Comparison of Safety and Efficacy of Erysipelas Inactivated and Attenuated Vaccines in Mice and Pigs

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ABSTRACT Mice and pigs were used to test the safety and efficacy of mono-and poly-valent inactivated vaccines and mono-valent attenuated vaccine of erysipelas. The results showed no side reactions on either experimental animals, even when 10 times doses for pigs were used in mice. When the mice were immunized, subcutaneously, with 1.4×10^4 CFU of attenuated vaccine they resisted to the challenge of 100 MLD of a virulent *Erysipelothrix rhusiopathiae* (Er). Although the inactivated vaccine is very safe, a vaccination dose of 3×10^{10} CFU was required for the protection from the 100 MLD challenge. On the other hand, the growth agglutination (GA) titers of four pigs were 1 : 32 when the sera were collected at two weeks after vaccination using attenuated vaccine. In addition, no distinct changes in the GA titers were found at two weeks after challenge (4 weeks after vaccination). However, the GA titers were 1 : 8 for other 6 pigs when the sera were tested at 2 weeks after vaccination with inactivated vaccine. The titers increased to 1 : 128 to 1 : 512 when the sera were tested at two weeks after challenge. These increased titers were apparently due to the exposure of pigs to challenge with Er. The results indicated that the efficacy of the attenuated vaccine is better than that of the inactivated bacterin. [*Chen C, Lu CC, Lin SC, Kuo NW and Chan IP. Comparison of safety and efficacy of erysipelas inactivated and attenuated vaccines in mice and pigs. J Chin Soc Vet Sci 24 (2) : 73 — 81, 1998. *Corresponding author TEL : 02-2621-2111-231, FAX : 02-2622-5345]

Keywords: Erysipelas , Inactivated bacterin , Attenuated vaccine. Immunity potency

INTRODUCTION

Since the attenuated vaccine and penicillin were developed, the swine Erysipelas outbreak has been markedly reduced[5]. Inevitably, there were always some cases reported in Taiwan[1]. Recently, antimicrobial additives are commonly used in feed, resulting in interfering the immunity effects of the attenuated vaccine in pigs. The serotype of imported inactivated bacterin may not be the same as to that of our local bacterial strain. The concentration of inactivated bacterin and the result of its application in

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Reprinted from the J. Chinese soci. Vet. Sci. 24 (2) : 73—81, 1998
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the field should be studied. Therefore, the recent study was to test and evaluate the immune efficacy of both inactivated and attenuated vaccines.

MATERIALS AND METHODS

Methods of vaccine preparation

1. *Attenuated vaccine :*

Attenuated vaccine strain, *Erysipelothrix rhusiopathiae (Er)* 65～0.15 koganei strain (la), was kindly provided by the Japanese Association of Veterinary Biologics and grown on the Tryptose phosphate broth (Difco, USA) containing 0.1 % Tween 80 (Merck, Germany) at 37 ℃ for 16～18 hrs. The culture suspension was harvested ; the pH was adjusted to 7.2, with sodium hydroxide (Mallinckrodt, USA) ; 0.02 % of Acriflavine (Sigma, USA) was added and incubated for another 48 hrs. The bacterial concentration was adjusted before being used as the attenuated vaccine.

2. *Inactivated bacterin :*

a. Monovalent bacterin preparation :

An Er virulent strain (la) which was isolated from a diseased pig in a pig farm where an outbreak of erysipelas occurred in Taiwan, and bacteria serotypes la and 8 strains stored in our laboratory were cultured separately at 37 ℃ for 16 hrs. The cell concentrations were adjusted to 3 × 10^{11} CFU / mL, inactivated with a final concentration of 0.3 % Formalin (Merck, Germany), and preserved with 0.02 % Thimerosal (Sigma, USA). A 10 % (v / v) of adjuvant of Emulsigen (MVP. USA) was added to prepare two kinds of monovalent bacterins which were stored at 4 ℃ before use.

b. Polyvalent bacterin preparation :

Several virulent Er strains belonging to various serotypes (la, lb, 2a, 2b, 8 and 10) stocked in our laboratory, were used as seed strains for poly-valent bacterin preparation and / or for challenge. The procedures for bacterin production were the same as those for making monovalent bacterin. The resultant mono-valent bacterial suspensions were combined and adjuvanted to prepare the polyvalent bacterins and were stored at 4 ℃ before use.

