

Review

Classical Swine Fever—An Updated Review

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Abstract: Classical swine fever (CSF) remains one of the most important transboundary viral diseases of swine worldwide. The causative agent is CSF virus, a small, enveloped RNA virus of the genus *Pestivirus*. Based on partial sequences, three genotypes can be distinguished that do not, however, directly correlate with virulence. Depending on both virus and host factors, a wide range of clinical syndromes can be observed and thus, laboratory confirmation is mandatory. To this means, both direct and indirect methods are utilized with an increasing degree of commercialization. Both infections in domestic pigs and wild boar are of great relevance; and wild boars are a reservoir host transmitting the virus sporadically also to pig farms. Control strategies for epidemic outbreaks in free countries are mainly based on classical intervention measures; i.e., quarantine and strict culling of affected herds. In these countries, vaccination is only an emergency option. However, live vaccines are used for controlling the disease in endemically infected regions in Asia, Eastern Europe, the Americas, and some African countries. Here, we will provide a concise, updated review on virus properties, clinical signs and pathology, epidemiology, pathogenesis and immune responses, diagnosis and vaccination possibilities.

Keywords: porcine viruses; *Pestivirus*; classical swine fever; clinical signs; pathogenesis; epidemiology; diagnosis; control; vaccination; marker strategy

1. Introduction

Classical swine fever (CSF) is one of the most important viral diseases of domestic pigs and wild boar. It has tremendous impact on animal health and pig industry and is therefore notifiable to the World Organization for Animal Health (OIE) [1]. After implementation of strict control measures, several countries succeeded in eradicating CSF. Nevertheless, in most parts of the world with significant pig production, CSF is at least sporadically present. Endemicity can be assumed in several countries of South and Central America, parts of Eastern Europe and neighboring countries, as well as Asia, including India. Little is known about the African situation.

A binding legal framework exists for the surveillance and control in most countries. 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 often also strictly prohibited. However, in Europe, where affected wild boar populations were shown to be an important reservoir for the virus, and acted as a source for reintroduction into the domestic pig population [2,3], emergency vaccination of wild boar has been practiced to control the disease [4–7]. Emergency vaccination is also among the options to combat CSF in domestic animals. Furthermore, vaccination is still in use to reduce the disease burden in endemically affected countries.

Design of control measures and risk assessment depends on the knowledge of factors that influence disease dynamics and epidemiology. For this purpose, the presented review aims at providing an updated overview on the disease and the underlying mechanisms but also control and diagnostic options.

2. Virus Properties

2.1. Virus Organization and Replication

Classical swine fever virus (CSFV) belongs to the genus *Pestivirus* within the *Flaviviridae* family [1]. Other members of this genus are *Bovine viral diarrhea virus 1 and 2* (BVDV-1 and -2), *Border disease virus* (BDV) and a growing number of unclassified and so-called atypical pestiviruses, from giraffe-virus over HoBi-like viruses to recently discovered Bungowannah virus and atypical porcine pestivirus [2–13].

The enveloped viral particles consist of four structural proteins, namely the core protein (C), and envelope glycoproteins E1, E2, and E^{ms} [14–18]. The core encloses the positive single-stranded RNA genome of approximately 12.3 kb [19–22] which is translated into one polyprotein. The coding region is flanked by non-translated regions (NTR) at both ends. Co- and post-translational processing of the precursor protein by viral and cellular proteases results in 13 mature proteins, the above-mentioned structural proteins and non-structural proteins N^{pro}, p7, NS2-3, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The latter have various functions in the viral replication, e.g., NS5B represents the RNA-dependent RNA polymerase [23] and NS3 acts as protease [24,25].

Virus replication takes place in the cytoplasm after receptor mediated endocytosis and does normally not lead to a cytopathic effect in cell culture (naturally occurring CSFV strains were found to be non-cytopathic) [26]. A putative receptor is the porcine complement regulatory protein cluster of differentiation (CD) 46 that was shown to play a major role in CSFV attachment, together with heparan sulfates [27]. Upon cell culture adaptation an increased usage of heparin sulfates is observed for cell-virus interaction [28]. The mutation responsible for the adaptation lies within the E^{ms} encoding region [8], namely in the C-terminus where a Ser residue is replaced by an Arg residue at amino acid 476 in the polyprotein of CSFV.

In any case, glycoproteins E2 and E^{ms} are necessary for viral attachment [9,10], and the initial contact with the host cell is mediated through the E^{ms} which interacts with glycosaminoglycans [10,11]. For receptor binding and subsequent endocytosis, the E2-E1 heterodimer is essential [12]. After fusion of the virus envelope with the endosomal membrane, the virus core is released into the cytoplasm [13–15]. Thereafter, viral RNA is released into the cytoplasm and translation takes place. The binding of ribosomes at the rough endoplasmic reticulum is realized through an internal ribosomal entry site (IRES) at the 5' NTR, which allows a cap-independent translation [16–18]. The processing of the resulting viral polyprotein precursor occurs with the help of viral and cellular proteases [19]. Initially, autoprotease N^{pro} is cleaved from the polyprotein [20,21]. Subsequently, cellular proteases cleave the C-protein and E^{ms}, E1 and E2, E2 and p7 as well as NS2-3. NS2-3 is then partially processed through the autocatalytic cysteine protease activity of NS2 into NS2 and NS3. In this way NS2 generates its own C-terminal ending [22,23]. The serine protease activity of NS3 leads to the cleavage of the rest of the NS3-NS5 region [24]. While replication progresses, negative-stranded RNA is generated, which serves as template for the synthesis of the positive stranded RNA. The positive stranded RNA is then packed into the capsid [25]. Virion assembly and maturation takes place in the endoplasmic reticulum and the Golgi apparatus after which the progeny virions bud at the cell membrane through exocytosis [26,27].

