Serological profile and calculation of half-life time with IBD ELISA in a commercial broiler breeder flock in the Netherlands

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The ELISA test has been widely accepted as a practical test to evaluate the success of vaccination. Also, the ELISA test is commonly used to predict the optimal times for vaccination with live vaccines for Infectious Bursal Disease (IBD).

The IBD ELISA vaccination date prediction method (Deventer log 2 Method) uses the half-life of maternal antibodies to estimate the optimal vaccination time. The half-life of maternal antibodies has been estimated to be 4.5 days in Broiler Breeders. However, these values are mostly based on experimental antibody decline rates in controlled experiments and have never been based on real data from flocks in the field. This paper describes the IBD serological ELISA results of a Broiler Breeder flock monitoring from birth to the end of production of hatching eggs. Apart from the general IBD serological profile made, particular studies were initiated to calculate the half-life time of Maternal Antibodies (MA's), and the transfer rate of MA's from Parent to young chicks from real field data. Also the accuracy of vaccination date prediction was determined by comparing predicted titer distributions with actual titer distribution measured from a Broiler Breeder Flock.

Materials and methods

Experimental animals

The flock monitored consisted of Ross 308 Parent Stock. The birds were housed at a commercial farm in The Netherlands.

Vaccines

The following IBD vaccines were used:

- Live vaccine, Gallivac IBD vaccine applied at 22D of age through drinking water.
- Inactivated vaccine, Nobilis IB3+G+ND vaccine, applied at 15W of age trough I.M. injection.

Serology

Antibody IBD titers in the serum were determined by ELISA using the BioChek IBD ELISA kit.

MA half-life determination

Serum samples were collected from 40 randomly chosen female Broiler Breeder chicks in house number 1. Serum samples were taken from the 01st day of life till 22nd day of age (= age of vaccination). Serum samples were frozen and send to BioChek service laboratory for ELISA testing. The resulting IBD ELISA titers were used to calculate the MA half-life's for each day from 01D till the fist negative mean titer at 21D. The MA half-life time was calculated using the following formula:

$$\text{MAB Half Life} = \frac{\text{No. Days to first negative titer}}{\text{Log}_2 \text{ Beginning titer} - \text{Log}_2 \text{ End titer (first negative titer)}}$$

Accuracy of vaccination date prediction

Vaccination date prediction was done for Gallivac IBD using the Deventer Log2 formula with an OTV (Optimal Titer of Vaccination) of 500 (as recommended by the vaccine producer), and vaccination cover of 75%. The predictions were done on ELISA results obtained from serum samples taken at 01D, 04D, 06D, and 10D. For

the prediction a value of 4.5 days for the MA Half-was used for samples taken at 01D, and an MA Half-life value of 4 days for samples taken at 04D, 06D, and 10D respectively. The vaccination cover and MA Half-life values were according the guidelines of the BIOCHEK ELISA producer. The accuracy of the predicted age of vaccination was then determined by taking a serum sample at the day of predicted vaccination and calculating the real % of samples at or below the optimal titer for vaccination (OTV). The real % of titers at or below the OTV were then compared with the predicted 75 % of titers at or below OTV. The accuracy of prediction was then calculated as follows:

$$\% \text{ Accuracy of Prediction} = \frac{\text{Real \% titers} \leq \text{OTV at predicted vaccination age}}{\text{Predicted \% titers} \leq \text{OTV at predicted vaccination age}} \times 100$$

Serological profile of vaccination

Serum samples were collected from 40 randomly chooses female birds at the ages of 9, 11, 14, 17, 18, 20, 24,26, 32,38, 46, 54, and 61 Weeks of age. Serum samples were frozen and send to BioChek service laboratory for ELISA testing.

