

Phylogenetic and geographic analysis of fowl adenovirus field strains isolated from poultry in Poland

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Abstract Fowl adenoviruses (FAdVs) are widely distributed in chickens in Poland and throughout the world. FAdV infections have been reported in the United States, Australia, Europe, and the Mediterranean basin. Detection of FAdVs strains is very important from the epidemiological point of view and for monitoring disease outbreaks and developing strategies for vaccine development. Several molecular epidemiology and phylogenetic studies have been performed, but the results obtained are still limited, because FAdV strains, even of the same serotype, have very diverse characteristics. Some strains are pathogenic and some are nonpathogenic. This report describes the successful isolation of 96 FAdV field strains from chickens in Poland. A PCR assay specific for the L1 loop region of the hexon gene was conducted, and the products were subjected to sequence analysis. The sequences were analysed using BLAST and Geneious 6.0 software and compared to adenovirus field and reference strain sequences from different parts of the world that are accessible in the NCBI GenBank database. The sequences of the adenovirus strains indicated that they belonged to five species, *Fowl aviadenovirus A-E*, represented by eight serotypes FAdV-1, FAdV-4, FAdV-5, FAdV-7, FAdV-8a, FAdV-8b, and FAdV-2/11 (FAdV-D). The relationships between FAdVs isolated in Poland and isolates from other regions of the world were determined.

Introduction

Fowl adenoviruses (FAdVs) are distributed worldwide in chicken flocks [2]. Every year, the number of adenovirus infections in birds increases [14], but the role of these viruses in pathogenesis is not yet clear. Adenoviruses have been isolated from sick birds as well as from birds without any clinical signs of infection [6, 13]. FAdVs may cause disease independently or may be one of multiple factors leading to disease. Our knowledge about the pathogenic role of adenoviruses has improved. Some FAdV strains can be responsible for clinical manifestations of diseases such as inclusion body hepatitis (IBH) without the presence of other viruses [1]. Almost all FAdVs serotypes can be aetiological agents. Strains belonging to serotype FAdV-1, representing FAdV-A and serotype FAdV-8 of FAdV-E are responsible mainly for gizzard erosion and ulceration (GEU) [15, 16]. GEU has been associated with FAdV-A infections in chickens in Japan, Europe [9, 20], and Korea [8]. Serotype FAdV-4 (FAdV-C) can cause hydropericardium hepatitis syndrome [2]. There appears to be considerable variation in the virulence properties of members of the different FAdV species [Niczyporuk, unpublished]. Adenoviruses may induce immunodeficiency or influence the results of vaccination of chickens [14]. The serotypes of fowl adenovirus strains in Poland remain unknown. All serotypes are related in various regions of the genome but also have some specific variable regions (HVR1-7).

Due to the lack of a clear classification system, proper classification of adenoviruses can be very problematic. Many countries, including the United States, Australia, Japan, and European countries, have created their own classification systems. The serotypes are often identified by numbers such as FAdV-1 or FAdV-4 without particular specifying the classification system to which they refer.

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This situation has led to inaccuracy. Studies have been based on the International Committee on Taxonomy of Viruses (ICTV) classification system while simultaneously indicating the differences between them.

The aim of this study was to investigate the similarities and differences in the loop L1 region of the hexon gene nucleotide sequence of FAdVs isolated from poultry in Poland and those from other parts of the world.

Materials and methods

Sequences of standard FAdV strains are presented in Table 1

Ninety-six field sequences of adenovirus strains were of serotypes FAdV-7-23, 15 of serotype FAdV-8a, seven of serotype FAdV-8b, seven of serotype FAdV-5, 36 of serotype FAdV-2/11 FAdV-D, four of serotype FAdV-1, and four of serotype FAdV-4. Data concerning field adenovirus strains isolated in Poland are presented in Table 2.

Adenovirus sequences were obtained from the following countries: USA, three strains; Italy, 19 strains; Brazil, three strains; Hungary, 12 strains; France, one strain; South Korea, nine strains; South Africa, four strains; Slovenia, one strain; India, 29 strains; Malaysia, one strain; Canada, four strains; Australia, one strain; Germany, six strains; Thailand, one strain; Sweden, two strains; Iran, one strain;

Table 1 Sequences of standard adenovirus strains obtained from the NCBI GenBank database

FAdV-1	PL/060/08
FAdV-2	P7-AVR-827 (AF339915) FAdV-2 P7-A
FAdV-3	VR 828 (AF339916)
FAdV-4	KR5 (AF508951)
	J2-A VR-829 (AF339917)
	HHS
FAdV-5	T8-A VR-830 (AF339919)
	FAdV-5 T8-A
	340 (AF508952)
	TR22 (AF508953)
FAdV-6	CR119 (AF508954)
FAdV-7	X11A VR-835 (AF339920)
	YR36 (AF508955)
	B-3A VR-382 (AF339922)
	FAdV-7 VR832
FAdV-8	VR833
FAdV-8a	TR59 (AF508956)
FAdV-9	A2-A (AF339918)
FAdV-10	C-2B VR-834 (AF339923)
FAdV-11	C2B (AF508959)
FAdV-12	380 (AF339925)

UK, one strain; Austria, six strains; Pakistan, four strains; Peru, one strain; Ecuador, one strain; Kuwait, one strain; Mexico, one strain; Russia, one strain.

