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CLINICAL AND SEROLOGICAL EXAMINATION OF A PARENTAL FLOCK LATENTLY INFECTED WITH CHICKEN ANEMIA VIRUS

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At the beginning of etiopathogenic research on chicken anaemia virus (CAV) it was thought that groups of chickens at risk for CAV infection were those coming from a parental flock infected before laying. Therefore it is important to know the level and persistence of transfered maternal antibodies (MaAt) and to measure specific antibody development during rearing.

The goal of this research was to assess the necessity for prophylactic measures by determining the humoral immune response to CAV and any clinical changes in breeder chickens.

Chickens from the parental Hybro flock were examined from the first day until the end of production. Maternal antibodies for CAV, which were present initially, were not detected at 4 weeks old. At 6 weeks old specific antibodies for CAV were found in 45% of the serum samples. These antibodies increased until the 18th week when the experiment was terminated. The state of health of the parental flock in the period when MaAt antibodies could not be detected and until specific antibodies appeared did not differ significantly. The results of these investigations are the first evidence of CAV infection in Yugoslavia, based on serological examination.

Key words: chicken anaemia virus, maternal antibodies, parental flock

INTRODUCTION

The causative agent of chicken anaemia, Gifu virus – 1 strain, was isolated for the first time in 1979 in Japan by Yuasa *et al.* (1979).

Contagious chicken anaemia is spread in all countries, especially in those with intensive production of heavy and light poultry hybrids. Infection was diagnosed on the basis of the isolated virus (Yuasa *et al*., 1980, 1985; Bülow, 1988; Lucio *et al*., 1989; Farkas *et al*., 1992). Serological examination showed a high content of antibodies towards chicken anemia virus (CAV) in blood sera of older poultry (Jorgensen, 1990; Kapetanov *et al*., 1999), as well as in many broiler chickens (Yuasa *et al.*, 1980, 1985; Lucio *et al.*, 1990; Dren *et al*., 1996). Antibodies towards CAV were also found in SPF chickens (Yuasa *et al.*, 1985; Bülow, 1988; Nicholas *et al.*, 1989; Godwin *et al.*, 1990; Kapetanov *et al.*, 2002). In the majority of flocks

most small chickens have maternal antibodies (MaAt) towards CAV, which initially prevent infection (Yuasa *et al*., 1980; Bülow *et al.*, 1986). These antibodies disappear from the blood by 3 weeks of age. Many chickens, become seropositive at 8 – 12 weeks old most probably due to horizontal infection, which occurs subclinically (McNulty *et al*., 1988).

Since there has been no complex research on the appearance, prevalence, prophylaxis and immunoprophylaxis in Serbia and Montenegro, we decided to investigate this disease.

MATERIAL AND METHODS

Chicken flock selected for observation and sampling

Blood samples were taken from a flock of 23000 chickens originating from grandparents vaccinated in Holland at 19 weeks old with live attenuated vaccine (CV5). The day-old broiler breeder chickens imported from Holland were held in three houses. In two houses 10000 female chickens were located and in the third house there were 3000 male chickens. The technical and technological characteristics of production on the selected farm were modern. Proper therapeutic and prophylactic measures were applied during production of the broiler breeder flock. Preventive measures conformed with a standard program which included checking the immunological status of the imported day-old chickens, vaccination, prophylactic medication and proper sanitation. Any infections and parasitic diseases were diagnosed at the Scientific Veterinary Institute in Novi Sad.

Blood sampling

Blood samples were taken from the wing vein of twenty day-old broiler breeder chickens in order to determine the immune status of the flock. This included antibody titers against Newcastle disease virus, infectious bursal disease virus, infectious bronchitis, Mycoplasma and Salmonella galinarum pulorum, as well as maternally derived CAV antibodies. Blood was also sampled from twenty chickens at 4, 6, 8 and 18 weeks of age in order to determine the level and persistence of specific antibodies for CAV.

ELISA test

The groups of blood samples were delivered to the virology laboratory at the Scientific Veterinary Institute in Novi Sad where the serum was separated. Antibodies for CAV were detected by ELISA (IDEXX PM).

The optical density (OD) of the negative control at 650 nm should be greater or equal to 0.60, while the OD of the positive control was expected to be equal to or below 0.50. SN was calculated as the quotient of the OD of the negative control and the OD of the sample. Samples with SN higher than 0.60 were considered negative. Samples with SN lower or equal to 0.60 were considered positive.

Data from the serological examination for CAV were evaluated with the usual statistical methods as follows: mean value ($\bar{X}$), standard deviation (SD), coefficient of variation (CV) and interval of variation (IV).

RESULTS

Maternal antibodies for CAV were found in all twenty sera from day-old broiler breeder chicks. The mean value was 0.192 and IV ranged from 0.071 to 0.460 (Table 1).

Table 1. Examination of serum taken from day-old broiler breeders for the presence of maternal antibodies using an ELISA.

