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Short communication

FOWL ADENOVIRUS INFECTION – POTENTIAL CAUSE OF A SUPPRESSED HUMORAL IMMUNE RESPONSE OF BROILERS TO NEWCASTLE DISEASE VACCINATION

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Fowl adenovirus infections have a significant economic impact, especially in the production of broilers. It is considered the leading cause of three syndromes: adenoviral gizzard erosions and ulcerations, inclusion body hepatitis, and hepatitis-hydropericardium syndrome. A critical feature of this virus is its immunosuppressive effect, via suppressing humoral and cellular immunity.

In this study, we examined the humoral immune response after administration of the Newcastle disease vaccine in broiler flocks with previously confirmed seroconversion against Fowl adenovirus. The study was conducted on 5 farms. A total of 220 chickens, five weeks of age, showing no clinical signs of the disease, were included in this study. The control group consisted of 20 chickens from a negative farm. Chickens were vaccinated with commercially available live NDV vaccines between 11 and 13 days of life. ELISA determined the presence of specific antibodies against FAdV in a total of 130/200 (65%) blood sera. Depending on the farm, seroprevalence ranged from 30-100%. The presence of specific antibodies against NDV was determined three weeks after vaccination using the hemagglutination inhibition assay. A positive hemagglutination inhibition (HI) titer (≥ 16) was found in 41/200 (20.5%) sera, which was significantly less compared to the control farm, where a positive HI titer was found in 20/20 (100%) sera.

The results of our study indicate the immunosuppressive effect of FAdV in subclinically infected birds and highlight the need for its diagnosis, prevention, and control.

Keywords: Fowl adenoviruses, Newcastle disease, vaccination, immunosuppression

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INTRODUCTION

According to World Organization for Animal Health, diseases induced by Fowl adenoviruses (FAdVs) are reported worldwide and considered of most concern, because of their severe economic effects on meat-oriented commercial bird production and their negative effects on trade [1]. In recent decades, it has been observed that the implementation of rigorous biosecurity measures may be the reason for the higher incidence of Fowl adenovirus disease in poultry. The elevated standards of biosecurity in the breeder flocks prevent the possibility of contact and generate a natural immune response against FAdV, and therefore the absence of maternally-derived immunity in the progeny. Further, on broiler farms, biosecurity measures are not so strict and the highest incidence of FAdV-induced diseases in one-day-old chicks that are without maternally-derived immunity is often observed [2-4].

FAdVs are non-enveloped, double-stranded DNA viruses that belong to the genus Aviadenovirus within the family Adenoviridae. According to the International Committee on Taxonomy of Viruses (ICTV), they are grouped into 5 different species, FadV A to FadV E, that are further subdivided into 12 serotypes (Fowl adenovirus 1, 2, 3, 4, 5, 6, 7, 8a, 8b, 9, 10, 11) [5]. The virus can induce acute infection with sudden onset, or subclinical infection, and therefore can be isolated from both sick and clinically healthy chickens. The virus is highly contagious. Vertical transmission is most important since the infection turns into a subclinical form in adults, but causes severe disorders at the young age of progenies. Viruses can be transmitted horizontally, directly, or indirectly via contaminated fomites due to very high titers in the feces of clinically infected chickens or carriers [6,7]. Fowl adenovirus infection is associated with several diseases that are usually caused by specific virus types: adenoviral gizzard erosions and ulcerations syndrome (GEUS), inclusion body hepatitis (IBH), and hepatitis-hydropericardium syndrome (HHS) [6-9]. Results from different studies confirmed that gizzard erosion is associated with FAdV-1 infection (species Fowl adenovirus -A), IBH cases could be associated with FAdV-2 and -11 (species Fowl adenovirus - D), and FAdV-8a and -8b (species Fowl adenovirus - E), whereas HHS case is associated with FAdV-4 (species Fowl adenovirus -C). Also, concurrent infections with multiple FAdV types are commonly detected [10-19].

