


Research Article

Molecular Epidemiology of Human Adenovirus in Asturias (2011–2023)

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Abstract

Human Adenoviruses (HAdV) are implicated in multiples pathologies causing mild to severe disease. The predominant genotypes detected in association with disease differ among different countries or regions, and change over time. In Spain and elsewhere little is known about the molecular epidemiology of HAdV. From a total of 250 HAdV, members of five species were present: A (1), B (126), C (87), D (27) and E (9). The most found genotypes were B3 (119), C2 (48), C1 (29) and D8 (26). Genotypes E4, C5, C6, B7, A31, B35 and D56 were also detected. HAdV diversity increases over the years until the B3 genotype displaces all other types in 2016 and 2023. HAdV detected in Asturias were similar to those already described in other countries, no new local genotype was observed. Genotypes 1-7 were more frequent in children under 15 years of age, while types 8-56 were more frequent in the elderly. Multiple HAdV introductions must have occurred given that only small transmission clades can be inferred. The diversity of the epidemic increased with the years until it disappeared one year periodically. The COVID-19 epidemic accelerated the loss of diversity suggesting that interventions during the pandemic were able to reduce HAdV transmission.

Keywords: Human Adenovirus, Molecular epidemiology pattern, HAdV Genotypes

Background

Human Adenoviruses (HAdV) are a leading pathogen of clinical diseases, such as gastroenteritis, conjunctivitis, respiratory illnesses, hemorrhagic cystitis, and systemic infections. HAdV have been categorized into seven species (A-G) [1-3] and have more than 113 types. Types were initially distinguished by serotyping the neutralization loops of the hexon capsid protein (types 1–51) and later by sequencing all three major capsid proteins: penton, hexon, and fiber (types 52–105) [4,5]. HAdV infection can occur sporadically, endemically, or epidemically and often is influenced by HAdV species and type. Transmission of novel strains between countries or across continents and replacement of dominant serotypes by new strains may occur. A full understanding of the epidemiology of HAdV is important to help clinicians identify infections early and take preventive measures [6]. The search for HAdV in clinical specimens has been increasing in recent years, however few hospitals routinely carry out HAdV culture and typing tests. Here, we conducted a 13-year study detecting, isolating, and genotyping HAdV in clinical samples in Asturias from 2011, to gain a better understanding of HAdV infection through long-term investigation. The aim of this study are to enrich the poor data of epidemiological molecular studies on HAdV in Spain, to support the benefit of molecular surveillance as a tool to determine

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the epidemiology of viruses circulating in each community, and to underline the need for the design and support of similar long-term studies.

Materials and methods

Samples and patients

From January 2011 to February 2023, 179,081 samples (respiratory samples of all types, stool, blood, tears, corneal scrapings, biopsies, etc.) for diagnosis of HAdV among other pathogens were processed (Table 1). In 250 samples, belong same number of patients hexon gene were sequenced. These specimens were chosen at random among the positive samples detected by a real time PCR, with cycle threshold lower than 25, specially in young and old people, and with apparent clinical significance. Of them, 188 (75%) were nasal/pharyngeal swabs, 37 (15%) were conjunctival and the remaining 25 (10%) were from various sources (blood, cutaneous, endomyocardial biopsy, genital ulcer, pleural fluid, sputum, stool, urine, vaginal and wound). These samples belonged to 134 (53.6%) male and 116 (46.4%) female. In age groups, 58 (23%) were younger than 2 years, 87 (35%) between 2-4 years, 50 (20%) between 5-14 years, 44 (17.6%) were between 15-68 and 11 (4.4%) older. Demographic data (age and sex) from those patients and type of sample are in

Virus detection and sequencing

Nucleic acids were extracted/purified by using an automated nucleic acid purifier Magnapure 96 (Roche Diagnostics, Mannheim, Germany/Switzerland) following manufacturer's instructions. Adenoviral genome was detected by a real time (RT)-PCR according to Lu et al [32]. For characterization, a fragment of the *hexon* gene were amplified by a nested-PCR using primers and protocol described by Lu et al. [32] PCR products were analyzed by

agarose gel electrophoresis, extracted by using Montage DNA Gel Extraction Kit (Millipore, USA) and sequenced with Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) using inner primers.

Phylogenetic reconstructions

The genotyping analysis of HAdV was used to characterize which types circulated in Asturias and to investigate whether phylogenetic clusters occurred in the study period. Nucleotide sequences were translated and aligned using the MUSCLE algorithm implemented in MEGA. Sequences (until 862 nucleotides) generated in this work have been deposited in GenBank with the following accession numbers: OQ680228-OQ680477. For type characterization, phylogenetic trees were constructed using ModelFinder, tree reconstruction and ultrafast bootstrap (1000 replicates) with IQ-TREE 2.1.3. HAdV complete genomes (1090) available in the NCBI Database www.ncbi.nlm.nih.gov; accessed on December 2022) (accession numbers are in Supplementary File S2) were used as reference. The best-fit nucleotide substitution model GTR+F+I+G4 was identified according to Bayesian information criterion. Bootstrap values were estimated using the SH test and ultrafast bootstrap. Diversity ($D=1-\sum f^2$) of HAdV genotypes in Asturias was analyzed over time, a measure of variability that takes into account the frequencies (f) of all types. To investigate the possible transmission clades that occurred in Asturias. One sequence of each type identified used for the Basic Local Alignment Search Tool (BLAST) in January 2023 and the same phylogenetic analysis was performed for each type independently using the downloaded sequences. Transmission clusters were defined as viral sequences circulating in Asturias grouped into a single and well supported monophyletic clade with >80% bootstrap values.

Table 1: Samples processed incidence and distribution of HAdV types from 2011 to 2023.

