CLASSICAL SWINE FEVER PATHOLOGY
In late 1992, there were outbreaks of chronic CSF in Thailand. Clinical signs were mild with low mortality. Haemorrhagic papular dermatitis was the only gross lesion observed. Isolation of low virulent CSFV is very difficult. The objectives of this study were to investigate gross and histopathological patterns of low virulent CSFV infection and to identify suitable specimens for viral isolation.

Materials and Methods

Experimental designs
Twenty-seven 3–4 week-old weaning pigs were tested for antibody to CSFV by neutralising peroxidase linked assay (Parchariyanon et al. 1997) and divided into three groups (nine pigs each): group 1, seropositive to CSFV pigs; group 2, seronegative to CSFV pigs; and group 3 (control group), seronegative to CSFV pigs. Pigs were housed separately. Pigs in groups 1 and 2 were inoculated intranasally with \(10^{6.5}\) TCID\(_{50}\) of low virulent swine fever virus strain Kampangpetch 1/1993 but pigs in group 3 received only culture medium. Three pigs (one from each group) were sacrificed at 1, 3, 6, 9, 12, 15, 18, 21 and 24 weeks post-inoculation (p.i.). There were no differences in clinical signs and pathological findings between groups 1 and 2. CSFV antigen was detected in lymphoid organs of all experimentally-infected pigs. CSFV was successfully isolated from blood and serum samples, but not from the tissue samples. The study indicates that viral isolation from blood and serum samples can be done for confirmation of chronic swine fever.
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<th>Organs</th>
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* Group 3 — data not shown, p.i.= post-inoculation, SSE = Starry-sky effect
- no remarkable changes, very mild, mild, moderate, severe
Results
Most inoculated pigs in groups 1 and 2 (eight of nine and seven of nine, respectively) had intermittent fever (39.8–40.8 °C) starting from nine days p.i.

Gross lesion observed was mild to moderate haemorrhagic papillary dermatitis (about 0.5 mm in diameter) on ears and body trunks of pigs in groups 1 and 2 (8 out of 9 each) starting at 3 weeks p.i.

Microscopic lesions found in brain, lymphoid organs, kidneys, bone marrow and skin of experimentally-inoculated pigs are summarised in Table 1. CSFV antigen was detected in lymphoid organs of all pigs in groups 1 and 2 from one week p.i. until 24 weeks p.i. Virus isolation, however, was successful from blood and serum samples, but not the tissue samples, during 3–16 weeks p.i.

Discussion
An experimental infection of chronic swine fever with low virulent strain Kampangpetch 1/1993 CSFV was reported. Observed clinical features: intermittent fever, mild skin lesions, endothelial swelling in brain and mild lymphoid depletion in lymphoid organs in agreement with studies by other investigators (Okaniwa et al. 1969; Van Oirschot 1980, 1992). In contrast, haemorrhages in kidneys, lymph nodes and urinary bladder, spleen infarction, ulcer in colon and leucopenia previously documented were not found (Mengeling et al. 1969; Okaniwa et al. 1969 and Van Oirschot 1992).

Unlike blood and serum samples from which the causative virus could be isolated successfully, the tissue samples were not suitable specimens for low virulent CSFV re-isolation. The unsuccessful re-isolation of the virus from tissue samples may be due to the much lower level of CSFV in tissues than in blood or sera.

Conclusion
The present experiment confirmed that, even in the presence of CSFV antibodies, pigs showed mild clinical signs and developed mild pathological changes when infected with low virulence CSFV. The examination of skin, brain, lymphoid organs and bone marrow for pathological lesions is very important. Samples of lymphoid organs are appropriate for the detection of the CSFV antigen, whereas blood and serum are suitable for isolation of the CSFV.

Acknowledgment
We would like to thank Dr Wiwat Chaichanasiriwithaya of the National Institute of Animal Health for assistance in preparing the English manuscript.

References
CLASSICAL SWINE FEVER
DIAGNOSIS
Diagnosis of and Emerging Diagnostic Technologies for Classical Swine Fever

T.W. Drew

Abstract

Pestiviruses can be broadly divided into four genotypes: Classical Swine Fever (CSF), Bovine Viral Diarrhoea (BVD) I and II, and Border Disease (BD) of sheep. While there is some propensity for the different viruses to be found in their respective hosts, they are not exclusive, so any detection of a pestivirus infection in a pig will require further identification before swine fever can be unambiguously diagnosed. The clinical signs of CSF are very variable and are not pathognomonic, so laboratory diagnosis is essential.