Furthermore, there is evidence that infection with immunosuppressive pathogens such as infectious bursal disease virus (IBDV), chicken infectious anemia virus (CAV), reovirus, and Marek's Disease virus (MDV) can enhance the pathogenicity of FAdV and promote the clinical manifestation of FAdV infection [19-24]. Previous research has reported that co-infection could postpone the innate immune response in chickens, which subsequently induces replication without restraint in the early stage and the induction of a cytokine storm and death [23]. Virulent FAdV-4 has a tropism for lymphoid tissue causing the depletion of B and T cells in lymphoid organs (thymus, bursa of Fabricius, spleen), structural and functional damage of immune organs with induction of a severe inflammatory response [3,25]. Immunosuppression induced by

FAdV 4 serotype occurs at the early stage after infection [3,23]. It has been reported that other serotypes of Fowl adenovirus compromise the immunological capabilities of infected chickens [26]. The capability of the immune system to respond against Newcastle disease virus (NDV) infection determines the severity of the disease. The key component in the protection against NDV is humoral immunity, maintaining high antibody (Ab) levels to NDV in the flocks [27]. Birds that are infected with FAdV experience suppression of the immune response to some vaccines reducing their protective effect [25,28,29].

The aim of this study was to investigate if the FAdV infection suppresses the immune response of broilers to the NDV vaccine.

MATERIAL AND METHODS

The study was conducted on 5 broiler farms (1, 2, 3, 4, and control farms), from different locations in the Belgrade city region. The capacity of farm 2 and farm 3 was 15000 broilers and the capacity of farm 1 and farm 4 was 25000 commercial broilers. The capacity of the control farm (C) was 10000 commercial broilers at the time of sampling. Birds were at the age of 5 weeks, had two different proveniences, and originated from two breeder farms. The chickens were apparently healthy with no clinical signs of the disease and were vaccinated against IBDV. At the age of 11-13 days, the birds were vaccinated by spraying against the Newcastle disease virus with 0.1 ml of live vaccine from different manufacturers, with the Newcastle disease lentogenic virus (10^6 to 10^7 $TCID_{50}$ - 50% Tissue culture infective dose). Three weeks after vaccination, the birds were sampled in order to assess antibody (Ab) response following vaccination. Depending on the farm capacity, the blood samples were collected from wing veins in the following quantities: from 40 broilers per farm (farm 2 and farm 3), from 60 broilers per farm (farm 1 and farm 4), and from 20 broilers from the control farm. The control farm was tested and declared free from Ab against FAdV. For antibody detection against NDV and titer determination haemagglutination inhibition (HI) test was used according to the OIE procedures using 4 hemagglutinating units where serum samples with titers ≥ 1:16 were considered positive [30]. The measure of antibody level to Fowl adenovirus was used with an indirect ELISA (Fowl Adenovirus 1 Antibody test kit, BioChek Netherlands). ELISA test is performed according to the manufacturer's instructions. The test detects specific antibodies against non-specific serotype common group antigen that poses all 12 serotypes. Titers ranging from 1070 or less were considered negative and titers ranging from 1071 or greater were considered positive. The immune response to the vaccination against the Newcastle disease virus was evaluated and compared between the commercial broiler chickens and the control group.

Statistical analysis was done by statistical software GraphPad PRISM 5.0 (GraphPad Software, Inc., San Diego, CA). The generated results were evaluated by the chi-square test (χ^2 –test), independence test based on statistics, and Fisher's exact test.

