EMERGING INFECTIOUS DISEASES®

Novel circovirus in European catfish (Silurus glanis)

Journal:	*Emerging Infectious Diseases*
Manuscript ID:	EID-11-1872
Manuscript Type:	Dispatch
Date Submitted by the Author:	19-Dec-2011
Complete List of Authors:	Lőrincz, Márta Dán, Ádám Láng, Mária Csaba, György Tóth, Ádám Székely, Csaba Cságola, Attila Tuboly, Tamás; Szent István University Faculty of Veterinary Science,
Keywords:	fish circovirus, phylogenetic analysis, catfish

EMERGING INFECTIOUS DISEASES®

Centers for Disease Control and Prevention
1600 Clifton Rd, NE
Mail stop D61
Atlanta, GA 30333
Phone: 404 498-2660
Fax: 404 639-1954

Checklist for Authors

First Author and Manuscript Title: Márta Lőrincz: Novel circovirus in European catfish

☑	This manuscript (or one with substantially similar content) has not been published and is not being considered for publication elsewhere.
☑	Corresponding author is the primary contact for proofing the manuscript and galleys.
☑	Financial support for this research is clearly disclosed in the manuscript.
☐	Any organization with a financial interest in the subject matter is disclosed in the manuscript.
☑	Authors have disclosed any conflict of interest related to this article.
☐	Research has been approved by appropriate human or animal subjects research review boards, which are named in the text of the manuscript.
☑	DNA and amino acid sequences have been submitted to a sequence database and accession numbers are used to refer to the sequences.
☑	All persons who have made substantial contributions to this work but did not fulfill the authorship criteria are named in the Acknowledgments.
☑	Written permission has been obtained from all persons listed in the acknowledgments.
☑	Written permission has been obtained from all persons listed as authors on this manuscript.
☐	Written permission has been obtained from the publishers of any figures or tables previously published or adapted from published figures or tables.
☐	Written permission has been obtained from persons identifiable in photographs, case descriptions, or pedigrees.
☐	Written permission has been obtained from persons named in personal communications (oral or written) stating that they agree to be named and that the information cited is accurate.
☑	All pages are double-spaced, numbered, and left justified (ragged right margin).
☑	All references are cited in the text, follow Uniform Requirements (http://www.icmje.org/index.html), and have been checked for accuracy and completeness.
☑	Legends for figures are at the end of the text.
☑	Each figure is in a separate file.
☑	Abstract and article meet word count, which is strictly enforced.
☑	All units of measure are expressed in SI units per Instructions to Authors.
☑	A short (2-3 sentence) biography is provided for the first author or both if two authors.
☑	Authors agree that if accepted for publication in Emerging Infectious Disease, their manuscript will upon publication be in the public domain and can be used without liability for copyright infringement.

Additional notes or statements:

Novel circovirus in European catfish (*Silurus glanis*)

Márta Lőrincz, Ádám Dán, Mária Láng, György Csaba, Ádám György Tóth, Csaba Székely, Attila Cságola, and Tamás Tuboly

Author affiliations: Faculty of Veterinary Science, Szent István University, Budapest, Hungary (M. Lőrincz, A. Cságola, T. Tuboly); Central Agricultural Office, Veterinary Diagnostic Directorate, Budapest, Hungary (Á. Dán, M. Láng, G. Csaba, Á. G. Tóth); Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary (C. Székely)

Address of correspondence: Tamás Tuboly, Szent István University Faculty of Veterinary Science, Department of Microbiology and Infectious Diseases, H-1143, Budapest, Hungária krt. 23-25, Hungary; e-mail: Tuboly.Tamas@aotk.szie.hu

Running Title:

Novel circovirus in catfish

Keywords:

Fish circovirus, phylogenetic analysis, catfish

Article Summary Line:

Newly identified circovirus of European catfish showed close relationship with barbel circoviruses and circular viruses detected in human stool samples.

Word count in abstract: 44

Word count in text: 1147

Abstract

PCR amplification and sequencing were used to identify a novel circular DNA virus in European catfish (*Silurus glanis*). Full genome characterization and phylogenetic analysis showed that the virus belonged to the *Circoviridae* family and it was closely related to the previously described barbel circovirus.

Members of the *Circoviridae* family are among the smallest known viruses and have a single stranded circular DNA genome of 1.7-2.3 kilobases. They are present in several avian species and pigs, and have also been detected most recently in fish (barbel, *Barbus barbus*). Besides the members of the *Circovirus* genus of this family new circular genomes of similar nature were described in different environmental and fecal samples (*1-5*) showing that they were more widespread than previously thought and the establishment of a new genus (*Cyclovirus*) for a group of these viruses was proposed (*4*).

