INFECTION WITH A NEW PORCINE RESPIRATORY CORONAVIRUS IN DENMARK: SEROLOGIC DIFFERENTIATION FROM TRANSMISSIBLE GASTROENTERITIS VIRUS USING MONOCLONAL ANTIBODIES

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ABSTRACT

In 1984 neutralizing antibodies against transmissible gastroenteritis virus (TGEV) were detected in pig herds in a small geographical area in the southern part of Denmark. No clinical symptoms were observed and accumulating epidemiological evidence gradually pointed towards a respiratory infection. In 1986 a TGE-like virus, tentatively named porcine respiratory coronavirus (PRCV), was isolated from the lungs of swine. The virus was partially characterized using monoclonal antibodies against TGEV and this showed that some (mainly non-neutralizing) epitopes of the peplomer glycoprotein E2 were absent in PRCV, whereas the major neutralizing domains were conserved. These findings allowed the design of competitive antibody immunoassays either discriminating or not discriminating the immune responses against the two viruses. However, the discriminating epitopes studied so far have shown minor immunodominance and some steric interference from non-discriminating epitopes.

INTRODUCTION

Until 1984 transmissible gastroenteritis (TGE) had never been diagnosed in Denmark either virologically or serologically, nor had firm clinical evidence of TGE been observed. The absence of the infection was confirmed by periodic serological surveys. During the spring of 1984 seropositive animals were identified in a few herds in the southern part of Jutland. Comprehensive serological examinations of in-contact herds revealed a number of seropositive herds. No clinical disease could be associated with seroconversion in any of these herds. Initially a voluntary eradication programme based on serological examinations was undertaken. However, in the beginning of 1986 the infection spread massively to other parts of the country and PRCV can now be considered enzootic in Denmark with a prevalence of seropositive animals of 75 - 80 %. Similar infections have been described in several other European countries.[1-4] Despite intensive efforts, the putative TGE-like

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virus was not isolated until June 1986, thus confirming the contagious nature of the seroreactions. Conventional cross-neutralization did not reveal significant differences between PRCV and TGEV. Therefore, studies employing anti-TGEV monoclonal antibodies were undertaken to characterize PRCV in more detail with the aim of identifying possible antigenic differences that might be used as markers in differential diagnosis.

METHODS

Virus Strains

The following TGEV strains were used: FS 216 (kindly provided by Dr. S. Cartwright, Central Veterinary Laboratory, Weybridge), Purdue (kindly provided by Professor M. Pensaert, Faculty of Veterinary Medicine, Ghent) and Riems (kindly provided by Professor W. Bathke, Friedrich Loeffler Institute, Insel Riems). PRCV strain DK 1/86 was isolated from the lungs of a pig that died of pneumonia (courtesy of Dr. L. Rønsholt, State Veterinary Institute for Virus Research, Lindholm). All virus strains were grown in primary porcine kidney cells in roller bottles. Virus titrations were performed in microplates using primary porcine thyroid cells.

Monoclonal Antibodies

Eight monoclonal antibodies against the peplomer protein E2 and 4 monoclonal antibodies against the transmembrane protein E1 of Purdue strain of TGEV[5] were kindly provided by Dr. H. Laude, INRA, Jouy-En-Josas. Two neutralizing monoclonal antibodies against TGEV were kindly provided by Dr. N. Juntti, Biomedicum, Uppsala. The latter monoclonals were subsequently shown to correspond to domain A of the peplomer protein according to the classification of Delmas et al.[6] (data not shown).

Immunofluorescence Test

Reactivity of monoclonal antibodies against each virus strain was tested on acetone-fixed monolayers of primary porcine kidney cells on multiwell slides. FITC-conjugated rabbit anti-mouse IgG (DAKO) was used at a dilution of 1/50 in PBS.

