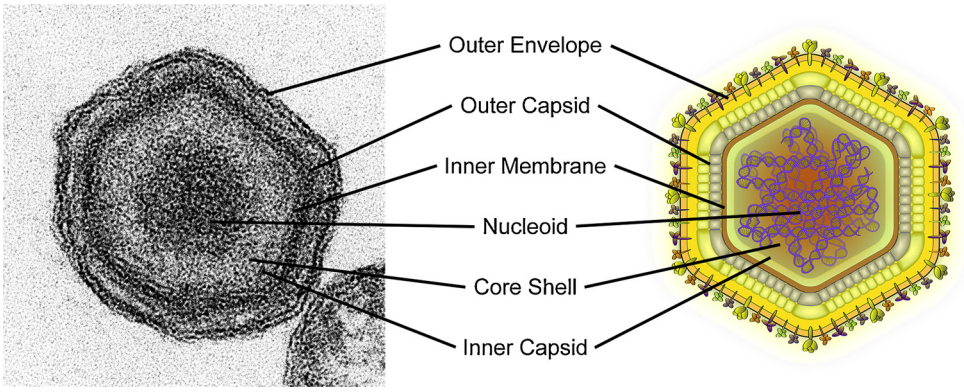

Fig. 1. Structure of African swine fever virus. Left hand side, Electron Micrograph of a chemically fixed ASFV particle embedded in resin. Right hand side, schematic overview of particle structure. The particle shows the typical icosahedral symmetry with a nucleoid containing the double-stranded DNA genome. The nucleoid is surrounded by the core shell and an inner and outer capsid, each capsid is enveloped by a lipid membrane (inner and outer membrane).

major implications for the outcome. The reason is practical. So far, a pure and large population of infected macrophages has been difficult to produce. However, sorting, synchronization and improved maturation protocols are under investigation for future studies.

African swine fever virus replicates primarily in the cells of the mononuclear-phagocytic system and entry takes place through clathrin-mediated and dynamin-dependent endocytosis and macropinocytosis (Galindo et al., 2015; Hernaez and Alonso, 2010; Sanchez et al., 2012). It has been shown that actin-dependent endocytosis and endocytic flux involving microtubule activity are also implicated, pointing to classical phagocytosis (Basta et al., 2010). The identity of the cellular receptor and its viral ligand are still unknown. A putative receptor, CD163, was controversially discussed (Lithgow et al., 2014; Sanchez-Torres et al., 2003), but CD163-knock-out pigs were still fully susceptible to ASFV challenge (Popescu et al., 2017). Among the surface markers that correlated with the susceptibility of adherent porcine bone marrow cells in another study was CD45 (Lithgow et al., 2014).

Upon entry, the entire endosomal pathway is needed for uncoating and the capsid disassembles at the acidic pH of the endosomal lumen (Cuesta-Geijo et al., 2012). After capsid degradation and membrane fusion, mediated by pE248R, viral cores are released into the cytoplasm. For release of the viral DNA, the ubiquitin-proteasome system is required. Replication and assembly take place in special virus factories close to the nucleus and the newly built virions are released from the infected cells by budding. For the strongly regulated transcription and RNA processing, virus encoded enzymes are needed. Classes of immediate-early, early, intermediate and late transcripts can be differentiated (Almazan et al., 1992, 1993). While the main part of viral replication takes place in the cytoplasm, an early nuclear stage is described (Rojo et al., 1999) that involves localized disassembly of the lamina network and redistribution of nuclear proteins (Ballester et al., 2011). Recently, the role of the nuclear phase has been reviewed in more detail and current knowledge compiled (Simões et al., 2019). It could be shown that ASFV infection activates DNA damage response (DDR) pathways that alter the nuclear landscape and cellular epigenetic signatures and facilitate effective infection and progeny formation (Simões et al., 2013, 2015). As an example, efficient ASFV replication depends on the interaction with the promyelocytic leukaemia nuclear bodies that are associated with cell cycle control, apoptosis and immune responses. Moreover, heterochromatin formation is induced, probably to silence host genes encoding proteins that could be detrimental for virus replication. These virus-induced rearrangements could probably explain the lamina breakdown mentioned above (Simões et al., 2019). Taken together, ASFV disrupts sub-nuclear domains and chromatin texture and induces nuclear architecture changes to enhance a repressive nuclear environment that allows efficient virus replication in the host cell. However, the full role of the nucleus is still unclear and needs further investigations.

Replication in general has recently been reviewed in more detail by Galindo and Alonso (2017). Transcription in particular was reviewed in detail by Rodríguez and Salas (2013).

2.1.1. Gaps and research needs

- Little is known about virulence genes, host range, and virus-vector-host interaction. High quality full-length genomic sequences of a broad spectrum of ASFV genotypes are needed to get deeper insights.
- The majority of the 150–170 ASFV ORFs are only predictions, and very few have any experimental evidence on either the RNA or protein level. Therefore, studies for a better understanding on viral proteins and their expression under different conditions are needed.
- There is also still a need to understand the host response side, especially viral receptors, innate immune responses and the interaction of the virus with the host on a cellular level.
- Research into the functionality of ASFV proteins should be promoted.

2.2. Tenacity and virus inactivation

The virus is highly stable in the environment and in raw pork products. Cool, moist and protein-rich environment favors survival. Thus, ASFV remains infectious in refrigerated meat for up to 15 weeks, up to six months in preserved ham, and 399 days in Parma ham (EFSA, 2009; McKercher et al., 1978; Mebus et al., 1993). In liquid manure, stability was observed for over 100 days. In liquid blood, the virus survives 18 months at room temperature and up to six years at 4 °C. A detailed listing is given in the "Scientific Report on African swine fever" published by the European Food Safety Authority (EFSA, 2009). Recent and ongoing studies have dealt with materials relevant for indirect transmission like feed, food, soil, and carcasses. For feed ingredients, studies by Stoian et al. targeted virus stability upon transoceanic shipping. The calculated half-life under these conditions ranged from 9.6 to 14.2 days depending on the matrix (Stoian et al., 2019). In addition, experimental studies showed that transmission through contaminated liquid and dry feed is possible (Niederwerder et al., 2019). Dose and repetition are important factors when looking at the outcome of these studies and additional studies are needed. In a laboratory scale study on the stability of ASFV on contaminated crops, the effect of drying and heat inactivation was explored. In summary, ASFV was already inactivated by drying at room temperature for two hours (Fischer et al., 2020). With regard to stability in carcasses, a study has been carried out recently in Lithuania. Carcasses of ASFV infected wild boar that had been buried at different time points and locations, were excavated and retested for the presence of infectious ASFV by *in vitro* assays and for viral genome by qPCR. Unexpectedly, only viral genome was found while all virus isolation attempts gave negative results (Zani et al., 2020). The role of soil was targeted by a recent, so far unpublished study (Carlson et al., submitted). In brief, different soil matrices spiked with ASFV-positive blood from infected wild boar were investigated for viral stability. Soil pH, structure, and ambient temperature played a significant role for the stability of infectious ASFV. Re-isolation was possible for a few weeks from sand or garden soil and for some days from soil of a swampy area. No virus was re-isolated from rather acidic forest soils. Mitigation attempts with citric acid or calcium hydroxide resulted in complete inactivation in all soil types.

