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Detection of porcine respiratory coronavirus and transmissible gastroenteritis virus by an enzyme-linked immunosorbent assay

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Abstract

An enzyme-linked immunosorbent assay (ELISA) for the detection of transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) was developed. A bovine TGEV-specific polyclonal antibody was purified by affinity chromatography with the TRIO Bioprocessing System and was used as the capture antibody, at a concentration of 1.5 μg/well. The F5.39 monoclonal antibody was obtained by the fusion of spleen lymphocytes from TGEV immunized mice with NS-1 myeloma cells. This mAb was used as a second antibody for the ELISA. The ELISA detected 40 ng of TGEV and 407 ng of PRCV. To study the ability of ELISA to detect TGEV in field cases, 53 intestinal samples were taken from pigs exhibiting clinical signs of transmissible gastroenteritis. All the positive samples detected by the ELISA were confirmed as positive by immunofluorescence or cell culture immunofluorescence. To study the ability of this ELISA to detect PRCV in nasal swabs and lung samples, 20 seven-day-old piglets were inoculated with a Quebec strain of PRCV. The ELISA was able to detect PRCV in both kinds of samples.

Keywords: Porcine respiratory coronavirus; Pig; Porcine transmissible gastroenteritis virus; Diagnosis, virus; ELISA

1. Introduction

Transmissible gastroenteritis (TGE) was first reported in the U.S. in 1945 (Doyle and Hutchings, 1946) and since then it has been shown to have an almost worldwide distribution (Saif and Heckert, 1990). TGE is characterized by vomiting, a profuse watery diarrhea, and a high mortality rate (often 100%) in piglets under 2 weeks of age, and manifests itself

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as transitory enteritis in adult pigs (Saif and Heckert, 1990; Moxley et al., 1993). Some serological surveys revealed that sampled swine from 5% to 57% of farms in Europe and North America were seropositive for transmissible gastroenteritis virus (TGEV) (Toma and Benet, 1976; Egan et al., 1982; Cubero et al., 1993).

In 1986, a TGEV-related porcine respiratory coronavirus (PRCV) was identified (Pensaert et al., 1986). PRCV has been detected in Europe and North America. PRCV replicates almost exclusively in the upper respiratory tract of swine, causing mild respiratory disease, but no gastroenteritis (Laude et al., 1993; Pensaert et al., 1993). In Quebec, the 1Q90 strain of PRCV has recently been isolated in three-week-old piglets with bronchointerstitial pneumonia (Elazhary et al., 1992).

The diagnosis of TGE may be accomplished by viral detection or rising titers of antibody in serum. The latter method takes two weeks because of the interval required between the two samples. The most widely used technique to detect TGEV is probably immunofluorescent (IF) staining of small intestinal sections or smears from infected pigs (Pensaert et al., 1970), but its use is limited to dead pigs. Problems which may be encountered in IF tests include a lack of sensitivity or specificity of antibody reagents and loss of infected epithelial cells in pigs killed at a later stage of infection (Saif and Heckert, 1990). Other methods frequently used to detect TGEV include cell culture immunofluorescence (CCIF) (Bohl, 1979), and negative contrast or immune electron microscopy. The first method is cumbersome for use in routine diagnosis, especially because wild strains of TGEV are difficult to cultivate in cell cultures; the second one is expensive and time-consuming. Other less commonly used procedures include: agar gel precipitation (Bohac et al., 1975), immunoelectrophoresis and counterimmunoelectrophoresis (Bohac and Derbyshire, 1975), indirect hemagglutination (Skalinskii et al., 1977) and immunoperoxidase staining of infected tissues (Becker et al., 1974; Chu et al., 1982). The ELISA has been demonstrated to be useful for detecting TGEV from feces (Bernard et al., 1986; van Nieuwstadt et al., 1988). However, according to our knowledge, its use to detect PRCV from nasal swabs and lungs has never been described.

In this paper we describe a double-sandwich ELISA using a monoclonal antibody (mAb) to detect PRCV and TGEV from lung, nasal swabs, and intestine samples. We also determined the kinetics of antigen detection of PRCV in the lung and nasal secretions from suckling piglets after experimental inoculation.

