Young Pigeon Disease Syndrome (YPDS) in Turkish Pigeons (Columba livia domestica) stemming from coinfection with Pigeon aviadenovirus A and Pigeon circovirus: The first isolation and molecular characterization

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Abstract

Pigeon aviadenovirus A and Pigeon circovirus are both viruses that can cause disease in pigeons. This study reports the identification of a natural co-infection of Pigeon aviadenovirus A and Pigeon circovirus in a breeding pigeon flock in central Anatolia, Turkey. Both viruses were isolated from pooled internal organs of pigeons using primary chicken embryo kidney cell cultures (CEKC) and specific pathogen free (SPF) embryonated chicken eggs. Both viruses were identified by PCR amplification followed by Sanger sequencing, while histopathological examination showed degenerated hepatocytes with basophilic intranuclear viral inclusions. The viruses have similar transmission characteristics and common clinical manifestations, but it is possible that coinfection may exacerabate disease. This is the first report of its kind in Turkey and is important for the protection against disease in pigeons.

Introduction

Viral infections of pigeons, particularly under the age of 1, are associated with high morbidity and mortality. It has been reported that Pigeon aviadenovirus A (Duchatel et al., 2000; De Herdt et al., 1995) and Pigeon circovirus (Todd, 2000) are associated with an immuno supression syndrome called "Young Pigeon Disease Syndrome" (YPDS).

Pigeon aviadenovirus A (PiAdV-A) is a member of the genus Aviadenovirus with in the family *Adenoviridae*(Raue et al., 2002). To date, the Aviadenovirus genus is made up of 14 different members incluiding, Duck aviadenovirus B, Falcon aviadenovirus A, Fowl aviadenovirus A-E, Goose aviadenovirus A, Pigeon aviadenovirus A-B, Psittacine aviadenovirus B and Turkey aviadenovirus B-D.

Pigeon circovirus (PiCV) is a member of the Circovirus genus, family Circoviridae (Mankertz et al., 2000) and within this family there are 10 viruses that infect and cause disease in birds; incluiding, Pigeon circovirus, Beak and feather disease virus, Canary circovirus, Goose circovirus, Duck circovirus, Gull circovirus, Raven circovirus, Swan circovirus, Chicken associated cyclovirus 1 and Duck associated cyclovirus 1.

Pigeons with adenovirus infection were originally reported by McFerran et al. in 1976 (McFerran et al., 1976a), although PiAdV infection was firstly isolated and identified in 1984 in Belgium. Pigeons infected

with PiAdV have since been observed all around the world (Vereecken et al., 1998). Although pigeons of any age can be infected, young pigeons under one year of age are particularly badly affected by PiAdV-A, showing acute watery diarrhoea, vomiting and anorexia. PiAdV-B affects pigeons of all ages and is characterized by sudden death and intensive hepatic necrosis (Duchatel et al., 2000; De Herdt et al., 1995; Vereecken et al., 1998). The annual pigeon mortality rate is very high, with approximately 30% due to PiAdV, but in some cases it can reach 100% in pigeon lofts with necrotizing hepatitis infections (Vereecken et al., 1998).

PiCV was first diagnosed in Canada in 1986, although it is known to be present across the World (Hess et al., 1998, Woods et al., 1993) and, similar to PiAdV-A, it is younger pigeons that are mainly affected by it (2 months - 1 year of age) (Pare et al., 1999; Takase et al., 1990; Tavernier et al., 2000; Todd, 2000; Woods et al., 1994). PiCV infection can induce a wide spectrum of non-specific clinical signs including lethargy, weight loss, respiratory distress and diarrhoea (Woods et al., 1994). Until recently, it was unclear what effect many aviadenoviruses had on animals, but now the impact of viruses such as the Fowl aviadenoviruses (FAdV) is well recognised (Hess, 2013). These FAdV strains can cause gizzard erosion (GE), hydropericardium syndrome (HS) and severe liver damage leading to the condition known as inclusion body hepatitis (IBH) and serotypes 2, 4, 5, 6, 8, 10 and 12 have been isolated from both diseased and healthy pigeons (Goryo et al., 1988; Hess et al., 1998a; Hess et al., 1998b; McFerran et al., 1976a). In this study we report the isolation and analysis of both PiAdV and PiCV from a co-infection in pigeons in central Anatolia, Turkey.

Materials and Methods

History, Gross Findings and Sampling

A pigeon flock (N=45, 4-5 month old domestic pigeons) had a history of increased mortality (20-25%) during November-December 2019. According to the pigeons' owner, the first sign was inapppetance followed within a couple of days by vomiting. This was followed within 24 hours by dark green watery diarrhea that continued for approximately 2 days with many pigeons dying during this time. A total of 5 pigeons were submitted to the Pathology Department of the Faculty of Veterinary Medicine, Kırıkkale University, Turkey for examination. All pigeons were necropsied, following natural death after a period of chronic weight loss. The birds had severe intestinal ascariasis and the intestinal wall presented with gross lesions. Tissue samples from different internal organs, including liver, kidney, spleen, gut and pancreas, were collected for virus isolation and identification under aseptic conditions.

Isolation of Viruses

Two different groups of pigeons' internal organs were pooled and mixed with PBS (Sigma-Aldrich) containing penicillin (2000 units/ml), streptomycin (2 mg/ml), and gentamicin (50 µg/ml) antibiotics and Mycostatin (1000 units/ml). The organs were homogenized, followed by centrifugation (4000 rpm for 10 min). The supernatant was collected for screening by PCR using primers specific for PiAdV-A and PiCV (Freick et al., 2008; Raue et al., 2002). Supernatants that were positive for PiAdV-A and PiCV were firstly inoculated into the chorioallantoic cavity of 10 day-old specific pathogen-free (SPF) embryonated chicken eggs and the yolk sac of 6 day old SPF eggs to observe and compare the viruses adaptation and propagation according to the protocol of the Villegas Laboratory Manual (Villegas, 2006). The eggs were incubated at 37 °C for 5 to 10 days according to inoculation route, respectively. The inoculated eggs were examined daily until embryos stopped moving and were presumed dead under ovoscope light. At this point the allantoic fluid was removed and inoculated into a fresh egg following the same procedure as before. This was repeated until each supernatant passaged through 5 eggs.

