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SEROLOGICAL SURVEY ON THE PREVALENCE OF CHICKEN ANEMIA VIRUS IN BACKYARD POULTRY FLOCKS IN BULGARIA

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Summary

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A total of 529 serum samples from backyard chickens originating from 22 villages in Western Bulgaria were tested for presence of chicken anemia virus (CAV) antibodies by means of commercial enzyme-linked immunosorbent assay (ELISA) kit. Three hundred ninety one samples were submitted to the laboratory in a screening programme for surveillance of avian influenza (AI) and originated from birds with unknown production type, age and sex. Another set of 138 samples was collected for the purposes of the study as a representative part of the home-reared indigenous chickens in three villages (82 hens and 5 roosters above one-year old, and 51 chickens aged 4–18 weeks). CAV reagents were detected in all villages surveyed with seroprevalence rates ranging from 84.4% to 100%. Significant association between the seropositivity and age of birds tested was found, with more CAV reagents detected among adult hens and roosters than among chickens aged up to 18 weeks. The results from the study indicate that CAV is widespread amongst backyard poultry population in Bulgaria. However, additional investigations are necessary to evaluate the epidemiological impact of infection among home-reared chickens and productivity losses due to CAV-related immunosuppression.

Key words: antibodies, backyard chickens, chicken anemia virus, immunosuppression

INTRODUCTION

Chicken infectious anemia virus (CAV), a member of *Gyrovirus* genus of the family *Circoviridae* (Prigle, 1999), is a small (22 nm), non-enveloped virus with icosahedral symmetry and circular DNA genome (Noteborn *et al*., 1991). In susceptible 2–4 week-old chicken having no maternal antibodies, CAV causes severe aplastic anemia (haematocrit values <0.27 L/L), often associated with haemorrhagic syndrome and atrophy of the thymus and bone marrow, resulting in retarded growth and increased mortality (Schat, 2003). In older birds the infection is mainly subclinical. Besides of the disease it causes itself, CAV infection has been associated with severe immunosuppression in affected young birds, due to the ability of the virus specifically to target and kill via apoptosis both precursor T lymphocytes in the thymus and erythroid and myeloid progenitor cells in the bone

marrow (Adair, 2000; Todd, 2000). As a result, affected birds show enhanced susceptibility to other infectious disease agents and reduced protective response to vaccinations (Otaaki *et al.,* 1988; Cloud *et al*., 1992, Ragland *et al.,* 1998*).*

CAV is ubiquitous and there are serological evidences indicating CAV presence in most commercial poultry farms, with a high seroprevalence within the chicken flocks (Schat, 2003). At the same time, the epidemiological status of CAV infection in backyard poultry remains largely unknown. Exceptions are small-scale serological surveys, detecting CAV-specific antibodies in Nigerian indigenous chickens (Emikpe *et al*., 2005; Oluwayelu & Todd, 2008), Chinese live-bird markets (Ducatez *et al*., 2008) and in fancy chicken breeds (De Wit *et al*., 2004). In addition to serological testing of 115 birds, Oluwayelu & Todd (2008) also demonstrated a presence of CAV DNA in sera from Nigerian indigenous chickens by polymerase chain reaction, and showed that they contained a mixed population of CAV strains with different restriction endonuclease patterns.

Extensive farming of village chickens, even without significant economical importance is a common practice in a lot of countries. Due to the low levels of biosecurity, however, these birds are commonly exposed to infectious viral, bacterial and fungal organisms through the environment, people and food. Moreover, the close contact between free-range reared chickens with waterfowl and wild bird's populations allows intensive circulation of different avian pathogens, some of which having the potential to overwhelm the host defences and to evolve into highly pathogenic forms; the AI viruses being those of biggest epidemiological and economical importance (Alexander, 2000). This process would be facilitated in immunocompromised birds, in which viruses would replicate more efficiently and shed in large quantities in faeces and respiratory secretions. Thus, the presence of backyard flocks of birds with weakened immune defences may promote the emergence and introduction into the industrially managed poultry farms of new infectious agents or strains with enhanced virulence.

