

Evaluation of the enzyme-linked immunosorbent assay for the rapid screening and detection of classical swine fever virus antigens in the blood of pigs

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Summary: *A workshop was convened, at which seven enzyme-linked immunosorbent assays (ELISAs) were compared with virus isolation for the detection of viraemia in serial blood samples collected from six pigs at up to fourteen days after inoculation with classical swine fever virus. All ELISAs were of the double antibody sandwich type, using monoclonal and/or polyclonal antibodies to detect a variety of viral proteins in leukocytes, or in anti-coagulated blood or serum. Compared to virus isolation, specificity of the ELISA was good: only one sample found negative by virus isolation yielded a positive result in a single ELISA. Some false-negative results occurred with samples collected at up to eight days after inoculation, but all tests found samples collected between nine and fourteen days post-inoculation to be positive. The ELISAs require less-specialised facilities and can be performed much more rapidly than virus isolation. They are therefore extremely promising tools for screening large numbers of live pigs.*

KEYWORDS: Antigen – Classical swine fever – ELISA – Hog cholera – Viraemia.

INTRODUCTION

Classical swine fever (CSF) is an economically important disease which is controlled, in general, by statutory measures. In view of the serious implications of a diagnosis of CSF, and because clinical signs are never pathognomonic, laboratory confirmation of diagnosis is very important. As with other viral diseases, laboratory diagnosis is based

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on either detection of the agent or serology. Serology is the relatively more simple technique, but is inappropriate for use in recently-infected pigs which may be shedding virus but have not yet developed serum antibodies. Virus detection has traditionally been performed by a combination of fluorescent antibody tests on tissue sections and virus isolation in cell cultures from either tissues or leukocytes. For diagnosis in live pigs, tissues are not readily available, and therefore virus isolation from leukocytes has been the method of choice. Although sensitive and specific, virus isolation is both time-consuming and laborious, and is therefore unsuitable for large-scale screening over a long period of time. The need for a sensitive, rapid and practical test for the detection of CSF virus (CSFV) in blood has become all too evident during recent CSF epidemics in Belgium and Germany, when a large number of animals had to be tested.

One way to overcome these problems has been through the recent development of enzyme-linked immunosorbent assay (ELISA) techniques for detecting CSF viral antigens (14). Sensitive and specific antigen-capture ELISA systems are already successfully used in the routine diagnosis of the related pestivirus diseases of cattle and sheep, namely bovine virus diarrhoea (BVD) and Border disease (BD), respectively.

The present report summarises the findings of a workshop on antigen detection procedures for CSFV, held under the auspices of the Commission of the European Union (EU) at the Community Reference Laboratory (CRL) for CSF, Institute of Virology, Hanover Veterinary School, from 19 to 21 March 1995. Representatives of the CRL and of the National Swine Fever Laboratories of Belgium, France, Germany, Italy and the United Kingdom attended the workshop to evaluate various ELISA techniques for detecting CSF viral antigen in blood. Emphasis was put on evaluating the ability of the tests to detect viraemia in pigs in the early phase of infection before classical signs of CSF were present.

MATERIALS AND METHODS

Materials available for antigen detection

Each workshop participant was invited to test 55 porcine blood samples (either blood with ethylenediaminetetraacetic acid [EDTA], or serum), using his/her own ELISA system or a commercial ELISA. Twenty of the samples originated from pigs which were free from CSFV, and 35 samples were taken from six weaned pigs at various time intervals after they had been inoculated with CSFV. One blood sample from a calf persistently infected with BVD virus was also included.

Principles of evaluated ELISAs

Seven different ELISAs were evaluated. Some had already been extensively tested for the detection of CSFV, whilst others were prototypes which had not been fully validated. All used a double antibody sandwich principle, with an antigen-capture antibody coated to ELISA plate microtitre wells and a detector antibody revealing bound antigen. The type and specificity of the antibodies used are compared in Table I, together with other test characteristics. The negative samples provided were used for the calculation of the cut-off values for each ELISA. Values higher than the means obtained with negative samples plus three times the standard deviation were scored positive. Values equivalent to the mean of negatives plus between two and three times the standard deviation were interpreted as inconclusive. The results of the 'Serelisa

HCV-Ag' test were calculated according to the instructions of the manufacturer. The sensitivity of each ELISA was calculated by dividing the number of positive and doubtful positive ELISA results by the number of positive virus isolations.

