



<u>Virus</u>	<u>Polyclonal</u>		<u>Antisera</u>	
	Anti BVDU1-Singer	Anti BVDU2-890	Anti BDU-BD31	Anti CSFU-Ames
BVDU1-NADL	2048	32	256	1024
BVDU2-125c	512	2048	128	512
BDU-CB5c	128	16	2048	8192
CSFU-NDSL	16	16	1024	224,000

Questions and Answers

Three Genotypes Detected in Pestiviruses Isolated from Ruminants in U.S. and Canada

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Abstract:

To examine the relationship between ruminant pestiviruses isolated in the U.S. and Canada, we surveyed 326 pestiviruses isolated from cattle, goats, sheep, and llamas. Based on differential polymerase chain reaction (PCR) amplification of sequences from the 5' untranslated region, 160 of the 326 viruses were characterized as bovine viral diarrhea virus (BVDV) 1 isolates and 152 were characterized as BVDV 2. Of the original 326 pestiviruses, 14 viruses could not be amplified using PCR primers specific for BVDV isolates. All 14 of these pestiviruses were isolated from small ruminants. Phylogenetic analysis, based on sequences from the 5' UTR, segregated these 14 viruses into a genotype separate from BVDV 1, BVDV 2, and hog cholera virus. The virus BD31, available from the American Type Culture Collection as a border disease-type virus (BDV), was also segregated into this genotype. Viruses from this BD31-like genotype (BDV genotype) could be differentiated from viruses from other pestivirus genotypes by virus neutralization using polyclonal sera and by binding of monoclonal antibodies

Introduction:

Viruses belonging to the Pestivirus genus, of the Flavivirus family, are enveloped viruses with a positive-sense single-stranded RNA genome. The genome, which varies from 12.3 to 12.7 Kb in length, contains one large open reading frame (ORF) bracketed by relatively large 5' (360-390 bases) and 3' (200-240 bases) untranslated regions (UTR). The three currently recognized pestivirus species are bovine viral diarrhea virus (BVDV), hog cholera virus (HCV) [also called classical swine fever virus (CSFV)], and border disease virus (BDV) of sheep. Because hog cholera has been eradicated from the U.S. and Canada, BVDV and BDV are the only recognized pestivirus species present in these countries. Assignment of pestiviruses to the BVDV or BDV species is often based on the animal hosts from which they are isolated. This type of classification is problematic because some pestiviruses may cross species barriers. Recently phylogenetic analysis has segregated ruminant pestiviruses into three genotypes, the BVDV1 genotype, the BVDV2 genotype, and the BDV genotype (Fig. 1). A fourth genotype, the HCV genotype (also known as the CSFV genotype), primarily infects pigs. To survey the types of pestiviruses isolated by diagnostic laboratories in the U.S. and Canada and the host species with which they are associated, we characterized 326 pestiviruses isolated from cattle, goats, sheep, and llama samples submitted to diagnostic laboratories in the U.S. and Canada.

Materials and Methods:

The 326 pestiviruses originated in either the U.S. or Canada. Of these pestiviruses, 305 were isolated from cattle and 21 were isolated from small ruminants (sheep, goat, and llama). All viruses originated as field strains from diseased animals or from fetal bovine serum and were initially propagated in bovine turbinate (BT) cells. Viruses that grew to a titer of less than 10⁶ virions/ml BT cells, were subsequently propagated in ovine fetal turbinate (OFTU) cells.

Viral isolates were segregated to the BVDV1, BVDV2 or BDV genotypes based on differential polymerase chain reaction amplification of sequences from the 5' UTR. Viral genomic RNA templates were generated by extracting total RNA from pestivirus-infected BT or OFTU cells.

Comparison of selected ruminant pestiviruses at the antigenic level was done using a monoclonal antibody (Mab) binding assay and by neutralization using polyclonal antisera. All Mabs reacted with the pestivirus glycoprotein gp53

and possessed viral neutralizing activity. Mab binding assay was performed with 30 pestiviruses propagated in either BT cells or OFTU cells grown in 96-well microtitration plates. Infected cell monolayers were fixed and Mab binding assessed by indirect immunoperoxidase staining.

Viral neutralization assays were done using antisera raised in cattle against BVDV1-Singer, BVDV2-890, and BDV-31; or antisera raised in swine against CSFV-Ames. The viruses used in the neutralization assays were the cytopathic viruses BVDV1-NADL, BVDV2-125c, BDV-CB5c, and the noncytopathic virus CSFV-NVSL. The assays were done in triplicate, using serial twofold dilutions of antiserum. End points of viral neutralization for cytopathic viruses were determined on day five of the assay by observing cells for cytopathic effect. Endpoints of viral neutralization for noncytopathic viruses were determined by immunoperoxidase staining.

Results:

The 305 pestiviruses isolated from cattle were segregated into the BVDV1 (157 viruses) and BVDV2 (149) genotypes. Both BVDV1 and BVDV2 viruses were isolated from animals with reproductive failure, persistent infections, enteric disease, and outbreaks of classic mucosal disease.

The 21 viruses isolated from small ruminants were segregated into three genotypes, the BVDV1 (3 viruses), BVDV2 (3 viruses) and BDV (14 viruses) genotypes.

Previously we have shown that viruses from the BVDV1 and BVDV2 genotypes are antigenically distinct. To determine if the 14 viruses that were grouped in the BDV genotype were antigenically distinct from viruses belonging to the BVDV1 and BVDV2 genotypes viruses from the three genotypes were compared by Mab binding analysis (Fig. 2). The pattern of Mab binding was distinctly different for viruses from all three genotypes. We also conducted a limited serological comparison using viruses from the BVDV1, BVDV2, BDV and CSFV genotypes (Fig. 3). Although viruses from the four genotypes appeared to be antigenically distinct, cross-reactivity, as measured by neutralization using polyclonal antisera, was observed between viruses from all four genotypes.

Discussion and Conclusions:

Viruses from three pestivirus genotypes (BVDV1, BVDV2 and BDV genotypes) were found in diagnostic samples from ruminants originating in the U.S. and Canada. Viruses from the BVDV1 and BVDV2 genotypes were isolated from both cattle and small ruminants. Viruses from the BDV genotype were only isolated from small ruminants. Viruses from the BDV genotype were antigenically distinct from viruses belonging to the BVDV1 and BVDV2 genotypes.

These results suggest that viruses from the BVDV1 and BVDV2 genotypes infect and cause disease in both cattle and small ruminants. In contrast, viruses from the BDV genotype predominantly infect small ruminants and do not appear to be a significant pathogen of cattle.

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References:

Comments:

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