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RESEARCH ARTICLE

Efficacy of Four Commercial Infectious Coryza Vaccines on Prevention of *Avibacterium paragallinarum* serovar A, B and C Infection in Thailand

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ABSTRACT

Efficacy of four commercial Infectious Coryza vaccines available in Thailand were examined for protection rate against Thai field isolates serovar A, B, and C. Three hundred and thirty six male, layer chickens were divided into 25 groups. Groups 1-18 were vaccinated twice at 9 and 13 weeks old. Groups 18-24 served as positive controls, and group 25 served as a negative control. Then, groups 1-24 were challenged with *Avibacterium paragallinarum* at 15 weeks old. The result showed that vaccines 1 and 2 provided 100% protection for the birds against serovar A, B, and C Thai field isolates. Vaccine 3 provided 100% protection against serovar B and C, while Vaccine 4 provided 100% protected against only serovar B. No adverse reaction of vaccines was observed in any group. This study revealed that the protection rate of Infectious Coryza vaccines depended on the strains isolated from each country.

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INTRODUCTION

Infectious Coryza is an acute respiratory disease in chickens caused by gram-negative bacteria, *Avibacterium paragallinarum* (Blackall *et al*., 2005). The disease causes high morbidity but low mortality. The clinical signs of the upper respiratory tract include nasal discharge, facial edema, swollen wattles, and conjunctivitis (Chukiatsiri and Chansiripornchai, 2007). The signs become evident within 24-72 hr after contact with other infected chickens. The chickens may also have diarrhea as well as decreased feed and water consumption, which result in a decrease in growth performance of the chickens, lower egg production in layers, and complications with other pathogens (Blackall and Soriano-Vargas, 2013). In case of no complication, the infected birds should be recovered within few weeks. These drawbacks can then lead to economic loss (Chukiatsiri *et al*., 2010).

A. paragallinarum has been classified by 2 schemes, the Page and Kume schemes. The Page scheme divides *A. paragallinarum* into 3 major serovars: A, B, and C, by the plate agglutination test (Page, 1962). The Kume scheme divides the bacteria into 3 major serogroups: I, II, and III by the Hemagglutinin Inhibition (HI) test (Kume *et al*., 1983). However, Page serovars can be classified by the HI test and correlated with the Kume serogroup. As a result, Page serovars A, B, and C match the modified Kume serogroups I, II, and III, respectively. Currently, 9 Kume serovars have been classified: A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3, and C-4 (Kume *et al*., 1983; Blackall *et al*., 1990). Importantly, 3 Page serovars are distinct from each other as the antibodies from each serovar are unable to protect chickens from 2 other serovars, while they can provide protection against the serovars within the same group. For example, bivalent vaccine, which contains *A. paragallinarum* serovar A and C, is unable to protect serovar B-1 infected chickens but can protect the chickens with A-1, A-2, A-3, A-4, C-1, C-2, C-3, and C-4 infection (Soriano *et al*., 2004).

A. paragallinarum has spread worldwide. Some countries, including Thailand, China, Taiwan, the United States, Mexico, Germany, and South Africa, have reported all 3 serovars of *A. paragallinarum*. Australia and Japan reported only serovar A and C but no serovar B (Soriano *et al*., 2004; Chukiatsiri *et al*., 2012). Prevention and control of Infectious Coryza can achieve through strict biosecurity, antimicrobial application (Noonkhokhetkong *et al*. 2013) and relevant vaccination, as commercial inactivated bacterin in aluminum hydroxide gel or mineral oil vaccines against Infectious Coryza are available. For

example, the bivalent vaccines such as *A. paragallinarum* serovars A and C, trivalent vaccines containing serovars A, B, and C, and tetravalent vaccines containing serovars A, B, C, and B variant (Soriano *et al*., 2004; Fernandez *et al*., 2005; Chukiatsiri *et al*., 2009). Suitable vaccines should be matched with reported serovars as there is no guaranteed cross protection between different serovars. Although, the HI test is used for classified serovars of *A. paragallinarum*, the HI titer does not represent the level of host immunity. Hence, a challenge study is still the best method to evaluate the protection efficacy of Infectious Coryza vaccines (García *et al*., 2008). The aim of this study was to evaluate the efficacy of the commercial trivalent vaccines against *A. paragallinarum* isolated from local Thai strains.

