EFFECT OF FOWL ADENOVIRUS (FAdV-7) INFECTION ON THE REPLICATION OF TURKEY HERPESVIRUS FC126 IN CHICKEN EMBRYO FIBROBLAST CULTURES

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Received: September 28, 2012
Accepted: November 27, 2012

Abstract

The aim of the study was to determine the influence of simultaneous infection of chicken embryo fibroblasts (CEF) with different doses of adenovirus field strain serotype 7 (FAdV-7 JN-5/10j) and turkey herpesvirus strain FC126 (FC126 HVT) on replication of the herpesvirus in in vitro cultures. Three experiments were performed: simultaneous infection of CEF with adenovirus and HVT; inoculation of CEF culture with adenovirus, followed by infection with HVT after 24 h; and inoculation of CEF with HVT, followed by the infection with adenovirus 24 h later. In order to detect the presence of HVT and adenovirus strains in CEF culture, SORF 1 and hexon genes were determined, respectively. The infection with adenovirus lowered replication of FC126 HVT in chicken embryo fibroblast.

Key words: adenovirus, herpesvirus strain, CEF culture, replication.

Adenoviruses are icosahedral non-enveloped dsDNA viruses with capsid of 74-90 nm in diameter, composed of 252 capsomers, which surround the core of 60-65 nm in diameter (7). They can be infectious for fish, birds, reptiles, mammals, and amphibian, and were isolated from over 40 vertebrate species (2, 6, 7). Fowl adenoviruses (FAdV) are the ubiquitous poultry viruses existing commonly in poultry flocks. Adenoviral infection is the most dangerous in young birds with a predilection for birds less than 35 d of age (5, 7, 21).

The pathogenic role of all strains of adenoviruses is not entirely clear, but they can cause the diseases, such as inclusion body hepatitis (IBH) (4), hydropericardium syndrome (HP) (17), haemorrhagic enteritis (HE), and egg drop syndrome (EDS), and can play an crucial role during the polyetiological infections of the respiratory and digestive tracts (5, 7). They can be also responsible for the immunosuppression in infected birds (8, 9, 12, 16, 20).

For molecular diagnostics, the hexon gene (103,000 kDa) was used due to the conservative domains, which are responsible for creating the basement of the molecule (3, 10). Additionally, the use of highly variable domains located mainly outside of the virion, responsible for antigenic variation of adenoviruses, has been reported (3, 10). The size of the protein depends on the adenovirus species, as well as, the serotype and ranges between 930 and 950 aa (5, 11, 15).

Marek’s disease virus (MDV) belongs to herpesviruses and is responsible for Marek’s disease (MD) in poultry (22). The live vaccines based on turkey herpesvirus from serotype 3 and attenuated Rispens strain belonging to serotype 1, have been used during the immunisation. Nowadays, an increase in the number of cases of MDV infections in birds vaccinated against the disease is observed. The adenovirus strains were also isolated from these cases. The viruses exist in the environment and can decrease the effectiveness of immunisation.

The aim of the study was to determine the influence of simultaneous infection of chicken embryo fibroblasts (CEF) with different doses of adenovirus field strain and turkey herpesvirus strain on the replication of the herpesvirus in in vitro cultures.

Material and Methods

Chicken embryo fibroblast (CEF) cultures. CEF cultures were prepared from 9-11-day-old SPF chicken embryos (Lohman, Germany) according to the standard procedure. Eagle’s growth medium (MEM) was used with addition of 10% foetal bovine serum and 0.1% antibiotic mixture (Antibiotic–Antimycotic, Gibco, U.K.). The maintaining medium consisted of MEM with 0.1% of antibiotic-antimycotic mixture. A monolayer of
CEF was obtained after about 24 h of incubation at 37.5°C.

**Virus reference strain.** The reference adenovirus strain, belonging to the serotype ATCC FAdV-7, was obtained from a commercial company (Charles River Laboratory, USA), and was used as a positive control in real-time PCR.

**Field strain.** The field adenovirus strain JN-5/10j was derived from 7-week-old chickens with adenovirus infection and with MD clinical signs. The chickens were vaccinated against MD, and the protection after the vaccination was suppressed. On the basis of phylogenetic analyses of the L1 fragment of the hexon gene, this strain was classified as serotype FAdV-7. The 3rd passage of the strain with the titre $10^{4.5}$ TCID<sub>50</sub> was used for infection of CEF.

**Vaccine strain.** Turkey herpesvirus strain FC126 (FC126 HVT), representing serotype 3, was obtained from commercial vaccine (Merial, France) and propagated in CEF cultures.