Serelisa HCV-Ag (Rhône-Mérieux)

This commercial ELISA used a mixture of three monoclonal antibodies (MAbs) which recognise epitopes on the p120/80 protein (1). These antibodies were coated to the solid phase and captured antigen from anti-coagulated blood or serum. An anti-p120/80 rabbit antiserum was used to detect bound antigen in combination with a goat anti-rabbit horseradish peroxidase (HRPO)-conjugate. The antiserum had been prepared by immunisation of a rabbit with a recombinant p120/80 protein. This test was originally established for use with EDTA blood but, in the present exercise, sera or unseparated EDTA blood were dispensed into the ELISA plate microtitre wells together with a lysis buffer. This test is panpestivirus-specific and can be performed in five hours. The ELISA was introduced by J.C. Thibault (Rhône-Mérieux) and performed by F. Koenen (*Institut national de recherches vétérinaires*, Belgium), C. Crucière and M. Gonzague (*Centre national d'études vétérinaires et alimentaires*, France).

CVL-1 and CVL-2 (Central Veterinary Laboratory, United Kingdom)

The CVL-1 ELISA is based on capturing antigen from leukocytes using an anti-p120/80 MAb. The bound antigen was detected with a second anti-p120/80 MAB which is biotin-conjugated. Both MAbs were raised against BVDV, but the test is panpestivirus-specific and can be performed in three hours. The principle of the CVL-2 ELISA is the same as for CVL-1, except that the capture MAB and the biotinylated MAB are CSFV-specific, reacting with separate epitopes on gp55. The test is CSFV-specific, but does not recognise all strains of CSFV. Leukocytes were prepared by expressing 5 ml of EDTA blood through a syringe-end filter. The trapped leukocytes were lysed and eluted simultaneously by passage through the filter of 0.5 ml of 2% octyl- β -D glucopyranoside in phosphate-buffered saline (5). Eluate was added at 0.05 ml per well of the ELISA plate as the test sample. Both ELISAs were introduced and performed by D.J. Paton.

Perugia ELISA (Istituto Zooprofilattico, Perugia, Italy)

In this ELISA, antigen from lysed buffy coats was captured using a strongly reactive swine polyclonal pestivirus antiserum. Bound antigen was detected with an HRPO conjugate produced from the same antiserum. Antigen was prepared from leukocytes pipetted from the buffy coat fraction of centrifuged EDTA blood samples by treatment with 1% Nonidet P-40. This test is panpestivirus-specific and can be completed within five hours following separation of the buffy coat. The ELISA was introduced and performed by G.M. De Mia.

CSFV E0-SADA (Tübingen, Germany)

The CSFV E0-SADA (SADA: serum antigen detection assay) is based on capturing antigen from serum using a strongly reactive rabbit-monospecific polyclonal antiserum against the E0 (gp44/48) protein of CSFV. The bound antigen was detected with a biotin-conjugated version of the same antiserum. The test is panpestivirus-specific and can be performed in four hours. This ELISA was introduced and performed by R. Stark.

HAN-1 and HAN-2 (Community Reference Laboratory, Germany)

These two ELISA kits are based on capturing antigen from anti-coagulated blood. The HAN-1 test uses a panpestivirus-specific MAB reactive with p120/80, while the

TABLE I

Characteristics of various antigen enzyme-linked immunosorbent assays (ELISAs)

ELISA	Capture antibody	Specificity of capture	Revealing antibody	Specificity of revealing	Pestivirus specificity	Sample tested	Time taken
Serelisa	MAb (× 3)	p120/80	PAb (rabbit)	p120/80	Pan	Blood/serum	5 h
CVL-1	MAb	p120/80	MAb	p120/80	Pan	Leukocytes	3 h
CVL-2	MAb	gp55	MAb	gp55	CSFV	Leukocytes	3 h
Perugia	PAb (pig)	CSFV	PAb (pig)	CSFV	Pan	Leukocytes	5 h
E0-SADA	PAb (rabbit)	gp44/48 (E0)	PAb (rabbit)	gp44/48 (E0)	Pan	Serum	4 h
HAN-1	MAb	p120/80	PAb (pig)	CSFV	Pan	Blood	4 h
HAN-2	MAb (× 2)	p120/80+ gp55	PAb (pig)	CSFV	Pan	Blood	4 h

