Serological Studies and Isolation of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus in Thailand

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Abstract

Swine serum samples from field cases during 1988-1996 and from imported pigs during 1991-1996 submitted to National Institute of Animal Health were tested for PRRS virus-specific antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) test kit. Of total 797 serum samples from field cases, 400 samples were seropositive to PRRS virus. The earliest detection of seropositive animals was in 1989. The percentage of seropositive animals increased annually from 8.6 in 1991 to 56 in 1996. Of total 804 serum samples from imported pigs, 39 samples were seropositive to PRRS virus. PRRS virus was first isolated from sera and tissue homogenates of suckling and nursery pigs with severe chronic respiratory distress from a farm in the central part of Thailand using primary swine alveolar macrophages. The indentification of the virus was confirmed by immunostaining with antisera specific to PRRS virus. Molecular characterization studies indicated that Thai isolate was more closely related to American isolate than Lelystad virus.

Key words : PRRS virus, serological studies, virus isolation.

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Introduction

Porcine reproductive and respiratory syndrome (PRRS), a relatively new disease of swine was first reported in the United States in 1987 (Keffaber, 1989). In early 1990, a similar disease referred to as porcine epidemic abortion and respiratory syndrome (PEARS) was described in Western Europe, where it spread rapidly (Wensvoort et al., 1992a). The disease was characterized by severe reproductive failure in sows (late-term abortions, increased numbers of stillborn, mummified and weakborn piglets,and increased preweaning mortality rate) and respiratory distress affecting pigs of all ages, but mainly suckling piglets. The disease was initially called by various names such as 'mystery swine disease' or 'swine infertility and respiratory syndromes (SIRS)' in the US, 'Seuchenhafter Spatabort der Schweine' in Germany, 'abortus blauw' in the Netherlands, 'blue-eared pig disease' in England, or 'Heko-Heko disease' in Japan (Collins et al., 1992; Christianson et al., 1992; Pol et al., 1991; Wensvoort et al., 1992a; Shimizu et al., 1994). The causative agent was first isolated in the Netherlands and then in the US and was named as Lelystad virus and ATCC VR-2332, respectively (Wensvoort et al., 1991; Collins et al., 1992).

PRSS virus is small, enveloped, and single-stranded RNA. The genomic RNA is approximately 15 kb long with positive polarity (Meulenberg et al., 1993). Based on the nucleotide sequence, genomic organization, replication strategy, and preference for infection of macrophages, both *in vitro* and *in vivo*, the virus is classified into the new family, the Arteriviridae, which consists of lactate dehydrogenase elevating virus, equine arteritis virus, and simian hemorrhagic fever virus (Plagemann and Moenning, 1992; Meulenberg et al., 1993; Conzelmann et al., 1993; Meulenberg et al., 1994). Although PRRS virus isolates share similar morphological and physiochemical properties, they are antigenically different. The PRRS virus isolates could be divided into two distinct antigenic classes, US and European. The US isolates are more antigenically diverse than European isolates (Wensvoort et al., 1992b; Nelson et al., 1993). Virus propagates preferentially in swine alveolar macrophages (SAM) and in a limited range of established cell lines, such as CL-2621 or MARC-145, the derivative cell lines from the MA-104 monkey kidney cells (Burtista et al., 1993; Kim et al., 1993). The detection of the virus is essentially based on virus isolation in SAM and final identification of the virus is confirmed by immunostaining with specific antisera (Wensvoort et al., 1992b; Burtista et al., 1993). Recently reverse transcription and polymerase chain reaction (RT-PCR) has been employed for the detection of PRRS virus nucleic acid from infected SAM cultures or tissue homogenates of infected pigs (Mardassi et al., 1994 a, b; Suarez et al., 1994). The indirect fluorescent antibody (IFA) test (Yoon et al., 1992), serum virus neutralization (SVN) test (Benfield et al., 1992; Yoon et al., 1994), immunoperoxidase monolayer assay (IPMA) (Wensvoort et al., 1991) and enzyme-linked immunosorbent assay (ELISA) (Albina et al., 1992) have been described for the detection of specific antibodies against PRRS virus. Currently, most North American veterinary diagnostic laboratories are using the IFA and/or the SVN test to detect PRRS virus-specific antibodies, whereas European laboratories have relied on the IPMA using PRRS virus-infected SAM (Zimmermann, 1993). However, the recent licensure of a commercial ELISA, a highly specific and sensitive method is widely used in many laboratories (Albina et al., 1992, 1994; Yoon et al., 1995).

The purposes of this study were to report serological evidences, virus isolation, and molecular characterzation of PRRS virus in Thailand

Materials and Methods

Serological Studies

Swin serum samples collected from field cases during 1988-1996 and from imported pigs during 1991-1996 submitted to National Institute of Animal Health for diagnosis were tested for PRRS virus-spccific antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) test kit* The test was performed according to the specification of manufacturer. Results from the ELISA were expressed as the ratio of the optical density of the test serum to the optical density of the positive control serum. Serum samples were considered positive when the ratio was greater than or equal to 0.4.

