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A blocking ELISA to differentiate hog cholera virus antibodies in pig sera from those due to other pestiviruses

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Summary — The blocking ELISA technique was extended to comparative serology by using 3 different pestivirus strains: Hog cholera virus (HCV) Alfort strain propagated in PK15 cell line, Border disease virus (BDV) Aveyron strain in PK15 and BVD NADL** strain in fetal calf kidney (FCK) primary cells. Rabbit antisera to the Alfort HCV strain and Aveyron BDV strain were raised for use in the test. A bovine hyperimmune serum to BVD virus was also used for detecting antibodies specific to BVD virus. The ELISA was compared with the neutralisation test on various groups of field and experimental porcine sera. The results obtained with the ELISA were well correlated with the neutralisation test. Therefore the ELISA may be recommended as a differential serological test between HCV and other pestivirus antibodies in pig sera.

hog cholera / pestivirus / ELISA

Résumé — *Utilisation d'un test ELISA par compétition pour la différenciation des anticorps antipeste porcine classique de ceux dirigés contre les autres pestivirus. La technique ELISA par compétition, déjà utilisée pour rechercher les anticorps sériques dirigés contre le virus de la peste porcine classique, a été étendue à la sérologie différentielle des anticorps antipestivirus chez le porc. La souche Alfort de virus peste porcine classique et la souche Aveyron de virus border disease cultivées sur cellules PK15 et la souche NADL du virus de la maladie des muqueuses cultivée sur cellules primaires de rein de fœtus bovin, ont été choisies comme antigènes. Des sérums hyperimmuns de lapins préparés contre le virus de la peste porcine classique souche Alfort et contre la souche Aveyron de virus border disease, et un sérum hyperimmun de bovin dirigé contre le virus de la maladie des muqueuses ont été utilisés comme signal. Les résultats obtenus en ELISA concordent avec ceux obtenus dans le test de séroneutralisation utilisant les mêmes souches virales, pour les différents groupes de sérums de porcs étudiés. Le test ELISA peut en conséquence être recommandé pour le diagnostic sérologique différentiel des anticorps antipeste porcine classique d'avec les anticorps dirigés contre les autres pestivirus.*

peste porcine / pestivirus / ELISA

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** NADL : National Animal Diseases Laboratory

INTRODUCTION

The differentiation between Hog cholera virus (HCV) antibodies and antibodies to other pestiviruses in the pig population assumes increasing importance as the Classical swine fever (CSF) eradication campaign progresses in European countries. Furthermore, in CSF-free countries or free areas, it can be of importance in documenting the absence of the disease by serological surveys of the pig population. Under such conditions, cross-reactions with HCV due to antibodies to other pestiviruses, such as Bovine viral diarrhoea virus (BVDV) may create problems (Holm Jensen, 1981). Several differential tests are available for this purpose, of which the most common is serum-virus neutralisation: BVDV antibodies have been demonstrated using the neutralization peroxidase linked assay in 6.4% of samples in a group of 3 000 pig sera submitted to the laboratory in Denmark, while all the sera were found to be free from antibodies to HCV (Holm Jensen, 1985).

However, all the neutralisation tests are rather laborious and therefore inadequate for large-scale serological testing. Owing to the non-cytopathic character of the virus, the HCV neutralisation test is particularly expensive and time-consuming.

Enzyme-linked immunosorbent assays (ELISA) have therefore been developed for HCV serology in Denmark and a blocking ELISA has been used since 1985 in the Danish surveillance programme for HCV antibodies (Have, 1984, 1987). This test was also applied in France where vaccination against Swine fever has been banned since April 1983; but it was not possible with the HCV ELISA to distinguish with certainty antibodies to HCV from antibodies to other pestiviruses in an

individual serum: this differentiation could only be achieved with the seroneutralisation test using BVD or BD viruses (Leforban *et al*, 1987).

Therefore a new ELISA including the 3 pestivirus antigens (HCV, BVDV and BDV) has been developed for this purpose. The present report will describe this new test and emphasize the comparative results obtained in ELISA and seroneutralisation tests.

