Virology 509 (2017) 232–238

Contents lists available at ScienceDirect

Virology

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

Evolution of Bovine viral diarrhea virus in Canada from 1997 to 2013

Adam Chernick*, Frank van der Meer*

Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Dr NW, Calgary, AB T2N 1N4, Canada

ARTICLE INFO

Keywords:
Bovine viral diarrhea virus
Evolution
Next-generation sequencing
ssRNA viruses
Phylogenetics
Canada

ABSTRACT

Bovine viral diarrhea virus (BVDV) is a rapidly evolving, single-stranded RNA virus and a production limiting pathogen of cattle worldwide. 79 viral isolates collected between 1997 and 2013 in Canada were subjected to next-generation sequencing. Bayesian phylogenetics was used to assess the evolution of this virus. A mean substitution rate of 1.4×10^{-3} substitutions/site/year was found across both BVDV1 and BVDV2. Evolutionary rates in the E2 gene were slightly faster than other regions. We also identified population structures below the sub-genotype level that likely have phenotypic implications. Two distinct clusters within BVDV2a are present and can be differentiated, in part, by a tyrosine to isoleucine mutation at position 963 in the E2 protein, a position implicated in the antigenicity of BVDV1 isolates. Distinct clustering within all sub-genotypes, particularly BVDV2a, is apparent and could lead to new levels of genotypic classification. Continuous monitoring of emerging variants is therefore necessary.

1. Introduction

Bovine viral diarrhea virus (BVDV) is a single-stranded RNA (ssRNA) virus that is a production limiting pathogen of cattle with a global distribution (Ridpath, 2010). It can be categorized in two genotypes or species, BVDV1 and BVDV2. Numerous sub-genotypes of BVDV1 have been reported as well as two proposed BVDV2 sub-genotypes (Vilček et al., 2001; Flores et al., 2002). In Canada and the United States BVDV1a, BVDV1b and BVDV2a are currently in circulation. Previous studies have described notable genetic diversity within and between each of these sub-genotypes (Vilcek et al., 2004) as well as within BVDV1a in western Canada (Chernick et al., 2014). In the latter study, the substitution rate of BVDV1a isolates was estimated to be 1.26×10^{-3} substitutions/site/year in the E1-E2 gene across all codon positions. This rapid evolution drives many important phenotypic changes in BVDV including extensive antigenic variability (Ridpath et al., 2000). High substitution rates are not unique to BVDV and are often noted in other ssRNA viruses including type 1a Hepatitis C virus (Yuan et al., 2013), HIV (Suzuki et al., 2000) and others (Duffy et al., 2008). This rapid evolution stems largely from the inherent properties of these viruses and their error-prone replication. In the case of BVDV specifically, the unique immune environment associated with persistent infections also appears to play an important role in generating novel viral variants (Dow et al., 2015).

BVDV is a *Pestivirus* within the family *Flaviviridae*. It shares a genome structure similar to these related viruses with 5′ and 3′ untranslated regions (UTRs), structural genes towards the 5′ end and most nonstructural genes to the 3′ end. The UTRs are both highly conserved with the 5′UTR acting as a ribosomal entry site (Fletcher and Jackson, 2002) and 3′UTR also essential to replication (Yu et al., 1999). Following the 5′UTR is the NPro, a viral protease responsible for cleaving some viral proteins from one another (Wiskerchen et al., 1991). The structural proteins follow and include the envelope proteins (Erns, E1 and E2) and the capsid. Erns is secreted from infected cells (Rumenapf et al., 1993) and binds to host cell surfaces (Iqbal et al., 2000) while E1 and E2 are both surface glycoproteins important to host cell binding and entry (Ronecker et al., 2008) as well as being highly antigenic (Paton et al., 1992; Deregt et al., 1998a, 1998b). The p7 gene sits between the structural and non-structural genes and is required for infectivity (Harada et al., 2000). NS2 and NS3 are non-structural genes encoding for a protein that performs multiple functions including a serine protease (Tautz et al., 1997) and helicase (Warrener and Collett, 1995). NS4A is a co-factor for the NS2/3 serine protease activity (Xu et al., 1997) and NS4B appears to play a role in viral genome replication and host membrane remodelling (Weiskircher et al., 2009). NS5A (Isken et al., 2014) and NS5B (Zhong et al., 1998) are both components of the RNA-dependent RNA polymerase complex.

