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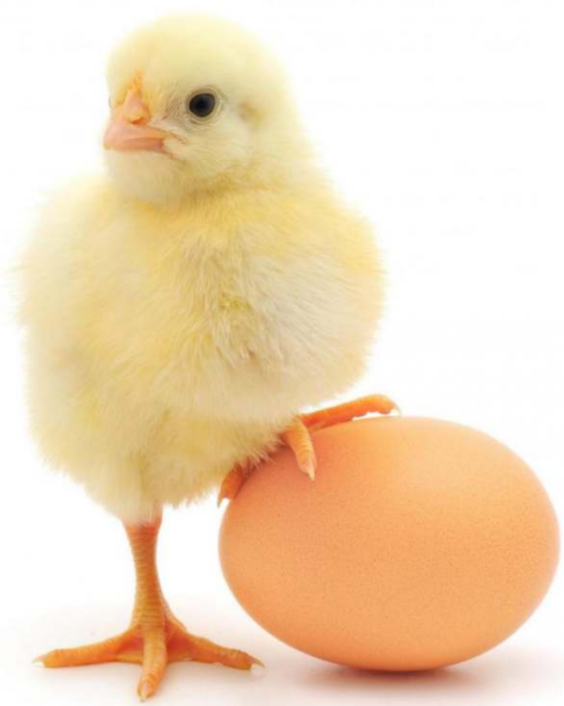

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Effects of Vaccination Routes Against IB on Performance and Immune Responses of Broiler Chickens

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Abstract: Vaccination is often considered as an appropriate option in prevention most of poultry viral diseases worldwide. This study was conducted to evaluate effects of current routine vaccination routes (spray, eye-drop and drinking water) of live vaccines against infectious bronchitis (IB) on performance and humoral immune responses of broiler chickens. The results of this study indicated that Vaccination significantly (P<0.05) affects performance of the broiler chickens and effects on weight gain and FCR, did not differed significantly among these routes. Immune responses of vaccinated chickens were significantly (P<0.001) differed from those of control chickens. Comparison of various vaccination routes revealed that eye-drop group had the highest antibody titer with the closest range. There were also positive significant (P<0. 01) degrees of correlation among chickens vaccinated with spray and eye-drop, spray and drinking water, eye-drop and drinking water (r = 0.84, r = 0.80 and r = 0.82, respectively). In conclusion, eye-drop method induced the highest antibody titers with the closest range.

Key word: Vaccination, IB, spray, eye-drop, drinking water

Introduction

The art and science of vaccinology aimed at prevention of infectious diseases responsible for the huge economic losses in poultry industry worldwide. Among viral poultry diseases, infectious bronchitis (Cavanagh and Naqi, 2003) is acute, highly contagious and economically important diseases of chickens and in most countries, control of this disease is largely through the vaccination. Isolation of different strains or serotypes of IB virus (Cook, 2001) indicates that vaccination policy could be effective if the vaccines contain local strains and delivered via the best application route. Different types of vaccine including live, inactivated, DNA (Kapczynski *et al.*, 2003) and recombinant (Song *et al.*, 1998; Johnson *et al.*, 2003) vaccines have been studied. On the other hand, effects of some vaccination routes with different types of vaccine on the outcome of immune responses have also been investigated (Ratanasethakul and Cumming, 1983; Cholakova, 1985; Wakenell and Sharma, 1986; Halvorson *et al.*, 1991; Toro *et al.*, 1997; Kapczynski *et al.*, 2003). Previous studies support that routes of application affect receiving the proper dose of vaccine, time of stimulating protective immunity and range of immunity in a vaccinated flock.
Differences between this study and the previous reports are application of three routes as a routine vaccination program and in drinking water routes. During this study, level of antibody, uniformity of immune responses and effects of secondary vaccination on humoral immunity induced by different methods of vaccination were compared.

Materials and Methods

Chickens: Eighty one-day-old broiler chicks (Arbor-Acres strain) were divided into 4 groups (A, B, C and D, 20 chicks in each group). The chicks of each group were leg labeled, kept in a separate cages, fed *ad libitum* with a diet prepared based on Arbor-Acres husbandry catalogue.

Vaccine and vaccination: Live attenuated Massachusetts H_{120} strain vaccine was used for primary vaccination of 1-day-old chicks on arrival day and for re-vaccination at age 18 days against IB via the most routine routes of application including spray, eye-drop and drinking water methods as follows:

Drinking water: A vial of vaccine was diluted with distilled water and serial dilutions were made until to get 1 dose of vaccine in 1 ml distilled water. The chickens of group A were given 1ml containing 1 dose of the vaccine via mouth using 1ml syringes.

Eye-drop method: The vaccine was diluted with distilled water and serial dilutions were made until to get 400 dose of vaccine in 10 ml distilled water. One drop (0.25 µl containing 1 dose) was delivered for one-eye of each chicks of group B using a droplet dividing 1ml into 40 drops.

