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Detection of Adenovirus from Respiratory and Alimentary Tract in Pusan, 1999

Kyung-Soon Cho*† and Young-Hee Kim

**Pusan Institute of Health and Environment*
Dept of Microbiology, Dongeui University, Pusan, Korea

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Abstract Adenovirus which is an important infectious viral agent in respiratory and alimentary tract was investigated in Pusan, 1999. Fifteen cases of adenovirus were detected from stools and throat swabs of suspected patients. Two cases of enteric adenovirus were detected from a 5 years old boy and a 6-month-old boy. Thirteen cases of respiratory adenoviruses were detected from children aged under 10 years old and one adult. From respiratory specimens, 1 case of adenovirus type 2, 1 case of type 5, and 11 cases of type 3 were found. Enterotype 41 was detected from fecal preparations. Adenoviruses appeared mostly during winter months, January, February and December. Adenovirus showed a slowly progressive cytopathic effect on HEp-2 cells, Vero cells and BGM cells at 37℃, in a 5-7% CO_2 incubation. An electron microscopic observation exhibited non-enveloped icosahedron with a diameter of 70 nm. No significant differences on cytopathic effect and morphological features have been found from specimens of either alimentary tract or respiratory secretions.

Key words: adenovirus, serotype, cytopathic effect

Introduction

Adenovirus is a DNA virus which infects humans as well as every species of placental mammal, birds and amphibian [6,11]. Adenoviruses have been recognized as the second most important viruses associated with gastroenteritis in infants and young children, after rotaviruses [8]. In human, adenoviruses were established as the etiological cause of acute respiratory disease as well as diarrhea in alimentary tract, and the persistent infection of lymphatic tissue were also recognized at an early stage [1]. Adenoviruses are now also suggested to be important causal agents of myocarditis [1]. The adenovirus particle is stable to low pH, bile and proteolytic enzymes. For these reasons, adenoviruses can replicate to high titers in the intestinal tract [2,4,5].

In man 49 different serotypes were recognized up to 1999. A clinical isolate is identified as a serotype on the basis of distinct antigenic epitopes which are capable of inducing neutralizing antibodies. Subgenus F adenoviruses, serotype 40 and 41, have been described as enteric adenoviruses. They were observed in large amounts in diarrheal fecal samples and are prevalent in more than 50% of the adenovirus positive samples [3,10].

Recently increasing of new virus is threatening the health of humans and the occurrence of diverse serotype has been a problem. Until now the routes by which virus is spreading among human population are not known and proper application of virus eradication is not available [3,9]. It was assumed that the outcome of virus is mainly water transmission either directly or indirectly through contaminated food. Although vaccination has been applied in some viral infection, the multiplicity of antigenic types and the usually mild nature of the diseases makes the application of vaccine impractical.

Detection of infectious virus is strongly recommended in advance of the next pandemic and also in preventive surveillance system of viral infection. We attempted to detect adenovirus to provide a part of a nationwide distribution of this virus outbreak as well as to stimulate antigenicity diversity study from the virus appearing on this peninsula since current detection work is very limited [7,8].

We report the occurrence of adenoviruses to establish the role of adenoviruses in infantile gastroenteritis as well as in respiratory system from a survey conducted in the Metropolitan City of Pusan, 1999.

Materials and Methods

Collection of specimens

Specimens from suspected patients were provided from 10 designated hospitals in Pusan from January to December

†Corresponding author
Phone: 82-51-757-6936, Fax: 82-51-757-2879
E-mail: viruscho@chollian.net

in 1999. The samples were transported on ice, on the same day, to the laboratory. Throat swabs were kept in Minimum Essential Medium (MEM, Gibco. BRL. USA)) containing 10% bovine albumin.

