

## **GUMBORO DISEASE:**

### **ESTIMATION OF OPTIMAL TIME OF VACCINATION BY THE DEVENTER FORMULA**

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Gumboro vaccines are in use throughout the world but all have a common problem i.e. when is the best time (age) to vaccinate? If you administer a live IBD vaccine to chickens that still have too many maternal derived antibodies (MDA), the vaccine will be neutralised by those antibodies. As a result the vaccine will not induce protection. On the other hand, one doesn't want to wait too long before vaccinating as this will leave the flock unprotected against early challenge.

It is not possible to know accurately without testing how much MDA a chicken receives from the hen. In theory, the MDA will be much higher in chickens from hens that have been vaccinated with an oil-based killed Gumboro vaccine than in chickens from hens that were vaccinated with a live vaccine only. In practice however, if a (sub-clinical) infection has occurred during the life of a hen, the antibody titre of the hen will rise, particularly in hens that have not been re-vaccinated with a killed vaccine. The progeny of these hens will need to be vaccinated later than normally expected.

When the Gumboro virus is in the neighbourhood, one wants to vaccinate as soon as possible. For these cases help in estimating the optimal time of vaccination has been developed to facilitate vaccination date estimation. The principle behind estimating the optimal age of vaccinating is simple, i.e. measure the level of MDA at a very young age and, as there is a regular decline (log<sub>2</sub> scale) of the MDA in the chick, it can be predicted when the level of MDA will be low enough to allow vaccination.

Several formulas for estimating the optimal age of vaccinating are used in the field. One of the first formulas was developed in the late eighties by Dr Ben Kouwenhoven (Poultry Health Institute, Doorn, The Netherlands, now integrated into the Animal Health Service, Deventer, The Netherlands). This formula was developed for the use of 'intermediate plus' vaccines and it helped overcome the Gumboro problems at that time. As the situation in the field has changed, this formula has been replaced in the Netherlands by 'the Deventer formula' of the Animal Health Service. This formula has been used in The Netherlands since 1990.

When estimating the optimal age for vaccinating against IBD, a few important factors for the formula need to be considered as follows:

- (1) Number of samples per flock. A minimum of 18 samples per house is required to obtain a representative sample of the flock. Estimations based on less than 18 samples are less reliable. It is false economy to try and save money by taking only 10-15 samples per flock. If the progeny of two houses originate from 1 breeder flock, sampling of 1 house will be sufficient.
- (2) Quality of chickens used for sampling. High quality chicks should be selected for sampling. Samples should not be taken from poor quality, ill or stressed chicks, as these do not represent the flock as a whole.

If these conditions are not met, estimate of the optimal date of vaccination will be unreliable.

#### **Advantages of the Deventer formula versus the older formulas:**

1. It is suitable for all types of birds: broilers, breeders and layers.
2. There are flexible bleeding dates for testing 1-10 days post hatch.

3. It allows estimations for flocks with both uniform and non-uniform titre distributions.
4. It is suitable for most Gumboro vaccines.

**Theoretical basis of the Deventer formula:**

1. The decline of the level of maternal antibodies (MDA's) varies between types of chicken. The decrease is a factor of the metabolism and the growth rate. The measured half-life time, as measured by the 'golden standard' i.e. the Virus Neutralisation test, is 3 to 3.5 days for broilers, 4.5 days for breeders and about 5.5 days for layers. Any half-life time can be used in the Deventer formula. Table 1 and 2 give the number of days one needs to wait from the day of testing to the time when successful vaccination can take place, (see paragraph 4 below). Vaccination will only be successful when the level of MDAs has declined to a certain level.

2. The level (titre) of maternal antibodies remains about the same during the first 4 days of life (absorption of yolk compensates for the decrease of titre by metabolism and growth). From 4 days of age, the titre decreases by 1 log<sub>2</sub> step per half-life time. In the Deventer formula, estimations made on the sera of chickens younger than 4 days are compensated to overcome these phenomena. For example, when the calculation is made from blood samples of 2 day-old chickens and the decrease of measured antibodies will take 13 days before the level is low enough to vaccinate, 2 extra days are added as compensation for their age (i.e. vaccinate 15 days after the blood sampling). If the same titre was measured from 4 day old or older chickens no extra days would be added (i.e. vaccinate 13 days after blood sampling).

