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Comparison of respiratory pathogenesis of porcine reproductive and respiratory syndrome virus isolates *in vitro* and *in vivo*

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Abstract : Respiratory pathogenic effects of several porcine reproductive and respiratory syndrome virus(PRRSV) isolates were examined in swine tracheal ring(STR) cultures by examining their effect on ciliary activity. One high and one low pathogenic PRRSV isolates were then selected and their pathogenicity investigated in 3-week-old conventional PRRSV-seronegative pigs. Ten pigs each were inoculated intranasally with the high or low pathogenic PRRSV isolate and 6 pigs were sham inoculated as negative controls. Two pigs each from the inoculated group and one pig each from negative control group were killed on 4, 7, 14, 21 and 28 days post-inoculation(PI). At necropsy, degrees of gross lung lesion was determined. Turbinate, tonsil, trachea and lung samples were collected for virus isolation or histopathology. Gross lung lesions were observed mainly on 14 days PI with high and low pathogenic isolates inducing moderate diffuse and mild gross lung lesions, respectively. Inoculation of either the high or low pathogenic virus resulted in loss of cilia in ciliated epithelium of turbinates and trachea between 7 and 28 days PI. High pathogenic virus caused increased number of Goblet cells in the tracheal epithelial layer between 4 and 21 days PI whereas the low pathogenic virus did it between 14 and 28 days PI and with a lesser degree. Although both viruses produced interstitial pneumonia, the lesion was less severe with the low pathogenic virus. The isolation of high pathogenic virus from tissues and sera was earlier and more consistent than that of the low pathogenic virus. The agreement between *in vitro* and *in vivo* tests indicates that STR cultures may be used as a routine method to determine the respiratory pathogenicity of PRRSV isolates.

Key words : PRRSV, respiratory pathogenesis.

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Introduction

Porcine reproductive and respiratory syndrome(PRRS) has been recognized as an important pig disease worldwide. It is caused by porcine reproductive respiratory syndrome virus (PRRSV) which is classified as a member of the arterivirus group[1]. The pathogenesis of PRRSV has been well documented in pregnant sows and in 2- to 10-week-old pigs. The virus induces reproductive failure at all stages of gestation in pregnant sows and causes chronic respiratory disease in young pigs[2,3]. The respiratory problems are manifested by chronic pneumonia, neonatal losses due to respiratory and systemic disease, and retarded growth from the nursery through the finishing stages of pigs[2~4], but vary in severity from farm to farm. Studies suggest that PRRSV isolates may cause respiratory disease of different severity in conventional pigs[5~7]. Although respiratory pathogenicity of PRRSV isolates can be determined by inoculating them into pigs, such experiments are costly and cumbersome.

We believe that an *in vitro* method which utilized swine tracheal ring(STR) cultures to measure respiratory virulence of PRRSV isolates would be rapid and less expensive. The STR cultures have been employed to study the respiratory pathogenic effects induced by different viruses and bacteria[8,9]. The present study was undertaken to compare the respiratory pathogenesis of PRRSV isolates *in vitro* and *in vivo*.

Materials and Methods

PRRSV isolates : Several PRRSV isolates were inoculated in STR cultures and their effect on ciliary activity was observed. Two PRRSV isolates were selected; one with high(isolate 110; STR score 4.0) and one with low pathogenicity(isolate 135; STR score 0.5) *in vitro*. The two viruses were propagated in MARC-145 cells[10] for inoculation in pigs.

Experimental design : Twenty-six 3-week-old conventional pigs were purchased from a PRRSV-negative Minnesota swine farm. Randomly selected 10 pigs each were housed in isolation rooms and were inoculated intranasally with high or low pathogenic PRRSV isolate(2ml each pig; $10^{4.0}$TCID $_{50}$/0.1ml). Six pigs were housed in another isolation room and served as negative controls. Following inoculation, groups of pigs were killed and necropsied as shown in Table 1. Blood samples were collected from all pigs at 0, 4, 7, 14, 21 and 28 days postinoculation(PI). At the time of necropsy, degree of gross lung lesion was determined, and the right and left lungs from each pigs were separated. The right lung, right turbinate, half tonsil, upper and lower tracheas were immersed in 10% neutral buffered formalin for 24 hours. Tissue blocks from anterior, middle and caudal lobes of the lung and the other organs were then prepared and immersed in fresh 10% neutral buffered formalin. Pieces of turbinate, tonsil, trachea and left lung samples were also collected for virus isolation.

