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Immune response and efficacy of pigeon pox virus vaccine and fowl pox virus vaccine in chickens

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Abstract

The humoral immune response of chicken vaccinated with fowl and pigeon pox virus vaccines was determined with the protective potentiality of the two vaccines in field condition of Bangladesh. Different aged Fayoumi chicks were subjected for the study. To assess the relationship with better immune response among experimental groups, the average percentage of 'take reaction' was examined and recorded to 97.77% in group A, 93.33% in group B and 100.0% in group C. The level of immune status induced by different vaccinated group was measured by passive hemagglutination (PHA) microplate test method. The mean PHA titer levels after primary vaccination were 33.06 ± 14.13 in group A, 32.0 ± 14.81 in group B, and 33.0 ± 13.66 in group C. Following booster vaccination, the mean PHA titer levels in prior of challenge were increased to 55.46 ± 14.64 in groups A and C, and 46.93 ± 16.52 in group B. The recorded PHA titer levels of each group at two weeks after challenge were significantly increased to 106.66 ± 31.22, 93.86 ± 33.04 and 110.93 ± 29.29, respectively. The PHA titer levels after vaccination and challenge were significantly increased compared to pre-vaccination titer levels ($P<0.01$). Although the PHA titer levels among three groups administrated different vaccine combinations in prior of challenge were

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significantly varied ($P<0.01$), it was observed that all of the vaccinated chicks were highly protected against challenge infection.

Key Words: humoral immune response, chicken, fowl and pigeon pox virus vaccine, passive hemagglutination (PHA) titer.

Introduction

Smallholder Livestock Development Project (SLDP 2) in five southern district in Bangladesh is underway to improve the rural poultry production system by applying sustainable modern technologies to help them to create self employment opportunities of women and poor people and to improve their nutritional status and social conditions. In relation to disease management in the project area of SLDP-2, the preventive measures were practiced by vaccination, but incidence of different diseases are likely to be seen, and fowl pox is an important viral endemic disease with mortality reaching as high as 60-70% per incident. The occurrence of such incidents have been frequently reported annually in Bangladesh[1].

Fowl pox is a common slow-spreading economically important viral disease of chicken, turkey, pigeons, canaries and wild birds characterized by discrete nodular proliferative skin lesions on nonfeathered parts or fibrino-necrotic lesions in mucous membranes of mouth, esophagus with intracytoplasmic inclusion bodies. It terminates with the formation of scabs and desquamation of degenerated epithelium[2].

Economic losses caused by this virus lead to the mild weight reduction and drop of egg production in laying chicken. This loss due to fowl pox has been minimized by chicken vaccination with fowl pox and/or pigeon pox vaccines[3] and also practiced in the country. On the other hand, vaccination schedule and combination, vaccine type, maternal derived antibody (MDA) in chicks and pathogenecity of the field challenge are the important factors.

Materials and Methods

1. Virus

Local strain of fowl pox virus isolated from field outbreak was used and propagated in 10 to 12 days embryonated eggs via chorioallantoic membrane (CAM) route for 3 to 5 passages[4]. The dead embryos within 24 hours of inoculation were discarded and all of alived and dead embryos upto 5 days were chilled at 4℃ for 18 hours. The CAM with discrete or confluent growth of pocks were selected and processed for further passage. The egg-infective-does of fifty percents (EID_{50}) of the test viruses were calculated following the method of Reed and Muench[5].

2. Experimental design

The study was carried out in the project area of SLDP 2. One hundred and eighty Fayoumi chicks were divided into three groups namely A, B and C.

Equal number of birds were assigned into each group, and subdivided into 4 groups. The first 3 subgroups were used as test replicates and the last group was used as a control group. Groups A and B were primarily vaccinated with pigeon pox virus (PPV) vaccine, and Group C was vaccinated with fowl pox virus (FPV) vaccine at their 3rd week of age by wing web puncture. Groups A and C were boosted with FPV vaccine, and Group B was vaccinated with PPV vaccine at 9th week of age.