In summary with blood from a day-old chicken – add 4 days; with blood from a 1 day-old chicken – add 3 days; with blood from a 2 day-old chicken – add 2 days; with blood from a 3 day-old chicken – add 1 day (Table 3). When high titres are expected, it is advisable to take blood samples at an older age. For example, day old layers usually have much higher MDAs than broilers and some ELISAs aren't capable of detecting antibodies above a certain level. Sampling therefore at 10 to 14 days will give a more reliable estimation than sampling at day 0.

3. The older formulae all produce estimation based on the mean titre of the blood samples. In a flock with uniform titres, this is not a problem. In reality, the uniformity of the titres is often not very high. This may occur when the chickens originate from several breeder flocks, or from flocks that have not been re-vaccinated with a killed vaccine.

**In the Deventer formula, the estimation is not based on the mean titre of the flock, but on the titre level when a certain percentage of the flock can be successfully vaccinated.**

Based on field experience the Deventer formula uses 75% as a default percentage. The general principle is that vaccination cannot be delayed until the last chicken will respond. This would place the whole flock at risk for too long. Also, it is not necessary to wait for the last responsive chicken, because the vaccine will spread for several days after the vaccination through the flock. This means chickens that are missed by the vaccination (because of higher MDAs) will be vaccinated by the others (assuming that 75% of the flock has been successfully vaccinated by proper administration of the vaccine). For determining what percentage of the birds have to be vaccinated successfully to ensure flock-wide spread, it is important to remember that Gumboro vaccines spread from bird to bird more easily on deep litter than in cages.

If one wanted to wait for the moment when 90% of the flock can be vaccinated successfully, the formula can be adjusted easily. When the uniformity of MDAs at the time of testing is poor or a decision has been made to vaccinate twice, the formula can give the ages when for example 40% and 90% or 20% and 70% of the flock can be vaccinated successfully.

4. Vaccines differ in their break-through titres. 'Intermediate plus' vaccines can break through a higher level of maternal antibodies than intermediate vaccines. The break-through titre of the vaccine is used in the formula. For the 'intermediate plus' vaccines like LZ 228E the break-through titre, as determined by the producer and Dr B Kouwenhoven is 500 (IDEXX standard ELISA). For the intermediate vaccine D78 the break-through titre is about 125 (IDEXX standard

ELISA). If other vaccines or ELISAs are used, the break-through titres should be established with the manufacturer of the vaccines and the ELISA kits.

**The Deventer formula is as follows:**

$$\text{Vaccination age} = \{ (\log_2 \text{ titre bird\%} - \log_2 \text{ breakthrough}) \times t_{\text{ }} \} + \text{age at sampling} + \text{correction 0-4}$$

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

t = half-life time (ELISA) of the antibodies in the type of chickens being sampled

Age sampling = age of the birds at sampling

Correction 0-4 = extra days when the sampling was done at 0 to 4 days of age.

To demonstrate how the Deventer formula works 5 examples are given below. The ELISA titres for each example are given in Table 4.

**Example A**

20 samples from 2 day-old broilers are tested in the IDEXX standard ELISA and the titres range from 2294 to 5304 (Table 4). The vaccine that the customer wants to use is an intermediate vaccine with a break-through titre of 125.

If the samples had been taken at day 4, the chicken with the lowest titre (2294) could have been vaccinated at 13 days (see table 1) after sampling. As bloods were taken from 2 day-old chicks, 2 days are added as compensation (see table 3), so the recommended vaccination age is 13+2=15 days after sampling (17 days of age).

The chicken with the highest titre (5304) can be vaccinated at 17 (see table 1) + 2 (see table 3) = 19 days after sampling. The difference is only 4 days, which is a sign of a very good uniformity.

To determine when 75% (default percentage) of the flock can be vaccinated, the highest 5 titres (representing 25% of the flock) are skipped. The highest titre that remains represents the 75% of the flock. This sample has a titre of 4584 so one has to wait for 16 + 2 = 18 days after sampling before vaccinating.

When the owner of the chicken would like to vaccinate with an "intermediate plus" vaccine (break-through titre of 500), the vaccination could be done 6 days earlier (see Table 1).

**Example B**

18 samples of 1-day-old broilers are tested in the IDEXX standard ELISA and the titres range from 235 till 4886 (Table 4). The vaccine that the customer wants to use is an intermediate vaccine with a break-through of 125.

