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Minireview

Small round structured viruses (SRSVs) and transmission electron microscopy

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HISTORICAL BACKGROUND

Every winter from November through March/April, the outbreaks and/or sporadic cases of sudden vomiting at the initial stage and developed diarrhea of infants accompanied by watery diarrhea often occur in some area, schools and other facilities. These occurrence have been paid attention by many researchers in the world. In 1929 Zahorsky proposed “winter vomiting” for these syndromes based on his over 30 years of clinical observation. After this proposal, medical terms such as “diarrhea of infants”, “vomiting and diarrhea”, “winter diarrhea”, “epidemic diarrhea and vomiting”, “epidemic diarrhea” based on clinical syndrome of non-bacterial gastroenteritis were used for similar features of diseases (Zahorsky, 1929).

In October 1968, outbreak of winter vomiting occurred in an elementary school, Norwalk, Ohio, USA. More than half of the school children and teachers were affected with acute non-bacterial gastroenteritis. Secondary infections were reported 32% of the families of patients. A joint research program was conducted with three institutes, NIH, Medical Department of Maryland University and Walter-Reed Army Medical Center. They succeeded in developing typical syndromes with volunteers taking oral administration of filtrated suspension of feces taken from one of secondary infected patients. In 1972 Kapikian and coworkers detected virus particles of 27 nm in diameter using immune-electron microscopy (IEM) from patients’ feces. They reported this virus particle as the causative agent of winter vomiting outbreaks in Norwalk (Kapikian et al., 1972). This is the remarkable landmark study of non-bacterial gastroenteritis viruses, especially for small round structured viruses (SRSVs). After that, many viruses were identified with transmission electron microscopy. This technique allows direct detection of virus particles taken from patient’s feces. These virus particles could not be cultivated either in cells or experimental animals. Figure 1 shows representative non-bacterial gastroenteritis viruses; Human Rota A virus, Human Adeno virus, Caliciviruses (Norovirus, Sapovirus), Astrovirus, ECHO virus, and hepatitis A virus. Figure 2 shows categories for morphological and genealogical classification of SRSVs (Tajiri-Utagawa, 2002) which was first proposed by Caul et al. (1982).

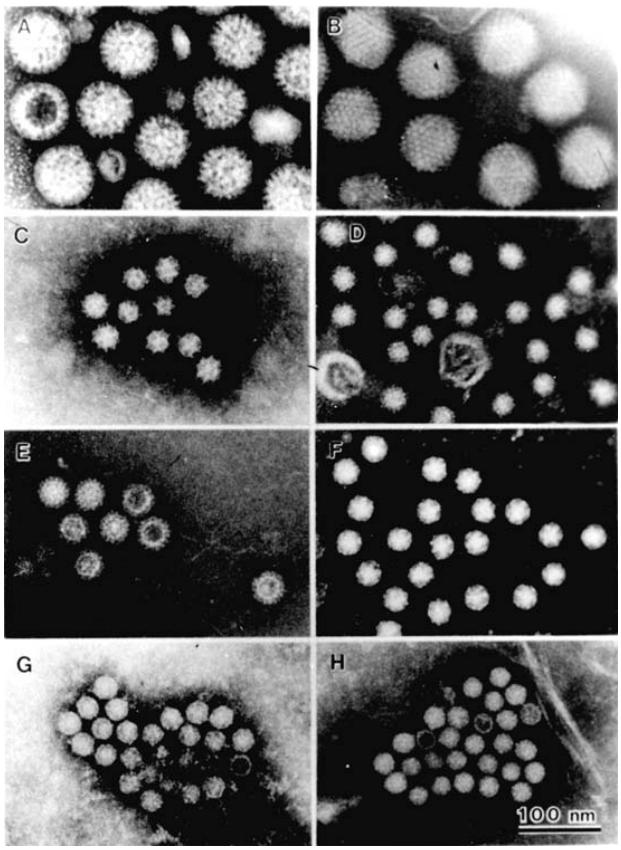

Figure 1. Representative Gastroenteritis Viruses. A, Human Rota virus; B, Human Adeno virus ; C, Sapovirus (Caliciviridae); D, Norovirus (Caliciviridae); E, Otofuke Virus (Norovirus group); F, Human Astrovirus; G, ECHO Virus; H, Hepatitis A virus. Bar represents 100 nm.

Morphology based on electron microscopy alone, however, often run into difficulties for a classification of viruses, especially for the SRSVs .