No. of samples	OD [a]	SN [b]	Results
Neg. cont. Pos. cont.	1.051 0.136	– –	– +
1	0.117	0.111	+
2	0.075	0.071	+
3	0.116	0.110	+
4	0.220	0.209	+
5	0.217	0.206	+
6	0.283	0.269	+
7	0.193	0.183	+
8	0.197	0.187	+
9	0.154	0.146	+
10	0.288	0.274	+
11	0.484	0.460	+
12	0.176	0.167	+
13	0.111	0.105	+
14	0.236	0.224	+
15	0.352	0.334	+
16	0.116	0.110	+
17	0.171	0.162	+
18	0.207	0.196	+
19	0.097	0.092	+
20	0.243	0.231	+
$\bar{X}$ 0.192	Sd 0.093	CV 0.465	IV 0.071 -0.460

a OD mean value of the optical density from sera tested in duplicate
b SN quotient of the mean value of sample and negative control

At 4 weeks old maternal antibodies were not found, so the results were negative for all examined sera (Table 2).

Table 2. Examination of serum taken from 4 week old broiler breeders for the presence of maternal antibodies for CAV using an ELISA.

No. of samples	OD	SN	Results
Neg. cont. Pos. cont.	1.118 0.134	– –	– +
1	1.098	0.982	
2	1.137	1.016	–
3	0.916	0.819	
4	0.955	0.854	–
5	1.158	1.035	
6	1.180	1.055	–
7	1.174	1.050	
8	0.878	0.785	–
9	0.961	0.859	
10	1.086	0.971	–
11	0.812	0.726	
12	1.027	0.918	–
13	0.944	0.844	
14	0.886	0.792	–
15	1.057	0.945	–
16	0.948	0.847	–
17	1.090	0.974	–
18	0.819	0.732	–
19	1.140	1.019	–
20	1.083	0.968	–
$\bar{X}$ 0.909	Sd 0.106	CV 0.53	IV 0.726 -1.055

Specific antibodies for CAV were detected in the 6th week of age in nine (45%) serum samples, while 11 (55%) were negative. Positive serological results for CAV antibodies suggest field infection in this broiler breeder flock.

The mean value for specific antibodies was 0.6 and IV ranged from 0.089 to 0.94 (Table 3).

Table 3. Examination of serum taken from 6-week-old broiler breeders for the presence of CAV antibodies.

No. of samples	OD	SN	Results
Neg. cont. Pos. cont.	1.378 0.124	– –	– +
1	1.00	0.727	–
2	0.240	0.174	+
3	0.123	0.089	+
4	1.207	0.875	–
5	0.184	0.133	+
6	0.377	0.273	+
7	0.223	0.161	+
8	0.333	0.241	+
9	0.372	0.269	+
10	1.224	0.888	–
11	1.242	0.901	–
12	0.722	0.523	+
13	0.424	0.307	+
14	1.110	0.805	–
15	1.227	0.890	–
16	1.279	0.928	–
17	1.296	0.940	–
18	1.218	0.883	–
19	1.230	0.892	–
20	1.260	0.914	–
$\bar{X}$ 0.60	Sd 0.338	CV 1.69	IV 0.089 -0.94

At the age of 8 weeks antibodies for CAV were found in all 20 serum samples (100%). The mean value for post infection antibodies was 0.148 with IV ranging from 0.096 to 0.236 (Table 4). The state of health in this parental flock did not differ from the period of absence of maternal antibodies until post infection antibodies were detected. Clinical and pathological examination of the carcasses yielded no signs of CAV infection. The mean value for maternal antibodies in the day-old chickens originating from a vaccinated flock was lower (29.73%) than the mean value of the post infection antibody level of the flock at 8 weeks old.

Table 4. Examination of serum taken from 8-week-old broiler breeders for the presence of CAV antibodies.

No. of samples	OD	SN	Results
Neg. cont. Pos. cont.	1.110 0.082	– –	– +
1	0.133	0.119	+
2	0.119	0.107	+
3	0.170	0.153	+
4	0.262	0.236	+
5	0.195	0.175	+
6	0.221	0.199	+
7	0.219	0.197	+
8	0.107	0.096	+
9	0.120	0.108	+
10	0.138	0.124	+
11	0.205	0.184	+
12	0.157	0.141	+
13	0.243	0.218	+
14	0.226	0.203	+
15	0.115	0.103	+
16	0.119	0.107	+
17	0.149	0.134	+
18	0.153	0.137	+
19	0.111	0.100	+
20	0.133	0.119	+
$\bar{X}$ 0.148	Sd 0.0443	CV 0.222	IV 0.096 -0.243

The mean value for post infection antibodies for CAV in the selected flock (0.061) was significantly higher in the 18th week compared to all the other examination periods. The IV ranged from 0.038 to 0.124 (Table 5).

Table 5. Examination of serum taken from 18 week-old-broiler breeders for the presence of CAV antibodies.