Blocking ELISA

The Purdue strain of TGEV was grown in primary porcine kidney cells in roller bottles. After 48 h the supernatant was clarified by low-speed centrifugation followed by pelleting of virus in a Beckman JA 14 rotor for 4 h. The pellet was resuspended in 1/100 of the original vol. in PBS and stored at -80°C. Microplates were coated overnight at 4°C with a predetermined dilution of virus, washed and stored ready for use at -20°C. Appropriate dilutions of test sera in PBS-0.1% Tween 20 (PBST) were incubated overnight at 4°C. The test was completed by incubation with a predetermined dilution of monoclonal antibody (E4 or 44-4) in PBST containing 10 % normal bovine serum followed by incubation with peroxidase-conjugated rabbit anti mouse IgG (DAKO) diluted 1/800 in the same buffer.

Test Sera

The following porcine sera have been included in the present study: 748 TGEV antibody negative sera from another country (sampled 1988), 440 randomly selected Danish sera (sampled 1987), 32 sera from pigs experimentally infected with Riems strain of TGEV, 35 sera sampled in France during 1979-85 and 141 sera sampled in France in 1987-88 (both sets kindly provided by Dr. P. Vannier, Ploufragan).

RESULTS

Indirect Immunofluorescence

The reactivity of monoclonal antibodies with strains of TGEV and PRCV is shown in Table 1. Of the monoclonal antibodies directed against the peplomer protein E2 only 4 fail to react with PRCV. These are 40 (domain D) and 6.179, 67.9 and 44.4 (outside domain A-D). Monoclonal antibodies 6.179 and 67.9 seem to be very strain specific in that they only react with the Purdue strain which was used to generate these antibodies. Two monoclonal antibodies (9.34 and 49.22) against the transmembrane protein E1 showed no reaction with PRCV and 1 monoclonal antibody (25.22) showed a very weak reaction with PRCV. The results are in concordance with those published by Laude et al.[7] From these results only monoclonal antibodies 40 and 44.4 against E2 could be considered possible candidates as specific markers of TGEV antibodies in competition assays. The monoclonal antibodies 49.22, 25.22 and 9.34 against E1 could not be used as markers since they did not react with all strains of TGEV.[7]

ELISA

Initially, all monoclonal antibodies against E2 were titrated in ELISA. Subsequently, each monoclonal antibody was tested in blocking assays against TGEV and PRCV reference sera

TABLE 1. Reactivity of monoclonal antibodies (MAB) with strains of TGEV and PRCV.

MAB Protein		V I R U S FS216	Purdue	PRCV	Riems
E2	11	20480	1280	5120	20480
	25	40960	20480	40960	40960
	40	5120	5120	<20	5120
	48	20480	5120	20480	10240
	51	40960	40960	81920	40960
	6.179	<20	320	<20	<20
	67.9	<20	80	<20	<20
	44.4	20480	81920	<20	81920
	E3	20480	5120	320	2560
	E4	81920	81920	81920	81920
E1	3.60	5120	5120	5120	1280
	25.22	320	1280	20	5120
	9.34	320	320	<20	1280
	49.22	5120	5120	<20	320