Spray method: In this method, a vial of vaccine was diluted with distilled water and serial dilutions were made until to get 10 dose of vaccine in 10 ml distilled

Table 1: Performance of chickens during eight weeks of husbandry period

Age (week)	Control Group			Routes of Vaccination								
	Feed	Average	FCR	Spray Group			Eye-drop Group			Drinking water Group		
				Feed (gr/week)	Average Weight (gr)	FCR	Feed (gr/week)	Average Weight (gr)	FCR	Feed (gr/week)	Average Weight (gr)	FCR
0	---	38	---	---	37.5	---	---	38	---	---	37.5	---
1	112	125	1.28	112	120.5	1.34	112	120	1.36	112	121	1.34
2	224	275	1.49	224	268	1.51	224	267	1.52	224	270	1.50
3	420	540	1.58	420	530	1.60	420	527	1.61	420	533	1.59
4	630	895	1.77	630	877	1.81	630	875	1.81	630	880	1.81
5	770	1320	1.81	770	1300	1.82	770	1300	1.81	770	1305	1.81
6	840	1750	1.95	840	1725	1.97	840	1725	1.97	840	1715	2.04
7	1050	2175	2.47	1050	2150	2.47	1050	2155	2.44	1050	2150	2.41
8	1260	2676	2.51	1260	2620	2.68	1260	2630	2.65	1260	2628	2.63
Total	5306	2676	2.011	5306	2620	2.054	5306	2630	2.047	5306	2628	2.048

$$\text{Weekly FCR (food conversion ratio)} = \frac{\text{Feed eaten during a week/chicken}}{\text{Weight at end of week - Primary weight}}$$

$$\text{Final FCR} = \frac{\text{Total feed eaten during 8 weeks/chicken}}{\text{Weight at end of 8-week- chick's weight}}$$

water. After gathering the chickens of group C in a cage, the 10 ml vaccine was sprayed (coarse spray) inside the cage in a relatively calm condition using a small hand-spray apparatus.
The chickens of group D were kept un-vaccinated as a control group in a separate room.

Blood sampling and serum preparation: On day 0, half of chicks of each group were scarified as previously described (Olorede and Longe, 1999; Alcorn, 2001) and their blood was considered as one-day-old samples for the half remaining chicks of each group. On day 7, blood samples were taken from jugular veins (Zander *et al.*, 1997) using 1ml insulin syringes and on day 14 and weekly intervals, blood samples were taken from the main brachial vein (wing vein) of the chickens as previously described (Zander *et al.*, 1997). Blood samples were labeled by number of birds and date, kept in room temperature until clotted (almost 30 minutes), the clots were denatured and kept in water-bath with 56^{o}C in order to separate the sera for serological tests.

Evaluation of immune responses: On day 0 (before vaccination) and weekly intervals, serum samples were used to evaluate maternal antibodies of the chicks and to assess humoral immune response derived from different vaccination routes. ELISA (using IDEXX kits), as a useful tool to monitor the humoral immune responses to vaccination against IB (Zellen and Thorsen, 1986; Cavanagh and Naqi, 2003), was used to evaluate antibody titers of the serum samples and to determine range of immunity among the chickens of each group.

Evaluation of performance: Different factors including weight gain and food efficiency ratio (FCR) were determined as performance of the chickens for each week and the whole experimental duration.

Statistical analysis: In SPSS program, ANOVA, Spearman's correlation and Two-tailed t-test were used as appropriate to analyze the data.

Results

Performance of different groups during husbandry period: As shown in Table 1, weight gain and food efficiency ratio (FCR) of the chickens for each week and the whole experimental duration varied among the groups. The differences of weight gain of vaccinated and control chickens were significant ($P<0.01$), but differences in weight gain among vaccinated groups were not significant ($P>0.05$).

Maternal immunity: maternal antibody titers of the unvaccinated chicks declined with a mean half-life of five to six days and reached from 5367 to 209 at end of 3rd week, while maternal antibody titers of vaccinated chickens declined gradually and reached to 534.8, 689.1, and 627.7 (spray, eye-drop and drinking water methods, respectively) at age of 21 day.

