Detection of bovine rotavirus C and study on its genetic properties

Summary of Doctoral Thesis

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Rotaviruses (RV), members of genus Rotavirus, family Reoviridae, are a major causative pathogenic microorganism of gastroenteritis such as diarrhea and vomiting in a wide range of animals including humans, cattle, pigs and birds. The Rotavirus genome consists of 11 double-stranded RNA segments encoding six viral proteins (VP1-VP4, VP6 and VP7) and five or six nonstructural proteins (NSP1-NSP5 / 6). Also, because it is a segmented genome, recombination of RNA segments (reassortment) occasionally occurs with mixed infection of different strains. RV are currently divided into ten species, commonly termed groups A-J, on the basis of their distinct antigenicity and genetic characterization of the major capsid protein, VP6. Rotavirus A (RVA), a conventional and general RV, are most frequently detected and clinically important in humans and animals. Rotavirus C (RVC) was first reported in the United States in 1980 as a causal virus of diarrhea in mammal pigs, since then, outbreaks and sporadic cases of diarrhea have been reported in humans, cows, pigs, dogs and ferrets. Over the world, the first detection of RVC from cattle is a Shintoku strain isolated from diarrhea of adult cattle in Hokkaido in 1991. And that, it was the only evidence to suggest that bovine is the natural host of RVC. Therefore, in the taxonomically of viruses, bovine was generally not included in the natural host of RVC. Therefore, the generation status of diarrhea caused by bovine RVC in the field and genetic characterization of the virus have been hardly clarified.

From the above background, in order to clarify the generation status of diarrhea caused by bovine RVC in the field and the genetic characterization of the virus, the author conducted detection and genetic analysis of bovine RVCs from bovine diarrhea cases occurring in Yamagata Prefecture.

In the first chapter, in April 2002, an outbreak of diarrhea in dairy cattle

occurred on a combined management farm of a dairy and beef cattle in Yamagata prefecture, and as a result of investigating the cause of diarrhea, only RVC was detected from diarrhea feces, and on the other hand no other major viruses, bacteria, protozoa causing diarrhea were detected. Furthermore, from diarrhea feces that detected RVC, RV-like particles were observed by electronic microscope, while other virus particles were not observed. In addition, the typical 4-3-2-2 pattern of RVC was confirmed by RNA polyacrylamide gel electrophoresis (RNA-PAGE), and it was very similar to that pattern of RVC Shintoku strain. From these results, it was revealed that RV confirmed in diarrhea feces were RVC, which named Yamagata strain. As a result of the homology analysis of the VP6 and VP7 genes, the Yamagata strain had much higher homology with the Shintoku strain than the porcine and human derived strains. In addition, as a result of molecular phylogenetic analysis of VP6 gene, Shintoku and Yamagata strain belonged to the same cluster and clearly distinguished from clusters formed by human and porcine strains. These results supported that RVC is involved in diarrhea of adult cattle and bovine is a natural host of RVC. Consequently, Shintoku and Yamagata strains were proposed to be called bovine RVC.

Since then, the presence of bovine RVC was recognized, and in addition, as multiplex RT-PCR for the purpose of detecting major diarrhea-related viruses including bovine RVCs was developed, diarrhea cases on bovine RVC were reported one after another. And, the occurrence state of bovine RVC in the field has been gradually elucidated, but it is not clear whether age and breed are involved in diarrhea caused by bovine RVC. In Chapter 2, in order to elucidate the occurrence state of bovine RVCs in the field, the author tried the detection of major diarrhea-related pathogenic microorganisms from the cases of diarrhea

occurred on farms in Yamagata Prefecture during the decade from 2002 to 2011, and in addition, the author organized the obtained information such as the occurrence situation and clinical symptoms. As a result, bovine RVC was detected in 7 cases (5.7%) from only dairy cattle, and on the other hand not from beef cattle, dairy and meat calves. In addition, the occurrence situation and the clinical symptoms of bovine RVC disease can be summarized four common characteristics as follows: ①the point of causing outbreaks, ②the point that occur from autumn to early spring, ③diarrhea feces show brownish wateriness and ④the points of reduction of milk production. Therefore, it was suggested that bovine RVC have the above four common characteristics and are involved in diarrhea of dairy cattle. Also, as an interesting point, the detection frequency of bovine RV including bovine RVA, bovine RVB and bovine RVC in dairy cattle diarrhea accounted for 25 cases (20.3%), and this detection frequency occupied the next largest proportion to 73 cases of BCoV (59.3%). The above four characteristics were also observed with respect to the occurrence situation and the clinical symptoms of bovine RV disease. Therefore, it was suggested that bovine RV also have the above four characteristics and are involved in diarrhea of dairy cattle occupying a larger proportion than previously thought. While having the above four characteristics even in BCoV disease, although it is not necessarily seen, slight differences, which have clinical symptoms such as hematochezia and nasal discharge, are observed. Among the seven cases in which bovine RVC was detected, diarrhea of dairy cattle on a farm in two years repeatedly occurred three times which was detected different pathogens (bovine RVB, BCoV and bovine RVC), respectively. As a common point of these three times of diarrhea, the above four characteristics was observed. On the other hand, when BCoV was detected, there were also