Virus isolation and serologic test : Sera collected at known intervals were inoculated in MARC-145 cells for virus isolation[11]. Tissues(turbinate, tonsil, trachea and lung) were homogenized in Eagle's minimal essential medium(1g tissue in 2ml medium). After centrifugation, supernatants were inoculated into MARC-145 cells in 24-well tissue culture plates.

Table 1. Experimental design

PRRSV	Inoculum*/route	Days postinoculation					Total no. pigs
		4	7	14	21	28	
Isolate 110	2ml/IN	2**	2	2	2	2	10
Isolate 135	2ml/IN	2	2	2	2	2	10
Control	-	2	1	1	1	1	6

* $10^{4.0}$TCID$_{50}$/0.1ml

** No. pigs killed, IN = intranasally.

Antibody against PRRSV was detected by an indirect immunofluorescent antibody(IFA) test[12] as described previously.

Microscopic pathology and immunohistochemical test : Turbinate, trachea and lung were routinely processed in an automated tissue processor. Tissues were embedded in paraffin blocks within 72 hours of necropsy. The tissues were fixed, embedded, sectioned at 4μm, and stained with hematoxylin and eosin(HE). The sections of trachea mounted on aminoalkylsilane-coated glass slides(Sigma Chemical Co., St. Louis, MO) were used for the detection of PRRSV antigen by a streptavidin-biotin complex(ABC) immunoperoxidase method[13]. The primary antibody used was a monoclonal ascites fluid(SDOW-17 supplied by Dr. Eric Nelson, South Dakota State University, Brooklings, SD). Commercially available biotinylated goat anti-mouse antisera(Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) and peroxidase-conjugated streptavidin(Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) were utilized.

Results

Gross lesions : Lung lesions produced by low and high pathogenic isolates were first observed at 14 days PI for both groups and were significantly different at 14 days PI(3 vs 1 in score) and 21 days PI(2 vs 1 in score)(Table 2). Individual severity ranged from no lesion to moderate diffuse pneumonia for isolates 110(high pathogenicity) group and from no lesion to mild pneumonia for the isolate 135(low pathogenicity) group. The lesions were predominant in the anterior, middle, and accessory lobes and the ventromedial portion of the caudal lobes. The pneumonia was characterized by diffuse petechiasis and multifocal, tan-mottled

Table 2. Gross lung lesion scores of necropsied inoculated intranasally with a high(H) or low(L) virulent porcine reproductive and respiratory syndrome virus(PRRSV)

Pig No.	PRRSV	Days postinoculation				
		4	7	14	21	28
2/7	H	-/-				
3/5	H		-/-			
4/10	H			3/3		
1/6	H				2/2	
8/9	H					1/1
17/19	L	-/-				
13/16	L		-/-			
11/15	L			1/1		
14/18	L				1/1	
12/20	L					1/1
95/96	C	-/-				
97	C		-			
99	C			-		
98	C				-	
94	C					-

H = isolate 110(high pathogenic); L = isolate 135(low pathogenic); C = control; - = no lesion; 1 = mild; 2 = moderate; 3 = moderate diffuse.

areas, with irregular and indistinct borders(Fig 1). The isolate 110 group had more extensive pneumonia. There was no pleuritis and no grossly visible pus in airways.

