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Original Article

Cholera Outbreak in Southeast of Iran: Routes of Transmission in the Situation of Good Primary Health Care Services and Poor Individual Hygienic Practices

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SUMMARY: Within the years 2001 to 2004, Sistan-va-Baluchestan was the only province with transmission of cholera in Iran. The objective of this study was to determine the epidemiological characteristics of the cholera outbreak that occurred in 2004 in the Sarbaz district in the southern parts of this province. The surveillance data were analyzed, and a matched case-control study was performed. From 22 October to 15 November 2004, from 2,242 diarrhea cases that were sampled for stool culture, 90 cases were positive for *Vibrio cholerae* O1 El-Tor biotype, serotype Ogawa. Multivariate analysis showed that risk factors for cholera were drinking beverages from street vendors (OR = 10.16, 95% CI: 2.55 - 40.50), illiteracy (OR = 5.76, 95% CI: 2.63 - 30.09), no hand washing with soap after toilet use (OR = 22.06, 95% CI: 2.91 - 167.11), no hand washing with soap before meals (OR = 3.64, 95% CI: 1.03 - 12.82), sex (OR = 3.73, 95% CI: 1.17 - 11.89) and eating food left over from previous meals without reheating (OR = 4.03, 95% CI: 1.23 - 13.18). The source of drinking water showed weak association with cholera only in univariate analysis (OR = 2.83, 95% CI: 1.12 - 7.19). The development of primary health care, even though it can improve the conditions that control the spread of an epidemic, is not enough of a control measure as long as the social hygienic standards are low and people do not follow the basic personal hygiene regulations.

INTRODUCTION

"Asiatic cholera," as it is sometimes called, has been endemic in south Asia, especially the Ganges delta region, from the time of recorded history. The current seventh pandemic began in 1961 when the *Vibrio cholerae* O1, biotype El Tor, first appeared as a cause of epidemic cholera in Celebes (Sulawesi), Indonesia. The disease spread rapidly and reached the USSR, Iran and Iraq in 1965 - 1966 (1). During the years 2001 to 2003, cholera showed a decreasing trend in Asia, but in 2004 officially reported cases of cholera from Asia increased by 66%. During these years the number of reported cases from Iran has remained stable within the range of 90 to 110 cases annually (2-5), and all confirmed cholera cases from Iran have been from Sistan-va-Baluchestan province, the largest province of Iran, with subtropical – tropical climatic condition, located in the southeast part of the country, near Afghanistan and Pakistan. The territory of this province has divided into 8 districts. In 2001 and 2002, 105 and 118 confirmed cases of cholera where reported, respectively, from 6 districts (Zahedan, Khash, Saravan, Iranshahr, Sarbaz and Chabahar) (2,3,6). In 2003, 96 confirmed cases were reported from Iran, and of these 75 (78.12%) were from Zahedan district (the capital of Sistan-va-Baluchestan province) and the remaining cases were from Khash district about 140 km south of Zahedan (4,6). In 2004, 94 confirmed cases were reported from Iran, and of these 90 were from Sarbaz district, located about 350 km south of Zahedan. The last mentioned outbreak that lasted about 25 days began in October 22.

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The outbreaks had a few common points. First, the focal points of all these outbreaks were located in rural areas. Second, the mean age of symptomatic confirmed cases usually was below 20 years. Third, all these outbreaks occurred in summer and early fall. The seasonality seems to be related to the ability of *Vibrios* to grow rapidly in warm environmental temperatures (7).

In Iran, primary health care (PHC) coverage has been increased markedly within the last decade, and some of the sanitary services such as safe drinking water are under strict supervision even in deprived rural areas of Sistan-va-Baluchestan province. In this situation, cholera outbreaks comprise special features. As mentioned previously, the last outbreak in Sarbaz began on October 22, 2004, when cholera was first reported in Jangal village, near the Pakistan border. The disease crept rapidly up to the Ashar area. Within 2 weeks of detection of the first case, the outbreak began to subside, and the last case was detected on November 15, 2004 in the Pishin region (Figure 1). The outbreak lasted about 25 days, and enforced surveillance activities continued for another 30 days, during which time no additional cholera case was detected.