Cell culture was also used. The supernatants were passed through a 0.22-µm microfilter directly before the inoculation. Following filtration, the samples were inoculated at the same time into primary chicken embryo fibroblast (CEF) and primary chicken embryo kidney cell (CEKC) cultures to compare cytopathic effects (CPE) and virus propagation.

Primary CEF and CEKC cultures were prepared from 10 day-old and 18 day-old SPF embryonated chicken eggs according to the protocol of the Villegas Laboratory Manual, respectively (Villegas, 2006). For cell

propagation, MEM (Gibco, UK) containing Earle's balanced salts, 10% fetal calf serum, 100 IU of penicillin (Sigma-Aldrich, USA) and 100 µg of streptomycin (Sigma-Aldrich, USA) antibiotics per ml was used. The cells were incubated at 37 °C for 24-48 hours until a confluent monolayer of CEFs and CEKCs had formed. For inoculation, the cell culture supernatants were removed and filtered supernatants from two groups of organ samples were overlaid onto the cell cultures, which were then incubated at 37 °C for 1 hour. Following this, fresh MEM supplemented with 2% fetal bovine serum was added to the cultures, and they were incubated at 37 °C in 5% CO_2 for 7 days. Once strong CPE was observed, the cultures were freeze thawed,centrifuged at $4000 \times g$ for 10 minutes, and the supernatants were stored at -20 °C until DNA extraction was performed.

Pathologic Examinations

c.1) Necropsy and Histopathological Examinations

After necropsy of the pigeons, collected systemic tissue samples were fixed in 10% neutral formalin (pH 7.4) and processed according to routine tissue processing procedures (Leica TP1020). After embedding in paraffin wax, tissue sections were cut at 5 µm thickness from paraffin blocks and stained with Haematoxylin-Eosin (H&E) (Luna, 1968). The histopathological changes of the lesioned organs were semiquantitatively scored and their photomicrographs were taken (Olympus BX51, DP25 digital camera).

c.2) Fowl Adenovirus-4 Specific Immunoperaxidase Test

The avidin biotin complex peroxidase (ABC-P) method was performed according to manufacturer's instructions (ab64264, Abcam, HRP/DAB, MA, USA). Briefly, tissue sections were deparaffinized, hydrated and then boiled in antigen retrieval solution (citrate buffer pH 6.0) for 20 minutes. The sections were incubated in 3% hydroperoxide-methanol mixture at room temperature for 20 minutes before blocking was performed using normal goat serum at 37°C for 7 minutes. The sections were incubated with polyclonal rabbit anti chicken FAdV-4 hyperimmune serum (kindly provided from Prof. Dr. Hafez) diluted at 1:40 at 37°C for 1 hour. After this, the sections were incubated with a biotinylated, HRP labelled anti-rabbt secondary antibody (Abcam kit, USA) followed by exposure to the AEC substrate chromogen solution (Dako). For counterstaining, sections were kept in Gill's haematoxylin (Sigma) for 2 minutes and cover slipped with aquous mounting medium. Washing procedures were carried out with Tris-HCl twice for 5 minutes excluding the blocking step. As a negative control, the sections were treated with Phosphate Buffered Saline (PBS) instead of primary antibodies. As a positive control, the same protocol was performed on chicken liver and brain sections that are obtained hyperimmun serum from ''Hafez Mohamed Hafez in Freie Universität Berlin-Germany", against FAdV-4 virus infection study in chickens.

DNA extraction and PCR

DNA was extracted from 200 µL of supernatant from two groups of samples separately, using a High Pure Viral Nucleic Acid Kit (Roche) according to the manufacturer's protocol. The extracted DNAs were stored at -20 °C until PCR was performed.

PCR for PiAdV-A and PiCV was carried out in a total volume of 25 µl containing 5 µl of DNA, 0.4 µM of each primer, and PCR master mix (Grisp, Portugal). The PCR for PiAdV-A was carried out in a thermocycler (Techne, UK) with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 20 seconds, 58 °C for 30 seconds, and 72 °C for 1 min with a final elongation step at 72 °C for 5 min. The PCR for PiCV was carried out in the same thermocycler with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 20 seconds, 62 °C for 30 seconds, and 72 °C for 30 seconds with a final elongation step at 72 °C for 5 min. The PCR amplicons were visualised by agarose gel electrophoresis. The PCR amplicons were purified according to the manufacturer's protocol (ExoSAP-IT PCR Product Cleanup Reagent, USA), before sequencing.

The PiAdV-A primers (F1-s and F2-as) and PiCV (PiCV2-s and PiCV2-as) primers were used in the study (Table 1) bind to a conserved region of the fiber 2 gene of PiAdV-A and capsid gene of PiCV. The samples were also tested by other primers which are listed in Table. 1, to investigate FAdV, PiAdV-B and PiHV to detect the presence of other possible viruses.

TABLE 1. Primers used in this study

The viruses	Target gene	Primer names	Sequence (5'–3')	product size (bp)	Reference
Fowl aviadenoviruses	hexon	Hexon A	CAARTTCAGRCAGATCGGT	894	(Meulemans et al., 2001).
		Hexon B	TAGTGATGMCGSGACATCAT		
Fowl aviadenoviruses	hexon	H1	TGGACATGGGGGTCCTGACCTA	1209	(Raue & Hess, 1998).
		H2	AAGGGATTGACGTTGTCCA		
Fowl aviadenoviruses	hexon	H3	AACGTCAACCCCTTCGTAACCACC	1310	
		H4	TTGCCTGTGGCGAAAGGCG		
PiAdV-1 (PiAdV-A)	fiber-2	PiAdV-1 F1-s	ATCAACTACGACAGCCGAAGGC	467	(Raue et al., 2002).
		PiAdV-1 F2-as	CGGTAGAGTTACGGGGAAATT		
PiAdV-2 (PiAdV-B)	hexon	PiAdV-2 Hex-3-F	GTAACATGAGCGGCTGTTTG	643	(Teske et al., 2017).
		PiAdV-2 Hex-3-R	CTGAGAAACGAAACCCGAATTG		
PiHV	Polymerase	PiHV-s	GGGACGCTCTGAATTAAGGAAT	217	(Raue et al., 2005).
		PiHV-as	CTTGGTGATCAGCAGCAGCTTG		
PiCV	Capsid	PiCV2-s	TTGAAAGGTTTTCAGCCTGGC	324	(Freick et al., 2008).
		PiCV2-as	AGGAGACGAAGGACACGCCTC		