**Design of experiments.** The study was conducted in three experiments. Monolayer of CEF was infected with strain FC126 HVT in a dose of $10^{3.0}$ TCID<sub>50</sub> and co-infected with different doses of adenovirus field strain JN 5/10j ($10^{0.0}$-$10^{3.0}$ TCID<sub>50</sub>) in the following manner: a) simultaneously, b) first the infection with FC126 HVT strain, followed by infection with adenovirus after 24 h, and c) first the infection with adenovirus, followed by infection with FC126 HVT 24 h later. CEF infected with FC126 HVT strain in a dose of $10^{0.0}$ TCID<sub>50</sub> were used as a positive control. After 18, 24, 48, 72, and 96 h of incubation, total DNA was isolated and collected. Afterwards, the number of copies of SORF 1 gene of FC126 HVT strain was determined by real-time PCR (Fig. 1). The presence of hexon gene of FAdV-7 field strain was determined also using real-time PCR (Fig. 2).

**DNA extraction.** Total DNA of both viruses was extracted using DNA Mini Kit (Qiagen, Germany) according to the manufacturer’s procedure. The DNA was isolated directly from the CEF cultures infected with adenovirus field strain or herpesvirus strain. DNA was also extracted from non-infected CEF and used as a negative control. CEF infected with reference strain FAdV-7 were used as a positive control sample. DNA samples were then stored at -20°C for the next step of the experiment.

**Plasmid standard.** pSORF 1 plasmid was constructed by cloning 196 bp fragment of SORF 1 gene into pGEM-T Easy Vector (Promega) and Blue/White X-Gal/ IPTG selection. pSORF1 was then amplified in E. coli DH5α (Invitrogen) in liquid LB medium with additive of 100 µg/mL of ampicillin in 37°C Max 4000Q apparatus (Barnstead/Lab-line). Isolation of plasmid standard was performed from 10 ml of liquid night culture of E. coli cells using Plasmid Maxi Kit (Qiagen).

**Real-time PCR for quantification of SORF 1 gene of FC126 HVT strain.** Primers, amplifying the specific sequence of the SORF 1 gene of FC126 HVT were designed using GeneBank database and Primer 3 software. The primers’ sequences were as follows: HVT F 5’ CGTCTTCAACACGTTAGGG 3’, HVT R 5’ CCAAGCCTTTAGCAAGAT 3’, respectively. Real-time PCR was conducted using QuantiTect PCR SYBR Green (Qiagen, Germany). The final volume of reaction mix was 25 µL. It contained: 12.5 µL of 2x QuantiTect Probe PCR Master Mix, 1 µL (0.4 µM) of pair of primers for SORF 1 gene of FC126 HVT, and 8 µL of deionised water. The reaction was carried out in plastic 96-well plates. As a negative control, DNA isolated from non-infected CEF was used. Two microlitres of DNA of the tested samples were added to each well. The conditions were as follows: 50°C for 2 min, 95°C for 15 min, then 40 cycles at 94°C for 15 s, and at 60°C for 1 min. The quantity of SORF 1 gene copies was analysed in Applied Biosystems 7500 version 2.0.1 on the basis of standard curve.

**Determination of the quantity of SORF 1 gene copy number.** The standard curve method was used to evaluate the exact number of copies of the SORF 1 gene of HVT in the examined samples (Applied Biosystems 7500 Version 2.0.1). Quantification of viral copy number was performed according to the procedure previously described by Baigent et al. (1) and Woźniakowski et al. (23). On the basis of serial ten-fold dilutions of pSORF 1 plasmid from $10^2$ to $10^4$ DNA copies/µL, 4 point standard curves were prepared and used to calculate the number of SORF 1 gene copies.

**Real-time PCR for identification of the hexon gene of adenovirus strain.** The sequences of nucleotide primers specific for adenovirus were as follows: AdV JSN (sense primer): 5’ AATGTCACCGAGGACGC 3’, AdV JSN (antisense primer) 5’ CBGCBTRCATGTACTGGTA 3’, AdV JSN (sense primer): 5’ AATGTCACCGAGGACGC 3’, respectively.

**Results.** The results are presented on Fig. 3 a-c. The presence of adenovirus strain in all experimental variants decreased the replications of HVT virus. In the experiment a, adenovirus introduced to the CEF culture
simultaneously with FC126 HVT decreased the number of copies of the SORF 1 gene, which was between $10^{5.0}$ and $10^7$, in comparison to the results obtained in the control CEF ($10^{7.0}$ and $10^{8.0}$ copy number). Depending on the dose of the adenovirus strain, less by 1.0 log to 2.5 log copies of SORF1 gene was observed, comparing to the results in control cultures (Fig. 3a).