An additional question that was raised was inactivation of ASFV in biogas plants that use pig slurry. The stability of ASFV in faeces and urine of experimentally infected animals was investigated by Davies et al. (2017). Based on the calculated half-lives, it can be assumed that ASFV remains infectious for almost four (urine) or three (faeces) days at 37 °C. In the context of a biogas plant, secondary factors such as pH and metabolites would be added, so that these times could be corrected downwards. In a work by Turner and William it was shown that at 40 °C inactivation in pig manure is achieved after 4 h. A PhD thesis published in 2001 (Moss, 2001) came to the conclusion that ASFV is inactivated in a correctly operated biogas plant within hours (thermophilic plant process) or days (mesophilic plant process). Nevertheless, it must be taken into consideration that these plants are usually not designed in such a way that a strict black/white separation can take place (in contrast to high security laboratories or modern rendering plants). In plants, which are e.g. followed by a fertilizer plant, it can be assumed that recontamination would also be eliminated, because the fermentation substrate is reduced under vacuum.

Various disinectants are in use worldwide. Agents based on peracetic or formic acid have shown good efficacy under experimental conditions (Fauser-Leiensetter, 2000). In general, ASFV is sensitive to lipid solvents and detergents as well as oxidizing agents. The EFSA published a Scientific Report (EFSA, 2009) on the available data on notified biocides efficacy under field conditions (compared to sodium hydroxide and sodium carbonate). In principle, it can be assumed that agents which show good efficacy for other enveloped DNA viruses are

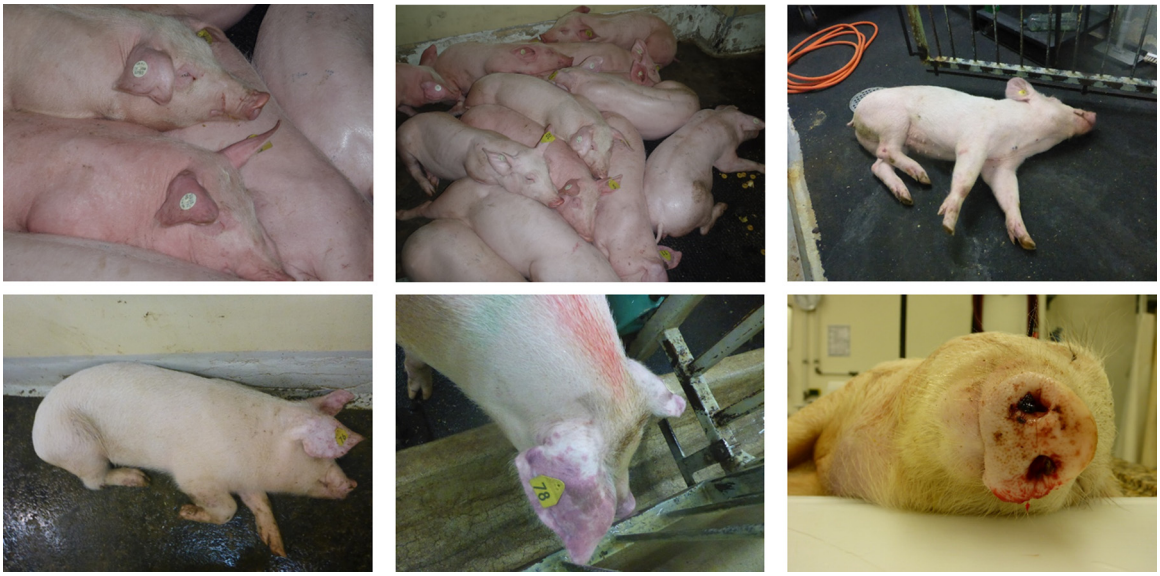

Fig. 2. Clinical signs of domestic pigs upon infection with highly virulent ASFV strains. First signs are observed app. four days post infection. They include high fever, reluctance to move, inappetence, and huddling (upper row left and center). Some animals develop conjunctivitis and gastro-intestinal signs (vomiting, diarrhea). With progression of the disease, animals become somnolent (lower row left), appear desorientated, and show dyspnea. In the final phase, affected animals may show seizures (upper row right) and haemorrhages (skin haemorrhages in the lower row center, epistaxis lower row right).

also effective against ASFV.

Gaps and research needs:

- The role of feed, water, and bedding for ASFV transmission is still controversially discussed and needs further research.
- The need of disinfection measures for carcass sites is still an open question and further stability tests e.g. using different soil types are necessary for improved risk assessments.
- While disinfectants against enveloped viruses work against ASFV under standard conditions, there is a need to find practical ways to test and select disinfectants for ASFV in resource limited settings and difficult surface structures (like wood, open concrete or other types of flooring).

2.3. Genetic diversity and typing

Over the past decades, different genetic regions have been used to type ASFV strains by partial sequencing of small DNA fragments. The region used for genotype denomination is most often the p72 encoding region. Enhanced discrimination is obtained through sequencing of the p54, p72, and pB602L genes (Gallardo et al., 2009). Based on this system, 24 genotypes of ASFV have been identified in Africa (Boshoff et al., 2007). The genetic diversity is promoted through the sylvatic cycle and does not exist outside these regions (Wade et al., 2019). Moreover, genotypes do not relate to virulence or pathogenicity. "International" genotypes are I and II.

In general, ASFV is very stable and shows a very low mutation rate (Dixon et al., 2020). This leads to low genetic variability in affected regions and even the use of next-generation sequencing does usually not allow molecular tracking of virus strains in a higher resolution e.g. for molecular epidemiology in an epidemic situation (Forth et al., 2019b). However, only full-genomes are probably able to show a certain discrimination and to deduce virulence factors. Also new strain variants with deletions are best identified by whole-genome sequencing (Zani et al., 2018). Several protocols have been optimized over the last decade including long-fragment Oxford Nanopore MinION sequencing (O'Donnell et al., 2019).

In the current epidemic situation, new sequences are generated every day and quality control is of upmost importance. Otherwise, the error-rate of sequencing could be higher than the error-rate during virus replication, and sequencing errors are mis- and over-interpreted as significant differences. Recently, a deep-sequencing workflow has been established for reliable and high-quality whole-genome sequences (Forth et al., 2019a). This workflow is based on target enrichment and use of different sequencing platforms to circumvent particular drawbacks. International collaboration is key to provide reliable data for future research.

Gaps and research needs:

- Efforts must be made to optimize and harmonize protocols for sample selection, sequencing, bioinformatics workflows, and data documentation to use the financial and technical resources most efficiently.
- In a targeted approach, whole-genome sequences for all available genotypes and host species should be generated as a basis for further studies.
- The discovery of viruses related to the family *Asfarviridae* should be expedited to allow e.g. evolutionary analyses.

3. Clinical signs and pathomorphological lesions

3.1. Clinical signs in susceptible hosts

The clinical signs of ASF are highly variable and depend on the virulence of the strain and the age and immune status of the animals. Apart from acute diseases resembling haemorrhagic fever, chronic and subclinical courses can occur.

The causative virus strains in Europe (apart from Sardinia) and Asia are of genotype II, highly related, and show high virulence for both domestic pigs and European wild boar under experimental conditions (Blome et al., 2012; Gabriel et al., 2011; Guinat et al., 2016; Mur et al., 2014; Nurmoja et al., 2017; Pietschmann et al., 2015; Pikalo et al., 2020). Highly virulent strains cause acute to peracute disease with up to 100 % lethality within 7–10 days. The clinical signs are often non-specific and include high fever, anorexia, respiratory and gastro-intestinal signs, cyanosis, ataxia, and peracute death. Pregnant sows can abort due to severe disease and high fever. Hemorrhagic symptoms have also been observed in a few cases. Images of experimentally infected animals are shown in Fig. 2 (domestic pigs) and Fig. 3 (wild boar). The experimental studies have been reviewed and summarized in

Fig. 3. Clinical signs in wild boar. The signs resemble the courses in domestic pigs. Depression and reduced liveliness are seen in most animal (see upper row left and center, lower row right). The same is true for dyspnea (the animal in the upper row right showed severe respiratory distress). Hind leg paresis (lower row left) and seizures (lower row center) can be observed in the final stage.

two publications recently (Pikalo et al., 2019; Sanchez-Cordon et al., 2019).