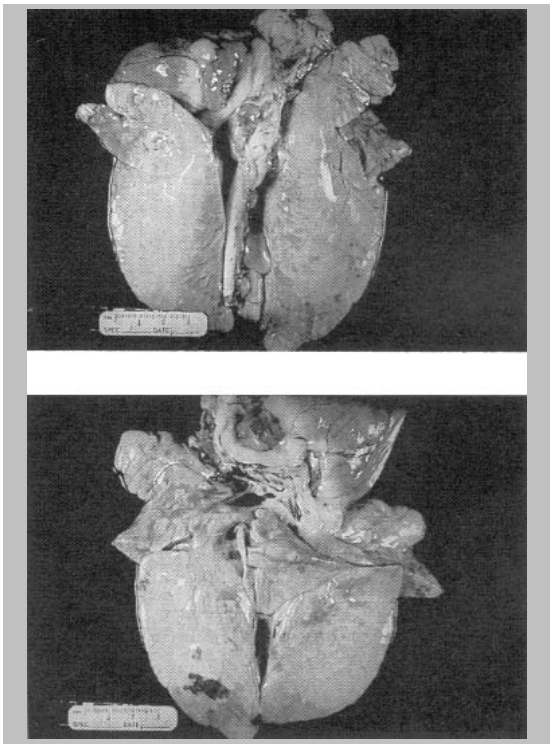

Fig 1. Lung lesion of a pig inoculated with a PRRSV, isolate 110 at 14 days PI. The pneumonia was characterized by diffuse petechiasis and multifocal, tan-mottled areas, with irregular and indistinct borders. Top ; dorsal Bottom ; ventral.

Microscopic lesions :

Turbinate : Nasal turbinate lesion was similar in type, severity and frequency in the two groups of pigs. A low number of the control pig and inoculated pigs had mild rhinitis observed at 7~28 days PI. The rhinitis was characterized by patchy dysplasia of the epithelium with loss of cilia and mild multifocal subepithelial lymphohistiocytic and suppurative inflammation with slight edema and congestion. Lesions were mild at 7~14 days PI but moderate by 28 days PI. The inflammation was most intense near the location where the ducts of submucosal mucous glands extended to the surface. Leukocytic exocytosis, especially of neutrophil, was frequently observed in dysplastic surface epithelium and along mucous ducts. By 28 days PI, the lesions had become moderate.

Trachea : Tracheal lesions was similar in type but differed in severity and frequency in the two groups of pigs. More of the isolate 110-inoculated pigs had tracheitis. Lesions of upper trachea were mild at 4~21 days PI but those of lower trachea were mild to moderate at 4~21 days PI (Table 3). In the isolate 135-inoculated pigs, lesions of upper and lower tracheas were observed later(7~28 days PI) and in lesser degree. The tracheitis was characterized by increased numbers of Goblet cells(Fig 2) with loss of cilia and multifocal infiltrate of lymphocyte, plasma cells, macrophages and neutrophils with slight subepithelial edema.

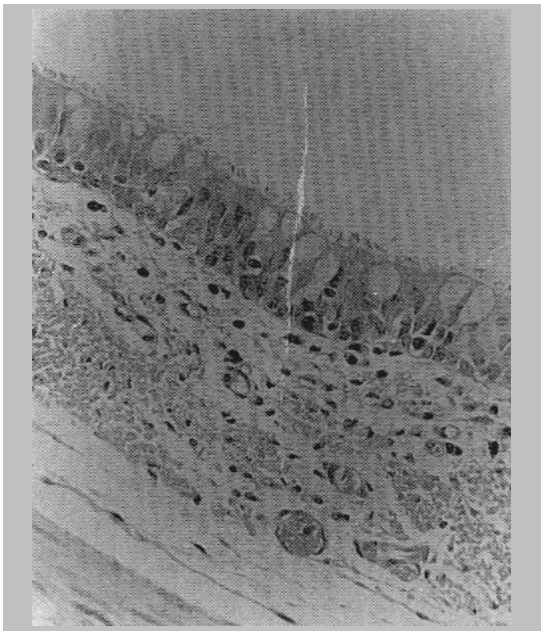

Fig 2. Tracheal lesion of the isolate 110-inoculated pig. The tracheitis was characterized by increased numbers of Goblet cells with loss of cilia.

