

Field study of seroconversion of three different commercial vaccines of chicken infectious anemia virus in Thailand

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Abstract

The aim of this study was to evaluate the effects of different chicken infectious anemia virus (CIAV) vaccine strains in breeders and progeny under field conditions. For this experiment, one commercial poultry company was selected, and independent management for each house was applied to avoid vaccine cross contamination. No natural infection was found before vaccination. BioChek ELISA kits were used, and a minimum of 20 birds per flock were sampled in each sampling time. Three commercial CIAV vaccine strains that are available in Thailand were selected for the study: 26P4 was vaccinated subcutaneously, Cux-1 was vaccinated orally, and Del Ros was vaccinated via wing web. The vaccine strains were administered to different flocks at 8, 8 and 6 weeks of age, respectively, following the manufacturers' instructions. At 14 weeks of age, average antibody titers against 26P4, Cux-1 and Del Ros were 1,650.78, 3,993.94 and 2,409.89; %coefficients of variance were 60, 19 and 40%; and vaccination indices were 28, 210 and 60, respectively. At 14, 23, 27, 32 and 50 weeks of age, the birds that received Cux-1 revealed significantly higher antibody titers than the birds that received 26P4 at the same age ($p < 0.05$). The birds vaccinated with Cux-1 showed 100% positive serum samples 6 weeks after vaccination. Birds that hatched from the broiler breeders vaccinated with Cux-1 at the ages of 27, 32, 50 and 61 weeks revealed significantly higher antibody titers than those of the birds vaccinated with 26P4 ($p < 0.05$).

Keywords: antibody titers, chicken infectious anemia virus, seroconversion, vaccines

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Introduction

Chicken infectious anemia virus (CIAV) is a causative agent of chicken infectious anemia (CIA) (Yuasa et al., 1979). CIAV is an immunosuppressive disease in chickens which causes serious economic losses. It is a ubiquitous and highly resistant chicken virus causing anemia and death in chicks less than 3 weeks of age and immunosuppression in chickens older than 3 weeks (Goryo et al., 1985; McConnell et al., 1993). The disease is characterized by aplastic anemia and generalized lymphoid atrophy with concomitant immunosuppression with secondary viral, bacterial or fungal infections (Schat and van Santen, 2008). There are approximately 50 billion chickens raised every year worldwide (Kaiser, 2010). CIA causes serious economic loss to the commercial broiler industry (McIlroy et al., 1992). Vertical transmission of the virus plays an important role in infection among young chickens (Miller et al., 2003). Detection of seroconversion in breeder flocks should be conducted before egg production because maternal antibodies provide complete protection of young chicks against CIAV-induced anemia (Yuasa et al., 1980). The risk for hatcheries lies in poorly vaccinated flocks that transmit insufficient quantities of maternal antibodies to the progeny or, even worse, become infected during lay and, hence, transmit the disease to the unprotected chicks. In Thailand, CIAV was first reported in 1996 (Tantaswasdi et al., 1996); since then many outbreaks have occurred and vaccine efficacy has frequently been questioned. A phylogenetic analysis of 13 CIAV strains isolated in Thailand on amino acid analysis of VP1 genes in the hypervariable region (139-151) showed a close relation to the Chinese and Japanese strains (99.2-99.4% sequence similarity) (Wanasawaeng et al., 2013). Therefore, a vaccine-based strategy to control clinical and subclinical diseases associated with CIAV infection is necessary (Todd et al., 1995) and should be broadly practiced in Thailand. Heng et al. (2001) reported that the prevalence of CIAV infection in Thai broiler and broiler breeder farms by PCR in 1999 was 89% (48/54) and 71% (17/24), and in 2000, 78% (29/37) and 49% (28/57), respectively. The high prevalence of CIAV infection is still found in the Thai poultry

industry. However, there is concern about the efficacy of CIAV vaccines in field condition. Therefore, the aim of this study was to evaluate the effects of three commercial CIAV vaccines on antibody responses against CIAV in breeders and progeny under field condition.

