Effect of Attenuated *Erysipelothrix rhusiopathiae* Vaccine in Pigs Infected with Porcine Reproductive Respiratory Syndrome Virus

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ABSTRACT. Twenty 2nd specific pathogen-free pigs were divided into 4 groups: Group A were infected with porcine reproductive and respiratory syndrome (PRRS) virus at 6 weeks of age and treated with available swine erysipelas and swine fever combined vaccine (vaccinated) at 7 weeks of age; Group B were vaccinated at 7 weeks of age and infected with PRRS virus at 8 weeks of age; Group C were vaccinated at 7 weeks of age: Group D were neither vaccinated nor infected with PRRS virus. All pigs were challenged to *Erysipelothrix rhusiopathiae* C42 strain at 10 weeks of age. No clinical signs appeared after vaccination of group A and B pigs, thus confirming that the safety of the vaccine was not influenced by infection with PRRS virus. None of the pigs in Groups A and C developed erysipelas after challenge exposure to *E. rhusiopathiae*. In contrast, fever and/or urticaria appeared transiently in all pigs of Group B after challenge exposure. At the time of challenge exposure to *E. rhusiopathiae*, the PRRS virus titer was high in sera of Group B, but was low in those from Group A. However, vaccination of pigs with attenuated *E. rhusiopathiae* was effective in dual infection with PRRS virus and *E. rhusiopathiae*, because the clinical signs were milder and the *E. rhusiopathiae* strain was less recovered from these pigs compared to pigs of group D. — KEY WORDS: *Erysipelothrix rhusiopathiae*, experimental infection, PRRS, swine, vaccine.

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Erysipelothrix rhusiopathiae is a causative pathogen for swine erysipelas, which causes enormous economic losses in pig production. The clinical signs of swine erysipelas can be divided into three types: acute (septicemia), subacute (urticaria), and chronic (arthritis and endocarditis). Lyophilized live vaccine prepared from an acriflavine-fast attenuated *E. rhusiopathiae* [15] has been widely used for prevention of swine erysipelas in Japan.

Reproductive disturbance in sows and respiratory distress in growing pigs were encountered around 1987 due to the outbreaks of porcine reproductive and respiratory syndrome (PRRS), and PRRS virus was isolated in 1993 [6, 12]. It has been believed that PRRS was associated with other microbial infections in growing pigs [10, 12]. Since 1988, in several large-scale pig farms infiltrated with PRRS virus, swine erysipelas broke out frequently even in 2–6-month-old pigs that had been vaccinated with commercially available attenuated *E. rhusiopathiae* vaccine (Hara, 1996, unpublished data). It was, therefore, suspected that the efficacy of the attenuated *E. rhusiopathiae* vaccine was diminished by PRRS virus infection in pig. The object of this study was to determine whether PRRS virus infection in pigs inhibited the effect of the attenuated *E. rhusiopathiae* vaccine.

MATERIALS AND METHODS

Pigs: Twenty 6-week-old 2nd specific-pathogen-free (SPF) pigs (produced at the Zen-Noh Central Institute for Feed and Livestock Research) were used. The pigs were separated into groups which were housed separately in clean pig rooms.

Vaccine: A commercially available swine erysipelas and swine fever combined live vaccine (Scientific Feed Laboratory Co., Ltd., Tokyo, Japan) was used. The reconstituted vaccine contained acriflavine-fast attenuated strain Koganei 65–0.15 of *E. rhusiopathiae* ($\geqq 1 \times 10^8$ viable bacteria/m*l*) [15] and strain GPE of hog cholera virus ($\geqq 10^3$ median tissue culture infective dose ($TCID_{50}$)/m*l*) [7].

Cell cultures: Swine alveolar macrophage (SAM) [3, 16] cells obtained from 4- to 6-week-old SPF pigs, were used throughout this experiment. A cell line derived from rhesus monkey kidney, MARC-145 cells [4] which was kindly provided by Murakami (National Institute of Animal Health, Japan), was also used for serological examination.

Virus and bacterial strains: PRRS virus strain E4 and *E. rhusiopathiae* strain C42 were used for challenge exposure of swine. PRRS virus strain E4 was isolated from a severely affected pig in 1993 [9]. Strain E4 was grown in fresh cultures of SAM cells at 37°C in 5% CO_2 incubator, and was diluted to a $10^{5.8}$ $TCID_{50}$/m*l*.