Studies about cholera in these regions have mostly focused on descriptive epidemiology of cholera, and the risk factors and routes of transmission have mostly remained unrecognized (8-10). The main objective of this study, therefore, was to determine the epidemiological characteristics of the 2004 cholera outbreak in Sarbaz district in Sistan-va-Baluchestan province. The study was conducted as an outbreak investigation within 2 days of the report of an outbreak at the province health center. We have tried to highlight the risk factors for transmission identified by the results of a population-based case-control study, and discuss the new

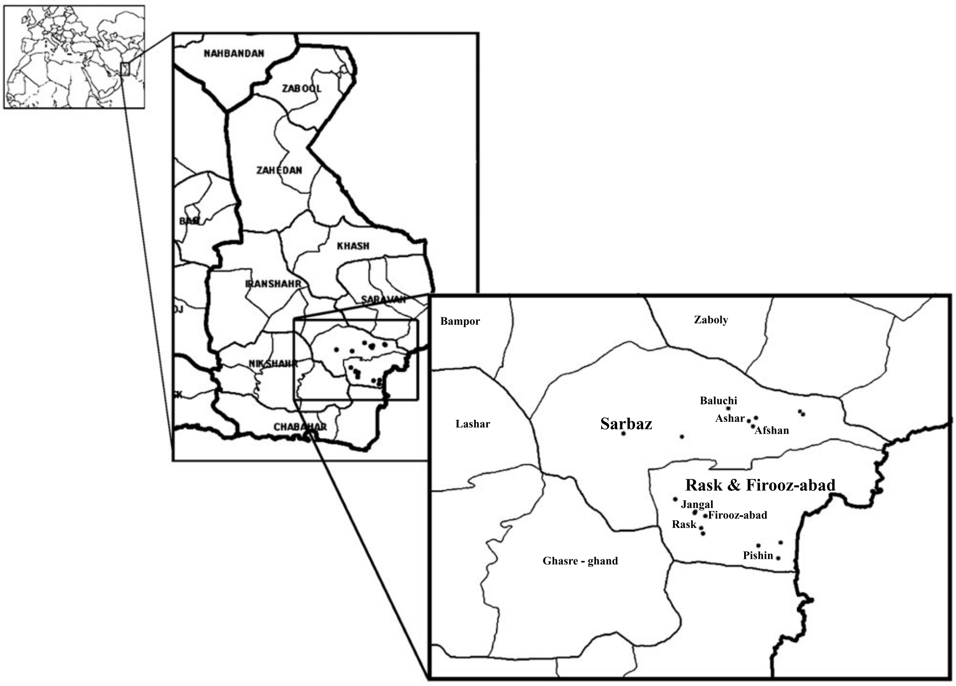

Fig. 1. Map of the involved regions of the Sarbaz district in the cholera outbreak of October and November 2004, Sistan-va-Baluchestan province, Iran. The points show the main involved villages.

aspects of cholera outbreaks in a rural society with good health services and poor individual hygienic practice. Even though the routes of transmission of cholera are well defined in most references, each outbreak has its own characteristics and deserves investigation.

MATERIALS AND METHODS

Sarbaz is a rural region bordering Pakistan, with a population of approximately 132,000 people living in 541 villages. The residents are primarily subsistence farmers and traders. At the beginning of the outbreak, the district had 12 rural health clinics and 60 health houses. A general practitioner was in charge of every rural health clinic, and the coverage of PHC services was about 63%. Only 65% of houses in the epidemic area had sanitary toilets, and 8 villages in the involved area did not have a water pipe network.

The study design was a hybrid of a descriptive epidemiological study and a case-control study. For the descriptive part, we used the information gathered by the surveillance system and for the case-control part, every cholera case who was positive in stool culture was compared with one healthy control (see below for details).

The surveillance for diarrheal diseases that is a routine activity and usually covers only the population under coverage of PHC was strengthened during the outbreak by organization of temporary mobile health care teams and a temporary field hospital. The population of the involved areas that was considered as the population at risk in this outbreak was about 41,000.

The surveillance system gathers information about demographic characteristics of all diarrheal cases including age, sex, address, the date of appearance of clinical signs, the date of stool sampling and the result of stool culture. For the purpose of surveillance in Sarbaz, all patients after October 22, 2004 with a history of watery diarrhea for any duration were considered as suspected cases of cholera and were sampled for stool culture. There was no age limitation for stool sampling. This policy was continued for 30 days after isolation of *V. cholerae* from the last culture positive case (i.e., until December 15, 2004). After November 15, 2004 none of the stool specimens was positive for *V. cholerae*.