The replication of HVT was also decreased when the adenovirus was introduced into the culture 24 h after the infection with HVT FC126 (Fig. 3b). The obtained results were similar to the results described above.

Adenovirus inhibited the replication of HVT FC126 to a lesser extent in experiment c. The number of copies of SORF1 gene was lower by 1.5 log in comparison to the control cultures (Fig. 3c).

The presence of hexon gene was detected in all samples infected with adenovirus strain in all variants of infection, independently of the infection dose of the virus.

![Amplification Plot](image1)

**Fig. 1.** The fluorescence curve indicates the amplification of specific fragment of the examined SORF 1 gen FC126 HVT.

![Amplification Plot](image2)

**Fig. 2.** The fluorescence curve indicates the amplification of specific fragment hexon gen of FAdV-7.
Fig. 3a. Copy number of SORF 1 gene of strain FC126 HVT

Legend:  
10^{1.0} FAdV-7 + HVT,  
10^{2.0} FAdV-7 + HVT,  
10^{3.0} FAdV-7 + HVT,  
K+ HVT.
Discussion

During the last years, the occurrence of clinical cases of Marek’s disease in vaccinated birds has increased. One of the reasons of this situation can be the adenovirus infections, which are commonly diagnosed in sick birds. The previously obtained results indicated that FAdV strains, which were isolated from MD affected birds, most commonly represented serotype FAdV-7. This serotype was used in the experiments (data not published yet), which allowed to establish the correlation and influence of adenovirus infections in birds vaccinated against MD. The results may indicate that poorer replication of FC126 HVT strain may occur in case of adenovirus infection in birds, which may influence the effectivenss of immunoprophylaxis, and may cause the suppression after vaccination. This assumption was confirmed in the present study. The obtained results revealed that FAdV-7 field strain lowered the replication of FC126 HVT strain in the in vitro studies. On the other hand, no influence of strain FC126 HVT on the replication of adenovirus was demonstrated.

The literature data indicate that the infections with different serotypes of adenovirus strains, which are the factors of IBH occurring mainly in broiler chickens, are also the reason of immunity suppression (13). They may be also correlated with the presence of different diseases. Adenovirus infection can be the factor decreasing the amount of lymphocytes in lymphoid organs (19) or inducing a noticeable immunosuppression in birds, which may result in a lowered body weight in infected chicks. In another experiment, a significant decrease in the percentage of IgM-producing B cells in the bursa of Fabricius of chickens infected with serotype FAdV-4, and lowered bursal index were observed (20). The study by Samorek-Salamonowicz (14), who used an in vitro and in vivo approach in her experiments, demonstrated that in the presence of adenovirus the replication of HVT strain was lowered.

The role of adenoviruses as immunosuppression factors was determined on the example of the immunity of birds vaccinated against Newcastle disease. The immunosuppressive influence on the humoral response was examined at 21st d after infection of chickens by adenovirus, which caused inhibition of the immunity against the Newcastle disease virus (NDV), in comparison to controls (9). In the later studies, the effect of the adenovirus on main lymphoid organs (spleen, thymus, bursa of Fabricius, and caecum tonsils) and replication of the virus in different periods of the infection was established. The virus was found in lymphoid organs to which it had predilection.

Shivachandra et al. (20) indicated that the high mortality rates and more expressed histopathological changes appear in birds exposed to mycotoxins, and immunosuppression during the simultaneous infections with IBH/HPS. Adenovirus strains, as well as, mycotoxins can cause immunosuppression in young chickens.

Immunosuppression in birds was also determined by the estimation of immune response of infected birds to Brucella abortus. Birds showed lower IgM response to B. abortus. (T-cell-dependent antigen), as well as, depletion of peripheral blood lymphocytes (PHA-P) and phytohaemagglutinin 2 and 3 weeks post-infection, compared to the control groups. Atrophy of the bursa of Fabricius and depletion of lymphocytes in infected birds were also observed (18).

The results obtained by Singh et al. (18) confirmed the infection with adenovirus strain FAdV-1 caused immunosuppression, affecting both humoral and cellular immune competency of the chicks. The immunosuppression can be the reason of an increasing poultry loss in flocks. Schonewille et al. (16) indicated that virulent adenovirus field strain, serotype FAdV-4, is responsible for HP infections in broiler chickens and influences immune system functions (16).

The results obtained in the experiments will be further confirmed during the in vitro studies on chickens.

References


