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Assessment of Porcine Reproductive and Respiratory Syndrome Virus RNA Load in Sera and Tissues during Acute Infection

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) RNA load in sera and tissues during acute phase of infection was evaluated using a PCR-based quantitative assay. More than 80% of infected pigs (21/25) showed the peak level of viral RNA concentrations in serum (up to 8.6 × 108 copies/ml) at day 5 postinfection (PI), and started to clear the virus from the systemic circulation thereafter. Regression analysis using the viral RNA concentrations in sera obtained from days 5 to 14 PI showed that the viral RNA was cleared at the rate of 0.37 log reduction in the number of PRRSV RNA copies per day. It was estimated to be day 27 PI when the viral RNA in the serum of infected pigs becomes undetectable. When correlation analysis was performed between the systemic clearance rate and viral RNA concentrations in tissues of 9 infected pigs obtained at day 14 PI, moderately strong negative correlation was observed in the thymus (r = - 0.62) and brain stem (r = - 0.48), suggesting the capability of host animal to clear PRRSV from the systemic circulation appears to be related to the viral activity in the thymus and brain stem.

Keywords : PRRSV, Quantitation, Serum, Tissue, Viral load

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is characterized by the late-term abortion of the sows and the respiratory distress of the pigs in the nursery and grower[5, 28, 29]. The PRRSV infection of young pigs generally manifests multisystemic lesions characterized by interstitial pneumonia, lymphadenopathy, conjunctivitis, ocular edema, vasculitits, myocarditis, and mild encephalitis [3, 5, 20]. Several lines of evidence have suggested that the clinicopathologic pictures are directly linked to active virus replication in specific organs, as examined by virus isolation, immunohistochemistry, and in situ hybridization[8, 11, 12, 15, 17, 21, 26, 27].

PRRSV is well known to have a strong predilection for monocyte/macrophage-lineage immune cells[18]. Immunohistochemistry and in situ hybridization studies have shown that PRRSV antigen or RNA exists in resident tissue macrophages such as the lung, liver, heart, kidney, brain, and lymphoid organs including lymph nodes, tonsil, thymus, and spleen[11, 12, 15, 21, 26, 27]. Although PRRSV antigen or RNA has been detected in cell types other than macrophages, such as endothelial cells, pneumocytes, bronchiolar epithelium, and testicular germ cells, the virus replication in porcine cells has only been observed in primary monocyte/macrophage-lineage cells including PAM, monocyte-derived macrophages, microglia, and splenic macrophages in vitro[18, 22]. The preferential replication of PRRSV in monocyte/macrophage-lineage cells suggests that PRRSV can establish infections in any type of tissues that contain resident macrophages.

Monocytes/macrophages are highly heterogeneous morphologically and functionally. Resident macrophages in different organs may be continuously affected by systemic or local physiopathologic, immunologic environments, so that macrophage susceptibility to PRRSV infection may reside in a distinctive stage of functional, morphologic maturation and differentiation of macrophages. Several studies have suggested that macrophage tropism of PRRSV are restricted within only some subpopulations representing some specific states of differentiation and activation (C.S. Choi et al. 1994. Proc.13th Congress IPVS, Bangkok, Thailand, p 67)[7, 18]. Restricted tropism for subpopulations of macrophages by PRRSV is suggestive of a quantitatively heterogeneous distribution of the virus in different organs or tissues.

Quantitative evaluation of viral activity provides valuable information for monitoring of disease progress, antiviral therapy, and disease prognosis because disease progression in general correlates to viral activity[4]. In addition, quantitative

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evaluation of viral activity in systemic circulation or local sites would play a contributory role in the better understanding of virus-induced clinical and pathologic manifestations as well. In a study on the quantitative relationship of systemic virus concentration on growth and immune response in pigs, it was shown that the serum concentration of PRRSV was quantitatively related to body weight gain and feed intake[10].

Quantitative information on PRRSV infectivity or infected cells in systemic or local body compartments has been obtained by infectivity titration assay, in situ hybridization and immunohistochemistry. Previously, we were able to establish PCR-based quantitative assay for estimating PRRSV load in semen[25]. In this study, the PCR-based quantitative assay for viral load in sera and tissues was applied to assess the rate of PRRSV clearance in systemic circulation, and to gain insights into relationship between viral clearance rate and viral RNA load in different tissues.

Materials and Methods

Collection of sera and tissues

A total of 30, 6 to 8 wk-old healthy pigs were obtained from a commercial farm herd seronegative for PRRSV. Animals were housed in the isolation facility, College of Veterinary Medicine. Animals were fed and well cared for following guidelines according to the Institutional Animal Care and Use Committee (IACUC), University of Minnesota. Twenty-five pigs were infected intranasally with 10^5 $TCID_{50}$ of PRRSV VR-2332 per pig, and five pigs served as uninfected controls. Serum samples were collected at 0, 2, 5, 7, 10, 12, and 14 days postinfection (dpi), and stored at -70℃. Animals were anethesized with Tilazol, and euthanized with overdose of pentobarbital sodium at 14 dpi. Following exanguination, tissue samples including lung, liver, heart, kidney, thymus, spleen, tracheobronchial lymph node (TBLN), tonsil, eye, brain, brain stem, and adrenal gland were placed into TRIzol reagent (Gibco BRL, Grand Island, NY), immediately frozen in dry ice/ethanol bath, and kept frozen at -70℃.