DNA sequencing and phylogenetic analysis

The PCR products of the fiber 2 gene of PiAdV-A and capsid gene of PiCV were sequenced with PCR primers by Microsynth (Switzerland). Sequencing was performed using the Sanger dideoxy sequencing method with an ABI 3730XL automatic sequencing apparatus and a Big Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA) in each direction (forward and reverse) for each isolate to ensure the validity of the data. Partial nucleotide sequences of the fiber 2 gene and capsid gene were aligned and edited using MEGA X (Molecular Evolutionary Genetics Analysis, version 10.1.8) software (The Biodesign Institute, Arizona, USA). The sequence data were then compared to complete genome reference sequences of aviadenovirus and circovirus species available from the National Center for Biotechnology Information (NCBI) and their phylogenetic relationships were investigated. Phylogenetic trees were built using the neighbor-joining method with the Hasegawa-Kishino-Yano and Kimura-2 parameter substitution model and gamma distribution using the maximum-likelihood statistical method in MEGA X, respectively (Hasegawa et al., 1985; Kimura, 1980; Kumar et al., 2018).

Results

Necropsy and histopathological findings

Macroscopically,the pigeons were dehydrated and emaciated with pectoral muscle atrophy while postmortal watery vomite was detected in two pigeons (Figure 1). The most prominent gross findings were severe liver necroses and hemorrhages (n=3). Enlargement and fragility of the affected livers showed association with pale grey-yellow necrotic areas (Figure 1), and widespread hemorrages. The proventriculus and stomach mucosae were thickened and oedematous in appearance, and in some instances they were coated with a

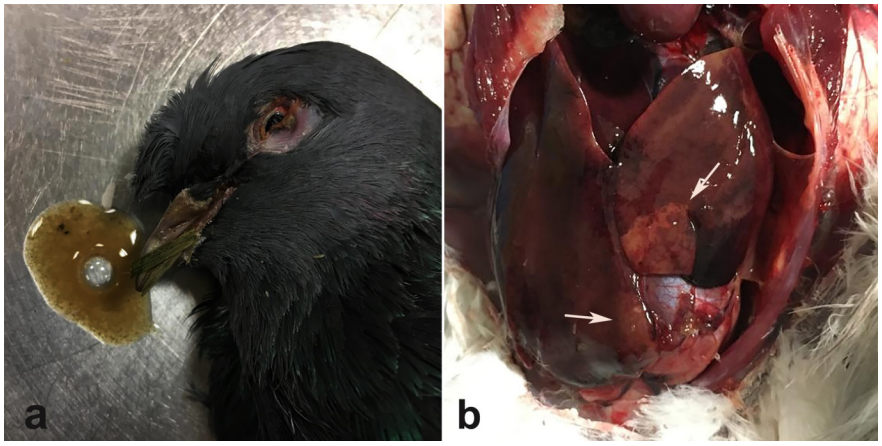
sticky mucous. The kidneys were also enlarged and contained multifocal grey foci that were detectable on the serosal surfaces.

TABLE 2. Semiquantitative scoring of histopathologic and Immunoperoxidase test findings; (-) None, (+) Mild, (++) Moderate, (+++) Severe

IPT: Immunoperoxidase test

Case#	Severity of Histopathologic Findings	Severity of Histopathologic Findings	Severity of Histopathologic Findings	IPT (FAdV-4)
	Liver	**Kidneys**	**Lungs**	
1	+++	++	-	+++
2	++	+++	-	++
3	+	+	-	+
4	++	+	+	++
5	+++	++	-	+++

FIGURE 1. a) Vomiting characterized by mucous and watery content; shrunken eye indicating dehydration and poor condition of the diseased pigeon. b) Widespread discoloration indicates severe necroses (white arrows) and hemorrhages on the liver.



Histopathologically, multifocal coagulation necroses characterized by acellular homogenous eosinophilic appearance and mononuclear cell infiltrations surrounded necrotic cell debris. In severe cases, widespread hemorrhages showed association with hepatic necroses. Hepatocytes mostly showed parenchymatous degeneration, cytoplasmic swelling and they had clear, hyperchromatic nuclei. In some nuclei of the hepatocytes, intranuclear basophilic viral inclusions were observed (Figure 2). In kidneys, multifocally localized interstitial lymphocytes and plasma cell infiltrations in the cortex, tubular hydropic degenerations were detected. In two cases, interstitial pnemonia, alveolar epithelia hyperplasia, and bronch/iolitis were seen. In proventriculus mucosae, numerous heterophilic leucocyte infiltrations were present in the submucosae. FAdV-4 antigen specific immunoreactions were observed in degenerated hepatocytes and in the necrotic areas, kidney tubul epithelia, interstititial inflammatory cells and in urethelial epithelia, alveolar and bronchial epithelia in the lungs.

FIGURE 2. a) Severe coagulation necrosis, homogeneous eosinophilic appearance and some structural remnants of hepatic cords were visible but the hepatocytes were completely necrotic (arrows). At the

periphery, focal lymphocyte and macrophage accumulations (arrow heads) were seen in the liver, H&E, Bar= 300 µm.