Virus Isolation

Swine alveolar macrophage (SAM) preparation. A 4-week old pig obtained from a herd free from PRRS, swine fever (SF), and Aujeszky's disease (AD) was used for the source of swine alveolar macrophages. The pig was euthanized and lung was removed aseptically. Lung was lavaged with phosphate buffer saline (PBS) and massaged thoroughly. The lavage fluid was collected by filtering through 4-layer sterile gauze. The collected fluid was centrifuged at 400 g for 10 min and the cell pellet was washed three times by centrifugation with PBS. Cells were collected and resuspended in complete media (CM) at appropriate concentrations. CM consisted of Eagle's minimum essential medium supplemented with 5% fetal calf serum, 10% tryptose phosphate broth, 50 ug/ml gentamicin, 1 mM L-glutamine, and 12 mM sodium bicarbonate. SAM were either used immediately for virus isolation or stored for later use in liquid nitrogen.

Field samples. A total of 7 piglets at the age of 3-8 weeks old with severe chronic respiratory distress from a farm in the central part of Thailand were submitted to our laboratory for diagnosis. Serum samples were collected and complete necropsies were performed. Tissue homogenates and serum samples were processed for the isolation of the disease-associated pathogens. Ten percent tissue homogenates (pools of tonsil, lymph nodes, spleen, kidney, liver, lung, and brain) and serum samples were processed for the isolation of PRRS virus. Serum samples were also tested for PRRS virus-specific antibodies using ELISA test kit. To rule out other viral infections, the tissue homogenates were also processed for virus isolation using PK-15 cell line and the cells were immunostained with antisera specific to SF and AD viruses as routinely performed in our laboratory.

Isolation of PRRS virus. SAM suspension was seeded into a 24-well plate at the final concentration 10^6 cells per well. The cultures were incubated at 37°C for at least 1 h to allow the cells to attach to the culture plate. Serum samples and 10% tissue homogenates (100 ul) were inoculated onto the cells and further incubated for 2 h. The cells were then washed twice with media and fresh CM (1ml) was added into the cultures. The cultures were incubated at 37°C in a 5% CO_2 incubator and observed daily for cytopathic effect (CPE). The cells were harvested at 48 h post-infection (p.i.) and fixed with absolute ethanol for 10 min. The fixed cells were prepared for an indirect immunofluorescent staining using anti-LV and anti-Chiba pig serum (kindly provided by Dr. G. Wensvoort, Central Veterinary Institue, Lelystad, Netherlands, and Dr. M. Shimizu, National Institute of Animal Health, Tsukuba, Japan, respectively) followed by rabbit anti-swine IgG antibody conjugated with fluorescein isothiocyanate. Chiba isolate of PRRS virus was closely related to American isolates (Murakami et al., 1994) so anti-Chiba serum was used instead of antiserum ot American isolates which was not available at that time of this study. The

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specific immunofluorescent staining in the cytoplasm of SAM indicated the presence of PRRS virus in the samples. The samples were disignated as negative after the cells were blind passaged twice.

PRRS virus isolated from the field samples was propagated in SAM in a 75 cm^2 cell culture flask. Infected cultures were frozen and thawed, aliquoted, titrated, and stored at -70 °C as virus stock.

Electron-microscopic examination. The PRRS virus stock was propagated in SAM in a 75 cm^2 cell culture flask. The cells were harvested at 36 h p.i. and pellet at low speed centrifugation. They were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide in a phosphate buffer and dehydrated in alcohol series. The pellets were embedded in epoxy resin, ultra-thin section sized 700 °A, and stained with lead citrate and uranyl acetate and examined for virus particles under a JEOL 1200 transmission electron microscope.

Molecular Characterization of PRRS Virus Thai Isolate

RNA sample preparation. Seven 1-month old pigs were obtained from a herd free from PRRS, SF, and AD. Pigs were bled and serum samples were tested that they were seronegative to PRRS virus before the experiment started. Five pigs were inoculated intranasally with $10^{3.5}$ $TCID_{50}$/2 ml of virus stock and the other two controls were sham infected intranasally with SAM cultures. Pigs were monitored daily for clinical signs and rectal temperature. Blood samples were collected at various times p.i. for virus isolation and PRRS virus-specific antibody responses. Serum samples from experimentally infected pigs and the controls were serially processed for the isolation of PRRS virus in SAM cultures. Infected and non-infected SAM cultures (100 ul/samples) inoculated with serum samples collected on day 7 p.i. were selected and processed for RNA extraction using TRIzol LS reagent* according to the specification of manufacturer. Lelystad and VR-2332 viruses (kindly provided by Dr. G. Wensvoort, Central Veterinary Institute, Lelystad, Netherlands, and Dr. T. Molitor, University of Minnesota, USA) as the representatives of European and American isolates were included for positive controls. RNA samples finally resuspended in 10 ul diethyl pyrocarbonate (DEP-C)- treated water

Reverse-transcription and polymerase chain reaction (RT-PCR). The common primer pair 1010 PLS-1011PLR which was specitic to the nucleocapsid protein (N) gene of both strains of PRRS virus (Mardassi et al., 1994a) was selected for RT-PCR. Their nucleotide sequences are as follows:

1010PLS 5' ATGGCCAGCCAGTCAATCA 3'

1011PLR 5' TCGCCCTAATTGAATAGGTG 3'