Years	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2022	2023	Total	
Positive Samples	217	298	495	277	877	1611	1250	1691	2893	1238	3240	948	15035	
Incidence %	4.8	7.9	10.7	5.5	12.8	14.8	9.2	11.4	13.5	4.6	9.2	12.6	9.2	
HAdV types	C1	1	2	4	1	1	1	9	3	1	6		29	
	C2	2	6	2	3	2	3	7	4	7	12		48	
	B3			9		2	10	1	5	16		60	11	114
	E4	1					1	2	5					9
	C5			1	1	1			3	1		1		8
	C6									1		1		2
	B7								1	10				11
	D8		9	3	7	1			4	1	1			26
	A31					1								1
	B35					1								1
	D56				1									1
	Total	4	17	19	13	9	10	6	31	41	9	80	11	250

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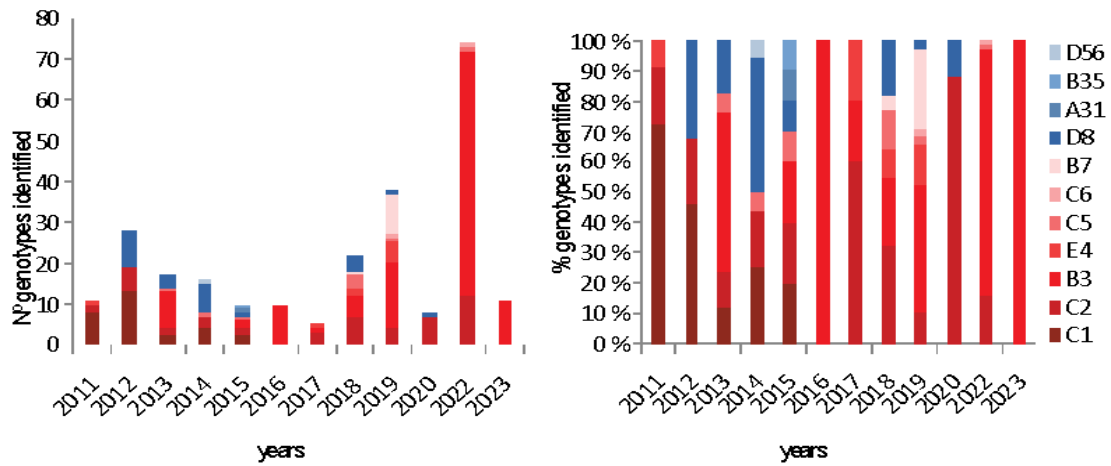


Figure 1: Distribution of HAdV genotypes by year in ordinary scale and as 100% rate. Genotypes 1-7 are in shades of red .Genotypes 8-56 are in shades of blue

Table 2: Distribution of HAdv genotypes by sex and age, and most common genotype by year

HAdv Genotype	Sex Male/Female	Age (years)			
		<2	02-Apr	May-14	≥15
A31	1/-			1	
B3	65/49	20	49	34	11
B7	05-Jun	3	2	3	3
B35	1/-				1
C1	Dec-17	8	13	2	6
C2	28/20	22	16	3	7
C5	04-Apr	3	4	1	
C6	2/-	1			1
D8	Oct-16	1	1	2	22
D56	1/-				1
E4	05-Apr		2	4	3
Most common genotype by year					
D8-2012	03-Jun			1	8
B3-2013	06-Mar	2	5	1	1
D8-2014	04-Mar				7
B3-2016	02-Aug	1	3	2	4
C1-2018	01-Aug	4	1		4
B3-2019	08-Aug	2	8	4	2
C2-2020	04-Mar	7			
B3-2022	37/23	7	29	22	2
B3-2023	06-May	2	3	5	1

Statistical analysis

Statistical tests (Chi-square, Fisher’s, t-Student’s, etc.) were performed using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA).

Results

In the study period (January 2011 to February 2023), HAdV was detected in 15035 (9.2%) samples. Table 1 and Fig 1 show the annual distribution of HAdV types. In 2021 no samples were sequenced and in 2023 they were only sequenced up to February.

During the period studied, five species (11genotypes) of HAdV were identified: A (1- 0,4%;genotype 31), B (126 -50,4%; genotypes 3, 7, 35), C (87 -34,8%; genotypes 1, 2, 5, 6), D (27 -10.8%, genotypes 8, 56) and E (9 -3.6%; genotype 4). HAdV types according age and sex, and most common genotype are showed in Table 2 by year. Distribution of HAdV genotypes by age is showed in Fig 2.

Genotypes 1 to 7 were found in 190 (97.4%) children less than 15 years of age and in 31 (56%) elders. Moreover, genotypes 8 to 56 were found in 5 (2,6%) children under 15 years of age, in 19 (43%) patients between 15 and 70 years and in 5 (45%) elders. (p<0.0001) The most common B3 genotype was found in 103 (90.4%) of children under 15 years old, and C1/C2 in 59 (76.6%) of children under 4 years old. In opposite, D8 was detected in 22 (84.6%) of people older than 14 years old. The phylogenetic genotyping of the HAdVs studied is shown in Fig 3.

On the other hand, and Fig 4 shows the annual diversity and incidence of the HAdV genotypes throughout the time of the study.

Analyzing the HAdV genotypes circulating, seven well-supported monophyletic clade (bootstrap support of >80%) were identified by phylogeny (Table 3, Supplementary Fig. S1)