MA transfer rate determination

Serum samples and hatching eggs were collected at the same time from 40 randomly chooses female birds at the ages of 24,26, 32,38, 46, 54 Weeks of age. From the day-old chicks hatched from the collected eggs serum samples were taken. Serum samples were frozen and send to BioChek service laboratory for ELISA testing. The % transfer rate of MA titers from parent to progeny was calculated as follows:

$$\% \text{ MA transfer rate} = \frac{\text{Mean Titer at 01D}}{\text{Mean Titer of Parent Flock}} \times 100$$

Relation of age of parent and 01D MA titers

Serum samples (23 samples) were taken from day old chicks derived from 32 Broiler Breeder parent flocks of different ages ranging from 30 weeks to 65 Weeks. All flocks did receive the same IBD vaccination program. Serum samples were frozen and send to BioChek service laboratory for ELISA testing. The mean MA titers of 01D chicks with the corresponding ages of the parents were analyzed to see if a relationship exists between Mean 01D titer and Age of Parent.

Results

Accuracy of vaccination date prediction

The resulting predictions, utilizing the Deventer Log2 Method have been summarized in *Table 1*.

Table 1. Predicted ages of vaccination with live IBD vaccine from serum samples taken at various ages from a Broiler Breeder flock

Age Days	Half–life Days	Predicted Age of vaccination
01D	4.5	20D
04D	4.0	20D
06D	4.0	19D
10D	4.0	20D

The predicted vaccination ages did not seem to be affected by the sampling ages (01D to 10D), at which the serum samples were taken. The prediction remained fairly constant throughout the sampling ages, being 19 to 20 days. This means that at the age of 19D and 20D it was predicted that 75% of the birds will have a titer ≤ 500.

The real (not predicted) distribution of % birds with a titer ≤ 500 is shown in *Figure 1*. The average % of birds (n=40) at or below the Optimal Titer to Vaccinate (OTV) of 500, was 78% at 19 Days and 73% at the predicted vaccination age 20 days. This compared very well to the predicted 75% of birds with the Deventer log2 Method, and the prediction was calculated to be 104 % and 97% accurate at 19 days and 20 days, respectively.

Figure 1. Percent of Maternal Antibody (MA) ELISA titers which are at , or below, the optimal titer of 500 for vaccination, during the first 25 days of age in a Broiler Breeder flock. (n = 40 birds/day)

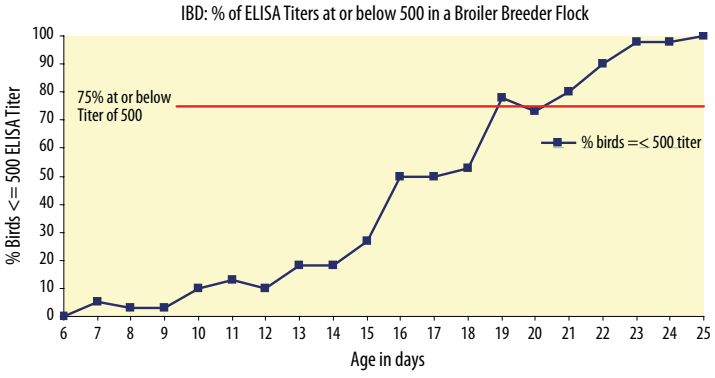
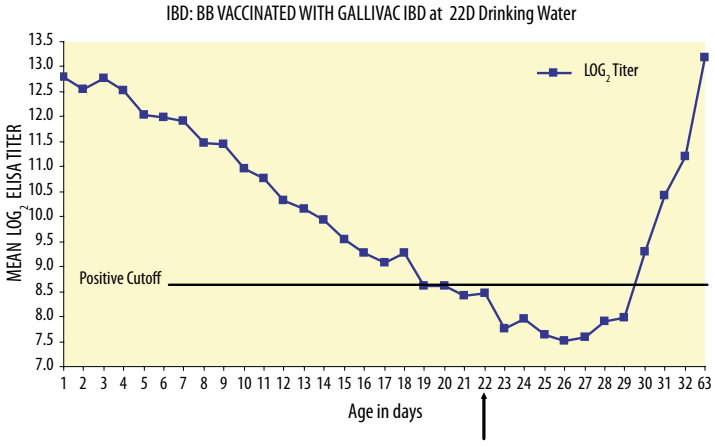


Figure 2. The Maternal antibody decline curve and titer response after live vaccination with Gallivac IBD vaccine after vaccination at 22D through drinking water in a Broiler Breeder Flock



Serological profile of vaccination

Although predicted at 20D, the flock was actually vaccinated at 22D with Gallivac IBD.

