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Estimation of Age of Infectious Bursal Disease Vaccination in Broiler Chickens in Haryana, India

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ABSTRACT

Infectious bursal disease (IBD) or Gumboro causes heavy economic losses in poultry industry. Farmers are facing the problems of Gumboro even in the vaccinated flocks. The control of IBD depends on appropriate immunization schedule and maintenance of good hygienic conditions on the farm. For estimating optimum day of vaccination in broiler chickens serum samples were collected from 18 hatcheries situated in different parts belonging to Jind, Panipat and Hisar district of Haryana. Maternal antibody (MAb) titre of all the serum samples collected from different hatcheries along with day of vaccination was calculated as per Deventer formula. Statistics was applied on this raw data and the uniformity of day-old broiler chicks was expressed as percentage of coefficient of variation (CV %) which was in the range of 28.2% to 75.3%. The vast range of MAb titre (2 to 13128) in different serum samples gave wide range of day of vaccination as calculated for individual samples of all the hatcheries and it came to be in the range of 7 to 19 days of age of vaccination. According to Deventer formula a flock showing variation of > 4 days in the age of vaccination range needs to be vaccinated twice. Due to wide range of MAb titre, twice vaccination is advisable and the age of first vaccination came to be 9th day and age for booster vaccination came to be 16th day. However, further studies are required to strengthen the observations.

Keywords
Infectious bursal disease, Maternal antibody, Titre, Vaccination.

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Introduction

Infectious bursal disease (IBD) or Gumboro disease is a highly contagious viral infection of poultry which causes heavy mortality and immunosuppression (Van den Berg et al., 1991). Infectious bursal disease virus (IBDV) belongs to genus Avibirnavirus under the Birnaviridae family and it is a ds-RNA, non-enveloped bi-segmented virion (Dobos et al., 1979). The disease is clinically characterized by dullness, depression, loss of appetite, white watery diarrhoea, soiled vent, ruffled feathers, severe prostration followed by death. The virus infects dividing IgM+ B lymphocytes and the main site of viral replication is the bursa of Fabricius, where B cells are produced (Nakai and Hirai, 1981; Palmquist
et al., 2006). Infectious bursal disease virus (IBDV) can also infect macrophages (Palmquist et al., 2006, Kim et al., 1998, Khatri et al., 2005). Infection spread orally via contaminated feed and water (Sharma et al., 2000).

Since the first report of IBD outbreak from Uttar Pradesh in India by Mohanty et al., (1971), the major work on the disease has been combined to diagnosis and characterization of virus. The disease causes heavy economic losses in poultry industries due to immunosuppression in subclinical cases (Jackwood and Sommer, 2010) and in acute cases; it is associated with mortality, haemorrhages and also bursal damage (Jackwood et al., 2009). Some strains of IBDV can cause mortality as high as 100% in a susceptible flock (Cao et al., 1998). Immuno-suppression makes birds susceptible to secondary diseases and lead to vaccination failures (Allan et al., 1972).

The emergence of variant or newer strains of the virus in the recent times has also been reported to cause vaccination failures (Moemen et al., 2014). Moreover, various attenuation levels of commercially available live vaccines for IBD lead to varying levels of immunosuppression increasing the birds’ vulnerability to various infections.

Control of IBD poses one of the most challenging tasks in the poultry health management. The control of IBD depends on appropriate immunization schedule and maintenance of good hygienic conditions on the farm (Farooq et al., 2003). Vaccinating breeding hens with live attenuated or inactivated virus vaccine most effectively controlled the disease.

Induced antibodies are transferred to the young chicks via the egg yolk and protect the newly hatched chicks for the critical first few days of their life (Wyeth and Cullen, 1976). A high level of maternal antibodies will protect most young chickens against challenge by vvIBD virus for up to 3 weeks after hatching (Van den Berg, 2000). The half-life of maternal antibodies varies between breeds and it is approximately three days for broilers and five days for laying hens (Brandt et al., 2001). Thus, if the antibody titre of a chick at hatch is known, the time of maximum flock susceptibility to the wild or vaccinal virus can be determined. This information is very important when establishing the timing of vaccination programs (Van den Berg, 2000).

Other experimental studies have shown that IBD vaccine virus may even be completely neutralized by maternally derived antibodies (MDAs) (Tsukamoto et al., 1995; Alam et al., 2002; Hair-Bejo et al., 2004; Moraes et al., 2005). Ahmed et al., (2003) reported that immune response against different IBD vaccines varied in accordance with the vaccine schedule and levels of maternal antibody against IBDV in the chicks. Chickens from more than one parent flock may be mixed in the same broiler house, adding to the variation in the level of maternally derived antibodies (MDA).

