Review of Different Strategies for Preventing and Controlling Classical Swine Fever

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ABSTRACT

Classical Swine Fever (CSF) caused by CSF virus (CSFV) which is single-stranded-RNA Pestivirus, belonging to the family Flaviviridae, leads to severe economic losses in the pig industry especially in developing countries. Many researchers have been involved in research in order to find better and strategies to control its outbreaks. Although prophylactic vaccination is banned within the European Union, emergency vaccination, allowing differentiation of vaccinated from infected animals, is still a key component of CSF control. Lessons learned from CSF epidemiology will expand our understanding of the ways in which virus co-opt host cells during the course of infection and help find approaches to control the disease. This review integrates research findings in CSF epidemiology and explores different strategies to prevent and control CSF.

Key words: Classical Swine Fever, CSFV, epidemiology, vaccination, control

INTRODUCTION

Classical Swine Fever (CSF), also called Hog Cholera or European Swine Fever, 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 (1). Although eradicated from most European Union (EU) member states, CSF continues to cause serious problems in different parts of the world, including South-Eastern European countries (2, 3) and Asian countries (4, 5). Studies conducted over the past several decades have improved our knowledge of the mechanisms of CSFV translation and replication. New methodologies have facilitated advances in our understanding of the RNA elements and viral and host factors that modulate CSFV replication and translation. In recent years, with the development of molecular techniques, diagnosis of CSF has become more rapid and accurate, however the control of CSF is still in question due to many complex factors in Asia and several countries of Central and South America. Antiviral drug (6), small interfering RNA (siRNA) (7), vaccination against CSF using inactivated or attenuated live virus vaccines (8) and marker vaccines (9) have been used to prevent CSF outbreaks or chronic infection in domestic pigs in these countries. CSF is a disease whose accurate diagnosis is difficult when based solely on clinical evidence. Thus, there is a growing demand for strategies that can provide timely clinical treatment and epidemiological control.

MOLECULAR EPIDEMIOLOGY OF CSF

Genetic typing of CSFV

Genetic typing of CSFV genotypes, subgroups and types shows a regional distribution and has proven to be a useful method for supporting epidemiological investigations. This information helps researchers to trace the origin of the virus and to follow the viral spread. Phylogenetic analysis of CSFV strains and isolates originating from different continents has
proved that CSFV can be divided into three groups with three or four subgroups: 1.1, 1.2, 1.3; 2.1, 2.2, 2.3; 3.1, 3.2, 3.3, 3.4; based on the partial sequences of the E2 and NS5B genes (10). Furthermore, different genotypes of CSFV are still spreading gradually and evolving worldwide.

Epidemics of CSFV
Molecular epidemiology based on nucleotide sequence diversity is a useful tool for tracing virus spread and for developing disease control strategies. For instance, epidemiological data from field investigations combined with genetic typing identified seven unrelated epidemics and a number of sporadic outbreaks in domestic pigs and in the wild boar population in Germany (11). Although not present in the United States, CSF is distributed worldwide. Several studies covering the molecular epidemiology of CSFV have been performed, including analysis of isolates from Asia, Europe and South America.

Europe
Several epidemics have occurred in the EU over the past decade. The phylogenetic tree shows that the Russian CSF virus isolates from outbreaks that occurred between 1994 and 1999 were all in subgroup 1.1 (12). Viruses of subgroup 2.1 only sporadically occurred in the EU in 1989, 1993, 1997, and 2000. The virus that occurred in 1993 in Austria was detected in wild boar meat illegally imported from China (13). The virus of The Netherlands in early 1997 was most probably newly introduced into the EU, as it belonged to subgroup 2.1 (14), which then spread from The Netherlands to Belgium in June 1997, starting a smaller epidemic (15). In 2000, CSFV was introduced into the United Kingdom. Genetic typing showed that the East Anglian CSFV also belonged to subgroup 2.1 (16).

