Korean J Vet Res(1999) 39(4) : 760~764

Prevalence of tissue antigen and serum antibody for porcine reproductive and respiratory syndrome in Cheju

Seong-hyun Ho, Yong-chul Jun, Choi-kyu Park*, Chang-hee Lee*, Jong-hee Bae

Veterinary Diagnostic Laboratory, Department of Veterinary Medicine, College of Agriculture, Cheju National University, Cheju 690-756, Korea
*National Veterinary Research and Quarantine Service, Anyang 430-016, Korea**
(Received Jul 12, 1999)

Abstract : A totoal of 219 pigs, 109 necropsy-pigs at the diagnostic laboratory of Cheju National University and 110 slaughter-pigs in Cheju, were evaluated for the prevalence of tissue antigen and serum antibody for spontaneus porcine reproductive and respiratory syndrome(PRRS). Tissues from 219 pigs examined for PRRS viral antigen by immmunohistochemistry included lung(cranio-ventral lobes and dorso-caudal lobes), tonsil, tracheobronchial lymph node, mesenteric lymph node, heart, kidney, liver, spleen, testis, ovary, brain, and spinal cord. Sera from 180 pigs were tested for the presence of antibody to PRRS virus by the indirect fluorescent antibody assay (IFA).

In the examination of serum antibody and tissue antigen for PRRS virus, serum antibody titers were considered as positive in 10%(18/180) of animals tested and PRRS viral antigen was detected in tissues of 4%(9/219) of the pigs. PRRS virus tissue antigen was most commonly detected by immunohistochemistry in the cranio-ventral lobe and tonsil.

We also confirmed the distribution of tissue antigen and prevalence of serum antibody to PRRS virus in Cheju. The detection of viral antigen by immunohistochemistry in tonsils and cranio-ventral lobes proved to be a very useful method for PRRS diagnosis.

Key words ; PRRS, antigen prevalence, seroprevalence, immunohistochemistry.

This research was funded by Problem Oriented Technology Development Project for Agriculture and Forestry in the Republic of Korea.

Address reprint requests to Dr. Jong-hee Bae, Veterinary Diagnostic Laboratory, Department of Veterinary Medicine, College of Agriculture, Cheju National University, Cheju 690-756, Republic of Korea.

Introduction

Porcine pneumonia can be induced due to primary pathogens (porcine reproductive and respiratory syndrome virus, porcine respiratory corona virus, swine influenza virus, Aujeszky's disease virus, *Mycoplasma hyopeumoniae* and so on.) and secondary pathogens (*Pasteurella multocida*, *Hemophilus parasuis* and others)[1]. Recently, a complicated respiratory diseases syndrome of swine was named PRDC (porcine respiratory disease complex)[2].

Porcine reproductive and respiratory syndrome (PRRS), a component of PRDC, was first reported in the United States in 1987[3]. The disease causes severe reproductive failure in sows, respiratory disease and increased preweaning mortality in suckling pigs, and a mild flu-like disease in grower-finisher pigs[3,4]. The etiologic agent of PRRS is a member of the *Arteriviridae* family of the order *Nidovirales*[5]. Swine are susceptible to PRRS virus(PRRSV) by a number of routes of exposure, including oral, intranasal, intramuscular, intraperitoneal, and vaginal[6]. The oronasal route appears to be the primary route of transmission. The virus then replicates in macrophages and dendritic cells in tonsils, upper respiratory tract, and lungs, resulting in viremia by 6-12 hours which may persist for several weeks. Further replication occurs in lymph nodes, spleen, thymus, bone marrow, and lungs. PRRSV is shed via the respiratory tract, saliva, feces, urine, and semen[2,6]. Previous studies have demonstrated that interstitial pneumonia is the most common type of pneumonia in pigs experimentally infected with PRRSV[6,7].

A definitive diagnosis of PRRS can be provided by antigen detection using techniques such as immunohistochemistry(IHC) of formalin-fixed tissue section[8], virus isolation[9], PCR[10] or antibody detection by indirect fluorescent antibody assay (IFA), ELISA, or a blocking ELISA utilizing monoclonal antibody[11,12]. Seroprevalence of PRRSV antibody was first reported as 12.7% in the Republic of Korea in 1993[13], and later reported as 45.2% in 1997[14]. PRRSV tissue antigen has been detected by IHC in lung, tonsil, spleen, heart, lymph nodes of experimentally PRRSV-infected pigs[7,15].