2.2. Tenacity and Virus Inactivation

The survival of CSFV under different ambient conditions varies considerably and is influenced especially by the temperature but also by the matrix in which it is found. Generally, survival times

are higher under cold, moist and protein rich conditions [28]. The dependence of viral survival and temperature is well studied [29–31].

For animal disease control, survival in excretions (left in the pen or stored as slurry) and stability in meat products are crucial parameters. For CSFV in excretions, survival times were demonstrated that range from a few days at room temperature to several weeks at 5 °C [32]. If temperatures are higher than 35 °C, survival times are dramatically reduced, and inactivation occurs in hours or even minutes from temperatures above 50 °C [33]. This is an important factor when biogas plants and other industry parts are discussed. Along the same lines, Botner and Belsham [34] could show that survival of CSFV in slurry was short when heated and remained infective for weeks at cool temperature. Turner showed that complete inactivation was achieved at 60 °C for 3 min under lab conditions [35]. However, homogeneity of the mixture that is to be inactivated and thus temperature distribution is crucial [36]. For contaminated pig pens, this can mean virus survival for at least several days [37] to one month under cold winter conditions [38]. Under laboratory conditions, freeze-thawing has a negative impact on viral titers which can however be prevented by some chemical compounds such as dimethyl sulfoxide [39]. With regard to pH values, CSFV is relatively stable between pH 5 and 10. Half-lives at low pH levels are temperature dependent with mean half-lives that are more than ten-fold lower at room temperature compared to 4 °C (70 h at 4 °C compared to 5 h at 21 °C for pH 3). Overall variability is high and shows some strain dependence [40]. Another important matrix is meat or downstream products. Farez and Morley [30] report virus survival over years in meat frozen at –70 °C and of days to years in different meat products. Survival of 4.5 years in frozen meat was also reported by Edgar (reviewed in the EFSA scientific report 2009, [28]). Curing and smoking alone have little effect on the virus while higher temperatures readily inactivate the virus [31]. Survival times of more than 75 days were reported for salami [41] and over 120 days for Iberian loins or shoulders [42].

2.3. Genetic Diversity and Virulence Factors

Classical swine fever virus strains can be divided into three genotypes with three to four sub-genotypes. The most recently added sub-genotype 1.4 was only very recently described for CSFV strains from Cuba. These strains had so far been placed into sub-genotype 1.2 but formed a distinct cluster when compared based on longer genome fragments, e.g., full-length E2, N^{pro}, C, E1, and E^{ms} [43]. Further divisions that have been proposed concern sub-genotypes 2.1 and 2.3 [44–47]. However, these systems of clusters or clades were not further harmonized and did not enter routine use. The genetic diversity does not result in true serotypes and does not impact vaccine efficacy. In general, CSFV is highly stable, especially for an RNA virus [48].

Up to very recently, phylogenetic studies were mainly based on two short fragments, namely a 150 nucleotide (nt) fragment of the 5'NTR and a 190 nt fragment of the E2 encoding region [49]. Moreover, a 409 nt fragment of the region coding for the polymerase gene NS5B was employed [50]. With the advent of affordable sequencing technologies for longer fragments or even full genomes, in-detail analyses are now more often based on more than the traditional fragments. The European Union (EU) Reference Laboratory for CSF nowadays recommends using full-length E2 encoding sequences for reliable CSFV phylogenies [51]. The latter resulted, e.g., in the designation of the above-mentioned new sub-genotype 1.4. Full-length sequences are being employed for quasispecies analyses, investigation of virulence determinants but also high resolution molecular epidemiology [52–55].

The distribution of genotypes shows a distinct geographical pattern [50,56]: Whereas isolates belonging to group 3 seem to occur solely in Asia, all European CSFV isolates of the 1990s and later belonged to one of the subgroups within group 2 (2.1, 2.2, or 2.3) [45,51,57–64] and were clearly distinct from former CSF reference viruses, which belong to group 1 [50,65]. On the global scale, the most prevalent genotype over the last decades was undoubtedly genotype 2 [66]. However, all field isolates from the American continent belong to genotype 1 with only 1.1 strains from Argentina, Brazil, Colombia, and Mexico; 1.3 strains from Honduras and Guatemala; and the above-mentioned

