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REVIEW ARTICLE

The main DNA viruses significantly affecting pig livestock

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Abstract

Swine DNA viruses have developed unique mechanisms for evasion of the host immune system, infection and DNA replication, and finally, construction and release of new viral particles. This article reviews four classes of DNA viruses affecting swine: porcine circoviruses, African swine fever virus, porcine parvoviruses, and pseudorabies virus. Porcine circoviruses belonging to the *Circoviridae* family are small single-stranded DNA viruses causing different diseases in swine including poly-weaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, and porcine respiratory disease complex. African swine fever virus, the only member of the *Asfivirus* genus in the *Asfarviridae* family, is a large double-stranded DNA virus and for its propensity to cause high mortality, it is currently considered the most dangerous virus in the pig industry. Porcine parvoviruses are small single-stranded DNA viruses belonging to the *Parvoviridae* family that cause reproductive failure in pregnant gilts. Pseudorabies virus, or suid herpesvirus 1, is a large double-stranded DNA virus belonging to the *Herpesviridae* family and *Alphaherpesvirinae* subfamily. Recent findings including general as well as genetic classification, virus structure, clinical syndromes and the host immune system responses and vaccine protection are described for all four swine DNA virus classes.

Keywords: DNA viruses, circoviruses, African swine fever virus, parvoviruses, pseudorabies virus.

Introduction

The genetic material of DNA viruses is either single-stranded (ss) or double-stranded (ds) deoxyribonucleic acid. Virus DNA genomes are variable in size, ranging from small with a size of 1 kilobase pairs (kbp) to large examples of several megabase pairs. DNA viruses use host cells for replication and subsequent infection. The first viral genes to be expressed, which are made by larger viruses, are called early genes. Genes encoding DNA polymerase and proteins incorporated in DNA replication often belong in this group. After DNA replication, viruses change the expression profile to the so-called late genes. Those genes are essential for the production of structural proteins used for coating the replicated DNA genome and forming new viral particles. At the end of the proliferation process, viral particles are released from the cell to infect new sites. In this article, the four main groups of DNA viruses significantly affecting swine are reviewed: porcine circoviruses, African swine fever virus, porcine parvoviruses, and pseudorabies virus. The genetic diversity inside a particular group and family classification, the structures of virus particles, the clinical syndromes, the course of infection, and recent progress in vaccine development as an effective means of protection against infections with swine DNA viruses are described.

Porcine circoviruses (PCVs)

Porcine circoviruses are the smallest autonomously replicating swine viruses containing circular single-stranded DNA (ssDNA) with a size of 1.76 kbp (73, 75). PCVs were first discovered by Tischer *et al.* (113) in 1974 when PCVs were mistaken for picornavirus-like particles in a contaminated PK-15 pig kidney cell line. Those circoviruses were non-pathogenic, and after they

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were classified into the *Circoviridae* family, their apparent harmlessness caused them to merit little attention so only a few articles concerning the topic were written until around 20 years later, when a new pathogenic type of porcine circovirus appeared called PCV2 (75). In 2015, PCV3 was discovered and associated with porcine dermatitis and nephropathy syndrome (PDNS) (90), and in 2019, a new type of PCV (type 4) was found in China (124).

Virus structure. PCV virions are small isometric particles with a diameter of 17 nm containing circular ssDNA which only contains three protein-coding genes (114). The virus particle of both PCV1 and PCV2 is composed of a single structural protein called the capsid protein (Cp), with a molecular mass of 30 kDa and which is responsible for spontaneous capsid formation (62) (Fig. 1).

PCV1. This was the first identified porcine circovirus. It was designated PCV PK-15 after its discovery and characterisation as a contaminant in the PK-15 porcine kidney cell line (113). Interestingly, it was also found in lymph nodes from piglets affected by a wasting syndrome in France (4, 60).