Moderately virulent strains lead to an acute clinical picture with high fever, anorexia, fatigue and non-specific respiratory and gastrointestinal symptoms. Pregnant animals can abort. The mortality rate in this case is 30–70 %. Low virulent strains show subclinical and chronic courses with unspecific symptoms and low mortality. Antibodies are formed after 7–10 days, but these are not predictive for disease outcome and are not able to completely neutralize the virus.

3.2. Pathomorphological lesions

The pathological-anatomical findings depend on the course of the disease and reflect the above-mentioned variability in the clinical presentation (Sanchez-Vizcaino et al., 2015). Among the findings recorded after infection with the Eurasian ASFV isolates were enlarged haemorrhagic lymph nodes in the liver/stomach area and varying degrees of splenomegaly. In addition, petechiae in kidneys, bladder and stomach wall as well as pulmonary edema and haemorrhagic gastritis have been observed. It is not too rare that only a few hemorrhagic lymph nodes are found (Sanchez-Vizcaino et al., 2015). To harmonize pathological investigations for pathogenesis studies and vaccine trials, Galindo-Cardiel et al. (2013) developed a score that can help to make these trials more comparable.

Gaps and research needs:

- Factors influencing the ASFV-related disease outcome and course are still poorly understood and research should target both major beneficial and detrimental host responses.
- The controversially discussed potential role of survivors and carriers in disease epidemiology must be studied in more detail.
- Improved clinical and pathological scoring schemes and use of state-of-the art diagnostic techniques would be most helpful for comparative pathogenesis and vaccine studies.
- A harmonized standard ASFV challenge model for both domestic pigs and wild boar should be developed.

4. Current situation and epidemiology

4.1. Current situation

When ASF was introduced into Georgia in 2007 and subsequently into several Trans-Caucasian countries and the Russian Federation, an exotic disease became a tangible threat to the European Union's pig industry and wild boar population (Sanchez-Vizcaino et al., 2013). Today, several European countries are affected and the virus has spread to Asia where it has been wreaking havoc since autumn 2018. The following countries are currently affected in the EU: Belgium, Bulgaria, Estonia, Greece, Hungary, Latvia, Lithuania, Poland, Romania, Serbia and Slovakia (Animal Disease Notification System of the European Commission, visited online 13th of April 2020; https://ec.europa.eu/food/animals/animal-diseases/not-system_en). Moreover, Ukraine, Moldova and Russia still report outbreaks. By the beginning of April 2020, the following Asian countries had reported ASF: China, Hong Kong, North Korea, South Korea, Laos, Vietnam, Myanmar, Cambodia, Indonesia, Philippines, Timor-Leste,Papua New Guinea, and India (OIE WAHIS, visited online July 5th 2020, see Figs. 4 and 5).

4.2. Epidemiology

The host range of ASFV is very narrow with suids as sole vertebrate hosts, and soft ticks of the genus *Ornithodoros* as competent arthropod vector (Jori and Bastos, 2009; Jori et al., 2013). ASF has no zoonotic potential and there are no indications that this might change (Dixon et al., 2020). Among the reasons for the assumption that the evolution of a zoonotic potential is unlikely are the accurate proofreading of the DNA polymerase and virus-encoded base excision DNA repair system that leads to low mutation rates and the lack of possible recombination partners (there is no known related virus in suids that ASFV could recombine with).

The disease has its roots in sub-Saharan Africa where it is transmitted in an ancient sylvatic cycle among warthogs and Ornithodoros soft ticks, making ASFV the only DNA arthropod-borne (ARBO) virus. This cycle is not accompanied by overt disease or mortality in warthogs and would probably go unnoticed. Other African wild suids (especially bushpig species) also show resistance (Jori et al., 2013). However, any introduction of the disease into the domestic pig sector via ticks or

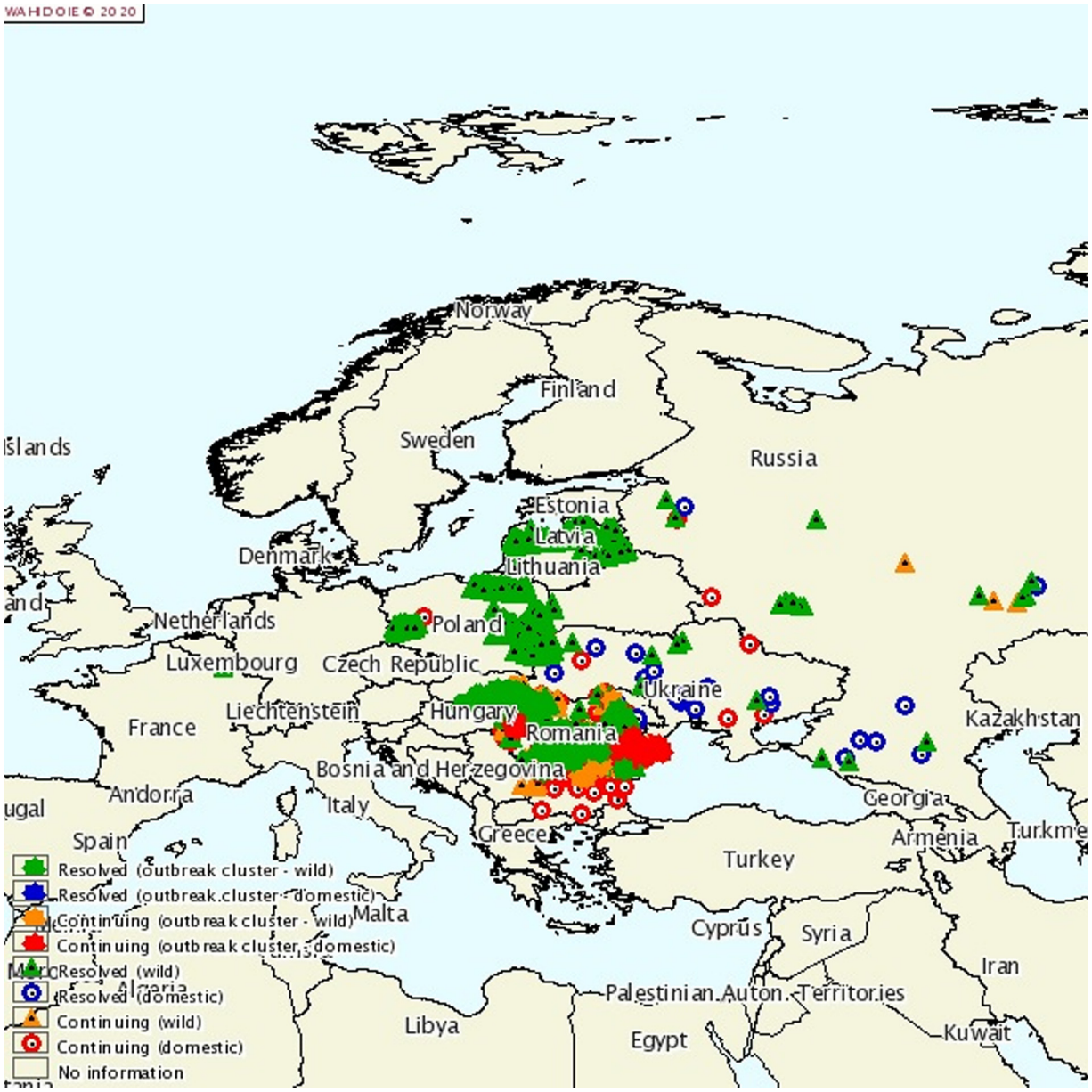

Fig. 4. ASF distribution in Europeas of July 2020 (source: OIE WAHIS, visited online July 5th 2020).

fomites leads to the above-described severe multi-systemic disease with high lethality. Ornithodoros soft ticks of another species complex (*O. erraticus*) were involved in outbreak scenarios on the Iberian Peninsula (Boinas et al., 2011; Oleaga-Perez et al., 1990). For the current global outbreak scenario, tick involvement does not seem to play any role. However, with the involvement of new countries and the emergence of tick species in new habitats, this might change.