An extensive research was initiated after the first detection of a fish circovirus (*6*) in order to assess the presence and importance of these viruses in other fish species. The present study describes the identification and genetic analysis of a new circovirus identified in European catfish (*Silurus glanis*).

The Study

During the spawning season of 2011 the number of fully developed European catfish found dead in Lake Balaton of Hungary was unexpectedly higher than in previous decades. Dead and moribund catfish of 6-50 kg body weight were collected and examined for possible causes of the unusual phenomenon. The routine bacteriological, parasitological, virological examinations and toxicological tests did not show the presence of any known cause to explain

the excessive fish death. Pathology and histopathology revealed skin lesions, vascular dilations in the skin, inflammation of the gastrointestinal tract and nuclear fragmentation in the hematopoietic cells raising the suspicion of some kind of undetected toxicosis.

Tissue samples of 6 European catfish were used for the detection of circoviruses in the same approach as it was previously done for the barbel circovirus (BaCV) (*6*). Liver, spleen, gills, kidneys and gonads (approximately 0.1 g of each) were processed individually and viral DNA was extracted using the Viral Gene-spin™ DNA/RNA Extraction Kit (Intron Biotechnology Inc., Korea) as recommended by the manufacturer. The nested PCR system of Halami et al. (*7*) was used for the detection of circovirus DNA and 3 out of the 6 examined fish were found positive. The amplicons were sequenced and a primer pair (HT-F: 5'-CAGACCATGCTTCCGGTACT-3', HT-R: 5'-GGGCTTCCTCGAAGGTTATC -3') was designed using the Primer3 program (*8*) for the amplification of the remaining part of the circular genome. Positive samples from 2 fish were selected for full circovirus genome amplification and analysis. DNA was multiplied with the TempliPhi™ 100 Amplification Kit (GE Healthcare, UK) with Exo-Resistant Random Primers (Fermentas, Lithuania) and amplified by PCR with the newly designed primer pair. The amplicons were sequenced and aligned with the sequences obtained from the initial PCR amplicons with the CAP3 program (*9*). The full genome nucleotide sequences (CfCV-H5 and CfCV-H6, GenBank no.: JQ011377 and JQ011378) were analyzed by the BioEdit Sequence Aligment Editor version 7.0.5.3 (*10*) and by NCBI tools (www.ncbi.bnlm.nih.gov). Phylogenetic trees were constructed with the neighbor-joining method of the MEGA 5 software (*11*) using 1000 bootstrap values.

The size of both CfCV genomes was 1966 nucleotides (nt), forming a covalently closed circular molecule. The sequences were 99.4% identical to each other. Two major open reading frames (ORF) of opposite orientations, separated by short intergenic sequences (282

bases at the 5' end and 60 bases at the 3' end) were predicted. ORF1 is located between 251 and 1193 nt and has a potential coding capacity of 314 amino acids (aa), whereas ORF2 spans from 1934 to 1253 nt encoding 217 aa. Besides ORF1 and 2, two additional ORFs of smaller sizes, ORF3 (158 aa, 472 bases, from nt 473 to 1) and ORF4 (136 aa, 408 bases, from nt 27 to 435) were identified in the genome. The sequence allowing for a stem loop structure characteristic to circoviruses was predicted by Mfold (*12*) and was located within the 5' intergenic region. The conserved nonanucleotide sequence (5'-TAGTATTAC-3') is identical to that of several members of the *Circovirus* genus including BaCV, porcine circovirus type 1 and some avian circoviruses. The nonamer of the NG13 virus of human origin reported by Li et al. (*4*) was also the characteristic circovirus sequence and not the 5'-TAATACTAT-3' typical for cycloviruses. The stem length of CfCV is 9 basepairs, the same as for NG13 and shorter than the stem of most circoviruses.

Blast analysis of ORF1 indicated homology with circovirus replication associated protein (Rep) sequences both at nucleotide and amino acid levels, and the putative protein is a member of the circovirus Rep family according to the Pfam database search. Based on ORF1 derived aa Blast comparisons the closest relative of CfCV is NG13 with 55% identity, whereas the rest of known circovirus Reps including BaCV showed lower similarities (49-52 %). The amino-terminal part of the potential capsid (Cap) protein (coded by ORF2) carries a 31 aa long arginine rich stretch (RRRTFRRPIRRRMHRRTRGRRMIRRRSRRSR) starting at residue 4, homologous to the circovirus Cap nuclear localization signal, and the closest relative of CfCV based on Cap aa comparison is also NG13 (35% similarity). The barbel and swan (*7*) circovirus Caps together with the RW-C sequence from reclaimed water (*2*) showed 27-28% similarity, but the value for other circoviruses was below that. GenBank search of ORF3 and 4 sequences did not reveal any homology with known viral genes.