In Germany, 424 outbreaks of CSF in domestic pigs and a great number of cases in wild boar were recorded between 1990 and 1998. Genetic typing of isolates from these outbreaks using the 5'NTR (150 nt) revealed the existence of seven regional groups of CSFV isolates within subgroup 2.1, 2.2, and 2.3 (17). A CSFV of subgroup 2.2 was responsible for CSF cases in wild boar in Switzerland (18), which also caused outbreaks in Austria (19). In Italy, viruses of subgroup 2.2 were occurred between 1985 and 2000 in the wild boar population (20, 21) and sporadically caused outbreaks in domestic pigs (22). CSF viruses of genotype 2.3 were endemic in domestic pigs as well as in a unique wild swine population (23), most probably due to widely diffused Sardinian tradition of "free pig" farming, where reared pigs are allowed occasional contact with wild boars. As a result of large quantities of pig and wild boar meat imported in some parts of Italy (24) local strain was introduced into mainland Italy. The epidemics in 1988, 1990 and between 1993 and 1994 in Belgium were studied. Apart from one CSF virus isolate from 1988 which belonged to subgroup 1.1, the isolates formed a discrete cluster within subgroup 2.3 (25). The possibility of a long-term persistence of genotype 2.3 CSFV strains existing at an almost undetectable level in affected regions, even after long-term oral vaccination campaigns with intensive monitoring is a possibility. Hence, regional persistence in German wild boar populations has to be taken into account as an important factor in the continual outbreaks in affected areas (26). Molecular epidemiology of 97 CSF virus isolates available from these countries, from outbreaks that occurred between 1994 and 2007, was performed. The findings suggested that most of the isolates were from Romania and Bulgaria, and belonged to genotype 2.3 (27). The viruses isolated in the outbreak in Spain were 100 percent homologous and belonged to subgroup 2.3 (28). In Poland, Slovakia, Hungary, Estonia (29) and the Czech Republic (19), virus isolates also belonged to subgroups 2.3.

On the basis of the former findings, we may concluded that group 1 consists of all CSFV strains (30) prior to the 1980's, whereas all CSFV strains isolated from different European countries except Russia in the 1990's and recent epidemics in the EU belongs to one of the subgroups within group 2 (2.1, 2.2, or 2.3) (10-12,20,25,29,31). CSF is still currently endemic in various European countries.

Asia
In Asia, CSF epidemics are also fairly ubiquitous. Strains of genotypes 1, 2, and 3 have been isolated in different Asian countries (10,32). Furthermore, isolates belonging to group 3 seem to occur solely in Asia (12). In Taiwan, by analyzing the E2 sequences of CSFV from field outbreaks during 1993-2001, CSF viruses have been classified into two subgroups 3.4 and 2.1 (33, 34). Furthermore the Taiwanese strains of sub-genotype 2.1 were divided into two different genotypes termed 2.1a and 2.1b which may have be introduced from different origins (33). Another finding suggested that all Taiwanese CSFV strains were sub-genotypes 3.4 prior to
1996. However, genotype 2 strains of CSFV, identified as sub-genotype 2.1 or 2.2, have been isolated since 1994 and have gradually replaced the sub-genotype 3.4 strain (35). One hundred and ten clinical specimens representing 109 epizootic sites from 1986-1999 in China were also analyzed. A phylogenetic tree showed that 103 of the 110 field viruses were clustered within group 2 and subdivided into three subgroups, while the remaining seven viruses were clustered into subgroup 1.1 within group 1. It is evident that sub-groups 2.1 and 2.2 have predominated in the more recent epizootics in China, while group 1 viruses have caused only limited epizootics (36). A novel isolated CSFV in China in 2004 belonged to 2.3 topotype (37).

CSFV isolates in Japan are divided into three genotypes, CSFV-1, CSFV-2 and CSFV-3 (41). 24 isolates of CSFV obtained from CSF outbreaks during 1988 and 2003 in the Republic of Korea were genetically characterized for partial E2 gene, compared with CSFV viruses reported by other countries. Phylogenetic analyses classified Korean field isolates between 1988 and 1999 into subgroup 3.2, and the viruses isolated during 2002-2003 CSF epidemics were classified into a different subgroup 2.1 (5).

PREVENTION AND CONTROL OF CSF

Cutting off transmission routes of CSF

CSF can spread via various routes. Direct transmission of CSF is undoubtedly the most efficient way of CSFV transmission. Epidemiological data found that most of primary CSF outbreaks were due to direct contacts with wild boar infected with CSFV or contaminated swill feeding (43). Trade of living infected pigs bears the highest risk of transmitting the virus; international trade especially may lead to the spread of the virus over long distances. Furthermore, neighborhood contacts to infected farms and other contacts via contaminated persons and vehicles are important modes especially in areas with high pig and pig farm density (44). However, the role of airborne transmission remains debatable (31). In EU Countries, boars for artificial insemination (AI) must be CSF-free. Furthermore, control of the influx of meat and meat products from countries, a commercial development towards more local marketing systems, the reduction of the pig density in critical areas and strict hygiene measures on pig farms as well as vehicles used for animal transportation would be also beneficial to avoid the spread of CSF.