Lung : In the isolate 110-inoculated pigs, microscopic lesions were first detected at 14 days PI. The lesions, when present, were multifocal, mild, generally at 14 days PI. The multifocal interstitial pneumonia was characterized mainly by septal thickening with mononuclear cells, type 2 pneumocyte hypertrophy and hyperplasia, and accumulation of normal and necrotic macrophages in alveolar spaces. These changes were present from 14 to 28 days PI. Mild peribronchiolar and perivascular lymphohistiocytic cuffing was

Table 3. Microscopic lesion scores of tissues from pigs inoculated intranasally with a high(H) or low(L) respiratory virulent porcine reproductive and respiratory syndrome virus(PRRSV)

Pig No.	PRRSV	DPI	Trachea				Lung lobes[a]		
			Upper		Lower		Cranial	Middle	Caudal
			G	L	G	L			
2/7	H	4	-/-	1/-	1/3	-/-	-/-	-/-	-/-
3/5	H	7	-/-	-/2	1/-	-/3	-/-	-/-	-/-
4/10	H	14	1/-	-/-	1/1	-/-	1/1	1/2	1/1
1/6	H	21	2/1	-/-	2/-	-/-	1/1	1/1	1/1
8/9	H	28	-/-	-/-	-/-	-/-	1/-	1/1	-/1
17/19	L	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-
13/16	L	7	-/-	-/2	-/-	-/1	-/-	-/-	-/-
11/15	L	14	-/1	1/-	-/2	1/-	1/-	1/1	1/-
14/18	L	21	1/-	-/-	1/-	-/-	1/-	1/-	-/-
12/20	L	28	1/-	-/-	1/-	-/-	1/1	1/1	1/1
95/96	C	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-
97	C	7	-	-	-	-	-	-	-
99	C	14	-	-	-	-	-	-	-
98	C	21	-	-	-	-	-	-	-
94	C	28	-	-	-	-	-	-	-

[a] proliferative interstitial pneumonia, DPI = days of postinoculation; L = loss of cilia, G = increased numbers of Goblet cells, H = isolate 110(high pathogenic), L = isolate 135(low pathogenic), C = control, - = no lesion, 1 = mild, 2 = moderate, 3 = severe.

observed in most pigs examined at 14 days PI but had resolved by 28 days PI. Lung lesions were seldom observed in sections taken from the caudal lung lobe. The isolate 135-inoculated pigs had microscopic lung lesions very similar in distribution, type, and severity to those of the isolate 110-inoculated pigs. Lung lesions were first observed at 7 days PI and persisted throughout the 28-day period(Table 3). The most severe lesions were seen in pigs necropsied at 14 days PI but were less consistent and less severe than those observed in the isolate 110-inoculated pigs.

Virus isolation and serology : PRRSV was isolated from sera from 4 to 21 days PI(Table 5) and from the turbinate, tonsil, trachea and lung from 4 to 28 days PI(Table 4) from both groups of inoculated pigs. High pathogenic PRRSV was consistently isolated earlier from tissues and sera as compared to low pathogenic PRRSV. All pigs were negative for PRRSV antibody prechallenge and remained so through 7 days PI. By 14 days PI, nearly all pigs were seropositive, with antibody titers ranging from 1 : 16 to 1 : 256 (Table 5). No PRRSV antibody was detected in control pigs.

Immunohistochemical detection : Specific viral antigen was observed by ABC technique in trachea(Fig 3). Intense and specific staining was observed in the cytoplasm of infected cells located in ciliated epithelium of trachea. Comparison with hematoxylin and eosin-stained sections from the same block indicated that most of the cells were macrophages.

