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Segmental configuration and putative origin of the reassortant orbivirus, epizootic hemorrhagic disease virus serotype 6, strain Indiana

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ABSTRACT

In 2006, an exotic reassortant orbivirus, epizootic hemorrhagic disease virus serotype 6 (EHDV-6) [strain (Indiana)], was first detected in the United States. To characterize the reassortment configuration of this virus and to conclusively determine the parental virus of each RNA segment, the complete genome of EHDV-6 (Indiana) was sequenced, in addition to the genomes of representative EHDV-6 and EHDV-2 isolates. Based on genomic comparisons to all other EHDV serotypes, we determined that EHDV-6 (Indiana) originated from a reassortment event between the Australian prototype strain of EHDV-6 (CSIRO 753) and the North American topotype of EHDV-2 (Alberta). Additionally, phylogenetic analysis of all EHDV-6 (Indiana) isolates detected in the United States from 2006 to 2010 suggests that the virus may be undergoing continual reassortment with EHDV-2 (Alberta). In 2010, EHDV-6 (CSIRO 753) was detected in Guadeloupe, demonstrating that the parental virus of the reassortment event is circulating in the Caribbean.

Introduction

Epizootic hemorrhagic disease virus (EHDV) is an arthropod-borne virus of the genus *Orbivirus*, family *Reoviridae*, that is maintained in nature in a transmission cycle involving hematophagous *Culicoides* midges and ruminant vertebrate hosts (e.g., cattle, deer) (Nettles et al., 1992). Like all orbiviruses, the genome of EHDV is composed of 10 segments of double-stranded RNA that encode seven structural (VP1–VP7) and four nonstructural (NS1–NS3/NS3a) proteins (Mertens et al., 2005). As the genome is segmented, orbiviruses are capable of undergoing reassortment with different serotypes of the same species (serogroup) during co-infection. Recently, based on antigenic and genetic analyses of the two outer capsid proteins (VP2 and VP5), the EHDV serogroup has been proposed to be condensed from ten to seven serotypes (Anthony et al., 2009b). Based on this newly proposed classification scheme, EHDV-318, a virus initially isolated in Bahrain in 1983 and formerly regarded as a separate serotype (Mertens et al., 2005), was demonstrated to be serologically and genetically related to the prototype strain of EHDV-6 isolated in Australia in 1981 (CSIRO 753) (Anthony et al., 2009b). Consequently, the 318 strain was grouped with CSIRO 753 to comprise two related, but genetically distinguishable, strains or topotypes of EHDV-6.

Historically, only two serotypes, EHDV-1 (New Jersey strain) and EHDV-2 (Alberta strain), were endemic to the United States (Chalmers et al., 1964; Shope et al., 1955). However, in 2006, an exotic strain of EHDV-6 was isolated from moribund and dead white-tailed deer (*Odocoileus virginianus*) in Indiana and Illinois (Allison et al., 2010). Since its initial discovery, the virus has been repeatedly isolated on an annual basis from additional states including Missouri, Kansas, Texas, Michigan, and Arkansas, suggesting that it may now also be endemic in the United States. Preliminary genetic analysis of the virus, designated as EHDV-6 [strain (Indiana)], suggested it was a reassortant derived from an exotic strain of EHDV-6 and endemic EHDV-2 (Alberta) (Allison et al., 2010). The detection of a reassortant EHDV derived from an indigenous serotype and a virus previously never reported in the New World not only provided a unique example of genetic shift among exotic and endemic viruses, but also demonstrated the plasticity of orbiviruses in

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general, not only in terms of the ability of different serotypes to reassort with one another, but also in their ability to enter, and become established in, novel environmental niches previously never occupied.