DNA extraction

The DNA of 96 field FAdV strains was extracted using a QIAamp Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Negative DNA controls for PCR were extracted from uninfected chicken embryo fibroblasts. The DNA was stored at -20 °C for the next step of the study as the templates for sequencing.

Primer design

Primers were designed using the programme Primer 3 on the basis of the loop L1 region of the hexon gene and were as follows: FAdVF JSN, 5'aatgtcacnaccgaraagc3'; FAdVR JSN, 5'cbgcbtrcatgtactgta3'. The position of the region of the hexon gene that was amplified was nt 178-1017.

PCR

The reaction was carried out in a final volume of 25 µL. The mixture contained 2.5 µL of 10x PCR buffer, 1 µL of dNTP (10 mM), 1.5 µL of each primer (10 µM), 2 µL of DNA template, and 11.5 µL of sterile water. After pre-denaturation at 95 °C for 5 min, 35 replication cycles were performed, with denaturation at 94 °C for 45 s, primer annealing at 55 °C for 1 min, and product elongation at 72 °C for 2 min. This was followed by a final elongation at 72 °C for 10 min. The amplification was done in a basic gradient thermocycler (Biometra, Germany).

Analysis of PCR products

After amplification, electrophoresis (150 V and 80 mA) was carried out in a 2 % agarose gel containing 1 µg of ethidium bromide per mL in Tris borate EDTA buffer (pH 8.2) for 50 min in a Mini-Sub Cell (Bio-Rad, USA). After gel electrophoresis, the sizes of the amplification products were compared with a 1031-bp MassRuler DNA ladder marker (Fermentas, USA). Bands were visualised using a UV transilluminator and then photographed and analysed. Results were considered positive if the DNA product had the predicted size of 830 bp.

Sequencing and geographical analysis

After the amplification reaction, PCR products were purified using a NucleoSpin Extract II Kit (Marcherey-Nagel, France) and sequenced using a GS FLX/Titanium

Table 2 Overview of collected adenovirus field strains at the Department of Poultry Viral Diseases, NVRI, Poland

