TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2017, vol. 2, No 2(3): 17–22

COINFECTION OF CHICKEN ANAEMIA VIRUS, MYCOPLASMA GALLISEPTICUM, AVIAN METAPNEUMOVIRUS AND AVIAN REOVIRUS IN FANCY CHICKEN BREEDS

Mariya Hristova, Reneta Petrova

National Diagnostic Science-and-Research Veterinary Medical Institute, Sofia, Bulgaria
E-mail: mariya.hristova_7@abv.bg

ABSTRACT

In this report, a case of concomitant Chicken anaemia virus (CAV), Mycoplasma gallisepticum (MG), Avian metapneumovirus (aMPV) and Avian reovirus (ARV) infection in fancy chicken breeds is presented. It concerns fancy chickens at the age from 2 weeks to 4 months with repetitive respiratory symptoms such as difficulty breathing, whistling sounds and conjunctivitis. The 5 chickens submitted at the Institute were with depression, rales and dyspnea as 2 of them had anaemia. The necropsy was performed after their humane euthanasia. The 2 older necropsed birds showed fibrinous airsacculitis with gathering caseous exudate and serous tracheitis. The air sacs of the younger chickens were with initial signs of opacity. Atrophy of the thymus was found in one of the examined chickens with anaemia. The 5 serum samples tested by rapid serum agglutination test with MG antigen, were positive for MG. CAV was confirmed in the five fancy chickens by polymerase chain reaction (PCR) and in the both chickens with anaemia histologically. The enzyme-linked immunosorbent assay (ELISA) determined the presence of antibodies to aMPV and ARV in the five tested birds. This study is a case of confirming co-infection of CAV with MG, aMPV and ARV.

Key words: chicken anaemia virus, mycoplasma gallisepticum, avian metapneumovirus, avian reovirus, co-infection, fancy chicken breeds.

Introduction

Raising rare bird breeds is a hobby, practiced in various places in the world. The participation of the fancy bird breeds in exhibitions, while they are getting into contact with birds from other places, is a conducive factor to exchanging various infectious agents. The all-in all-out management system is not applied as fancy birds of different age are mixed, which is precondition for transovarial transmission and persistence of infections. There are taken no other preventive measures such as hygiene improvement and disease monitoring. When fancy chickens are bred, in most cases, no vaccination is performed, which leads to high susceptibility to infections. These are conductive factors to transmitting, persisting and sustaining a number of infectious diseases in them [1, 4]. Therefore from veterinary medical aspect, problems by breeding fancy birds are more different than those that exist in the poultry industry.

CAV is economic important avian pathogen as the infection with it has been described in most countries with a developed chicken industry [10]. Mycoplasma gallisepticum is also an important agent causing chronic respiratory disease in chickens, the infection with which as well as the infection with the Avian reovirus is widespread among the poultry flocks [2, 6, 7]. Avian metapneumovirus causes acute respiratory tract infection and reductions in egg production in various avian species as the infection primary affects the upper respiratory tract of young birds, while also decreases egg production of adult hens [8]. There are data which demonstrate the seroprevalence of these pathogens among fancy poultry [1, 4, 12].

In this report we describe a case of simultaneous confirming CAV, MG, aMPV and ARV in fancy chicken breeds.

Materials and methods

Case presentation. Five fancy chickens at the age from 2 weeks to 4 months originating from fancy chicken-breeding farm, were submitted at the Institute for routine diagnostic examination. According to the owner great percent of the fancy birds in the flock have shown persisting respiratory symptoms such as hard breathing, whistling sounds and conjunctivitis. The birds have been treated with antibiotics Rodotium (Tiamulin), Roxacin (Enrofloxacin) and Doxyvit (Doxycyclin and Ascorbic acid). The chickens submitted to the laboratory were with depression (Fig. 1), rales and dyspnea, while anaemia was found only in two of them (Fig. 1).

Figure 1: Fancy chickens with anaemia and depression

Samples. After blood samples for serological testing were taken, birds were humanly euthanized. Necropsy was performed and tissue samples from thymus and bone marrow were collected for histological and by PCR examinations.

DNA extraction and PCR. CAV DNA was extracted from the thymuses of the five fancy chickens using the Tissue and Cell Genomic DNA Mini Kit (Guangzhou Geneshun Biotech, China) according to the manufacturer's instructions. Amplifications were carried out as described previously [11], but in a total volume of 25 μL (12,5μL mastermix, 2μL of primer S.1.1., 2μL of primer S.1.2., 2μL of target DNA and 6,5μL nuclease-free water), in automatic thermocycler (QB-96, LKB).

The steps and conditions of thermocycling for PCR are presented in Table 1.

Table 1: Cycling parameters of amplification

Steps	Temp.	Time
Initial denaturation	95ºC	2 min
Denaturation	95ºC	1 min
Annaeling	56ºC	2 min
Extension	74ºC	2 min
30 cycles		
Final extension	74ºC	10 min

After the amplification, 7μl of each obtained PCR product was analysed by electrophoresis on a 1.5% agarose gel, and the expected band was visualized by staining with ethidium bromide. A 100-bp DNA ladder served as a size marker.

