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Comparative serology of porcine reproductive and respiratory syndrome in eight European laboratories, using immunoperoxidase monolayer assay and enzyme-linked immunosorbent assay

T.W. DREW *

Summary: *The members of a European Community Concerted Action participated in a study to compare in-house and commercial tests for the serodiagnosis of porcine reproductive and respiratory syndrome (PRRS). These tests included the immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA). The trials involved assays on experimentally-produced reference sera, a blind trial of sera of known status, and a comparative study of negative and 'problem' field sera. The results showed a high level of agreement among IPMA tests used in the participating laboratories at the herd level, with only minor inconsistencies in testing early post-infection sera. A blocking ELISA, used in experimental work by one laboratory, was almost as sensitive as IPMA in assays of reference and validation trial sera. Commercial ELISAs were generally less sensitive than IPMA when used to assay ten-day post-infection sera. Discrepant results among certain field sera are likely to be due to antigenic variation among PRRS viruses in Europe. This may lead to increasing instances of misdiagnosis using tests which rely solely on test antigens derived from single PRRS virus isolates.*

KEYWORDS: Comparative trial – Diagnosis – ELISA – European Community (Union) – Immunoperoxidase monolayer assay – Porcine reproductive and respiratory syndrome – Serological techniques.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) has been recognised in the United States of America (USA) since 1987 (8). The disease is manifest as both reproductive failure and respiratory disease, and can affect pigs of any age, though more severe respiratory symptoms are seen in younger animals. Although PRRS was known to be of viral aetiology, the causative agent remained elusive for many years. The disease later appeared in Europe, first being observed in Münster (Germany) in November 1990 and thereafter spreading rapidly into other European countries. In July 1991, workers at the ID-DLO (Institute for Animal Science and Health,

* Central Veterinary Laboratory (Weybridge), Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom.

Research Branch) in Lelystad (The Netherlands) announced the discovery of a new virus, isolated using pig alveolar macrophage (PAM) cells, which they called 'Lelystad virus' (10). This virus was shown to fulfil Koch's postulates of causation and is now therefore accepted as the primary agent of PRRS. The first serological test for this virus was also described by the Lelystad group, and is called the immunoperoxidase monolayer assay (IPMA) (10). In 1992, Albina *et al.* (1) described another serological test for the virus, utilising an enzyme-linked immunosorbent assay (ELISA). In the USA, a test detecting neutralising antibody has also been described, using CL2621 cells (13), but the inability of many European isolates to grow in this cell-line renders such a test unsuitable for use in the assay of European sera. It is not possible to demonstrate neutralisation in PAM cells, as the presence of Fc receptors on the surface of these cells actually enhances infectivity by the uptake of immune-complexed virus (4, 14).

Since the discovery of PRRS virus, several laboratories in Europe have set up research and diagnostic facilities to work with the disease. In 1993, the European Community (EC: now European Union [EU]) established an EC Concerted Action (ECCA) – involving representatives from The Netherlands, the United Kingdom, France, Germany, Belgium, Denmark and Spain – with the aim of exchanging research information and standardising the serological tests for PRRS, to ensure uniform diagnosis throughout the EC (Table I). Latterly, a representative laboratory in Italy participated in one of the validation exercises reported here. Most laboratories are

TABLE I

Participating laboratories in the European Union

Code	Country	Laboratory
Be	Belgium	Universiteit Gent, Faculty of Veterinary Medicine, Laboratory of Virology, Salisburylaan 133B, 9280 Merelbeke
Da	Denmark	Statens Veterinaire Institut for Virusforskning, Lindholm, 4771 Kalvehave
Fr	France	Station de Pathologie Porcine, Les Croix, BP 53, 22440 Ploufragan
Ge	Germany	Bundesforschungsanstalt für Viruskrankheiten der Tiere, Paul-Ehrlich Straße 28, D-72076 Tübingen
It	Italy	Instituto Zooprofilattico Sperimentale, Via Bianchi 7, 25124 Brescia
NL	The Netherlands	ID-DLO (Institute for Animal Science and Health, Research Branch), Virology Department, Houtribweg 39, P.O. Box 365, NL-9200 AJ Lelystad
Sp	Spain	Laboratorio de Sanidad Animal (MAPA), Zona Franca, Circunvalación – Tramo 6, Esquina Calle 3, 08004 Barcelona
UK	United Kingdom	Central Veterinary Laboratory (Weybridge), Woodham Lane, New Haw, Addlestone, Surrey GU21 1PS

using tests developed in-house, although two ELISAs are now commercially available (HerdChek® from IDEXX, and Ingenzim® from Ingenaza).

This report summarises the findings of an evaluation of serological tests used by ECCA member countries. A limited evaluation of the HerdChek and Ingenzim ELISA kits was also made.

