Global Veterinaria 14 (3): 425-431, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.03.93111

Impact of BCG Vaccination on Productive Performance and Immune Response To avian Influenza Vaccine in Two Breeds of Ducks

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Abstract: The present study was conducted on A total of 200 unsexed day old ducklings of two breeds of ducks, Peckin and Cherry valley ducks each breed subdivided into BCG vaccinated group (50 ducklings) and control non vaccinated one (50 ducklings). All ducklings were vaccinated with both Avian Influenza and BCG vaccine at 14thdays of age. Body weights, body weight gain and immune response were measured for BCG injected group and the control one. The results were showed that, the Cherry valley duck was significantly higher than Peckin duck in both initial body weight and body weight gain during the first period (0-2 weeks of age). Also, Cherry valley ducks were significantly higher in final body weight than Peckin. But, Peckin ducks were significantly higher at second week of age than Cherry valley one. BCG vaccine was expressed its effect on body weight by a significant reduction in body weight at 4th and 6th weeks of age, but, body weight at other ages and body weight gain at all periods were non-significant. The interaction between BCG and breed revealed that body weight and body weight gain were significantly lower in BCG vaccinated Peckin ducks at 8th week of age (2825.19±61.81 and 2781.04±63.10 g). Antibody titer against AI was significantly improved only at 15 days post-vaccination in BCG vaccinated group compared with control one. Phagocytic activity in Cherry valley ducks were significantly improved for BCG vaccinated group at 3rd day post-vaccination than control one (20.30±0.63vs18.42±0.57). Phagocytic activity was significantly increased in BCG vaccinated group for both breeds at 7th day post vaccination. It could be concluded that, immune response (Humeral and Cellular) to Avian Influenza vaccine could be improved by BCG vaccination, but it has a worth effect on productive performance and its intense was varied with the genotype of ducks.

Key words: Avian Influenza · BCG · Cherry Valley · Peckin · Immune response

INTRODUCTION

The duck meat market has grown significantly in recent years and is likely to continue with genetic and husbandry advances, making duck increasingly competitive to other poultry and meat products. Vaccines are the most cost-effective medical intervention known to prevent disease in poultry. A series of observations suggest that non-specific immunomodulation by agents such as BCG, could represent a simple means of controlling diseases [1]. BCG is a widely used live vaccine against tuberculosis without significant adverse effects and a vaccine vehicle for the presentation of heterologous antigens with advantages of excellent cellular immune adjuvant properties, long-persisting effects, safety and low production costs [2, 3]. A wide range of recombinant BCG vaccine candidates containing foreign viral, bacterial, parasitic or immunomodulatory genetic materials have been developed and evaluated for stimulation of immune response to the foreign antigen [4-6]. BCG can stimulate the differentiation of the bone marrow pluripotent stem cells along with the proliferation of macrophage,

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significantly improving the cellular immunity [7]. Qiuyue *et al.* [7] also, study the effect of BCG vaccination on body weight in chicken and stated that, there is no significant difference for the body weight were detected between groups before immunization and 2 weeks after the booster immunization. The effect of BCG vaccination on the body weight gain in chicken was noted to be higher in the BCG vaccinated group than the control one but the difference is not statistically significant. Ducks mount a poor antibody response to influenza in comparison to mammals or even chickens and the reasons for the much weaker immune response of ducks to this vaccine are not clear [8]. So vaccination of ducks with reverse-genetics engineered inactivated oil emulsion AI vaccine, while efficacious, required larger doses and a second boost of vaccine in comparison to chickens.

Li *et al.* [9] evaluate the capacity of *Bacillus Calmette-Guerin* (BCG) to deliver apical membrane antigen1 (AMA1) of *Eimeria maxima* to stimulate specific cellular and Humoral immune responses in chickens and the challenge experiments demonstrated that rBCG vaccination via intranasal or subcutaneous routes could increase weight gain, decrease intestinal lesions and reduce fecal oocyst shedding and the subcutaneous and intranasal routes were superior to the oral route based on the immune effects. Furthermore, intranasal rBCG immunization could also lead to a significant increase in serum antibody. In this study, BCG vaccine was used to investigate its effect on duck performance (Body weight, body weight gain) and immunity (Cellular or Humeral immune response) to Avian Influenza vaccine in two breeds of ducks.