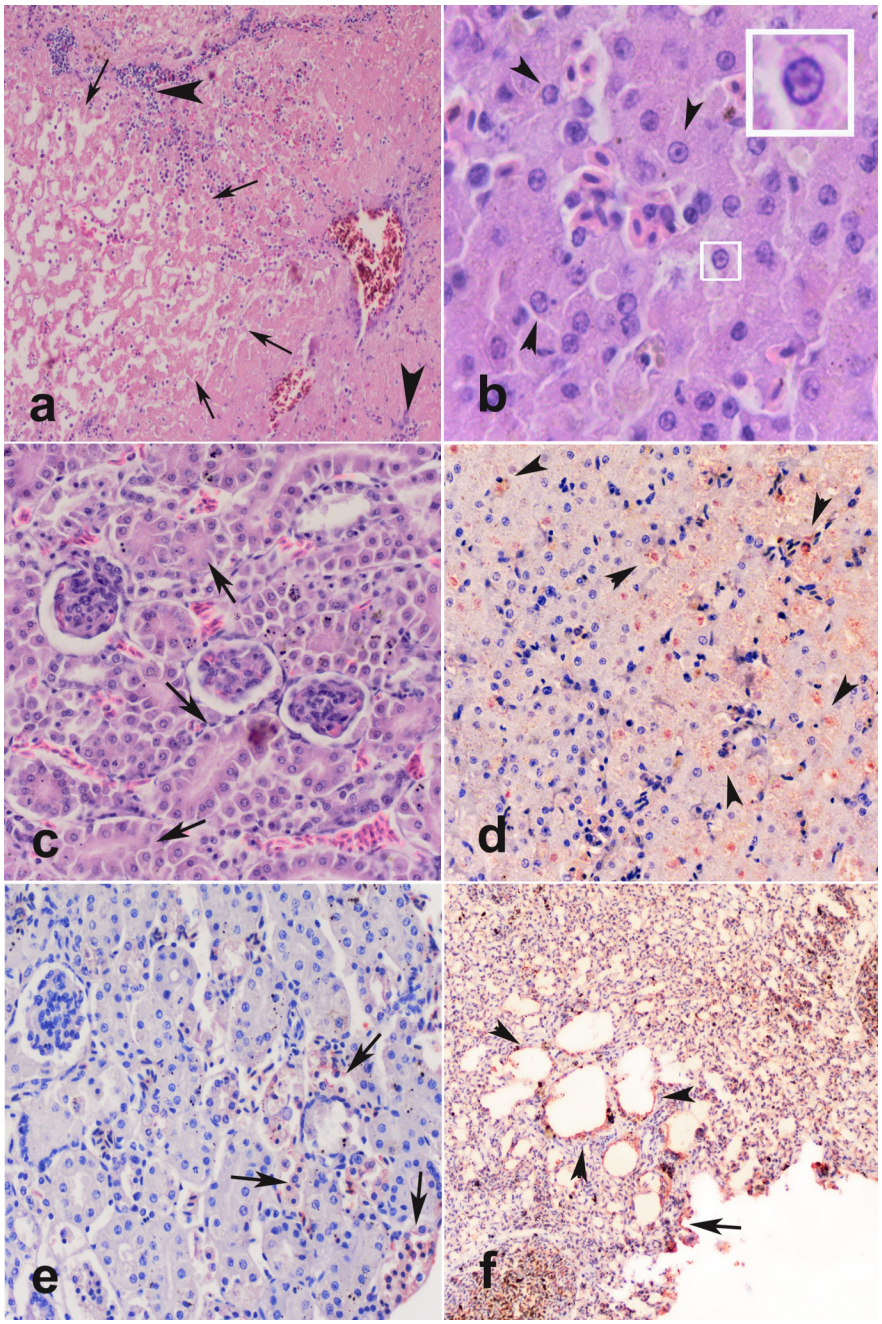
b) Clear nucleus due to characteristic circular margination of the chromatin in the hepatocytes. Some degenerated hepatocytes had basophilic intranuclear viral inclusions (arrow heads and inset). Liver, Hematoxylin-eosin, Bar= 45 µm.

c) Hydropic degeneration and necrosis of the cortical tubule epithelia, and in healthy tubular epithelia nuclei seemed clearbut contained numerous intranuclear basophilic inclusions. Hematoxylin-eosin, Bar= 90 µm.

d) FAdV-4 antigen immunopositive reactions, red dots, in the necrotic areas and degenerated hepatocytes (arrowheads). Avidin biotin complex immunoperoxidase test, FAdV-4 antibody, Gill's hematoxylin counterstaining, Bar= 180 µm.

e) Immunoperoxidase positive reactions in the degenerative and necrotic tubule epithelia of the kidney. Avidin biotin complex immunoperoxidase test, FAdV-4 antibody, Gill's hematoxylin counterstaining, Bar= 180 µm.

f) Strong viral antigen immunopositivity on the bronchiolar epithelia (arrowheads= and alveolar walls. Avidin biotin complex immunoperoxidase test, FAdV-4 antibody, Gill's hematoxylin counterstaining, Bar= 300 µm.



Virus isolation

The supernatants of PiAdV-A and PiCV positive samples from a pigeon flock were inoculated into SPF embryonated chicken eggs. All samples were passaged 5 times in SPF chicken eggs. It was not possible to propogate PiAdV-A virus from the PiAdV-A PCR positive sample in eggs, but PiCV virus was propagated from the PiCV PCR positive sample in the SPF embryonated chicken eggs and caused extensive damage to chicken embryos regardless of the inoculation route (chorioallantoic cavity and yolk sac) (Figure.3). PiAdV-A and PiCV positive samples did not lead to any propogation of virus in primary CEFCs, but both samples caused extensive rounding, clumping and detatchment of cells in the CEKC cultures 48-72 hours post-

inoculation (Figure. 4).

FIGURE 3. a-b-c) Extensive malformation and hemorrhaging was caused in chicken embryos by PiCV (TR/SKPC20) and compared with normal chicken embryo (d). Livers with multiple petechial haemorrhages (black arrows)

