Short communication

Molecular epidemiological analyses of Cryptosporidium parvum virus 1 (CSpV1), a symbiotic virus of Cryptosporidium parvum, in Japan

Fumi Murakoshi a, f, Madoka Ichikawa-Seki b, Junya Aita b, Seiko Yaita c, Aiko Kinami d, Katsuhisa Fujimoto e, Yoshifumi Nishikawa a, Shin Murakami l, Taisuke Horimoto l, Kentaro Kato a, f, l

a National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
b Laboratory of Veterinary Parasitology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan
c Kumage Agricultural Mutual Aid Association, 6410-8 Noma, Nakatane-cho, Kumage, Kagoshima 891-3664, Japan
d Veterinarian of Iwate Prefecture, Japan
e Veterinary Clinical Center, Ishikari Agricultural Mutual Aid Association, 7–4–1 Seiryu, Chitose, Hokkaido 066-0081, Japan
l Department of Veterinary Microbiology, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

A R T I C L E   I N F O

Article history:
Received 29 June 2015
Received in revised form 27 September 2015
Accepted 28 September 2015
Available online 9 October 2015

Keywords:
dsRNA viruses
Cryptosporidium parvum
Cryptosporidium parvum virus 1
Partitiviridae
Epidemiology

A B S T R A C T

We show that Cryptosporidium parvum virus 1 (CSpV1), a member of the family Partitiviridae, genus Cryptovirus that can infect Cryptosporidium parvum, is a new candidate for high-resolution tool for tracing C. parvum. CSpV1 was detected in all C. parvum-positive samples tested. Phylogenetic analysis of dsRNA1 sequence from CSpV1 can distinguish infected areas of C. parvum on the national level. Sequences detected in samples from Iwate prefecture and other islands (Tanegashima, and Okinawa) belonged to a single clade. This system can differentiate the samples from Hokkaido and south part of Japan as well as from other countries. Samples from Iwate, Tanegashima, and Okinawa belonged to a single subclade, respectively. Therefore, the CSpV1 dsRNA sequences reflect the regional distribution of their host and have potential as a high-resolution tool to trace C. parvum IaA15G2R1 subtype.

© 2015 Elsevier B.V. All rights reserved.

Cryptosporidium parvum belongs to the phylum Apicomplexa and has been reported to be a zoonotic species that causes severe diarrhea (Sulaiman et al., 2005) and no effective therapy has yet been established. It can cause waterborne or foodborne outbreaks. Therefore, studies on the species/subspecies distribution and transmission routes of C. parvum are important for public health and food production. The 60-kDa glycoprotein (GP60) gene is the major subtyping gene of Cryptosporidium hominis and C. parvum (Leav et al., 2002; Glaberman et al., 2002; Abe et al., 2006) because the GP60 gene is the most polymorphic marker identified to date in the Cryptosporidium genome. Moreover, GP60 is located on one of the dominant targets for neutralizing antibody responses in humans (Cevallos et al., 2000). On the basis of this gene, the sequences of C. parvum and C. hominis have been subtyped with following designations: Ia–Ig for C. hominis; and Ia–Ilo for C. parvum (Xiao, 2010; Valenzuela et al., 2014; Wang et al., 2014). The Ila subtype is the most common subtype found in humans and cattle in many countries (Santín et al., 2008). GP60 based subtype can usually be further characterized based on the number of trinucleotide repeats (TCA or TCG) coding for the amino acid serine (Sulaiman et al., 2005). In Japan, IlaA15G2R1 subtype is the only subtype of C. parvum that has been detected to date (Wu et al., 2003; Abe et al., 2006; Murakoshi et al., 2013, 2014; Ichikawa-Seki et al., 2014; Aita et al., 2015). Therefore, there are no tools that can trace infection and contamination sources in outbreaks.

Recently, double-stranded RNA (dsRNA) viruses, belonging to the family Partitiviridae, have been identified in Cryptosporidium and classified as members of the genus Cryptovirus (Nibert et al., 2009, 2014). These viruses contain two unrelated, linear dsRNA segments of 1.7 kbp (dsRNA1) and 1.4 kbp (dsRNA2) that...
are encapsidated separately. dsRNA1 encodes the RNA-dependent RNA polymerase (RdRp) and dsRNA2 encodes the capsid protein (CP). The molecular mass of RdRp and CP are 62 kDa and 37 kDa, respectively (Khramtsov and Upton, 2000). Crypsovirus detected from C. parvum has been named Cryptosporidium parvum virus 1 (CpV1). This virus has also been detected from Cryptosporidium felis, C. hominis, and C. meleagridis (Leoni et al., 2003, 2006). Most species classified with the family Partitiviridae infect fungi and plants, and are transmitted from cell to cell during cell division. Therefore, CpV1 is transmitted only by intracellular routes, although its exact mode of transmission remains unclear. There are a few studies of the host effects by CpV1, and its virulence. Only a correlation between CpV1 dsRNA2 levels and parasite fecundity of C. parvum has been reported (Jenkins et al., 2008). RNA viruses show higher mutation frequencies than their hosts because they lack proofreading enzymes (Holland et al., 1982). An attempt to sub-type C. parvum using CpV1 dsRNA sequences has previously been reported by two research groups, suggesting that CpV1 sequences may be useful for tracing Cryptosporidium infection sources because of their sequence diversity (Xiao et al., 2001; Leoni et al., 2003). Consequently, we were interested in whether CpV1 dsRNA could serve as novel C. parvum trace markers.

