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Investigation of Risk Factors Associated with a Bacillary Dysentery Outbreak in a Primary School in Taoyuan County

Introduction

Shigellosis is a common bacterial disease of the intestine characterized by watery diarrhea, and accompanied by fever, cramps and stools containing blood and mucus. The bacillus was isolated by Dr Shiga in 1898. The *Shigella* bacillus is divided into four main groups: *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*. It is believed that a neurotoxin, the Shiga toxin, produced by *Shigella* bacillus. is responsible for the serious symptoms such as the inflammation and secretion of mucus of the intestines [(1)].

Shigellosis is one of the notifiable diseases in Taiwan. The disease is transmitted through fecal-oral route from an infected person, and by eating contaminated food or drinking contaminated water [(2-9)]. Direct personal contact is also a major route of transmission. The disease is self-limited, and mild cases and inapparent infections are numerous. Serious infections will bring about high fever, stools with blood and diarrhea of different degrees. Even a small amount of 10-200 microbes can cause infection through personal contact. Infections occur more often in overcrowding places such as schools, nursing homes, home care centers, mental institutions, jails and military compounds [(10-12)]. The four groups of *Shigella bacillus* show different levels of toxicity, *S. dysenteriae* being the most toxic with a fatality as high as 25%. An epidemic occurred in Japan at one time ending in thousands of infections [(1)].

The Background

On 18 November 1995, the Taiwan Provincial Taoyuan Hospital reported a confirmed bacillary dysentery case of a primary school student hospitalized for treatment for high fever and diarrhea on 11 November 1995. On 22 November, the bacillus was verified by the National Institute of Preventive Medicine as *S. sonnei.* Soon after, specimens from the 12 students of this class for retarded children in a primary school in Pingtsen City of Taoyuan County and their families were collected by the

National Quarantine Service and the Taoyuan County Health Bureau. Specimens from home and school environments (washbasins, drinking fountains, toilets, tap water and underground water) were also collected. These places were disinfected with 10% Lysol solution and the No. 1 well with bleaching powder though not the No. 2 and 3 wells as they were sealed. The 12 classmates were found negative; and the father (69 years) and the sister (17 years) of the case were inapparent carriers. The second confirmed case of the same primary school was reported again by the Taiwan Provincial Taoyuan Hospital on 23 November. This student with high fever, abdominal pain and mild diarrhea was admitted to the pediatrics department for the emergency care of suspected appendicitis. After operation, perforating appendicitis and peritonitis were noted. The abdominal fluid culture later showed *S. sonnei* positive.

In the period between August and October of the year in the greater Taoyuan area, 14 confirmed cases had been reported from Chungli, Pingtsen, Kueishan, Lungkang and Yangmei. Four of them came from the same family and before the onset of the disease, all of them attended a wedding party in Chienshih township of Hsinchu County. The distribution of these 14 cases was relatively wide, they could have been sporadic individual cases. However, to find two cases in the same school in a month was rather unusual. The school uses, for a part of the water supply, underground water and also supplies school lunches. Of the two confirmed cases, one was from the high risk group of the retarded children. It was then decided that the National Quarantine Service together with the local health bureau give prophylactic medication to all students of the class for retarded children and general disinfection of the school environment. On 30 November, the National Quarantine Service and the local health bureau, upon visit to the school, noted that several students visited the school health center on the day for the treatment of vomiting, fever and diarrhea. The local health bureau had thus far referred 42 students to the Provincial Taoyuan Hospital for medical care, and this number increased to 144 by 1 December. They were all confirmed by the Provincial Taoyuan Hospital and the National Institute of Preventive Medicine to be *S. sonnei* infections. To understand the possible sources of infection of this outbreak, an epidemiological investigation was taken place on 3 December.

