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# Current epidemiological situation in the context of inclusion body hepatitis in poultry flocks in Poland

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ABSTRACT

The research has been undertaken to understand the spreading of adenovirus strains in Poland's poultry flocks in the last six years. One hundred and forty-nine herds suspected of infection with adenoviruses were tested and the presence of poultry adenoviruses was found in 86 studied herds which were about 57,71% of examined flocks. Thirty-eight (44.18%) strains were connected with the infection of inclusion body hepatitis, 11 (12.79%) strains were isolated from digestive system dysfunction, 33 (38.37%) strains had been obtained from the flocks with no symptomatic changes/behaviour, and four (4.65%) strains were obtained from flocks with the manifestation of depression. Sequencing analysis was based on Loop L1 region of the HVR1–4 fragment of the hexon gene. The adenovirus strains were classified into five species FAdV-A-E, belonging to the following eight serotypes: FAdV-1/A, FAdV-5/B, FAdV-3/D, FAdV-2/11/D, FAdV-10/C, and FAdV-7/8a/E. The most common serotype in poultry turned out to be type/species FAdV-2/11/D, FAdV-5/B, and FAdV-7/8b/E while the least frequent was type/species FAdV-10/C (only two strains respectively of this type were isolated with the following range: FAdV-1/A 6 (6.97%), FAdV-5/B 24 (27,90%), FAdV-3/D 4 (4,65%), FAdV-10/C 2 (2,32%), FAdV-2/11/D 36 (41,86%), and FAdV-E 14 (16.27%). The understanding of genetic diversity, geographic distribution, and antigenic properties of fowl adenovirus strains (FAdVs) isolated in Poland have been evaluated.

## 1. Introduction

The first avian adenoviruses had been isolated from *Colinus virginianus* over 71 years ago during the bronchitis infection (Ono et al., 2001) and since that time the viruses have been investigated. The problem of adenovirus infections from the *Adenoviridae* family, widespread in poultry flocks in the country, is an ongoing (critical) issue and the epidemiological situation shows an upward trend. The pathogenic role of poultry adenoviruses is still not clear. Strains belonging to the same type show diverse pathogenicity (Grafl et al., 2012). Fowl adenoviruses (FAdVs) are non-enveloped, double-stranded DNA (dsDNA) viruses with a icosahedral virion, about 80 nm in diameter (Fitzgerald, 2020), belongs to the genus *Aviadenovirus*, and can infect different avian species of poultry and wild birds worldwide (Fitzgerald, 2020). Adenoviruses in poultry were classified into five different species FAdV (A-E) based on the restriction enzyme digestion pattern [47] and using their molecular/genetic structure further divided into 12 serotypes (FAdV-1–8a-8b-11) [(Hess, 2017; Kajan et al., 2013), 46].

The association of FAdVs with chickens present inclusion body hepatitis, (IBH) mainly due to species D and E (Hess, 2017; Kumar et al., 2016; Reece and Pass, 1985), gizzard erosion and ulceration (GE) associated with species A (Harrach et al., 2019; Kumar et al., 2010; Oliver-Ferrando et al., 2016; Redondo et al., 2018), hydropericardium hepatitis syndrome (HHS) with species C (Kiss et al., 2021), immunosuppression as a primary aetiologic agents (Hess, 2017), and even as secondary infections by other viruses/companion viruses (Eregae et al., 2014; Niczyporuk et al., 2013) is well established. Some of the adenovirus strains are ubiquitous among poultry and can be isolated from healthy birds.

The most common disease caused by FAdVs is IBH, with lesions that are pale, friable, and swollen on the liver (Fitzgerald, 2020; Mo, 2021; Zhao et al., 2015). In broiler chickens, we can observe lesions including atrophy of the bursa of Fabricius and thymus. Chickens presenting poor feed conversion and low weight gain might have slightly elevated mortality, however, in some cases, might reach 30% especially in younger birds. Small white foci can be seen on the liver surface

*Abbreviations:* FAdV, Fowl adenoviruses; IBH, inclusion body hepatitis; GE, gizzard erosion and ulceration; HHS, hydropericardium hepatitis syndrome; HVR, hyper variable regions; aa, amino acid; nt, nucleotide.

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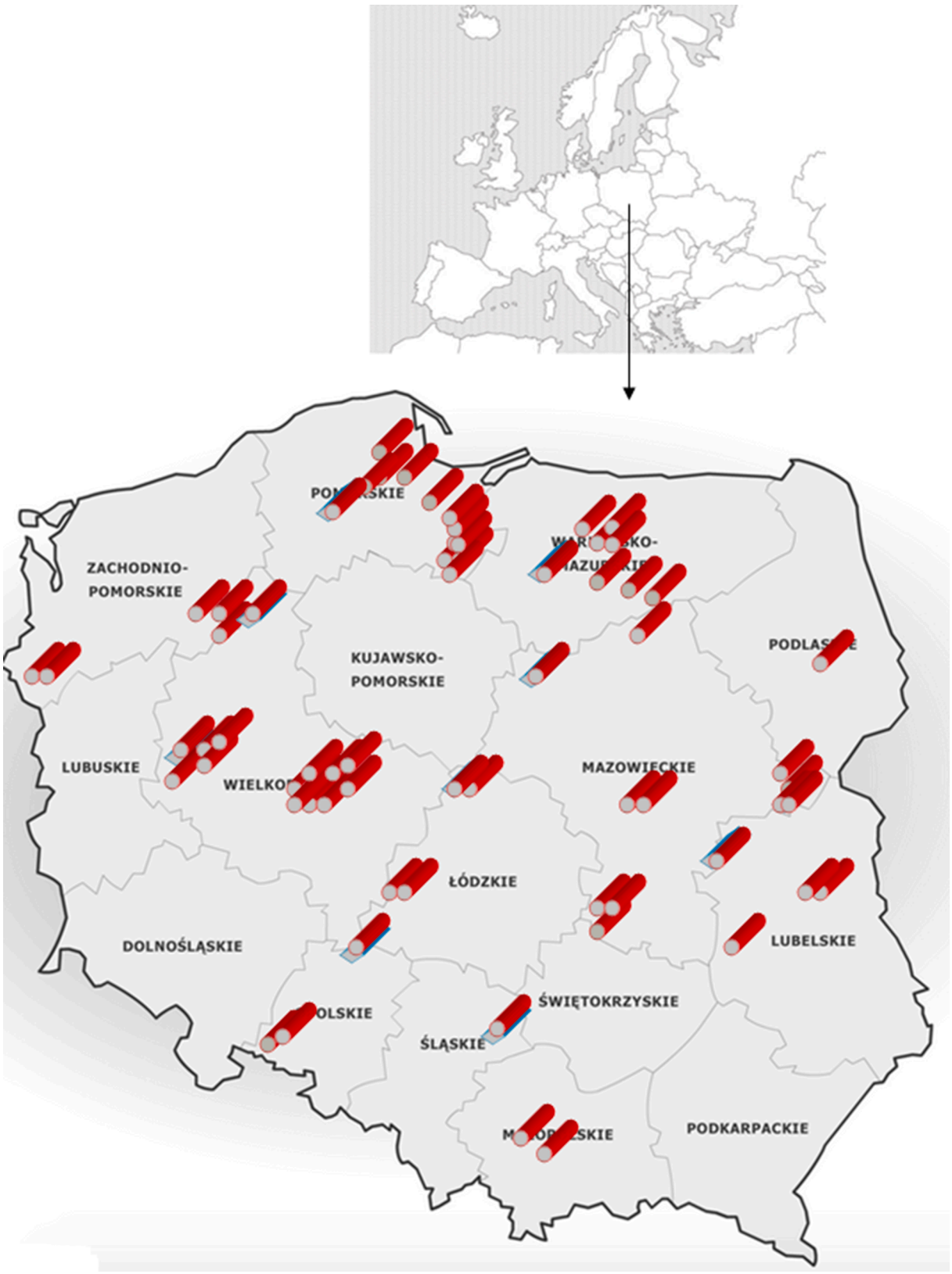


