SHORT COMMUNICATION

Comparative sero evaluation of live and killed Gumboro vaccine in broilers.

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ABSTRACT

Infectious Bursal Disease (IBD) is a fatal disease, which is caused by a Birnavirus. Vaccination is one of the most effective ways to prevent virus. Two types of vaccines (live and killed) are normally used to induce immunity. In the present experiment, comparative sero evaluation of live and killed Gumboro vaccine in broilers was carried out. For comparing the titers obtained with the two vaccines, one broiler group was vaccinated with live and the other with killed vaccine. At four points in time serum was collected from these broilers. Serum samples were collected on day 0 and day 14 of age (i.e. before vaccination was administered at 14 days of age) and subsequently on day 28 and day 42 (i.e. 14 and 28 days after vaccination). Collected serum samples were examined to determine antibody titer using the indirect ELISA method. The average maternal antibody titers were 2888.80 and 224.80 on day 0 and day 14 of age, respectively. The average combined titer of killed vaccine was 3582.1 on day 28 and day 42 and the average combined titer of live vaccine was 1513 on days 28 and 42. The efficacy of killed vaccine was higher than that of live vaccine. It was observed that live vaccine induced the desired immunity for a limited period of time. Higher antibody titers were obtained from killed vaccine after live vaccine administration. For these reasons, it is suggested to administer live vaccine first and subsequently the killed vaccine in order to obtain an immediate immune response and subsequently to maintain high antibody titers for a long period.

Key words: Antibody titer, Live and killed vaccine, Infectious Bursal Disease, ELISA.
gruppi di broilers trattati; i campioni di siero venivano prelevati a 0 e 14 giorni, cioè prima della vaccinazione praticata a 14 giorni di vita, e successivamente a 28 e 42 giorni post vaccinazione. I sieri ottenuti sono stati esaminati in ordine alla determinazione dei titoli anticorpali, utilizzando un ELISA indiretto. Il titolo medio di anticorpi materni era di 2888,80 e di 224,80 rispettivamente al giorno 0 ed al 14° giorno di vita. Il titolo anticorpale medio del gruppo trattato con vaccino spento era di 3582,1 per entrambi i prelievi eseguiti a 28 e 42 giorni post vaccinazione, mentre quello ottenuto nei broilers vaccinati con il vivo era di 1513 per entrambi i prelievi sierologici effettuati. Il vaccino spento ha dimostrato una maggiore efficacia rispetto a quello attenuato ed è stato verificato che quest’ultima evoca l’immunità desiderata per un periodo di tempo limitato. Titoli anticorpali più elevati si ottengono dalla somministrazione di un vaccino attenuato seguito da una vaccinazione di richiamo con inattivato. Per tali ragioni si suggerisce di seguire questo schema vaccinale così da ottenere una risposta immunitaria immediata che venga mantenuta ad alti titoli per un lungo periodo di tempo.

Parole chiave: Titoli anticorpali, Vaccino vivo e spento, Bursite infettiva aviare, ELISA.

Introduction

Bangladesh is an agricultural country where 80% of the people live on agriculture. As an integral part of agriculture, poultry and livestock play a vital role in the economic development of the country. Therefore, consideration should be given to the maintenance of domestic resources, especially poultry production, for promoting the lifestyle and livelihood of commercial entrepreneurs, landless and marginal farmers. It has been shown that poultry and livestock wealth provides about 9.5% of the Gross National Product (GNP) which is 6.5% of Gross Domestic Product (GDP) (Ahmed, 1992; Rahman and Rahman, 1998).

Generally, the unemployed persons, landless farmers and rural women of this country rear broilers. Due to tremendous demand for animal proteins, poultry enterprises have become the target of increasing capital investment for commercial broiler production. Broiler farming has now been recognized as a specialized field. Poultry industries have thus taken an operational role in all developing countries to overcome the scarcity of protein for the malnourished people of Third World countries, including Bangladesh.

Therefore, production, vaccination, treatment, and other management practices of the poultry industries need special attention in a country like Bangladesh.

