Veterinary Microbiology 160 (2012) 463–467

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Molecular detection of murine noroviruses in laboratory and wild mice☆

Tibor Farkas [a,b,*], Brittney Fey [a], Gary Keller [a], Vito Martella [c], Laszlo Egyed [d]

[a] Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA
[b] University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA
[c] University of Bari Aldo Moro, S.p. per Casamassima km 3, 70010 Valenzano, Bari, Italy
[d] Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1143 Budapest, Hungária krt. 21, Hungary

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 31 May 2012
Accepted 5 June 2012

Keywords:
Mouse
Calicivirus
Murine norovirus

ABSTRACT

Fecal specimens collected from 121 laboratory mice, 30 striped field mice (*Apodemus agrarius*), 70 yellow-necked mice (*Apodemus flavicollis*), and 3 bank voles (*Myodes glareolus*) were tested in sample pools for the presence of murine noroviruses (MNV). Ten of 41 laboratory mice and 2 of 3 striped field mice pooled samples were positive for MNV. All laboratory mouse MNVs were closely related to previously described MNVs. The complete ORF2 (VP1) of both striped field mouse MNVs identified in this study was 1623 nt (541 aa) long and differed at 12% nt (8% aa) positions from each other, at 22–24% nt (15–18% aa) positions from the laboratory mouse MNVs and at 20–22% nt (13–14% aa) positions from the recently described wood mouse (*Apodemus sylvaticus*) MNVs. This study provides further evidence for the circulation of novel, genetically diverse MNVs in wild mice.

1. Introduction

The *Norovirus* genus of the *Caliciviridae* family contains both human and animal pathogens and is further subdivided into five genogroups (GI–V), from which GV represents the murine NoVs (MNV). The ~7.5 kb positive sense, single stranded norovirus RNA genome is organized into three major open reading frames (ORF): ORF1 encodes a large polyprotein that is cleaved to individual non-structural proteins; ORF2 encodes the viral capsid protein (VP1); and ORF3 encodes a minor structural protein (VP2). In the MNV genome, an additional ORF4 has also been identified (Thackray et al., 2007). Since the discovery of the first MNV in 2003 (Karst et al., 2003) it became evident that MNV infection is the most prevalent viral infection in laboratory mice (Hsu et al., 2005; Kim et al., 2011; Perdue et al., 2007). MNV infection in immunocompetent mice is subclinical but in impaired mice such as STAT1−/−, MNV establishes systemic infection that leads to high mortality (Karst et al., 2003). Due to its ability to grow in tissue culture, MNV became the most widely used surrogate model for human norovirus research (Wobus et al., 2006). All MNV strains isolated from laboratory mice worldwide belong to a single genotype with ~13% nt and ~7% aa diversity in the ORF2 (VP1) region indicating a very limited genetic diversity compared to the genetic diversity observed among human noroviruses (Thackray et al., 2007). Recent studies, by either serology or molecular detection suggest that MNVs are also circulating in wild rodents (Parker et al., 2009; Smith et al., 2012). However, the extent of their genetic diversity and biological similarities to MNVs is not well established. In this study, wild and laboratory mice were screened for MNVs, and two novel MNVs from striped field mice (*Apodemus agrarius*) were identified and characterized.

2. Materials and methods

2.1. Sample collection

Thirty striped field mice (*A. agrarius*), 70 yellow-necked mice (*Apodemus flavicollis*), and 3 bank voles (*Myodes glareolus*) were live trapped between July and October

☆ The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences determined in this study are: JQ408727–JQ408738.

* Corresponding author at: Division of Infectious Diseases, Children's Hospital Medical Center, ML7017, 3333 Burnet Avenue, Cincinnati, OH 45229, USA. Tel.: +1 513 636 0131; fax: +1 513 636 7655.
E-mail address: tibor.farkas@cchmc.org (T. Farkas).

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http://dx.doi.org/10.1016/j.vetmic.2012.06.002

2010 in South-West Hungary. Two pellets collected from each of 10 individual animals of the same *Apodemus* species were mixed together and made into 20% suspensions in RPMI-1640, yielding 3 striped field mice and 7 yellow-necked mice sample pools. The bank vole samples were processed individually. In addition 121 stool samples were collected from sentinel and research mice between May and July 2011 at the CCHMC animal facility, housing over 40,000 mice. Two to five pellets were collected from each animal, made into 20% (w/v) suspensions and stored at −80 °C. For MNV testing 3 individual samples were pooled together yielding 40 pooled and one individual laboratory mouse samples.

2.2. RNA extraction and cDNA synthesis

Viral RNA was extracted with the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Wild mice RNA samples were converted to cDNA using an oligo-dT primer (Reverse transcription system; Promega, Madison, WI), ethanol precipitated, vacuum dried and shipped to Cincinnati where they were reconstituted in 20 μl molecular biology grade water (Thermo Fisher Scientific Inc., Waltham, MA). RNA and cDNA samples were stored at −80 °C.