The present study investigated prevalence of serum antibody and tissue antigen of PRRSV in Cheju. As well as a distribution of PRRSV antigen in tissues of naturally infected pigs was determined.

Materials and Methods

Animals : A total of 219 pigs were evaluated in this study, 109 necropsy-pigs submitted to the diagnostic laboratory of Cheju National University from 1995 to 1998 and 110 slaughter-pigs from 5 herds in Cheju in 1998. Preweaner (59 pigs), weaner (50 pigs) and finisher (110 pigs) were evaluated for tissue antigen by IHC. Sera from 180 non-vaccinated pigs were tested for the presence of antibody of PRRSV by IFA.

Serological test : Antibody to PRRSV was detected using PRRSV, Korean isolate PL96-1 infected MA-104 cells[16]. Briefly, confluent monolayers of MA-104 cells were infected with virus then fixed with cold methanol at 48 hours after infection. Serum samples were diluted 1 : 10 with phosphate buffered saline (PBS, pH 7.2) and added to fixed, virus infected cells and incubated for 60 minutes followed by washing three times with PBS. Anti-swine IgG antibody labeled with FITC was applied to the washed cells and incubated for 60 minutes followed by washing as in the previous step. PRRSV specific cytoplasmic immuno-fluorescence was observed under an ultra violet light microscope.

Immunohistochemistry : Sections of cranioventral lung lobe, dorso-caudal lung lobe, tonsil, tracheobronchial lymph node, liver, kidney, spleen, heart, small intestine, testis, ovary, brain of 219 pigs were cut at 3~5μm and mounted on poly-L-lysine-coated slides. Endogenous peroxidase was blocked for 30 minutes by 3% hydrogen peroxide. Blocking was followed by digestion with 0.05% protease in PBS for 30 minutes at 37℃. Blocking was done for 30 minutes with a 10% solution of normal goat serum in PBS, then endoavidin and endobiotin was blocked for 15 minutes with an avidin-biotin blocking kit. Primary monoclonal antibody ascites fluid diluted 1 : 2000 in PBS was added for 13 hours at 4℃ in a humidified chamber. After primary antibody incubation and three 5 minute changes in PBS, the slides were flooded with biotinylated goat anti-mouse linking antibody[e] for 90 minutes at 37℃. The sections were washed with PBS and

treated with peroxidase-conjugated streptavidin for 30 minutes and incubated with 3,3'-diaminobenzidine tetrahydrochloride for 1~2 minutes. Sections were then stained with hematoxylin. Antigen amount was evaluated as the number of positive cells per cm^2 of tissues by image analysis.

Results

Distribution of antibody and antigen to PRRSV : Serum antibody titers were considered as positive in 10% (18/180) of animals tested and PRRSV antigen was detected in tissues of 4% (9/219) of the pigs. In age comparison studies, the highest levels of serum antibody and tissue antigen detection was 29% and 10%, respectively, in weaner and grower pigs (Table 1, Fig 1).

Table 1. Prevalence of antigen and antibody for spontaneous PRRS

Age	IFA*	IHC**
Preweaner	3/38(7.9)***	2/59(3.4)
Weaner and Grower	10/34(29)	5/50(10)
Finisher	5/108(4.6)	2/110(1.8)
Total	18/180(10)	9/219(4.1)

* IFA : Indirect fluorescent antibody assay.
** IHC : Immunohistochemistry.
*** No. of positive pigs/No. of pigs examined(%).

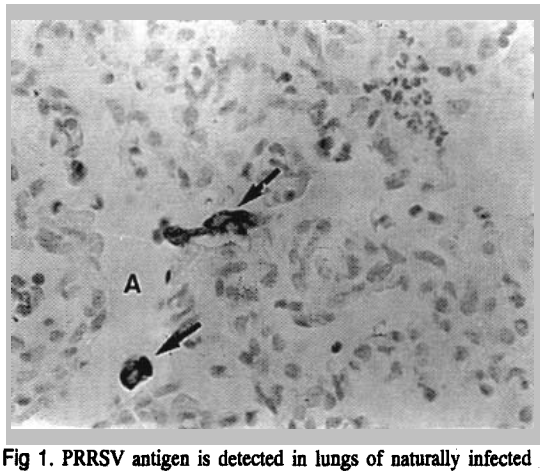

Fig 1. PRRSV antigen is detected in lungs of naturally infected pigs by immunohistochemistry. Labeled antigen(arrows) is seen within the cytoplasm of macrophages in alveolar spaces (A) and alveolar septa. ABC stain, × 400.