MATERIALS AND METHODS

Antigen

The antigens were prepared using the technique described for the HCV blocking ELISA (Have, 1984, 1987). Briefly, the virus strains were propagated on cell monolayers: Alfort HCV and Aveyron BDV were cultivated on the PK15 cell line, Thiverval HCV on RPL₂ cell line and BVDV on fetal calf kidney (FCK) primary cells. The viral antigens were extracted from the cell pellets by the action of a 0.2% octyl 1-D glycopyranoside (Fluka AG, Buchs, Switzerland) solution in PBS for 15 min. Cell debris was sedimented by centrifugation at 10 000 *g* for 15 min, and the supernatant was used as antigen in ELISA.

Several strains of pestivirus were tested: Thiverval vaccine HCV strain (Coglapest[®]: Sanofi Santé Animale, France), reference Alfort HCV strain, reference NADL BVDV strain, Oregon C24V BVDV vaccine strain (kindly furnished by Professor Liess, Hannover, FRG), Aveyron field French strain of Border disease virus (Chappuis *et al*, 1984). Mock antigens were also prepared using the 3 types of non-infected cells by the same technique as the viral antigens. Each antigen was initially titrated in comparison with its respective mock antigen: the dilution of the antigen was chosen as the highest dilution providing the maximal inhibition with both a high positive pig serum and a weak positive serum diluted 1/2.

Rabbit and bovine antisera

Rabbit antisera to the HCV Chinese vaccine strain and Aveyron BDV strain were raised for use in the ELISA as positive signal. They were obtained by intravenous inoculation of 8–12 rabbits with each viral strain. The neutralising antibody titres were measured at 2-week intervals for each animal. The inoculation schedule was adapted to the serological response of the rabbits (Have, 1984).

Antisera to the Chinese vaccine strain of HCV (Vadimum®; Smith Kline) were collected after one or two inoculations of this virus (Leforban *et al.*, 1987).

The Border disease virus antiserum was collected 7 days after the third inoculation of $10^{5.5}$ TCID₅₀ of Aveyron strain, propagated on IR04 cell line (kindly furnished by Dr G Chappuis, Rhône-Mérieux), and inoculation carried out with crude material on days 0, 30 and 37.

After exsanguination of each rabbit the final sera were tested individually in Seroneutralisation and ELISA and only the serum of the rabbit with the highest titre was used for the positive signal in the blocking ELISA.

For BVDV, the positive signal was obtained with a hyperimmune gnotobiotic bovine serum

which was raised against 4 field isolates of ruminant pestivirus.

Test samples

The porcine test sera comprised firstly samples collected from pigs experimentally infected with different strains of pestiviruses, and secondly samples collected in the field, either in abattoirs or on farms.

Sera collected from experimentally infected animals

Characteristics of the pigs are described in table I.

Field sera

The field sera were selected from samples collected on breeding farms or at abattoirs during the French national serological surveillance programme for HCV in 1986. These sera were included within 3 groups :

The first group consisted of 35 randomly chosen sow sera collected in abattoirs. These sera

Table I. Characteristics of experimentally infected pigs.

	<i>Pigs infected with viruses</i>		
	<i>Hog cholera</i>	<i>Bovine diarrhoea</i>	<i>Border disease</i>
No of pigs	13	2	2
Weight of pig (kg)	30	70	70
Infecting strain of virus (dose) :			
First inoculation	Chinese CL (1 dose)	NADL (10^4 TCID ₅₀)	Aveyron –
Booster or challenge	Manche (10^4 LD ₅₀)	NADL (10^4 TCID ₅₀)	– –
No of injections	2	3	–
Route of infection	IM	IM	contact
Interval of injections (days)	14	20	–
Interval of serum collection (days)	15	7	7

were tested in a HCV comparative ELISA using 2 different antigens, Alfort and Thiverval strains.

The second group comprised a further 29 sow sera collected in the same abattoirs and tested first by seroneutralisation for the detection of neutralising antibodies to the 3 species of pestivirus (HCV, BVDV, BDV) and then with the ELISA using the 3 antigens prepared with the same strains of virus. These sera were initially classified within 3 subgroups according to their seroneutralisation results and to the known details of pestivirus exposure of the corresponding animals.