CVL: Central Veterinary Laboratory (Weybridge, United Kingdom)

SADA: serum antigen detection assay

HAN: Hanover Veterinary School, Germany

MAb: monoclonal antibody

PAb: polyclonal antibody

CSFV: classical swine fever virus

Pan: panpestivirus-specific (i.e. recognises CSFV, bovine virus diarrhoea virus and Border disease virus)

HAN-2 test employs a mixture of two MAbs specific for p120/80 and gp55, respectively. The bound antigen was detected with a panpestivirus-specific polyclonal HRPO-conjugated antiserum. This antiserum had been prepared by immunisation of a pig with CSFV. Antigens were prepared by treatment of EDTA/dextran blood samples with 1% Triton X-100® detergent. Both tests are panpestivirus-specific and can be performed in four hours. The ELISAs were introduced by A. Müller and K. Depner, and were performed by U. Behr (Institute of Virology, Hanover Veterinary School, Germany).

Experimental infection of pigs with CSFV

Six weaned pigs weighing approximately 15 kg and free from neutralising antibodies against pestiviruses (BVDV strain NADL [National Animal Disease Laboratory] and CSFV strain Alfort/187) were used for the provision of viraemic pigs. Each animal was inoculated intranasally with 5 ml of pig kidney (PK)15 cell-culture suspension containing 1,000 TCID₅₀ (50% tissue culture infective dose) of the recent German CSFV isolate Diepholz 1/Han94. Pairs of pigs were inoculated twelve days (nos 1 and 2), eight days (nos 3 and 4) and five days (nos 5 and 6) before the workshop commenced. The stage of infection of the pigs during the three days of the workshop was between 5 and 14 days post-inoculation (d.p.i.).

Clinical signs and body temperatures were recorded daily, and blood samples (EDTA blood and serum) were collected three times weekly prior to the workshop and daily during the workshop. Blood samples taken more than two days in advance were stored at -20°C prior to use.

Virus isolation and serology

Re-isolation of CSFV from buffy coats and serum was attempted using PK15 cell cultures, as described elsewhere (3).

Serum samples were tested for the presence of neutralising antibodies against the homologous CSFV isolate Diepholz 1/Han94, the CSFV strain Alfort/187 and the BVDV strain NADL, using a direct neutralising peroxidase-linked assay (NPLA) in PK15 and fetal calf kidney cells, respectively (9).

RESULTS

Clinical course of the disease

All animals became febrile between 6 d.p.i. and 8 d.p.i. (Fig. 1). No clinical signs typical of CSF (e.g. skin haemorrhages, nervous signs) were visible during the workshop period. Pig 1 died due to a rupture of the aorta cranialis at 11 d.p.i., before the workshop started. The remaining five pigs were euthanised one day after the workshop.

Virus isolation and serology

Results for virus isolation from buffy coats and serum are given in Figure 1 and Table II, respectively, and show that virus was detected sooner in buffy coats in five of six animals. Virus was not recovered from the serum of pig 5. CSFV was first re-isolated from leukocytes at between 5 d.p.i. and 7 d.p.i. It is noteworthy that CSFV could be re-isolated from the blood of pigs 3, 4 and 6 during the incubation period, one to three days before the onset of fever.