One hundred and seventeen calf fecal samples were collected from farms located in Hokkaido (Kushiro and Ishikari) in 2014. C. parvum positive bovine fecal samples from Ashoro (Hokkaido), Iwate, Okinawa, and Tanegashima (Kagoshima) prefectures were kindly provided by numerous scientists (Ichikawa-Seki et al., 2014; Aita et al., 2015). The C. parvum HNJ-1 strain (kindly provided by Dr. Yagita) was first isolated from an infected woman in Japan (Satoh et al., 2005), and passaged in our laboratory by using nude mice (BALB/c Slc- nu/nu, SLC Japan). C. parvum was detected in the fecal samples using both sugar flotation microscopic observation and a molecular procedure. For DNA extraction, 0.3–0.4 g of fecal specimen was used. Cryptosporidium spp. were detected and subtyped by nested polymerase chain reaction (PCR) amplification targeting a ~830 bp and a ~850 bp fragment of the small subunit (SSU) rRNA and 60-kDa glycoprotein (GP60) genes, respectively, as described previously (Feng et al., 2007).

Oocysts were purified from C. parvum-positive feces by using the sucrose gradient method. To extract RNA easily, oocysts were excised by modifying a method previously described (Rochelle et al., 2002). Total RNAs were extracted from sporozoites with the TRIZOL reagent (Life Technologies) and total RNA was used for cDNA synthesis with a SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions with the following primers: CPVL ORF_R (5′-TCTAAATTTTGGTACTGCTG-3′) and CPVS ORF_R (5′-ATGGAGGGCATCIGGCTACAC-3′). The resulting cDNA was amplified using KOD FX Neo (TOYOBO, Japan) with primers CPVL ORF_F (5′-AAGTTGTTCAATCTATCGATAC-3′) and CPVS ORF_F (5′-ATCATAACTTTGATGACCC-3′) for amplifying CpV1 dsRNA1 ORF (−1576 bp), and CPVs ORF_F (5′-ATTTAACAGTTTTGATGACCC-3′) and CPVS ORF_R (5′-ATGGAGGGCATCIGGCTACAC-3′) for CpV1 dsRNA2 ORF (−954 bp). Then, all PCR products were cloned (Zero Blunt TOPO PCR Cloning Kit, life technologies) and were sequenced.

The sequences were aligned using Clustal X2 (Larkin et al., 2007) and the computed sequences were edited by hand with BioEdit 7.0.5.3 (Hall, 1999). All gaps were eliminated and dsRNA1 sequences were used for the phylogenetic analysis. A maximum likelihood (ML) analysis was performed using MEGA 6.0.6 (Tamura et al., 2011). The used substitution model and optional parameter sets were evaluated by MEGA 6.0.6, and the most suitable sets were selected according to the Akaike information criterion (AIC). To calculate the bootstrap values, 500 ML trees were constructed using the same datasets. To investigate whether CpV1 dsRNA1 sequence can trace sampling region, in Fig. 1, we constructed phylogenetic tree which substitution model and optional parameters used is T92 (Tavaré, 1986) + Gamma distribution (five categories) option. CpV1 sequences from America and Australia were retrieved from the NCBI database, and Japanese CpV1 sequences were obtained in this study. Most of CpV1 reference sequences registered with NCBI were partial. Because of the short reference sequences, Fig. 1 has a small amount of information (low-resolution). To acquire more detailed information, we reconstructed a phylogenetic tree with a focus on Japanese samples using longer sequences (1446 bp) with the T92 substitution model incorporating the invariable site and Gamma distribution (five categories) options (Fig. 2). We remove LC014998 sequence because we cannot read full length of its ORF.

Of the 117 fecal samples collected from Ishikari and Kushiro (Hokkaido), 4 samples (3.4%) were positive for Cryptosporidium. The parasites in all of the PCR-positive specimens were identified as C. parvum and all of the GP60 nucleotide sequences were identical to each other and had similarity with C. parvum subtype IlaA15G2R1. Ashoro (Hokkaido) (7 samples), Iwate (5 samples), Okinawa (5 samples) prefectures and Tanegashima Island (2 samples) samples were previously reported and also subtyped C. parvum IlaA15G2R1 (Ichikawa-Seki et al., 2014; Aita et al., 2015). In total, all 24 C. parvum-positive specimens were CpV1 dsRNA-positive by RT-PCR. Partitivirus sequences were found integrated into host chromosomes and some of them were transcribed (Liu et al., 2010; Chiba et al., 2011). Therefore, we did PCR using C. parvum genomic DNA as a template with CpV1-specific primers. As a result, no DNA was amplified. We also performed Local BLAST search using whole genome of C. parvum, however, CpV1 sequence...
دانلود مقاله

http://daneshyari.com/article/3427974

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات