ISSN 1466-2523

Animal Health Research Reviews **15**(1); 87–101
doi:10.1017/S1466252314000024

Classical swine fever in pigs: recent developments and future perspectives

Vishal Chander[1]*, S. Nandi[1], C. Ravishankar[1], V. Upmanyu[2] and Rishendra Verma[3]

[1]*Virology Laboratory, Centre for Animal Disease Research and Diagnosis (CADRAD), Indian Veterinary Research Institute (IVRI), Izatnagar 243122, U.P., India*
[2]*Division of Biological Standardization, IVRI, Izatnagar 243122, U.P., India*
[3]*CADRAD, IVRI, Izatnagar 243122, U.P., India*

Received 6 March 2013; Accepted 4 April 2014

Abstract

Classical swine fever (CSF) is one of the most devastating epizootic diseases of pigs, causing high morbidity and mortality worldwide. The diversity of clinical signs and similarity in disease manifestations to other diseases make CSF difficult to diagnose with certainty. The disease is further complicated by the presence of a number of different strains belonging to three phylogenetic groups. Advanced diagnostic techniques allow detection of antigens or antibodies in clinical samples, leading to implementation of proper and effective control programs. Polymerase chain reaction (PCR)-based methods, including portable real-time PCR, provide diagnosis in a few hours with precision and accuracy, even at the point of care. The disease is controlled by following a stamping out policy in countries where vaccination is not practiced, whereas immunization with live attenuated vaccines containing the 'C' strain is effectively used to control the disease in endemic countries. To overcome the problem of differentiation of infected from vaccinated animals, different types of marker vaccines, with variable degrees of efficacy, along with companion diagnostic assays have been developed and may be useful in controlling and even eradicating the disease in the foreseeable future. The present review aims to provide an overview and status of CSF as a whole with special reference to swine husbandry in India.

Keywords: classical swine fever, *Pestivirus*, diagnosis, vaccines, control

Introduction

Classical swine fever (CSF) or hog cholera is an important infectious disease of pigs, with considerable economical implications in the swine industry worldwide. It affects domestic pigs, wild boars and feral pigs. The first outbreak of the disease, observed in France in 1822 (Cole *et al.*, 1962), was thought to be caused by a bacterium termed the 'hog cholera bacillus'. It is now known that the CSF virus (CSFV) is closely related to the bovine viral diarrhea virus (BVDV) and ovine border disease virus (BDV). Infection with CSFV is listed as reportable to the World Organisation for Animal Health (OIE), so every suspected case should be investigated and the OIE should be notified of positive cases (OIE, 2014).

Acute cases of CSF caused by virulent virus can be diagnosed relatively easily, but not the infections caused by less virulent viruses. In some cases, dullness and poor reproductive performance are noticed. Due to a wide range of clinical signs and similarity to other diseases, it has become a challenging task to diagnose the CSF accurately. In spite of a number of efficacious and safe vaccines available to control CSF, the disease is prevalent in Europe, Asia and South America. However, the African continent considered CSF-free except for Madagascar. The disease is endemic to India, where it is prevalent in most of the states. This review is aimed to provide the latest information of all the aspects of CSF including the status of

*Corresponding author. E-mail: drvishal1@gmail.com

the disease in India, latest diagnostic tools, immunoprophylactic measures and control strategies for the benefit of the swine industry.

Etiological agent

CSFV is classified under the genus *Pestivirus* within the family *Flaviviridae* (ICTV, 2012). The capsid of this enveloped virus is hexagonally shaped, 45 nm in diameter and encloses the single-stranded positive sense RNA genome of 12.3 kb. The CSFV genome contains one open reading frame (ORF), which encodes one large polyprotein that is cleaved by proteases to yield mature viral proteins (Moormann *et al.*, 1990). The ORF is flanked by a 5′ untranslated region (UTR) of almost 400 nt and a 3′-UTR of about 200 nt. The 5′-UTR functions as an internal ribosome entry site (IRES) for translation of the polyprotein of about 3900 amino acids. The order of the gene products is N–C–E^{rns}–E1–E2–P7–NS2.3–NS4a–NS4b–NS5A–NS5B–COOH, with coding regions for the capsid protein and envelopes the glycoprotein E^{rns}–E1–E2 at the N terminus and non-structural proteins at the C terminus. The 5′ and 3′ NTRs of the CSFV are thought to act together *in cis* to induce apoptosis, and this NTR-mediated apoptosis requires double-stranded RNA and translation shutoff through activation of protein kinase *R* (Hsu *et al.*, 2014). The E^{rns} protein shows RNase activity and is immunosuppressive in nature *in vitro*. The E2 is the most immunodominant protein and is composed of two independently formed antigenic domains. The NS2.3 is the most conserved among the pestiviruses. Other members under the genus *Pestivirus* include BVD-1, BVD-2, BDV and a pestivirus isolated from a giraffe. CSFV differs from BVDV and BDV in sequences at the 5′-UTR and the E2 gene (Katz *et al.*, 1993; Vilcek *et al.*, 1994; Van Rijn *et al.*, 1997). CSFV is genetically more stable than BVDV and different strains can be grouped on the basis of differences in the nucleotide sequences of the 5′ NTR, the N terminal of E2 and a region of NS5B. The amino acid similarity between CSFV and BVDV is about 70% with the highest homology between NS3 of the NS2.3 protein and the lowest in the E2 protein (Paton, 1995).

There is only one CSFV serotype, but a number of strains of variable virulence and antigenicity can be distinguished with monoclonal antibodies (Wensvoort *et al.*, 1989; Edwards and Sands, 1990; Edwards *et al.*, 1991). Classification of CSFV strains and isolates currently is mainly based on 190 nt of the E2 envelope glycoprotein gene, but also with 150 nt of the 5′-UTR and 409 nt of the NS5B polymerase gene. On the basis of phylogenetic analysis, CSFV can be divided into three groups with three or four subgroups: 1.1, 1.2, 1.3; 2.1, 2.2 and 2.3; 3.1, 3.2, 3.3 and 3.4. These groups and subgroups show distinct geographical distribution patterns. All of the groups have been found in Asia, but group 3 mainly occurs in Asia, group 2 in the European Union and group 1 in South America, Central America and the Caribbean (Paton *et al.*, 2000a). CSFV genomes can also be clustered on the basis of their codon pair bias, which correlates with the genotype rather than with the virulence of the isolates (Leifer *et al.*, 2011). Most of the highly virulent CSFV strains and the vaccine strains belong to genotype 1. Genotypes 2 and 3 are the moderately virulent strains, and the genetic variability within this strains is comparatively higher than genotype 1 (Uttenthal *et al.*, 2003; Rasmussen *et al.*, 2010). Both 1.1 and 2.2 subgroups have been reported in India, with the predominance of 1.1 and involvement of both subgroups in outbreaks (Patil *et al.*, 2010).

Pathology and pathogenesis

Under natural conditions, CSFV enters the host through oral, nasal, conjunctival and genital mucous membranes. The spread of highly virulent CSFV strains in pigs is characterized by lymphatic, viremic and visceral phases. The virus initially infects epithelial cells of the tonsillar crypts, followed by the regional lymph node and the efferent blood capillaries, leading to viremia. Virus proliferation occurs in the spleen, visceral lymph node, lymphoid tissues and bone marrow in acute CSF and persists until the death of the animal. The virus has distinct affinity for vascular endothelium and cells of the immune system. The widespread hemorrhages in acute CSF are due to degeneration and necrosis of endothelial cells, thrombocytopenia and disturbances in fibrinogen synthesis (Pauly *et al.*, 1998; Gómez-Villamandos *et al.*, 2001).

Moderate or low-virulence CSFV strains can induce persistent infections designated as chronic or late onset swine fever. Initially, the virus disseminates throughout the body at a slower rate with low concentrations of virus in serum and organs. Secondly, the virus is limited to epithelial cells of the tonsils, ileum, salivary glands and kidney. The antigen–antibody complex is deposited in the kidney leading to glomerulonephritis. Finally, the virus disseminates throughout the body with secondary bacterial infections (Cheville *et al.*, 1970).

Late onset CSF occurs when low virulent CSFV infects a fetus during the first 40 days of gestation. Such pigs have a lifelong viremia with spread of the virus in epithelium, lymphoid and reticuloendothelial tissues (van Oirschot, 1979). The pathogenesis of CSFV is depicted in Fig. 1.

The multiplication of low-virulence CSFV following postnatal infection is mainly restricted to tonsils and lymph nodes. In pregnant sows, the outcome of the fetal infection depends on the age of the fetus and the virulence of the virus (Radostitis *et al.*, 2007). Transplacental infection with both field and vaccine strains of the virus may induce abnormalities such as hypoplasia of lungs, malformation of the pulmonary artery,