sub-genotype 1.4 strains from Cuba [43,67–69]. Little is known about the CSFV situation in Africa and the Middle East. Exceptions are the 2005 outbreak in South Africa and the 2009 outbreak in Israel that were both caused by 2.1 CSFV strains [70,71]. Reports from India are increasingly detailed and demonstrate that sub-genotypes 1.1, 2.1, and 2.2 are co-circulating [72–79]. This changes the historical situation where genotype 1.1 strains predominated. From Nepal, strains of sub-genotype 2.2 were reported [80]. The situation in China is characterized by high variability of strains that belong mainly to sub-genotypes 1.1, 2.1, 2.2, and 2.3 [81–84]. Taiwan is also experiencing a change in sub-genotypes. It seems that the historical 3.4 strains are replaced by the Chinese 2.1 strains [85]. However, Taiwanese reports include all the above-mentioned sub-genotypes [85–87]. Sub-genotype 2.1 and 2.2 strains are also reported from Laos [88,89]. From Korea, strains of sub-genotypes 3.2 and 2.1 were reported [44], and, for Japan, indications exist that genotype 3 is found [90]. Generally, endemicity is accompanied or driven by strains of moderate or low virulence. These strains have been found in several regions with long-term circulation of CSFV (e.g., Cuba [91]), and mathematical models have shown that these strains may represent the viral optimum for long-term persistence [92]. An overview of the genotype distribution is provided in Figure 1.

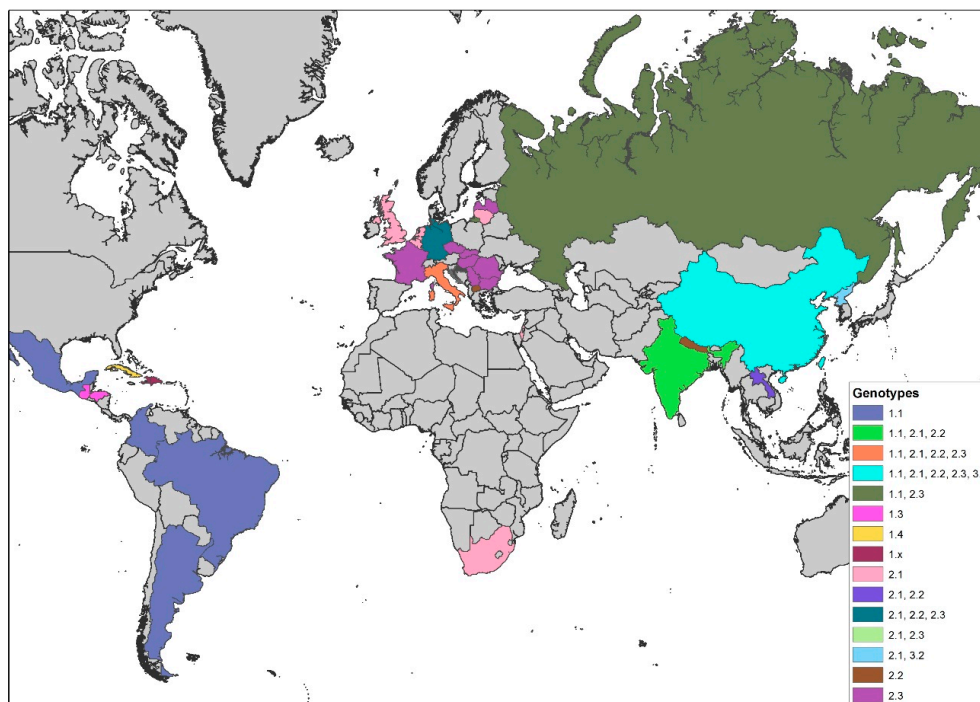


Figure 1. Global distribution of classical swine fever virus (CSFV) sub-genotypes (map based on Global Administrative Areas (GADM database 2.8; November 2015)).

European CSFV sequences were collected and made available through the semi-public CSF-database (DB) at the EU and OIE reference laboratory for CSF in Hannover, Germany [49,93–95]. Since the Institute of Virology became European Reference Laboratory for CSF more than 30 years ago, the virus isolates involved in European outbreaks but also other accessible sequence data were collected and stored. The database includes the above-mentioned fragments and also partial NS5B, full E2, and full-length CSFV sequences. It also allows automated typing and retrieval of sequences for in-detail analyses [95].

The search for virulence markers indicated a role of the N^P₁₀ [96], the E2 [97], the ribonuclease activity and dimerization of the E^{rn}₅ [98,99], and NS4B [100]. Furthermore, glycosylation of structural proteins was shown to affect virulence [101–105]. However, these determinants are still far from being understood and do not seem to be transferrable among strains. Even the direct comparison of vaccine

strains and their virulent ancestors did not reveal clear pattern [100,106]. Investigations into the role of quasispecies composition did not lead to the establishment of a clear correlation between variability and virulence [52]. There were also no predictors for different disease courses found [107].

3. Clinical Signs and Pathomorphological Lesions

Classical swine fever can be divided into the following forms of the disease: an acute (transient or lethal), a chronic and a persistent course, which usually requires infection during pregnancy [65]. In general, the same clinical signs are seen in both domestic pigs and wild boar, and show up after an incubation period of four to seven (seldom 10) days after the infection. The progression is dependent on strain virulence, host responses, and secondary infections and may vary considerably. However, infection of young pigs (weaners) with a moderately virulent CSFV strain may serve as an example for the acute disease course: During the first two weeks upon infection, the acute phase is characterized by unspecific (often referred to as “atypical”) clinical signs like high fever, anorexia, gastrointestinal symptoms, general weakness, and conjunctivitis [108]. Around two to four weeks after infection neurological signs can occur including incoordination, paresis, paralysis and convulsions. At the same time, skin hemorrhages or cyanosis can appear in different locations of the body such as the ears, limbs, and ventral abdomen. These late signs are the textbook cases and are therefore referred to as “typical” CSF signs. Examples of clinical signs can be found in Figure 2.