MATERIALS AND METHODS

Experimental Colony: A total of 200 unsexed ducklings of two different breeds of ducks; Peckin ducks (French strain stare 53) and Cherry valley ducks (The world's first hybrid egg-type duck known as CV2000 was developed at Cherry Valley Farms, England) were used in this experiment at duck farm of Faculty of Veterinary Medicine of Damanhur University, Damanhur, Egypt. The experimental design and procedures were approved by the Committee for Animal Care and Faculty of Veterinary Medicine, Damanhur University. The birds were obtained from the French company at El Sadat City,, Al Menofeia Governorate, Egypt. One hundred wing banded day old ducklings (Metal wing bands from Fath Allah Group Company©, Alexandria, Egypt)from each genotype were used in thisstudy in two replicates n=25); they were housed and brooded in an open-sided house until 3 weeks of age. At the third week of age birds within each breed were allowed to an outdoor access yard from the third week of age (A soft earth yard of 10 m length × 10 m width) the yard was supplied with a tunnel of running water (10 m length × 1 m width × 0.3 m depth).The stocking density in the outside yard was 3 kg of live weight /m^2 of the yard floor space while, at the confinement part (10 m length × 5 m width) it was 12 kgof live weight /m^2 of floor space. All birds were healthy and vaccinated with Avian Influenza (AI) vaccine at 14 days of age with 0.5 ml by intramuscular injection.

Flock Management: The birds were housed in a clean and well-ventilated house and provided with a gas heater, in addition to incandescent lamps. Birds were bedding with a fresh and clean wheat straw litter and equipped with a suitable water and feeders. Feed and clean water were supplied *ad libitum*. Ducklings of all breeds were fed the same ration (Alex-Feed Company©, Al Behira Governorate, Egypt) as starter ration containing 20% crude protein for the first two weeks of age then grower feed of 18% crude protein and then fed 16% crude protein until marketing. Temperature was started at brooding as 30 °C then gradually decreased to be 21 °C at the third week of age.

BCG Vaccination: BCG vaccine (*Bacille calmette-Guerin*); *Mycobacterium Bovis* BCG vaccine was kindly provided by BCG Research unit, Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt, inform of vials containing 1 mg lyophilized material (Contains 50 human doses);in the BCG-vaccinated group each duckling had received two human doses of BCG vaccine [10]. Each breed was subdivided into two groups according to BCG vaccination into;

- BCG-Vaccinated group; 50 birds from each breed were vaccinated with two human doses of BCG vaccine by subcutaneous injection at 14 days of age.
- Control group; 50 birds from each breed (Injected with 0.5 ml of phosphate buffer saline)

Traits of Concern

Productive Performance: Average body weight of ducks was determined at 0 day (Initial weight) and then biweekly until the end of the experimental period. Feed was withdrawn for 12 hours with water being provided *ad libitum* before each weighing of ducks. The gain in body weight was calculated biweekly by finding the difference in weight between two successive weights.

Immunological Traits: The blood samples from all groups were taken randomly for humeral immune response against AI vaccine after 14 days and 28 days of injection (BCG and AI) and serum was separated and frozen at-20 ºC until assay. On the other hand, whole blood samples were collected in heparinized tubes (Anticoagulant) after three and seven days of BCG injection to investigate the cellular immune response (Differential leukocytes count, phagocytic activity and index). Blood films were prepared from collected blood samples according the method described by Lucky [11] for measuring of Differential Leukocytes count (DLC). Phagocytic activity was determined according to Kawahara *et al.* [12]

Statistical Analyses: The statistical analyses of the data were carried out utilizing statistical analysis system [13]. Numbers of preliminary analyses were done, using SAS, (For checking normality, homogeneity and equality of variances listing all data and testing the significance of higher order interactions for any of the dependent variables). Significance of the effects was tested at the level ($P< 0.05$). Most of data were analyzed by adapting the following model; $Y_{ijk} = \mu + B_i + T_{ij} + e_{ijk}$

Where; Y_{ijk}= the k^{th} observation in the i^{th} breed j^{th} treated group. μ = Overall mean. B_i = Effect of i^{th} breed. T_{ij} = Effect of j^{th} injected group within the i^{th} breed. e_{ijk} = Random error.

But data for immune response and blood parameters were analyzed by adapting the following model; $Y_{ijk} = \mu + B_i + T_{ij} + M_{ijk} + e_{ijkl}$

Where; Y_{ijk}= the k^{th} observation in the j^{th} injected group. μ = Overall mean. B_i = Effect of i^{th}. T_{ij}= Effect of j^{th} injected group within the j^{th} breed. M_{ijk} = Time of blood sampling. e_{ijkl} = Random error.