2. Materials and methods

2.1. Viruses and cells

The attenuated Purdue strain of TGEV and the Quebec-1Q90 strain of PRCV were used (Elazhary et al., 1992). The viruses were propagated in monolayers of swine testis (ST) cells as previously described (Jabrane and Elazhary, 1994). The viruses were partially purified or purified as described by Gebauer et al. (1991) and Correa et al. (1988) respectively.

2.2. Bovine TGEV-specific IgG

Two cows were inoculated four times at one week intervals with the Purdue strain of TGEV using aluminium hydroxide (20%) and Quil-A (1 mg/dose) as adjuvants. The dose was 3 ml inoculated intramuscularly in three different places. Prior to inoculation, the two animals were seronegative for the following viruses: TGEV, PRCV, bovine respiratory syncytial virus, bovine viral diarrhea virus, and parainfluenza type 3 virus.

The IgG fraction from the Purdue TGEV-specific bovine antiserum was purified and concentrated with the TRIO Bioprocessing System (Sepracor, Massachusetts, USA). The purification was carried out by affinity chromatography using a protein G module. Concentration of the antibody sample was attained by ion-exchange chromatography using an SP-Trisacryl synthetic matrix (IBF Biotechnics Inc, Columbia, USA).

2.3. TGEV/PRCV-specific monoclonal antibodies

BALB/c mice were immunized with partially purified TGEV (Gebauer et al., 1991) emulsified in Freund's adjuvant. Each mouse was injected three times intraperitoneally at 2-week intervals. Four, two, and one day before fusion, intraperitoneal injections containing 0.2 ml of partially purified virus were given. Mouse myeloma cells (NS-1) were fused with spleen cells from immunized BALB/c mice as previously described (Cornaglia et al., 1990). The antibody-secreting hybridomas were detected by ELISA using non-purified TGEV and PRCV as antigens. Hybridomas were then cloned three times by use of an end-point dilution technique (Zola, 1988).

2.4. Field samples

A total of 53 small intestines of piglets with TGE clinical signs were obtained from the Pathology Laboratory of the Faculty of Veterinary Medicine (University of Montreal, Canada).

2.5. PRCV experimentally induced infection

A total of 20 seven-day-old specific pathogen free (SPF) piglets were inoculated by the intratracheal route with 3 ml of PRCV 1Q90 strain containing 3×10^7 $TCID_{50}$. Ten control piglets were inoculated in the same way with 3 ml of Iscove's modified Dulbecco's medium (IMDM). Two PRCV-inoculated piglets and one control piglet were killed each day, over a nine day period. For each piglet, nasal swabs, feces, and eight lung pieces were sampled. Lung pieces were taken from the following right and left lobules: apical, ventrodiaphragmatic, dorsodiaphragmatic and caudal. Tissues collected at necropsy were prepared as 10 per cent (w/v) homogenates in IMDM medium containing 100 IU penicillin and 0.1 mg streptomycin per ml. After clarification, by centrifugation at 1500 *g* for 15 minutes, the supernatants were stored at $-70°C$ until tested. Nasal swabs were immediately suspended in 2 ml of IMDM medium and then treated as for tissue homogenates.

2.6. ELISA test

Purified bovine IgG was treated for 1 h with 0.1 M citric acid and then diluted in PBS (pH 7.2) to a concentration of 1.5 μg/100 μl. The coating was done for 1 h at 37°C using PBS, pH 7.2. Plates were washed with 0.05% (v/v) Tween 20 in PBS and were blocked for 30 min at 37°C with 0.5% casein, 0.05% Tween 20 in PBS. Samples were mixed (50:50) with the F5.39 mAb and were incubated for 20 min. The mixture was added to the wells and was incubated for 30 min. After three washes, a 1:1000 dilution of anti-mouse IgG-peroxidase conjugate (Jackson Immunoresearch) was added to the wells and was incubated for 30 min. The substrate was 0.05% (w/v) urea peroxide and the chromogen 3,3',5,5'-tetramethyl-benzidine at a concentration of 100 mg per ml in 0.1 M sodium citrate buffer, pH 5. After 10 min, the reaction was stopped by addition of 2N SO_4H_2. The optical density of the solution was measured at 450 nm.