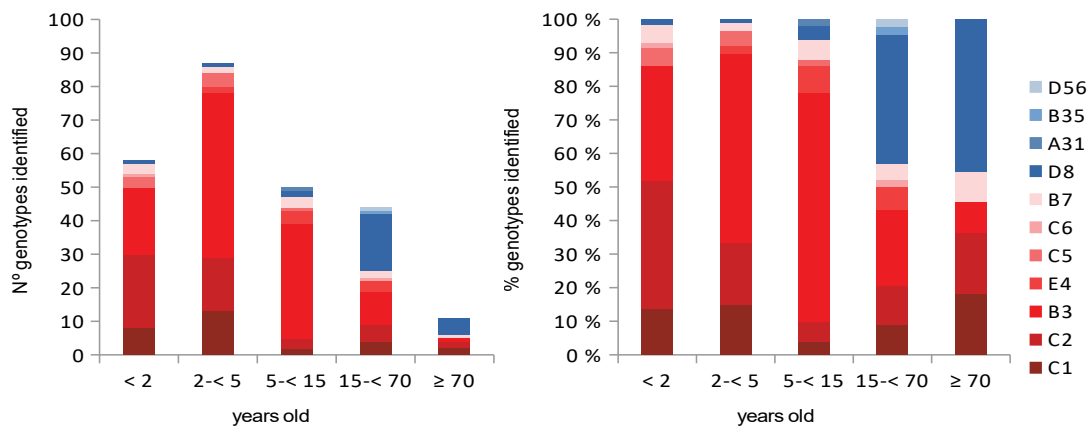


Figure 2: Distribution of HAdV genotypes by year in ordinary scale and as 100% rate. Genotypes 1-7 are in shades of red. Genotypes 8-56 are in shades of blue.

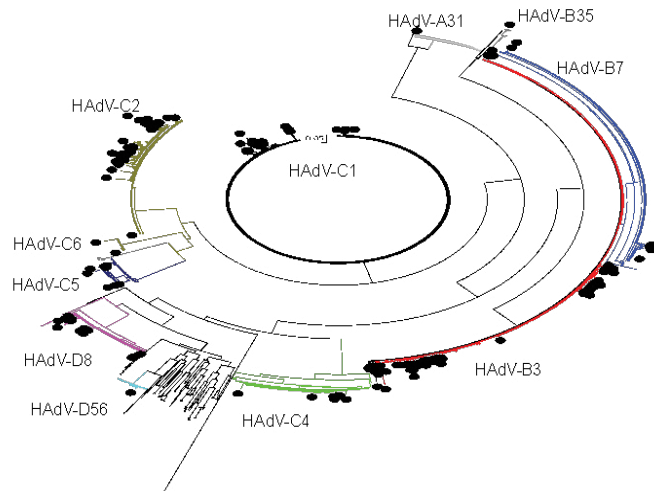


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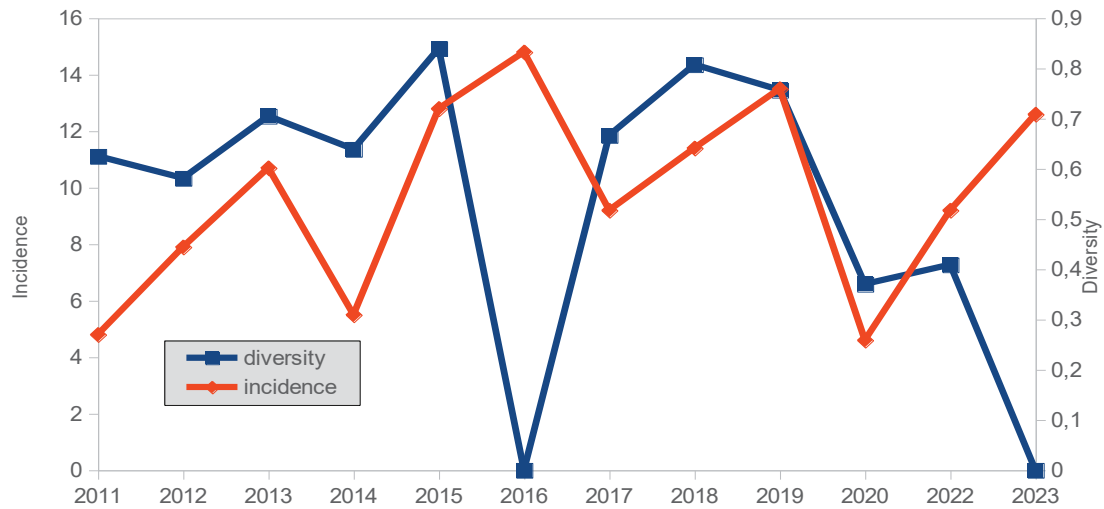


Figure 3: Phylogenetic tree for genotype characterization constructed with IQ-TREE 2.1.3, using HAdV sequences available in the NCBI Database. The colors indicate the genotypes found and the circles our sequences.

Table 3: Characteristics of the samples belonging to the monophyletic cluster found in Asturias

Transmission Node	genotype	Sex	Age	Interval Sequenced Sample
231301261HUCA/231301862HUCA	C1	Male	2-<5	19/01/2013-26/01/2013
2522171137HUCA/252222479HUCA	C1	Male	<2	20/05/2022-29/05/2022
231936627HUCA/231936639HUCA	C2	Male	<5	25-10-2019
252230519HUCA/232006322HUCA/ 232010517HUCA/232011466HUCA	C2	Male/Female	<5	23/01/2020-13/06/2022
252281392HUCA/252281393HUCA/ 252280912HUCA/2522 82234HUCA/262201697HUCA/252281862HUCA	B3	Male/Female	2-<5	10/10/2022-18/12/2022
252297106HUCA/252297647HUCA/252297823HUCA	B3	Male/Female	<5	01/12/2022-02/12/2022
231201797HUCA/ 231203150HUCA_/231311851HUCA	D8	Male/Female	>30	26/01/2012-13/06/2013

Discussion

Human Adenoviruses, implicated in various pathologies, have seen an increase in the search due to their importance. For example, in our laboratory we have gone from processing around 3000 specimens per year to 3000 samples per month. In them, around 10% HAdV was detected. Adenovirus outbreaks are more likely to cause serious disease in infants and immune-compromised hosts, as well as in adults with respiratory or cardiac issues [7,8], but infect anyone and can alter biochemical markers [9]. The predominant HAdV types detected in association with clinical presentation differ between different geographic regions and may shift over time according to the year of surveillance. Thus, monitoring the molecular epidemiology of HAdV in different sites and over the years is of high importance [6,10,11].