Materials and Method

1. Questionnaire Interviewing:

A structured questionnaire on risk factors was administered to all students of the school on 4 December. The questionnaire contained: background information of the student, time of onset, symptoms, medication, whether having school lunch during 25 November and 1 December (the week of outbreak), and risk factors such as the use of drinking fountains, hand washing, brushing teeth and/or rinsing mouth after meal with water from the washbasin, etc.

A shigellosis case was defined as: a student of the primary school since early

November 1995, and was laboratory-confirmed to be *S. sonnei* positive, or had one of the following conditions:

diarrhea (more than twice a day) and two of the following symptoms:

a. fever
b. abdominal pain, cramps
c. vomiting
d. nausea
e. weakness

2. Laboratory Testings:

Rectal swabs were collected from all students, teachers and employees (including cooks) of the school for laboratory testings by the Division of Bacteriology of the National Institute of Preventive Medicine. Specimens were first placed on either SS agar or DHL agar for culture; suspected colonies were then removed to TSI agar, LIM or SIM agar, or chistonsen citrate agar for the confirmation of *Shigella* bacillus. Serological testings were conducted to identify the group and serotypes of the bacillus.

Bauer-Kirby test for antibiotic sensitivity was also conducted. Paper tablets of antibiotics: cefuroxime, nalidixic acid, amikacin, chloramphenical, ceftriaxone, ampicihin, cephalothin, cortrimoxazole and oxacihine, were placed on the strains. The strains were decided as either susceptible or resistant to antibiotics from the size of the inhibition ring with reference to the NCCLS standards. This is a commonly used method for the testing of antibiotic sensitivity.

To detect any cross infection of amoebic dysentery, fresh stool specimens were collected from the patients in the hospital for laboratory testings with the formalin-ether sedimentation and direct smear methods.

3. Investigation of Environment and Testings of Environment Specimens:

The pipelines of the underground water, the distance between the well and the septic tank and toilet, the water tower and others were inspected. A plan of the school showing the cases was prepared to see any clustering of patients. Environment specimens such as waste water from the toilets and ditches, tap water, detergent for kitchen use, water on the floor of the kitchen, the drinking fountains, and underground water of the three wells were collected for the testings of coli form, *Escherichia coli* and *Shigella*. To understand whether the underground water was polluted by the septic tanks, on 7 December, 3 kg of the No. 6 Red was thrown into the septic tank of each of the two toilets near the well in the backyard and the toilet of a neighboring house. An additional 12 kg was thrown into them on 8 December. Specimens were collected each day for laboratory testings.

Results

1. The Questionnaire Interview:

2,796 copies of the questionnaire were distributed to the students from grade one through grade six; 2,408 copies were returned, giving a return rate of 86.1%. Of them, 1,226 were male (50.9%) and 1,180 female (49.0%). 715 students. 363 (50.8%) male and 352 (49.2%) female, met the criteria of a case, giving an attack rate of 29.7%. The attack rates by grade were: 23.1 % for the first grade, 30.6% for the second grade, 25.7% for the third grade, 48.9% for the fourth grade, 36.6% for the fifth grade, and 15.7% for the sixth grade (see Table 1). For spot map by grade, see Figure 1. Major symptoms were: fever (66.1%), diarrhea (72.8%), abdominal pain (54.3%), vomiting (48.6%), nausea (27.9%), weakness (40.7%) and tenesmus (18.8%) (Table 2). 505 of the 715 patients had sought for medical care, giving a medication rate of 69.7%. Of the 715 students who met the criteria of cases and others confirmed by the National Institute of Preventive Medicine as *Shigella* positive (totaling 887 cases), 172 did not turn in the questionnaires. They were reported to the National Quarantine Service by the Hospital through the disease surveillance system. Their time of onset was according to which reported through the disease surveillance system. Of the total, some failed to give the time of onset, giving 737 valid cases for the epidemiological curve as shown in Figure 2. The first date of onset was 1 November and the peak being around 1 December.

