



Rate of Transfer of Infectious Anaemia Maternal Antibodies from Broiler Breeders To the Progeny: a Field Evaluation

■ Author(s)

Rodrigues EE^I  <https://orcid.org/0000-0003-4553-9862>
Imperatori F^{II}  <https://orcid.org/0000-0002-8592-4709>
Back A^{III}  <https://orcid.org/0000-0002-8941-9814>
Kuana SL^{IV}  <https://orcid.org/0000-0003-2598-0431>
Brehmer M^V  <https://orcid.org/0000-0001-6582-0155>

^I Veterinarian, Cascavel, PR, Brazil.

^{II} MercoLab Laboratories, Cascavel, PR, Brazil.

^{III} Technical Consultant, Curitiba, PR, Brazil.

^{IV} IDEXX Laboratories, São Paulo, SP, Brazil.

ABSTRACT

Clinical manifestation of the disease caused by the chicken anaemia virus (CAV) occurs when chicken chicks are vertically contaminated or before the second week of life. CAV control is based on the vaccination of broiler breeders in order to promote progeny protection through maternal antibodies. This work aims to evaluate, under field conditions, the antibody titre in commercial broiler breeders at 28, 48, and 68 weeks of age, the rate of transference to the progeny, as well as the duration of antibodies in the progeny up to 21 days of age. Thus, a total of 92 sera samples from 93,000 broiler breeders vaccinated with a live vaccine for CAV at 14 weeks of age and 366 sera samples from their respective progeny were analyzed using ELISA. Breeders' antibody titre for CAV ranged between 5051 and 8660, and these titres may provide sufficient protection for their progeny. On average, 63% of the maternal antibodies were transferred to the progeny and lasted up to the second week of chick's life. It is possible to conclude that the vaccine and the vaccination procedure used by this company for breeders against CAV seems to be effective in inducing high antibody levels in the breeders and transferring protective maternal antibodies to the progeny.

INTRODUCTION

Chicken anaemia virus (CAV) causes an acute immunosuppressive disease in young chickens, characterized by anaemia, generalized atrophy of the lymphoid tissues, anorexia, lethargy, depression, and consequently, growth retardation and death (Fatoba & Adeleke, 2019; Brentano *et al.*, 2000; Kamdi *et al.*, 2020). The economic impact of the disease is due to mortality, which usually varies between 1 and 5%, but can reach up to 30%. Losses are aggravated by stunting and low feed conversion, as well as possible secondary and subclinical infections (Zhang *et al.*, 2015; Back, 2019; Brentano, 2020). Birds of all ages are susceptible to infection through ingestion or inhalation of the virus, but only chicks without maternal antibodies develop the disease (McNulty, 1991). According to Dhama *et al.* (2008), chicks that are contaminated on the first day of life are susceptible to clinical infection and develop the disease within two to five weeks. In birds infected after two weeks of life, CAV may cause subclinical diseases (Back, 2019). Due to their immunosuppressive effect, CAV outbreaks are often followed by opportunistic secondary bacterial infections (Schat, 2003; Zhang *et al.*, 2015; Fatoba & Adeleke, 2019), which can aggravate the severity of other diseases and cause vaccine failures (Otaki *et al.*, 1992; Orakpoghenor, 2019). Vertical transmission plays a major role, but horizontal transmission may occur between infected birds and those without humoral immunity (Yuasa *et al.*, 1980; McNulty, 1991; Schat, 2003; Fatoba & Adeleke, 2019). Hoop (1992) demonstrated

■ Mail Address

Corresponding author e-mail address

Éverton Eilert Rodrigues

Mercolab Laboratórios, R. Maringá, 2388 -
Bairro São Cristóvão, Cascavel - PR, 85816-
280, Brazil.

Phone: (45) 3218-0000

Email: article.cav@gmail.com

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that CAV is eliminated in faecal material, as well as in fertile eggs at the time of laying. The period of vertical transmission occurs between three and six weeks after the initial infection of the breeders (Brentano, 2020).