Serological testing. Sera were tested using commercial ELISAs (Avian Reovirus Antibody Test Kit and Avian Pneumovirus Antibody Test Kit; IDEXX Laboratories). Serological testing was performed according to the manufacturer's recommendations as the optical density (OD) was measured at 450 nm using ELISA Reader LKB 50660-006, Shimagzu. The rapid serum agglutination test for MG was carried out using a MG antigen (Sanofi). A drop of serum was mixed with a drop of antigen suspension and observed for visible agglutination.

For ***histological examination***, thymus and bone marrow samples from the both chickens with anaemia were fixed in 10% neutral phosphate-buffered formalin. They were dehydrated and embedded in paraffin wax, sectioned (4-μm-thick), stained with hematoxylin and eosin (HE) and evaluated under a light microscope.

Results

At necropsy, the most prominent changes were found out in the air sacs as the 2 older birds showed fibrinous airsacculitis with gathering caseous exudate (Fig. 2) and serous tracheitis. The air sacs of the younger chickens were with initial signs of opacity. Thymus atrophy was found in one of the examined chickens with anaemia (Fig. 3).

Figure 2: Fibrinous aersacculitis with gathering caseous exudate (arrow)

Figure 3: Atrophy of the thymus (arrow)

The 5 serum samples tested by the rapid serum agglutination test with MG antigen, were positive for MG (Fig. 4).

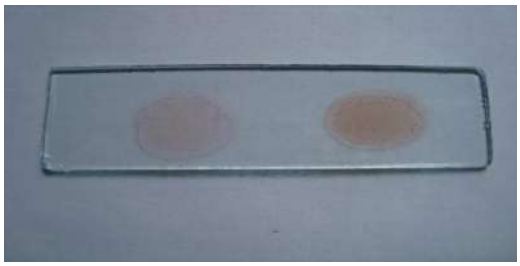

Figure 4: Positive control serum (left) and positive serum sample (right) tested by the rapid serum agglutination test with MG antigen

CAV PCR performed with DNA extracted from the thymuses of the five birds resulted in an amplification of a product with a predictable size of 583 bp (Fig. 5).

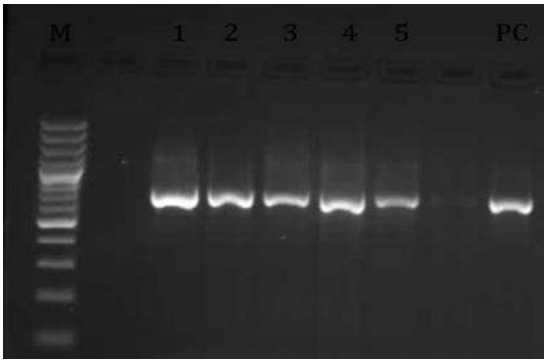

Figure 5: Agarose gel showing PCR product from amplification of thymus samples, as described: Lane M: 100 bp DNA marker; lane 1: positive thymus sample 1; lane 2: positive thymus sample 2; lane 3: positive thymus sample 3; lane 4: positive thymus sample 4; lane 5: positive thymus sample 5; lane PC: positive control

Histologically, hypoplasia of bone marrow (Fig. 6) as well as depletion of lymphocites from subcapsular thymic cortex was detected, which indicated the presence of CAV in the both chickens with anaemia.

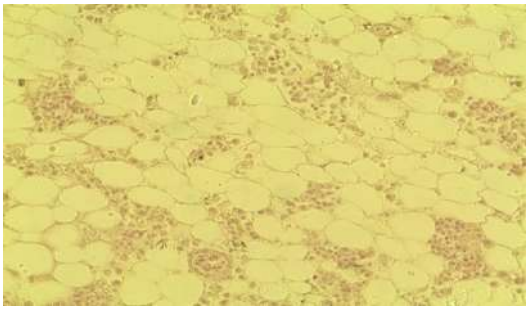

Figure 6: Hypoplasia of the bone marrow: destruction of the haemocytoblasts, which have been replaced by adipose tissue

The ELISA test detected antibodies against Avian reovirus and Avian metapneumovirus in the five tested fancy chickens.

Discussion and conclusions

In this study we confirmed the presence of CAV, MG, aMPV and ARV in fancy chicken breeds in Bulgaria and described a case of co-infection of CAV with MG, aMPV and ARV.

Considering the persisting respiratory symptoms such as difficulty breathing, whistling sounds and conjunctivitis, which have been shown by great percent of the fancy birds in the flock, as well as the observed during the necropsy gross pathological lesions including fibrinous airsacculitis with gathering caseous exudate and air sacs with initial signs of opacity, we suggested that in this case it concerned to MG infection. Thus, we confirmed MG serologically by the rapid serum agglutination test in the five tested fancy chickens. The control of the pathogenic avian mycoplasmas can be carried out in three ways: maintaining the flocks free of infection (all-in all-out management system, good biosecurity including improvement of the hygiene practices and effective monitoring system), vaccination and medication [5]. The practice of maintaining the flocks free of infection, though, by extensively bred birds in yards conditions including fancy birds is as a whole impracticable. That is the reason to rely on treating the sick birds and applying vaccination. The tested by us birds have been treated, but with no effect, because mycoplasma develops antibiotic resistance [3].