No of samples	OD	SN	Results
Neg. cont. Pos. cont.	1.265 0.061	– –	– +
1	0.069	0.054	+
2	0.158	0.124	+
3	0.056	0.044	+
4	0.049	0.038	+
5	0.064	0.050	+
6	0.068	0.053	+
7	0.082	0.064	+
8	0.073	0.058	+
9	0.054	0.042	+
10	0.099	0.078	+
11	0.059	0.047	+
12	0.150	0.118	+
13	0.064	0.050	+
14	0.127	0.100	+
15	0.089	0.070	+
16	0.119	0.094	+
17	0.064	0.050	+
18	0.062	0.049	+
19	0.115	0.090	+
20	0.069	0.054	+
$\bar{X}$ 0.061	Sd 0.0257	CV 0.129	IV 0.038 -0.124

The mean values for specific antibodies for CAV in the parental flock increased in time during rearing (Figure 1).

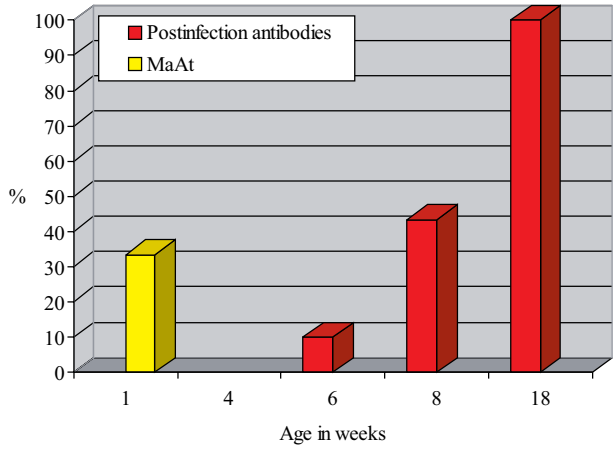

Figure 1. The mean value of maternal and post infectious antibodies from parental flock during rearing in %.

DISCUSSION

The high level of maternal antibodies found in our breader flock at one day old was a result of vaccination of the grandparent flock and subsequent transfer of maternal antibodies to the progeny (Steinhuisenm *et al.* 1994). However, at 4 weeks old maternal antibodies for CAV could not be detected, as found earlier by Pages *et al.* (1997). Their chickens also originated from a vaccinated grandparent flock and had maternal antibodies until 3 or 4 weeks of age. Yuasa (1994) pointed out that a lack of antibodies creates a dangerous situation for chickens, particularly in the third and fourth week, when the level of maternal antibodies tends to drop and they become susceptible to CAV. In our study antibodies for CAV were found in the 6th week in 45% of the tested chickens while 55% of the samples were negative. These results provide evidence of spread of infection by the horizontal route (Kapetanov *et al*., 1999).

At 8 weeks of age 100% of the serum samples were positive, indicating continued infection with CAV. This confirms the results of McNulty *et al*. (1988), who provided evidence that MaAt disappears from the circulation of chickens in the 3rd week and that from the 8th to the 12th week CAV antibodies appear as a result of horizontal infection passed in a subclinical form. The mean value of CAV antibodies for our parental flock was the highest in the 18th week. However, during monitoring of the state of health of chickens on the selected farm no deviation was noticed.

Pages *et al.*, (1997) suggested vaccination of chickens in the 20th week despite the high level of protective antibodies, because they are continuously exposed to the virus. The highest antibody responce was found 10 weeks after vaccination, namely in the 30th week. We decided not to vaccinate our chickens be-

cause the antibody level was very high at 18 weeks old, 12 weeks after the first specific antibodies were discovered.

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KLINIČKA I SEROLOŠKA ISPITIVANJA LATENTNO INFICIRANOG RODITELJSKOG JATA SA VIRUSOM ZARAZNE ANEMIJE PILIĆA

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SADRŽAJ

U početku istraživanja etiopatogeneze Virusne zarazne anemije pilića (VZAP) smatralo se da su rizične grupe pilića samo one koje potiču od roditeljskih jata inficiranih u periodu pronošenja jaja. Smatra se da je poznavanje nivoa i perzistiranja transfera maternalnih antitela (MaAt) i sticanje specifičnih antitela u toku odgoja veoma bitno i to je bila osnovna ideja ovih istraživanja. Takođe, dobijeni rezultati ispitivanja predstavljaju i prve podatke serološkom načinu dokazivanja infekcije ovim virusom u Jugoslaviji.

Cilj ovih istraživanja je bio da se dođe do saznanja kako da se sprovode mere opšte i specifične profilakse u uslovima savremenog živinarstva.

Zadatak ovog istraživanja je bio da se nakon infekcije prati klinička slika i humoralni imuni odgovor.

Za istraživanja su korišćeni jednodnevni pilići roditeljskog jata "Hybro" koji su praćeni sve do kraja odgoja.

Kod svih jednodnevnih pilića roditeljskog jata dokazano je prisustvo MaAt do uzrasta od 4 nedelje. U 6. nedelji uzrasta dokazano je u 45% uzoraka krvnih seruma prisustvo specifičnih antitela za virus ZAP, što ukazuje da je došlo do infekcije terenskim virusom. Specifična antitela su dokazana do kraja odgoja, odnosno do 18 nedelje uzrasta.

Klinička slika roditeljskog jata u periodu odsutnosti MaAt pa do pojave specifičnih antitela nije se bitno razlikovala u odnosu na prethodni period.