Finally, the third group comprised 85 sera, from 74 sows or their piglets, which were collected from 8 breeding units known either to be infected with a non-HCV pestivirus or to have kept sows which were vaccinated against HCV before 1983. These farms were identified as having positive sera to HCV or to other pestiviruses on the occasion of the national serological surveillance programme for Classical swine fever in 1986. Eleven of the 74 animals tested were resampled 2–4 months after the initial sample was taken.

ELISA procedure

The blocking ELISA was based on previously described methods (Have, 1984; Sorensen and Lei, 1986; Leforban *et al*, 1987). In brief, flat bottomed 96-well polystyrene plates (reference CML-M29 L) were optimally coated by passive adsorption with 100 μ l of HCV, BVDV or BDV stock antigen preparations diluted in PBS pH 7.2 (Na_2HPO_4 0.015 M, KH_2PO_4 0.005 M, NaCl 0.12 M) for at least 18 h at room temperature. When not utilized immediately coated plates were kept either in the refrigerator (+ 4 °C) without emptying or at – 20 °C after emptying and sealing with plastic film.

Each antigen was initially titrated in comparison with the mock antigen. The mock antigen, prepared with non-inoculated cells and diluted by the same procedure as positive viral antigen, did not react in blocking ELISA and the optical density (OD) value obtained with this antigen was equal to the background OD value of the microplate (below 0.1). This value was similar when tested with either seropositive or seronegative pig sera and was not affected by the anti-

gen dilution. As a result, the mock antigen was not included in the test itself. The dilution of the viral antigen in the test was chosen as the highest dilution providing the maximal inhibition with both a high positive pig serum and a weak positive serum diluted 1/2.

Pig sera were tested at a single dilution (1/2) in PBS-Tween 20, 0.1% (PBS-T). 100- μ l serum samples were applied in duplicate wells of the antigen coated microplate after rinsing. Plates were resealed, and following incubation for 18 h at 4 °C, the wells were emptied; then without any washing step, 100 μ l of the virus specific signal antisera were applied, optimally diluted in PBS-T + 10% SPF pig serum.

Following an incubation period of 1 h in the incubator at 37 °C, the wells were emptied and washed 3 times with PBS-T for 5 min each, then filled either with 100 μ l of 1/400 dilution of horseradish peroxidase-labeled pig anti-rabbit immunoglobulin (Sebia P217, Dako, Denmark) or with 100 μ l of 1/4000 dilution of horseradish peroxidase-labelled rabbit anti-bovine immunoglobulin (Sebia P 159, Dako, Denmark). After incubation for 30 min at 37 °C the conjugate solution was discarded and the plates were washed 3 times for 5 min in PBS-T. Then wells were filled with 100 μ l of substrate solution containing 4.6 mM OPD (ortho phenylene diamine)–9 mM H_2O_2 in 38 mM citric acid, sodium phosphate buffer pH 5. After 15 min the reaction was stopped with sulphuric acid 0.5 N. Optical densities (OD) were then recorded at 490 nm with a Dynatech MR 700 plate reader.

The amount of antibodies in the unknown serum was expressed as the mean percentage inhibition (I%) of the positive signal OD for 2 duplicate wells. The positive signal was measured by substituting a seronegative SPF pig serum in place of test serum. Mathematically, the calculation was as follows:

$$I\% = 100 - (\text{OD obtained with test serum} / \text{OD obtained with negative SPF serum}) \times 100$$

When OD obtained with the test serum was higher than OD obtained with the negative serum the I% was expressed by a negative value. The OD signal obtained with negative pig serum was between 0.7–1.2 depending on the antigen strain and batch.

For diagnostic application in HCV serology, samples diluted 1/2 were considered positive if

they inhibited 25% or more of the signal. However, for the comparison of the 3 pestivirus antigens, inhibition below 25% could also be considered as specific, especially when an increase in this inhibition was observed during the kinetic studies of antibody response in experimentally infected pigs.