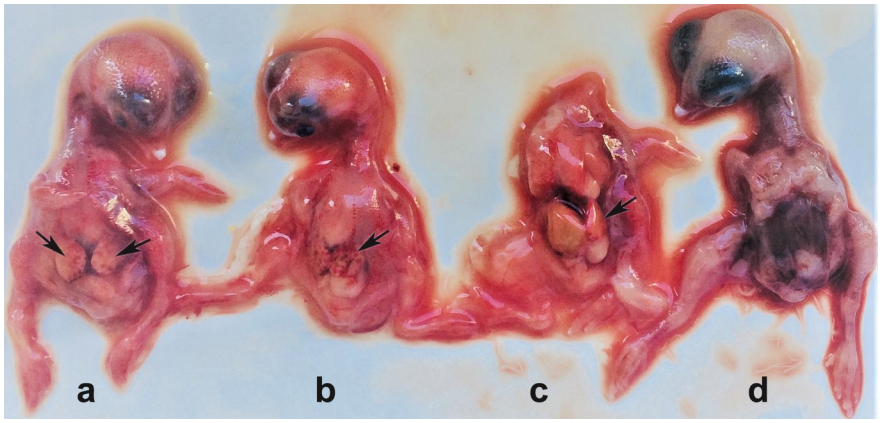
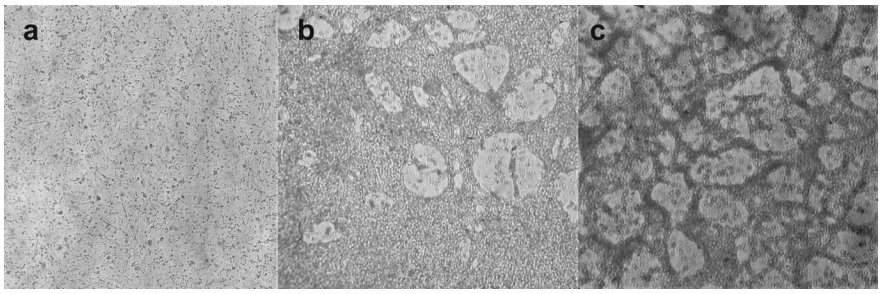


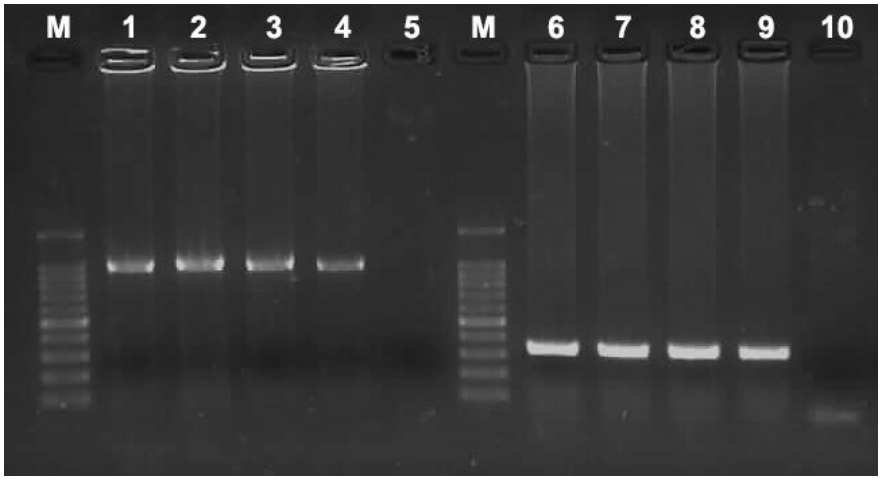
FIGURE 4. CPE formed by PiAdV-A field isolate (TR/SKPA20) and PiCV field isolate (TR/SKPC20) in CEKC cultures. a) Healthy, mock-infected CEKCs. b-c) PiAdV-A and PiCV infected cultures displaying CPE, respectively.



PCR

The PiAdV-A fiber-2 and PiCV capsid genes were partially amplified by PCR using the F1-s/F2-as and PiCV2-s/PiCV2-as primers, respectively. The expected 967-bp and 325-bp specific DNA fragments were amplified (Figure. 3). The specific PCR products were identified as PiAdV-A and PiCV by sequencing and BLAST analysis.

FIGURE 5. a) Lane M, 100 bp ladder marker; lane 1, TR/SKPA20 (liver); lane2, TR/SKPA20 (kidney) ;lane 3, TR/SKPA20 (spleen); lane 4, TR/SKPA20 (gut and pancreas); lane 5, negative control (Raue et al., 2002) (967 bp.). Lane 6, TR/SKPC20 (liver); lane7, TR/SKPC20 (kidney) ; lane 8, TR/SKPC20 (spleen); lane 9, TR/SKPC20 (gut and pancreas); lane 10, negative control (Freick et al., 2008) (325 bp.).



DNA sequencing and phylogenetic analysis

The amplified fiber-2 gene and capsid gene fragments were sequenced directly. The PiAdV-A isolate was named "TR/SKPA20" and the PiCV isolate was named "TR/SKPC20". A BLAST search (https://blast.ncbi.nlm.nih.gov) was used to compare these sequences with the complete genomes of aviadenovirus and circovirus species. The newly detected field isolate TR/SKPA20 was found to be related with only one PiAdV-A ''IDA4" strain, with 99.03% similarity at the amino acid level. The TR/SKPC20 field isolate was found to be closely related with strains previously isolated in Poland (MK994767, KC691682), Hungary (JF330097) and Taiwan (GO844278) (Figures. 6 and 7). The nucleotide sequences of the field isolates presented here (TR/SKPA20 and TR/SKPC20) have been submitted to the NCBI-GenBank database under the following accession numbers: MN985817, MT130538.

FIGURE 6. Phylogenetic tree based on the amino acid sequence of the PiCV capsid gene. The tree shows circovirus species which affect fowl. The tree shows the names and GenBank accession numbers of each isolate. The PiCV isolate from this study is indicated by a red dot.

