RAPID RISK ASSESSMENT

Cyclovirus in cerebrospinal fluid of patients with central nervous system infection

5 July 2013

Summary

In 2013, studies conducted on human clinical samples in Vietnam and Malawi indicated for the first time that cycloviruses might be associated with central nervous system infection in humans. A definitive causal link to neurological diseases was not established and would require further studies. Cycloviruses belong to the *Circoviridae* family together with the genera circovirus and gyrovirus. They have been identified in a variety of animal and human samples. Circoviruses have been shown to cause disease in pigs and poultry.

The pathogenicity and routes of transmission of the virus are uncertain. The detection of cyclovirus in animal samples points to a potential animal reservoir and consequently a zoonotic infection. According to published studies, the virus seems to be widely spread in nature but cycloviruses have not yet been reported in human or animal samples collected from Europe.

At this point, epidemiological data on cyclovirus infections in humans are very limited. There are insufficient data to assess the risk for disease occurrence in humans or potential of human-to-human transmission. Further studies should be encouraged in Europe and elsewhere to investigate the possible pathogenicity, epidemiology, and transmission patterns of cycloviruses.

Source and date of request

ECDC internal decision, 19 June 2013.

Public health issue

This assessment is related to the publication of the identification of a new cyclovirus species, tentatively named cyclovirus-Vietnam (CyCV-VN), in cerebrospinal fluid (CSF) of patients with acute central nervous system infection [1]. A review of the current state of knowledge of this type of virus and an assessment of the potential impact for human health was proposed.

ECDC internal response team

Tarik Derrough, Josep Jansa, Diamantis Plachouras, Bertrand Sudre

Consulted experts

Marion Koopmans, Matthias Niedrig

Background information

The information below is based on a scientific literature review using database queries (key words: 'cyclovirus' or 'novel ssDNA virus'; databases: Web of Knowledge, PubMed, Embase, and Scopus; date: 20 June 2013). A manual selection based on title and abstract was performed on 20 June 2013, and 26 articles of interest were retrieved for review. Full text articles were reviewed together with their list of bibliographic references when available. The main information presented below was used as background information for this rapid risk assessment. Additional references were reviewed with regard to the list of articles yielded during the initial scientific database queries.

Cycloviruses are members of the cyclovirus genus and belong to the *Circoviridae* family [2], together with the genera circovirus and gyrovirus. Cycloviruses have been recently characterised as single-stranded DNA (ssDNA) viruses, with a genome size of between 1.7 and 1.9 Kb, presenting two major bidirectionally transcribed open reading frames (ORFs): (a) one for replication-associated protein (Rep), and (b) one for capsid protein (Cap).

Cycloviruses are distinguished from other viruses in the family *Circoviridae* by specific genetic markers [2]. Cycloviruses are considered the smallest autonomous replicating viral genomes.

Since 2009, multiple sequences of cyclovirus genomes have been available in public genome databases. This increased the scientific knowledge about this virus and allowed genetic comparison analyses between samples [3]. According to International Committee on Taxonomy of Viruses, a cyclovirus species is defined by sequence homology of at least 75% nucleotide identity and 70% identity in the Cap protein [3].

It is of importance to note that the conserved (Rep) region can be used to assess the prevalence of circovirus/cyclovirus genome presence in individual samples; however, additional sequencing steps and genomic organisation studies are needed to differentiate both genera [2].

There is a high diversity of ssDNA viruses, including cyclovirus among insect populations and some mammals. New viral species have been recently identified among vertebrates and are more are likely to be identified as the metagenomic approach is now more frequently used [2, 4, 5]. The viral genome identification from animal tissues supports the hypothesis of animal infection.

Tables 1 and 2 below summarise the key findings of the literature review, focusing on cyclovirus identification in human, animals and insects. Viral genome isolated from non-haematophagous insects supports a wider distribution in arthropods.

Li et al. studied the genetic diversity among cycloviruses in human stool samples and local cow, goat, sheep, camel, and chicken meat samples. Positive faecal samples cannot discriminate individual intestinal infections from virus ingested with contaminated food items. The study showed that there is no major overlap between isolated human and animal genomes; however, the human and animal samples obtained at various geographical regions show no similarities, which makes the interpretation of the findings difficult [3]. In addition, closely related viruses were found in cows and goats form Pakistan [3]. Due to the apparent wide distribution of the virus, human exposure to the virus through diet (meat) or exposure to animal faeces seems likely.