HAdV as one of the causative agents of severe acute respiratory infection can involve all ages, however, their infections occur more frequently in children younger than 5

years old [12]. In this study HAdV mainly was sequenced in children under 15 years of age in up to 78% of cases, and half of them occur in children under 4 years of age. In this paediatric age group (children under 15 years of age) genotypes 1 to 7 accounted for almost 90% of the cases according to other studies [13]. In older patients, these genotypes accounted for half of the cases. And in patients older than 70 years, it almost reached 60%, indicating that these genotypes increase with age. In total, eleven different HAdV genotypes were found throughout the study. However, diversity usually increases from year to year. Even the majority one-year genotypes changed in subsequent years, at least before the SARS-Cov-2 pandemic. After 2020, HAdv-B3 became predominant two years in a row.

Genotype B3 and, in general, those belonging to species B were the most frequently detected in our sampling, although there are years in which it was not detected. HAdv-B causes more severe respiratory tract infections than other genotypes and is more likely to result in hospitalization. For

example, HAdV-B3 is considered to be related to severe and complicated pneumonia [11]. Our findings are consistent with the global predominance of B3 (28% before the COVID-19 pandemic and 71% after) as the most common genotype implicated in reportable adenoviral infections in children and adults [14]. This was the most identified genotype every 3 years and is the predominant one in the current year.

Other HAdV-B genotype found, but not in a high rate, was B7. Several reports linking HAdV-B7 with more severe acute respiratory disease [13,15-17], and in South America has been a predominant strain [7]. In this study, HAdV-B7 was only found just before the onset of the COVID-19 epidemic, and stopped circulating. Finally, one HAdV-B35 was found in an urine of a man of 50 years old. This genotype has been described and associated with hemorrhagic cystitis [18], as in our case. Other HAdV that are associated with symptomatic respiratory infections worldwide include species C (types 1, 2, and 5) and E (type 4) [10,19-21]. Infections with HAdV-C often are endemic, mild, and most commonly seen in young children, but are important in immunocompromised patients [19,22].

In this work, HAdV-C was the second most frequent species, accounting for one in three of the cases, and basically in children younger than 5 years, specially HAdV-C2 and HAdV-C1. HAdV-C2 was the first mainly in patients under 2 years of age and the second in patients between 2 and 5 years of age. Others HAdV-C types found was C5 and C6, both related to acute respiratory illnesses [23]. Before SARS-Cov-2 pandemic, HAdV-C species were the most identified in America [24], but not in our region, that followed the incidence reported in United Kingdom of 12% for C1 and 19% for C2 [25]. With respect to the HAdV-E specie, E4 is associated with respiratory infections but mostly among military recruits and at a low frequency in the general population [26]. HAdV-E4 was reported with sporadic circulation, but increased in many countries in recent years [27]. In contrast to other types, HAdV-E4 was found in people older than two years. In this context, in people older than 15 years, the most frequently detected HAdV genotype was HAdV-D8. HAdV-D species cause keratoconjunctivitis, and the most common cause of adenoviral keratoconjunctivitis has been associated to type 8 [28]. In our study all of D8 genotypes were isolated from conjunctival swabs (smears) except one. In addition to the HAdV-D genotypes, we found a case of D56, also associated with conjunctivitis. D56 has been reported in cases of epidemic keratoconjunctivitis as well as in cases of male urethritis with concurrent conjunctivitis [29].

Finally, only one case was found in our study of HAdV-A31 in a stool of a male of 8 year old. A31 is associated with gastroenteritis in children, circulates worldwide and has a high seroprevalence [30]. In the only

study found carried out in Spain (Madrid) between the years 2005-2013 where 154 sequences were included, the most frequent genotypes were B3 (24%), C6 (21%), C5 (20%) and C2 (19%) [31]. The distribution of the genotypes is different from that of our region. As previously mentioned, the appearance of the COVID-19 epidemic accelerated the loss of diversity. Before the SARS-Cov-2 pandemic, the five species with the majority of C but predominance of type B3 were found. After 2020, the three main species (B, C, D) were maintained with a clear dominance also of type B3. Analyzing the occurrence of the different types in each year, of the eleven HAdV characterized, only four (C1, C2, B3 and D8) were found more frequently in each year of study. And if the distribution of genotypes over time is observed, the diversity seems to show a pattern every six years, increasing with the years until decreasing due to the dominance of the B3 genotype. Establishing transmission chains in viral infections is a challenge: infections are usually mild or asymptomatic and viruses may persist in the environment for long periods. In this case, no major transmission clades were identified. Only one C2 variant and one D8 variant were detected that could be transmitted in more than one year. This suggests that multiple HAdV introductions must have occurred and the dissemination of HAdV infections is controlled among immunocompetent hosts. Furthermore, the results do not fully prove transmission, as the genetic diversity of circulating HAdV remains obscure without sufficient sampling of circulating strains and sequencing of short fragments. Globally, C1, C2, B3, E4, C5, C6, B7, B14, and B55 were the main genotypes in causing outbreaks [7]. In our study, phylogenetically related (possible outbreaks) viral sequences were identified in genotypes C1, C2, B3 and D8; the first three cases related to individuals younger than 7 years and D8 to individuals older than 15 years.

Our study is limited to a geographic region. Due to the small sample size, the results may not accurately reflect the diversity circulating in this area; for the same reason we did not analyse whether HAdV types show any seasonality. In addition, molecular characterization based on more than one gene is currently advised to better identify recombination phenomena and new types, but sequencing was done before methods based on more than one gene became available. The construction of these data sets is limited due to the lack of sequence submission to GenBank. Despite the small number of sequences, they represent 1% of all those available in GenBank.

Conclusions

In summary, up to 5 species and 11 HAdV genotypes were found in the last decade in Asturias. Diversity followed a pattern decreasing every 6 years. The COVID-19 epidemic accelerated the loss of diversity suggesting that interventions

during the pandemic were able to reduce HAdV transmission. Genotypes 1-7 were associated with children and the rest with older individuals. Each type of HAdV has been associated mainly with a specific presentation of the disease and in our sampling we have found that the associations already described are fulfilled in all cases. In Spain and elsewhere, there are a limited number of studies in regards to the incidence and molecular epidemiology of HAdV cases. Due to the involvement of HAdV in several pathologies, and the relationship with certain ages, as demonstrated in this study, the implementation of a laboratory-based surveillance system that includes genotyping should be considered.