Nucleic acid isolation from sera and tissues

Isolation of nucleic acids from sera was performed using guanidium thiocyanate (GTC)/silica method according to Boom et al. (1990)[2]. Briefly, 0.1 ml of serum was mixed with 0.9 ml of lysis buffer (5 M GTC, 0.1 M Tris-HCl, 25 mM EDTA, 2% Triton X-100, final pH, 6.4). After 1 cycle of freezing and thawing of the mixture, 0.05 ml of 2 M sodium acetate, pH 4.0 and 0.02 ml of size-fractionated silica particle was added to the mixture and vortexed for 5 s. After 10 min incubation at room temperature, tubes were centrifuged at 14,000×g for 30 s. Supernatants were promptly disposed and silica-nucleic acid pellets were washed once with washing buffer (5 M GTC, 0.1 M Tris-HCl, final pH, 6.4), once with 70% ethanol (ice-cold), and once with acetone (ice-cold). Silica-nucleic acid pellets were dried at 50℃ for 10 min. Nucleic acid was eluted with 0.1 ml of 0.1% diethyl pyrocarbonate (DEPC)-treated water containing 20 U/ml of RNase inhibitor at 56℃ for 10 min. Ten microliter of eluted nucleic acids solution was used in RT-PCR.

Isolation of total RNA from tissues was performed using TRIzol reagent according to the manufacturer's instruction. The isolated total RNA was further treated with 0.1 ml of 0.5 U/ml DNase I solution (Boehringer Mannheim, Indianapolis, IN) and reisolated using GTC/silica method as described above. The quantity of total tissue RNA was determined using a spectrophotometer at a wavelength of 260. Microgram of RNA was aliquoted in 0.1 ml DEPC-treated water, and 100 ng RNA was used in RT-PCR.

Quantitative competitive RT-PCR

A QC-RT-PCR for the quantitation of PRRSV RNA in sera and tissues was performed using slightly modified method described previously[25]. Briefly, wild-type PRRSV RNA and synthetic competitor RNA (1,000 copies/reaction) were co-reverse transcribed with 0.2 μM of gene-specific primer, VR7.3 (antisense, 5'-TGA CGC GGA TCA GGC GCA C-3') in the presence of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.25 mM $MgCl_2$, 0.2 mM each dNTP, 500 μg/ml trehalose (Sigma, St. Louis, MO), 25 μg/ml cresol red, 10 U RNase inhibitor, and 20 U SuperScript RNase H- reverse transcriptase (Gibco BRL, Grand Island, NY) in a 0.5 ml PCR tube for 45 min at 50℃. After inactivation of reverse transcriptase at 95℃ 5 min, 2 μl of primer-polymerase mixture containing 50 pmol each of PCR primers (VR7.1.1, sense, 5'-ATG GCC AGC CAG TCA ATC A-3'; VR7.2.2, antisense, 5'-CGG ATC AGG CGC ACA GAT ATG-3') and 0.25 U AmpliTaq Gold (Perkin-Elmer, Foster city, CA) were added into the reaction tubes. After activation of *Taq* polymerase at 95℃ for 9 min, amplification reaction was performed for 45 times at 94℃ for 1 min and 62℃ for 2 min. All reactions were performed using Perkin-Elmer Model 480 thermocycler.

Synthetic competitor RNA was prepared as previously described[25]. The copy number of synthetic competitor RNA molecules was calibrated using calibrator cDNA obtained from a plasmid vector which contains an insertion of PRRSV open reading frame 7 gene. Diluted competitor RNA was mixed with 250, 500, 2500, 5,000, 25,000 and 50,000 copies of double-stranded calibrator cDNA in a separate tube and RT-PCR was performed as described below. Based on calibration data, 10,000 copies/tube of synthetic competitor RNA was aliquoted and stored at -70 ℃. A standard curve (X = log wild-type-band density vs. competitor-band density ratio; Y=log initial number of wild-type RNA) was generated QC-RT-PCR assay.

Infectivity titration

Serial 10-fold dilution of sera was made in RPMI-1640 medium containing 10% fetal bovine serum and 100 μg/ml gentamicin. Inoculum was added onto CL-2621 cell monolayers seeded in 96-well microtiter plates. Infectivity titer was calculated according to Reed and Muench (1938)[19], and expressed as median tissue culture infectious dose/ml.

Statistical analysis

Regression analysis was used to estimate the rate of viral clearance in systemic circulation using infectivity titers and copy numbers of viral RNA in sera from days 5 to 14 postinfection. Pearson correlation analysis was performed to estimate the extent of correlation between copy numbers of viral RNA and infectivity titer in sera, and between copy numbers of viral RNA in sera and tissues. The extent of correlation was interpreted based on correlation coefficient: strong ($r \geq 0.7$), moderately strong ($0.4 \leq r < 0.7$), weak ($0.2 \leq r < 0.4$), and no correlation ($r < 0.2$).

