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Chapter 7

Adenoviruses and Their Diversity in Poultry

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Additional information is available at the end of the chapter

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Abstract

An investigation into the aetiology of fowl adenovirus strains and their distribution worldwide in populations of poultry flocks industry has been conducted. Pathogenic role of the viruses is not always clear. They can cause latent infection or several diseases and are the reason of economic losses in poultry flock industry. Ubiquity of adenovirus strains was commonly described, and stand-alone pathogenicity for a long time has been disputed. A globally emerging trend of adenoviruses and adenovirus-associated diseases has been increasing from year to year in all over the world. Mainly, type FAdV-4 is responsible for hydropericardium hepatitis syndrome (HP), type FAdV-1 for gizzard erosion and ulceration (GEU), and types FAdV-2, 8a, 8b, and 11 seem to be responsible for inclusion body hepatitis (IBH). Defining the spreading of the avian adenovirus strains in different types of fowl profile production, recognising their property and determining their types and molecular characterisation are very important from the epidemiological point of view and are considered as excellent basis for vaccine development and gene therapy implementation. This chapter provides a comprehensive review of FAdVs, including their epidemiology, pathogenesis, diagnostic, detection, and molecular characterisation. This comprehensive review is needed to better understand the latest progress in study of the viruses and prospects regarding disease control and implementation of gene therapy.

Keywords: CPA, FAdVs, HVRs region, LAMP, molecular characteristic, pathogenicity, RSCU

1. Introduction

Adenoviruses are a very diverse group of pathogens, causing diseases in domestic and wild birds. Fowl adenoviruses are common in healthy birds and can cause different diseases with pathogenicity from 10 up to 90%, depending on strain virulence, as demonstrated in poultry flocks in all over the world [44, 62]. Adenoviruses have been isolated from different types

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of fowl profile production, and infections in fowl seem to be commonly widespread. Recent outbreaks of adenovirus infections (FAdV) in poultry flocks have been determined in many countries such as Europe, USA, Asia, and Australia, and are connected with economic loss in poultry production. Wild birds play some role in the transmission of several adenovirus types/species, which represent an important problem for poultry production [35]. Our knowledge about the role of wildlife reservoirs in the transmission of the adenoviruses is under exploration [35]. Worldwide distribution and outbreaks of adenovirus infection in 31 wild bird species have been reported by many authors, and types FAdV-1 and 4 infective for poultry are not infective for wild birds [45, 48]. To understand more, the evolution and transmission of FAdV viruses and detailed codon usage analysis was performed for FAdV strains representing five species FAdV-A-E and 12 types FAdV-(1–81-8b-11). High effective number of codons and indication of the presence of relative synonymous codon usage have been determined. The presence of mutations and their influence on codon usage was confirmed by the correlation between nucleotide compositions at the third codon position, HVRs1–4, and ENCs. This indicates some influence of natural selection and antigenic properties of examined FAdVs strains (Niczyporuk, Vol. 21 no3 (September 2018) of PJVS).

2. Morphology and biology/epidemiology

Adenoviruses, belonging to *Adenoviridae* family, are non-enveloped double-stranded DNA viruses [67]. The conserved domains are responsible for basement creation of the molecule and are responsible for trimmer formation [54]. The highly variable domains are mainly located outside the virion and are responsible for antigenic variation of the strains [7, 10]. Schematic concept of adenovirus genome is presented in **Figure 1**. The International Committee on Taxonomy of Viruses [32] separates the *Adenoviridae* into five genera: *Mastadenovirus*, *Aviadenovirus*, *Siadenovirus*, *Atadenovirus*, and *Ichtadenovirus*. Fowl adenoviruses are separated into five species designated as FAdV-A-E with 12 types of FAdV (1-8a-8b-11) [45, 48]. Adenoviruses are very divergent pathogens generally with low level of virulence; however,

