Assessment of classical swine fever diagnostics and vaccine performance

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Summary
Rapid and accurate diagnosis is of the utmost importance in the control of epizootic diseases such as classical swine fever (CSF), and efficacious vaccination can be used as a supporting tool. While most of the recently developed CSF vaccines and diagnostic kits are mostly validated according to World Organisation for Animal Health (OIE) standards, not all of the well-established traditional vaccines and diagnostic tests were subject to these validation procedures and requirements. In this report, data were compiled on performance and validation of CSF diagnostic tests and vaccines. In addition, current strategies for differentiating infected from vaccinated animals are reviewed, as is information on the control of CSF in wildlife. Evaluation data on diagnostic tests were kindly provided by National Reference Laboratories for CSF in various European countries.

Keywords
Classical swine fever – Diagnosis – DIVA strategy – Performance – Vaccine – Validation – Wild boar.

Background and objective

Rapid and accurate diagnosis is of the utmost importance in the control of epizootic diseases such as classical swine fever (CSF), and it contributes significantly to safeguarding animal welfare by limiting the spread of the infection. Efficacious vaccination, including emergency vaccination, can be used as a supporting tool.

While most of the recently developed CSF vaccines and diagnostic kits are validated according to World Organisation for Animal Health (OIE) standards, not all of the well-established traditional vaccines and diagnostic tests were subject to these validation procedures and requirements. To investigate this discrepancy, an OIE ad hoc group on CSF recommended that a full assessment of CSF diagnostics and vaccine performance be carried out by the OIE Biological Standards Commission.

The OIE Reference Laboratory for CSF at the Institute for Virology of the University of Veterinary Medicine in Hannover, Germany, which also acts as the European Union (EU) Reference Laboratory for CSF, was asked to provide a report including:

– updated information on performance and validation of CSF diagnostics and vaccines

– published or otherwise presented data or information on test validation and related relevant issues

– updated information on the control of CSF in wildlife

– current strategies for the differentiation of infected from vaccinated animals (DIVA) during vaccination and discriminatory testing.

Assessment of classical swine fever vaccine performance and recent developments

Vaccines

The most widely used vaccines for the control of CSF in countries where the disease is still endemic are live attenuated virus strains. These vaccines are highly efficacious but do not allow discrimination of infected from vaccinated animals. Control measures relying on serology are, therefore, not applicable. To circumvent these problems, marker vaccines which allow DIVA have been developed, including protective peptides, single expressed proteins, naked DNA, and chimeric viruses (19, 30); however, so far, only two subunit marker vaccines based on the E2 glycoprotein have been licensed. The discriminatory enzyme-linked immunosorbent assays (ELISAs) produced as an accompaniment to these vaccines are designed to detect antibodies against the E^{rns} glycoprotein of CSF.

The European Commission's Scientific Committee on Animal Health and Animal Welfare compiled a report on 'Diagnostic Techniques and Vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases' (24). Characteristics of vaccine performance in terms of vaccine types, efficacy, and safety are thoroughly discussed in this report.

In the aforementioned report, CSF live attenuated vaccines and the commercially available E2 subunit marker vaccines are described in detail. Other marker vaccines and second generation marker vaccines that are currently under development, such as recombinant and replicon vaccines, are also mentioned. In addition, the report contains details of the methods used for CSF antibody and antigen detection, DIVA strategies, and information on the application of vaccines in the field (domestic and feral pigs). The information compiled in the report on live attenuated and E2 subunit marker vaccines is briefly summarised in the following sections of this paper.

Live attenuated vaccines

Worldwide, different attenuated virus strains are used as live vaccines. The most common strain is the Chinese (C) strain. The Japanese guinea-pig exaltation-negative (GPE⁻)

strain, the Thiverval strain, and the Mexican PAV strains are regionally used.

The efficacy of these vaccines is tested according to the standards of the European Pharmacopoeia or other regulatory standards in vaccination-challenge experiments in the target host. In a model in which swine received an intramuscular injection of the test vaccine and were then challenged with the CSF virus, it was shown that vaccines should have a potency of at least 100 PD$_{50}$ (protective dose) to induce sterile immunity (40). It was demonstrated that animals vaccinated with 160 PD PD$_{50}$ were protected against oronasal challenge with a highly virulent CSF virus (CSFV) strain as early as one week post vaccination (4).

For C-strain vaccines it was shown that beginning at about four days post vaccination challenged pigs did not show clinical signs or replication of the challenge virus. Protection with C-strain vaccines has been proven to last longer than one year and most likely provides lifelong immunity (4, 50). As with all vaccines, maternal antibodies interfere with the induction of vaccinal immunity (50). In experiments, C-strain vaccines were able to block transmission of the challenge virus from at least seven days post vaccination (15). Data indicate that congenital infections with field virus would be similarly prevented.

With respect to safety, in no case should the vaccine virus cause any damage. The C-strain does not interfere with gestation, nor is it harmful to the foetuses, although passage across the placental barrier seems to be possible (51, 53). Vaccination appears to be safe even in (experimentally) immunosuppressed pigs (5).