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

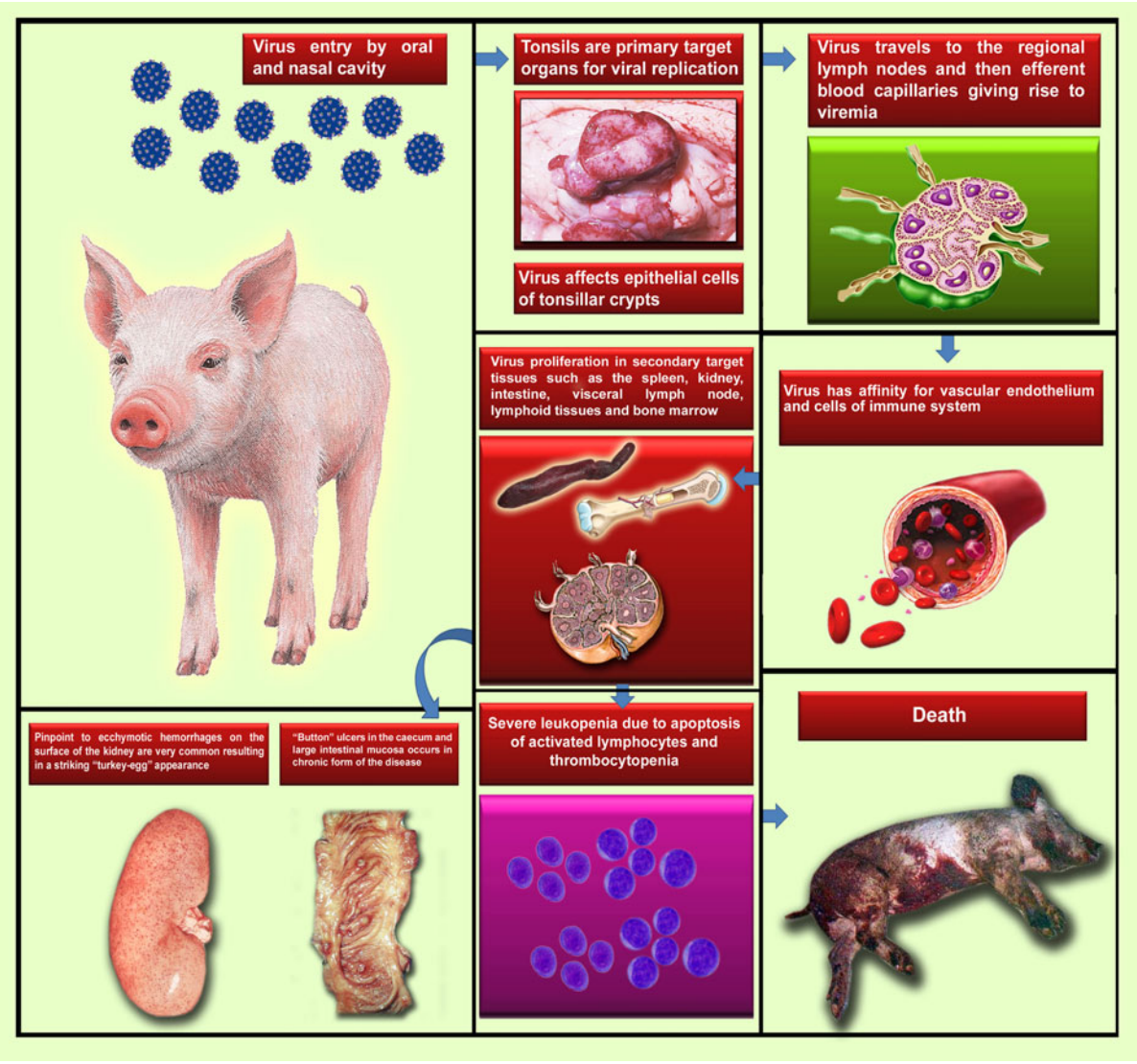

Fig. 1. Pathogenesis of classical swine fever virus.

micrognathia, arthrogryposis, fissures in the renal cortex, multiple septa in the gall bladder and malformations in the brain. Earlier infection leads to more severe abnormalities and may result in abortions, stillbirths, mummification and malformations, whereas infections at 50–70 days leads to birth of persistently viremic piglets that are clinically normal at birth but die after several months. These animals have retarded growth (runts) and shed a large number of virus particles. Infection occurring at a later stage of gestation may lead to development of immunotolerance (van Oirschot, 1979; Meyer *et al*., 1981). Immunity develops rapidly in immunocompetent swine that survive acute infections (Susa *et al*., 1992; Summerfield, 1998a, b; van Oirschot, 2004; van Oirschot et al., 2004; Radostitis *et al*., 2007).

In peracute cases, no gross changes are found. Hemorrhages occur in various organs throughout the body in case of acute and subacute infection due to capillary endothelium degeneration and thrombocytopenia. Lymph nodes are swollen, edematous and appear as red or black due to diffuse hemorrhages. The heart, larynx, urinary bladder, intestinal mucosa, serosa and skin show petechial to echhymotic hemorrhages. It has also been observed that disseminated intravascular coagulation (DIC) caused by the CSFV may not have an effective role in the pathogenesis of the disease (Blome *et al*., 2013). Kidneys on removal of the capsule show petechiae in the cortex and have an appearance known as 'turkey egg'. The skin may be cyanotic. The presence of splenic infarcts up to 10 mm in size is considered to be pathognomonic for acute swine fever. Intestines appear hyperemic with diphtheroid inflammation, and large intestines show characteristic 'button ulcers' in subacute and chronic CSFV infection. Lesions occur in the brain

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

and spinal cord, and congestion occurs in the liver, bone marrow and lungs. Hemorrhages and infarctions are less pronounced or absent in persistent infection. Thymic atrophy and depletion of lymphocytes in lymph nodes, tonsils and spleen are also observed (Van Oirschot, 2000, 2004).

In a proteomic analysis study of serum from pigs with CSF, lower levels of apolipoprotein A-I (apo A-I), vitamin D-binding protein (VDBP) and haptoglobin (Hp) were observed, and proteins observed at higher levels included retinol-binding protein 4 (RBP4) and serotransferrin. Up and downregulation of these proteins has different implications on the pathogenesis of the disease due to various functions of these proteins, as Apo A-I may affect vascular endothelium repair. Hp, which is a positive acute phase protein, affects angiogenesis and vascular repair. RBP4 in CSFV-infected pigs may be involved in kidney injury caused by CSFV infection. Serotransferrin in CSFV-infected serum suggests that iron is needed for efficient CSFV replication (Sun Jin-fu *et al.*, 2011).

Epidemiology

Both domestic pigs (*Sus scrofa domesticus*) and wild boar (*Sus scrofa scrofa*) are equally susceptible to CSFV infection. All the secretions and excretions, notably saliva, nasal and ocular discharges, urine, semen and feces, are rich sources of the virus, and pigs are susceptible to infection through ingestion and inhalation of contaminated products. Movement of the pigs which are incubating the disease or persistently infected is the most common cause of spreading the infection (de Smit *et al.*, 1999). The virus can survive for long periods as it is quite resistant to many chemical and physical conditions. Pigs infected with the virulent virus may shed the virus before the onset of clinical signs causing high morbidity and mortality in a herd until they die or recover. Pigs are also infected during transport in contaminated trucks, through inanimate objects and mechanical vectors like flies and mosquitoes (de Smit *et al.*, 1999). Experimental transmission of CSFV to goats, sheep, cattle, peccaries (*Tayassu tajacu*) and rabbits was successful whereas other vertebrates such as racoons, mice and pigeons did not support the propagation of the virus. Pork and pork products may also harbor the virus for several months under frozen conditions (Dunne, 1975).

Immune mechanisms

CSF affects the immune system, mainly by causing generalized leukopenia. Neutralizing antibodies are observable after 9 days in recovering animals and after 15 days in fatally infected animals. Neutralizing antibodies are important in terms of protection and are detectable during the partial recovery phase, 3–6 weeks after infection in chronic cases. Maximum antibody titers occur 3–4 weeks after infection and may persist for 6 months.

Lymphoid organs are also affected by CSFV infections, leading to a deficiency of B lymphocytes. The cell-mediated immune response plays a key role in CSF pathobiology and prevention through quantitative changes in the T-lymphocyte population and qualitative changes in cytokine expression by these cells in the serum, thymus and spleen (Gómez-Villamandos *et al.*, 2001; Sánchez-Cordon *et al.*, 2002, 2003). The lymphoid depletion is caused by lymphocyte apoptosis induced by chemical mediators from monocyte–macrophage cells (Sánchez-Cordón *et al.*, 2002). In CSF, differentiation and maturation of T lymphocytes in the thymic cortex are potentiated, although T-lymphocyte apoptosis impairs the effectiveness of the non-specific immune response (Sánchez-Cordón *et al.*, 2005). The non-arrival of T lymphocytes from the thymic cortex might prevent any recovery of T-lymphocyte populations in the spleen. From the onset of the disease, an increase is noted in the number of $CD4^+$ and $CD8^+$ lymphocytes (Narita *et al.*, 1996, 2000), and the $CD8^+$ population was found to be relatively high compared to $CD4^+$ T cells at advanced stages (Narita *et al.*, 1996, 2000; Pauly *et al.*, 1998; Lee *et al.*, 1999; Sánchez-Cordón *et al.*, 2005). This increase, despite intense lymphoid depletion, may be due to activation and differentiation of lymphocytes still remaining in the organs examined, which may have played a role in inducing a cell-mediated response to the virus (Hernández *et al.*, 2001). The increase in the number of $CD8^+$ T cells is more closely linked to expression of IL-6 (Van Snick, 1990; Sánchez-Cordón *et al.*, 2002) and may also be related to the clonal expansion (Tizard, 1998). $CD8^+$ T cells are cytotoxic for CSFV-infected cells, and their increase during the disease may therefore be a part of the defense mechanism (Doherty *et al.*, 1992; Pauly *et al.*, 1995; Summerfield *et al.*, 1996). This has been demonstrated when vaccinated swine remained protected from the virus despite the absence of neutralizing antibodies (Rümenapf *et al.*, 1991; Suradhat *et al.*, 2001). IL-2 synthesis may be enhanced by secretion of IL-1 and IL-6 from macrophages, with IFN-γ inducing an autocrine effect on the production of this cytokine (Biron and Sen, 2001). Early apoptosis of infected monocyte–macrophage cells and phagocytosis of apoptotic bodies by other monocyte–macrophage cells may play a decisive role in the spread of the virus and in its initial evasion of the immune response (Gómez-Villamandos *et al.*, 2001; Sánchez-Cordón *et al.*, 2003). A general dysfunction of the T-lymphocyte activity has also been observed. Follicular depletion of lymphocytes and/or necrosis in pigs infected with a virulent CSFV strain are observed in histopathological examinations.

Antibodies are produced against NS3, E2 and E^{rns} viral proteins. Antibodies against E2 and E^{rns} are protective. Although CSFV has little tendency to accumulate mutations in contrast to other RNA viruses, antigenic variation

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

is observed among CSFV isolates due to the highly variable antigenic region of the E2 gene but not to the extent observed with ruminant pestiviruses. Convalescent animals therefore have a stable and long-lasting immunity against all variants of CSFV (Moennig and Greiser-Wilke, 2008).

The number of $CD4^+$ and $CD8^+$ T lymphocytes is significantly higher in pigs infected with virulent strains of CSFV. Mature granulocytes ($SWC3^+$; $SWC8^+$) are not susceptible to CSFV infection, whereas the less differentiated myeloid progenitor cells ($SWC3^{low}$; SWC^{8-}) are infected, thus explaining the presence of CSFV in peripheral blood mature $SWC8^+$ cells (Summerfield *et al.*, 1998a, b, 2001). As much as 90% of total T cells are depleted in the final stages of the disease, depending on the virulence of the viral strain (Pauly *et al.*, 1998). Increased numbers of necrotic and apoptotic uninfected cells have been identified in the bone marrow of CSFV-infected pigs (Summerfield *et al.*, 2000). Immunosuppression can be detected much earlier than seroconversion and clinical signs of the disease, which is relevant both for early diagnosis and for the study of viral pathogenesis (Pauly *et al.*, 1998; Summerfield *et al.*, 1998a, 2001; Ganges *et al.*, 2005). In one study, it was observed that during CSFV infection there may be inhibition of expression of MHC class II molecule SLA-DR, which is primarily involved in antigen presentation (Feng *et al.*, 2012).