**Fig. 1.** Geographic distribution of the examined flocks.

(Niczyporuk, 2016). We can mainly observe petechial and ecchymotic haemorrhages on the liver. Birds have been more susceptible to higher mortality which is strictly connected with the severe metabolic imbalance and possible destruction of the pancreas. Both vertical and horizontal transmission are possible (Fitzgerald, 2020).

Flock mortality increases due to starvation and dehydration in IBH-infected cases. These clinical manifestations are presented in birds from 3 to 8 weeks old. Therefore, FAdVs is a serious health problem for broiler

**Table 1**
A Table summarizing the adenovirus strain sequences detected and investigated presented/described in connection with the description of the metadata.

| | Number | Date of Isolation | Symptoms | Poultry type | type/species |
|---|---|---|---|---|---|
| 1 | 1-ZCHD-PL18–1-L | 2018 | IBH clinical case | Broiler 9 days | FAdV-1/A |
| 2 | 2-ZCHD-PL21–1-L | 2021 | IBH clinical case | Layer 3 weeks | FAdV-5/B |
| 3 | 3-ZCHD-PL21–2-L | 2021 | Nonspecific symptoms | Broiler 3 weeks | FAdV-5/B |
| 4 | 4-ZCHD-PL21–3-G | 2021 | IBH clinical case | Broiler 5 weeks | FAdV-5/B |
| 5 | 5-ZCHD-PL 18–2-I | 2018 | Nonspecific symptoms | Broiler 6 weeks | FAdV-5/B |
| 6 | 6-ZCHD-PL18–3-G | 2018 | Nonspecific symptoms | Broiler 2,5 weeks | FAdV-1/A |
| 7 | 7-ZCHD-PL18–4-L | 2018 | IBH clinical case | Broiler 9 days | FAdV-5/B |
| 8 | 8-ZCHD-PL18–5-I | 2018 | IBH clinical case | Broiler 3,5 weeks | FAdV-3/D |
| 9 | 9-ZCHD-PL21–4-L | 2021 | Nonspecific symptoms | Broiler 14 days | FAdV-2/11/D |
| 10 | 10-ZCHD-PL21–5-S | 2021 | IBH clinical case | Broiler 3 weeks | FAdV-5/B |
| 11 | 11-ZCHD-PL18–6-L | 2018 | IBH clinical case | Broiler 7 days | FAdV-2/11/D |
| 12 | 12-ZCHD-PL18–7-S | 2018 | IBH clinical case | Broiler 14 days | FAdV-2/11/D |
| 13 | 13-ZCHD-PL18–8-S | 2018 | Digestive system dysfunction | Broiler 3 weeks | FAdV-7/E |
| 14 | 14-ZCHD-PL18–9-L | 2018 | Digestive system dysfunction | Broiler 3 weeks | FAdV-7/E |
| 15 | 15-ZCHD-PL20–1-S | 2020 | Digestive system dysfunction | Broiler 3 weeks | FAdV-5/B |
| 16 | 16-ZCHD-PL19–1-L | 2019 | Depressed | Broiler 5 weeks | FAdV-D |
| 17 | 17-ZCHD-PL20–2-L | 2020 | Nonspecific symptoms | Layer 36 weeks | FAdV-5/B |
| 18 | 18-ZCHD-PL-20–3-L | 2020 | Nonspecific symptoms | Broiler 2 weeks | FAdV-5/B |
| 19 | 19-ZCHD-PL1–21–6-A-L | 2021 | IBH clinical case | Layer 40 weeks | FAdV-5/B |
| 20 | 20-ZCHD-PL-21–7-G | 2021 | Nonspecific symptoms | Broiler 14 days | FAdV-2/11/D |
| 21 | 21-ZCHD-PL-20–4-L | 2020 | IBH clinical case | Broiler 5 weeks | FAdV-2/11/D |
| 22 | 22-ZCHD-PL-18–10-S | 2018 | Digestive system dysfunction | Broiler 3 weeks | FAdV-2/11/D |
| 23 | 23-ZCHD-PL-18–11-L | 2018 | Digestive system dysfunction | Broiler 3 weeks | FAdV-2/11/D |
| 24 | 24-ZCHD-PL-18–12-L | 2018 | Depressed | Broiler 2 weeks | FAdV-7/E |
| 25 | 25-ZCHD-PL-18–13-L | 2018 | Nonsymptomatic | Broiler 7 days | FAdV-1/A |
| 26 | 26-ZCHD-PL-21–14-G | 2018 | Digestive system dysfunction | Broiler 3 weeks | FAdV-2/11/D |
| 27 | 27-ZCHD-PL-17–1-L | 2017 | IBH clinical case | Broiler 14 days | FAdV-2/11/D |
| 28 | 28-ZCHD-PL-18–15-S | 2018 | Depressed | Layer 43 weeks | FAdV-2/11/D |
| 29 | 29-ZCHD-PL-18–16-L | 2018 | IBH clinical case | Broiler 9 days | FAdV-2/11/D |
| 30 | 30-ZCHD-PL-20–5-L | 2020 | IBH clinical case | Broiler 42 days | FAdV-5/B |
| 31 | 31-ZCHD-PL-20–6-G | 2020 | IBH clinical case | Broiler 42 days | FAdV-7/E |
| 32 | 32-ZCHD-PL-20–7-I | 2020 | IBH clinical case | Broiler 42 days | FAdV-7/E |
