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Classical swine fever: Pathogenesis and prevention

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Abstract
Classical swine fever virus (CSFV) is a highly contagious viral disease of domestic pigs, wild boar and feral pigs. In Europe, the wild boar population is an important reservoir for the virus, and represents a source for reintroduction of the disease in domestic pigs. It is a listed disease by world Organization for Animal Health (OIE). Classical swine fever virus (CSFV) infection of pigs causes disease courses from life-threatening to asymptomatic, depending on the virulence of the virus strain and the immunocompetence of the host. The disease is characterized by acute, subacute, chronic, late onset, or in apparent course, depending on a variety of viral and host factors of which the age of the animals, the virulence of the virus and the time of infection (pre- or post-natal) are of greatest importance. At present, live-attenuated vaccines are routinely used. These are often based on either the 'Chinese' (C) strain, on the cell culture adapted Japanese guinea-pig exaltation-negative (GPE−) strain or on the French cell culture adapted Thiverval strain. Massive vaccination with live attenuated vaccines, such as C-strain has been implemented routinely as a major control strategy.

Keywords: Classical swine fever, pig, vaccine, OIE, C-strain

1. Introduction
Classical Swine Fever (CSF), also called Hog Cholera or European Swine Fever, caused by CSF virus is one of the most important infectious diseases of pigs and wild boar, causing significant economic losses to the pig industry all over the world (Westergaard *et al*., 1990) [51]. It is a serious, often fatal, economically damaging disease of swine which can spread in an epizootic form as well as establish enzootic infections in domestic and wild pig populations (Edwards *et al*. 2000) [8]. It is one of the listed diseases of the World organization for animal health or Office International des Epizooties (OlE). Successful eradication has been achieved in many countries, including North America, Australia, and parts of Northern Europe in absence of vaccination, for rest of the pig producing countries CSF remains a serious threat, because of the globalisation and intensification of pig trade and transport, the increase in pig densities in many areas, increased numbers of wild boar, which act as reservoirs of CSF virus (CSFV), and the feeding of improperly sterilized swill (Van, 2003) [49].

2. History
CSF was first recorded in Ohio, USA in 1833 but an epizootic resembling CSF became reported in France in 1822 (Cole *et al.* 1962) [5]. The disease became widespread in Europe and America by 1866. Brich reported that the development of railways during the mid 19 th century facilitated the spread of virus. In India the first suspected case of CSF occurred in Aligarh in 1944 (Krishnamurthy 1964).

3. Etiology
CSFV is a member of the genus pestivirus within the Flaviviridae family. It is a spherical/icosahedral shaped virus particle of 40–60 nm in diameter, consisting of a lipid envelope surrounded by a nucleocapsid packaging a positive-strand RNA genome. The 12.5 kb CSFV genome consists of one large open reading frame (ORF) that encodes an approximately 4000-aminoacid polyprotein which is co- and post-translationally processed into 11–12 final cleavage products (NH2-Npro-C-Erns-E1-E2-p7- NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH) using cellular and viral proteases (Rice, 1996). The ORF is flanked by untranslated regions (UTRs) that are highly conserved among virus isolates (Risatti *et al*., 2003) [24]. Despite the availability of various CSFV genomic sequences representing varying virulence phenotypes, the genetic basis of CSFV virulence in the natural host remains poorly understood (Van 1999) [47]. However, several viral determinants of virulence have been:

identified in Npro (Mayer *et al*., 2004; Meyers *et al*., 1999; Moser *et al*., 2001; Risatti *et al*., 2005a, b, 2006, 2007a,b; Ruggli *et al*., 2005, 2003; Tratschin *et al*., 1998; Van Gennip *et al*., 2002, 2004, 2005; Van Rijn *et al*., 1994) [14, 15, 43, 23, 25-28, 30, 43, 45-49].