When the immune responses are insufficient to clear the virus from the body, persistence of CSFV in the host may occur. Persistent infections can be established even in the presence of neutralizing antibodies. Congenital infection with CSFV can lead to persistently infected animals that do not develop specific antibodies against the virus (van Oirschot, 1979; de Smit *et al.*, 2000), probably due to the immunotolerance developed during fetal lymphocyte differentiation. Specific immune unresponsiveness may occur during intrauterine infection of the piglets which are persistently viremic and may live for several weeks, but usually die at 3 weeks of age (Radostitis *et al.*, 2007). The animals with persistent infection continuously shed the virus and are a potential source of new CSF outbreaks (Vannier *et al.*, 1981; Carbrey, 1989), as well as creating problems in diagnosis. Viral destruction of the germinal centers in lymphoid tissues leads to the B-lymphocyte deficiency and is the most significant immunopathologic consequence of acute infection with the virulent strain of CSFV. Thus, CSFV appears to alter the immunity of the pigs by causing the B-cell reaction and increased T lymphocytes in chronic stages (Narita *et al.*, 2000).

Diagnosis

Recognition of clinical signs by veterinary practitioners in the field, and of gross pathological lesions after *post mortem* examination, is important in diagnosis of CSF. A laboratory-based diagnostic test validates the presence of the CSFV and helps to differentiate the disease in the presence of similar signs attributable to others diseases.

Clinical diagnosis

The diversity of clinical signs in CSF under field and experimental conditions makes it difficult to diagnose the disease. Furthermore, BVDV and BDV infections can seriously interfere with the clinical and laboratory diagnosis of the disease.

Laboratory diagnosis

Antigen detection

CSFV antigens can be detected by the direct fluorescent antibody test (DFAT) or immunohistochemistry by demonstration of the virus in the tonsillar crypt and germinal center besides the spleen, lymph node, kidney, thymus, tonsil, brain and lower ileum. To rule out BVDV and BDV, a panel of monoclonal antibodies (MAbs) is required. Antigen can also be tested with the agar gel precipitation test (AGPT) (Nandi *et al.*, 2011b), MAbs detected with an avidin–biotin complex (ABC) immunoperoxidase test (Porntrakulpipat *et al.*, 1998) and sandwich enzyme-linked immunosorbent assays (ELISAs) with polyclonal or monoclonal antibody to capture and detect CSFV antigen in serum, blood or WBCs for rapid herd screening (Leforban *et al.*, 1990; Moser *et al.*, 1996; Clavijo *et al.*, 1998). The sensitivity of ELISA is comparatively higher and less cumbersome than that of virus isolation (Clavijo *et al.*, 1998).

Detection of infectious virus

Virus can be isolated from tissue or blood and is a sensitive *in vitro* method for detection of CSFV in the early phase of infection. The porcine kidney cell lines (PK-15, SK-6) or swine testicular cells are commonly used for isolation of virus. CSFV is almost always non-cytopathic, so fluorescent antibody tests (FAT) or immunoperoxidase staining after incubation for 1–3 days is required to detect the CSFV antigen (OIE, 2008). The virus isolation is more sensitive than DFAT on frozen tissue sections (de Smit, 2000).

Detection of antibodies

CSFV infection mainly induces antibodies against viral proteins E2, E^{rns} and NS3. Detectable levels of antibodies

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

appear 2–3 weeks post-infection and persist lifelong. Paired sera samples are to be collected from convalescent pigs for testing. Among diagnostic techniques, the virus neutralization test (VNT), namely the neutralization peroxidase linked assay (NPLA), is most commonly used in Western Europe. In Japan, the END method (exaltation of the Newcastle disease virus) to assay most field viruses has been used, including the END neutralization test (Shimizu *et al.*, 1964). The immunoperoxidase monolayer assay (IPMA) is often used in North America and Latin America (Afshar *et al.*, 1989). To avoid false-positive results, parallel assays with BVDV/BDV and analysis by differences in the antibody titer are usually taken into consideration. OIE–recommended tests for international trade use blocking or indirect ELISAs to detect antibodies to E2, E^{rns} or NS3 protein (Muller *et al.*, 1996; Langedijk *et al.*, 2001). The sensitivity and specificity of E2-based ELISA are 90–99 and 99%, respectively, compared to VNT. In general, ELISAs are cross-reactive leading to false positive results but are well suited for mass screening of animals, and testing of individual animals should be avoided. Tissue, blood and serum samples can be diagnosed by an antigen capture ELISA based on the CSFV E^{rns} glycoprotein.

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR is a more rapid, sensitive and specific test than FAT, ELISA or virus isolation and is useful in preclinical and clinical diagnosis of CSF (McGoldrick *et al.*, 1998; Paton *et al.*, 2000b; Risatti *et al.*, 2003, 2005; Hoffmann *et al.*, 2005). Care should be taken to avoid false-positive results due to contamination during processing and false-negative results due to nucleic acid degradation and inhibitors in the sample (OIE, 2008). RT-PCR followed by nucleotide sequencing is helpful in differentiating other pestivirus infections beside grouping and tracing the outbreak strain during epidemics for molecular epidemiology (Lowings *et al.*, 1996; Vilcek *et al.*, 1996; Greiser-Wilke *et al.*, 2006; Sarma *et al.*, 2011; Patil *et al.*, 2012). Nested RT-PCR is generally considered the most sensitive method for the detection of CSFV. A multiplex RT-PCR assay is useful for rapid and differential diagnosis of CSFV among other *Pestivirus* infections based on the NS5B gene and 5′-UTR (de Arce *et al.*, 2009). A pan-*Pestivirus*-specific single-tube nested PCR and a CSFV-specific fluorescent probe allow detection of pestiviruses as well as CSFV in clinical samples (McGoldrick *et al.*, 1998). A real-time RT-PCR has also been developed for detection and genotyping of CSFV without inter-genotypic cross-reactivity among different CSFV strains or with other swine pathogens (Huang *et al.*, 2009). TaqMan-based real-time PCR using lyophylized RT-PCR reagents and portable instruments has been developed as a pen-side assay for rapid on-site detection of CSFV (Risatti *et al.*, 2003). RT-PCR is less vulnerable to sample degradation with on-site detection, because the average half-life of viral RNA is 1–3 days. The tonsil and spleen are appropriate samples for the detection of infectious virus and viral RNA both in fresh and degraded samples (Weesendorp *et al.*, 2010), but blood may be considered as the most appropriate sample for early detection of CSFV by RT-PCR (Shivaraj *et al.*, 2013). Whole blood and tonsil scrapings may be considered as the samples of choice for quick and early CSFV detection in live pigs irrespective of the virulence of the CSFV strain (Donahue *et al.*, 2012).

A reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay targeting the CSFV 5′-UTR was more recently developed (Wongsawat *et al.*, 2011). The benefits of LAMP compared with other nucleic acid amplification techniques are ease of operation, no need for specialized equipment like a thermocycler or electrophoresis apparatus, superior sensitivity, lower risk of contamination, suitability for high-throughput DNA detection and visualization of results by naked eye (Mori *et al.*, 2001).

The OIE-prescribed tests for serological diagnosis or surveillance and international trade include NPLA, fluorescent antibody virus neutralization (FAVN) and ELISA. The virus neutralization test (VNT), although slow and laborious, is considered to be the best method for antibody detection in terms of sensitivity and the ability to distinguish antibodies directed against CSFV from antibodies against other pestiviruses. VNT does not discriminate infected from vaccinated animals, although it may be used as a confirmatory test in ELISA-positive cases from areas free of CSF. Thus, to screen large amounts of sera for antibody, ELISA is the best method and the doubtful cases may be confirmed by VNT (Blome *et al.*, 2006).

Differential diagnosis

CSF needs to be differentiated from several viral and bacterial diseases that affect swine. Splenomegaly, edema of the gall bladder and bile ducts, subpleural and interlobular lung edema and hematoma-like lymph nodes are indicative of African swine fever (ASF), a disease that is similar to CSF. Other diseases affecting swine such as pasteurellosis, erysipelas, streptococcosis, porcine reproductive and respiratory syndrome (PRRS), cumarin poisoning, porcine dermatitis and nephropathy syndrome (PDNS), salmonellosis and Glasser's disease (due to *Haemophilus parasuis* infection) have CSF-like signs. In the case of runt pigs, diarrhea, occurring usually in the chronic phase of CSF, must be differentiated from other etiologies such as *Escherichia coli* and *Salmonella* infections. CSF intestinal inflammatory lesions must be differentiated from those due to campylobacteriosis, clostridial infections and swine dysentery. Reproductive

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

inefficiencies such as abortions, mummified fetuses and stillbirths can also be found in the cases of pseudorabies infection, parvovirus and PRRS. CSF and pasteurellosis often are concurrently associated with swine mortality (Kumar *et al.*, 2007). Based on the epidemiology of CSF in a particular area, differentiating tests can be used reliably to effectively diagnose the disease in the presence of other diseases.

Vaccines and vaccination

In endemic countries, the introduction of CSFV can be prevented by purchasing pigs from CSF-free herds and implementing a quarantine period of 4 weeks followed by testing for CSFV. Strict hygiene and thorough cleaning and disinfection of pens also help in preventing virus entry into a herd. Furthermore, live attenuated vaccines (LAV), namely the lapinized Chinese (LC) strain, Japanese GPE-strain, French PK-15 cell-adapted Thiverval strain or PAV-250 strain, are used to control classical swine fever. The LC strain has an insertion of 13 nucleotides in the 3′-UTR compared to virulent virus whereas the GPE strain differs in 225 nucleotides from its parental strain. The LC strain, attenuated by 100 passages in rabbits, is very efficacious and widely used as it provides solid immunity against clinical signs, virus replication and excretion within a week of vaccination (Bironet *et al.*, 1987); this is a vaccine of choice for an emergency vaccination (Van Oirschot, 2000, 2004). This vaccine can block transmission of virulent CSFV to in-contact pigs 7 days after vaccination, it does not persist in pigs beyond 2–3 weeks (Lorena *et al.*, 2001) and it confers protection for 3 years to life time (van Oirschot, 2004; van Oirschot *et al.*, 2004). The vaccine types available in India, and that are mostly used, include the tissue culture LC strain vaccine, although it is still in the developmental phase (Bett *et al.*, 2012). Piglets vaccinated in the presence of maternal antibodies need to be revaccinated at 8 weeks of age. Multiplication of the vaccine strains is mainly restricted to lymphoid organs, notably tonsils, and can cross the placenta without any abnormalities in fetuses (van Oirschot, 2004; van Oirschot *et al.*, 2004). Vaccination does not always prevent replication of virulent CSFV upon challenge.