The serum neutralisation test

Neutralising antibodies to HCV and BDV were investigated by the neutralisation-immunofluorescence test using the Alfort and Aveyron strains respectively (Liess and Prager, 1976; Costes *et al*, 1982). Briefly, the sera were inactivated at 56 °C for 30 min. Equal 50- μ l volumes of 1/5 dilution of the serum or if an exact titre

was required, serial 2-fold dilutions of serum and suspension of virus containing approximately 100 TCID₅₀ were mixed in the wells of a microtitre plate and incubated at 37 °C for 1 h. Similar control mixtures with virus replaced by medium were incubated in the same way. Then 150 μ l of a suspension of 150 000 PK15 cells/ml were added to each well and the plates sealed with plastic film and incubated for 2 days. The test was read by carrying out an indirect immunofluorescence staining of the virus infected cells. Titres were expressed as the reciprocal value of the serum dilution at the 50% end point.

Antibodies to the NADL strain of BVD were measured by the standard neutralisation test using the principle of inhibition of the cytopathic effect (Vannier, 1985). The initial dilution of the test serum was also 1/5 and FCK cells replaced the PK15 cell line in the test.

Table II. Comparative titration in cell culture and in blocking ELISA of 5 antigen preparations.

<i>Viral strains</i>	<i>Titre in cell culture (log₁₀)</i>	<i>Titre in blocking ELISA (inverse of dilution)</i>
Hog cholera virus		
Alfort	7.5	2 000
Thivernal	4	50
Bovine viral diarrhoea virus		
NADL	4.5	200
Oregon	4	50
Border disease virus		
Aveyron	5.5	150

Table III. Comparison of Alfort and Thivernal virus strains in blocking ELISA tested with 35 field sow sera. (a) = (+) sera positive, (-) sera negative, using a cut-off value of 25% inhibition.

<i>ELISA tested with Thivernal antigen</i>		
	<i>+</i> (a)	<i>-</i>
No of sera		
ELISA tested with Alfort antigen		
+	7	1
-	1	26

RESULTS

ELISA antigen

The optimal dilution of the ELISA antigens was roughly proportional to the titre of the corresponding viruses in cell culture (table II). The comparison of titres between the 2 HCV antigens (Alfort reference strain and Thiverval vaccine strain) using the same set of field sow sera showed very few differences except for 2 sera which presented discrepancies in ELISA results between Alfort and Thiverval strains. When the cut-off value of ELISA was set at 25% inhibition, the qualitative ELISA results with the 2 HCV antigens were very similar (table III). Seroneutralisation titres to HCV Alfort strains were also in agreement with Alfort antigen ELISA scores (not shown).

Comparison of the blocking ELISA and the seroneutralisation

Sera of pigs experimentally vaccinated with Chinese strain and challenged with a virulent strain of HCV reacted in seroneutralisation positively with HCV (mean titre : 200 ± 100) and negatively to BVD and BD viruses (all titres < 10). They gave concordant results in ELISA when tested against the 3 antigens (mean 1% : HCV : 51.9 ± 8.2 ; BVDV : 8.69 ± 3.37 ; BVD : 3.38 ± 3.66) BVDV and BDV being below the cut-off value of 25% inhibition.

When the same methods (trivalent seroneutralisation test and trivalent ELISA) were used for testing field sera the results again corresponded. The same 3 groups of animals were identifiable by both SNT and ELISA (table IV): one group included sera containing HCV antibodies and corre-

Table IV. Comparison of titres seroneutralisation and ELISA obtained with 29 sow sera collected in abattoirs and classified according their seroneutralisation titres. ^a HCV: Hog cholera virus; BVDV: Bovine viral diarrhoea virus; BDV: Border disease virus.

Mean \pm SD					
<i>Seroneutralisation titres (inverse of dilution)</i>			<i>Blocking ELISA values (1%)</i>		
<i>HCV^a</i>	<i>BVDV</i>	<i>BDV</i>	<i>HCV</i>	<i>BVDV</i>	<i>BDV</i>
Positive sera to HCV (6 pigs)					
38.3 ± 24.0	< 10	< 10	54 ± 13.7	23.6 ± 13.3	27.3 ± 33.3
Positive sera to BVD or/and BVDV (11 pigs)					
< 10	10.6 ± 4.8	27.3 ± 20.6	17.6 ± 5.7	33.7 ± 9.3	48.6 ± 11.6
No neutralising antibodies to pestivirus (12 pigs)					
< 10	< 10	< 10	6.3 ± 7.8	5.4 ± 5.5	7.7 ± 8.27

Table V. Comparison of titres in seroneutralisation and blocking ELISA obtained with 85 pig sera collected from 8 breeding farms and classified according their seroneutralisation titres. ^a HCV: Hog cholera virus; BVDV: Bovine viral diarrhoea virus; BDV: Border disease virus.