Materials and Methods

Animal experiments, vaccines and vaccination: Broiler breeders (ROSS 308) raised in the same hatchery were divided into 3 evaporative-cooling system broiler breeder houses (Groups A-C) in a farm belonging to Top Agribusiness Co., Ltd. in Thailand. The birds in all 3 houses received the same vaccination program except CIAV vaccine. Three kinds of live commercial CIAV vaccines which were available in Thailand were used for this experiment: 26P4 (Nobilis CAV-P4®, Boxmeer, Netherlands), Cux-1 (AviPro® Thymovac, Cuxhaven, Germany) and Del Ros (Circomune®, Lenexa, USA). Group A was vaccinated with 26P4; one dose (10^3 TCID₅₀) was administered subcutaneously at the age of 8 weeks. Group B was vaccinated with Cux-1; one dose ($10^{4.5}$ - $10^{5.5}$ TCID₅₀) was administered via drinking water at the age of 8 weeks. Group C was vaccinated with Del Ros; one dose ($10^{4.9}$ TCID₅₀) was injected via the wing web at the age of 6 weeks.

Blood sampling: Serum samples were collected from a minimum of 20 broiler breeders of a specific flock from each group at intervals as shown in Table 1 and then tested for CIAV-specific antibodies with the BioChek ELISA (Reeuwijk, The Netherlands). Twenty sera of chickens hatched from fertile eggs from each group at 27, 32, 50 and 61 weeks of age were tested for CIAV-specific antibodies at the first day of age. Vaccination index (VI) describes vaccination response and is expected to give a high score for a good vaccination and a low score for a poor vaccination. VI was calculated from mean titers/coefficient of variance (CV). Data were analyzed and compared by ANOVA and Duncan's multiple range test. Percent CV was analyzed with Chi-square test.

Table 1 Program of random serum collection (20 samples/group/week) for ELISA test (BioChek) of broiler breeders and progeny vaccinated with 3 different commercial CIAV vaccines

Age (wk)	Breeder sampling	Progeny sampling	Age (wk)	Breeder sampling	Progeny sampling
5	Random serum collection from groups A, B and C before vaccination	No	27	21 weeks post vaccination for Del Ros and 19 weeks post vaccination for 26P4 and Cux-1	Yes
11	5 weeks post vaccination for Del Ros and 3 weeks post vaccination for 26P4 and Cux-1	No	32	26 weeks post vaccination for Del Ros and 24 weeks post vaccination for 26P4 and Cux-1	Yes
14	8 weeks post vaccination for Del Ros and 6 weeks post vaccination for 26P4 and Cux-1	No	50	44 weeks post vaccination for Del Ros and 42 weeks post vaccination for 26P4 and Cux-1	Yes
23	17 weeks post vaccination for Del Ros and 15 weeks post vaccination for 26P4 and Cux-1	No	61	55 weeks post vaccination for Del Ros and 53 weeks post vaccination for 26P4 and Cux-1	Yes

Results and Discussion

Chicken infectious anemia has been recognized as an economically detrimental disease in chickens since it was discovered as a vaccine contaminant (Yuasa et al., 1979). The disease is characterized by immunosuppression by induction of thymic T-cell apoptosis. Successful vaccination of CIAV can provide solid protection against CIAV infection. The outbreaks of CIA in the field often correlate with the absence of anti-CIAV antibody in respective parent flocks (Engström, 1998). Maternal-derived antibody to progeny is important for clinical protection from field challenge with chicks less than 3 weeks old. The efficacy of vaccines, as a result, could influence field challenge. The field investigation into chickens vaccinated with 3 commercial vaccine strains, 26P4, Cux-1 and Del Ros, at 8, 8 and 6 weeks of age via subcutaneous injection, drinking water and wing web, respectively, revealed differences in antibody titers. According to BioChek guidelines for chicken anemia virus antibody ELISA (CAV, 2016), titer ranges of no protection, moderate protection and protection are 724, 724-2295 and >2296, respectively. No natural infection was found before the vaccination based on the test of 5-week-old serum samples. At 14, 23, 27 and 32 weeks of age, the birds that received Cux-1 revealed significantly higher antibody titers than the birds that received 26P4 and Del Ros at the same age ($p < 0.05$). At 50 weeks of age, the antibody titers of the birds receiving Cux-1 vaccine were significantly higher than those of the birds receiving 26P4 vaccine ($p < 0.05$) (Table 2). At all ages of serum collection, the birds vaccinated with Cux-1 revealed higher antibody titers than the birds vaccinated with the other CIAV vaccines. For percentage of positive serum samples at all ages of sample collection, the birds vaccinated with Cux-1 showed 100% positive serum samples at 6 weeks after vaccination. At the ages of 11 and 14 weeks old, the percentage of positive serum samples of the birds vaccinated with Cux-1 was significantly higher than that of the birds vaccinated with 26P4 ($p < 0.05$) (Table