CONVENTIONAL methodologies for the laboratory diagnosis of CSF are well documented and are provided in detail by the OIE (Anon. 1996). CSF is a list A disease and a number of OIE reference laboratories exist to provide reagents and consultancy. Initial tests include the examination of frozen sections or impression smears of tonsil, spleen, kidney and ileum for viral antigen, followed by virus isolation in a PK15 cell line or other suitable cells. Cultures are examined for CSF virus by immunofluorescence, initially using a labelled polyclonal antibody. Further characterisation of isolates is performed using a panel of differential monoclonal antibodies. Laboratories undertaking isolation must ensure freedom of cell lines and culture media, particularly calf serum, free of adventitious pestivirus infection. In countries where the C-strain vaccine is used, its isolation must be a consideration in diagnosis and can be identified by inoculation into rabbits.

Serological tests are particularly useful in monitoring for disease in low-incidence areas and providing proof of CSF-free status. Serology is also sometimes used in tracing exercises in the event of an outbreak. The serum neutralisation test is the only serological test that can differentiate among pestiviruses. Such tests can employ fluorescent or peroxidase-conjugated antibody to visualise the virus. A number of antibody ELISAs have also been described, but current configurations cannot discriminate. The use of vaccine in a country can severely limit the value of serological tests, since no current tests can discriminate between antibodies induced by a vaccine and those induced by field infection.

Emerging Technologies

In the sphere of serology, ELISA tests are under development, designed to differentiate antibodies induced by different pestivirus genotypes. The tests will utilise recombinant proteins from the major envelope glycoprotein, E2. Along with the development of E2-based vaccines, companion tests are under development that hope to differentiate E2 vaccinal antibody from that induced by field infection, either by detecting antibody to Erns, or to E2 itself, if a deletion E2 protein vaccine is used (van Rijn et al. 1999).

A significant emerging technology for the detection of CSF is the reverse transcriptase–polymerase chain reaction (RT-PCR). This technique detects viral RNA in a number of types of sample and is very rapid and sensitive. In the past, the technique suffered from a high incidence of false positives, generated by contamination with PCR DNA. This, along with difficulties associated with scale-up, has hampered earlier introduction of this technique, but a novel one-tube method (van Rijn et al. 1999), combined with the use of a molecular probe (McGoldrick

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et al. 1999), has finally allowed this technique to enter the sphere of routine diagnosis.

In this new modification, an RT-PCR reaction is performed, followed by a second-round PCR, or nested PCR (nPCR), all within the same tube. The one-tube method involves drying the second-round reagents in a polysaccharide, trehalose, in the lid of the tube, obviating the need to open the tube. After the RT and first-round PCR, the tubes are inverted a few times and the second-round PCR is then performed. Primers which recognise all pestiviruses are used in this assay, while a fluorogenic probe confers the specificity of the reaction, providing a signal that can be read automatically using a TaqMan reader (McGoldrick et al. 1998).

The fluorogenic signal is generated by the specific action of the polymerase during successful PCR, so the amount of signal is proportional to the amount of PCR product. The presence of a signal indicates that the probe must have bound, confirming that the product of the reaction is derived from a pestivirus. Probes have been designed that allow the detection of all pestiviruses or a particular genotype, so a series of reactions can be simultaneously performed that both detect and type the pestivirus in question.

Experiments have shown the TaqMan RT-nPCR test to be up to 1000 times more sensitive than conventional virus isolation using CSF-infected blood, serum and tissues.

In the immediate future, it is anticipated that the TaqMan RT-nPCR will become widely used within Europe, where validation trials have already been undertaken and where harmonisation is now underway. With time, as it gains international recognition, it is likely to become the ‘Gold Standard’ for the detection of CSF and other pestiviruses worldwide.

**References**


Considerations Regarding the Transport of Samples and Development of Diagnostic Protocols for the Detection of Classical Swine Fever Virus under Endemic Conditions

Zhang Fuqing1, Syseng Khounsy2, Zhang Nianzu1 and S.D. Blacksell2

Abstract

Many factors influence the reliability of laboratory techniques to diagnose classical swine fever virus (CSFV). These include its close relationship to other members of the pestivirus genus, such as bovine viral diarrhoea virus (BVDV) and border disease virus (BVD), and the prevailing transport infrastructure of a developing country to transport safely animal samples to the laboratory for diagnosis. Then there is the choice of the best test procedure to employ, given the problem of finding the balance between technical issues, financial considerations and the expectations of stakeholders. Against this background, two laboratory techniques for the diagnosis of CFS, the antigen trapping enzyme-linked immunosorbent assay (AT-ELIZA) and the reverse transcription polymerase chain reaction (RT-PCR), were assessed to ascertain their application to differing sets of endemic CSF circumstances.