E. rhusiopathiae strain C42 of serotype 1a was isolated from a growing pig which was dying due to dual infection with PRRS virus and *E. rhusiopathiae* in 1995. Strain C42 was grown on nutrient broth (Eiken Chemical Co., Ltd., Tokyo, Japan), and adjusted to $10^{6.5}$ colony-forming-units (CFU)/m*l*.

Experimental design: The pigs were divided into 4 groups (Table 1). Pigs of Groups A–C were injected subcutaneously with a commercially available swine erysipelas and swine fever combined vaccine at 7 weeks of age. All pigs were challenge-exposed to 0.1 m*l* of bacterial suspension of *E. rhusiopathiae* C42 strain administered intracutaneously in the side at 10 weeks of age (post-vaccination week (PVW) 3). A 1 m*l* suspension of PRRS

Table 1. Experimental design

Group	Pig No.	Passage of week						
		0[a]	1	2	3	4	5	6
A	51–55	PRRS[b]	Vac[c]			*E. r*[d]		Necropsy
B	56–60		Vac	PRRS		*E. r*		Necropsy
C	61-65		Vac			*E. r*		Necropsy
D	66–70					*E. r*		Necropsy

a) Six weeks of age. b) Inoculated with PRRS virus. c) Treated with swine erysipelas and swine fever combined vaccine. d) Challenged with *E. rhusiopathiae*.

virus was introduced intranasally at 6 weeks of age (pre-vaccination week 1) to pigs of group A, and at 8 weeks of age (PVW 1) to pigs of group B.

Clinical observation: The pigs were observed daily for body temperature, clinical signs of PRRS, erysipelas and other abnormalities.

Serological tests: Blood samples were collected from all pigs at intervals of one week to determine the antibody titers of serum. The indirect immunofluorescence assay (IIFA) test of PRRS virus [6] and growth agglutination (GA) test of *E. rhusiopathiae* [8] were carried out by methods described previously.

Recovery of PRRS virus: The SAM cells suspended in Eagle's minimum essential medium supplemented with 10% of fetal calf serum (FBS-MEM) at $10^{6.2}$ cells/m*l* were seeded in 96-well plates in aliquots of 0.1 m*l* per well. Two hr after seeding, the cell cultures were inoculated with 10% tissue homogenates or 0.05 m*l* undiluted serum. After adsorption at 37°C for 1 hr, each well was washed twice with FBS-MEM and then fed with 0. 1 m*l* of fresh FBS-MEM. The plates were incubated at 37°C in a 5% CO_2-incubator for 6 days. The supernatant was inoculated onto fresh cultures of SAM cells if a cytopathic effect (CPE) was not observed during the first passage. Virus titration was performed by the microtiter method using the SAM cell cultures prepared as described above.

Recovery of E. rhusiopathiae: Specimens of the heart, lung, liver, spleen, kidney, lymphocentrun subiliacum and cutis (segment of challenged to *E. rhusiopathiae*) were plated on Tryptic Soy (TS) agar (Difco Lab., Detroit, MI., U.S.A.) containing 10% sheep blood and TS agar containing 5% horse serum, 50 μg/m*l* of gentamicin (GM) and 500 μg/m*l* of kanamycin (KM) (selective agar of *E. rhusiopathiae*), and were incubated for 2 days at 37°C. Swabs of the tonsil, hip joint and genu joint were cultured using TS broth (Difco Lab.,) containing 0.1% Tween 80, 50 μg/m*l* of GM and 500 μg/m*l* of KM and were incubated for 2 days at 37°C, and the culture medium was transferred to selective agar of *E. rhusiopathiae*, and was incubated for 2 days at 37°C. Suspected colonies of *E. rhusiopathiae* were identified using conventional biochemical tests [17]. The isolates were tested for resistance to acriflavine and pathogenicity in mice [15].

Necropsy findings: The pigs were sacrificed by intravenous overdose of thiopental sodium (Tanabe Pharmaceutical Co., Ltd., Osaka, Japan) at 12 weeks of age.

RESULTS

Clinical signs: Two or 3 pigs showed a local skin reaction at the site of vaccination, but no clinical signs or fever resulted from vaccination in all pigs of Groups A, B and C. In all pigs of Groups A and B, transient fever above 40°C was observed after inoculation with the PRRS virus.

After-challenge exposure to *E. rhusiopathiae*, fever occurred in all pigs with the exception of pigs in Groups A and C. In Groups B and D, urticaria was noted in 2 of the 5 pigs, respectively. On the other hand, no clinical signs were recognized in any pigs of Groups A and C (Table 2).

