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Molecular Characterization of Peruvian Fowl Adenovirus (FAdV) Isolates

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Abstract

Forty seven clinical samples of Fowl adenovirus (FAdV) associated with Inclusion Body Hepatitis (IBH) from Peruvian broilers received between July 2006 and April 2013 were genotyped using sequencing of L1 Loop of Hexon gene. All 47 clinical samples presented macroscopic and histopathology lesions consistent with IBH, and amplified a specific fragment of Hexon gene by Polymerase Chain Reaction (PCR). A unique nucleotide sequence of 789 base pairs of Hexon gene (position 273 to 1061) was obtained in all 47 clinical samples analyzed. This sequence showed a high level of conservation in amino acid and nucleotide sequence (>99%) with a Fowl Adenovirus C serotype 4 previously identified. Sequence and phylogenetic analysis indicate no genotypic variation in Peruvian isolates. The presence of a unique genotype very closely related with genotype C1 previously reported in Peru and Ecuador (>99%), suggests the presence of FAdV C serotype 4 genotype C1 in clinical cases of IBH from Peruvian broilers.

Keywords

Fowl Adenovirus C, Molecular Characterization, Inclusion Body Hepatitis, Peru

1. Introduction

Fowl Adenovirus (FAdV) includes 12 serotypes [1], with a high diversity of strains for each serotype and a wide range of clinical and pathological presentations (pneumonias, tracheitis, gizzard erosions, proventriculitis, pancreatitis, inclusion body hepatitis and hydropericardium) [2]. The most important sanitary problem in North America and South America is inclusion body hepatitis/hydropericardium syndrome due to high levels of morbidity and mortality in broilers resulting in significant economic losses [3]-[7]. This great diversity exhibited by FAdV required the use of additional techniques to make a correct identification of levels of species and serotypes and characterization of genetic diversity. An alternative to microbiologic and serologic techniques are

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molecular techniques based on DNA analysis. Several studies have reported the feasibility of molecular genetic tools for genetic differentiation and molecular typing of FAdV. The use of PCR for Hexon gene, RFLP analysis and direct sequencing of Hexon and Penton genes [8]-[10] have permitted a correct identification and characterization of clinical isolates. The lack of information available for molecular characterization of isolates from Peru do not allow the identification of the type of serotypes currently present in poultry production systems. This is a key requirement for establishing actions to control and prevent IBH. The objective of this study was to determine FAdV genotypes/serotypes isolated from IBH clinical cases in broilers from Peru.

2. Materials and Methods

2.1. Sources of Samples

A total of 47 suspected clinical cases of Inclusion body hepatitis (IBH) from broilers received in the Microbiology Laboratory-Bioservice SRL between July 2006 and April 2013 were included in this study (**Table 1**). Agar gel immune-diffusion (AGID) tests for serotypes 4, 8 and 9, liver histopathology analysis and Hexon gene PCR were performed to confirm the presence of fowl adenovirus (FAdV) in all suspected clinical cases.

2.2. Viral DNA Extraction and PCR Amplification of Hexon Gene

Viral DNA was extracted from liver lysate using DNeasy Tissue kit (Qiagen) according to manufacturer. PCR for L1 loop of the Hexon gene was carried out using primers HA and HB as described previously [9]. In brief, the PCR reaction was performed using a final volume of 20 uL containing 10 ng genomic DNA, 1X PCR buffer (20 mM Tris-HCl, 20 mM KCl, 5 mM $(NH4)_2SO_4$), 2 mM MgCl2, 5 pmol of each primer, 0.2 mM of dNTPs, 0.5 U Maxima HotStart-Taq DNA polymerase (Fermentas). Thermal cycles were the following: initial denaturation of 94˚C for 3 minutes, 30 cycles of 94˚C for 30 seconds, 53˚C for 60 seconds, 72˚C for 30 seconds and a final extension of 72˚C for 5 minutes. The PCR amplification was carried out using a Veriti Thermal cycler (Applied Biosystems). The PCR products were separated in 1% agarose gel TBE 1X electrophoresis and visualized by fluorescence using an ethidium bromide solution (0.1 ug/mL) and UV light.

2.3. Sequencing and Phylogenetics Analysis of L1 Loop of Hexon Gene

The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up kit, cloned with pGEM-T Easy (Promega) and submitted for sequencing to Macrogen Inc, Korea. The sequencing reaction was performed in both directions using Big Dye sequencing kit (Applied Biosystems) and the primers HA and HB. Partial nucleotide sequence of the L1 Loop of hexon gene from FAdV's was submitted to Gene Bank with the following accession number: KF601685.

Forward and reverse sequences were aligned together using Clustal program [11] to create consensus sequences. The consensus nucleotide sequences were aligned by the Clustal W method using MEGA 5.0 software [12]. Pairwise amino acid and nucleotide identity and pairwise evolutionary distances were computed using

Table 1. Collection year and city of origin of 47 clinical cases of Inclusion body hepatitis (IBH) from broilers from Peru.