Table 1. Specimen Collection and Attack Rate by Grade

Grade	No.	No. of specimens Collected	Collection Rate (%)	No. Positive	Positive Rate (%)	No. of Questionnaire Returned	Return Rate (%)	No. Meeting Criteria of Case	Attack Rate (%)
I	443	395	89.2	44	11.1	368	83.1	85	23.1
II	443	354	79.9	76	21.5	346	78.1	106	30.6
III	397	350	88.2	51	14.6	342	86 1	88	25.7
IV	447	412	92.2	89	21.6	401	89 7	196	48.9
V	525	472	89.8	81	17.2	434	82 7	159	36.6
VI	541	279	51.6	23	8.2	517	95 6	81	15.7
Total	2,796	2,22	80.9	364	16.1	2,408	86 1	715	29.7

Table 2. Distribution of Symptoms

Symptoms	No. of Cases	%
Fever	479	66.1
Diarrhea	528	72.8
Abdominal pain	394	54.3
Vomiting	352	48.6
Nausea	202	27.9
Weakness	295	40.7
Tenesmus	136	18.8

Analysis of Risk Factors:

Possible risk factors such as the school lunch, tooth-brushing and mouth-rinsing with the underground water of the washbasins, use of the drinking fountains, and drinking water and washing hands from washbasins were analyzed by the single variate analysis method. For school lunch, with the exception of Monday, the other days of the week showed negative correlation (RR 1, $p<0.05$). Carrying drinking water to school had a significant protective effect against dysentery (RR=0.56, 95% CI: 0.46 - 0.67). Drinking of water either from washbasins or drinking fountains was found a risk factor significantly related to Shigellosis (RR=1.36, 95% CI: 1.09 - 1.70; RR=1.37, 95% CI: 1.05 - 1.37). Tooth-brushing and mouth-rising with water from washbasins were also found to be significantly related to the infection (RR= 1.79, 95% CI: 1.49 - 2.16). Hand-washing after toilet, however, was not related to the infection (RR= 1.43, 95% CI: 0.67 - 3.10) (Table 3). When multiple logistic regression was applied for further analysis, drinking water from washbasins and drinking fountains, tooth-brushing and mouth-rinsing with water from washbasins, and hand-washing after toilet were all found to be significantly related to the Shigellosis infection, whereas school lunch as a factor was not found to be so related (Table 4).

Dose-Response Effect:

As the use of underground water is one of the risk factors of the Shigellosis infection, an investigation into any likely dose-response effect was also made. The findings were that more of those who more frequently tooth-brush and mouth-rinse with underground water and more frequently hand-wash after toilet was infected. The χ^2 test was significant at $p<0.05$.

Fig1. Map of cases with shigellosis in the primary school in Taoyuan, Nov 30, 1995 (N=403)