All reagents were added in a volume of 100 μl and the incubations were at room temperature unless indicated otherwise. Dilutions of samples were made in PBS containing 0.05% Tween 20 and 10% fetal bovine serum. Dilution of the conjugate was made in 0.5% casein, 0.05% Tween 20, 0.015 M TRIS, pH 7.2. All samples were tested in the presence or absence of the F5.39 mAb in order to detect any non-specific reactions.

2.7. Immunofluorescence (IF)

Frozen sections of intestine were examined by the direct immunofluorescent method as previously described (Pensaert et al., 1970). A hyperimmune serum against TGEV conjugated with fluorescein isothiocyanate was used.

2.8. Cell culture immunofluorescence (CCIF)

Four-day-old confluent ST cell monolayers were inoculated with samples at 1:10, 1:100 and 1:1000 dilutions. After three days at 37°C, plates were fixed with 80% acetone and stained with a hyperimmune serum against TGEV conjugated with fluorescein isothiocyanate. Cells were examined for immunofluorescence by use of a fluorescence microscope.

3. Results

3.1. Monoclonal antibody

A total of 34 TGEV/PRCV mAbs were obtained. Only five of these mAbs showed a good reactivity by the double-sandwich ELISA. Different combinations of mAbs were assayed and none showed an increase in the reactivity of the ELISA, even with the reference viruses or with TGEV or PRCV positive samples. The highest reactivity was obtained using mAb F5.39 which was chosen as the second antibody for the ELISA (results not shown). mAb F5.39 is an IgG1 that recognizes the N protein as demonstrated by immunoprecipitation.

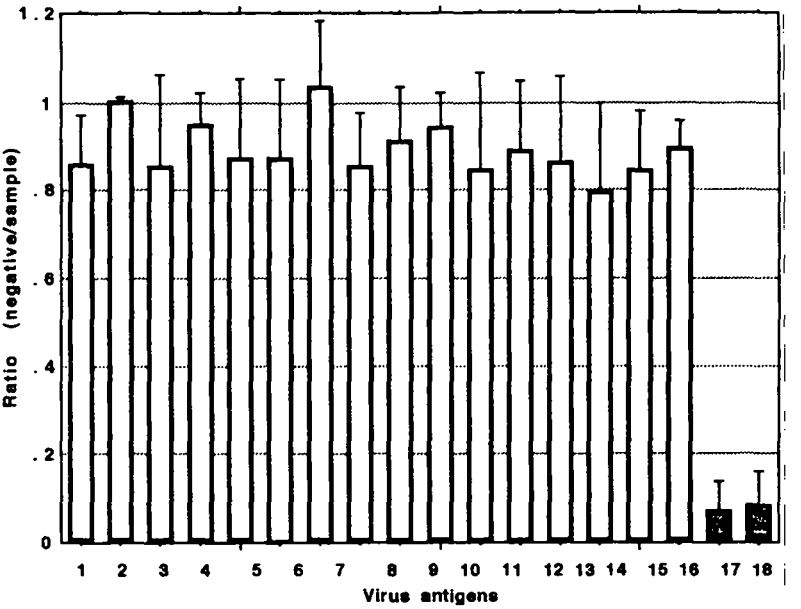

Fig. 1. Immunological specificity of the ELISA. The TGEV-specific bovine IgG was used as a capture antibody. The following viruses were used as antigens: (1) Bovine adenovirus; (2) Infectious bovine rhinotracheitis virus; (3) Bovine herpesvirus type 4; (4) Bovine diahrrea virus; (5) Bovine coronavirus; (6) Bovine rotavirus; (7) Bovine respiratory syncytial virus; (8) Bovine parainfluenza virus; (9) Equine influenza virus; (10) Feline leukaemia virus; (11) Canine parvovirus; (12) Porcine arterivirus; (13) Encephalomyocarditis virus; (14) Porcine parvovirus; (15) Porcine influenza virus (H3N2); (16) Porcine influenza virus (H1N1); (17) TGEV; (18) PRCV. The F5.39 mAb was used as the second antibody and the reaction was detected by a goat anti-mouse IgG-peroxidase conjugate (Jackson Immunoresearch).

3.2. *Immunological specificity and sensitivity of the ELISA*

ELISA revealed a specific reactivity for TGEV and PRCV (Fig. 1).

Of the 240 lung samples and 113 nasal swabs or feces, only one sample showed a non-specific reaction, a positive reaction when the mAb F5.39 was not added to the wells.