Year of isolation	Place of isolation (City, Province)	Number of isolates
2006, 2007, 2011, 2012, 2013	Lima, Lima	12
2011, 2012, 2013	Huaral, Lima	20
2011, 2012, 2013	Chilca, Lima	1
2011	Iquitos, Loreto	2
2011	Arequipa, Arequipa	1
2011	Tacna, Tacna	1
2011	Chanchamayo, Junin	2
2011	Nazca, Ica	1
2006, 2007, 2012, 2013	Trujillo, La Libertad	7

Mega v5.0. A phylogenetic analysis for the distance method was performed using partial sequences of L1 loop of Hexon gene and Hexon protein previously published in Gene Bank and FAdV Peruvian sequence (**Table 2**)

Table 2. ID sequence, accession number, country of origin, species, serotype and genetic cluster of 37 FAdV using phylogenetic analysis.

ID Sequence	Accession number[1]	Country[2]	Species[3]	Serotype[4]	Genetic Cluster[5]
FAdV_1_(CELO)	AAL13217	NR[6]	A	EU/US 1	A
FAdV_2_(SR48)	AAN77072	NR[6]	D	EU 2	D2
FAdV_3_(75)	AAN77075	NR[6]	D	EU 3	D3
FAdV_4_(KR5)	AAN77077	NR[6]	C	EU 4	C
FAdV_5_(VR-830)	AAL13222	NR[6]	E	US 5	D1
FAdV_5_(TR22)	AAN77079	NR[6]	B	EU 5	E
FAdV_5_(340)	AAN77078	NR[6]	B	EU 5	B
FAdV_6_(VR-831)	AAL13224	NR[6]	D	US 6	D3
FAdV_6_(CR119)	AAN77080	NR[6]	E	EU 6	D1
FAdV_7_(YR36)	AAN77081	NR[6]	E	EU 7	D1
FAdV_7_(VR-832)	AAL13225	NR[6]	E	EU 7	D1
FAdV_8_(TR59)	AAN77082	NR[6]	E	EU 8	D1
FAdV_8_(VR-833)	AAL13221	NR[6]	D	US 8	D3
FAdV_9_(VR-834)	AAL13226	NR[6]	C	US 9	C
FAdV_9_(764)	AAN77084	NR[6]	E	EU 9	D1
FAdV_10_(VR-835)	AAL13227	NR[6]	E	US 10	D1
FAdV_11_(X11)	AAL13223	NR[6]	E	US 11	D1
FAdV_11_(C2B)	AAN77085	NR[6]	C	EU 11	D2
FAdV_12_(380)	AAL13228	NR[6]	D	US 12	D2
(922-1)_Germany	FN869978.1	Germany	C	EU/US 4	C
(09-2602)_Austria	FN869977.1	Austria	C	EU/US 4	C
(K31)_Pakistan	FN869976.1	Pakistan	C	EU/US 4	C
(09-584)_Austria	FN869975.1	Austria	C	EU/US 4	C
(53)_Peru	FN869973.1	Peru	C	EU/US 4	C
(K1013)_Ecuador	FN869972.1	Ecuador	C	EU/US 4	C
(Da60)_Germany	FN869971.1	Germany	C	EU/US 4	C
(K99-97)_Kuwait	FN869970.1	Kuwait	C	EU/US 4	C
(AG234)_Mexico	FN869969.1	Mexico	C	EU/US 4	C
South_Korea	HQ697593.1	South Korea	C	EU/US 4	C
(04-50388)_Canada	EF685395.1	Canada	C	EU/US 4	C
(488)_Russia	AY581295.1	Russia	C	EU/US 4	C
(4158)_Italy	HM592284.1	Italy	C	EU/US 4	C
(5997)_Italy	HM592281.1	Italy	C	EU/US 4	C
(6169)_Italy	HM592277.1	Italy	C	EU/US 4	C
(5670)_Italy	HM592274.1	Italy	C	EU/US 4	C
(488)_Indian	AY581295.1	India	C	EU/US 4	C
(HARYANA-07)_Indian	EU847626.1	India	C	EU/US 4	C

[1]Accesion number for Hexon protein or sequence in database Genbank or EMBL. [2]Country of origin of strain. [3]Fowl Adenovirus species according with [10]. [4]Fowl Adenovirus serotype according with [1]. [5]Fowl Adenovirus phylogenetic cluster according with [10]. [6]No reported in [10].

using Kimura-2-parameter method [13] and Dayhoff matrix based method [14]. A dendogram was constructed with the Neighbor-joining method [15] using 1000 bootstrap [16] replicate values with Mega v5.0.

3. Results

3.1. Clinical Cases of Inclusion Body Hepatitis

All clinical cases presented liver histopathology consistent with IBH lesions hepatocyte cytoplasmic vacuolation, multifocal to coalescing areas of hepatocytes necrosis, irregular shape hepatocyte nuclei, presence of basophilic intranuclear inclusion bodies without presence of HPS. The presence of FAdV was confirmed by PCR amplification of Hexon gene fragment in 47 suspected clinical cases of IBH.