CSF monitoring strategies in wild boars and domestic pigs

The wild boar population should be monitored for CSF outbreaks. The CSF status of wild boar populations is still unknown in several parts of Europe although a recommended monitoring program (oral immunization together with special hunting strategies) is available (45). When an outbreak of CSF in wild boar is detected, early measures must be taken to protect domestic pigs. The control of endemic infections in wild boar populations appears to be difficult. The basic requirement is the identification of both risk and surveillance zones, taking into account geographical conditions and the structure and the size of the wild boar populations. A complete ban on hunting activities was introduced in Switzerland...
for 2 months after the detection of CSF in wild boar in order to limit movement of potentially infected animals outside their natural habitat (21).

Increased monitoring of the local domestic pig population should detect possible CSF outbreaks at an early stage. Surveillance programs aim to keep the number of infected herds as small as possible, by shortening the so-called “high risk period”. For instance, it was concluded leukocyte counts (31) could be an effective method for individual herds—but probably not for large-scale surveillance. It is probable that the Dutch CSF epidemic of 1997/1998 would have been detected earlier if routine serological surveillance had been applied (46). Additionally, computer-based models help to understand disease dynamics and can support decision-makers in case of an outbreak. Mass-action and state-transition models, branching processes and Monte Carlo methods (44), spreadsheet models (47) and spatial and stochastic models (48) have been used to study virus spread and to evaluate control strategies. However, due to changes in the biology of CSFV and pig trading schemes, it has become more difficult to completely control this disease, indicating that newer methods have to be developed for this purpose.

Eradication of CSF from wild boars and domestic pigs

Within the EU, different strategies are used for the eradication of CSF in wild boar and in domestic pigs. The combination of prophylactic mass vaccination and culling of infected pigs in endemic regions has made it possible to almost eradicate the disease in the EU. The control of CSF epidemics comprises of eradication of infected herds and preventive emptying of herds was effective within a radius of 750-1000 m of infected areas (49).

However, several epidemics which occurred in Europe during the last decade have pointed out that eradication according to the present legislation may be very costly and ethically unacceptable, particularly in areas with a high pig density (50,51). For example, CSF occurred in Netherlands form 1997 to 1998 where millions of pigs were slaughtered and direct losses were calculated to amount to 2 billion US dollars for eradication (14). The epidemiological characteristics of the 1997 CSF outbreak that occurred in the Limburg Province of Belgium between 30 June and 17 July 1997 resulted in a total of 46,561 pigs being slaughtered to control the spread of the infection. Another 27,579 pigs were slaughtered in the framework of the market support. The total direct costs of the episode were estimated at 10,893,337 Euros (15,52). Spain suffered an outbreak of CSF between June 14, 2001 and May 7, 2002; a total of 291,058 animals were slaughtered (28).

VACCINATION

Though many countries including EU member states pursue a non-vaccination stamping-out policy, however for some developing countries such as China, India and Africa, there is still a difficult method to eradicate this complex disease, even though vaccination against CSFV has been applied. Therefore, under certain conditions, emergency vaccination combined with control measures might be a future option for disease eradication and control (45).

Live attenuated virus strain vaccines

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 in China as well as many other developing countries. The C-strain of the CSFV is considered as the gold standard vaccine for the control of CSF (53). The attenuated lapinized CSFV strains such as PAV-250, LPC, and HCLV, the attenuated CSFV by deletion of the viral N(pro) gene (54), can also induce virtually complete protection against the disease (55). The live CSFV strain vaccines mentioned above provide protection for pigs for CSF, however, they do not permit the serological discrimination between infected and vaccinated animals and its use can therefore impose severe trade restrictions. To solve these problems, advanced vaccines against CSF and discriminatory tests have been developed.

DNA-based Vaccines

A number of efforts are in progress to develop DNA vaccines in recent years. DNA vaccines are an attractive prospect because plasmid DNA can be highly purified and there is less opportunity for adventitious viruses to contaminate the vaccine preparation. Furthermore these vaccines are very stable at ambient temperatures and can be delivered in very small quantities under optimized route of injection. The vaccines developed were tested for safety and efficacy in animal models and claimed to be immunogenic and safe. The investigations showed that a DNA vaccine expressing the complete E2 protein of CSFV such
as the Semliki Forest virus (SFV) replicon-derived DNA vaccine, conferred total protection against a severe viral challenge in domestic pigs immunized three times with 600 µg or twice with 100 µg (56-58). However, the results generated by scientists from different laboratories need to be pooled and evaluated scientifically to select a few potential vaccine candidates for large-scale trials to proof efficacy and safety.