The poultry business may be profitable with proper management in any country. The major problem of this business is the outbreak of infectious diseases, including Infectious Bursal Disease (IBD). Although IBD vaccines are available on the commercial market, the quality of vaccines, transportation, storage, distribution, time interval of vaccinations, presence of maternal antibodies, age of vaccination, stress, immuno-suppression, route of vaccination etc. are often causes for vaccination failure (Phatak, 2000). Two types of vaccines are used in broilers: live IBD vaccines and killed IBD vaccines. It has been observed that killed vaccines induce higher antibody response and last for a longer period than live vaccines (Phatak, 2000).

In Bangladesh, there are no research data to compare the sero evaluation of live and killed vaccines after the vaccination of broilers. Therefore, the present research was undertaken with the following objectives:

1. To determine the antibody titer of Gumboro vaccinated broiler flocks using Enzyme Linked Immunosorbent Assay (ELISA) test.
2. To compare the antibody titer of serum samples of vaccinated flocks by live and killed Gumboro vaccines using the ELISA test.
3. To suggest the measures for protecting the parental stock of broilers from loss of immune response.

Material and methods

Laboratory Organization

The experiment was set at the virology laboratory of Bangladesh Livestock Research Institute (BLRI). The necessary equipment and chemicals
were provided by the Animal Health Research Division, BLRI and Usha Poultry and Feed Company, respectively.

**Vaccination**

During the experiment, a pure breed broiler named broiler 14 was selected for the administration of vaccine. Two types of vaccines were used in two groups of that breed of broilers. Live IBD vaccine was administrated through the nasal route in one group of 14-day-old broilers. Killed IBD vaccine was administrated by injection to another group of broilers of the same age. At 28 days of age (i.e. 14 days after 1st vaccination), booster doses of vaccines were administrated in the same way and at the same time in both groups of broilers.

**Serological Test**

Randomly, 10 serum samples were collected from both groups of vaccinated broilers at 4 time points. First, two serum samples were collected at 14 days before vaccination; second, serum was collected at 28 days after first vaccination; and finally, serum was collected at 42 days after the booster dose vaccination. It should be mentioned that for broilers that were vaccinated for the second time at 28 days of age, the time point “28 days after first vaccination” corresponds to 14 days after second (booster) vaccination.

**Sampling and Serological ELISA**

The serum samples (except control) were diluted five hundred fold (1:500) with sample diluents prior to assay. Separated tips were used for each sample. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect the relative level of antibody to IBD in chicken serum. IDEXX Laboratory, USA, supplied the ELISA kit. Test sample serum was added to an antigen coated 96-well plate. After incubation, the antibody of the test sample binds with specific IBD antigen and forms a complex, which can be detected by color development when conjugate and TMB substrate are added. Each serum is double tested. The ELISA Test was performed following the producer’s indications.

The presence or absence of antibody to IBD was determined by relating the (A650) value of each tested serum to the positive control mean. The positive control was standardized and represented significant antibody levels to IBD in chicken serum. The relative level of antibody in the tested sera was determined by calculating the sample to positive (S/P) ratio. Endpoint titers were calculated using the equation described in the calculation section. The difference between the positive control (PC) mean and the negative control (NC) mean (PCx - NCx) should be greater than 0.075. The negative control mean absorbance should be less than or equal to 0.150 (IDEXX Laboratory, USA, UK and Australia).

**Results and discussion**

The maternal antibody titers obtained from broiler serum on day 0 are shown in Table 1: the average optical density (OD) of PC and NC and that of testing samples. The average PC and NC values were 0.148 and 0.15, respectively. The average OD values for testing samples were 0.175, 0.207, 0.165, 0.150 and 0.20, respectively. Samples to positive ratio (S/P) were 1.203, 1.440, 1.128, 1.015 and 1.391, respectively, but PC and NC had no S/P ratio. Maternal antibody titers were 2803, 3419, 2612, 2328 and 3283, respectively, and average titer value was 2888.80.