Challenge test

The efficacy of the vaccines prepared as described above test on mice was conducted by the exposure to bacterial challenge according to the standard assay for animal drugs[2]. On the other hand, the experiment on immunized pigs, the body weight 35～40 kgs, which had never been previously inoculated with a bacterial vaccine was conducted according to the reports by Chen et al. [1]. The virulent bacterial suspension was inoculated into pigs, subcutaneously with 100,000 MLD of mouse doses for challenge.

Titration of growth agglutination titers

The growth agglutination (GA) titers of sera collected from the immune pigs were carried out according to the methods described by Sawada et al.[3] and Chen et al.[1]. A local isolate of Er (la) was used as the antigen for the test.

RESULTS

1. Experimental results on the safety and immunity tests of both *Erysipelothrix rhusiopathiae* polyvalent inactivated and attenuated vaccines in mice:

Following challenge, no side reactions were observed from the mice immunized with polyvalent inactivated vaccine with 0.5 ml of 3 × 10^{11} CFU / mL during the 2 weeks of safety test period. These mice could resist the challenge of a virulent strain (la) with 100 MLD. On the other hand, when the mice were inoculated with 10 times of pig dosage (7 × 10^{9} CFU / mouse) of attenuated vaccine, no any side reactions were observed. When the mouse was injected subcutaneously, 1.4 × $10^{7\text{-}9}$ CFU they were able to resist the challenge with 100 MLD doses of Er strain (Table 1).

2. Relationship between the potency of the doses, immunity period and survival rates of mice inoculated with both inactivated and / or attenuated *E. rhusiopathiae* vaccines:

The inactivated bacterin and attenuated vaccine were inoculated with different doses in mice, then these mice were challenged at the same time at 2, 3 or 4 weeks after immunization. The results indicated that inoculation with more than 3×10^{10} CFU of inactivated vaccine was required for the resistance to the challenge with 100 MLD Er. The survival rate mice were especially higher for those that have been immunized for 2 and 3 weeks. Mice immunized with less than 3×10^{10} CFU doses and mice that were already immunized for 4 weeks did not show satisfactory potency after challenge exposure (Table 2).

On the other hand, the mice inoculated with attenuated vaccine needed a dose of only 1.4×10^4 CFU for resistance to the challenge with 100 MLD of a virulent strain. Furthermore, they all had a good protection potency at 2, 3 and 4 weeks after immunization (Table 3).

3. Relationship between the dosage, antibody titers and potency of pigs vaccinated with inactivated and attenuated *E. rhusiopathiae* vaccines:

Various dosages of the inactivated polyvalent bacterin and the attenuated monovalent vaccine were used to inoculate the pigs. Two weeks after the vaccination, the sera of the immunized pigs were sampled for GA titer test. The GA titers at two weeks after inoculation with 5 mL and / or 10 mL of inactivated vaccine (3×10^{11} CFU / mL) were only 1 : 8. After the challenge exposure, 6 pigs survived but various degrees of fever reactions and / or urticaria were found. Two weeks after the challenge exposure, the GA titer increased to higher levels ranging from 1 : 128 to 1 : 512. On the other hand, the vaccinated group with the attenuated did not show any side reactions, despite the dosages used. The GA titer at two weeks after inoculation was 1 : 32. After challenge using a similar strain, the 4 pigs showed no side reactions, and two weeks after the challenge, their GA titer did not increase. On the contrary, two control pigs showed typical symptoms of Erysipelas. One of them died on the 5 day after challenge (Table 4).