In countries free of CSF, preventive vaccination is very rarely practiced. However, if an outbreak occurs, regional vaccination may be necessary for effective control of CSF. Oral CSF-MLV vaccination was introduced by the European countries for the purpose of controlling CSF in wild boars (Van Oirschot, 2003). To obtain complete protection, a higher dose of the oral CSF vaccine along with a stabilizer is necessary (Kaden *et al.*, 2000). Subunit vaccines based on the E2 protein in a double water–oil emulsion also reduced clinical signs and mortality in pigs challenged with virulent CSFV. A recombinant truncated E2 protein conferred protection with reduced clinical signs and mortality, but was unable to prevent transplacental transmission (Reimann *et al.*, 2004).

Marker vaccines

The concept of marker vaccines has come into existence to differentiate infected from vaccinated animals with the help of companion serological tests. One such vaccine is based on CSFV E2, which elicits a neutralizing antibody response in pigs. The N-terminal half of E2 forms two structural subunits, one consisting of domains B and C and the other consisting of domains A and D (van Rijn *et al.*, 1994). Antigenic domains B and C are non-conserved while antigenic domain A is highly conserved (van Rijn *et al.*, 1996). Neutralizing MAbs have shown that synergistic neutralization effects have been observed in domains B and A, and in domains C and A, but not in domains B and C. The baculovirus-expressed recombinant E2 protein, in which the antigenic unit B/C or A has been deleted, is capable of protecting pigs from lethal CSFV challenge (van Rijn *et al.*, 1996). Bouma *et al.*, (1999) reported that 3 weeks after a single vaccination with 32 μg of baculovirus-expressed E2 in a water–oil–water emulsion, clinical signs and mortality can be prevented after challenge with virulent CSFV. This E2 subunit marker vaccine could significantly reduce transplacental transmission of a moderately virulent CSFV challenge for up to 13 months (Ahrens *et al.*, 2000; de Smit *et al.*, 2000, 2001). Two commercially produced ELISAs which detect antibodies to E^{rns} could also be successfully used as a companion test (Floegel-Niesmann, 2001).

The next generation of marker vaccine candidates for the control of CSF includes recombinant porcine adenovirus expressing the CSFV E2 gene (Hammond *et al.*, 2000, 2001a, b), live attenuated chimeric C strain viruses containing marker antigens and replicon vaccines that are non-replicating virus particles produced by infecting the appropriate trans-complementing cell lines with CSFV-E2 and CSFV-E^{rns} deletion mutants (van Gennip *et al.*, 2002; Frey *et al.*, 2006). Experimental DNA vaccines encoding the full-length CSFV E2 glycoprotein have also been used (Andrew *et al.*, 2000; Yu *et al.*, 2001; Ganges *et al.*, 2005; Wienhold *et al.*, 2005; Andrew *et al.*, 2006). To enhance the immunogenicity, co-administration of cytokine genes (IL-3, IL-12 and IL-18) or regulatory cell surface molecule genes (CD154 or CD40) was performed (Wienhold *et al.*, 2005; Andrew *et al.*, 2006). However, to protect pigs against lethal CSFV challenge infection, high dosages and several applications were needed (Wienhold *et al.*, 2005). Commercially available E^{rns} or NS3-based antibody ELISAs are also being used as a DIVA (Differentiating Infected from Vaccinated Animals) strategy (Beer *et al.*, 2007). Enhanced IFN-γ and persistent, high IgG2 levels are induced using the CP7_E2alf marker vaccine which

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

was based on the cytopathogenic BVDV strain, 'CP7', that carries the structural protein E2 of the CSFV strain, 'Alfort/187' (Reimann *et al.*, 2004), suggesting an important role of cell-mediated immunity in long-term protection against CSFV (Renson *et al.*, 2014). Also, in another study, this DIVA vaccine was proposed as an alternative to C-strain-based bait vaccines, after its successful assessment (Feliziani *et al.*, 2014). CP7_E2gif is another efficient DIVA vaccine which can be used in combination with detection of anti-CSFV E2-specific antibodies (Rosen *et al.*, 2014).

Viral vectors based on the pseudorabies virus (PRV) (van Zijl *et al.*, 1991; Mulder *et al.*, 1994; van Iddekinge *et al.*, 1996; Peeters *et al.*, 1997), porcine adenovirus (PAV) (Hammond *et al.*, 2000, 2001a, b, 2003, 2005), swinepox virus (Hahn *et al.*, 2001), vaccinia virus (Konig *et al.*, 1995), parapoxvirus (Voigt, 2005) and alphavirus (Zhao *et al.*, 2009; Sun *et al.*, 2010, 2011), expressing the E2 and/or E^{rns} glycoproteins (rPRV-E2, rPAV-E2 or rPPV-E2) have been tested. Chimeric pestiviruses based on the infectious DNA copy of the CSFV vaccine strain C or the BVDV strain CP7 were constructed and characterized *in vitro* and *in vivo* (van Gennip *et al.*, 2000; Reimann *et al.*, 2004). Pigs immunized with these chimeras were completely protected against lethal CSFV infection, with no virus transmission to contact animals (van Gennip *et al.*, 2000; de Smit *et al.*, 2001).

Epitope-based vaccines are one of the current focuses in the development of new vaccines against CSF. Recombinant rE2-ba-based E2 glycoprotein immunized pigs have shown a good response against CSF (Zhou *et al.*, 2011). A yeast-expressed CSFV glycoprotein E2 has been shown to induce a protective immune response against the virus (Cheng *et al.*, 2014). The heterologous DNA prime and recombinant adenovirus pSFV1CS-E2/rAdV-E2 boost strategy can induce solid protective immunity with high titers of CSFV-specific neutralizing antibodies and comparable increases in numbers of $CD4^+$ and $CD8^+$ T cells, compared to the pigs receiving immunizations with rAdV-E2 twice (Sun *et al.*, 2010). A double antigenic marker, live attenuated CSFV strain FlagT4v obtained by combining two genetic determinants of attenuation FlagT4v (a synthetic Flag® epitope positive antigenic marker introduced through a 19mer insertion in the E1 glycoprotein) and a negative marker resulting from mutations of the binding site of the MAb WH303 (mAbWH303) epitope in the E2 glycoprotein have been utilized. Intranasal or intramuscular administration of FlagT4v protected swine against the virulent CSFV Brescia strain at early exposure (2 or 3 days) (Holinka *et al.*, 2009).

Recombinant proteins can be used as vaccines. Highly immunogenic mixtures were found to induce protective immunity against CSFV challenge infection. However, in most cases the peptide vaccines failed to provide complete protection from clinical signs, viremia and virus shedding (Dong *et al.*, 2006; Dong and Chen, 2006).

Mutations were introduced to dampen the immunogenicity of the A-domain to render the C-strain suitable as a DIVA vaccine. Antibody response analysis in rabbits elicited shielding of the A-domain by an *N*-linked glycan had a minor effect on the immune response against the A-domain, whereas a single amino acid targeted deletion severely dampened this response. LC-strain mutants with larger deletions were highly debilitated and incapable of sustained growth *in vitro*. Genetically stable and replicating LC-strain mutant was produced by virtue of the compensatory evolution that can be serologically differentiated from wild-type CSFV (Kortekaas *et al.*, 2010). By integration of B and T antigenic sites of CSFV and displaying the B epitopes, three peptide-based systems were designed and produced, and shown to bring about significant enhancements in immunogenicity over the peptides in monomeric form (Zhao *et al.*, 2009; Monso *et al.*, 2010).

Conventional LAV are still used successfully to control CSF, and are regarded as a 'gold standard' because they have proven to be very efficacious and safe for inducing a high level of protection a few days after application. However, LAV does not comply with the DIVA principle, and there is a need to develop vaccines that comply with the DIVA strategy. As the first step, subunit marker vaccines based on the baculovirus-expressed E2 glycoprotein of CSFV have been developed and are available on the market. However, the immune response is delayed and less protective compared with conventional LAV. The same disadvantages can also be seen with the immunogenic peptides, DNA vaccines and *trans*-complemented replicons. The most promising candidates at the moment are vaccines based on viral vectors or chimeric pestiviruses. They have the potential of inducing a similarly strong immunity as conventional LAV with the DIVA strategy. Nevertheless, all of these new vaccines are genetically modified prototypes and there are many problems with regard to their acceptance and registration, which means the focus of disease control will have to be shifted during an outbreak situation from an indirect (serological testing) to a direct approach (antigen detection) (Beer et al., 2007). Under such conditions, conventional modified live and novel marker vaccines might regain importance.

Prevention and control

There is no specific treatment for CSF. With consistent implementation of zoosanitary control measures, many countries such as United States, Canada, Australia, New Zealand and several European countries have eradicated this disease by adopting a test-and-slaughter policy. This also includes banning the importation of live pigs and pig products from CSF-endemic countries. Furthermore, kitchen leftovers and swill must be treated to destroy CSFV before feeding to pigs. When CSF is diagnosed in a

country previously free of it, stamping out policy along with cleaning and disinfection of the premises and bans on movement of pigs are generally followed. Both Japan and Brazil are in the final stages of eradication after carrying out similar control programs in the past decade. In CSF-endemic countries, it is common practice to vaccinate pigs. As per EU guidelines, pig and pig products can only be imported from countries where no CSF has occurred and vaccination is carried out for 12 months. Despite continued efforts to control CSF, outbreaks have occurred intermittently in several European countries and large numbers of pigs were culled. Notably, emergency vaccination has not been used in Western Europe. Oral MLV-based vaccination of wild hogs can prevent the spread of disease to domestic animals under experimental conditions (Gers *et al.*, 2011; Everett *et al.*, 2011).