Once introduced into the domestic pig population, the virus does not need its arthropod vector for transmission. ASFV can be transmitted by direct contact between infected and susceptible animals and by indirect contact to contaminated objects or feed (Chenais et al., 2019). Contaminated pork (swill feeding) and also blood products used as protein source can play an important role (Wen et al., 2019). Moreover, fomites such as clothing, trucks and veterinary equipment (especially vaccine pistols and similar objects) may act as source of infection. In the wild boar habitat, carcasses are crucial in maintaining infection cycles (Depner et al., 2016). Moreover, persistently infected carrier animals have been discussed as an important factor for viral maintenance, especially in an endemic situation. The role of such persistently infected animals in long-term transmission is still controversially discussed (Stahl et al., 2019). Some of the controversy around "persistence" of ASFV is probably rather a matter of definition. Beyond doubt, virus and especially viral genome can be detected in surviving animals for a rather long time (Petrov et al., 2018). In the absence of truly neutralizing antibodies, virus can still be isolated from survivors for roughly 60–70 days. Viral genome can be detected even longer (~100 days). However, there is no evidence for a major role of such carriers from field experience and long-term studies (Stahl et al., 2019). The latter showed a) no transmission to sentinels and b) no virus in survivors beyond 100 days (Nurmoja et al., 2017; Petrov et al., 2018).

For surveillance actions, there are different categories of animals that should be clearly defined (at present data bases do not distinguish between pathogen and antibody detection): Animals that are positive only in pathogen detection methods should be regarded as animals in the early phase of infection (from experimental data this would be between days three and ten post infection). With high certainty, these animals can transmit the virus. Animals that show both virus and antibodies have a certain probability to shed and transmit virus. From

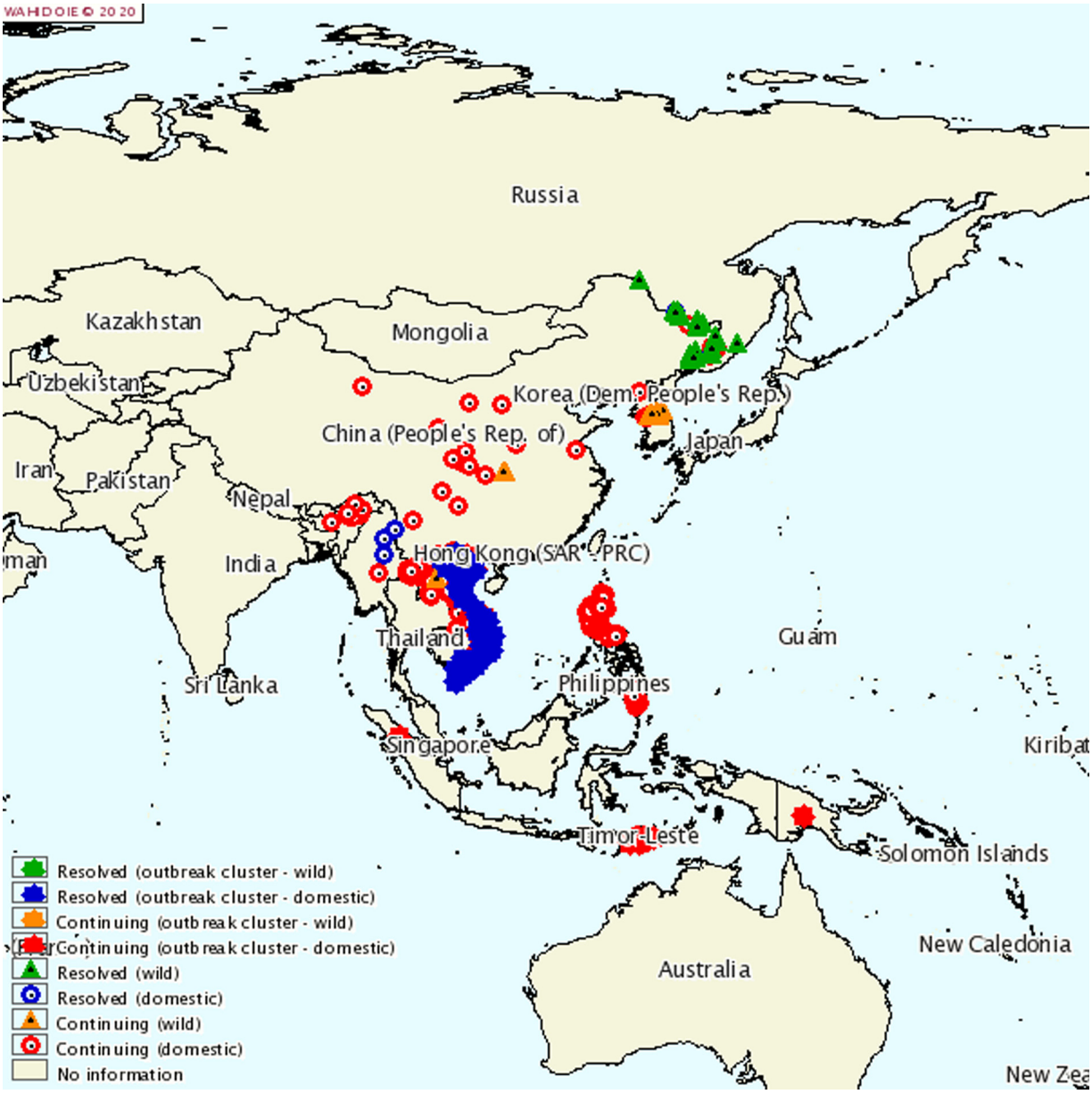

Fig. 5. ASF distribution in Asia as of July 2020 (source: OIE WAHIS, visited online July 5th 2020).

seven to ten days post infection to almost 100 days post infection, animals can show such a behavior. As antibodies do not have a clear predictive value for disease outcome, these animals can still die from ASF, but they have at least survived for one week or more. The last category are animals with only antibodies. These animals are the true long-term survivors and should not be called persistently infected, as no virus was detected in relevant samples. These animals are probably protected from reinfection and thus very safe. It cannot be excluded that a couple of genome copies are still present somewhere in the lymphatic tissues. However, given the rather high dose usually needed for oral infection, i.e. 10,000 heamadsorbing units (McVicar, 1984), the impact should be low. However, both duration of immunity and possible reactivation scenarios need further investigation

While it is very clear that soft ticks do not play a major role for the current epidemic (at least so far), mechanical vectors including carrion-eating predators, birds and blood-sucking arthropods are controversially discussed. Detailed studies on predators and birds are missing, but the virus does not survive the conditions of a gastro-intestinal passage and these animals are not considered a host. A mechanical function cannot be completely excluded but was considered a minor risk factor after evaluation of game camera data (Probst et al., 2019). Given the summer peaks observed in several countries, arthropods are in the focus of investigations (Fila and Wozniakowski, 2020). Flies, tabanids, mosquitoes, and hard ticks were collected in Baltic areas with ASF in wild boar. In this context, viral genome was not detected (unpublished results). Under experimental conditions, no indications were found that e.g. larvae of blowflies would play a major role (Forth et al., 2018). Other studies showed that ingestion of ticks and stable flies can lead to infection (Olesen et al., 2018; Pereira De Oliveira et al., 2020). However, to be precise, these studies only show that highly contaminated objects can be a source of infection, which is not surprising and is not a special characteristic of the used arthropods. Any other surface, e.g. a cotton ball, could have the same effect, and a particular vector function for a particular arthropod species other than soft ticks is not shown. The stable fly itself is relevant for ASF research, since only for this fly it was shown many years ago that it carries infectious virus over a certain period of time and can transmit it mechanically (Mellor et al., 1987). The proboscis of this biting fly is large

enough to temporarily store a sufficient amount of blood for an infection (for parenteral infection, as little as 0.13 heamadsorbing units 50 % are needed, (McVicar, 1984)). A relevant period of 24 h is specified for ASF. However, the stable fly usually does not fly over long distances. In another study, viral genome was confirmed in arthropods from affected stable units (Herm et al., 2020). This is also not surprising. At present, one could summarize that the role of arthropods seems rather limited for disease spread across holdings or areas. Nevertheless, within a pen or stable of the same farm, or a smaller affected region, their role cannot be excluded.