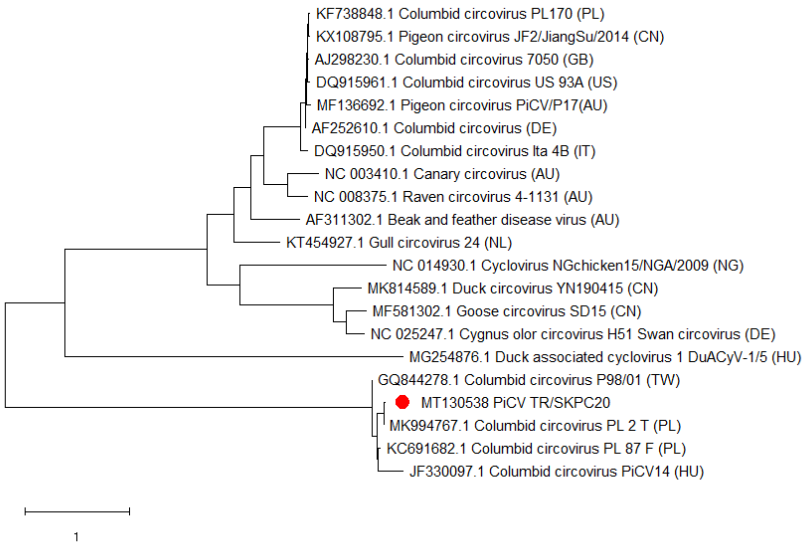
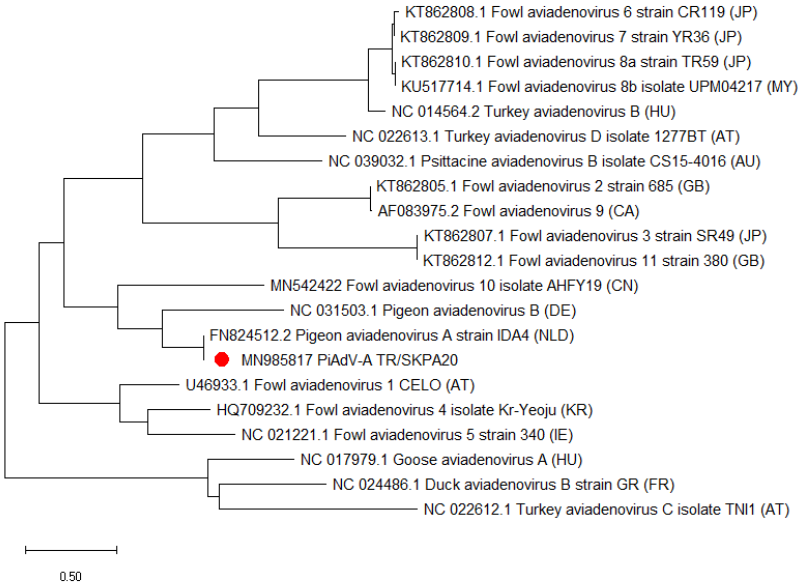


FIGURE 7. Phylogenetic tree based on the amino acid sequence of the PiAdV fiber-2 gene. The tree shows aviadenovirus species which affect fowl. The tree shows the names and GenBank accession number of each isolate. The PiAdV-A isolate from this study is indicated by a red dot.



DISCUSSION

PiAdVs are classified into two species pigeon aviadenovirus A and pigeon aviadenovirus B within the genus aviadenovirus. (De Herdt et al., 1995). PiCV is the only circovirus that only affects pigeons. In the present study, we detected a PiCV, PiAdV co-infection, in a pigeon flock in Kirikkale province, Turkey. The genetic material of both PiAdV-A and PiCV were found in the same flock of young pigeons under six months old with severe clinical signs (crop vomiting, watery diarrhea, anorexia and sudden death) that are typically associated with Young Pigeon Disease Syndrome (YPDS).

Primers based on the PiAdV-A fiber-2 gene (Raue at al., 2002) and PiCV capsid gene (Freick et al., 2008), were used for the detection and amplification of the isolates. This was then sequenced and phylogenetic analyses were performed.

Several FAdV serotypes 2, 4, 5, 6, 8, 10 and 12 have been previously isolated from diseased and healthy pigeons (Goryo et al., 1988; Hess at al., 1998a; Hess et al., 1998b; McFerran et al., 1976a). In this present study, other viruses such as fowl aviadenovirus (FAdV 1-7, -8a and -8b, -9-11) and PiAdV-B and PiHV were all screened for but not detected (Table. 1). In a recent study, adenoviral poultry diseases, especially IBH were reported for the first time in broiler and broiler-breeder flocks in Turkey (Sahindokuyucu et al., 2020). PiAdV-A adenovirosis is sometimes called IBH due to the intranuclear basophilic bodies seen by histopathology (McFerran et al., 1976b; Abadie et al., 2001) and it is possible that misdiagnosis has previously occured. This study illustrates the importance of molecular diagnosis of infection.

The sequence analysis carried out showed that one of the isolates belonged to the PiAdV-A species. The isolate matched with a strain called ” Pigeon adenovirus 1 complete genome, strain IDA4” in the NCBI-Genbank database. The percent identity at the amino acid level between our sample and IDA4 was 99.03%. To the best of our knowledge, this study reports the first identification and isolation of PiAdV-A in a pigeon flock in Turkey.

In this study both PiAdV-A and PiCV positive samples were isolated onto CEK cell cultures. An attempt was also made to use primary CEF cell cultures to isolate positive PiAdV and PiCV samples, but it was unsuccessful. PiAdV serotypes are generally quite challenging to grow in cell culture (Duchatel et al., 2000; Marlier & Vindevogel, 2006; Vereecken et al., 1998). The isolation of pigeon avidenovirus strains have been achieved in primary chicken embryo kidney cells (McFerran et al., 1976a) and primary chicken embryo liver cells (Takase et al., 1990; Vereecken et al., 1998). However, even with these techniques isolation does not always succeed. The isolates reported in this study can now be used for further molecular and virulence studies.

Isolation of PiAdV was only successful in CEKC cultures. PCR of the fiber-2 gene was used for identification. Although little is understood about the fiber-2 protein of PiAdV, the fiber protein of FAdV is known to play a crucial role in viral infection and pathogenesis (Lu et al., 2019; Pallister et al., 1996; Zhang et al. 2018). It is responsible for initial attachment of the virus to cellular receptor, which is followed by binding to cell surface integrins (Wickham et al., 1993). It is likely that the PiAdV fiber-2 protein plays a similar role. When the region of the fiber-2 gene that was amplified and sequenced was compared to IDA4 reference strain, there were 6 amino acid differences present (Table 3). The consequence of these changes is currently unclear and an area for future investigations.

TABLE 3. Location of amino acid differences between the TR/SKPA20 (MN985817) and IDA4 (MT130538) strains in the section of the Fiber-2 protein that was amplified and compared.