Mean \pm SD					
Seroneutralisation titres (inverse of dilution)			Blocking ELISA values (%)		
HCV ^a	BVDV	BDV	HCV	BVDV	BDV
Pestivirus infected pigs (69)					
2 \pm 5.5	34.1 \pm 6	47.8 \pm 43.4	17.9 \pm 11.2	39.8 \pm 7.7	44.6 \pm 9.5
HCV vaccinated sows (16)					
118 \pm 56	< 10	< 10	67 \pm 13.3	20.5 \pm 8.7	15.9 \pm 10.7

sponded to HCV vaccinated old sows, another group of sera contained antibodies to non-porcine pestivirus, whereas the last group represented pestivirus antibody-free sera.

The 85 sera collected from 8 breeding units previously shown to have pestivirus serological reactors also gave concordant results in seroneutralisation and ELISA when tested with the two trivalent serological tests (table V). When tested in seroneutralisation, most of the sera had a higher titre to BDV than to BVDV. Within the animals of 3 particular units, both tests were able to distinguish the old sows vaccinated against HCV, which gave high seroneutralisation and ELISA values to HCV, from heterologous pestivirus-infected animals which gave higher SN titres and ELISA values with BVD or BD viruses or antigens than with HCV.

Within a group of 11 animals resampled and retested after an interval of 2–4 months, the seroneutralisation titres and ELISA values were very similar in all pairs of sera. However, a decrease in neutraliz-

ing antibodies was observed with a few sera, while their ELISA scores tended to remain constant. The HCV seroneutralisation titres were found to be negative or low positive (< 20) when sows were infected with heterologous pestivirus, while they were usually high positive (> 40) in HCV vaccinated sows.

Kinetics of ELISA antibodies after experimental inoculation

BVD inoculation

The two pigs infected with BVD-NADL strain developed ELISA antibodies after the first booster on day 20, with a maximum level obtained on day 60 after which the antibody response began to fall. The shapes of the curves obtained with both antigens tested were similar; however, the level of antibodies to the heterologous Oregon antigen was lower than that to the homologous NADL strain (fig 1).

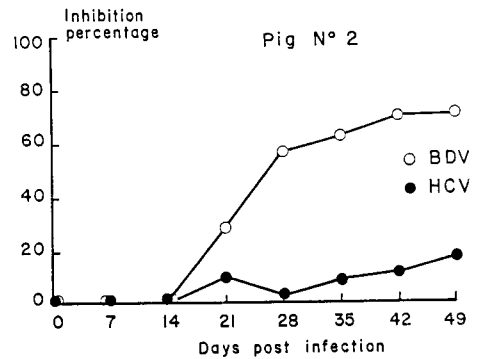
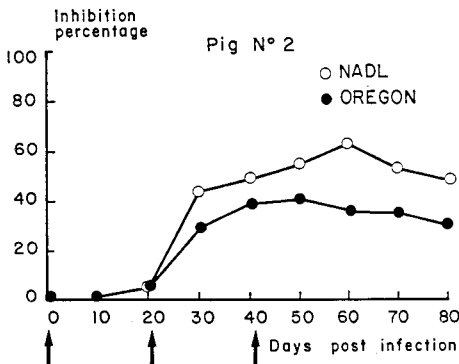
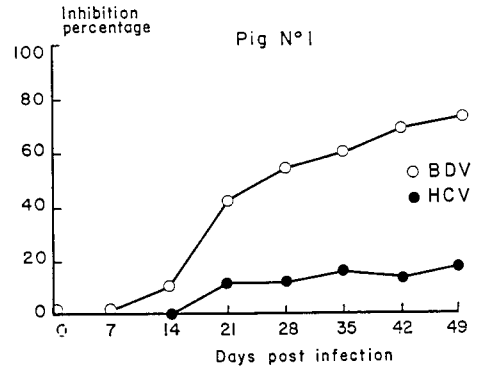
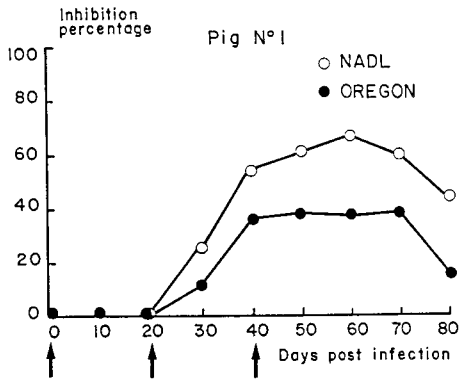