Status in India

CSF is enzootic to most of the pig producing states particularly in the North Eastern (NE) region of India. Pigs are reared in the majority of households in NE India (80%), where pork is a key part of their diet. In an epidemiological study conducted in Assam, Mizoram and Nagaland by the International Livestock Research Institute (ILRI), it was shown that Indian pig farmers incur huge economic losses from mortality, treatment, replacement costs, etc., amounting to more than 2 billion Indian rupees (INR) each year (Bett *et al.*, 2012).

In India, the first suspected case of CSF occurred in Aligarh in 1944 (Krishnamurty, 1964). The first documented report on CSF in India dates back to 1962, where an outbreak in a piggery unit in Morol, a suburb of Mumbai, led to widespread outbreaks in other parts of the city (Sapre *et al.*, 1962). The disease has been reported thereafter in a number of states of India (Sarma *et al.*, 2008). The CSFV diagnosis has been confirmed by RT-PCR and nucleotide sequence data showed the presence of 1.1 and 2.2 subgroups (Chakraborty *et al.*, 2011). According to the OIE website data compilation, it could be concluded that there were 1308 outbreaks of CSF in India from 1996 to 2008, which is indicative of the disease burden in this country (Patil *et al.*, 2010). Because of the sporadic nature of the disease and the lower preference for the pig husbandry (barring NE states) in India, CSF has not been studied systematically and the epidemiology of the disease is largely unknown. In one study, phylogenetic analysis revealed the presence of subgroup 1.1 (Patil *et al.*, 2010). This was different from the situation prevailing in other Asian countries and E2 and NS5B region analysis placed the Indian isolates in a clearly separated clade within subgroup 1.1 (Sarma *et al.*, 2011). In another study, a total of 594 sera samples from 12 states and 287 tissue samples from 13 states of India were tested using commercial ELISA kits. The mean prevalence of antibodies against CSFV in suspected sera was found to be 63.3% (376/594) and 76.7% (220/287) for the antigen in the CSFV-suspect samples. This suggests that CSF is endemic to India (Nandi *et al.*, 2011a).

CSFV strains have also been isolated from pigs in India. Nine CSFV field isolates were collected from the union territory of Andaman and Nicobar Islands and from the states of Assam, West Bengal and Uttarakhand; and phylogenetic analysis indicated that three isolates belonged to genotype 2.1 and were in close relation to European CSFV strains, and six isolates belonged to genotype 1 based on 5′-UTR sequence analysis and subsequent genetic classification. Based on this study, circulation of both genotypes 1 and 2.1 in NE India was observed (Desai *et al.*, 2010). An isolate was obtained from an outbreak in Mizoram, a NE state of India; the analysis of the isolate was determined to be Chinese strain Shimen–HVRI, which may be due to the proximity of this state to China and Myanmar (Barman *et al.*, 2010). CSF is also known to cause disease in the pygmy hog which is a rare, small and highly endangered mammal belonging to the *Suidae* family, found only in the Assam state of India. During investigation of death of pygmy hogs, it was confirmed that they were susceptible to and died as a result of contracting CSF. The phylogenetic analysis revealed that CSFV 5′-UTR sequences were grouped in the Indian CSFV genotype 1.1 cluster, and thus the strains causing infection were closely related to CSFV isolates circulating in domestic pigs, which shows the threat of 'spillover transmission' of infectious agents from reservoir domestic populations to sympatric wildlife and vice versa (Barman *et al.*, 2012). In a study on 16 CSFV isolates from Assam, India, the genetic typing based on 5′-UTR, E2 and NS5B gene sequences showed that all isolates only belonged to subgroup 1.1, indicating their common origin which could have occurred due to slaughter of CSFV infected pigs sold at local markets. These outbreaks occurred in small sized backyard pig farms in Assam, where garbage containing infected pork scraps, feeding swill, is common and vaccination is not practiced (Sarma *et al.*, 2011). Likewise various CSFV isolates recovered from field outbreaks in various parts of India were used for genetic analysis in the NS5B gene region (409 nt), which indicated the continued dominance of subgroup 1.1 strains, and subgroup 2.2 virus detailed analysis indicated the probable Chinese origin of this subgroup. This also provides indirect evidence of routes of CSFV movement within the South East Asia region (Patil *et al.*, 2012). Recently, in an outbreak among zovawk pigs indigenous to Mizoram, CSFV was confirmed by RT-PCR on representative tissue samples targeting the genomic region of CSFV that encodes the 5′-UTR, NS5B, Erns and E2 genes (Rajkhowa *et al.*, 2013). Out of 12 outbreaks attended in nine different districts of Karnataka suspected for CSF, 9 were confirmed CSF, by antigen-capture ELISA and RT-PCR CSFV NS5B gene-specific primers (Shivaraj

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

Fig. 2. Map of India showing classical swine fever virus-affected states and union territories based on reported disease outbreaks and serological evidence.

et al., 2013). CSFV-affected states and union territories of India, based on reported disease outbreaks and serological evidence up to 2013, are illustrated in Fig. 2.

A LC strain vaccine is currently being used in India, but the quantity is not sufficient to immunize even 1% of the total pig population (Sarma *et al.*, 2008). The northeast part of India requires 7.64 million doses of the CSF vaccine, while the total requirement per year in India amounts to 22.26 million doses, and only 1.2 million doses (<1%) are currently available (Bett *et al.*, 2012). CSF outbreaks due to inadequate attenuation of the virus have also been reported in various NE states (Sarma *et al.*, 2008). The presence of CSFV in the tissues of pigs slaughtered for human consumption has also been reported.

Conclusion

Worldwide, CSF is a highly contagious viral disease of swine with high morbidity and mortality, particularly in young animals. The diversity of clinical symptoms, under both natural and experimental conditions, and similarities to other hemorrhagic diseases make it difficult to diagnose CSF. Secondary infections are common, and may overshadow the presence of CSFV. Furthermore, other pestiviruses are antigenically closely related, and their serology with polyclonal antibody-based diagnostic tests can be confusing. MAb-based serological tests and molecular techniques are useful in diagnosing and differentiating exposure to CSFV from other pestiviruses and pathogens. It is important to check the disease at

https://doi.org/10.1017/S1466252314000024 Published online by Cambridge University Press

small scale or village level, especially in parts of India where backyard pig farming is common. Furthermore, surveillance programs should be adopted for laboratory testing of ailing pigs and materials from abattoirs. The application of excellent biosecurity measures in pig farms needs to be emphasized. In all the pig-farming sectors, a proper level of data collection, processing and recording along with the combined effort of the owner, private practitioners, veterinary authorities and welfare organizations should be adopted. In CSF-free counties with intensive pig industries, the control of CSF outbreaks is expected to change from mass culling of pigs to control based on real-time RT-PCR-based diagnosis followed by vaccination with marker vaccines. In endemic areas, mass vaccination with modified live virus vaccines, control of pig movement and epidemiological surveillance might help to control the disease to a great extent. Endemicity of CSFV infections of wild boar populations may also be an important threat to domestic pigs and can pose a threat in reintroducing the disease. Due to the inadequate availability of vaccine doses, other factors that increase the incidence of CSF in India include the lack of timely diagnosis, unrestricted movement of pigs within the country and across the border surrounding the NE states, and poor public awareness. Thus, detailed epidemiological investigations with proper preventive and control measures are needed to fully contain the disease throughout the country. Finally, increased understanding and awareness of CSF among concerned parties would help to control this disease in the near future, which in turn will help to avoid enormous economic losses.

References

Afshar AI, Dulac GC and Bouffard A (1989). Application of peroxidise labelled antibody assay for detection of porcine IgG antibodies to hog cholera and bovine diarrhoeal viruses. *Journal of Virological Methods* **23**: 253–262.

Ahrens U, Kaden V, Drexler C and Visser N (2000). Efficacy of the classical swine fever marker vaccine Porcilis Pesti in pregnant sows. *Veterinary Microbiology* **77**: 83–97.

Andrew ME, Morrissy CJ, Lenghaus C, Oke PG, Sproat KW, Hodgson AL, Johnson MA and Coupar BE (2000). Protection of pigs against classical swine fever with DNA-delivered gp55. *Vaccine* **18**: 1932–1938.

Andrew M, Morris K, Coupar B, Sproat K, Oke P, Bruce M, Broadway M, Morrissy C and Storm D (2006). Porcine interleukin-3 enhances DNA vaccination against classical swine fever. *Vaccine* **24**: 3241–3247.

Barman NN, Gupt RS, Bora DP, Kataria RS, Tiwari AK and Roychoudhury P (2010). Characterization of Classical swine fever virus involved in the outbreak in Mizoram. *Indian Journal of Virology* **21**: 76–81.

Barman NN, Bora DP, Tiwari AK, Kataria RS, Desai GS and Deka PJ (2012). Classical swine fever in the pygmy hog. *Revue Scientifique et Technique (International Office of Epizootics)* **31**: 919–930.

Beer M, Reimann I, Hoffmann B and Depner K (2007). Novel marker vaccines against classical swine fever. *Vaccine* **25**: 5665–5670.

Bett B, Deka R, Padmakumar V and Rajasekhar M (2012). Classical swine fever in northeast India: prevention and control measures. *ILRI Policy Brief* 1–4. Available oline at http://cgspace.cgiar.org/bitstream/handle/10568/21152/Policy_brief_7_print.pdf?sequence=9.

Biron CA and Sen GC (2001). Interferon and other cytokines. In: Knipe DM, Howley PM, Griffin DE, Marti M Roizman B and Straus SE (eds) *Fields Virology*, 4th edn. Philadelphia: Lippincott Williams and Wilkins, pp. 321–351.

Bironi P, Leunen J and Vandeputte J (1987). Inhibition of virus replication in the tonsils of pigs previously vaccinated with a Chinese strain vaccine and challenged oronasally with a virulent strain of classical swine fever virus. *Veterinary Microbiology* **14**: 105–113.

Blome S, Meindl-Böhmer A, Loeffen W, Thuer B and Moennig V (2006). Assessment of classical swine fever diagnostics and vaccine performance. *Revue Scientifique et Technique (International Office of Epizootics)* **25**: 1025–1038.

Blome S, Meindl-Böhmer A, Nowak G and Moennig V (2013). Disseminated intravascular coagulation does not play a major role in the pathogenesis of classical swine fever. *Veterinary Microbiology* **162**: 360–368.