The GPE⁻ vaccine strain was developed in 1969 by the Department of Exotic Diseases at the National Institute of Animal Health in Tokyo, Japan. Serial passage of the virulent ALD strain through sequential cell cultures at 30°C resulted in expression of different *in vitro* markers, e.g. interference with the growth of Newcastle disease virus in swine testicle cell culture. These alterations allow differentiation of the GPE⁻ strain from the field virus (48, 55). Vaccines based on the Japanese GPE⁻ strain are often used in Asian and Pacific countries. According to the information released by the OIE Regional Representation for Asia and the Pacific (29), there are no published trials comparing vaccine performance of GPE⁻ and C-strain based vaccines, but field evidence suggests that the safety and efficacy of both products are similar. The GPE⁻ strain rarely produces viraemia in inoculated swine and is not shed in excretions. Protection can be observed beginning at about three days post vaccination (29).

The cell culture adapted Thiverval strain, produced in France, has been derived from the virulent Alfort strain

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through more than 170 serial passages at 29°C to 30°C, and can also be identified by several *in vitro* markers (3, 55).

The Mexican PAV-250 vaccine was derived from the 250th passage of the A-PAV-1 strain. The vaccine is licensed in Mexico. A study on the epidemiology of CSF in the central Mexican highlands revealed that the efficacy of the vaccine differed markedly in field conditions compared to under laboratory conditions (13).

E2 subunit marker vaccines

Two research groups independently developed subunit vaccines using the viral E2 glycoprotein expressed in a baculovirus vector (32, 42, 44). Several experiments demonstrated protection of piglets against the clinical course of CSF two weeks after double vaccination or six weeks after single vaccination with the E2 subunit vaccine (37, 45, 55). It was shown that 32 µg of E2 protein in a water-oil-water adjuvant induced protective immunity three weeks after single vaccination (6). However, as Van Oirschot has noted, in order to prevent or minimise the spread of the virus in case of an outbreak, the efficacy of the vaccine to reduce replication and shedding is obviously more relevant than the clinical protection induced (54). Several experiments addressing horizontal (6, 7, 17, 52) and vertical transmission (1, 14, 16, 18) of challenge virus gave varying results.

E2 subunit vaccines have been shown to be highly safe. No side effects were observed in swine following administration of the vaccine, except for some local tissue reactions at the injection site (6, 17, 41).

Current research efforts

At the present time, there are three EU-funded projects in progress addressing new developments in CSF vaccines (26). Two of the projects are aimed at vaccine development and are entitled 'Immunological mechanisms of protection against CSFV: towards the development of new efficacious marker vaccines' (12) and 'Identification of efficacious delivery systems for recombinant and nucleic acid construct vaccines' (9). The third EU-funded project involves investigation of vaccination strategies in wild boar. The project, called 'Epidemiology and control of CSF in wild boar and potential use of a newly developed live marker vaccine' (36), is briefly described in this paper in the section on CSF control in wildlife.

The first project seeks to determine the mechanisms involved in early protection against clinical signs of CSF, and involves characterisation of the protective immune response against CSFV by means of defining the correlates of clinical protection and the immunomodulatory elements of CSFV. To achieve this objective, the interactions between antigen-presenting cells containing the vaccine virus are being assessed, as are the role of natural killer cells in innate immunity against CSFV, and the involvement of major histocompatibility complex (MHC) class II restricted T helper cells (stimulated upon vaccination) in the immune response. The CSFV specific cytotoxic immune response will also be characterised using MHC I tetramer prototypes. Construction of viral mutant C strain viruses will allow characterisation of viral proteins and epitopes that might be involved in the protective immune response. Based on this knowledge, new efficacious vaccines, immunostimulants, and diagnostics can be developed in the future. The project has not been completed.

The project on the identification of efficacious delivery systems focuses on a comparative evaluation of novel experimental marker vaccines with respect to immunomodulatory activation of defence mechanisms by the vaccines. In addition to development and application of novel-type marker vaccines, qualitative and quantitative analysis of the immune response is also being performed. So far, four prototype vaccines have been generated. Two of the novel vaccines are nucleic acid vaccines (complementary DNA and messenger RNA encoding CSFV-E2/NS3 combined with DNAs that encode cytokines). The other vaccines are a viral vector (recombinant Orf virus expressing CSFV protein E2) and a bacterial lipoprotein-based delivery system (recombinant subunit protein vaccine based on *Pseudomonas aeruginosa* outer membrane fusion lipoprotein and CSFV proteins E2 and NS3). The prototypes and second generation vaccines (modified prototypes) are tested in animal experiments and the immune response is evaluated. Several publications have already resulted from this project (2, 10, 11, 46, 49, 56, 57).

Conclusions

The assessment of CSF vaccine performance can be summarised as follows:

– live attenuated vaccines are most widely used for the control of CSF in countries where the disease is still endemic. These vaccines are highly efficacious and have the potential to induce sterile immunity;

– marker vaccines that allow DIVA have been developed. To date, two subunit marker vaccines based on the E2 glycoprotein of CSFV have been licensed. Enzyme-linked immunosorbent assays designed to detect antibodies against the E^{rns} glycoprotein are used as discriminatory assays;

– current research projects are aimed at the development and improvement of new marker vaccines and possible delivery systems. Efforts are being made to design a marker vaccine for use in wild boar.