RNA samples (5ul) were processed for RT-PCR using RT-PCR kit** in a final volume of 25 ul of reaction buffer containing 1x AMV/Tfl buffer, 1mM $MgSO_4$, 0.2 mM (each) dATP, dCTP, dGTP, DTTP, 10 mM of each primer, 2.5 U of AMV reverse transcriptase, and 2.5 U of Tfl DNA polymerase. The samples were heated up at 48° C, 45 min to allow reverse transcription of RNA to cDNA and followed by 94°C, 2 min to get rid of AMV reverse transcriptase activity. The PCR reaction was programmed in an automated DNA Thermal cycler 9600*** as followed : 95° C, 20 sec, 60° C, 30 sec, and 68° C, 50 sec. After 35 cycles, the reaction was held at 68 °C for 7 min in order to elongate any uncompleted products. PCR procucts were analysed by electrophoresing 10 ul aliquots through 2% agarose gels in TAE buffer (0.04 M Tris-acetate [pH 8.5], 0.002M EDTA) in the presence of ethidium bromide for approximately 30 min at 10 V/cm and the gels were photographed under UV illumination.

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* Gibco BRL Life Technologies, Grand Island, NY, USA.

** Promega, Madison, WI, USA.

*** Perkin-Elmer, Norwalk, CT, USA.

Results

Serological Studies

Of total 797 swine serum samples from field cases submitted to National Institute of Animal Health for diagnosis during 1988-1996, 400 samples were seropositive to PRRS virus. The earliest detection of seropositive animals was in 1989. The percentage of seropositive animals increased annually from 8.6 in 1991 to 55.3 in 1996 (Table 1). The percentage of seropositive animals was not shown during 1988-1990 due to the limited number of available serum samples. Of total 224 serum samples from imported pigs during 1991-1995, 39 samples were seropositive to PRRS virus (Table 2). The seropositive animals were imported from both Europe and Northern America. No seropositive imported pig has been detected since January-June 1996.

Virus Isolation

No specific gross lesions were observed from the pigs affected with severe chronic respiratory distress. Histopathological examination revealed that all infected pigs had proliferative and interstitial pneumonia (Fig. 1) and some had lymphoid necrosis and lymphocyte depletion in spleens and lymph nodes. Immunoflorescent staining of PK-15 cells with antisera against SF and AD viruses was all shown negative. No other pathogenic bacteria was isolated except *E. coli*. Serum samples and tissue homogenates were further processed for PRRS virus isolation by inoculating the samples on SAM cultures. Cytopathic effects characterized by the appearance of fine granules in the cytoplasm and cellular shrinkage were observed in SAM cultures at 36 h p.i. (Fig. 2). The identification of PRRS virus was confirmed by immunostaining of the cells with antisera specific to LV and Chiba isolate. PRRS Thai isolate reacted strongly with antiserum against Chiba virus while it reacted weakly or none with antiserum against LV. PRRS virus was isolated from the sera and tissue homogenates of infected pigs as indicated by immunofluorescent staining in the cytoplasm of SAM (Fig. 3). PRRS virus was isolated from some pigs at the same time with the detection of PRRS virus-specific antibodies (Table 3).

For electron-microscopic examination, the spherical enveloped virions were observed with the size 45-55 nm in diameter containing 30-35 nm nucleocapsids (Fig. 4).

Molecular Characterization of PRRS Virus Thai Isolate

Following experimentally induced infection, PRRS virus was isolated from serum samples of all infected pigs collected on days 4, 7, and 10 p.i. and one infected pig remained viremia until day 20 p.i. Details of this experimentally induced infection will be reported elsewhere. SAM cultures of serum samples from infected and non-infected pigs at day 7 p.i. were selected and processed for RNA extraction and RT-PCR. The common primer pair 1010 PLS-1011 PLR which could amplify the nucleocapsid protein (N) gene of both strains of PRRS virus was selected for RT-PCR. PCR product of LV migrated more rapidly (398 bp expected product, lane 8) than that of American isolate (433 bp expected size, lane7) due to the deletion of 35 nucleotides confined to the 3' end of the N gene and the beginning of the downstream non-coding region (Mardassi et al., 1994b). No amplified product was detected from SAM cultures of the two controls (lane 1 and 9). PCR products of SAM cultures of 5 infected animals (lane 2 to 6) yielded the same electrophoretic mobility as that of American isolate (Fig. 5).

Table 1. Detection of PRRS virus-specific antibodies from field cases submitted for diagnosis during 1988-1996.

Year	No. cases positive/tested	No. pigs positive/tested (%)
1988	0/2	0/9
1989	1/1	3/5
1990	2/3	3/15
1991	3/8	3/35 (8.6)
1992	7/9	14/39 (35.8)
1993	13/14	29/63 (46)
1994	11/13	29/63 (46)
1995	25/38	272/483 (56.3)
1996	4/4	47/85 (55.3)
Total	**66/92**	**400/797**

Table 2. Detection of PRRS virus-specific antibodies from imported pigs during 1991-1996.

Year/country import	No. cases positive/tested (Country positive)	No. Pigs positive/tested
1991/Belgium, England	2/2 (Belgium, England)	9/9
1992/Sweden	0/1	0/5
1993/Canada, England,Finland,Norway	4/7 (Canada, England)	15/30
1994/Australia, Belgium,Denmark,England	2/7 (Belgium,England)	3/29
1995/Belgium,Denmark,England,Ireland	3/8 (Belgium, England)	12/151
1996/Belgium,Denmark,England,Finland,Ireland	0/9	0/580
Total	**11/34**	**39/804**

Table 3. Virus isolation and PRRS virus-specific antibodies detected from sick pigs with severe chronic respiratory distress from a farm in the central part of Thailand.