Fig 1. BVD blocking ELISA results obtained with the sera of 2 pigs experimentally infected with NADL-BVD strain using two different antigens for the test (days of inoculation).

Fig 2. HCV and BDV blocking ELISA results obtained with the sera of 2 pigs experimentally infected with Aveyron BDV strain.

BD virus infection

ELISA antibodies to the BDV Aveyron strain antigen appeared 1 week and 2 weeks respectively after the contact infection in the 2 pigs and continued to rise up to the end of the observation on day 49 (fig 2). Neutralizing antibodies to the BD virus appeared at the same time in both animals (results not shown).

ELISA antibodies to HCV Alfort strain antigen were detected after 2 weeks, but

their level remained low (1% score below 20). No neutralising antibody (titres < 10) to HCV Alfort was detected in the same set of sera (results not shown).

DISCUSSION

Preliminary tests of the positive signal sera on mock antigen prepared from non-inoculated cell cultures are essential in order to guarantee the specificity of the ELI-

SA. When a positive signal is detected with mock antigen it could be related either to pestivirus contamination of the cell culture utilised for antigen production or to lack of specificity of the positive signal serum itself. Many cell lines including those of non-bovine origin may be chronically infected by a non-cytopathic strain of BVDV (Wellemans and Van Opdenbosh, 1987). Such cell lines, whose source of contamination is very often BVDV contaminated bovine calf serum, are unsuitable for pestivirus propagation and for the preparation of ELISA antigens.

The levels of background signal in the blocking ELISA is related to the quality of the antiserum used as positive signal: it must be prepared with potent antigen and it appears particularly important not to pool the sera but to collect and test the serum of each animal individually. The species origin of the cell culture support utilised for virus propagation both for the antigen preparation and for immunisation of the animals for signal serum production may also be of importance for the sensitivity and specificity of the ELISA. The same cell support should not be used for both, otherwise the positive signal serum could possibly contain antibodies to the cell proteins which will be shown by reaction with the mock antigen.

Other methods to prevent these non-specific reactions in the test would be the immunisation of the signal serum producer animals with a purified virus, or the use of one or several monoclonal antibodies to a major protein of the virus as positive signal serum (Have, personal communication, 1989).

The dilution of each ELISA viral antigen to be used in the test was determined in an initial chess-board titration including antigens and positive signal serum. The absence of background signal ($OD < 0.1$), whatever the dilution of the antigen, is a

characteristic of the blocking ELISA, presumably related to the use of species-specific conjugates against rabbit or bovine immunoglobulins, lacking cross-reactivity with porcine immunoglobulins.

Antigen prepared from Thiverval HCV vaccine strain gave very similar results to those obtained with Alfort reference strain toward the field sow sera tested: the 2 quantitative differences observed could possibly be explained by vaccination of one sow with the Thiverval strain and the other with Chinese strain, both vaccines being used in France up to April 1983.

Concerning the BVDV ELISA, the quantitative difference in the level of the response of the 2 pigs inoculated with NADL strain toward the 2 antigen preparations could be related to antigenic differences between the NADL and Oregon strains or to the specificity of the bovine serum used as positive signal in the test, although neither of these two BVDV strains was utilised to raise the positive signal bovine serum.