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Recommendations

Development and commercialisation of efficacious marker vaccines should be encouraged. Further research should seek to improve marker vaccines and the accompanying discriminatory assays.

Assessment of classical swine fever diagnostic tests

Methods for the detection of CSFV antigen and antibodies are described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* (58). For the identification of the CSF agent it lists the following test procedures:

– fluorescent antibody test (FAT) for the detection of CSF antigen in cryostat sections

– immunoperoxidase staining using monoclonal antibodies for differentiation of pestiviruses (in cryostat sections)

– antigen-capture ELISAs

– virus isolation in cell culture

– reverse transcription-polymerase chain reaction (RT-PCR).

For serological tests, the listed methods are:

– fluorescent antibody virus neutralisation test (VNT)

– neutralising peroxidase-linked assay (NPLA)

– antibody ELISAs.

Commercial ELISA test kits for the detection of CSF antibodies and CSF antigen, as well as commercially available antibodies for use in the diagnostic tests, are tested and validated according to the national regulations of the respective countries during the process of registration and batch release. However, in-house ELISAs, VNTs, FATs, RT-PCRs, and virus isolation techniques have to be evaluated and validated by the laboratories using these tests as diagnostic tools. This also applies to the reagents used in the tests, i.e. monoclonal and polyclonal antibodies, conjugates, primers, probes, cell lines, and reagents for in-house ELISAs.

Difficulties in standardising and validating these methods are attributed to potential inconsistencies in the materials and reagents used in the tests. For example, different cell lines are used for the cell culture based methods, such as virus isolation and VNTs. Although all of the cell lines should, in principle, be suitable for the purpose, standardisation and inter-laboratory validation are needed. However, it is often difficult to meet these requirements because the same cell line might show different characteristics depending on the number of passages that have been performed. Information obtained on validation and standardisation of CSF diagnostics, including problems and recommendations, is discussed in the following sections of this paper.

Virus neutralisation test

Although the VNT has a long history and serves as the traditional gold standard for serological investigations, almost no published information on standardisation and validation can be found. Nevertheless, it has proven to be a very valuable and sensitive tool in the diagnosis of CSF. Test conditions are often modified to meet specific demands in different laboratories, and such modifications are a reasonable tool to improve diagnosis and differential diagnosis. For example, inclusion of additional test virus strains that represent the epidemiological situation in the country in question may facilitate detection.

For the evaluation of VNTs, the majority of the European National Reference Laboratories for CSF use control samples from negative pigs, laboratory specific experimental CSFV Ab-positive sera, and sera provided for the annual inter-laboratory comparison tests (ILCT). Samples used for the annual ILCT are produced and distributed by the European Community Reference Laboratory (CRL) and originate from well-defined and tested pig sera. The sera chosen are negative control sera, positive control sera, cross-reacting sera to border disease virus (BDV) or bovine viral diarrhoea virus (BVDV), and serum samples with low or inconclusive antibody titres. The samples provided for the ILCT are also used for validation of virus isolation, RT-PCR, and ELISAs. To validate single diagnostic VNT, a well-characterised control sample is tested together with the diagnostic material. Titre differences up to ± log 2 are tolerated in the quality control. Back titration of the test virus suspension ensures homogenicity in the quantity of virus used for the neutralisation assay. As with all living systems, tolerance limits have to be chosen carefully.

Validation carried out at the Swiss National Swine Fever Laboratory showed that VNTs are suitable for serum and plasma samples. As long as bacterial contamination is absent, even samples kept at room temperature or haemolysed samples can be used. The test is more sensitive than ELISA tests analysed in parallel with the VNT (in-house ELISA and Chekit-CSF-Sero®, former Dr Bommeli AG, Switzerland) especially when used for the detection of antibodies in samples that are taken 10 to 14 days post infection. The test has a moderate sensitivity of detection in samples with low antibody titres taken in the late phase

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of infection. Nevertheless, distinct antibody titres are detected. The VNT recognises all CSFV genotypes, but the genotype of the causative virus strain influences the test sensitivity. The best results are achieved with virus strains that are homologous or closely related to the virus strains used to produce the VNT. As antibodies against other pestiviruses are detected with this system as well, differential diagnosis has to be performed using BVDV and BDV strains. Problems with back titration and reproducibility are often associated with the CSF-VNT. Contaminated sera can sometimes not be analysed due to toxic effects on the cell culture system. Samples obtained from wild boar are often of very poor quality, making diagnosis difficult.

