Epidemiological Observation of Coxsackieviruses Group B in Sewage

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Abstract

Different types of coxsackieviruses group B were isolated from the sewage consecutively annually from January 1982 to March 1987: type 3 in 1982, type 4 in 1983, type 2, 4 and 5 in 1984, type 3 in 1985, and type 4 in 1986 were found. These viruses caused large epidemics among children aged 1 to 4 and proximities. Herald waves for the epidemics were observed six times during this period, and the viruses were detected in a brief period of isolation.

With respect to the periodicity of these viruses, the cycles for types 3 and 4 were 2 to 3 years, and longer cycles seemed to be exhibited by types 1, 2, and 5. The onset and termination of the epidemics will only be grasped by surveys throughout the year. HEp-2 cells exhibited the best sensitivity to the viruses.

Introduction

Although the apparent infection by coxsackieviruses group B causes a wide variety of clinical pictures, the general trend is that the infection results in a subclinical course\cite{10}. Therefore, it is estimated that it would take an enormous expense and labor if we try to grasp the whole aspect of the viral epidemic: the present status of the epidemic, the onset to termination of the process, from the side of infected humans. However, since these viruses invade humans via the oral route, grow in the pharynx and intestinal lumen, and are finally excreted into the sewage together with the feces, routine monitoring of sewage will, to some extent, permit grasping the rise and fall of the process. It is true that there have been many articles reporting the epidemiological analysis of the isolated or detected coxsackieviruses group B, but, since these lack sufficient sampling frequencies and only short periods of study are adopted, no such epidemiological observation as is stated above has been reported. The present authors, in this context, attempted weekly sampling of sewage from January 1982 to March 1987. The present article intends to report some findings obtained from this study. With respect to the presence or absence of an epidemic, the authors conducted a seroepidemiological examination, and the results obtained will be developed at the same time.

Materials and Methods

Materials

One liter of sewage in a sewage treatment plant was collected by the grab method\cite{3}, and this was used as the test sample. The test sample was thus obtained every week, totaling to 237 samples. To add, the area covered by this sewage contained a population of approximately 100,000 habitants.

Treatment of samples

The sewage samples were centrifuged at 3000 rpm for 20 min at 4°C. The supernatants were concentrated about 100-fold by polyethylene glycol 6000 hydroextraction\cite{4-5}. The concentrated superna-
tants were centrifuged at 15000 rpm for 30 min at 4°C and were subjected to virus isolation after addition of penicillin and streptomycin to a final concentration of 1 mg/ml and 5 mg/ml, respectively. The sediments obtained were suspended in a 3% beef extract solution (10 ml, DIFCO), shaken vigorously for 10 min and allowed to stand at 4°C overnight. Then, the suspensions were centrifuged at 3000 rpm for 20 min and then, at 15000 rpm for 30 min at 4°C. The supernatants obtained were subjected to virus isolation after addition of penicillin and streptomycin.

**Isolation and identification of viruses**

The cells used for the viral isolation were HEp-2, FL and Vero cells (Dainippon Seiyaku Co.). These cells were grown and maintained in Eagle’s minimum essential medium (MEM/Nissui Seiyaku, Japan) with 10% or 2% fetal bovine serum (GIBCO).

Aliquots in amounts of 0.2 ml of the treated samples prepared as described above were inoculated onto monolayer cells prepared in culture tubes and incubated at 36°C for 2 h. After the inoculum was discarded, Eagle’s MEM containing 2% fetal bovine serum (1 ml) was added to each tube. Then, the tubes were incubated at 36°C and observed daily for viral cytopathic effect (CPE) for 8 days. A secondary passage was performed by inoculating new culture tubes with 0.2 ml of the culture fluid harvested 8 days after inoculation and CPE was also observed for 8 days. A third passage was carried out in the same manner as above. A suspension was considered to be virus free if there was no observable CPE in the culture tubes for not less than 24 days after the first inoculation. Cultures having shown CPE were usually passaged at least once to increase the infectivity titer before identification tests. The isolates were identified by the neutralization test employing immune serum pools prepared according to the modification of “intersecting serum scheme” of Schmidt et al\(^6\). These antisera were kindly supplied from the National Institute of Health of Japan.

**Neutralization test of sera against coxsackieviruses group B**

The incidence of antibodies against prototype strains of coxsackieviruses group B in the sera (1:4 dilutions) were examined by the neutralization test using HeLa cells: sera (1:4 dilutions), inactivated at 56°C for 30 min, were mixed with equal quantities of a virus suspension containing about 100 TCID\(_{50}\) per 0.1 ml. After incubation at 36°C for 2 hr, 0.2 ml of the mixture was inoculated into each of two HeLa cell culture tubes. The cultures were incubated at 36°C, and CPE was read and recorded 8 days postinoculation. Sera were collected at a hospital in this study area. The number of the serum samples collected were as follows: 259 during February-April 1983 (54/aged 0 to 1, 50/aged 1 to 4, 52/aged 5 to 9, 52/aged 10 to 14 and 51/aged 15 to 19), 246 during February-April 1984 (44/aged 0 to 1, 51/aged 1 to 4, 49/aged 5 to 9, 49/aged 10 to 14 and 53/aged 15 to 19), 253 during March-May 1985 (50/aged 0 to 1, 50/aged 1 to 4, 54/aged 5 to 9, 50/aged 10 to 14 and 49/aged 15 to 19), 263 during February-April 1986 (44/aged 0 to 1, 51/aged 1 to 4, 56/aged 5 to 9, 57/aged 10 to 14 and 55/aged 15 to 19) and 271 during February-April 1987 (30/aged 0 to 1, 53/aged 1 to 4, 65/aged 5 to 9, 66/aged 10 to 14 and 57/aged 15 to 19).