To understand disease dynamics in the different transmission cycles, detailed knowledge on actors and value chains is needed. This is particularly important for the domestic pig cycle and, due to the variability in farming practices, there is no blueprint that fits all situations. A detailed review on epidemiology and control of ASF has recently been published (Dixon et al., 2020). The following section uses some of the main points discussed there.

In the absence of a vaccine, biosecurity is key in preventing introduction and spread of ASF. Unfortunately, the path of introduction can often not be determined with certainty. This leads to the wrong perception that biosecurity has not been effective after all and that there is no need to change behavior. Good communication strategies are necessary. The same applies to the fact that the virus persists in the environment and also in pork products for such a long time that even after a longer period of time it still poses a risk. Unfortunately, the picture is not black and white and many transmission paths only become significant through the high frequency of exposure. These facts render precise predictions impossible.

Socio-economic factors are also crucial. There are still many parts of the world with small-scale pig production, often for own consumption. Under these settings, biosecurity is low and swill feeding common practice. FAO and OIE have drafted manuals on how to mitigate disease risks under these circumstances and control strategies should find appropriate solutions for traditional farming (Good practices for biosecurity in the pig sector, http://www.fao.org/3/a-i1435e.pdf; Preparation of African swine fever contingency plans, http://www.fao.org/3/a-i1196e.pdf).

Gaps and research needs:

- The potential role of mechanical vectors for ASFV transmission must be studied furtherincluding e.g. arthropods, birds, and predators
- The role of different environmental factors on the presence of ASFV in the habitat of wild boar needs additional in detail research.
- The role of ASFV survivors and potential carriers is understudied as well as the duration of immunity and the duration of maternally derived antibodies.
- There is a need to continue molecular epidemiology studies to monitor both captive and wild suid populations as well as soft ticks for the phylogeny and evolution of ASFV and related viruses.

5. Diagnosis

5.1. State-of-the art and obstacles

Rapid and reliable diagnosis is of paramount importance when it comes to early warning, timely intervention, and monitoring of ASF (Arias et al., 2018). The optimal assay should allow detection of all ASFV genotypes and variants, detection of infected animals with high sensitivity and specificity, should be validated according to the OIE guidelines, should be easy to handle, should allow swift interpretation, should be available at reasonable costs at all times, should be adaptable to high-throughput application. Such assays would also be needed to accompany potential DIVA (differentiating infected from vaccinated animal) vaccines. It is clear that not all characteristics can be met at the same time, and for this reason diagnostic workflows should be established that consider the advantages and disadvantages of the implemented assays. This may include the use of pen-side approaches but also alternative and/or non-invasive sampling strategies (Carlson et al., 2017). The latter are of special interest for the diagnosis of ASF in the wild boar population. In this context, oral-fluid based methods, swab samples, and baiting strategies have recently been investigated and have shown potential for swine fevers (Blome et al., 2014; Carlson et al., 2017; Dietze et al., 2017; Mur et al., 2013; Nieto-Pelegrin et al., 2015; Petrov et al., 2014).

Due to the international notification requirement, laboratory diagnosis is regulated in recommendations and legal requirements. Methods and protocols can be found in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2019) or, for the European Union, on the website of the European Union Reference Laboratory for ASF (https://asf-referencelab.info/asf/en/procedures-diagnosis/sops). Given the historical importance of ASF in some European Union Member States, in particular Spain and Portugal, guidelines and legal documents are quite mature and can act as blueprint also for scenarios outside Europe.

In general, reliable tools for the direct and indirect diagnosis of ASF exist that work with appropriate samples from both domestic pigs and wild boar. There is no difference in test performance when testing high quality samples from either domestic pigs or wild boar (they are in fact the same species). These assays include virus isolation on porcine macrophages, (real-time) polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), immuno-blotting, and indirect immunostaining techniques (OIE, 2019). However, up to very recently, a majority of these test systems was based on “in-house” protocols and comparability was only guaranteed through the use in inter-laboratory comparison tests. For resource limited countries, fully validated and easy-to-use test systems are beneficial. Here, commercial kits with reasonable pricing and low need of extra consumables are key.

The current situation and methodology-oriented research projects have improved the situation and several ELISA, qPCR and also Lateral Flow Assay (LFA) kits have been brought to the market. In fact, there is a growing number of commercial PCR and ELISA tests for the diagnosis of ASF. Manufacturers are distributed all around the globe. While most of the tests will probably work fine, comparable data are not always present. As Germany has an official licensing process for any kits applied in the country for notifiable and reportable animal diseases, basic data exist for the licensed kits and an updated list can always be found in the German Official collection of methods for notifiable diseases (https://www.fli.de/en/publications/amtliche-methodensammlung/). So far, eight commercial real-time PCR systems have been approved in Germany and are applied in regional laboratories in the context of exclusion diagnostics and monitoring. All these tests have been tested at the German national reference laboratory (NRL) with a defined set of experimental samples representing different genotypes (mainly I and II), host species, matrices, and infection status. The following PCR tests have been found fit-for-purpose: INgene q PPA (Ingenasa), virotype ASFV (Indical Bioscience, former Qiagen Leipzig), ID Gene ASF Duplex (IDvet), RealPCR ASFV (IDEXX), SwineFever combi (gerbion), ViroReal Kit ASF Virus (Ingenetix), Kylt ASF Real-Time PCR (Anicon), VetMAX African Swine FeverVrius Detection Kit (Thermo Fisher Scientific). Some of these commercial PCR tests and three routinely used automated extraction methods have been compared at the German NRL in more detail (Schlottau, unpublished). Nucleic acids were extracted using the NucleoMag® VET (Macherey - Nagel), MagAttract Virus M48 (Qiagen), and MagMAX™ CORE (Thermo Fisher Scientific, workflow C) kits on the KingFisher extraction platform (Thermo Fisher Scientific) according to the manufacturer’s instructions. In general, all kits were suitable and gave reliable results in downstream applications. In terms of PCR kits, the virotype ASFV PCR Kit (Indical Bioscience), the INgene q PPA Kit (Ingenasa), the RealPCR ASFV DNA (IDEXX), and the ID Gene™ African Swine Fever Duplex (ID.vet) were compared among each other and to the OIE recommended test by King et al. (2003). All kits gave reliable results with slight variability when using weak samples.

Recently, data have been published on the comparison of seven commercially available PCR kits and three polymerase reaction mixes (Schoder et al., 2020). Here, the following kits were included: virotype ASFV 2.0 PCR kit, (Indical Bioscience), Adiavet ASFV Fast Time (Adiagen), Bio-T kit ASFV (Biosellal), VetMax ASFV Detection kit (Thermofisher), RealPCR ASFV DNA Test, (IDEXX), VetAlert ASF PCR Test Kit (Tetracore), and the ID Gene™ African Swine Fever Duplex (ID.vet). In brief, sensitivity and specificity were tested on 300 well-characterized wild boar samples collected in Belgium during the 2018–2019 outbreak. This study confirms that all commercial kits and two out of three Taq polymerases (AgPath-ID™ One-Step RT-PCR Reagents, Applied Biosystems, and TaqPathTM 1-Step Multiplex Master Mix, ThermoFisher) are suitable for ASFV detection in diagnostic laboratories. This is in line with the above experience and confirms the suitability of commercial kits for rapid and user-friendly diagnosis.

