

Prevalence of antibodies against chicken anaemia virus (CAV) in broiler breeders in Southern Brazil¹

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ABSTRACT.- Canal C.W., Ferreira D.J., Macagnan M., Fallavena L.C.B., Moraes H.L.S. & Wald V.B. 2004. **Prevalence of antibodies against chicken anaemia virus (CAV) in broiler breeders in Southern Brazil.** *Pesquisa Veterinária Brasileira* 24(2):89-92. Centro de Diagnóstico e Pesquisa em Patologia Aviária (CDPA), Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil. E-mail: claudio.canal@ufrgs.br

Chicks infected during the first two weeks of life with chicken anaemia virus (CAV) manifest clinical disease that can be avoided if the breeder hens transfer enough antibodies to their progeny. The objective of the present work was to establish the prevalence and titer of anti-CAV antibodies in some Brazilian broiler hen breeder flocks and verify in which phase of life the birds were infected. A total of 1,709 serum samples from 12 broiler hen flocks vaccinated against CAV and 64 unvaccinated flocks were analyzed for CAV antibodies with an enzyme-linked immunosorbent assay (ELISA). All non-vaccinated breeder flocks were found to be infected with CAV, with 89% of the hens tested presenting antibodies, 52% of these with titers considered high enough to protect their progeny against CAV infection. Likewise, all vaccinated hens had antibody titer to CAV capable of conferring protection to their progeny. Thus, vaccination of hens seems capable of conferring protection to chicks against clinically apparent CAV-associated disease.

INDEX TERMS: Antibodies, avian pathology, chicken anemia virus, CAV, epidemiology, prevalence.

RESUMO.- [Prevalência de anticorpos contra o vírus da anemia das galinhas (CAV) em matrizes de corte no Sul do Brasil.] A doença clínica causada pelo vírus da anemia das galinhas (CAV) ocorre quando os pintos são infectados durante as primeiras duas semanas de vida e pode ser prevenida se as matrizes transferirem anticorpos suficientes para a sua progênie. Em vista disso, este estudo foi realizado visando determinar a prevalência de anticorpos contra o CAV em alguns lotes de matrizes pesadas no Brasil. Buscou-se ainda verificar em que fase da vida as reprodutoras seriam infectadas e quais seriam os títulos de anticorpos nessas aves. Um total de 1709 amostras de soro de 64 lotes de reprodutoras não vacinadas e 12 lotes de

reprodutoras vacinadas contra o CAV foram analisados por ELISA. Todos os lotes de aves não vacinadas apresentaram anticorpos. Dentre esses, 89% dos indivíduos foram positivos, 52% com títulos de anticorpos capazes de conferir proteção a sua progênie contra o CAV. Igualmente, todos os lotes de matrizes vacinadas apresentaram anticorpos contra o CAV em títulos considerados protetores para a progênie. Conclui-se que a vacinação das reprodutoras parece capaz de conferir proteção aos pintinhos contra doença associada ao CAV.

TERMS DE INDEXAÇÃO: Vírus da anemia das galinhas, CAV, epidemiologia, patologia aviária, prevalência, anticorpos.

INTRODUCTION

Chicken anemia virus (CAV), a non-enveloped virus with a circular single-stranded DNA genome belonging to the family *Circoviridae* (Murphy 1996), was first isolated and described by Yuasa and colleagues (Yuasa et al. 1979). In young chickens, CAV causes a disease characterized by aplastic anaemia, generalized lymphoid atrophy and concomitant immune-depression (Todd 2000). Clinical disease takes place when chicks are infected during the first two weeks of life but can be avoided if hens

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transfer sufficient antibodies to their progeny. After two weeks of age, although chicks can be infected with the virus they do not develop clinical symptoms of the disease. Breeder hens infected during the laying period do not demonstrate clinical signs or changes in the number of eggs produced, fertility or embryo viability (Bülow & Schat 1997). After some weeks, infected hens develop antibodies against CAV that are able to control the infection and can be transferred to the egg, thus conferring either partial or total protection to chicks from clinical disease when they are most susceptible (Hoop 1992). To prevent economical losses due to CAV infections, the immune status of parent flocks can be monitored before the start of the laying period in order to determine whether vaccination should be carried out. The objective of the present work was to perform a serological survey to establish the prevalence of anti-CAV antibodies in some commercial breeder hen flocks and verify if antibody titers were high enough to confer protection to chicks, as well as to determine in which phase of the life breeders were infected.