Serological tests: IIFA antibody to the PRRS virus was not detected in all pig sera collected before inoculation with PRRS virus, but was observed after inoculation in all pigs of Groups A and B, and the titers reached 1:40 to 1:320.

GA antibody to *E. rhusiopathiae* was not detected in all pigs at vaccination, but increased after vaccination in Groups A, B and C, up to geometric mean (GM) titers of 12.1, 9.2 and 10.6, respectively at PVW 3. The titers in these groups increased up to levels ranging from 24.3 to 32.0 at necropsy.

Recovery of PRRS virus: In Groups A and B, a large number of PRRS virus were recovered from sera of almost all pigs during post-inoculation weeks (PIW) 1 to 3, and a small amount of virus was recovered after PIW 4. At necropsy, the PRRS virus was recovered from the tonsil and/or lung of 2 pigs in Group A and all pigs of Group B. On the other hand, in Groups C and D, the PRRS virus was not recovered at any time from the serum, tonsil and lung of any pigs (Table 3).

Recovery of E. rhusiopathiae: At necropsy, *E. rhusiopathiae* was not recovered from any pigs of Groups A and C, but was recovered from 2 and 4 pigs in Groups B and D, respectively (Table 4). All of the recovered organisms were sensitive to acriflavine (minimal inhibitory concentration: 0.001–0.002% for all the isolates) and fatal to mice (50% lethal dose (LD_{50}): $10^{3.2}$–$10^{2.5}$ CFU), which were used in challenge exposure.

Necropsy findings: The necropsy findings in all pigs of Groups A, B and C were nearly normal. Two of the 5 pigs in Group D had muddy hip joint liquid.

DISCUSSION

Field evidence, strongly suggests a role of PRRS virus in predisposing animals to secondary infection [5, 10, 12]. In some cases, the pigs, which had already been vaccinated with attenuated *E. rhusiopathiae*, were found moribund due to acute swine erysipelas and dual infection with PRRS virus. The strain C42 of *E. rhusiopathiae* was isolated in these cases, and this strain was used for challenge in order to confirm the field evidence in this study. The field evidence aroused a doubt about the safety of attenuated *E. rhusiopathiae* vaccines in PRRS virus-infected pigs. The

Table 2. Body temperature and clinical signs in pigs after challenge with *E. rhusiopathiae*

Group[a)]	Pig No.	Body temperature[b)]	Urticaria on the skin[c)]	Depression and loss of appetite[d)]
A	51	–	–	–
	52	–	–	–
	53	–	–	–
	54	–	–	–
	55	–	–	–
B	56	40.6 (4)	–	–
	57	41.9 (5)	++ (4)	–
	58	41.9 (6)	+ (2)	+ (1)
	59	40.6 (4)	–	–
	60	40.3 (4)	–	–
C	61	–	–	–
	62	–	–	–
	63	–	–	–
	64	–	–	–
	65	–	–	–
D	66	40.1 (1)	–	–
	67	> 42.0 (6)	++ (6)	++ (8)
	68	40.6 (6)	–	+ (1)
	69	41.0 (3)	–	–
	70	> 42.0 (6)	++ (9)	++ (10)

a) See Table 1. b) Maximum temperature, –: < 40°C. ( ): Number of days with fever ≧40°C. c) –: No lesion, +: Lesion partially on body, ++: Lesion on whole body. ( ): Number of days with symptom. d) –: No clinical signs, +: Slight depression, ++: Severe depression and loss of appetite. ( ): Number of days with symptom.

Table 3. Recovery of PRRS virus from serum during six weeks and from tissues at necropsy

		Recovery of PRRS virus from serum							Recovery of PRRS virus at necropsy	
	Pig	passage of week								
Group[a)]	No.	0	1	2	3	4	5	6	tonsil	lung
		↓				▼				
A	51	–[b)]	4.30[c)]	2.05	>4.80	2.05	–	–	–	–
	52	–	3.30	1.80	2.30	+[d)]	+	+	3.05	+
	53	–	3.80	2.30	+	+	–	–	–	–
	54	–	4.30	+	2.80	+	–	–	–	–
	55	–	4.55	2.05	2.55	2.80	1.80	–	3.80	–
				↓		▼				
B	56	–	–	–	2.05	+	3.05	–	2.80	–
	57	–	–	–	3.80	3.30	2.55	–	3.30	2.80
	58	–	–	–	4.05	4.05	3.05	+	3.30	–
	59	–	–	–	4.30	2.55	3.05	+	4.05	4.30
	60	–	–	–	4.30	3.80	3.80	3.05	2.55	2.30

a) See Table 1. b) Negative. c) Log $TCID_{50}$/m*l*. d) Positive (< log 1.8 $TCID_{50}$/m*l*). ↓ Inoculated with PRRS virus. ▼ challenged with *E. rhusiopathiae* C42.

