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Review

# The challenges of classical swine fever control: Modified live and E2 subunit vaccines

Yu-Liang Huang[a], Ming-Chung Deng[a], Fun-In Wang[b], Chin-Cheng Huang[c], Chia-Yi Chang[a,*]

[a] Animal Health Research Institute, Council of Agriculture, 376 Chung-Cheng Road, Tansui, New Taipei City 25158, Taiwan
[b] School of Veterinary Medicine, National Taiwan University, No. 1, Section 4, Roosevelt Road, Taipei 10617, Taiwan
[c] Pingtung Agriculture Biotechnology Park, Council of Agriculture, No. 1 Shennong Road, Dehe Village, Changjhih Township, Pingtung County 90846, Taiwan

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ABSTRACT

Classical swine fever (CSF) is an economically important, highly contagious disease of swine worldwide. CSF is caused by classical swine fever virus (CSFV), and domestic pigs and wild boars are its only natural hosts. The two main strategies used to control CSF epidemic are systematic prophylactic vaccination and a non-vaccination stamping-out policy. This review compares the protective efficacy of the routinely used modified live vaccine (MLV) and E2 subunit vaccines and summarizes the factors that influence the efficacy of the vaccines and the challenges that both vaccines face to CSF control. Although MLV provide earlier and more complete protection than E2 subunit vaccines, it has the drawback of not allowing differentiation between infected and vaccinated animals (DIVA). The marker vaccine of E2 protein with companion discriminatory test to detect antibodies against $E^{rns}$ allows DIVA and is a promising strategy for future control and eradication of CSF. Maternal derived antibody (MDA) is the critical factor in impairing the efficacy of both MLV and E2 subunit vaccines, so the well-designed vaccination programs of sows and piglets should be considered together. Because of the antigen variation among various genotypes of CSFV, antibodies raised by either MLV or subunit vaccine neutralize genotypically homologous strains better than heterologous ones. However, although this is not a major concern for MLV as the induced immune responses can protect pigs against the challenge of various genotypes of CSFVs, it is critical for E2 subunit vaccines. It is thus necessary to evaluate whether the E2 subunit vaccine can completely protect against the current prevalent strains in the field. An ideal new generation of vaccine should be able to maintain the high protective efficiency of MLV and overcome the problem of antigenic variations while allowing for DIVA.



**Contents**



* Corresponding author at: 376 Chung-Cheng Road, Tansui, New Taipei City 25158, Taiwan. Tel.: +886 2 26212111#343; fax: +886 2 26265345.
*E-mail address:* cychang@mail.nvri.gov.tw (C.-Y. Chang).

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## 1. Introduction

Classical swine fever (CSF) is a highly contagious multisystemic hemorrhagic viral disease of pigs that can present as acute, subacute, chronic or late onset forms, but it can also remain subclinical. *Sus scrofa* species including domestic pigs, wild boars, and feral pigs such as warthogs and bushpigs are susceptible to CSF (Everett et al., 2011; Gers et al., 2011).

The etiological agent of CSF is classical swine fever virus (CSFV), which is an enveloped RNA virus belonging to the genus *Pestivirus* of the family *Flaviviridae* (King et al., 2011). The CSFV genome consists of a single, positive-stranded RNA of approximately 12.3 kb encoding for a polyprotein of 3898 amino acids, which is flanked by 5′ and 3′ non-translated regions (NTR). The translated polyprotein is processed by viral as well as cellular proteases to the mature viral proteins of four structural (C, $E^{rns}$, E1, and E2) and eight nonstructural proteins ($N^{pro}$, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Lindenbach et al., 2007).

The genotypes of CSF viruses are classified into three major groups with ten subgroups (Paton et al., 2000). Group 1 contains primarily historical strains isolated from many regions of the world and includes all live-attenuated vaccine strains. Group 2 contains most of the currently circulating strains, whose prevalence has increased and caused epidemic infection since the 1980s. Group 3 contains most of the strains distributed in separated geographic regions such as Taiwan, Korea, Japan, Thailand and the United Kingdom. Recent phylogenetic analyses have indicated a "switch" of field CSFVs from the historical group 1 or 3 to the more recently prevalent group 2 in Europe and Asia (Cha et al., 2007; Deng et al., 2005; Greiser-Wilke et al., 2000; Tu et al., 2001).

