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OCCURRENCE OF SHIGELLOSIS IN THE YOUNG AND ELDERLY IN RURAL CHINA: RESULTS OF A 12-MONTH POPULATION-BASED SURVEILLANCE STUDY

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Abstract. In 2002, population- and treatment center-based surveillance was used to study the disease burden of shigellosis in rural Hebei Province in China. A total of 10,105 children with diarrhea or dysentery were enrolled. Infants were treated most frequently for diarrhea (1,388/1,000/year) followed by children ≤ 5 years old (618/1,000/year). Shigellosis was treated most often in children 3–4 years old (32/1,000/year) and people ≥ 60 years of age (7/1,000/year). Fifty-six percent (184 of 331) Shigella isolates were detected in patients who had non-bloody diarrhea. Shigella flexneri was identified in 93% of 306 isolates. The most common S. flexneri serotypes were 1a (34%), X (33%), and 2a (28%). More than 90% of the Shigella isolates were resistant to cotrimoxazole and nalidixic acid, but remained susceptible to ciprofloxacin, norfloxacin, and gentamicin. Widespread resistance to antibiotics adds urgency to the development and use of vaccines to control shigellosis.

INTRODUCTION

Bacillary dysentery remains a leading cause of mortality and morbidity in China according to the Disease Surveillance Point System1, ranking fourth as cause of death and second as cause of morbidity among communicable diseases. With the widespread use of hepatitis A and B vaccines, diarrhea has replaced hepatitis as a leading public health issue in China, especially in the population less than five years of age. However, China is undergoing rapid social and economic changes. Water supply and sanitation, factors intimately linked to the prevalence of enteric diseases, have rapidly improved in urban and rural areas, except in the more remote western regions.

Although the national incidence rate of bacillary dysentery has decreased from 1.2/1,000/year in 1991 to 0.4/1,000/year in 2000,2 a substantial proportion of cases are missed by the surveillance system. Studies on underreporting at the provincial level indicate that 38–77% of bacillary dysentery cases are missed by the government reporting system.3–5 The incidence rate and the disease burden of shigellosis are difficult to estimate due to the absence of population-based studies. Data on the burden of disease by age group is essential for rational decisions regarding the introduction of potential vaccines against shigellosis. Since immunity to shigellosis is likely to be species and serotype specific, information on the relative distribution of these species and serotypes is crucial for vaccine development. In this study, we investigated the burden of diarrhea and shigellosis by age group and the relative distribution of Shigella species and serotypes in a rural area of China.

METHODS

Study population. The catchment area consisted of 29 villages in four rural townships in Zhengding County, Hebei Province, China, where the residents depend mostly on agriculture for income. The national census of 20006 showed a total population in the catchment area of 75,630 of which 2,997 (4%) were children less than 60 months of age.

Health care system. In the catchment area, 101 village clinics, 4 township hospitals, and 5 county hospitals provide health care. All health care providers, with the exception of the county hospitals, which provide secondary health care services, were included in the shigellosis surveillance system. All village doctors sell medications, which is their major source of income. In 10 villages, pharmacies sell allopathic as well as traditional drugs. The use of health care in Zhengding County was recently reviewed.7

Study design. We estimated the burden of diarrhea and shigellosis through a population- and treatment center–based surveillance system. The study followed a standardized protocol, which was based on a generic protocol8 and was reviewed and approved by the World Health Organization (WHO) Institutional Review Board. Consenting patients of all age groups with diarrhea or dysentery coming to the participating health care providers were included in the study. Diarrhea was defined as three or more loose bowel movements during a 24-hour period. Dysentery was defined as one or more loose bowel movements with visible blood. Persistent diarrhea was defined as diarrhea for more than 14 days. Diarrhea following three or more days of normal bowel movements was considered a new diarrhea episode. Fever was defined as an axillary temperature ≥ 37.5°C. The disease duration was defined as number of days from onset of symptoms to recovery, and was calculated as number of days from onset of symptoms to presentation plus number of days from presentation to recovery. Recovery was defined as three consecutive days free of diarrhea.