3.2. Sequencing and Phylogenetic Analysis of L1 Loop of Hexon Gene

All 47 Peruvian FAdV isolates did not show genotype variation, a unique nucleotide sequence of 789 bp corresponding to position 273 and 1061 of Hexon gene amplified showed high nucleotide identity level (99.5% identity, E value = 0) and high conservation level in the amino acidic sequence (99.5%) in relation with a FAdV C serotype 4 previously identified [10]. The pairwise matrix of evolutionary divergence (nucleotide and amino acid) showed less divergence between Peruvian FAdV with FAdV C serotype 4 (0.005, 0.005) and FAdV C serotype US 9 (0.029, 0.070) in contrast to FAdV D serotype 8 (0.365, 0.709) and serotype 12 (0.378 and 0.663) (**Table 3**). Phylogenetic analysis from a segment of 770 nucleotide (260 amino acids) suggests the presence of FAdV group C serotype 4 present in all clinical cases from IBH of Peruvian broilers (**Figure 1(a)** and

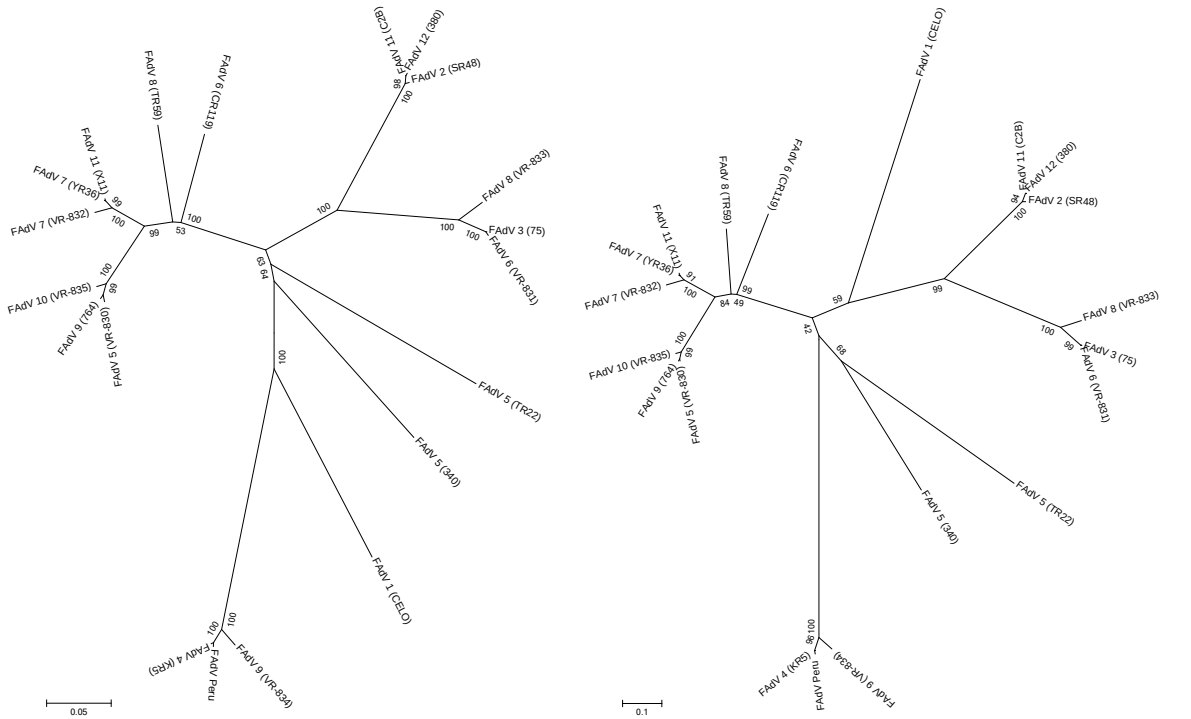

Figure 1. (a) Phylogenetic tree of L1 loop of the hexon nucleotide sequence from 12 species of FAdV's. The evolutionary relationships of taxa were inferred using Neighbor-Joining method [17]. The evolutionary distances were computed using the Kimura 2-parameter method [13]. The analysis involved 20 nucleotide sequences and 770 nucleotide positions in the final dataset, the strain names are according to **Table 2**. Bootstrap [16] test (1000 replicates) is expressed in % values. The scale bar indicates the evolutionary distance between sequences; (b) Phylogenetic tree of L1 loop of the hexon protein sequence from 12 species of FAdV's. The evolutionary relationships of taxa were inferred using Neighbor-Joining method [17]. The evolutionary distances were computed using the Dayhoff matrix method [14]. The analysis involved 20 amino acid sequences and 199 amino acid positions in the final dataset, the strain names are according to **Table 2**. Bootstrap [16] test (1000 replicates) is expressed in % values. The scale bar indicates the evolutionary distance between sequences.

