JOURNAL OF CLINICAL MICROBIOLOGY, July 2003, p. 3078–3088 Vol. 41, No. 7
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.7.3078–3088.2003

Molecular Epidemiology of *Shigella* in a Taiwan Township during 1996 to 2000

Jiann-Hwa Chen,[1]* Chien-Shun Chiou,[2] Pei-Chun Chen,[1]† Tung-Liang Liao,[2] Tsai-Ling Liao,[2] Jen-Ming Li,[1]‡ and Wen-Bin Hsu[1]

Institute of Molecular Biology, National Chung Hsing University,[1] and The Third Branch Office, Center for Disease Control, Department of Health,[2] Taichung, Taiwan 402, Republic of China

Received 9 October 2002/Returned for modification 8 April 2003/Accepted 1 May 2003

A previously identified *Shigella flexneri* serotype 2a strain was responsible for an outbreak of shigellosis in a Taiwan township in August 1996. In order to find the relationship between this outbreak strain and subsequent *Shigella* infections in the area, 59, 47, 35, and 20 *Shigella* isolates recovered in 1997, 1998, 1999, and 2000, respectively, were collected and typed by serological and pulsed-field gel electrophoresis (PFGE) techniques. Of these 161 isolates, 139 isolates were *S. flexneri* serotype 2a, and one-third of them (47 isolates) exhibited the outbreak pattern. The remaining 92 *S. flexneri* serotype 2a isolates displayed 49 different *Not*I-PFGE patterns. Forty-five patterns were closely related to the outbreak pattern, with deletions of three specific *Not*I fragments occurring with high frequency. While the outbreak strain remained the main cause of shigellosis after the outbreak, the continuous emergence of closely related though poorly transmissible strains from the outbreak strain contributed to the observed annual decrease of shigellosis in the area.

Shigellosis is an acute gastroenteritis caused by *Shigella* species, including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*. It is one of the major causes of diarrheal diseases worldwide. In Taiwan, about 250 to 500 cases of shigellosis were reported annually from 1995 to 2000, with an average annual incidence rate of 1 to 3 cases per 100,000 persons (4). Infections are usually caused by *S. flexneri* and *S. sonnei*, with the former mostly found in mountainous central Taiwan and the latter found in western industrial Taiwan. Infections caused by *S. dysenteriae* and *S. boydii* are rare in Taiwan and are observed only in imported cases.

An outbreak of shigellosis occurred in the Renai Township in central Taiwan in August 1996. The outbreak lasted 3 months, and 10 villages from the township were affected. During the outbreak, 37 *Shigella* isolates were collected from Renai and neighboring townships, and all were shown to be *S. flexneri* serotype 2a. By genotyping with *Not*I- and *Xba*I-pulsed-field gel electrophoresis (PFGE), we further demonstrated that this serotype was endemic in the area and that a single strain with a distinct PFGE pattern and a person-to-person mode of transmission was responsible for the outbreak (2).

After the outbreak, however, the number of shigellosis cases still occurring in the Renai Township was considered high. The purpose of this study is to understand whether the high incidence had any relation to the outbreak in 1996 and, in particular, to the outbreak strain. We collected 161 *Shigella* isolates that had been recovered from Renai and neighboring townships from 1997 to 2000 and typed them by serological and PFGE methods. Since *Not*I-PFGE could differentiate almost all the strains that were previously differentiated by using *Not*I- and *Xba*I-PFGE with the exception of isolate SH4799 (2), we chose to use only *Not*I-PFGE for this study. While the results indicate that the outbreak strain continued to be the main strain causing shigellosis during the 4-year period, the percentage of outbreak strain isolates among *S. flexneri* serotype 2a isolates recovered in the year decreased annually. Many closely related strains emerged during this period but were shown to not transmit as well as the outbreak strain.

MATERIALS AND METHODS

Bacterial isolates. During 1997 through to 2000, stool specimens from patients with either diarrhea or dysentery (bloody diarrhea) in Nantou County were collected and screened for *Shigella* species by conventional biochemical methods (17) in two local hospitals (Puli Christian Hospital and Puli Veteran Hospital) and our laboratories. *Shigella* species recovered were serotyped by a slide agglutination test with commercial polyclonal antiserum (Denka Seiken Co. Ltd., Tokyo, Japan). The isolates were cultured in Luria broth and were stored in 15% glycerol at −70°C. *Shigella* isolates recovered from Renai and neighboring townships (Fig. 1) were used in the present study. Some of the isolates recovered in 1996 were used as reference strains. Characteristics of *S. flexneri* serotype 2a isolates from 1996 to 2000 are described in Table 1, whereas those of non-*S. flexneri* serotype 2a isolates will be provided upon request. These non-*S. flexneri* serotype 2a isolates were *S. sonnei* (SH7150, SH7156, SH8255, SH8289, SH8542, SH9397, SH10657, SH11620, SH14974), *S. boydii* (SH7571); *S. flexneri* serotypes 1a (SH18504), 1b (SH19453, SH19455, SH19700), 3a (SH20928, SH20904, SH20907), 3b (SH5619, SH5621), y (SH7396); and nontypeable *S. flexneri* (SH16934, SH19406).

Epidemiological data. Epidemiological data from the patients were obtained from standardized case report forms filled in by the county public health authorities. The reports included basic patient information, such as date of onset, sex, age, residency, symptoms, medical treatment, and travel history.

PFGE of bacterial isolates. Genomic DNA of *Shigella* species recovered from 1997 to 2000 were digested with *Not*I and were analyzed by PFGE, as in our previous study with the 1996 isolates (2). Yeast chromosomal DNA (New England BioLabs, Inc., Beverly, Mass.) was used as a size standard. DNA bands on the gel were visualized by ethidium bromide staining and UV and were photographed.

PFGE pattern analysis. PFGE patterns were analyzed by visual inspection of the photographs of the strained gels. An index isolate was included in each

* Corresponding author. Mailing address: Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan 402, Republic of China. Phone: 886-04-22851885. Fax: 886-04-22874879. E-mail: jhchen@dragon.nchu.edu.tw.

† Present address: The Reproduction Laboratory, Changhua Christian Hospital, Changhua, Taiwan, Republic of China.

‡ Present address: Graduate School, National Defense Medical Center, Taipei, Taiwan, Republic of China.