Results

Generation of a standard curve by QC-RT-PCR

Previously we demonstrated that a simplified standard curve method using RT-nested PCR for the quantitation of PRRSV RNA could be achieved a single tube reaction for each sample. The same approach was employed in this study using RT-PCR instead of RT-nested PCR. Competitive RT-PCR was performed with the known number of copies of PRRSV RNA and 1,000 copies of competitor RNA. Inverted image with distinct sizes of amplified products from wild-type and competitor RNA was obtained from ethidium bromide-stained analytical agarose gel (Fig. 1, panel A).

A least-squares regression line with $y = 3.185 + 1.877x$ (r^2=0.91) was obtained from log band intensity ratio (X) of wild-type over competitor versus log initial copy number of wild-type RNA (Y) (Fig.1, panel B), and used as a standard curve for quantitation of PRRSV RNA in sera and tissues. Comparable sensitivity and accuracy could be achieved by QC-RT-PCR over 100 copies of wild-type RNA compared to QC-RT-nested PCR (Fig.1).

Quantitation of PRRSV RNA in serum samples and estimation of viral clearance rate in systemic circulation

To determine the copy number of PRRSV RNA in sera obtained from 5 uninfected and 5 infected pigs at day 0, 2, 5, 7, 10, 12, and 14 postinfection, competitive RT-PCR was performed using isolated RNA from 0.01 ml equivalent volume of serum and 1,000 copies of competitor RNA. The copy number of PRRSV RNA in each serum were calculated based on band intensity ratio obtained from inverted gel image (Fig. 2, panel A). The mean copy number per ml of serum at each time point was plotted (Fig. 2, panel B). The copy number of PRRSV RNA peaked at day 5 postinfection in 4 out of 5 infected pigs. The mean number of PRRSV RNA in sera from 5 infected pigs reached 5.2×10^8 copies/ml of serum. The maximum quantity was 8.6×10^8 copies/ml when 25 infected pigs were examined. Thereafter, a continuous decrease in the copy number of PRRSV RNA was observed until the termination of the experiment at day 14 postinfection. The infectivity titration performed on CL-2621 cell monolayers using the same serum samples showed a peak titer at day 5 and a decrease in the titer thereafter (Fig. 2).

To determine the rate of PRRSV clearance in systemic circulation, viral RNA quantity and infectivity titer in sera were analyzed by regression analysis. Regression analysis of the quantity data and postinfection days from days 5 to 14 postinfection showed that the number of viral RNA was cleared away from serum at the rates of 0.308 on a log10 scale per day (Fig. 2, panel B). Viral infectivity titer was decreased at the rate of 0.183 on a log10 scale per day. In other words, it took 3.2 or 5.6 days for the infected pigs to reduce one log10 copy number of viral RNA or infectious virus, respectively. From the equations using mean values, the estimated time point when undetectable (zero) level of viral RNA and infectious virus in one milliliter of sera was predicted to be 31.3 and 17.5 days postinfection, respectively (Fig. 2). When regression analysis was performed using viral RNA quantity data obtained from a total of 25 infected pigs, it showed that viral RNA was cleared away at the rate of 0.37 day on a log10 scale (i.e., 2.7 days to reduce one log10 copy number of viral RNA), with predicted days of 27.2 at which infected pigs become free of viral RNA in one milliliter of serum (data not shown).

The QC-RT-PCR assay could provide sensitive and effective means of estimating the rate of viral clearance in serum with wide range of quantitative values (up to 109 copies vs. 104 TCID50) and greater r square value (0.94 vs. 0.62) than infectivity titration (Fig. 2).

Estimation of clearance rate of viral RNA in pigs grouped by the level of viremic states at day 14 postinfection

As shown in Fig. 2, pig no. 10, some animals showed different level in viral RNA concentrations at 14 dpi. To examine the rate of viral clearance in some pigs showing relatively high level of viral RNA concentrations, 4 out of 25 infected pigs, showing two distinct values with at least 2 orders of magnitude in the copy number of viral RNA in serum at 14 dpi, were selected and grouped into high and low viremic pigs. The rate of viral clearance for each group was determined (Fig. 3, panel A and B). The mean rates of viral RNA clearance in serum samples were 0.16 (slow rate) and 0.49 (fast rate) for each of high and low viremic groups, respectively.

Quantitation of PRRSV RNA in tissue samples

QC-RT-PCR was performed for the quantitation of PRRSV RNA in tissues from 12 distinct anatomical sites per animal.

A total of 60 individual tissue samples were obtained from 2 uninfected and 4 infected pigs grouped into fast and slow rates of viral clearance as determined in Fig. 3. All of 24 individual tissue samples from uninfected pigs were negative for PRRSV RNA with the mean numerical value of 0.26 ± 0.21 copies/100 ng tissue RNA. Tissue samples from infected animals showed a wide range of copy number of PRRSV RNA from negative to 1065 copies/μg tissue RNA (Fig. 4).