Table 4. Virus isolation from tissues of pigs inoculated intranasally with a high(H) or low(L) respiratory virulent porcine reproductive and respiratory syndrome virus(PRRSV)

Pig No.	PRRSV	Days PI	Turbinate	Tonsil	Trachea	Lung
2/7	H	4	+/+	+/+	-/+	+/+
3/5	H	7	+/+	+/+	+/+	-/+
4/10	H	14	+/+	+/+	+/+	+/+
1/6	H	21	-/-	-/-	-/+	+/+
8/9	H	28	-/-	-/-	+/-	+/-
17/19	L	4	+/-	+/-	+/-	-/-
13/16	L	7	+/-	+/NT	-/-	-/-
11/15	L	14	-/-	+/+	+/-	+/+
14/18	L	21	-/-	-/+	-/+	+/+
12/20	L	28	-/-	-/+	+/-	+/+
95/96	C	4	-/-	-/-	-/-	-/-
97	C	7	-	-	-	-
99	C	14	-	-	-	-
98	C	21	-	-	-	-
94	C	28	-	-	-	-

H = isolate 110(high pathogenic), L = isolate 135(low pathogenic), C = control, + = positive PRRSV isolation, - = negative PRRSV isolation, NT = not tested.

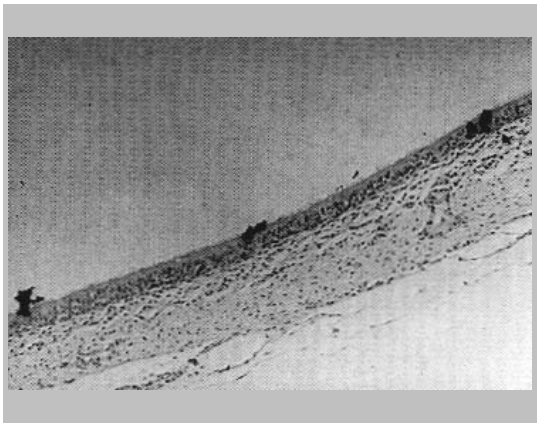

Fig 3. Immunohistochemical stain of swine trachea infected with PRRS virus(isolate 110) at 7 days PI. PRRS viral antigen in cytoplasm of macrophages in tracheal epithelial layer(arrow). ABC staining with hematoxylin counterstain.

Discussion

The results indicate some agreement between *in vitro* and *in vivo* tests in determining respiratory pathogenicity of PRRSV isolates. *In vitro* pathogenic effects of different PRRSV isolates were scored in swine tracheal ring(STR) cultures by examining the ciliary activity. The STR culture examination has been suggested as a method to determine respiratory pathogenicity of PRRSV isolates[14].

Specific and consistent, experimentally reproductible gross lung lesions have often not been observed in PRRSV-inoculated pigs[7,15~17]. However, it has been proposed that PRRSV infection might impair the respiratory clearance mechanism and decrease resistance to bacterial infection[18]. The 3-week old commercial pigs with intranasal inoculation appear to be a practical model for reproduction of mild to moderate gross and microscopic lung lesions and for studying differences in respiratory pathogenicity. Using this model, we found significant differences in pathogenicity between

Table 5. Porcine reproductive and respiratory syndrome virus(PRRSV) isolation/indirect immunofluorescence antibody(IFA) detection from sera collected at different days of postinoculation