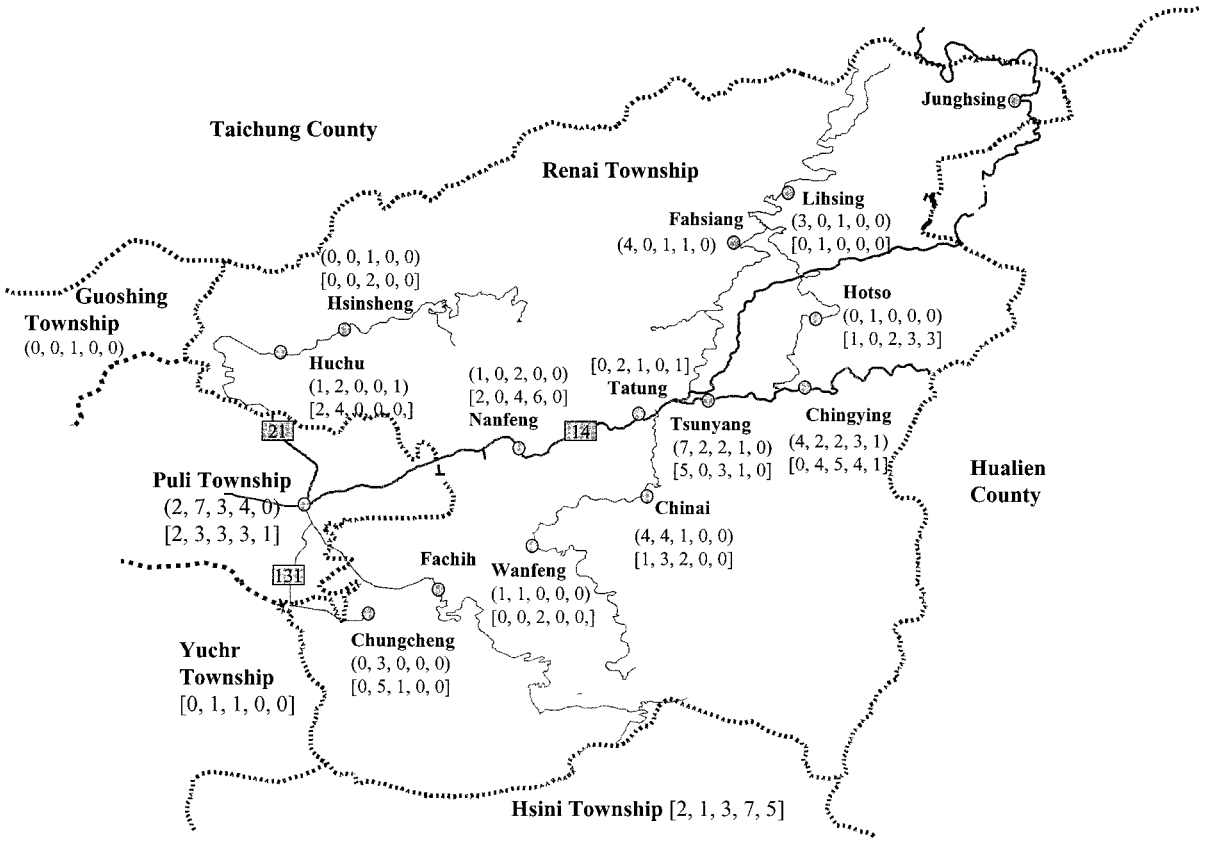


FIG. 1. Distribution of isolates of the outbreak strain and closely related strains. Numbers of isolates of the outbreak strain recovered in a specific village or township in 1996, 1997, 1998, 1999, and 2000 are indicated from left to right in parentheses next to the village or township, whereas numbers of isolates of the closely related strains are indicated in brackets.

photograph for comparison of isolates in separate photographs. In our previous study we identified a single strain with a distinct *Not*I-PFGE pattern as the cause of the 1996 outbreak (2). Since isolates with the outbreak pattern were still the most prevalent among isolates recovered from 1997 to 2000, the classification described by Tenover et al. (21) was also used for interpretation of isolates recovered from 1996 to 2000 and their PFGE patterns. Difference in one to three bands is considered to represent a single genetic event, and difference in four to six bands is considered to represent two genetic events. Isolates with the same *Not*I-PFGE pattern as the 1996 outbreak strain (2) were designated the outbreak strain and were the most prevalent in the present study. The PFGE pattern is reported as type A. Isolates with PFGE patterns different from the outbreak pattern in one to three bands or four to six bands are considered probably or possibly related to the outbreak strain. Their patterns are defined as subtypes of type A and are designated type A1, A2, etc. Isolates with PFGE patterns different from the outbreak pattern in more than six bands are considered unrelated to the outbreak strain, and their patterns are designated type B, C, etc.

Estimation of similarity among isolates and construction of a dendrogram. Genetic similarities between pairs of patterns were calculated by Nei and Li's *F* statistic (18). A matrix of *F* values for all pairs of patterns was prepared and was used for construction of a dendrogram by the NTSYS-PC software (Numerical Taxonomy and Multivariate Analysis System, version 1.50) from Applied Biostatistics, Inc. (Setauket, N.Y.).

RESULTS

***Shigella* species recovered in Nantou County from 1995 to 2000.** Bacteria of *Shigella* species were recovered from patients in Nantou County from 1997 to 2000 for confirmation of shigellosis, and the numbers of *Shigella* species recovered monthly are listed in Table 2. The numbers for 1995 and 1996 (2) are also listed and analyzed together. From 1995 to 2000, 8, 41, 60, 57, 35, and 20 *Shigella* isolates were recovered yearly. Of the total of 221 isolates, 210 isolates (95%) were from Renai and its neighboring townships (Hsini, Yuchr, Puli, and Guoshing townships; see Fig. 1), 1 isolate was from Nantou city, and 10 isolates were from the Jushan Township. Of the total, 185 isolates (84%) were *S. flexneri* serotype 2a, and the remaining 36 isolates (16%) were *S. sonnei* (20 isolates), *S. boydii* (1 isolate), *S. flexneri* serotypes 1a (1 isolate), 1b (3 isolates), 3a (4 isolates), 3b (2 isolates), and y (3 isolates), and nontypeable *S. flexneri* (2 isolates).

All of the 185 *S. flexneri* serotype 2a isolates recovered were from Renai and neighboring townships. Of them, 5, 41, 49, 44, 33, and 13 isolates were recovered yearly from 1995 to 2000. Two outbreaks of *S. flexneri* serotype 2a occurred in Renai Township during the 6-year period with sporadic cases before, between, and after the two outbreaks. The first outbreak occurred from August to October 1996, and the second occurred in October 1999. Thirty-four and 12 *S. flexneri* serotype 2a isolates were recovered in the two outbreaks, respectively. There were more sporadic cases between and after the two outbreaks than before (Table 2).

PFGE of the *S. flexneri* serotype 2a isolates. *Not*I-PFGE of the 161 *S. flexneri* serotype 2a isolates recovered in the area from 1997 to 2000 was performed, and 50 *Not*I-PFGE patterns were identified, including one pattern identical to the *Not*I-

TABLE 1. Characteristics of 180 *S. flexneri* serotype 2a isolates recovered from Renai and the neighboring townships during 1996 to 2000[a]