In acute-lethal courses, death usually occurs 2–4 weeks after CSFV infection. Mortality can reach up to 100% from 10 to 30 days depending on the age of the animal and the virulence of the virus strain [65,109–111]. Due to the immunosuppressive character of CSF infection, severe respiratory and gastrointestinal secondary infections can complicate the disease course and overlay the CSF signs. This is particularly important for clinical diagnosis. Infections with highly virulent CSFV strains such as “Margarita” or “Koslov” (the ones that are often used for vaccine testing) show a less age-dependent clinical course and may result in 100% mortality in all age classes of animals and severe neurological signs within 7 to 10 days (see, e.g., [112]).

Chronic course occurs when an infected pig is not able to mount an adequate immune response. In general, only non-specific clinical signs are observed in infected animals like remittent fever, depression, wasting and diffuse dermatitis (see Figure 3). It is acknowledged opinion that all chronically infected animals will eventually die. However, they can live for month in which they constantly shed high amounts of virus. Affected animals may develop antibodies that are in some cases only intermittently present and do not effect viral clearance. This, together with persistent infection, can play a role especially for affected wild boar populations [113–115], but also in endemically affected regions with constant virus circulation. Host rather than viral factors seem to play a role for the establishment of chronic infection [107].

Clinical signs of acute classical swine fever infection

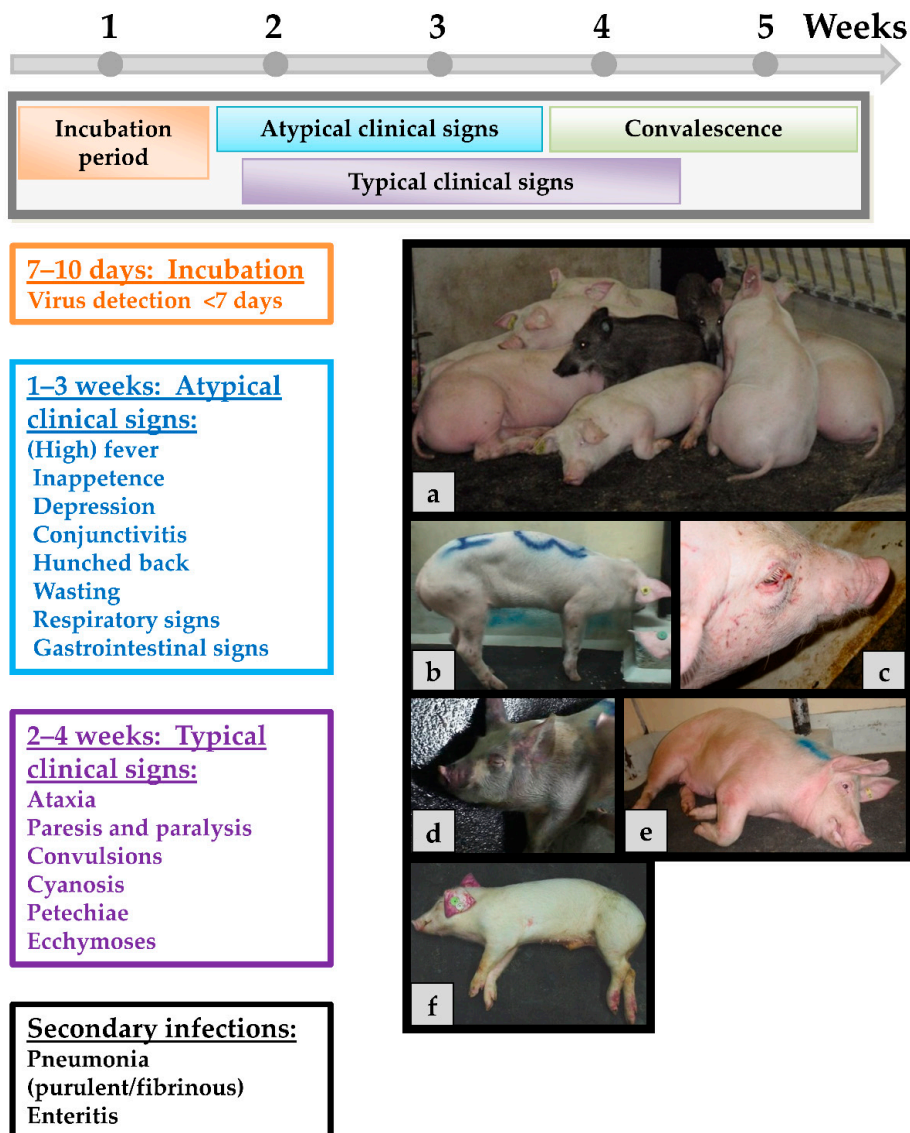
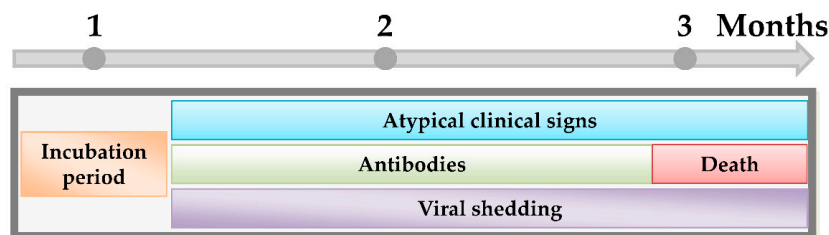