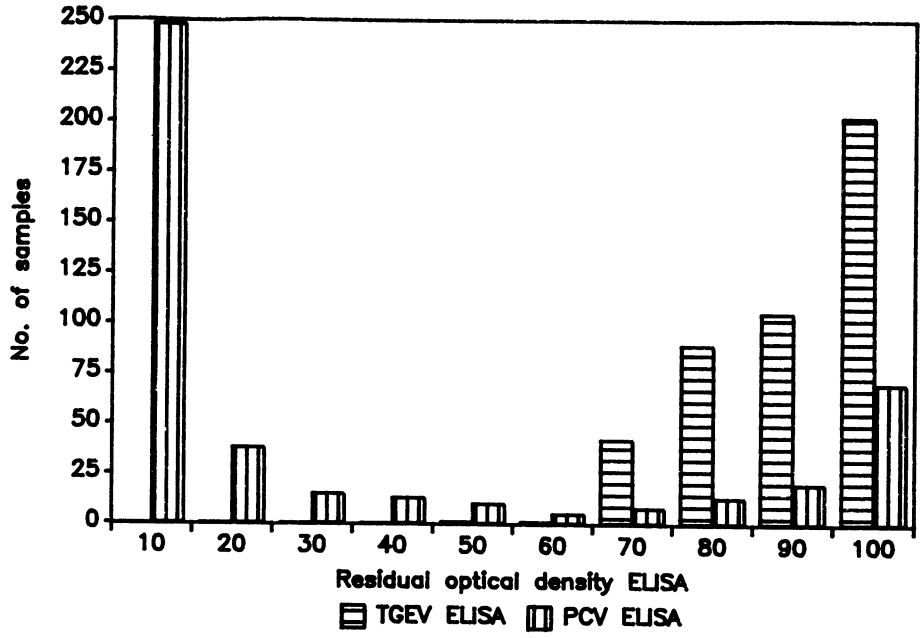

Fig. 1. Distribution of 440 porcine sera in E4 ELISA (termed PCV) and in 44.4 ELISA (termed TGEV).

to evaluate its usefulness as marker antibody. Monoclonal antibodies E4 and 44.4 proved to be especially useful as markers of common and TGEV-specific antibodies, respectively (data not shown).

The specificity of E4 ELISA has been evaluated using 748 TGEV and PRCV antibody negative sera. These showed on the average 0 % inhibition with a standard deviation of ±11 %. One hundred PRCV antibody positive sera were used to compare the sensitivity of E4 ELISA (50 % cut-off level) with virus neutralization test using TGEV. The two tests showed a high correlation (r=0.88) and E4 ELISA was on the average 2-fold more sensitive than neutralization test.

The specificity of 44.4 ELISA (TGEV-specific) was evaluated using 440 randomly sampled Danish sera of which most were PRCV antibody positive but assumed to be free of TGEV antibodies. The results (see Fig. 1) showed that up to 40 % inhibition may be encountered by PRCV antisera in 44.4 ELISA (single determinations). Regression analysis of these data showed that PRCV antibodies do interfere with the 44.4 epitope (average of 20 % inhibition for PRCV positive sera, slope 0.2).

The sensitivity of 44.4 ELISA was studied using sera from 4 experimentally TGEV-infected pigs. These results showed that the TGEV-specific response was delayed up to 1 week and remained 2-4 fold lower than that of the E4 response even though this study used a cut-off level for 44.4 ELISA of 25 % inhibition.

The 44.4 ELISA has been further evaluated (cut-off level 25 %) using sera from various sources. Thus, the 100 PRCV

antibody positive sera used to evaluate E4 ELISA were all negative in 44.4 ELISA. Of 130 French sera sampled in 1987 and suspected to be PRCV positive, 127 were positive in E4 ELISA and only 1 was positive in 44.4 ELISA. In contrast, of 35 sera sampled in France during 1979-85 34 were positive in E4 ELISA and 30 were positive in 44.4 ELISA which is to be expected if these sera were sampled before the introduction of PRCV. Furthermore, 11 sows vaccinated against TGEV were all strongly positive in both tests.

DISCUSSION

The E4 ELISA has been shown to be a sensitive and specific test for antibodies against TGEV and PRCV. The 44.4 ELISA has proven useful for serologic differentiation between TGEV and PRCV infections. However, the specificity of this test is limited due to some interference from PRCV antibodies. This requires the cut-off level to be adjusted accordingly, leading to decreased sensitivity which is furthermore influenced by the apparent low avidity of antibodies against the 44.4 epitope and incomplete immune response against this epitope (data not shown). A similar test has been described by Callebaut et al.[8] which supports these findings and conclusions. Thus, the 44.4 test is useful for differentiating TGEV and PRCV infections but indiscriminate use in individual pigs is not warranted at present.

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