Veterinary Microbiology 179 (2015) 299–303

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

Identification of amino acid changes in the envelope glycoproteins of bovine viral diarrhea viruses isolated from alpaca that may be involved in host adaptation☆

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ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 22 May 2015
Accepted 4 June 2015

Keywords:
Persistent infection
Host adaptation
Genetic change
Phylogenetic analysis
Camelid

ABSTRACT

Bovine viral diarrhea viruses (BVDV) are most commonly associated with infections of cattle. However, BVDV are often isolated from closely related ruminants with a number of BVDV-1b viruses being isolated from alpacas that were both acutely and persistently infected. The complete nucleotide sequence of the open reading frame of eleven alpaca-adapted BVDV isolates and the region encoding the envelope glycoproteins of an additional three isolates were determined. With the exception of one, all alpaca isolates were >99.2% similar at the nucleotide level. The Hercules isolate was more divergent, with 95.7% sequence identity to the other viruses. Sequence similarity of the 14 viruses indicated they were isolates of a single BVDV strain that had adapted to and were circulating through alpaca herds. Hercules was a more distantly related strain that has been isolated only once in Canada and represented a separate adaptation event that possessed the same adaptive changes. Comparison of amino acid sequences of alpaca and bovine-derived BVDV strains revealed three regions with amino acid sequences unique to all alpaca isolates. The first contained two small in-frame deletions near the N-terminus of the E2 glycoprotein. The second was found near the C-terminus of the E2 protein where four altered amino acids were located within a 30 amino acid domain that participates in E2 homodimerization. The third region contained three variable amino acids in the C-terminus of the E^{rns} within the amphipathic helix membrane anchor. These changes were found in the polar side of the amphipathic helix and resulted in an increased charge within the polar face. Titration of bovine and alpaca viruses in both bovine and alpaca cells indicated that with increased charge in the amphipathic helix, the ability to infect alpaca cells also increased.

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1. Introduction

Bovine viral diarrhea viruses (BVDV) are members of the *Pestivirus* genus of the *Flaviviridae*. BVDV can be divided into two different species, BVDV1 and BVDV2, as well as multiple subgroups (Simmonds et al., 2012). BVDV are ubiquitous pathogens of cattle and have a major economic impact for producers. BVDV establish both transient and persistent infections. However, BVDV infections are not limited to cattle. Infections have been identified in white tailed deer (Chase et al., 2008; Passler et al., 2008), mountain goats (Nelson et al., 2008), elk (Tessaro et al., 1999) and camelids (Belknap et al., 2000; Wentz et al., 2003). BVDV are able to establish persistent infections in ruminant species other than cattle, including alpaca (Carman et al., 2005; Kim et al., 2009). BVDV was first reported in alpaca where a BVDV1b virus was isolated from a stillborn cria (Goyal et al., 2002). The first BVDV persistently infected (PI) alpaca was reported in Canada (Carman et al., 2005). A BVDV1b virus was isolated from a stillborn cria and from a second cria at birth from a single herd. The second cria was positive for BVDV at birth, 3 days and 26 days of age by reverse transcription PCR, immunohistochemistry and antigen detection ELISA. A large study looking at the prevalence of PI alpaca in North America found 46 PI alpaca in a total of more than 12,000 alpaca tested (Kim et al., 2009). All PI viruses isolated were BVDV 1b, as determined by sequencing of the 5′ untranslated region (UTR) and

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http://dx.doi.org/10.1016/j.vetmic.2015.06.007
0378-1135/Published by Elsevier B.V.

N^{pro} sequences. A subsequent report (Topliff et al., 2009) presented results from a second survey of alpaca herds in the United States where four PI cria were identified in 63 herds.

Information regarding genetic changes that allow BVDV to adapt to infection and replication in other host species is limited. The E2 protein possesses receptor-binding activity and is the major antigenic determinants as the majority of neutralizing antibodies are targeted to the E2 protein (Hulst and Moormann, 1997 Yu et al., 1996). Serial persistent infections between cattle and sheep (Paton et al., 1997) revealed that changes were introduced into the E2 protein as the virus replicated in different hosts as shown by alterations in monoclonal binding patterns.

This study was done to gain a greater understanding of the genetic changes introduced into the genomic RNA of BVDV in adaptation to alpaca. Here, BVDV 1b isolated from PI and transiently infected alpaca were sequenced and compared to the genomic sequences of BVDV1b isolated from cattle to determine if differences exist that increase fitness of BVDV in alpaca.