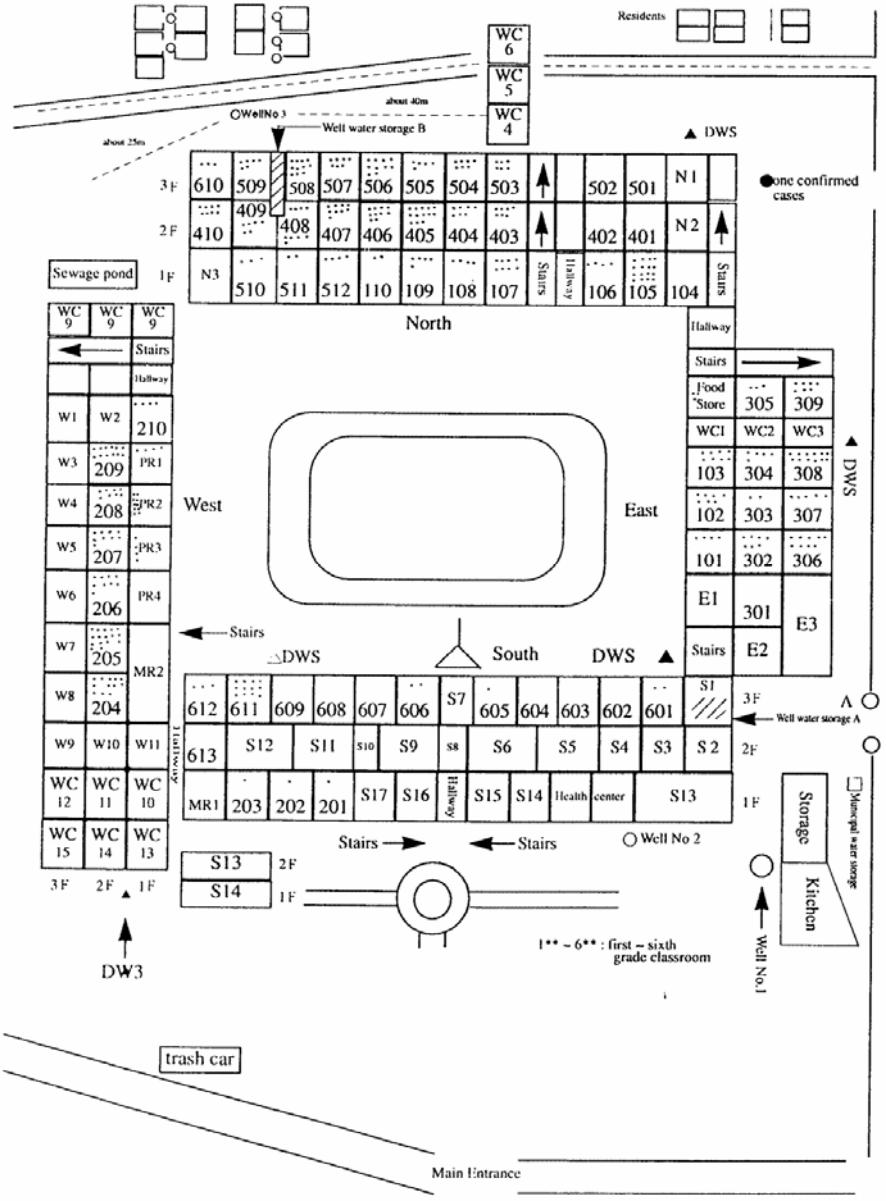
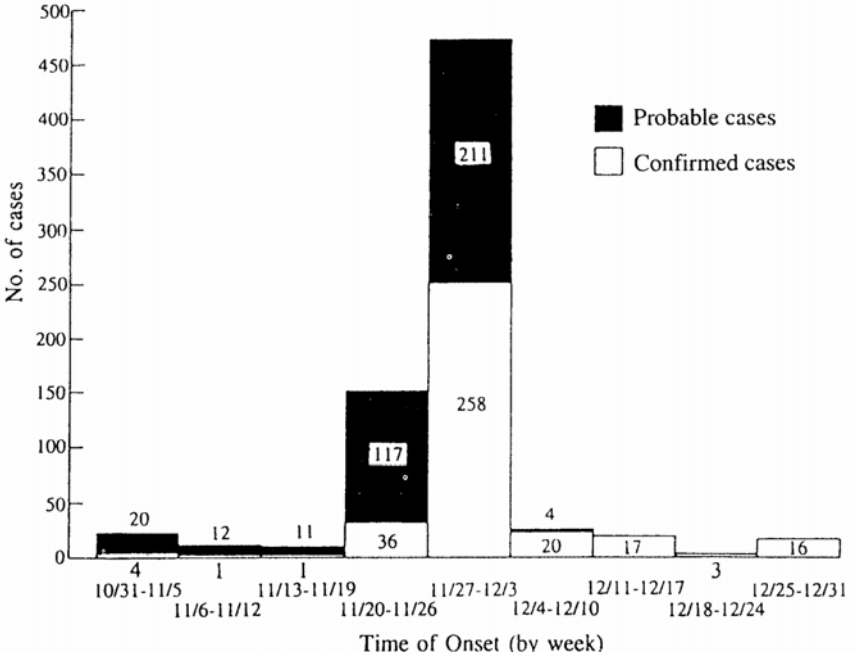