Pig No.	PRRSV	Days of postinoculation					
		0	4	7	14	21	28
2	H	-/〈16	+/〈16*				
7	H	-/〈16	+/〈16*				
3	H	-/〈16	+/〈16	+/〈16*			
5	H	-/〈16	+/〈16	+/〈16*			
4	H	-/〈16	+/〈16	-/〈16	+/256*		
10	H	-/〈16	+/〈16	+/〈16	+/64*		
1	H	-/〈16	+/〈16	-/〈16	+/256	-/256*	
6	H	-/〈16	+/〈16	+/〈16	+/64	-/64*	
8	H	-/〈16	+/〈16	+/〈16	-/16	-/256	-/256*
9	H	-/〈16	+/〈16	+/〈16	+/64	+/256	-/256*
17	L	-/〈16	-/〈16*				
19	L	-/〈16	-/〈16*				
13	L	-/〈16	-/〈16	+/〈16*			
16	L	-/〈16	+/〈16	-/〈16*			
11	L	-/〈16	-/〈16	+/〈16	+/16*		
15	L	-/〈16	-/〈16	+/〈16	+/16*		
14	L	-/〈16	-/〈16	+/〈16	+/16	+/16*	
18	L	-/〈16	-/〈16	+/〈16	+/256	+/64*	
12	L	-/〈16	-/〈16	+/〈16	+/16	+/16	-/256*
20	L	-/〈16	-/〈16	-/〈16	+/16	+/64	-/64*
95	C	-/〈16	-/〈16*				
96	C	-/〈16	-/〈16*				
97	C	-/〈16	-/〈16	-/〈16*			
99	C	-/〈16	-/〈16	-/〈16	-/〈16*		
98	C	-/〈16	-/〈16	-/〈16	-/〈16	-/〈16*	
94	C	-/〈16	-/〈16	-/〈16	-/〈16	-/〈16	-/〈16*

* when sacrified; H = highly respiratory virulent PRRSV(isolate 110); L = low respiratory virulent PRRSV(isolate 135); C = control; + = positive virus isolation, - = negative virus isolation.

high pathogenic and low pathogenic viruses in STR cultures. However, it is not known whether the isolates originated from herds with different disease severity. The severity of experimentally reproduced clinical disease and lesions does not mimick always that observed in the herd of origin due to concurrent infection of pigs in the herd[19]. Gross lung le-

sions in 3-week-old conventional pigs inoculated intranasally with the high or low pathogenic PRRSV isolates were observed mainly from 14 days PI and generally most severe, characterized by diffuse petechiasis and mottled-tan changes. In highly controlled experiments, 4~5-week-old cesarian-derived colostrum-deprived(CDCD) pigs inoculated with different PRRSV isolates showed multifocal mottled-tan consolidation with different severity at 10 days PI[19,20]. The severity of the gross lung lesions generally correlated well with severity of clinical disease[19].

PRRSV-induced microscopic lesions are unique and may be detected when gross lesions are absent. Between 4 and 14 days PI, inoculation of the high pathogenic virus resulted in loss of cilia, formation of vacuoles in the tracheal epithelium and increased numbers of Goblet cells which produce mucin. Low pathogenic virus caused similar lesions, but the lesions were observation later(7 and 14 days PI) with lesser degree. The isolate 110-inoculated pigs had earlier onset of lung lesions, significantly more severe lesions, and more persistent lung lesions. We failed to explain direct effects between PRRSV-infection and cilia loss or increased number of Goblet cells. The detection of PRRSV with a monoclonal antibody (SDOW-17) was limited in macrophages of tracheal epithelium. However, this impairment of mechanical clearance mechanism such as reduced ciliary movement with hypersecretion of mucin may induce respiratory failures by itself in pigs infected with PRRSV or be able to allow secondary infections easily. Histopathological lesions of turbinate and lung produced in these pigs were similar to those described previously[7,15,17,19,20]. The rhinitis was characterized by patchy dysplasia of the epithelium with loss of cilia, mild multifocal subepithelial lymphohistiocytic and suppurative inflammation with slight edema and congestion. Characteristic microscopic lung lesions were septal infiltration with mononuclear cells, type 2 pneumocyte hypertrophy and hyperplasia, and accumulation of necrotic macrophages and debris in alveolar spaces.

PRRSV was readily isolated from turbinate, tonsil, trachea and lung and sera of all inoculated pigs over the 28-day period. However, the isolation of high pathogenic virus from tissues and sera was earlier and more consistent than that of the low pathogenic virus. The discrepancy of virus isolation from the tissues may explain the different affinity of various strains of the virus for respiratory tissues.

In consequence, the results of this study and those of Halbur *et al*[19,20] could explain different pathogenicity of PRRSV in the respiratory system and various clinical severity of respiratory disease on PRRSV infected farms. Further experiments would be desired to observe the difference of molecular level of PRRSV isolates.

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