Cyclovirus DNA has also been identified in blood from patients with unexplained fever. In a study of serum samples from 123 Nicaraguan children with symptoms resembling dengue but testing negative for dengue, cyclovirus sequences were identified using metagenomic methods in 13/123 (10.6%) of samples [6]. However, it was not possible to recover full genome sequences. Consequently, it was not possible to exclude that these sequences were the result of an environmental artefact or derived from other organisms encoding similar sequences.

Vietnamese study. Using metagenomic methods to test CSF samples from adult patients with central nervous system (CNS) infections of unknown cause in Vietnam, a 190-bp sequence was identified in a pool consisting of five CSF samples that showed 82% identity to a replication-associated gene (Rep) of cycloviruses [1]. A specific PCR targeting this sequence was subsequently employed to confirm the findings and successfully detect a fragment of the expected size in two samples. Following this, the full genome of the virus was amplified and sequenced. The cyclovirus species of the completed genome was tentatively named cyclovirus-Vietnam (CyCV-VN). The single-stranded circular DNA genome revealed a genomic structure characteristic of the genera *Circovirus* and *Cyclovirus* in the family *Circoviridae*. Phylogenetic analysis showed that CyCV-VN was genetically distinct from known cycloviruses, although most closely related to CyCV-TN18 and CyCV-TN25, both derived from human stool samples from Tunisia.

Subsequently, CyCV-VN was detected in 26 of 642 (4%) acute-infection CSF specimens (collected from 1999 to 2009), including:

- 10 of 273 (3.7%) CSF specimens from adults and children with acute CNS infections of unknown etiology; and
- 16 of 369 (4.3%) samples from adults and children in whom laboratory-confirmed CNS infection (encephalitis and meningitis) with other pathogens was established.

The clinical presentation of the cases without other laboratory-confirmed infections was generally mild and all patients recovered. The identification of the virus in the CSF of patients with laboratory confirmed CNS infection caused by other pathogens, indicates the possibility of co-infection.

CyCV-DNA could not be detected in 122 CSF specimens collected from Vietnamese patients with non-infectious neurological disorders. CyCV-VN DNA was also detected in 8/188 (4.2%) faecal specimens from healthy children. When specimens from poultry and pigs in the same area were tested, the virus was detected in 38/65 (58%) specimens. Pairwise comparison of the obtained sequences from animals and humans revealed sequence similarity of >97% between the detected strains.

The findings of this study suggested a possible causal relationship. Further studies are needed in order to definitively establish causality. Such studies would explore whether CyCV-VN fulfils Koch's postulates or adaptations thereof.

Malawian study. The presence of cyclovirus in humans was also investigated as part of a research project in Malawi conducted in 2010 and 2011 [7]. This study was undertaken to analyse serum and CSF from adult patients who sought hospital care when presenting with unexplained paraplegia. Fifty-eight adults were enrolled in the research; serum and CSF samples were investigated to determine the prevalence of cyclovirus. A full cyclovirus genome was sequenced from one patient (cyclovirus VS57000009). The genome origin of the isolated and sequenced cyclovirus strain was different (less than 85% amino-acid homology) from previously known human cyclovirus. It was most closely related to strains detected in faeces from children with non-polio-associated acute flaccid paralysis in another investigation, namely strains CyCV-TN18 and CyCV-TN25 in the investigation conducted in Vietnam.

A specific PCR for the detection of human cyclovirus identical to the strain detected in patient VS57000009 was performed in the available serum and CSF samples of the 58 patients. The phylogenetic comparison with the cyclovirus-Vietnam (CyCV-VN) was not performed. However, according to the comparison with previous sequences (humans and animal), the cyclovirus VS5700009 represents a new cyclovirus species.

The results of the investigation indicated that samples were found to be positive for human cyclovirus:

- in eight of the 54 (15%) serum samples; and
- in four of the 40 (10%) of the CSF samples.

The causal role of human cycloviruses in the occurrence of paraplegia in this study could not be established as there was no control group – but it confirmed other reports indicating the presence of these viruses in different organs of the humans. Further investigations are warranted to establish the prevalence of carriage of the virus in healthy people.

Advances in techniques for the discovery of new viruses have helped characterise a number of emerging infectious disease threats in a timely manner. However, increased sensitivity may on occasion lead to spurious associations between a microorganism and a nosological entity due to environmental contamination or amplification of ubiquitous sequences. Causal relationship needs verification with criteria that are suitably adapted to modern methodologies [8].