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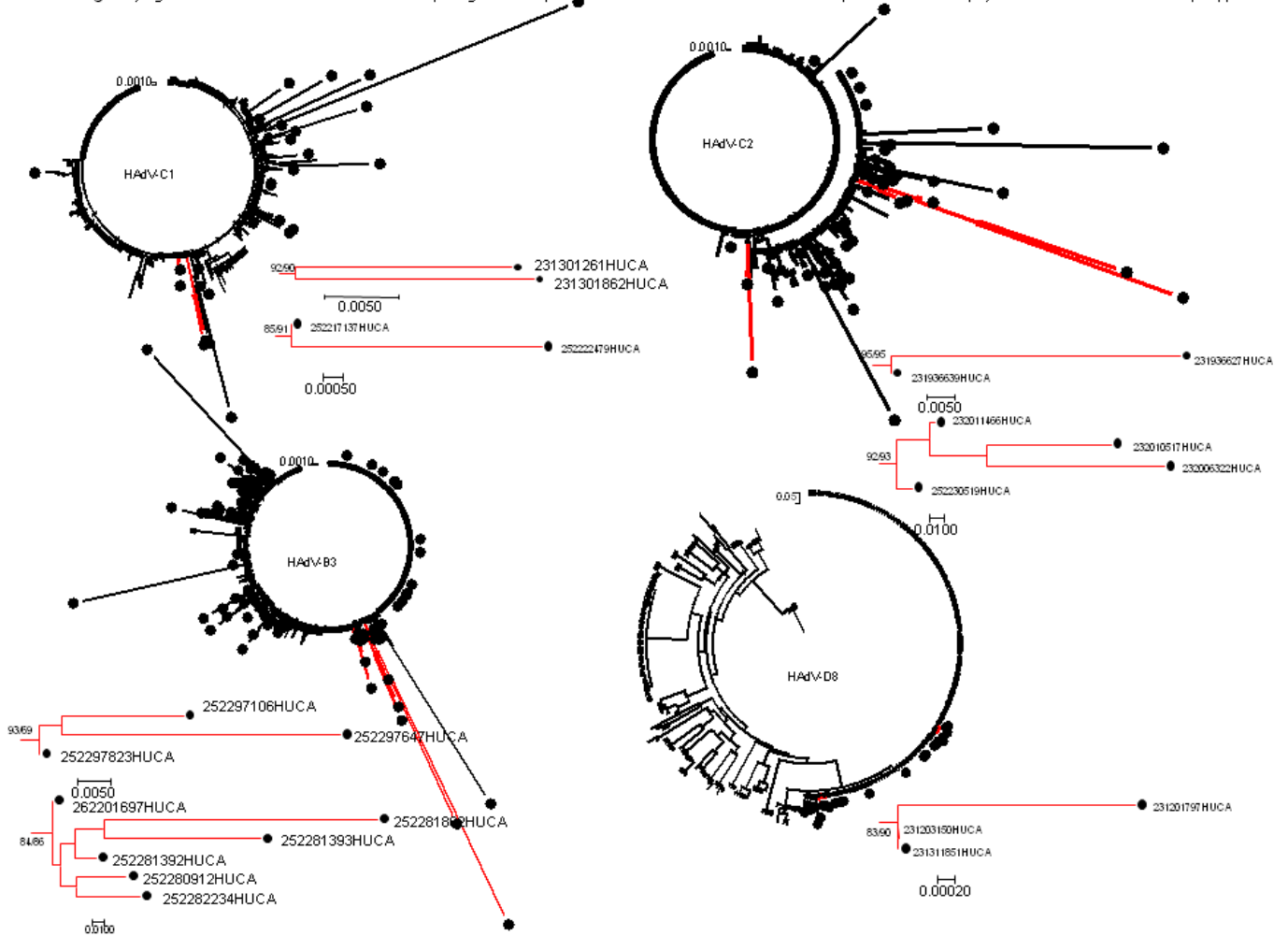
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Supplementary Fig. S1

S1 Fig.- Phylogenetic tree constructed with IQ-TREE 2.1.3, using HAdV sequences available in the NCBI Database. In red, the Asturian monophyletic clades with >80% bootstrap support.



Supplementary File: S2

Sequence_ID	isolate_source	Collection_Date	Age	Sex	Genotype
231112888HUCA	pharyngeal exudate	29-07-11	1	Female	1
231113147HUCA	blood	04-08-11	0	Male	2
231117445HUCA	conjunctival exudate	18-10-11	32	Female	4
231117854HUCA	urine	24-10-11	28	Female	2
231200908HUCA	pharyngeal exudate	16-01-12	2	Male	2
231201797HUCA	conjunctival exudate	26-01-12	78	Male	8
231201959HUCA	pharyngeal exudate	30-01-12	1	Male	1
231202275HUCA	conjunctival exudate	02-02-12	32	Female	8
231202664HUCA	pharyngeal exudate	07-02-12	3	Female	2
231202709HUCA	conjunctival exudate	08-02-12	64	Female	8
231202726HUCA	conjunctival exudate	08-02-12	54	Female	8
231202819HUCA	conjunctival exudate	09-02-12	56	Female	8
231202919HUCA	conjunctival exudate	10-02-12	59	Female	8
231203150HUCA	conjunctival exudate	14-02-12	39	Male	8
231204488HUCA	nasal wash	01-03-12	39	Male	2