Even though small quantitative differences may be observed in the ELISA scores according to the choice of the viral strains used for the preparation of the antigens, this choice seems to be of relatively minor importance provided one HCV and one ruminant pestivirus strain are used, and their comparative scores for the same pig serum are taken into account.

The blocking ELISA sensitivity and specificity are dependent on the selected cut-off value; a high value will eliminate false positive results, but will also decrease the sensitivity leading to a risk of false negative results. In this respect the cut-off value of the Danish HCV ELISA was set at 25%. In these conditions, out of 4 741 samples tested in Denmark, 34 (0.7%) were found to be false positive (*ie* containing heterologous pestivirus antibodies). It was consequently recommended in this HCV blocking ELISA, that sera giving

an 1% value in the range of 20–50% should be examined for antibodies against heterologous pestiviruses (Have, personal communication).

When dealing with ruminant pestivirus antibodies in pig sera it is of less significance than for HCV serology to establish an official cut-off value between positive and negative sera. Since the main problem encountered is the differentiation between antibodies to HCV and antibodies to ruminant pestiviruses, the level of antibodies to ruminant pestiviruses in pig sera could always be evaluated in comparison with the level of HCV antibodies. However, the 25% cut-off value could be considered as a useful rule of thumb for positivity for all pestivirus blocking ELISAs.

Nevertheless, with respect to antigenic relationships within the pestivirus genus, our results confirm the results of previous studies concerning the serological cross-reactivity of pestivirus (Corthier *et al*, 1974; Laude *et al*, 1979).

With the methods used (seroneutralisation and ELISA against the 3 antigens HCV, BVDV, BDV) it was possible to differentiate between sera, and constitute 3 groups: sera containing HCV antibodies, sera containing antibodies to non-porcine pestivirus and sera free of pestivirus antibodies. However, the test did not appear to differentiate between BDV and BVDV antibodies.

After enquiry on the pig farms with ruminant pestivirus reactors, most of them were suspected to have been contaminated with non-porcine pestivirus by the use in 1984 of a batch of a live Pseudorabies vaccine accidentally contaminated with a Border disease virus strain (Vannier and Carnero, 1985; Vannier *et al*, 1988).

The need to use different host species to raise sera giving satisfactory positive signals for the 3 pestiviruses, and the

consequent requirement for different anti-species conjugates, renders the test somewhat cumbersome and open to operator error. An alternative system has been evaluated (Edwards and Iyata, unpublished data) in which the rabbit and bovine positive signal sera were labelled with biotin (Guesdon *et al*, 1979), thus requiring only a single conjugate, namely streptavidin peroxidase complex (Amersham). It was evaluated on convalescent sera following experimental intranasal inoculations of pigs with field isolates of HCV, BVDV or BDV respectively. The results were very similar to those described in this paper with a clear differentiation between antibodies to HCV on the one hand, and BVDV and BDV on the other. This variant of the test also removed all species limitations on the test sera so that it could be used for example to test bovine sera even though one of the signal sera was also bovine.

The polyclonal-based blocking HCV ELISA offers a rapid, practical and economical screening test which has been used since 1985 in the Danish surveillance programme for classical swine fever and is now available in kit form in France. An alternative monoclonal-based system, the complex-trapping-blocking ELISA (Wensvoort *et al*, 1988) is highly specific for HCV but offers no information on BVDV/BDV antibodies. The new test we have described, incorporating BVDV or BDV antigens, extends the ELISA to differential serology previously possible only by seroneutralisation combined with fluorescent or enzyme labelling. It enables differentiation between HCV antibodies and those due to the other pestiviruses which frequently infect pigs, even in Hog cholera-free areas. It is anticipated that future developments will lead to the substitution of HCV-, BVDV- and/or BDV-specific monoclonal antibodies as positive signal reagents in

the test, enabling a highly standardised test format to be set up and utilised.

CONCLUSION

In conclusion, it has been shown that the blocking ELISA using HCV, BVDV and BDV antigens and appropriate positive signal sera offers a discriminatory capacity for pestivirus serology equivalent to that of fluorescent or peroxidase-linked seroneutralisation tests but with greater speed and economy, enabling accurate surveillance of large numbers of samples.

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