Figure 2. Acute CSFV infection with moderately virulent strains. The incubation period in most cases is from 7 to 10 days. Atypical clinical signs range from one to two weeks. Typical clinical signs occur around 2 to 4 weeks post-infection. The convalescent period is from 3 to 4 weeks. Death can occur as late as five weeks post-infection. (a) Swine are huddling, 10–15 days post-infection; (b) swine are presenting with hunched back; (c) severe conjunctivitis; (d) severe cyanosis of the skin around the face, ears, and limbs; (e) neurological signs, swine was unable to stand; and (f) dead swine with classic cyanosis of the ears.

Clinical signs of chronic classical swine fever infection



Up to 1 month:
Incubation

1–3 months: Atypical signs

Remittent fever
Depression
Stunted growth
Wasting
Diffuse dermatitis
Respiratory signs
Gastrointestinal signs

1–3 months:
Viral shedding

Common secondary infections:

Pneumonia
(purulent/fibrinous)
Enteritis

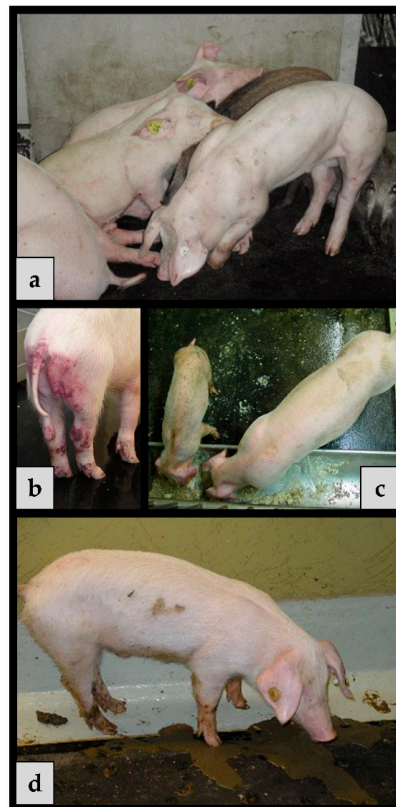


Figure 3. Chronic CSFV infection. The incubation period is the same as with the acute course. However, it may take up to a month until they are truly recognized. Atypical clinical signs can be present throughout and until death, occurring up to three months or even later after the infection. Antibodies can be detected at low levels after two weeks or later but do usually not persist. Viral shedding is observed from about four days post infection till the death of the animal. (a) Pigs are depressed, hunched over, and anorexic; (b) pig with petechial bleedings and ecchymosis in the anogenital region; (c) stunted and wasting pig beside a normally developed one of the same age; and (d) pig with diarrhea, shedding high viral loads until death.

When infection occurs during pregnancy, the virus can also infect the fetus in the womb due to its ability to pass the placental barrier which in turn might lead to persistent infection in the piglets. While the sows often show only mild clinical signs, an infection depending on the stage of gestation, leads to absorption or mummification of the fetuses and to abortions or stillbirth [114,116–123]. When infected between days 50 and 70 of pregnancy, an immunotolerance phenomenon can be induced and persistently infected offspring are born. The problem is that those piglets seem to be healthy and survive for several months but die due to the so-called late onset form of CSF. During that period they shed high viral loads which are sufficient for transmission. Recent studies discuss that persisting infection can also be induced when infecting newborn piglets within the first eight hours of life or even

48 h after birth [124,125]. This was shown to impact on the efficacy of vaccines and may complicate control in endemically affected countries.

The pathological findings (Figure 4) depend on the course of the viral infection. In the acute course of CSF, pathology often reveals enlarged lymph nodes, hemorrhages and petechiae on serosal and mucosal surfaces of different organs such as the, lungs, kidneys, intestines and urinary bladder. Tonsillitis, necrotic ulcers in the intestines, lesions in the lymphoreticular system, and non-purulent encephalitis can be observed [126] Splenic infarctions can occur and are considered pathognomonic for CSF [127]. Infected piglets develop leukopenia, thrombocytopenia and immunosuppression, which increases the risk for secondary infections and thus to diseases of the gastrointestinal and respiratory system [128]. In the chronic form, pathological lesions include atrophy of the thymus, depletion of the lymphoid organs, necrosis and ulceration of the small intestine, colon, and ileocecal valve. It is important to consider that these clinical signs and pathological lesions should be considered as differentials for a number of swine pathogens. These unspecific clinical signs and lesions can vary among animals depending on host factors and the virulence of the CSFV strain. Often, the age, breed and immune status play a role in the outcome of the disease [65,108,129].