With the unprecedented incursion of multiple *bluetongue virus* (BTV) serotypes into northern Europe since 2006 (Saegerman et al., 2008; Wilson and Mellor, 2009) and the recognition of EHDV-associated morbidity and mortality in cattle from Réunion Island in 2003 and 2009 (Bréard et al., 2004; Sailleau et al., 2011), Morocco, Tunisia, Algeria, and Israel in 2006 (Yadin et al., 2008; http://www.oie.int/eng/info/hebdo/AIS_64.htm), and western Turkey in 2007 (Temizel et al., 2009), concern regarding the recent geographical expansion of pathogenic orbiviruses has been growing steadily. Historically, other than the Ibaraki strain of EHDV-2 (Omori et al., 1969; Uchinuno et al., 2003), EHDV has not been considered to be a major threat to domestic animal health, whereas the closely-related BTV has been the focus of in-depth research for many years (for a review, see Tabachnick, 1996), primarily due to its pathogenic potential in sheep (and to a lesser extent cattle), in association with trade restrictions on ruminants and associated germplasm from BTV endemic areas. However, with numerous recent EHDV outbreaks involving serotypes previously never recognized as pathogens (i.e., EHDV-6, EHDV-7), coupled with the incurrence of significant economic losses during such outbreaks (Kedmi et al., 2010), EHDV may no longer be regarded as an anecdotal pathogen of cattle and its introduction into new areas should be perceived as it having the potential of becoming a disease agent of clinical and economic importance. Concomitant with the detection and repeated isolation of EHDV-6 (Indiana) in the United States, we instituted a number of research initiatives to comprehensively characterize the virus from a genetic and evolutionary standpoint, determine the susceptibility of a known North American EHDV vector (*Culicoides sonorensis*) for the reassortant, and to assess the potential risk that the virus may become an emerging pathogen of domestic and wild ruminants in the United States. In this report, we present the genomic sequence of EHDV-6 (Indiana), along with additional representative strains of EHDV-2 and EHDV-6, to more precisely determine the parental viruses responsible for the segmental configuration of the novel reassortant and explore its genetic diversity and evolutionary history within the United States.

Results and discussion

Comparative analysis of EHDV-6 (Indiana) with other EHDV-6 isolates from South Africa (M44/96, strain 318), Australia (AUS 1981/07, strain CSIRO 753), and Bahrain (BAR1983/01, strain 318) revealed that the parental virus of RNA segments 2 and 6 (which encode the outer capsid proteins VP2 and VP5, respectively) in EHDV-6 (Indiana) was very similar to the Australian prototype CSIRO 753 strain, with the two viruses sharing 97–98% nucleotide identities in their VP2 and VP5 sequences and a strong phylogenetic relationship (Tables 1 and 2; Fig. 1). Conversely, the RNA segments encoding the four nonstructural proteins (NS1, NS2, and NS3/NS3a), the three replicative enzymes (VP1, VP4, and VP6), along with the structural proteins that comprise the inner subcore (VP3) and core surface layer (VP7) were derived from EHDV-2 (Alberta) (Tables 1 and 2; Fig. 2). Thus, although EHDV-2 was the majority parental virus, contributing eight of the 10 RNA segments, the reassortant retained the serotype specificity (and hence serotype designation) of EHDV-6, as the outer surface antigens (VP2 and VP5) responsible for the induction of neutralizing antibodies during infection in vertebrate hosts (Iwata et al., 1991, 1992) were retained from EHDV-6 (CSIRO 753).

The genome of EHDV-6 (Indiana) is 19,407 base pairs (bp) in length, with the RNA segments ranging in size from 810 bp for segment 10 (NS3) to 3942 bp for segment 1 (VP1) (Table 1). Based on analysis of the terminal ends of each of the 10 RNA segments in positive-sense orientation, the 5′ and 3′ UTR sequences that are strictly conserved are the hexanucleotide 5′-GUUAAA-3′ and the pentanucleotide 5′-CUUAC-3′, respectively (Fig. 3). The 5′ UTRs ranged in size from eight (VP4) to 32 (NS1) nt, while the 3′ UTRs were, on average, considerably longer, ranging from 22 (VP1) to 115 (NS1) nt (Table 1 and Fig. 3). The 10 RNA segments of the EHDV genome and their associated genes have been described in detail elsewhere (Anthony et al., 2009a, 2009b, 2009c; Cheney et al., 1995, 1996; Iwata et al., 1991, 1992; Jensen and Wilson, 1995; Le Blois et al., 1991; Mecham et al., 2003; Wilson, 1994).