PCV2. In 1998, Meehan *et al.* (74) observed that monoclonal antibodies raised to circoviruses causing post-weaning multisystemic wasting syndrome (PMWS) were different from those raised to the PCV PK-15 isolate. They also published the first nucleotide sequences of the circoviruses associated with PMWS, which showed less than 80% identity with the PCV PK-15 isolate, and they thus provided evidence for a new pathogenic type of porcine circovirus, referred to as PCV2 (74). Based on the results of the phylogenetic study using the capsid protein gene region as a marker, PCV2 sequences were divided into two main groups: the first group, which subdivides into three clusters 1A to 1C, and the second group, which branches into five clusters 2A to 2E (40, 85). There is also another grouping method considering the geographic localisation of the virus, dividing PCV2 into PCV2a for the North American-like isolates (which also fall into the first capsid protein gene region-differentiated group of PCV2), and PCV2b for the European-like isolates (also in the second capsid protein group of PCV2) (86).

PCV3. This is a recently discovered type, and yet it has been detected and characterised in many countries throughout the world, including China (55), Italy (31), Brazil (115), and Sweden (121). PCV3 was first identified in 2015 in North Carolina (USA) in isolates from sows showing high mortality, low conception rates and typical signs of PDNS (90). Therefore, PCV3 was associated with PDNS and reproductive failure (90) and it has also been linked to congenital tumours in piglets as well after Chinese PDNS cases were investigated (21). This new type of PCV shares only a small percentage of homology in genomic DNA sequence with those of PCV1 and PCV2 (90). The homology between PCV3 and PCV2 found by sequencing in the *rep* gene sequence is 55% and in the *cp* gene only 37% (90). In China, PCV3 was divided into two groups (a and b) and five subgroups (a1–a3, b1, and b2) by a phylogenetic study using full-length sequences of PCV3 DNA (22). In a phylogenetic study conducted in Germany where only open reading frame (ORF) 2 (coding for the Cp protein) was used for grouping, the number of subgroups differed; group a was not divided but group b was, into three subgroups (22, 39). The difference is caused by the usage of whole-genome sequences in the Chinese study, while ORF2 was considered a critical phylogenetic marker in Germany (85).

PCV4. This type was only discovered in April 2019 (124). Type 4 contains 1.77 kbp long DNA and shares 67% homology with mink circovirus, which is the highest homology across circoviruses, and 43–52% homology with other porcine circoviruses (124). The size of two crucial genes was predicted at 891 nucleotides for the *rep* gene and 687 nucleotides for the *cp* gene (124). For the understanding of porcine circovirus' pathogenicity and infection, further investigations will be necessary.

Clinical syndromes. Postweaning multisystemic wasting syndrome was first described in 1996 and a year later was associated with PCV2 (46). The precise definition of PMWS was proposed by Sorden in 2000 (109). For pigs to be diagnosed with PMWS, they must show all of the following conditions: firstly, clinical signs like wasting, weight loss or failure to thrive; secondly, histological lesions, which are signs of depletion of lymphoid tissues and organs, and inflammation of the lungs and lymphoid tissues in usual cases and less often the liver, kidneys, pancreas or intestine; and thirdly, PCV2 infection inside the lesions. The effect of PMWS on the host immune system is pronounced, causing virus-induced lymphocyte depletion. In the work of Mandrioli *et al.* (69), the presence of activated macrophages was described as an essential factor for the development of the syndrome. Although mainly CD4+ T-lymphocyte counts were decreased during the infection, a dramatic decline in CD8+ and CD4+/CD8+ T-lymphocyte and B-lymphocyte numbers was also observed, associated with the loss of lymphoid follicles (69). The reduced proliferation of lymphocytes thus results in a reduction of cytokines as positive growth factors, which can affect the further expression of major histocompatibility complex I antigens type I and II (MHC I and MHC II) and thus impair the immune response (72). Interestingly, apoptosis was not observed in lymphoid tissues that showed a decreased rate of virus proliferation (69). However, the work of Shibahara *et al.* (106) showed that apoptosis occurred only in B-lymphocytes and not in macrophages (106). This can be explained by the yet-unknown cause of the apoptosis in lymphoid tissues of PMWS in swine (69).

Another disease associated with porcine circoviruses is PDNS. Pigs affected by this syndrome are slightly febrile, depressed, and have ventrocaudal subcutaneous oedema (100). The incubation time of this

disease is very short, and most swine die within three days. There are some similarities between PMWS and PDNS, such as lymphoid depletion and the presence of syncytial cells and others, suggesting that PCV2 may be responsible for this disease. Typically, this disease leads to skin lesions on the hind legs, however PCV2 has not been confirmed as the causative agent of this phenomenon (100).