MATERIALS AND METHODS

Chickens and experimental designs: Three hundred and thirty-six, one-day old male, layer chickens (Izabrown) were obtained from a commercial hatchery. The chickens were kept in the laboratory facility at the Animal Research Unit, Chulalongkorn University, Thailand. Birds were provided feed and water *ad lib* and raised under ethical approval for animal experimentation by the Chulalongkorn University Animal Care and Use Committee no. 13310021. At 9 weeks old, the birds were divided into 25 groups. Groups 1-18 were vaccinated groups of 13 birds each. Groups 19-24 were positive controls of 12 birds each. Group 25 was a negative control of 30 birds. Birds in groups 1-18 were subcutaneously vaccinated with dosage according to the manufacturer's recommendation at 9 and 13 weeks old. After vaccination, chickens were individually observed for 7 days for any adverse effect of the vaccines. At 15 weeks old, birds in groups 1-24 were intranasally inoculated with 0.2 ml (10^8 cfu/ml) of *A. paragallinarum* (Table 1). After bacterial inoculation, clinical signs of Infectious Coryza, including nasal exudates, sinus swelling, facial edema, and swollen wattles, were observed on 1-5 days post inoculation (DPI). At 5 and 7 DPI, sera were collected for the Hemagglutination Inhibition (HI) test (Chukiatsiri *et al*., 2010), and birds from each group euthanized for testing the presence of *A. paragallinarum* from infraorbital sinus culture. The left and right infraorbital sinuses were swabbed and cultured for *A. paragallinarum*. The protection rate was analyzed for the presence of *A. paragallinarum* in the culture.

Vaccines: Four commercial vaccines were administered to groups 1-18 chickens at 9 and 13 weeks old. Commercial vaccines, including Vaccine 1, trivalent inactivated vaccine in mineral oil emulsion, and Vaccine 2, trivalent inactivated vaccine in aluminium hydroxide gel, were administered 0.5 ml intramuscular. Vaccine 3, trivalent inactivated vaccine water in oil emulsion, was administered 0.25 ml intramuscular. Vaccine 4, trivalent inactivated vaccine in oil emulsion, was administered 0.5 ml subcutaneously.

Inoculated bacteria: *A. paragallinarum* isolates previously characterized by Chukiatsiri *et al*. (2012) were used in this study. The study used 3 serovars of *A. paragallinarum*, including serovars A (B1E1), B (CMA0509), and C (102943). Some reports suggested that serovar B, the latest reported serovar in Thailand, was not clearly pathogenic in chickens (Page, 1962; Kume *et al*., 1980). So, an additional 3 isolates of serovar B were used for the challenge, including 1687, 211108, and 102984 in birds vaccinated with vaccine 1 (groups 4-6), vaccine 2 (groups 10-12), and positive control (groups 22-24).

Hemagglutination inhibition (HI) test: The samples were tested using the HI Kitasato Institute method. Briefly, the tested sera were absorbed by adding 10% (v/v) GA (Glutaraldehyde)-fixed chicken erythrocytes to make a final dilution of 1:5. The mixture was allowed to stand for 2 hr at room temperature or overnight at 4°C, then centrifuged, and the supernatant was used as the five-fold diluted serum for the HI test. The five-fold diluted serum was diluted by a two-fold dilution method using BSA-PBS (0.1% bovine serum albumin in phosphate buffer saline) to produce dilutions of 1/10-1/640 (each well contained 0.2 ml of each diluted serum). The same amount (0.2 ml) of the antigen with 4 hemagglutinating units/0.2 ml was added to each well. Lastly, 0.4 m*l* of 1% (v/v) GA-fixed chicken erythrocyte was added and shaken well. The maximum serum dilution completely inhibiting hemagglutination was regarded as an HI titer.