3). The positive antibodies in breeder hens against CIAV can prevent vertical transmission to progeny, however, viral DNA can still be transmitted (Cardona et al., 2000; Miller et al., 2003; Brentano et al., 2005; Hailemariam et al., 2008). Many outbreaks of CIAV in the field often correlate with the absence of anti-CIAV antibody in respective parent flocks (Yuasa et al., 1987; Vielitz and Landgraf, 1988; Chettle et al., 1989; Engström, 1998). According to the vaccination index (VI) of the BioChek guidelines for CIAV vaccination, only the broiler breeders vaccinated with Cux-1 revealed VI that matched the guidelines (VI = 100-300). The antibody titers of the vaccinated birds could last at least 53 weeks after receiving one shot of vaccination; this result agrees with a previous report (Imai et al., 1993). One-day-old broilers derived from the specific ages of broiler breeders vaccinated with Cux-1 showed the highest antibody titers among the vaccinated groups. The birds hatched by the broiler breeders vaccinated with Cux-1 at the ages of 27, 32 and 61 weeks revealed significantly higher antibody titers than those that hatched by the birds vaccinated with 26P4 ($p < 0.05$) (Table 4). For the percentage of positive serum samples at all ages of sample collection, the birds vaccinated with Cux-1 showed 100% positive serum samples at 6 weeks after vaccination (Table 5). Maternal-derived antibodies in young chicks against CIAV is quite important for protection against CIAV-induced anemia in progeny (Yuasa et al., 1980). In conclusion, the broiler breeders vaccinated with Cux-1 revealed the highest antibody titers when compared to those vaccinated with 26P4 and Del Ros. Moreover, they showed 100% positive samples. The one-day-old broilers hatched by the birds vaccinated with Cux-1 revealed higher antibody titers than those hatched by the birds vaccinated with the other vaccines. The commercial CIAV vaccine strains provided different antibody titer levels for the vaccinated birds in these field trials. Therefore, choosing the best vaccine will influence antibody titer level and maternal-derived antibody to protect progeny.

Table 2 Antibody titers (Mean±SD) of broiler breeders receiving different CIAV vaccines

Age (wk)	Antibody titers (Mean±SD)			Age (wk)	Antibody titers (Mean±SD)		
	26P4	Cux-1	Del Ros		26P4	Cux-1	Del Ros
5		69.67±40.08*		27	1638.22±1013.81 ^a	3329.89±820.16 ^b	2401.50±1008.41 ^{a,c}
11	1623.15±1085.28	2448.60±1770.67	2110.15±801.77	32	1017.33±590.84 ^a	3152.33±1139.92 ^b	1622.17±924.30 ^{a,c}
14	1650.78±995.06 ^a	3993.94±769.81 ^b	2409.89±956.51 ^c	50	1908.35±1302.38 ^a	3694.40±1139.24 ^b	2898.80±1328.56 ^{b,c}
23	1922.47±1138.07 ^a	3208.73±897.14 ^b	2157.93±851.83 ^{a,c}	61	2534.30±1564.63	3246.95±846.04	2221.94±1450.64

Note: The different superscripts in each age mean significant difference at $p < 0.05$.

*Serum samples were randomly collected from 3 CIAV-vaccinated flocks as representatives before vaccination.

Table 3 Percent CV, percent positive serum samples and vaccination index (VI) of broiler breeders receiving different CIAV vaccines

Age (wk)	%CV/%positive serum samples/VI			Age (wk)	%CV/%positive serum samples/VI		
	26P4	Cux-1	Del Ros		26P4	Cux-1	Del Ros
5		57/0/1*		27	62/88/26	25/100/133	42/88/57
11	67/85 ^{a,b} /24	72/70 ^a /34	38/100 ^b /56	32	58/61 ^a /18	32/100 ^b /99	57/83 ^{a,b} /29
14	60/77 ^a /28	19/100 ^b /210	40/100 ^b /60	50	68/85/28	31/100/119	46/100/63
23	59/80/33	28/100/115	39/100/55	61	62/100/41	26/100/125	65/88/34

Note: The different superscripts in each age mean significant difference at $p < 0.05$.

*Serum samples were randomly collected from 3 CIAV-vaccinated flocks as representatives before vaccination.