4. Epidemiology

CSFV isolates from different parts of the world have been placed in to various genomic groups/subgroups (Lowings *et al.,* 1996) [13]. Major genetic groups being 1, 2, 3 and 10 with subgroups. In Asia, CSF epidemics are also fairly ubiquitous. Strains of genotypes 1, 2, and 3 have been isolated in different Asian countries (Paton *et al*., 2000, Blacksell *et al*., 2005) [18, 3]. Furthermore, isolates belonging to group 3 seem to occur solely in Asia (Vlasova *et al*., 2003) [50]. CSF is endemic in India. Phylogenetic analysis revealed that all CSFV isolates during 2005-2007 from domestic pigs in different districts of Assam belonged to group 1 and subgroup 1.1 in contrast to the situation in other Asian countries (Sarma *et al*., 2011) [34]. Seventeen CSFV isolates recovered during the period of 3 years (2006-2008) from India could be grouped into two subgroups, 1.1 and 2.2 (Patil *et al*., 2010) [17]. Another study demonstrated that CSFV field isolates from India (3 isolates) belonged to genotype 2.1 and were closely related to European CSFV strains, and the remaining 6 isolates belonged to genotype 1 that contained old and new strains. It also indicated circulation of both genotypes 1 and 2.1 in north-eastern part of India (Desai *et al*., 2010) [6]. One of the isolates from Mizoram was closely related to Chinese strain Shimen (Barman *et al*., 2010) [1]. Phylogenetic characterization of Indian virulent (CSFV/ MP) and lapinised strain (CSFV/LAP) at I.V.R.I Izatnagar placed both the strains into genogroups 1.1(Gupta *et al* 2011) [9]

5. Pathogenesis

CSFV has tropism for vascular endothelial and immune system cells, mainly those that are derived from the monocyte-lineage (Summerfield *et al*., 1998 a, 2001) [36]. After oronasal infection, CSFV passes through the epithelial cells and cells of the tonsilar crypts, the primary target tissue for virus replication. Thereafter, the virus is found in the tonsils and local oropharyngeal lymph nodes (Ressang *et al*., 1973 and Trautwein *et al*., 1988) [21, 44]. A particular affinity of the virus for the reticuloendothelial cell system has been noted with macrophages. Macrophage, dendritic cells (DC) and endothelial cells (EDC) being the primary targets (Summerfield *et al*., 1998; Knoetig *et al*., 1999) [36, 11]. From these primary sites of replication, the virus spreads to other lymphoid organs. Such secondary target organs include the spleen, lymph nodes, gut associated lymphoid tissue, bone marrow and thymus. CSFV has also been found in the pancreas, brain, heart, gall and urinary bladders, mandibular salivary and adrenal glands, thyroid, liver and kidney, particularly in association with EDC and macrophages. The invasion of CSFV to the host immune system can cause severe lymphopenia which is the hallmark of CSFV infection, resulting in immunosuppression (Jamin *et al*., 2008) [10]. CSFV can also penetrate the placenta to establish an infection in the developing fetus, resulting in the birth of persistently infected animals. The per acute and acute disease is characterised by pyrexia, anorexia, central nervous disorders, diarrhea and in some cases also haemorrhages of the skin, mucosa and various other organs.

The virulent CSFV strains can induce a typical hemorrhagic fever with immunological characteristics common to all viral hemorrhagic fevers. The disease is associated with severe lymphopenia and lymphocyte apoptosis (Summerfield *et al*., 1998) [36] thrombocytopenia (Trautwein *et al*., 1998) [44], platelet aggregation (Bautista *et al*., 2002) [2], bone marrow depletion affecting myelopoiesis and magakaryocytopoiesis and thymus atrophy as well as thymocyte apoptosis. Lymphoid depletion is generalized, not only affecting peripheral blood and lymph nodes but also the mucosal tissue. At later stages, disseminated intravascular coagulation (DIC), petechial bleedings and haemoconcentration can be found which can result in a circulation failure hypotension and death. A recent study however suggests that the hemorrhagic lesions observed in the late stages of the disease are not attributable to DIC. Inhibition of diffuse fibrin and thrombi formation did not influence the extent of haemorrhagic lesions. From this, it was concluded that DIC was not the cause for the thrombocytopenia and haemorrhages observed in acute-lethal CSF (Blome *et al*., 2013) [4].

Very high levels of serum interferon (IFN)-α are a hallmark of the acute disease phase induced by virulent CSFV. It appears that the levels of IFN-α found in the serum correlate with disease severity and the virulence of the isolate used for infection (Ruggli *et al*., 2009) [31]. In younger animals a correlation between serum IFN-α levels and the degree of lymphopenia induced by CSFV was found. The onset of severe lymphopenia was concomitant with the IFN-α responses, and all animals with serum IFN-α had depleted peripheral B and T lymphocytes (Summerfield *et al*., 2006) [35]. These observations concluded that high levels of IFN-α cannot control the virus but may rather mediate aberrant responses leading to immunopathology. Microarray analyses of PBMC isolated from infected pigs confirmed not only the dominance of IFN stimulated genes but also of cell death receptor and apoptosis pathways such as TRAIL, FAS and TNF relating to previous studies performed with peripheral blood cells using flow cytometry (Summerfield *et al*.,1998) [36]. Type I interferon (IFN) has broad antiviral and immunomodulatory effects and is part of the innate immune response against viruses. Type I IFN has beneficial effects in viral infections, restricting viral dissemination and promoting immunopathological events when released at high levels over a longer duration (Summerfield *et al*., 2006) [35]. However, CSFV exacerbates the IFN-α response, which is detected in the serum of infected pigs; this response been hypothesized to be related to disease severity rather than to protective immune responses (Summerfield *et al*., 2006; Tarradas *et al*., 2010) [35, 42].