Statistical analysis: Analysis was calculated on data using non–parametric (a Kruskal Wallis Test) and one-way ANOVA for Avibacterium isolation and antibody titers, respectively. The difference in means was considered statistically significant when $P<0.05$.

Table 1: Experimental design of vaccination, and inoculation strains

Groups	Vaccines	Inoculation serovars (strains) of *A. paragallinarum*	Groups	Vaccines	Inoculation serovars (strains) of *A. paragallinarum*
1	Vaccine 1	A (B1E1)	13	Vaccine 3	A (B1E1)
2	Vaccine 1	B (CMA0509)	14	Vaccine 3	B (CMA0509)
3	Vaccine 1	C (102943)	15	Vaccine 3	C (102943)
4	Vaccine 1	B (1687)	16	Vaccine 4	A (B1E1)
5	Vaccine 1	B (211108)	17	Vaccine 4	B (CMA0509)
6	Vaccine 1	B (102984)	18	Vaccine 4	C (102943)
7	Vaccine 2	A (B1E1)	19	Positive control	A (B1E1)
8	Vaccine 2	B (CMA0509)	20	Positive control	B (CMA0509)
9	Vaccine 2	C (102943)	21	Positive control	C (102943)
10	Vaccine 2	B (1687)	22	Positive control	B (1687)
11	Vaccine 2	B (211108)	23	Positive control	B (211108)
12	Vaccine 2	B (102984)	24	Positive control	B (102984)
			25	Negative control	no inoculation

RESULTS

Protection efficacy of the vaccines against *A. paragallinarum* serovar A (B1E1): Birds in groups 1, 7, and 25 (vaccinated with vaccine 1 and 2 and the negative control group) showed no clinical signs, and the bacterial culture from infraorbital sinus swabs were negative for *A. paragallinarum* on 5 and 7 DPI. In contrast, 8, 5, and 10 birds from groups 13, 16, and 19, respectively, showed clinical signs of *A. paragallinarum* infection at 1-5 DPI. One bird from group 13 showed *A. paragallinarum* positive from the infraorbital sinus culture on 5 DPI but no positive sample on 7 DPI. In group 16, 3 birds showed a positive infraorbital sinus culture on 5 DPI and 1 birds on 7 DPI. Four and two birds from group 19 showed a positive infraorbital sinus culture on 5 and 7 DPI, respectively. Birds in groups 1 and 7 had high antibody titers against *A. paragallinarum* serovar A on 5 DPI (217.14 and 104.29 HI unit, respectively), and the titers were slightly higher on 7 DPI (240 and 113.33 HI unit). Birds in groups 13 and 16 had antibody titers at 43 and 79 HI units on 5 DPI, and 53 and 69 HI units on 7 DPI. The antibody titer of groups 19 and 25 were negative on 5 and 7 DPI. From the infraorbital sinuses culture result, the protective rate of vaccines 1 and 2 against *A. paragallinarum* serovar A (B1E1) was 100% on 5 and 7 DPI (Table 2).

Protection efficacy of the vaccines against *A. paragallinarum* serovar B (CMA0509): Clinical signs of *A. paragallinarum* infection were not found in birds of groups 2, 8, 14, 20, and 25. Only 2 of 13 birds from group 17 showed clinical signs of *A. paragallinarum* infection, but the infraorbital sinus bacterial culture for *A. paragallinarum* was negative. Only 3 of 5 birds from group 20 were positive with *A. paragallinarum* infraorbital sinus culture at 5 DPI. The antibody titers against *A. paragallinarum* serovar B (CMA0509) in all 4 vaccines were high on 5 DPI (172.86, 1097.14, 508.57, and 421 HI units, respectively). Antibody titer of group 2 was higher on 7 DPI (286.67 HI unit), while, antibody titers of birds in groups 8, 14, and 17 were lower on 7 DPI (450.67, 320.0, and 25.0 HI unit, respectively). Birds in groups 20 and 25 showed low antibody titer (<10) on 5 and 7 DPI. The protection rate of all 4 vaccines against *A. paragallinarum* serovar B (CMA0509) was 100% at 5 and 7 DPI (Table 2).