2.3. Molecular detection of murine noroviruses

Detection of caliciviruses in the wild mice samples were attempted with generic calicivirus primers (P289/P290) targeting nucleotide sequences encoding conserved amino acid motifs in the RNA dependent RNA polymerase (RdRp) region of ORF1 (Jiang et al., 1999) and with a MNV specific primer pair targeting a 396 bp region at the 5′ end of MNV ORF2 (VP1) (Kim et al., 2011). Two microliters of cDNA was used in 25 μl PCR reactions using the GoTaq Green Master Mix (Promega, Madison, WI) according to the manufacturer's protocol. Laboratory mouse RNA samples were tested only with the MNV specific primers in one step RT-PCR reactions (AccessQuick RT-PCR system; Promega,

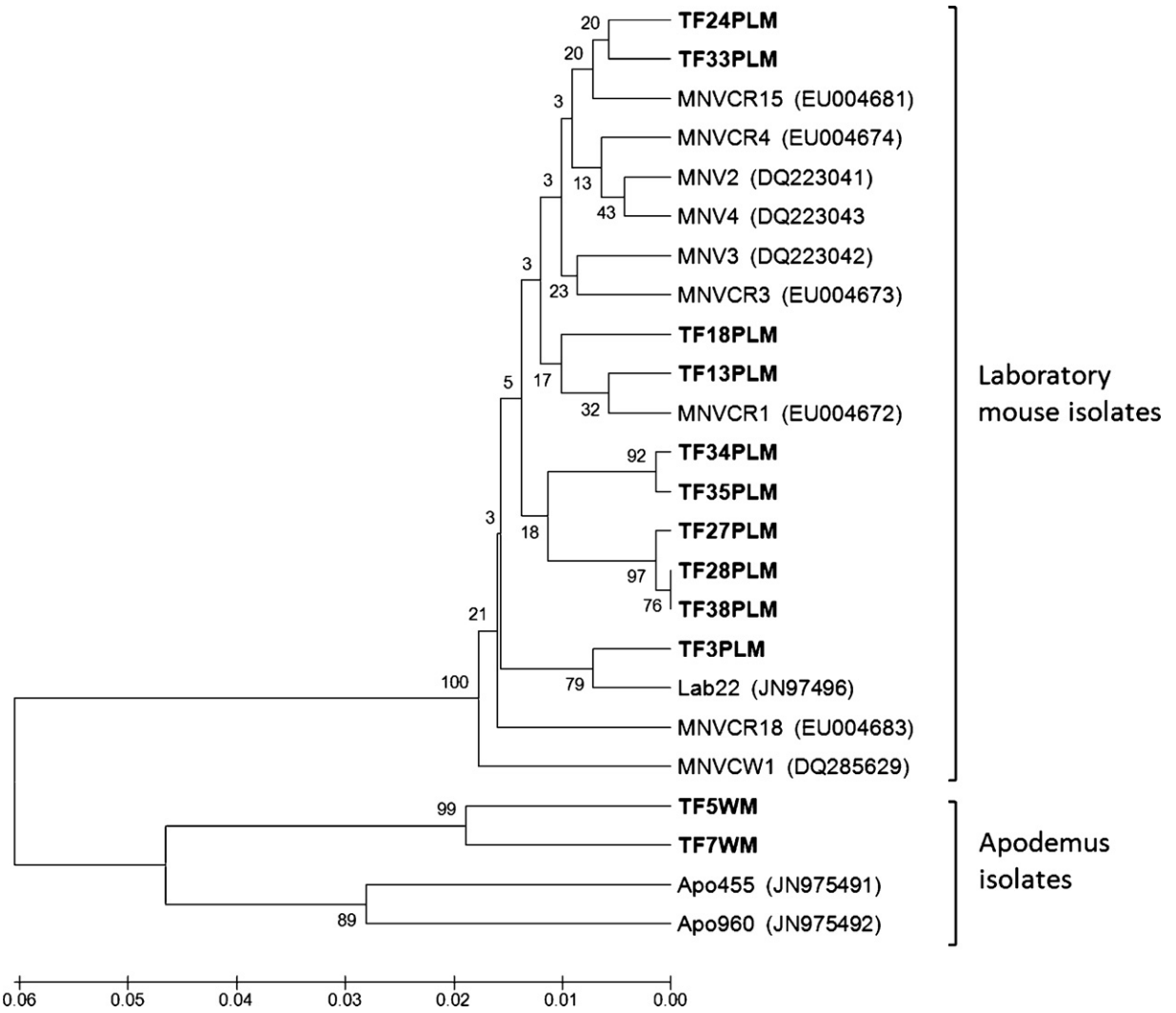

Fig. 1. Phylogenetic analysis of 356 nt partial ORF2 sequences of laboratory and wild mouse MNV isolates. The dendrogram was constructed by the UPGMA clustering method of MEGA version 3.1 with Jukes–Cantor distance calculations and 1000 bootstrap analyses. Strains described in this study are in bold.