For every patient presenting with diarrhea, a case report form (CRF), which included demographics, medical history, physical examination, and management plan, was completed and rectal/stool swabs or a stool specimen were obtained. The swab was placed in buffered glycerol saline (BGS). The specimens were stored refrigerated until they were transported in a cool box to the central laboratory by motorcycle, usually within four hours. A trained community health worker visited...
The specimens in BGS were collected from…n < 5 years of age, 83 for those with Shigella –e < 5 years of age, 5 isolates collected during the surveillance were cultured on MacConkey agar. A’/H23041°’/H11350The study was reviewed and approved by the local institutional review board. All CRFs were double-processed for the patient were asked whether the patient may have had symptoms consistent with persistent diarrhea, rectal prolapse, ileus, other gastrointestinal manifestations, pneumonia, seizure, or encephalopathy. No additional specimens were obtained during the follow-up visits. The study procedures were discussed with and taught to the study staff. During training sessions and monitoring visits, the WHO guidelines for the treatment of diarrhea were emphasized by the investigators. The procedures were adapted and implemented during a pilot phase from August through December 2001.

Microbiologic procedures. The specimens in BGS were plated on MacConkey agar and Salmonella-Shigella agar. Biochemical reactions of colonies were evaluated in Kliger’s iron agar and motility indole urease medium. Serologic confirmation of colonies was performed by slide agglutination with appropriate group-specific polyvalent antiserum, followed by type-specific monovalent antisera. Standardized commercial antisera (Denka Seiken Co., Tokyo, Japan) were used for identification. In cases where no agglutination occurred with live bacteria, the test was repeated with boiled suspensions of bacteria. Antimicrobial susceptibility testing was done by the disk diffusion method using nationally standardized disks (National Institute for Control and Production of Biologic Products, Beijing, People’s Republic of China). Strains were stored at -70°C for confirmation. The species, serotype, subtype, and antimicrobial resistance profile of all Shigella isolates collected during the surveillance were confirmed at a reference laboratory in Shanghai after study completion (Fudan University) using the same standardized commercial antisera. The reported findings are based on the final strain identification by Fudan University.

Data management and analysis. All CRFs were double-entered into a custom-made data entry programs (FoxPro®, Microsoft, Redmond, WA). The data management programs included error as well as consistency check programs. We used the SAS program (SAS Institute Inc., Cary, NC) for statistical analysis. Incidence rates were calculated based on the population residing in the catchment area in 2000 as the denominator. Since the observation period was 12 months, it was assumed that each individual residing in the study area contributed 12 person-months of time to the denominator. Since a person is not at risk for a new disease episode during a current episode, the duration of time of each episode was subtracted from the person time in the denominator. The age-specific number of disease episodes was used as the numerator. Monthly average rainfall and temperatures were obtained from the Zhengding Weather Station (Zhengding, People’s Republic of China). The mean of highest and lowest temperature measured each day was used to estimate the average temperature for each month.

Chi-square tests were used for the analysis of binary data. For nonparametric data, the Wilcoxon rank sum test was used for comparison of two groups and the Kruskal-Wallis equality of populations rank test was used if more than two groups were compared. A test for trend (chi-square) was applied to assess the statistical significance of increasing incidence rates with increasing age after the age of 30 years. To explore the potential correlation between temperature and shigellosis incidence rate as well as rainfall and incidence rate, the Spearman rank correlation coefficient was calculated. P < 0.05 (two-tailed) was considered statistically significant.

Ethics. The study was reviewed and approved by the local government of Hebei Province and the Secretariat Committee for Research Involving Human Subjects (WHO, Geneva, Switzerland).

RESULTS

Incidence. Between January 1 and December 31, 2002, 16,550 individuals with diarrhea came to one of the participating health care providers (5,208 persons [32%] refused to participate in the study and to provide a rectal swab or stool specimen; for study population assembly, see Figure 1). A total of 11,342 individuals had a CRF completed and provided a stool sample or a rectal swab, but 1,237 persons did not fulfill the inclusion criteria (three loose bowl movements within a 24-hour period or an episode of dysentery) and were excluded from the analysis.

Of the 10,105 diarrhea patients included in the analysis, 1,851 (18%) were less than five years of age. The incidence rate of treated diarrhea was highest in infants (1,388/1,000/year). The incidence rate per thousand population per year by age group was 618 in children < 5 years of age, 83 for those 5–14 years of age, and 123 in those ≥ 15 years of age. Among 10,105 diarrhea cases, 451 (5%) reported a history of dysentery. The incidence rate per thousand per year of treated dysentery was 25, 3, and 6 for those < 5 years of age, 5–14 years of age, and ≥ 15 years of age, respectively.

