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Short Paper

Molecular survey of avian circoviruses in some non-psittacine birds and detection of a novel canary circovirus in a pigeon

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Abstract

Background: Circoviruses are small, non-enveloped, single stranded DNA viruses. There is scarce information about these agents in non-psittacine birds. **Aims:** It is attempted to detect and characterize circoviruses in non-psittacine birds. **Methods:** Forty-five samples were collected from different non-psittacine species belonging to seven avian orders. A nested polymerase chain reaction (nested-PCR) for the detection of *rep* gene of circoviruses was applied. **Results:** Two different types of circoviruses were detected in two pigeon samples (2/11, 18.2%). One of the detected circoviruses was placed in clade A next to a polish strain based on phylogenetic analysis. Interestingly, the other detected circovirus was closely related to canary circoviruses (CaCVs). **Conclusion:** In addition to the molecular diagnosis of a pigeon circovirus (PiCV), this is the first report of the detection of CaCv in a pigeon. The possible hypotheses of such circumstance are discussed.

Key words: Birds, Canary, Circovirus, Pigeon

Introduction

Circoviruses are small non-enveloped and icosahedral viruses with a circular, non-segmented, single stranded DNA. Circoviral agents convey two major open reading frames (ORFs); ORF V1 located in viral strand and encode the replication-associated proteins (Rep) and ORF C1, located in the complementary sense strand, encoding the viral capsid protein (Cap) (Todd, 2000). The family Circoviridae is composed of two genera including Cyclovirus and Circovirus. The genus Circovirus consists of 39 species. There were 11 known avian circoviruses. Beak and feather disease virus (BFDV), duck circovirus (DuCV), goose circovirus (GoCV), starling circovirus (StCV), canary circovirus (CaCV), pigeon circovirus (PiCV), swan circovirus (SwCV), raven circovirus (RvCV), zebra finch circovirus (ZfiCV), finch circovirus (FiCV), and gull circovirus (GuCV) are the established circoviruses (ICTV Virus Taxonomy, 2018). Because the virus is difficult to isolate (Todd, 2000), several features, including the host specificity are still debatable.

Most circoviruses such as SwCV, StCV, FiCV, and GuCV were discovered accidentally and associated with secondary infections (Todd, 2000; Johne *et al*., 2006; Halami *et al*., 2008). Pigeon circovirus infection is responsible for "young pigeon disease syndrome" (YPDS) (Raue *et al*., 2005). Circoviruses play a role in a condition called "Black spot" in neonatal canaries with distended abdomen and gall bladder congestion (Goldsmith, 1995).

In this study, a nested broad-spectrum polymerase chain reaction (nested-PCR) was used which should be capable of sensitive detection of a broad range of different circoviruses including circoviruses not currently known. According to the author's knowledge, this is the first report of multi-species molecular survey of avian circoviruses in Iran.

Materials and Methods

Clinical samples were collected from non-psittacine birds in Tehran, Iran, during 2014. Samples were collected either individually (from faeces or triple sampling from conjunctiva, choanal cleft and cloaca) or mixed faeces from several birds with the same aviary bird species in three zoological collections. Blood samples and tissue specimens were collected individually from birds which have been referred to a veterinary clinic (Table 1). No anticoagulant was used for taking blood samples.

The viral DNA was extracted using High Pure PCR template preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). A nested broad spectrum PCR using degenerated primers was applied for the detection of circoviruses (Halami *et al*., 2008). The primers

targeted the *rep* gene with an expected size of 350 bps. Polymerase chain reaction products were evaluated using electrophoresis in a 2% agarose gel containing RedSafe ™ (iNtRON BIOTECHNOLOGY, South Korea). The PCR products of the expected length were sequenced for confirmation (Fig. 1). Psittacine BFDV from the previous study was used as positive control DNA (Haddadmarandi *et al*., 2018).

The positive products of the PCR were subjected to DNA sequencing by Bioneer Biotechnology (South Korea). Nucleotide sequences were submitted in GenBank (Table 2). For sequence analysis Basic Local Alignment Search Tool (BLAST), BioEdit (version 7.2.5) and MEGA 7 (Kumar *et al*., 2016) softwares were applied.

Results

Forty-five samples belonging to seven orders including Anseriformes (14/45), Columbiformes (11/45), Passeriformes (10/45), Galliformes (4/45), Gruiformes (4/45), Struthioniformes (1/45), and Phoenicopteriformes (1/45) were collected (Table 1). Nineteen samples were taken from various referred bird species with a variety of clinical manifestations and the remaining were gathered from three different aviaries in Tehran named A1, A2 and A3.