Accession No	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids	Sequence of Amino Acids
	40	**75**	**91**	**142**	**151**	**162**				
MN985817	S	D	I	S	R	A				
MT130538	G	N	T	I	Q	E				

From this study it is clear that co-infections of PiAdV and PiCV is possible and can lead to serious consequences for the birds infected. The prevelance of both viruses in pigeon populations needs further investigation to determine their pathogenic burden. Moreover, further studies should focus on the whole genome sequence and the virulence of the PiAdVs and PiCV in diseased pigeons as well as their potential to jump species into economically important fowl. This study paves the way for these future studies and offers the potential for a better understanding of viral infections of birds in Turkey.

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Ethical statement: No culling has been done for the sampling process.

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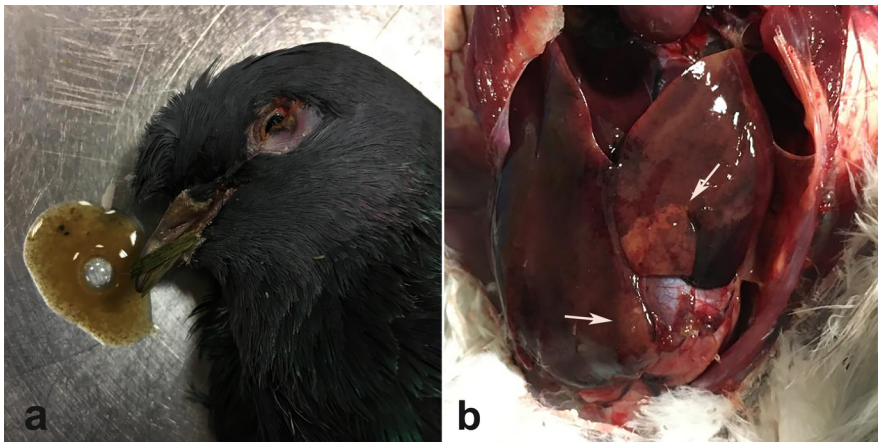