Validation tests that were carried out at the Dutch National Swine Fever Laboratory investigated the precision (repeatability and reproducibility), specificity, sensitivity, selectivity, limit of detection, and robustness of the VNTs performed as a neutralising peroxidase-linked assay. To express repeatability, the variation coefficient (VC%) was calculated. A VC lower than 10% was assumed to be an indication of sufficient test performance. Two sera, one with a high and one with a low antibody titre, were analysed in n = 30 tests. For both sera, the VC was lower than 10%. The VC for the serum sample with the low antibody titre (VC = 7.1%) was twice as high as the VC for the serum sample with the high antibody titre (VC = 3.0%). Reproducibility was tested in an intra-laboratory trial using sera sent for the ILCT. Again, the VC was considered a measure of the precision of the test. Results showed that the test precision associated with reproducibility was lower than that associated with repeatability. These results indicate that the precision of the test depends on the consistency of the test conditions. The criterion for test specificity is the ability of a test to correctly identify a negative sample. For the CSF-VNTs, cross-reacting antibodies directed against BVDV and BDV are of particular importance as they may lead to false positive results. The trial included BDV and BVDV positive samples. The specificity of the tests was approximately 99%. Sensitivity, in terms of the ability of the test to correctly identify a positive sample, was estimated using a panel of test sera obtained from experimentally infected pigs at seven different time points after exposure. Sera sent out by the CRL for the annual ILCT were also tested.

Reverse transcription-polymerase chain reaction

So far, only commercially available test kits, reagents, and recently developed ready to use RT-PCR kits are validated according to OIE guidelines. None of the 'in-house' PCR for the detection of CSFV nor any of the commercially available test kits are fully validated according to the five-stage procedure described in Chapter 1.1.4 of the *Terrestrial Manual* (58). Specifically, data on diagnostic sensitivity and specificity are lacking, as are data for stages four and five of the validation procedure.

To a certain extent, PCR assays routinely used in the European National Swine Fever Laboratories and other international laboratories are validated during the ILCT. During the last few years, samples distributed exclusively for PCR testing were included in the trials. So far, only recommendations on minimum sensitivity and specificity requirements can be given for PCRs for the detection of CSFV. In any case, the PCR assay has to be at least as sensitive as the 'gold standard': virus isolation. To ensure specificity, primers and probes should be designed to detect as many viral sequences as necessary to cover genetic diversity.

A recently developed and validated PCR for the detection of CSFV utilised consensus sequences of the 5' non-translated region of 78 different CSFV strains (31).

Diagnostic evaluation of another recently developed fluorogenic-probe hydrolysis (TaqMan)-RT-PCR for CSFV demonstrated 100% sensitivity and 98.9% specificity on clinical samples (nasal swabs) obtained from the Dominican Republic (47).

At the 2005 Annual Meeting of the European National Swine Fever Laboratories, the evaluation of new commercial real-time RT-PCR kits for the detection of the CSFV genome was presented (38). Criteria assessed in the study included sensitivity, specificity, reproducibility, and ease of handling. The evaluation was carried out in three steps with different sample panels. Only test kits that passed all three steps were selected. One commercial PCR kit (LSI Kit Taqvet, Laboratoire Service International, Lissieu) was approved and it has subsequently been used in epidemiological surveys of the wild boar population in France (38).

Enzyme-linked immunosorbent assays

Commercially available ELISAs are validated upon registration and batch release according to different national regulations. The requirements and regulations are not harmonised on an international level. In-house ELISAs that are not validated according to these rules exist in many laboratories. These tests are compared with other diagnostic methods by the laboratories that produce the tests.

National Reference Laboratories of EU Member States must regularly perform quality control on each batch of tests (sensitivity and specificity testing), according to Commission Decision 2002/106/EC, Chapter VII (22), using a panel of reference sera provided by the CRL. The

panel comprises well-characterised sera from experimentally infected animals and includes sera from pigs in the early phase of CSFV infection (< 21 days post infection), sera from reconvalescent pigs (> 21 days post infection), and sera from pigs infected with ruminant pestiviruses. All ELISAs used for serological diagnosis of CSF must recognise CSF antibodies in sera from reconvalescent pigs. Furthermore, the tests must detect all positive samples from the early phase of infection and have minimum cross-reactions with sera from pigs infected with ruminant pestiviruses.

At the 2005 Annual Meeting of the European National Swine Fever Laboratories in Brussels, preliminary results were presented on the evaluation of five commercially available CSF-ELISA kits. The evaluation, which was carried out at the Dutch National Swine Fever Laboratory, included the Herdcheck CSFV Antibody test kit (IDEXX Laboratories), the Chekit-CSF-Sero® and Chekit-CSF-Marker® ELISAs (former Dr Bommeli AG), and the Ceditest CSFV and Ceditest CSFV 2.0 test kits (Cedi-Diagnostics). The samples tested represented different antibody titres, different days post infection, different negative field samples, and samples containing antibodies against non-CSF pestiviruses. Preliminary results showed that, in general, all of the tests performed well. Specifically, the Herdchek CSFV Antibody ELISA was the only test to correctly detect all of the negative field samples investigated in the trial. The Ceditest CSFV 2.0 gave only two false positive results. The Chekit-CSF-Sero® ELISA had the lowest specificity, reporting 48 (3.1%) of the negative field samples as false positives. The Chekit-CSF-Marker® ELISA had the lowest specificity with regard to BVD positive samples. The Chekit-CSF-Sero® was the most sensitive test in the trial on days 21 and 28 post infection, outperforming even the VNT. The sensitivity of the other tests was comparable; however, the Chekit-CSF-Marker® was slightly better on day 14 post infection.