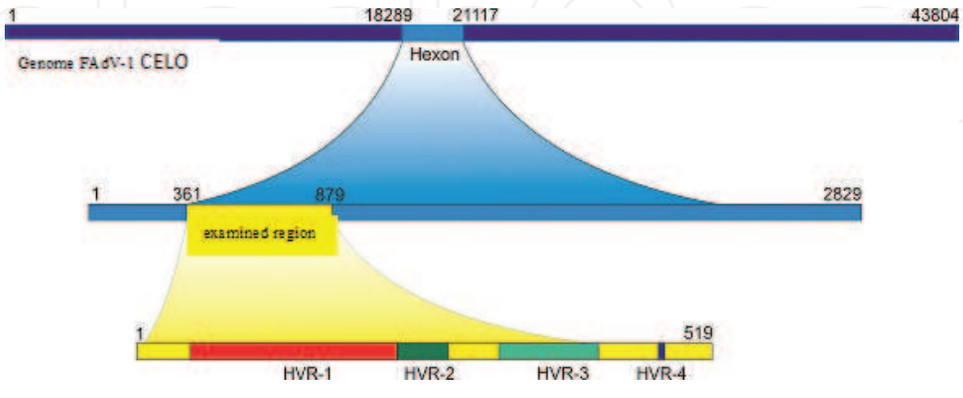

Figure 1. Schematic concept of adenovirus genome structure. Yellow colour indicates examined region of loop L1 hexon gene additionally divided into sections representing hypervariable regions HVR1–4. Niczyporuk, Vol. 21 no3 (September 2018) of PJVS.

under certain conditions, they can cause a variety of disorders in domestic and wild birds [33, 35]. Adenoviral infections can be found as asymptomatic or a complication factor in the course of different diseases [7].

Data connected with adenovirus structure, different protein functions, genome organisation, and replication are based mostly on human adenovirus research [58]. The adenovirus capsid is formed by three major proteins: hexon, penton base, and fibre. The hexon plays an important role in the genome organisation. Predicted amino acid sequences in hexon gene from human Ad2 and Ad5 and bovine adenovirus type 3 presented regions with high homology at the end of N and C regions, separated by central part with low homology. Both hexon proteins have a common structure. Hexon is the major capsid protein presented from 40 to 820 copies per virion. Every hexon has two characteristic parts: triangular peak with three towers and a basis with central part. To construct from conservative component: regions P1 and P2 are basements created of the particle and takes part in trimmer formation. Hypervariable regions (HVRs) of hexon protein creates loops L1, L2, and L4, which are located in external part of virion towers created; loop L1 is presented in (**Figure 1**). We can identify seven hypervariable regions HVRs1–7 [14]. Four of them are located in loop L1, two HVRs in loop L2, and one HVR in loop L4. Type-specific antigenic determinants are coded by HVRs from loops L1 and L2, existed on the hexon surface, and are strictly responsible for induction of immunological response. In loop L3, there are no such determinants [37]. This region can help in distinguishing the differences between strains of different types and point out their diversity. In a study conducted by Niczyporuk [52], the geographic analysis of adenovirus strains isolated from Poland, based on loop L1 region of hexon gene, has been described, and all the relative synonymous codon usages (RSCUs) in HVRs1–4 were designated (Vol. 21 no3 (September 2018) of PJVS). Alignment of consensus sequences clearly indicated the differences in hypervariable regions (HVRs) as described by Raue (2005). Four HVRs in loop L1 were identified as the regions of the highest sequence variability: HVR1 of about 191 bp long, HVR2 of about 50 bp long, HVR3 of about 90 bp, and HVR4 of about 18 bp long. The DNA sequences of HVRs are constant for every type, but there are major differences between FAdV types. Singh [64] indicated that timer stability in TAdV-3 fibre head monomer has the surface area in trimmer, and that fibre head from other adenovirus strains concerned with the stability of the trimmer are comparable. The melting temperatures of virulent and avirulent forms of the protein are 80°C at pH = 6, which indicates high protein stability. Stewart [68] indicated that adenovirus capsid amino acids are in symmetrical location in the inner and outer sides with the icosahedral symmetry. Each hexon has six nearest neighbours, and every hexon takes part in the construction of three hexon rows. Every triangular facet participates in forming three vertices, and every facet has three nearest neighbouring facets. Lenong [41] indicated that three structures of Ad type2 (Ad2) of species CE3-19 K/HLA-A2 complex showed the adaptation of a novel tertiary fold and uses a new binding surface on HLA-A2. Significant differences in genome size may have an influence on the genome organisation [10, 23]. The genome of avian adenoviruses is about 44–45 kb long, depending on the species/type. Adenovirus strains from the genus *Siadenovirus* with a genome of 26 kb length, genus *Mastadenovirus* with 31–36 kb, and the genus *Atadenovirus* with 27.7 kb [3, 38, 41] are analysed for comparisons. The shape of the trimeric hexon is not common and is divided into a hexagonal basis, which is reach in

β-structure, and every triangular top creates secondary structure [8]. They can be infectious for fish, reptiles, amphibians, birds, mammals, and were isolated from over 40 vertebrate species [7, 29]. Wild bird species [35] with adenovirus infection were documented by other authors and were found in falcons [65], common buzzards [22], *Milvus migrans* [24], tawny frogmouth [59], pigeons [67], and psittacines [40]. It is possible that under some conditions, adenoviruses can be more virulent in non-host-adapted species than in their typical host [24]. Some virulent FAdV strains can pass species barrier and can also infect new organisms [24].