Preparation of specimens

A portion of collected samples were processed immediately for several enteropathogen diagnostic procedures, and the rest was kept frozen at -20℃ for further tests. A sample of each stool specimen collected was diluted (1:10) in PBS buffer (NaCl 8.0g, KCl 0.2g, NaHPO4 1.15g, KH_2PO_4 0.2g, 800 ㎖ DDW, pH 7.2-7.4, $MgCl_2 \cdot 6H_2O$ 0.1 in 100 ㎖, $CaCl_2$ 0.1g in 100 ㎖), vortexing was followed and centrifugation at 1,000 $\times g$, 20 min. 1/10 of chloroform was added to the fecal supernatant and well mixed. Sample volume of 6-7 ㎖ was harvested and 0.1 ㎖ of 1X, 10X, 100X was used and the rest was frozen at -20℃. Throat swabs were autoclaved and squeezed with forceps, amphotericin B (0.25 ㎍/㎖) was added and the antibiotics [penicillin (5 units/㎖), stereptomysin (5 ㎍/㎖)] fungizone (5 ㎍/㎖)] were added and left for 1 hr at 4℃. The supernatants were inoculated in a volume of 0.1 ㎖ into 24-well plate containing monolayers of cells. The plates were maintained for 1 hr at 37℃ for viral adsorption and then added with 0.3-0.5 ㎖ of maintenance medium.

Propagation of cells

To isolate the virus, HEp-2 (Human epidermoid carcinomas) cells in DMEM (Dulbecco's modified Eagles medium), Vero (Africa green monkey) cells and BGM (Buffalo green monkey) cells in MEM (Minimum Essential Medium) were used. The cells were propagated in medium supplemented with 5% fetal serum. Monolayer was obtained by cultivation of the cells in a 24-well plate and 0.1 ㎖ of prepared stool specimens were inoculated whereas 0.3 ㎖ of throat swab preparations were inoculated. The inoculated cultures were incubated at 34℃, in a 5-7% CO_2 with medium changes at intervals of 3 or 4 days and examined daily for cytopathic effect (CPE) up to 10 days post-infection. Subsequent passages of at least three times were made from each virus by the same procedure. Positive specimens were subcultured for the elevation of activity for the next step. And final serotyping was performed at the Virus Division of National Institute of Health in Seoul.

Electron microscopy

Concentrated virus was used for electron microscopy. Negative stained with 4% uranyl acetate was examined under magnification of 120K with an electron microscope (JEM1200 EX 2, JEOL).

Results

Detection of virus

We observed 15 cases of adenoviruses as seen in Table 1.

Table 1. Detection of adenoviruses from Pusan, 1999

Month	Sex	Age(years old)	Sero Type	Specimens
1	F	46	2	Throat swab
1	F	5	3	Throat swab
1	M	3	3	Throat swab
1	F	4	3	Throat swab
2	F	5	3	Throat swab
2	F	4	3	Throat swab
2	F	5	3	Throat swab
2	M	6	3	Throat swab
2	M	5	3	Throat swab
2	M	1	3	Throat swab
2	F	6	3	Throat swab
12	M	8	5	Throat swab
12	M	5	3	Throat swab
12	M	5	entero 41	Stool
8	M	6 month	entero 41	Stool

F: Female, M: Male

Adenoviruses were detected during winter months, January, February and December except one entero adenovirus was found in August. Thirteen cases of respiratory adenoviruses were examined from 1,686 throat swab samples, and 2 entero adenoviruses from 575 fecal preparations. One out of thirteen respiratory adenoviruses was adult and the rest was children under 10 years old. One child including a six-month-old infant contained entero type adenovirus. The serotype obtained were 2, 3, 5 from throat swabs and 2 of entero 41 type from fecal specimens.

Cytopathic Effects

Positive cytopathic effect was observed after 72 hr of incubation. The typical positive appearance of cytopathic effect was shown on HEp-2 cell showing grape-like cluster. Susceptibility on three types of cells, HEp-2, Vero and BGM were shown in Fig. 1.

Microscopic observation

Two different isolation sources of virus showed a similar appearance with almost the same size with diameter of 70 nm, non-enveloped, icosahedron by an electron microscopic observation as seen in Fig. 2.