Porcine respiratory disease complex (PRDC) is a disease that affects mainly 2–8-month-old pigs. PRDC is characterised by poor appetite, weight loss, or weak growth accompanied by clinical signs like anorexia, fever, cough and dyspnoea (19, 52, 86).

Development of vaccines. PCVs are highly resistant to conventional detergents and disinfectants, which makes decontamination problematic (4). To cope with the negative impacts on pig livestock, scientists have developed vaccines for combating these viruses. The first step in producing an efficient vaccine against pathogenic PCV2 is creating and characterising monoclonal antibodies against the pathogen. In 2001, McNeilly *et al.* (72) prepared and characterised monoclonal antibodies against six PCV2 isolates. One year later, Fenaux *et al.* (32) reported the first construction of a DNA clone containing an inserted infectious PCV2 genome and its subsequent use for *in vivo* transfection of pigs. The results from transfection testing showed that the cloned PCV2 genomic DNA could be used for future pathogenesis testing, replacing the virulent virus for greater safety (32). The same research group observed that not only PCV2 genomic DNA could enhance the production of specific monoclonal antibodies, but also that a DNA clone containing a capsid gene from PCV2 inserted into the backbone of PCV1 could achieve the same (34). This DNA clone was further tested as a live attenuated vaccine, which enhanced cell-mediated immune response and thus protected pigs against a pathogenic PCV2 challenge (33).

The first preparation which came onto the market (Circovac®, now produced by Ceva, France) successfully vaccinated sows and piglets older than three weeks (87). Interestingly, the two-dose vaccine was observed to enable the transfer of specific PCV2 antibodies from sow to offspring via colostrum (66). This type of vaccination was named dam vaccination. Another preparation used for immunisation of pregnant sows was a baculovirus-expressed PCV2 vaccine (Ingelvac CircoFLEX®, Boehringer Ingelheim, Germany). Only a single dose of the vaccine could develop neutralising antibodies against PCV2, but 10% of the piglets born to those vaccinated sows contracted *in utero* infection (67, 68). These studies also suggest that the timing of vaccination is crucial, selection of the life stage for administration depending on the desired result. For example, if a farm with sows wants to prevent *in utero* infection in the next generation, they will specify pre-breeding and post-farrowing vaccinations (66, 68). As another example, in the case of protecting piglets in the early stage of growth, the vaccination should be administered pre-farrowing, when colostrum contains more specific antibodies (66). The two vaccines described are currently used frequently for controlling PCV2 infection.

A useful way of combating PCV can also be the application of vaccines or drugs which could block the attachment of viral particles to host cells. Recently two studies have reported two different components which can accomplish that. Li *et al.* (63) found that epigallocatechin gallate from green tea can inhibit the infection of PCV by interfering with the capsid protein and thus inhibiting its binding to the host cells. Another option could be therapeutically neutralising antibodies. In the study of Huang *et al.* (49), a new neutralising monoclonal antibody was prepared capable of blocking the capsid protein attachment to PK15 cells. These findings can provide useful information for the development and synthesis of new vaccines and drugs against porcine circoviruses.

Recent approaches to vaccines mostly target the sole capsid protein (Cp), recognising it as the most important. This protein was either expressed in bacterial strains (*Lactobacillus lactis*) (116) or viruses (adenoviruses) (127) or used to produce PCV2 virus-like particles in insect cells in a baculoviral expression system (18, 70).

African swine fever virus (ASFV)

ASFV is a large DNA virus that is the sole member of the *Asfivirus* genus within the *Asfarviridae* family (64) affecting all species of swine and predominantly vectored by ticks from the *Ornithodoros* genus (37). ASFV causes a highly infectious disease called African swine fever (ASF). Even though ASF was first identified in 1921, its first occurrence had already been observed in 1910 in British East Africa (the Kenya Colony) as an infectious disease affecting domestic pigs (78).