Tonsil, lung, TBLN, and adrenal gland tissues showed the highest copy number of PRRSV RNA (≥104 mean number of copies/μg RNA), whereas the least copy number of PRRSV RNA were found in kidney, brain, and brain stem tissue samples (≤102 mean number of copies/μg RNA) in infected pigs (Fig. 4), irrespective of the rates of viral clearance in sera (Fig. 3). However, spleen, eye, and thymus tissue samples from pigs with the slow rate of viral clearance in sera showed a difference in the copy number of PRRSV RNA with at least 1.5 order of magnitude at the mean level, suggesting these sites may relate to the clearance rate in systemic circulation. Since tissues taken from the liver and kidney, which are closely associated with the circulation system, showed extremely low number of copies of PRRSV RNA in infected animals, a possibility of contamination with virus or infected cells from blood source during tissue sampling might be scarce.

Correlation analysis with the rate of viral clearance and viral RNA load in selected tissues

To determine whether the rate of viral clearance correlates to viral RNA load in selected tissues of the eye, brain stem, thymus, spleen, and tonsil, tissues taken from 9 infected animals were examined for viral RNA by QC-RT-PCR. The copy number of viral RNA in serum sample from the corresponding pig was also obtained, and the rate of viral RNA clearance in individual animals was calculated by regression analysis (Table 1).

Correlation analysis between the rate of viral RNA clearance and viral RNA load in serum and tissue samples obtained at day 14 postinfection showed negative correlation coefficients (Table 2). Moderately strong inverse correlation between the rate of viral RNA clearance and viral RNA load in tissue samples was observed in the thymus (r = - 0.62), and to a lesser extent, in the brain stem (r = - 0.48) (Table 2). There was very week correlation with the rate of viral RNA clearance (r = -0.05), although the copy number of PRRSV RNA in the tonsil was highest. Correlation coefficient among viral RNA loads of different tissues was also obtained (Table 2). Strong positive correlation was observed between viral RNA loads in thymus and spleen tissue samples (r=0.78). The copy numbers of PRRSV RNA in tissue samples from the brain stem showed strong correlation with those of the spleen (r=0.70) and of thymus (r=0.68). Taken together, the correlation coefficient data suggested that the slower rate of PRRSV clearance in systemic circulation and the higher viral load in the brain stem may coincide with higher viral load in the thymus.

Discussion

The quantitative evaluation of PRRSV RNA concentrations and infectivity titers in sera from infected pigs during the acute phase of infection clearly indicates that most infected pigs initiated virus clearance from systemic circulation at day 5 postinfection. The rate of virus clearance from 5 to 14 dpi had a linear relationship with postinfection days as determined by both QC-RT-PCR and infectivity titration assays. The quantitative information on PRRSV RNA concentrations in different tissues indicates that clinical signs or pathologic lesions may directly relate to virus replication in particular organs. As it was reported previously[13, 16, 17], the tonsil, lung, and TBLN were the most preferred sites for PRRSV replication in young pigs. The highest virus load in the tonsil, lung, and TBLN may explain why respiratory distress is the major clinical symptom in acutely affected young pigs, although the mechanism of the clinicopathology remains unresolved. The brain and brain stem showed the most restricted sites of PRRSV spread in the body. The CNS lesions in PRRSV-infected pigs, largely described as perivascular cuffings, have been inconsistently observable[5, 13]. The virus load in the liver and kidney was as low as that in the CNS. This may be the reason why obvious clinicopathologic symptoms in the liver and kidney have rarely been described in the literature[6]. An interesting finding in this study was relatively higher virus load in the heart and eye. Myocarditis, conjunctivitis and ocular edema have frequently been described in PRRSV-infected young pigs[3, 13]. Depending on the age, physiologic status, and genetics of the host, involvement of secondary infections, virus strains, or time postinfection, there may exist altered patterns of PRRSV load in different anatomical locations and clinicopathologic outcomes as well[6, 13, 14, 23]. The correlation analysis using the rates of viral RNA clearance and viral RNA concentrations in tissue samples suggests that the delayed rate of viral clearance from the systemic circulation appears to coincide with higher viral load in the thymus. PRRSV antigen and RNA in the thymic medula and cortex have been demonstrated by immunohistochemistry and in situ hybridization[13, 16, 20, 26]. Pathogenetic role of PRRSV infection in the thymus is still in question. In a study on in utero infection by PRRSV, most striking effects were found in the thymus of infected piglets[9]. It was shown that PRRSV-infected piglets had thymic lesions of cortical involution, nearly complete disappearance of cortical thymocytes in some piglets causing a 52% reduction in thymic weights[9].