MATERIALS AND METHODS

Immunoperoxidase monolayer assay

The principle of the IPMA used in all countries was similar. A summary of variations within the techniques used is given in Table II. The methods used were essentially those described by Wensvoort *et al.* (10). Briefly, pig alveolar macrophages were first seeded into microplates, and cultures in alternate rows were then infected with an isolate of the virus. After incubation to enable virus replication, the cultures were fixed. Dilutions of test sera were applied to infected and control rows and, after a period at 37°C, these were washed off. A signalling reagent, usually rabbit anti-pig antibody conjugated to horseradish peroxidase (HRPO), was then applied and incubated for a further period at 37°C. A substrate was then applied, incubated for a period at room temperature, and washed off. Periods of time were those determined by individual laboratories as providing good specific staining with a positive control serum (usually approximately one hour). The cultures were then examined under an optical microscope. Specific staining of infected cells indicated the presence of antibody to PRRS virus, with a titre being the end-point dilution of this staining.

Enzyme-linked immunosorbent assays

The French and Danish in-house ELISAs generally followed the method of Albina *et al.* (1). Cells supporting the replication of PRRS virus were used to produce an antigen, which was then coated onto ELISA microplates. Control antigen was also prepared from uninfected cultures. After washing, a dilution of test serum was then applied to positive and negative wells, and was incubated for a period of time. Control positive and negative sera were also included in the test. After washing, an anti-pig antibody conjugated to HRPO was then applied, incubated for a further period of time and again washed off. Finally, a substrate (*ortho*-phenylenediamine or tetramethylbenzidine) was applied and the plate was read in an ELISA reader at the appropriate optical density (OD), and the ΔOD was calculated by subtracting the OD of the negative well from that of the positive well. A sample to positive control (S/P) ratio was then calculated. Periods of time were those determined by individual laboratories as providing maximal ΔOD with positive control antiserum.

If the S/P ratio (i.e. $\Delta OD_{test\ sample}\ \Delta OD_{positive\ control}$) was ≥ 0.3, the test sample was considered positive for antibody to PRRS virus. A value between 0.2 and 0.3 was considered borderline positive.

The Belgian ELISA was unique among PRRS ELISAs, in that it detected the blocking of a polyclonal antibody-HRPO conjugate by sample antibody, and the results obtained were therefore inverse (i.e. reduction in colour indicated the presence of antibody). The antigen was a Belgian PRRS virus-infected cell extract, bound to the

TABLE II

Test variables for the immunoperoxidase monolayer assay (IPMA) for porcine reproductive and respiratory syndrome

Variables	Belgium	Denmark	France	Germany	Italy	Netherlands	Spain *	United Kingdom
Age of donor pig	5-6 weeks	3-6 weeks	4-7 weeks	4-12 weeks	4 weeks	4-8 weeks	4-6 weeks	2-4 weeks
Cells per well	500,000	450,000	180,000	120,000	100,000	100,000	300,000	500,000
Strain of virus	Lelystad	111/92	SDRPI/SDRPII	2.46	2156/2	Lelystad	5710	H2
Origin of virus	Netherlands	Denmark	Spain/France	Germany	Italy	Netherlands	Spain	United Kingdom
MOI	0.003	0.035	0.028	0.5	0.01	0.003	0.001	0.05
Pre-infection time	18-24 h	1 h	3-6 h	24 h	24 h	none	none	4 h
Post-infection time	24-36 h	22 h	18 h/26 h	18-24 h	30-35 h	24 h	48 h	20 h
Fixative	4% paraform -aldehyde	100% cold ethanol	4% paraform-aldehyde	100% cold ethanol	80% cold ethanol	4% paraform-aldehyde	4% paraform-aldehyde	4% paraform-aldehyde
Blocker	4% horse serum	5% skim milk	2% skim milk	2% casein	5% fetal calf serum	4% horse serum	4% horse serum	4% horse serum
Conjugate	RAP-HRPO	RAP-HRPO	RAP-HRPO	RAP-HRPO	RAP-HRPO	RAP-HRPO	PRO-A HRPO	RAP-HRPO

* IPMA is also performed in Spain using a clone of MA104 cells
MOI: multiplicity of infection

RAP-HRPO: rabbit anti-pig horseradish peroxidase
PRO-A HRPO: protein A horseradish peroxidase

plate via capture antibody. Both capture and signalling antibody were polyclonal, being derived from a pig inoculated with the Belgian isolate of PRRS virus. A range of dilutions of sera were tested, starting at 1:5, and results were expressed as the reciprocal of the highest dilution effecting a 50% or greater decrease in OD when compared to a control well. This value, expressed as percentage inhibition (PI), followed the guidelines adopted by the Standards Commission of the Office International des Epizooties. This test has been previously described by Houben *et al.* (6), who showed it to be more sensitive than IPMA in detecting early active antibody and passive antibody; it also detected higher numbers of positive animals in infected herds. In the study presented here, a result of 5 was classified as borderline positive, although in practical diagnosis this would be regarded as positive.

The HerdChek® ELISA kit (IDEXX) is of the indirect format, and comprised an antigen mixture derived from both European and USA isolates of PRRS virus. A corrected value was obtained as described above but with greater stringency, as the manufacturers recommended a positive/negative cut-off of 0.4 with no stated borderline result. The Ingenzim® ELISA kit (Ingenasa) is also an indirect test, using an antigen derived from viral proteins of a Spanish isolate of PRRS expressed in a baculovirus expression system. Results were expressed as a corrected OD value, derived by subtracting the absorbance of a PRRS virus positive antigen from that of a control cell antigen. Control positive and negative sera were also provided, and acted as validation for tests. The criteria recommended by the manufacturer defined a sample as positive if the corrected value was > 0.300, negative if < 0.200 and borderline if between these two values.

Viruses and sera

Viruses used by individual laboratories in the IPMA tests are detailed in Table II. These were usually isolated from serum obtained from field cases in the country performing the test, using PAM cells in culture. Reference antisera were prepared by participating laboratories in each country, through intranasal inoculation of specific pathogen-free (SPF) pigs using a reference virus from the same country. The pigs were killed approximately 10 days post-infection (d.p.i.) or 2-3 weeks post-infection (w.p.i.). Serum was collected, inactivated and sent to the Central Veterinary Laboratory in Weybridge (United Kingdom), where it was aliquoted and freeze-dried.

For validation sera, laboratories from France, The Netherlands and the United Kingdom provided experimental sera of known origin, from which virus isolation had been attempted. Other sera, consisting of hyperimmune antisera to other viral agents and negative pig sera, were also included in the validation. The origin and status of these sera is shown in Table III. The sera were aliquoted and code-labelled by an independent third party to achieve blind trial status. Participating laboratories were invited to assay the samples by any tests which were available in their laboratory. In addition, each participating laboratory provided approximately fifty sera to a serum bank. These sera were aliquoted, freeze-dried and sent to all laboratories for use in test validation. While a comprehensive assay of these sera was not planned as part of the ECCA activity, certain of these sera were tested by all participating laboratories. These comprised eighteen sera collected from farms classified as PRRS-negative and twenty-five Danish sera which had been identified as 'problem sera' by the Danish laboratory, as they had given discrepant results in IPMA, in-house ELISA and a 1993 version of the HerdChek ELISA.

TABLE III

Origin of validation trial serum samples

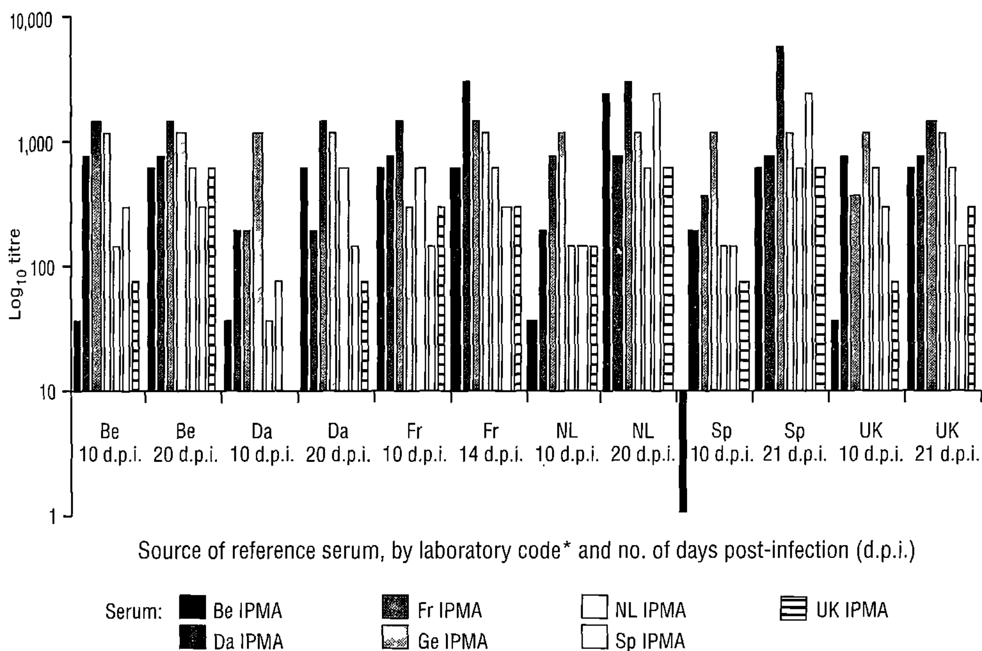
Serum code	Serum label	History	Virus isolation	Country of origin
1	SDRP FB	PRRS – experimental infection	+	France
2	NL4	PRRS – 35 days post-contact	+	Netherlands
3	NL7	PRRS – 35 days post-contact	+	Netherlands
4	SH1AujPI IV	Aujeszky's disease antiserum	ND	France
5	D1153	PRRS – 86 days post-contact *	–	United Kingdom
6	3613	PRRS – experimental infection	+	France
7	NL11	SPF	ND	Netherlands
8	NL10	SPF	ND	Netherlands
9	SHBD 60011	Border disease antiserum	ND	United Kingdom
10	SH SDRP 134 5.3.92	PRRS – 42 days post-infection	+	France
11	D1155	PRRS – 86 days post-contact *	–	United Kingdom
12	D1270	PRRS – 17 days post-contact	+	United Kingdom
13	NL9	SPF	ND	Netherlands
14	NL5	PRRS – 35 days post-contact	+	Netherlands
15	EPOS 6000004	SPF	–	France
16	SHI 80134	PRRS – 21 days post-contact	+	France
17	NL6	PRRS – 35 days post-contact	+	Netherlands
18	D1154	PRRS – 86 days post-contact *	–	United Kingdom
19	SHIS DRP134 13.3.92	PRRS – 35 days post-infection	+	France
20	D1300	PRRS – 17 days post-contact	+	United Kingdom
21	D1196	PRRS – 11 days post-infection	+	United Kingdom
22	NL8	PRRS – 35 days post-contact	+	Netherlands
23	SHI SDRP10	PRRS – experimental infection	+	France
24	D1152	PRRS – 87 days post-contact *	–	United Kingdom
25	NL3	PRRS – 35 days post-contact	+	Netherlands

PRRS: porcine reproductive and respiratory syndrome
ND: not done
SPF: specific pathogen-free
* from pigs demonstrably seronegative by sequential sampling and monitoring by immunoperoxidase monolayer assay and virus isolation
\+ virus isolated
– virus not isolated

RESULTS

Assay of reference sera

All laboratories were asked to assay the reference sera (produced by inoculating SPF pigs and obtaining serum samples at 10 d.p.i. and 2-3 w.p.i.). Variation between the laboratories in titres obtained by IPMA is shown in Figure 1. All but one laboratory detected the 10 d.p.i. sera as positive, the Belgian IPMA failing to detect the Spanish



* see Table I for an explanation of codes

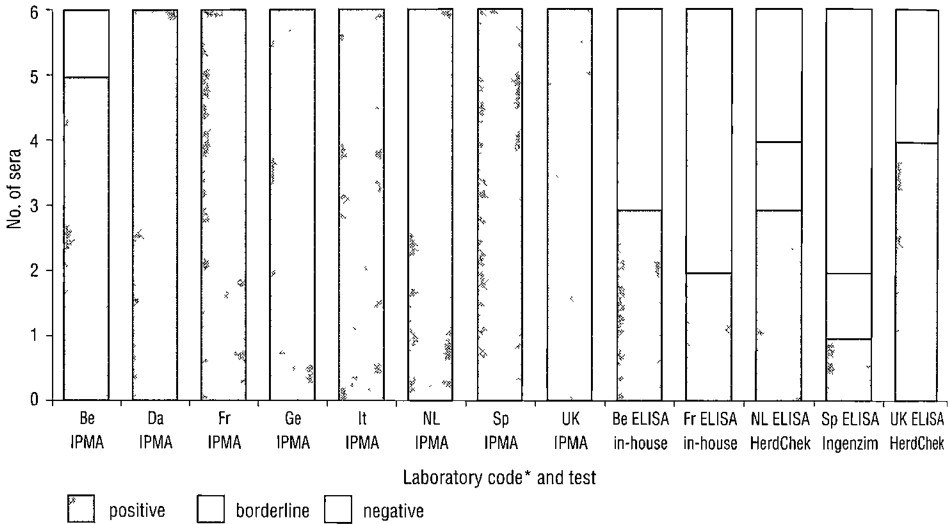
Fig. 1

European Union reference sera for diagnosis of porcine reproductive and respiratory syndrome: comparison of immunoperoxidase monolayer assay (IPMA) titres

10 d.p.i. serum. The United Kingdom IPMA test detected only a very slight specific staining with the Danish 10 d.p.i. serum at a dilution of 1:10. These results are summarised in Figure 2.

There was greater variation among results obtained with the 10 d.p.i. reference sera using the various ELISAs (Fig. 2). Of the six 10 d.p.i. sera, the Belgian blocking ELISA detected Danish, Spanish and United Kingdom sera as positive, and Belgian, French and Netherlands sera as borderline positive at a value of 5. The French ELISA diagnosed Danish and French sera as positive and the other four sera as borderline positive. The Netherlands and United Kingdom laboratories each obtained similar results using the HerdChek ELISA: Belgian, French and United Kingdom sera tested positive; the Netherlands serum tested positive or borderline positive; and Danish and Spanish sera tested negative. The Ingenzim kit detected only French serum as positive and United Kingdom serum as borderline positive, with all other sera testing negative.