Several phylogenetic and genetic diversity studies of pestiviruses

Abbreviations: BVDV, Bovine viral diarrhea virus; E, envelope; ESS, effective sample size; Gbp, gigabase pairs; GTR, general time-reversible; HIV, human immunodeficiency virus; HPD95, 95% highest probability density; MRCA, most recent common ancestor; NS, non-structural; RB, reversible-jump based; ssRNA, single-stranded RNA; SYM, symmetrical; TIM, transition; UK, United Kingdom; UTR, untranslated region; VSACC, Veterinary Sciences Animal Care Committee

* Corresponding authors.
E-mail addresses: a.chernick@ucalgary.ca (A. Chernick), frank.vandermeer@ucalgary.ca (F. van der Meer).

http://dx.doi.org/10.1016/j.virol.2017.06.024
Received 8 April 2017; Received in revised form 20 June 2017; Accepted 21 June 2017
Available online 29 June 2017

and BVDV have also been conducted, although none exhaustively examine Canadian field isolates. With respect to the age of the entire BVDV lineage, all BVDV1 and BVDV2 isolates are believed the share a most recent common ancestor about the year 1743 (95% highest probability density (HPD95) 1373–1926) (Liu et al., 2009). Other studies have examined the spatial movement of BVDV while inferring evolutionary rates about 9.3×10^{-3} substitutions/site/year in the 5′ untranslated region (5′UTR) among Italian isolates (Luzzago et al., 2012). Phylogenetic and genetic diversity studies of European isolates are available in large part due to the need to inform BVDV-eradication and control programs in those countries. For example, isolates in Denmark span BVDV1 sub-genotypes a, b, d, e, f, g and h (Uttenthal et al., 2005) while a collection from France included sub-genotypes b, d, e and l (Jackova et al., 2008).

Prior to these phylogenetic studies numerous experiments were conducted using anti-BVDV antibodies to assess strain variation. Although many antibodies were found to be broadly neutralizing against genetically distinct isolates (Deregt et al., 1990), significant differences that correlated with genetic distance were also noted (Deregt et al., 1992). Panels of antibodies capable of distinguishing BVDV1 from BVDV2 as well as some sub-genotypic variation have also been generated (Deregt et al., 1998a, 1998b). Although there is merit in classifying isolates based on antibody binding assays, it has been noted that such a practice is “suggestive at best”, implying that sequence data is a more reliable tool even if it does not produce such an explicit connection to the phenotypic/antigenic properties of a given isolate (Dubovi, 1992). Sequence data also allows for more comprehensive evolutionary studies that describe the origins and evolutionary rates of BVDV.

In this study we describe the evolution of BVDV in Canada using a collection of viral isolates archived from 1997 to 2013 and obtained from academic and diagnostic laboratories across the country as well as from active field sampling efforts. We employed a next generation sequencing (NGS) approach in conjunction with Bayesian phylogenetics to generate an integrated model of BVDV1 and BVDV2 evolution within the Canadian cattle population. This both describes the evolutionary history of this pathogen in Canada as well as important variability among currently circulating BVDV isolates. Apart from the practical use of this information in the development and maintenance of virus control programs including vaccination, these results also contribute to the understanding of the evolutionary capacity of ssRNA viruses which include a number of important human and animal pathogens.