ELISA detected 40 ng of purified TGEV and 407 ng of purified PRCV.

3.3. *Relative sensitivity of the ELISA to detect TGEV*

When the results obtained by ELISA and IF were compared, a high correlation between the intensity of the reaction by both tests was observed (Fig. 2). The relative sensitivity and specificity of the ELISA were of 100% and 75% respectively, using IF as the reference test (Table 1). The low specificity found was caused by two samples that were positive by the ELISA but negative by IF. These two IF negative samples were analyzed by CCIF and both were found to be positive. This result confirms that the lower specificity expressed above is only due to the higher sensitivity of the ELISA as compared to IF.

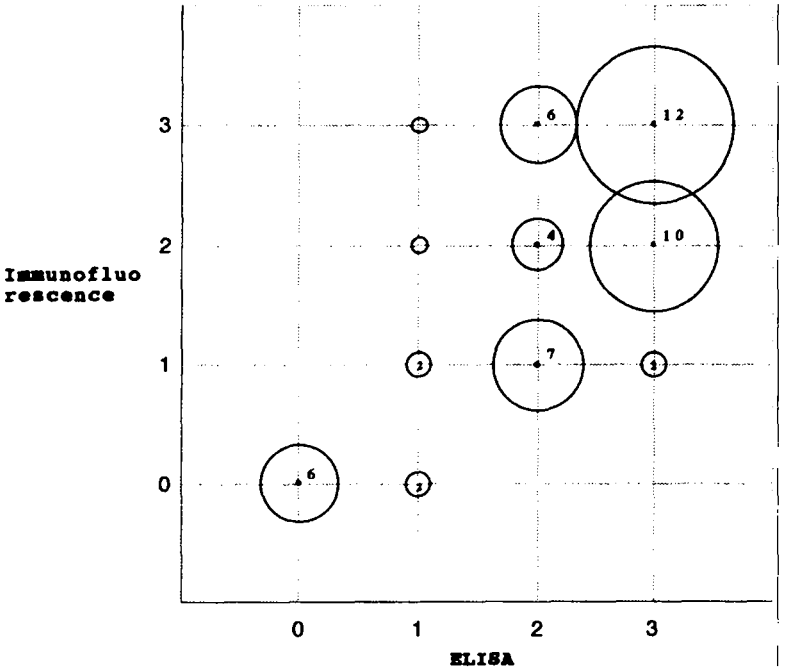

Fig. 2. Examination of piglet small intestines by immunofluorescence and ELISA tests to detect TGEV. ELISA: (0) negative samples; (1) titer of the sample = 10^1; (2) titer of the sample = 10^2; (3) titer of the sample = 10^3. The titer was expressed as the reciprocal of the last dilution giving an optical density ≥ 0.2. Immunofluorescence: (0) negative samples; (1) $\cong$ 10 to 30 fluorescent cells; (2) $\cong$ 30 to 100 fluorescent cells; (3) $\cong$ > 100 fluorescent cells.

3.4. PRCV experimentally induced infection and PRCV detection by ELISA

The 10 piglets inoculated with IMDM medium remained healthy during the experiment and did not present any macroscopic lesions, whereas 15 of the 20 inoculated piglets showed respiratory signs including polypnea and abdominal respiration, although coughing was not observed. In addition, one piglet (#11) died at day five post-inoculation. Macroscopic lesions in lungs were observed in all inoculated piglets except in those sacrificed at days one and two post-inoculation. Lesions included congestion and emphysematous areas.

Table 1
Relative specificity and sensitivity of the ELISA for TGEV detection taken immunofluorescence (IF) as the reference test

	IF +	IF −	Total
ELISA +	45	2	47
ELISA −	0	6	6
Total	45	8	53
Sensibility: 100%			
Specificity: 75%			