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231206599HUCA	pharyngeal exudate	28-03-12	1	Female	2
231209922HUCA	conjunctival exudate	14-05-12	11	Female	8
231212324HUCA	nasal wash	14-06-12	15	Female	2
231212745HUCA	pharyngeal exudate	21-06-12	100	Male	1
231219650HUCA	pharyngeal exudate	21-10-12	2	Female	2
231224240HUCA	conjunctival exudate	27-12-12	64	Male	8
231300484HUCA	conjunctival exudate	09-01-13	26	Female	8
231301050HUCA	aspirate	17-01-13	1	Male	2
231301261HUCA	nasal exudate	19-01-13	3	Male	1
231301637HUCA	pharyngeal exudate	23-01-13	4	Male	3
231301861HUCA	pharyngeal exudate	26-01-13	1	Female	1
231301862HUCA	pharyngeal exudate	26-01-13	2	Male	1
231303015HUCA	nasopharyngeal exudate	12-02-13	0	Female	5
231304103HUCA	pharyngeal exudate	27-02-13	35	Female	3
231304802HUCA	pharyngeal exudate	08-03-13	4	Male	3
231305133HUCA	pharyngeal exudate	13-03-13	3	Male	1
231305178HUCA	pharyngeal exudate	14-03-13	8	Female	2
231305188HUCA	pharyngeal exudate	14-03-13	3	Female	3
231311851HUCA	conjunctival exudate	13-06-13	100	Female	8
231312106HUCA	pharyngeal exudate	17-06-13	4	Female	3
231312326HUCA	pharyngeal exudate	20-06-13	7	Male	3
231312737HUCA	nasal exudate	26-06-13	0	Male	3
231313679HUCA	conjunctival exudate	11-07-13	40	Female	8
231313817HUCA	pharyngeal exudate	13-07-13	2	Male	3
231314692HUCA	nasopharyngeal exudate	29-07-13	0	Male	3
231402626HUCA	conjunctival exudate	10-03-14	89	Male	8
231402723HUCA	conjunctival exudate	10-03-14	73	Female	8
231403901HUCA	conjunctival exudate	10-03-14	19	Male	8
231405710HUCA	conjunctival exudate	10-03-14	51	Female	8
231405872HUCA	conjunctival exudate	19-03-14	67	Male	8
231406074HUCA	conjunctival exudate	19-03-14	36	Male	8
231407089HUCA	pharyngeal exudate	16-03-14	2	Male	2
231407615HUCA	conjunctival exudate	22-03-14	0	Male	2
231407717HUCA	pharyngeal exudate	24-03-14	1	Male	2
231408083HUCA	conjunctival exudate	28-03-14	63	Female	8
231422013HUCA	conjunctival exudate	18-11-14	49	Male	56
231422873HUCA	pharyngeal exudate	01-12-14	3	Male	5
231422955HUCA	pharyngeal exudate	02-12-14	1	Female	1
231500133HUCA	stool	02-01-15	0	Male	5
231506651HUCA	pharyngeal exudate	23-03-15	3	Male	1
231507200HUCA	urine	30-03-15	61	Male	2
231507521HUCA	pharyngeal exudate	05-04-15	0	Female	2
231508624HUCA	pharyngeal exudate	16-04-15	3	Male	3
231517460HUCA	nasopharyngeal exudate	19-08-15	1	Female	3
231519497HUCA	pharyngeal exudate	20-09-15	0	Female	8
231519946HUCA	stool	27-09-15	8	Male	31

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231521960HUCA	urine	22-10-15	64	Male	35
231607316HUCA	wound exudate	08-03-16	17	Female	3
231608400HUCA	pharyngeal exudate	17-03-16	1	Female	3
231610616HUCA	pharyngeal exudate	10-04-16	5	Female	3
231611442HUCA	wound exudate	19-04-16	28	Male	3
231612761HUCA	pleural fluid	04-05-16	84	Male	3
231612996HUCA	stool	06-05-16	3	Female	3
231613523HUCA	urine	12-05-16	8	Female	3
231613812HUCA	stool	16-05-16	3	Female	3
231613862HUCA	pharyngeal exudate	17-05-16	3	Female	3
231614112HUCA	conjunctival exudate	19-05-16	32	Female	3
231711597HUCA	stool	09-04-17	76	Male	2
231712928HUCA	pharyngeal exudate	24-04-17	1	Male	2
231713057HUCA	pharyngeal exudate	26-04-17	2	Female	2
231718312HUCA	pharyngeal exudate	22-06-17	0	Male	3
231719290HUCA	conjunctival exudate	05-07-17	24	Female	4
231720177HUCA	nasopharyngeal exudate	17-07-17	1	Male	1
231802985HUCA	conjunctival exudate	18-01-18	31	Male	8
231810967HUCA	nasopharyngeal exudate	26-03-18	33	Female	3
231812092HUCA	conjunctival exudate	07-04-18	2	Female	8
231812098HUCA	nasopharyngeal exudate	08-04-18	4	Female	1
231814699HUCA	sputum	02-05-18	72	Male	2
231815448HUCA	pharyngeal exudate	09-05-18	2	Female	5
231815465HUCA	blood	10-05-18	84	Female	1
231816976HUCA	conjunctival exudate	25-05-18	35	Male	8
231817051HUCA	pharyngeal exudate	26-05-18	0	Male	2
231817497HUCA	blood	31-05-18	54	Female	1
231817542HUCA	pharyngeal exudate	31-05-18	2	Male	2
231817634HUCA	pharyngeal exudate	31-05-18	0	Male	2
231817907HUCA	urine	04-06-18	54	Female	1
231818170HUCA	nasopharyngeal exudate	06-06-18	2	Male	2
231818202HUCA	nasopharyngeal exudate	06-06-18	6	Female	5
231818217HUCA	blood	07-06-18	54	Female	1
231818308HUCA	nasopharyngeal exudate	07-06-18	2	Male	5
231818705HUCA	nasopharyngeal exudate	13-06-18	2	Male	2
231818892HUCA	pharyngeal exudate	14-06-18	9	Male	4
231818968HUCA	pharyngeal exudate	15-06-18	11	Female	1
231825910HUCA	nasopharyngeal exudate	12-09-18	0	Male	3
231828826HUCA	conjunctival exudate	15-10-18	70	Female	8
231828839HUCA	nasopharyngeal exudate	15-10-18	65	Female	4
231833746HUCA	nasopharyngeal exudate	29-11-18	2	Female	1
231834056HUCA	nasopharyngeal exudate	03-12-18	2	Male	1
231834363HUCA	conjunctival exudate	05-12-18	1	Male	3
231834369HUCA	pharyngeal exudate	05-12-18	3	Female	1
231834370HUCA	pharyngeal exudate	05-12-18	1	Male	3
231835154HUCA	nasopharyngeal exudate	13-12-18	0	Male	2