In this paper, the authors discuss the relative benefits of two popular diagnostic technologies, the antigen-capture enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) for their suitability for diagnosis in CSF endemic situations.

Materials and Methods

Samples

In Lao PDR, samples were transported to the ACIAR project laboratory via the provincial sample submission network. Samples of spleen were collected at post-mortem and placed in a transport buffer (50% PBS pH 7.2 + 50% glycerol) in a glass or plastic tube (usually a clean, previously used, evacuated blood tube) which was subsequently placed inside a specimen transport container of locally constructed PVC plumbing fittings construction (Figure 1a, 1b). The sample was sent to the laboratory via road, post or air transport. In PR China, samples were submitted to the laboratory via a structured field network. The samples are collected post-mortem and are transported on ice to the laboratory.
Figures 1a and 1b. Collecting a spleen sample for CSFV diagnosis in Lao PDR. The PVC specimen transport container is shown on the left. (Photographs by Jim Holmes).
Diagnosis of CSF infection by the CSF antigen-trapping ELISA

The classical swine fever antigen-trapping ELISA (CSF AT-ELISA) is similar to that described by Shannon et al. (1993) with local modifications described by Blacksell et al. (1999). The assay employs three monoclonal antibodies, a pestivirus group, bovine viral diarrhoea virus (BVDV)—specific and negative, to specifically detect the presence of CSF antigen or otherwise by inference.

Sample preparation

Two grams of tissue were minced into small pieces in a 20 mL universal bottle followed by the addition of 5 mL of a solution of 1% NP-40 in PBSA. The preparation was mixed thoroughly by vortexing and allowed to stand at 25 °C for 2 hours, mixing every 10 minutes. Following incubation, the tissue preparation was centrifuged at 2000 r/min for 10 minutes and the supernatant tested undiluted. Samples not tested immediately were stored at -80 °C.

CSF AT-ELISA methodology

This method required the use of a 96 well U-bottom polystyrene microtitre plate (low protein binding) that was used as a liquid-phase incubation plate (referred to as the LP plate) and a 96 well flat-bottom polystyrene microtitre plate (Maxisorb, Nunc, Denmark) used for the ELISA procedure (referred to as the ELISA plate). The LP plate was blocked for potential immunoglobulin binding with 5% skim milk powder (SMP) + 5% Normal Goat Sera (NGS) in carbonate buffer (blocking solution A) and incubated at 4 °C overnight. An ELISA plate was coated with goat anti-CSFV IgG at a dilution of 1 in 5000 and incubated at 4 °C overnight. Following incubation, the ELISA plate was washed 3 times with washing buffer (PBSA + 0.05% (v/v) Tween 20) with 1 minute soak between each wash. To block any potential adverse immunoglobulin binding, the ELISA plate was incubated with blocking solution A for 90 minutes at 37 °C. Following incubation, the LP plate was washed 3 times with washing buffer with a 1-minute soak between each wash. One hundred microlitres of each test sample, QC control. CSF positive and negative control samples to three appropriate wells (see Figure 2 for plate format). Add 100 μL/well of Pestivirus Group-reactive, BVDV-reactive and negative monoclonal antibodies (MAb) to the appropriate column wells (see Figure 3 for plate format). Incubate the LP plate stationary at 37 °C for 1 hour. During the incubation of the LP plate, the ELISA plate was washed 3 times with washing buffer and blocking solution added and the plate incubated for 1 hour at 37 °C. Following the completion of the respective incubations, the ELISA plate was washed by 3 washing cycles and 95 μL volumes of sample/MAb mixtures transferred from the LP plate to the ELISA plate in the appropriate format wells (see Figure 4 for plate format) and incubated for 90 minutes at 37 °C. The LP plate was discarded at the completion of this transfer step. At the completion of the incubation, the ELISA plate received a 5-cycle washing procedure and 100 μL/well of rabbit anti-mouse IgG – horseradish peroxidase conjugate diluted 1 in 1000 in PBSGT (i.e. PBSA + 1% gelatine + 0.05% Tween 20) and incubated for 60 minutes at 37 °C with shaking. At the completion of the incubation, the ELISA plate received a 5-cycle washing procedure and 100 μL/well TMB substrate was added and incubated for 10 minutes at room temperature and the reaction with 50 μL of 1M H2SO4 and read at 450 nm on a microplate reader.