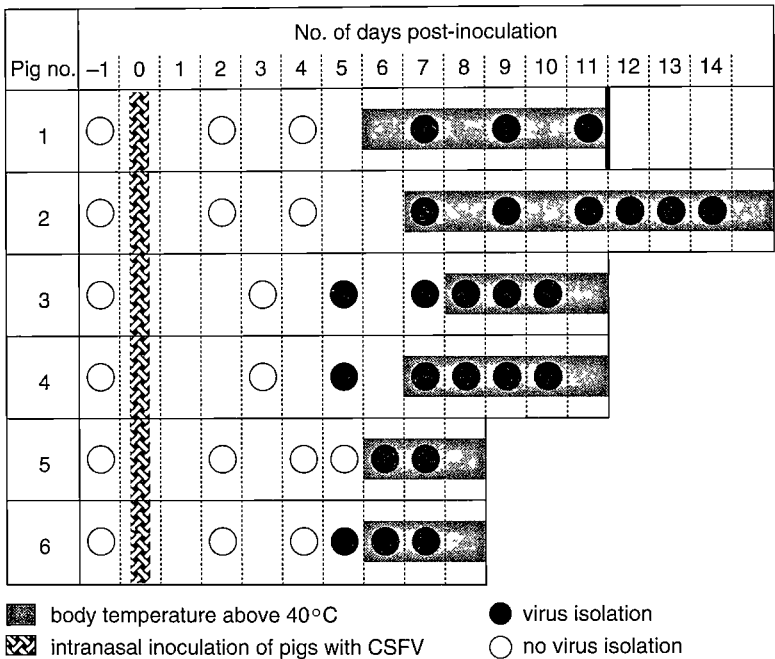


FIG. 1

Virus isolation from buffy coat and body temperatures of six pigs intranasally inoculated with classical swine fever virus (CSFV) isolate Diepholz 1/Han94

None of the pigs developed neutralising antibodies against BVDV or CSFV during the test period.

ELISA results

A single 3 d.p.i. sample from pig 4 was positive with the HAN-1 test. Otherwise, all samples found to be negative by virus isolation also tested negative by ELISA. Pigs 1-4 were detected as positive by all ELISAs (Table III). Samples taken from pig 1 at 11 d.p.i. and from pig 2 at 11, 12, 13 and 14 d.p.i. were positive in all tests. Samples taken from pigs 3 and 4 at 10 d.p.i. were not tested with all ELISAs, but were positive in those used. For pigs 5 and 6, blood was available during the workshop period up to 7 d.p.i. Pig 5 tested negative by all ELISAs throughout the observation period, except for a doubtful positive result with CVL-2 at 7 d.p.i. Pig 6 tested positive at 6 d.p.i. only by E0-SADA. At 7 d.p.i., this animal tested positive by CVL-2 and doubtfully positive by HAN-1 and HAN-2. No single ELISA was able to detect early viraemia as consistently as virus isolation from leukocytes.

The sensitivity of the ELISAs is compared in Table IV. Compared to virus isolation, false-negative results were obtained with blood samples taken during the first 8 d.p.i. By 8 d.p.i., however, the sensitivity of the ELISAs increased to up to 100%.

• The blood sample from the calf which was persistently-infected with BVDV scored positive in all ELISAs, except in the CSFV-specific CVL-2 ELISA.

TABLE II

Re-isolation of classical swine fever virus from serum of pigs at various times post-inoculation (shaded area indicates workshop period)*

d.p.i.	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
4	–	–	NT	NT	NT	NT
5	NT	NT	–	–	–	–
6	NT	NT	NT	NT	–	0.7
7	0.7	–	2.0	2.0	–	1.5
8	NT	NT	2.7	3.0	NT	NT
9	2.7	2.5	2.7	3.3	1.7	2.7
10	NT	NT	3.3	3.0	NT	NT
11	3.0	2.5	NT	NT	NT	NT
12	NT	3.3	NT	NT	NT	NT
13	NT	2.0	2.7	NT	NT	NT
14	NT	2.0	NT	2.3	3.3	3.3

* \log_{10} titre of virus as 50% tissue culture infective dose per 0.1 ml of serum

d.p.i.: no. of days post-inoculation

NT: not tested

– no virus isolated

This study concentrated on evaluation of viraemia in the early post-inoculation period, as this is the time when clinical signs may be inapparent or non-specific and when serological tests are generally inappropriate. The Diepholz 1/Han94 isolate was chosen for inoculation of the pigs, as it is a recent field isolate. It has been shown to cause 90% mortality in inoculated weaners, although deaths did not occur until 17-25 d.p.i. (4). In the present study, the pigs were killed at 15 d.p.i. and, although they became pyretic from 6 d.p.i., they did not develop marked signs of illness or become moribund. As previously reported, virus isolation from leukocytes was more sensitive than isolation from serum and, using the former technique, three of six pigs became viraemic before the onset of fever. Viraemia persisted in all six pigs for as long as samples were taken and up to 14 d.p.i. In view of the range of virulence among CSFV isolates, other challenge strains would be expected to produce viraemia and clinical signs of variable duration and intensity.