RESULTS AND DISCUSSION

Productive Performance: Summary statistics of productive traits (Body weight and body weight gain) in both breeds were presented in tables 1 and 2 revealed that, the initial body weight for Cherry valley ducks were significantly higher than Peckin ducks (65.70±0.83 vs56.08±0.81 g) also, body weight gain was significantly higher in the same breed (422.07±7.19 vs 373.61±7.42 g) for the first period (0-2 weeks of age).

On the other hand the Peckin ducks were significantly higher at two weeks of age than Cherry valley ducks. However, at the fourth and sixth weeks of age the breed has no significant effect on body weight. The variation in body weight at market age (8 weeks) revealed that the Cherry valley was significantly higher in body weight than Peckin. The evolution on the performance of duck breeds has been more pronounced in recent decades and resulted in significant genetic gains and, therefore, in differences among current commercial breeds according to the selection process they were submitted to. The tendency with the progress in breeding programs on ducks is that the interaction between genotype and environment become more and more important, aiming selection, management and nutrition of breeds for more specific market objectives. So, these differences in body weights could be attributed to the high genetic potential of the Cherry valley ducks than Peking ducks [14].Genetic polymorphisms of exon 2 and partial intron of preproinsulin gene in Peking ducks and Cherry Valley ducks and they showed significant differences in productive performance traits between the different genotypes of both breeds of ducks [15].

Table 1: Least square means ± standard error (SE) of the body weights (g) in two breeds of ducks injected with BCG vaccine compared with the control

		Biweekly body weights ± SE				
Item		Initial	BW2	BW4	BW6	BW8
Duck Breed						
Cherry valley		65.70±0.83[a]	440.00±7.72[b]	1347.37±15.52[a]	2267.37±25.09[a]	3056.83±45.19[a]
Peckin		56.08±0.81[b]	478.20±7.53[a]	1360.34±14.86[a]	2271.63±23.89[a]	2884.84±42.60[b]
Treatment						
BCG		60.82±0.83[a]	453.15±7.67[a]	1338.03±15.24[a]	2236.55±24.5[b]	2932.68±43.31[a]
Control		60.96±0.81[a]	465.05±7.58[a]	1369.68±15.15[a]	2302.45±24.5[a]	3008.99±44.51[a]
Breed* Treatment						
Cherry valley	BCG	66.48±1.18[a]	433.63±10.92[b]	1334.23±21.94[a]	2244.62±35.48[a]	3040.18±60.7[a]
	Control	64.93±1.17[a]	446.38±10.92[b]	1360.51±21.94[a]	2290.13±35.48[a]	3073.48±66.97[a]
Peckin	BCG	55.17±1.15[b]	472.68±10.78[a]	1341.83±21.14[a]	2228.49±33.79[a]	2825.19±61.81[b]
	Control	57.00±1.13[b]	483.72±10.53[a]	1378.84±20.90[a]	2314.77±33.79[a]	2944.50±58.64[a]

-Means carrying different litters within the same column within the same parameter are statistically non-significant ($p \leq 0.05$)

Table 2: Least square means ± standard error (SE) of the body weights gain (g) in two breeds of ducks injected with BCG vaccine compared with the control.

		Biweekly body weight gain ± SE				
Item		0-2	2-4	4-6	6-8	Total (0-8)
Duck Breed						
Cherry valley		422.07±7.19[a]	905.09±11.54[a]	922.81±19.02[a]	834.07±29.21[a]	2988.45±45.83[a]
Peckin		373.61±7.42[b]	888.07±11.19[a]	909.98±17.84[a]	618.62±26.85[b]	2834.24±43.11[b]
Treatment						
BCG		392.36±7.32[a]	886.94±11.40[a]	907.23±18.31[a]	711.94±27.32[a]	2878.79±43.82[a]
Control		403.33±7.29[a]	906.22±11.34[a]	925.56±18.57[a]	740.75±28.76[a]	2943.90±45.16[a]
Breed * Treatment						
Cherry valley	BCG	417.56±10.29[a]	898.33±16.22[a]	928.68±26.53[a]	821.48±38.64[a]	2976.54±60.81[a]
	Control	426.58±10.05[a]	911.84±16.43[a]	916.94±27.26[a]	846.67±43.81[a]	3000.36±68.60[a]
Peckin	BCG	367.15±10.42[b]	875.55±16.01[a]	885.79±25.23[a]	602.41±38.64[b]	2781.04±63.10[b]
	Control	380.08±10.55[b]	900.60±15.63[a]	934.17±25.23[a]	634.83±37.28[b]	2887.43±58.74[a]

-Means carrying different litters within the same column within the same parameter are statistically non-significant ($p \leq 0.05$)

Differences in body weight and body weight gain between different breeds of ducks have been previously reported by many authors [16-20].