MATERIALS AND METHODS

Serum samples

Serum samples were collected from April 2000 to February 2001 from broiler hens in 64 non-vaccinated breeder flocks belonging to major farms in the southern Brazil (States of Rio Grande do Sul, Santa Catarina, and Paraná). Sera were kept at -20°C until tested. Additionally, 270 serum samples from 12 vaccinated breeder flocks were collected.

The minimum number of flocks (60 for non-vaccinated hens) needed for statistical analysis was determined using the Win Episcope 2.0 program (Blas et al. 1998), assuming that the total breeder hen population of Rio Grande do Sul, Santa Catarina, and Paraná was 15,205,000 and the estimated prevalence of CAV-positive sera 90% (99% confidence interval, 10% acceptable error). Flocks were considered CAV-positive when there was a minimum of one hen positive for antibodies to CAV. In practice, 64 unvaccinated flocks were actually analyzed, four more than the minimum number required statistically. The minimum number of serum samples needed to be taken from each flock for a valid statistical analysis (19) was also determined with the Win Episcope 2.0 program (Blas et al. 1998), assuming that the average number of breeder hens per flock was 5,000 and the estimated prevalence (Brentano et al. 2000) of CAV-positive sera of 92% (90% confidence interval and 10% acceptable error). In practice, depending on the age of the birds 22 or 23 hens were actually analyzed (three or four sera above the minimum number statistically required). Apparent prevalence was expressed as the percentage of positive sera that was transformed to true prevalence using the sensitivity and specificity values of the ELISA as calculated using the Win Episcope 2.0 program (Blas et al. 1998). A total of 1,440 sera were examined from unvaccinated flocks and 270 sera from vaccinated flocks.

To verify in which phase of life breeder hens were infected with CAV, the unvaccinated flocks were collected at four distinct ages for each company, one flock being at the rearing stage and one each at the first, second and third breeding periods (Table 1). Vaccinated flocks were also analyzed to compare the result with those of the unvaccinated flocks. A chi-square analysis was used to detect significant differences in the prevalence of seropositive birds.

Based on data provided by KPL, breeder hens with anti-CAV antibody titers above 5,000 were considered as capable to confer

protection to their progeny from CAV-induced clinical disease. Hens with no detectable antibodies or with anti-CAV antibody titers below 5,000 were considered to confer either partial protection or no protection at all to their chicks from clinical disease during the susceptible period of the chicks' life.

Enzyme-linked immunosorbent assay (ELISA)

Sera were analyzed by indirect ELISA using the Chicken Anemia Virus Antibody Test Kit (Kirkegaard and Perry Laboratories (KPL), optical densities of ELISA products being determined in an ELISA Bio-TEK ELX 800 reader and processed and statistically analyzed using the Profile for Windows program (KPL). The character (positive or negative), titer, geometric mean titer and coefficient of variation per flock were determined. Sensitivity (100%), specificity (96.66%), predictive positive value (99.58%) and predictive negative value (100%) of the kit were calculated with Win Episcope 2.0 program (Blas et al. 1998) using data from KPL that correlates ELISA values with virus neutralization results.

RESULTS

The ELISA results showed that in unvaccinated flocks anti-CAV antibodies were present in from 39% to 100% of hens, with coexisting CAV-positive and CAV-negative hens being found in 55% of flocks. The apparent prevalence of antibodies to CAV in unvaccinated hens was 89.23% and the true prevalence was 88.86%. All the breeders from the vaccinated flocks were CAV-positive.

The prevalence of unvaccinated hens with antibodies to CAV decreased from almost 92% in the second breeding period to about 84% in the third breeding period (Fig. 1). Chi-square analyses showed that there was no significant difference between the rearing period and the first and second breeding periods, although the number of CAV seropositive hens was significantly lower in the third breeding period flocks ($p=0.001$).

In unvaccinated flocks, about 52% of the hens had anti-CAV antibody titers above 5,000 while about 47% of hens had titers below 5,000, the progeny of hens with titers less than 5,000 being susceptible, or partially susceptible, to CAV clinical infection. In vaccinated flocks, 99% of hens had anti-CAV antibody titers above 5,000 although two serum samples (1%) were found in which titers were below 5,000 but above 4,000.