It is unknown how PRRSV is cleared from the circulation during the acute phase of infection. Humoral immunity may not play an important role in the virus clearance during

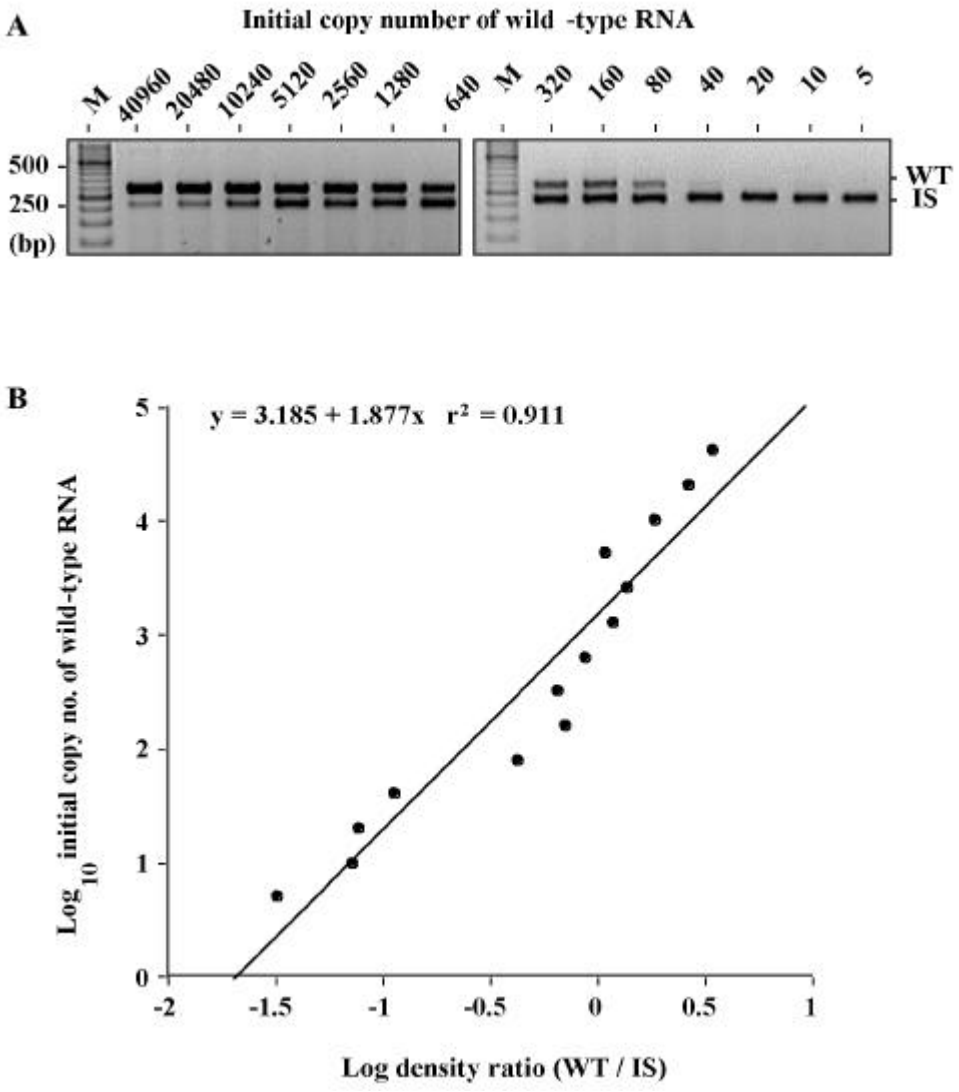

Fig. 1. Generation of standard curve by QC-RT-PCR for quantitation of PRRSV RNA. PRRSV RNA was isolated from two-fold serial dilutions of PRRSV VR 2332. Each of isolated viral RNA was co-reverse transcribed and co-amplified with 1,000 copies of competitor RNA internal standard for 45 cycles. **A**. Ethidium bromide-stained gel, inverse image. Upper band: wild-type PRRSV RNA (WT); lower band: internal standard competitor RNA (IS). **B**. Standard curve plot. A standard curve with y = 3.185 + 1.877x (r2=0.91) was obtained by regression analysis.