Number	Date of isolation	Symptoms	Poultry type	Serotype/species	
1	23/07	2007	Nonsymptomatic	Layer 16 weeks	FAdV-7/FAdV-E
2	45/09	2009	Nonsymptomatic	Layer 18 weeks	FAdV-7/FAdV-E
3	50/03	2003	Nonsymptomatic	Layer 22 weeks	FAdV-7/FAdV-E
4	17/09	2009	Sadness	Broiler 4.5 weeks	FAdV-7/FAdV-E
5	72/08	2008	Nonsymptomatic	Layer, 39 weeks	FAdV-7/FAdV-E
6	19-8/10wF	2010	Depressed	Layers 24 weeks	FAdV-7/FAdV-E
7	15-14-10kij	2010	IBH clinical case	Broiler 19 weeks	FAdV-7/FAdV-E
8	14-7-10j5F	2010	Depressed	Broiler 29 days	FAdV-7/FAdV-E
9	13-7-10z4F	2010	IBH clinical case	Broiler 29 days	FAdV-7/FAdV-E
10	12-7-10w4F	2010	IBH clinical case	Broiler 29 days	FAdV-7/FAdV-E
11	11-6-10wF	2010	Crouching position	Layer 9 weeks	FAdV-7/FAdV-E
12	10-5-10jF	2010	Nonsymptomatic	Layer 17 weeks	FAdV-7/FAdV-E
13	9-5-10wF	2010	Nonsymptomatic	Layer 17 weeks	FAdV-7/FAdV-E
14	8-2-10F	2010	IBH clinical case	Broiler 6 weeks	FAdV-7/FAdV-E
15	4-82-09F	2009	Depressed	Layer 25 weeks	FAdV-7/FAdV-E
16	12/10j	2010	Depressed	Layer 37 weeks	FAdV-7/FAdV-E
17	11/10j	2010	Depressed	Layer 25 weeks	FAdV-7/FAdV-E
18	36/09	2009	Nonsymptomatic	Layer 17 weeks	FAdV-7/FAdV-E
19	119/10j	2010	Depressed	Layer 33 weeks	FAdV-2/11/FAdV-D
20	35/08	2008	IBH clinical case	Broiler 12 days	FAdV-7/FAdV-E
21	20-8-10jF	2010	Nonsymptomatic	Layer 24 weeks	FAdV-7/FAdV-E
22	20-8-10j F(2)	2010	Nonsymptomatic	Layer 24 weeks	FAdV-7/FAdV-E
23	34/08	2008	IBH clinical case	Broiler 12 days	FAdV-7/FAdV-E
24	75/08	2008	IBH clinical case	Broiler 7 weeks	FAdV-8b/FAdV-E
25	32/07	2007	Depressed	Layer 27 weeks	FAdV-8b/FAdV-E
26	47/09	2009	IBH clinical case	Broiler 7 weeks	FAdV-7/FAdV-E
27	60/10z	2010	Depressed	Layer 12 weeks	FAdV-8b/FAdV-E
28	102/10j	2010	Digestive system dysfunction	Layer 35 weeks	FAdV-8b/FAdV-E
29	5-81-09F	2009	Nonsymptomatic	Layer 26 weeks	FAdV-8a/FAdV-E
30	5-81-09F(2)	2009	Nonsymptomatic	Layer 26 weeks	FAdV-8a/FAdV-E
31	51/04	2004	Nonsymptomatic	Layer 27 weeks	FAdV-8a/FAdV-E
32	96/10j	2010	IBH clinical case	Broiler 6 weeks	FAdV-8a/FAdV-E
33	17/10jF	2010	Nonsymptomatic	Layer 20 weeks	FAdV-8a/FAdV-E
34	38-10(1)R	2010	Nonsymptomatic	Layer 38 weeks	FAdV-8a/FAdV-E
35	7-1-10F	2010	IBH clinical case	Broiler 3 weeks	FAdV-8a/FAdV-E
36	37/10z	2010	IBH clinical case	Broiler 8 weeks	FAdV-8a/FAdV-E
37	6-86-09BF	2009	Digestive system dysfunction	Layer 28 weeks	FAdV-8a/FAdV-E
38	35/09	2009	IBH clinical case	Broiler 12 days	FAdV-8a/FAdV-E
39	48/08	2008	IBH clinical case	Broiler 8 days	FAdV-8a/FAdV-E
40	8/08	2008	Digestive system dysfunction	Layer 26 weeks	FAdV-8a/FAdV-E
41	94/10j	2010	Digestive system dysfunction	Layer 12 weeks	FAdV-8a/FAdV-E
42	94/10z	2010	Nonsymptomatic	Layer 12 weeks	FAdV-8a/FAdV-E
43	46/10jR	2010	IBH clinical case	Broiler 5 weeks	FAdV-8a/FAdV-E
44	10/10jF	2010	Nonsymptomatic	Layer 19 weeks	FAdV-8b/FAdV-E
45	16-14-10k2jF	2010	IBH clinical case	Broiler 29 days	FAdV-8b/FAdV-E
46	16-1410k2jF(2)	2010	IBH clinical case	Broiler 19 weeks	FAdV-8b/FAdV-E
47	43/10w	2010	IBH clinical case	Broiler 6 weeks	FAdV-2/11, FAdBV-D
48	22/10wF	2010	IBH clinical case	Broiler 2, 5 weeks	FAdV-2/11/FAdV-D