4. Efficacies of monovalent and polyvalent inactivated *E. rhusiopathiae* bacterins in mice:

Monovalent (la) bacterin and polyvalent (1a, 1b, 2a, 2b, 8, 10) bacterins were used to immunize the mice. Two weeks after inoculation, they were challenged with 100 MLD of different serotypes of *Er.* strains. The results indicated that mice vaccinated with the bacterin prepared by using la strain was only resistant to challenge with homologous strain and its potency reached to 80 % which was minimum of the national standard. It exhibited 50 % potency when challenged with heterologous strains 1b or 2b of *Er.* Whereas the potencies resulting from challenge with serovar 8 and / or 10 strains were not satisfactory. On the other hand, the monovalent inactivated bacterin prepared with serovar 8 strain only had a 30 % potency resistant to challenge with homologous strain. It showed poor results when challenge with different strains. From the experimental results of monovalent bacterin, the immunity potency of the inactivated bacterin was very specific to the derived strain. However, when the challenge strain and the vaccine produced bacterin strain are the same, it does not necessarily mean that the potency will meet the 80 % national standard. Similarly, mice immunized with polyvalent inactivated vaccine were challenged with various serotype strains, the results showed that when serotypes 1a, 1b and 2b were individually used for challenge, immunity effects met 80 % of the national standard. However, when challenge with serotypes 8 and 10, the immunity effects did not meet the national standard, however immunity effects of 50 % and 60 % was achieved (Table 5).

Table 1. Safety and immunity tests of *Erysipelothrix rhusiopathiae* vaccines in mice.

Vaccine	Dose and route inoculated (CFU / mouse / sc)[3]	Survival rate after safety test period of 2 weeks	Survival rate after challenged with 100 MLD for 2 week[4]
Inactivated	1.5×10^{10}	10 / 10	0 / 10
bacterin[1]	1.5×10^{11}	10 / 10	9 / 10
Attenuated	7×10^{7}	10 / 10	10 / 10
vaccine[2]	7×10^{8}	10 / 10	10 / 10
	7×10^{9}	10 / 10	10 / 10
Control	placebo (saline) 0.5 mL	10 / 10	0 / 10

[1] Original inactivated bacterin contains 3×10^{11} CFU / mL.
[2] Attenuated vaccine (original suspension) contains 1.4×10^{10} CFU / mL.
[3] CFU : Colony forming unit : SC : Subcutaneously.
[4] Virulent strain of *Erysipelothrix rhusiopathiae* (1a) isolated from Chia-yi county, TPB media culture suspension was incubated at 37 ℃ for 16 hours and then diluted for challenge use.

Table 2. Relationship between the potency of the doses, immunity period, and survival rate of mice inoculated with inactivated *E. rhusiopathiae* bacterins[1].

Group	Dose and route vaccinated (CFU / mouse / sc)	Survival rate from challenge with 100 MLD on various weeks of immunization period[2]		
		2	3	4
Experimental	1.5×10^{10}	4 / 10	8 / 10	0 / 10
	3.0×10^{10}	10 / 10	10 / 10	7 / 10
	1.5×10^{11}	10 / 10	10 / 10	7 / 10
Control		0 / 10	0 / 10	0 / 10

[1] Original polyvalent (1a, 1b, 2a, 2b, 8, 10) inactivated bacterin contains 3×10^{11} CFU / mL : SC, Subcutaneously.
[2] Virulent strain of *Erysipelothrix rhusiopathiae* (1a) was used for the challenge.

Table 3. Relationship between the potency of the doses, immunity period, and survival rate of mice inoculated with attenuated *E. rhusiopathiae* vaccine[1].

Group	Dose and route vaccinated (CFU / mouse / sc)	Survival rate from challenge with 100 MLD on various weeks of immunization period[2]		
		2	3	4
Experimental	1.4×10^{3}	10 / 10	5 / 10	7 / 10
	1.4×10^{4}	10 / 10	10 / 10	10 / 10
	1.4×10^{5}	10 / 10	10 / 10	10 / 10
Control		0 / 10	0 / 10	0 / 10

[1] *E. rhusiopathiae* Koganei strain (1a) was used for attenuated vaccine preparation. The original suspension contains 1.4×10^{10} CFU / mL : SC, Subcutaneously.
[2] *E. rhusiopathiae* virulent strain (1a) isolated from diseased pig in Chia-yi county was used for challenge.

Table 4. Relationship between the dosage, antibody titers and potency of pigs vaccinated with *E. rhusiopathiae* vaccines.