The results were interpreted by first calculating a signal to noise ratio (S/N) for each sample thus:

\[ \frac{\text{average OD}_{450} \text{ nm with positive MAb}}{\text{average OD}_{450} \text{ nm with negative MAb}} \]

As recommended by Shannon et al. (1993), the following interpretation was made for each sample:

- S/N ratio >2.00 Positive
- 1.50–1.99 Doubtful (repeat test)
- <1.50 Negative

RT-PCR for the diagnosis of CSF infection

RNA extraction

The methodology for RNA extraction using TRizol® reagent was essentially the same as that described by the manufacturer with some minor modifications (Christian Mittlehozer, pers. comm.). Spleen tissue from CSF-positive animals was homogenised in PBSA with 1% NP-40 to give a 20% (w/v) solution, mixed well and incubated at 25 °C for 1 hour. To clarify, the homogenate was centrifuged at 6500 r/min for 1 minute and 250 μL of the supernatant transferred to a new 1.5 mL microfuge tube. To the supernatant, 750 μL of TRizol® reagent was added and incubated for a minimum of 5 minutes at room temperature followed by the addition of 200 μL of chloroform, vortexed for 15 seconds, incubated at room temperature for 3 minutes and centrifuged at 12000 r/min at 4 °C for 15 minutes. To precipitate the RNA, 450 μL of the aqueous phase was transferred to a new 1.5 mL microfuge tube to which 500 μL of isopropanol was added, mixed by inversion, incubated at room temperature for 15 minutes and centrifuged at 12000 r/min at 4 °C for 15 minutes. To wash the RNA
Figure 2. Layout of sample and controls on LP plate for the classical swine fever antigen trapping ELISA.

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Figure 3. Layout of monoclonal antibodies on the LP plate for the classical swine fever antigen trapping ELISA. Group-Pestivirus group reactive. BVDV = Bovine Viral Diarrhea Virus reactive. Neg = Negative. N/U = Not Utilised.

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Figure 4. Layout of sample monoclonal antibody mixtures when transferred to the ELISA plate for the classical swine fever antigen trapping ELISA. Group-Pestivirus group reactive. BVDV = Bovine Viral Diarrhoea Virus reactive. Neg = Negative. N/U = Not Utilised.

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<td>–ve</td>
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</table>
pellet, the supernatant was carefully decanted and 1000 µL of 80% (v/v) ethanol/water was added to the resultant pellet, briefly mixed and centrifuged at 12000 r/min at 4 °C for 5 minutes. The supernatant was carefully decanted and the pellet air-dried and finally resuspended in 25 µL of DEPC-treated water and stored at -85 °C.

**Description of oligonucleotide primers**

Two primer sets were employed for routine CSFV diagnosis. The first primer set amplified a 288 bp region located in the 5’ non-coding region (5’NCR) of the CSF genome. The primer sequences were as follows: forward primer 324, 5’ -ATG CCC TIA TA GTA GGA CTA GCA-3' (positions 108-128 bases in BVDV NADL strain), reverse primer 326, 5’-TCA ACT CCA TGT CiCC .4TG TAC-3' (positions 395-375 bases in BVDV NADL strain) (Vilcek et al. 1994). The second primer set was based on those described by Lowing et al. (1996) with minor modifications (P. Lowings, pers. comm.) to amplify an expected product size of 271 bp of the 5’ end of the CSFV E2 gene. The primer sequences were as follows: forward primer – 5’ TCR WCA ACC AAY CiACi .4TA GCiCi-3' (positions 2467-2487 in Alfort strain) and reverse primer – 5’ CAC AGY CCR AAY CCR AAC; TCA TC 3’ (positions 273-1716 in Alfort strain).