At 22D, the actual measured % of birds with a MA titer ≤ 500 was 90%. So at 22D 90% of the birds were considered to be susceptible to vaccination. This should result in a successful titer response, following vaccination, as normally 75% of susceptibility is presumed to be sufficient.

The real maternal antibody decline curve and titer response after live vaccination with Gallivac IBD vaccine after vaccination is shown in Fig, 2. The Mean log2 MA titers declined in a fairly linear fashion, becoming first negative at 21 days of age. The positive cutoff titer for the ELISA was a Log2 titer of 8.6, corresponding to a titer of 391. Flock was vaccinated at 22 days, showing the first positive mean titer at 30D, being 8 days after vaccination. Mean log2 titers ranged from 9.3 (= 631 titer, 25% positive) at 30D to log2 13.2 (=9 249 titer, 100% positive) at 63D, indicating successful seroconversion after live vaccination with Gallivac IBD vaccine. The complete serological profile after live priming and vaccination with inactivated vaccine is shown in *Figure 3*. After vaccination with inactivated vaccine (nobilis IB3 + G + ND) at 15W, mean log2 titers peaked at 14.4 (= 21 937 Titer), 5 weeks after vaccination with inactivated vaccin. Thereafter Mean log2 titers remained stable, ranging from 13.9 (=15 228 Titer) at 31W to 14.0 (=16 527 Titer) at 61W.

MA half-life determination

The MA decline curve plotted in mean titers and mean log2 titers is shown in *Figure 4*. Mean ELISA titers did not decline in a linear fashion during the first 6 days. The best linear titer decline was noted between 7 and 15 days, but flattened out thereafter. However, when titers were converted into Log2 titers, the decline rate was fairly linear during the entire age span (01D- 21D) measured.

The linearity, and thus predictability, is also the reason why the Deventer Method for vaccination age prediction uses the conversion of titers into Log2 titers.

The calculated half-life time of MA antibodies is shown in *Table 2*.

Table 2. Calculated Half–life of MA titers from 01D till 21 days of life in a Broiler Breeder flock.
(Mean BioChek IBD ELISATiters, n=40)

Age (days)	Mean titer	Log_2 titer	Ma half–life (days)	Age interval
1	7072	12,8	4,6	1d–21d
2	6002	12,6	4,6	2d–21d
3	7021	12,8	4,1	3d–21d
4	5868	12,5	4,1	4d–21d
5	4193	12,0	4,4	5d–21d
6	4025	12,0	4,2	6d–21d
7	3878	11,9	4,0	7d–21d
8	2858	11,5	4,3	8d–21d
9	2782	11,4	4,0	9d–21d
10	2002	11,0	4,3	10d–21d
11	1727	10,8	4,3	11d–21d
12	1286	10,3	4,7	12d–21d
13	1145	10,2	4,6	13d–21d
14	969	9,9	4,7	14d–21d
15	742	9,5	5,4	15d–21d
16	618	9,3	5,9	16d–21d
17	537	9,1	6,2	17d–21d
18	616	9,3	3,6	18d–21d
19	393	8,6	10,2	19d–21d
20	394	8,6	5,0	20d–21d
21	343	8,4		

The half-life time of the MA ELISA titers were calculated till the 21st day of life, as at this point the first negative mean MA titers (< 391) were observed. The calculated half-life values vary according to sampling age. The average calculated half-life time varies from 4.6 – 4.0 days during the first 10 days of age. The highest half-life time observed was during the first 2 days of age, being 4.6 days. Thereafter, between 03D and 10D, the half-life values fluctuated around a fairly constant decline rate of 4.0 days.