Figure 2. Epidemiological Curve of Confirmed Positive Cases of *Shigellosis* (N=737, November 1995-January 1996)



2. Findings of Laboratory Testings:

Of the 6,421 rectal swabs collected, 649 were found positive. Of the 237 strains sent by the Hospital, 228 were found positive. Less repetitions, the total number of confirmed Shigellosis positive cases was 404: 364 students of grades one through six (positive rate 16.1 %), 21 children of the kindergarten, 4 teachers and employees (3.5% of the 114 teachers and employees), and 15 family members.

Tests for amoebic dysentery did not detect any trophozoites or cysts of the protozoa.

Antibiotic sensitivity tests showed that the *S. sonnei* strain of this outbreak was resistant to ampicillin, cephalothin, cotrimoxazole and oxaciline; the diameters of their inhibition rings being 15mm, 12mm, 12mm and 12mm respectively. This strain was still sensitive to ceftriaxone, chloramphnicol, amikacin, nalidixic acid and cefuroxime; the diameters of their inhibition rings being 17mm, 24mm, 24mm, 24mm and 26mm respectively.

Table 3. Single Variate Analysis of Risk Factors (N=2,445)

Variables	Yes		No		RR	CI	p value
	Case	Not Case	Case	Not Case			
had school lunch:							
on 11/25	682	1,603	26	48	0.79	0.47-1.31	0.396
on 11/26	679	1,609	31	41	0.56	0.34-0 92	0.015*
on 11/27	675	1,602	35	47	0.57	0.35-0.91	0.011*
on 11/28	619	1,601	78	47	0.23	0.16-0.34	0.000*
on 11/29	535	1,550	144	88	0.21	0.16-0.28	0.000*
tooth-brushing using washbasin water	380	644	302	917	1.79	1.49-2.16	0.000*
use drinking fountain	533	1,029	150	394	1.36	1.09-1.70	0.005*
drink from washbasin	126	178	499	964	1.37	1.05-1.77	0.015*
bring own drinking water	304	849	340	529	0.56	0.46-0.67	0.000*
hand-wash after toilet	697	1,614	10	33	1.43	0.67-3.10	0.328

* significant at $p<0.05$

3. Survey of Environment and Testings of Environmental Specimens:

The primary school is located at Lungkang where Chungli City and Pingchen Township border. The environmental sanitation of the area is relatively poor. The school, with a history of 34 years, has a total enrollment of 2,880 students in 64 classes, three classes of kindergarten and two classes for retarded children; six workers in the bread workshop, four guards and two workers in the school cooperative. The school has an area of around 20,000 square meters. The toilets have been in use for more than 20 years.

Water sources are the tap water and three underground wells. Well water is used for toilets, washbasins and kitchen washing. The tap water is for cooking and drinking after boiling. Underground water is pumped up to the water towers for use. Water of tower A comes from wells 1 and 2; and tower B is supplied by well 3. The two towers are connected and water can come through freely. Towers have not been washed for years and are filled with dirt (see Figure 1 for the layout). The second and the third floors of the north wing where more cases clustered are supplied by tower B; the rest classrooms are supplied by towers A and B.