Table 3. Pairwise estimates of evolutionary divergence[1] of nucleotide sequence (below diagonal) and amino acid sequence (above diagonal) between 12 species of FAdV[2].

	FAdV Peru	FAdV1	FAdV2	FAdV3	FAdV4	FAdV5A	FAdV5B	FAdV5C	FAdV6A	FAdV6B	FAdV7A	FAdV7B	FAdV8A	FAdV8B	FAdV9A	FAdV9B	FAdV10	FAdV11A	FAdV11B	FAdV12
FAdVPeru		0.628	0.663	0.704	0.005	0.603	0.663	0.573	0.704	0.568	0.578	0.573	0.548	0.709	0.070	0.603	0.598	0.583	0.663	0.663
FAdV1	0.300		0.573	0.608	0.628	0.583	0.648	0.598	0.608	0.603	0.553	0.563	0.578	0.603	0.618	0.583	0.598	0.553	0.563	0.563
FAdV2	0.374	0.335		0.432	0.663	0.558	0.663	0.563	0.432	0.508	0.543	0.528	0.528	0.427	0.658	0.558	0.553	0.538	0.030	0.030
FAdV3	0.366	0.323	0.203		0.704	0.568	0.588	0.588	0.000	0.588	0.563	0.558	0.578	0.101	0.724	0.568	0.573	0.563	0.417	0.417
FAdV4	0.005	0.299	0.374	0.365		0.603	0.663	0.573	0.704	0.568	0.578	0.573	0.548	0.709	0.070	0.603	0.598	0.583	0.663	0.663
FAdV5A	0.353	0.321	0.278	0.273	0.352		0.573	0.543	0.568	0.317	0.216	0.246	0.271	0.563	0.613	0.010	0.035	0.221	0.563	0.563
FAdV5B	0.361	0.340	0.303	0.264	0.360	0.278		0.533	0.588	0.573	0.558	0.568	0.543	0.598	0.653	0.573	0.568	0.558	0.653	0.653
FAdV5C	0.332	0.312	0.295	0.305	0.331	0.284	0.271		0.588	0.503	0.523	0.518	0.528	0.583	0.588	0.543	0.538	0.518	0.563	0.563
FAdV6A	0.369	0.326	0.205	0.003	0.368	0.275	0.266	0.308		0.588	0.563	0.558	0.578	0.101	0.724	0.568	0.573	0.563	0.417	0.417
FAdV6B	0.339	0.313	0.240	0.258	0.339	0.151	0.279	0.262	0.261		0.286	0.281	0.302	0.573	0.563	0.322	0.312	0.286	0.533	0.533
FAdV7A	0.336	0.301	0.262	0.268	0.336	0.096	0.278	0.265	0.270	0.117		0.055	0.231	0.568	0.578	0.221	0.211	0.010	0.553	0.553
FAdV7B	0.331	0.305	0.257	0.264	0.332	0.105	0.277	0.264	0.266	0.116	0.021		0.266	0.568	0.573	0.241	0.241	0.065	0.543	0.543
FAdV8A	0.339	0.310	0.249	0.274	0.339	0.145	0.270	0.268	0.277	0.140	0.122	0.135		0.573	0.543	0.271	0.256	0.236	0.533	0.533
FAdV8B	0.365	0.323	0.200	0.044	0.364	0.271	0.275	0.308	0.047	0.260	0.265	0.264	0.279		0.714	0.563	0.573	0.568	0.412	0.412
FAdV9A	0.029	0.301	0.371	0.368	0.031	0.353	0.355	0.334	0.370	0.340	0.339	0.336	0.338	0.364		0.613	0.613	0.583	0.668	0.668
FAdV9B	0.355	0.322	0.277	0.271	0.353	0.009	0.278	0.283	0.274	0.151	0.094	0.100	0.145	0.271	0.355		0.035	0.226	0.563	0.563
FAdV10	0.356	0.327	0.274	0.274	0.355	0.021	0.278	0.283	0.277	0.144	0.090	0.099	0.138	0.273	0.357	0.021		0.216	0.568	0.568
FAdV11A	0.338	0.300	0.260	0.266	0.338	0.097	0.277	0.262	0.269	0.117	0.003	0.023	0.123	0.264	0.340	0.095	0.091		0.548	0.548
FAdV11B	0.378	0.332	0.010	0.199	0.378	0.281	0.296	0.295	0.201	0.248	0.269	0.262	0.252	0.196	0.378	0.279	0.277	0.266		0.000
FAdV12	0.378	0.335	0.010	0.201	0.378	0.282	0.299	0.296	0.204	0.248	0.270	0.262	0.253	0.199	0.378	0.281	0.278	0.268	0.003	

[1]Analyses were conducted using p-distance [18]. This distance is the proportion (p) of amino acid (nucleotide) sites at which the two sequences to be compared are different. [2]The FAdV strain accession number: FAdV1 (AAL13217), FAdV2 (AAN77072), FAdV3 (AAN77075), FAdV4 (AAN77077), FAdV5A (AAL13222), FAdV5B (AAN77079), FAdV5C (AAN77078), FAdV6A (AAL13224), FAdV6B (AAN77080), FAdV7A (AAN77081), FAdV7B (AAL13225), FAdV8A (AAN77082), FAdV8B (AAL13221), FAdV9A (AAL13226), FAdV9B (AAN77084), FAdV10 (AAL13227), FAdV11A (AAL13223), FAdV11B (AAN77085), FAdV12 (AAL13228) and FAdVPeru (this study).