Table 2 continued

Number		Date of isolation	Symptoms	Poultry type	Serotype/species
49	46/09	2009	Nonsymptomatic	Layer 49 weeks	FAdV-2/11/FAdV-D
50	32/10j	2010	Nonsymptomatic	Layer 16 weeks	FAdV-2/11/FAdV-D
51	18-15-10jF11F	2010	IBH clinical case	Broiler 4, 5 weeks	FAdV-2/11/FAdV-D
52	121/10j	2010	Nonsymptomatic	Layer 17 weeks	FAdV-2/11/FAdV-D
53	117/10j	2010	Digestive system dysfunction	Broiler 6, 5 weeks	FAdV-2/11/FAdV-D
54	24-6-11w	2011	Nonsymptomatic	Layer 3 weeks	FAdV-2/11/FAdV-D
55	22-4-11w	2011	Nonsymptomatic	Layer 20 weeks	FAdV-2/11/FAdV-D
56	54-10w3	2010	IBH clinical case	Broiler 4 weeks	FAdV-2/11/FAdV-D
57	25/09	2009	Nonsymptomatic	Layer 29 weeks	FAdV-2/11/FAdV-D
58	Imielno	2007	Nonsymptomatic	Layer 18 weeks	FAdV-2/11/FAdV-D
59	17-15-10jF6F	2010	Digestive system dysfunction	Broiler 4, 5 weeks	FAdV-2/11/FAdV-D
60	2-68-09F	2009	IBH clinical case	Broiler 7 week	FAdV-2/11/FAdV-D
61	42-10wR	2010	IBH clinical case	Broiler 21 days	FAdV-2/11/FAdV-D
62	86/09j	2009	Nonsymptomatic	Layer 28 weeks	FAdV-2/11/FAdV-D
63	49-10F-6-	2010	Nonsymptomatic	Broiler 6 weeks	FAdV-2/11/FAdV-D
64	38-10(2)	2010	Nonsymptomatic	Layer 38 weeks	FAdV-2/11/FAdV-D
65	105-10TF	2010	Nonsymptomatic	Broiler 8 weeks	FAdV-2/11/FAdV-D
66	87/10j	2010	IBH clinical case	Broiler 6, 5 weeks	FAdV-2/11/FAdV-D
67	87/10z	2010	IBH clinical case	Broiler 6, 5 weeks	FAdV-2/11/FAdV-D
68	25-119-10guz-w	2010	Nonsymptomatic	Layer 33 weeks	FAdV-2/11/FAdV-D
69	65/10w	2010	IBH clinical case	Broiler 3 weeks	FAdV-2/11/FAdV-D
70	66-10-(1-60)	2010	IBH clinical case	Broiler 2 weeks	FAdV-2/11/FAdV-D
71	67-10zoltko	2010	nonsymptomatic	Layer 17 weeks	FAdV-2/11/FAdV-D
72	69/10w	2010	IBH clinical case	Broiler 4, 5 weeks	FAdV-2/11/FAdV-D
73	70/10z	2010	Nonsymptomatic	Layer 14 weeks	FAdV-2/11/FAdV-D
74	74/10j	2010	Nonsymptomatic	Layer 14 weeks	FAdV-2/11/FAdV-D
75	75/10z	2010	IBH clinical case	Broiler 8 days	FAdV-2/11/FAdV-D
76	49-10N	2010	IBH clinical case	Broiler 6 weeks	FAdV-2/11/FAdV-D
77	29/07	2007	Nonsymptomatic	Layer 27 weeks	FAdV-2/11/FAdV-D
78	19/10j	2010	Dead birds	Broiler 8 days	FAdV-2/11/FAdV-D
79	20/10F	2010	Dead birds	Broiler 13 days	FAdV-2/11/FAdV-D
80	21-10F1F	2010	IBH clinical case	Broiler 2 weeks	FAdV-2/11/FAdV-D
81	21-10F2F	2010	IBH clinical case	Broiler 2 weeks	FAdV-2/11/FAdV-D
82	131-10Tj	2010	IBH clinical case	Broiler 5 weeks	FAdV-5/FAdV-B
83	127-10TF	2010	IBH clinical case	Broiler 5 weeks	FAdV-5/FAdV-B
84	126/10j	2010	Nonsymptomatic	Broiler 5 weeks	FAdV-5/FAdV-B
85	88/10z	2010	Nonsymptomatic	Broiler 6 weeks	FAdV-5/FAdV-B
86	88/10j	2010	Nonsymptomatic	Broiler 6 weeks	FAdV-5/FAdV-B
87	21-133-10j	2010	IBH clinical case	Broiler 6 weeks	FAdV-5/FAdV-B
88	45/10jR	2010	IBH clinical case	Broiler 4, 5 weeks	FAdV-5/FAdV-B
89	1-66-09F	2009	IBH clinical case	Broiler 6, 5 weeks	FAdV-1/FAdV-A
90	27/10jF	2010	Nonsymptomatic	Layer 14 weeks	FAdV-1/FAdV-A
91	110/10z	2010	IBH clinical case	Broiler 2 days	FAdV-1/FAdV-A
92	14/08	2008	Nonsymptomatic	Layer 27 weeks	FAdV-1/FAdV-A
93	35/10w	2010	IBH clinical case	Broiler 2 weeks	FAdV-4/FAdV-C
94	62/10z	2010	Nonsymptomatic	Layer 12 weeks	FAdV-4/FAdV-C
95	31/10z	2010	Nonsymptomatic	Layer 27 weeks	FAdV-4/FAdV-C
96	64/10j	2010	Nonsymptomatic	Layer 14 weeks	FAdV-4/FAdV-C

Fig. 1 Phylogenetic and geographic analysis based on the 560-nt loop L1 region of the hexon gene. Nine unique reference sequences representing serotypes FAdV-7 VR832, FAdV-11 X11, FAdV-7 YR36, FAdV-7 B-3A, FAdV-6 CR119, FAdV-8a TR59, FAdV-10 VR835, FAdV-5 VR830, and FAdV-5 T8-A and 46 field FAdV strains from Poland and 23 strains from different countries were analyzed. The phylogenetic tree was generated by the NJ method. The robustness of the tree was determined by bootstrapping of multiple sequence alignments (1000 sets)