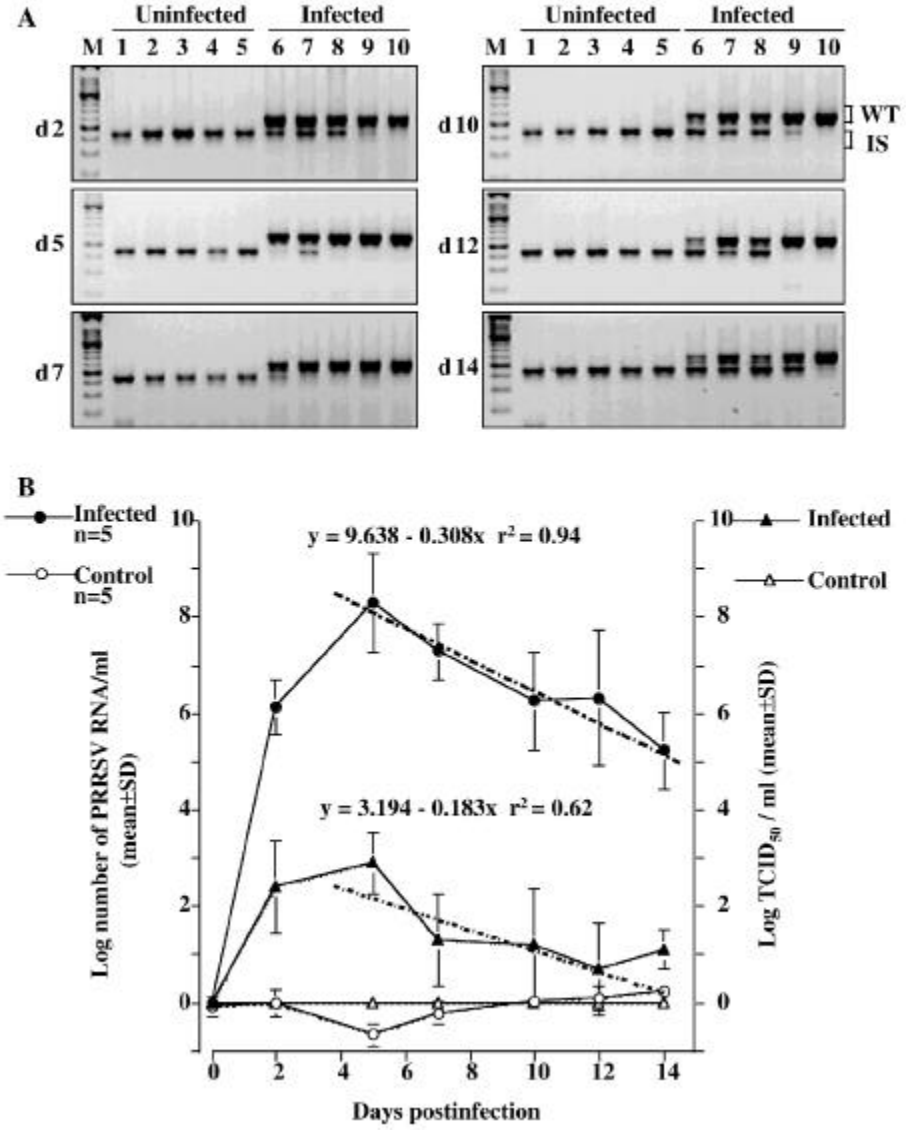

Fig. 2. Quantitation of PRRSV RNA in serum sample and estimation of clearance rate of viral RNA and infectious virus. Five pigs were infected with PRRSV VR-2332, and 5 pigs were uninfected control. Serum sample was collected at days 0, 2, 5, 7, 10, 12, and 14 postinfection. QC-RT-PCR and infectivity titration were performed as described in *Materials and Methods*. **A**. Inverted image of ethidium bromide-stained gel obtained by competitive RT-PCR. **B**. A line plot for the copy number of PRRSV RNA per ml equivalent volume of serum sample and infectivity titer expressed as median tissue culture infectious dose/ml serum sample. Regression analysis was performed to estimate the rate of viral clearance in systemic circulation using infectivity titers and copy numbers of viral RNA in serum samples from days 5 to 14 postinfection.