231836966HUCA	nasopharyngeal exudate	29-12-18	16	Female	7
231837001HUCA	nasopharyngeal exudate	29-12-18	1	Male	3
231904615HUCA	pharyngeal exudate	29-01-19	3	Male	3
231906748HUCA	nasopharyngeal exudate	08-02-19	4	Female	3
231909530HUCA	pharyngeal exudate	23-02-19	0	Male	7
231909842HUCA	conjunctival exudate	25-02-19	3	Female	3
231909848HUCA	nasopharyngeal exudate	26-02-19	3	Male	3
231911751HUCA	nasopharyngeal exudate	08-03-19	1	Male	7
231912457HUCA	pharyngeal exudate	13-03-19	1	Male	1
231913746HUCA	nasopharyngeal exudate	21-03-19	0	Male	3
231914121HUCA	conjunctival exudate	25-03-19	4	Female	3
231914124HUCA	nasopharyngeal exudate	25-03-19	5	Female	7
231914915HUCA	pharyngeal exudate	30-03-19	5	Female	7
231915364HUCA	nasopharyngeal exudate	03-04-19	3	Female	7
231915591HUCA	pharyngeal exudate	04-04-19	3	Male	3
231915681HUCA	pharyngeal exudate	04-04-19	3	Female	3
231917121HUCA	vaginal	05-06-19	58	Female	3
231918578HUCA	conjunctival exudate	02-05-19	47	Male	7
231920562HUCA	pharyngeal exudate	05-06-19	37	Female	3
231922132HUCA	conjunctival exudate	31-05-19	12	Male	8
231922134HUCA	nasopharyngeal exudate	31-05-19	3	Male	4
231922135HUCA	pharyngeal exudate	31-05-19	3	Male	3
231922136HUCA	pharyngeal exudate	31-05-19	0	Female	7
231922142HUCA	pharyngeal exudate	01-06-19	8	Male	3
231922179HUCA	pharyngeal exudate	02-06-19	2	Female	1
231922783HUCA	nasopharyngeal exudate	06-06-19	3	Male	7
231922961HUCA	pharyngeal exudate	07-06-19	9	Male	7
231922976HUCA	pharyngeal exudate	08-06-19	4	Female	4
231923660HUCA	nasopharyngeal exudate	14-06-19	1	Female	2
231924319HUCA	nasal exudate	20-06-19	7	Male	3
231924526HUCA	pharyngeal exudate	22-06-19	5	Female	3
231924846HUCA	pharyngeal exudate	25-06-19	2	Female	1
231925749HUCA	nasopharyngeal exudate	04-07-19	8	Male	3
231926141HUCA	nasopharyngeal exudate	09-07-19	8	Male	4
231926142HUCA	conjunctival exudate	09-07-19	8	Male	4
231926170HUCA	nasopharyngeal exudate	09-07-19	10	Male	4
231929561HUCA	nasopharyngeal exudate	20-08-19	1	Female	5
231929685HUCA	pharyngeal exudate	21-08-19	1	Female	3
231932486HUCA	genital ulcer exudate	19-09-19	73	Female	7
231936627HUCA	nasal exudate	25-10-19	1	Male	2
231936639HUCA	endomyocardial biopsy	25-10-19	43	Male	2
231937414HUCA	pharyngeal exudate	03-11-19	4	Female	2
231941472HUCA	nasopharyngeal exudate	03-12-19	52	Male	6
232003159HUCA	conjunctival exudate	13-01-20	28	Female	8
232006322HUCA	nasopharyngeal exudate	23-01-20	0	Male	2

232009890HUCA	pharyngeal exudate	06-02-20	0	Female	2
232010517HUCA	pharyngeal exudate	10-02-20	1	Female	2
232010665HUCA	pharyngeal exudate	10-02-20	1	Male	2
232011434HUCA	pharyngeal exudate	13-02-20	0	Male	2
232011466HUCA	pharyngeal exudate	13-02-20	0	Male	2
232015270HUCA	cutaneous	05-03-20	65	Female	1
232015566HUCA	nasopharyngeal exudate	06-03-20	0	Female	2
232302445HUCA	conjunctival exudate	18-01-23	0	Male	3
232303183HUCA	nasopharyngeal exudate	20-01-23	10	Male	3
232305783HUCA	conjunctival exudate	31-01-23	33	Female	3
232306242HUCA	pharyngeal exudate	02-02-23	6	Female	3
232306407HUCA	nasopharyngeal exudate	02-02-23	1	Male	3
232306611HUCA	nasopharyngeal exudate	03-02-23	5	Male	3
232306613HUCA	pharyngeal exudate	03-02-23	6	Female	3
232306614HUCA	pharyngeal exudate	03-02-23	2	Female	3
232306797HUCA	pharyngeal exudate	04-02-23	3	Male	3
232306799HUCA	pharyngeal exudate	04-02-23	4	Female	3
232307689HUCA	nasopharyngeal exudate	08-02-23	10	Male	3
242242022HUCA	pharyngeal exudate	20-02-22	3	Female	2
242248319HUCA	nasopharyngeal exudate	26-02-22	1	Male	6
242256621HUCA	pharyngeal exudate	07-03-22	2	Female	2
242266224HUCA	nasopharyngeal exudate	18-03-22	2	Male	2
252209368HUCA	nasopharyngeal exudate	10-05-22	0	Male	2
252216986HUCA	pharyngeal exudate	19-05-22	2	Female	1
252217137HUCA	nasopharyngeal exudate	20-05-22	1	Male	1
252217536HUCA	pharyngeal exudate	20-05-22	2	Female	3
252222479HUCA	pharyngeal exudate	29-05-22	0	Male	1
252227760HUCA	pharyngeal exudate	07-06-22	4	Male	5
252227935HUCA	nasopharyngeal exudate	08-06-22	9	Female	3
252228809HUCA	nasopharyngeal exudate	09-06-22	2	Male	1
252229369HUCA	pharyngeal exudate	11-06-22	2	Male	2
252230519HUCA	nasopharyngeal exudate	13-06-22	2	Female	2
252273752HUCA	nasopharyngeal exudate	12-09-22	1	Male	3
252273768HUCA	nasopharyngeal exudate	12-09-22	2	Female	1
252277437HUCA	nasopharyngeal exudate	27-09-22	9	Female	1
252279435HUCA	pharyngeal exudate	05-10-22	2	Female	2
252280912HUCA	nasopharyngeal exudate	10-10-22	2	Male	3
252281383HUCA	nasopharyngeal exudate	12-10-22	5	Male	3
252281392HUCA	pharyngeal exudate	12-10-22	3	Female	3
252281393HUCA	pharyngeal exudate	12-10-22	3	Male	3
252281600HUCA	pharyngeal exudate	13-10-22	2	Female	3
252281862HUCA	conjunctival exudate	14-10-22	50	Female	3
252282209HUCA	pharyngeal exudate	15-10-22	1	Female	2
252282234HUCA	pharyngeal exudate	15-10-22	4	Male	3
252282277HUCA	pharyngeal exudate	15-10-22	2	Female	3