In order to have chickens protected from IBDV field challenge, it is crucial to determine the optimal timing for IBD vaccine delivery (Tsukamoto et al., 1995). The optimal timing is often predicted based on serological data following detection of IBDV-MDA by an ELISA system during the first week post hatch (Kouwenhoven and Van den Bos, 1994).

The “Deventer formula” was developed to estimate the optimal vaccination time based on the half-life time of the MDA, the age of the chicken at sampling, genetic background, breakthrough titre of the vaccine, and the requested percentage of the flock having
antibody levels below the breakthrough titre of the vaccine at the time of administration (De Wit, 2001). Generally vaccination is done at 12-15 day of age in broiler chickens. Inspite of extensive control measures the farmers are still facing the problems of Gumboro even in the vaccinated flocks (Hasan et al., 1998; Dias et al., 2009; Anonymous, 2015). Therefore, estimation of maternal antibody titre and age of vaccination in broiler chicken still remains a major area of concern and forms a prime area of research.

Materials and Methods

Collection of samples

Blood samples from minimum of ten chicks (day old) from 18 hatcheries in Haryana was collected to evaluate the maternal antibody titre. 2-3 ml blood was collected directly from heart of day old chicks. Blood samples were left at room temperature for 15-20 minutes and then samples were kept in refrigerator or cold area for 10 minutes. Serum was separated efficiently from the clotted blood samples. Serum vials were marked for identity and serum samples were preserved at -20º C for maintaining its quality till further use.

Test procedure

An indirect enzyme linked immunosorbent assay was performed to detect the IBD antibody levels using IDEXX-IBD-ELISA kit (IDEXX, Westbrook, USA). The test was performed as per the manufacturer’s instructions.

Serum samples were diluted five hundred fold (1:500) with serum diluent and 100 μl of the diluted samples were added to the antigen coated wells. Positive and negative controls were included every time in the test. The plates were incubated at 18-26ºC for 30 min.

The contents of the wells were removed and the wells were washed three times with distilled water. Care was taken to avoid the drying of plate in between plate washings and prior to the addition of the next reagent. Plates were tapped onto absorbent material after the final wash to remove any residual wash fluid and after that 100 μl of Goat Anti chicken Horse radish peroxidase conjugate was added to each well. The plates were incubated at 18-26ºC for 30 minutes. The plates were emptied and washed thrice with distilled water.

The residual droplets were removed by lightly tapping the plate over a filter paper pad. One hundred microliters of TMB substrate was added to each well and incubated in dark at 18-26ºC for 15 minutes. Later, 100 μl of stop solution was added to each well to stop the colour reaction. The absorbance values were measured at 650 nm wavelength in an ELISA reader.

Assay was considered to be valid only when the difference between positive control mean and negative control mean (PCX - NCX) was greater than 0.075. The negative control mean absorbance should be less than or equal to 0.150. Serum samples with S/P ratios of greater than or equal to 0.2 (titers greater than 396) were considered as positive. The presence or absence of antibody to IBD was determined by relating the A (650) value of the unknown to the positive control mean. Antibody titre was calculated using the equations provided in the ELISA kit.

Calculation

Controls

Negative control mean,

$$\text{NCX} = \frac{\text{NC1}_A(650) + \text{NC2}_A(650)}{2}$$
NC1 - Negative Control 1 value, NC2 - Negative Control 2 value

Positive control mean,

\[ PC_{\bar{x}} = \frac{PC1_{\bar{x}} + PC2_{\bar{x}}}{2} \]

PC1 - Positive Control 1 value, PC2 - Positive Control 2 value

**Validity criteria**

\[ PC_{\bar{x}} - NC_{\bar{x}} > 0.075 \]

\[ NC_{\bar{x}} \leq 0.150 \]

**Samples**

The relative level of antibody in the sample was determined by calculating sample to positive (S/P) ratio.

Sample to positive ratio, S/P =

\[ \frac{\text{Sample Mean} - NC_{\bar{x}}}{PC_{\bar{x}} - NC_{\bar{x}}} \]

**Endpoint titres were calculated using equation**

\[ \log_{10} \text{Titer} = 1.09 (\log_{10} S/P) + 3.36^* \]

* relates S/P at a 1: 500 dilution to an end point titre

**IDEXX ELISA standard for IBD antibody titre**

| S/P value | Titre range | Antibody status |
|-----------|-------------|-----------------|
| <0.20     | <396        | negative        |
| ≥0.20     | ≥396        | positive        |

The optimal age of vaccination was determined by the Deventer formula (De Wit, 1998).