2. Methods

2.1. Sample collection and processing

Viral isolates were obtained from diagnostic and research laboratories across Canada (Table S1). This was the result of an extensive survey of laboratories working on BVDV and likely represents most historical isolates available for evaluation. All samples were collected in accordance with the animal care protocol VSACC AC11-0081/AC14-0144. All isolates had been passaged at least once at the laboratory of origin in Madin Darby Bovine Kidney or Bovine Turbinate cell culture prior to further characterization in our laboratory. RNA was extracted from an aliquot of each isolate using the E.Z.N.A. Viral RNA kit (Omega Bio-tek, Norcross, USA). A series of four reverse transcriptase PCRs were used to amplify overlapping fragments of the protein-coding region of the genome. See Table 1 for primer sequences and amplicon regions (Ridpath and Bolin, 1998; Couvreur et al., 2002; Greiser-Wilke et al., 1993; Gilbert et al., 1999). The amplicons were gel purified using the E.Z.N.A. Gel Extraction kit (Omega Bio-tek, Norcross, USA) and quantified using the Qubit 3.0 and the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, USA). Amplicons were pooled in equimolar amounts, adjusted for amplicon length. Each pool was subjected to library preparation using the Nextera XT DNA Library Preparation kit (Illumina, San Diego, USA). All libraries were prepared with dual indices and sequenced with paired-end, 250 bp reads on a MiSeq using a 500-cycle v2 cartridge (Illumina, San Diego, USA).

Table 1
Primer sequences and references for the four amplicon method used to generate DNA for next-generation sequencing.

Name	Sequence	Reference	Position
Frag1_fwd	CATGCCCATAGTAGGAC	(Harada et al., 2000)	107
Frag1_rev	GGGCAWACCATYTGGAAGGCYGG	(Tautz et al., 1997)	2853
Frag2_fwd	TGATAACAGGGGTACAAGGGC	(Warrener and Collett, 1995)	2442
Frag2_rev	RTTGYCCCTAGCGGTATAT	N/A	5314
Frag3_fwd	GCAGATTTTGAAGAAAGACACTA	(Warrener and Collett, 1995)	4934
Frag3_rev	AAGAAGCCATCATCMCCACA	(Xu et al., 1997)	11547
Frag4_fwd	AAGATCCACCCTTATGARGC	(Xu et al., 1997)	10385
Frag4_rev	CTGTGTGCATTRARTGTAGTGTT	N/A	12511

Primer sequences and references for the four amplicon method used to generate DNA for next-generation sequencing. All positions are relative to the NADL reference genome (GenBank Accession M31182).

2.2. Data Analysis

Raw reads were imported into Geneious R9 (Kearse et al., 2012) for pre-processing and assembly. Reads were paired and assembled using the Geneious assembler set to high sensitivity and iterated up to 5 times. Consensus sequences were extracted from assemblies for all individual genes except p7 (leaving a total of 10 genes). These are available in GenBank via accession numbers KX169935 through KX170703 along with the relevant protein translations (Table S2). Gene-specific consensus sequence alignments were built using MUSCLE (Edgar, 2004). Alignments were visually inspected and corrected for errors. The HyPhy software package (Kosakovsky Pond et al., 2005) running on the Datamonkey server (Delport et al., 2010) was used to test for recombination. Specifically, the single breakpoint recombination (SBP) analysis (Kosakovsky Pond et al., 2006) was run using the HKY85 substitution model on each gene-specific alignment.

Sequence alignments were imported into BEAUti to prepare the run parameter files for BEAST 2 (Bouckaert et al., 2014). Viral genes were analyzed independently to explicitly accommodate any differences in their phylogenies. All sequences were dated with their year of collection and separated into three partitions for the codon positions. Year was used as it was the most precise date data available for all isolates. A reversible-jump based (RB) substitution model accounting for ambiguities in the consensus sequences was employed to simultaneously explore different substitution models and infer the phylogeny using the mixture that best fit the data (Bouckaert et al., 2014, 2013). Six substitution models were evaluated including the Felsenstein (F81) (Felsenstein, 1981), HKY (Hasegawa et al., 1985), Tamura and Nei (TN93) (Tamura and Nei, 1993), transition (TIM), symmetrical (SYM) (Zharkikh, 1994) and generalized time-reversible (GTR) (Lanave et al., 1984) models. Separate relaxed exponential molecular clocks were used for each codon partition. Runs were allowed to continue until adequate convergence (ESS > 200 for most parameters) was reached. Phylogenetic trees were built using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and Tracer was used to extract parameters of interest while verifying run quality.

Two additional trees were built. The first using only the BVDV2a isolates in addition to BVDV2a and BVDV2b reference strains to further refine the clusters observed in the larger trees using partial 5′UTR sequences. The second includes all isolates in this study as well