Fluorescent antibody tests

In recent years, the FAT has been replaced in many laboratories by RT-PCR. The test's quality mainly depends on experienced personnel and well-defined test conditions. Reagents and antibodies have to be validated in intra-laboratory trials. In 2001, Bouma *et al.* (8) published an evaluation of the diagnostic use of the FAT in the detection of CSF. It was shown that the test had only a moderate sensitivity level of detection. On a herd basis, the test is suitable for diagnosis, provided that an appropriate sample size is taken per farm.

Validation data on test precision, sensitivity, and specificity were provided by the Dutch National Swine Fever Laboratory. Using samples from experimentally infected pigs, repeatability and reproducibility of the test were 99%.

Specificity was estimated using field samples assumed to be free of CSFV and was calculated to be 99%. Sensitivity determined using tonsil samples from experimentally infected pigs was also 99%.

Note: CSFV could be detected from 2 to 15 days post infection in tonsil samples by the FAT and the immunoperoxidase test. The probability of detecting virus in the tonsils is much higher in pigs with clinical signs of CSF than in pigs without any symptoms of CSF.

Virus isolation

As with the VNT, the traditional method of virus isolation has proven to be useful in practice for many years, but validation data are rare. Standardisation and validation are extremely difficult to perform as storage and transport conditions can influence the amount of viable virus in the test samples and reference material. Thus, the quantitative result of virus isolation cannot be verified objectively by repeating the test in another laboratory. Nevertheless, all laboratories should be able to correctly detect if a sample is CSFV positive or negative. Cell lines, antibodies, and conjugates need to be validated.

Validation of virus isolation in terms of precision (repeatability and reproducibility), specificity, sensitivity, and robustness was carried out by the Dutch National Swine Fever Laboratory in 1998. The VC was calculated to estimate repeatability. Test performance was considered to be sufficient if the VC was < 10%. The trial showed a VC of 3%. The same procedure was applied and results obtained for the estimation of reproducibility. Specificity was tested with pre-experimental blood samples from specific pathogen-free (SPF) pigs. Under these conditions, the test specificity was estimated to be 99%. Sensitivity was estimated using test samples from experimentally infected pigs. Samples were collected at regular intervals after infection and tested for the presence of CSFV by virus isolation. Depending on the virus strain, virus could be detected from one to nine days after infection. After deliberate implementation of test variations, such as different incubation periods, viral titres were compared. Virus isolation proved to be robust within certain limits. No differences in virus titres were observed between incubation for three versus four days of culture.

Other tests

Regarding miscellaneous tests, a presentation was given during the meeting of European National Swine Fever Laboratories in Brussels in 2005 on the evaluation of an avidin-biotin complex monoclonal based assay that was used to test over 900 tonsil samples (43). The trial comprised CSFV strains of low, moderate, and high

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virulence from Mexico, the Caribbean, and Central America and used CSFV strains Brescia and Ames as reference strains. Specificity was determined using tonsil samples from pigs infected with BDV and BVDV and organ material from field submissions negative for CSFV. The monoclonal antibody used in the trial was directed against the A1 domain of the E2 protein (V3, Cedi-Diagnostics). An avidin-biotin alkaline phosphatase reagent from Vector Kit® was used in the test (Vector Laboratories, Canada). The protocol was modified for automation. Preliminary results showed that the sensitivity determined using tonsil samples from experimentally infected animals was 92.1%. The overall sensitivity is currently being determined using additional material of different origin that was pre-tested by real-time PCR.

The test showed high specificity using tonsil samples collected from non-CSF pestivirus-infected pigs and field submissions submitted for CSFV testing.

The immunoperoxidase test was validated in much the same way as the FAT by the Dutch National Swine Fever Laboratory (unpublished data). Repeatability was estimated using positive and negative reference samples obtained from experimentally infected and uninfected SPF pigs. Under these conditions, repeatability was 99%. Reproducibility was tested on the same material and was calculated to be 99%. Sensitivity determined using positive material obtained from experimentally infected pigs was also 99%.

Inter-laboratory comparison tests

The ILCTs for the diagnosis of CSF have been established by the National Swine Fever Laboratories of the EU Member States. Several other countries from various parts of the world also participate in these trials. The trials provide a method of assessing both the quality of the results of the diagnostic tests performed by the laboratories and the laboratories' proficiency (28). They are also an appropriate tool to measure test performance in terms of sensitivity and specificity. In recent years the trials were comprised of reference sera to be tested for CSFV and viral antibodies using routine diagnostic tests and samples for the detection of CSFV using PCR assays. Sensitivity of the serological diagnosis for the detection of CSF antibodies was good. It was shown that the antibody titres varied within a tolerance level of one dilution step (the dilution steps are preset by the assay design). Following the methods described in both the *Terrestrial Manual* (Chapter 2.1.13) and the EU manual (Technical Part), this means that the antibody titres varied within one log 2 step using an initial serum dilution of 1:5 (22, 58). The interpretation of results from the VNTs was mostly correct, although the reported CSF antibody titres varied within the limits. The variation between the CSF antibody titres reported for a single serum sample was greater in sera with a low CSF antibody titre, indicating that these types of samples are the most difficult to correctly diagnose. Commercial and 'in-house' CSF antibody ELISAs used in these trials have improved over the last few years. However, at the present time ELISAs are still less sensitive than VNTs (28). Virus neutralisation test and ELISA results from identical samples tested over two consecutive years were similar, proving repeatability. The performance of CSFV isolation was satisfactory, showing only occasional minor problems.