Vaccination age = \{((\log_{2} \text{titre bird}\% - \log_{2} \text{breakthrough}) \times t_\text{t}) + \text{age at sampling} + \text{correction0-4} \}

in which Bird\% = ELISA titre of the bird representing a certain percentage of the flock.

Breakthrough = breakthrough (ELISA) titre of the vaccine to be used (500 for intermediate plus vaccine)

\[ t_\text{t} = \text{half-life time (ELISA) of the antibodies in the type of chickens being sampled (3.0 days)} \]

Age at sampling = age of the birds at sampling (1 day of age)

Correction 0-4 = extra days when the sampling was done at 0 to 4 days of age (3 for day old chicks).

**Statistical analysis**

Maternal antibody titres of different hatcheries were analyzed using the one-way analysis of variance (ANOVA) test, followed by Duncan post hoc test. Difference between groups were considered significant at \( P<0.05 \). The data was expressed as mean ± SE. The coefficient of variation (CV %) of maternal antibody titre with in a hatchery was calculated. Alpha was set at 95%. Statistical software SPSS\textsuperscript{TM} 20.0 (IBM, Corp. USA) was used.

**Results and Discussion**

A total of 291 serum samples of day old chicks were collected from 18 hatcheries situated in different parts belonging to Jind, Panipat and Hisar district of Haryana, India. All the samples were tested by using IDEXX ELISA kit (Fig. 1). Maternally derived antibody titres of all the serum samples collected from different hatcheries along with day of vaccination calculated as per Deventer
The mean titres of hatcheries A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q and R were 5089.26, 5548.03, 4488.87, 263.40, 7006.25, 5332.42, 1231.25, 3083.54, 6716.11, 3281.88, 3378.90, 3307.08, 5364.99, 6899.31, 4585.66, 4031.42, 2368.09 and 5022.55 respectively. The highest mean titre was in hatchery E (7006.25) while lowest titre was in hatchery D (263.40). There was a vast variation in maternal antibody titres of day old chicks within and between hatcheries.

The uniformity of day-old broiler chicks can be estimated by serological profiling and expressed as a percentage of coefficient of variation (CV %).

Good and poor uniformity are the coefficients of variation < 30% or > 30%, respectively. Coefficient of variation was 53% in hatchery A, 58.7% in B, 35.8% in C, 46.1% in D, 41.5% in E, 47.9% in F, 75.3% in G, 55.9% in H, 52.5% in I, 67.4% in J, 67.1% in K, 61.85 in L, 45.1% in M, 34.4% in N, 28.2% in O, 47.9% in P, 73.3% in Q and 50.8% in hatchery R. Coefficient of variation was very high in all the hatcheries except O which indicate non-uniform MAb titres in them.

The vast range of MAb titre in different serum samples gave wide range of day of vaccination as calculated for individual samples of all the hatcheries and it ranged from 7-19 days (Table 1-6) as titre varied from 2-13128.

The mean of MAb titres and age of vaccination calculated as per Deventer formula is presented in Table 7.

Under conditions where in the range of day of vaccination is less than 4 days, one could estimate the day of single vaccination on the basis of MAb titres of 75% flock excluding upper 25% MAb titres. As per Deventer formula, the age of vaccination according to Ab titre of 75% flock came to be 17 days of age for hatchery E and N; 16 days of age for hatchery B, F and M; 15 days of age for hatchery A, C, O, P and R; 14 days of age for hatchery H, J, K and L; 12 days of age in hatchery Q; 8 days of age for hatchery G and 7 days of age for hatchery D (Table 7).

If we took average of lowest and highest age for day of vaccination calculated for individual samples of different hatcheries, the age of first vaccination came to be 9th day and age for second vaccination came to be 16th day as depicted in column 4 of the Table 7. However if we calculate age of first and age for second vaccination as per Deventer formula on the basis of 40% and 90% flock, the age of first and second vaccination came to be 12.1≈ 13 and 16th day respectively.

One way ANOVA revealed significant difference between MAb titres of serum samples from all the 18 hatcheries. Mcllroy et al., (1992) suggested that a high variation in MDA levels between birds make advisable to vaccinate a broiler flock twice to induce homogeneous protection in birds. Van den Berg and Meulemans, (1991); Tsukamoto et al., (1995) reported that to protect chickens against IBD, determination of optimal time for IBD vaccination is important.