differences as follows: ① the points of hematochezia, ② the points of nasal discharge, ③ the percentage of milk yield reduction, ④ the period of diarrhea, ⑤ the period of onset of diarrhea. These differences may help clinically distinguish bovine RVB disease and bovine RVC disease from BCoV disease. However, since there are many commonalities, it is necessary to differentiate causative pathogens by laboratory tests when diarrhea occurs.

The occurrence state of bovine RVC in the field has been gradually elucidated, but genetic properties of bovine RVC have not been hardly clarified because of little genetic information. Therefore, in Chapter 3, the author analyzed whole genome of RVC 6 strain detected in Chapter 2, and discussed the genetic diversity, genetic dynamics and ecology of bovine RVC in the field. As a result of homology analysis, molecular phylogenetic analysis and genotyping, the RVC 6 strain was found to be bovine RVC, furthermore it was also revealed that all gene segments have not been caused reassortant by strains derived from other animal species. And the RVC 6 strain were named Y / 03, Y / 1/04, Y / 2/04, Y / 3/04, Y / 08 and Y / 10. In addition, it was clarified that all 11 segments of bovine RVC strain, including previously reported strain, belong to one genotype. In compliance with the classification method proposed by RCWG, the genotypes of bovine RVC strain were classified as G2 - P [3] - I3 - R3 - C3 - M4 - A3 - N3 - T3 - E3 (G3), in response to VP7 - BP4 - VP6 - VP1 - VP2 - VP3 - NSP1 - NSP2 - NSP3 - NSP4 - NSP5 - H3. From this, it was suggested that circulation of infection have been basically formed in cattle. As a point of interest, VP4 gene could be clearly divided into two clusters within the genotype. These two clusters showed homology of 83.7-84.8% at the nucleotide sequence level and 88.9-89.9% at the amino acid level, and Y / 1/04 strain and Y / 2/04 strain belonging to one cluster

had one amino acid deletion and three amino acid insertions, which differed to the remaining strain belonging to the other cluster. For this reason, the author has provisionally defined these two clusters as lineage I and II. As described above, although it cannot be said as rearrangement in the VP4 gene, insertion and deficiency were observed, and diversity of bovine RVC was first confirmed. In addition, each segment of the bovine RVC strains are not divided with the same tendency according to the detected areas or years, and it seems that its each segment is randomly divided into plural lineages independently. From the above, it has been shown that strains belonging to the same genotype have been acquired genetic diversity by repeating reassortants independently for each segment. Therefore, it has become clear that several strains with different genetic backgrounds are widely distributed in Japan and are involved in bovine diarrhea. The 6 cases detected of bovine RVC have been organized about the obtained information such as the occurrence situation and clinical symptoms at Chapter 2. As an interesting point among them, it is confirmed the first time that diarrhea outbreak repeatedly occurred on epidemiologically related neighboring farms and reoccurred on the same farm 4 years later. By analyzing these cases, it was confirmed for the first time at the genetic level that the same strain or new strain invaded the farm. As a result, a part of the ecology in bovine RVC has become clear.

Thus, in this study, the author supported that RVC is involved in diarrhea of adult cattle and bovine is a natural host of RVC. Consequently, Shintoku and Yamagata strains were proposed to be called bovine RVC. The author also organized the occurrence state of the diarrhea caused by bovine RVC in the field using the diarrhea cases occurred on a farm in Yamagata prefecture during the

decade. Furthermore, the author analyzed whole genome of RVC 6 strain, and discussed the genetic diversity, genetic dynamics and ecology of bovine RVC in the field. In conclusion, this study provides useful information for the control of bovine RVC disease.