3. Clinical features and pathogenesis

Adenovirus replication is based on two different mechanisms of the interaction between cell and the virus strains. Virus interaction with the cell starts from the entering into the pathway of the endocytosis to the host cells [49]. Domains, which are located at the end of the fibre, recognise the receptors, which are located on membrane, and binding process begins. We can suspect that the proteins from major histocompatibility complex (MHC) are also involved in this process. In the next step, the proteins, which are basis for penton, are involved in interactions with integrin cells that help in endocytosis (49, 70). During the next step, capsid disintegration takes place and genome that is connected with histone infiltrates into cell nucleus. This process is coordinated by microtubule of host cells (32, 39, 49). The replication is divided into early and late stages. Firstly, the transcript, which is created during the splicing, creates monocistronic mRNA, having the information about one protein. Next, the translation processes starts. Early genes are responsible for non-structural proteins, which are responsible for replication, regulation of cell metabolism, and changing the cell for the production of DNA viral protein and apoptosis prevention. During the beginning of replication, protein pre-terminal (pTP), polymerase DNA (pol), and protein DNA (DBP) binding are created. In the next step, heterodimer pol-pTP is created and is connected with 5′DNA, and replication gets started. The cell regulation transcripts like NFI/CTFI and NFIII/Oct-1 [43] are also involved. After the DNA replication starts, the transcription for late genes and formation of structural protein gets started. Virions stay in the nucleus and sometimes can create crystals and chromatin accumulation. The latency period for formation and maturation of the virus particles persists and the lysis of infected cells begins and virion particles are released [32, 39, 70]. Virus particles can appear after 14 h of post-infection—the first stage of particles releasing and increasing quick copy number of the virus particles with the highest point after 48–72 h. After 72 h, quantity of copy number of the virus particles decreases [45].

Adenoviruses can be transmitted horizontally [45, 48] and vertically [28]. Infections are most dangerous in younger birds with a predilection for birds less than 35 days of age. These viruses have the minor role in apoptosis, as type FAdV-1 has genes encoding anti-apoptotic peptides [28]. In embryonated eggs, virus can replicate causing death of the embryo or can be in latency period till hatching. Cell cultures, which are prepared from infected embryos, have the adenovirus infection. In chickens, hatched from these eggs, the infection can stay in latent phase and is indicated in the next chicken generation [18, 46, 69] which was indicated for types FAdV-4 [25] and FAdV-1 [28]. During the horizontal transmission with poultry litter,

water, or feeding staff in front of infected birds, adenovirus infections can have different courses of action. The infection can be latent, can be the implication factors for different diseases, or can be the reason of infection by themselves [48, 55]. In infected birds, the virus was found in faeces and in organs like trachea, kidneys, liver, spleen, and intestines. Adenovirus tropism depends on the age of the bird, its immunological status, and strain pathogenicity. Pathogenic activity in birds is not always clear. Even isolates from the same species show different pathogenicity depending on the strain, infective dose, and different environmental factors. In some cases, adenoviruses can be the opportunistic viruses [19], with a low level of virulence [61]. This mechanism is presented, e.g., FAdV-1 type for example pathogenic strains from that type are responsible for gizzard erosion and ulceration like strain FAdV-99ZH, but others like Japanese non-pathogenic strain FAdv-1-ote [19] did not induce indicated changes. Some strains cannot induce the disease by themselves; however, their pathogenic role is still important during the multi-aetiological syndromes. Adenoviruses can be isolated from sick and healthy birds. From one side the viruses can be the reason of the infections without any signs or can be the aetiological factor of syndromes like inclusion body hepatitis (IBH) [2, 17, 36, 55], hydropericardium hepatitis syndrome (HPS) [13, 34], haemorrhagic enteritis (HE) [56], marble spleen disease (MSD) [20], egg drop syndrome (EDS) [1], and gizzard erosion and ulceration (GEU) [54]. Period from first signs of infection is different and can be short 24–48 h. Antibodies produced after the infection with one virus type do not have any protection against the other adenovirus types [32]. However, serum with antibodies against type FAdV-9 is capable to neutralise the type FAdV-3. No cross-reactivity in opposite direction was noted [66]. For the adenovirus pathogenicity, genotype does not decide the type selection [27, 48] and pathogenic properties depend on the interaction between hexon proteins.