Table 2
Detection of PRCV in nasal swabs and lungs from infected piglets

Days[a] PI	Pigs	Nasal swabs		Lungs		
		ELISA[b]	CCIF[c]	ELISA[d]	CCIF	IF[e]
1	1	(A)[f] − (0.08)	ND	+ (0.62 ± 0.25)	+	+ / + + +[g]
	2	(B) − (0.08)	−	− (0.09 ± 0.00)	−	+ / −
2	3	(A) − (0.14)	−	+ (0.23 ± 0.03)	+	±
	4	(B) − (0.08)	−	+ (0.21)	+	−
3	5	(A) ND	+	+ (0.36 ± 0.05)	+	±
	6	(B) + (0.59)	ND	+ (0.31 ± 0.08)	+	+ / + + +
4	7	(A) + (0.63)	+	+ (0.39 ± 0.08)	+	+
	8	(B) + (0.33)	+	+ (0.25 ± 0.02)	+	± / ±
5	9	(B) + (0.44)	+	+ (0.21)	+	−
	10	(A) + (0.72)	+	+ (0.25 ± 0.03)	+	+
	11	(A) ND	+	+ (0.44 ± 0.08)	+	+ + / −
6	12	(A) + (0.48)	+	+ (0.24 ± 0.01)	+	− / −
	13	(B) − (0.09)	+	− (0.12 ± 0.00)	+	± / ±
7	14	(A) + (0.21)	+	+ (0.29 ± 0.04)	+	+ +
	15	(B) − (0.07)	−	− (0.13 ± 0.00)	−	− / −
8	16	(B) − (0.08)	−	− (0.11 ± 0.00)	−	−
	17	(B) + (0.38)	+	− (0.11 ± 0.03)	−	−
	18	(A) + (0.38)	+	+ (0.31)	−	+ / + +
9	19	(A) + (0.31)	+	+ (0.28 ± 0.04)	−	−
	20	(B) + (0.38)	+	+ (0.26)	+	± / −

[a]Days post-inoculation;
[b]The samples were considered positive when the optical density was ≥ 0.158 (twice the negative value). The mean of 40 negative samples was 0.079 ± 0.019;
[c]CCIF: tissue culture isolation. The presence of the virus was detected by IF;
[d]The samples were considered positive when the optical density was ≥ 0.206 (twice the negative value). The mean of 40 negative samples was 0.103 ± 0.021;
[e]IF: (−)negative sample; (+) ≅ 10 to 30 fluorescent cells; (+ +) ≅ 30 to 100 fluorescent cells; (+ + +) ≅ > 100 fluorescent cells;
[f]Litter A or litter B (optical density);
[g]Two different samples were analyzed;
ND: Not done.

Infection experimentally induced with PRCV was verified by recovery of the virus from the lungs of piglet #14. Monoclonal antibodies to the nucleoprotein of influenza virus (supplied by Dr Weber, CDC Ottawa), polyclonal antisera specific for Lelystad virus (provided by Dr. Pensaert), porcine parvovirus, porcine haemagglutinating encephalomyelitis virus, and bovine diarrhoea virus did not react by indirect immunofluorescence with infected ST cells. A positive cytoplasmic reaction was observed when the mAb 6AC3 directed against the peplomer glycoprotein (E2) of TGEV and PRCV was used, whereas no reaction was observed with the TGEV E2 specific-mAb 1DB12 (mAbs kindly provided by Dr. Enjuanes, Universidad Autonoma, Madrid, Spain).

The ELISA was able to detect PRCV in nasal swabs and lung samples of piglets experimentally inoculated with PRCV and a good correlation with CCIF and IF was observed (Table 2). All fecal samples were negative by ELISA and CCIF tests.

Each time the virus was detected in lungs, it was also detected in nasal swabs, except for the first two days post-inoculation (PI). This could indicate a primary viral multiplication in the lungs before viral excretion in nasal secretions, or it could be a consequence of the inoculation route (intratracheal). The detection of PRCV in lungs was not associated with any particular lung piece. Both results indicate that the viral detection in lungs is related to viral concentration but not to viral distribution.

All the ELISA positive nasal swabs were also positive by CCIF.

In four cases, the ELISA detected positive lung samples which were negative for IF (piglets #4, #9, #12 and #19), and in two cases, IF detected positive lung samples which were negative for the ELISA (piglets #2 and #13). However, CCIF confirmed the results obtained by ELISA. Only one sample (piglet #19) positive by ELISA and negative by IF could not be detected by CCIF.