**RT-PCR protocols**

A single-step RT-PCR kit, PCR ACCESS (Promega, USA) was employed and the method as described by the manufacturer was followed. The kit allowed the RT and PCR to take place in the same reaction tube that employed a single proprietary buffer for both reactions. The RT-PCR reaction contained 4.0 µL of RNA, 1.0 µL of 10 mM dNTP, 3.0 µL of 10 µM forward primer, 3.0 µL of 10 µM reverse primer, 10.0 µL of 5 × AMV/IFL reaction buffer (composition is proprietary information), 2.0 µL of 25 mM MgSO₄, 26 µL of DEPC-treated water, 1.0 µL of of avian myeloblastosis virus (AMV) reverse transcriptase (5 units/µL) and 0.5 µL Thermus flavus (Tfl) polymerase (5 units/µL). The reverse transcription of RNA to cDNA took place at a temperature of 48 °C for 45 minutes followed by heating to 94 °C for 2 minutes to inactivate the AMV reverse transcriptase. The PCR immediately followed for 40 cycles of 94 °C for 30 seconds, 54 °C for 1 minute, 68 °C for 2 minutes followed by a final extension of 68 °C for 7 minutes.

To assess samples at the completion of the RT-PCR protocols, 5 µL of sample was mixed with a 5 × loading buffer (Biorad, USA) and loaded into a 2% agarose gel containing ethidium bromide (0.5 µg/mL) in 40 mM Tris-Acetate, 1 mM EDTA (TAE) buffer and subject to electrophoresis at 100 volts for 60 minutes. A 100 bp molecular size marker (Biorad Easyload) containing 10 fragments from 100–1000 bp in 100 bp increments was run in parallel on all gels. To visualise samples, the agarose gel was subjected to ultraviolet transillumination.

**Comparison of ELISA and RT-PCR methodologies**

The RNA extraction and RT-PCR methodology was compared against the CSF AT-ELISA by assessing eight Chinese samples and 70 Lao samples. Both the E2 and 5’NCR primer sets were employed in the assessment of the RT-PCR.

**Comparison of the relative sensitivity of AT-ELISA and RT-PCR methodologies to the detection of CSFV in decomposed samples**

To determine the relative sensitivity of AT-ELISA and RT-PCR methodologies to the detection of CSFV in decomposed samples, a sample of experimentally-infected CSFV spleen was placed in a standard sample transport tube and subjected to ambient temperature in the shade during the month of April 1998. Samples were taken daily and processed in the usual manner for RT-PCR or AT-ELISA assessment.

**Results**

**Sample transport**

In Lao PDR, the average submission time to the laboratory was 4 days although some specimens reached the laboratory more than 7 days following dispatch. The average annual temperature in Vientiane city during 1998 was 27.4 °C (Anon. 1999) although at certain times of the year such as the hot season the temperature can be much higher. On initial examination of the samples on arrival at the laboratory, most were found to be in decomposed but acceptable condition for diagnosis. Samples that had been subjected to longer periods of transport were generally in a putrid state.

Samples submitted to the laboratory in PR China were in a generally better condition due to the refrigeration of the sample during transport.

**Comparison of AT-ELISA and RT-PCR methodologies with routine diagnostic submissions**

The optimised RT-PCR system of TRizol® RNA extraction and the single-step RT-PCR with both the E2 and 5’NCR primer sets was compared against the
CSF AT-ELISA. Results are presented in Table 1 for Chinese samples and Table 2 for Lao PDR samples. From the results presented, it is apparent that the AT-ELISA was able to detect a greater number of positive samples in the Lao samples than the RT-PCR with either primer set. Overall, the 5’NCR primer set detected a greater number of CSFV positives than the E2 primer set.

**Table 1.** Comparison of Chinese CSF samples in RT-PCR and AT-ELISA systems

<table>
<thead>
<tr>
<th>Result</th>
<th>RT-PCR (5’NCR)</th>
<th>RT-PCR (E2)</th>
<th>AT-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2.** Comparison of Lao CSF samples in RT-PCR and AT-ELISA systems

<table>
<thead>
<tr>
<th>Result</th>
<th>RT-PCR (5’NCR)</th>
<th>RT-PCR (E2)</th>
<th>AT-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>56</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>22</td>
<td>6</td>
</tr>
</tbody>
</table>

**Discussion**

Classical swine fever virus is a somewhat difficult virus to diagnose from a laboratory viewpoint. The CSFV is antigenically closely related to other members of the pestivirus genus, BVDV and border disease virus (BDV) and therefore must be discriminated from these related viruses before a confident result may be reported.

CSFV does not naturally lend itself to classical virological techniques such as cell culture isolation, as the agent, with a few exceptions, does not produce cytopathic effect (CPE) and the ubiquitous nature of BVDV contamination of cell lines via infected media supplements is an overriding concern.