Bouma A, de Smit AJ, de Kluijver EP, Terpstra C and Moormann RJM (1999). Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Veterinary Microbiology* **66**: 101–114.

Carbrey EA (1989). Diagnostic procedures. In: Liess B (ed.) Classical Swine Fever and Related Viral Infections. Boston: Nijhoff, pp. 94–114.

Chakraborty S, Veeregoda BM Chandra Naik BM, Rathnamma D, Isloor S, Venkatesha MD, Leena G, Veeresh H and Patil SS (2011). Molecular characterization and genogrouping of classical swine fever virus isolated from field outbreaks. *Indian Journal of Animal Sciences* **81**: 803–806.

Cheng CY, Wu CW, Lin GJ, Lee WC, Chien MS and Huang C (2014). Enhancing expression of the classical swine fever virus glycoprotein E2in yeast and its application to a blocking ELISA. *Journal of Biotechnology* http://dx.doi.org/10.1016/j.jbiotec.2014.01.007.

Cheville NF, Mengeling WL and Zinober MR (1970). Ultrastructural and immunofluorescent studies of glomerulonephritis in hog cholera. *Laboratory Investigation* **22**: 458–467.

Clavijo A, Zhou EM, Vydelingum S and Heckert R (1998). Development and evaluation of a novel antigen capture assay for the detection of classical swine fever virus antigens. *Veterinary Microbiology* **60**: 155–168.

Cole CG, Henley RR, Dale CN, Mott LO, Torrey JP and Zinober MR (1962). History of hog cholera research in the US Department of Agriculture 1884–1960. *Agriculture Information Bulletin No. 241*. Washington DC: USDA.

de Arce HD, Perez LJ, Frias MT, Rosell R, Tarradas J, Nunez JL and Ganges L (2009). A multiplex RT-PCR assay for the rapid and differential diagnosis of classical swine fever and other pestivirus infections. *Veterinary Microbiology* **139**: 245–252.

Desai GS, Sharma A, Kataria RS, Barman NN and Tiwari AK (2010). 5′-UTR-based phylogenetic analysis of classical swine fever virus isolates from India. *Acta Virologica* **54**: 79–82.

de Smit AJ, Bouma A, Terpstra C and van Oirschot JT (1999). Transmission of classical swine fever virus by artificial insemination. *Veterinary Microbiology* **67**: 239–249.

de Smit AJ (2000). Laboratory diagnosis, epizootiology, and efficacy of marker vaccines in classical swine fever: a review. *Vet Q* **22**: 182–8.

de Smit AJ, van Gennip HG, Miedem GK, van Rijn PA, Terpstra C and Moormann RJ (2000). Recombinant classical swine fever (CSF) viruses derived from the Chinese vaccine strain

(C-strain) of CSF virus retain their avirulent and immunogenic characteristics. *Vaccine* **18**: 2351–2358.

de Smit AJ, Bouma A, de Kluijver EP, Terpstra C and Moormann RJ (2001). Duration of the protection of an E2 subunit marker vaccine against classical swine fever after a single vaccination. *Veterinary Microbiology* **78**: 307–317.

Doherty PC, Allan W and Eichelberger M (1992). Roles of alpha, beta and gamma delta T cell subsets in viral immunity. *Annual Reviews of Immunology* **10**: 123–151.

Donahue BC, Petrowski HM, Melkonian K, Ward GB, Mayr GA and Metwally S (2012). Analysis of clinical samples for early detection of classical swine fever during infection with low, moderate, and highly virulent strains in relation to the onset of clinical signs. *Journal of Virological Methods* **179**: 108–115.

Dong XN and Chen YH (2006). Candidate peptide-vaccines induced immunity against CSFV and identified sequential neutralizing determinants in antigenic domain A of glycoprotein E2. *Vaccine* **24**: 1906–1913.

Dong XN, Qi Y, Ying J, Chen X and Chen YH (2006). Candidate peptide-vaccine induced potent protection against CSFV and identified a principal sequential neutralizing determinant on E2. *Vaccine* **24**: 426–434.

Dunne HW (1975). Hog cholera. In. Dunne HW and Leman AD (eds) *Diseases of Swine*, 4th edn. Ames, IA: Iowa State University Press, pp. 189–255.

Edwards S and Sands JJ (1990). Antigenic comparison of hog cholera virus isolates from Europe, America and Asia using monoclonal antibodies. *Deutsche Tierartzliche Wochenschrift* **97**: 79–91.

Edwards S, Moennig V and Wensvoor G (1991). The development of an international reference panel of monoclonal antibodies for the differentiation of hog cholera virus from other pestiviruses. *Veterinary Microbiology* **29**: 101–108.

Everett H, Crooke H, Gurrala R, Dwarka R, Kim J, Botha B, Lubisi A, Pardini A, Gers S, Vosloo W and Drew T (2011). Experimental infection of common warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus*) with classical swine fever virus. I: susceptibility and transmission. *Transboundary and Emerging Diseases* **58**: 128–134.

Feliziani F, Blome S, Petrini S, Giammarioli M, Iscaro C, Severi G, Convito L, Pietschmann J, Beer M and Mia GMD (2014). First assessment of classical swine fever marker vaccine candidate CP7 E2alf for oral immunization of wild boar under field conditions. *Vaccine* http://dx.doi.org/10.1016/j.vaccine.2014.02.006.

Feng L, Li XQ, Li XN, Li J, Meng XM, Zhang HY, Liang JJ, Li H, Sun SK, Cai XB, Su LJ, Yin S, Li YS and Luo TR (2012). *In vitro* infection with classical swine fever virus inhibits the transcription of immune response genes. *Virology Journal* **9**: 175. doi: 10.1186/1743-422X-9-175.

Floegel-Niesmann G (2001). Classical swine fever (CSF) marker vaccine. Trial III. Evaluation of discriminatory ELISAs. *Veterinary Microbiology* **83**: 121–136.

Frey CF, Bauhofar D, Ruggli M, Summerfield A, Hoffman MA and Tratschin JD (2006). Classical swine fever virus replicon particles lacking the E^{rns} gene : a potential marker vaccine for intradermal applications. *Veterinary Research* **37**: 655–670.

Ganges L, Barrera M, Nunez JI, Blanco I, Frias MT, Rodriguez F and Sobrino F (2005). A DNA vaccine expressing the E2 protein of classical swine fever virus elicits T cell responses that can prime for rapid antibody production and confer total protection upon viral challenge. *Vaccine* **23**: 3741–3752.

Gers S, Vosloo W, Drew T, Lubisi BA, Pardini A and Williams M (2011). Experimental infection of common warthogs (Phacochoerus africanus) and bushpigs (Potamochoerus larvatus) with classical swine fever virus II: A comparative Histopathological study. *Transboundary and Emerging Diseases* **58**: 135–144.

Gómez-Villamandos JC, Ruiz-Villamor E, Bautista MJ, Sánchez CP, Sánchez-Cordón PJ, Salguero FJ and Jover A (2001). Morphological and immunohistochemical changes in splenic macrophages of pigs infected with classical swine fever. *Journal of Comparative Pathology* **125**: 98–109.

Greiser-Wilke I, Dreier S, Haas L and Zimmermann B (2006). Genetic typing of classical swine fever viruses – a review. *Deutsche Tieraztliche Wochenschrift* **113**: 134–138.

Hahn J, Park SH, Song JY, An SH and Ahn BY (2001). Construction of recombinant swinepox viruses and expression of the classical swine fever virus E2 protein. *Journal of Virological Methods* **93**: 49–56.

Hammond JF, McCoy RJ, Jansen ES, Morrissy CJ, Hogson ALM and Johnson MA (2000). Vaccination with a single dose of a recombinant porcine adenovirus expressing the classical swine fever virus gp55 (E2) gene protects pigs against classical swine fever. *Vaccine* **18**: 1040–1050.

Hammond JM, Jansen ES, Morrissy CJ, Goff WV, Muhan GC, Williamson MM, Lenghaus C, Sproat KW, Andrew ME, Coupur BE and Johnson MA (2001a). A prime boost vaccination strategy using naked DNA followed by recombinant porcine adenovirus protects pigs from classical swine fever. *Veterinary Microbiology* **80**: 101–119.

Hammond JM, Jansen ES, Morrissy CJ, Williamson MM, Hodgson AL and Johnson MA (2001b). Oral and sub-cutaneous vaccination of commercial pigs with a recombinant porcine adenovirus expressing the classical classical swine fever virus gp55 gene. *Archives of Virology* **146**: 1787–1793.

Hammond JM, Jansen ES, Morrissy CJ, Hodgson AL and Johnson MA (2003). Protection of pigs against 'in contact' challenge with classical swine fever following oral or subcutaneous vaccination with a recombinant porcine adenovirus. *Virus Research* **97**: 151–157.

Hammond JM and Johnson MA (2005). Porcine adenovirus as a delivery system for swine vaccines and immunotherapeutics. *Veterinary Journal* **169**: 17–27.

Hernández J, Garfias Y, Nieto A, Mercado C, Montaño LF and Centeno E (2001). Comparative evaluation of $CD4^{+}CD8^{+}$ and $CD4^{+}$ $CD8^{+}$ lymphocytes in the immune response to porcine rubulavirus. *Veterinary Immunology and Immunopathology* **79**: 249–259.

Hoffmann B, Beer M, Schelp C, Schirrmeier H and Depner K (2005). Validation of a real-time RT-PCR assay for sensitive and specific detection of classical swine fever. *Journal of Virological Methods* **130**: 36–44.

Holinka LG, Fernandez-Sainz I, O'Donnell V, Prarat MV, Gladue DP, Lu Z, Risatti GR and Borca MV (2009). Development of a live attenuated antigenic marker classical swine fever vaccine. *Virology* **384**: 106–113.

Hsu WL, Chen CL, Huang SW, Wu CC, Chen IH, Nadar M, Su YP and Tsai CH (2014). The Untranslated regions of classic swine fever virus RNA trigger apoptosis. *PLoS ONE* **9**: e88863. doi: 10.1371/journal.pone.0088863.

Huang YL, Pang VF, Pan CH, Chen TH, Jong MH, Huang TS and Jeng CR (2009). Development of a reverse transcription multiplex real-time PCR for the detection and genotyping of classical swine fever virus. *Journal of Virological Methods* **160**: 111–118.

ICTV (2012). The International Committee on Taxonomy of Viruses. http://www.ictvonline.org/virusTaxonomy.asp.