Lesions of classical swine fever infection

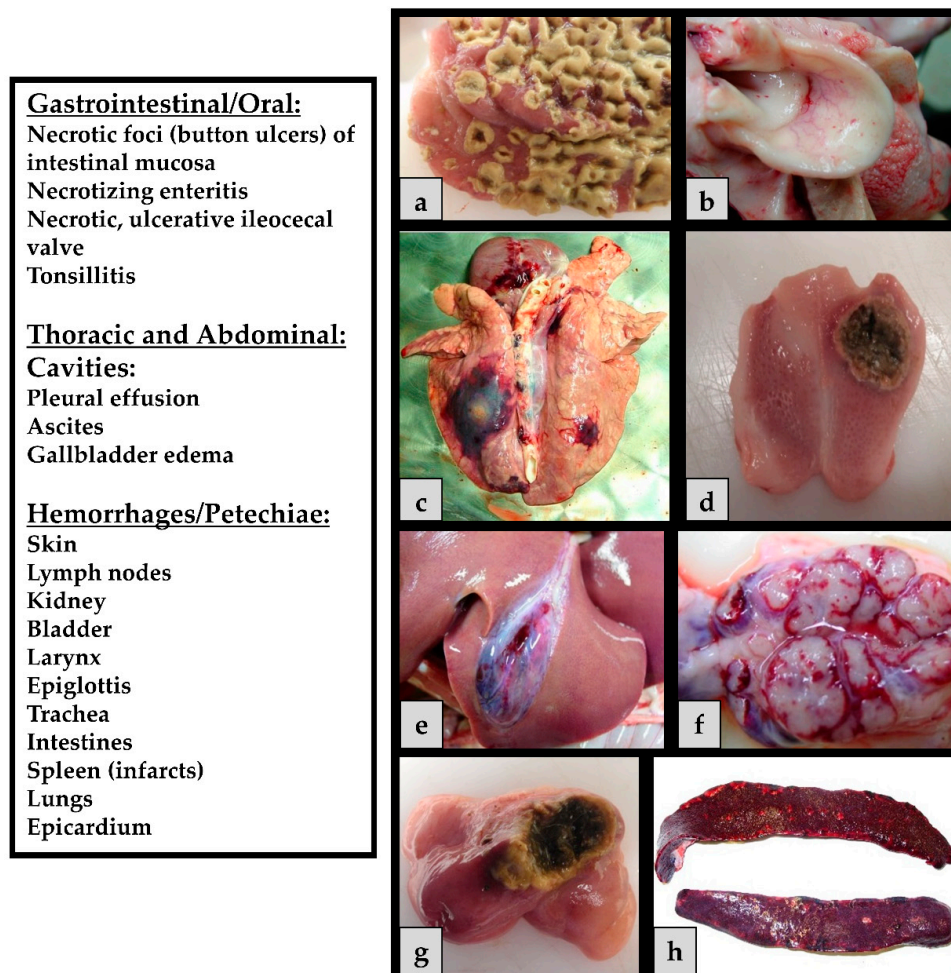


Figure 4. CSF related lesions: (a) Diphtheroid-necrotizing enteritis; (b) hemorrhages on the epiglottis; (c) severe secondary infections of the lung (*Actinobacillus pleuropneumoniae*); (d) necrotic tonsillitis with an ulcer; (e) gallbladder edema; (f) hemorrhagic lymph node; (g) necrotizing ileocecal valve; and (h) splenic infarcts.

4. Pathogenesis and Immune Responses

As mentioned above, clinical signs of CSFV infections can vary considerably from peracute deaths to unapparent courses depending on virulence of the virus strain involved and different (partly unknown) host factors [65]. Unspecific clinical signs predominate, and differentiation from several other infectious diseases of swine is only possibly based on laboratory diagnosis. Acute-lethal forms can be viral hemorrhagic fever-like with severe thrombocytopenia, pulmonary edema, petechial bleedings, and increased vascular leakage [130]. Cytokine involvement is discussed for many lesions observed in acute CSF [131].

Infection with CSFV is followed by primary replication in the tonsils and subsequently spread to surrounding lymphoid tissues [132]. The virus reaches the regional lymph nodes through lymphatic vessels. Here further replication takes place and the virus is spread via blood to secondary replication sites such as spleen, bone marrow, and visceral lymph nodes [133–135]. Apoptotic reactions as well as phagocytic and secretory activation can be observed in several macrophage populations [136–144]. These activated macrophages seem to play a crucial role in (immuno-)pathogenesis while direct damage by the virus could be almost excluded for many lesions occurring in the course of CSFV infection. Moreover, dendritic cells are targeted and disturbance of the interferon system contributes to the pathogenesis [136–140]. There seems to be a correlation between high interferon (IFN)- α in the serum and disease severity and virulence of the strain involved [140,141]. High IFN- α concentrations are found as early as two days post infection, prior to the onset of clinical symptoms [112]. These findings are confirmed by microarray analyses of peripheral blood monocyte cells derived from CSFV-infected pigs [142].