The specimens from 331 (3%) of 10,104 diarrhea and dysentery patients were positive for Shigella; this bacteria was assayed for by an identification system entering into a custom-made data entry programs (FoxPro®, Microsoft, Redmond, WA). The data management programs included error as well as consistency check programs. We used the SAS program (SAS Institute Inc., Cary, NC) for statistical analysis. Incidence rates were calculated based on the population residing in the catchment area in 2000 as the denominator. Since the observation period was 12 months, it was assumed that each individual residing in the study area contributed 12 person-months of time to the denominator. Since a person is not at risk for a new disease episode during a current episode, the duration of time of each episode was subtracted from the person time in the denominator. The age-specific number of disease episodes was used as the numerator. Monthly average rainfall and temperatures were obtained from the Zhengding Weather Station (Zhengding, People’s Republic of China). The mean of highest and lowest temperature measured each day was used to estimate the average temperature for each month.

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The specimens from 331 (3%) of 10,104 diarrhea and dysentery patients were positive for Shigella; this bacteria was assayed for by an identification system

16550 presented with a history of diarrhea

5208 refused to provide a stool sample

11342 consented and were enrolled in the study

1237 less than three bowel movements or no blood in stool

10105 enrolled on Day 0

9774 specimen did not grow Shigella

331 shigellosis cases detected

26 lost to follow up

305 followed up on Day 3

305 followed up on Day 7

305 followed up on day 14

301 followed up on day 90

isolated from 147 (33%) of those with a history of dysentery. The incidence rate of treated shigellosis was highest in children 3–4 years of age (32/1,000/year) and lowest in persons 10–30 years of age (3/1,000/year) and increased significantly after the age of 30 ($P < 0.001$). Individuals > 60 years of age had a shigellosis rate of 7/1,000/year (Figure 2).

Shigellosis cases peaked during the hottest months of the year (July through September) (Figures 3 and 4) and were lowest during the winter months (November through February), when the temperature frequently drops below the freezing point. A statistically significant correlation between average temperature and shigellosis incidence for each month was observed ($r = 0.73, P = 0.007$). An even greater statistically significant relationship was observed between shigellosis incidence and mean monthly rainfall ($r = 0.90, P < 0.001$).

Clinical features. Thirty-two percent (107 of 331) of Shigella patients had fever at the time of presentation, in contrast to 9% (831 of 9,774) of non-Shigella patients ($P < 0.001$). The proportion of febrile shigellosis patients was similar in the three age groups (< 5 years old, 5–14 years old, and ≥ 15 years old) ($P = 0.12$). Patients with $S. \text{flexneri}$ infections had diarrhea for a longer period (median duration = 5 days, 95% confidence interval [CI] = 4–5 days) compared with patients with $S. \text{sonnei}$ infections (median = 4 days, 95% CI = 2–4 days).
days, \( P = 0.006 \). The time from onset to presentation of both *Shigella* and non-*Shigella* patients was one day or less.

Of 331 culture-confirmed shigellosis patients, 301 (91%) could be followed until day 90. During the 90-day follow-up, two patients had a second episode of diarrhea that yielded a *Shigella* serotype different from the first episode. The first patient had an *S. flexneri* 1a infection followed by an *S. flexneri* 2a infection three months later. The second patient had an *S. flexneri* x variant infection followed by an *S. flexneri* 1a infection one month later. Two shigellosis patients had acute respiratory tract infections. One patient required hospitalization for cerebral hemorrhage due to an accident. Clinical sequelae were not detected, including the sequelae related to *Shigella* infection specifically looked for during each follow-up visit: persistent diarrhea, rectal prolapse, ileus, other gastrointestinal manifestations, pneumonia, seizure, and encephalopathy. None of the shigellosis patients died within 90 days of presentation. No case of persistent diarrhea was detected.

The most frequently prescribed therapies for diarrhea in the catchment area were antibiotics, oral rehydration solution, intravenous fluids, and antidiarrheal medications (Table 1). The three most commonly used antibiotics were oral gentamicin (33 of 100 episodes), norfloxacin (14 of 100 episodes), and ampicillin (9 of 100 episodes).