The nasal swabs or lung samples from all the piglets sacrificed at days 3, 4 and 5 PI were positive, indicating the best moment for sample collection. After day 6 PI, some litter and individual variations in viral presence were observed. Two animals from litter B (piglets #15 and #16) were negative for all samples, whereas two other piglets (#13 and #17) were weakly positive by IF at days 6, 7 and 8 PI. However, all the piglets from litter A were positive in nasal swabs and lung samples after day 3 PI (Table 2). In addition, the piglet sacrificed from litter B at day 1 PI was negative, whereas that from litter A was positive. In most cases, the number of positive lung pieces was higher for litter A than for litter B (Table 2).

4. Discussion

The ELISA has previously demonstrated its usefulness to detect TGEV in fecal samples (Bernard et al., 1986; van Nieuwstadt et al., 1988; van Nieuwstadt et al., 1989). However, the detection of PRCV by ELISA has never been described. In this study, we describe a double sandwich ELISA that is sensitive and specific for the detection of PRCV and TGEV in nasal swabs, lung and intestine samples.

Protein N shares many antigenic determinants among coronavirus isolates (Enjuanes et al., 1992). Moreover, Vaughn and Paul (1993) found that an anti-N mAb reacted with 24 field isolates of TGEV. Consequently, we chose to use an N-specific mAb (mAb F5.39) in this study to detect the presence of TGEV and PRCV. The ELISA showed that a pre-incubation step of the mAb F5.39 with samples favoured a stronger reaction, probably due to the increase of the interaction antigen-mAb.

False-positive results in the ELISA may have been caused by preexisting antibodies in the animals used to prepare the anti-TGEV hyperimmune serum or in the anti-mouse conjugate. Such non-specific antibodies may react with other microorganisms present in samples. Another cause of false positive results is the presence of immunoglobulin-binding materials, such as rheumatoid arthritis factor and immunoglobulin-binding bacteria in samples (Yolken, 1982). However if this were the case, in all instances the control well included in the test where the mAb F5.39 was not added would give a positive reaction. The ELISA showed a very low percentage (0.28%: one of 353) of false positive reactions detected in this way.

The immunological sensitivity of ELISA, 40 ng/ml, is similar to that described by Bernard et al. (1986) and van Nieuwstadt et al. (1988). However, the sensitivity of the ELISA was 10 times lower for detection of PRCV, suggesting that mAb F5.39 may have a greater affinity for TGEV than for PRCV. Nevertheless, when the ability of the ELISA to detect PRCV was analyzed, the results showed a good correlation between the ELISA and CCIF or IF.

Results demonstrated a real difference in viral sensitivity between the two litters inoculated with PRCV. This difference could not be explained by the presence of PRCV-specific maternal antibodies, since none were detected in either sows or piglets from the two litters.

The pathogenicity reports of PRCV isolates are variable (Duret et al., 1988; O'Toole et al., 1989; van Nieuwstadt and Pol, 1989; Cox et al., 1990a; Bourgueil et al., 1992; Halbur et al., 1993 Lanza et al., 1992). In the present study, the pathogenicity of 1Q90 strain of PRCV was confirmed. After the experimental inoculation, respiratory signs, mortality and macroscopic lesions in lungs were observed. These observations were according to the observations described in a previous report (Jabrane and Elazhary, 1994). The existence of pathogenic strains of PRCV has to be taken into consideration when PRCV inoculation is proposed as a vaccination strategy to control TGE (Cox et al., 1993; Wesley and Woods, 1993). Finally, even though the replication of PRCV in low titers in the gut has previously been reported by other (O'Toole et al., 1989; Cox et al., 1990a; Cox et al., 1990b; Halbur et al., 1993) in this experiment, as well as in a previous one (Jabrane and Elazhary, 1994), we did not detect PRCV in feces.

In conclusion, according to our knowledge, it is the first time that an ELISA to detect PRCV from lungs and nasal swab samples has been described. The results obtained by ELISA, IF and CCIF test in a total of 353 nasal swabs, lung, feces and intestine samples correlated well with each other. Consequently, the ELISA appeared to be sensitive and specific for the detection of PRCV or TGEV. The detection of PRCV in samples from experimentally infected piglets showed that nasal swabs or any piece from the lungs can be used to detect PRCV by the ELISA. Finally, the 1Q90 was confirmed to be a pathogenic strain of PRCV since mortality and lung lesions were observed in the experimentally infected piglets.