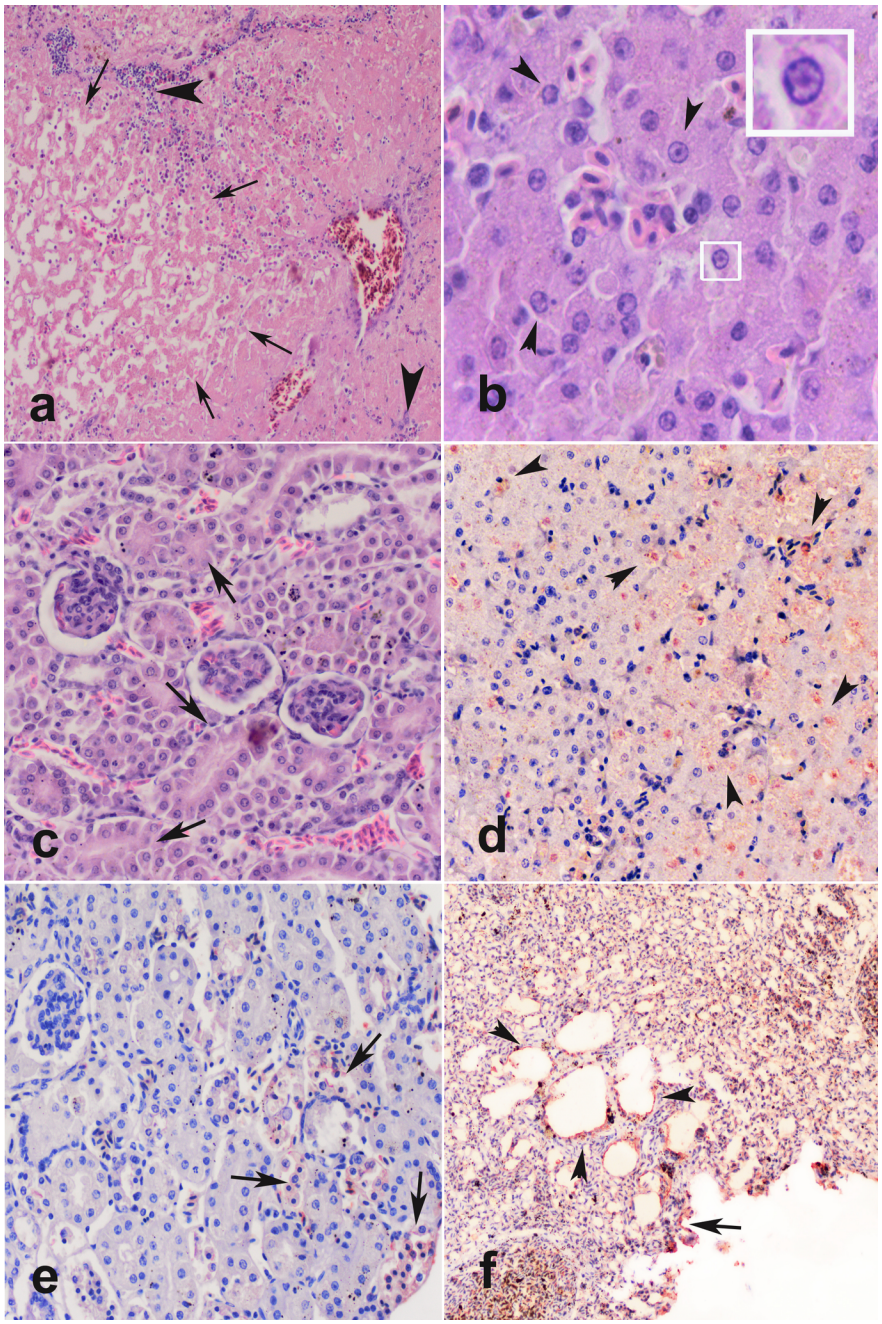

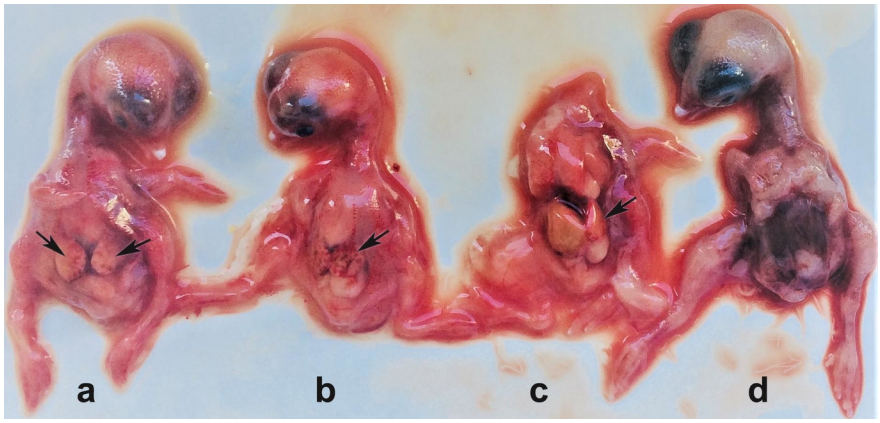

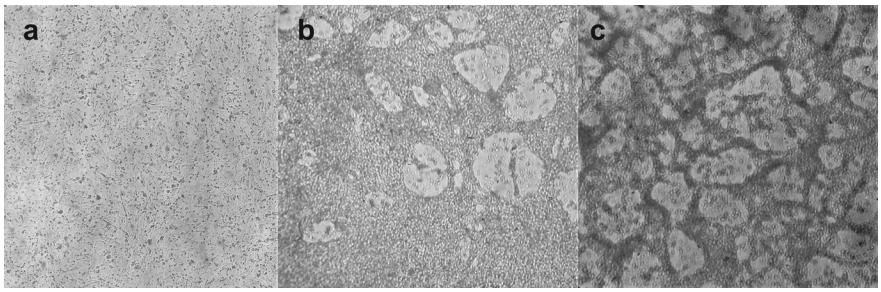

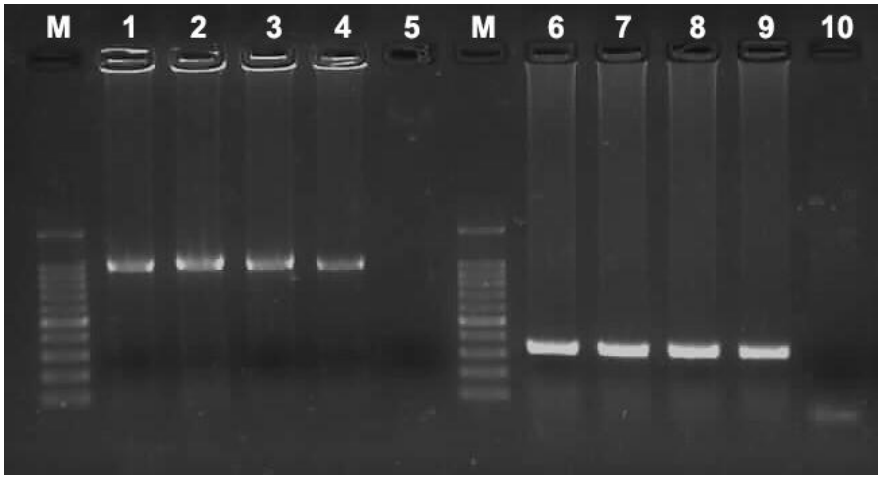

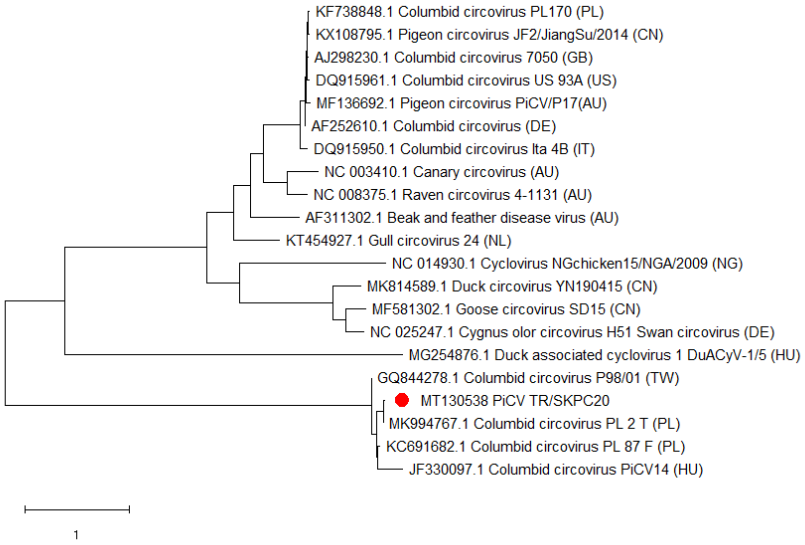

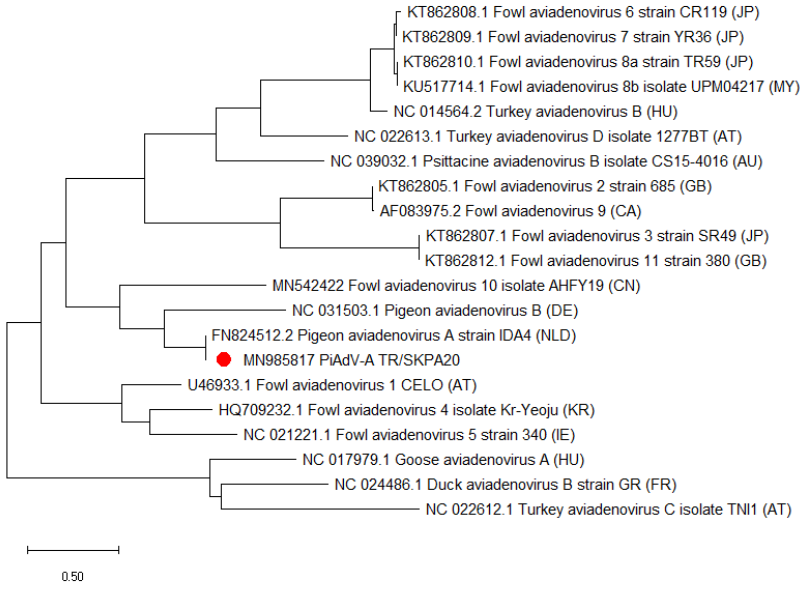

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