Phylogenetic analysis of the newly identified CfCV-H5 and -H6 was performed for complete genome sequences and also for the Rep and Cap proteins using available GenBank data, including selected sequences from the *Circovirus* and the *Cyclovirus* genera. The full genome comparisons indicated that CfCV was a new virus within the *Circoviridae* family (Figure 1) closer related to the *Circovirus* genus than to the cycloviruses. Similar results were obtained when the tree was generated based on Rep protein sequences (not shown). The Cap sequence analysis indicated that CfCV-H5 and -H6 formed a separate group together with the recently identified BaCV and NG13 (Figure 2).

The similarity to the other only known fish circovirus (BaCV) was not surprising but NG13 was detected in human stool samples of children with acute flaccid paralysis (*4*). NG13 together with the partial Rep sequence of NG24 (*4*) were the only ones that could not be grouped in that study either to the *Circovirus* or the *Cyclovirus* genera. Although the similarity of NG13 to CfCV and BaCV was low, it was detected not only in the case of the Cap sequence but also for the entire genome, indicating a closer relation among these viruses.

Conclusions

The results of the study showed that circoviruses were present in European catfish. The role of the virus as a pathogen is still to be determined, but circoviruses are generally considered to be immune-suppressive, so CfCV may play such role in the development of diseases, especially during the exhaustive spawning season. The similarity of CfCV (or BaCV) to the NG13 of human origin raises several possibilities, namely, that NG13 may be a food born contamination from fish source or that these viruses may have a common but so far unknown origin.

Acknowledgements

The study was financed in part by the Hungarian research grant OTKA K 71837 and by the Bolyai Research Fellowship of the Hungarian Academy of Sciences. The skilled help of Imre Balogh, Ágnes Juhász and Mária Ottingerné is appreciated.

Author Bio:

Dr. Lőrincz is a PhD student of the Szent István University, Faculty of Veterinary Science. Her research interest is circovirus evolution, focusing on new hosts of known circoviruses and viral discovery.

References

1. Blinkova O, Victoria J, Li Y, Keele BF, Sanz C, Ndjango JB, et al. Novel circular DNA viruses in stool samples of wild-living chimpanzees. J Gen Virol. 2010; 91: 74-86.
2. Rosario K, Duffy S, Breitbart M. Diverse circovirus-like genome architectures revealed by environmental metagenomics. J Gen Virol. 2009; 90: 2418-24.
3. Rosario K, Nilsson C, Lim YW, Ruan Y, Breitbart M. Metagenomic analysis of viruses in reclaimed water. Environ Microbiol. 2009; 11: 2806-20.
4. Li L, Kapoor A, Slikas B, Bamidele OS, Wang C, Shaukat S. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol. 2010; 84: 1674-8.
5. Li L, Shan T, Soji OB, Alam MM, Kunz TH, Zaidi SZ. Possible cross-species transmission of circoviruses and cycloviruses among farm animals. J Gen Virol. 2011; 92: 768-72.
6. Lőrincz M, Cságola A, Farkas SL, Székely C, Tuboly T. First detection and analysis of a fish circovirus. J Gen Virol. 2011; 192: 1817-21.

7. Halami MY, Nieper H, Muller H, Johne R. Detection of a novel circovirus in mute swans (*Cygnus olor*) by using nested broad-spectrum PCR. Virus Res. 2008; 132: 208-212.
8. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000; 132: 365-86.
9. Huang X, Madan A. CAP3: A DNA Sequence Assembly Program. Genome Res. 1999; 9: 868-77.
10. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser. 1999; 41: 95-8.
11. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 2011; 28: 2731-39.
12. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 2003; 31: 3406-15.

Figure legends:

Figure 1. Phylogenetic analysis of the European catfish circovirus (CfCV-H5 and -H6) based on complete genomic DNA sequences. The viruses included are: canary circovirus (CaCV), columbid circovirus (CoCV), gull circovirus (GuCV), beak and feather disease virus (BFDV), duck circovirus (DuCV), goose circovirus (GoCV), barbel circovirus (BaCV1, BaCV2), porcine circovirus (PCV1, PCV2a, PCV2b), human stool-associated circular virus (NG13), cycloviruses (TN25, Chimp 12, NG 12, NGchicken15, PK 5222, PK5006), circovirus-like genome from reclaimed water (RW-C). Scale represents estimated phylogenetic distance. GenBank accession numbers are shown on the tree.

Figure 2. Phylogenetic analysis of the predicted Cap protein amino acid sequences. Names are as in Figure 1. The *Circovirus* genus is divided into 2 groups, A and B. **A** representing the traditional circoviruses and **B** including the fish viruses and NG13.