Figure 1(b)). The FAdV genotype identified is closely related with the genotype C1 [19] previously reported in Peru (evolutionary divergence = 0, 100% nucleotide identity) and Ecuador (evolutionary divergence = 0.002, 99.9% nucleotide identity), however the two isolates showed 100% amino acid identity with FAdV isolated in this study (**Table 4**); this relationship is corroborated by phylogenetic clustering analysis (**Figure 2(a)** and **Figure 2(b)**).

4. Discussion

The nucleic acid technology has demonstrated the utility to detect differences in structural proteins related with immune response as Hexon protein. The sequencing of Hexon gene permits a correct identification of species level and serotype in FAdV, with similar results as reported for RFLP genome analysis [2]. Inside Hexon gene, 7 hypervariable regions were identified exclusively located in Loop 1 (L1) and Loop 2 (L2) regions [20]. The loop 1 in Hexon gene of FAdV reported higher variability [8], and has demonstrated additional applications than include genetic variability analysis of FAdV isolates [6] [10] [21].

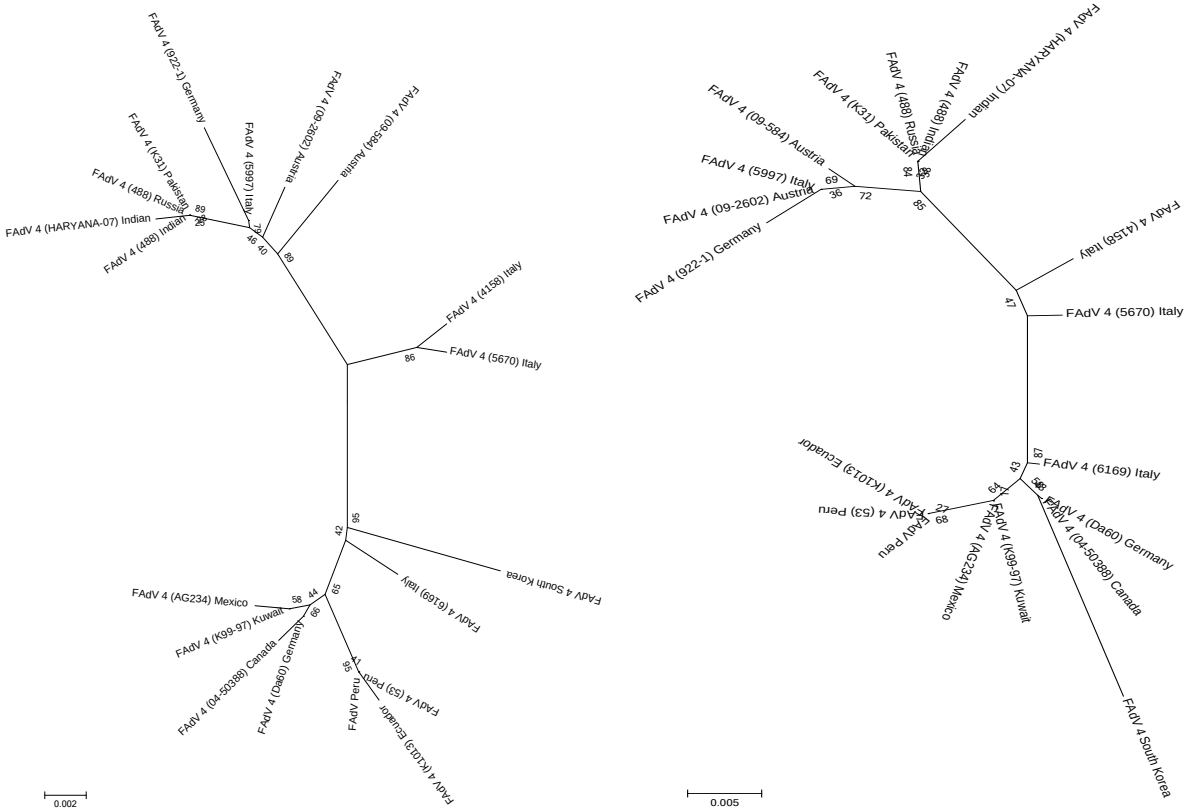

Figure 2. (a) Phylogenetic tree of L1 loop of the hexon nucleotide sequence from FAdV C serotype 4. The evolutionary relationships of taxa were inferred using Neighbor-Joining method [17]. The evolutionary distances were computed using the Kimura 2-parameter method [13]. The analysis involved 19 nucleotide sequences and 611 nucleotide positions in the final dataset, the strain names are according to **Table 2**. Bootstrap [16] test (1000 replicates) is expressed in % values. The scale bar indicates the evolutionary distance between sequences. (b) Phylogenetic tree of L1 loop of the hexon protein sequence from FAdV C serotype 4. The evolutionary relationships of taxa were inferred using Neighbor-Joining method [17]. The evolutionary distances were computed using the Dayhoff matrix method [14]. The analysis involved 19 amino acid sequences and 202 amino acid positions in the final dataset, the strain names are according to **Table 2**. Bootstrap [16] test (1000 replicates) is expressed in % values. The scale bar indicates the evolutionary distance between sequences.

During this study we analyzed 47 FAdV isolates from IBH clinical cases, examined their Hexon gene loop 1 sequences and compared them with reference strains previously published sequences. Our results suggest the absence of genetic variability in FAdV C in Peru, similar to previous reports [19]; however this study only has access to one Peruvian isolate. Similar results were reported from India [22] [23] and South Korea [24] for clinical cases of IBH associated with the presence of FAdV C serotype 4 (>99% clinical cases of IBH). However, FAdV C serotype 4 (strain K531) is also associated with hydropericardium syndrome outbreaks (HPS) from South Korea [24] [25] and Germany [26] in contrast with Peruvian FAdV C serotype 4 than only reports IBH clinical lesions. The amino acid sequence analysis shows a slight differentiation between isolates from Peru and South Korea (**Table 4**) and phylogenetic analysis corroborates the different genetic clustering (**Figure 2(B)**).