RESULTS AND DISCUSSION

In this study, using ELISA, the presence of specific antibodies against FAdV was determined in 130/200 (65%) blood sera. Depending on the farm, the average seroprevalence ranged from 0-100% (Table 1). The highest rate of FAdV seropositivity in 60/60 (100 %) was recorded on farm 4, while farm 1 had the lowest rate of 20/60 (30 %). Fowl adenovirus infection may be involved in the altered response of the immune system against different viral infections. Also, it can decrease the humoral immune response of the host [25,28]. As the virus has a tropism for lymphoid tissue inducing depletion, the FAdV group presented lymphocytes of the bursa of Fabricius and thymus, interstitial edema in the thymus and lymphocytes necrosis in the cortex of the spleen, it was concluded that the virus may affect both humoral and cell-mediated immunity [25]. In addition, Naeem et al. (1995) confirmed the immunosuppressive effect in the experimental birds that survived the infection [28]. The host immune response provoked by FAdV reacts with an increase in the expression of the mRNA levels for genes which encode cytokines (IFN-γ, IL-1β, IL-2, IL-6, IL-8, and IL18). It is presumed that over secretion of cytokines in infected tissues (spleen, the bursa of Fabricius, liver, bone marrow, and cecal tonsils) might be responsible for tissue damage induced by FAdV serotype 4 [23]. The co-infection of IBDV and FAdV induces the delay of the expression of interleukin (IL)-6, IL-1β, and interferon-γ mRNAs, and subsequent to the lower antibody response [23]. According to the result of the current study, the combined positive hemagglutination inhibition (HI) NDV titer (≥ 16) was found in 41/200 (20.5%) blood serum samples from all tested farms, which was significantly less compared to the control farm, where a positive HI titer was found in 20/20 (100%) blood sera.

Table 1. The number of tested, Ab FAdV positive birds, mean titer per farm and number of Ab NDV protected birds

Farm	No tested	No Ab FAdV* pos (%)	Mean ELISA titer for FAdV positive	No Ab NDV** (%)
1	60	20 (30 %)	2113 (1101-7179)	0 (0)
2	40	15 (37.5 %)	1613 (1151-3490)	5 (17.5)
3	40	35 (87.5 %)	6586 (1350-16424)	12 (30)
4	60	60 (100 %)	20853 (9654-25956)	19 (31.4)
5 (control)	20	0 (0)	1070 <	20 (100)

*number of birds that has specific antibodies against FAdV (FAdV – Fowl adenovirus)
**number of bird with the protective level of antibodies against NDV (NDV – Newcastle disease virus)

The determined values of antibody titers to NDV in the serum showed that on farm 1 all sera had non-protective titers (<16), while on the control farm (C) all sera had protective titer values (≥16). According to the results of the chi-square test (χ^2 – test) on the other farms, a statistically significantly lower number of individuals was

protected ($p \leq 0.011$). Results of χ^2 –test of independence ($\chi^2=73.98$; $p<0.0001$) indicated that the ratio of individuals that were protected and that were not protected, depends very significantly from farm to farm, i.e. if compared on all farms at the same time, it differed statistically very significantly (Figure 1.).

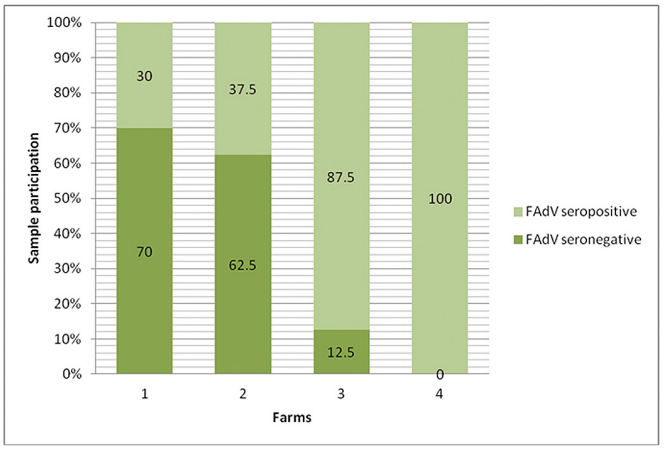

Figure 1. The ratio between FAdV seropositive and FAdV seronegative samples on the observed farms

Taking into account that the four experimental farms vary in proveniences, and sources of one-day-old chicks, also they vary in the capacity and level of biosecurity measures (internal and external) they have implemented, statistical analysis of the obtained results within farms was performed.