| 33 | 33-ZCHD-PL-20–8-G | 2020 | Nonspecific symptoms | Broiler 17 days | FAdV-7/E |
| 34 | 34-ZCHD-PL-21–8-I | 2021 | Nonspecific symptoms | Broiler 4 weeks | FAdV-2/11/D |
| 35 | 35-ZCHD-PL-21–9-I | 2021 | Nonspecific symptoms | Broiler 14 days | FAdV-2/11/D |
| 36 | 36-ZCHD-PL-21–10-G | 2021 | Nonspecific symptoms | Broiler 4 weeks | FAdV-2/11/D |
| 37 | 37-ZCHD-PL-21–11-G | 2021 | Nonspecific symptoms | Broiler 14 days | FAdV-2/11/D |
| 37 | 38-ZCHD-PL-20–9-S | 2020 | IBH clinical case | Broiler 42 days | FAdV-7/E |
| 39 | 39-ZCHD-PL-20–10-S | 2020 | Depressed | Layer 14 weeks | FAdV-8a/E |
| 40 | 40-ZCHD-PL-18–17-G | 2018 | IBH clinical case | Broiler 23 days | FAdV-2/11/D |
| 41 | 41-ZCHD-PL-18–18-G | 2018 | IBH clinical case | Broiler 5 weeks | FAdV-D |
| 42 | 42-ZCHD-PL-18–19-I | 2018 | Nonspecific symptoms | Layer 17 weeks | FAdV-D |
| 43 | 43-ZCHD-PL-18–20-L | 2018 | Nonspecific symptoms | Layer 17 weeks | FAdV-D |
| 44 | 44-ZCHD-PL-18–21-I | 2018 | Nonspecific symptoms | Broiler 3 weeks | FAdV/2/11/D |
| 45 | 45-ZCHD-PL-18–22-L | 2018 | IBH clinical case | Broiler 42 days | FAdV/8a/E |
| 46 | 46-ZCHD-PL-18–23-I | 2018 | IBH clinical case | Broiler 9 days | FAdV/3/D |
| 47 | 47-ZCHD-PL-20–11-S | 2020 | IBH clinical case | Broiler 5 weeks | FAdV/2/11/D |
| 48 | 48-ZCHD-PL-18–24-L | 2018 | IBH clinical case | Broiler 3 weeks | FAdV/1/A |
| 49 | 49-ZCHD-PL-21–12-I | 2021 | IBH clinical case | Broiler 2 weeks | FAdV-5/B |
| 50 | 50-ZCHD-PL-21–13-I | 2021 | IBH clinical case | Broiler 3 weeks | FAdV-5/B |
| 51 | 51-ZCHD-PL-21–14-I | 2021 | IBH clinical case | Broiler 42 days | FAdV-2/11/D |
| 52 | 52-ZCHD-PL-21–15-C-L | 2021 | IBH clinical case | Broiler 12 days | FAdV-5/B |
| 53 | 53-ZCHD-PL-21–16-L | 2021 | Nonsymptomatic | Broiler 5 weeks | FAdV-5/B |
| 54 | 54-ZCHD-PL4–19–2-I | 2019 | Digestive system dysfunction | Broiler 14 days | FAdV-11/D |
| 55 | 55-ZCHD-19–3-L | 2019 | Digestive system dysfunction | Broiler 14 days | FAdV-11/D |
| 56 | 56-ZCHD-PL-20–12-L | 2020 | Digestive system dysfunction | Layer 8 weeks | FAdV-5/B |
| 57 | 57-ZCHD-PL-20–13-L | 2020 | Digestive system dysfunction | Broiler 3 weeks | FAdV-5/B |
| 58 | 58-ZCHD-PL-21–17-S | 2021 | Digestive system dysfunction | Broiler 42 days | FAdV-8a |
| 59 | 59-ZCHD-PL-15–1-G | 2015 | Nonspecific symptoms | Layers 41 weeks | FAdV-D |
| 60 | 60-ZCHD-PL-20–14-S | 2020 | Nonspecific symptoms | Broiler 42 days | FAdV-7/E |
| 61 | 61-ZCHD-PL-18–25-I | 2018 | Nonspecific symptoms | Broiler 8 days | FAdV-1/A |
| 62 | 62-ZCHD-PL-20–15-L | 2020 | IBH clinical case | Broiler 3 weeks | FAdV-5/B |
| 63 | 63-ZCHD-PL-19–4-S | 2019 | Nonspecific symptoms | Broiler 44 days | FAdV-11/D |
| 64 | 64-ZCHD-PL-18–26-L | 2018 | IBH clinical case | Broiler 28 days | FAdV-11/D |
| 65 | 65-ZCHD-PL-18–27-I | 2018 | IBH clinical case | Broiler 3 weeks | FAdV-2/11/D |
| 66 | 66-ZCHD-PL-18–28-L | 2018 | IBH clinical case | Layers 32 weeks | FAdV-2/11/D |
| 67 | 67-ZCHD-PL-18–29-L | 2018 | IBH clinical case | Broiler 17 days | FAdV-11/D |
| 68 | 68-ZCHD-PL-20–16-I | 2020 | Nonspecific symptoms | Layer 37 weeks | FAdV-5/B |
| 69 | 69-ZCHD-PL-15–2IB-L | 2015 | Nonspecific symptoms | Broiler 3 weeks | FAdV-5/B |
| 70 | 70-ZCHD-PL-20–17-L | 2020 | IBH clinical case | Broiler 3 weeks | FAdV-5/B |
| 71 | 71-ZCHD-PL-20–18-S | 2020 | IBH clinical case | Broiler 5 weeks | FAdV-7/E |
| 72 | 72-ZCHD-PL-20–19-S | 2020 | IBH clinical case | Layer 37 weeks | FAdV-7/E |
| 73 | 73-ZCHD-PL-18–30-I | 2018 | IBH clinical case | Brojler 3 weeks | FAdV-11/D |
| 74 | 74-ZCHD-PL-21–18-L | 2021 | IBH clinical case | Broiler 3 weeks | FAdV/5/B |

(*continued on next page*)