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References

Becker, W., Teufel, P. and Mields, W., 1974. The immune-peroxidase method for detection of viral and chlamydial antigens. III. Demonstration of TGE antigen in pig thyroid cell cultures. Zentralbl. Veterinarmed., B, 21: 59–65.

Bernard, S., Lantier, I., Laude, H. and Aynaud, J.M., 1986. Detection of transmissible gastroenteritis coronavirus antigens by a sandwich enzyme-linked immunosorbent assay technique. Am. J. Vet. Res., 47: 2441–2444.

Bohac, J. and Derbyshire, J.B., 1975. The demonstration of transmissible gastroenteritis viral antigens by immunoelectrophoresis and counterimmunoelectrophoresis. Can. J. Microbiol., 21: 750–753.

Bohac, J., Derbyshire, J.B. and Thorsen, J., 1975. The detection of transmissible gastroenteritis viral antigen by immunodiffusion. Can. J. Comp. Med., 39: 67–75.

Bohl, E.H., 1979. Diagnosis of diarrhea in pigs due to transmissible gastroenteritis virus or rotavirus. In: F. Bricout and R. Scherrer (Editors), Viral Enteritis in Humans and Animals, Vol. 90. INSERM, Paris, pp. 341–344.

Bourgueil, E., Hutet, E., Cariolet, R. and Vannier, P., 1992. Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion. Vet. Microbiol., 31: 11–18.

Chu, R.M., Li, N.J., Glock, R.D. and Ross, R.F., 1982. Applications of peroxidase-antiperoxidase staining technique for detection of transmissible gastroenteritis virus in pigs. Am. J. Vet. Res., 43: 77–81.

Cornaglia, E., Elazhary, Y., Roy, R.S. and Talbot, B., 1990. Monoclonal antibodies to Quebec strain (Q17) of bovine rotavirus. Vet. Microbiol., 23: 283–294.

Correa, I., Jimenez, G., Suné, C., Bullido, M.J. and Enjuanes, L., 1988. Antigenic structure of the E2 glycoprotein from transmissible gastroenteritis coronavirus. Virus Res., 10: 77–94.

Cox, E., Hooyberghs, J. and Pensaert, M.B., 1990a. Sites of replication of a porcine respiratory coronavirus related to transmissible gastroenteritis virus. Res. Vet. Sci., 48: 165–169.

Cox, E., Pensaert, M.B., Callebaut, P. and van Deun K., 1990b. Intestinal replication of a porcine respiratory coronavirus closely related antigenically to the enteric transmissible gastroenteritis virus. Vet. Microbiol., 23: 237–243.

Cox, E., Pensaert, M.B. and Callebaut, P., 1993. Intestinal protection against challenge with transmissible gastroenteritis virus of pigs immune after infection with the porcine respiratory coronavirus. Vaccine. 11: 267-272.

Cubero, M.J., Leon, L., Contreras, A., Astorga, R., Lanza, I. and Garcia, A., 1993. Transmissible gastroenteritis in pigs in south east Spain: prevalence and factors associated with infection. Vet. Rec., 132: 238–241.

Doyle, L.P. and Hutchings, L.M., 1946. A transmissible gastroenteritis in pigs. J. Am. Vet. Med. Assoc., 108: 257–259.

Duret, C., Brun, A., Guilmoto, H. and Dauvergne, M., 1988. Isolement, identification et pouvoir pathogène chez le porc d'un coronavirus apparenté au virus de la gastro-entérite transmissible. Rec. Méd. Vét., 164: 221–226.

Egan, I.T., Harris, D.L. and Hill, H.T., 1982. Prevalence of swine dysentery, transmissible gastroenteritis and pseudorabies in Iowa, Illinois and Missouri swine. In: Proc. 86th Ann. Meet. United States Animal Health Association, 7–12 november 1982, at Nashville, TN, USA. pp. 497–502.

Elazhary, Y., Jabrane, A. and Talbot B.G., 1992. Porcine respiratory coronavirus isolated from young piglets in Quebec. Vet. Rec., 130: 500.

Enjuanes, L., Suné, C., Gebauer, F., Smerdou, C., Camacho, A., Anton, I.M., Gonzalez, S., Talamillo, A., Mendez, A., Ballesteros, M.L. and Sanchez, C., 1992. Antigen selection and presentation to protect against transmissible gastroenteritis coronavirus. Vet. Microbiol., 33: 249–262.