Especially in the acute-lethal course, CSF is accompanied by severe lymphopenia and resulting immunosuppression as well as granulocytopenia [143–146]. Moreover, a marked thrombocytopenia starts very early after infection [147–149]. The mechanisms leading to this platelet decrease are not yet understood but disseminated intravascular coagulation (DIC), degeneration of megakaryocytes, bone marrow lesions, and accelerated deterioration have been discussed [130]. In addition, massive activation and subsequent phagocytosis of platelets has been discussed as an etiological factor [147] while DIC related correlates were not observed upon infection with a genotype 2.3 CSFV strain [150]. At least in vitro, endothelial cells are also activated and expression levels of pro-inflammatory and pro-coagulatory factors are increased [151]. The pathogenic mechanism involved in hemorrhagic lesions include damage of endothelial cells, causal involvement of thrombocytopenia (and DIC), erythrodiapedesis, and capillary vasodilatation and increased permeability [146,148,149,152,153]. However, several factors remain unclear and studies with different strains have given conflicting results.

Despite the immunopathogenesis of most CSF-related lesions, pigs recovering from CSFV infection mount an effective immune response with E2-specific antibodies detectable after 10–14 days. The E2 antibodies are able to neutralize CSFV in vitro and induce protective immune responses [154,155]. These antibodies and protection against re-infection persist probably lifelong. In addition to E2, antibodies are raised against the E^{ms} and the non-structural protein NS3 [156,157]. Immunization with live attenuated CSFV can be efficient as early as 3–5 days post vaccination [158–160]. Thus, protection is possible without neutralizing antibodies and even before specific T-cell responses can be seen. Despite the fact that this very early protection is far from being understood, IFN- γ secreting T-cells seem to play a role [161–163].

5. Epidemiology

Susceptible hosts are different members of the *Suidae* family, particularly domestic pigs (*Sus scrofa domestica*) and European wild boar (*Sus scrofa scrofa*) [113,164]. Moreover, the susceptibility of common warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus*) was recently demonstrated [165].

Classical swine fever virus can be transmitted both horizontally and vertically. Horizontal transmission takes place through direct or indirect contact between infected and susceptible pigs. Important indirect routes include feeding of virus contaminated garbage/swill and mechanical transmission via contact to humans or agricultural and veterinary equipment [127]. Aerogenic transmission was reported under experimental conditions [166–168], and it can probably play a role for within herd transmission [169].

Upon contact, infection usually occurs through the oronasal route, or less frequently via conjunctiva, mucus membranes, skin abrasions, insemination, and the use of contaminated instruments [170–173]. Infected pigs show high-titer viremia and shed virus at least from the beginning of clinical disease until death or specific antibodies have developed. The main excretion routes are by saliva, lacrimal secretions, urine, feces, and semen [127,135,173]. As mentioned above, chronically infected pigs shed the virus continuously or intermittently until death [65]. Vertical transmission from pregnant sows to fetuses is possible throughout all stages of gestation and can lead to persistently infected offspring (see above).

Classical swine fever affected wild boar populations can serve as reservoir of the virus and present a constant risk for domestic pigs. Fritzemeier et al. [2] could show that almost 60% of the primary CSF outbreaks in Germany between 1993 and 1998 were linked to infected wild boar. This link was particularly important for holdings with low biosecurity or problems in biosafety management.

Over the last decades, a decreasing virulence was observed for the CSFV strains involved in many outbreaks among wild boar and domestic pigs. In Europe, the most prevalent genotype 2.3 strains showed moderate virulence with a highly age-dependent clinical picture and rather unspecific clinical pictures in older animals (see above). These strains showed potential to establish endemicity in affected wild boar populations rather than showing the self-limiting behavior of the historical highly virulent CSFV strains. It was discussed whether these strains are somewhat the ideally adapted variants of CSFV for long-term perpetuation in wildlife [92].

In endemically affected countries with official but imperfect vaccination, circulation of less virulent CSFV strains is often masked by partial protection. In combination with management and biosecurity issues (swill feeding, contacts, shared equipment), the virus is maintained over prolonged periods in the domestic pig population.

6. Diagnosis

Rapid and reliable diagnosis is of utmost importance for the timely implementation of control measures against CSF. On the international level, laboratory methods as well as sampling and shipping guidelines can be found in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals and the respective EU Diagnostic Manual (European Commission Decision 2002/106/EC).

For CSFV, primary detection is performed using well established real-time reverse transcription polymerase chain reaction (RT-qPCR) systems [174–183], of which many are available commercially. Recently, field applicable RT-PCRs [184] but also alternatives have been designed such as loop-mediated isothermal amplification (LAMP) assays [185–190], primer-probe energy transfer RT-qPCR [191,192] or recently insulated isothermal RT-qPCR [193]. Moreover, CSFV can be isolated on different permanent cell lines such as porcine kidney cell lines PK15 or SK6 (Technical Annex to Commission Decision 2002/106/EC). In addition, detection of antigen on fixed cryosections of tissues is possible using fluorescence antibody or immune-peroxidase assays [194,195]. The available antigen ELISAs are recommended for the use with herd-based testing only. While the sensitivity of panpestis-specific assays (based on the E^{tns}) is usually at least comparable with virus isolation, most CSF specific assays lack sensitivity [196]. Serological screening can be performed using different commercially available E2 antibody enzyme-linked immunosorbent assays (ELISAs). In addition, neutralization assays allow, to a certain extent, differentiation of pestivirus antibodies and are used for confirmation [197].