**Microbiologic features.** Of 331 *Shigella* strains isolated during the surveillance period, 306 (93%) were *S. flexneri* and 25 were *S. sonnei*. No *S. dysenteriae* or *S. boydii* were detected. Of the *S. flexneri* isolates, the three most common serotypes were 1a (34%), type X (33%), and 2a (28%). Seven (2%) strains were identified as serotypes 4 and five (1.6%) strains as type Y. There was one (< 1%) strain each of serotypes 2b, 3a, and 5b. Two strains were nontypeable.

Of 306 *S. flexneri* strains, 305 (99.7%) were resistant to cotrimoxazole. Less than 10% of the *S. flexneri* strains were resistant to ciprofloxacin, gentamicin, and norfloxacin. Twenty-four (96%) of 25 *S. sonnei* strains were resistant to both cotrimoxazole and nalidixic acid. Less than 10% of the *S. sonnei* strains were resistant to ampicillin, amoxicillin, ciprofloxacin, gentamicin, and norfloxacin (Table 2).

**DISCUSSION**

The shigellosis incidence rate observed in Zhengding was approximately 10 times higher than that reported by National Noticeable Infectious Diseases Report System. This is a passive surveillance system and the incidence rate of bacillary dysentery is based on clinical diagnosis. The most likely reason for the observed difference in incidence is the underreporting of shigellosis by the government report system, which varies between 0% and 100% depending on year and province. Furthermore, only 44% of the shigellosis cases detected in our study in Zhengding presented with dysentery. If

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-4</th>
<th>5-14</th>
<th>&gt; 15</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral rehydration solution</td>
<td>449</td>
<td>24</td>
<td>310</td>
<td>23</td>
</tr>
<tr>
<td>Intravenous fluids</td>
<td>238</td>
<td>13</td>
<td>190</td>
<td>14</td>
</tr>
<tr>
<td>Antidiarrheal</td>
<td>1,351</td>
<td>73</td>
<td>1,038</td>
<td>77</td>
</tr>
<tr>
<td>Other*</td>
<td>55</td>
<td>3</td>
<td>48</td>
<td>4</td>
</tr>
</tbody>
</table>

* No documentation found for specific therapy.
Table 2
Antimicrobial susceptibility pattern of Shigella isolates, Zhengding, Hebei Province, China, 2002

<table>
<thead>
<tr>
<th>Organisms treated/antibiotic</th>
<th>S. flexneri (n = 25)</th>
<th>S. sonnei (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone size (mm) No.</td>
<td>Zone size (mm) No.</td>
<td>Zone size (mm) No.</td>
</tr>
<tr>
<td>≤ 13</td>
<td>14-16</td>
<td>16-18</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>292</td>
<td>13</td>
</tr>
<tr>
<td>≤ 13</td>
<td>14-17</td>
<td>16-18</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>237</td>
<td>49</td>
</tr>
<tr>
<td>≤ 12</td>
<td>13-14</td>
<td>15-16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>≤ 15</td>
<td>16-20</td>
<td>18-20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>≤ 13</td>
<td>14-18</td>
<td>16-20</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>305</td>
<td>1</td>
</tr>
<tr>
<td>≤ 12</td>
<td>13-16</td>
<td>15-16</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>≤ 10</td>
<td>11-15</td>
<td>13-15</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>206</td>
<td>4</td>
</tr>
</tbody>
</table>

Increasing susceptibility to shigellosis due to a less effective immune response of older people. Differences in hygiene and education are another explanation for these observations. In previous studies, the household economic status and education levels correlated with diarrheal diseases in that household.16–19 The younger generation is likely to have benefited more from health education than their elders. An alternative explanation is referral bias due to differences in use of health care. It is possible that older people make more frequent use of health care providers than younger people. However, this explanation is less likely because no increase in overall diarrhea rates with age was detected. Since the highest incidence of shigellosis was in children < 5 years old and in those ≥ 60 years old, the elderly as well as children should be targeted for future preventive strategies such as vaccinations.

This study detected few clinical sequelae; no shigellosis-related hospitalizations or deaths were reported. This relatively benign presentation of shigellosis could be related to the care the patients received. Individuals residing in this rural area have access to a health care provider within 10 minutes. The village doctor-population ratio is very low, on average 500 residents share one village doctor.7 Diarrhea episodes, including shigellosis episodes, are treated early, nearly always within 24 hours of onset and 75% of patients reported receiving antibiotic treatment. Some severe diarrhea cases could have escaped the surveillance network and presented directly to county hospitals, although only a small fraction (< 5%) of patients make use of county hospitals as their first-line health care provider.7 Patients referred from village doctors to county hospitals would have been detected during follow-up visits.