Protection efficacy of the vaccines against *A. paragallinarum* serovar C (102984): Birds in groups 3, 9, 18, and 25 showed no clinical signs. However, 1 and 5 birds from groups 15 and 21 showed clinical signs of *A. paragallinarum* infection, respectively. One bird from group 18 and 5 birds from group 21 had *A. paragallinarum* positive in an infraorbital sinus culture on 5 DPI. All vaccinated groups showed high antibody titers on 5 DPI (297.14, 382.86, 291, and 101.43 HI unit, respectively) and even higher on 7 DPI (720, 685.71, 373.33, and 170.0, respectively). From the bacterial culture result, vaccines 1, 2, and 3 showed 100% protection against *A. paragallinarum* serovar C (102984) (Table 2).

Protection efficacy of the vaccines against *A. paragallinarum* serovar A, B, and C: The overall result of vaccine protection against *A. paragallinarum* serotypes A, B, and C is shown in Table 3. Birds vaccinated with vaccines 1 and 2 (groups 1-3 and 7-9), and group 25 showed no clinical signs and were negative for *A. paragallinarum* in the infraorbital sinus culture. Nine of the birds vaccinated with vaccine 3 (group 13-15) showed clinical signs, and one bird was positive for infraorbital sinus culture on 5 DPI. In groups 16 - 18, 7 birds showed clinical signs, but 4 and 1 birds were positive for infraorbital sinus culture at 5 and 7 DPI. Infraorbital culture result indicated that vaccine 1 and vaccine 2 provided 100% protection for chickens against *A. paragallinarum* serovar A (B1E1), B (CMA0509), and C (102984).

Table 2: Clinical signs, result of *A. paragallinarum* serogroup A (B1E1) culture (groups 1, 7, 13, 16, 19 and 25), serogroup B (CMA0509) culture (groups 2, 8, 14, 17, 20 and 25) and serogroup C (102943) culture (groups 3, 9, 15, 18, 21 and 25) from infraorbital sinus, and antibody titers at 5 and 7 days post inoculation (DPI)

Groups	Clinical signs	Positive No. / Total of chicken					
		5 DPI			7 DPI		
		Avibacterium in sinuses	Protection rate (%)	Antibody titer	Avibacterium in sinuses	Protection rate (%)	Antibody titer
1	0/13[a]	0/7[a]	100	217.14±205.08[a]	0/6[a]	100	240±214.67[a]
7	0/13[a]	0/7[a]	100	104.29±106.45[a,b]	0/6[a]	100	113.33±53.17[a,c]
13	8/12[b]	1/7[a,c]	85.7	42.86±26.9[b]	0/5[a]	100	34.0±28.81[b,c]
16	5/13[b,c]	3/7[a,d]	57.14	78.57±61.76[a,b]	1/6[a]	83.3	55±52.82[b,c]
19	10/12[b]	4/6[b,c,d]	33.3	<10±0[b]	2/6[a]	66.7	<10±0[b,c]
25	0/10[a]	0/5[a]	100	<10±0[b]	0/5[a]	100	<10±0[b,c]
2	0/13[a]	0/7[a]	100	172.86±145.46[a]	0/6[a]	100	286.67±215.28[a]
8	0/13[a]	0/7[a]	100	1097.14±780.72b,c	0/6[a]	100	450.67±293.36[a]
14	0/12[a]	0/7[a]	100	508.57±399.15a,c	0/5[a]	100	320±195.96[a,c]
17	2/13[a]	0/7[a]	100	421.43±944.36[a,c]	0/6[a]	100	25.0±27.39[b,c]
20	0/11[a]	3/5[b]	40	<10±0[a]	0/6[a]	100	<10±0[b]
25	0/10[a]	0/5[a]	100	<10±0[a]	0/5[a]	100	<10±0[b]
3	0/13[a]	0/7[a]	100	297.14±171.05[a,c]	0/6[a]	100	720±914.94[a]
9	0/13[a]	0/7[a]	100	382.86±414.32[a]	0/6[a]	100	685.71±850.23[a]
15	1/13[a,c]	0/7[a]	100	291.0±196.93[a,c]	0/6[a]	100	373.33±218.60[a]
18	0/13[a]	1/7[a]	85.7	101.43±108.69[a,c]	0/6[a]	100	170±260.61[a]
21	5/12[b,c]	5/7[b]	28.6	<10±0[a,b]	0/5[a]	100	<10±0[a]
25	0/10[a]	0/5[a]	100	<10±0[a,b]	0/5[a]	100	<10±0[a]