CAV control is based on the presence of maternal antibodies (Dhama *et al.*, 2008; Kamdi *et al.*, 2020), as long as they are not immunosuppressed by other factors (Schat, 2003; Orakpoghenor, 2019). For this reason, breeder vaccination and serology are important tools for ensuring progeny protection, avoiding the risk of clinical disease (Todd *et al.*, 1999). CAV vaccination for breeders is a well-established procedure in the Brazilian poultry industry, but interestingly the results of the monitoring have not yet been published in the literature.

Therefore, CAV maternal immunity is of fundamental importance for protecting chicks in the first and second weeks of life – the most critical periods for the disease. On the other hand, there is a lack of better documentation on how long this circulating immunity remains and on the transmission rate from breeders to the progeny at different ages. In this context, our aim is to investigate, under field conditions, the antibody title of chicken breeders at 28, 48, and 68 weeks of production, their transmission rate to the progeny, and the duration of antibodies in the progeny up to 21 days old.

MATERIAL AND METHODS

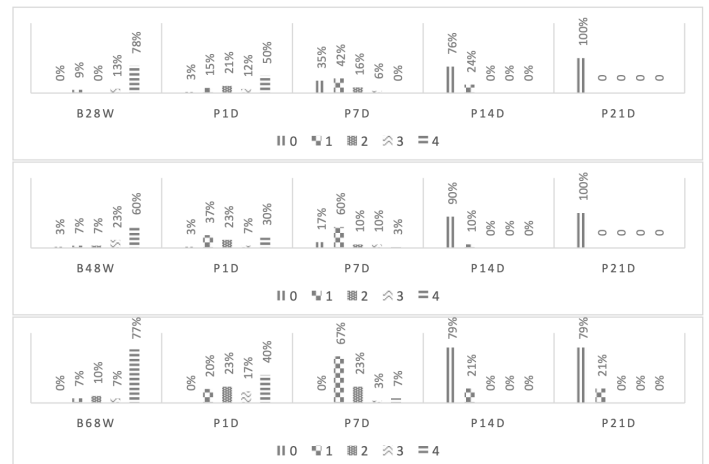
Study design

The present study was performed between August 2014 and April 2015 in a breeder flock (n=93,000; Cobb Slow) located in the Southern Brazil that received the company's standard vaccine program, including the live vaccine for CAV (AviPro Thymovac®, Lohmann Animal Health, Germany) at 14 weeks of age, via drinking water, following the manufacturer's guidelines.

Sampling

Breeders blood samples (2 mL) were collected by venopuncture of the wing vein using Eppendorf tubes at 28, 48, and 68 weeks of age; and their corresponding progeny at 1, 7, 14, and 21 days of age. For each sampling, 35 animals were bled and sera samples were sent under refrigeration to a private laboratory (MercoLab Laboratories), where they were stored (-20 °C) until analysis. In total, 458 sera were analyzed, around 30 sera per sample. The number of sera within each group was counted, transformed into a percentage, and distributed

according to the age of the breeder and the progeny (Figure 1).



B28W, B48W, B68W: broiler breeders at 28, 48, 68 weeks; P1D, P7D, P14D, P21D: progeny at 1, 7, 14, and 21 days; 0,1,2,3,4: groups of ELISA titles.

Figure 1 – Title distribution in groups of ELISA titles in breeders at 28, 48, and 68 weeks and their corresponding progeny at 1, 7, 14, and 21 days of age.

Serology

Antibody level against CAV was detected by ELISA blocking kit (IDEXX CAV Ab Test), using the 1:100 dilution and following the manufacturer's kit. The optical density was determined at 650nm in an ELISA reader (Biotek ELX 800) and the results were interpreted using the IDEXX xChek® software. The sera were classified into groups of titles, according to Table 1:

Table 1 – Titles and interpretation for ELISA IDEXX CAV 1:100.

Group	Title	VN log ₂	Interpretation
0	<1000	<7	Negative or positive
1	1000 - 2460	8 – 10	Positive protected
2	2461 - 5050	8 – 10	Positive protected
3	5051 - 8660	8 – 10	Positive protected
4	≥8661	≥11	Positive protected

Table 1 shows the criteria reported by Malo & Weingarten (1995), where flocks with antibody levels between 1000 and 8660 are considered protected (groups 1, 2, 3 with VN 8 to 10 log₂, respectively). The title group 0 (ELISA result <1000 and VN title <7log₂) means low or negative title. That is, they are birds that could transmit the field virus vertically to the susceptible progeny if infected. Titles belonging to groups 1, 2, and 3 confer protection against CAV infection to the progeny at different levels. The highest protection titles for the progeny are in group 4 (≥8661), as the maternal antibodies have a longer half-life and delay horizontal infection with the field virus (Malo & Weingarten, 1995).



