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The prevalence of antibodies against chicken anemia virus in unvaccinated broilers and broiler breeders in Croatia

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ABSTRACT

The prevalence of antibodies against infectious chicken anemia virus (CAV) in unvaccinated broiler and broiler breeder flocks in Croatia was investigated in this study. Five broiler breeder flocks from four farms and six broiler flocks from four farms were serologically tested for the presence of antibodies against CAV. A total of 147 blood sera were collected and tested using enzyme-linked immunoabsorbant assay (ELISA). The ELISA results showed the presence of antibodies against CAV in 94.7% broiler breeder sera, while 26.6% broilers were found positive. The obtained results indicate a high prevalence of antibodies against CAV in broiler breeder flocks and naturally occurring horizontal CAV infection in four out of six broiler flocks without clinical symptoms of disease. This study indicates that there is no need for vaccination against CAV as long as broiler flocks are protected against infectious bursal disease and Marek's disease that provides normal development of immunocompetency and age resistance to CAV infection.

Key words: chicken infectious anemia, broiler breeders, broilers, antibodies, vaccination

Introduction

Chicken infectious anemia virus (CAV) is a small, ichosaedral, non-enveloped virus belonging to the family *Circoviridae,* and has been recently classified as the sole member of the genus *Gyrovirus* (PRINGLE, 1999). CAV is the causative agent of chicken anemia, which mostly affects chicks of 2 to 4 weeks, although all categories of chicken are prone to the infection. The virus spreads vertically from parents to progeny and horizontally by contact exposure to infected chickens (CANAL et al., 2004). The clinical

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disease is characterized by aplastic anemia and generalized atrophy of hematopoietic and lymphoid organs and concomitant immunosuppression (TODD, 2000). High resistance to disinfection and heath makes CAV ubiquitous and retainable in poultry premises (YUASA et al., 1979).

The immunosuppression is responsible for decreased vaccine response (BOX et al., 1988; DE BOER et al., 1994; VON BÜLOW and SCHAT, 1997) and decreased resistance to viral and bacterial diseases in the breeding period (McILROY et al., 1992). Chicken infectious anemia appears mostly in subclinical form (McCONNELL et al., 1993) and is complicated by viral, bacterial, fungal and parasitic diseases (OTAKI et al., 1989). Economic losses arise from reduced weight gain and the increased mortality of chicks by 10 to 20%, or even up to 60% in some outbreaks (VON BÜLOW, 1991).

The synergistic effect between infectious bursal disease virus (IBDV) and CAV was first reported by YUASA et al. (1980). It was determined that chicks infected with IBDV became CAV positive after contact exposure and the emergence of antibodies against CAV was prolonged for several weeks. Early investigations of interaction between CAV and Marek's disease (MD) showed higher morbidity and mortality of chicks, increased pathogenicity of chicken anemia virus and decreased humoral response after vaccination against MD (VON BÜLOW et al., 1983; YUASA and IMAI, 1986; OTAKI et al., 1987).

This serological study describes the prevalence of antibodies against CAV as a result of natural infection in broiler breeders and broiler flocks in Croatia.

Materials and methods

Flocks. The study was performed on 5 flocks from 4 broiler breeder (Hybro and Ross) farms (A, B, C, D) and 6 flocks from 3 farms of their progeny (E, F, G, H). Broilers were 32 to 45 days old, and hens were 18, 34, 51 and 52 weeks old. Broiler breeder flocks were vaccinated against MD, Newcastle disease (ND), infectious bronchitis (IB), infectious bursal disease (IBD) and egg drop syndrome (EDS), while broilers were vaccinated against ND, MD and IBD according to vaccination programs. Neither of them was vaccinated against chicken anemia. The birds from all the flocks were apparently healthy, without any clinical sign of disease. Broilers were raised in conditions of intensive production with food and water provided *ad libitum*, while broiler breeder flocks were reared according to the corresponding intensive breeding program.

Serology. Serum samples were collected from 147 chickens (57 hens and 90 broilers). Blood was collected from 9 to 18 birds randomly selected from each house on every farm. Serum was stored at -30 °C until it was tested.