**Results**

**Virus isolation and seroepidemiological study**

The results of virus isolation from sewage are shown in Table 1. It is seen that, for the five years, the types of coxsackieviruses group B which were isolated successively for a long period changed from year to year: type 3 in 1982, type 4 in 1983, type 2, 4, and 5 in 1984, type 3 in 1985, and type 4 in 1986, respectively, suggesting that these viruses caused large epidemics. In addition, incidence of antibodies against the coxsackieviruses group B (Fig. 1) found in the serum samples collected at a hospital in this study area indicated that the types which caused large epidemics every year caused epidemics dominantly among children of 1 to 4 years of age.
| Type B1 | | Type B2 | | Type B3 | | Type B4 | | Type B5 |
|--------|--------|--------|--------|--------|--------|--------|--------|
| 1982   |    1  |      |      |      |      |      |      |      |      |      |      |      |
| 1983   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1984   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1985   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1986   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1987   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1982   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1983   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1984   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1985   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1986   |      |      |      |      |      |      |      |      |      |      |      |      |
| 1987   |      |      |      |      |      |      |      |      |      |      |      |      |

Table 1  The number of coxsackieviruses group B isolations from weekly sewage samples (Jan. 1982—Mar. 1987)
Herald wave for epidemic virus

Type 3 caused epidemic in 1982, type 4 in 1983, type 5 in 1984, type 3 in 1985, and type 4 in 1986 had been detected in a brief period in the preceding year or several months before the onset of each epidemic. Type 4 which caused the epidemic in 1984 was isolated for a long period in the preceding year. As stated above, the herald wave was observed six times from January 1982 to March 1987 (Table 1).

Onset and termination of epidemic

Most of the viruses causing epidemics had its onset in summer, and terminated in autumn to winter. However, there a few exceptions such as the type 3 which caused epidemics from October 1985 to April 1986. The duration of the epidemic was between five and seven months (Table 1).

Periodicity of epidemic virus

During the approximately five years of survey, types 2 and 5 presented their respective epidemic once, while type 3 caused epidemics twice and type 4 caused epidemics three times. From this, it was considered
that types 3 and 4 had comparatively short cycles of epidemics that continued for two to three years. While longer cycles were exhibited by types 1, 2, and 5 (Table 1).

**Sensitivity of cells**

The comparative data of isolated viruses from HEp-2, FL and Vero cells (Table 2) shows that HEp-2 cells yielded most isolation, which was followed by FL and vero cells in this order. Type 1 was best isolated with HEp-2 cells.

**Discussion**

The principal enteric viruses isolated from sewage include polio, echo, adeno and reoviruses as well as coxsackieviruses group B. We have been surveyed enteric viruses in water since 1982. In our survey polio, adeno and reoviruses were isolated from water almost similarly every year. On the other hand, the types of coxsackieviruses group B which were isolated successively for a long period changed from year to year, namely the types of coxsackieviruses group B which caused large epidemics were different every year (Table 1). From this, it was concluded that coxsackieviruses group B were non-resident in nature also in sewage. In addition, the routine monitoring, like that conducted in the present study, of sewage will help grasp the enteric viral epidemic. However, there are some viruses, like echoviruses type 9 and 30 plus adenovirus type 7, that are difficult to isolate from sewage. Thus it is desired that caution be taken with respect to this point in a survey where enteric viral epidemic is to be grasped.

Glezen et al. reported herald waves for the epidemic of influenza virus. The present authors also observed herald waves for group B coxsackieviral epidemic six times. This success in detecting the herald waves lay probably in weekly sampling of sewage which covered the area of a comparatively small population of habitants (approximately 100,000): the viruses isolated as a herald wave consisted only of several strains.

This means that, if less frequent sampling were conducted, no isolation would have been possible. On the other hand, population were larger, the species and types of viruses in the sewage would have been copious, which might bring about results that herald wave viruses showing rapid growth may be isolated while those showing slow growth may not.

Coxsackieviruses group B are thought to cause epidemics mainly in summer. The present survey also showed peak viral epidemics in summer. However, in some cases, the epidemic continued until the next spring. Therefore, survey throughout the year will be required if the termination of epidemic is to be identified.

Dahling et al. recommended BGM cells and Irving et al. Hela cells to isolate coxsackieviruses group B from water. The present study revealed that the highest isolation rate was attained with HEp-2 cells. Judging from this, the authors recommend HEp-2 cells for the isolation of coxsackieviruses group B.

**References**