Isolate	Isolation date (mo-day-yr)	Source of isolation		PFGE types and subtypes	Relationship among members of the same epidemiological groups
		Township	Village		
SH1105	1-3-1996	Puli		A1	
SH2182[b]	8-12-1996	Renai	Tsunyang	A	
SH3896	8-18-1996	Renai	Chingying	A	
SH2214[b]	8-19-1996	Renai	Tsunyang	A	
SH2229	8-20-1996	Renai	Chingying	A	
SH2276	8-23-1996	Renai	Tatung	B	
SH2302	8-27-1996	Renai	Tsunyang	A	
SH2286	8-29-1996	Renai	Fahsiang	A	
SH2291	8-31-1996	Renai	Chingying	A	
SH2308[c,d]	9-4-1996	Renai	Tsunyang	A	A2 has addition of band 21 from A
SH2557[c]	9-4-1996	Renai	Tsunyang	A	
SH2590[c]	9-4-1996	Renai	Tsunyang	A2	
SH2343[b]	9-5-1996	Renai	Tsunyang	A	
SH2371[e]	9-5-1996	Renai	Chinai	A	
SH2372[e,f]	9-5-1996	Renai	Chinai	A	
SH2374[f]	9-5-1996	Renai	Chinai	A	
SH2576	9-5-1996	Renai	Tsunyang	A3	
SH2558	9-7-1996	Puli		A4	
SH2594	9-9-1996	Renai	Hotso	A4	
SH2585[c]	9-10-1996	Renai	Nanfeng	A	
SH3151	9-12-1996	Hsini		A5	
SH3160	9-12-1996	Renai	Tsunyang	A	
SH2683	9-13-1996	Renai	Chinai	A6	
SH3010	9-13-1996	Renai	Tsunyang	A3	
SH3162	9-13-1996	Renai	Nanfeng	A7	
SH3006[g]	9-13-1996	Renai	Tsunyang	A3	
SH2955[g]	9-14-1996	Renai	Tsunyang	A3	
SH2953	9-14-1996	Renai	Nanfeng	A8	
SH3094[d]	9-14-1996	Renai	Chingying	A	
SH3364	9-19-1996	Hsini		A4	
SH4029	9-25-1996	Renai	Wanfeng	A	
SH4232	10-12-1996	Renai	Lihsing	A	
SH4217	10-14-1996	Renai	Lihsing	A	
SH4347[h]	10-18-1996	Renai	Fahsiang	A	
SH4377[i]	10-23-1996	Renai	Chinai	A	
SH4332	10-24-1996	Renai	Lihsing	A	
SH4418[i]	10-31-1996	Renai	Huchu	A	
SH4798[h]	10-31-1996	Renai	Fahsiang	A	
SH4520	11-14-1996	Puli		A	
SH4799	12-30-1996	Renai	Fahsiang	A	
SH4785	12-31-1996	Puli		A	
SH4795	1-7-1997	Puli		A	
SH4797[j]	1-8-1997	Puli		A	
SH4834[j]	1-14-1997	Puli		A	
SH4903[k]	1-23-1997	Renai	Chinai	A9	
SH4916[k]	1-30-1997	Renai	Chinai	A	
SH4966	2-5-1997	Renai	Chingying	A	
SH4968	2-11-1997	Puli		A10	
SH4991	2-20-1997	Hsini		C	
SH5268	3-19-1997	Puli		A11	
SH5518	3-21-1997	Puli		A	
SH5598	3-31-1997	Puli		A	
SH5620	4-8-1997	Yuchr		A12	
SH5977[l]	5-6-1997	Renai	Chungcheng	A11	
SH5978	5-6-1997	Puli		A	
SH6337[m]	5-13-1997	Renai	Chungcheng	A	
SH7032	5-22-1997	Renai	Chinai	A	
SH7056[m]	5-23-1997	Renai	Chungcheng	A	
SH7080[l]	5-23-1997	Renai	Chungcheng	A	
SH7149	5-29-1997	Renai	Chingying	A4	
SH7227	6-6-1997	Renai	Chingying	A13	
SH7228	6-6-1997	Renai	Chinai	A	
SH7291	6-13-1997	Hsini		A4	
SH7292	6-13-1997	Renai	Hotso	A	
SH7344	6-17-1997	Renai	Tsunyang	A	
SH7372	6-19-1997	Renai	Chungcheng	A14	
SH7408	6-27-1997	Renai	Chinai	A15	
SH7565	7-8-1997	Renai	Chinai	A16	
SH7734	7-21-1997	Renai	Wanfeng	A	
SH7773	7-25-1997	Renai	Tsunyang	A	
SH7594	8-6-1997	Renai	Chinai	A	
SH7826	8-6-1997	Renai	Chinai	D	

Continued on following page

TABLE 1—*Continued*

Isolate	Isolation date (mo-day-yr)	Source of isolation		PFGE types and subtypes	Relationship among members of the same epidemiological groups
		Township	Village		
SH7845	8-7-1997	Renai	Hotso	E	
SH7932	8-20-1997	Renai	Chungcheng	A17	
SH8188	9-9-1997	Puli		A	
SH8189	9-9-1997	Renai	Chingying	A18	
SH8254	9-12-1997	Renai	Chingying	A	
SH8357	9-22-1997	Renai	Chungcheng	A19	
SH8500	9-26-1997	Renai	Chingying	A20	
SH8670	10-1-1997	Renai	Lihsing	A21	
SH8732	10-1-1997	Renai	Tatung	A4	
SH8653	10-9-1997	Renai	Tatung	A18	
SH8922	11-1-1997	Renai	Chungcheng	A22	
SH8925	11-1-1997	Renai	Huchu	A	
SH8923[n,o]	11-1-1997	Renai	Huchu	A	A21 has deletion of band 32 from A, All has deletion of band 19 from A21
SH8924[n]	11-1-1997	Renai	Huchu	A21	
SH8957[n,p]	11-2-1997	Renai	Huchu	A11	
SH8981	11-2-1997	Puli		A23	
SH9154[n,p]	12-1-1997	Renai	Huchu	A24	A25 has difference of 5 bands from A24
SH9155[n,o]	12-1-1997	Renai	Huchu	A25	
SH9658[q]	2-4-1998	Hsini		A26	A27 has addition of band 16 and deletion of band 14 from A26
SH9672[q]	2-6-1998	Hsini		A26	
SH9677[q]	2-6-1998	Hsini		A27	
SH9729	2-11-1998	Renai	Wanfeng	A12	
SH9981	2-15-1998	Renai	Chinai	A11	
SH10004[r]	2-24-1998	Renai	Chinai	A11	
SH10008[r]	3-27-1998	Renai	Chinai	A	
SH10200	3-27-1998	Puli		F	
SH10245[s]	4-7-1998	Renai	Chingying	A	
SH10262[s]	4-10-1998	Puli		A	
SH10279	4-16-1998	Renai	Wanfeng	A28	
SH10326	4-24-1998	Puli		A29	
SH10492[t]	5-11-1998	Renai	Tsunyang	A	
SH10592[t]	5-20-1998	Renai	Tsunyang	A	
SH10823	6-3-1998	Renai	Fahsiang	A	
SH10862	6-10-1998	Renai	Nanfeng	A30	
SH10900	6-16-1998	Puli		A	
SH11119[u,v]	7-22-1998	Renai	Nanfeng	A	A30 has addition of band 31 and deletion of band 32 from A
SH11143[u]	7-24-1998	Renai	Nanfeng	A30	
SH11174	7-28-1998	Puli		A31	
SH11201[u,v]	8-5-1998	Renai	Nanfeng	A	
SH11209	8-6-1998	Renai	Chungcheng	A32	
SH11221	8-11-1998	Renai	Tsunyang	A4	
SH11229	8-13-1998	Renai	Lihsing	A	
SH11350	8-19-1998	Renai	Tatung	A23	
SH11356	8-20-1998	Renai	Nanfeng	A30	
SH11365	8-25-1998	Renai	Nanfeng	A33	
SH11575	8-25-1998	Renai	Chingying	A23	
SH11430[w]	9-1-1998	Renai	Chingying	A23	
SH11443[w]	9-2-1998	Renai	Chingying	A23	
SH11491	9-5-1998	Yuchr		A3	
SH11492	9-6-1998	Puli		A	
SH11543[w]	9-9-1998	Renai	Chingying	A	
SH11662[x]	9-18-1998	Renai	Hsinhseng	A23	A34 has addition of band 24 from A23
SH11646[x]	9-20-1998	Renai	Hsinhseng	A34	
SH11661	9-20-1998	Renai	Chingying	A35	
SH11668	9-20-1998	Kuoshing		A	
SH11710	9-20-1998	Renai	Tsunyang	A23	
SH11684	9-25-1998	Renai	Hotso	A36	
SH11787	9-30-1998	Renai	Hotso	A37	
SH11788	10-1-1998	Renai	Tsunyang	A23	
SH12581	10-29-1998	Renai	Chingying	A38	
SH13917	11-21-1998	Renai	Hsinhseng	A	
SH14092	12-2-1998	Puli		A23	
SH15112	1-22-1999	Puli		A4	
SH15111[y]	1-24-1999	Hsini		A39	
SH15279[y]	2-8-1999	Hsini		A39	
SH15744	6-8-1999	Hsini		A40	
SH15779	6-20-1999	Puli		A41	
SH15778	6-21-1999	Puli		A	
SH16000	7-8-1999	Renai	Tsunyang	A	
SH16026	7-11-1999	Renai	Hotso	A23	
SH16027[z]	7-11-1999	Hsini		A42	
SH16053[z]	7-14-1999	Hsini		A42	

Continued on following page

TABLE 1—*Continued*

Isolate	Isolation date (mo-day-yr)	Source of isolation		PFGE types and subtypes	Relationship among members of the same epidemiological groups
		Township	Village		
SH16286[z]	7-21-1999	Hsini		A42	
SH16492[a1]	7-21-1999	Puli		A	
SH16475	7-25-1999	Renai	Chingying	A43	
SH16566	8-5-1999	Renai	Chingying	A	
SH16632	8-16-1999	Puli		A23	
SH16633[a1]	8-16-1999	Puli		A	
SH16776	9-8-1999	Hsini		A44	
SH16893[b1]	10-1-1999	Renai	Nanfeng	A23	
SH17015[b1]	10-5-1999	Renai	Nanfeng	A23	
SH17081[b1]	10-9-1999	Renai	Nanfeng	A23	
SH17247[c1,d1,e1]	10-9-1999	Renai	Chingying	A	
SH17155	10-10-1999	Renai	Nanfeng	A30	
SH17279[f1]	10-12-1999	Renai	Nanfeng	A45	A23 has difference of 5 bands from A45
SH17289[f1]	10-12-1999	Renai	Nanfeng	A23	
SH17274	10-13-1999	Puli		A	
SH17384[d1]	10-15-1999	Renai	Hotso	A46	
SH17656[c1,e1]	10-15-1999	Renai	Chingying	A4	
SH17669[e1]	10-15-1999	Renai	Chingying	A4	
SH17802	10-18-1999	Renai	Chingying	A	
SH17843	10-20-1999	Renai	Chingying	A47	
SH18145	11-1-1999	Renai	Fahsiang	A	
SH18237	11-15-1999	Renai	Hotso	A4	
SH18509	12-31-1999	Renai	Tsunyang	A21	
SH18661	2-3-2000	Puli		A48	
SH18682	2-11-2000	Hsini		A42	
SH18742	2-18-2000	Renai	Tatung	A49	
SH19325	5-29-2000	Renai	Huchu	A	
SH19699[g1]	7-1-2000	Renai	Hotso	A4	A3 has addition of band 20 and deletion of band 19 from A4
SH19698[g1]	7-1-2000	Renai	Hotso	A3	
SH19723[g1]	7-2-2000	Renai	Hotso	A4	
SH20107	8-2-2000	Hsini		A42	
SH20185	8-10-2000	Renai	Chingying	A	
SH20693	8-16-2000	Hsini		A42	
SH20764	8-16-2000	Hsini		A42	
SH20974	12-7-2000	Hsini		A50	
D21072	12-22-2000	Renai	Chingying	A51	

[a] Twelve epidemiological groups are boxed. The superscripts b, e, j, l, m, n, q, r, z, a1, and c1 indicate family members; c, g, and i, indicate close relatives and friends; d and d1 indicate classmates; f, h, k, b1, e1, f1, and g1 indicate neighbors; o, p, t, v, and x indicate same persons; s, u, w and y indicate contacts.

PFGE pattern previously identified with the outbreak strain (2). A representative photograph showing 17 *Not*I-PFGE patterns from 19 *S. flexneri* serotype 2a isolates is shown in Fig. 2A. By using 0 or 1 to represent the absence or presence of the individual *Not*I bands, respectively, the 50 *Not*I-PFGE patterns were coded in binary format and are shown in Table 3. For comparison, seven additional nonoutbreak *Not*I-PFGE patterns identified previously with the 1996 *S. flexneri* serotype 2a isolates were also binary coded and are shown in Table 3. By using this process, a total of 57 patterns and 39 *Not*I fragments were identified from the isolates recovered from 1996 to 2000. The classification described by Tenover et al. (21) was used to designate the 57 patterns, and a main type (type A), 51 subtypes of type A (types A1 to A51), and 5 types unrelated to type A (types B to F) were identified. Patterns for each of the *S. flexneri* serotype 2a isolates recovered from 1996 to 2000 are listed in Table 1. A dendrogram depicting the genetic similarity of the 51 subtypes and the 6 types was constructed and is shown in Fig. 2B. It shows genetic similarities of greater than 80% among type A and the 51 subtypes. Except for type A and 11 subtypes, only one isolate was recovered for each of these types and subtypes. The numbers of isolates of type A and the 11 subtypes recovered each year from 1996 to 2000 are also shown in Fig. 2B.

Isolates of type A pattern (the outbreak pattern) were recovered each year and accounted for 41% (74 out of 180) of the *S. flexneri* serotype 2a isolates recovered from 1996 to 2000. Numbers of the type A isolates recovered decreased annually. Percentages of the type A isolates among *S. flexneri* serotype 2a isolates recovered in the year also decreased annually, i.e., 66% (27 out of 41) in 1996, 45% (22 out of 49) in 1997, 35% (14 out of 44) in 1998, 27% (9 out of 33) in 1999, and 15% (2 out of 13) in 2000.

Isolates of the 51 subtypes (A1 to A51) and the other 5 types (B to F) accounted for the remaining 59% (106 out of 180) of the *S. flexneri* serotype 2a isolates recovered from 1996 to 2000. Compared with the number of type A isolates, the numbers of isolates recovered for each subtype and the other types are low. Only one isolate was recovered for 40 of the 51 subtypes and all of the other 5 types during the 5-year period, while 2 to 15 isolates were recovered for the other 11 subtypes (Fig. 2B). Only isolates of 8 subtypes were recovered in more than one year. This includes isolates of subtype A4 that were recovered in each year of the 5 years studied, isolates of subtypes A3 and A23 in 3 years, and isolates of subtypes A11, A12, A21, A30, and A42 in 2 years (Fig. 2B). The yearly distributions of isolates of type A and the 51 subtypes in villages of the Renai Township and in four neighboring townships are depicted in Fig. 1. This figure shows that 10 villages in Renai Township and Puli Township had isolates of type A and its subtypes recov-

ered from 1996 to 2000. Locations that had the most isolates of type A recovered, such as Chingying, Tsunyang, and Chinai villages and the Puli Township, also had the most isolates of the subtypes recovered.

Relationship among members of the same epidemiological groups. Many isolates of type A and the subtypes were recovered from family members, neighbors, classmates, or contacts within a couple of days. They are considered epidemiologically related. We arbitrarily defined those isolates that were epidemiologically related and were recovered within 1 to 3 days as the same epidemiological group. This yielded 12 groups from the 180 *S. flexneri* serotype 2a isolates recovered from 1996 to 2000, which are boxed and described in Table 1. For four groups (SH2371, SH2372, and SH2374; SH3006 and SH2955; SH11430 and SH11443; SH17656 and SH17669), isolates of the same group demonstrated the same PFGE pattern. For two groups (SH9154 and SH9155; SH17279 and SH17289), isolates of the same group demonstrated PFGE patterns different in five bands, indicating two genetic events between the isolates (21). For the remaining six groups (SH2308, SH2557, and SH2590; SH8923, SH8924, and SH8957; SH9658, SH9672, and SH9677; SH11119 and SH11143; SH11662 and SH11646; SH19698, SH19699, and SH19723), however, isolates of the same group demonstrated PFGE patterns only different in one or two bands, indicating a single genetic event between them (21).

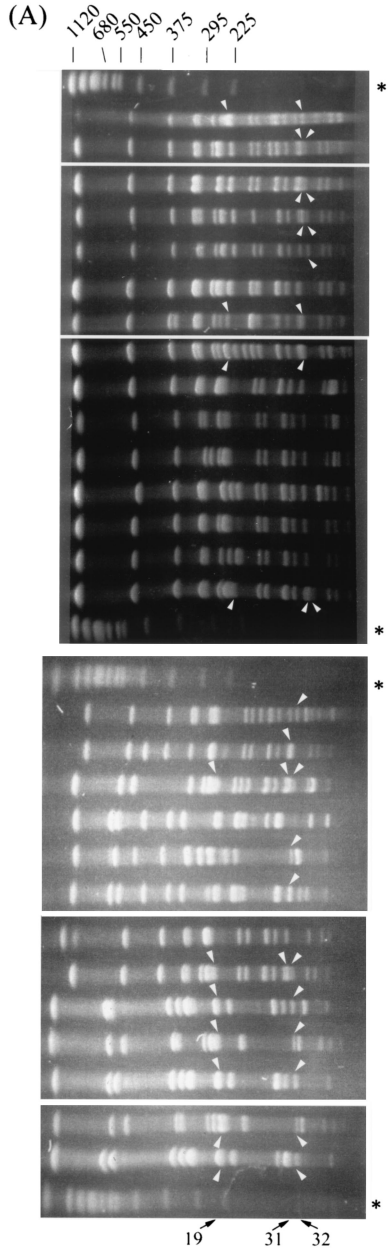
Classification of the 51 subtypes by presence or absence of three *Not*I bands. Detailed examination of the PFGE patterns of the 51 subtypes and type A indicates that three specific bands in the type A pattern were deleted at high frequency (Table 3). These are bands 19, 31, and 32. Based on the presence or absence of these three bands, the 51 subtypes can be divided into six classes (classes I to VI; Table 3). Subtypes that have bands 19 and 32 but not band 31 belong to class I. Subtypes that have bands 19 and 31 but not band 32 belong to class II. Subtypes that have bands 31 and 32 but not band 19 belong to class III. Subtypes that have only one of the three bands belong to class IV. Subtypes that do not have any of the three bands belong to class V. Subtypes that have all of the three bands belong to class VI. By using this classification, 15, 7, 7, 8, 6, and 8 subtypes belong to classes I, II, III, IV, V, and VI, respectively. Fifty-three percent (10 out of 19) of subtypes involving a deletion in band 19 also had an addition of band 20. For classes I, II, and III, subtypes of the same class were mostly placed in the same genetic cluster with similarity of 0.90 or greater (Fig. 2B).

PFGE of other *Shigella* isolates in Renai and the neighboring townships. Isolates of *Shigella* spp. other than *S. flexneri* serotype 2a also seemed to be recovered at a relatively high frequency in Renai and neighboring townships after the outbreak in 1996 (Table 2). In order to identify possible relationships between the outbreak strain and the non-*S. flexneri* serotype 2a isolates, PFGE was also performed with the non-*S. flexneri* serotype 2a isolates recovered in Renai and neighboring townships from 1997 to 2000. The results indicate that all of them had differences of more than 10 bands in their *Not*I-PFGE patterns compared with that of the outbreak pattern. They are not related to either type A or any of the 51 subtypes, and no major pattern can be found among them. A representative photograph showing the *Not*I-PFGE patterns of 8 non-*S. flexneri* serotype 2a isolates is shown in Fig. 2A.

TABLE 2. Numbers of *Shigella* spp. isolated monthly from townships of Nantou County from 1995 to 2000

Month	No. of *Shigella* isolates recovered in indicated township or city in the year[a]																																							
	1995 (n = 8)			1996 (n = 41)				1997 (n = 60)						1998 (n = 57)								1999 (n = 35)			2000 (n = 20)																
	Renai	Hsini	Yuchr	Renai	Puli	Hsini	Renai	Puli	Hsini	Yuchr	Nantou	Renai	Puli	Hsini	Yuchr	Guoshing	Jushan	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini	Renai	Puli	Hsini
January					1		2	3				3										1	1																		
February							1	1						3									1																		
March								3				1	1																												
April	1						1 (3b)	1 (3b)				2	2																												
May							6	1	2 (2:Ss)			2	1 (Ss)											1																		
June		1 (y)					7 (1:y)					2	1											2	1	1 (N)														
July							3	1 (Sb)				2	1					3						1	3	3															
August	2	1		8			4				1 (Ss)	8						1						2	1	1														
September			1 (3a)	19	1	2	6 (2:Ss)	1				9	2 (1:Ss)			1							1																		
October				7			3	1 (Ss)				2					6 (6:Ss)	13 (1:N)	1																					
November				1			5	1				1					4 (4:Ss)	2																						
December	1 (y)	1		1	1		3 (1:Ss)					1				1 (Ss)		2 (1:1a)								3 (3:3a)														
Total	4	3	1	35	4	2	41	13	4	1	1	32	9	3	2	1	10	21	7	7	11	4	5																

[a] *S. flexneri* isolates with serotypes other than 2a are indicated with 3a, 3b, Y, 1a, 1b, and N (nontypable) in parentheses; species of *S. boydii* and *S. sonnei* are also indicated in parentheses with Sb and Ss, respectively.



Isolate	Species / Serotype	PFGE type / subtype	Class
SH4991	*S. flexneri* 2a	C	
SH11646	*S. flexneri* 2a	A34	III
SH11661	*S. flexneri* 2a	A35	III
SH11787	*S. flexneri* 2a	A37	III
SH11491	*S. flexneri* 2a	A3	VI
SH8653	*S. flexneri* 2a	A18	V
SH7932	*S. flexneri* 2a	A17	II
SH11209	*S. flexneri* 2a	A32	II
SH9658	*S. flexneri* 2a	A26	V
SH9672	*S. flexneri* 2a	A26	V
SH9677	*S. flexneri* 2a	A27	V
SH7565	*S. flexneri* 2a	A16	V
SH7227	*S. flexneri* 2a	A13	V
SH8189	*S. flexneri* 2a	A18	V
SH4795	*S. flexneri* 2a	A	
SH8357	*S. flexneri* 2a	A19	IV
SH7826	*S. flexneri* 2a	D	
SH10200	*S. flexneri* 2a	F	
SH5621	*S. flexneri* 3b		
SH7396	*S. flexneri* y		
SH5619	*S. flexneri* 3b		
SH7845	*S. flexneri* 2a	E	
SH4795	*S. flexneri* 2a	A	
SH7156	*S. sonnei*		
SH9397	*S. sonnei*		
SH7150	*S. sonnei*		
SH8255	*S. sonnei*		
SH8289	*S. sonnei*		

FIG. 2. (A) Photograph of *Not*I-PFGE patterns of 19 *S. flexneri* serotype 2a strains and 8 non-*S. flexneri* serotype 2a strains. Arrows indicate bands 19, 31, and 32. An asterisk indicates the yeast chromosomes used as molecular size standards with sizes of selected chromosomes indicated in kilobases. (B) Dendrogram of types A, B, C, D, E, and F and 51 subtypes (A1 to A51) of type A. For the type and subtypes with more than one isolates recovered from 1996 to 2000, numbers of isolates are indicated.

DISCUSSION

Nantou County is located in central Taiwan and consists of 12 townships and 1 city, of which Renai and Hsini townships are the two largest and are the only two mountainous townships in Nantou County. Shigellosis was rare in Nantou County before August 1996 but was frequently found in Renai and neighboring townships since an outbreak of *S. flexneri* serotype 2a in Renai Township in August 1996 (Table 2). An *S. flexneri* serotype 2a outbreak strain with the type A pattern was the main strain observed during and after the outbreak. Seven and 45 closely related strains with subtypes A2 to A8 patterns and A3, A4, and A9 to A51 patterns, respectively, appeared during and after the outbreak. They were recovered far less frequently than the outbreak strain, indicating that transmission of these closely related strains occurred less frequently than transmission of the outbreak strain. Compared to the outbreak strain, 84% (42 out of 50) of the closely related strains had deletions of at least one of three specific *Not*I bands in their PFGE patterns, suggesting deletion hot spots in the genome of the outbreak strain.

Of the 180 *S. flexneri* serotype 2a isolates recovered from 1996 to 2000, 12 epidemiologically related groups were iden-

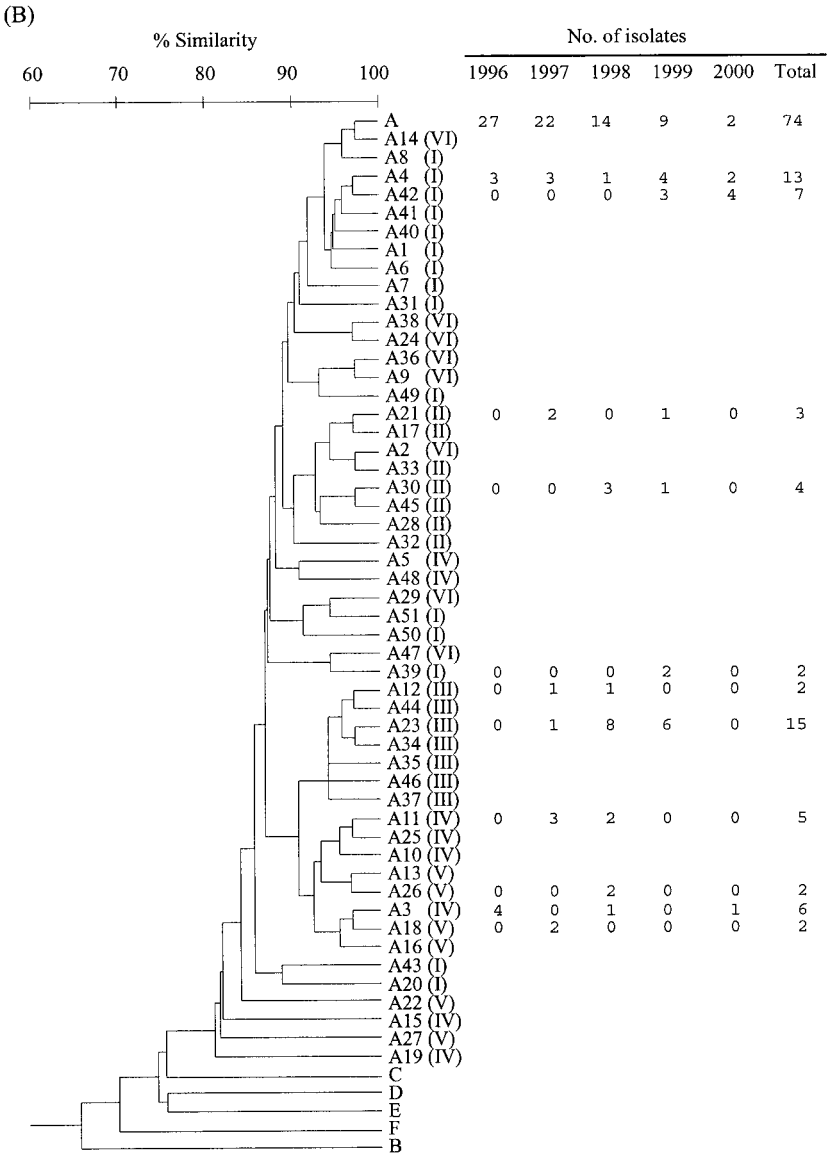


FIG. 2—*Continued.*

tified (Table 1). For four groups, isolates of the same group showed identical PFGE patterns, indicating transmission of the same strain among members of the group. For the other two groups (SH9154 and SH9155; SH17279 and SH17289), isolates of the same group showed a difference of five bands in their PFGE patterns. While SH17279 (subtype 45) and SH17289 (subtype 23) were recovered from two neighbors during the outbreak in 1999 (Table 1) and were probably not truly epidemiologically related, the relationship between SH9154 and SH9155 was somewhat complicated. SH9154 (subtype A24) and SH9155 (subtype A25) were recovered from two family members. One month earlier, three separate strains (type A and subtypes A21 and A11) had been recovered from the same two persons and another family member, respectively. Although the possibility of independent origins can not be excluded, the two isolates were likely to have evolved from complex genetic rearrangements among isolates of type A and subtypes A21 and A11 during the 1-month period in the family. Furthermore, for the remaining 6 groups, isolates of the same group showed differences of only one or two bands in their PFGE patterns, indicating a single genetic event among them (21). It appears that for these six groups one isolate had evolved directly from the other isolate of the same group. Based on these epidemiological observations and the presence of deletion hot spots in the genome of the outbreak strain, we hypothesized that many, if not all, of the 50 closely related strains recovered during and after the 1996 outbreak were derived from the outbreak strain or other closely related strains by various genetic events, such as tandem duplication, deletion, translocation, and inversion (see below).