The presence of CAV antibodies was analyzed by enzyme-linked immunoabsorbant assay (ELISA) using the Flocksreen ™ CIA Antibody ELISA Kit (IDEXX, Portland, Maine, USA). Sera were diluted 1:500, and obtained results were computerized by the

program of the same manufacturer (FlockCheck Manager Software). The presence or absence of CAV antibodies was determined by the Sample to Negative (S/N) ratios for each sample. Samples with S/N values of equal to or greater than 0.15 are considered negative, and samples with a S/N ratio equal or greater than 0.2 are considered positive. The presence of CAV antibodies indicates exposure to chicken anemia virus.

Statistics. Data were statistically analyzed by the software program (StatSoft, Inc., 2008, version 8.0). The titer values obtained are presented as the arithmetical mean (Anm), standard deviation (SD) and coefficient of variation (CV) for each tested flock.

Results

Broiler breeder flocks. The ELISA results showed the presence of specific CAV antibodies in all the broiler breeder flocks tested. Out of 57 samples from a total of 5 flocks, 54 (94.7%) were found positive while only 3 (5.2%) were observed to be negative. At the flock level, 3 flocks (60%) had 100% positive results, while 2 other flocks from the same farm were 83.3% and 90.9% positive for specific CAV antibodies.

The ELISA results for each broiler breeder flock are shown in table (Table 1).

Table 1. Specific antibodies for CAV in unvaccinated broiler breeder flocks in Croatia

Farm	Age/ weeks	No. Positive/ total tested serum samples	Positive %	Antibody titer mean	Antibody titer (SD)	Antibody titer (CV)
A	18	10/12	83.3	0.276	0.064	23.1
A	34	10/11	90.9	0.955	0.893	93.5
B	18	11/11	100	1.136	0.169	14.8
C	51	11/11	100	1.06	0.224	21.1
D	52	12/12	100	0.33	0.053	16.0

Broilers. A total of 90 blood sera from commercial broilers were serologically tested by ELISA. Positive results were observed in 24 (26.6%) samples, while 66 (73.3%) were negative for the presence of specific CAV antibodies. Out of 6 broiler flocks, 4 (66.6%) were found positive and 2 flocks (33.3%) had 100% negative results.

The ELISA results for each tested broiler flocks are shown in table (Table 2).

Table 2. Specific antibodies for CAV in unvaccinated broiler flocks in Croatia

Farm	Age/ days	No. Positive/ total tested serum samples	Positive %	Antibody titer mean	Antibody titer (SD)	Antibody titer (CV)
E	32	5/18	27.7	0.319	0.249	78.0
F	42	9/18	50	0.313	0.125	39.9
G	42	5/9	55.5	0.476	0.401	84.2
G	43	0/9	0	0.18	0.05	27.7
G	45	5/18	27.7	0.24	0.093	38.7
H	35	0/18	0	0.17	0.045	26.4

Discussion

This serological study demonstrates natural CAV infection in broiler breeder and broiler flocks. Results obtained by ELISA reading showed 94.7% broiler breeder sera positive to antibodies against CAV indicating the widespread occurrence of CAV infection in unvaccinated breeder flocks in Croatia. The tested broiler flock samples were 26.6% positive, suggesting that passive immunity had been developed congruently to maternal antibody status.

The control of CAV disease is based on maternal immunity, resulting either from vaccination of breeder flocks a few weeks before sexual maturity, or natural exposure (VON BÜLOW, 1991; SCHAT and VAN SANTEN, 2008). The clinical disease takes place when chicks are infected during the first two weeks of life but this may be avoided if the breeder hens transfer enough antibodies to their progeny (CANAL et al., 2004). Maternal antibodies have been shown to be completely protective against CAV-induced disease by 2-3 weeks of age (OTAKI et al., 1992; VON BÜLOW and SCHAT, 1997) when they disappear and chicks are able to develop age resistance to CAV disease.

Naturally occurring horizontal infection in the broiler breeder flocks detected in our study caused heterogeneous production of specific CAV antibodies that were transmitted to their progeny in analogous proportions. In the case of the broiler flocks tested, exposure to CAV is most likely to have occurred from the environment. Our assumption is based on the detection of specific CAV antibodies in tested blood sera (26.6%) at the age when maternal antibodies are normally not present in the blood sera of chicks. That confirms naturally occurring horizontal CAV infection among broilers from 4 out of 6 (Table 2) unvaccinated broiler flocks at the age of 32 to 45 days, while it is considered that most flocks became infected with CAV between 8 and 12 weeks (McNULTY et al., 1988). These infections arise through horizontal transmission of the virus and do not result in CAV disease (ADAIR, 2000).