Conventional CSF diagnostic methods in smaller laboratories have therefore relied upon the technically simpler immunofluorescence techniques employing polyclonal or monoclonal antibodies on cryostat cut sections. A more complex assay with increased sensitivity and specificity is the CSF antigen-capture ELISA initially described by Shannon et al. (1993) which has been successfully employed at regional laboratories in Thailand (Blacksell et al. 1999). The test employs a panel of monoclonal antibodies to discriminate between CSFV and BVDV antigens. The most sophisticated assay for the laboratory detection of CSFV is the polymerase chain reaction that enables the specific detection of CSFV in clinical samples (Vilcek et al. 1994). Reports of the CSFV PCR to clinical applications have concentrated mainly on primer sets with the 5’NCR (Vilcek et al. 1994).

The quality and method of sample transportation is an important factor in the overall diagnostic outcome. Samples compromised by delays in transit to the laboratory and/or high ambient temperature will decompose rapidly. Ribonucleases (Rnas) naturally present in the sample will degrade RNA rapidly making the RT-PCR less reliable or unable to generate a product. In general, the ELISA technologies are less affected by degraded samples as only CSFV antigen is detected. Financial constraints on developing...
countries in general do not allow the luxury of specimen refrigeration. This factor, coupled with high ambient temperatures such as in Lao PDR, results in rapid sample decomposition. Chemical stabilisers such as using TRIzol® in the transport medium (Trevor Drew, pers. com.) is one solution to the problem but is expensive to purchase and potentially harmful to the inexperienced field operative.

In this study, we have assessed two diagnostic technologies for CSFV diagnosis. The AT-ELISA was able to reliably detect CSFV antigen in decomposed samples. The RT-PCR using the 5'NCR primer set was less reliable in amplifying CSFV genetic regions than the AT-ELISA. A nested or secondary PCR was not attempted because of the potential of cross-contamination of the sample due to limited designated PCR areas in the laboratory. A nested or secondary PCR may have provided the additional sensitivity required to make the test as sensitive as the ELISA but this must be balanced against the potential for amplifying a false positive due to a lack of PCR infrastructure.

The choice of diagnostic techniques for any infectious agent must maintain a balance between technical issues (i.e. sensitivity, specificity and methodology), financial considerations (i.e. cost per sample and overall available budget) and expectations of stakeholders (i.e. speed of testing and reporting to authorities). In a disease-free situation or regions where authorities are aiming for that achievement, technical issues and expectations of the stakeholders are of paramount importance when considering the choice of diagnostic assay. The cost of the assay may be of lesser importance given the importance placed on the ‘correctness’ and rapidity of the result given the potential financial losses in the case of an incorrect result. Conversely, in the case where a disease is endemic, the technical, financial and stakeholder issues take on the perspective of disease monitoring and require a different level of consideration.

Scientists have the responsibility to employ the most suitable laboratory technologies to the disease situation. There are examples of 'gold standard’ assays employed at great cost to developing country institutions in terms of establishment costs, only to find later that the cost of consumables and management issues are beyond the scope of the laboratory. PCR is a case in point of a highly sensitive diagnostic technology that has important management issues especially in terms of quality control. The problem of false positives due to contamination exacerbated by lax specimen processing or a lack of basic laboratory infrastructure can have dire consequences. Furthermore, knowing the limitations of assays such as PCR is an important step in understanding the performance capabilities of a test.

While PCR may not be the most suitable assay for developing country laboratories at this point in time, it still has an important role to play in the area of molecular epidemiology. The amplification of genomic regions for future downstream applications such as sequence determination can be performed at relatively low cost and tailored to medium level throughput in a small laboratory. The important considerations of false positives due to cross-contamination of samples is still of major concern but is somewhat offset by determining sample identity following nucleotide sequencing.

Acknowledgments

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References

Retrospection of Research into Classical Swine Fever, National Institute of Veterinary Research, Vietnam

Nguyen Tien Dzung

Abstract

Classical swine fever (CSF) has been and still is a main theme of research in the National Institute of Veterinary Research (NIVR) of Vietnam due to its importance in the pig production of that country. Results of research conducted in the NIVR since its foundation (1968) are described. Studies of epidemiology showed a wide distribution of CSF in the country and the disease has the tendency to a chronic evolution. The presence of infection by the low virulent CSFV was demonstrated by ELISA. Research into the CSF virus strain C (Chinese strain), the vaccination and the level of maternal antibodies suggested that vaccination had to be practiced on piglets born to vaccinated sows when they were 35–45 days old. The maternal antibody level examined by the commercial antibody-detecting ELISA was found different from one piglet to another piglet at the same age.