Virus structure. ASFV is a large virus, of which the viral particle has icosahedral symmetry (Fig. 1). The size of ASFV derives from the trilayer viral envelope protecting the core that contains linear dsDNA. Each of the layers is composed of different structural proteins playing not only a protective role but also an infective one. A brief description of each envelope layer and the most important structural proteins follows.

Outer envelope. The outer layer is composed of the structural proteins p12 (*pO61R*), p22 (*KP177R*) and CD2v (*EP402R*) (3, 17, 98). The p12 protein (*pO61R*) is a late structural protein which attaches the viral particle to the host cell (3), and the p22 (*KP177R*) protein is an early structural protein which is localised on the outer envelope of the viral particle (17). CD2v is a more complex protein which plays different roles during ASFV infection. It is a transmembrane protein containing 402 amino acids showing a high degree of similarity to CD2, an adhesion receptor of T lymphocytes, particularly

sharing the immunoglobulin Ig domain with 28–30 highly glycosylated sites (76, 99). This protein functions in the adsorption of red blood cells on the surface of infected host cells (13, 99) and was found to interact with an adaptor protein complex (AP-1) through the diLeu motif in the C-terminal domain (91). Adaptor protein complex 1 is a group of cytosolic heterotetramers which sort membrane proteins to endosomes by the formation of clathrin-coated vesicles using clathrin as a scaffold protein (80). In this way, CD2v helps ASFV to enter into the host cells.

Capsid envelope. The major capsid p72 protein (encoded by the viral *B646L* gene) is knowable by its assembly in the area of the inner core matrix and outer capsid layer of the viral particle (24). This assembly is mediated by a chaperone encoded by *B602L* and takes place on the membrane of the endoplasmic reticulum, where the process of envelopment is localised (24). Another crucial structural protein is p49 (*B438L*), which forms the icosahedral shape of the viral particles by localising in the vertices of the capsid (29).

Inner envelope. The inner envelope contains five structural proteins: the abundant transmembrane p17 (*D117L*); the late structural pE248R (*E248R*), j5R (*H108R*) and j18L (*E199L*); and p54 or j13L (*E183L*) (15, 96, 97, 111, 112). Their functions have also been characterised, and it was learned that j5R and j13L/p54 are involved in the assembly of viral particles in which j13L is accumulated on the endoplasmic reticulum membrane, and involved in recruiting viral membrane precursors (15, 98). Protein p17 is also involved in recruiting viral precursors (111). Although the function of pE248R is not precisely known, it has been ascertained that it is an actor in the early phase during virus entry into the host cell (97).

Core layer. The first step in forming the viral particle is protecting the genomic DNA with a core layer of proteins. This layer is composed of structural proteins, which originate from polyproteins pp62 (*CP530R*) and pp220 (*CP2475L*) (107, 108). Both polyproteins are processed by SUMO-like protease (*S273R*) yielding different structural proteins, which in the case of pp62 are p15 and p35 and in the case of pp220 are p14, p34, p37 and p150 (107, 108).

Genomic DNA. ASFV genome is 170 kbp long and contains 151 ORFs (20, 120). The genome contains multiple genes with different functions. There are genes involved in DNA replication, genes encoding enzymes and factors involved in transcription and processing, genes encoding structural proteins and proteins involved in the assembly of viral particles, genes encoding proteins involved in host defences, and last but not least, multigene families, which correspond to the 30% of the genome (29).

Genetic classification. Distinct ASFV genotypes were identified based on the p72 structural protein. Phylogenetic analysis of the C-terminal end of the p72 gene showed the presence of 22 different genotypes (I–XXII) (14). Recently two new genotypes were added, XXIII and XXIV (2, 94), of which XXIII shares a common ancestor with the genotypes IX and X (2). In Europe, two types of genotypes caused outbreaks: genotype I on Sardinia and genotype II in Eastern Europe (11).

Clinical syndromes. The clinical signs caused by ASFV infection include lesions, high fever, skin haemorrhages and neurological diseases (117). Although these clinical signs may be similar to those of other diseases like classical swine fever virus and porcine reproductive and respiratory syndrome, African swine fever is manifested by additional symptoms including depression, apathy, anorexia, vomiting, and red skin on the ears, abdomen and chest (117).