In vivo macrophage infection and morphological signs of activation were found in various organs like spleen, kidney, lung, liver and the intestine. The infection of pigs was associated with macrophages producing pro-inflammatory cytokines, such as IL-1α, IL-1β, IL-6 and TNF-α. There is also evidence for macrophage activation leading to the production of vasoactive mediators including prostaglandin E2 (Knoetig *et al*., 1999) [11] and platelet activation. Therefore, MΦ infection and activation has been proposed to play an important role in CSF pathogenesis, in particular through release of pro-inflammatory and vasoactive mediators.

Despite the severe lymphoid depletion, acute CSF is also associated with a pronounced anergy of T lymphocytes in the acute phase of the disease (Pauly *et al*., 1998) [19]. Similarly, indication of B-cell activation has been described in terms of

an increase in cells expressing the lambda light chain and IgM. In separate studies it was seen that IL-2, in conjunction with antigen and T cells, promotes the proliferation of B cells (Murtaugh, 1994) [16] whose activation is completed through the participation of various cytokines including IL-4. The division and differentiation of B cells into immunoglobulin-producing plasma cells is induced by IL-4 (Van Miert, 1995; Roitt *et al*., 1998) [46, 29]. In CSF, this differentiation mechanism appears to be enhanced by an increase in the amount of IL-4 released by T lymphocytes, and by the eventual predominance of IL-4 over IL-2; this, together with a late decrease in IFN-Y, emphasizes replacement of a type 1 immune response by a type 2 response as the disease progresses (Sanchez-Cordon *et al*., 2005a) [33].

6. Prevention and Control

In the highly endemic areas routine vaccination against CSF is the most common means used for prevention and control. Massive vaccination with live attenuated vaccines, such as C-strain, developed in China in mid-1950s has been implemented routinely as a major control strategy The C strain, modified live vaccine (MLV) has been regarded as one of the most effective CSF vaccines that provides complete clinical and virological protection, i.e. sterile immunity, within a week of vaccination (Suradhat *et al*., 2001; Van 2003) [40, 49]. It is considered as the gold standard vaccine for the control of CSF (Dewulf *et al*., 2004) [7]. Several strains of commercial CSF-MLV, mostly derived from genogroup 1, are available in the market. Although, CSF-MLV could effectively induce protective immunity in pigs, certain conditions are required to achieve complete viral protection. Maternally derived antibodies (MDA) is the most common factor affecting the induction of protective immunity against CSFV in the field. It should be noted that this protective effect was observed on the condition that the pigs had low levels of MDA (<32) at the time of vaccination (Suradhat *et al*., 2001; Suradhat and Damrongwatanapokin, 2003) [40]. Apart from MDA, age at the time of primary vaccination (Suradhat and Damrongwatanapokin, 2002) [38] and complication by other pathogens also influences protective immunity against CSFV. It has been demonstrated that PRRSV infection significantly interfered with induction of CSFV-specific immunity which resulted in vaccine failure (Suradhat *et al*., 2006) [41].

Recent years have witnessed a growing interest in a field of vaccinology that we have named vaccinomics. Vaccinomics was defined by Poland *et al*., (2011) [20] as "the integration of immunogenetics and immunogenomics with systems biology and immune profiling". The overall idea behind vaccinomics is to identify genetic and other mechanisms and pathways that determine immune responses, and thereby provide new candidate vaccine approaches. Considerable data show that host genetic polymorphisms act as important determinants of innate and adaptive immunity to vaccines. The influence of HLA genes, non-HLA, and innate genes in inter-individual variations in immune responses to viral vaccines are examined using population-based gene/SNP association studies. The ability to understand relationships between immune response gene variants and vaccine-specific immunity may assist in designing new vaccines as well as in selecting disease resistant animals.

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