Vaccinated birds were observed up to 6 to 9 days for the "take", the typical lesions of pox at the site where vaccine was applied. Blood was collected for serum at the 3 weeks (pre-vaccination), 9 weeks (pre-booster), 12weeks (pre-challenge), and 14 weeks (post-challenge) of age from the five birds of each vaccinated and unvaccinated sub groups. Ten birds of each vaccinated sub group and five from each unvaccinated control were challenged with virulent FPV at 12 weeks of age at $10^{6.0}$ EID_{50}/0.1 ㎖ following feather follicle method as described by Winterfield and Reed[6]. Antibody titer levelsof the collected serum were determined by passive hemagglutination (PHA) test to assess the efficacy of the two vaccines.

3. PHA test

The test was conducted by following the method described by Tripathy et al[7]. FPV antigensare coupled to chemically modified erythrocytes (sheep red blood cells; SRBC) and then antigen-coated erythrocytes readily reacted with specific antibodies and resulted hemagglutination. Briefly, 10 ㎖ of sensitized SRBC were prepared by mixing 1.5㎖ of 2.5% suspension of tannic acid treated cells with 0.5㎖ of 1:10 dilution of FPV suspension and phosphate-buffered saline (PBS), and incubated at 37℃ for 30 min by diluting with 1% normal rabbit serum diluents.

Fifty microliters of PBS was first poured in each well up to 8th well of horizontal row of microtiter plate. The equal amount of test scrum was added in the 1st well. Two fold dilutions of serum ranging from 1 : 2 to 1 : 256 were prepared. Then 50㎕ of 0.5% FPV sensitized SRBC was added in each of the eight wells. Proper control in three horizontal rows was also maintained. The plates were kept at room temperature for 4 to 5 hours.

The agglutination results were recorded by deposition of diffuse thin layer of clumping of RBC on the bottom of the well. The end point was determined by observing the highest dilution at which cells were agglutinated.

Results

1. Propagation of FPV in chicken embryonated eggs

Presence of FPV was confirmed by characteristic pock lesions on CAM. Discrete pock lesions along with thickening of CAM were observed after harvesting at 5 days of inoculation in the chicken embryo fibroblast cell cultures (data not shown).

2. Post vaccinal observation

"Take" reaction was observed after the vaccination by wing web puncture in birds of all groups characterized by the formation of pimples that became further swollen and turned to nodular form lesion typical of pox. Later scab was found over the vaccinated area. The lesion was subsided within 5 to 10 days of its appearance. No adverse reaction was observed in the birds following the formation of the lesions. The average percentage of 'take reaction' of vaccinated birds of groups A, B and C were 97.77 %, 93.33% and 100.0%, respectively (Table 1).

Table 1. Relationship of 'take reaction', titer levels of serum at different vaccination schedule and protection percentage against challenge

Group	Take reaction (%)	Antibody titer			Protection (%)
		Pre-booster	Pre-challenge*	Post-challenge	
A	97.77	33.06 ± 14.0	55.46 ± 14.64	106.66 ± 31.22	100.0
B	93.33	32.0 ± 14.81	46.93 ± 16.52	93.86 ± 33.04	100.0
C	100.0	33.0 ± 13.66	55.46 ± 14.64	110.93 ± 29.29	100.0

*significantly differed at 1% level

3. PHA titer levels of sera collected at pre-vaccination stage

The serum of randomly selected 5 chicks of each subgroup including control were tested to determine the antibody titer levels by PHA microplate test at pre vaccination stage and the average recorded result of PHA titer level was $\geqq 4.0 \pm 0.0$.

4. PHA titer levels of sera collected at pre-booster vaccination

At 9 weeks of age, the observed mean PHA titer levels of sera of subgroups A1, A2 and A3 vaccinated with PPV vaccine as priming were 35.2 ± 17.52, 28.8 ± 7.15 and 35.2 ± 17.52, respectively. On the other hand, the titer levels of birds of subgroups B1, B2 and B3 vaccinated with the same vaccine were 28.8 ± 7.15, 35.2 ± 17.52 and 32.0 ± 1959, respectively. In case of subgroups C1, C2 and C3 vaccinated with FPV vaccine the mean titer levels were found to be 28.8 ± 7.15, 44.8 ± 17.52 and 25.6 ± 8.76, respectively. The average PHA titer levels of groups A, B and C were 33.06 ± 14.13, 32.0 ± 14.81 and 33.0 ± 13.66, respectively.