As most of the ELISAs utilised had not been fully validated, positive/negative cut-off levels were established by reference to the results obtained with the pre-challenge samples. More extensive validation, as had been conducted in the case of the Serelisa, might have required different cut-off points to be used. In fact, if the same method was used for calculating the cut-off for the Serelisa as for the other tests, only a marginal qualitative difference in the Serelisa readings resulted, with one sample becoming positive instead of doubtful positive and one sample becoming doubtful positive instead of negative. Compared to virus isolation from leukocytes, the evaluated antigen-capture ELISAs were specific, but less sensitive for the detection of very early viraemia, often requiring an extra day or two to have elapsed before a positive result was obtained from individual pigs. By 9 d.p.i., however, all samples were consistently positive with all assays. It should be borne in mind that the relatively low sensitivities of some antigen-capture ELISAs shown in Table IV reflect the many weakly positive samples

TABLE III

Detection of viral antigens in selected samples collected from pigs at various intervals post-inoculation

Assay	Fig 1		Fig 2		Fig 3		Fig 4			
	4 d.p.i.	7 d.p.i.	4 d.p.i.	7 d.p.i.	5 d.p.i.	7 d.p.i.	8 d.p.i.	3 d.p.i.	5 d.p.i.	7 d.p.i.
Virus isolation ^(a)	-	+	-	+	+	+	+	-	+	+
Virus isolation ^(b)	-	+	-	-	-	+	+	-	-	+
Serelisa ^(c)	-	-	-	+/-	-	+	+	-	-	+
Serelisa ^(d)	-	-	-	+/-	-	-	+/-	-	-	-
CVL-1	NT	-	-	-	-	-	NT	-	-	+
CVL-2	-	+	-	+	-	+	+	-	-	+
Perugia	-	-	-	-	-	+/-	+	-	-	+
E0-SADA	NT	+	-	+	-	+	+	-	+	+
HAN-1	NT	-	-	+	-	+	+	+	+	+
HAN-2	NT	-	NT	+	-	-	+	-	-	+

a) from buffy coats

b) from serum

c) using blood with ethylenediaminetetraacetic acid

d) using serum

d.p.i.: no. of days post-inoculation

- negative

+/- doubtful positive

+ positive

NT: not tested

TABLE IV

Sensitivity of different antigen enzyme-linked immunosorbent assays (ELISAs) in testing for classical swine fever at various times post-inoculation

Assay	Proportion of samples positive at different days post-inoculation						Overall sensitivity *
	1-4 d.p.i.	5 d.p.i.	6 d.p.i.	7 d.p.i.	8 d.p.i.	9-14 d.p.i.	
Virus isolation ^(a)	0/6	3/4	2/2	6/6	2/2	11/11	100% (24/24)
Virus isolation ^(b)	0/10	0/4	1/2	4/6	2/2	17/17	75% (18/24)
Serelisa ^(c)	0/10	0/4	0/2	3/6	2/2	10/10	65% (15/23)
Serelisa ^(d)	0/10	0/4	0/2	0/6	1/2	10/11	45% (11/24)
CVL-1	0/9	0/4	0/2	1/6	1/1	10/10	60% (12/20)
CVL-2	0/10	0/4	0/2	6/6	2/2	10/10	78% (18/23)
Perugia	0/8	1/4	0/2	2/4	2/2	8/8	68% (13/19)
E0-SADA	0/7	1/4	1/2	3/4	2/2	8/8	78% (15/19)
HAN-1	1/9	1/4	0/2	4/6	2/2	11/11	75% (18/24)
HAN-2	0/6	1/4	0/2	3/6	2/2	10/10	66% (16/24)

d.p.i.: no. of days post-inoculation

* no. of samples positive or doubtful positive as a proportion of no. positive by virus isolation from buffy coat samples, excluding results where virus isolation from buffy coats was not performed

a) from buffy coats

b) from serum

c) using blood with ethylenediaminetetraacetic acid

d) using serum

examined, and greater sensitivity could probably be achieved with field samples from naturally-occurring cases. A previous comparison between virus isolation and an antigen-capture ELISA, using a hyper-immune BVDV antiserum as capture antibody and anti-p120/80 MAbs as immunological detectors, also concluded that ELISA was highly specific but slightly less sensitive (14). A challenge inoculation with a low virulent CSFV strain (Baker A) was found to produce a viraemia which was not detected at all by ELISA.