Table 4. Multivariate Analysis of Risk Factors (N=2,445)

	Coefficient	SD	OR	CI	p value
	−1.0636	0.3943			0.007
had school lunch:					
on Monday	0.8296	0.6491	2.292	8.17-0.64	0.2012
on Tuesday	0.5552	0.7909	1.742	8.21-0.37	0.4827
on Wednesday	0.3108	0.5552	1.365	4.05-0.46	0.5756
on Thursday	−1.1814	0.3696	0.307	0.63-0.15	0.0014*
on Friday	−1.6166	0.2168	0.199	0.35-0.30	0.0001*
tooth brushing using					
washbasin water	0.2692	0.0516	1.309	1.45-1.18	0.0001*
use drinking fountain	0.3615	0.1438	1.435	1.90-1.08	0.0120*
drink from washbasin	0.3856	0.1560	1.470	2.00-1.08	0.0134*
bring own water	−0.0890	0.1297	0.915	1.18-0.85	0.4926
hand-wash after toilet	0.3001	0.1023	1.350	1.65-1.10	0.0034*

SD: standard error; OR: odd ratio; CI: confidence interval
* $p<0.05$

Table 5. χ^2 Analysis of Tooth-Brushing/Mouth Rinsing with Underground Water and Bacillary Dysentery Infections (N=2,243)

	Never	Sometimes	Often	Always	M-H value	p value
Case	302	122	153	105	63.22	0.000*
Non-case	917	305	215	124		

* $p<0.05$

Only from well 3 was *S. sonnei* identified. *E coli* above the permissible amount ((60 MPN/100 mL) was found only in the boiled water in the kitchen. In washbasins in the east and south ends only, a total number of bacterial colonies above the permissible amount (100/mL) was found.

Table 6. χ^2 Analysis of Hand-Wash After Toilet and Bacillary Dysentery Infections (N=2243)

	Never	Sometimes	Often	Always	M-H value	p value
Case	10	22	85	590	4.735	0.0029*
Non-case	33	68	235	1,311		

* $p<0.05$

School lunch was supplied every day to the teachers and all students except the first and the second graders who were on half-day schedule. 11 workers worked in the kitchen. Plates were sterilized centrally before use. Food was placed on the plates and carried by students to classrooms. There were both tap water and underground water in the kitchen, and both were used for food preparation and utensil washing. Underground water was used for washbasins. Both the teachers and the students used the water that was boiled and pumped up to the tower for drinking.

Discussion

Worldwide but excluding mainland China, around 140 million children under five years of age are infected by Shigellosis each year, resulting in 576,000 deaths [(1)]. The incidence and prevalence of Shigellosis vary from place to place. A follow-up study of the newborns in Guatemala showed a 1.6% prevalence rate of the newborns in their first week; this rate increased with age, and reached 20% at the age of three [(1)]. The prevalence of Shigellosis is generally higher in the developing countries. Studies in Egypt give a prevalence rate of apparent infection of 101,200 person/times per 100,000 population per year; the prevalence rate of inapparent infections is at about the same level. In other words, the prevalence rate of Shigellosis in Egypt, apparent and inapparent, is 202,400 person/times per 100,000 population per year; or, on average, two infections per person per year [(13.14)]. In USA and other developed countries, Shigellosis is still one of the major causes of gastro-intestinal diarrhea. In 1987 alone, 16,567 cases were reported through disease surveillance systems to US CDC; of them, 67.7% were of *S. sonnei*, and then, *S. flexneri* [(15)].

The most common serotypes in Taiwan are *S. flexneri* (B *type*) and *S. sonnei* (D *type*). In the present outbreak, the agent was found to be *S. sonnei* (D *type*); the attack rate for students was 29.7% and that for teachers, 3.5%. The attack rate for students was significantly higher than that of teachers. This corresponds to findings of other surveys showing that attack rate of children (67%) is often higher than that of adults (27%)[(8-10)].In the current survey, 385 children (of both primary school and kindergarten)

were confirmed positive; of them, 321 had developed symptoms (83.4%), 48 (12.5%) without symptoms, and 16 (4.2%) not known of their time of onset. If these 16 were considered symptomless, the number of inapparent infections would be 64 (16.6%). The questionnaire interview identified 715 children who met the definition for a case. This was far more than the 385 confirmed positive. The likely reasons are: 1) to prevent further transmission, DOH immediately gave prophylaxes to all students at the initial stage of the outbreak; specimens of some students could have been collected after the medication and therefore gave negative reaction; 2) the culture used was the Cary-Blair media which is a common transportation medium and the survival rate of bacteria in it is moderate; however, specimen collection rate by rectal swab is often lower than by fresh stool, this could have resulted in some false negative reactions[17,18].