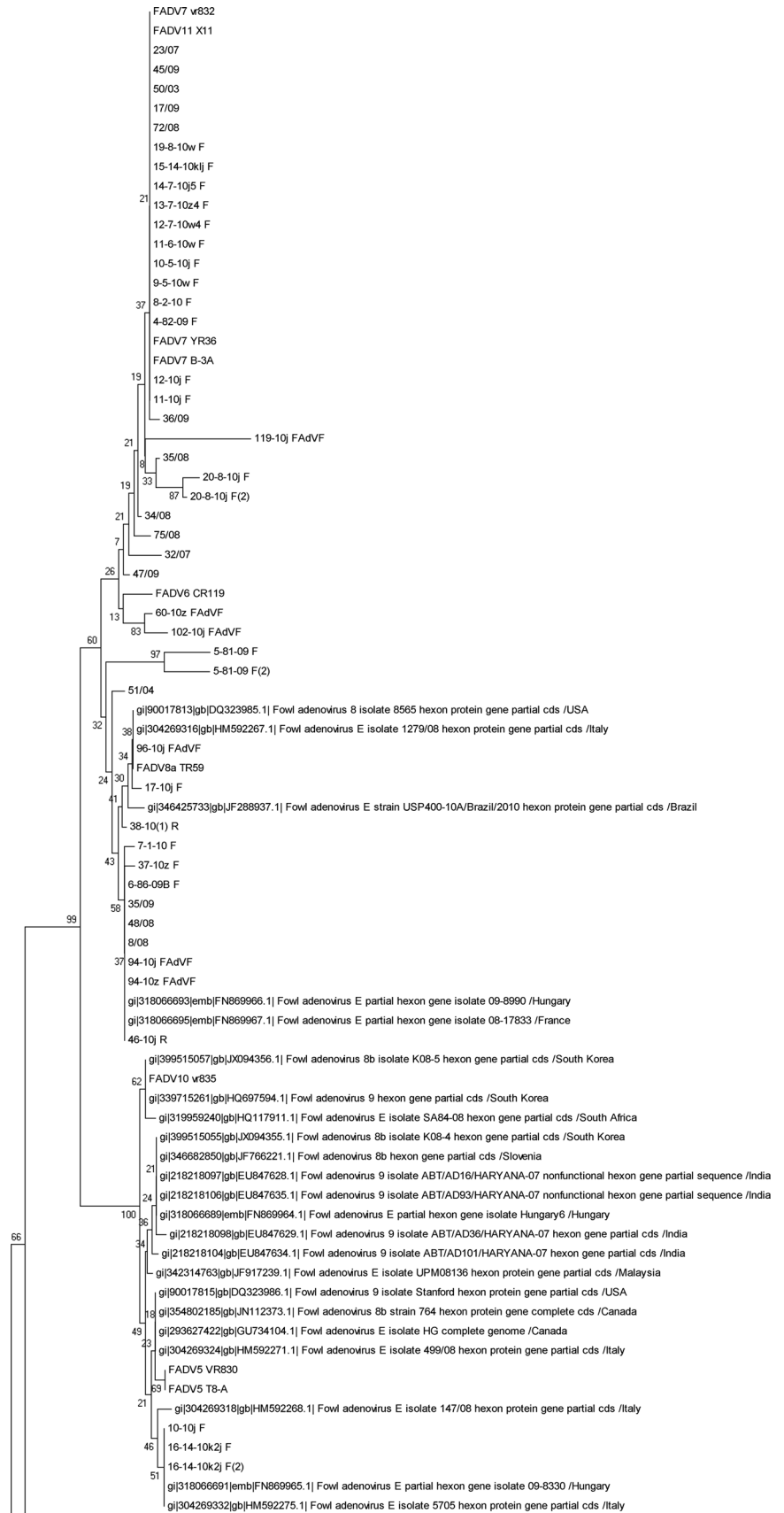
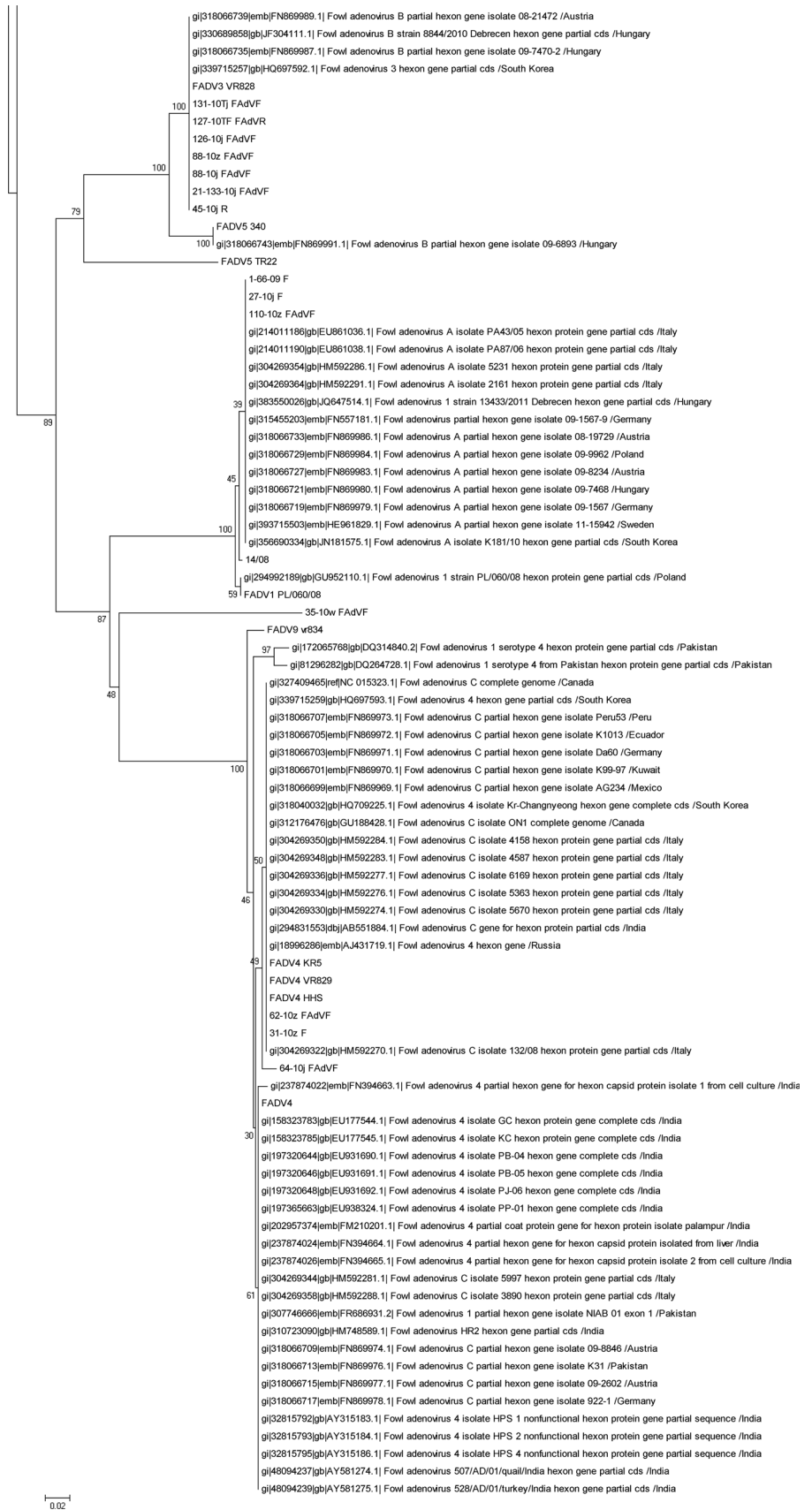