Pig No.	Viurs isolation		PRRSV antibodies[a]
	Serum	10% Tissue homogenates	(S/P)
1	+	+	0.144 (-)
2	-	-	0.682 (+)
3	+	+	0.449 (+)
4	+	-	0.268 (-)
5	+	NT[b]	0.614 (+)
6	NT	+	2.02 (+)
7	NT	-	0.3 (-)

[a] PRRS virus-specific antibodies detected by an ELISA test kit (S/P ≥ 0.4 is positive)
[b] Not tested

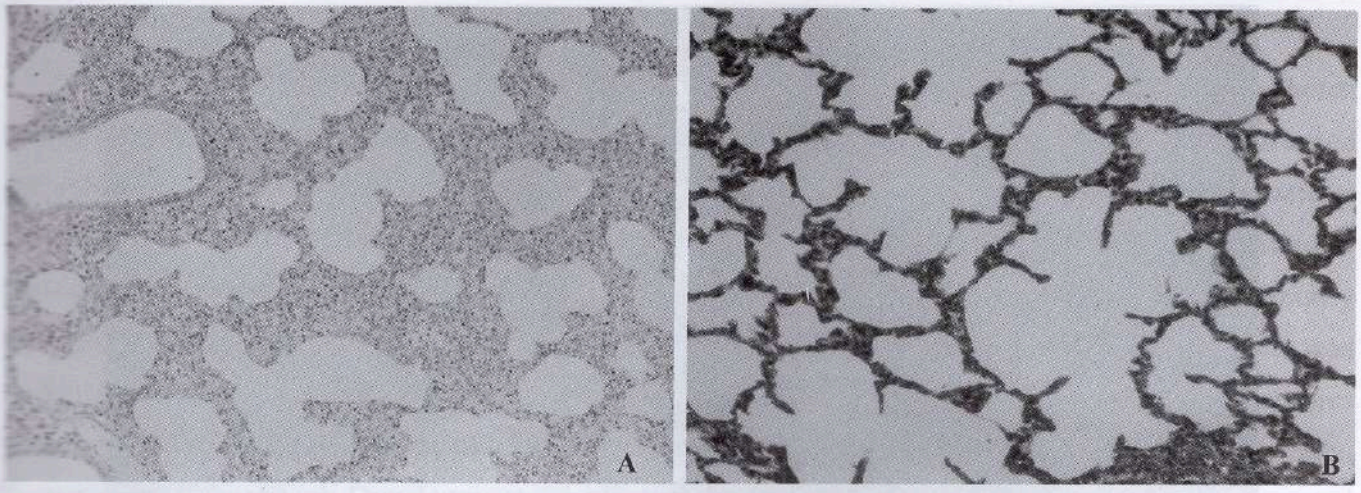

Figure 1. Proliferative and interstitial pneumonia in the lung of PRRS virus-infected pig (A) compared with lung of the control (B).

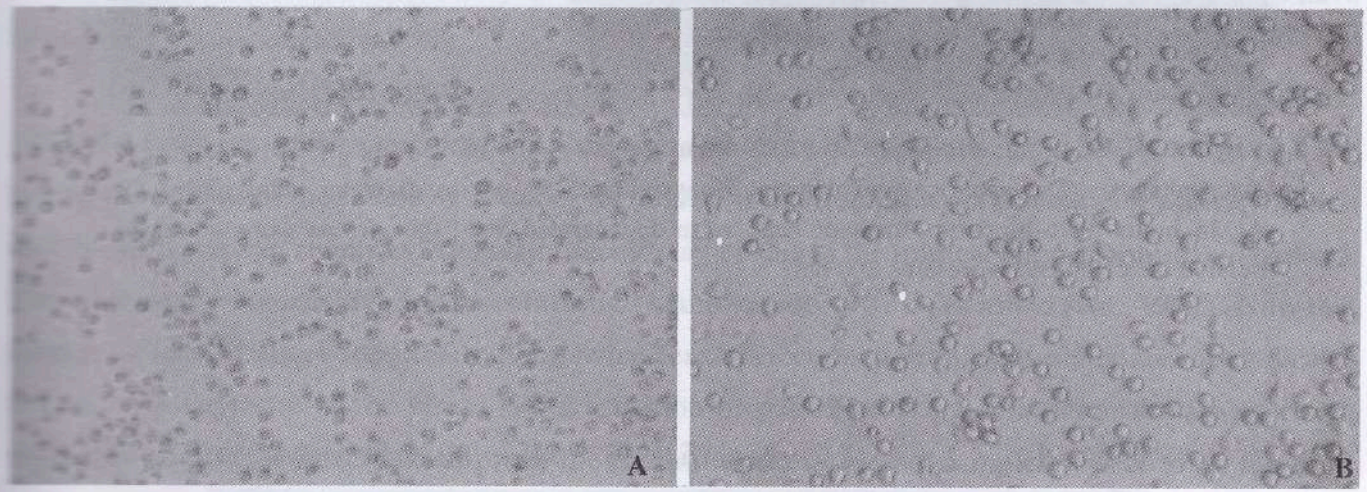

Figure 2. Cytopathic effect (CPE) caused by PRRS virus infection in primary swine alveolár macrophages (A) at 36 hours post-infection compared with non-infected cells (B).