Block et al., (2007) performed a field study on the significance of vaccination against IBD at the optimal time point in broiler flocks with maternally derived IBDV antibodies. It was estimated that flocks vaccinated between 1 day before, at, or up to 3 days after the estimated optimal time point developed detectable humoral immunity up to 14 days post vaccination.

If birds had been vaccinated more than 1 day before the calculated optimal day of vaccination, the humoral immune response was delayed or non-detectable until slaughter.
### Table 1: Maternal antibody titre and age of vaccination in serum samples of hatcheries A, B, C

| Sr. No. | Hatchery A ELISA titre | Day of vaccination | Hatchery B ELISA titre | Day of vaccination | Hatchery C ELISA titre | Day of vaccination |
|---------|-------------------------|--------------------|-------------------------|--------------------|-------------------------|--------------------|
| 1       | 1668.12                 | 10                 | 678.29                  | 7                  | 1661.03                 | 10                 |
| 2       | 2910.15                 | 12                 | 712.40                  | 7                  | 2130.53                 | 11                 |
| 3       | 2963.95                 | 12                 | 1094.44                 | 8                  | 2345.16                 | 11                 |
| 4       | 3143.84                 | 12                 | 2342.99                 | 11                 | 3024.10                 | 12                 |
| 5       | 3151.57                 | 12                 | 2670.61                 | 12                 | 3331.17                 | 13                 |
| 6       | 3456.78                 | 13                 | 2747.94                 | 12                 | 3391.80                 | 13                 |
| 7       | 3575.08                 | 13                 | 3163.94                 | 12                 | 3551.12                 | 13                 |
| 8       | 3827.03                 | 13                 | 3458.07                 | 13                 | 3733.22                 | 13                 |
| 9       | 3921.38                 | 13                 | 3841.35                 | 13                 | 3913.59                 | 13                 |
| 10      | 4086.94                 | 13                 | 5621.25                 | 15                 | 4150.83                 | 14                 |
| 11      | 4088.26                 | 13                 | 5972.57                 | 15                 | 4278.85                 | 14                 |
| 12      | 4169.94                 | 14                 | 6530.12                 | 16                 | 4645.20                 | 14                 |
| 13      | 4313.85                 | 14                 | 6890.75                 | 16                 | 4739.69                 | 14                 |
| 14      | 4625.45                 | 14                 | 7088.87                 | 16                 | 4956.88                 | 14                 |
| 15      | 4754.58                 | 14                 | 7424.24                 | 16                 | 5527.45                 | 15                 |
| 16      | 5053.88                 | 14                 | 7895.58                 | 16                 | 5939.28                 | 15                 |
| 17      | 5454.33                 | 15                 | 8075.02                 | 16                 | 5973.62                 | 15                 |
| 18      | 5528.51                 | 15                 | 8999.57                 | 17                 | 6172.31                 | 15                 |
| 19      | 5598.72                 | 15                 | 9612.37                 | 17                 | 6393.17                 | 15                 |
| 20      | 5769.14                 | 15                 | 10075.18                | 17                 | 7183.29                 | 16                 |
| 21      | 7159.15                 | 16                 | 11613.02                | 18                 | 7223.90                 | 16                 |
| 22      | 7554.31                 | 16                 | -                       | -                  | -                       | -                  |
| 23      | 12238.50                | 18                 | -                       | -                  | -                       | -                  |
| 24      | 13128.76                | 19                 | -                       | -                  | -                       | -                  |
| Mean±SE| 5089.26±550.62          | 13.54±0.58         | 5548.03±711.71          | 13.81±0.73        | 4488.87±350.77          | 13.62±0.36         |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

| Flock   | Hatchery A | Hatchery B | Hatchery C |
|---------|------------|------------|------------|
| 75%     | 5528.51    | 7895.58    | 5939.28    |
| 40%     | 4086.94    | 3841.35    | 3913.59    |
| 90%     | 7554.31    | 9612.37    | 6393.17    |