**Marker vaccines**
Marker vaccines against CSFV infection which allow differentiation of infected from vaccinated animals have been

**Table 1: Molecular epidemiology of CSF**

<table>
<thead>
<tr>
<th>Continent</th>
<th>Country</th>
<th>Group or subgroup</th>
<th>Time</th>
<th>Animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>Russia</td>
<td>2.1</td>
<td>1994-1999</td>
<td>DP</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>Austria</td>
<td>1.1, 2.2</td>
<td>1993, 1992,94</td>
<td>WB, DP</td>
<td>(13, 19)</td>
</tr>
<tr>
<td></td>
<td>The Netherlands</td>
<td>2.1</td>
<td>1997</td>
<td>DP</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>Belgium</td>
<td>2.1, 1.1, 2.3</td>
<td>1997, 1988,90</td>
<td>DP, DP</td>
<td>(15, 25)</td>
</tr>
<tr>
<td></td>
<td>UK</td>
<td>2.1</td>
<td>2000</td>
<td>DP</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>2.1, 2.2, 2.3</td>
<td>1990-1998</td>
<td>DP, WB</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>Switzerland</td>
<td>32.2</td>
<td>1998</td>
<td>WB</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td>The Czech Republic</td>
<td>2.2, 2.3</td>
<td>1991-1998</td>
<td>DP, WB</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>2.2, 2.3</td>
<td>1985-2000</td>
<td>DP, WB</td>
<td>(20-22, 23-24)</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>2.3</td>
<td>2010</td>
<td>WB</td>
<td>(26)</td>
</tr>
<tr>
<td></td>
<td>Romania</td>
<td>2.3</td>
<td>1994-2007</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Bulgaria</td>
<td>2.3</td>
<td>1997-2008</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Kosovo</td>
<td>2.3</td>
<td>2006</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Montenegro</td>
<td>2.3</td>
<td>2000</td>
<td>DP, WB</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Serbia</td>
<td>2.3</td>
<td>2000-2007</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Croatia</td>
<td>2.1</td>
<td>1996-2007</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Macedonia</td>
<td>2.2</td>
<td>2000</td>
<td>DP</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>2.3</td>
<td>2001-2002</td>
<td>DP</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>Estonia</td>
<td>2.3</td>
<td>1990s</td>
<td>DP</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Hungary</td>
<td>2.3</td>
<td>1990s</td>
<td>DP</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Poland</td>
<td>2.3</td>
<td>1990s</td>
<td>DP</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Slovakia</td>
<td>2.3</td>
<td>1990s</td>
<td>DP</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Lao People's Democratic Republic</td>
<td>2.1, 2.2</td>
<td>1997, 1999</td>
<td>DP</td>
<td>(32)</td>
</tr>
<tr>
<td></td>
<td>Taiwan</td>
<td>3.4, 2.1, 2.2</td>
<td>Prior to 1994, After 1994</td>
<td>DP</td>
<td>(33, 34, 35)</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>1.1, 2.2, 2.3</td>
<td>1986-1999, 2004</td>
<td>DP</td>
<td>(36, 37)</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td>1.2, 2.3</td>
<td>Prior to 1999</td>
<td>DP</td>
<td>(41)</td>
</tr>
</tbody>
</table>

WB: wild boar; DP: domestic pigs
developed, including protective peptides, single expressed proteins and chimeric viruses (59-80) (Table 2).

CSFV glycoproteins E2, E(rns) and E1 are structural proteins detected on the external part of viral particles and play a major role in the initial stages of viral infection. Studies on these structural proteins have also opened a new ways to develop new vaccines. For example, the absence of the antigenic portion of glycoprotein E(rns), deletion of E2 gene, the A-domain of E2 or E2-encoding region of classical CSFV can be used as non-transmissible, modified, live-attenuated marker vaccines which protects pigs from a lethal challenge dose of the highly virulent strain or differentiate between infected and vaccinated animals (59-62). In addition, the carboxy-terminal domain of E1 glycoprotein provides the basis for a rationally designed and efficacious live-attenuated CSF vaccine (63-65).