Different superscript in the same column means statistical significance (P<0.05).

Table 3: Combination of clinical signs, result of *A. paragallinarum* sero group A (B1E1), B (CMA0509), and C (102943) culture from infraorbital sinus, and protection rate at 5 and 7 days post inoculation (DPI)

Groups	Clinical signs	Positive no./total of chicken			
		5 DPI		7 DPI	
		Avibacterium in sinuses	Protection rate (%)	Avibacterium in sinuses	Protection rate (%)
1 – 3	0/39[a]	0/21[a]	100	0/18[a]	100
7 – 9	0/39[a]	0/21[a]	100	0/19[a]	100
13 – 15	9/36[b,c]	1/21[a,c]	99.95	0/17[a]	100
16 – 18	7/39[b]	4/21[b,c,d]	99.81	1/18[a]	99.94
19 – 21	15/35[b,c]	11/17[b]	35.29	2/18[a]	88.89
25	0/30[a]	0/15[a,d]	100	0/15[a]	100

Different superscript in the same column means statistical significance (P<0.05).

Table 4: Clinical signs, result of *A. paragallinarum* serovar B; strain B (1687) (group 4, 10, 22, and 25), strain B (211108) (group 5, 11, 23, and 25), and strain B (102984) (group 6, 12, 24, and 25) culture from infraorbital sinus, and antibody titers at 5 and 7 days post inoculation (DPI)

Groups	Clinical signs	Positive no./total of chicken					
		5 DPI			7 DPI		
		Avibacterium in sinuses	Protection rate (%)	Antibody titer	Avibacterium in sinuses	Protection rate (%)	Antibody titer
4	0/10[a]	0/6[a]	100	120±43.82[a]	0/4[a]	100	120±40.0[a,b]
10	0/13[a]	0/7[a]	100	550±535.82[b]	0/6[a]	100	416.67±477.73[a]
22	3/12[a]	2/7[a]	71.4	<10±0[a]	1/5[a]	80	<10±0[a,b]
25	0/10[a]	0/5[a]	100	<10±0[a]	0/5[a]	100	<10±0[a,b]
5	0/12[a]	0/7[a]	100	165.7±114.14[a]	0/5[a]	100	176±87.64[a]
11	0/13[a]	0/7[a]	100	560±383.67[b]	0/6[a]	100	700±502[b]
23	0/12[a]	2/6[a]	66.7	<10±0[a]	0/6[a]	100	<10±0[a]
25	0/10[a]	0/5[a]	100	<10±0[a]	0/5[a]	100	<10±0[a]
6	5/13[a]	0/7[a]	100	571.4±181.42[a]	0/7[a]	100	426.67±240.89[a,b]
12	2/12[a]	0/7[a]	100	548.57±156.14[a]	0/5[a]	100	960±986.31[a]
24	5/12[a]	2/6[a]	66.7	<10±0[b]	0/6[a]	100	<10±0[a,b]
25	0/10[a]	0/5[a]	100	<10±0[b]	0/5[a]	100	<10±0[a,b]

Different superscript in the same column in each challenge strains means statistical significance (P<0.05).

Protection efficacy of the vaccines against *A. paragallinarum* strain B (1687): Birds in groups 4, 10, and 25 showed no clinical signs and were negative for *A. paragallinarum* in the infraorbital sinus culture. Only 3 of 12 birds in group 22 showed clinical signs of *A. paragallinarum* infection, and the infraorbital sinus culture was positive in 2 birds on 5 DPI and 1 bird on 7 DPI. Birds in groups 4 and 10 showed high antibody titer on 5 DPI (120 and 640 HI unit) and 7 DPI (120 and 416.67 HI unit). The protection rate of vaccines 1 and 2 against *A. paragallinarum* serovar B (1687) was 100% (Table 4).