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Table 2: Maternal antibody titre and age of vaccination in serum samples of hatcheries D, E, F

| Sr.No. | Hatchery D | Hatchery E | Hatchery F |
|--------|------------|------------|------------|
|        | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
| 1      | 67.53      | 7.0         | 1504.33    | 9               | 1715.85    | 10               |
| 2      | 109.05     | 7.0         | 1955.26    | 10              | 2120.13    | 11               |
| 3      | 125.08     | 7.0         | 2773.33    | 11              | 2561.29    | 11               |
| 4      | 130.70     | 7.0         | 4136.97    | 13              | 2883.29    | 12               |
| 5      | 179.19     | 7.0         | 5871.90    | 15              | 3203.13    | 12               |
| 6      | 214.85     | 7.0         | 5989.51    | 15              | 3589.40    | 13               |
| 7      | 229.55     | 7.0         | 7431.55    | 16              | 4414.43    | 14               |
| 8      | 244.33     | 7.0         | 7754.25    | 16              | 4685.32    | 14               |
| 9      | 244.33     | 7.0         | 7771.97    | 16              | 5675.77    | 15               |
| 10     | 285.34     | 7.0         | 7993.06    | 16              | 5732.59    | 15               |
| 11     | 290.60     | 7.0         | 8055.53    | 16              | 5847.74    | 15               |
| 12     | 329.12     | 7.0         | 8274.06    | 17              | 6362.02    | 15               |
| 13     | 347.39     | 7.0         | 8339.48    | 17              | 6736.98    | 16               |
| 14     | 364.20     | 7.0         | 9687.50    | 17              | 6824.82    | 16               |
| 15     | 371.86     | 7.0         | 10280.23   | 17              | 8448.88    | 17               |
| 16     | 426.63     | 7.0         | 10389.23   | 17              | 9248.52    | 17               |
| 17     | 518.14     | 7.0         | 10898.09   | 17              | 10600.9    | 17               |
| mean±SE | 263.40±29.42 | 7.0±0.0    | 7006.25±705.91 | 14.99±0.64 | 5332.42±619.89 | 14.12±0.55 |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

| Flock  | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
|--------|-------------|--------------------|-------------|--------------------|-------------|--------------------|
| 75% flock | 347.39    | 7.0         | 8339.48    | 17              | 6736.98    | 16               |
| 40% flock | 229.55    | 7.0         | 7431.55    | 16              | 4414.42    | 14               |
| 90% flock | 371.86    | 7.0         | 10389.23   | 17              | 9248.52    | 17               |
Table 3: Maternal antibody titre and age of vaccination in serum samples of hatcheries G, H, I

| Sr. No. | Hatchery G | Hatchery H | Hatchery I |
|---------|------------|------------|------------|
|         | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
| 1       | 271.86     | 7           | 844.39     | 7           | 331.02     | 7           |
| 2       | 317.75     | 7           | 1447.19    | 9           | 612.21     | 7           |
| 3       | 399.55     | 7           | 1451.48    | 9           | 3564.94    | 13          |
| 4       | 402.64     | 7           | 1598.83    | 9           | 4101.43    | 14          |
| 5       | 514.99     | 7           | 1784.82    | 10          | 5461.84    | 15          |
| 6       | 805.07     | 7           | 2212.11    | 11          | 5529.85    | 15          |
| 7       | 829.62     | 7           | 2424.41    | 11          | 6154.67    | 15          |
| 8       | 981.49     | 7           | 2775.59    | 12          | 6335.71    | 15          |
| 9       | 1138.68    | 8           | 2984.47    | 12          | 7248.09    | 16          |
| 10      | 1294.30    | 8           | 3173.50    | 12          | 9205.67    | 17          |
| 11      | 1469.51    | 9           | 4262.05    | 14          | 9840.19    | 17          |
| 12      | 1509.07    | 9           | 4415.17    | 14          | 10233.84   | 17          |
| 13      | 2659.90    | 11          | 4735.98    | 14          | 10525.60   | 18          |
| 14      | 2909.86    | 12          | 4976.82    | 14          | 10786.64   | 18          |
| 15      | 2964.44    | 12          | 7166.29    | 16          | 10809.93   | 18          |
| mean±SE| 1231.25±239.65 | 9±0.46     | 3083.54±445.6 | 11.43±0.65 | 6716.11±912.06 | 14.74±0.9 |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

|     | Hatchery G | Hatchery H | Hatchery I |
|-----|------------|------------|------------|
| 75% flock | 1294.30 | 8 | 4415.17 | 14 | 10233.84 | 17 |
| 40% flock | 805.07 | 7 | 2212.11 | 11 | 5529.85 | 15 |
| 90% flock | 2909.86 | 12 | 4976.82 | 14 | 10786.64 | 18 |
Table 4: Maternal antibody titre and age of vaccination in serum samples of hatcheries J, K, L