**Table 1.** Maternal antibody titer obtained from broiler serum on day 0.

| Sample | PC  | NC  | Sample A | Sample B | Sample C | Sample D | Sample E |
|--------|-----|-----|----------|----------|----------|----------|----------|
| OD     | 0.148 | 0.015 | 0.175 | 0.207 | 0.165 | 0.150 | 0.200 |
| S/P Ratio | - | - | 1.203 | 1.440 | 1.128 | 1.015 | 1.391 |
| Titer | - | - | 2803 | 3419 | 2612 | 2328 | 3283 |
| Average titer | 2888.8 |

PC: Positive Control; NC: Negative Control; OD: Optical Density; S/P ratio: Sample to Positive ratio.
Table 2 shows the maternal antibody titer obtained from broiler serum on day 14: the average optical density (OD) of PC and NC and that of testing samples. The average PC and NC values were similar to those shown in Table 1. The average OD values were 0.030, 0.035, 0.025, 0.032 and 0.033, respectively. Samples to positive ratio (S/P) were 0.105, 0.150, 0.075, 0.128 and 0.135, respectively, but PC and NC had no S/P ratio. Maternal antibody titers were 196, 290, 136, 244 and 258, respectively, and average titer value was 224.6.

The antibody titer of vaccinated broiler serum (killed) on day 28 are shown in Table 3. The average optical density (OD) of PC and NC were similar to those shown in Table 2 and average OD values of testing samples were 0.245, 0.239, 0.180, 0.205 and 0.173, respectively. Samples to positive ratio (S/P) were 1.729, 1.677, 1.241, 1.429 and 1.180, respectively. The antibody titers were 4161, 4025, 2899, 3381 and 2744, respectively, and average titer value was 3442.

Table 4 shows the antibody titer of vaccinated broiler serum (live) on day 28. The average optical density (OD) of PC and NC were similar to those shown in Table 3. The average OD values of testing samples were 0.102, 0.113, 0.099 and 0.097 on day 28, respectively. S/P was 0.647, 0.729, 0.654, 0.624 and 0.617, respectively. S/P ratio of PC and NC was similar to those shown in Tables 1 to 3. The antibody titers were 1425, 1623, 1442, 1370 and 1353, respectively, and average titer value was 1442.6.

The antibody titers of vaccinated broiler serum (killed) on day 42 are shown in Table 5. The average optical density (OD) of PC and NC was similar to those shown in Table 4. The average OD values of testing samples were 0.273, 0.249, 0.199, 0.210 and 0.183, respectively. Samples to positive ratio (S/P) were 1.932, 1.759, 1.383, 1.459 and 1.263, respectively. The antibody titers were 4693, 4240, 3262, 3458 and 2955, respectively, and average titer value was 3722.2.

Table 6 shows the antibody titers of vaccinated broiler serum (live) on day 42. The average optical density (OD) of PC and NC was similar to those shown in Table 5. The average OD values of testing samples were 0.107, 0.119, 0.113, 0.103 and 0.101, respectively. Samples to positive ratio (S/P) were 0.684, 0.782, 0.729, 0.654 and 0.647, respec-

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### Table 2. Maternal antibody titer obtained from broiler serum on day 14.

| Sample  | PC  | NC  | Sample A | Sample B | Sample C | Sample D | Sample E |
|---------|-----|-----|----------|----------|----------|----------|----------|
| OD      | 0.148 | 0.015 | 0.030    | 0.035    | 0.025    | 0.032    | 0.033    |
| S/P Ratio | -   | -   | 0.105    | 0.150    | 0.075    | 0.128    | 0.135    |
| Titer   | -   | -   | 196      | 290      | 136      | 244      | 258      |
| Average titer | 224.6 |

PC: Positive Control; NC: Negative Control; OD: Optical Density; S/P ratio: Sample to Positive ratio.

### Table 3. Antibody titer killed vaccine obtained from broiler serum on day 28.

| Sample  | PC  | NC  | Sample A | Sample B | Sample C | Sample D | Sample E |
|---------|-----|-----|----------|----------|----------|----------|----------|
| OD      | 0.148 | 0.015 | 0.245    | 0.239    | 0.180    | 0.205    | 0.173    |
| S/P Ratio | -   | -   | 1.729    | 1.677    | 1.241    | 1.429    | 1.180    |
| Titer   | -   | -   | 4161     | 4025     | 2899     | 3381     | 2744     |
| Average titer | 3442 |

PC: Positive Control; NC: Negative Control; OD: Optical Density; S/P ratio: Sample to Positive ratio.
The antibody titers were 1514, 1752, 1623, 1442 and 1389, respectively, and average titer value was 1544.