Gebauer, F., Posthumus, W.P.A., Correa, I., Suné, C., Smerdou, C., Sanchez, C.M., Lenstra, J.A., Meloen, R.H. and Enjuanes, L., 1991. Residues involved in the antigenic sites of transmissible gastroenteritis coronavirus S glycoprotein. Virology, 183: 225–238.

Halbur, P.G., Paul, P.S., Vaughn, E.M. and Andrews, J.J., 1993. Experimental reproduction of pneumonia in gnotobiotic pigs with porcine respiratory coronavirus isolate AR310. J. Vet. Diagn. Invest., 5: 184–188.

Jabrane, A. and Elazhary, Y., 1994. Pathogenicity of porcine respiratory coronavirus isolated in Quebec. Can. Vet. J., 35: 86–92.

Lanza, I., Brown, I.H. and Paton, D.J., 1992. Pathogenicity of concurrent infection of pigs with porcine respiratory coronavirus and swine influenza virus. Research in Veterinary Science. 53: 309-314.

Laude, H., Van Reeth, K. and Pensaert, M., 1993. Porcine respiratory coronavirus: molecular features and virus–host interactions. Vet. Res., 24: 125–150.

Moxley, R.A., LeRoy, D.O. and Davis, A.P., 1993. Experience with a planned exposure program for the control of enzootic transmissible gastroenteritis in swine. J. Am. Vet. Med. Assoc., 202: 1861–1864.

O'Toole, D., Brown, I; Bridges, A. and Cartwright, S.F., 1989. Pathogenicity of experimental infection with "pneumotropic" porcine respiratory coronavirus. Res. Vet. Sci., 47: 23–29.

Pensaert, M., Haelterman, E.O. and Burnstein T., 1970. Transmissible gastroenteritis of swine: virus-intestinal cell interactions. I. Immunofluorescence, histopathology and virus production in the small intestine through the course of infection. Archiv für Gesamte Virusforschung. 31: 321–334.

Pensaert, M., Callebaut, P. and Vergote, J., 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. Vet. Q., 8: 257–261.

Pensaert, M., Cox, E., van Deun, K. and Callebaut, P., 1993. A sero-epizootiological study of porcine respiratory coronavirus in belgian swine. Vet. Q., 15: 16–20.

Saif, L.J. and Heckert, R.A., 1990. In: L.J. Saif and K.W. Theil (Editors). Viral diarrhea of man and animals. Boca Raton, Florida, pp. 190–204.

Skalinskii, E.I., Mel'nikova, L.A., Kasyuk, I.I. and Boiko, A.A., 1977. Diagnosis of porcine transmissible gastroenteritis virus. Veterinaria, 9: 104.

Toma, B. and Benet, J.J., 1976. A technique of research on microplates of the antibodies neutralizing transmissible gastroenteritis virus of swine. Rec. Méd. Vét., 152: 565–568.

van Nieuwstadt, A.P. and Pol, J.M.A., 1989. Isolation of a TGE virus-related respiratory coronavirus causing fatal pneumonia in pigs. Vet. Rec., 124: 43–44.

van Nieuwstadt, A.P., Cornelissen, J.B.W.J. and Zetstra, T., 1988. Comparison of two methods for detection of transmissible gastroenteritis virus in feces of pigs with experimentally induced infection. Am. J. Vet. Res., 49: 1836–1843.

van Nieuwstadt, A.P., Zetstra, T. and Boonstra, J., 1989. Infection with porcine respiratory coronavirus does not fully protect pigs against intestinal transmissible gastroenteritis virus. Vet. Rec., 125: 58–60.

Vaughn, E.M. and Paul, P.S., 1993. Antigenic and biological diversity among transmissible gastroenteritis virus isolates of swine. Vet. Microbiol., 36: 333–347.

Wesley, R.D. and Woods, R.D., 1993. Immunization of pregnant gilts with PRCV induces lactogenic immunity for protection of nursing piglets from challenge with TGEV. Vet. Microbiol., 38: 31–40.

Yolken, R.H., 1982. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. Rev. Infect. Dis., 4: 35–68.

Zola, H. (Editor), 1988. Monoclonal antibodies: a manual of techniques. CRC Press, Boca Raton, Florida, pp. 54–56.