The presence of multiple FAdV species in clinical cases of IBH were reported in Canada [6] with a fixation of FAdV serotypes 8 (45%) and 11 (45%) and South Korea [27] with FAdV serotype 4 (60%) and 11 (40%), however South Korean FAdV serotype 4 showed clinical hydropericardium/ IBH lesions in contrast to the Canadian report [6].

This study is the first report of genetic characterization of FAdV C isolates from Peru that includes samples from a coastal region (Ica, La Libertad and Lima regions) which concentrates 80% of poultry production systems in Peru and provides important information than can serve as starting point for further investigations related to pathogenicity, antigenic properties and specific vaccine development for prevention and control of IBH

Table 4. Pairwise estimates of evolutionary divergence[1] of nucleotide sequences (below diagonal) and amino acid sequence (above diagonal) between 19 strain of FAdV-C serotype 4.

	FAdV Peru	Germany A	Austria A	Pakistan	Austria B	Peru	Ecuador	Germany B	Kuwait	Mexico	South Korea	Canada	Russia A	Italy A	Italy B	Italy C	Italy D	Indian A	Indian B
FAdV Peru		0.050	0.045	0.035	0.045	0.000	0.000	0.010	0.005	0.005	0.025	0.010	0.035	0.030	0.045	0.010	0.025	0.035	0.040
Germany A	0.029		0.005	0.015	0.010	0.050	0.050	0.040	0.045	0.045	0.045	0.040	0.015	0.030	0.005	0.045	0.030	0.015	0.020
Austria A	0.023	0.008		0.010	0.005	0.045	0.045	0.035	0.040	0.040	0.040	0.035	0.010	0.025	0.000	0.040	0.025	0.010	0.015
Pakistan	0.023	0.008	0.007		0.010	0.035	0.035	0.035	0.030	0.030	0.050	0.035	0.000	0.020	0.010	0.035	0.020	0.000	0.005
Austria B	0.026	0.011	0.007	0.010		0.045	0.045	0.040	0.040	0.040	0.045	0.040	0.010	0.020	0.005	0.035	0.020	0.010	0.015
Peru	0.000	0.029	0.023	0.023	0.026		0.000	0.010	0.005	0.005	0.025	0.010	0.035	0.030	0.045	0.010	0.025	0.035	0.040
Ecuador	0.002	0.031	0.025	0.025	0.028	0.002		0.010	0.005	0.005	0.025	0.010	0.035	0.030	0.045	0.010	0.025	0.035	0.040
Germany B	0.007	0.028	0.020	0.023	0.023	0.007	0.008		0.005	0.005	0.015	0.000	0.035	0.025	0.035	0.005	0.020	0.035	0.040
Kuwait	0.005	0.029	0.021	0.021	0.025	0.005	0.007	0.002		0.000	0.020	0.005	0.030	0.025	0.040	0.005	0.020	0.030	0.035
Mexico	0.007	0.031	0.023	0.023	0.026	0.007	0.008	0.003	0.002		0.020	0.005	0.030	0.025	0.040	0.005	0.020	0.030	0.035
South Korea	0.016	0.028	0.026	0.026	0.026	0.016	0.018	0.010	0.011	0.013		0.015	0.050	0.040	0.040	0.020	0.035	0.050	0.054
Canada	0.008	0.029	0.021	0.025	0.025	0.008	0.010	0.002	0.003	0.005	0.011		0.035	0.025	0.035	0.005	0.020	0.035	0.040
Russia A	0.023	0.008	0.007	0.000	0.010	0.023	0.025	0.023	0.021	0.023	0.026	0.025		0.020	0.010	0.035	0.020	0.000	0.005
Italy A	0.025	0.020	0.018	0.015	0.015	0.025	0.026	0.021	0.023	0.025	0.021	0.023	0.015		0.025	0.020	0.010	0.020	0.025
Italy B	0.026	0.005	0.003	0.003	0.007	0.026	0.028	0.023	0.025	0.026	0.023	0.025	0.003	0.015		0.040	0.025	0.010	0.015
Italy C	0.011	0.026	0.021	0.021	0.025	0.011	0.013	0.005	0.007	0.008	0.011	0.007	0.021	0.016	0.021		0.015	0.035	0.040
Italy D	0.021	0.020	0.018	0.015	0.015	0.021	0.023	0.018	0.020	0.021	0.018	0.020	0.015	0.003	0.015	0.013		0.020	0.025
Indian A	0.023	0.008	0.007	0.000	0.010	0.023	0.025	0.023	0.021	0.023	0.026	0.025	0.000	0.015	0.003	0.021	0.015		0.005
Indian B	0.025	0.010	0.008	0.002	0.011	0.025	0.026	0.025	0.023	0.025	0.028	0.026	0.002	0.016	0.005	0.023	0.016	0.002	