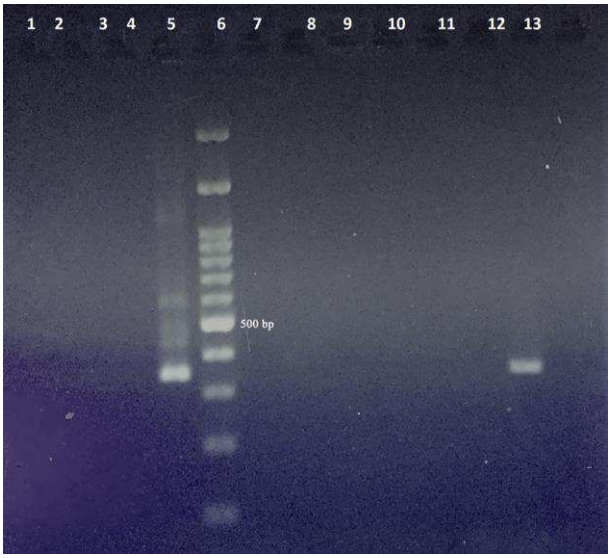

Fig. 1: Agarose gel electrophoresis of broad-spectrum degenerated PCR product targeting circovirus *rep* gene approximately 350 bp. Lanes 1-4: Negative samples, Lane 5: Pigeon sample, Lane 6: DNA ladder 100 bp, Lanes 7-12: Negative samples, Lane 13: BFDV positive sample from previous study. DNA smear and non-target faint band in Lane 5 are because of using degenerated broad spectrum primers. 350 bp DNA band was isolated from the gel and sequenced for confirmation

Table 1: Clinical specimens from different avian species which were used for the molecular detection of avian circoviruses

Order	Species	Sample				Number of birds tested (total= 45)	Number of positive birds
		Blood	Triple swabs[b]	Faeces	Tissue		
Columbiformes	*Columba livia*	2	7	2[a]	-	11	2 (Faeces, Blood)
Passeriformes	*Serinus canaria*	-	-	-	5	5	-
	Corvus cornix	-	-	-	2	2	-
	Acridotheres tristis	1	-	-	-	1	-
	Podoces pleskei	-	-	1	-	1	-
	Pyrrhocorax pyrrhocorax	-	-	1	-	1	-
Anseriformes	Mixed species of ducks[c]	-	-	3[a]	-	3	-
	Mixed species of swans[c]	-	-	2[a]	-	2	-
	Mixed species of geese[c]	-	-	3[a]	-	3	-
	Anas platyrhynchos	1	-	-	-	1	-
	Tadorna ferruginea	-	1	-	-	1	-
	Cairina moschata	-	-	2[a]	-	2	-
	Cygnus olor	-	-	-	1	1	-
	Cygnus atratus	-	-	-	1	1	-
Galliformes	*Meleagris gallopavo*	-	-	-	3	3	-
	Pavo muticus	-	-	-	1	1	-
Phoenicopteriformes	*Phoenicopterus roseus*	-	-	1[a]	-	1	-
Struthioniformes	*Struthio camelus*	-	-	-	1	1	-
Gruiformes	*Balearica regulorum*	-	-	4	-	4	-

[a] Each sample related to mixed faeces of several birds with same species that were kept in one cage, [b] The sample was taken with a single swab from conjunctiva, choana and cloaca respectively, and [c] Mixed species of the same genus kept in a single enclosure

Table 2: GenBank accession numbers of circovirus *rep* gene sequences detected in pigeons

Strain name	Host species	Accession No.
CaCV-MH-IR-Ch49-Rep-2014	*Columba livia domestica*	KU521520
CoCV-MH-IR-C12-Rep-2014	*Columba livia domestica*	KT962830

The isolate names were coded according to the scheme: 'A'-MH-IR-'B'-Rep-'C', where 'A' denotes the species of circovirus, MH refers to the name of the author (Mohammadreza Haddadmarandi), IR indicates the country (Iran), 'B' denotes the laboratory code, the Rep shows the replication part of circoviral genome, and the last 'C' shows the year of detection