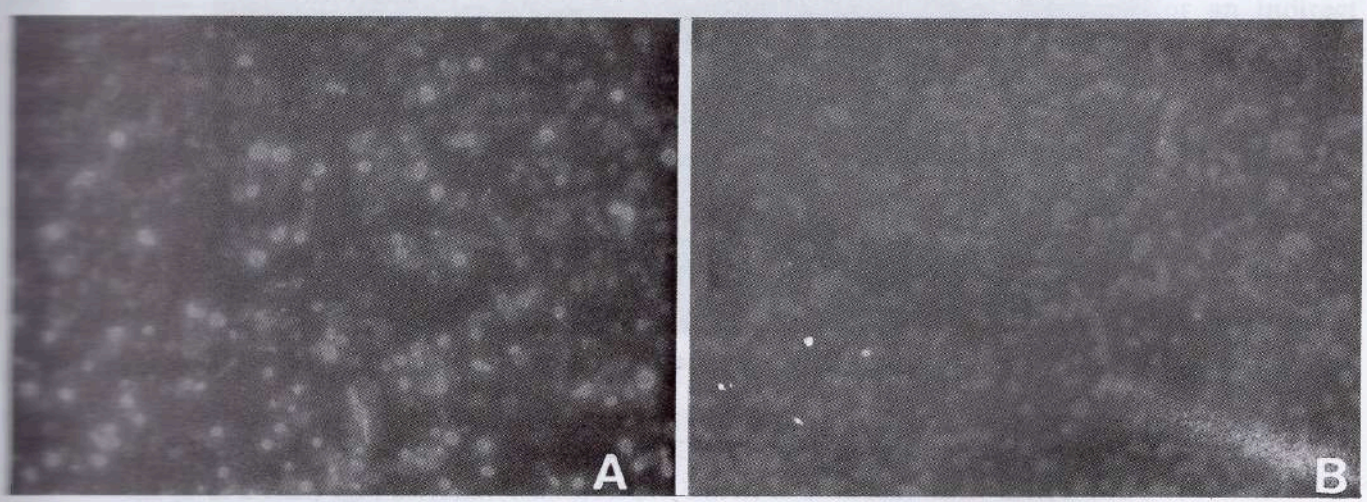

Figure 3. Primary swine alveolar macrophages infected (A) and uninfected (B) with PRRS virus. Specific antigen was detected in the cytoplasm of infected cells by indirect immunofluorescent staining.