According to the results of Fisher's exact test, farm 1 was statistically very significantly different from the other farms (the level of significance of the difference, p, in relation to the second farm is 0.0025, and in the relation to the others is less than 0.0001). Also, the control farm differed statistically very significantly ($p<0.0001$) from farm 2, farm 3, and farm 4. The ratio of NDV-protected and NDV-unprotected chickens on farm 2 was not statistically significantly different from the ratio on the third ($p=0.293$) and fourth ($p=0.122$) farms. The difference was not statistically significant between farm 3 and farm 4 ($p>0.999$).

A significantly higher number of negative FAdV responses was found in chickens from farm 1 ($\chi^2=8.000$; $p=0.0047$), and a statistically significantly higher number of positive FAdV responses was detected in chickens from farm 2 ($\chi^2=22.50$; $p<0.0001$). The difference in the number of positive and negative FAdV responses obtained for farm 3 was not statistically significant ($\chi^2=2.50$; $p=0.1138$). All chickens examined from farm 4 had antibodies against FAdV.

According to the results of the χ^2-test a statistically significant difference ($\chi 2=89.46$; $p<0.0001$) in the immune response to the vaccine was found between the farms where seroconversion against FAdV was confirmed (farms 1, 2, 3, and 4). According to the

results of Fisher's exact test, the difference in the structure of the response is not statistically significant between farm 1 and farm 2 ($p=0.5044$). It was determined that farm 1, as well as farm 2, differs statistically very significantly compared to farm 3 ($p<0.0001$) and farm 4 ($p<0.0001$). Also, farm 3 and farm 4 differ statistically very significantly ($p=0.0054$) in the immune response to the vaccine.

These results are not weird knowing the fact that farm 1 and farm 2 have better overall biosecurity programs and management practices that are key to reducing the risk of transmission of infectious diseases.

Research conducted in 2021 and 2022 in the epizootiological area of Belgrade revealed the widespread of FAdV in apparently healthy broilers [31]. In Serbia, vaccination against FAdV has never been practiced. Therefore, our finding of FAdV group-specific antibodies was proof of natural exposure of broiler chickens to the field virus.

In the present study, we confirmed seroconversion against FAdV on all farms, except on the control farm and presumed that the FAdV infection could induce a lower humoral response to the NDV vaccine since in the past few years (data not published), there were reports from commercial broilers on very low titer level 2-3 weeks after NDV vaccination. According to the results obtained in this study, chickens that have confirmed seroconversion against Fowl adenovirus experienced greater inhibition of antibody responses to live vaccines against NDV, compared to the group where seroconversion was not confirmed. Namely, the immune response against the vaccine was reduced compared to the control farm, i.e. all samples from the control farm had HI titers higher than 1:16 compared to the examined farms (farms 1, 2, 3, and 4), where protective HI titers had a significantly lower number of samples. Results from the observed farms, regarding the ratio of the number of samples with protective titers and the number of samples with no protective titers, differed significantly from farm to farm. Those differences were probably due to the sample size, proveniences, and breeding technology, but it may also depend on the FAdV serotype and its capability to induce a potent antibody response and high infection rate. This can explain differences in FAdV antibody levels from farm to farm. Also, we proved significant differences between farms regarding the number of samples with seroconversion against FAdV and significant difference in the immune response to the vaccine. Our results are in agreement with previous findings related to the compromised immune capacity of the infected host [25,26,28]. It has been confirmed that serotype FAdV 8 and the high virulent serotype FAdV 4 induce growth impairment in the thymus and the bursa of Fabricius, and lymphocyte apoptosis, depletion of lymphocytes in the medullary area of the bursa of Fabricius and thymus, and necrosis of lymphocytes in the cortex of the spleen. As a consequence of those pathological effects, suppression of the humoral immune response, as well as the cellular immune response occurs [25,26,28,32]. FAdV decreases the antibody response of birds to T-cell-independent antigens by decreasing IgM responses [32]. FAdV infection induces a significant decrease in the blastogenesis response of peripheral blood lymphocytes [32]. This can explain our results that infected birds had a reduced humoral response to the NDV vaccine. Actually, in the

present study, suppression of humoral immunity or cell-mediated immunity at the time of vaccination or at the time of challenge had an impact on the level of serum antibodies against NDV. In this study, the flocks were not analyzed for the presence of other immunosuppressive viruses or other causes of immunosuppression. Still, knowing the immunosuppressive potential of FAdV, it could be supposed that the presence of FAdV influenced NDV antibody levels.