In the current study, the effect of BCG vaccine was expressed as reduced body weight significantly at 4th and 6th weeks of age, moreover, body weight at other ages and body weight gain at all periods were non-significant differences. These results were in agreement with Qiuyue *et al.* [7] who reported that,there is no significant difference for the body weight were detected between groups before immunization and 2 weeks after the booster immunization of BCG vaccine. However, the effect of BCG vaccination on the body weight gain in chicken was noted to be higher in the BCG vaccinated group than the control one but the difference is not statistically significant.

As shown in Table 1 and 2, the interaction between BCG and breed (Breed*treatment) revealed that the effect of the interaction on body weight and body weight gain were significantly lower in BCG vaccinated Peckin duck at the last week of the experimental period (2825.19±61.81 and 2781.04±63.10 g). Although, Li *et al.* [9] stated that vaccination via recombinant BCG either intranasal or subcutaneous routes could increase weight gain, decrease intestinal lesions and reduce fecal oocyst shedding in chickens.

Immunological Traits

Humeral Immunity: The data of Humeral immune response were shown in table 3 and the breed of duck has no significant effect on humeral immune response to Avian Influenza vaccine at both ages of the study (15 and 28 days of age) it may be attributed to that, infection or vaccination of ducks with Avian Influenza typically results in weak antibody responses and short-lived memory [8]. In contrast; Rana *et al.* [21] concluded that the immune responses varied according to breed.

Antibody titer against AI at 15 days post-vaccination was improved significantly in BCG vaccinated group than control one in Cherry valley (2.38±0.15vs 1.96±0.16) and Peckin ducks (2.41±0.15vs2.03±0.14) at $P \leq 0.05$ but, at 28 days of age the differences were not significant. These results indicating that, effect of BCG vaccination on immune response to AI vaccine was effective for a short period of time. These results were in agreement with Katharine [8] who stated that, the increase in specific antibody was very transient, lasting only a week or two.

Cellular Immunity: Phagocytic activity in Cherry valley ducks breed were significantly improved for BCG vaccinated group at 3rd day post-vaccination than control one (20.30±0.63vs18.42±0.57).The cellular immune response in form of phagocytic activity was observed to be significantly increased in BCG vaccinated group for both breeds at 7th day post vaccination. BCG can stimulate the differentiation of the bone marrow pluripotent stem cells along with the proliferation of macrophage, significantly improving the cellular immunity [7]. So, a series of observations suggest that non-specific immunomodulation by BCG, could represent a simple means of controlling diseases [1].

Tables 3: Log-geometric mean ± standard error (SE) of the antibody titer, phagocytic activity and index against Avian Influenza vaccine in two duck breeds injected with BCG vaccine

		HI ± SE		PA± SE		PI ± SE	
		15 day post	28 day post	3rd day post	7th day post	3rd day post	7th day post
Item		vaccination	vaccination	vaccination	vaccination	vaccination	vaccination
Duck Breed							
Peckin		2.18±0.07[a]	1.33±0.07[a]	19.08±0.24[a]	20.32±0.52[a]	1.69±0.06[a]	1.52±0.03[a]
Cherry valley		2.17±0.07[a]	1.39±0.06[a]	19.21±0.26[a]	20.38±0.53[a]	1.65±0.07[a]	1.59±0.03[a]
Treatment							
BCG		2.31±0.07[a]	1.37±0.07[a]	19.06±0.24[a]	20.53±0.53[a]	1.73±0.06[a]	1.58±0.03[a]
Control		2.06±0.07[b]	1.34±0.07[a]	19.22±0.26[a]	19.17±0.52[b]	1.61±0.07[a]	1.53±0.03[a]
Breed* Treatment							
Peckin	BCG	2.41±0.15[a]	1.32±0.14[a]	19.90±0.63[ab]	19.88±0.87[a]	1.70±0.13[a]	1.69±0.09[a]
	Control	2.03±0.14[b]	1.30±0.13[a]	19.13±0.50[ab]	18.60±0.73[b]	1.80±0.15[a]	1.54±0.08[a]
Cherry valley	BCG	2.38±0.15[a]	1.39±0.14[a]	20.30±0.63[a]	21.50±0.65[a]	1.65±0.12[a]	1.70±0.09[a]
	Control	1.96±0.16[b]	1.28±0.15[a]	18.42±0.57[b]	18.38±0.68[b]	1.66±0.13[a]	1.69±0.09[a]