**Table 1** (*continued*)

| | Number | Date of Isolation | Symptoms | Poultry type | type/species |
|---|---|---|---|---|---|
| 75 | 75-ZCHD-PL-21–19-I | 2021 | IBH clinical case | Broiler 3 weeks | FAdV/5/B |
| 76 | 76-ZCHD-PL-21–20-L | 2021 | IBH clinical case | Broiler 14 days | FAdV/5/B |
| 77 | 77-ZCHD-PL-20–21-S | 2020 | Nonspecific symptoms | Layer 34 weeks | FAdV/7/E |
| 78 | 78-ZCHD-PL-19–5_G | 2019 | Nonspecific symptoms | Broiler 8 days | FAdV/D |
| 79 | 79-ZCHD-PL-18–31-G | 2018 | Nonspecific symptoms | Broiler 30 days | FAdV/1/A |
| 80 | 80-ZCHD-PL-21–21-L | 2021 | Nonspecific symptoms | Broiler 27 days | FAdV-3/D |
| 81 | 81-ZCHD-PL-21–22-S | 2021 | Nonspecific symptoms | Broiler 27 days | FAdV-3/D |
| 82 | 82-ZCHD-PL-21–23-S | 2021 | Nonspecific symptoms | Broiler 3 weeks | FAdV-10/C |
| 83 | 83-ZCHD-PL-21–24-S | 2021 | Nonspecific symptoms | Broiler 36 days | FAdV-10/C |
| 84 | 84-ZCHD-PL-21–25-W | 2021 | Nonspecific symptoms | Broiler 36 days | FAdV-2/11/D |
| 85 | 85-ZCHD-PL-21–26-S | 2021 | Nonspecific symptoms | Broiler 39 days | FAdV-2/11/D |
| 86 | 86-ZCHD-PL-21–27-S3 | 2021 | Nonspecific symptoms | Broiler 39 days | FAdV-2/11/D |

B. Table summarizing the adenovirus strain sequences (reference and field strains) which have been used to molecular studies and obtained from database GenBank NCBI

| | Sequence name and number | Date of Isolation | Type/species |
|---|---|---|---|
| 1 | Fowl adenovirus 6 CR119 | 2018 | FAdV-6/E |
| 2 | Fowl adenovirus 8 TR-59 8a JQ034216 | 2011 | FAdV-8a/E |
| 3 | Fowl adenovirus 10 C2B EU979377 | 2009 | FAdV-10/C |
| 4 | Fowl adenovirus 5 340EU979371 | 2013 | FAdV-5/B |
| 5 | Fowl adenovirus 8 VR-833 A-2A AF339918 | 2001 | FAdV-8/E |
| 6 | Fowl adenovirus 4 VR-829 J2-A AF339917 | 2001 | FAdV-4/C |
| 7 | Fowl adenovirus 9 VR-834 C-2B AF339923 | 2001 | FAdV-9/D |
| 8 | Fowl adenovirus 3 VR-828 IBH-2A AF339916 | 2001 | FAdV-3/C |
| 9 | Fowl adenovirus 7 VR-832 B-3A AF339922 | 2001 | FAdV-7/E |
| 10 | Fowl adenovirus 10 VR-835X-11A AF339924 | 2001 | FAdV-10/C |
| 11 | Fowl adenovirus E NC 014,969 USA | 2001 | FAdV-E |
| 12 | Fowl aviadenovirus 2 AF339915 | 2001 | FAdV-2/D |
| 13 | Fowl aviadenovirus 5 TR22 EU FAdV 5 | 2002 | FAdV-5/B |
| 14 | Fowl aviadenovirus 1 Phelps(ATCC VR-432) U46933 | 1996 | FAdV-1/A |
| 15 | Fowl adenovirus 8b ADL17 816 MN737080 SOUTH KOREA | 2019 | FAdV-8b/E |
| 16 | Fowl adenovirus 8b ADL19 0569 MN737085 South Korea | 2019 | FAdV-8b/E |
| 17 | Fowl adenovirus 8b 764 FAdV-8b EU979375 | 2008 | FAdV-8b/E |
| 18 | Fowl adenovirus 8b JF766221 | 2011 | FAdV-8b/E |
| 19 | Fowl adenovirus 8b KY229185 BRAZIL | 2016 | FAdV-8b/E |
| 20 | Fowl aviadenovirus E SA62–08 HQ117904 SOUTH AFRICA | 2010 | FAdV-E |
| 21 | Fowl aviadenovirus E SA78–08 SOUTH AFRICA | 2010 | FAdV-E |
| 22 | Fowl aviadenovirus ESA84–08 SOUTH AFRICA | 2010 | FAdV-E |
| 23 | Fowl aviadenovirus E T-8AMK572854 | 2019 | FAdV-E |
| 24 | Fowl adenovirus 8b UPM04217 KU517714MALAYSIA | 2016 | FAdV-8b/E |
| 25 | Fowl aviadenovirus E VS41BR 8b Brasil | 2018 | FAdV-E |
| 26 | Fowl aviadenovirus E HM592275 ITALY | 2010 | FAdV-E |
| 27 | Fowl adenovirus FAV-LS-170,123 CHINA | 2017 | FAdV-E |
| 28 | Fowl adenovirus FAV-QDLX-1-CHINA | 2017 | FAdV-E |
| 29 | Fowl adenovirus FAV-QDLX-CHINA | 2017 | FAdV-E |
| 30 | Fowl aviadenovirus 7 YR36 KT862809 | 2015 | FAdV-7/E |
| 31 | 1× 11 AF339920 | 2001 | FAdV-11/D |
| 32 | Fowl aviadenovirus E FAdV-7-B2 MT459109 | 2020 | FAdV-E |
| 33 | Fowl adenovirus 8a MN453821 BRAZIL | 2019 | FAdV-8a/E |
| 34 | Fowl adenovirus 8a KT781517EGYPT | 2015 | FAdV-8a/E |
| 35 | Fowl adenovirus LN907533 GERMANY | 2015 | FAdV-8a/E |
| 36 | Fowl aviadenovirus E HM592290 ITALY | 2010 | FAdV-E |
| 37 | Fowl aviadenovirus 5 TR22 EU FAdV 5 AF508953 | 2002 | FAdV-5/B |
| 38 | Fowl aviadenovirus 11 AY683552 | 2004 | FAdV-11/D |
| 39 | Fowl aviadenovirus 7 AY683548 USA | 2004 | FAdV-7/E |
| 40 | Fowl adenovirus FAdVB CPON040 KP274037 GERMANY | 2014 | FAdV-5/B |
| 41 | Fowl adenovirus FAdVE CDAO182 GERMANY KP274038 | 2014 | FAdV-E |
| 42 | Fowl adenovirus India AD/528/01 AY581275 | 2004 | FAdV-11/D |
| 43 | Fowl aviadenovirus D Tokushima2010/IBH JAPAN LC650578 | 2021 | FAdV-11/D |
| 44 | Fowl aviadenovirus 4 KR5 FAdV-4 | 2008 | FAdV-4/C |
| 45 | Fowl adenovirus subcluster A MN165282 AUSTRIA | 2019 | FAdV-1/A |
| 46 | Fowl aviadenovirus A Fown adenovirus 1 AC 000,014 | 2004 | FAdV-1/A |
| 47 | Fowl adenovirus subcluster B MN165283 AUSTRIA | 2019 | FAdV-5/B |
| 48 | Fowl aviadenovirus A HM592291 ITALY | 2019 | FAdV-1/A |
| 49 | Fowl aviadenovirus D HM592292 ITALY | 2019 | FAdV-11/D |
| 50 | Fowl adenovirus 15–14,088 LN907542 GERMANY | 2015 | FAdV-D |
| 51 | Fowl Adenovirus FAdV/SP/42/2013 SPAIN | 2016 | FAdV-D |
| 52 | Fowl adenovirus FAdV/SPAIN KU647792 | 2016 | FAdV-D |
| 53 | Fowl adenovirusKr/ADLFAdV/2012 KC593424 SOUTH KOREA | 2013 | FAdV-11/D |
| 54 | Fowl adenovirus MG008492 SPAIN | 2017 | FAdV-11/D |
| 55 | Fowl adenovirus FAdV D/serotype2/Ind-TN/CL1/2017 hexon INDIA | 2019 | FAdV-11/D |
| 56 | Fowl aviadenovirus D SA58–08 SOUTH AFRICA | 2010 | FAdV-11/D |
| 57 | Fowl aviadenovirus D KU01/07 EU678792 THAILAND | 2008 | FAdV-11/D |
| 58 | Fowl aviadenovirus 2 SR48 FAdV-2 EU979368 | 2008 | FAdV-2/D |
| 59 | Fowl aviadenovirus 11 C2B EU FAdV 11 | 2003 | FAdV-11/D |
| 60 | Fowl aviadenovirus D 380-CORR MK572873 | 2019 | FAdV-11/D |