Fig. 2 Phylogenetic and geographic analysis based on the 560-nt loop L1 region of the hexon gene. Nine reference nucleotide sequences representing serotypes FAdV-12 380, FAdV-11 C2B, FAdV2 VR827, FAdV-2 P7-A, FAdV-6 VR831, FAdV-8 VR833, FAdV-3 VR828, FAdV-5 340, and FAdV-5 TR22 and 42 field FAdV strains from Poland and 32 strains from different countries were analyzed. The phylogenetic tree was generated by the NJ method. The robustness of the tree was determined by bootstrapping of multiple sequence alignments (1000 sets)



Fig. 3 Phylogenetic and geographic analysis based on the 560-nt loop L1 region of the hexon gene. Nine reference nucleotide sequences representing serotypes FAdV-3 VR828, FAdV-5 340, FAdV-5 TR22, FAdV-1 PL/06/08, FAdV-9 VR834, FAdV-4 KR5, FAdV-4 VR829, FAdV-4 HHS, an FAdV-4 and 15 field FAdV strains from Poland and 61 strains from different countries were analyzed. The phylogenetic tree was generated by the NJ method. The robustness of the tree was determined by bootstrapping of multiple-sequence alignments (1000 sets)



sequencer (Roche, Switzerland) at GENOMED (Warsaw). The sequences were aligned with fowl adenovirus reference sequences and field adenovirus sequences isolated from different countries and continents obtained from the GenBank database, identified according to the general obligatory classifications. A phylogenetic tree was generated by the neighbour-joining method, using the p-distance method (on 1000 bootstrapped datasets). The analyses were performed using the computer software MEGA5, Genius 6.2, and BLAST. On the basis of this analysis, the phylogenetic tree and the relationship between the field adenovirus strains isolated in Poland and those obtained from different countries were determined.