PRRS virus was also suspected of interfering with the effect of this attenuated vaccine. In this study, no clinical evidence of swine erysiplelas appeared after vaccination of pigs in Groups A and B, thereby confirming that a commercially available swine erysipelas and swine fever combined vaccine was safe and that its efficacy was not influenced by the inoculated PRRS virus. No pigs in Group A developed swine erysipelas after challenge exposure to the *E.*

Table 4. Recovery of *E. rhusiopathiae* from several tissues at necropsy

Group[a)]	Pig No.	Tonsil	Heart	Hip-joint	Others
A	51	–	–	–	–
	52	–	–	–	–
	53	–	–	–	–
	54	–	–	–	–
	55	–	–	–	–
B	56	–	–	–	–
	57	–	–	–	–
	58	–	+	–	–
	59	–	–	+	–
	60	–	–	–	–
C	61	–	–	–	–
	62	–	–	–	–
	63	–	–	–	–
	64	–	–	–	–
	65	–	–	–	–
D	66	–	–	–	–
	67	+	–	+	–
	68	+	–	–	–
	69	+	–	–	–
	70	–	+	+	+ (spleen, kidney, lymphocentrum,genu-joint)

a) See Table 1. –: Negative, +: Positive.

rhusiopathiae C42 strain. In contrast, transient fever and/or urticaria appeared in all pigs of Group B. At the time of challenge exposure to *E. rhusiopathiae* strain C42, PRRS virus recovered from sera of pigs in Group B had a high titer, but that in Group A had low titer. These results indicate that the outbreak of swine erysipelas is due to infection of virulent *E. rhusiopathiae* and severe PRRS viremia in pigs vaccinated with attenuated *E. rhusiopathiae*. However, vaccination of pigs with attenuated *E. rhusiopathiae* was effected by dual infection with PRRS virus and *E. rhusiopathiae*, because the clinical signs were milder and the *E. rhusiopathiae* strain, used in challenge exposure, was recovered in less amounts from these pigs, compared to pigs that were unvaccinated and infected with *E. rhusiopathiae* alone. It was suggested that immuno compromising occurred by infection with PRRS virus. However, little is known about the propensity of PRRS virus for alveolar macrophages [5, 11], and the suppression for systemic immunopathy is unknown.

All pigs of Group D (neither vaccinated nor treated with the PRRS virus) developed fever after challenge exposure to the *E. rhusiopathiae* C42 strain. However, clinical evidence of swine erysipelas was recognized in only 2 of 5 pigs. Takahashi *et al.* [13] reported that LD_{50} of a virulent strain of *E. rhusiopathiae* in inoculated mice was $<10^2$ CFU and that of avirulent strain $> 10^7$ CFU. LD_{50} of the C42 strain in inoculated mice was $10^{2.7}$ CFU (unpublished data). These results suggest that the C42 strain is a mesovirulent strain, and may indicate that even a mesovirulent strain of *E. rhusiopathiae* can cause acute swine erysipelas in pigs affected with PRRS in the field.

It has been reported that PRRS virus infection in pigs exacerbated bacterial diseases. For example, PRRS virus predisposes piglets to clinical disease caused by *Streptococcus suis* serotype 2 [2]. The interaction between PRRS virus and *Mycoplasma hyorhinis* in hysterectomy-produced and colostrum-deprived pigs has been recognized [12]. In other experimental studies, no differences have been found in clinical signs and lung lesions of pigs coinoculated with PRRS virus and *Mycoplasma hyopneumoniae* [14] or other bacteria [1]. Future studies on PRRS virus titers at secondary infection with microorganisms are awaited to define evidence contradictory to these reports.

It was considered that the susceptibility induced by PRRS virus to secondary diseases does not last for a long time and the risk period seems to be short, and limited to the time when the PRRS virus is remarkably proliferated in the affected pig. The results of this study support the safety and efficacy of the attenuated *E. rhusiopathiae* vaccine in pigs infected with PRRS virus.

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