4. Detection

The presence of genetic material of adenovirus strains can be detected in samples of internal organs from sick or suspected for the diseased chickens. The sections of the liver, spleen, gizzard, kidneys, and intestines are collected and prepared as 10 w/v homogenates in a phosphate buffered saline (PBS). The homogenates for virus isolation are centrifuged and filtered through the 450-nm filters.

4.1. Virus neutralisation test

Serum samples from infected or suspected chickens are heat inactivated for 30 min at 56°C, and virus neutralisation test (VNT) is performed in 96-well plates containing CEF cells. After incubating for 5 days at 37°C in 5% CO_2 atmosphere, the cells are examined for cytopathic effects (CPE), virus titres are calculated, and titres above 3 $\log_2$ are considered positive.

4.2. Cell culture and virus isolation

Chicken embryo fibroblasts (CEFs) or chicken embryo kidney cells (CEKs) are prepared from 11-day-old or 19-day-old SPF chicken embryos (Lohmann-Tierzucht, Cuxhaven, Germany).

The cell suspensions are inoculated onto monolayer cultures, in which 0.2 mL suspension contains usually from 10^6 to 10^7 of live cells. Inoculated and control cultures are incubated at 37°C in a humid incubator containing 5% CO_2. Areas of cytopathic effects appear within 5–6 days; thereafter, the virus particles can be isolated.

4.3. Immunofluorescence assay

Usually, CEF cultures are infected with the third passage of the adenovirus strains. When CPE are observed after 5–6 dpi, CEFs are covered with 90% acetone cooled to −20°C. After 30 min, the acetone is removed and the plates are allowed to dry for the next 24 h. Then, the CEFs are washed three times with PBS buffer followed by the addition of 500 μL of blocking mix: 1× PBS, 5% bovine serum, and 0.3% Triton X-100. The plates are incubated for 1 h at 18–24°C, the blocking mix is removed, and 500 μL of mouse primary FAdV antibody, diluted 1:100 with PBS, are added. After incubation at 37°C for 18 h, the plates were washed three times with PBS (BioLab, Poland), a 1:200 PBS dilution of a secondary rabbit antibody against mouse IgG_1 conjugated with fluorescein isothiocyanate (FITC) was used and incubated at 18–24°C for 2 h in the dark. The fluid is removed and the plates are washed thrice with PBS buffer. The cells are observed under a fluorescence microscope. The presence of fluorescent cells of different sizes indicated a positive result of the IFA (**Figure 2**).

4.4. Molecular techniques

Molecular techniques such as restriction endonuclease analysis (REA) [50, 60], in situ hybridisation using DNA probes, polymerase chain reaction (PCR) [23, 52], real-time PCR [27], and real-time PCR Gunes, 2012 for the detection and quantitation of FAdV(A-E) species have been used. However, in the studies, primers were based on conserved nucleotide sequences within the 52 K gene with the efficiency of 98%, and regression squared values of $R^2 = 0.999$. Different real-time PCR and subsequent high-resolution melting curve analysis (HRM) of 191-bp region of the hexon gene and restriction enzyme analysis have been performed by

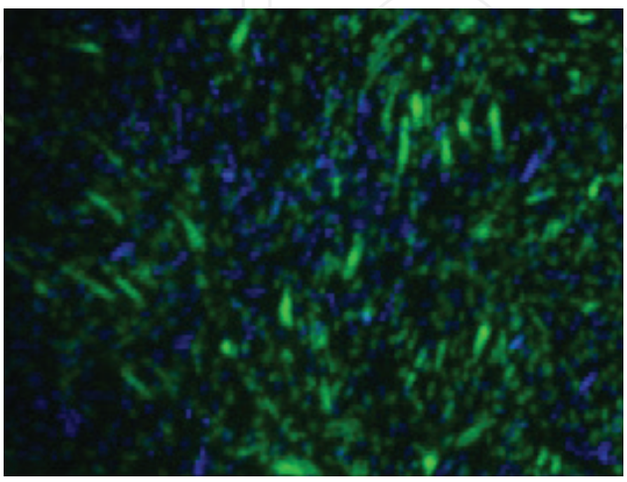

Figure 2. IF cytopathic effect of adenovirus strain type/species 1/A, IIIp. 96 h.p.i.