Discussion

In Korea, adenoviruses are one of the most prevalent infectious virus causing gastroenteritis as well as in respiratory infection in young children [4,7,8]. The role of adenoviruses was investigated as etiological agents in acute diarrhea as well as respiratory tract among suspected children during a survey in 1999 conducted in Pusan. The clinical and epidemiological characteristics of the children infected with enteric adenovirus were a 5-year-old boy and from a 6-month-old boy. Both cases were occurred in

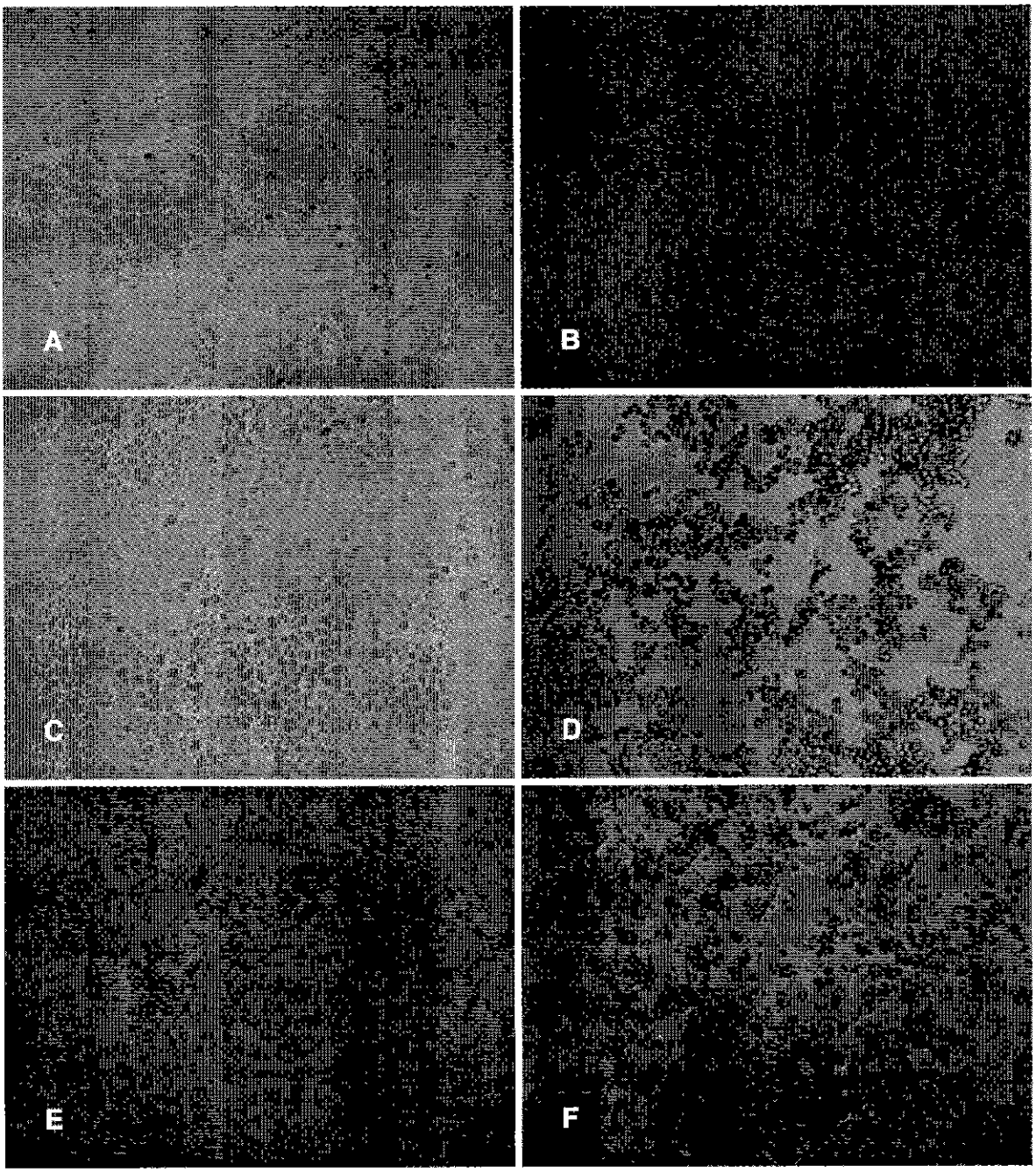

Fig. 1. Cytopathic effect by adenovirus on susceptible cells. A; Vero uninfected, B; CPE on Vero cells, C; HEp-2 uninfected, D; CPE on HEp-2 cell, E; BGM uninfected, F, CPE on BGM cell.