Several lines of evidence further support our hypothesis. First, during the two outbreaks in 1996 and 1999 isolates that had no apparent epidemiological relationships showed a single genetic event among them. For example, isolates of subtypes

TABLE 3. Binary-coded PFGE patterns of type A, B, C, D, E and F, and the 51 subtypes of type A (A1 to A51)

Type or subtype	Binary code for PFGE band number[a]																																					Class
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
A	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	
A1	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	**0**	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	I
A2	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	**1**	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A3	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	IV
A4	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	I
A5	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	**1**	1	0	1	**0**	**0**	0	1	0	1	0	1	0	IV
A6	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	**0**	0	1	**0**	1	0	1	0	1	0	1	0	I
A7	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	**0**	**1**	1	0	I
A8	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	**1**	I
A9	0	0	1	0	0	1	0	0	0	1	0	0	**1**	1	**0**	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A10	0	0	1	0	0	1	0	0	**1**	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	IV
A11	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	IV
A12	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A13	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	0	1	0	V
A14	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	**1**	VI
A15	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	1	**0**	**1**	**0**	0	1	0	1	**1**	IV
A16	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	**0**	**0**	**1**	1	0	1	0	1	0	V
A17	0	0	1	0	0	1	0	0	0	1	**1**	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	II
A18	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	0	1	0	V
A19	0	0	**0**	**1**	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	1	**1**	1	0	1	0	1	0	IV
A20	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	**0**	1	1	**1**	0	1	1	0	0	1	0	1	0	1	**0**	1	**1**	1	0	1	0	1	0	I
A21	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	II
A22	0	0	1	0	0	1	0	0	0	1	**1**	0	0	1	**0**	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	0	1	0	V
A23	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A24	0	0	1	0	0	1	0	0	0	1	0	0	0	**0**	1	0	1	**0**	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A25	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	IV
A26	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	**1**	1	0	V
A27	0	0	1	0	0	1	0	0	0	1	0	0	0	**0**	1	**1**	1	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	**1**	1	0	V
A28	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	**1**	1	**1**	1	0	II
A29	0	0	1	0	0	**0**	**1**	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A30	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	**0**	**1**	1	0	1	0	1	0	II
A31	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	**0**	**1**	1	0	1	0	I
A32	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	**1**	**1**	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	**1**	II
A33	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	**1**	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	II
A34	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	1	**1**	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A35	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	**0**	1	**0**	**1**	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A36	0	0	1	0	0	1	0	0	0	1	0	0	**1**	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A37	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	1	**0**	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A38	0	0	1	0	0	1	0	0	0	1	0	0	0	**0**	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A39	0	0	1	0	0	1	0	0	0	**0**	**1**	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	**1**	1	0	1	0	1	0	I
A40	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	**0**	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	I
A41	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	**1**	1	0	1	0	1	0	I
A42	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	**1**	1	0	I
A43	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	**0**	**1**	1	**0**	1	**1**	1	0	1	0	1	0	I
A44	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	0	0	1	1	**1**	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A45	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	**0**	**1**	1	0	1	**1**	1	0	II
A46	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	1	**0**	**1**	0	**0**	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	III
A47	0	0	1	0	0	1	0	0	0	**0**	**1**	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1	0	VI
A48	0	0	1	0	0	1	0	0	0	1	0	**1**	0	1	1	0	**0**	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	1	0	1	0	IV
A49	0	0	1	0	0	1	0	0	0	1	0	0	**1**	1	0	0	1	1	1	0	**1**	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	I
A50	0	0	1	0	0	**0**	0	0	0	1	0	**1**	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	0	1	0	1	0	1	0	I
A51	0	0	1	0	0	**0**	**1**	0	0	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	1	**0**	1	**1**	1	0	1	0	1	0	I
B	**1**	0	**0**	0	0	**0**	0	0	0	1	0	**1**	0	1	1	**1**	**0**	1	**0**	0	0	1	1	0	0	1	0	**0**	**1**	1	**0**	**0**	0	1	0	1	0	1	0	
C	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	1	**0**	1	**1**	0	1	1	0	**1**	1	0	1	0	1	**0**	1	**1**	**0**	0	**0**	**1**	1	0	
D	0	0	**0**	**1**	0	1	0	**1**	0	1	0	0	0	1	**0**	0	**0**	1	**0**	0	0	1	1	0	0	1	0	1	0	1	1	**0**	0	1	0	1	0	1	0	
E	0	**1**	**0**	0	0	1	0	0	0	1	0	0	0	1	1	0	**0**	1	**0**	0	0	1	1	0	0	1	0	1	0	1	**0**	**0**	0	1	0	**0**	**1**	1	0	
F	0	0	1	0	**1**	**0**	**1**	0	0	**0**	0	0	0	**0**	1	0	1	1	1	0	**1**	1	1	0	0	1	0	1	0	1	1	1	0	1	**1**	**0**	0	1	0	

[a] Codes different from the code of the same band in the type A pattern are in bold face.

A3 to A8 were recovered during the outbreak in 1996. Subtypes A3, A5, A6, A7, and A8 differed from subtype A4 in two, two, one, two, and one band, respectively. Isolates of subtype A3, A5, A6, A7, and A8 can thus be explained by separate single events from isolates of subtype A4 (Table 1). Similarly, isolates of type A and subtypes A4, A23, A30, A45, A46, and A47 were recovered during the outbreak in October 1999. Subtypes A4 and A47 differed from type A in one and two bands, respectively, subtype A46 differed from subtype A23 in one band, and subtype A45 differed from subtype A30 in one band. Isolates of subtype A4, A47, A46, and A45 can thus be explained by single events from isolates of type A and subtypes A23 and A30 (Table 1). Second, the distribution of isolates of the subtypes roughly correlates with the distribution of the outbreak strain (Fig. 1). Third, numbers of *S. flexneri* serotype 2a isolates recovered decreased yearly from 1997 to 2000. The decrease is related to yearly decrease in the actual numbers of the outbreak strain isolates and also to the relative numbers of the outbreak strain isolates to all *S. flexneri* serotype 2a isolates recovered in that year. As the outbreak strain transmitted much better than the closely related strains, one possible explanation for the correlation would be de novo generation of closely related strains in human colons from the outbreak strain. Inside human colons the newly generated, closely related strains might be preferentially selected over the outbreak strain, as adaptive immunity would initially be elicited by infection with the outbreak strain.

Rearrangements of large DNA pieces are usually caused by homologous recombination between long repeat sequences, such as rRNA operons, transposons, and IS (insertion sequence) elements. Depending upon whether the two repeat sequences are the same or are in reversed orientation, tandem duplication, deletion, translocation, and inversion of the intervening sequence could occur. Mobility of IS elements would increase the variety of rearrangements (10). *Shigella* species carry multiple copies of insertion sequences, such as IS*1*, IS*600*, and IS*629* in their genomes (14). IS-mediated deletions and tandem amplifications of DNA of 10 to 100 kb have been reported for *S. dysenteriae* and *Yersinia pestis* (5, 15). Futhermore, *S. flexneri* serotype 2a carry at least four pathogenicity islands (1, 13, 16, 19, 20, 22). Pathogenicity islands are large (often more than 30 kb), unstable DNA fragments that carry many virulence-associated genes and mobility genes (including insertion sequences, integrases, transposases, and origin of plasmid replication) (6). It has been demonstrated that pathogenicity islands are involved in spontaneous deletions of 51 and 99 kb of DNA in *S. flexneri* serotype 2a (1, 20). Both insertion sequences and pathogenicity islands could account for the frequent generation of closely related strains and deletion hot spots observed in this study. We propose that some important factors present in the outbreak strain were lost or inactivated during the course of generating the closely related strains, leading to poor transmission of the strains. These factors might be associated with the prolonged survival of the bacteria in the environment, as recently reported for poly P kinase of *Shigella* and *Salmonella* spp. (11), or for intracellular persistence of the bacteria in human enteric cells.