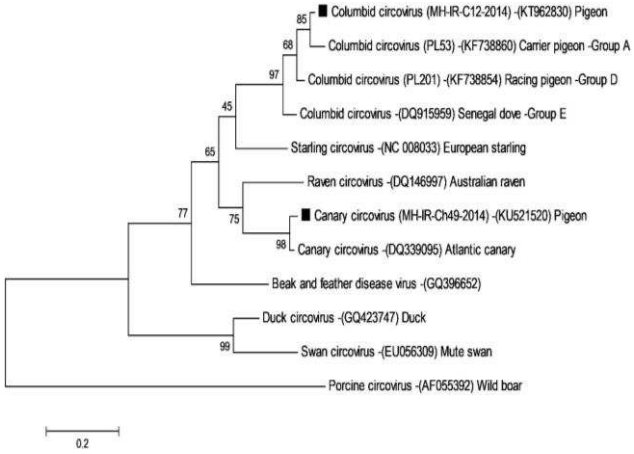

Fig. 2: Molecular phylogenetic analysis by maximum likelihood tree of *rep* gene partial sequences (350 bp) of different circoviruses. The evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The Iranian circoviruses (IR) are marked with black square. All samples are named according to the scheme: Circovirus species (strain name) - (GenBank accession number) host species followed by - Columbid circovirus geno-group, if applicable

Two samples were positive for circoviruses (4.4%). Both positive samples belonged to domestic pigeons (*Columba livia*). No characteristic clinical signs were seen in these two specimens. One of them which had been detected in the blood sample of a young pigeon was placed in clade A, next to Polish PiCV strain PL53 (GenBank accession No. Kf738860). Interestingly, the other circovirus was identified as CaCV (Fig. 2). It was detected in a group of pigeons housed in a single species pigeon loft.

Discussion

In the present study, two types of circoviruses were detected in pigeons using a previously established nested broad-spectrum PCR (Halami *et al*., 2008). The characterization of detected circoviruses was performed using the partial sequencing of *rep* gene.

Pigeon circoviruses could be genetically grouped in five different genogroups (A-E). The reported infection rates were 57% in Hungary (Cságola *et al*., 2012), 32% in Belgium (Hattermann *et al*., 2002), 48% in Germany (Soike *et al*., 2001), 70% in Poland (Stenzel and Pestka, 2014), and 75% in China (Zhang *et al*., 2015). The first report of PiCVs in Iran showed the prevalence of 24% of PiCV in Chaharmahal va Bakhtiari province (Mahzounieh *et al*., 2014). The only positive PiCV of the present study placed with those classified as group "A" PiCVs were closely related to the strain which was detected in Poland (Stenzel *et al*., 2014). According to the limited sample size in the present study, it is not possible to show the prevalence of PiCV infection in Tehran.

The other positive sample was detected a single species aviary which only pigeons were kept in. It was identified as CaCV. As the aviary was not a completely closed enclosure, contamination of the specimen with canary or other wild bird droppings was also possible. Circoviruses are highly resistant to chemical and physical agents (Todd, 2000) and therefore can survive for a long time in the environment or even gastrointestinal tract of the pigeon. Such assumption was previously reported as StCV was detected in an Estuarine Mollusc (*Amphibola crenata*) as a passenger (Dayaram *et al*., 2013). There is a debate in host specificity among circoviruses and very few studies have been performed on CaCV (Phenix *et al*., 2001; Todd *et al*., 2001; Rampin *et al*., 2006). Hence the host specificity of the virus remains uncertain. The detected CaCV has only 98% identity with other CaCV in the GenBank, so it could be an intermediate strain which might pass the host barrier. A self-limiting viral host-switching of BFDV between Psittaciformes and Coraciiformes has recently been shown (Sarker *et al*., 2015). There could be high mutation rate within circoviruses (Halami *et al*., 2008) and these mutations can possibly change the host specificity of circoviruses. Another hypothesis for the presence of the canary-like circovirus in a pigeon sample could be the viral genome mutations or recombinations (Bassami *et al*., 2001; Rau *et al*., 2004; Hughes and Piontkivska, 2008). Full genome sequencing is needed to determine the potential capability of these viral changes. Unfortunately, it was not possible to find the exact positive pigeon in the aviary to re-sample and re-examine it for the presence of circoviruses.

Circoviruses were detected in the present study in clinical specimens of pigeons. Further studies with larger sample sizes from different geographical regions are needed to elaborate host specificity and the incidence of the infection in the country.

References

Bassami, MR; Ypelaar, I; Berryman, D; Wilcox, GE and Raidal, SR (2001). Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. Virology. 279: 392-400.

Cságola, A; Lorincz, M; Tombácz, K; Wladár, Z; Kovács, E and Tuboly, T (2012). Genetic diversity of pigeon circovirus in Hungary. Virus Gen., 44: 75-79.