[1]Analyses were conducted using p-distance [18]. This distance is the proportion (p) of amino acid (nucleotide) sites at which the two sequences to be compared are different. [2]The FAdV strain accession number: Germany A (FN869978.1), Austria A (FN869977.1), Pakistan (FN869976.1), Austria B (FN869975.1), Peru (FN869973.1), Ecuador (FN869972.1), Germany B (FN869971.1), Kuwait (FN869970.1), Mexico (FN869969.1), South Korea (HQ697593.1), Canada (EF685395.1), Russia A (AY581295.1), Italy A (HM592284.1), Italy B (HM592281.1), Italy C (HM592277.1), Italy D (HM592274.1), Indian A (AY581295.1), Indian B (EU847626.1) and Fad VPeru (this study).

associated with FAdV.

5. Conclusion

In conclusion, our results indicate the presence of FAdV C, serotype 4, genotype C1 circulating in clinical cases of IBH from Peruvian broilers.

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References

[1] McFerran, J.B. and Smyth, J.A. (2000) Avian Adenoviruses. *Revue Scientifique et Technique*, **19**, 589-601.

[2] Hess, M. (2000) Detection and Differentiation of Avian Adenoviruses: A Review. *Avian Pathology*, **29**, 195-206. http://dx.doi.org/10.1080/03079450050045440

[3] Nakamura, K., Mase, M., Yamaguchi, S., Shibahara, T. and Yuasa, N. (1999) Pathologic Study of Specific-Pathogen-Free Chicks and Hens Inoculated with Adenovirus Isolated from Hydropericardium Syndrome. *Avian Diseases*, **43**, 414-423. http://dx.doi.org/10.2307/1592638

[4] Toro, H., Prusas, C., Raue, R., Cerda, L., Geisse, C., Gonzalez, C. and Hess, M. (1999) Characterization of Fowl Adenoviruses from Outbreaks of Inclusion Body Hepatitis/Hydropericardium Syndrome in Chile. *Avian Diseases*, **43**, 262-270. http://dx.doi.org/10.2307/1592616

[5] Chandra, R., Shukla, S.K. and Kumar, M. (2000) Thehydropericardium Syndrome and Inclusion Body Hepatitis in Domestic Fowl. *Tropical Animal Health and Production*, **32**, 99-111. http://dx.doi.org/10.1023/A:1005230703093

[6] Ojkic, D., Martin, E., Swinton, J., Vaillancourt, J.P., Boulianne, M. and Gomis, S. (2008) Genotyping of Canadian Isolates of Fowl Adenoviruses. *Avian Pathology*, **37**, 95-100. http://dx.doi.org/10.1080/03079450701805324

[7] Alvarado, I.R., Villegas, P., El-Attrache, J., Jensen, E., Rosales, G., Perozo, F. and Purvis, L.B. (2007) Genetic Characterization, Pathogenicity, and Protection Studies with an Avian Adenovirus Isolate Associated with Inclusion Body Hepatitis. *Avian Diseases*, **51**, 27-32. http://dx.doi.org/10.1637/0005-2086(2007)051[0027:GCPAPS]2.0.CO;2