Shigella species, like *Salmonella* species, are facultative intracellular pathogens. Both bacteria not only cause gastrointestinal diseases but also survive in enteric epithelium cells. Studies with *Salmonella* spp. in a mouse model indicated that gastroenteritis symptoms could be separated from intracellular accumulation and systemic spreading of the bacteria and that genes for gastroenteritis symptoms were mainly located on pathogenicity island SPI1 and that genes for intracellular accumulation and systemic spreading of the bacteria were located on pathogenicity island SPI2 (8). Clements et al. (3) demonstrated that a single mutation in a global regulator gene, the polynucleotide phosphorylase gene, in *Salmonella enterica* could switch the acute gastroenteritis symptom into a systemic infection in the mouse model. It is likely that a similar regulation system might also exist in *S. flexneri*. Recovery from gastroenteritis symptoms caused by *S. flexneri* usually takes about a week, but survival of the bacteria in human enteric cells of up to 17 months has been reported (12). These asymptomatic carriers are suspected as the source of transmission of shigellosis (7, 9). Although we did not screen the asymptomatic contacts as was done in a previous study, the five *S. flexneri* serotype 2a isolates recovered from asymptomatic contacts in 1996, indeed, were the outbreak strain (2). This and the fact that only the outbreak strain could be recovered twice from the same persons within a 2-week period (SH10492 and SH10592 and SH11119 and SH11201; Table 1) indicated that the outbreak strain had a better surviving capability than the closely related strains had, though they might have similar capabilities in eliciting the gastroenteritis symptom in patients.

In summary, our PFGE results show that a single *S. flexneri* serotype 2a strain responsible for the outbreak in 1996 continued to be the main strain causing shigellosis in the area from 1997 to 2000. Forty-five closely related but poorly transmissible strains emerged from the outbreak strain during the 4-year period. Compared to the outbreak strain, 87% (39 out of 45) of them had deletions of at least one of three specific *Not*I bands in their PFGE patterns. Characterization of these three *Not*I bands is in progress.

ACKNOWLEDGMENTS

This work was supported by research grant DOH-90-DC-2018 from the Department of Health, Taiwan, Republic of China.

We thank the staff from the Renai Township Health Station for assistance in the epidemiological investigation and from the Puli Christian Hospital and the Puli Veteran Hospital for providing the *Shigella* isolates.

REFERENCES

1. **Al-Hasani, K., K. Kajakumar, B. Dieter, R. Robin-Browne, B. Adler, and H. Sakellaris.** 2001. Genetic organization of the she pathogenicity island in *Shigella flexneri* 2a. Microb. Pathog. **30:**1–8.
2. **Chiou, C.-S., W.-B. Hsu, H.-L. Wei, and J.-H. Chen.** 2001. Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. J. Clin. Microbiol. **39:**1048–1056.
3. **Clements, M. O., S. Eriksson, A. Thompson, S. Lucchini, J. C. D. Hinton, S. Normark, and M. Rhen.** 2002. Polynucleotide phosphorylase is a global regulator of virulence and persistency in *Salmonella enterica*. Proc. Natl. Acad. Sci. USA **99:**8784–8789.
4. **Department of Health, Taiwan, R. O. C.** 2002. Statistically important characteristics in public health in Taiwan, Republic of China. Department of Health, Taipei, Taiwan, Republic of China.
5. **Fetherston, J. D., P. Schuetze, and R. D. Perry.** 1992. Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element. Mol. Microbiol. **6:**2693–2704.
6. **Hacker, J., G. Blum-Oehler, I. Muhldorfer, and H. Tschape.** 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23:**1089–1097.
7. **Haider, K., M. I. Huq, A. R. Samadi, and K. Ahmad.** 1985. Plasmid charac-

terization of *Shigella* spp. isolated from children with shigellosis and asymptomatic excretors. J. Antimicrob. Chemother. **16:**691–698.
8. **Hanson-Wester, I., and M. Hensel.** 2001. Salmonella pathogenicity islands encoding type III secretion systems. Microb. Infect. **3:**549–559.
9. **Hossain, M. A., K. Z. Hasan, and M. J. Albert.** 1994. *Shigella* carriers among non-diarrhoeal children in an endemic area of shigellosis in Bangladesh. Trop. Geograph. Med. **46:**40–48.
10. **Hughes, D.** 2000. Evaluating genome dynamics: the constraints on rearrangements with bacterial genomes. Genome Biol. **1:**0006.1–0006.8.
11. **Kim, K. S., N. N. Rao, C. D. Fraley, and A. Kornberg.** 2002. Inorganic polyphosphate is essential for long-term survival and virulence factors in *Shigella* and *Salmonella* spp. Proc. Natl. Acad. Sci. USA **99:**7675–7680.
12. **Levine, M. M., H. L. Dupont, M. Khodabandelou, and R. B. Hornick.** 1973. Long-term *Shigella*-carrier state. N. Engl. J. Med. **288:**1169–1171.
13. **Luke, S. N., S. A. Turner, K. Kajakumar, H. Sakellaris, and B. Adler.** 2001. Ferric dicitrate transport system (Fec) of *Shigella flexneri* 2a YSH6000 is encoded on a novel pathogenicity island carrying multiple antibiotic resistance genes. Infect. Immun. **69:**6012–6021.
14. **Matsutani, S., and E. Ohtsubo.** 1993. Distribution of the *Shigella sonnei* insertion elements in Enterobacteriaceae. Gene **127:**111–115.
15. **McDonough, M. A., and J. R. Butterton.** 1999. Spontaneous tandem amplification and deletion of the Shiga toxin operon in *Shigella dysenteriae* 1. Mol. Microbiol. **34:**1058–1069.
16. **Moss, J. E., T. J. Cardozo, A. Zychlinsky, and E. A. Croisman.** 1999. The selC-associated SHI-2 pathogenicity island of *Shigella flexneri*. Mol. Microbiol. **33:**74–83.
17. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and F. C. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology Press, Washington, D.C.
18. **Nei, M., and W. H. Li.** 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76:**5269–5273.
19. **Rajakumar, K., C. Sasakawa, and B. Adler.** 1996. A spontaneous 99-kb chromosomal deletion results in multi-antibiotic susceptibility and an attenuation of contact haemolysis in *Shigella flexneri* 2a. J. Med. Microbiol. **45:**64–75.
20. **Rajakumar, K., C. Sasakawa, and B. Adler.** 1997. Use of novel approach, termed island probing, identifies the *Shigella flexneri* she pathogenicity island which encodes a homolog of the immunoglobulin A protease-like family of proteins. Infect. Immun. **65:**4606–4614.
21. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
22. **Vokes, S. A., S. A. Reeves, A. G. Torres, and S. M. Pane.** 1999. The aerobactin iron transport system genes in *Shigella flexneri*. Mol. Microbiol. **33:**63–73.