ASFV and host immune system. The primary target cells of ASFV include macrophages and monocytes (45). ASFV uses macropinocytosis and clathrin-mediated endocytosis as two different mechanisms to enter the host cells (47). When the virus enters the cell, the lower pH inside late endosomes causes the disruption of the outer envelope and capsid (47). Thus, the inner envelope is exposed and subsequently fused with the endosomal membrane to release the viral genome into the cytosol (6). This fusion is mediated by the pE248R transmembrane protein of the inner envelope (6). Cholesterol from the endosome is also essential for the ASFV genome release to the cytosol (26). The further transport of the genome is mediated by p54 protein, which interacts with the light chain of dynein until it reaches the perinuclear spot near the microtubular organizing centre (MTOC), where DNA replication and transcription take place (5). Interestingly, the ASFV genome replicates independently on the host cell (29). The next step of ASFV infection is forming viral factories. These are formed near the nucleus at the MTOC, where virus proteins and DNA are assembled to form new viral particles (41). The integrity of the microtubules is necessary for the formation of viral factories (41). The last step is the release of completed viral particles outside the cells. The pE120R virus protein helps in the microtubule-mediated transfer of viral particles from the viral factory to the plasma membrane (7). The protein is attached to the surface of intracellular virions by binding to the p72 major capsid protein, which helps to incorporate pE120R into the viral particle (7).

Evasion from the host immune system. ASFV contains multiple genes that inhibit the function of interferon type I (IFN I), which results in inhibition of the antiviral state in infected host cells (30). One study suggests that the MGF 360 and 505 multigene families are involved in evasion from the antiviral state, due to the sensitivity of the virus to IFN I when MGFs were deleted (42). The essential part of the escape from the host immune system includes inhibition of cell death by apoptosis. Here, many proteins from ASFV can disable the apoptosis mechanism of the host cell. One of these is a protein encoded by the *A179L* gene, which belongs to the B-cell lymphoma Bcl2 family (10). This family is characterised by an anti- or pro-apoptotic function

depending on the type of homology region (BH1–BH4) and the protein interactions (56, 122). This protein is known for its interaction with proteins containing the BH3 domain (such as Bak and Bax) and resultant inactivation of them (10). Bak and Bax are primary gatekeepers, which upon activation by apoptosis inducers cause disruption of mitochondrial membranes, and the subsequent release of cytochrome *c* activates the caspase cascade resulting in apoptosis (56, 122). However, their inactivation by the *A179L* gene–encoded protein causes the inhibition of apoptosis in infected host cells. Another protein which can inactivate apoptosis is that encoded by the *A224L* gene. This protein belongs to the inhibitors of apoptosis protein family, which is recognised by the BIR motif, and uses tumour necrosis factor alpha (TNF-α) as a stimulus for inhibition of apoptosis (30). That inhibition by this protein is accomplished by inhibition of caspase 3 and activation of the NF-κB nuclear factor (30), which then activates the expression of cFLIP, an inactivated caspase 8 homologue that subsequently blocks caspase 8 activity (30). However, this protein is not essential for growth or viral virulence (81), which suggests that inhibition of apoptosis by TNF-α is not necessary for the replication of ASFV.

Development of vaccines. The development of vaccines for combating ASFV began in the 1960s (9). During those early years, multiple vaccines were developed, but none of them proved effective enough for commercial purposes. There are three main types of vaccines which were designed against ASFV: inactivated vaccines with a killed virus, live attenuated vaccines and subunit vaccines. Inactivated vaccine approaches were not successful at all; such vaccines could not enhance the immune response in pigs, even with the addition of different types of adjuvants (12).