The chicken with the lowest titre can be vaccinated at 3 (see Table 1) + 3 days (compensation for blood sampling at 1 days of age (see table 3) is 6 days after sampling. The chicken with the highest titre of 4886 can be vaccinated at 16 + 3 is 19 days after sampling. The difference is 13 days. This is a sign of a bad uniformity. Depending on the Gumboro risk on the farm, it can be wise to vaccinate twice, for example when 40% and 90% of the flock can be vaccinated. To calculate these data the Deventer formula has to be applied for the first 40% (bird 7 with a titre

of 1171) and for 90% (bird 16 with a titre of 3968) . Result: vaccinate at 13 {10 (Table 1) + 3 (Table 3)} and 18 {15 (Table 1) + 3 (Table 3)} days after sampling.

### **Example C**

20 samples of 7-day-old layers of which the titres range from 245 till 4792 (Table 4). The vaccine that the customer prefers to use only one dose of "intermediate plus" vaccine with a break-through of 500.

To determine when 75% of the flock can be vaccinated, the highest 5 titres are skipped. The highest titre that remains is 2761. Using the intermediate plus vaccine, this part of the flock can be vaccinated at 20 days of age ( 7 days of age at sampling + 13 (Table 2) + 0 (Table 3)) days after sampling.

### **Example D**

8 samples of 0 day-old broilers. The number of birds is too small to give a good estimation of the best day of vaccination. Advice on what day to vaccinate should be guarded and the owner should be warned in writing that the number was too small.

### **Example E**

18 samples of 1-day-old broilers are tested in the IDEXX standard ELISA and the titres range from 1570 till 5722 (Table 4). The customer prefers to vaccinate at 10 and 17 days of age using an intermediate vaccine with a break-through titre of 125. In his last few flocks, several blood samples, collected at slaughter (39 days of age), were negative in the ELISA.

When chickens that were vaccinated against Gumboro are still negative in ELISA at slaughter, it's good to check the vaccination programme and the vaccine application. ELISA-negative chickens at slaughter are a strong indication of an inadequate protection against challenge.

If a flock was vaccinated at 10 days of age using the intermediate vaccine, birds with titres below 512 at 1 day of age would be susceptible for the vaccine (6 days of waiting for the decrease of 512 to 125 (table 1) + 3 days (compensation for blood sampling at 1 days of age (see table 3))).

Looking at the titres from this flock (table 4), non of the birds would be susceptible for the vaccine at 10 days of age. Although not all birds were sampled, it is very likely that only a very minor part (if any) of the flock will be successfully vaccinated at day 10.

If a flock was vaccinated at 17 days of age using the intermediate vaccine, birds with titres below 2580 at 1 day of age would be susceptible for the vaccine (14 days of waiting for the decrease of 2580 to 125 (table 1) + 3 days (compensation for blood sampling at 1 days of age (see table 3))).

Looking at the titres from this flock (table 4), 9 of 18 birds would be susceptible for the vaccine at 17 days of age.

Summarizing it can be concluded that a vaccination of this flock at 10 days would be too early. Even the vaccination at 17 days of age is relatively early, possibly causing an inadequate protection against field challenge. When the previous flocks had comparable titres of maternally derived antibodies, this vaccination schedule could have been the cause of the inadequate vaccination as shown by the negative ELISA results at slaughter.

**Table 1.** Number of days to wait between a measured titre (IDEXX ELISA) at sampling in a broiler and the time when break-through titre of the Gumboro (intermediate plus and intermediate ) vaccine is reached according to the Deventer formula.

<b>DAYS TO WAIT AFTER SAMPLING</b>	<b>TITRE OF BROILER T_ = 3 days, break-through titre is 500</b>	<b>TITRE OF BROILER T_=3days,break-hrough titre is 125</b>
3	0 - 1024	0 - 256
4	1025 - 1290	257 - 322
5	1291 - 1629	323 - 407
6	1630 - 2048	408 - 512
7	2049 - 2580	513 - 645
8	2581 - 3258	646 - 815
9	3259 - 4096	816 - 1024
10	4097 - 5159	1025 - 1290
11	5160 - 6502	1291 - 1629
12	6503 - 8192	1630 - 2048
13	8193 - 10319	2049 - 2580
14		2581 - 3258
15		3259 - 4096
16		4097 - 5159
17		5160 - 6502
18		6503 - 8192
19		8193 - 10319

**Table 2.** Number of days to wait between a measured titre (IDEXX ELISA) at sampling in a layer and the time when break-through titre of the Gumboro (intermediate plus and intermediate) vaccine is reached according to the Deventer formula.