Fig. 1. True prevalence of CAV antibody-positive hens in unvaccinated flocks during the four periods of life.

Table 1. Geometric mean titer (GMT) and mean percentage coefficient of variation (CV%) in the different periods of life of vaccinated and unvaccinated breeder hen flocks

Period of life	Age (weeks)	Vaccinated		Unvaccinated	
		GMT	Mean CV (%)	GMT	Mean CV (%)
Rearing	06-21	17,586	24.84	7,653	63.06
First breeding	22-35	17,467	31.50	7,638	54.61
Second breeding	36-45	15,601	36.38	7,321	57.59
Third breeding	46-55	16,022	29.88	7,185	65.39
Mean		16,669	30.65	7,449	60.16

The geometric mean anti-CAV antibody titer (GMT) for unvaccinated flocks was greater than 5,000 in all periods of life (Table 1), the GMT varying from 23,243 in a flock that was in the rearing period to 1,483 in a flock that was in the third breeding period (data not shown). In vaccinated flocks, the GMT was more than twice that seen in unvaccinated flocks (Table 1). The mean coefficients of variation (CV) for unvaccinated flocks were about twice as large as for vaccinated flocks (Table 1), while individual CV values for unvaccinated flocks were higher than for vaccinated flocks, varying from 44% to 142%. Only 17% of unvaccinated flocks presented CV values between 31% to 42%, similar to those found in vaccinated flocks, where CV values for individual flocks varied from 22% to 44% (data not shown).

DISCUSSION

The aim of the research presented in this paper was to verify the degree of protection against CAV that the progeny of breeder hens receive during the age at which they are susceptible to the clinical disease. The serum titers of anti-CAV antibodies in flocks and individual hens was determined in order to ascertain the situation in the southern Brazilian states of Rio Grande do Sul, Santa Catarina and Paraná. These determinations were based on epidemiological procedures and performed using computer programs specific designed for this type of analysis, producing data that can be considered representative of the actual field situation in the major Brazilian poultry-producing region.

Our work shows that all the breeder hen flocks analyzed contained birds with antibodies to CAV, the true antibody prevalence being about 89% for individual hens from unvaccinated flocks, findings which confirm that CAV infection is widespread in Brazilian commercial broiler flocks.

Other anti-CAV antibody surveys have been carried out on breeder hen flocks from various countries, for example in flocks from 12 USA states antibodies against CAV were detected in 23 out of 29 flocks (birds 10 to 78 weeks of age) tested by indirect immuno-fluorescent assay (IIFA), the apparent prevalence of anti-CAV antibody positive hens being about 79% (Lucio et al. 1990). Goodwin et al. (1990) analyzed the prevalence of anti-CAV antibodies using IIFA from 52 flocks and 861 heavy breeder hens from three USA states, and found that 98% of the flocks and 62% of the birds were positive for antibodies against CAV, with the percentage of CAV positive hens varying from zero to 100% within flocks. In contrast with the findings reported by us in this paper, Goodwin et al. (1990) found a significant minor percentage of anti-CAV antibody positive hens with less than 19 weeks of

age. In agreement with our findings, Drén et al. (1996) reported that in Hungary 100% of the unvaccinated broiler breeder flocks studied using IIFA were positive for antibodies against CAV, the percentage of positive birds in the flocks being 40% to 93%, with a mean of 73%. In China, Zhou et al. (1996) found that 82% of 28 flocks and 42% of the 185 sera subjected to IIFA were CAV positive in broiler and layer breeder hens, layer hens and broilers, while in Japan, Farkas et al. (1998) found an apparent CAV prevalence of 60% in individual birds and 69% in 13 flocks using a virus neutralization test. One source of differences between our work and some of the studies cited above may be due to the fact that IIFA does not detect low titers of antibody (Chettle et al. 1991).