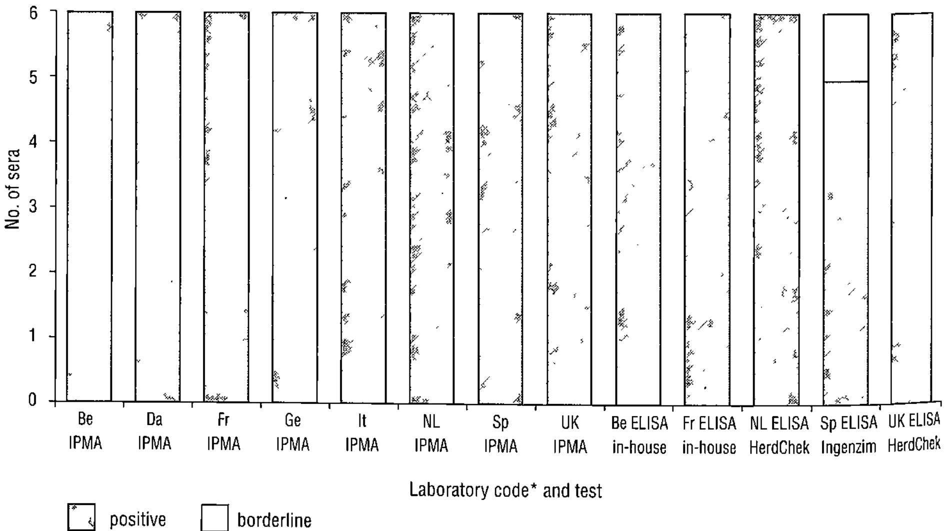
Using the IPMA, all 2-3 w.p.i. sera were found to be positive by all laboratories (Fig. 3). The ELISA results with these sera were much more consistent than those obtained with 10 d.p.i. sera. All ELISAs diagnosed all sera as positive or borderline positive with the exception of the Ingenzim kit, which diagnosed the Belgian serum as borderline positive (Fig. 3).



* see Table I for an explanation of codes

FIG. 2

Immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA) results on 10 d.p.i. (days post-infection) European Union reference sera



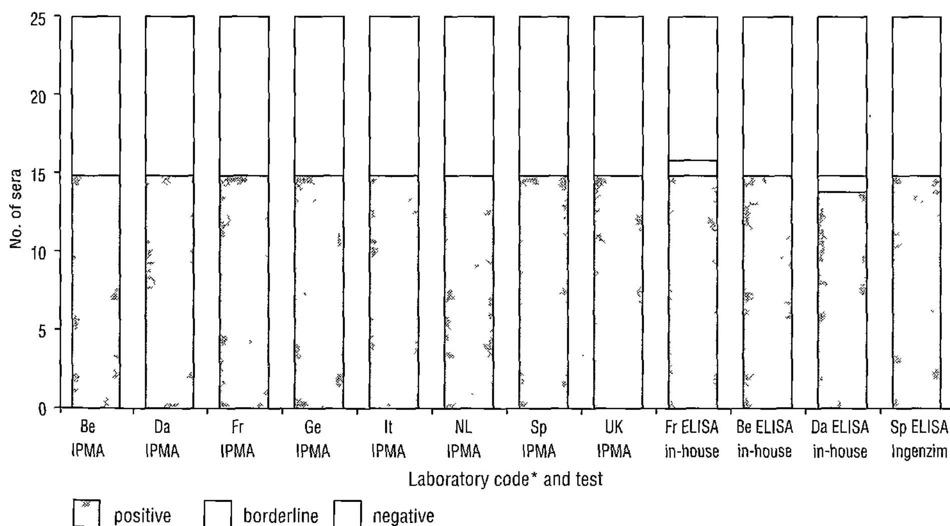
* see Table I for an explanation of codes

FIG. 3

Immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA) results on 2-3 w.p.i. (weeks post-infection) European Union reference sera

Assay of validation sera

All participating laboratories obtained the same qualitative results by IPMA, although a wide range of titres was obtained (data not shown). In the ELISA test, there was similarly a high degree of concordance in results. The Danish in-house ELISA diagnosed one of the positive sera as borderline positive. The only anomalous result was obtained with the French ELISA, which diagnosed serum no. 24 – a PRRS 87-day post-contact serum – as borderline positive. All other IPMA and ELISA tests classified this serum as negative. This animal had been monitored throughout the contact period and had been consistently negative by IPMA, using virus homologous to that with which the animal had been in contact. A summary of the IPMA and ELISA results obtained with these validation sera is displayed in Figure 4.