Vaccination with the CSFV E2 subunit marker vaccine was an efficacious tool in the control program during an outbreak of CSF. Vaccination 10 days after outbreak significantly reduced the vertical transmission of moderate-virulent strain of CSFV from the pregnant sow to its offspring (66-69) and decreased the horizontal virus transmission of weaning piglets (53). However, another study showed that double vaccination with an E2 subunit marker vaccine only protected pregnant gilts from the clinical course of the disease but did not prevent horizontal nor vertical spread of the CSF virus (70). The E(rns) which possessed enzymatic activity and retained antigenicity might provide useful material for developing a marker vaccine (71,72).

An envelope glycoprotein D/E-negative Pseudorabies Virus (PRV) recombinant, Vaccinia Virus Recombinant (VVR) (62,73) or a recombinant Porcine Adenovirus (rPAV) (74,75) which expressed envelope glycoprotein E2 of CSFV was constructed as a biologically safe vaccine vector. Vaccination of pigs showed that the recombinant virus was able to protect pigs against CSF. The other observations demonstrated that a deletion of E2 protein in combination with a modified CTB (76), eukaryotic expression plasmid with only 5’ signal sequence of E2 (77), synthetic peptide vaccine using E2 N-terminal antigenic units B/C (54,78) and a multi-peptide-vaccine (MPV) using aluminum adjuvant (79) or E2 emulsified in Freund’s adjuvant (80) were all suitable to act as a candidate marker vaccine against CSFV.

### Development of Antiviral drugs

A few effective antiviral drugs are currently available against CSFV infections (Table 3). At a concentration of 5 µg/ml,
Classical Swine Fever

Prostaglandin (PGA1) was found to inhibit the multiplication of CSFV in 99% in cultures of PK-15 cells (81,82). Tyborowska et al. (2007) found that very low doses of tunicamycin drastically reduced CSFV spread and the virus yield in SK6 (swine kidney) cell cultures (83). Some glycosylation inhibitors, such as two of newly designed uridine derivatives of 2-deoxy sugars, IW3 and IW7 mimicking part of tunicamycin could effectively arrest viral growth without significant toxicity for mammalian cells. Moreover, IW3 and IW7 reduced the formation of viral glycoproteins E2 and E\textsuperscript{rms} in a dose-dependent manner (84). Due to the observed antiviral effect accompanied by low cytotoxicity, these inhibitors are potential candidates for the inhibition of the spread of CSFV.

Capsid-targeted viral inactivation (CTVI) approach might be applicable to CSFV inhibition as a novel antiviral strategy. Zhou et al. (2010) found that the CSFV capsid protein (Cap) fused with the nuclease of Staphylococcus aureus (SN) in Escherichia coli could inhibit effectively the replication of CSFV in a dose-dependent manner, whereas the Cap fused with an enzymatically inactive SN (Cap-SN\textsuperscript{a}) showed no nuclease activity or antiviral effects (85). Some proteins with ribonuclease (RNase) activity have been shown to suppress viral replication and may be valuable therapeutic approaches against CSFV. Exogenous treatment of 3D8 single-chain variable fragment (3D8 scFv) prior to or post-CSFV infection, could suppress CSFV replication at the viral RNA level (86).

**Small interfering RNA (siRNA)**

Small interfering RNA (siRNA) can be used to control genome replication and viral production (Table 3). For example, three species of siRNA, targeting different regions of CSFV: Npro and NS5B genes, were prepared by in vitro transcription. After transfection of PK-15 cells with each of the siRNAs followed by infection with CSFV, the results showed that treatment with the siRNAs caused a 4-12-fold reduction in viral genome copy number and suppressed the production of infectious virus by up to 467-fold for 72-84 h (87). The use of synthetic siRNA which corresponded to nucleotides 1130-1148 of the CSF virus strain Alfort, targeting the nucleocapsid protein (C) was investigated to inhibit viral replication (7). Therefore, the application of RNAi strategy for controlling CFSV could become a promising alternative to conventional eradication measures.

**CONCLUSION**

CSF epidemics in Asia and several countries of Central and South America have clearly shown that preventing the introduction of CSFV deserves high priority. Development of suitable marker vector vaccines to allow the vaccination of pigs, as well as better control strategies in disease outbreak countries is also important. Although many techniques and strategies have been developed for detection and control of CSF, there still remains many efforts that should be taken: 1) study of the efficacy and stability of vaccines against CSFV;
2) virological surveys to be carried out to establish the distribution of CSFV among domestic pigs and wild boar; 3) phylogenetic analyses and genealogical relationships among members of the CSFV; 4) the study of atypical infection in piglets; 5) the investigation of persistent infection even after vaccination and (6) the differentiation between vaccinated and infected pigs.

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