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**Table 1** (*continued*)

B. Table summarizing the adenovirus strain sequences (reference and field strains) which have been used to molecular studies and obtained from database GenBank NCBI

| | Sequence name and number | Date of Isolation | Type/species |
|---|---|---|---|
| 61 | Fowl aviadenoviru 11 ADL14 1036 11 MN737051 | 2019 | FAdV-11/D |
| 62 | Fowl adenovirus 13–19,590 AUSTRIA | 2015 | FAdV-11/D |
| 63 | Fowl aviadenovirus D MX95-S11 KU746335 MEXICO | 2016 | FAdV-11/D |
| 64 | Fowl aviadenovirus 9 A-2A AF083975 CANADA | 1998 | FAdV-9 |
| 65 | Fowl aviadenovirus D A-2A 9 NC 000,899 | 1998 | FAdV-9 |
| 66 | Fowl adenovirus HB1505 MZ054256 CHINA | 2021 | FAdV-11/D |

producers (Fitzgerald, 2020). Histopathologically, hepatic & spleen necrosis with basophilic intranuclear inclusion bodies and haemorrhage are used to be design and present during the IBH symptoms in affected birds. Many different adenovirus types/species have been responsible/associated with IBH infections, mainly Fowl adenovirus species E (type 6, 7, 8a, 8b) and Fowl adenovirus species D (type 2, 9, and 11). Broiler and broiler breeder flocks have not been vaccinated in many countries in Europe, as well there is no commercial vaccine for IBH infections in Poland. In some flocks auto vaccines are prepared from the strains which have been isolated from affected flocks (Hess, 2017; Kumar et al., 2010; Tamura and Nei, 1993).

FAdVs horizontal transmission occurs by the shedding of virus particles through the digestive tract, since most birds are likely to become infected via the faecal-oral route, but transmission by the respiratory tract may also occur. Vertical transmission to the progeny was also reported at a low rate (Fitzgerald, 2020). Infections by low pathogenic or apathogenic strains are usually asymptomatic (Fitzgerald, 2020). Free-living birds were demonstrated to carry virulent FAdVs genetically related/identical to chicken FAdVs strains, implicating free-living birds as reservoirs for the virus transmission (Forbes et al., 1997; Frolich et al., 2005; Meulemans et al., 2004; Schachner et al., 2020). An adenoviruses can developed as viruses causing a latent infection, during that time they can go undetected, and after some factors such stimulates immunodeficiency in immunocompromised chickens could be reactivated with the use of immunosuppressants (Fadly et al., 1980; Grafl et al., 2012; Niczyporuk, 2017). The opportunistic infection can be a cause of secondary infection (adenovirus infection) in immunocompromised birds (Hoffmann et al., 1975; Niczyporuk et al., 2021).

Hexon gene is the largest gene in adenovirus genome and represents crucial/ important region of adenovirus immunity indicated/presented (HVR-hyper variable regions). This sequences phylogenetically informative sequences have been used for molecular studies (Ganesh et al., 2001; Masaji et al., 2020; Matos et al., 2016; Niczyporuk et al., 2012).

This study aimed to investigate the clinical and molecular epidemiology and characterisation of different FAdVs strains from affected chicken flocks in Poland and investigated the geographic correlation and their genetic type/species recognitions.

## 2. Materials and methods

### 2.1. Chicken samples

Samples from various organs (liver, spleen, gizzard, and caecal tonsils) of examined sick chickens have been collected from 86 affected flocks located in geographically distant regions in Poland. Specimens from liver tissue corresponding/presented necrotic focal lesions, and have been collected from birds during the anatomopathological examinations were obtained. The 200 μL of homogenate prepared from liver, spleen, gizzards, and caecal tonsils collected were prepared in a PBS ratio of 1:10. Than using the DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany), and DNA extractions have been performed for molecular analysis. Samples were stored at −20 °C until use.

### 2.2. PCR amplification methods

#### 2.2.1. Molecular studies

Genomic DNA Loop L1 HVR1–4 region of the hexon gene that is strictly variable among FAdV types/species, at nucleotide position 178–1017 (based on FAdV-1,GenBank accession number AC_000014) was amplified in accordance as described previously (Niczyporuk et al., 2010). After the DNA amplification the obtained hexon gene sequence had been used for genetic/molecular studies. The obtained samples were also analysed using the methods previously developed for type/ species 1/A, 5/B, 8a/E and 7/E (Niczyporuk, 2017; Niczyporuk et al., 2021; Niczyporuk et al., 2012) in order to exclude mixed infections in the tested samples in case of infection with two types/species of adenoviruses at the same reaction mix.

The nucleotide sequence (nt) and, after translation, deduced amino acid (aa) sequence similarities were determined by using the ClustalW v.2.1 including the BioEdit v. 7.2.4 software. Algorithm implemented in the megAlign v. 7.1.0. The PhyML 3.0 v of maximum likelihood algorithm was used for phylogenetic analysis. The phylogenetic tree was performed by the MEGA7 program choosing the "root on midpoint" option and neighbour-joining method with 1000 bootstrap replicates based on the Loop L1, HVR1–4 of the hexon gene was performed (Kataria et al., 2013).