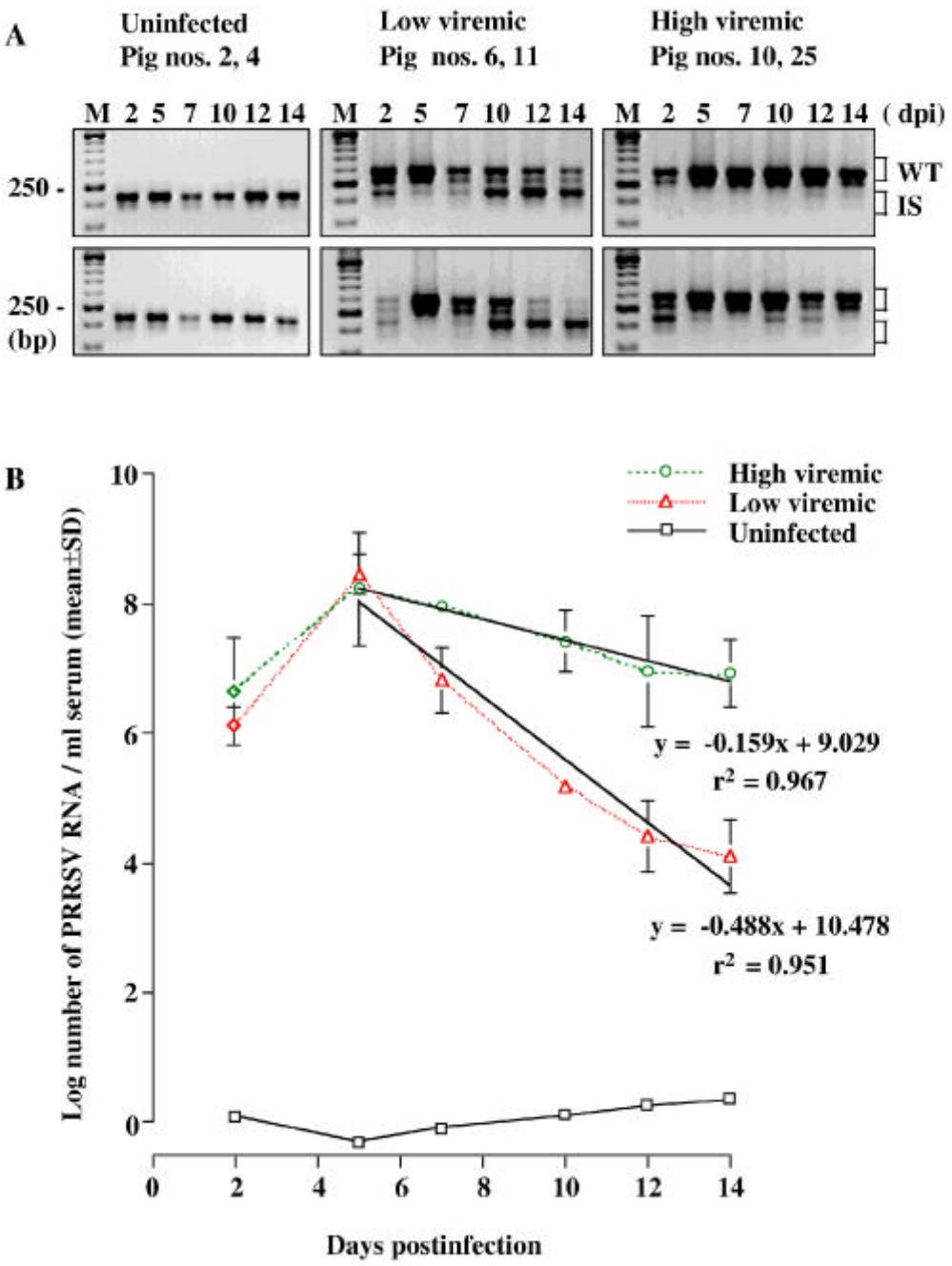

Fig. 3. Estimation of clearance rate of viral RNA in systemic circulation in infected pigs grouped by the level of viremia at day 14 postinfection. Four infected pigs, showing two distinct values with at least 2 orders of magnitude in copy number of viral RNA in serum sample at day 14 postinfection were selected and grouped into high and low viremic pigs. The rate of viral RNA clearance for each group was determined from the slope of regression equation. Serum samples from 2 uninfected pigs were included to validate the specificity of competitive RT-PCR. **A**. Ethidium bromide-stained gel obtained from competitive RT-PCR. **B**. Line plots and regression equations for each group.