The recognition that both VP2 and VP5 (which are the most genetically divergent EHDV proteins) of the prototype reassortant (CC 304-06) were derived from a single serotype (EHDV-6) suggests that their homologous reassortment may have been necessary to form a stable outer capsid, such that a virus containing heterotypic VP2 and VP5 (i.e., from two different serotypes, such as EHDV-2 and EHDV-6) may be structurally and/or functionally debilitated and thus would be less likely to persist in nature (Mertens, 1999). However, based on the structure of the closely-related BTV, heterotypic protein interactions in EHDV-6 (Indiana), such as VP7 interacting with VP2 and VP5 during inner/outer capsid formation (Nason et al., 2004; Zhang et al., 2010), NS3 binding to VP5 or VP2 during viral assembly and egress, respectively (Beaton et al., 2002; Bhattacharya and Roy, 2008), along with heterotypic protein-RNA interactions such as EHDV-2-derived NS2 recognizing the two EHDV-6 RNAs during genome assembly (Lymperopoulos et al., 2006; Roy, 2008), apparently did not compromise the ability of EHDV-6 (Indiana) to survive in nature. However, whether these chimeric protein–protein, protein–RNA, or possibly even RNA–RNA interactions affect the fitness level (e.g., replication, assembly, and transmissibility) of EHDV-6 (Indiana) relative to non-heterotypic viruses is unknown and requires further study. Additionally, whether similarly structured reassortant viruses derived from sympatric endemic serotypes (e.g., VP7 of EHDV-1 and VP2/VP5 of EHDV-2) may also circulate in the United States has not been investigated extensively (Mecham et al., 2003).

Analysis of partial VP2, VP5, and VP7 sequences from 14 EHDV-6 (Indiana) isolates (see Table 3 for list) recovered in the United States

Table 1
Genomic characteristics of EHDV-6 (Indiana), showing the length of the open reading frames (ORFs) and untranslated regions (UTRs) of each segment, along with the predicted length and molecular weights (MW) of the cognate proteins.

Segment[a]	Gene	5′ UTR (nt)[b]	ORF (nt)	3′ UTR (nt)	Length (nt)	Length (aa)	MW (kDa)	Parental virus
1 (L1)	VP1	11	3909	22	3942	1302	149.7	EHDV-2 (Alberta)
2 (L2)	VP2	16	2919	36	2971	972	112.6	EHDV-6 (CSIRO 753)
3 (L3)	VP3	17	2700	51	2768	899	103.1	EHDV-2 (Alberta)
4 (M4)	VP4	8	1935	40	1983	644	76.0	EHDV-2 (Alberta)
5 (M5)	NS1	32	1656	115	1803	551	64.6	EHDV-2 (Alberta)
6 (M6)	VP5	28	1584	30	1642	527	59.1	EHDV-6 (CSIRO 753)
7 (S7)	VP7	17	1050	95	1162	349	38.1	EHDV-2 (Alberta)
8 (S8)	NS2	19	1122	45	1186	373	43.2	EHDV-2 (Alberta)
9 (S9)	VP6	14	1080	46	1140	359	39.8	EHDV-2 (Alberta)
10 (S10)	NS3	20	687	103	810	228	25.5	EHDV-2 (Alberta)

[a] RNA segments are designated as large (L), medium (M), or small (S) based on electrophoretic mobility.
[b] The position of the UTRs corresponds to the genomic RNA in positive-sense orientation.