To calculate the similarities of adenovirus strain sequences the Loop L1 region of the hexon gene was analysed. The fowl adenovirus strains obtained sequences were edited with the CHROMAS software. Molecular analyses have been performed with reference sequences of adenovirus strains representing the twelve types that had been obtained from the GenBank database (NCBI), the 86 sequences of adenovirus Polish field strains together with 46 adenovirus strain sequences representing different countries and continents. A total of 153 nucleotide sequences of the FAdVs hexon gene were included in the phylogenetic and geographic analyses. Obtained sequences were aligned with CLUSTAL/ W using BioEdit software 7.1.3.0 and the phylogenetic tree was created with the Maximum Likelihood method based on the Tamura-Nei model (Zadravec et al., 2013) with MEGA7 software (Kataria et al., 2013; Marek et al., 2010).

### 2.3. Statistical analysis

The relationship between adenovirus type/species detection and the predilection of infected birds (the predilection of internal organs) were assessed by fitting a linear model and were analysed using Mann-Whitney-Wilcoxon test. Statistical significance level was determined with the P-value was $<0.05$ for all examined values.

## 3. Results

### 3.1. Epidemiological analysis

One to three samples per flock were collected and examined. Samples have been tested individually, no samples were pooled. All examined flocks were suspected of adenovirus infection due to the mortality rates and presentation of clinical signs of infection. The necropsy showed an enlarged discoloured liver with characteristic foci of haemorrhage and necrosis indicative of liver dysfunction and organ lesions

**Table 2**
Current spectrum of detected fowl adenoviruses*.

| species/type | % |
|---|---|
| A/1 | 6 (6,9) |
| B/5 | 24 (27,90) |
| D/3 | 4 (4,65) |
| C/10 | 2 (2,32) |
| E/7 | 11 (16,27) |
| E/8a | 3 (3,48) |
| D/2/11 | 36 (41,86) |

* The FAdVs type/species were identified by genomic sequencing and molecular analysis.

characteristic for adenovirus infection. The mortality rates were indicated between 10% and in some cases 30%. Epidemiological analysis showed that adenovirus strains have been isolated from vivodeships: some of them are in the North-East and North-West part of the country (Fig. 1). However nearly 60% of the strains were isolated from the North Poland. Two adenovirus strains were isolated in 2015, one adenovirus strain was isolated in 2017, thirty one adenovirus strains were isolated in 2018, five adenovirus strains were isolated in 2019, twenty adenovirus strains were isolated in 2020, and twenty-seven adenovirus strains were isolated in 2021 (Table 1 **A** and 1 **B)**. In Table 1**A** are summarising the adenovirus Polish field strains detected and investigated. In Table 1**B** the reference of adenovirus strains and strain sequences isolated from other countries/continents were indicated. The% of adenovirus type/species contribution have been presented in Table 2 and tissue/species predilection have been presented in Fig. 2**.** Phylogenetic analysis and case numbers (fowl adenovirus strains) which were detected in NVRI Department of Poultry Diseases from 2015 to 2021 have been presented in Table 3.

Sequences of reference adenovirus strains and strain sequences represented different countries and continents such as: South Korea (3 sequences), South Africa (2 sequences), US (1 sequence), Brazil (2 sequences), Malaysia (1 sequence), Italy (1 sequence) China (3 sequences), and reference sequences of type/species FAdV-E/6/7/8a, and type/species FAdV- C/10 were located in one main branch divided into five sub-branches. The first sub-branch was created by eight field sequences indicated phylogenetic similarities with type/species 7/E with the highest similarities with a 100% between strains: 31-ZCHD-PL-20–6-G and FAdV-7-B2 MT459109.

The next branch was created by three field strains: 39-ZCHD-PL-20–10-S, 45-ZCHD-PL-18–22-L, and 58-ZCHD-PL-21–17-S, and phylogenetic analysis indicated type/species 8a/E closely related with strain sequences isolated from Egypt, Germany and more distantly one sequence from Italy represented species E HM592290 related to three Polish strains: 13-ZCHD-PL-18–8-S, 14-ZCHD-PL-18–9-L, 24-ZCHD-PL-18–12-L, and other phylogenetically distanced strains species B. The next clad was created by two field strains: 6-ZCHD-PL-18–3-G, and 25-ZCHD-PL-18–13-L which are closely related with type/species 1/A.

The second big branch was formed by four field sequences: 46-ZCHD-PL-18–23-I, 80-ZCHD-PL-21–21-L, 81-ZCHD-PL-21–22-S, and 8-ZCHD-PL-18–5-I which are similar to species D type 3 and for two sequences from Germany represented species E and B. The% of similaritity of those strain sequences was estimated between (79–53%−97.42%). The second sub-branch was created by two reference sequences: 10 C2B and 9 C-2B with the similarities with fled sequences: 82-ZCHD-PL-21–24-S and 83-ZCHD-PL-21–24-S of about 95.41%. The third sub-branch was created by sequences from Japan (1 sequence), Italy (1 sequence) and Austria (2 sequences), with reference sequences represented species C and A. The similarities between field strains: 82-ZCHD-PL-21–23-S, and 83-ZCHD-PL-21–24-S to type 9 and 10 was about 95.42%. The sequence from Austria MN165282 was similar to the sequence from Poland 61-ZCHD-PL-18–25-I at about 99.55%. Three Polish sequences: 79-ZCHD-PL-18–31-G, 48-ZCHD-PL-18–24-L, and 1-ZCHD-PL-18–1-L in this same branch were similar with Austria (2 sequences) and two sequences from Italy.

The next main branch was created by strains from South Korea (1 sequence), Spain (3 sequences), India (1 sequence), South Africa (1 sequence), Thailand (1 sequence), Austria (1 sequence), Mexico (1 sequence), Canada (1 sequence), and were compared with the 24 sequences of Polish strains: 51-ZCHD-PL-21–14-I, 28-ZCHD-PL-18–15-S, 40-ZCHD-PL-18–17-G, 44-ZCHD-PL-18–21-I, 11-ZCHD-PL-18–6-L, 12-ZCHD-PL-18–7-S, 21-ZCHD-PL-20–4-L, 22-ZCHD-PL-18–10-S, 23-ZCHD-PL-18–11-L, 29-ZCHD-PL-18–16-L, 65-ZCHD-PL-18–27-I, 47-ZCHD-PL-20–11-S, 84-ZCHD-PL-21–25-W, 27-ZCHD-PL-17–1-L, 9-ZCHD-PL-21–4-L, 20-ZCHD-PL-21–7-G, 26-ZCHD-PL-21–14-G, 85-ZCHD-PL-21–26-S, 86-ZCHD-PL-21–27-S3, 34-ZCHD-PL-21–8-I, 35-ZCHD-PL-21–9-I, 66-ZCHD-PL-18–28-L, 36-ZCHD-PL-21–10-G, and 37-ZCHD-PL-21–11-G creating type/species FAdV-2/11/D. Since some sequences of the own field strains were so similar to each other that on the molecular level it turned out to be impossible to classify them to type 2 or 11, therefore such sequences were marked as FAdV-2/11/D in accordance with ICTV classification. However there may be an assumption that in these samples mixtures of types 2 and 11 were