<b>DAYS TO WAIT AFTER SAMPLING</b>	<b>TITRE OF LAYER T_=5.5 days, break-through titre is 500</b>	<b>TITRE OF LAYER T_=5.5 days, break-through titre is 125</b>
5	0 - 1024	0 - 256
6	1025 - 1160	257 - 294
7	1161 - 1314	295 - 329
8	1315 - 1499	330 - 372
9	1500 - 1698	373 - 423
10	1699 - 1924	424 - 481
11	1925 - 2180	482 - 545
12	2181 - 2469	546 - 617
13	2470 - 2798	618 - 699
14	2799 - 3169	700 - 798
15	3170 - 3591	799 - 904
16	3592 - 4096	905 - 1024
17	4097 - 4640	1025 - 1160
18	4641 - 5257	1161 - 1314
19	5258 - 5997	1315 - 1499
20	5998 - 6794	1500 - 1698
21	6795 - 7697	1699 - 1924

22	7698 – 8719	1925 - 2180
23	8720 - 9878	2181 - 2469
24	9879 - 11191	2470 - 2798
25	11192 - 12678	2799 - 3269
26	12679 - 14362	3270 - 3591
27	14363 - 16384	3592 - 4096
28		4097 - 4640
29		4641 - 5257
30		5258 - 5997
31		5998 - 6794
32		6795 – 7697

**Table 3.** Number of extra days (broilers, breeders and layers) before vaccination when the blood sampling is done between 0 and 4 days of age.

Age of sampling	Extra waiting days
0	4
1	3
2	2
3	1
4 or older	0

**Table 4.** Measured Gumboro ELISA titres (IDEXX) of 20 broilers

Number of samples	Example A titres	Example B titres	Example C titres	Example D titres	Example E titres
1	2294	235	245	2912	1570
2	2556	379	632	3267	1831
3	2821	802	710	3799	1160
4	3030	885	1165	3943	2533
5	3287	938	1237	4182	2975
6	3322	1075	1247	4840	3093
7	3415	1171	1294	5312	3111
8	3439	1299	1435	5998	3129
9	3451	1332	1471		3229
10	3462	1342	1565		3747
11	3698	1364	1601		3956
12	3900	1658	1877		4165
13	4007	3724	2132		4240
14	4126	3802	2191		4356
15	4584	3835	2761		4426
16	5174	3968	3099		4547
17	5247	4328	3339		5567
18	5259	4886	3661		5722
19	5300		4195		
20	5304		4795		
	Broilers 2 days old	Broilers 1 day old	Layers 7 days old	Broilers 0 days old	Broilers 1 day old

When the logic of the Deventer formula becomes clear, it becomes simple. Because of its flexibility (all types of chicken, age of sampling, adjustable percentage of flock and different vaccines) it can be adjusted to every field situation. The next two points are however most important.

#### **NOTE**

1. The Deventer formula is based on half-life times of antibodies that are measured by the gold standard test i.e. the Virus Neutralisation test. The principle of the Deventer formula is valid as long as there is a good correlation between the half-life time as measured by the ELISA and the VN-test. Different ELISAs can have a different correlation with the VNT. The break-through titres are determined for the IDEXX standard ELISA and cannot be used for other ELISAs unless approved by the manufacturer. If the Deventer formula is used for other ELISAs without approval (requiring comparison of the half-life time to VN-test, break-through titres, reproducibility etc), discrepancies will occur. The producer of the ELISA should be contacted for more information on how to use the Deventer formula with other ELISA tests.
2. An estimation of the correct Gumboro vaccination date (whatever formula is used) is only a tool to apply the vaccine at the optimal age of the flock. It is not a guarantee. It cannot compensate for poor hygiene, very high field-pressure (velogenic field virus has a higher break-through titre than any vaccine), poor application of the vaccine etc. One should therefore always seek expert veterinary advice on vaccination matters.