| Sr.No. | Hatchery J | Hatchery K | Hatchery L |
|--------|------------|------------|------------|
|        | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
| 1      | 814.07     | 7           | 2          | 7                 | 822.66     | 7                 |
| 2      | 1351.36    | 9           | 1156.78    | 8                 | 848.90     | 9                 |
| 3      | 1630.86    | 9           | 1182.07    | 8                 | 1420.18    | 9                 |
| 4      | 1707.26    | 10          | 1443.33    | 9                 | 2087.56    | 11                |
| 5      | 1794.42    | 10          | 2023.97    | 10                | 2191.24    | 11                |
| 6      | 2005.89    | 10          | 2341.71    | 11                | 2396.22    | 11                |
| 7      | 2316.28    | 11          | 2721.77    | 12                | 3693.61    | 13                |
| 8      | 2408.30    | 11          | 4399.65    | 14                | 4105.17    | 14                |
| 9      | 4471.65    | 14          | 4964.94    | 14                | 4271.90    | 14                |
| 10     | 4707.59    | 14          | 5065.72    | 14                | 4459.88    | 14                |
| 11     | 5451.79    | 15          | 5111.41    | 14                | 6111.18    | 15                |
| 12     | 5885.88    | 15          | 5940.33    | 15                | 7276.52    | 16                |
| 13     | 8114.17    | 16          | 7573.09    | 16                |            |                   |
| mean±SE | 3281.88± 614.19 | 11.5± 0.78 | 3378.90± 629.51 | 11.55± 0.86 | 3307.08± 590.34 | 11.89± 0.79 |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

| Flock   | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
|---------|-------------|--------------------|-------------|--------------------|-------------|--------------------|
| 75% flock | 4707.59    | 14                 | 5065.72    | 14                 | 4271.90    | 14                 |
| 40% flock | 1794.42    | 10                 | 2023.97    | 10                 | 2191.24    | 11                 |
| 90% flock | 5885.88    | 15                 | 5940.33    | 15                 | 6111.18    | 15                 |
### Table 5 Maternal antibody titre and age of vaccination in serum samples of hatcheries M, N, O

| Sr. No. | Hatchery M | Hatchery N | Hatchery O |
|---------|------------|------------|------------|
|         | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
| 1       | 2553.68     | 11          | 3040.98    | 12              | 1803.59     | 10          |
| 2       | 3733.22     | 13          | 4116.86    | 14              | 3423.75     | 13          |
| 3       | 3834.93     | 13          | 4293.01    | 14              | 3704.73     | 13          |
| 4       | 3948.03     | 13          | 4959.72    | 14              | 3704.73     | 13          |
| 5       | 4646.27     | 14          | 5386.72    | 15              | 3751.12     | 13          |
| 6       | 4813.34     | 14          | 6372.06    | 15              | 4046.29     | 13          |
| 7       | 5528.63     | 15          | 7390.81    | 16              | 4394.95     | 14          |
| 8       | 6678        | 16          | 7407.50    | 16              | 4402.47     | 14          |
| 9       | 6787.74     | 16          | 7744.89    | 16              | 4517.34     | 14          |
| 10      | 11126.05    | 18          | 8144.35    | 16              | 4939.30     | 14          |
| 11      | -           | -           | 8251.28    | 17              | 5186.68     | 15          |
| 12      | -           | -           | 8696.55    | 17              | 5740.07     | 15          |
| 13      | -           | -           | 8758.75    | 17              | 6163.38     | 15          |
| 14      | -           | -           | 12026.88   | 18              | 6351.22     | 15          |
| 15      | -           | -           | -          | -               | 6655.25     | 16          |
| mean±SE| 5364.99±765.22 | 13.3±0.63 | 6899.31±634.72 | 15.41±0.43 | 4585.66±333.90 | 13.64±0.39 |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

|        | Hatchery M | Hatchery N | Hatchery O |
|--------|------------|------------|------------|
| 75% flock | 6678       | 16          | 8251.28    | 17          | 5740.07     | 15          |
| 40% flock | 3948.03    | 13          | 6372.06    | 15          | 4046.29     | 13          |
| 90% flock | 6787.74    | 16          | 8758.75    | 17          | 6351.22     | 15          |