Conclusions and recommendations

The assessment of CSF diagnostic tests can be summarised as follows:

– although the data for commercial and in-house tests is available in many laboratories worldwide, validation and evaluation data from diagnostic tests for CSF are rarely published

– some publications are difficult to obtain as they are available only in Russian or Chinese

– the ILCTs are a valuable tool for assessing the quality of results obtained in diagnostic tests used by different laboratories and for evaluating test performance in terms of sensitivity and specificity.

General recommendations

– Every laboratory should participate in ILCTs

– data on validation and evaluation of test kits should be collected and made accessible to the international community

– standardised reference material should be produced and made accessible to CSFV laboratories.

Test specific recommendations

Virus neutralisation tests

– To achieve worldwide standardisation and validation, VNTs used for diagnosis and differential diagnosis of CSF should be validated with standardised and, if possible, certified reference material. Following an OIE request in 1997, a first attempt to prepare international standard sera for CSF was made. A number of international reference laboratories participated in the evaluation process that followed, which was presented at the Annual Meeting of European National Swine Fever Laboratories in Vienna in June 1997 (20). The evaluation should be repeated;

– CSFV strains used for testing should at least include the standard reference strain Alfort 187 and, in addition,

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should include virus strains reflecting the current epidemiological situation of the country;

– to ensure specific results and to eliminate differential diagnoses, other pestiviruses (BDV and BVDV) must be included in the test;

– following general principles of cell culturing, cell lines used in VNTs should be well characterised and tested. At regular intervals the cell lines should be investigated for the presence of *Mycoplasma* and viral contamination.

Reverse transcription-polymerase chain reactions

– An adequate number of positive and negative controls must be included in each test;

– the use of external and internal controls should be considered to ensure that the test results are reliable;

– as many laboratories have recently included RT-PCR in their diagnostic repertoire, intra- and inter-laboratory trials with well-characterised reference material are essential. Assessment of tests must include reproducibility, sensitivity, and specificity data, as well as assessment of possible contamination;

– so far, only recommendations on minimum sensitivity and specificity values can be given for RT-PCRs used for the detection of CSFV. In any case, the test has to be at least as sensitive as the standard method, i.e. virus isolation;

– in the future, minimum requirements for sensitivity and specificity should be agreed upon;

– to ensure specificity, primers and probes should be designed to detect as many viral sequences as necessary to encompass genetic diversity.

Enzyme-linked immunosorbent assay

– Quality control on sensitivity and specificity should be carried out regularly using a panel of standardised reference sera

– the reference panel should comprise sera from the early phase of CSFV infection (< 21 days post infection), sera from reconvalescent pigs (> 21 days post infection), and sera from pigs infected with ruminant pestiviruses.

Virus isolation

– Attempts should be made to harmonise cell culture systems and validate reagents and antibodies used in the test.

Inter-laboratory comparison test

– Every diagnostic laboratory should participate in ILCT.

Classical swine fever in wildlife

Control of classical swine fever in wildlife

Classical swine fever is still endemic in the feral pig population in some European countries/regions and presents a constant threat to domestic livestock. Contact with infected wild boar was identified as the most probable source of infection of domestic pigs and is regarded as the main risk factor in endemic areas. About 80% of primary outbreaks of CSF in domestic pigs in Europe occurred in regions where CSF is endemic in wild boar, stressing the importance of controlling CSF in wildlife.

General and specific information on CSF in wild boar was compiled in a report by the European Commission's Scientific Committee on Animal Health and Animal Welfare that was adopted on 10 August 1999 (21). The report outlines the criteria for the surveillance of CSF in domestic pigs and control measures for the movement of domestic pigs kept in areas where infected wild pigs reside. Criteria for monitoring CSF in wild boar in an infected area, the effects of wild boar population levels on the disease, factors that influence the evolution of CSF in wild boar in a given area, and control measures related to the domestic pig are also discussed.

Different approaches to controlling CSF infections in wild boar have been tried, including hunting measures, hunting combined with vaccination, and natural immunisation (33). Natural immunisation means the acquisition of natural immunity against CSF virus by adult animals after an outbreak of CSF and during the progression of an epidemic. Increasing herd immunity reduces the number of susceptible animals and thus contains CSF infection. In small confined areas this might lead to the elimination of the disease.

Although of considerable debate when first implemented, vaccination proved to be a valuable tool for the control of CSF in wild boar (34, 35). To date, only vaccine baits containing C-strain have been used in vaccination trials (34) and programmes (35). The disadvantage of current vaccination programmes is that young wild boar (< 4 months), which are most susceptible to CSF, often do not consume the baits and are, therefore, not vaccinated. Also, C-strain vaccines do not allow discrimination between vaccinated and naturally infected pigs in serology-based surveillance programmes. In Germany, the baits are now being modified to increase their uptake by piglets.