Live attenuated vaccines (LAVs). These vaccines, containing viruses with deleted genes responsible for host invasion, infectivity or immune system inhibitors, were found to enhance cellular and humoral immunity and further protected pigs against the virulent virus type (102). There are three successful LAVs, which derive from the OURT88/3, NH/P68 and BA71ΔCD2v isolates (61, 77, 79). The OURT88/3 strain has been observed to enhance the production of CDβ8+ lymphocytes, the part of CD8+ lymphocytes confirming the importance of cellular immunity in the resistance to ASF (89). Interestingly, using the OURT88/3 isolate, it has been found that deletion of genes involved in virulence such as *DP71L*, *DP96R* and the IFN I interferon modulators MGF 360 and MGF530/505 weakened the infectivity of and conferred subsequent protection against the OURT88/1 virulent strain (1, 95). However, MGF360/505 and 9GL deletion in the ASFV Georgia 2007 isolate also reduced the virulence of the isolate but without affording protection against the parental virus (84). A similar result was observed using the Georgia isolate with the deletion of the thymidine kinase gene involved in the virulence of ASFV (104). It has also been noted that cross-protection provided by the non-virulent OURT88/3 isolate and virulent OURT88/1 isolate used in combination induced protection against two isolates, Benin 97/1 and genotype X Uganda 1965 (53). Interestingly, the mutant virus BA71ΔCD2v conferred protection to both parental BA71 and heterologous E75 virulent strains, which are two genotype I strains (77). Furthermore, pigs also survived a lethal challenge with the virulent Georgia 2007/1 genotype II strain (77). In the study of Sánchez-Córdon *et al.* (103), the immunisation technique was observed to be crucial for protection against ASFV: vaccination through the intranasal route was markedly more effective than the intramuscular route (103).

Subunit vaccines. Subunit vaccines use biomacromolecules for immunisation, such as DNA or protein antigens. DNA vaccines have one main disadvantage, which is their reduced immunogenicity in large animals. This fact was confirmed by failed immunisation with a DNA vaccine containing ASFV genes (8). The study of Argilaguet *et al.* (8) attempted the construction of a new DNA clone encoding ASFV genes fused with a fragment of an antibody specific to a swine leukocyte antigen II and yielded the observation that targeting antigens to the antigen-presenting cells induced an immune response in pigs. Unfortunately, protection against lethal challenge was not achieved (8). There was also protection by a DNA vaccine containing ASFV genes encoding p54, p30 and the HA extracellular domain fused to ubiquitin against challenge with the virulent E75 strain (57). Protein antigens are, however, more effective than DNA vaccines; even if they do not confer protection in all cases. For example, immunisation with baculovirus-expressed p30, p54, p72 and p22 ASFV antigens showed only a temporal delay in the onset of disease and reduced viremia (82). It has been observed that neutralising antibodies were raised to p54 and p72 antigens inhibiting virus attachment to the surface of the host cells (44). Neutralising antibodies specific to the p30 antigen, which is the most immunogenic among ASFV antigens, were found to inhibit virus internalisation (44, 92). Recently, new p30-specific monoclonal antibodies were prepared, and their binding epitopes were mapped (92). It was found that immunisation with either p30 or p54 recombinant antigen was not successful because pigs were not protected and eventually died. However, when the antigens were used together as a cocktail, immunisation was successful and pigs raised neutralising antibodies, which delayed the disease and even stopped the infection (43). The study of Ruiz-Gonzalvo *et al.* (101) conducted in 1996 showed that immunisation with recombinant CD2v antigen inhibited the haemagglutination, restricted the infection temporally and in some cases also conferred protection against lethal disease. A more recent study from 2016 reports a similar result, which was that serotype-specific CD2v or C-type lectin induced haemadsorption-inhibition serotype-specific protective immunity. This shows that these antigens could be used for future vaccine development (16).