The epidemiological curve shows that the outbreak began in early November to reach two peaks on and around 22 November and 1 December, and particularly on 1 December. Cases could have discharged pathogenic agents to contaminate the underground water. The agents multiplied on the weekend to become susceptible on the following Monday when the students had contacts with the water. Infections occurred after an incubation period of 1-3 days in two peaks. This finding is supported by: 1) the identification of *S. sonnei* in the environmental specimens from well 3 water; 2) the findings from the questionnaire interview of risk factors that use of underground water being significantly related to infections and that school lunch having nothing to do with infections; and 3) the clustering of cases on the second and the third floors of the north wing with a higher attack rate as noted from the distribution of cases (Figure 1).

Resistance to antibiotics is a common problem encountered in the treatment of *Shigellosis* in both developing and developed countries. Clinicians often are left with no choice but to use expensive medicines of the third generation such as cephalosporins. In US alone, it has been found that the resistance of Shigella to ampicillin and SXT (sulfamethoxazole-trimethoprim) had increased from 5% in 1983 to 25% in 1986. Hence, in the US, nalidixic acid is used as the front-line medicine for the treatment of Shigellosis [1]. Resistance to medicine is produced by the plasmid in *Shigella*, which makes bacteria produce exotoxins and also serves as a medium of drug resistance. In the present survey, *S. sonnei*, as also found in other surveys, was found to be resistant to several antibiotics such as ampicillin, caphalothin, cotrimoxazole and oxaciline[1]. Three medicines, ampicillin, nalidixic acid, cefixime and ceftriaxone, were used for treatment. In the initial stage, ampicillin was used as the front-line medicine. It was replaced by nalidixic acid and cefixime in mid-December when drug resistance was noted. Some cases developed adverse reactions to nalidixic acid and cefixime. This could have been the difference in the antibiotics tests in vivo and in vitro, and they continued to show positive reactions upon follow-ups. Of the 404 positive cases, one was resistant to cefixime, ceftriaxone intravenous injection was applied instead.

In the control of an outbreak such as this, in addition to the problem of drug resistance of *Shigella* to various antibiotics, the compliance of students to medication

and the cooperation of the parents are also important. It was found that for either prophylaxes or medicines for treatment, students terminated medication soon after the disappearance of symptoms and became chronic carriers. They, therefore, showed positive in the follow-ups. The school itself was a reservoir, infection within school therefore
increased and some sporadic cases were found even after the peak of 1 December as seen in the epidemiological curve.

Recommendations

1. Tap water should be used for both drinking and cleaning. Underground water should only be used for toilet washing and gardening. Water towers should be cleaned regularly.
2. Worn-out toilets and septic tanks should be repaired.
3. Local health authorities should set up a disease control command center soon after the occurrence of a notifiable disease to handle the outbreak and to collect relevant information for disease control.
4. Coordination and support of agencies concerned should be sought for.
5. School, parents and persons concerned should be informed of the disease conditions and also involved in its prevention.
6. Surveillance systems should be strengthened. At time of outbreaks, local laboratories should be responsible for the laboratory testings. Task forces should be created to handle the vast amount of testings in good time.

Acknowledgement

The size of infection, the duration of infection and the manpower involved in the control program in the present outbreak were comparable to those of the outbreak in 1993 in Taichung. Thanks are due to the help of the staffs of the health bureau and the health station, to Mr. H.C. Lin, to the Taiwan Provincial Government, and particularly to Governor Soong who inspected the site personally and instructed the school to improve water supply. The laboratory testings by the National Institute of Preventive Medicine and the disinfection of environment and the collection of specimens by the National Quarantine Service are appreciated.

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