Dayaram, A; Goldstien, S; Zawar-Reza, P; Gomez, C; Harding, JS and Varsani, A (2013). Identification of starling circovirus in an estuarine mollusc (*Amphibola crenata*) in New Zealand using metagenomic approaches. Genome Announc., 1: e00278-13.

Goldsmith, TL (1995). Documentation of passerine circoviral infection. In Proceedings: Annual Conference of the American Association of Avian Veterinarians. Philadelphia, USA. PP: 349-350.

Haddadmarandi, MR; Madani, SA; Nili, H and Ghorbani, A (2018). Molecular detection and characterization of beak and feather disease virus in psittacine birds in Tehran, Iran. Iran. J. Vet. Res., 19: 22-26.

Halami, MY; Nieper, H; Müller, H and Johne, R (2008). Detection of a novel circovirus in mute swans (*Cygnus olor*) by using nested broad-spectrum PCR. Virus Res., 132: 208-212.

Hattermann, K; Soike, D; Grund, C and Mankertz, A (2002). A method to diagnose Pigeon circovirus infection *in vivo*. J. Virol. Methods. 104: 55-58.

Hughes, AL and Piontkivska, H (2008). Nucleotide sequence polymorphism in circoviruses. Infect. Genet. Evol., 8: 130-138.

ICTV Virus Taxonomy (2018). The Online (10th) Report of International Committee on Taxonomy of Viruses. Available at: https://talk.ictvonline.org/ictv-reports/ictv_online_report.

Johne, R; Fernández-de-Luco, D; Höfle, U and Müller, H (2006). Genome of a novel circovirus of starlings, amplified by multiply primed rolling-circle amplification. J. Gen. Virol., 87: 1189-1195.

Kumar, S; Stecher, G and Tamura, K (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol., 33: 1870-1874.

Mahzounieh, M; Heidari Khoei, H; Ghasemi Shamsabadi, M and Dastjerdi, A (2014). Detection and phylogenetic characterization of Columbid circoviruses in Chaharmahal va Bakhtiari province, Iran. Avian Pathol., 43: 524-528.

Phenix, KV; Weston, JH; Ypelaar, I; Lavazza, A; Smyth, JA; Todd, D; Wilcox, GE and Raidal, SR (2001). Nucleotide sequence analysis of a novel circovirus of canaries and its relationship to other members of the genus Circovirus of the family Circoviridae. J. Gen. Virol., 82: 2805-2809.

Rampin, T; Manarolla, G; Pisoni, G; Recordati, C and Sironi, G (2006). Circovirus inclusion bodies in intestinal muscle cells of a canary. Avian Pathol., 35: 277-279.

Raue, R; Schmidt, V; Freick, M; Reinhardt, B; Johne, R; Kamphausen, L; Kaleta, EF; Müller, H and Krautwald-Junghanns, ME (2005). A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. Avian Pathol., 34: 418-425.

Sarker, S; Moylan, KG; Ghorashi, SA; Forwood, JK; Peters, A and Raidal, SR (2015). Evidence of a deep viral host switch event with beak and feather disease virus infection in rainbow bee-eaters (*Merops ornatus*). Sci. Rep., 5: 14511.

Soike, D; Hattermann, K; Albrecht, K; Segales, J; Domingo, M; Schmitt, C and Mankertz, A (2001). A diagnostic study on columbid circovirus infection. Avian Pathol., 30: 605-611.

Stenzel, T and Pestka, D (2014). Occurrence and genetic diversity of pigeon circovirus strains in Poland. Acta Vet. Hung., 62: 274-283.

Stenzel, T; Piasecki, T; Chrząstek, K; Julian, L; Muhire, BM; Golden, M; Martin, DP and Varsani, A (2014). Pigeon circoviruses display patterns of recombination, genomic secondary structure and selection similar to those of beak and feather disease viruses. J. Gen. Virol., 95: 1338-1351.

Tamura, K and Nei, M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512-526.

Todd, D (2000). Circoviruses: immunosuppressive threats to avian species: a review. Avian Pathol., 29: 373-394.

Todd, D; Weston, J; Ball, NW; Borghmans, BJ; Smyth, JA; Gelmini, L and Lavazza, A (2001). Nucleotide sequence-based identification of a novel circovirus of canaries. Avian Pathol., 30: 321-325.

Zhang, Z; Dai, W; Wang, S and Dai, D (2015). Epidemiology and genetic characteristics of pigeon circovirus (PiCV) in eastern China. Arch. Virol., 160: 199-206.