[8] Raue, R. and Hess, M. (1998) Hexon Based PCRs Combined with Restriction Enzyme Analysis for Rapid Detection and Differentiation of Fowl Adenoviruses and Egg Drop Syndrome Virus. *Journal of Virological Methods*, **73**, 211-217. http://dx.doi.org/10.1016/S0166-0934(98)00065-2

[9] Meulemans, G., Boschmans, M., van den Berg, T.P. and Decaesstecker, M. (2001) Polymerase Chain Reaction Combined with Restriction Enzyme Analysis for Detection and Differentiation of Fowl Adenoviruses. *Avian Pathology*, **30**, 655-660. http://dx.doi.org/10.1080/03079450120092143

[10] Meulemans, G., Couvreur, B., Decaesstecker, M., Boschmans, M. and van den Berg, T.P. (2004) Phylogenetic Analysis of Fowl Adenoviruses. *Avian Pathology*, **33**, 164-170. http://dx.doi.org/10.1080/0307945031000165208 6

[11] Thompson, J.D., Givson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The Clustal_*X* Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. *Nucleic Acids Research*, **24**, 4876-4882. http://dx.doi.org/10.1093/nar/25.24.4876

[12] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739. http://dx.doi.org/10.1093/molbev/msr121

[13] Kimura, M. (1980) A Simple Method for Estimating Evolutionary Rate of Base Substitutions through Comparative Studies of Nucleotide Sequences. *Journal of Molecular Evolution*, **16**, 111-120. http://dx.doi.org/10.1007/BF01731581

[14] Schwarz, R. and Dayhoff, M. (1979) Matrices for Detecting Distant Relationships. In: Dayhoff, M., Ed., *Atlas of Protein Sequences*, National Biomedical Research Foundation, 353-358.

[15] Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*, **4**, 406-425.

[16] Felsenstein, J. (1985) Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution*, **39**, 783-791. http://dx.doi.org/10.2307/2408678

[17] Tamura, K., Nei, M. and Kumar, S. (2004) Prospects for Inferring Very Large Phylogenies by Using the Neighbor-Joining Method. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 11030-11035. http://dx.doi.org/10.1073/pnas.0404206101

[18] Nei, M. and Kumar, S. (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York, 18.

[19] Marek, A., Günes, A., Schulz, E. and Hess, M. (2010) Classification of Fowl Adenoviruses by Use of Phylogenetic Analysis and High-Resolution Melting-Curve Analysis of the Hexon L1 Gene Region. *Journal of Virological Methods*, **170**, 147-154. http://dx.doi.org/10.1016/j.jviromet.2010.09.019

[20] Crawford-Miksza, L. and Shnurr, D.P. (1996) Analysis of 15 Adenovirus Hexon Proteins Reveals the Location and Structure of Seven Hypervariable Regions Containing Serotype-Specific Residues. *Journal of Virology*, **70**, 1836-1844.

[21] Ojkic, D. and Nagy, E. (2000) The Complete Nucleotide Sequence of Fowl Adenovirus Type 8. *Journal of General Virology*, **81**, 1833-1837.

[22] Gaba, A., Parmar, H., Pal, J.K. and Prajapati, K.S. (2010) Isolation, Identification and Molecular Characterization of Inclusion Body Hepatitis Virus. *Veterinary World*, **3**, 415-417.

[23] Parthiban, S., Manoharan, S., Roy, P., Chandranm, N., Aruni, A.W. and Koteeswaran, A. (2005) Nucleotide Sequence Analysis of the L1 Loop Variable Region of Hexon Gene of Fowl Adenovirus 4 Isolates from India. *Acta Virologica*, **49**, 65-68.

[24] Lim, T.H., Lee, H.J., Lee, D.H., Lee, Y.N., Park, J.K., Youn, H.N., Kim, M.S., Youn, H.S., Lee, J.B., Park, S.Y., Choi, I.S. and Song, C.S. (2011) Identification and Virulence Characterization of Fowl Adenoviruses in Korea. *Avian Disease*, **55**, 554-560. http://dx.doi.org/10.1637/9730-032011-Reg.1

[25] Kim, J.N., Byun, S.H., Kim, M.J., Kim, J., Sung, H.W. and Mo, L.P. (2008) Outbreaks of Hydropericardium Syndrome and Molecular Characterization of Korean Fowl Adenoviral Isolates. *Avian Disease*, **52**, 526-530. http://dx.doi.org/10.1637/8178-112207-Case

[26] Hess, M., Raue, R. and Prusas, C. (1999) Epidemiological Studies on Fowl Adenoviruses Isolated from Cases of Infectious Hydropericardium. *Avian Pathology*, **28**, 433-439. http://dx.doi.org/10.1080/03079459994443

[27] Choi, K.S., Kye, S.J., Kim, J.Y., Jeon, W.J., Lee, E.K., Park, K.Y. and Sung, H.W. (2012) Epidemiological Investigation of Outbreaks of Fowl Adenovirus Infection in Commercial Chickens in Korea. *Poultry Science*, **91**, 2502-2506. http://dx.doi.org/10.3382/ps.2012-02296

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