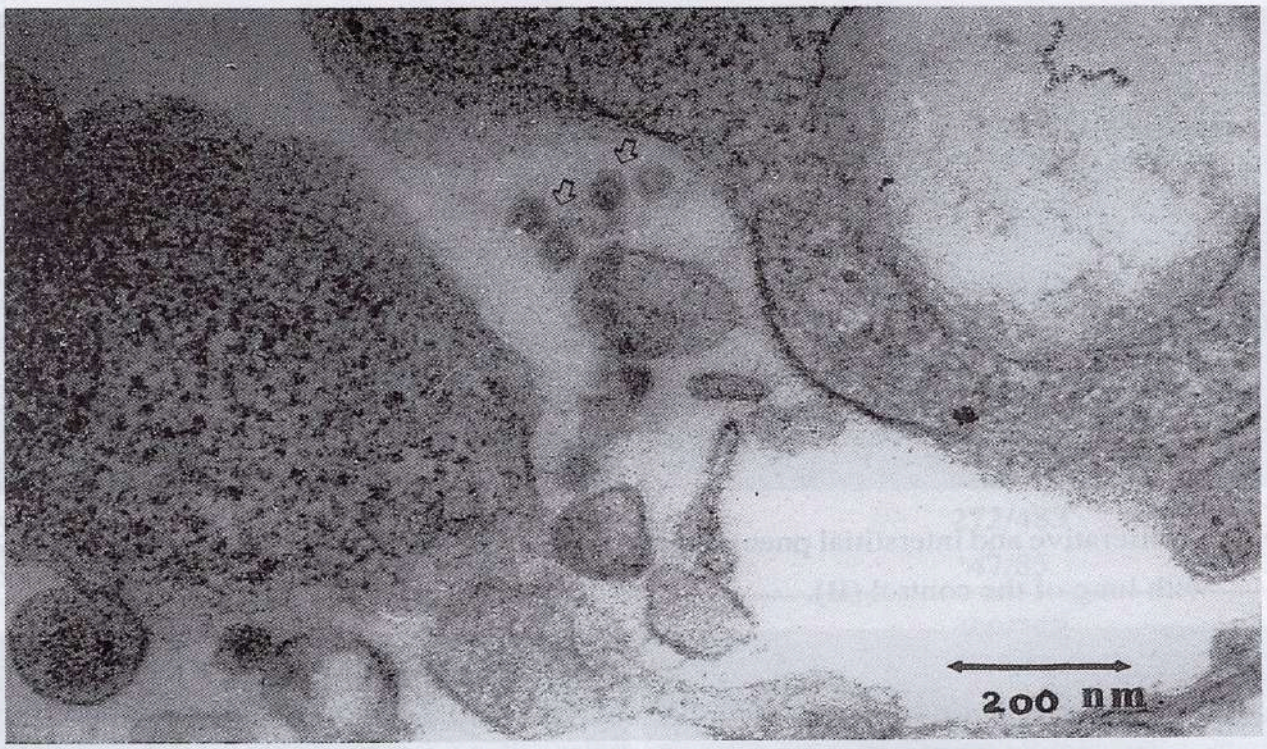

Figure 4. Electronmicrograph of PRRS virus (arrows) in the culture of swine alveolar macrophages. The spherical enveloped virions with 45-55 nm in diameter were observed. At 36 hours post-infection most of cells were degenerated and the virions were observed outside the cells. Bar represents 200 nm.

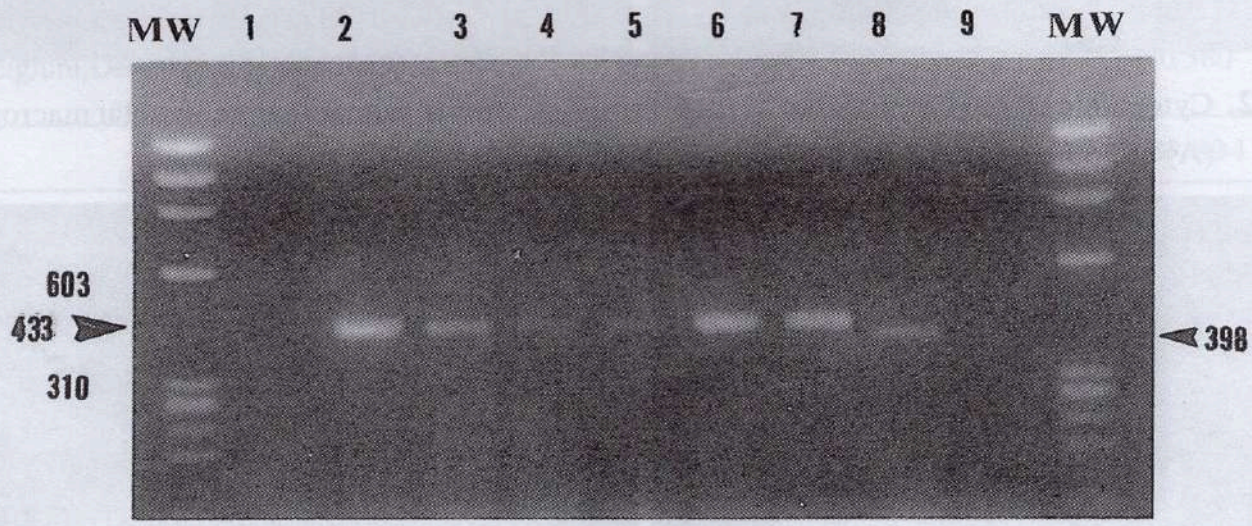

Figure 5. Agarose gel electrophoresis of RT-PCR products obtained using the primers 1010PLS and 1011 PLR. PRRS virus Thai isolates from 5 experimentally-infected pigs (lane 2 to 6) and American isolate (lane 7) yielded RT-PCR products of the same size (433 bp). LV (lane 8) yielded RT-PCR product of 398 bp. No amplified product was observed from RNA extracted from SAM cultures of the two control pigs (lane 1 and 9). MW lane, molecular weight size marker.

Discussion

In the present study, we could confirm the presence of PRRS virus infection among swine herds in Thailand by both serological and virological evidences. In this study, we successfully demonstrated the existence of PRRS virus in pigs from a herd affected with severe chronic respiratory distress. Retrospective studies of swine serum samples from field cases for PRRS virus-specific antibodies indicated the presence of seropositive pigs at least since 1989. PRRS virus might spread into the country through imported gilt replacement and boars that were infected or acted as carriers. However, there was no serum sample from imported pigs before 1991 in the serum bank to trace back. The serological studies were conducted at the beginning of 1995 and the results were reported to Disease Control Division, Department of Livestock Development. The quarantine regulation was established and all imported swine had to be tested for PRRS virus-specifie antibodies in order to prevent the introducing of new strains of PRRS virus into the country. Because of this regulation, no seropositive imported pigs have been detected since January 1996.

Results from our serological studies and the previous seroprevalence report (Oraveerakul et al., 1995) indicated the widespread of the virus exposure among swine herds in Thailand. Fortunately, there has been no report of the severe outbreak of reproductive and respiratory problems as it was reported in the US and European countries. The major factor may be due to the tropical climate nature of Thailand. Virus is temperature sensitive and loses infectivity quite rapidly at 37°C (Benfield et al., 1992). Now that the disease is becoming widespread among swine herds, detection of seropositive animals only indicates the previous exposure to the virus. Virus isolation or paired serology and demonstration of rising antibodies should be performed to prove the recent infection. Serum samples appear to be the most satisfactory specimens for attempting virus isolation from field cases, especially the serum samples collected from pigs in the early stage of infection. Because of the antigenic variation among PRRS virus isolates, antisera specific to American isolate and LV should be included for an indirect immonofluorescent staining of field samples in order to prevent misinterpretation. Results from immunoflurescent staining and RT-PCR indicated that PRRS Thai isolate was more closely related to American isolate than LV. However, it may be too early to draw this conclusion since only one isolate of PRRS virus was characterized. More PRRS virus isolates from the field outbreaks should be confirmed.