For antibody detection, three commercial ELISA systems are currently licensed in Germany for the serological diagnosis of ASF. The test systems use different antigens and may therefore be used in parallel. In addition to a blocking ELISA based on p72 (INGEZIM PPA COMPAC, Ingenasa), an indirect test system is available that uses an antigen mixture of p72, p62 and p32 (ID Screen® African Swine FeverIndirect ELISA, IDvet). In addition, a competitive ELISA based on p32 (ID Screen African Swine Fever Competition, IDVet) has recently been approved. Internationally, there are various test systems whose performance is not easy to assess.

Gaps and research needs:

- Virus isolation techniques must be improved and further optimized cell lines should be developed to enable detection and sufficient replication of field virus strains.
- Antibody and virus growth kinetics in ASFV survivors as well as persistently infected domestic pigs and wild boar should be investigated in more detail.
- Virus persistence in the environment should be further analysed.
- Harmonized genetic markers of ASFV and further agreement on genome regions for harmonized analyses are needed.
- Improved non-invasive sampling techniques and point-of-care/pen-site diagnostics are necessary for an optimized disease control
- In general, diagnostic methods, protocols and techniques should be further harmonized, standardized and disseminated. Standard operating procedures (SOPs) for ASFV-diagnostics should be available as a global standard.

In conclusion, our tool box of diagnostic tests has grown considerably over the past years but there is still a need for harmonization, situation-adapted diagnostic workflows, and general knowledge of disease biology that helps us in further adjusting our methodologies (see also GARA gap analysis report, https://www.ars.usda.gov/GARA/).

6. Control

6.1. Control options

A binding legal framework exists for surveillance and control in most countries with substantial pig production. Integral parts of the control measures are timely and reliable diagnosis, stamping out of infected herds, establishment of restriction zones, movement restrictions, and tracing of possible contacts. Prophylactic vaccination and other treatments are still not available but would be strictly prohibited in the EU and other countries. An example of the legal framework is the legislation at EU level that is based on the experience gained during the outbreaks in the 1960ies to 1990ies. While it may not be applicable in resource limited settings, the basic principles can be applied worldwide. For this reason, some general points are outlined here. At present, Council Directive 2002/60/EC lays down the minimum Community measures for the control of ASF. Chapters are dedicated to measures in case of a suspicion, measures in case of a confirmed outbreak, measures in contact holdings and epidemiological inquiries, the establishment of protection and surveillance zones, cleaning and disinfection, measures that are taken to lift restrictions and for repopulation, and details special cases such as suspicions at slaughterhouses. Commission Decision 2003/422/EC details the control measures and provides guidelines and minimum requirements on diagnostic procedures, sampling methods and criteria for evaluation of the results, biosafety requirements, and principles and applications of laboratory tests. The legal framework at EU level is under revision and will be replaced by a new animal health law in 2021. However, the basic principles will stay in place.

Implementation of control measures depends on the resources of the veterinary service and compliance with these measures. A critical point for compliance with measures is a timely and appropriate compensation scheme. As can be seen in Africa and Asia, the lack of compensation leads to selling of sick pigs to slaughterhouses and markets resulting in large outbreak scenarios (Dixon et al., 2020).

For the wild boar cycle, local situations must be taken into consideration when designing control measures. Currently, the example of the Czech Republic is often used, as this is the only case where the disease was completely eradicated from a country's wild boar population (so far). Based on these successful measures (EFSA, 2018), a control strategy could involve the following: Zonation determining an infected zone and surrounding buffer and control zones. These zones should be established as soon as possible and should be sufficiently big. Carcasses should be searched and disposed of. Incentives for carcasses found can increase compliance and success. In the infected zone, strict hunting rest should be applied. Trapping can be allowed if established and appropriate. Fencing has proven successful and can be applied adapted to the local situation. Fences are not pig proof but can slow down the spread to a minimum. Where possible, bans on entry can be implemented. Reduction of wild boar in the surrounding area can be applied but must be decided individually for each case.

As soon as a more endemic phase is reached, zones should be adjusted and elimination of remaining wild boar can be discussed (where feasible). Applicable animal welfare and risk factors must be taken into consideration. The population of wild boar outside the infected zone should be reduced. A quite similar approach was taken by the Belgian authorities, with apparent success (https://www.wallonie.be/fr/peste-porcine-africaine).

7. Gaps and research needs

- Control in rural areas and under backyard settings has proven to be very difficult and adapted strategies - with a high level of acceptance in the respective regions - should be designed and applied.
- Control measures for affected wild boar populations need further optimization and must take different regional conditions into account.
- Lessons learned from successful eradication and control programs e.g. in the Czech Republic and Belgium should be analysed and used as blueprints for the control of future outbreak scenarios.

7.1. Vaccine approaches

7.1.1. African swine fever vaccines

To date, safe and efficacious vaccines against ASF are still lacking and production of most vaccine candidates is massively hampered by the simultaneous lack of a permanent cell line that is sufficiently susceptible to ASFV and does not force further genetic adaptations within the ASFV genome. However, vaccine design seems still feasible due to the fact that animals recovering from acute ASF are protected against challenge infection with closely related strains (Detray, 1957, Mebus and Dardiri, 1980, King et al., 2011, Penrith, 2009). Problems in

developing a vaccine are partly due to the fact that antibodies produced by the affected animal cannot really render the virus harmless (no complete neutralization). Furthermore, many components that would be important for the establishment of a favorable immune response are not yet fully characterized in localization and function, so that it is challenging to produce vaccines based on individual components or antigens expressed by vectors. Inactivated virus preparations have shown no substantial protective effect, although antibodies were formed against the virus (Revilla et al., 2018, Blome et al., 2014). Finally, it should also be mentioned that the virus is able to establish complex immune modulation and efficient immune evasion (Reis et al., 2017; Dixon et al., 2013), i.e. it brings along numerous factors that influence the host's immune system in such a way that virus replication can take place efficiently.

With the pandemic spread of ASF, research towards vaccine development has been intensified and some very promising results have been obtained. ASFV vaccines may be closer than they appear (Bosch-Camós et al., 2020).

As there are several recent reviews that summarize the approaches to create safe and efficient vaccines against ASF (Dixon et al., 2020, Sang et al., 2020, Teklue et al., 2020, Gaudreault and Richt, 2019, Sanchez et al., 2019, Revilla et al., 2018, Arias et al., 2017, Rock, 2017, Zakaryan and Revilla, 2016, Bosch-Camós et al., 2020), the section below is mainly focused on general features, recent publications and developments, and also some rather neglected issues.

7.1.2. Live attenuated vaccines

Live attenuated vaccines can be either based on naturally occurring ASFV strains of reduced virulence or virulent strains attenuated by deletion of virulence factors (Sang et al., 2020). Some approaches targeted rational improvement of naturally occurring strains (Dixon et al., 2013).

First vaccine approaches were already available in the 1960ies. These early live vaccines were based on attenuated ASFV strains and were used thousands of times under field conditions in both Portugal and Spain in the 1960s. Unfortunately, they induced debilitating chronic lesions in many vaccinated animals, months after vaccination, and led to an increase in case numbers in both countries (Petisca, 1965). In the end, vaccine withdrawal stopped field use. This experience explains why, in particular, the safety aspects of today's live ASF vaccine candidates require a very close examination. There should be no hasty solutions in ASF vaccine design (Gavier-Widen et al., 2020).