252282296HUCA	pharyngeal exudate	16-10-22	6	Female	2
252282304HUCA	pharyngeal exudate	16-10-22	0	Male	2
252285381HUCA	nasopharyngeal exudate	25-10-22	4	Male	3
252285553HUCA	nasopharyngeal exudate	26-10-22	5	Male	2
2522911786HUCA	nasopharyngeal exudate	15-11-22	3	Female	3
252293430HUCA	pharyngeal exudate	20-11-22	2	Male	3
252293434HUCA	nasopharyngeal exudate	20-11-22	2	Female	3
252296009HUCA	nasopharyngeal exudate	28-11-22	5	Male	3
252297106HUCA	nasopharyngeal exudate	01-12-22	6	Female	3
252297286HUCA	nasopharyngeal exudate	01-12-22	5	Male	3
252297296HUCA	pharyngeal exudate	29-11-22	12	Male	3
252297570HUCA	pharyngeal exudate	02-12-22	8	Female	3
252297645HUCA	pharyngeal exudate	02-12-22	2	Female	3
252297647HUCA	nasopharyngeal exudate	02-12-22	3	Male	3
252297664HUCA	pharyngeal exudate	02-12-22	2	Male	3
252297823HUCA	nasopharyngeal exudate	02-12-22	0	Male	3
252297854HUCA	nasopharyngeal exudate	03-12-22	2	Female	2
252297856HUCA	pharyngeal exudate	03-12-22	3	Female	3
252297857HUCA	pharyngeal exudate	03-12-22	2	Male	3
252298546HUCA	nasopharyngeal exudate	06-12-22	1	Female	3
252299395HUCA	pharyngeal exudate	10-12-22	3	Male	3
252299397HUCA	pharyngeal exudate	10-12-22	6	Male	3
252299398HUCA	pharyngeal exudate	10-12-22	6	Male	3
252299400HUCA	pharyngeal exudate	10-12-22	3	Male	3
252299401HUCA	pharyngeal exudate	10-12-22	2	Male	3
252299412HUCA	pharyngeal exudate	10-12-22	2	Female	3
252299525HUCA	pharyngeal exudate	11-12-22	8	Female	3
252299526HUCA	pharyngeal exudate	11-12-22	7	Female	3
252299560HUCA	nasopharyngeal exudate	11-12-22	6	Male	3
252299958HUCA	nasopharyngeal exudate	11-12-22	7	Male	3
262201243HUCA	nasopharyngeal exudate	16-12-22	4	Male	3
262201281HUCA	nasopharyngeal exudate	15-12-22	5	Male	3
262201497HUCA	pharyngeal exudate	17-12-22	1	Male	3
262201556HUCA	pharyngeal exudate	17-12-22	43	Female	3
262201566HUCA	pharyngeal exudate	17-12-22	3	Male	3
262201653HUCA	pharyngeal exudate	18-12-22	2	Male	3
262201683HUCA	pharyngeal exudate	18-12-22	6	Male	3
262201697HUCA	nasopharyngeal exudate	18-12-22	8	Male	3
262201712HUCA	pharyngeal exudate	18-12-22	3	Female	3
262203151HUCA	pharyngeal exudate	22-12-22	4	Male	3
262203157HUCA	nasopharyngeal exudate	22-12-22	1	Male	3
262203726HUCA	pharyngeal exudate	25-12-22	1	Female	3
262203746HUCA	nasopharyngeal exudate	26-12-22	8	Female	3
262203747HUCA	nasopharyngeal exudate	26-12-22	5	Male	3
262203756HUCA	pharyngeal exudate	26-12-22	7	Female	3

262203757HUCA	pharyngeal exudate	26-12-22	2	Male	3
262203790HUCA	pharyngeal exudate	26-12-22	5	Male	3
262204068HUCA	pharyngeal exudate	27-12-22	2	Male	3
262204069HUCA	nasopharyngeal exudate	27-12-22	3	Female	3
262204174HUCA	pharyngeal exudate	27-12-22	3	Female	3
262204224HUCA	pharyngeal exudate	27-12-22	7	Male	3
262204522HUCA	pharyngeal exudate	29-12-22	0	Male	3
262204698HUCA	nasopharyngeal exudate	29-12-22	8	Male	3