-Means carrying different litters within the same column within the same parameter are statistically non-significant ($p \leq 0.05$)

Tables 4: Least square means ± standard error (SE) of phagocytic activity and differential leukocytes counts (10^3/ml) in two breeds of duck injected with BCG vaccine compared with the control

			DLC ± SE				
3rd day post vaccination	Item		Lymphocytes	Monocytes	Basophils	Eosinophils	Neutrophils
	Duck Breed						
	Peckin		43.72±0.62[a]	1.52±0.08[a]	3.11±0.27[a]	7.90±0.18[a]	43.75±0.76[a]
	Cherry valley		42.82±0.66[a]	1.49±0.09[a]	3.38±0.29[a]	8.25±0.19[a]	44.07±0.80[a]
	Treatment						
	BCG		43.10±0.62[a]	1.43±0.08[a]	3.47±0.27[a]	8.17±0.18[a]	43.84±0.76[a]
	Control		43.44±0.66[a]	1.58±0.09[a]	3.02±0.29[a]	7.98±0.19[a]	43.98±0.80[a]
	Breed* Treatment						
	Cherry valley	BCG	44.27±0.87[a]	1.47±0.12[a]	3.43±0.38[a]	7.83±0.25[a]	43.00±1.06[a]
		Control	43.18±0.90[a]	1.57±0.12[a]	2.79±0.39[a]	7.96±0.26[a]	44.50±1.09[a]
	Peckin	BCG	41.93±0.90[a]	1.39±0.12[a]	3.50±0.39[a]	8.50±0.26[a]	44.68±1.09[a]
		Control	43.71±0.97[a]	1.58±0.13[a]	3.25±0.42[a]	8.00±0.28[a]	43.46±1.18[a]
7th day post vaccination	Duck Breed						
	Peckin		39.55±0.42[a]	1.87±0.12[a]	2.20±0.10[a]	8.23±0.15[a]	48.15±0.48[a]
	Cherry valley		40.08±0.43[a]	1.98±0.12[a]	2.37±0.10[a]	8.41±0.15[a]	47.17±0.48[a]
	Treatment						
	BCG		40.10±0.43[a]	2.03±0.12[a]	2.28±0.10[a]	8.19±0.15[a]	47.40±0.48[a]
	Control		39.53±0.42[a]	1.82±0.12[a]	2.28±0.10[a]	8.45±0.15[a]	47.92±0.48[a]
	Breed* Treatment						
	Cherry valley	BCG	40.80±0.60[a]	2.13±0.17[a]	2.07±0.14[b]	8.17±0.21[a]	46.83±0.67[b]
		Control	38.30±0.60[b]	1.60±0.17[b]	2.33±0.14[ab]	8.30±0.21[a]	49.47±0.65[a]
	Peckin	BCG	39.39±0.62[ab]	1.93±0.17[ab]	2.50±0.14[a]	8.21±0.22[a]	47.96±0.70[ab]
		Control	40.77±0.60[a]	2.03±0.17a	2.23±0.14[ab]	8.60±0.21[a]	46.37±0.67[b]

-Means carrying different litters within the same column within the same parameter are statistically non-significant ($p \leq 0.05$)

The data of differential leukocytes counts (DLC) were presented in table 4 and the results demonstrated that, at the third day of inoculation DLCs were non-significantly affected neither by the breed of ducks nor the treatment (BCG injection) these results disagreed with Benda *et al.* [22] who reported that the breeds of birds differed significantly in both humeral and cellular immune reactivity to sheep red blood cells and *Brucella abortus* crude antigen.. On the other hand, after 7th day of BCG vaccine inoculation, only the interaction between breed and treatment was showed a significant difference in Cherry valley ducks in BCG injected group compared with the control one in both Lymphocytes (Higher significant difference) (40.80±0.60 Vs. 38.30±0.60) and neutrophils (Lower significant difference) (46.83±0.67 Vs. 49.47±0.65).

CONCLUSION

It could be concluded that, immune response to Avian Influenza vaccine(Humeral and Cellular) could be improved by BCG vaccination, but generally it had an adverse effect on body weight and body weight gain and its worth was varied with the duck breed (It could be applied in Cherry Valley with no significant effect on performance). So, further investigations may be needed to judge its effect on other breeds of ducks.

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