Steer [66] for differentiating all FAdVs species, and melting curve profiles were found to be mainly related to GC composition and distribution through the amplicons. High-resolution melting (HRM)-curve analysis [60, 67] was developed and applied as a specific, sensitive, and efficient detection method without cross-reactivity. For the latest years, the new effective and chipset techniques have been developed like loop-mediated isothermal amplification (LAMP) [51]) or cross priming amplification (CPA) by Niczyporuk [51].

5. Molecular study on HVRs1–4 and RSCU diversity

Data concerning the adenovirus genome, structure, function of selected proteins, and replication are based on human adenovirus strains [8]. In Poland, the presence of five species with eight types of adenovirus strains: FAdV-1/A, 2/D, 4/C, 5/B, 7/E, 8a/E, 8b/E, and 11/D were described recently [51]. The biggest group was formed by the strains classified as type FAdV-2/11/species D and the smallest group by the type FAdV-4/C [51]. Moreover, the relationship between type/species and the internal organs from which strains were isolated was found. It was demonstrated that types/species FAdV-2/11/D were most commonly isolated from the liver and intestines while type FAdV-4/C was represented less frequently [51]. Avian adenovirus genome size is 44–45 kb and depends on type/species representation. For comparison, mastadenoviruses having a genome size of 31–36 kb is chosen. Genome of adenoviruses belonging to different species has differences in quantity a pair of (GC) content. The value in avian adenovirus genome is between 53.8 and 66.9% and in mastadenoviruses it is 43.6 and 63.9% [8, 32]. Different sizes of the genome have a influence on gene organisation, depending on the type/species, and have 23–46 genes, which code several proteins [10]. Number of genes and their functions are different among strain-type/species; however, their basic genome organisation is common for the *Adenoviridae* family especially in the central part. Genome contains over a dozen of transcriptional units, which code 1–8 sequences with open reading frames (ORF). Owing to the alternative splicing, 50–70 different adenovirus proteins can be created. Till now, not all the genes and their functions are known [32]. Schematic concept of adenovirus genome and the location of hexon gene and hypervariable regions, HVRs1–4, are presented in **Figure 1**. Avian adenovirus genome has unique transcriptional units, so has the parts which are not well characterised yet. Adenoviruses have the ability to induce interferon that was demonstrated in experimentally infected animals [5] and cell cultures [4]. Adenovirus genome can also be used as a system for gene expression analysis [71]. Natural selection and mutation pressure have the influence on synonymous codon used during protein translation [11].

The relative synonymous codon usage (RSCU) is a simple measure of the heterogeneity in the usage pattern of synonymous codons. The value was calculated and performed by [11, 21, 26, 30, 31, 63]. RSCU value >1 means that the codon is more frequently used than expected and over-represented, while the RSCU value <1 means that the codon is less frequently used than expected [11]. The effect of mutational pressure on codon usage was confirmed by correlation between nucleotide compositions at the third codon position [64]. Sharp and Li [63] also defined the RSCU value as the ratio of observed frequency of a specific codon to the frequency expected, and the effect of mutational pressure was assessed by correlations of the third nucleotide position

in the codon. The presence of RSCU values for types FAdV-1/A and FAdV-7/E was published by Niczyporuk [53] and for types FAdV-2/11/D, 4/C, 5/B, 8a/E, 8b/E was described by Niczyporuk (Vol. 21 No3 (September 2018) of PJVS). However, all the types/species are different and all the molecular characteristics of the strains indicate diversity in each of the adenovirus type.