5. PHA titer levels of sera collected at pre-challenge

At 3 weeks after booster vaccination, the recorded PHA titer levels of subgroups A1, A2 and A3 vaccinated with FPV vaccine were 57.6 ± 14.31, 57.6 ± 14.31 and 51.2 ± 17.52, respectively. In case of subgroups B1, B2 and B3 that were vaccinated with PPV vaccine, the

PHA titer levels were 44.8 ± 17.52, 51.2 ± 17.52 and 44.8 ± 17.52, respectively. Subgroups C1, C2 and C3 were vaccinated with FPV vaccine as booster and the antibody titer levels measured at that period were 57.6 ± 14.31, 51.2 ± 17.52 and 57.6 ± 14.31, respectively. The average antibody titer levels were 55.46 ± 14.64, 46.93 ± 16.52 and 55.46 ± 14.64, respectively, for the groups A, B and C.

6. PHA titer levels of sera collected at post-challenge

At 2 weeks post challenge, the PHA titer levels were 115.2 ± 28.62, 102.4 ± 35.05 and 102.4 ± 35.06, respectively, for subgroups A1, A2 and A3. The mean PHA titer levels of subgroups B1, B2 and B3 were 89.6 ± 35.06, 89.6 ± 35.05 and 102.4 ± 35.05, respectively. In case of subgroups C1, C2 and C3, the PHA titer levels were 102.4 ± 35.05, 115.2 ± 28.62 and 115.2 ± 28.62, respectively, for the subgroups. The average highest titer level was recorded in group C (110.93 ± 29.29) followed by groups A (106.66 ± 31.22) and B (93.86 ± 33.04).

7. Protective potentiality against challenge

Each bird received thigh feather follicle method by dosing predetermined one-chick-dose of 0.1㎖ of virulent FPV having a virus concentration of $10^{6.0}EID_{50}$ per 0.1㎖ at 12 weeks of age. It was observed that 10 challenged birds of each vaccinated subgroups of groups A, B, and C were completely protected (100.0%) against infection with virulent FPV, whereas all 5 challenged birds of each control groups could not withstand such exposure and therefore exhibited pox lesion.

Discussion

The humoral immune response plays an important role in protecting birds against fowl pox infection[2]. In this study, the humoral immune responses in selected group of chicks having been inoculated with PPV and FPV vaccines were investigated. The parameters of investigation included recording of initial reaction ('take reaction') on the site of inoculation, measurement of antibody titer level by PHA test and resistance of birds to challenge infection with a virulent field isolate of FPV. The characteristic features of the propagation of FPV in the CAM of 12 days old chicken embryonated eggs were also observed. and showed that characteristic discrete pock lesions on CAM was found during propagating local strain of fowl pox in 10-12 days old embryonated chicken eggs similar to the findings of Reed and Schrader[4].

Two vaccines of PPV and FPV manufactured at Livestock Research Institute of Bangladesh were used to asses the comparative efficacy. All birds were vaccinated at 3 weeks of age for priming and at 9 weeks of age for booster. It was possible to observe the 'take reaction' characterized by the formation of pimple among vaccinated birds, which was also reported by Tripathy and Reed[2]. Resultantly, no adverse reactions were observed in vaccinated birds following

appearance of 'take'[8]. As shown in Table 1, all the vaccinated birds resisted challenge infection with different level of 'take reaction'. It was reported that a lack of 'take' reaction did not necessarily indicate a lack of immunity[9].

In maximum cases, the recorded antibody titer level at different stage by PHA microplate method in present study was observed to be more than the findings of previous study, which might have been due to use of different vaccine virus strain, vaccination method, age of vaccination and strain of challenge virus[8]. Following booster vaccination at pre challenge stage, the PHA titer levels of groups A and C were more than group B, this may be due to FPV vaccine produced solid and durable immunity[2]. The PHA titer level increased following primary vaccination which again reached a significance level ($P<$ 0.01) after booster vaccination and challenge (Table 1). The rise in antibody titer level in challenged birds was suggestive of active immune response of the vaccinated birds. These results obtained herein corresponded to the result of Prabhakar et al[10].

Significant variation ($P<0.01$) was found among the groups A, B and C in regard to immune response at pre challenge level using different vaccines combination, but no significance variation was found in case of protection in three groups (Table 1). This might be due to both humoral and cell mediated immunity to play a critical role in the protection against FPV and in the capacity of individual bird to produce antibody to influence challenge. This observation supports the statement of Siddique et al[11].

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