3400
Table 6 Maternal antibody titre and age of vaccination in serum samples of hatcheries P, Q, R

| Sr.No. | Hatchery P | Hatchery Q | Hatchery R |
|--------|------------|------------|------------|
|        | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination | ELISA titre | Day of vaccination |
| 1      | 1410.76     | 9           | 24.97      | 7             | 1156.36    | 8             |
| 2      | 1421.04     | 9           | 33.72      | 7             | 2307.36    | 11            |
| 3      | 1672.07     | 10          | 1049.66    | 8             | 2402.04    | 11            |
| 4      | 1814.08     | 10          | 1479.57    | 9             | 2656.30    | 12            |
| 5      | 3057.86     | 12          | 1534.12    | 9             | 3397.51    | 13            |
| 6      | 3409.94     | 13          | 1535.34    | 9             | 3700.56    | 13            |
| 7      | 3555.70     | 13          | 1633.93    | 10            | 3907.79    | 13            |
| 8      | 3779.90     | 13          | 1969.72    | 10            | 4110.31    | 14            |
| 9      | 3966.03     | 13          | 1983.35    | 10            | 4233.92    | 14            |
| 10     | 4170.67     | 14          | 2198.79    | 11            | 4273.31    | 14            |
| 11     | 4941.20     | 14          | 2410.95    | 11            | 4439.64    | 14            |
| 12     | 5234.37     | 15          | 3196.68    | 12            | 4862.91    | 14            |
| 13     | 5561.49     | 15          | 3307.76    | 13            | 4880.93    | 14            |
| 14     | 5662.25     | 15          | 3500.94    | 13            | 5031.95    | 14            |
| 15     | 7407.01     | 16          | 5794.88    | 15            | 5627.21    | 15            |
| 16     | 7438.43     | 16          | 6235.16    | 15            | 5808.85    | 15            |
| 17     | -           | -           | -          | -             | 8022.21    | 16            |
| 18     | -           | -           | -          | -             | 9433.31    | 17            |
| 19     | -           | -           | -          | -             | 10095.26   | 17            |
| 20     | -           | -           | -          | -             | 10103.32   | 17            |
| mean±SE | 4031.42±483.43 | 12.8±0.59 | 2368.09±434.37 | 10.56±0.63 | 5022.55±570.78 | 14.3±0.5 |

As per Deventer formula - Age of vaccination according to antibody titre of (excluding birds of higher titre)

| 75% flock | 40% flock | 90% flock |
|-----------|-----------|-----------|
| 5234.37   | 3555.70   | 7407.01   |
| 15        | 13        | 16        |
| 3196.68   | 1633.93   | 5794.88   |
| 12        | 10        | 15        |
| 5627.21   | 4110.31   | 9433.31   |
| 15        | 14        | 17        |

mean±SE
**Table 7** Mean maternal antibody titre and age of vaccination in different hatcheries

| Hatchery | Number of samples (N) | Range of MAb titre | Range of day of vaccination | Mean ±SE | Age of vaccination according to antibody titre of (excluding birds with higher titre) |
|----------|-----------------------|---------------------|-----------------------------|---------|--------------------------------------------------------------------------------|
|          |                       |                     |                             |         | **75% flock** | **40% flock** | **90% flock** |
| A        | 24                    | 1668.12- 13128.76   | 10-19                       | 5089.26±550.62<sup>efgh</sup> | 15 | 13 | 16 |
| B        | 21                    | 678.29-11613.02     | 7-18                        | 5548.03±711.71<sup>fgi</sup> | 16 | 13 | 17 |
| C        | 21                    | 1661.03- 7223.90    | 10-16                       | 4488.87±350.77<sup>def</sup> | 15 | 13 | 15 |
| D        | 17                    | 67.53- 518.14       | 7                           | 263.40±29.42<sup>a</sup> | 7 | 7 | 7 |
| E        | 17                    | 1504.33- 10898.09   | 9-17                        | 7006.25±705.91<sup>i</sup> | 17 | 16 | 17 |
| F        | 17                    | 1715.85- 10600.97   | 10-17                       | 5332.42±619.89<sup>fgi</sup> | 16 | 14 | 17 |
| G        | 15                    | 271.86 – 2964.44    | 7-12                        | 1231.25±239.65<sup>ab</sup> | 8 | 7 | 12 |
| H        | 15                    | 844.39- 7166.29     | 7-16                        | 3083.54±445.6<sup>cd</sup> | 14 | 11 | 14 |
| I        | 15                    | 331.02 – 10809.93   | 7-18                        | 6716.11±912.06<sup>gh</sup> | 17 | 15 | 18 |
| J        | 13                    | 814.07 – 8114.17    | 7-16                        | 3281.88±614.19<sup>cde</sup> | 14 | 10 | 15 |
| K        | 13                    | 2- 7573.09          | 7-16                        | 3378.90±629.51<sup>cde</sup> | 14 | 10 | 15 |
| L        | 12                    | 822.66- 7276.52     | 7-16                        | 3307.08±590.34<sup>cde</sup> | 14 | 11 | 15 |
| M        | 10                    | 2253.68- 11126.05   | 11-18                       | 5364.99±765.22<sup>fgih</sup> | 16 | 13 | 16 |
| N        | 14                    | 3040.98 – 12026.88  | 12-18                       | 6899.31±634.72<sup>ghi</sup> | 17 | 15 | 17 |
| O        | 15                    | 1803.59 – 6655.25   | 10-16                       | 4585.66±333.90<sup>def</sup> | 15 | 13 | 15 |
| P        | 16                    | 1410.76 – 7438.43   | 9-16                        | 4031.42±483.43<sup>cde</sup> | 15 | 13 | 16 |
| Q        | 16                    | 24.97-6235.16       | 7-15                        | 2368.09±434.37<sup>bc</sup> | 12 | 10 | 15 |
| R        | 20                    | 1156.36 – 10103.32  | 8-17                        | 5022.55±570.78<sup>efgh</sup> | 15 | 14 | 17 |
| **Mean** |                       |                     |                             | 5098.26±550.62<sup>efgh</sup> | 15 | 13 | 16 |