As shown in some other investigations FAdV infection can cause significant inhibition of antibody responses to inactivated vaccines against Newcastle disease and avian influenza virus subtype H9 [33,34]. Also, it was reported that FAdV decreased the ability to mount an antibody response to unrelated antigens in infected birds [33]. Furthermore, Naeem et al. (1995) researchers demonstrated a significantly reduced ($P < 0.01$) serological response to a live NDV vaccine, and then to a killed vaccine, in the group that was experimentally inoculated with FAdV isolate compared to the uninfected control group [28]. This is an important fact as in the infected broiler flocks it could be expected a reduced humoral response during regular vaccination program that usually includes vaccines against infectious bursal disease virus and infectious bronchitis virus.

CONCLUSION

The results of our study indicate the immunosuppressive effect of FAdV in subclinically infected birds, and highlight the need for its diagnosis, prevention, and control. It is very important to raise the awareness of veterinarians and farmers about this feature of FAdV because the virus can be the primary pathogen in broilers which increases the possibility of outbreaks of diseases associated with other pathogens and leads to large economic losses. Also, it should be taken into consideration as one of the causes that can reduce the efficacy of vaccines in the field.

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Authors' contributions

JM conceived the study, participated in its design and coordination, interpreted the data, and wrote the manuscript. MM performed the statistical analysis and interpreted the data. LJS and BK conceived the study, participated in its design and coordination, and were involved in laboratory work. VM, JMZ, and LJV participated in the coordination of the study. All authors read and approved the final manuscript.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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INFEKCIJA ADENOVIRUSOM ŽIVINE – POTENCIJALNI UZROK SUPRESIJE HUMORALNOG ODGOVORA NAKON VAKCINACIJE PROTIV NJUKASTL BOLESTI

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Infekcije adenovirusom živine (FAdV) imaju značajan ekonomski uticaj, posebno u proizvodnji brojlera. FAdV se smatra glavnim uzrokom tri sindroma: adenovirusne erozije i ulceracije mišićnog dela želuca, hepatitis sa inkluzijonim telima i hepatitisa-hidroperikardijum sindrom. Veoma važna karakteristika ovog virusa je njegovo imunosupresivno dejstvo, koja se ogleda u supresiji i humoralnog i ćelijskog imuniteta.

U ovoj studiji smo ispitivali humoralni imuni odgovor nakon primene vakcine protiv Njukastl bolesti (NJD) u jatima brojlera sa prethodno potvrđenom serokonverzijom protiv adenovirusa živine. Studija je sprovedena na 5 farmi. U ovu studiju je uključeno ukupno 220 pilića, starosti od pet nedelja, bez kliničkih znakova bolesti. Kontrolnu grupu činilo je 20 pilića sa negativne farme. Pilići su vakcinisani komercijalno dostupnim živim vakcinama protiv virusa ND između 11 i 13 dana života. ELISA je utvrdila prisustvo specifičnih antitela protiv FAdV u ukupno 130/200 (65%) krvnih seruma. U zavisnosti od farme, seroprevalencija se kretala od 30-100%. Korišćenjem testa inhibicije hemaglutinacije, tri nedelje nakon vakcinacije, utvrđeno je prisustvo specifičnih antitela protiv ND virusa. Pozitivan titar inhibicije hemaglutinacije (HI) (≥ 16) pronađen je u 41/200 (20.5%) seruma, što je značajno manje u poređenju sa kontrolnom farmom, gde je pozitivan HI titar nađen u 20/20 (100%) seruma.

Rezultati našeg istraživanja ukazuju na imunosupresivno dejstvo FAdV kod subklinički inficiranih ptica i naglašavaju potrebu njegove dijagnoze, prevencije i kontrole.