Results

Phylogenetic analysis was conducted on the basis of the loop L1 region of hexon gene sequences of 96 field adenovirus strains and 113 sequences of adenovirus strains from different countries obtained from the GenBank database (NCBI). The database contained 191 fragments and complete sequences of hexon genes for which the country of origin had been identified. The phylogenetic relationships of such a large number of strains could be very difficult to analyze. Therefore, sequences lacking loop L1 were omitted, and one sequence per country was analyzed unless the particular serotype had differences in more than one locus. The remaining 116 sequences were analyzed by comparative methods. Alignment of the examined nucleotide sequences was done with ClustalW (MEGA5), and construction of a phylogenetic tree was done by the neighbour-joining method in MEGA5) Phylogenetic trees (Figs. 1, 2, 3) were analysed with regard to the similarity of sequences of field strains and sequences available in the GenBank database. Six of the adenovirus strains were classified as serotype FAdV-1 in a previous study [14], and four of them (66/09, 27/10, 110/10z, 14/08) are presented in a phylogenetic tree (Fig. 3). These were similar to 14 sequences obtained from GenBank representing serotype FAdV-1, species *Fowl aviadenovirus A*. The strains originated from Poland (one sequence), Germany (two sequences), Austria (two sequences), Hungary (two sequences), Sweden (one sequence), Italy (four sequences), and South Korea (one sequence). Similarity in this group was about 98.3 %. It is very interesting that the sequence of the strain obtained from South Korea was in 99.8 % identical to one from Germany despite the fact that they were from completely different geographical locations. Only one nucleotide sequence difference was detected at position 307.

A group of 50 strains, including four sequences of field strains (35/10w, 62/10z, 31/10z, and 64/1), were classified

as serotype FAdV-4 (species *Fowl aviadenovirus C*) and showed 97.4 % internal similarity. These included 41 strains from the GenBank database from Austria (two sequences), Ecuador (one sequence), India (17 sequences), Canada (two sequences), Kuwait (one sequence), Mexico (one sequence), Germany (two sequences), Pakistan (four sequences), Peru (one sequence), South Korea (one sequence), Russia (one sequence), and Italy (eight sequences), as well as five reference sequences: FAdV-4 KR5, FAdV-4 J2-A VR-829, FAdV-4 HHS, FAdV-4, and FAdV-9 C-2B VR-834. On the basis of the quantity of loop L1 sequences of adenovirus strains derived from birds from different regions of the world, the clade representing serotype FAdV-4 is very good example for the presence of variability sequences depending on geographical origin. Strains from India within the group showed 99.2 % identity, and strains from Europe showed 98.5 % identity. Strains from the USA, Canada, and Mexico showed 99.2 % identity, and strains from Asia, 96.3 %. The examined strains formed three subgroups. The first one included 17 sequences of adenovirus strains from eight countries obtained from GenBank database, three strains from this study (62/10z, 31/10z and 64/10j), and three reference strains (FAdV-9 C2B VR-834, FAdV-4 J2-A VR-829, and FAdV-4 KR5). The 23 sequences showed 97.9 % inter-group similarity. Another group was formed by 24 sequences of adenovirus strains with 99.1 % similarity between them. This group included strains from Europe, Asia, and the Middle East. Another small group included the two strains from Pakistan: GenBank DQ314840 and DQ264728, with 98.4 % similarity. The strains were not grouped according to their geographic regions. Strains originating from different geographical locations, for example, Mexico and India, showed closer similarity than some strains from the same country.

Three FAdV-B strains from Hungary, one from Austria, and one from South Korea, as well as seven FAdV field strains and reference strain FAdV-5 IBH-2A VR-828 showed 99.4 % similarity. One strain from Hungary, with 100 % identity to FAdV-5 340, belongs to the species *Fowl aviadenovirus B*. These strains formed a common group representing serotype FAdV-B, with 87.0 % similarity. However, differences were observed between the FAdV-5 340, FAdV-5 TR22 and FAdV-5 IBH-2A strains.

The group represented by FAdV-2/11 (species *Fowl aviadenovirus D*) was divided into separate subgroups. The first subgroup included 26 sequences of FAdV strains with inter-subgroup similarity of 96.1 % and nineteen field strains and strains from different countries: Brazil, Germany (one sequence), Iran, Sweden, Hungary, Great Britain (one sequence), Italy (one sequence). The second subgroup consisted of 52 strains, including 32 field strains from this study, 17 isolates from different countries

(Austria, one sequence; Brazil, one sequence; India, six sequences; Germany, one sequence; Poland, two sequences; South Africa, one sequence; South Korea, one sequence; USA, one sequence; Thailand, one sequence; and reference strains FAdV-2 P7-A and FAdV-12 380, FAdV-11 C2B, which are 100 % identical. The similarity among all 63 sequences belonging to the species *Fowl aviadenovirus D* was 92.9 %.

The group represented by serotype FAdV-8a (species *Fowl aviadenovirus E*) included 27 sequences: 21 field strains from this study, five each from Brazil, France, USA, Hungary, and Italy, as well as reference strain FAdV-8a TR59. The similarity in this group was about 91.9 % and was lower than in the other subgroups. The differences in the examined sequences in this group were due to the very low similarity between subgroups and strains (81/09j, 81/09w and 24/12z).