Research on the rational selection of attenuated strains for emergency vaccine development (vaccination to slaughter and safe processing) was done in Russia from the 1970ies to 1990ies (reviewed by Sereda et al., 2020). Several strains were tested and classified according to their properties and sero-immunotype as defined by haemadsorption inhibition and immunobiological testing. In summary, most of the candidates still caused rather prolonged viraemia and slight fever reactions. Protection was high, even after longer times, but not complete, especially in immunocompromised animals (Sereda et al., 2020).

Also recently, naturally occurring, attenuated virus variants have been tested as vaccine candidates. Such viruses have been recently found in the Baltic States (Zani et al., 2018, Gallardo et al., 2019). The virus strain Lv17/WB/Rie1 (patented in Spain under reference PCT/2018/000069) has been tested after initial trials reported by Gallardo et al. (2019), for its safety and efficacy profile after oral immunization of wild boar by Barasona et al. (2019). This strain displays a truncated CD2v (EP402R) and is non-haemadsorbing. Under the tested settings in an immunization – challenge trial with wild boar, a slight increase in body temperature was observed as only sign in most immunized and one early contact control animal. Low viral loads were detected in several animals by qPCR. Over the vaccination period, most immunized and all early contact controls developed antibodies against ASFV proving both immunogenicity but also transmission of vaccine virus. Upon challenge, 11 of 12 animals were protected from lethal challenge (92 %). The one that developed acute ASF was among the ones that had not developed antibodies or shown increased body temperatures upon vaccination. In summary, the results were promising but more precise data on the protective effect, safety and innocuousness are still lacking (Gavier-Widen et al., 2020). In the earlier study with the same virus, at least one animal showed joint swelling and lesions (Gallardo et al., 2019) that may indicate chronicity. Furthermore, there was definitively a transmission of vaccine virus that could have both beneficial and detrimental effects in affected populations and countries. The advantage of such a virus is its non-GMO nature.

Among the most promising candidates for ASFV vaccines are deletion mutants created by homologous recombination. Using this approach, rational deletion of virulence genes and interferon inhibitors has been attempted for several strains (Bosch-Camós et al., 2020). Among the virulence associated genes that were deleted are 9GL (B119L), UK (DP96R), CD2v (EP402R), DP148R, and different members of multigene families (MGF) (Teklue et al., 2020). Along these lines, deletion of different interferon type I inhibitors resulted in attenuated viruses with protection against challenge (O'Donnell et al., 2016; O'Donnell et al., 2017; Reis et al., 2016; Reis et al., 2017). The same held true for deletion of CD2v from BA71 (genotype I) (Monteagudo et al., 2017). However, the same deletion did not result in the same degree of attenuation in a genotype VIII strain (Borca et al., 1998). Another common target was the gene B119L (9GL). Attenuation and protection was shown with several backbones (Dixon et al., 2020) but problems were seen with genotype II ASFV Georgia (O'Donnell et al., 2015). For ASFV Georgia, full attenuation was achieved through additional deletion of DP96R (UK) (O'Donnell et al., 2017). Yet, one important lesson learned was that the genetic background of the virus affects the phenotype of deletion mutants (Dixon et al., 2020).

The fact that rational deletion does not always result in the wanted effects has recently been shown again by Reis et al. (2020). In an attempt to increase the OURT88/3 safety profile, the gene I329L was deleted, a gene previously shown to inhibit the host innate immune response. The resulting virus, OURT88/3ΔI329L, was tested in vitro to evaluate the replication and expression of type I interferon (IFN) and in vivo by immunisation and challenge experiments in pigs. While replication characteristics remained the same, increased amounts of IFN-β and IFN-α were observed. Unexpectedly, protection against challenge infection with the virulent OURT88/1 isolate was drastically reduced. Interestingly, the deletion of the I329L gene alone failed to attenuate the virulent Georgia/2007 isolate (Reis et al., 2020).

Very recently, a seven-gene-deleted live attenuated vaccine candidate was presented by Chen et al. (2020). This vaccine candidate, named HLJ/18-7GD, is based on the highly virulent Chinese ASFV strain HLJ/18 and lacks genes encoding MGF505-1R, MGF505-2R, MGF505-3R, MGF360-12L, MGF360-13L, MGF360-14L, and CD2v (EP402R). It was reported that this candidate is completely attenuated in pigs and confers dose-dependent protection against virulent ASFV HLJ/18 challenge (intramuscular and oral). In more detail, all animals immunized with a lower dose (10^3 tissue culture infectious doses 50 %, $TCID_{50}$) of HLJ/18-7GD (n = 4) developed fever for three to nine days upon challenge infection whereas only one animal of the higher dose group (n = 4 immunized with 10^5 $TCID_{50}$) developed fever for one day. All animals survived the observation period of 21 days. Intramuscular inoculation of six pigs with 10^7 TCID50 for serial passaging did not result in positive samples and blind passaging did not result in transmission. Upon inoculation with $10^{7.7}$ $TCID_{50}$ and sequential slaughtering, viral DNA was detected only in lymph nodes of some animals. In addition, the vaccine candidate was safe in pregnant animals at all stages of gestation. Duration of immunity was highly dose dependent. While high doses were able to confer long-term protection, double vaccination with moderate doses already failed to confer complete protection 80 days after the last vaccination. The observation of lacking long-term protection is in line with studies published by Sánchez-Cordón et al. (2020). In this study, no long-term protection was

observed after a single intramuscular immunization of pigs with either OURT88/3 or BeninΔMFG. Challenge at 130 days post immunization resulted in acute ASF in all animals. It appears that the duration of immunity remains a weakness of the ASF vaccines. This point therefore requires intensive research and clarification.

Another recently reported vaccine candidate is ASFV-G-ΔI177 L (Borca et al., 2020). In this vaccine candidate based on ASFV Georgia, only the I177 L gene is deleted by homologous recombination techniques. In culture, this variant showed reduced growth (kinetics and yield). The ORF I177L encodes a 177 amino acid protein of unknown function. Animals immunized once with the purified deletion mutant at doses of 10^2–10^6 haemadsorbing doses 50 % (HAD_{50}) remained clinically healthy in the 28 day observation period. While low to moderate viral DNA loads were detected over the entire observation period, no shedding to sentinel animals occurred and high antibody levels were detected by the end of the trial. Upon intramuscular challenge at 28 days post immunization, all vaccinees remained clinically healthy. Using tailored PCR systems, vaccine virus detection and lacking challenge virus detection was confirmed. Only one animal from the low dose group showed low challenge virus loads. Anti-ASFV antibody levels detected by an indirect full-virus ELISA correlated with protection as described previously (Carlson et al., 2016).

7.1.3. Vectored vaccines, DNA and subunit vaccines

Vectored vaccines and subunit vaccines could have a better safety profile than live attenuated vaccines and allow differentiation of infected from vaccinated animals (DIVA). Besides, vaccine production would usually not rely on primary cells. However, the design has been severely hampered by the lack of knowledge regarding protective antigens and their possible interaction. Moreover, subunit vaccines could not be used for oral vaccination of wild boar (this requires a live vaccine approach).

Based on serological responses of recovered animals, structural proteins p30 (encoded by CP204 L), p54 (E183L), p72 (B646L), pp62 (CP530R) and CD2v (EP402R) have been the main targets for rational design and were tested in protein, DNA and viral vectored ASFV vaccines in challenge studies (Goatley et al., 2020). In addition, in silico predicted antigens have been used in different projects. Unfortunately, results with similar antigens were not always conclusive. The inconsistency could be attributed to a variety of factors, including the type of vaccine, vaccination strategy, the antigens, and the immune response induced, as well as the challenge model, including factors like animal genetics, virus strain, and vaccine and challenge dose (Gaudreault and Richt, 2019). Most approaches yielded no or partial protection.