Reliable DIVA (differentiation of infected and vaccinated animals) assays are needed when using DIVA vaccines. Commercially available tests that can accompany both E2 subunit vaccines and

chimeric vaccines such as “CP7_E2alf”, target the detection of antibodies directed against glycoprotein E^{ms} [196,198,199]. Recently, additional diagnostic tests have been developed. One is a double-antigen ELISA format that was recently commercialized [200], another is an ELISA with a screening and a confirmation part [201]: Moreover, a microsphere immunoassay was also developed as a confirmatory test [202].

Due to the increased sensitivity of diagnostic tools (especially RT-qPCR), vaccine virus detections are quite common in oral vaccination campaigns of wild boar and vaccination programs of domestic pigs. For this reason, different RT-qPCR systems have been developed and tested, these allow differentiation between vaccine and field viruses (genetic DIVA) [203–208].

Sampling can be the bottleneck of swine fever diagnosis, especially in the case of wild boar, but also in remote areas. For this reason, alternative sampling strategies and sample matrices have been tested for CSF (often combined with African swine fever sampling) especially for wildlife specimens and under rural conditions [209–212]. However, most of them are not yet in routine use and need further validation.

7. Vaccination

Highly efficacious and safe live-attenuated CSF vaccines have existed for decades [160]. The underlying virus strains (e.g., the C-strain of CSFV, the Lapinized Philippines Coronel, the Thiverval or the Japanese guinea-pig exaltation negative GPE strain) were attenuated through serial passages in animals (rabbits) or cell culture. These vaccines have been implemented in mandatory control programs that led, together with strict hygiene measures, to the eradication of CSF from several regions of the world [213]. At this time, they are still in use in several Asian countries including China [84], countries of South and Central America, Trans-Caucasian Countries, and Eastern Europe (see Table 1). In 2016, 22 countries officially reported mandatory vaccination campaigns (OIE WAHIS [214]).

In addition, these vaccines were also adapted to a bait format for oral immunization of wild boar [6,215,216] and were recently explored for the vaccination of domestic pigs under backyard conditions [217–219]. While these vaccines usually have outstanding virtues in terms of onset, spectrum and duration of immunity [158,220–223], the main drawback is the lack of a serological marker concept [160] that would allow differentiation of field virus infected from vaccinated animals (DIVA concept). This is usually less important in endemically affected countries where prophylactic vaccination is carried out to reduce the disease burden and to ensure product safety. In general, there are also no legal obligations to use a certain type of vaccine for an emergency vaccination scenario. However, due to the trade restrictions that are imposed on pigs vaccinated with conventional live attenuated vaccines, only DIVA vaccines are considered a feasible option for domestic pigs [224]. Up to very recently, only E2 subunit marker (DIVA) vaccines were available on the market (at present, one E2 marker vaccine is commercially available, Porcilis[®] Pesti, MSD Animal Health, Unterschleißheim, Germany). These vaccines are safe and were shown to provide clinical protection and limit the spread of CSF [225–235]. However, they show drawbacks especially in terms of early protection [160,236] and protection against transplacental transmission [237]. Due to these problems, emergency vaccination was hardly implemented in domestic pigs (one exception being Romania). Several research groups have therefore sought to develop a next-generation marker vaccine candidate that would ideally answer all demands with regard to safety, efficacy, DIVA potential, and marketability [238]. Among the concepts that have been investigated are different vector vaccines based on vaccinia virus, pseudorabies virus or adenoviruses. Other vaccine designs include recombinant attenuated vaccines with chimeric constructs, subunit vaccines based on different expression systems, and RNA/DNA vaccines (recently reviewed by Blome et al., [239]. In 2014, the European Medicines Agency (EMA) licensed one of the chimeric marker vaccine candidates, “CP7_E2alf”, after extensive testing in the framework of an EU-funded research project [159,240–257]. This new marker vaccine is still under investigation and could be a powerful tool for both emergency vaccination of domestic pigs and also wild boar.

Oral emergency vaccination of wild boar with baits has proven to be a potent tool to control the disease in wildlife and to safeguard domestic pigs [3]. For this purpose, the above-mentioned C-strain formulations have been used in several European countries including Germany and France. To further optimize the strategy, a DIVA vaccine such as “CP7_E2alf” could be used. The latter was already tested for use in wild boar under both laboratory and field conditions and could be a medium term option [241,246,251].

Table 1. CSF vaccination: Countries that reported official vaccination campaigns through World Organization for Animal Health (OIE) in 2016 (their last reported outbreaks are presented in brackets; no reports for some countries since 2005) (WAHIS Interface [214]).

Country	Last reported CSF outbreak
Albania	no reports
Armenia	2006
Azerbaijan	no reports
Belarus	no reports
Bosnia and Herzegovina	2007
Bulgaria (wb)	2009 wb
China	2015
Colombia	2016
Cuba	2016
Dominican Republic	2016
Ecuador	2016
Macedonia	2008
Georgia	no reports
Hong Kong	2005
Madagascar	2016
Moldova	(no reports)
Mongolia	2016
Myanmar	2015
Peru	2016
Philippines	2016
Russia	2016
Ukraine	2015

Wb: Wild boar.

Conflicts of Interest: The authors were involved in the design and testing of some of the vaccines and received third party funds to carry out the studies (industry funding and EU framework programs FP6 and FP7 under grant agreement numbers 227003 CP-FP and SSPE-CT-2003-501559). No other conflicts of interest exist.

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