2. Materials and methods

2.1. Cells and viruses

Fetal alpaca turbinate (aTu) and kidney (aK) cells were maintained in Leibowitz MEM containing 1 g/l NaHCO3 and 10% fetal bovine serum (FBS) at 37 °C in a humidified 5% CO2 atmosphere. Bovine turbinate (bTu) cells were passaged using MEM as described above. All FBS used in these studies was tested to be BVDV and BVDV antibody free as previously described (Bolin et al., 1991).

The alpaca-adapted BVDV isolates Aries, Cepheus, Columba, Corona, Gemini, Hercules, Kurhah, Leo, Lyra, Mars, Naos, Pluto, and Scorpius were previously described (Kim et al., 2009). The alpaca BVDV isolate 10,270 was obtained from Dr. James Evermann (Washington State University). These viruses were originally isolated using fetal bovine testicular cells and were passed once on primary aTu or aK cells prior to RNA isolation and DNA sequence analysis in this study. Alpaca cells were infected at an MOI of approximately 0.5 and incubated for 4 days. The cells were lysed by 2 freeze-thaw cycles, the medium clarified by centrifugation and all virus stocks stored at −80 °C. The BVDV-1b isolate 23,909 was a gift from Dr. Sagar Goyal (University of Minnesota) and was passaged on bTu cells. The bovine-derived BVDV-1b strains NY-1 and TGAN were grown in bTu cells and stocks prepared as described above.

2.2. Virus titrations

Titrations of viruses on bTu and aTu cells to compare infection of bovine and alpaca cells by bovine and alpaca BVDV was done as previously described (Bauermann et al., 2012). The concentration of virus in $TCID_{50}$/ml was calculated as described (Reed and Muench, 1938).

2.3. RNA extraction, PCR and sequencing

Viral RNA was isolated using the RNeasy spin kit (Qiagen, Inc., Valencia, CA) according to manufacturer's specifications. One tube PCR reactions were done using PCR primers designed for amplification of BVDV 1b genomic RNAs as previously described (Neill et al., 2011). The resulting PCR products were gel purified using GENECLEAN spin column kits (MP Biomedicals, Inc., Solon, OH). DNA sequencing was done using ABI BigDye Terminator v. 3.1 chemistry with an ABI PRISM 3100 automated sequencer (ABI, Inc., Carlsbad, CA). All DNA sequences were aligned and analyzed using CodonCode Aligner software (Codoncode, Inc., Dedham, MA). The GenBank accession numbers for the virus sequences are shown in Table 1. All numbering of genomic nucleotide positions started from the ATG initiation codon of the open reading frame (ORF).

3. Results and discussion

3.1. Sequencing and phylogenetic analysis

The complete ORF of eleven alpaca-derived BVDV isolates (10270, Aries, Columba, Corona, Gemini, Hercules, Kurhah, Leo, Lyra, Mars, Scorpius) were amplified and sequenced (Table 1). The complete ORF sequences showed that ten of the alpaca isolates were closely related (≥99.2%) with Hercules being more distantly related. An ORF consensus sequence of the ten related viruses was derived and was compared to each individual virus, revealing that these viruses were multiple isolations of a single strain of BVDV-1b circulating through alpaca herds. These single strain viruses represent the second known BVD outbreak that was caused by single strain of virus (Ridpath et al., 2006). The number of differences from the consensus sequence is shown in Table 1. Of the remaining three isolates (Cepheus, Naos, Pluto), only the N^{pro} and

Table 1
Viruses used in this study.

Virus	Isolation source[a]	GenBank accession number	ORF sequence[b]	Differences from ORF consensus[c]
10270	P	JX297512	Y	24
Aries	D	JX297513	Y	35
Columba	P	JX297514	Y	10
Corona	A	JX297515	Y	5
Gemini	A	JX297516	Y	28
Hercules	P	JX297517	Y	763
Kurhah	P	JX306012	Y	13
Leo	P	JX297518	Y	10
Lyra	P	JX297519	Y	67
Mars	P	JX297520	Y	4
Scorpius	P	JX297521	Y	76
Cepheus	A	JX306011	N[d]	nd[e]
Naos	Ab	JX306013	N	nd
Pluto	P	JX306015	N	nd

[a] Isolated from acute infection (A), persistent infection (P), dead cria (D) or aborted fetus (Ab).
[b] Complete ORF sequence.
[c] ORF consensus sequence derived from alignment of single-strain BVDV nucleotide sequences.
[d] N—only sequences encoding N^{pro}, capsid E^{rns}, E1 and E2 proteins were determined.
[e] ND: not determined.