MA transfer rate determination

The calculated MA transfer rates from Parent to 01D Chick are presented in *Table 3*. The MA

Figure 3. Serological profile of a Broiler Breeder Flock vaccinated at 22D with Live IBD vaccine (Gallivac IBD) and vaccinated with inactivated IBD vaccine (Nobilis IB3+G+ND)

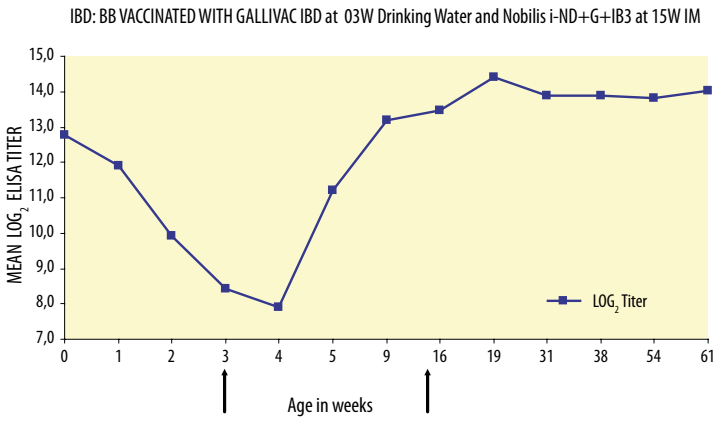
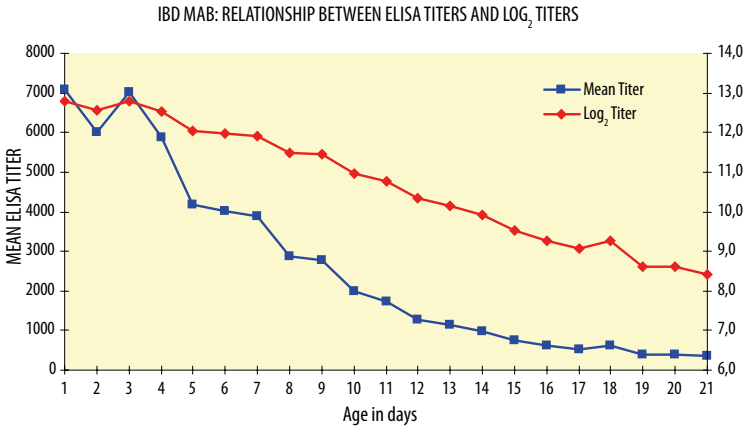


Figure 4. Relationship between maternal antibody ELISA titers and Log2 ELISA titers in a Broiler Breeder flock



transfer rates varied from 64% to 51%, with an average of 57% over all ages. The MA transfer rates declined with increasing age, being highest at 26W with 64%, and declining to 51 % at 61W. The observed 01D MA titers were fairly consistent over time, varying from 6 999 to 8 854, and did not seem to be affected by the age of the Parent .

Table 3. Maternal Antibody (MA) transfer rate from Parent to 01D Old Chick

	Mean Biochek Ibd Elisa Titer (N=40)		
Age W	Parent	01D Chick	MA Transfer Rate
26	13 745	8 854	64%
38	15 282	8 886	58%
46	11 846	6 999	59%
54	14 301	7 422	52%
61	16 527	8 482	51%
Average			**57%**