The fact that two of the submitted to the laboratoty fancy chickens had anaemia and at necropsy of one of them atrophy of the thymus was found out, turned us to investigation for CAV. By PCR we confirmed the presence of CAV in the five tested birds, that as an immunosuppressive agent causes co-infections with reoviruses (blue wing disease, haemorrhagic anaemia syndrome), adenoviruses (inclusion body hepatitis/hydropericardium syndrome), IBDV-Infectious Bursal Disease Virus, MDV-Marek´s disease virus (early mortality syndrome), IBV-Infectious Bronchitis Virus, Clostridium perfringens (gangrenous dermatitis), Staphylococcus aureus [9]. We assume that the CAV infection together with the MG infection in this case is the reason for the unsatisfied results of the therapy. We claim that the CAV infection is active because we observed anaemia in some of the birds, atrophy of the thymus, though it was found out in only one chicken, and we confirmed the presence of the virus in the five fancy birds by PCR and in the both chickens with anaemia histologically.

The respiratory symptoms of the examined chickens including rales and dyspnea as well as the serous tracheitis which we found out during the necropsy of the two older fancy birds, suggested that other respiratory pathogens might have been involved in the clinical manifestations and lesions, therefor we performed serological testing for presence of antibodies to aMPV. Although blue wing disease was not observed in the five examined chickens, we investigated them for antibodies against ARV. Thus, infections with aMPV and ARV were confirmed serologically by ELISA in the five tested fancy birds. We concider that in this case it concerns to complicated infection of CAV with MG, aMPV and ARV in which the four infectious agents mutually act and enhance their effects as the whole association of viruses exacerbates the clinical picture.

The present study demonstrates that fancy chicken breeds could be reservoir of CAV, MG, aMPV and ARV and play an important role in the transmission of these infectious agents. When persisting respiratory problems conditioned by mycoplasma are found out, a possible infection with CAV should always be considered in the diagnostic plan. The disease complex primarily resulted from the interplay between CAV and MG, subsequently could be aggravated by co-infection with other pathogens such as aMPV and ARV.

Acknowledgements

We would like to thank one anonymous reviewer for helpful discussions and critical reading of the case report, and also the staff in NDSRVMI-Sofia for their excellent technical assistance.

References

1. De Wit J. J., J. H. van Eck, R. P. Crooijmans, A. Pijpers. (2004). *A serological survey for pathogens in old fancy chicken breeds in central and eastern part of the Netherlands*. Tijdschrift Voor Diergeneeskunde, 129(10):324–327.
2. Erol N., and S. S. Şengül. (2012). *Seroprevalence of avian reovirus infections in chickens in western provinces of Turkey*. Kafkas. Univ Vet Fak Derg, 18, 653–656.
3. Gharaibeh S., M. Al-Rashdan. (2011). *Change in antimicrobial susceptibility of Mycoplasma gallisepticum field isolates*. Veterinary Microbiology, 150, 379–383.
4. Haesendonck R., M. Verlinden, G. Devos, T. Michiels, P. Butaye, F. Haesebrouck, F. Pasmans, A. Martel. (2014). *High seroprevalence of respiratory pathogens in hobby poultry*. Avian Dis, 58(4):623–627.
5. Kleven S. H. (2008). *Control of avian mycoplasma infections in commercial poultry*. Avian Diseases, 52(3):367–374.
6. Levisohn S., and S. H. Kleven. (2000). Avian mycoplasmosis (Mycoplasma gallisepticum). Review Scientific et Technic (International Office of Epizootics), 19(2):425–442.
7. Osman K. M., M. M. Aly, Z. M. Amin, B. S. Hasan. (2009). *Mycoplasma gallisepticum: an emerging challenge to the poultry industry in Egypt*. Rev Sci Tech, 28(3):1015–1023.
8. Park J. E., D. W. Lee, H. J. Shin. (2011). *Serological survey of antibodies against avian metapneumovirus in Korean chicken flocks*. Journal of Applied Poultry Research, 20(4):573–576.
9. Rosenberger J. K., and S. S. Cloud. (1998). *Chicken anaemia virus*. Poult Sci, 77(8):1190–1192.
10. Schat, K. A. (2009). *Chicken anemia virus*. Curr Top Microbiol Immunol, 331, 151–183.
11. Simeonov K. B., R. T. Petrova, B. I. Gyurov, R. D. Peshev, B. K. Mitov. (2014). *Isolation and PCR identification of chicken anaemia virus infection in Bulgaria*. Bulgarian Journal of Veterinary Medicine.
12. Wunderwald C., and R. K. Hoop. (2002). *Serological monitoring of 40 Swiss fancy breed poultry flocks*. Avian Pathology, 31, 157–162.