August and December. Twelve children aged from 1 to 8 years old including a six-month-old infant were infected with respiratory adenovirus. And a 46 years old lady was infected with the virus. Serotyping showed three different types from throat swabs and 2 of enterotype 41 were confirmed from the fecal specimens. From this result we can conclude that adenovirus is an important viral infectious agent in children. We can assume that nationwide surveillance will show a diversity in serotypes. Kim [8] also mentioned that adenovirus is a life threatening infection in children and their main symptoms were cough, fever, dyspnea, diarrhea and conjunctival injection. Careful attention with these symptoms will offer better samples of potency of virus.

Among adenoviruses, serotype 40/41 are the only enteric viruses and cause severe diarrhea while other types are

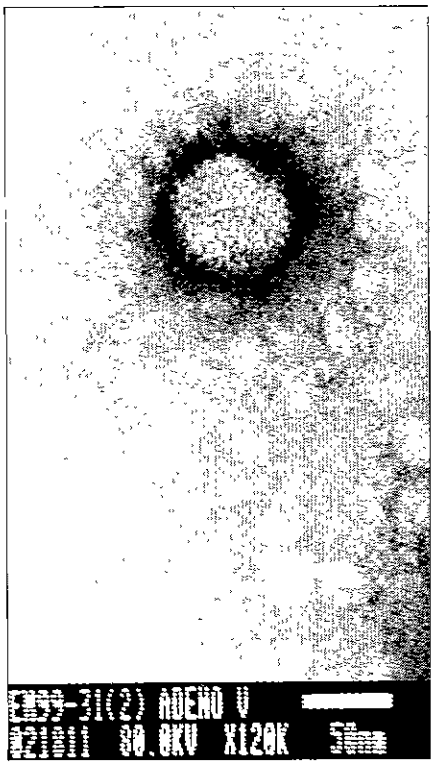

Fig. 2. Electron microscopic feature of adenovirus detected from feces of 7 years old boy. Bar represents 50 nm.

respiratory tract agents. And enteric adenoviruses (Ad 40/41) are the most common serotypes detected in children with diarrhea and this fact was consistent with our findings. From this study we confirmed a 5 year-old boy and a six-month-old boy containing enteric adenovirus 41. Until now 49 different human serotype of adenoviruses are known 31, 40 and 41 are the major agent of human gastroenteritis. Specifically serotype 40 and 41 are threatening infants and hygienes of these children are calling attention.

Virus spread is related to fecal-oral route, environmental source like water can be a suspicious factor in other country [9]. We could not find the exact source of virus transmission but our samples obtained were mainly from feces and throat swabs.

Recent methods for the identification of viruses by use of reverse-transcriptase (RT)PCR have also been applied and these methods are straight forward and can be of help when ambiguous results are obtained from serotyping methods [4,7]. In this study we adopted classical method of cell cultivation, immune fluorescence assay (IFA) test and electron microscopic observation to identify the virus. Three types of cells in HEp-2, Vero and BGM, the susceptibility was distinguishable and the isolated virus showed a typical appearance of adenovirus with non-enveloped, icosahedron, diameter of 70 nm. No significant differences on CPE or microscopic appearance were observed in comparison with both enteric and respiratory specimens.

Our detective studies showed that the occurrence of adenovirus is common. Therefore continuous infectious virus detection work has to be continued in advance of the next pandemic since viral genomic diversity has been approved [7]. And also our work will provide fundamental data for characterization of the diverse in viral antigenicity and geographic distribution which may support development in vaccine.

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