Relation of Age of Parent and 01D MA titers

No clear relation between Age of Parent bird and 01D Maternal Antibody (MA) titers could be detected from the results presented in *Table 4*. Throughout the age groups from 30-59W, Mean 01D MA titers varied from 9706 to 9992. Only in the age group of 60-65W, there appeared to be a marked decline in Mean MA titers, with a corresponding increase in Mean CV%. Because it was difficult to obtain samples from these age groups, the differences could also be due to the limited amount of flocks examined. The lack of evidence of a relationship between Age of Parent and corresponding 01D MA titers, was also consistent with results found in another independent study. (see section MA transfer rate determination**).**

Table 4. Relation between Age of BB Parents and Mean 01D Maternal antibody Titers.
(Mean BioChek IBD ELISA titers, n=23)

Parent	No.	Mean Titer	Mean Titer	Mean Titer	CV%	CV%	CV%
age (w)	*flocks*	*01D*	*min*	*max*	*mean*	*min*	*max*
30–36	15	9992	8315	14366	40	20	58
41–49	17	9930	8189	15837	42	20	86
53–59	5	9706	8474	13903	39	33	50
60–65	5	7315	4608	12662	49	33	57

Conclusions and discussion

Before this study was undertaken, the half-life times of Maternal Antibodies were estimated by the producers of the BioChek ELISA, to be ranging from 4.5 to 4.0 days, for samples taken at 01D to 10D, respectively.
The results in this study confirmed that these approximate half-life values are correct, varying from 4.6 to 4.0 days in the age interval of 01D to 10D.
An important point is that half-life time values vary and depend on the age of the bird when the sample is taken. This is important, because correct vaccination date prediction, also depends on the correct use of half-life values, which should correspond with the age at which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the serum samples have been taken. The highest half-life's observed were during 1st and 2nd day of age, being 4.6 days. Thereafter the half-life values fluctuated around a fairly constant decline rate of about 4.0 days, between 03D and 10D of age. The higher half-life values during the first 2 days in the life of a young chick, are thought to be the result of rising MA titers, due to the " Yolk-sac effect". The resorption of yolk, containing maternal antibodies, is highest during the first 3 days in the life of a young chick, resulting in rising (or stable) titers, rather than decreasing titers.

The vaccination date predictions based on the estimated half-life values, yielded a high level of accuracy (between 97 and 104 %), further validating the guidelines for half-life values as indicated by the producer of the BioChek ELISA.
It should be stressed however, that one should realize that vaccination date predictions are in fact estimations, will almost never be 100% accurate. They will help the veterinarian to apply live vaccines in the correct time window,

to ensure reasonable “vaccine take”, without the neutralizing effect of Maternal Antibodies. It is strongly recommended, for veterinarians who practice vaccination date prediction with ELISA systems, to check the accuracy of prediction, a few times a year. This can be done by taking samples at predicted vaccination age and calculating the % accuracy of prediction. If the % accuracy of prediction falls within the interval of 95-105 %, the predictions are considered to be valid. However, when prediction accuracy falls below 90 % (too early prediction), or above 110 % (too late prediction), corrective values for half-life’s should be considered to bring prediction back to an reasonable level of accuracy.
The additional monitoring of mean titers 3-5 weeks after live IBD vaccination, is also helpful to determine if vaccination (and prediction) has been ultimately successful or not. If mean titers are within normal expected range after vaccination, the vaccination and prediction are considered to be successful. If resulting titers are below expected levels and/or contain a high % of negatives, it is usually an indication of too early vaccination.

In two independent studies, there was no evidence found of a clear relationship between age of the Parent bird and the resulting MA titers in 01D old chicks. This was surprising, as it was assumed that older Parent birds produce lower and more erratic 01D MA levels in young chicks then young Parent birds. However, when looking at transfer rates of antibodies from Parent to 01D offspring, the MA transfer rates declined with increasing age, being highest at 26W with 64%, and declining to 51 % at 61W. The knowledge of transfer rates can be useful to estimate the 01D MA antibody titers, from serum taken from the Parent Breeder. Whether these estimations of 01D MA titers are accurate enough for vaccination date prediction, needs to be further investigated and validated. ■