Most previous publications on antigen-capture ELISAs for pestiviruses have reported on the detection of virus in tissues, leukocytes separated from peripheral blood (5, 6, 7, 8, 11, 12, 13, 14) or unseparated, anti-coagulated blood (1, 2, 15). Leukocyte separation has been achieved by centrifugation, flash lysis or filtration. In the case of ELISAs for the detection of cattle persistently infected with BVDV, most tests have utilised anti-p120/80 MAbs and do not reliably detect these proteins in serum (D.J. Paton, unpublished findings). J.C. Thibault (personal communication), however, reports that a p120/80-specific test can identify pestivirus antigen in sera collected from persistently-infected cattle, albeit less reliably than if leukocytes are included. Preliminary indications are that similar results can be achieved for the detection of CSFV in pig sera (2, 10). A test for BVDV viraemia has been reported using both anti-gp48 and anti-p120/80 MAbs (12) and, in this case, the authors stated that unseparated anti-coagulated blood did contain detectable levels of gp48.

In the present study, the different antigen-capture ELISAs utilised a variety of methods for blood sample preparation. Serum can be prepared readily from clotted blood, and it would be highly advantageous to be able to perform both serology and

antigen detection on a single preparation. Only two tests used serum, the E0-SADA test (which was specifically designed for this purpose) and the Serelisa (which was not). The E0-SADA is specific for the E0 or gp44/48 protein, which is present at a high level in serum as it is secreted from infected cells (R. Stark, unpublished findings). The Serelisa is p120/80-specific and is less sensitive at detecting this protein in serum than in leukocytes. The explanation for the apparent presence of the p120/80 non-structural protein in serum is not known. Serum samples from the six inoculated pigs were also tested by the gp55-specific CVL-2 ELISA (data not shown), but pigs testing positive by virus isolation could not be distinguished from the others. The remaining ELISAs utilised leukocytes and/or other blood components derived from EDTA blood. In the case of the HAN-1, HAN-2 and Serelisa tests, detergent-lysed, unseparated blood was examined after minimal sample preparation. A previous report indicated that use of separated leukocytes increased the sensitivity of the Serelisa (15). The Perugia, CVL-1 and CVL-2 tests used separated leukocytes prepared by either filtration or centrifugation. Filtration is very rapid but increases the cost of materials used in the test. Furthermore, all three tests require anti-coagulated blood which has not been previously frozen.

Only the gp55-specific CVL-2 test was specific for CSFV, and this test is of limited value in the present form, as it does not recognise all strains of CSFV. It was included in the evaluation in order to assess the potential of a gp55-specific assay. It could possibly be modified to detect all strains by replacement of the capture MAb with a polyclonal antiserum while retaining the detector MAb, which recognises all CSFVs. All of the other ELISAs detected BVDV in the blood of a persistently viraemic calf and might also detect BVDV or BD virus (BDV) in pigs. This lack of specificity is not considered crucial, however, as viraemia with BVDV or BDV is rare and, in most applications, a positive diagnosis by ELISA would need to be confirmed by subsequent virus isolation, when differentiation could be made.

All of the ELISAs are very rapid compared to virus isolation, which normally requires a minimum of three days. The ELISAs are also simple to perform, require little sophisticated equipment and are amenable to automated reading. For this reason, these tests are very promising tools for screening large numbers of blood samples from live pigs, where a rapid decision is required on whether or not the animals are infected. This situation has frequently arisen during recent epidemics of CSFV in Europe. For example, due to the limitations of virus isolation, outbreaks of disease in regions with dense pig populations have resulted in the preventive slaughter of pigs on neighbouring premises without even establishing their virological status. A rapid assessment by ELISA could provide additional epidemiological information and/or obviate the need for slaughter. The tests could also be used to reduce the risk of trading viraemic pigs. Deficiencies in the sensitivity of ELISA could be largely offset by testing a sufficient proportion of animals in any group. An alternative approach would be to test all pigs showing pyrexia, as most pigs in the present study became ELISA positive at or soon after the onset of fever. Further validation of antigen-capture ELISAs is still necessary, particularly with respect to detection of infection in different age groups of pigs, detection of infection with different strains of CSFV, detection of chronic and persistent infections, and full field evaluation.