Protection efficacy of the vaccines against *A. paragallinarum* strain B (211108): Birds in groups 5, 11, 23, and 25 showed no clinical signs. However, 2 birds from group 23 were positive for infraorbital sinus culture. Birds in vaccination groups showed high antibody titer on 5 DPI (165.7 and 560 HI unit) and 7 DPI (176 and 700 HI unit). The protection rate of vaccines 1 and 2 against *A. paragallinarum* serovar B (211108) was 100% at 7 DPI (Table 4).

Protection efficacy of the vaccines against *A. paragallinarum* strain B (102984): Five, two, and five birds in groups 6, 12, and 24 showed clinical signs of *A. paragallinarum* infection, respectively. Birds in groups 6, 12, and 25 were negative for infraorbital sinus culture, but 2 birds in group 24 were positive for *A. paragallinarum* in the infraorbital sinus culture. The antibody titers of vaccines 1 and 2 against *A. paragallinarum* serovar B (102984) were high on 5 DPI (571.4 and 548.57 HI unit) and 7 DPI (426.67 and 960 HI unit). The protection rate of vaccines 1 and 2 against *A. paragallinarum* serovar B (102984) was 100% at 7 DPI (Table 4).

DISCUSSION

The present study is the first published investigation on the efficacy of the commercial trivalent vaccines that compose of different kinds of adjuvants including mineral oil emulsion, aluminium hydroxide gel, water in oil emulsion and oil emulsion against local Thai strains of *A. paragallinarum*. As widely accepted concept that serogroups A, B and C belongs to three distinct immunovars and they are some limitation of cross-protection (Soriano *et al*., 2004). Apart from serovar specificity and inactivating agent, adjuvant suitability is the key point of efficacy to inactivated vaccine products and this issue still needs further investigation. Early reports revealed that oil-based adjuvant is effective as alum-gel based vaccines (Davis *et al*., 1976) while the others reported that oil based vaccines were less effective (Reid and Blackall, 1987). In the current study, although, these 4 commercial vaccines were trivalent vaccines, only Vaccine 1 and Vaccine 2 provided 100% protection against *A. paragallinarum* serovar A, B, and C on 5 and 7 DPI since no clinical signs were observed and *A. paragallinarum* was negative in the infraorbital sinus culture. Vaccine 3 provided 85.7% protection against serovar A (B1E1), as clinical signs were observed in birds challenged with serovar A and *A. paragallinarum* was positive in the infraorbital sinus culture. Vaccine 4 provided 57.14% and 85.7% protection against serovar A and C, respectively, as clinical signs were observed in the birds challenged with serovar A and *A. paragallinarum* was present in the infraorbital sinus culture for birds challenged with serovar A and C. Nevertheless, Vaccines 3 and 4 provided 100% protection against serovar B, and Vaccine 3 provided 100% protection against serovar C. In addition, all types of vaccines caused no adverse clinical reaction.

The comparative efficacy of oil-based and gel-based vaccine adjuvants has been studied by other researchers. Some studies demonstrated that an oil-based vaccine induced a higher antibody level and provided better protection against field strains (Jacobs *et al*., 1992; Fukanoki *et al*., 2000; Chukiatsiri *et al*., 2010; Gong *et al*., 2014). In contrast, other studies showed that gel-based vaccines induced higher antibody titer and better protection (Gong *et al*., 2014). In this study, oil-based and gel-based vaccines (Vaccines 1 and 2) from the same company showed similar levels of protection against field strains, and no adverse reaction were observed throughout the study.