Table 4 Antibody titers (Mean±SD) of one-day-old broilers (progeny) from broiler breeders receiving different CIAV vaccines

Age (wk)	Antibody titers (Mean±SD)			Age (wk)	Antibody titers (Mean±SD)		
	26P4	Cux-1	Del Ros		26P4	Cux-1	Del Ros
27	925.28±	2159.11±	1417.06±	50	1807.35±	2227.10±	1345.60±
	591.53 ^a	1089.28 ^b	1320.22 ^{a,c}		1519.26 ^a	893.72 ^{a,b}	745.82 ^a
32	616.30±	2086.05±	822.00±	61	882.75±	2572.20±	1113.89±
	419.87 ^a	1058.64 ^b	771.35 ^{a,c}		698.56 ^a	1347.18 ^b	746.48 ^{a,c}

Note: The different superscripts in each age mean significant difference at $p < 0.05$.

Table 5 Percent CV, percent positive serum samples and vaccination index (VI) in one-day-old broilers (progeny) from broiler breeders receiving different vaccinations

Age (wk)	%CV/%positive serum samples/VI			Age (wk)	%CV/%positive serum samples/VI		
	26P4	Cux-1	Del Ros		26P4	Cux-1	Del Ros
27	64/61 ^a /15	50/94 ^b /43	93/55 ^{a,c} /15	50	84/70 ^a /13	60/100 ^b /37	55/80 ^a /25
32	68/30 ^a /9	51/100 ^b /41	94/45 ^a /9	61	79/50 ^a /11	52/90 ^b /50	67/73 ^{a,b} /17

Note: The different superscripts in each age mean significant difference at $p < 0.05$.

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บทคัดย่อ

การศึกษาภาคสนามของการเปลี่ยนแปลงซีรัมของวัคซีนเชิงพาณิชย์สามชนิดที่แตกต่างกัน ของไวรัสเลือดจางติดต่อในไก่ในประเทศไทย

นิวัตร จันทรศิริพรชัย

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของวัคซีนไวรัสเลือดจางติดต่อในไก่ในไก่พันธุ์และไก่เนื้อในภาคสนาม โดยเลือกฟาร์มไก่ที่มีการจัดการโรงเรือนที่แยกอิสระ และทำการทดสอบแยกกันในแต่ละโรงเรือน เพื่อป้องกันการปนเปื้อนข้ามของวัคซีน ไม่พบการติดเชื้อตามธรรมชาติในไก่ก่อนเริ่มการทดลองด้วยการใช้ชุดทดสอบอิลูซา (BioChek) ในการตรวจซีรัม โดยการสุ่มแต่ละครั้งทำการเก็บเลือดไก่อย่างน้อย 20 ตัว ทำการทดสอบวัคซีนไวรัสเลือดจางติดต่อในไก่ที่มีจำหน่ายในประเทศไทย 3 ชนิด คือ สายพันธุ์ 26P4 ซึ่งให้โดยการฉีดใต้ผิวหนัง Cux-1 ซึ่งให้โดยการละลายน้ำ และ Del Ros ซึ่งให้โดยการแทงปีก ที่อายุ 8, 8 และ 6 สัปดาห์ ตามลำดับ ตามคำแนะนำของบริษัท ที่อายุ 14 สัปดาห์ระดับไตเตอร์เฉลี่ยต่อ 26P4, Cux-1 และ Del Ros เท่ากับ 1,650.78, 3,993.94 และ 2,409.89 เปอร์เซ็นต์ coefficient of variance เท่ากับ 60, 19 และ 40% และ vaccination index เท่ากับ 28, 210 และ 60 ตามลำดับ ที่อายุ 14, 23, 27, 32 และ 50 สัปดาห์ ที่อายุเดียวกันไก่ที่ได้รับวัคซีนสายพันธุ์ Cux-1 มีระดับแอนติบอดีไตเตอร์สูงกว่าไก่ที่ได้รับวัคซีนสายพันธุ์ 26P4 อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในไก่ที่ได้รับวัคซีนสายพันธุ์ Cux-1 พบตัวอย่างซีรัมที่ให้ผลบวก 100 เปอร์เซ็นต์ที่ 6 สัปดาห์หลังได้รับวัคซีน ไก่เนื้อที่ฟักจากไข่ไก่พันธุ์ที่ได้รับวัคซีนสายพันธุ์ Cux-1 ที่อายุ 27, 32, 50 และ 61 สัปดาห์มีระดับแอนติบอดีสูงกว่าไก่เนื้อที่ฟักจากไข่ไก่พันธุ์ที่ได้รับวัคซีนสายพันธุ์ 26P4 อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$)

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