CONCLUSIONS

Several ELISAs have been developed to detect CSFV antigens in blood, using double antibody sandwich methods and different types of antibody, directed at various virus-specific proteins. Some of the ELISAs can use serum, while others require anti-coagulated whole blood or leukocyte preparations. All ELISAs proved able to detect CSFV infections at approximately the time when fever develops, but none were quite as sensitive as virus isolation for detection of very early viraemia. All ELISAs presented (except CVL-2) are not CSFV-specific, also being able to detect persistent BVDV infection in cattle. Further validation of the tests is required, particularly in relation to:

- detection of infection in different age groups of pigs
- detection of infection with different strains of CSFV
- detection of chronic and persistent infections
- field evaluation.

These tests are promising tools for the early and rapid screening for infection of large numbers of live pigs. As herd tests, in combination with serology, ELISAs could offer an alternative to preventive slaughtering and could reduce the risk of trading viraemic pigs.

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ÉVALUATION DE LA TECHNIQUE IMMUNO-ENZYMATIQUE APPLIQUÉE AU DÉPISTAGE ET À LA DÉTECTION RAPIDES DES ANTIGÈNES DU VIRUS DE LA PESTE PORCINE CLASSIQUE DANS LE SANG DE PORCINS. – K. Depner, D.J. Paton, C. Crucière, G.M. De Mia, A. Müller, F. Koenen, R. Stark et B. Liess.

Résumé : Lors d'un atelier expérimental, sept épreuves immuno-enzymatiques (enzyme-linked immunosorbent assay : ELISA) ont été comparées à la méthode de l'isolement du virus pour la détection de la virémie dans une série de prélèvements sanguins effectués sur six porcs dans les quatorze jours suivant l'inoculation du virus de la peste porcine classique. Toutes les épreuves ELISA étaient de type « sandwich » à double anticorps et utilisaient des anticorps monoclonaux pour déceler un ensemble de protéines virales dans les leucocytes ou dans du sang ou du sérum non coagulé. Les épreuves ELISA présentaient une bonne spécificité par rapport à l'isolement du virus : un seul prélèvement, dans lequel le virus n'avait pas été isolé, a réagi positivement à un test ELISA. De faux résultats ont été observés jusqu'à huit jours après l'inoculation, mais toutes les épreuves ont donné des résultats positifs de neuf à quatorze jours après l'inoculation. Les tests ELISA nécessitent des installations moins spécialisées et sont plus rapides que la méthode d'isolement du virus. Ce sont, par conséquent, des outils extrêmement prometteurs pour le dépistage de la maladie dans de grands effectifs de porcs vivants.

MOTS-CLÉS : Antigène – ELISA – Peste porcine classique – Virémie.

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EVALUACIÓN DE LA TÉCNICA DE INMUNOENSAYO ENZIMÁTICO PARA UNA RÁPIDA EXPLORACIÓN Y DETECCIÓN EN SANGRE DE CERDO DE LOS ANTÍGENOS DEL VIRUS DE LA PESTE PORCINA CLÁSICA. – K. Depner, D.J. Paton, C. Crucière, G.M. De Mía, A. Müller, F. Koenen, R. Stark y B. Liess.

Resumen: Los autores describen un taller experimental en el que siete inmunoensayos enzimáticos (enzyme-linked immunosorbent assay: ELISA) fueron confrontados, en cuanto a su capacidad de detección de viremias, con la técnica de aislamiento del virus. Para ello se utilizó una serie de muestras sanguíneas tomadas de seis cerdos hasta catorce días después de inocularlos con el virus de la peste porcina clásica. Todas las pruebas ELISA aplicadas fueron del tipo «sandwich» mediante doble anticuerpo, con el empleo de anticuerpos monoclonales y/o policlonales para la detección de diversas proteínas víricas ya fuera en leucocitos, o en suero o sangre tratados con anticoagulantes. En comparación con el método del aislamiento en cultivo, la especificidad de las técnicas ELISA resultó buena: tan sólo en un caso una prueba ELISA dio un resultado positivo para una muestra identificada como negativa por la técnica de aislamiento del virus. También se produjeron algunos resultados falsos negativos con muestras recogidas hasta ocho días después de la inoculación, pero todas las pruebas arrojaron un resultado positivo para las muestras tomadas entre los nueve y los catorce días siguientes a la inoculación. Las técnicas ELISA requieren instalaciones menos especializadas y pueden practicarse con mucha más rapidez que el aislamiento del virus. Por ello constituyen una herramienta muy prometedora para la realización de exámenes serológicos masivos de cerdos.

PALABRAS CLAVE: Antígeno – ELISA – Peste porcina clásica – Viremia.

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