Table 1: Summary of cyclovirus genome identification from human samples

Origin	Type of sample	Year	Location	Main findings – cyclovirus	Reference
Human	Stool from children with AFP (non-polio infected)	2005–2008	Pakistan	9/57 (15.8%)	[3] [9]
	Stool from healthy contact children	2005–2008	Pakistan	3/9 in contact; 7/41 in control; (17.1%)	[3]
	Stool from children with AFP (non-polio infected)	2007	Nigeria	9/96 (9.4%)	[3]
	Stool from children with AFP (non-polio infected)	2005–2008	Tunisia	7/96 (7.3%)	[3]
	Stool from healthy contact children	2005–2008	Tunisia	7/96 (7.3%)	[3]
	Stool of patent with acute gastro-enteritis	2004–2006	USA	None (n=140)	[3]
	Stool healthy control	2004–2006	USA	None (n=107)	[3]
	Plasma specimen from blood donors	–	USA	None (n=96)	[3]
	Plasma specimen from bush hunter	–	Africa	None (n=113)	[3]
	CSF from patients with CNS infection	1999–2009	Vietnam	26/642 (4%)	[1]
	CSF from controls with non-infectious CNS disease	1999	Vietnam	None (n=122)	[1]
	Stool of healthy children	–	Vietnam	8/188 (4.2%)	[1]
	Serum	2010–2011	Malawi	8/54 (15%)	[7]
	CSF	2010–2011	Malawi	4/40 (10%)	[7]

Table 2: Summary of Cyclovirus genome identification from animals, arthropods and environmental samples

Origin	Type of sample	Year	Location	Main findings – cyclovirus	Reference
Pig	Commercial meat	2008	USA	None (n=13)	[3]
	Faecal samples		Vietnam	12/20 (60%)	[1]
Chicken	Commercial meat	2008–2009	Pakistan	None (n=13)	[3]
		2009	Nigeria	22/40 (55 %)	[3]
		2008	USA	None	[9]
	Faecal samples		Vietnam	12/12 (100%)	[1]
Duck	Faecal samples		Vietnam	14/33 (42%)	[1]
Beef	Commercial meat	2008–2009	Pakistan	4/26 (15.4 %)	[3]
		2009	Nigeria	3/25 (12 %)	[3]
		2009–2010	USA	None	[9]
Goat	Commercial meat	2008–2009	Pakistan	7/18 (38.9 %)	[3]
		2009	Nigeria	None	[3]
Camel	Commercial meat	2009	Nigeria	3/27 (11 %)	[3]
Sheep	Commercial meat	2009	Nigeria	1/29 (3%)	[3]
Chimpanzee	Stool form common chimpanzee	2002–2007	Central Africa	6/44 (13.6%)	[3]
Bat	Muscle, digestive tract and faecal specimen.	2009	Texas	Viral replication	[9]
Bat		2009–2010	China	2/199 (2%)	[5]
Bat guano		2009	Texas		[10]
Environmental sample	Sewage			*Circoviridae* genus	[11]
Insect (non-blood-feeding arthropods)	Dragonfly	2010	Tonga	95% of similarities between samples coming from different locations	[12]
	Dragonfly	2007–210	Australia, New Zealand, and the United States of America	Four different cyclovirus species isolated from three dragonfly species (*Orthetrum sabina*, *Xanthocnemis zealandica*, and *Aeshna multicolour*); intra- and inter-species recombination events among cycloviruses. Wide circulation in insect population is likely.	[4]
	Wood cockroach	2011	Florida (USA)	Florida woods cockroach (*Eurycotis floridana*)	[13]

Threat assessment for the EU

- Detection of cycloviruses in human or animal samples from Europe has not yet been reported. Further studies are needed to assess the geographical spread of the virus and its potential reservoir (wild and domestic animals).
- The detection of cyclovirus in animal samples points to a potential animal reservoir and, potentially, a zoonotic infection. There are insufficient data to assess the potential of human-to-human transmission.
- At this point, epidemiological data on cyclovirus infections and carriage in humans are very limited. Consequently, the risk for disease occurrence in humans cannot be assessed with any degree of accuracy.

Conclusions

- Cycloviruses belong to the *Circoviridae* family, together with the genera circovirus and gyrovirus. They have been identified in a variety of animal and human samples.
- In 2013, studies conducted on human samples in Vietnam and Malawi demonstrated for the first time that *Cyclovirus* may be associated with CNS infection in humans. A definitive causal link was not established and would require additional studies.
- At this point, epidemiological data on cyclovirus infections in humans are very limited. There are insufficient data to assess the risk for disease occurrence in humans or potential of human-to-human transmission. Further studies should be encouraged in Europe and elsewhere to investigate the possible pathogenicity, epidemiology, and transmission patterns of cycloviruses

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