RESULTS AND DISCUSSION

By monitoring a broiler breeder flock vaccinated against CAV and their progeny using an ELISA test, it was possible to verify the level of antibodies as well as the rate of antibody transfer (Table 2).

Table 2 – Sera distribution according to the title groups: breeder and progeny.

Title Group	B28W	P1D	P7D	P14D	P21D	B48W	P1D	P7D	P14D	P21D	B68W	P1D	P7D	P14D	P21D
0	0	1	11	25	32	1	1	5	26	29	0	0	0	23	23
1	3	5	13	8	0	2	11	18	3	0	2	6	20	6	6
2	0	7	5	0	0	2	7	3	0	0	3	7	7	0	0
3	4	4	2	0	0	7	2	3	0	0	2	5	1	0	0
4	25	17	0	0	0	18	9	1	0	0	23	12	2	0	0
Total Sera	32	34	31	33	32	30	30	30	29	29	30	30	30	29	29

B28W, B48W, B68W: broiler breeders at 28, 48, 68 weeks; P1D, P7D, P14D, P21D: progeny at 1, 7, 14, and 21 days.

the prevalence of antibodies against CAV in broilers flocks in Brazil, Canal *et al.* (2004) identified that vaccinated breeders showed antibodies against CAV in flocks with titles considered to be protective for the progeny. The results showed that, in vaccinated flocks, 99% of hens had anti-CAV antibody titers above 5000, although two serum samples (1%) showed titers between 4000-5000. Roussan (2006) investigated the seroprevalence of infectious avian anaemia virus in 32 commercial flocks of broilers and identified high

The average rate of maternal transfer at one day of age was 63%, ranging between 51 and 73% (Table 3). The average title for the three ages throughout the breeder's life was in group 3 (5051–8660) and in the average title transferred to the progeny was in group 2 (2461–5050) and 3 (5051–8660). When researching

protective titers in 1-4 days old chicks. In this study, the GMT ranged between 1145-5287, which is associated with the presence of maternal antibodies. Gharaibeh *et al.* (2008) performed serological monitoring of the progeny of a non-vaccinated broiler breeder's flock for CAV that had exposure to the field virus and identified GMT ranging between 1692–2944, which corresponds to groups 1 and 2 on the ELISA. According to the authors, this titration should be protective against clinical manifestation of CAV.

Table 3 – Transfer of maternal antibody (%)* to their respective progeny at the ages of 28, 48, and 68 weeks of the breeders.

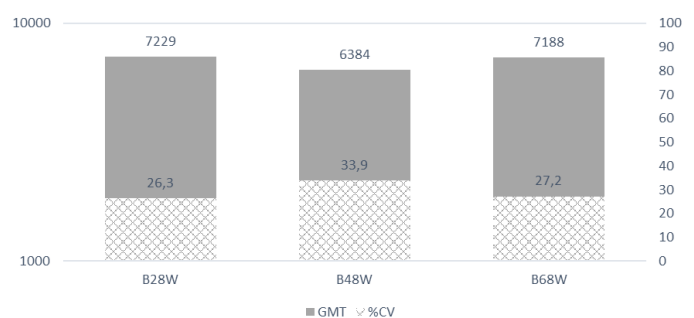
Age and immune status in the vaccinated broiler	Average title (GMT) of the broiler breeders	Average title (GMT) of the progeny at 1 day	Transfer rate of maternal antibodies
28 weeks (32/32)	7229	5280	73%
48 weeks (29/30)	6384	3269	51%
68 weeks (30/30)	7188	4733	66%

*Percentage of maternal antibody transfer: progeny title/broiler title x 100.

It is important to consider the title average and distribution in order to evaluate the vaccination program and make the necessary adjustments. With the CAV vaccination program used in this breeder flock, it was possible to observe >78% of the serum samples in the title group 4 at 28 weeks of life, showing good protection levels throughout the progeny with low coefficient of variation (Figure 1 and 2).