Separate branch were created by 28 sequences with 97.8 % similarity grouped in smaller branches. The group included eight sequences from this study, 18 sequences obtained from GenBank, and reference sequences of adenovirus strains FAdV-5 T8-A and FAdV-10 X11A. Most of the sequences from GenBank were denoted as serotype FAdV-9 or FAdV-8b, which indicates that all strains from this group are strains of FAdV-8b according to the ICTV classification. Strain T8A is usually classified as FAdV-8a rather than FAdV-5. The similarity to FAdV-8a T8-A is not correct, and it can be assumed that the serotype determination of FAdV-8a T8-A was wrong, especially considering the differences between serotypes FAdV-8a TR59 and FAdV-8a T8-A. It seems correct to classify these isolates as strains representing serotype FAdV-8b.

The group represented by serotype/species FAdV-7/ FAdV-E did not allow a lineage to be determined. It consisted of 22 field strains and three reference strains: FAdV-7 B3-A VR-832, FAdV-11 X11, and FAdV-7 YR36, with 92.4 % similarity.

Discussion

Phylogenetic trees based on the loop L1 sequences of the hexon gene confirmed the previously determined serotype classification. It also indicated differences among strains representing different serotypes [14]. This region was found in a previous study to be very useful for type-level identification of FAdVs [10, 12]. Close similarities between members of the species *Fowl aviadenovirus D* and *Fowl aviadenovirus E* were described previously by Grgic et al. [5] and Marek et al. [11], and, more recently, by Günes et al. [4].

Hexon gene sequencing analysis was conducted. The hexon gene is the longest gene in the adenovirus genome.

Because of its specific nature and structure (with regions that are strictly conserved and hypervariable regions located in Loop L1 (HVR1-4), the hexon gene is often a target of research on taxonomy and antigenic properties of FAdVs [3, 12, 17, 21]. Conserved sequences that are very similar or almost identical in every FAdV serotype cannot be used for strain discrimination [3, 12, 17, 18].

Ninety-six nucleotide sequences of the hexon gene were generated and compared to reference sequences of FAdVs and sequences from other countries. The results of this analysis indicated that all of the examined strains belonged to the genus *Aviadenovirus*, and their sequences match the sequences of loop L1 of adenovirus strains isolated from birds [18]. The next step of the study was nucleotide sequence alignment and construction of a phylogenetic tree, using a simpler method for identification of FAdV strains/serotypes [12, 18]. The sequences formed five main branches representing the species *Fowl aviadenovirus A-E* with eight serotypes. In a previous study in Poland, the presence of five serotypes was shown [19].

The biggest group included 51 field strains similar to serotype FAdV-2/11 (species *Fowl aviadenovirus D*). They were found mostly in broiler and breeder flocks. FAdV-7 and FAdV-8a serotypes (species *Fowl aviadenovirus E*) were less frequent. These results are similar to results from Hungary [7], where isolated adenovirus strains frequently belonged to the species *Fowl aviadenovirus D* or *Fowl aviadenovirus E*. Serotype FAdV-1 was even more infrequent [8]. The rarest serotype isolated in Poland was serotype FAdV-4, which was found in only 2.9 % of the examined strains; however, it was never isolated in Hungary [7]. On the other hand, serotypes FAdV-3, FAdV-6, FAdV-9, and FAdV-10 have not been identified in Poland.

The next step of the study was the analysis of isolated strains and comparison with the reference strains. The similarity of nucleotide sequences of strains belonging to the same serotype (within the group) was between 89.7 % and 93.0 %, which was comparable to the data reported by Kajan et al. [7]. However, differences among strains representing different species were found. The data confirmed the relationships and classification of the examined field strains to exact serotypes.

The study demonstrated very low differences among strains representing the same serotypes from different countries. The loop L1 fragment of the hexon gene is the region with HVR1, HVR2, HVR3, and HVR4 fragments examined by Niczyporuk [14] and also with the HVR5, HVR6, and HVR7 fragments examined by Pichla-Golon et al. [17]. HVR1 is the region responsible for eliciting neutralising antibodies. It has been noted that there are numerous differences in this region between strains from different countries and continents. However, the similarity between strains from countries as far away as Germany and

South Korea could be up to 99.8 %. The study indicated that the differences in loop L1 of hexon gene can be connected to serotypes, but a certain sequence is constant for a serotype and independent of the geographic distance.

To conclude, this study allowed the creation of a phylogenetic tree that confirmed the previous classification of FAdV strains [14] and indicated differences between loop L1 sequences of hexon genes from different serotypes/species. There was low divergence between strains of the same serotype from different countries. Loop L1 is the most variable region in the adenovirus genome, and HVR1 is the region that is strictly responsible for antibody binding. We could suspect more differences in this region between strains isolated from countries that are far away from each other like Germany and South Korea; however, the similarity between two of these strains was up to 99.8 %. These small differences exist independently of the serotype. This study demonstrated that the divergence of the examined loop L1 region of the hexon gene is strictly related to differences between serotypes, but each sequence is characteristic for its serotype rather than its geographic origin.

Compliance with ethical standards

Conflict of interest The author declares no competing interests.

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