As previously mentioned, the project 'Epidemiology and control of CSF in wild boar and potential use of a newly developed live marker vaccine' (36) aims to design an epidemiological and economic model for CSF eradication in wild boar and to develop a new generation of live

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attenuated vaccines (more details about this project can be found at http://www.csfvaccine.org/index.php). Addressing the recommendations given in the aforementioned report of the Scientific Committee, this project will provide a scientific basis for the control of CSF in wild boar by describing and comparing the available results and the newly acquired knowledge concerning CSF epidemiology in wild boar. Access to a European database as part of the project will support research activities by providing up-to-date information on wild boar populations (e.g. the dynamics and status of CSF epidemics and other relevant aspects, such as annual hunting bags).

Laboratory diagnosis of classical swine fever in wild boar

The possibilities and limitations of laboratory diagnosis have to be taken into account when considering control strategies and surveillance programmes for wild boar. This information is accessible in the report of the meeting of the OIE Working Group on Wildlife Diseases (59).

As there is no biological difference between CSF diagnosis in wild boar and domestic pigs, all of the established diagnostic tools can, in principle, be used. The diagnostic tests recommended by Commission Decision 2002/106/EC (22) and the 'Technical Part' accompanying it are also applicable to wild animals. However, problems frequently arise due to the poor quality of samples taken from wild boar. In these cases appropriate tests have to be chosen and results have to be interpreted with special care.

Regarding the options available to detect active CSF infection in wild boar, the combination of methods proposed by Le Potier and Mesplede (39) is recommended for detection of the infection in suspicious cases. The detection methods employed are RT-PCR and subsequent virus isolation in positive cases. Virus isolation is still considered to be the gold standard. Antigen capture ELISAs may be used for ongoing epidemics or endemics of CSF in wild boar in which a moderate sensitivity of detection is acceptable. The advantage of using the ELISA test is that it is fast and inexpensive and, therefore, suitable for screening large numbers of samples. The FAT may be used when skilled technical staff and the appropriate conditions are available in the regional laboratory. The last resort would be an animal inoculation experiment as envisaged in the procedures established by the United States of America; however, the authors do not support this method.

In summarising the possibilities for antibody detection, the best method in terms of sensitivity and the ability to distinguish antibodies directed against CSFV from antibodies against other pestiviruses is the neutralisation assay. However, because this technique is slow and labour intensive it should be used as a confirmatory test in cases that tested positive using the ELISA test from areas free of CSF or areas where infections with ruminant pestiviruses are assumed. Poor sample quality may interfere with the cell culture system used for the VNT and hamper diagnosis. Toxic damage to the cell culture system may be avoided by adding different mixtures of antibiotics and fungicides. Antibody ELISAs are the method of choice to screen large numbers of sera and other body fluids from wild boar. Positive or questionable results should be confirmed by neutralisation assays.

Conclusions and recommendations

The preceding discussion on CSF in wild boar can be summarised as follows:

– endemic CSFV infections of wild boar populations are an important threat to domestic pigs and are a dangerous source for the reintroduction of CSF

– oral immunisation is a valuable tool for the control of CSF in wild boar

– problems in laboratory diagnosis can arise from poor sample quality.

Recommendations

– The development of new live attenuated marker vaccines for oral application and the accompanying diagnostic assays should be supported

– oral immunisation procedures applied in combination with hunting measures should be refined.

Strategies for the differentiation of infected from vaccinated animals

Although various attempts to develop novel DIVA vaccines against CSF have been made during the last decade, to date, only the E2 subunit vaccines have entered the marketplace. Most DIVA vaccines contain the E2 glycoprotein because it is the most immunogenic protein of the CSFV. Differentiation of naturally infected from vaccinated pigs can be achieved by detecting antibodies against a protein that is not included in the DIVA vaccine (55).

Currently, there are two marker vaccines and accompanying ELISA tests licensed in Europe. Both marker vaccines are based on the E2 glycoprotein expressed in a baculovirus system. As vaccinated animals

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will generate only the E2 antibodies, the discriminatory tests are based on the detection of antibodies against another CSFV glycoprotein (E^{rns}) in naturally infected animals. Both commercially available subunit vaccines were assessed and validated by the European Agency for the Evaluation of Medicinal Products (EMEA). Based on the Assessment Reports adopted by the Committee of Veterinary Medicinal Products (CVMP), marketing authorisations were issued by the European Commission. The EMEA did not take responsibility for assessment and validation of the accompanying discriminatory assays. The lack of a licensing prerequisite requiring simultaneous licensing of a fully validated accompanying discriminatory assay can be seen as a weak spot in the licensing process of CSF marker vaccines. A fully validated discriminatory assay is necessary for a new marker vaccine to be considered as a valuable tool in the control of CSF.

The currently licensed CSF marker vaccines are Advasure® (Pfizer, UK) (formerly Bayovac CSF E2® [Bayer, Germany]) and Porcilis Pesti® (Intervet, the Netherlands). The corresponding discriminatory assays are the Chekit-CSF-Marker® ELISA (formerly Dr Bommeli AG, Switzerland) for the Bayovac CSF E2® vaccine and the Ceditest CSFV E^{rns}® ELISA (Cedi Diagnostics, Lelystad, the Netherlands) for the Porcilis Pesti® vaccine. Product profiles and scientific data that were presented during the licensing process can be obtained on the internet. For the Porcilis Pesti® and Advasure® vaccines the data on the scientific profiles are available through the EMEA website at http://www.emea.eu.int/vetdocs/PDFs/EPAR/Porcilispesti/041200en6.pdf and http://www.emea.europa.eu/vetdocs/vets/Epar/advasure/advasure.htm, respectively.