Our results demonstrated that the level of maternal antibodies with title ≥ 8 log₂ progressively declines, while the average of samples with low or negative titles increases (Figure 3 and Table 4).

The decline in maternal antibodies was linear up to 14 days of the progeny's age, with an approximate half-life of 7 days and a weekly reduction of 1.0 log₂ (figure 4). Half-life values were similar to those reported by Gharaibeh & Mahmoud (2013).



B28W, B48W, B68W: broiler breeders at 28, 48, 68 weeks.

Figure 2 – Geometric mean title (GMT) and the coefficient of variation (CV) for chicken anaemia virus of breeders at 28, 48, and 68 weeks of age.

In summary, under field conditions, the strategy using a live vaccine delivered by drinking water against CAV in broiler breeders at 14 weeks of age was effective to induce high antibody levels in breeders and their respective progeny, especially in the first week of



Table 4 – Transfer rate of maternal antibodies according to the age of the progeny.

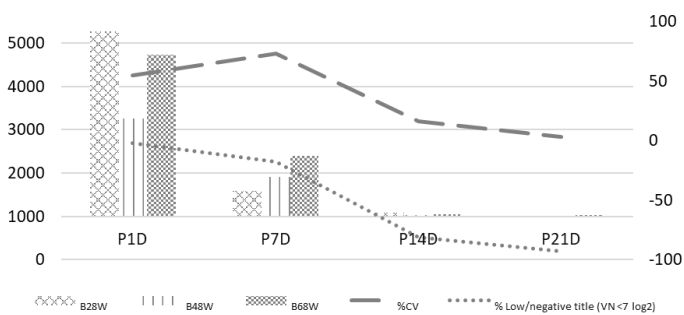
Immune status in the breeders	ELISA in progeny serum according to the age in days indicated				Half-life ¹
	1D	7D	14D	21D	Mean
28 week. – 7229 (32/32) ²	5280 (30/31)	1594 (20/31)	1080 (8/33)	999 (0/32)	5,08
48 week. – 6384 (29/30)	3269 (29/30)	1911 (25/30)	1040 (3/29)	999 (0/29)	8,75
68 week. – 7188 (30/30)	4733 (30/30)	2392 (30/30)	1057 (6/29)	1029 (6/29)	6,78
Mean 6384 (99%) ³	4427 (98%)	1966 (82%)	1059 (19%)	1009 (7%)	6,87

¹The calculation for maternal antibody half-life: $t_{1/2} = t * \ln(2) / \ln(N_0/N_t)$, which \ln , natural log; N_0 , initial antibody titer; N_t , final antibody titer and t , days.

²The number of positive samples with ELISA titer ≥ 1000 /total number of samples.

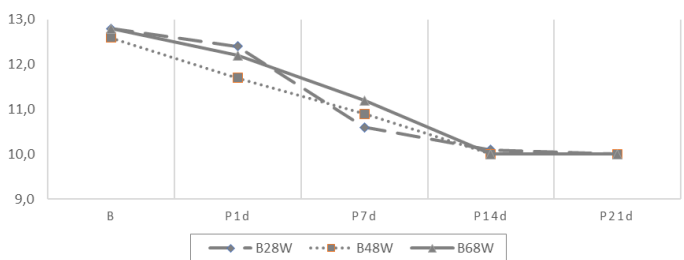
³Percentage of positive samples with ELISA titer ≥ 1000 .

life when birds are more susceptible to the disease. We conclude that the CAV vaccination program studied provides adequate antibody response and, along with serological surveillance, is an important tool to monitor and prevent chicken anaemia disease.



B28W, B48W, B68W: broiler breeders at 28, 48, 68 weeks; P1D, P7D, P14D, P21D: progeny at 1, 7, 14, and 21 days. CV: coefficient of variation.

Figure 3 – Transfer rate of maternal antibodies, the average percentage of samples with low/negative title, and the coefficient of variation according to their progeny.



B: breeders; B28W, B48W, B68W: broiler breeders at 28, 48, 68 weeks; P1D, P7D, P14D, P21D: progeny at 1, 7, 14, and 21 days.

Figure 4 – Reduction (log₂) profile of progeny's maternal antibodies.

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