6. Adenovirus diversity

The degeneracy of the genetic code is established with in a group of 2–6 synonymous codons and some codons can be preferred than the others. This specific usage of codons is called codon usage bias, and between the various factors, natural selection, mutational pressure, RNA structure, and gene length are most important for codon usage bias [64]. Codon usage analysis is a well-established technique for understanding the process of evolution on molecular level [11]. In a study conducted by Niczyporuk, the nucleotide frequency in codons has been determined by the content of (GC) pairs and was different for different types/species (data was not published yet). The obtained data are comparable to the data reported by Raue [60]. It may be possible that genes of higher transcriptional importance could have higher (GC) content [16, 31]. Similar researches concerning different species have been done by Halder [30]. Every single deviation in codon usage is based on the codon preference by using the exact codon during the translation process. Specific codons are used more frequently than the other synonymous codons in genes with the high expression. Such codons are chosen as preferable or optimal one. This adaptation forces the expression, gene size, genome structure, and percentage of (GC) content or frequency of recombinations. In 2017, RSCU for FAdV-1/A and 7/E have been published by Niczyporuk [53], and preference of codons was defined.

The loop L1 with HVRs-1-4 is the main indicator of variability that was confirmed by authors of Refs. [14, 57, 60, 72]. Not every amino acid substitution has the influence on the structure and adenovirus protein function. Most substitutions are of similar size, charge, or hydrophobic properties. These are the amino acids of conserving function. This fact can explain that protein can have self-structure and function with different amino acid sequences coded by highly different nucleotide sequences. We can establish, that every codon can appear with equal frequency; however, Niczyporuk (data not published yet) indicated that from few possibilities, one codon can be preferable than others. These preferences can appear in genes with strong expression [6, 31]. The frequency of the presence of some codons in front of others (codon synonymous) is called relative synonymous codon usage (RSCU). It is indicated that the optimal codon can lead to the fast and exact or accurate translation, which is extremely important for the proteins synthesised in higher amounts [71], which has an impact in evolution. A deep analysis of examined consensus sequences based on the evaluation of mutation quantity, their localization, and possibility of the influence on protein tertiary structure (data not published yet) has been performed. Many researchers suggest, (Behura and Severson [6], Crawford-Miksza and Schnurr [14], Epstein et al. [16]) that the most important for the study are the mutations which are located on the first and second codon places. These are the mutations that influence amino acid coding, which have the impact on differences in protein structure and function. Changes in variability have in consequence an influence on virus pathogenicity. Principal component

analysis also supported that most codons showed biased effect on (G) and (C) at the third codon position and at the preferred codons that end with either (G) or (C). Halder [30] also indicated that positive significant correlation in gene expression parameters with a few amino acids such as Val (V), Arg (A), Ser (S), and Ile (I) might influence the gene expression.

Gu [26] in their comparative study described the RSCU of ovine 287(OAdV287) and human HAdV2/5 adenovirus strains. Gu [26] presented that OAdV287 had more conservative codon usage than HAdV 2/5. The preferred codons of HAdV2/5 mostly had (GC) ends. Das [15] indicated that one of the major determinants is the (GC) content in the third codon position C3S and G3S, and significant variations are observed in synonymous codon choice in structural and non-structural genes of HAdV. In a previous study, Niczyporuk [53] described RSCU in the loop L1 region of the hexon gene for types/species FAdV-1/A and FAdV-7/E. The most important mutations were those in the first and second codon positions, because these mutations are more likely to result in an amino acid change, affecting the structure and protein function. Nucleotide sequence analysis indicated that different codons can code the same amino acid, but only some of them are preferred. Codon analysis of the loop L1 region of the hexon gene indicated differences in codon preference patterns between adenovirus strains representing diverse types. It was found that (C) was the most frequent nucleotide for each type ranging from RSCU 29.3 to RSCU 34.4. The analysis indicated antigenic properties of examined viruses, the presence of relative synonymous codon usage, and the presence of mutations. The effect of mutational pressure of the codon can be tested in the future in order to understand its impact on FAdV pathogenicity.

7. Adenoviruses in poultry

Inclusion body hepatitis (IBH) mainly existed in broiler chickens from certain breeder flocks and is associated with many different serotypes. Concerning the type definition is misleading because of three classification systems: US, EU and Australia [8]. These systems indicated the type number and not the species designation. In the past years, viruses reported for IBH infection belongs mainly to species/type D/2, 11, 9, 3 or E/6, 7, 8a, 8b primarily affecting the liver and in haematopoietic system and presented intra-nuclear inclusion bodies in the hepatocytes and lesions in other organs [13]. Mortality may reach 10 till 30% occasionally [2]. The other syndrome caused by adenovirus infection is hydropericardium syndrome (HS) occurring in broilers, breeding, and laying flocks in pigeons and quails [13, 42]. It is connected to an infection with an type/species C/4. In HS cases, we observed an accumulation of clear, straw-coloured fluid in the pericardial sac with pulmonary oedema and enlarged kidneys. The vaccination against C/4 is commonly used. Gizzard erosions (GE) in broilers are caused by a type/species A/1 and E/8 with first cases described in Japan [34]. Gizzards are distended with haemorrhagic fluid and contain multiple black patchy erosions within the koilin layer.