* see Table I for an explanation of codes

FIG. 4

Immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA) results on validation sera

Assays of the serum bank

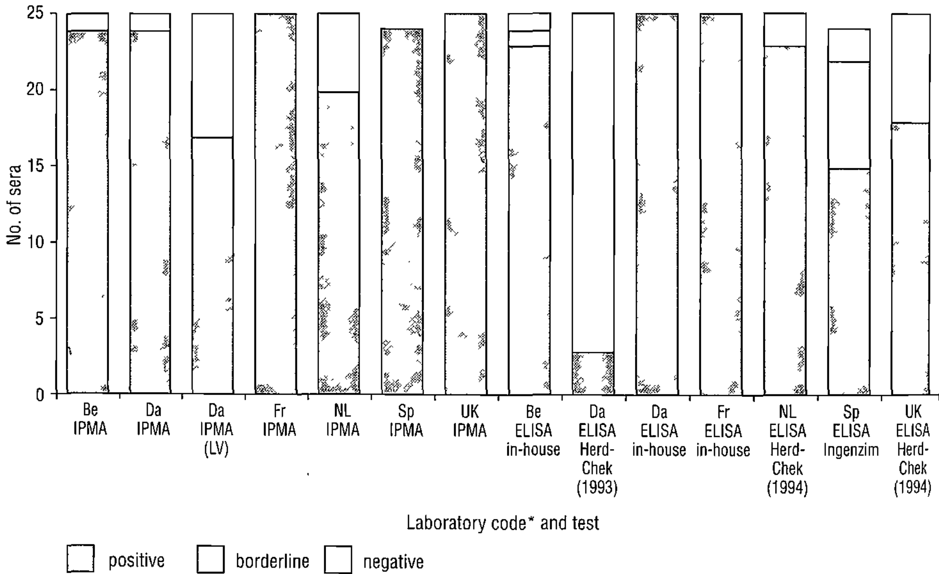
To assess the degree of concordance of the IPMA test with a large number of field sera, all laboratories tested eighteen reportedly negative sera and twenty-five sera which the Danish laboratory had identified as 'problem' sera by virtue of discrepant results in various IPMA and ELISA tests.

All laboratories found the eighteen negative field sera negative by both IPMA and ELISA, with the exception of the French ELISA, which encountered a high degree of non-specific binding – a phenomenon seen previously with some heat-inactivated or frequently freeze/thawed sera. This resulted in six positive and five borderline results.

Concerning the Danish 'problem' sera, the Danish workers had determined that 22 of these 25 sera were positive using an IPMA test with the Danish reference virus, but when they used the Lelystad isolate of PRRS, only 17 were positive. When these sera

were examined by the Danish in-house ELISA, all were positive, but only three were positive when tested with a 1993 version of the HerdChek kit. Accordingly, these sera were tested by all laboratories using IPMA, and by certain laboratories using various ELISA tests.

The IPMA results obtained with these sera were highly variable, ranging from 20/25 positive (NL) to 25/25 positive (United Kingdom). A summary of the group results is given in Figure 5.



* see Table I for an explanation of codes
LV: Lelystad virus

FIG. 5

Immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA) results on Danish 'problem' sera

One serum sample gave a negative result in four of the six tests, and three of the five sera found negative by the Netherlands IPMA were among those which gave negative results in the original IPMA performed in Denmark using the Lelystad isolate. The Belgian test, however, which also uses the Lelystad isolate in the IPMA, found 21/25 sera positive. Three of the four sera reported IPMA negative by the Belgian laboratory were reported as IPMA positive by all other laboratories, with only one of the 25 sera being consistently negative in four of the six laboratories, including the three laboratories using the Lelystad isolate. The ELISA results for the Danish 'problem' sera proved even more variable. The Belgian blocking ELISA diagnosed 23 sera as positive, one as borderline positive, and one as negative. The ELISA results for the 'problem' sera are summarised in Figure 5.

DISCUSSION

The results of testing the eighteen negative field sera show that the specificity of the tests used in the EC is generally very high. It is unfortunate that the French ELISA seemed to have problems assaying these particular sera, which had been freeze/thawed a small number of times and heat-inactivated in the trial preparation process. Published data from this test (1) indicate a high degree of sensitivity, specificity and concordance with the IPMA when testing sera from France. This test has been in commercial use since 1992 and has proved to be a reliable and valuable diagnostic assay for routine diagnosis with samples not only from France but also from other countries.

The IPMA test and the fluorescence-based equivalent – the indirect immunofluorescence assay (IFA) (12) – have been employed in the serological diagnosis of PRRS since the causative virus was first discovered (10). In North America, a clone of MA104 cells is often used for IFA, IPMA and virus isolation (2), but this cell-line is not routinely used by any of the eight European laboratories involved in this study. These two tests have proved to be robust and accurate; but they are labour-intensive and, therefore, expensive and difficult to perform in large numbers. In the limited comparative study presented here, the IPMA retained its position as the 'gold standard' test for antibody to PRRS virus, with only the Belgian blocking ELISA showing equal sensitivity.

Nevertheless, there was evidence of discordant IPMA results with certain field sera. There may be a number of explanations for this. While the IPMAs used in European laboratories are broadly similar, there are some differences which could explain discrepancies. The most obvious difference concerns the isolate of virus used in the test. Most laboratories use an isolate from their own country, and evidence of antigenic variation between PRRS virus isolates from Europe and North America (3, 9, 11), and within a single European country (5), raises the distinct possibility that IPMA tests used within the EC may vary in their ability to detect antibody induced by a different strain of European virus.