Assuming that CAV seropositivity relates to immunosuppression and that the available data on the prevalence of CAV in backyard flocks of fowl are quite limited, we designed a serological study as a first step for establishing the epidemiology of chicken anemia in domestic poultry in Bulgaria.

MATERIALS AND METHODS

Sera

A total of 529 serum samples were tested for antibodies against CAV. Three hundred ninety one of them were collected from backyard fowl from 19 villages in Western Bulgaria, with one to three birds per randomly selected household, during the winter of 2007 to spring of 2008. They were submitted to the National Reference Laboratory of Avian Influenza and Newcastle Disease (Department of Exotic Disease, National Diagnostic Research, Veterinary Institute, Sofia) in a screening programme for surveillance and control of the AI.

Another subset of 138 blood samples was specially collected for the purpose of this study during the summer of 2007 from birds, randomly selected from 21 households in 3 villages. Eighty-two of the samples were obtained from hens older than one year of age, five were from roosters and the other 51 sera originated

from immature chickens at the age of 4–18 weeks. In this case epizootological data, including the number and age of the birds in the flock, breeding practices and health status were also collected, depending on owner's cooperation. The serum samples were frozen at −20 °C until use.

ELISA

A commercial test kit was used to detect specific antibodies against CAV (IDEXX FlockChek CAV), following the instructions of the manufacturer. A serum dilution of 1:10 was used. Optical density (OD) values were read at 650 nm using a Tecam Sunrise ELISA reader. S/N was the ratio of OD of the sample (S) divided by the OD of the negative control (N). Samples with S/N values higher than 0.60 were considered negative, whereas these with S/N lower or equal to 0.60 were considered positive.

Statistical analysis

For birds (n=138) sampled specially for this study, the mean S/N value obtained from the sera collected from adult hens and rosters was compared to that of sera collected from chickens aged 4-18 weeks using Student's *t* test. *P* values less than 0.05 were considered significant.

RESULTS

Antibodies against CAV were detected in 379 (96.9%) of 391 serum samples, originating from 19 villages from Western Bulgaria, with S/N values ranging from 0.056 to 0.702. The occurrence of CAV antibodies was determined in the sera from all surveyed villages with seroprevalences from 85 to 100% (Table 1).

Table 1. Presence of CAV-specific antibody in sera, collected from backyard birds (n=391)

Serum sample set	Number positive/ number tested	Percentage positive
1	46/46	100.0
2	18/18	100.0
3	17/20	85.0
4	9/10	90.0
5	15/15	100.0
6	12/12	100.0
7	12/12	100.0
8	32/33	96.9
9	26/26	100.0
10	12/12	100.0
11	7/7	100.0
12	9/10	90.0
13	16/17	94.1
14	39/41	95.1
15	35/35	100.0
16	8/9	88.9
17	14/14	100.0
18	19/19	100.0
19	33/35	94.3

The serological investigation of the 138 samples, collected as a representative excerpt of home-reared birds in three villages identified 125 seropositive cases (90.6%) in all 21 households investigated, with seroprevalences of 94.9%, 91% and 84.4%, respectively (Table 2).

All sampled hens and four out of five sampled roosters around or above one year of age had antibodies against CAV, with a mean S/N value (S/Nmn) for this age group 0.126±0.091. In contrast, 12 out of 51 chicks were CAV-seronegative (seropositivity 76.5%). In addition, juvenile chickens showed significantly higher S/Nmn, i. e., lower titres of specific antibodies (S/Nmn=0.347±0.274), as compared to mature birds (S/Nmn=0.126± 0.091) (P<0.001) (Table 3).

Table 2. CAV antibody status of the backyard poultry flocks in three villages examined (n=138). S/N values are presented as mean ± standard deviation

Birds (age)	Number positive/ number tested	Percentage	S/N
Village A			
Chickens (4–18 wks)	12/14	85.7	0.311±0.257
Hens (>1 year)	25/25	100.0	0.139±0.089
Total	37/39	94.9	0.201±0.186
Village B			
Chickens (4–18 wks)	21/26	80.8	0.453±0.309
Roosters	3/4	75.0	0.271±0.280
Hens>1 year	37/37	100.0	0.109±0.055
Total	61/67	91.0	0.202±0.207
Village C			
Chickens (4– 8 wks)	6/11	54.5	0.323±0.263
Roosters	1/1	100.0	0.116
Hens>1 year	20/20	100.0	0.114±0.063
Total	27/32	84.4	0.230±0.245

Table 3. Relationship of CAV antibody levels in tested backyard chickens to their age

Birds (age)	Number positive/ number tested	Percentage	S/N*
Chickens (4–18 wks)	39/51	76.5	0.347±0.274**
Hens and roosters (>1 year)	86/87	98.9	0.126±0.091

* Mean S/N values calculated from all tested sera; ** significantly different from group of adult birds ($P<0.001$).