Up to the 1990s, about 20 years after the discovery of SRSVs using TEM, research did not make a rapid progress due to difficulty in cultivation of viruses. Jaing et al. (1992) reported on the Norwalk virus genome which opened a new page for the history of SRSVs research. Following this, gene analyses of various non-cultivatable SRSVs were actively performed throughout the world and a new classification of viruses depend on their gene organization was started. Both Norovirus (NV) and Sapovirus (SV) based on the gene organization classified in "*Caliciviridae*." On the other hand, Monroe et al. reported astroviruses belong to "*Astroviridae*". Based on the reports on many sequences, reverse transcriptase polymerase chain reaction (RT-PCR) method was developed using common primer pairs which was subsequently applied for the detection of viruses in patient's feces. Gene analysis of various viruses revealed genetic diversity of SRSVs. Since cultivated NV and SV are still not available for many practical method, ELISA, immunoelectron, western blot analysis, and other techniques has been difficult to make into commercially available reagents for diagnosis of patients and epidemiological studies. However, it has now become possible to make virus-like particles (VLPs) which are morphologically similar to that of native viruses. Animals immunized with these refined VLPs produced immuned sera against them. By using those VLPs antigen and anti-VLPs antibodies, antigen and/or antibody detection ELISA have been developed. However, there are still various kinds of viruses which lack information and

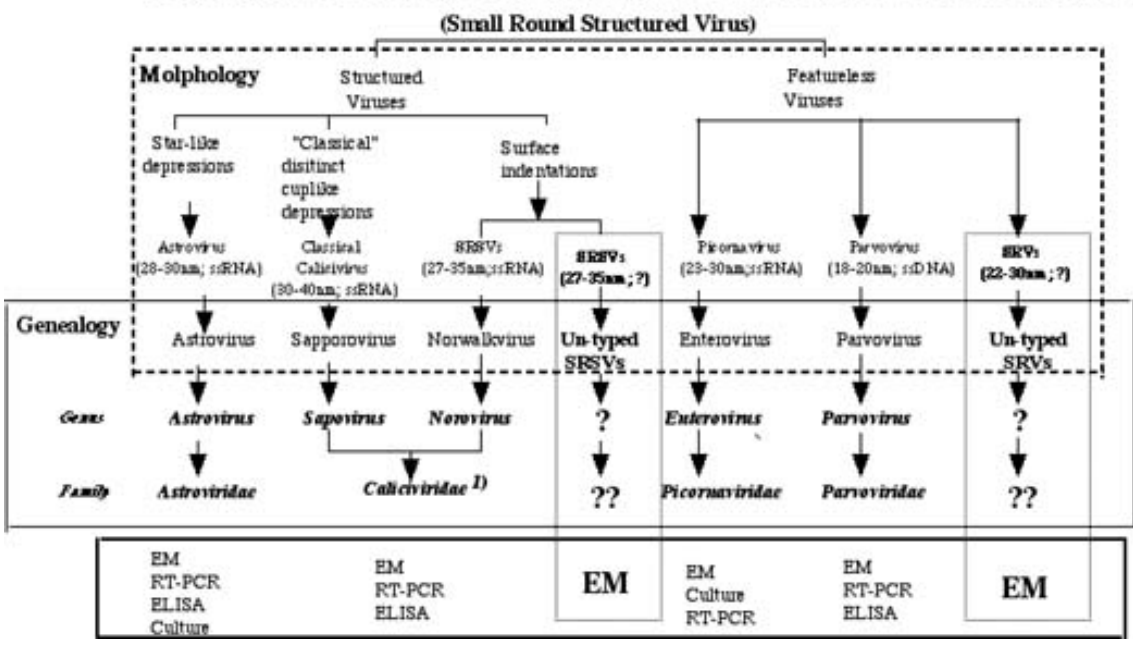

Figure 2. Morphological and Genealogical Classification of SRSVs (modified from Coul et al., 1982).

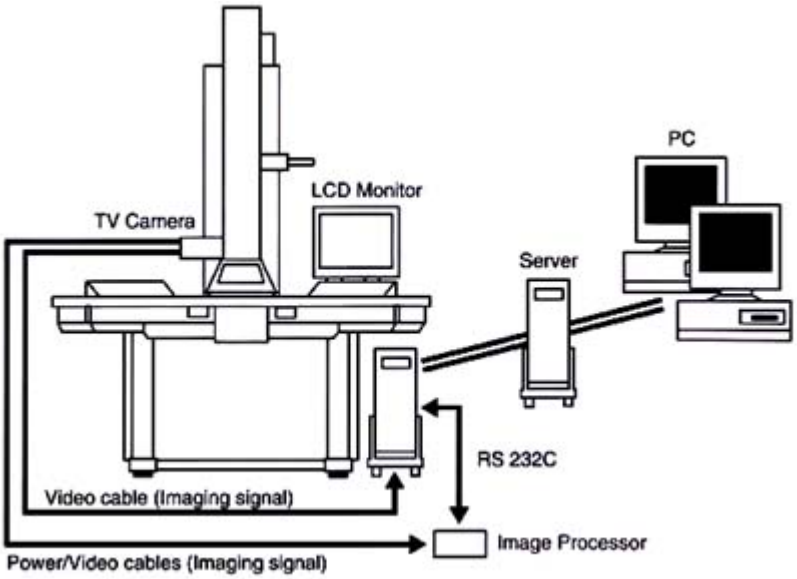

Figure 3. System configuration of specimen search function of ASS system in Hitachi H-7600 transmission electron microscope (TEM).