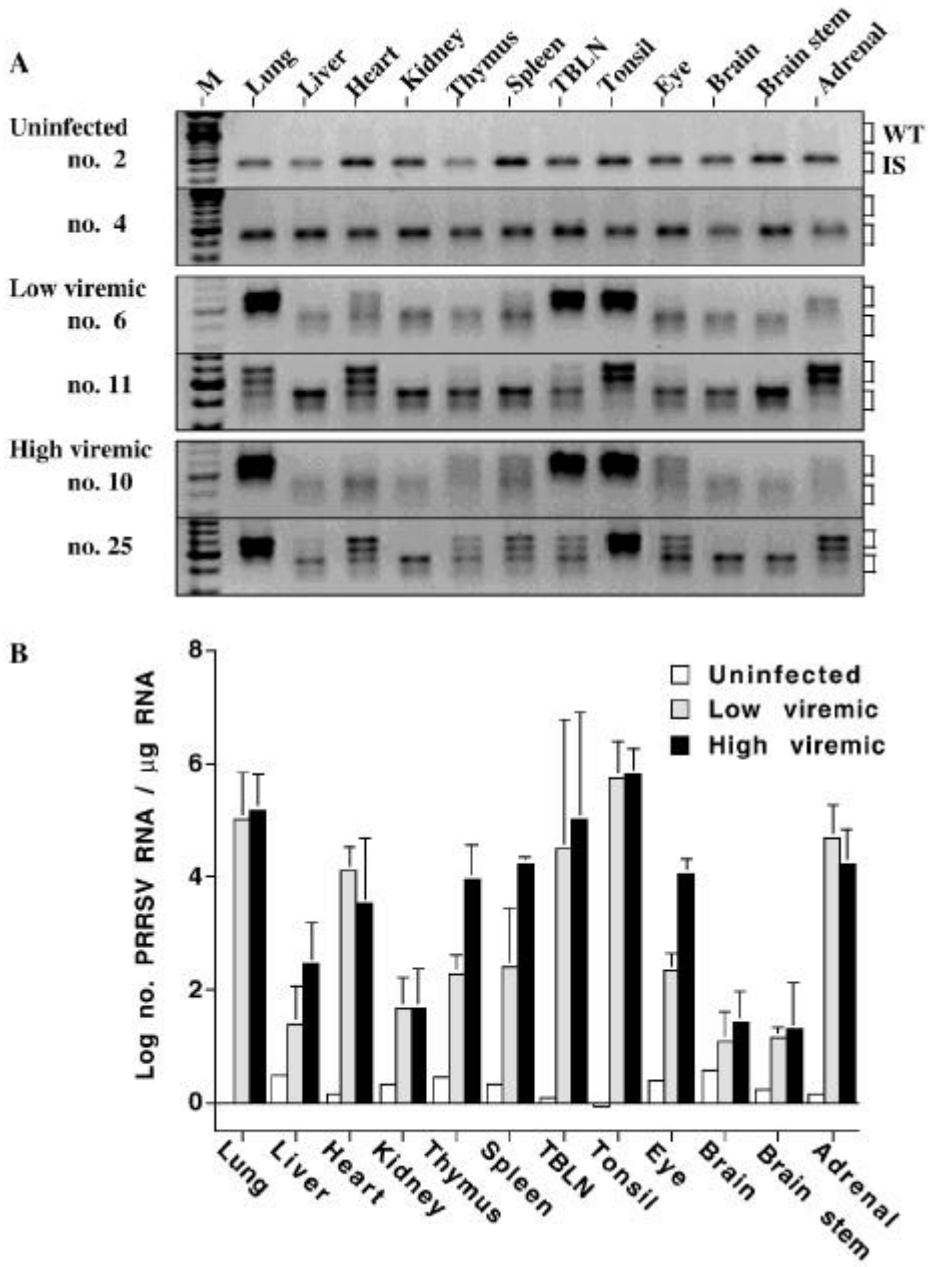

Fig. 4. Quantitation of PRRSV RNA in tissues of infected pigs grouped by the level of viremia at day 14 postinfection. All tissue samples were obtained at day 14 postinfection. RNA isolation and QC-RT-PCR were performed as described in *Materials and Methods*. **A**. Ethidium bromide-stained gel obtained from competitive RT-PCR. **B.** A histogram for tissue distribution of PRRSV RNA in 2 infected controls, 2 infected pigs with slow rate of virus clearance (high viremic), and 2 infected pigs with fast rate of virus clearance (low viremic).

Table 1. PRRSV RNA load in sera and tissues from 9 infected pigs with PRRSV VR-2332

Pig ID	Systemic viral RNA clearance rate*	Log10 number of copies of PRRSV RNA per 10 μℓ serum or 100 ng tissue RNA at day 14 postinfection					
		Serum	Eye	Brain stem	Thymus	Spleen	Tonsil
6	0.40	2.50	1.31	-0.02	1.13	1.81	5.98
9	0.28	3.29	1.06	0.17	3.84	4.19	5.56
10	0.22	4.55	2.70	1.26	3.37	3.29	5.68
11	0.57	1.70	1.49	-0.06	1.95	0.93	5.13
13	0.63	0.90	4.47	-0.01	2.20	1.85	5.68
15	0.59	3.81	4.47	0.34	2.39	4.33	5.81
22	0.32	4.03	3.51	1.12	6.02	6.27	5.78
23	0.75	0.50	-0.05	-0.34	1.38	2.17	5.80
25	0.13	5.28	3.30	-0.10	3.67	1.94	5.69

*The clearance rate of PRRSV RNA in serum of each infected pig was obtained from the slope of regression equation, the values of which represent logarithmic reduction of viral RNA concentrations in ml of serum per day.

Table 2. Correlation analysis using the rate of PRRSV RNA clearance and the viral RNA concentrations in serum and selected tissue samples at day 14 postinfection

	Clearance rate	Serum	Eye	Brain stem	Thymus	Spleen	Tonsil
Clearance rate	-	-0.98	-0.14	-0.48	-0.62	-0.29	-0.05
Serum	-0.98	-	0.40	0.58	0.64	0.47	0.14
Eye	-0.14	0.40	-	0.38	0.36	0.31	0.14
Brain stem	-0.48	0.58	0.38	-	0.68	0.70	0.11
Thymus	-0.62	0.64	0.36	0.68	-	0.78	-0.02
Spleen	-0.29	0.47	0.31	0.70	0.78	-	0.32
Tonsil	-0.05	0.14	0.14	0.11	-0.02	0.32	-

*Data represent Pearson correlation coefficient.

the acute phase of infection, since no detectable amounts of virus neutralizing antibodies were present in any of the sera obtained. Lymphoproliferation response to PRRSV has been demonstrated, but only later than 2 weeks postinfection, peaking at 4 and 7 weeks postinfection[1]. It remains to determine whether cytotoxic T lymphocytes (CTL) are necessary and sufficient for the virus clearance during the acute phase of infection, and whether the augmented virus load in the thymus is associated with the frequency or functions of CTL. A study on the peripheral blood lymphocytes of PRRSV infected specific pathogen-free pigs reported abnormal changes of T-cell subpopulation with reduced CD4+ cells (3 to 14 dpi) and remarkable increase of CD8+ cells . Although it was not demonstrated whether the CD8+ cells were specific to PRRSV, the increasing frequency of CD8+ cells during the convalescent stage of infection was suggestive of the possible role of CTL in the viral clearance.

The quantitative evaluation of PRRSV by a PCR-based method obviously has an additive value in the area of molecular diagnosis and viral pathogenesis study. A simple demonstration of the presence or absence of PRRSV RNA in specific tissues may have limitations in explanation of complex mechanisms of disease manifestation. Further understanding of the virus survival mechanisms in specific cell subsets and tissues, together with the strategies of the host to protect against the virus infection will be a promising advantage for the future development of more effective preventive and therapeutic regimens.

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