Vaccine	Pig No.	Concentration of vaccine used	Dose and route vaccinated (mL/pig/sc)	Side reaction during 2 weeks	G A titer (1 : X) and results after challenge			
					Before vaccination	2 weeks after vaccination	Challenge response[1]	2 weeks after challenge
Inactivated bacterin serovars 1a, 1b, 2a, 2b, 8, 10	1	3×10^{11} CFU / mL	5	–[2]	<2	8	++[4]	256
	2	ditto	5	–	2	8	++	512
	3	ditto	5	–	2	8	++	256
	4	3×10^{11} CFU / mL	10	–	<2	8	++	128
	5	ditto	10	–	2	8	+[3]	128
	6	ditto	10	–	2	8	++	512
Attenuated vaccine serovar 1a	7	1.4×10^{8} CFU / mL	2	–	<2	32	–	16
	8	ditto	2	–	2	32	–	32
	9	1.4×10^{9} CFU / mL	2	–	<2	32	–	32
	10	ditto	2	–	2	32	–	32
Control	11	placebo (saline)	2	–	<2	Typical Erysipelas symptoms were observed and died on the 5 day after challenge.		
	12	ditto	2	–	<2	Typical Erysipelas symptoms appeared and sacrificed on 10 day post challenge.		

[1] Local strain of *E. rhusiopathiae* (1a) isolated from Chia-yi county of diseased pig. 100,000 mouse MLD doses was used for challenge.
[2] No side reaction was observed.
[3] Slight degree of urticaria and fever.
[4] Moderate degree of urticaria and fever.

Table 5. Efficacies of monovalent and polyvalent inactivated bacterins of *E. rhusiopathiae* in mice.

Treatment Group	Dose and route vaccinated (CFU / mouse / sc)	Survival rate after challenge with 100 MLD bacterial suspension of various serotypes for 2 weeks (%) [1]				
		1a	1b	2b	8	10
mono-valent bacterin[2] serovar 1a	5×10^{10}	8 / 10[3] (80)	5 / 10 (50)	5 / 10 (50)	1 / 10 (10)	0 / 10 (0)
mono-valent bacterin[2] serovar 8	5×10^{10}	0 / 10 (0)	1 / 10 (10)	2 / 10 (20)	3 / 10 (30)	0 / 10 (0)
poly-valent bacterin[2] serovars 1a, 1b,2a,2b,8,10	5×10^{10}	9 / 10 (0)	8 / 10 (80)	9 / 10 (90)	6 / 10 (60)	5 / 10 (50)
Control		0 /10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)

[1] Challenge tests were conducted after 2 weeks of vaccination.

[2] The cell concentrations both monovalent and polyvalent inactivated bacterins were adjusted to the concentration of 2.5×10^{11} CFU / mL and adjuvanted with 1 / 10 oil adjuvant.

[3] Numbers of survived / numbers of tested.