In a serological survey using the Idexx ELISA kit and 2,355 serum samples from 127 unvaccinated breeder hen flocks from nine Brazilian states, Brentano et al. (2000) found anti-CAV antibodies in 92% of the birds, with 8% of hens being negative for anti-CAV antibodies and thus being at risk of infection. Although the sampling method and the size of the region analyzed were different, these results are still close to our findings. The occurrence of serum negative breeder hens at all periods of their life is a cause of concern because they are susceptible to infection and can vertically transmit CAV virus to their progeny, anti-CAV antibody negative new-born chicks being susceptible to clinical chicken anemia.

Brentano et al. (2000) and Herdt et al. (2001) noted an increase in the number of anti-CAV antibody positive hens with increasing age of the birds, but our results do not confirm this tendency. In our work, we found that flocks in the rearing period (06 to 21 weeks) had a prevalence of antibody positive chickens not significantly different from the first and second breeding periods, what indicates they were infected before the first period analyzed. We also found breeder hens in unvaccinated flocks that did not present antibodies during all periods of their life, although in the third (last) breeding period there was a significantly higher percentage of birds (16%) that were negative for anti-CAV antibodies. It may be that some of the anti-CAV antibody positive birds became anti-CAV antibody negative during the last breeding period due to the long elapsed time since the last antigenic stimulation (Hoop 1992).

Otaki et al. (1992) studied the protection that titers of maternal anti-CAV antibodies offered to progeny, and found that virus neutralization antibody titers higher than 40 could protect progeny until the second week after hatching. Although the virus neutralization technique is very sensitive as regards detection of antibodies, its use in large-scale antibody surveys is inappropriate due to the high cost of the test and the three weeks delay in obtaining results. Based on the correlation between virus neutralization and ELISA, we established that in breeder hens ELISA titers higher than 5,000 could completely protect progeny in the clinical disease critical period of life.

We also found that although the geometric mean titer (GMT) of unvaccinated breeder hens was in general above 7,000 at any period of their life, 47.67% of these hens presented antibody titers lower than 5,000. In general, vaccinated breeders had a GMT in excess of 15,000 during all periods of their life and 99% of hens had anti-CAV antibody titers above 5,000. Vaccinated flocks also presented a lower coefficient of variation (CV) in

regard to anti-CAV antibody titers than unvaccinated flocks at all stages of their life, the anti-CAV antibody titer CV values of unvaccinated flocks being twice that of vaccinated flocks. Of the 64 unvaccinated flocks studied by us, we found that only 10 flocks had anti-CAV antibody titer CV values similar to the anti-CAV antibody titer CV values (31% to 40%) found in vaccinated flocks, the other unvaccinated flocks having much higher CV values than vaccinated flocks. However, two of the ten unvaccinated flocks had 4% of anti-CAV antibody negative birds, demonstrating that although the anti-CAV antibody CV values of the unvaccinated flocks could be considered adequate, there was some potential to transmit the virus vertically. These findings confirm that breeder hens subject to natural CAV infection show great variation in anti-CAV antibody titers.

According to Brentano et al. (2000), in unvaccinated flocks consisting of breeder hens aged between six and 18 weeks about 42% of hens were anti-CAV antibody negative, 7% of hens were anti-CAV antibody positive but with low antibody titers and only 50% of hens were both anti-CAV antibody positive and had anti-CAV antibody titers considered as completely protective to their progeny. In the mating period, only 3.5% of breeder hens had very low anti-CAV antibody titers or were anti-CAV antibody negative. Engström (1999) found that 18 out of 94 unvaccinated Swedish flocks did not have anti-CAV antibodies before 18 to 20 weeks when the breeder hens were transferred to breeding pens, and also that in the breeding period there were (with one exception) antibodies to CAV in all flocks. In our work, the differences found might be due to differences in the sampling and the testing methods or to the criteria adopted to classify the CAV antibody status.

The present work aimed to indirectly determine the degree of protection against chicken anemia that the progeny of heavy breeder hens have under the rearing conditions present in Brazilian commercial aviaries. Our conclusion is that although all flocks were infected by the onset of reproduction some of the progeny are still either susceptible or partially susceptible to the clinical disease. The work presented in this paper has implications not only for the situation in Brazil but also supports the findings of several other research groups in different countries. Vaccination against CAV could be an efficient route for eliminating susceptible birds, but new experiments need to be carried out in which the costs and economic benefits are taken into account to define if vaccination really is desirable. We are currently undertaking studies to address this question.

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