The National Institute of Veterinary Research (NIVR) of Vietnam was established in 1968. The mandate of the institution is to conduct research in the veterinary sciences in Vietnam. Its facilities are mainly located in Hanoi and a branch in Nha Trang City (centre of Vietnam).

The research themes focus on infectious and parasitic diseases in commercial animals. In addition, they are strongly oriented to the reality of the disease situation in the country.

Classical swine fever (CSF) is the most devastating disease in pig-rearing in Vietnam (Dao et al. 1985). Consequently, it has been and still is one of the main subjects for NIVR since its foundation. This paper reports the results of research into CSF conducted by the NIVR. It is worth noting that the compilation of the data to write this paper is difficult since research achievements in Vietnam in general and in veterinary sciences in particular are poorly published.

Epidemiology of CSF

The epidemiology of CSF in Vietnam was studied and reviewed by Tran and Dao (1989). In summing up CSF occurrences during 20 years (1969–89), they found that 80% of outbreaks were recorded in the period from December to March of the following year and attributed the condition to the cold climate and the active circulation of the animals and their products during that time.

On the source of the contaminant, the authors considered that CSFV existed in the location wherever there was pig-raising. This implied the ubiquity of the virus in the pig herds. Interestingly, two disease patterns were described. The first one occurred in the unvaccinated or occasionally vaccinated regions. CSF was observed in pigs of all ages. The disease was expressed in its ‘pure form’ and carried the endemic character. Clinical signs and lesions were identical to those that had been described widely elsewhere and the infected animals normally died.

The second pattern was characterised by secondary bacterial infections, mainly Salmonella, E. coli, Pasteurella and Streptococcus, that complicated diagnosis. This pattern was recorded in the vaccinated regions and occurred mainly in pigs of weaning age.

The infection in the sows induced reproductive failures: abortion, mummification, stillborn and abnormalities. In the first quarter of 1985 in the farm of Binh Luc, 26 of 75 farrowings resulted in total loss due to CSF. The reproduction failures generalised later in the early 1990s.
Chronic CSF and/or infection by the low virulent CSFV were indicated by field veterinarians also at the beginning of the 1990s. The disease was characterised by stunting and constipation only. This latter explains the nickname ‘dry CSF’ to describe this form of CSF.

Confirmation of CSFV infection by the laboratory test was reported (Nguyen et al. 1999). Other studies using the antigen-detecting ELISA (SERELISA HCV Ag Mono Indirect, Synbiotics) indicated that in the industrial pig sector, the proportion of CSFV carrier sows was as high as 20% on the studied farms. (Nguyen 1988; Nguyen and Ngo 1999). The figure could be higher than that. We have found seroconverted sows during our observation, but tested by the same antigen ELISA kit, the animals were negative (unpublished data). Low sensitivity combined with poor viraemia may explain the finding.

In addition, with the collaboration of the Australian Animal Health Laboratory (Geelong, Victoria) we have confirmed the presence of congenital CSF. This form of CSF was characterised by the birth of apparently normal piglets. However, the piglets died during the first 10 days of life.

**Studies of CSFV**

Virulent CSFV were isolated from diseased pigs. Two isolates were studied extensively, not the viruses themselves, but rather their pathogenicity. The two isolates were named 73A or HY (the abbreviation of Hung Yen, the name of the province where the virus was isolated) and the 73B or HT (likely, for the name of Ha Tinh province). The isolates were kept by monthly passages in the susceptible pigs. They were titrated in pigs. The 73B was found to have a higher titre than the 73A (10^7.1 ID50/pig/mL of whole blood versus 10^5.5 ID50/pig/mL, respectively). Also, pigs inoculated with the 73B had a short (3–5 days post-inoculation) incubation time while those inoculated with the 73A had a long incubation time (6–7 days). The 73B was considered more virulent than the 73A strain and then used for virulent exposure in other studies.

The lapinised c-strain (Chinese strain) was introduced into Vietnam in 1960. This strain was also studied in the 1970s. The pathogenic stability of the strain for rabbits was confirmed after 110 passages in a total of more than 600 rabbits. The criteria used for the evaluation were incubation time, fever duration and fever intensity. On average, the fever of the inoculated rabbits began to rise 36 hours after inoculation, the duration of the fever was 23 hours and intensity was 1.5 °C higher than the rabbit body temperature before the inoculation (T.D. Nguyen, unpublished data). The avirulent nature of the virus toward the pigs was tested and confirmed as totally apathogenic for pigs of all categories, even for pregnant sows (Dao et al. 1979a). Transmission of the CSFV c-strain from sow to foetus was not found. The test used for the revelation of the condition was the inoculation into rabbits (three successive blind passages) using the spleen and the mesenteric lymph nodes of newborn piglets as the initial inoculum (Dao et al. 1979b).