Egg drop syndrome (EDS) or duck adenovirus 1 of genus atadenovirus of the *Adenoviridae family* is responsible for the syndrome in laying hens. Outbreaks of EDS in laying birds will cause significant loss of saleable eggs, and the disease is controlled by vaccination [1].

Other adenovirus disease in poultry is haemorrhagic enteritis (HE), which is an acute viral disease in turkeys characterised by depression, bloody droppings, and death. Marble spleen disease (MSD) is related to pheasants with 3–8 months of age [20]. The virus is serologically indistinguishable from HEV with diversity only at the genomic level, causing lung oedema, congestion, dyspnoea and death, and avian adenovirus splenomegaly (AAS) in broilers [8].

8. Discussion

Clear differences appeared between strains classified to different adenovirus types/species. All the data confirmed correctness in classification of the examined strains. The analysis of adenovirus genome was mostly based on properties of hexon gene, which is the biggest gene in adenovirus genome. Hexon gene has specific nature and structure with conservative and hypervariable regions HVR1–4, and is the object of most adenovirus studies based on taxonomy and characteristic antigenic properties [9, 16, 38, 50, 57, 60, 72]. It is very difficult or nearly impossible to conduct taxonomy studies based on conservative sequence, which is very similar and almost identical in all adenovirus types/species [23, 50, 57, 60]. That is why loop L1 HVRs1–4 region of hexon gene was used for analysis by many authors.

Mutations in genes, their quantity, and localisation can influence the protein structure. Mutations on the first and second places of codon are the most important, because these mutations can influence amino acid coding and subsequently change the structure and protein function. The differences are focused mainly in loops L1 and L2. Loop L1, together with HVR1–4 regions, is the longest loop in protein with complicated folding [14]. It also serves as the location of specific receptors [39, 58, 60]. Examinations on the regions that are responsible for the antibodies binding Pichla-Gollon [57] indicated that this bridge pile is responsible for it. Hypervariable regions indicate high differentiation between adenovirus species/types and between the adenovirus strains, which can infect different hosts [57]. Simultaneously comparison study on amino acid protein hexon sequences of adenovirus strains from different species conducted by Crawford-Miksza [14] indicated that amino acid sequences of HVR1 are specific for exact adenovirus host.

HVR1 region forms the structure called 'hairpin' [68, 71]. Analysis of the HVR1 structure suggests the presence of β-sheet structure, which is created by the 'hairpin'. This structure was found in all FAdV types. HVR3, similar to HVR1, also forms 'hairpin' structure, which forms above protein surface. HVR1 and HVR3 are positioned close to each other in shape of V. The HVR5 (sequence not in the loop 1) is positioned between these V-structure arms. Study by Pichla-Gollon [57] indicated that the side of hairpin structure of HVR1 is the main region for the neutralising antibody binding. All mutations, which are situated in HVR1–4 region, can led to avoidance of host mechanisms of immunity and face modifications of these regions, which can led to create vectors for clinical treatment.

9. Conclusions and future perspectives

The adenovirus area and their mechanisms of pathogenicity are still under the exploration and depend on virus type and species. To our knowledge, the pathogenicity is mediated by

interference with other antiviral immune responses, and fowl adenoviruses are widely considered as excellent platforms for vaccine development and gene therapy. The detailed outcome of the RSCU, (HVRs1–4) from adenovirus strains was examined for the presence of similarity and mutations on the first, second, and third codon positions as well as impact on the amino acid creation, tertiary structure, and spinal conformation. Analysis indicated specific sequence in loop L1 HVR1 region, which is strictly responsible for antibody binding. This review will help better understand the mechanisms of pathogenicity of adenovirus strains and provides a guide for disease control in birds. Further studies are needed to explain the possible predisposing factors, which may lead to the pathogenicity. Moreover, the detailed study on whole genome sequence and virulence of newly isolated strains should follow.

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