The clinical signs of PRRS are extremly variable depending on the strains of the virus, quality of management, health status, quality of housing system, and sanitation. The association with other pathogens accounts for the severity of field cases of PRRS. High standards of hygiene, management, and prevention of introducing new strains of virus into the farm are the best ways to control and minimize economic loss caused by the disease.

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References

Albina, E., Leforban, Y., Baron, T., Plana Duran, J. and Vannier, P. 1992. An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to the porcine reproductive and respiratory syndrome (PRRS) virus. Ann. Rech. Vet. 23:167-176.

Albina, E., Madec, F., Cariolet, R. and Torrison, J. 1994. Immune response and persistence of the porcine reproductive and respiratory syndrome virus in infected pigs and farm unit. Vet. Rec. 14:567-573.

Bautista, E.M., Goyal, S.M., Yoon, I.J., Joo, H.S. and Collins, J.E. 1993. Comparison of porcine alveolar macrophages and CL-2621 for the detection of porcine reproductive and respiratory syndrome (PRRS) virus and anti-PRRS antibody. J. Vet. Diagn. Invest. 5: 163-165.

Benfield, D.A., Nelson, E.A. and Collins, J.E. 1992. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC-VR 2332). J. Vet. Diagn. Invest. 4:127-133.

Christianson, W.T, Collins, J.E, Benfield, D.A., Harris, L., Gorcyca, D.E., Chladek, D.W, Morrison, R.B. and Joo, H.S. 1992. Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. Am. J. Vet. Res. 53: 485-488.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P.,Goyal, S.M., McCullough, S., Morrison, R.B., Joo, H.S., Gorcyca, D.E. and Chladek, D.W. 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J. Vet. Diagn. Invest. 4: 117-126.

Conzelmann, K.K., Visser, N., Van Woensel, P. and Thiel, H.J. 1993. Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the arterivirus group. Virology 193 : 329-339.

Keffaber, K.K. 1989. Reproductive failure of unknown etiology. Am. Assoc. Swine Pract. Newsl. 1:1-9.

Kim, H.S., Kwang. J., Yoon, I.J., Joo, H.S. and Frey, M.L. 1993. Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line. Arch. Virol. 133 : 477-483.

Mardassi, H., Wilson, L., Mounir, S. and Dea, S. 1994a. Detection of porcine reproductive and respiratory syndrome virus and efficient differentiation between Canadian and European strains by reverse transcription and PCR amplification. J. Clin. Microbiol. 32: 2197-2203.

Mardassi, H., Mounir, S. and Dea, S. 1994b. Identification of major differences in the nucleocapsid protein genes of a Quebec strain and European strains of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 75: 681-685.

Meulenberg, J.J.M., Hulst, M.M., De Meijer, E.J., Moonen, P.L.J.M., Den Besten, A., De Kluyver, E.P., Wensvoort, G. and Moormann, R.J.M. 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology 192: 62-72.

Meulenberg, J.J.M., Hulst, M.M., De Meijer, E.J., Moonen, P.L.J.M., Den Besten, A., De Kluyver, E.P., Wensvoort, G. and Moormann, R.J.M. 1994. Lelystad virus belongs to a new virus family, comprising lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus. Arch. Virol. (Suppl.) 9: 441-448.

Murakami, Y., Kato, A., Tsuda, T., Morozumi, T., Miura, Y. and Sugimura, T. 1994. Isolation and serological characterization of porcine reproductive and respiratory syndrome (PRRS) viruses from pigs with reproductive and respiratory disorders in Japan. J. Vet. Med. Sci. 56:891-894.

Nelson, E.A., Christopher-Hennings, J., Drew, T.,Wensvoort, G., Collins, J.E. and Benfield, D.A.1993. Differentiation of US and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. J. Clin. Microbiol. 31: 3184-3189.

Oraveerakul, K., Punarriwatana, D., Luangyosluechakul, S., Tuntasuparak, V. and Kunavongkrit, A. 1995. Seroprevalence of porcine reproductive and respiratory syndrome (PRRS) virus among swine breeding farms in the central and northeastern part of Thailand. Thai. J. Vet. Med. 25:233-240.

Plagemann, P.G.W. and Moenning, V. 1992. Lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus : a new group of positive-strand RNA viruses. Adv. Virus Res. 41: 99-192.

Pol, J.M.A., Van Dijk, J.E., Wensvoort, G. and Terpstra, C. 1991. Pathological, ultrastructural, and immunohistochemical changes caused by Lelystad virus in experimentally induced infections of mystery swine disease (synonym : porcine epidemic abortion and respiratory syndrome (PEARS)). Vet. Q. 13:137-143.

Shimizu, M., Yamada, S, Murakami, Y., Morozumi, T., Kobayashi, H., Mitani, K., Ito, N., Kubo, M., Kimura, K., Kobayashi, M., Yamamoto, K., Miura, Y., Yamamoto, T. and Watanabe, K. 1994. Isolation of porcine reproductive and respiratory syndrome (PRRS) virus from Heko-Heko disease of pigs. J. Vet. Med. Sci. 56:389-391.