Means with different superscripts are significantly different (p<0.05)
Similar study was done by Besseboua et al., (2015) who determined optimal time of vaccination against IBDV (Gumboro) in Algeria. It was noticed that on day 1 the chicks contained a high level ($6400.54 \pm 2993.67$) of maternally derived antibody that gradually decreased below a positive level within 21 days ($365.86 \pm 634.46$). It was observed that a high level of MDA interferes with the vaccine virus, resulting in no immune response. For better immune response, it was suggested that the chickens should be vaccinated at day 21, as the uniformity of MDA is poor (coefficient of variation [CV] $> 30\%$), and boosted at day 28. Indeed, two vaccinations are necessary to achieve good protection against infectious bursal disease virus of the entire flock. Fantay et al., (2015) also determined optimum time for administration of live intermediate vaccine of infectious bursal disease to chickens at Mekelle farm. The study was conducted from December 2013 to April 2014 to predict the proper age for vaccination of chickens against infectious bursal disease (IBD) and calculate the maternal antibody transfer rate. This study showed that the proper time for administration of live intermediate IBD vaccine is 18 days instead of 21 days with the management conditions in place at the farm. Suzuki et al., (2009) reported that, 15th day is the optimal vaccination time for Gumboro disease which was three days earlier than reported by Fantay et al., (2015). This difference might be due to variation in the type of vaccine used as they used live intermediate plus IBD vaccine instead of intermediate IBD vaccine only.

De Wit (1998) clearly stated that chickens vaccinated using live intermediate IBD vaccine had a capacity to breakthrough maternally derived antibody titre of 125 whereas those vaccinated using intermediate plus IBD had the capacity to break through maternal antibody titre of 500. These substantial differences could be ascribed to the amount of antibodies transferred from hen to chick through the egg (Hamal et al., 2006).

Due to wide range of MAb titre, twice vaccination is advisable for chicks belonging to different hatcheries of Haryana as per our study. The MAb titre calculation is not possible for individual hatchery at moment. Further studies will be required to see the response of IBD vaccination using different vaccines for this schedule.

Maternal antibody titres from the serum samples of day old chicks of 18 hatcheries showed a wide variation. Statistics was
applied on this raw data and the uniformity of day-old broiler chicks was expressed as percentage of coefficient of variation (CV %) which was in the range of 28.2% to 75.3%. Coefficient of variation was very high in all the hatcheries except hatchery O which indicate non-uniform maternal antibody (MAb) titres in them. The vast range of MAb titre (2 to 13128) in different serum samples gave wide range of day of vaccination as calculated for individual samples of all the hatcheries and it came to be in the range of 7 to 19 days of age of vaccination. According to Deventer formula a flock showing variation of > 4 days in the age of vaccination range needs to be vaccinated twice. Due to wide range of MAb titre, twice vaccination is advisable. For this we took average of lowest and highest age for day of vaccination calculated for individual samples of different hatcheries and the age of first vaccination came to be 9th day and age for booster vaccination came to be 16th day. However, further studies are required to strengthen the observations.

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