Kaden V, Lange E, Fischer U and Strebelow G (2000). Oral immunization of wild boar against classical swine fever: evaluation of the first field study in Germany. *Veterinary Microbiology* **73**: 239–252.

Katz JB, Ridpath JF and Bolin SR (1993). Presumptive diagnostic differentiation of hog cholera virus from bovine viral diarrhoea and border disease viruses by using a cDNA nested-amplification approach. *Journal of Clinical Microbiology* **31**: 565–568.

Konig M, Lengsfeld T, Pauly T, Stark R and Thiel HJ (1995). Classical swine fever virus: independent induction of protective immunity by two structural glycoproteins. *Journal of Virology* **69**: 6479–6486.

Kortekaas J, Vloet RP, Weerdmeester K, Ketelaar J, van Eijk M and Loeffen WL (2010). Rational design of a classical swine fever C-strain vaccine virus that enables the differentiation between infected and vaccinated animals. *Journal of Virological Methods* **163**: 175–185.

Krishnamurty D (1964). Paper presented at the *11th Conference on Animal Diseases*, Madras.

Kumar H, Mahajan V, Sharma S, Alka Singh R, Arora AK, Banga HS, Verma S, Kaur K, Kaur P, Meenakshi K and Sandhu KS (2007). Concurrent pasteurellosis and classical swine fever in Indian pigs. *Journal of Swine Health Production* **15**: 279–283.

Langedijk JP, Middel WG, Meloen RH, Kramps JA and de Smit JA (2001). Enzyme-linked immunosorbent assay using a virus type-specific peptide based on a subdomain of envelope protein E^{rns} for serologic diagnosis of pestivirus infections in swine. *Journal of Clinical Microbiology* **39**: 906–912.

Lee WC, Wang CS and Chien MS (1999). Virus antigen expression and alterations in peripheral blood mononuclear cell subpopulations after classical swine fever virus infection. *Veterinary Microbiology* **67**: 17–29.

Leforban Y, Edwards S, Ibata G and Vannier P (1990). A blocking ELISA to differentiate hog cholera virus antibodies in pig sera from those due to other pestiviruses. *Annales de Recherches Veterinaires* **21**: 119–129.

Leifer I, Hoeper D, Blome S, Beer M and Ruggli N (2011). Clustering of classical swine fever virus isolates by codon pair bias. *BMC Research Notes* **4**: 521. doi: 10.1186/1756-0500-4-521.

Lorena J, Barlic-Maganja D, Lojkić M, Madić J, Grom J, Cac Z, Roić B, Terzić S, Lojkić I, Polancec D and Cajavec S (2001). Classical swine fever virus (C strain) distribution in organ samples of inoculated piglets. *Veterinary Microbiology* **81**: 1–8.

Lowings P, Ibata G, Needham J and Paton D (1996). Classical swine fever diversity and evolution. *Journal of General Virology* **77**: 1311–1321.

Mcgoldrick A, Lowings JP, Ibata G, Sands JJ, Belak S and Paton DJ (1998). A novel approach to the detection of classical swine fever virus by RT-PCR with a fluorogenic probe (Taq Man). *Journal of Virological Methods* **72**: 125–135.

Meyer H, Liess B, Frey HR, Hermanns W and Trautwien G (1981). Experimental transplacental transmission of hog cholera virus in pigs. *Zentrallblatt fur Veterinarmedizin B* **28**: 659–668.

Moennig V and Greiser-Wilke I (2008). Classical swine fever virus. *Encyclopedia of Virology* **1**: 525–533.

Monso M, Tarradas J, de la Torre BG, Sobrino F, Ganges L and Andreu D (2010). Peptide vaccine candidates against classical swine fever virus: T cell and neutralizing antibody responses of dendrimers displaying E2 and NS2–3 epitopes. *Journal of Peptide Science* **17**: 24–31.

Moormann V, Schagemann G, Dahle J, Greiser-Wilke I and Leder L (1990). Molecular cloning and nucleotide sequence of hog cholera virus strain Brescia and mapping of the genomic region encoding envelope protein E1. *Virology* **177**: 184–198.

Mori Y, Nagamine K, Tomita N and Notomi T (2001). Detection of loop-mediated isothermal amplification re-action by turbidity derived from magnesium pyrophos-phate formation. *Biochemical and Biophysical Research Communications* **289**: 150–154.

Moser C, Ruggli N, Tratschin JD, Hofmann MA (1996). Detection of antibodies against classical swine fever virus in swine sera by indirect ELISA using recombinant envelope glycoprotein E2. *Veterinary Microbiology* **51**: 41–53.

Mulder WA, Priem J, Glazenburg KL, Wagenaar F, Gruys E, Gielkens AL, Pol JM, Kimman TG (1994). Virulence and pathogenesis of non-virulent and virulent strains of pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus. *Journal of General Virology* **75**: 117–124.

Muller A, Depner KR and Liess B (1996). Evaluation of gp 55 (E2) recombinant based ELISA for the detection of antibodies induced by classical swine fever virus. *Deutsche Tierarztliche Wochenschrift* **103**: 451–453.

Nandi S, Muthuchelvan D, Ahuja A, Bisht S, Chander V, Pandey AB, Singh RK (2011a). Prevalence of classical swine fever virus in India: A 6-year study (2004–2010). *Transboundary and Emerging Diseases* **58**: 461–463.

Nandi S, Kumar M and Manohar M (2011b). Detection of classical swine fever virus antigen in tissue samples by AGPT. *Indian Veterinary Journal* **88**: 10–11.

Narita M, Kawashima K and Shimizu M (1996). Viral antigen and B and T lymphocytes in lymphoid tissues of gnotobiotic piglets infected with hog cholera virus. *Journal of Comparative Pathology* **114**: 257–263.

Narita M, Kawashima K, Kimura K, Mikami O, Shibahara T, Yamada S and Sakoda Y (2000). Comparative immunohistopathology in pigs infected with highly virulent or less virulent strains of hog cholera virus. *Veterinary Pathology* **37**: 402–408.

Drew TW (2008). Classical Swine Fever (hog cholera). In: *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees)*, 6th edn. Paris, France: World Organisation for Animal Health. pp. 1092–1106.

OIE (2014). http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2014/

Patil SS, Hemadri D, Shankar BP, Raghavendra AG, Veeresh H, Sindhoora B, Chandan S, Sreekala K, Gajendragad MR and Prabhudas K (2010). Genetic typing of recent classical swine fever isolates from India. *Veterinary Microbiology* **141**: 367–373.

Patil SS, Hemadri D, Veeresh H, Sreekala K, Gajendragad MR and Prabhudas K (2012). Phylogenetic analysis of NS5B gene of classical swine fever virus isolates indicated plausible Chinese origin of Indian subgroup 2.2 viruses. *Virus Gene* **44**: 104–108.

Paton DJ (1995). Pestivirus diversity. *Journal of Comparative Pathology* **112**: 215–236.

Paton DJ, Mcgoldrick A, Greiser-Wilke I, Parchariyanon S, Song JY, Liou PP, Stadejek T, Lowings JP, Bjorklund H, Belak S (2000a). Genetic typing of classical swine fever virus. *Veterinary Microbiology* **73**: 137–157.

Paton DJ, Mcgoldrick A, Bensaude E, Belak S, Mittelholzer C, Koenen F, Vanderhallen H, Greiser-Wilke I, Scheibner H, Stadejek T, Hofmann M and Thuer B (2000b). Classical swine fever virus: a second ring test to evaluate RT-PCR detection methods. *Veterinary Microbiology* **77**: 71–81.

Pauly T, Elbers K, König M, Lengsfeld T, Saalmüller A and Thiel HJ (1995). Classical swine fever virus-specific cytotoxic T lymphocytes and identification of a T cell epitope. *Journal of General Virology* **76**: 3039–3049.

Pauly T, Konig M, Thiel HJ and Saalmuller A (1998). Infection with classical swine fever virus: effects on phenotype and immune responsiveness of porcine T lymphocytes. *Journal of General Virology* **79**: 31–40.

Peeters B, Bienkowska-Szewczyk K, Hulst M, Gielkens A and Kimman T (1997). Biologically safe, non-transmissible pseudorabies virus vector vaccine protects pigs against both Aujeszky's disease and classical swine fever. *Journal of General Virology* **78**: 3311–3315.

Porntrakulpipat S, Depner KR and Moennig V (1998). Detection of classical swine fever virus in peripheral blood leukocytes by flow cytometry. In: *Proceedings of the OIE Symposium on Classical Swine Fever* (Hog Cholera), Birmingham, U.K. p 44.

Radostitis OM, Gay CC, Hinchcliff KW and Constable PD (2007). Veterinary Medicine. 10th edn. USA: Saunders.

Rajkhowa TK, Hauhnar L and Jamlianthang J (2013). Clinicopathology and diagnosis of CSF in Zovawk pigs: an indigenus pig of Mizoram, India. *Indian Journal of Animal Sciences* **83**: 620–624.

Rasmussen TB, Reimann I, Uttenthal A, Leifer I, Depner K, Schirrmeier H and Beer M (2010). Generation of recombinant pestiviruses using a full-genome amplification strategy. *Veterinary Microbiology* **142**: 13–17.

Reimann I, Depner K, Trapp S and Beer M (2004). An avirulent chimeric pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. *Virology* **322**: 143–157.

Renson P, Le Dimna M, Gabriel C, Levai R, Blome S, Kulcsar G, Koenen F and Le Potier MF (2014). Cytokine and immunoglobulin isotype profiles during CP7_E2alf vaccination against a challenge with the highly virulent Koslov strain of classical swine fever virus. *Research in Veterinary Science* http://dx.doi.org/10.1016/j.rvsc.2014.01.002.

Risatti GR, Callahan JD, Nelson WM and Borca MV (2003). Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. *Journal of Clinical Microbiology* **41**: 500–505.

Risatti G, Holink L, Lu Z, Kutish G, Callahan JD, Nelson WM, Brea Tio E and Borca MV (2005). Diagnostic evaluation of a real-time reverse transcriptase PCR assay for detection of classical swine fever virus. *Journal of Clinical Microbiology* **43**: 468–471.

Rosen TV, Rangelova D, Nielsen J, Rasmussen TB, and Uttenthal A (2014). DIVA vaccine properties of the live chimeric pestivirus strain CP7_E2gif. *Veterinary Microbiology* http://dx.doi.org/doi:10.1016/j.vetmic.2014.02.018.