Breeder hens infected during the laying period do not demonstrate clinical signs or changes in the number of eggs laid, fertility or embryo viability (VON BÜLOW and SCHAT, 1997), also shown in this study, where all broiler breeder flocks were CAV positive (Table 1) but without any manifestations related to chicken anemia. CARDONA et al. (2000) reported that CAV infection can be activated as a consequence of hormonal changes, which may serve as or activate transcription factors leading to the onset of viral replication and subsequent seroconversion. In the same study it was also remarked that seroconversion occurred at or near sexual maturity. In accordance with this, our results showed increased titer levels in the pullet's sera at the point of laying, but also at the peak of egg production and at the end of production cycle. The real cause of concern in breeder production is the occurrence of serum negative breeder hens (5.2% in our study) because of their susceptibility to CAV infection and the possibility of vertical transmission of CAV to their progeny (CANAL et al., 2004).

Age resistance to CAV develops by 4 weeks of age in immunocompetent chicks (YUASA et al., 1979; YUASA and IMAI, 1986), while immunosuppression caused by dual infection with CAV and IBDV or MDV harms maternal immunity and the emergence of immunocompetency in chicks (VON BÜLOW et al., 1986).

Chicken anemia virus and IBDV are ubiquitous in commercial chicken operations (TORO et al., 2009) because of their resistance to chemical and physical agents (ETERRADOSSI and SAIF, 2008; SCHAT and VAN SANTEN, 2008). Beside compromised age resistance to CAV, IBDV infection increases the persistence of CAV in lymphocytes and/or monocytes and prolongs viral shedding by chickens infected at 6 weeks of age (IMAI et al., 1999).

The broilers used in our study were indirectly protected from immunossuppresion that is expected to appear after CAV infection, by vaccination against IBD and MD. The uninterrupted development of the immune system and immunocompetency enabled the production of specific antibodies against CAV and resistance to CAV infection that obviously occurred after the period of effective maternal immunity.

Conclusion

From the present study we can conclude the widespread prevalence of CAV antibodies in unvaccinated commercial broiler breeder farms and the occurrence of natural horizontal CAV infection in some tested broiler flocks. The findings of positive titers of antibodies against CAV in broiler sera at the age of 35 to 45 days led us to the conclusion that they were in contact with the virus in the period when maternal antibodies waned from the blood sera of the chicks. This indicates attained age resistance to CAV and no interference from immunosuppressive agents which would harm this development. Furthermore, we suggest that there is no need for vaccination against CAV in small poultry farming

operations, because vaccination against IBD and MD indirectly ensures the natural appearance of age resistance to CAV infection.

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SAŽETAK

Istražena je pojavnost protutijela za virus zarazne anemije pilića (VZAP) u necijepljenih teških hibrida kokoši i tovnih pilića u Hrvatskoj. Serološki je pretraženo pet jata s četiri farme rasplodnih kokoši i šest jata tovnih pilića s ukupno četiri farme. Prikupljeno je 147 uzoraka krvnog seruma i imunoenzimnom probom (ELISA) pretraženo na prisutnost specifičnih protutijela za virus zarazne anemije pilića. Protutijela za VZAP dokazana su u 94,7% uzoraka seruma rasplodnih jata i 26,6% tovnih pilića. Dobiveni rezultati dokazuju veliku pojavnost protutijela za VZAP u rasplodnih jata i prirodnu horizontalnu infekciju tovnih pilića u četiri od ukupno šest jata bez kliničkih simptoma bolesti. Provedeno istraživanje ukazuje na nepotrebnost cijepljenja protiv VZAP ako se jata zaštite od zarazne bolesti burze i Marekove bolesti, što omogućuje normalan razvoj imunosnog sustava i posljedično, dobne otpornosti na infekciju virusom zarazne anemije pilića.

Ključne riječi: zarazna anemija pilića, teški hibridi kokoši, tovni pilići, protutijela, cijepljenje