Before vaccination, the average antibody titer of collected serum sample was 2888.80 on day 0 and 224.6 on day 14. The titer at day 0 is greater than that at day 14 and this is in agreement with Phatak (2000), who stated that passively transferred antibodies from parent to chick usually wane after 7-14 days. Therefore, it is evident that the maternal antibodies of chicks are very protective on day 0 compared to day 14.

The average titer of non-vaccinated serum sample was 224.6 on day 14 and the titer of vaccinated (killed vaccine) serum sample was 3442 on day 28. This proves the increase of the titer in birds in comparison to non-vaccinated birds.

After vaccination, the average titer of serum samples induced by killed and live vaccines were 3442 and 1442 on day 28, respectively, and 3722.2 and 1551.2 on day 42, respectively. It was found that the efficacy of killed vaccine was higher compared to that of live vaccine. It is evident that Phatak (2000) also expressed a similar opinion that killed vaccines induced higher antibody response and lasted for a longer period.

| Sample  | PC  | NC  | Sample A  | Sample B | Sample C  | Sample D  | Sample E  |
|---------|-----|-----|-----------|----------|-----------|-----------|-----------|
| OD      | 0.148 | 0.015 | 0.102 | 0.113 | 0.103 | 0.099 | 0.097 |
| S/P Ratio | - | - | 0.647 | 0.729 | 0.654 | 0.624 | 0.617 |
| Titer  | - | - | 1425 | 1623 | 1442 | 1370 | 1353 |
| Average titer | 1442.6 |

PC: Positive Control, NC: Negative Control, OD: Optical Density, S/P ratio: Sample to Positive ratio.

| Sample  | PC  | NC  | Sample A  | Sample B | Sample C  | Sample D  | Sample E  |
|---------|-----|-----|-----------|----------|-----------|-----------|-----------|
| OD      | 0.148 | 0.015 | 0.273 | 0.249 | 0.199 | 0.210 | 0.183 |
| S/P Ratio | - | - | 1.932 | 1.759 | 1.383 | 1.459 | 1.263 |
| Titer  | - | - | 4696 | 4240 | 3262 | 3458 | 2955 |
| Average titer | 3722.2 |

PC: Positive Control, NC: Negative Control, OD: Optical Density, S/P ratio: Sample to Positive ratio.

| Sample  | PC  | NC  | Sample A  | Sample B | Sample C  | Sample D  | Sample E  |
|---------|-----|-----|-----------|----------|-----------|-----------|-----------|
| OD      | 0.148 | 0.015 | 0.107 | 0.119 | 0.113 | 0.103 | 0.101 |
| S/P Ratio | - | - | 0.684 | 0.782 | 0.729 | 0.654 | 0.647 |
| Titer  | - | - | 1514 | 1752 | 1623 | 1442 | 1389 |
| Average titer | 1551.2 |

PC: Positive Control, NC: Negative Control, OD: Optical Density, S/P ratio: Sample to Positive ratio.
Conclusions

It is evident from literature and experimental findings that killed vaccine induces an antibody titer more slowly than a live vaccine. Indeed live vaccine induces desired immunity for a short period of time (Phatak, 2000). This justifies the administered ratio of live vaccine first and subsequently of killed vaccine. In fact, the killed vaccine keeps the antibody titer in the chick at a higher level. In the present experiment, live vaccine was administered to commercial broilers to obtain the desired immunity for a short period of time, since they are usually slaughtered for marketing within 8-12 weeks of age. The experimental results suggest that chicks need to receive both live and killed vaccine, respectively, in order to obtain immediate immune response and subsequently to keep the high antibody titers.

Due to limitations of time and other required experimental resources, it was not possible to obtain the daily antibody titers of live and killed vaccine. However, further research in this area using recent molecular techniques would be worthy to investigate.

REFERENCES

Ahmed, N., 1992. Livestock program and forage development prospect in Bangladesh. Asian livestock. 17(1): 5-11.

Phatak, R.K., 2000. Vaccination failures and their solutions. N.500029 in Proc. Poultry Seminar, Hyderabad, India.

Rahman, M. A., Rahman, A. K, 1998. Study in poultry and livestock programme in Bangladesh. Asian livestock. 23(1): 9-15.