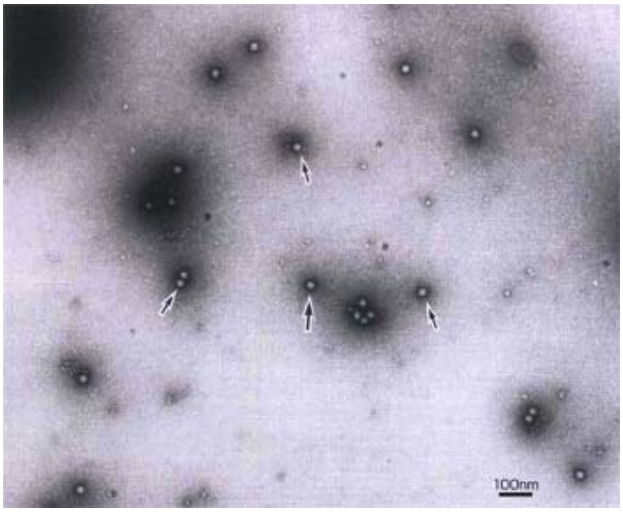

Figure 4. Negative stain micrograph of Norovirus particles Acc. Vol.: 100kV, Direct magnification: X 25,000.

cannot be identified using available primer pairs but detected directly using electron microscopes. Electron microscopy is still a major and indispensable inspection technique for SRSVs, which cannot still be cultured.

AUTOMATED INSPECTION OF SRSVS PARTICLES

For the detection of SRSVs in patient's feces, complicated specimen preparation is a mandatory requirement. Patient's feces need to be processed through an ultra-centrifugation treatment for concentration of viruses and elimination of all other materials. It is, however, difficult to eliminate all foreign materials leaving only viruses in the preparation process. These particles often prsent problems in the identification of SRSVs particles during microscopy. Therefore, experienced skills are still required on the part of microscope operations. Conventional TEM microscopy technique, therefore, are not convenient and useful for general inspection purpose. Some of these virus particles are detected only by morphological observation using TEM. This requires new electron microscopy software that can be used by beginners who do not have experienced skills in microscopy. In response to these urgent needs, we have developed an automated specimen search system (ASS system) for SRSVs inspection (Tajiri-Utagawa et al., 2002) which is mounted on the Hitachi H-7600 electron microscope operated on Windows® 95 (Figure 3). In this system, TEM images of inspection specimens are recorded using built-in TV camera. Particles are automatically detected from digitized images and input parameters such as diameters and roundness of particles of interest (Figures 4 and 5). The detected images are automatically stored in memory with their specimen positions (X,Y coordinates), operating magnifications, accelerating voltages and other TEM operating conditions into database. TEM images can be recorded using conventional silver-halide films as required. In addition to these functions, the system has an automated focusing as well as automated image recording functions. Once a field of interest is selected, the microscope takes care of focusing and recording automatically. The H-7600 is one of the most user-friendly microscopes available today for inexperienced operators.

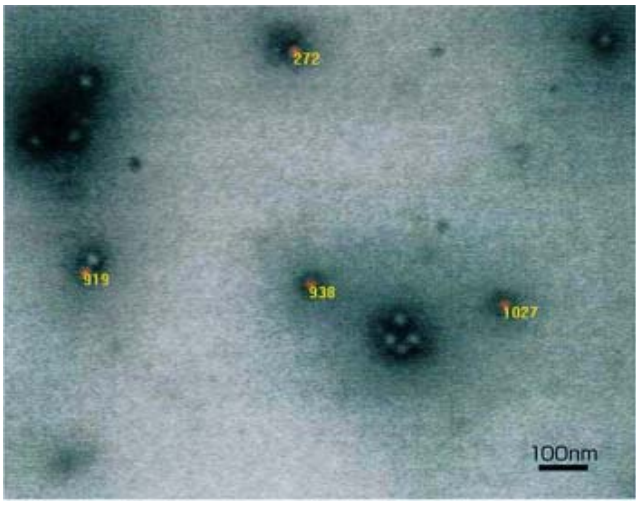

Figure 5. CG image of searched particles with ASS system of Hitachi H-7600. Acc. Vol.: 100kV, Direct magnification : x 25,000 bar represent 100 nm

FUTURE PROSPECTS TRANSMISSION ELECTRON MICROSCOPY

We have compared detection rates taken by using the above-mentioned ASS system and conventional electron microscopy. Both systems shows a good agreement of more than 95% for detection of a single SRSVs particle in a purified virus fraction. On the other hands a single particle in semi-purified sample matched less than 70%. By using electron microscopes with ASS system, it has become possible to automatically detect SRSVS particles that have been identified by operators based on their skills and experience. There is requirement for simultaneous detection of virus particles of various diameters and sizes such as rotavirus, adenovirus, SRSVs, as shown in Figure 1. At this present time, it is difficult to search virus particles of different diameter simultaneously and detect them individually. Sometimes, it is also difficult to detect virus particles that have specified parameter simply because of foreign particles or problems with staining. In the future, it will be necessary for us to study input parameters in addition to diameter and roundness of particles that have been used with the present system. It will also be necessary for us to develop better software that allows a comparison between an acquired image of identification of SRSVs without depending on operator skill and experience with electron microscopy.

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