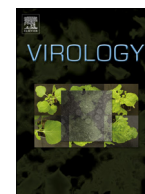




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## Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated sewage <sup>☆</sup>



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### ABSTRACT

Viruses with small circular ssDNA genomes encoding a replication initiator protein can infect a wide range of eukaryotic organisms ranging from mammals to fungi. The genomes of two such viruses, a cyclovirus (CyCV-SL) and gemycircularvirus (GemyCV-SL) were detected by deep sequencing of the cerebrospinal fluids of Sri Lankan patients with unexplained encephalitis. One and three out of 201 CSF samples (1.5%) from unexplained encephalitis patients tested by PCR were CyCV-SL and GemyCV-SL DNA positive respectively. Nucleotide similarity searches of pre-existing metagenomics datasets revealed closely related genomes in feces from unexplained cases of diarrhea from Nicaragua and Brazil and in untreated sewage from Nepal. Whether the tropism of the cyclovirus and gemycircularvirus reported here include humans or other cellular sources in or on the human body remains to be determined.

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### Introduction

*Cyclovirus* is a recently proposed genus of the *Circoviridae* family with small ssDNA circular genome of approximately 2-kb (Li et al., 2010a). Cycloviruses are a sister clade to the circoviruses, a genus known to infect a wide variety of birds (Todd, 2004) and mammals (Li et al., 2013, 2011). A third genus, krikovirus, was recently proposed from genomes detected in mosquitoes (Garigliany et al., 2015) and bat feces (Li et al., 2010b; Lima et al., 2015). The *Circoviridae* family is part of a much more diverse group of viruses with circular, replication initiator protein (Rep) encoding, single stranded DNA (CRESS-DNA) genomes, found in a wide range of hosts and environment (Delwart and Li, 2012; Rosario et al., 2012b). CRESS-DNA viruses, encoding only Rep and capsid (Cap) proteins, have the smallest genomes of autonomously

<sup>☆</sup>The GenBank accession numbers for new cycloviruses and gemycircularviruses are, KP133075, KP151567, KP133076–KP133080.

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replicating eukaryotic viruses. Cyclovirus genomes, initially found in the feces of Pakistani children with and without acute flaccid paralysis (Li et al., 2010a) have also been reported in the feces of bats (Ge et al., 2011; Li et al., 2010b), poultry (Li et al., 2010a, 2011; Tan le et al., 2013) and in meat samples of various farm animals in Pakistan and Nigeria (Li et al., 2010a, 2011). Cyclovirus genomes have also been detected in the abdomen of dragonflies and in the Florida cockroach (Dayaram et al., 2013; Padilla-Rodriguez et al., 2013; Rosario et al., 2011). In 2013 a cyclovirus was also reported in 4% of cerebrospinal fluid (CSF) from south and central Vietnamese children with unexplained central nervous system disorders, and in 4.2% of healthy children's feces (Tan le et al., 2013). Fifty eight percent of feces from nearby pigs were also positive for the same cyclovirus DNA (Tan le et al., 2013). A later study did not detect this virus in CSF of similar patients from northern Vietnam, Cambodia, Nepal and The Netherlands (Le et al., 2014). A recent study detected this cycloviral DNA in fecal samples from healthy children from Madagascar and from healthy pigs in Cameroon (Garigliany et al., 2014). In 2013 a related cyclovirus was found in 10% of CSF and 15% of sera from 58 paraplegia (leg paralysis) Malawian adult patients (Smits et al., 2013). These CSF associated