The virus amount in inoculated rabbits was also studied. The spleen and the mesenteric lymph nodes contained the highest amount of the virus (10^4.5 ID50/g for rabbits). Attempts to increase virus production in rabbits were not successful (T.D. Nguyen, unpublished data).

**Studies of Vaccination**

Maternal immunity and its influence on the immunogenic response to CSF vaccine in piglets born to vaccinated sows were investigated using 207 piglets of varying ages. The virulent exposure to the animals showed that 30-day-old piglets were 100% protected by maternal antibodies; 35-day-old piglets were 60% protected; and at 45 days old the piglets became quite susceptible. This suggested that piglets had to be vaccinated not later than at 45 days old.

Vaccinated piglets exposed to the virulent challenge developed vaccinal immunity in spite of the presence of maternal antibodies. The interesting thing was that the vaccinal immunity did not last long. Vaccination of piglets under 30 days old induced an immunity for up to two months. The 45-day-old or older piglets when vaccinated developed a solid immunity that lasted until the end of the experiment (six months post-vaccination) (Dao et al. 1990).

Based on these results, since 1980 vaccination has been recommended for piglets of 35–45 days in normal conditions and for pigs of all categories in a CSF outbreak area. Recent studies using simultaneously the antibody-detecting ELISA of AAHL (Geelong, Australia) (Shanon et al. 1993) and the ELISA kit from the Netherlands (CTB-ELISA, id-dlo, Netherlands) indicated that maternal antibodies could be detected only in piglets (446 individuals examined) under 50 days old born to vaccinated sows (98 sows). It is important to note that there was a great difference in maternal antibody level not only between litters of the same age but also between piglets of a litter. The results imply that it is difficult to get an homogenous vaccinal immunity in piglets if an active immunity to prevent the infection at early age is sought.

The anti-infection property of the vaccinal immunity was determined by exposing susceptible pigs to
the virulent challenged pigs which were previously vaccinated. The susceptible pigs became infected, having been put with the challenged pigs within 21 days post-challenge. This suggested the possibility of their infection and the excreting of virulent virus by vaccinated pigs. However, the vaccinated sows when infected by virulent CSFV did not transmit the virus to the foetuses (Dao et al. 1979b).

**General Comments**

Research conducted at the NIVR has responded to the problem of the existence of CSF, its distribution and its evolution in Vietnam. The research sometimes bore the character of certain diagnosis. Effectively, by clinical signs, CSF cannot be distinguished from other infectious diseases, especially infections by the low virulent CSFV. Moreover, the research by NIVR plays a guiding role in the fight against infectious diseases in the country. Research into vaccination is aimed at guiding field veterinarians in their vaccination campaigns.

Questions arising about the real situation of animal health are many. The above-mentioned results have answered some of them in a realistic fashion, and so contributed to pig industry development in Vietnam.

Differences between the above-mentioned results or conclusions and those published elsewhere may exist. The specific experimental conditions, the animals used and the experiment design could explain those differences.

However, results of the vaccination studies suggest that vaccination needs further study. The maternal antibody level is not the same in every pig, leaving some piglets unprotected either by maternal antibodies or by vaccinal antibodies. Moreover, well-vaccinated pigs could still be infected and excrete the virus, not to mention that not all pigs could be vaccinated in the vaccination campaigns. Assessment of vaccination efficacy by virulent exposure is an absolute and definite test but only valid for the exposed pigs. Furthermore, the vaccination practised in laboratories is quite different from that practised in the field. Therefore, there is an urgent need for a simple test for measuring the immunity against CSF created by the vaccination campaigns. It would help monitoring and improve the vaccination.

The fight against CSF in Vietnam might be complicated by the Bovine Viral Diarrhoea Virus. The presence of this pathogen in Vietnam remains obscure in terms of laboratory confirmation. The so-called ‘immune failures’ in pigs, meaning unresponsiveness to CSF vaccine, have been repeatedly reported by pig veterinary practitioners. The condition suggests an immunotolerant state that

**References**