This is not the only variable. Some laboratories use ethanol as a fixative. A study in the United Kingdom laboratory (data not shown) revealed that a comparative assay using ethanol-fixed plates gave apparently slightly higher titres than using paraformaldehyde, indicating that antigens are better preserved and/or better revealed with ethanol than with paraformaldehyde. The disadvantage of ethanol is that it causes the PAM cells to shrink, making differentiation between specific and non-specific staining more difficult. Other differences include the age of the donor pig, and the blocker and conjugate used in the test. It was also apparent that different laboratories applied different criteria to the determination of the end-point for positive results. Any of the above factors, alone or in combination, could explain the discrepancies encountered.

The relative lack of discrepancy among reference and validation sera, compared to field sera, is most likely to be due to the relatively long period post-infection, in most cases, and the good physical quality of reference and validation sera; by contrast, field sera are often haemolytic, dirty, and from pigs at different stages of infection which are exposed to a range of dietary factors. Other factors likely to be important in assays include whether the antibody is actively or passively acquired and the age of the pig from which the sample was obtained. Houben *et al.* (6) have observed background staining in IPMA which can mask specific staining in serum of sows and pigs younger

than six weeks. Many in the ECCA group observed weaker staining of PAMs in IPMA using heterologous sera, even at high concentration, suggesting lower antibody affinity.

The use of ELISA for PRRS diagnosis in Europe has an interesting history. Soon after the discovery of the virus, an ELISA test was described by Albina *et al.* (1) (see 'Introduction'). The use of uninfected PAM cells as a control antigen, however, has created many difficulties in ELISA development. Many groups have observed an increase in non-specific binding of antibody to uninfected PAM antigen which is a consequence of PRRS virus infection. This has the effect of reducing the ΔOD even in the presence of a large increase in binding to positive antigen. Tests which use cell-lines do not seem to encounter this problem. The reason for this binding phenomenon is not known, but it may possibly be due to the presence of transient auto-antibody to PAM antigens, induced as a consequence of PAM destruction during acute infection. Another explanation could be the presence of immune complexes in the serum of infected animals. In a related disease, Hu *et al.* (7) report the observation of immune complexes, in the sera of mice infected with lactate dehydrogenase-elevating virus, which bind to ELISA plates not coated with antigen.

The finding that 10 d.p.i. sera were consistently positive by IPMA, while a number of these sera were negative or borderline by ELISA, indicates that the IPMA is the superior test in detecting antibody in such sera. By 2-3 w.p.i., results for the two tests were broadly similar, showing that the ELISA is certainly a valuable test with an important role to play in the detection of serum antibodies to PRRS virus. No animals within negative herds were diagnosed as seropositive, with the exception of those assayed by the French ELISA; as explained above, the latter results were considered to be artefactual and due to the condition of the sera.

The field sera results of the Danish laboratory using the 1993 version of the HerdChek kit, where only three animals were diagnosed as positive, could be due to modifications subsequently made to the kit (M.L. Snyder, personal communication). These improvements could have resulted in the more concordant results obtained with the 1994 version of the kit used by the Netherlands and United Kingdom laboratories. The Ingenzim test kit seemed to produce a large number of borderline results, which could cause dilemmas in diagnosis. The Belgian blocking ELISA performed well in all tests – in particular, it was the only test to diagnose all six 10 d.p.i. reference sera as positive, although three of these were at the lowest PI of 5. This confirms the findings of Houben *et al.* (6), who found this blocking ELISA to be superior to their own IPMA. The slight disadvantage of this test is that the need to dilute sera can mean it takes a little longer to perform than IPMA or conventional ELISAs. Conventional ELISAs are cheaper and quick to perform, however, and the IDEXX ELISA has the added advantage of using antigens derived from both North American and European strains of PRRS virus; it would therefore be expected to perform better than an IPMA using European-type virus in detecting antibody induced by infection with an American-type virus.

CONCLUSION

The studies described here have demonstrated that there is a high degree of uniformity in serodiagnosis of PRRS among ECCA member countries, and that the IPMA using PAM cells is currently the test of choice within the EU for maximum sensitivity. There may be small discrepancies among tests used in the EU concerning the status of individual sera, but all tests are able to rapidly detect seroconversion in herds.

For this reason, no single test should be regarded as suitable for individual animal certification. Existing ELISAs, both commercial and in-house, have a valuable role in providing rapid, economical diagnosis, particularly in laboratories where tissue culture and virus containment facilities are not available.

Increasing knowledge of the variation among and within PRRS virus populations, combined with the use of recombinant PRRS antigens in future generation ELISAs, will undoubtedly result in the development of improved tests. Although this does not form part of the study presented here, European laboratories should be aware of the risk posed by American-type PRRS virus strains to the pig populations within the EU, by virtue of their potential for evading detection by some existing tests.