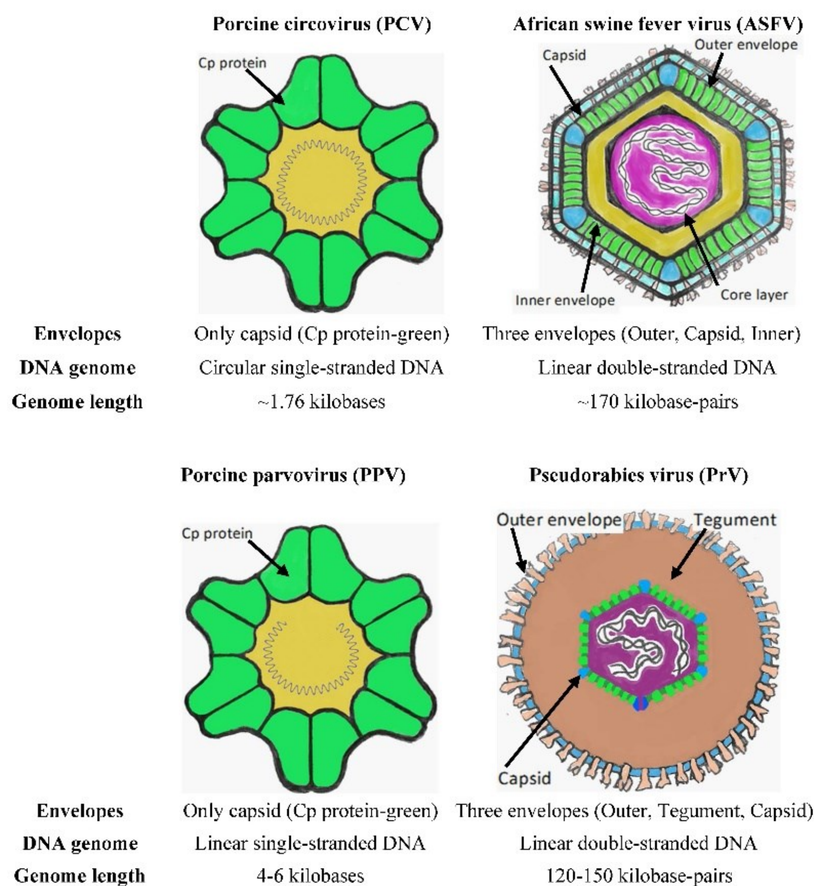

Fig. 1. Structural characteristics of viruses of interest

Porcine parvovirus (PPV)

PPV (58, 65) is a small ssDNA icosahedral nonenveloped virus (Fig. 1) with 5 kbp-long genomic DNA, which belongs to the *Parvoviridae* family, *Parvovirinae* subfamily and *Protoparvovirus* genus. PPV was first isolated in 1965 as a cell-culture contaminant (71) and this first isolate is designated PPV1. From 1965 onwards, different genotypes were identified, and recorded as PPV2 to PPV7, which were further classified based on their different characterisation as a separate genus within the family *Parvoviridae*.

Genetic classification. PPV1 genotype is the first identified genotype that was classified as the *Parvovirus* genus (65). PPV2 and PPV3 were both sorted into the *Tetraparvovirus* genus (27). PPV2 was identified for the first time during a study of the hepatitis E virus in swine sera collected in Myanmar in 2001 (48). The PPV3 genotype is closely related to human parvovirus 4 (PARV4) and porcine hokovirus that was identified for the first time in Hong Kong in 2008 (59). PPV4, PPV5 and PPV6 were classified in the *Copiparvovirus* genus (83, 110). Even though PPV4 belongs to the *Copiparvovirus* genus, it is closely related to the *Bocavirus* genus, containing an additional ORF3 (23) as *Bocavirus* does. The PPV5 and PPV6 genotypes were first identified in 2013 and 2014 in the USA and China, respectively (83, 105, 118). The first occurrence of PPV6 in Europe was observed in Poland in 2017 (28). The last identified genotype was PPV7, which was found in the USA, China and Korea in 2016 and 2017 (88, 119).

Clinical syndromes. The pathogenicity of PPV1 is the best known among the genotypes. PPV1 causes a reproductive failure disease in pregnant sows with clinical signs called SMEDI, an acronym of stillbirth, mummification, embryonic death and infertility (54). The route of infection in gravidity can influence the pathogenesis of the virus. The study by Joo *et al.* (50) shows that the intramuscular route facilitated the transfer of the virus from the dam through placenta and caused infection of foetuses earlier than oral routes of infection. However, the natural PPV entry path is oral, and such infections occur only when dams are exposed in the first part of the middle trimester of gestation (50).