Rümenapf T, Stark R, Meyers G and Thiel HJ (1991). Structural proteins of hog cholera virus expressed by vaccinia virus: further characterization and induction of protective immunity. *Journal of Virology* **65**: 589–597.

Sánchez-Cordón PJ, Romanini S, Salguero FJ, Núñez A, Bautista MJ, Jover A and Gómez-Villamandos JC (2002). Apoptosis of thymocytes related to cytokine expression in experimental classical swine fever. *Journal of Comparative Pathology* **127**: 239–248.

Sánchez-Cordón PJ, Romanini S, Salguero FJ, RuizVillamor E, Carrasco L, Gómez-Villamandos JC (2003). A histopathological, immunohistochemical and ultrastructural study of the intestine in pigs inoculated with classical swine fever virus. *Veterinary Pathology* **40**: 254–262.

Sánchez-Cordón, Salguero FJ, Carrasco L and Gómez-Villamandos JC (2005). Evolution of T lymphocytes and cytokine expression in classical swine fever (CSF) virus infection. *Journal of Comparative Pathology* **132**: 249–260.

Sapre SN, Moghe RG, Bhagwat SV, Chaudhry PG and Purohit BL (1962). A note on observations and investigations into an outbreak of swine fever in Bombay (Maharashtra). *Indian Veterinary Journal* **39**: 527–534.

Sarma DK, Krishna L and Mishri J (2008). Classical swine fever in pigs and its status in India: a review. *Indian Journal of Animal Sciences* **78**: 1311–1317.

Sarma DK, Mishra N, Vilcek S, Rajukumar K, Behera SP, Nema RK, Dubey P and Dubey SC (2011). Phylogenetic analysis of recent classical swine fever virus (CSFV) isolates from Assam. *Comparative Immunolology, Microbiolgy and Infectious Diseases* **34**: 11–15.

Shimizu T, Kumagai T, Ikeda S and Matumoto M (1964). A new *in vitro* method (END) for detection and measurement of hog cholera virus and its antibody by means of effect of HC virus on Newcastle disease virus in swine tissue culture. III.END neutralization test. *Archives Gesammte Virusforsch* **14**: 215–226.

Shivaraj DB, Patil SS, Rathnammal D, Hemadri D, Geetha S, Narayanaswamy HD, Manjunatha Reddy GB, Sharada R, Aralikatti SS and Rahman H (2013). Early detection of classical swine fever virus in blood samples of pigs in Karnataka. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **34**: 18–23.

Summerfield A, Rziha HJ and Saalmüller A (1996). Functional characterization of porcine $CD4^+$ $CD8^+$extrathymic T lymphocytes. *Cellular Immunology* **168**: 291–296.

Summerfield A, Knotig SM and McCullough KC (1998a). Lymphocyte apoptosis during classical swine fever: implication of activation induced cell death. *Journal of Virology* **72**: 1853–1861.

Summerfield A Hofmann MA and McCullough KC (1998b). Low density blood granulocytic cells induced during classical swine fever are targets for virus infection. *Veterinary Immunology and Immunopathology* **63**: 289–301.

Summerfield A, Knotig SM, Tschudin R and McCullough KC (2000). Pathogenesis of granulocytopenia and bone marrow atrophy during classical swine fever involves apoptosis and necrosis of uninfected cells. *Virology* **272**: 50–60.

Summerfield A, Zingle K, Inumaru S and McCullough KC (2001). Induction of apoptosis in bone marrow neutrophil-lineage cells by classical swine fever virus. *Journal of General Virology* **82**: 1309–1318.

Sun Jin-fu, Shi Zi-xue, Guo Huan-cheng, Li Su and Tu Chang-chun (2011). Proteomic analysis of swine serum following highly virulent classical swine fever virus infection. *Virology Journal* **8**: 107. doi: 10.1186/1743-422X-8-107.

Sun Y, Li N, Li HY, Li M and Qiu HJ (2010). Enhanced immunity against classical swine fever in pigs induced by prime-boost immunization using an alphavirus replicon-vectored DNA vaccine and a recombinant adenovirus. *Veterinary Immunology and Immunopathology* **137**: 20–27.

Sun Y, Li HY, Tian DY, Han QY, Zhang X, Li N and Qiu HJ (2011). A novel alphavirus replicon-vectored vaccine delivered by adenovirus induces sterile immunity against classical swine fever. *Vaccine* **29**: 8364–8372.

Suradhat S, Intrakamhaeng M and Damrongwatanapokin S (2001). The correlation of virus-specific interferon-gamma production and protection against classical swine fever virus infection. *Veterinary Immunology and Immunopathology* **83**: 177–189.

Susa M, Konig M, Saalmuller A, Reddehase MJ and Thiel HJ (1992). Pathogenesis of classical swine fever: B-lymphocyte deficiency caused by hog cholera virus. *Journal of Virology* **66**: 1171–1175.

Tizard IR (1998). Células T auxiliaries y su respuesta al antígeno. In: Tizard IR (ed.) Inmunología Veterinaria. 5th edn. Mexico: McGraw-Hill Interamericana Editores, pp. 119–131.

Uttenthal A, Storgaard T, Oleksiewicz MB and de Stricker K (2003). Experimental infection with the Paderborn isolate of classical swine fever virus in 10-week-old pigs: determination of viral replication kinetics by quantitative RT-PCR, virus isolation and antigen ELISA. *Veterinary Microbiology* **92**: 197–212.

van Gennip HG, van Rijn PA, Widjojoatmodjo MN, de Smit AJ and Moormann RJ (2000). Chimeric classical swine fever viruses containing envelope protein E (RNS) or E2 of bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a distinguishable antibody response. *Vaccine* **19**: 447–459.

van Gennip HG, Bouma A, van Rijn PA, Widjojoatmodjo MN and Moormann RJ (2002). Experimental non-transmissible marker vaccines for CSF by trans-complementation of E^{rns} or E2 of CSFV. *Vaccine* **20** (11–12): 1544–1556.

van Iddekinge HBJ, de Wind N, Wensvoort G, Kimman TG, Giel kens AL and Moormann RJ (1996). Comparison of the protective efficacy of recombinant pseudorabies viruses against pseudorabies and classical swine fever in pigs; influence of different promoters on gene expression and on protection. *Vaccine* **14**: 6–12.

van Oirschot JT (1979). Experimental production of congenital persistent swine fever infections. I. Clinical, pathological and virological observations. *Veterinary Microbiology* **4**: 117–132.

van Oirschot JT (2000). Hog cholera. *Infectious Diseases of Livestock* **0**: 975–986.

van Oirschot JT (2003). Vaccinology of classical swine fever: from lab to field. *Veterinary Microbiology* **96**: 367–384.

van Oirschot JT (2004). Hog cholera. In: Coetzer JAW and Tustin RC (eds) *Infectious Diseases of Livestock*, 2nd edn. South Africa: Oxford University Press. pp. 975–986.

van Rijn PA, Miedema GK, Wensvoort G, van Gennip HGP, Moormann RJM (1994). Antigenic structure of envelope glycoprotein E1 of hog cholera virus. *Journal of Virology* **68**: 3934–3942.

van Rijn PA, Bossers A, Wensvoort G and Moormann RJM (1996). Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. *Journal of General Virology* **77**: 2737–2745.

van Rijn PA, van Gennip HGP, Leendertse CH, Bruschke CJM, Paton DJ, Moormann RJM and Van Oirschot JT (1997). Subdivision of the pestivirus genes based on envelope glycoprotein E2. *Virology* **237**: 337–348.

Van Snick J (1990). Interleukin-6: an overview. *Annual Reviews of Immunology* **8**: 253–278.

van Zijl M, Wensvoort G, de Kluyver E, Hulst M, van der Gulden H, Gielkens A and Moormann R (1991). Live attenuated pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog cholera. *Journal of Virology* **65**: 2761–2765.

Vannier P, Plateau E and Tillon JP (1981). Congenital tremor in pigs farrowed from sows given hog cholera virus during pregnancy. *American Journal of Veterinary Research* **42**: 135–137.

Vilcek S, Herring AJ, Nettleton PF, Lowings JP and Paton DJ (1994). Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. *Archives of Virology* **136**: 309–323.

Vilcek S, Stadejek T, Ballagi-Pordany A, Lowings JP, Paton DJ and Belak S (1996). Genetic variability of classical swine fever virus. *Virus Research* **43**: 137–147.

Voigt H (2005). Rekombinante Parapockenvakzine gegen klassische Schweinepest: Vergleichende Charakterisierung der Immunreaktionen im Schwein. Inaugural-Dissertation LMU M̈unchen.

Weesendorp E, Willems EM and Loeffen WLA (2010). The effect of tissue degradation on detection of infectious virus and viral RNA to diagnose classical swine fever virus. *Veterinary Microbiology* **141**: 275–281.

Wensvoort G, Terpstra C and De Kluyver EP (1989). Characterization of porcine and some ruminant pestiviruses by cross-neutralisation. *Veterinary Microbiology* **20**: 291–306.

Wienhold D, Armengol E, Marquardt A, Marquardt C, Voigt H, Buttner M, Saalmuller A and Pfaff E (2005). Immunomodulatory effect of plasmids co-expressing cytokines in classical swine fever virus subunit gp55/E2-DNA vaccination. *Veterinary Research* **36**: 571–587.

Wongsawat K, Dharakul T, Narat P and Rabablert P (2011). Detection of nucleic acid of classical swine fever virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP). *Health* **3**: 447–452.

Yu X, Tu C, Li H, Hu R, Chen C, Li Z, Zhang M and Yin Z (2001). DNA-mediated protection against classical swine fever virus. *Vaccine* **19**: 1520–1525.

Zhao HP, Sun JY, Sun Y, Li N and Qiu HJ (2009). Prime-boost immunization using alphavirus replicon and adenovirus vectored vaccines induces enhanced immune responses against classical swine fever virus in mice. *Veterinary Immunology and Immunopathology* **131**: 158–166.

Zhou B, Liu K, Jiang Y, Jian-Chao Wei JC and Chen PU (2011). Multiple linear B-cell epitopes of classical swine fever virus glycoprotein E2 expressed in *E. coli* as multiple epitope vaccine induces a protective immune response. *Journal of Virology* **8**: 378. doi: 10.1186/1743–422X-8-378.