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Genetic Characterization of a New U.S. Bovine Rotavirus Isolate

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Abstract
We have identified a new group A rotavirus associated with diarrheic calves in the field. The VP7 gene of this virus (designated VMRI-29), appears to differ genetically from that of the reference strain NCDV-Lincoln. Studies are underway to determine the importance of this genetic variant in the etiology of rotavirus-induced calf diarrhea.

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Genetic Characterization of a New U.S. Bovine Rotavirus Isolate

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Summary

We have identified a new group A rotavirus associated with diarrheic calves in the field. The VP7 gene of this virus (designated VMRI-29), appears to differ genetically from that of the reference strain NCDV-Lincoln. Studies are underway to determine the importance of this genetic variant in the etiology of rotavirus-induced calf diarrhea.

Introduction

Group A rotaviruses play an important role in the etiology of neonatal calf diarrhea. A vaccine is currently available, but there are reports of vaccination failure. Two surface proteins of the virus VP4 (P-type) and VP7 (G-type) play an important role in eliciting the immune response to rotaviral infection. Previous studies in our laboratory and by other workers indicated that the P type of the vaccine virus differs from that of the field isolates and might be responsible for vaccination failure. However, the VP7 of the majority of the field isolates is similar to that of vaccine strain (G6 type). Recently, we have identified a rotavirus isolate designated VMRI-29 in which the VP7 gene appears to be a variant of the G-type 6 viruses.

Materials and Methods

Fecal samples were obtained from diarrheic calves from different herds. VMRI-29 was isolated from a three-week-old diarrheic calf in Michigan. The herd has a history of sporadic outbreaks of diarrhea and the calves are routinely vaccinated at birth with a modified live vaccine. Rotavirus RNA was extracted directly from fecal sample using a glass matrix method (RNaid, Bio 101, La Jolla, CA). Complementary DNA synthesis and polymerase chain reaction were performed according to standard procedures using rotavirus VP7 gene-specific oligonucleotide primers. The VP7 gene was sequenced using an automated fluorescent sequencer (Applied Biosystems, Foster City, CA) and sequence analysis was performed using GeneWorks® software (Intelligenetics, Mountain View, CA).

Results and Discussion

The VP7 genes of the field isolates of G6 type usually share a homology of more than 95% at the nucleotide level and 96% at the amino acid level. However, the VMRI-29 VP7 gene had only 81% nucleotide homology and 90% amino acid identity with that of the NCDV-Lincoln strain. Amino acid sequence analysis also revealed that several amino acids were substituted in the antigenically important regions in the VP7 of VMRI-29. We also found amino acid substitutions in the regions of VP7 protein that are thought to be important in cell-mediated immune response. The putative glycosylation sites, which might play an important role in the antigenicity of a protein, were also different between the VP7 proteins of VMRI-29 isolate and NCDV-Lincoln strain. Phylogenetic trees constructed from the amino acid sequences indicated that the VP7 sequence of VMRI-29 was more closely related to that of KN-4, a Japanese bovine rotavirus of G6 type. We do not completely understand the mechanisms which are responsible for the evolution of the this type of virus variant. One hypothesis is that widespread vaccination in the field might have lead to the selection of genetically different strains. Another hypothesis is that VMRI-29-like isolates might play an insignificant role in the epidemiology of calf diarrhea. We have developed strategies based on restriction endonuclease analysis to differentiate the VP7 genes from these different isolates. This will allow us carry out epidemiological studies and determine if VMRI-29 like isolates are widespread in the field.

Implications

Our study indicates variability in the VP7 genes of the G6 type rotaviruses. This might influence future strategies for control of rotavirus diarrhea through vaccination. Further studies are underway in our lab to understand rotavirus epidemiology.

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