PPV and host immune system. Induction of a cellular immune response to infection with PPV was observed (58). More specifically, CD4+ CD8+ T-cells were found to proliferate, while the activity of cytotoxic T-lymphocytes (CTL) was weak during the infection, indicating the role of humoral activity (58). The invasion by PPV also causes cell death by apoptosis, probably as a result of reactive oxygen species formation, which

activates the Bax apoptosis regulator and translocates it to near the mitochondrial membrane, triggering the subsequent release of cytochrome *c* and a caspase cascade (128). A recent study discovered that the NS1 PPV non-structural protein is responsible for the induction of apoptosis and thus involved in placental tissue damage and reproductive failure (125).

Development of vaccines. Vaccines designed against PPV infection are, in most cases, inactivated virus preparations based on PPV genotype 1 strains. It has been observed that inactivated vaccines can only prevent the disease but not the infection and virus shedding of PPV (35). In 2016, the study by Foerster *et al.* (35) showed that this applies both to homologous heterologous challenges with virulent PPV. Several approaches in vaccine development have been assessed. Vaccines based on genotype 1, including PPV-NADL2, PPV-IDT (MSV) and PPV-143a, and a vaccine based on the Stendal strain (51), are used for combating the disease caused by PPV1 (51, 123). It has been found that these vaccines were able to protect pigs against the disease but not against PPV-27a genotype 2 strain infection (51). PPV-27a was also used to prepare an inactivated vaccine, which likewise was only successful in providing protection from the disease and not from the infection and DNA replication (35). A vaccine against other genotype strains was not designed mainly due to inadequate information on the pathogenicity of these strains.

Pseudorabies Virus (PrV)

PrV is a large enveloped virus with a size of approximately 180 nm containing dsDNA (25). This virus was first described by Aujeszky in Hungary in 1902 as the agent of a disease, and although that disease was not related to rabies, its viral agent was named pseudorabies virus (the disease being termed Aujeszky's disease). The virus symptoms had already been observed previously, however, in the USA in the 1800s (25).

Virus structure. The viral particle appears in diagram form in Fig. 1. It is composed of morphologically different layers including a capsid protecting the dsDNA in the centre of the particle and thus forming a nucleocapsid and a protein matrix known as a tegument coated by the outer envelope, which contains a lipid membrane with distinct glycoproteins (93). A description of all structural proteins and their genes is given in detail in the article by Pomeranz *et al.* (93).

Genetic classification. Originally called suid herpesvirus 1 or Aujeszky's disease virus, PrV is classified into the *Herpesviridae* family and *Alphaherpesvirinae* subfamily containing a single serotype (36). A phylogenetic study based on sequences from the *UL44* gene encoding glycoprotein C (gC) divides PrV into five genotypes (A–E), which are neither country- nor continent-specific, in large part as a consequence of swine imports (36).

Clinical syndromes. Aujeszky's disease is typified by neurological and respiratory disorders resulting in weight loss, decreased growth and high mortality of piglets (93). Recently, it was found that the coinfection with PrV and PCV2 causes severe neurological and respiratory symptoms in pigs while damaging brain and lung tissue in piglets, resulting in higher mortality (126).

Development of vaccines. Two different vaccine types were developed for combating Aujeszky's disease. Inactivated and live attenuated vaccines were explored and live vaccines transpired to show higher efficiency and be more genetically stable than inactivated vaccines (38). Furthermore, live attenuated vaccines were observed to exhibit no or minimal residual virulence, suggesting their safety (38). The development of live attenuated vaccines against PrV is reviewed in the article by Freuling *et al.* (38).

The main DNA viruses significantly affecting swine are divided into four groups: PCVs, ASFV, PPVs, and PrV. Both porcine circoviruses and parvoviruses are small viruses having one capsid protein (Cp) and short genomic ssDNA. Vaccines against both viruses have been developed. However, a new vaccine should be designed, as a response to new genetically different genotypes having been identified which either have demonstrably different or yet unknown pathogenicity. In contrast, the African swine fever virus and pseudorabies virus are large viruses composed of a trilayer envelope and long linear genomic dsDNA. In the case of the African swine fever virus, there are many approaches to vaccine development. However, the effectiveness of every preparation was not sufficient for commercial purposes. In other words, there is no commercial vaccine for combating the viral infection and its disease. Further research is needed in this area to rectify this deficit. In the case of the pseudorabies virus, the majority of developed vaccines are live attenuated vaccines, due to their efficiency.

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