As an example, baculovirus-expressed p30, p54, p72, and p22 induced antibodies with neutralizing capacities but did not prevent fatal disease courses in six immunized animals upon homologues challenge with Pr4 (10^4 $TCID_{50}$ intramuscularly) after the fourth boost. A slight delay in disease onset and viremia was observed (Neilan et al., 2004). In contrast, a fusion protein of p30 and p54 expressed by a recombinant baculovirus in insect cells and in Trichoplusia ni larvae did induce partial protection and reduced viremia in two pigs that were immunized five times and intramuscularly challenged with 500 $TCID_{50}$ of ASFV strain E75 (Barderas et al., 2001). Approaches to use recombinant CD2v resulted in antibody responses and dose-dependent protection against challenge infection with 400 $TCID_{50}$ of ASFV E75 in three animals. High immunization doses prevented severe clinical signs and detectable viremia (Ruiz-Gonzalvo et al., 1996).

DNA vaccination with a fusion of p54, p30 and the extracellular domain of CD2v did not result in protection unless fused to ubiquitin (Argilaguet et al., 2012). This construct protected a proportion of triple immunized pigs (in total 3 of 12) from lethal challenge with ASFV E75 (10^4 HAU_{50}). Along these lines, a similar approach was taken with an expression library that resulted in partial or almost complete protection of 60 % of the animals (n = 5) challenged with 10^4 HAU_{50} of ASFV E75 after double vaccination (Lacasta et al., 2014). Despite the above mentioned results, DNA-prime followed by protein-boost using CD2v, p30 and p54 did not result in protection (Sunwoo et al., 2019). In this study, pigs (n = 5) were vaccinated three times with a cocktail that included ASFV plasmid DNA (CD2v, p72, p32, ± p17) and recombinant proteins (p15, p35, p54, ± p17). Vaccinated pigs were not protected from infection or disease with ASFV Arm07. Compared to the non-vaccinated controls, earlier onset of clinical signs, viremia, and death were observed for the vaccinated animals following virulent ASFV challenge. ASFV induced pathology was also enhanced in the vaccinated pigs.

Rational screening for viral proteins recognized by lymphocytes from ASF-immune pigs using a gamma interferon ELIspot assay led to a pool comprised of the ASFV genes A151R, B646L (p72), C129R, CP204L (p30), CP530R (pp62), E146L, I73R, I125L, L8L, M448R, MGF110-4L and MGF110-5L. These antigens vectored by replication-deficient human adenovirus 5 (rAd) prime and modified vaccinia Ankara (MVA) boost led to reduced clinical signs and reduced levels of viremia in a proportion of pigs after challenge with the virulent OUR T88/1 isolate (Netherton et al., 2019).

Adenovirus-vectored cocktails containing e.g. B646L (p72), CP204L (p30), CP530R (pp62), E183L (p54) in combination with the mature p37 product and two sections of the mature p150 protein of the pp220 polyprotein (CP2475L gene) were shown to induce antibody and T cell responses, including cytotoxic T lymphocytes (Lokhandwala et al., 2017, Lokhandwala et al., 2016). However, challenge infection showed that some vaccines (using an adjuvanted 9 antigen cocktail) had even more severe reactions relative to the controls and no significant protection was conferred (Lokhandwala et al., 2019).

Recently, immunogenicity and efficacy of thirty-five rationally designed adenovirus-vectored ASFV antigens were evaluated in wild boar (n = 9 treated in different schedules). The cocktail contained adenoviruses expressing the above-named antigens and others such as EP153R, p10, p15, CP80R, I329L, H108R, K196R, CP312R, F334L, NP419L, NP868R, B66L, H339R, and R298L, which had previously been shown to be immunogenic in domestic pigs. The rest (K145R, B385R, F165R, F778R, S273R, MGF100 − 1 L, A224L, MGF505-6R, and B175L) were selected based on the presence of putative T cell epitopes. Challenge was performed as shedder-animal challenge-exposure infection model with an intramuscular inoculation of 10 HAU in two seeders. In summary, no protection was observed upon challenge with ASFV Arm07. All immunized animals developed clinical signs and pathological lesions indicative for ASF (Cadenas-Fernández et al., 2020).

However, recently, a pool of eight ASFV antigens has been shown to protect pigs from lethal disease after ASFV genotype I challenge when vectored by a replication-deficient human adenovirus 5 (prime) and modified vaccinia Ankara (boost) (Goatley et al., 2020). The experiments were a follow-up of previous work where different antigens had been shown to be immunogenic but not protective in a DNA prime/vaccinia boost approach (Jancovich et al., 2018). The protective antigen cocktail comprised gene products of B602L, B646L (p72), CP204L (p30), E183L (p54), E199L (cysteine-rich protein), EP153R (lectin-like protein), F317L, and MGF505-5R. Despite the fact that the preparation was far from being an efficacious vaccine as all animals got sick and showed considerable viraemia, this approach is promising in terms of antigen choice and demonstration that a DIVA compatible subunit vaccine could confer protection. Unfortunately and again, no clear correlates of protection could be deduced.

Taken together, a viral-vectored vaccine against ASF is a feasible approach, however, the choice of antigens is key to success (Goatley et al., 2020), and convincing candidates are still missing.

7.1.4. Commercialization of vaccines and their implementation

Currently, headlines and tweets are chasing each other with potential candidates that are presented as success stories and suggest that a vaccine is feasible in the short term. However, due to the lack of data, safety issues and consumer concerns, not all (or rather, almost none) of

the vaccine candidates would be suitable as a short-term tool to control a highly effective notifiable disease, and for all modified live vaccine candidates presented up to now, production would be severely hampered by the lack of permanent cell lines that would ensure viral replication and production under standardized conditions (Gavier-Widen et al., 2020). All reported new and promising vaccine candidates depend on the production on primary cells, which makes vaccine production neither safe nor feasible.

Furthermore, for wild boar, a bait-based live vaccine is needed. Due to the fact that this virus is set lose in the field, safety data should be extensive and these studies are currently very rare.

Once vaccines become available, they can only be one tool to control the disease. Vaccination must be embedded in an ASF control and prevention strategy (Dixon et al., 2020). An example for the limited ability of vaccines to eradicate a disease under suboptimal conditions is Classical swine fever. While the live attenuated C-strain vaccine is highly efficacious, very safe and probably one of the best vaccines against an animal disease (van Oirschot, 2003), eradication of CSF in China has still been impossible to date (Luo et al., 2014). Vaccines will not replace the need for biosecurity, behavior changes, improved management, diagnostic approaches and targeted culling measures (Dixon et al., 2020).

Gaps and research needs:

- ASFV virology and functional genomics studies are needed to support vaccine discovery research with important additional data sets (e.g. protein structure and function,
- Harmonized challenge models are needed to compare candidate vaccines in immunization/challenge trials.
- Safety characteristics and minimum standards associated with experimental live attenuated vaccines must be developed and agreed on. A pharmacopeia chapter might be necessary.
- Genes that enable or enhance ASFV growth without major genetic changes should be developed for vaccine production.
- Gene-deleted ASFV are major vaccine candidates and should be developed and studied more intensively.

8. Outlook

The future fight against African swine fever must even more focus on enhanced classical control measures like early detection, strict hygiene and biosecurity measures, culling of infected farms, compensation of losses, stand still measures, epidemiological tracing and wild boar control programs. This is particularly necessary since an applicable, safe and effective vaccine that could be produced in large quantities and also be used in the wild animal reservoir will probably not be available in the short term. Therefore, future research should concentrate on filling the gaps listed in this review.

Declaration of Competing Interest

The authors declare no conflict of interest.

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