Two main strategies to control CSF epidemics are systematic prophylactic vaccination and a non-vaccination stamping-out policy. Compared to the huge costs and in some cases inadequacy of stamping out (Stegeman et al., 2000; Terpstra and de Smit, 2000), modified live vaccine (MLV) are inexpensive and can induce complete protection against virulent CSFV, so MLV are still widely used in the CSF endemic areas, including Asia and central-south America (Blome et al., 2006; Deng et al., 2005; Paton et al., 2000; Suradhat et al., 2007). In Japan, where MLV are used, no outbreaks occurred between 1993 and 2003, and with a non-vaccination policy, CSF was successfully eradicated in 2007 (Ozawa et al., 2006; Sakoda et al., 2012). In Taiwan, MLV have been used since the 1950s and proven efficiently protective, and no cases of CSF have been reported in recent years. Eradication of CSF based on the MLV vaccination alone is difficult because differentiation between infected and vaccinated animals (DIVA) is impossible based on the antibodies induced (Suradhat et al., 2007; van Oirschot, 2003). To overcome this problem, marker vaccine and companion discriminatory serological test have been developed. Two subunit vaccines based on expressed E2 protein entered large-scale trials in EU in 1999 and successfully induced solid immunity against CSFV in vaccinated pigs (Depner et al., 2001; Uttenthal et al., 2001). However compared to MLV, due to the expense, delayed induction of protection immunity and inferior efficacy of E2 subunit vaccines, they have not been successfully promoted in the CSF endemic areas.

In order to help controlling CSF, this article compares the protective efficacy of the routinely used MLV and E2 subunit vaccines and summarizes the influential factors and challenges of these two types of vaccines.

## 2. Pathogenesis of CSFV

The most common route of infection is the oronasal route. After exposure, CSFV first replicates in the tonsils, where it infects the crypt-epithelial cells, macrophages, lymphocytes, and endothelial cells by heparin sulfate and chondroitin sulfate B glycosaminoglycan receptors (Belak et al., 2008; Hulst et al., 2000; Liu et al., 2011; Ophuis et al., 2006; Ressang, 1973). It then spreads to regional lymph nodes and from there disseminates systemically *via* viremia (Belak et al., 2008; Liu et al., 2011; Ophuis et al., 2006; Ressang, 1973). The rate of CSFV spread to all systems is positively correlated with its virulence. Highly virulent CSFV can rapidly distribute throughout the body after challenge and can induce higher viral load in tissues, more shedding, and more severe pathological changes than moderately virulent strains (Belak et al., 2008; Summerfield et al., 2001a; Weesendorp et al., 2009a, 2009b).

In its acute form, CSF can induce systemic hemorrhaging and immunosuppression by down-regulating the cytokines and immune cells, including endothelial cells, lymphocytes, monocyte/macrophage lineage cells, dendritic cells (DCs), and bone marrow hematopoietic cells (BMHCs). The systemic hemorrhaging is the result of CSFV-induced necrosis of endothelial cells with subsequent vasculitis and hemorrhaging, accompanied by severe anemia, thrombocytopenia and disturbance of fibrinogen synthesis (Bensaude et al., 2004; Summerfield et al., 2001a, 2001b). The immunosuppression is the result of down-regulation and necrosis of BMHCs and lymphocytes including granulocytes, B-lymphocytes, $CD3^+CD4^+CD8^+$ memory T-helper, $CD3^+CD4^+CD8^-$ naïve T-helper, $CD3^+CD4^-CD8^+$ cytotoxic T-cells, and $CD3^+CD4^-CD8^{-/low}\gamma\delta$ T-cells (Summerfield et al., 1998a, 1998b, 2000, 2001a). The results of lymphocyte depletion have demonstrated that CSFV directly or indirectly induced the lymphocyte apoptosis by the reduction of mitochondrial transmembrane potential and $E^{rns}$ protein (Bruschke et al., 1997; Summerfield et al., 1998a, 1998b). Besides, TNF-α from CSFV-infected macrophage is able to induce the apoptosis of B-lymphocytes (Choi et al., 2004). In the T-lymphocytes, CSFV infection increases the CD49d, MHCII, and Fas expression that increase susceptibility to apoptosis mediated by Fas-FasL interaction (Summerfield et al., 1998b). In addition, the proliferation, cytolytic activities, and MHC-restricted lysis of T-lymphocytes are markedly reduced by CSFV infection and $E^{rns}$ after ConA stimulation (Pauly et al., 1998; Summerfield et al., 1998b; Susa et al., 1992). Taken together, these CSFV-induced lymphocytic changes would lead to lymphopenia and immunosuppression in CSFV-infected pigs.

## 3. Immune responses and protective efficacy of CSFV commercial modified live and E2 subunit vaccines (Table 1)

### 3.1. Modified live vaccines (MLV)

MLV have routinely been used in CSF endemic areas, including Asia and central-south America. These are often the Chinese (C) strain (C-strain), Japanese guinea-pig exaltation-negative ($GPE^-$)