Vaccine performance in terms of efficacy and safety is discussed in the report on 'Diagnostic Techniques and Vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases' (24). The report can be accessed through the EU Commission's website at http://ec.europa.eu/food/fs/sc/scah/out93_en.pdf. The corresponding discriminatory assays were evaluated in ring-trials conducted by the CRL. Trials conducted in 1999 showed that neither the Chekit-CSF-Marker® nor the Ceditest CSFV E^{rns}® fulfilled the requirements for vaccine licensing. Both tests showed deficiencies in sensitivity and specificity when compared to conventional CSF antibody ELISAs. No confirmatory test

was or is currently available to verify the results on the discriminatory ELISAs (27).

After revisions, the Chekit-CSF-Marker® ELISA was tested in a second evaluation trial in 2003 (25). Summarising the results obtained during the trial, the sensitivity of the Chekit-CSF-Marker® ELISA was slightly lower than the conventional E2-Ab-ELISAs. It was determined that the sensitivity value was acceptable if the discriminatory test is used on a herd basis with sufficiently high numbers of random samples. The test should not be used to diagnose individual animals. The Chekit-CSF-Marker® ELISA is not specific for CSF and also detects antibodies directed against BDV and BVDV. In regions with a high prevalence of BDV and/or BVDV infections this fact may cause problems, and, thus, the test may not be recommended in these regions (25).

Taking into account the aforementioned results, Commission Decision 2003/859/EC was enacted by the Commission of the European Communities in December 2003 amending Decision 2002/106/EC (23). The 2003 Commission Decision allows the use of the Chekit-CSF-Marker® discriminatory test in the event of emergency vaccination with a marker vaccine.

Conclusions and recommendations

The preceding discussion on the use of marker vaccines and discriminatory assays for DIVA can be summarised as follows:

– two E2 subunit marker vaccines are currently licensed for the European market: the Porcilis Pesti® and Advasure® (formerly Bayovac CSF E2®)

– discriminatory assays were designed to detect antibodies against the E^{rns} protein of CSFV. An evaluation trial showed that the discriminatory ELISA Chekit-CSF-Marker® is suitable for diagnosis on a herd basis.

Recommendations

– In the future, licensing procedures for CSFV marker vaccines should include the simultaneous assessment and validation of a discriminatory assay.

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Évaluation de la performance des épreuves de diagnostic et des vaccins vis-à-vis de la peste porcine classique

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Résumé

Pour être efficace, la prophylaxie d'épizooties telles que la peste porcine classique doit recourir à des épreuves diagnostiques rapides et fiables ainsi qu'à une vaccination d'appui. Si la plupart des vaccins et des trousses de diagnostic mis au point récemment ont été validés suivant les normes de l'Organisation mondiale de la santé animale (OIE), cela n'est pas toujours le cas des épreuves diagnostiques et des vaccins traditionnels d'usage bien établi, qui n'ont pas fait l'objet de ces procédures de validation. Ce rapport réunit les données sur les performances et la validation des épreuves diagnostiques et des vaccins vis-à-vis de la peste porcine classique. En outre, les stratégies visant à différencier les animaux infectés des animaux vaccinés sont passées en revue, ainsi que l'information disponible sur la prophylaxie de la peste porcine classique chez la faune sauvage. Les données d'évaluation des épreuves diagnostiques ont été aimablement transmises par les Laboratoires de référence nationaux pour la peste porcine classique de plusieurs pays européens.

Mots-clés

Diagnostic – Performance – Peste porcine classique – Sanglier sauvage – Stratégie de différentiation entre les animaux infectés et vaccinés – Vaccin – Validation.

■

Evaluación del diagnóstico de la peste porcina clásica y del rendimiento de la vacuna

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Resumen

Disponer de un diagnóstico rápido y exacto es de capital importancia en la lucha contra enfermedades epizoóticas como la peste porcina clásica (PPC), y en esa empresa una vacunación eficaz puede constituir una herramienta complementaria de inestimable valor. Aunque la mayoría de las vacunas y estuches de diagnóstico elaborados en fechas recientes han sido validados con arreglo a las normas de la Organización Mundial de Sanidad Animal (OIE), no todas las técnicas que se utilizan desde hace tiempo fueron sometidas en su día a tales requisitos y protocolos de validación. Para el presente estudio se reunieron datos sobre el rendimiento y la validación de pruebas de diagnóstico y vacunas contra la PPC. Los autores pasan revista además a las estrategias

empleadas actualmente para distinguir entre animales infectados y vacunados y a la información disponible sobre el control de la enfermedad en la fauna salvaje. Los datos correspondientes a la evaluación de pruebas de diagnóstico fueron amablemente facilitados por los laboratorios nacionales de referencia para la PPC de varios países europeos.

Palabras clave

Diagnóstico – Estrategias de diferenciación entre animales infectados y vacunados – Jabalí – Rendimiento – Peste porcina clásica – Vacuna – Validación.

■

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