**Table 3**
Case numbers (Fowl adenovirus strains) which have been detected in NVRI Department of Poultry Diseases from 2015 to 2021.

| Type/Species | 2015 | 2017 | 2018 | 2019 | 2020 | 2021 |
|---|---|---|---|---|---|---|
| FAdV-1/A | | | 6 | | | |
| FAdV-5/B | 1 | | 2 | | 9 | 12 |
| FAdV-3/D | | | 2 | | | 2 |
| FAdV-10/C | | | | | | 2 |
| FAdV-2/11/D | 1 | 1 | 17 | 5 | 2 | 10 |
| FAdV-7/E | | | 3 | | 8 | |
| FAdV-8a/E | | | 1 | | 1 | 1 |
| Total | 2 | 1 | 31 | 5 | 20 | 27 |

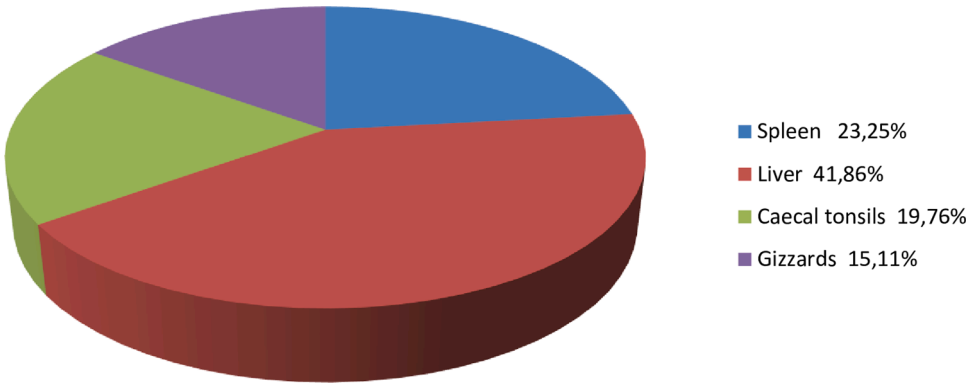


**Fig. 2.** Fowl adenovirus tissue samples predilection presented in%.

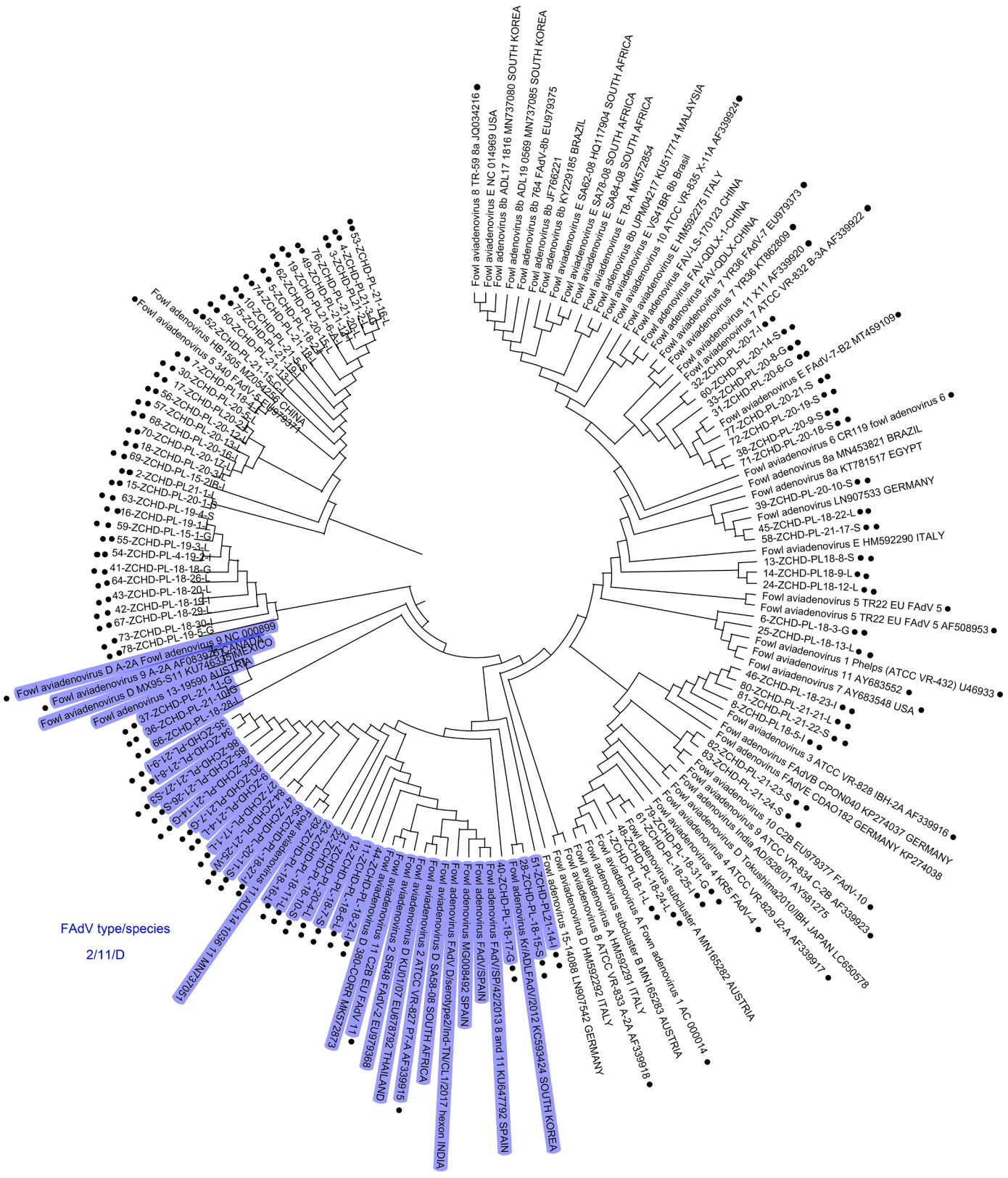


**Fig. 3.** Phylogenetic tree. Phylogenetic analysis of 830 bp long Loop L1 region of the hexon gene originating from 86 strain Polish sequences, 17 reference sequences and 50 adenovirus strain sequences derived from different countries/continents and their geographic correlations.
the sequences of the reference strains are marked in one dot
the sequences of Polish strains are marked with two dots
the sequences of FAdV-2/11/D strains are marked in purple.

detected, therefore their differentiation on the molecular level/background turned out to be impossible. The other remaining types and species of adenoviruses were also marked according to the ICTV classification system and showed a clear division into type and species.