Distribution of PRRSV antigen by tissue : IHC evaluation, for distribution of PRRSV antigen in tissues of naturally infected pigs, demonstrated tissue antigen in lungs and tonsils of antigen-positive pigs (9/9). PRRSV antigen was also detected in spleen (4/9), tracheobronchial lymph node (3/9), mesenteric lymph node (3/9), liver (1/9), kidney (1/9), heart (1/9) and testis (1/3) but was not detected in brain and spinal cord (Table 2).

Table 2. Distribution by organs of viral antigen for spontaneous PRRS

Organs	Age			
	Preweaner	Weaner and grower	Finisher	Total
C.V.	2/2	5.5	2.2	9/9(100)*
D.C.	1/2	2/5	0.2	3/9(33)
Tonsil	UK**	4/4	1/1	5/5(100)
Tb ln	1/2	1/5	1/2	3/9(33)
Mes ln	1/2	1/5	1/2	3/9(33)
Liver	0/2	1/5	0/2	1/9(11)
Kidney	0/2	1/5	0/2	1/9(11)
Spleen	1/2	2/5	1/2	4/9(44)
Heart	0/2	1/5	0/2	1/9(11)
Small Int.	1/2	1/4	1/2	3/8(37.5)
Testis	UK**	1/3	UK**	1/3(33)
Ovary	UK**	0/1	0/1	0/2(0)
Brain	0/2	0/2	UK**	0/4(0)

C.V. : cranio-ventral lobe ; D.C. : dorso-caudal lobe ; Tb ln : tracheobronchial lymph node ; Mes ln : mesenteric lymph node ; Int. : intestine.
* No. of positive pigs/Total no. of pigs examined (%).
**UK : unknown.

The highest number of PRRSV antigen-positive cells per cm^2 of tissue was up to 6 cells in cranioventral lobe of lungs. Antigen-positive cells were also detected in spleen (3 cells/cm^2), small intesine (3 cells/cm^2), tonsil (2 cells/cm^2), lymph nodes (2 cells/cm^2), and dorso-caudal lobe (1 cells/cm^2). Less than 1 antigen-positive cell/cm^2 was detected in liver and heart (Table 3).

Table 3. Mean score for PRRS virus antigen-positive cells by organs

Age	Lung		Tonsil	Tb ln	Mes ln	Liver	Heart	Spleen	Small Int.
	C.V.	D.C.							
Preweaner	10.9*	1.3	UK**	3.8	3.6	0	0	4	2.75
Weaner and grower	5.8	2.7	1.4	2	1.9	0.06	0.1	4.1	3.8
Finisher	2.4	0	5.6	1.5	0.9	0	0	1	1.4
Mean	6.18	1.81	2.26	2.28	2.03	0.03	0.05	3.35	3.02

C.V.: cranio-ventral lobe; D.C.: dorso-caudal lobe; Tb ln: tracheobronchial lymph node; Mes ln: mesenteric lymph node; Int.: intestine.
* No. of antigen-positive cells per cm^2.
** UK: unknown.

Discussion

The seroprevalence of PRRSV has recently been reported as 45.2% in the Republic of Korea[14] and our study indicated that the seroprevalence of PRRSV was 10% in Cheju, confirming our expectation that seroprevalence of PRRSV is lower in Cheju than in other provinces of Korea. Regional environment of Cheju island that limits the movement of contaminated pigs between provinces may be concerned with it. Prevalence of the viral antigen in tissue (4.1%) was less than seroprevalence (10%). Perhaps low virus numbers in tissues or cells that reduces sensitivity of IHC can be one of the reasons. The seropositive may also be due to past PRRSV infection thereafter viral antigen can no longer be detected. This study is the first to report the presence and prevalence of PRRSV in Cheju.