DISCUSSION

According to Wood's[10] reports in the U. S. A., the seed strains used for swine erysipelas inactivated bacterin preparation includes *Er* AN-4, SE-9. CN 3342, and CN 3461. All these strains belong to serotype 2. This may be attributed to the results of the investigation carried out by Wood and Harrington[9] in 1978. who found that serotype 2 was the highest isolated percentage of 41.2 % (670 / 1672). In Japan, Takahashi et al.[6] reported that 74 % (191 / 258) of serotype 2 was found among the isolates of *Erysipelothrix rhusiopathiae* collected from arthritis, lymph node, endocarditis and urticaria. In indonesia, Takahashi et al.[7] reported that the isolates of *Erysipelothrix rhusiopathiae* from the tonsil in apparently healthy pigs which were slaughtered had 23.7 % (58 / 245) of serotype 2, higher than other serovars. However, in Taiwan, Chen et al.[1] reported that serovar la had a higher ratio (21 / 26) than other types of *Erysipelothrix rhusiopathiae* isolated from diseased pigs in Taiwan. Sawada & Takahashi[4] reported that the experiments were carried out on mice and pigs with attenuated vaccine prepared from Koganei 65～0.15 strain (serovar 2). Two weeks after vaccination, the mice were challenged with 10 different serovar strains, 4, 6, 7, 8, 9, 10, 15, 16, 20, and N. The mortality rate was 30 % for the immunized mice challenged with serovar 20, but the rest of the immunized mice survived. On the contrary, all control mice were dead. Similar experiments were carried out on two immunized group of pigs using the 10 serovars. Among the groups challenged with serovar 8, 10, and 20, one pig in each of these group showed local urticarial lesions. No any sign was observed in the other immunized pigs. On the other hand, all the pigs in the control group showed the local lesions. Therefore, these results showed that immunization of mice and pigs with the attenuated vaccine provided resistance to the challenge of various serovar strains. A known serovar seed strain Koganei 65～0.15-la and an unknown serovar strain were used to prepare a live vaccine by chen et al.[1]. The immunized pigs developed similar levels of sera titers against the serovars la and 2. Sawada[5] also reported the efficacy of a live vaccine prepared from serovar seed strain la on mice. Twenty different serovar strains were used for challenge after immunization. Among the various mice group of size 10, only 2 and 1 mouse died from the groups challenged with serovar 10 and 20. The rest of the immunized mice survived. On the contrary, all control mice died. This result suggests that immunization with the live vaccine prepared from serovar seed la developed good resistance against challenge exposure to different serovars. According to the reports of Watarai et al.[8], there are two strains of the attenuated vaccine seeds in Japan : Koganei 65～0.15-la (strain Kg-la) and serovar 2 (strain Kg-2). At present, Koganei la is the only serovar strain used for the production of attenuated vaccine from the association of biological products manufacturers in the industry. In recent studies. The inactivated bacterins were also used to vaccinate the mice. Two weeks after vaccination with the prepared bacterin from serovar strain la, mice were challenged with various serovar strains. The group challenged with the homologous la strain had a 80 % survival rate which was up to the level of the national bioassay standard. The other groups challenged with serovars 1b and 2b had less than 50 % survival rate. Even when challenged with serovar strain 8 and 10, the resulting immune efficacies were not satisfactory. Similar experiment were conducted by using the inactivated bacterin prepared from serovar 8 seed strain. Once again, the results were not satifactory when challenged with homologous and heterologous serovar strains. Although these results indicated that the efficacies of the inactivated bacterins were restrictly type-specific, the bacterin prepared from the high virulent strain for mice did not necessarily develop good immune efficacy. Hence, the selection of seed strain for bacterin production is very important as shown in Table 5. As for polyvalent inactivated bacterins, they were not able to develop immuinty effects against heterologous

strain challenges to meet the national bioassay standard. Based on the results by Sawada reported [5], the immunity potency of the attenuated vaccine did not depend on the various serovars challenge. Hence, even though the inactivated vaccine could be used to develop immune potency when appropriate, a properly applied attenuated vaccine would produce a more satisfactory immunity effects.

ACKNOWLEDGEMENTS

Our sincere thanks are extended to Dr. H. J. Ko and J. S. Lai for their kind assistance on the experimental works.

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豬丹毒不活化菌苗與弱毒活菌苗之安全與免疫效力

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（收稿日期：86 年 10 月 27 日。接受日期：86 年 12 月 26 日）

摘要　試製之豬丹毒不活化菌苗與弱毒活菌苗，以小白鼠及豬隻作安全及免疫效力試驗之結果，得知兩者對供試動物均無不良之接種反應。小白鼠即使注射 10 倍豬免疫劑量之弱毒活菌苗亦無任何不良反應，而接種 1.4×10^4 CFU / Mouse / SC 即可耐過 100 MLD 之攻擊。另一方面不活化菌苗，其安全性雖甚佳，但免疫效力要達耐過 100 MLD之攻擊，則需 3×10^{10} CFU / Mouse / SC 以上。4 頭豬隻免疫接種弱毒活菌苗 2 週後血清，對豬丹毒強毒株（la）發育凝集抗體價（G A tiyer）達 1：32，攻擊後 2 週其抗體價亦無明顯改變。但 6 頭豬隻接種不活化菌苗 2 週後其血清中發育凝集抗體價均爲 1：8，攻擊後 2 週則達 1：128-1：512 不等。此等高抗體價顯然是攻擊耐過之免疫反應。由試驗結果顯示，弱毒活菌苗效果優於不活化菌苗。〔*陳清、呂清泉、林旭志、郭乃維、詹益波。豬丹毒不活化菌苗與弱毒活菌苗之安全與免疫效力。中華獸醫誌 24 (2)：73－81, 1998。* 聯絡人 TEL：02-2621-2111，FAX：02-2622-5345〕

關鍵詞：豬丹毒、不活化菌苗、弱毒活菌苗、免疫效力

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轉載自中華獸醫誌 J. Chin. Soc. Vet° Sci 24 (2)：73－81, 1998
台灣省家畜衛生試驗所