In the past, bivalent inactivated vaccines composed of serovar A and serovar C were commonly used in the Thai poultry industry, but in 2010, serovar B was first reported in an outbreak at a layer farm (Chukiatsiri *et al*., 2009). As a result, trivalent vaccine with serovar B has been employed in the Thai poultry industry up till now. Therefore, serovar B has been continuously isolated from the field, and several isolates have been used for challenge in this study. The infraorbital culture result revealed that vaccines 1 and 2 provided 100% protection against all serovar B isolates. However, some vaccinated birds challenged with B (102984) strain exhibited some clinical signs.

These 4 vaccines were able to induce antibodies measured by the HI test, but the antibody titers varied. Vaccines 3 and 4 induced lower antibody titers than vaccines 1 and 2 in birds challenged with serovar A, while, the antibody titers from the serovar B and serovar C challenged groups were not obviously different among these 4 vaccines. Hence, this study has shown that the antibody titer against Infectious Coryza does not show significant different on disease protection rate. However, the higher antibody titers showed the better protection.

Although all 4 commercial vaccines were inactivated vaccines, only vaccines 1 and 2 provided 100% protection for all Thai field isolates, as there was no positive infraorbital culture found in vaccines 1 and 2 vaccinated birds challenged with serovar A, B, and C. Nevertheless, each Kume serovar did not provide strong cross protection with all other serovars in the same Kume serogroup. For example, serovars A-1, A-2, and A-3 provided strong cross protection with each other, but serovar A-4 showed a lower level of cross protection, while serovar C-1 provided lower cross protection for serovar C-2 and serovar C-4 induced significantly lower cross protection for serovar C-1, C-2, and C-3 (Soriano *et al*., 2004). Similarly, this study showed that vaccines 3 and 4 did not provide 100% protection against Thai field serovar A, and vaccine 4 did not provide 100% protection against Thai field serovar C. It is possible that serovar A and C of Thai field strains were different from the vaccine seeds while belonging to the same serogroups. Moreover, Kume sero group B contains only serovar B-1. At the same time, these results show that all 4 commercial vaccines provide 100% protection for the birds in the serovar B field strain challenge.

The lower rate of protection of within the serovars had been observed by other studies as well. Those studies indicated that the same serovar collected from the same geographic area showed degrees of different response to the vaccination .In Korea, 7 field isolates were confirmed as serovar A. Birds in the study vaccinated with local commercial vaccine and challenged with 2 different isolates. However, the vaccine was effectiveness on decreased clinical signs and decreased number of re-isolated on 5 and 10 DPI, the lower effectiveness was observed when challenged with another isolate (Han *et al*., 2016). Another study showed 16 field isolates of serovar B-1 in Ecuador, Mexico, and Panama. But there were 10 genotypic distinguish pattern within 16 isolates. Non-commercial vaccine was use in this study. The challenged bacteria that have common genotype with the vaccine indicated higher protection than the different genotype) (Morales *et al*., 2014). Another work in Ecuador and Mexico studied efficacy of 4 different commercial trivalent vaccines against one isolate of serovar C. The result showed that vaccine 1 provided the highest protection rate compared to other 3 vaccines (Morales *et al*., 2015). As a result, the vaccines of infectious coryza disease with high protection against all serovars in one shot still develop. The highly variable genetic among each isolate even in the same serovar may cause vaccine failure in the field situation. Recently, efficacy of in-house recombinant vaccines were studies but they were proved to be effectiveness against the same serovar (Noro *et al.*, 2006; Hsu *et al*., 2007; Wang *et al.*, 2007; Noro *et al*., 2008; Sakamoto *et al*., 2013).

In Thailand, trivalent vaccine is necessary for protection against Infectious Coryza infection. In this study, vaccines 1 and 2 were most effective to protect chickens against Thai isolates. In addition, the efficacy of oil-based and gel-based adjuvant were shown to be no different. However, in Kume serogroups A and C, each consisting of 4 serogroups, some serogroups do not provide cross protection for the others. As a result, the use of Infectious Coryza vaccine in each country is complicated. It was also found that the antibody titers measured by HI test do not correlate with the protection rate. Therefore, vaccine efficacy of Infectious Coryza disease is necessary for effective control and prevention strategy in the poultry industry.

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