Suarez, P., Zardoya, R., Prieto, C., Solana, A., Tabares, E., Bautista, J.M. and Casto, J.M. 1994. Direct detection of the porcine reproductive and respiratory syndrome (PRRS) virus by reverse polymerase chain reaction (RT-PCR). Arch. Virol. 135: 89-99.

Wensvoort, G., Terpstra, C., Pol, J.M.A., Ter Laak, E.A., Bloemraad, M., De Kluyer, E.P., Kragten, C., Van Buiten, L., Den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.L.J.M., Zetstra, T., De Boer, E.A., Tibben, H.J., De Jong, M.F., Van't Veld, P., Groenland, G.J.R., Van Gennep, J.A., Voets, M.T.H., Verheijden, J.H.M. and Braamskamp, J. 1991. Mystery swine disease in the Netherlands : the isolation of Lelystad virus. Vet. Q. 13: 121-130.

Wensvoort, G., De Kluyver, E.P., Pol, J.M.A., Wagenaar, F., Moormann, R.J.M., Hulst, M.M., Bloemraad, M., Den Besten, A., Zetstra, T. and Terpstra, C. 1992a. Lelystad virus, the cause of porcine epidemic abortion and respiratory syndrome : a review of mystery swine disease research at Lelystad. Vet. Microbiol. 33: 185-193.

Wensvoort, G., De Kluyver, E.P., Luijtze, E.A., Den Besten, A., Harris, L., Collins, J.E., Christianson, W.T. and Chladek, D. 1992b. Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome (SIRS) virus. J. Vet . Diagn. Invest. 4: 134-138.

Yoon, I.J., Joo, H.S, Christianson, W.T., Kim, H.S., Collins, J.E., Carlson, J.H. and Dee, S.A.1992. An indirect flourescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. J. Vet. Diagn. Invest. 4:144-147.

Yoon, I.J., Joo, H.S., Goyal, S.M. and Molitor , T.W. 1994. A modified serum meutralization test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. J. Vet. Diagn. Invest. 6: 289-292.

Yoon, K.J., Zimmermann, J.J., Swenson, S.L., MacGinley, M.J., Eernisse, K.A., Brevik, A., Rhinehart, L.L., Frey, M.L., Hill. H.T. and Platt, K.B. 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. J. Vet. Diagn. Invest. 7: 305-312.

Zimmermann, J.J. 1993. An overview of porcine reproductive and respiratory syndrome (PRRS). Proc. Annu. Meet. Iowa. Vet. Med. Assoc. 11 : 75-86.

การศึกษาทางซีรั่มวิทยา และการแยกเชื้อไวรัส พี อาร์ อาร์ เอส ในประเทศไทย

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บทคัดย่อ

ได้ทำการตรวจหาแอนติบอดีต่อเชื้อไวรัส พี อาร์ อาร์ เอส โดยสุ่มตัวอย่างซีรั่มสุกรในท้องที่ ตั้งแต่ปี พ.ศ. 2531-2539 และซีรั่มสุกรนำเข้ามาจากต่างประเทศ ปี 2534-2539 ที่ส่งเข้ามาตรวจที่สถาบันสุขภาพสัตว์แห่งชาติ โดยใช้ชุดตรวจสำเร็จรูป (ELISA) พบว่าซีรั่มสุกรในท้องที่จำนวน 797 ตัวอย่าง ตรวจพบแอนติบอดีต่อเชื้อไวรัส พี อาร์ อาร์ เอส 400 ตัวอย่าง โดยตรวจพบแอนติบอดีต่อเชื้อไวรัสนี้ตั้งแต่ปี 2532 และซีรั่มสุกรที่ให้ผลบวกต่อการตรวจมีจำนวนเพิ่มมากขึ้นในแต่ละปี จาก 8.6% ในปี 2534 เป็น 56% ในปี 2539 ส่วนซีรั่มสุกรนำเข้าจากต่างประเทศจำนวน 804 ตัวอย่าง ให้ผลบวกต่อการตรวจ 39 ตัวอย่าง เชื้อไวรัส พี อาร์ อาร์ เอส แยกได้เป็นครั้งแรกจากซีรั่มและตัวอย่างเนื้อเยื่อของสุกรดูดนม และสุกรอนุบาลที่ป่วยด้วยโรคระบบหายใจเรื้อรัง จากฟาร์มแห่งหนึ่งในเขตภาคกลาง โดยใช้เซลล์แม็คโครฟาจ และตรวจยืนยันโดยวิธี immunostaining ด้วยแอนติซีรั่มจำเพาะต่อเชื้อไวรัส พี อาร์ อาร์ เอส จากการศึกษาคุณสมบัติทางด้านโมเลกุล บ่งชี้ว่าเชื้อไวรัส พี อาร์ อาร์ เอส ที่แยกได้ในประเทศไทย มีความใกล้เคียงกับเชื้อสายพันธุ์ทางอเมริกามากกว่าทางยุโรป

คำสำคัญ : เชื้อไวรัส พี อาร์ อาร์ เอส ซีรั่มวิทยา การแยกเชื้อไวรัส

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