Shigella strains resistant to multiple antimicrobial drugs have emerged in the study area, and ~100% of the Shigella strains in this study were resistant to nalidixic acid. Several Shigella strains resistant to ciprofloxacin and norfloxacin were also detected. The emergence of resistance against fluoroquinolones raises serious questions regarding adequate treatment of shigellosis in the future. The widespread use of oral gentamicin was surprising. The Shigella strains isolated by the study were susceptible to gentamicin, but the therapeutic efficacy of gentamicin for shigellosis if administered orally is increasing susceptibility to shigellosis due to a less effective immune response of older people. Differences in hygiene and education are another explanation for these observations. In previous studies, the household economic status and education levels correlated with diarrheal diseases in that household.16–19 The younger generation is likely to have benefited more from health education than their elders. An alternative explanation is referral bias due to differences in use of health care. It is possible that older people make more frequent use of health care providers than younger people. However, this explanation is less likely because no increase in overall diarrhea rates with age was detected. Since the highest incidence of shigellosis was in children < 5 years old and in those ≥ 60 years old, the elderly as well as children should be targeted for future preventive strategies such as vaccinations.

Our estimates underestimate the true shigellosis incidence rate for three reasons. First, 5,208 (32%) of 16,550 diarrhea patients presenting to health care providers refused to participate in the study. The most likely reason for refusal was the perceived indignity of having a rectal swab taken or having to provide a stool specimen. Second, passive surveillance based on detection in treatment centers was used. Patients who treat themselves, purchase drugs from pharmacies without medical consultation, or who had mild disease might not seek care and thus would be missed by the study. Similarly, patients using the county hospitals were not detected by the surveillance. Previous surveys of health care use in the study area indicated that no more than 5% of the residents would consider the county hospitals as first-line health care provider. Approximately 85% of all diarrhea patients would make use of the participating health care providers.7 Third, the standard laboratory methods used in this study could not detect all episodes of shigellosis, which are sensitive to changes in temperature and pH. Previous studies indicate that more sensitive laboratory methods could detect significantly higher numbers of Shigella isolates.14–15 Since the refusal of specific population groups could have biased our findings, we compared the age, sex, and socioeconomic and educational status of patients who participated and patients who refused to participate. There was no statistically significant difference between the two groups except that older patients more frequently refused to participate than younger patients. It is therefore likely that even more older individuals in the study area have shigellosis than we report here. An additional limitation is the 12-month study period. The serotype prevalence as well as other epidemiologic characteristics such as age distribution may change over time. Further studies over more extended periods would be desirable to get a better understanding of the epidemiology of shigellosis.

A surprising finding was that the incidence of shigellosis increased with age after age 30. Persons more than 60 years old had a shigellosis rate of 7/1,000/year, the second highest incidence rate in the study population. This may reflect an
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27. Sun D, Zhang F, Gen Y, 1997. Analysis on the serotype of controversial.20 The frequent irrational use of antibiotics documented in this study may have played a role in the emergence of resistant Shigella strains. The Chinese government is aware of this issue and has introduced legislation in August 2004 that forbids the sale of antibiotics without a prescription. Consistent with previous studies, the predominant Shigella species isolated in Zhengding was S. flexneri, followed by S. sonnei. Also consistent with previous studies, S. flexneri infections were followed by a more prolonged clinical course than S. sonnei infections.21,22 However, in contrast to previous observations,23–26 we found that S. flexneri serotypes X and 1a each represented one-third of all S. flexneri strains. These serotype profiles of S. flexneri have not been reported previously. Other studies from China have indicated the proportion of S. flexneri 2a as ≥ 70%.21,22,26,27 However, those studies on serotypes were hospital based and made used of locally produced antisera.

For long-term shigellosis control, ideal measures are improved water supply, sanitation, and general hygiene. An effective Shigella vaccine could help control shigellosis in the short and midterm.30,31 Keeping in mind the serotype-specific immunity of shigellosis, vaccine developers must consider the prevalent species as well as serotypes.32 Our findings highlight the importance of site-specific serotype profiles in the development of Shigella vaccines.