Effects of routes and re-vaccination on level of antibody titers: Immune responses of vaccinated chickens, regardless routes of application, differed significantly ($P<0.001$) from those of unvaccinated control chickens, however primary vaccination of groups

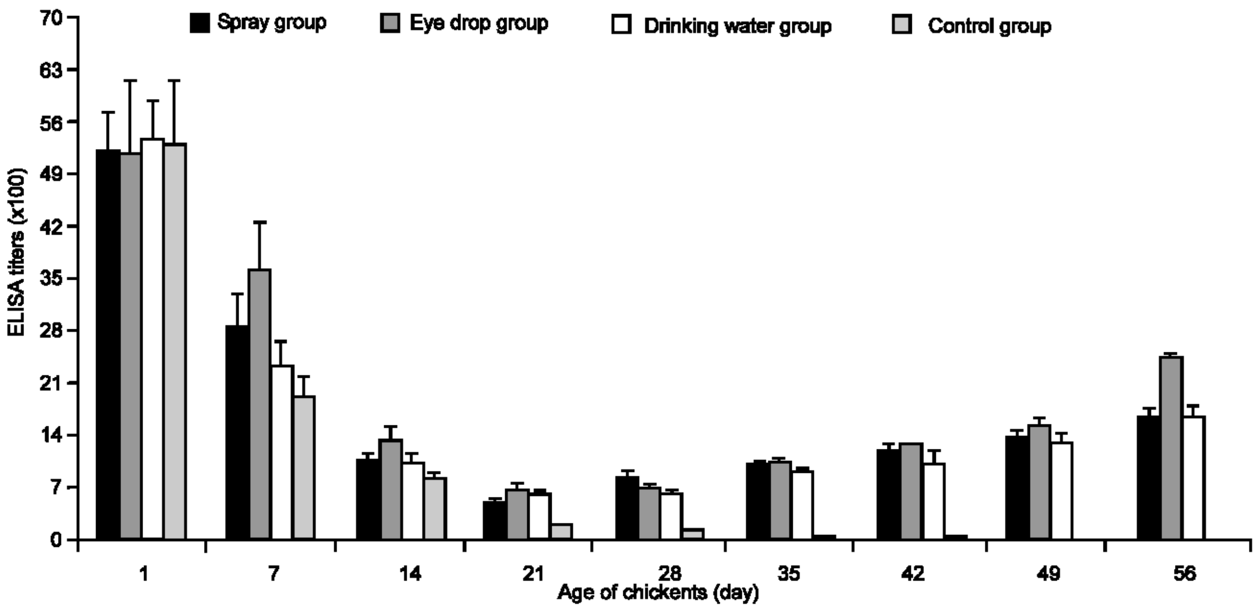

Fig. 1: Effects of different routes of application of infectious bronchitis H120 vaccine on antibody titers

A, B and D with H_{120} vaccine at one-day-old affected reduction rate of maternal immunity in contrast to control chickens. Re-vaccination at age of 18 day, boosted the immune responses and based on routes of application, increasing in antibody titers varied among chickens of the groups. Spray method of vaccination had the faster effects and increased the antibody titers from 534.8 at 21 day to 1652 at the end of experiment. Eye-drop method of vaccination increased antibody titers from 689.1 at day 21 to 2446 at age of 56 day. Drinking water method of vaccination increased antibody titers from 627.7 at day 21 to 1681 at age of 56 day. As shown in Fig. 1, chickens vaccinated with eye-drop route had the highest level of antibody titers with the closest uniformity (range) among the methods were applied.

Discussion

A successful vaccination comes up in terms of higher and stronger immunity and a logical criterion for the evaluation of any vaccination program is based on the assessment of production parameters including feed conversion ratio (Zander *et al.*, 1997).

As shown in the Table 1, presence of a significant differences in weight gain of control and vaccinated chickens, indicating that stress due to vaccination may affect feed conversion ratio (FCR) during vaccination period, but lack of significant differences in performance of vaccinated chickens indicating that all the routes of vaccination had some effects on FCR.

Maternally-derived antibodies are important in preventing or reducing severity of lesions in infectious bronchitis (Herdt *et al.*, 2001; Mondal and Naqi, 2001) as well as in timing of first vaccination. Meanwhile, high level of maternal antibodies has negative feed back effect on B-lymphocytes. Results of this study revealed that the different routes of vaccination of day-old-chicks with IB H_{120} vaccine had some effects on reduction rate of maternally-derived antibodies as its titer declined with a mean half-time as previously described (Darbyshire and Peters, 1985).

Spray method of vaccination increased antibody titer faster than eye-drop route and the latter than drinking water method when immune responses were compared at 28-day age. Overall, eye-drop method had produced the highest level of antibody response in primary and secondary vaccination (Fig. 1), indicating that eye-drop route is the best option for vaccination against IB as previously reported (Winterfield *et al.*, 1976; Ratanasethackl and Cumming, 1983; Al-Tarcha *et al.*, 1991). The results of this study approved that eye-drop route of vaccination induce higher level of antibody titer with the closest range in comparison to drinking water or spray method, and this is because intra-ocular route is performed on a bird-by-bird basis.

Conclusion: Eye-drop route of vaccination is the best method for primary and secondary vaccinating of broiler chickens with live IB H_{120} vaccine in order to obtain a high level and closer range of humoral immune response. In spray method, some of vaccine droplets were spoiled by contact to bodies of chickens or mixing with litters, therefore vaccine failure may occur due to inadequate dose of vaccine received.

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