In our comparison of the prevalence of PRRSV antigen and antibody by age, prevalence was the highest in weaner-grower pigs at 10% and 29%, respectively. We could presume that PRRSV is most frequently infecting weaner and grower pigs[17].

We also identified the presence of PRRSV tissue antigen in a sero-negative case. The animal may have been tested during the early stage of infection and prior to seroconversion. Therefore, the detection of viral antigen by IHC in these cases for diseases monitoring proved to be a very useful method for PRRS diagnosis[7,18].

The result of our examination of the distribution of PRRSV antigen in tissue was similar to previous reports involving experimental infection with a U.S. PRRSV strain[7]. We also demonstrated that PRRSV tissue antigen was most commonly detected by immunohistochemistry in the cranio-ventral lobe of lung and tonsil.

References

1. Christensen G, Soresen V, Mousing J. Diseases of the respiratory system, p 913-940, 1999. *In* Straw BE, D'Allaire S, Mengeling WL, Taylor DJ(ed.), Disease of Swine, 8th ed, Iowa State University Press, Ames, Iowa.
2. Halbur PG. Porcine Respiratory Disease Complex. *North Carolina Healthy Hogs Seminar*, 1997.
3. Keffaber KK. Reproductive failure of unknown etiology. *American Assoication Swine Pract News*, 1:1-10, 1989.
4. Wensvoort G, Terpstra C, Pol JMA, *et al*. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *J Vet Q*, 13(1):121-130, 1991.
5. Cavanagh D. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. *Arch Virol*, 142(3): 629-633, 1997.
6. Benfield DA, Collins JE, Dee SA, *et al*. Porcine reproductive and respiratory syndrome, p 201-232, 1999. *In* Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (ed.), Disease of Swine, 8th ed, Iowa State University Press, Ames, Iowa.

7. Halbur PG, Paul PS, Frey ML, *et al*. Comparison of the antigen distribution of two US PRRSV isolates with that of the Lelystad virus. *J Vet Pathol*, 33:159-170, 1996.
8. Pol JM, Van Dijk JE, Wensvoort G, *et al*. 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)). *J Vet Q*, 13(3):137-143, 1991.
9. Wensvoort G, Terpstra C, Pol JMA. Blue ear disease of pigs. *Vet Rec*, 128(24):574, 1991.
10. Suarez P, Zardoya R, Prieto C, *et al*. Direct detection of the porcine reproductive and respiratory syndrome (PRRS) virus by reverse polymerase chain reaction (RT-PCR). *Arch Virol*, 135(1-2):89-99, 1994.
11. Nelson EA. Benfield DA, Christopher Hennings J, *et al* : Development and evaluation of blocking and/or competitive ELISA for the improved sero-diagnosis of PRRS. *NPPC* 1997 *Research Investiment Report*, 1997.
12. Yoon I, Joo H, Christianson WT, *et al*. An indirect fluorescent antibody test for the detection of antibody to swine infertility and respiratory syndrome virus in swine sera. *J Vet Diagn Invest*, 4(2):144-147, 1992.
13. Shin J, Kang Y. Sero-epidemiological studies on porcine reproductive and respiratory syndrome in Korea. I. Detection of indirect fluorescent antibodies. RDA *J Agri Sci*, 35(2):572-576, 1993.
14. Cheon D, Chae C, Lee Y. Sero-prevalence of antibody to porcine reproductive and respiratory syndrome virus using enzyme linked immunosorbent assay in selected herd in Korea. *J Vet Diagn Invest*, 9:434-436, 1997.
15. Rossow KD, Goyal SM, Nelson EA, *et al*. Chronological immunohistochemical detection and localization of porcine reproductive and respiratory syndrome virus in gnotobiotic pigs. *J Vet Pathol*, 33:551-556, 1996.
16. Lyoo Y, Park C, Chang C. Diagnostic manual for animal diseases. *I-Kong World Press*, Seoul, Korea, 1997.
17. Done SH, Paton DJ, Brown I, *et al*. W. Pathology of porcine reproductive and respiratory syndrome(PRRS) in the UK. *Proceedings of the Congress International Pig Veterinary Society, The Netherlands*, (1):111, 1992.
18. Joo H, Park B, Dee SA, *et al*. Indirect fluorescent IgM antibody response of pigs infected with PRRSV. *Vet Microbiol*, 55:303-307, 1997.