An interesting observation was that the sequence 29-ZCHD-PL-18–16-L indicated 100% similarities with strain sequence MN737051, and sequence 28-ZCHD-PL-18–15-S indicated 100% similarities with the sequence KC593424 isolated from South Korea. Additionally Sequences: 36-ZCHD-PL-21–10-G and 37-ZCHD-PL-21–11-G have 100% similarities.

The last branch created by 14 Polish sequences indicated similarities with type/species 5/B together. The sequence 7-ZCHD-PL-18–4-L with sequence EU979371 indicated 99.99% similarities and was closely related with sequence isolated from China (MZ054256). The phylogenetic tree was presented in Fig. 3.

### 3.2. Statistical analysis

Virus strains were isolated from the liver which was (36 samples, 41.86%), from gizzard (13 samples, 15.11%), from caecal tonsils (17 samples, 19.76%), and from spleen (20 samples, 23,25%) with determined with the *P*-value was $<0.05$ with significance statistical level.

## 4. Discussion

Adenoviruses have been distributed worldwide, and in the next following years, adenovirus strains have been isolated from many bird species: poultry, goose, ducks, quails, wild birds and migrating birds. Fowl adenoviruses (FAdVs) have provided a variety of clinical settings and are an important cause of infections in broiler chicken flocks. The growing numbers of FAdVs types designated by genomic analysis required continuous diagnostic detection and monitoring of FAdVs infections.

An increased rate of FAdV-related diseases, caused by pathogenic strains of different type/species, are evident in Poland. In the last decade 7/8a/E, 1/A, 5/B, and 2/11/D type/species were detected and classified (Niczyporuk, 2016; JS Niczyporuk et al., 2021; Niczyporuk et al., 2010). In Brazil, previous reports demonstrated that for adenovirus infections were responsible mostly type/species 11/D and 8/E. In Saudi Arabia type/species 2/11/D and 7/8a/8b/E (Wilson et al., 2010). In Slovenia type/species D and E (Zsak and Kisary, 1984), Turkey indicated type/-species 8b/E and 11/D (Schachner et al., 2016). In Japan type/species 8b/E (Mirzazadeh et al., 2020) and in Spain type/species 8b/E and 11/D were determined (Reece and Pass, 1985). As in Egypt where the species D and type/species 8a/E were dominated, however in some cases types 1, 3, and 8b have been found lastly (Amany et al., 2021). As we can see, similar type/species of adenoviruses began to dominate in most countries or even continents.

Therefore, this study aimed to investigate the presence of FAdV strains that represent type/species in commercial poultry flocks in Poland in recent years. To provide adequate type/species identification, molecular analysis, and characterisation of these virus strains, molecular analysis have been conducted and were based on the Loop L1 region of the hexon gene.

Poland has been recorded an increasing number of IBH cases during the last years. We can suspect that some parental flocks do not have immunity. In some affected flocks birds died without any clinical signs of infection, a similar situation has been reported in Greece (Franzo et al., 2020) and Austria (Hess, 2017).

The adenovirus contamination/infection can have a huge influence on failures during the conduct of prophylactic vaccinations against Marek's disease (MD), and can have an impact on failure vaccinations in adenovirus infected flocks, what we tried to prove in conducted studies *in vitro* and *in vivo* (Olson, 1951; Ono et al., 2007).

Fowl adenovirus type 8b was isolated from chickens with inclusion body hepatitis (IBH) in Japan in 2018/2019 and were identical to foreign strains which can suggest that Japanese IBH strains might have been introduced from other countries (Mirzazadeh et al., 2020). In conducted studies Polish strain sequence 61-ZCHD-PL-18–25-I was in 100% similar/identical with a strain sequence isolated in Austria MN165282 which can suggest that this strain was probably introduced from Austria. This same situation was described with strain sequence 31-ZCHD-PL-20–6-G with a 100% similarity with sequence MT459109 of adenovirus strain isolated in Austria. A similar situation was found with the strain sequence 28-ZCHD-PL-18–15-S which is 100% similar to the sequence of adenovirus strain isolated in South Korea. These two strain sequences were detected in completely distant continents/countries. One can only suspect the introduction of this virus into the Polish region was probably due to migration birds? One only hopes that this suspicion can be more precisely verified in the future. Another similar situation is visibly indicated with the sequence of Polish strain 29-ZCHD-PL-18–16-L with a sequence of adenovirus strain isolated as well from South Korea MN737051.

The molecular analysis of FAdV strains in Marocco, indicative of the hexon gene, showed that FAdV were identical and closely similar to type 11 showing a maximal identity of (93.65%) and had lower similarity with the other references (less than 66.51%). One strain was grouped into type/species 8a/E showing a maximum identity (98.4%) with the FAdV-8a reference strain and less than 78% with the rest of the reference types (Scxahindokuyucu et al., 2020). In Iran the studies in breeders were conducted with a screening of FAdV-associated diseases in local broilers over a three-year period; twenty-six cases of inclusion body hepatitis (IBH) with dominant involvement of FAdV-11/FAdV-8b, one outbreak of adenoviral gizzard erosion (AGE) related to FAdV-1, and no evidence of hepatitis-hydropericardium syndrome (HHS) suggest that identical types are maintained in the local poultry industry (Niczyporuk and Czekaj, 2018).

Concerning the need for vaccination, Popowich, 2018 indicated that the efficiency of a bivalent vaccine in breeders flocks containing live adenovirus strains representing type/species 8a/E and 11/D was confirmed as a significant and useful tool against this type/species responsible for adenovirus infection. In Canada from the same farm during the next hatching period the same viral species were consistently detected. The efficiency of maternal antibodies has been studied and confirmed. Popowich, 2018 also indicated that IBH could be controlled by prophylactic vaccinations with high efficiency in poultry flocks.

## 5. Conclusion

This study showed that adenovirus type/species which have been identified in Poland were mostly responsible for IBH infections (38 poultry flocks with 44.18% of examined). The present molecular studies conducted in Poland indicated/identified 86 sequences of adenovirus strains representing five species (FAdV-A-E). Studies indicated/confirmed the high frequency of adenovirus infection in examined poultry flocks with 57.71%. The occurrence of various type/species of adenoviruses from year to year makes their full diagnostics and molecular characterization time-consuming, and the occurrence of new virulent FAdV strains is a challenge that should entail full monitoring and the assessment of the epidemiological situation of poultry flocks in Poland, and should be continued.

## Author agreement

Jowita Samanta Niczyporuk and Wojciech Kozdrun is a statement to certify that all authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

## Declaration of Competing Interest

Author A and B declares that they has no conflict of interest.

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Authors declare that they have no competing financial, professional or any non-financial competing interests that might be perceived to influence the interpretation of data or pre-sentation of information described in this manuscript.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2022.198825.

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