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LA TECHNIQUE DE L'IMMUNO-PÉROXYDASE MONOCOUCHE ET L'ÉPREUVE IMMUNO-ENZYMATIQUE APPLIQUÉES À LA SÉROLOGIE COMPARATIVE DU SYNDROME DYSGÉNÉSIQUE ET RESPIRATOIRE DU PORC DANS HUIT LABORATOIRES EUROPÉENS. – T.W. Drew.

Résumé : *Les membres d'une « Action concertée de la Communauté européenne » ont participé à une étude visant à comparer des épreuves élaborées au laboratoire ou proposées dans le commerce, appliquées au sérodiagnostic du syndrome dysgénésique et respiratoire du porc ; ces comparaisons ont notamment porté sur l'immuno-péroxydase monocouche (*immunoperoxydase monolayer assay *: IPMA) et sur l'épreuve immuno-enzymatique (*enzyme-linked immunosorbent assay *: ELISA). Les travaux ont consisté en l'application d'épreuves à des sérums de référence obtenus en laboratoire, en un test en double aveugle sur des sérums dont on connaissait le statut et en une étude comparative de sérums prélevés sur le terrain qui avaient donné des résultats négatifs et « à problème ». Les résultats des tests IPMA utilisés au niveau des élevages dans les laboratoires participants à l'expérience se sont révélés très homogènes ; seules quelques divergences ont été constatées lors des examens pratiqués sur des sérums provenant d'animaux récemment infectés. Une épreuve ELISA de compétition, utilisée en laboratoire et appliquée à des sérums de référence et aux sérums préparés pour les essais de validation, a été au moins aussi sensible que le test IPMA. Les épreuves ELISA proposées dans le commerce sont, en général, moins sensibles que les tests IPMA lorsqu'elles sont appliquées à des sérums dix jours après l'infection. Les écarts de résultats entre certains sérums prélevés sur le terrain s'expliquent vraisemblablement par des variations antigéniques entre les virus du syndrome dysgénésique et respiratoire du porc en Europe. Les épreuves qui se fondent uniquement sur des antigènes obtenus à partir d'un seul isolat du virus peuvent ainsi donner lieu à des erreurs de plus en plus nombreuses de diagnostic.*

MOTS-CLÉS : Communauté (Union) européenne – Diagnostic – ELISA – Essai comparatif – Syndrome dysgénésique et respiratoire du porc – Technique de l'immuno-péroxydase monocouche – Techniques sérologiques.

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SEROLOGÍA COMPARADA DEL SÍNDROME DISGENÉSICO Y RESPIRATORIO PORCINO EN OCHO LABORATORIOS EUROPEOS, CON EMPLEO DEL ENSAYO DE INMUNOPEROXIDASA EN MONOCAPA Y DEL INMUNOENSAYO ENZIMÁTICO. – T.W. Drew.

Resumen: *Los miembros de una Acción Concertada de la Comunidad Europea participaron en un estudio destinado a la comparación entre pruebas comerciales y pruebas de elaboración propia para el serodiagnóstico del síndrome disgenésico y respiratorio porcino (*porcine reproductive and respiratory syndrome*: PRRS). Las pruebas en cuestión fueron el ensayo de inmunoperoxidasa en monocapa (*immunoperoxidase monolayer assay*: IPMA) y el inmunoensayo enzimático (*enzyme-linked immunosorbent assay*: ELISA). Los experimentos se efectuaron realizando dichos ensayos sobre sueros de referencia producidos experimentalmente, así como un test ciego sobre un suero de condición conocida y un estudio comparado de sueros negativos y sueros «problema» salvajes. Los resultados pusieron de manifiesto un elevado grado de coincidencia, a nivel de rebaño, entre las diversas pruebas IPMA utilizadas en los laboratorios participantes, con sólo algunas incoherencias menores en el diagnóstico de los sueros justo después de su infección. Una técnica ELISA de bloqueo, aplicada de forma experimental por uno de los laboratorios, se reveló casi tan sensible como los ensayos IPMA para los sueros tanto de referencia como de control positivo. Las técnicas ELISA comerciales resultaron en general menos sensibles que las IPMA para sueros extraídos diez días después de la infección. Es probable que las discrepancias observadas entre ciertos sueros salvajes obedezcan a la variación antigénica existente en Europa entre los virus del PRRS. Ello puede conducir a un número creciente de diagnósticos erróneos cuando se apliquen pruebas basadas exclusivamente en antígenos tomados de una sola cepa del virus del PRRS.*

PALABRAS CLAVE: Comunidad (Unión) Europea – Diagnóstico – ELISA – Ensayo de inmunoperoxidasa en monocapa – Experimento comparativo – Inmunoensayo enzimático – Síndrome disgenésico y respiratorio porcino – Técnicas serológicas.

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