The clinical observation of the flocks revealed neither visible signs suggestive for CAV, nor other significant disturbances in the performance and the productivity of birds.

DISCUSSION

Serological surveys have shown that CAV infection is widespread in industrial poultry (Schat, 2003). The results from the present study indicate that this is the case also for the backyard poultry flocks. Three hundred ninety one local birds from 19 villages were examined and 96.9% of them were found to have antibodies against CAV. Moreover, antibodies against CAV were found in birds from all the surveyed villages with prevalence rates ranging from 85% to 100%. The small differences between the seropositivity rates observed in Nigeria – 88.9% (Emikpe *et al.,* 2005) and 66.2% (Oluwayelu & Todd, 2008), and Bulgaria may reflect the differences in the environment conditions, husbandry practices and samples size. The high seroprevalence of CAV among the backyard fowl was not surprising, taking into account the high virus resistance in the environment and the multiple ways for transmission. Comingling of birds from different generations in the same flock, a common

practice for the most of the backyard farms and popularity of the live-bird markets, additionally promote intensive virus circulation and facilitate horizontal transmission of CAV. On the other side, vertical transmission, which is an important way of viral spread at the conditions of industrial poultry farming due to the ability of CAV to persist in the ovaries of laying hens even at a presence of circulating antibodies (Hoop, 1992; Brentano *et al.,* 2005), also may play a role in CAV epidemiology in the backyard poultry in the cases, where chickens are hatched by brood hens or in hatchery from eggs, collected from infected parents.

Even though the low stocking density and large spatial distance between the households may at least particularly prevent the spread of virus, our study detected CAV seroreagents in all flocks surveyed. These results are indicative for the high rate of environmental contamination and reflect the possibility for easy virus transmission between farms by environmental factors, people or other birds. However, the duration of virus persistence in separately raised flocks and the possibility for self-cleaning within the flock due to immunity events or physical decontamination remain to be established.

Outbreaks of clinically manifested CAV infection in the backyard chickens have not been reported so far. Presumably, single chickens with low levels of maternal antibodies may develop clinical disease, but it is not recognized as such. Neither detectable clinical manifestations of infectious anemia, nor other associated clinical symptoms, such as gangrenous dermatitis (Engstrőm & Luthman, 1984) were observed in the households investigated. Thus, the high percentage of seropositivity indicates predominantly subclinical infection in sampled birds, since vaccination against CAV is not in practice in village poultry.

Serological surveys in broiler breeder flocks have shown an age dependence of seroprevalence, which increase gradually and reach levels of almost 100 % at or near sexual maturity (McNulty *et al.,* 1988). There was no information available for the sex and age of the birds whose 391 samples were submitted to the laboratory. However, it is a common practice the new backyard flocks to be renewed, or present ones completed with new birds during the spring, and therefore most of the birds at the time of sampling should be at least 8–10 months old. In this respect, the high percentage of CAV antibody positive reagents may reflects the investigation of predominantly older birds. This presumption is confirmed, given the age profile of the birds, sampled especially for this study. In this case the number of CAV-positive reagents was statistically significantly higher in mature birds, moreover, they showed higher titres of CAV-specific antibodies as compared to chickens aged 4–18 weeks (1–4.5 months).

Although seropositivity to CAV does not necessarily relate to overt disease, it indicates that birds have undergone at least subclinical infection with possible consequent immunosuppressive effects such as increased predisposition to other viral or bacterial infections or suboptimal response to vaccination. Thus, considering its widespread occurrence, CAV infection must be taken into account as a risk factor related to the epizootological picture of backyard chicken flocks.

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