As stated previously, we performed a case-control study at the involved villages in Sarbaz, enrolling nearly all culture positive cholera patients identified through surveillance and matching controls by age (index case's age ± 3 years). Controls were selected from the same villages of the residence of patients within 2 days of presentation by the cases and were excluded if they described any diarrheal episode (more than 3 loose stools per 24 h) in the preceding 30 days. An identical 2-page questionnaire (especially designed for this study) was filled out for cases and controls during an interview by two interviewers adept in local language to gather information about demographics, food and water consumption, and hygienic practices of cases and controls in the 5 days preceding their clinic visit (or their interview for controls). It was not possible to blind interviewers to the case-control status of the subjects. Cases were interviewed within at most 24 h of confirmation of their disease by the laboratory (usually within 3 days after presentation).

The following variables were checked: age, sex, nation, education level, household density per room (household size divided by number of living spaces), the source of drinking water, whether there was a refrigerator in the house, preservation method of food left over from previous meals, preparation method of foods left over from previous meals, individual hygiene after toilet use and before meals, eating and drinking from street vendors, getting ice from street vendors, chlorine test of drinking water, history of traveling of the subject or one of his or her relatives (e.g., father or brother) within the week before disease to one of the neighboring countries (Afghanistan and Pakistan), presence of soap in the hand-washing place, sanitary condition of the house-toilet and general knowledge about cholera (using multiple choice questions).

Laboratory surveillance for diarrheal pathogens, including *V. Cholerae* O1 and other vibrios, was conducted at three of the health facilities. Whole stools or rectal swabs were obtained from patients with diarrhea, and all specimens were immediately placed in Cary-Blair transport medium (MIRMEDIA, Khorramshahr, Iran) and were kept either in a refrigerator or on ice for same-day transport to microbiology laboratories. All the specimens arrived at the laboratory within 8 h of collection and were plated on thiosulfate citrate bile salt sucrose (TCBS) agar (Merck KGaA, Darmstadt, Germany) on the same day. *V. cholerae* was identified by previously published methods (11). Strains were serotyped by using polyvalent O1 and monospecific Inaba and Ogawa antisera (Pasteur Institute of Iran, Tehran, Iran). Rectal swabs were obtained from all controls and, using the same methods as used for all cases, were sent for culture in the same laboratories.

We defined a household as the group of people who live together in a house and cook their food in the same bowl. Most of the subjects (both cases and controls) were children who were not able to respond to many of the questions appropriately. In such instances one of the parents (almost always the mother) responded instead of the subject.

Data were analyzed using Statistical Package for Social Scientists (SPSS) v. 9 and Stata v. 6. For univariate analysis the odds ratios (OR) and their 95% confidence intervals (CI) were used. For multivariate analysis, we used conditional logistic regression modeling and calculated adjusted OR and 95% CI. To select a model, we used the backward elimination procedure, starting with a complex model and successively taking out terms. At each stage the term in the model that had the largest *P*-value was eliminated, and we checked that its parameters equaled zero. Maximum likelihood estimation was calculated by using the likelihood ratio test. Asymptotic standard errors were used to find confidence intervals for parameters in the model (12).

RESULTS

From October 22 through November 15, 2004, a total of 2,242 suspected cholera patients in Sarbaz district were reported to the province health center of Sistan-va-Baluchestan from 12 health clinics in the district (Figure 2). All patients were from rural areas. The population of the affected villages (the population at risk) was about 41,000, giving an attack rate of 5.7%.

All 2,242 patients were identified in rural health clinics where stool specimens were also collected. Ninety of these specimens yielded *V. cholerae* O1, and another 29 were identified as containing non-agglutinating vibrios. All *V. cholerae* O1 isolates were biotype El Tor, serotype Ogawa. Among the laboratory confirmed patients, there was only one cholera-related death reported, giving a case fatality rate of 1.11%. There was no other diarrhea-related death reported during the outbreak period.

Of the 90 patients confirmed as being affected by *V. cholerae* O1, 85 (94.4%) were matched to one healthy control from the village of residence of the patients, as was explained above. Table 1 shows some of the most important demographic characteristics of the enrolled subjects. Although in univariate analysis seven factors were associated with increased risk or protection from cholera, in multivariate analysis, six variables were independent risk factors for illness (Table 2).

The age range of cases was from 9 months to 70 years (median = 9 years), and 34 (37.6%) of all 90 culture-confirmed patients were less than 5 years old (32 of them were enrolled in the case control study).

A separate geographic analysis of all culture-confirmed cases revealed that patients from whom *V. cholerae* O1 was isolated were mostly clustered in three foci. Figure 1 shows the geographic location, and Figure 3 shows the time sequence of emergence of cholera in these three foci. Among 90 patients from whom *V. cholerae* was isolated, 40 (44.4%) lived in the Ashar region, 39 (43.3%) were from another cluster in

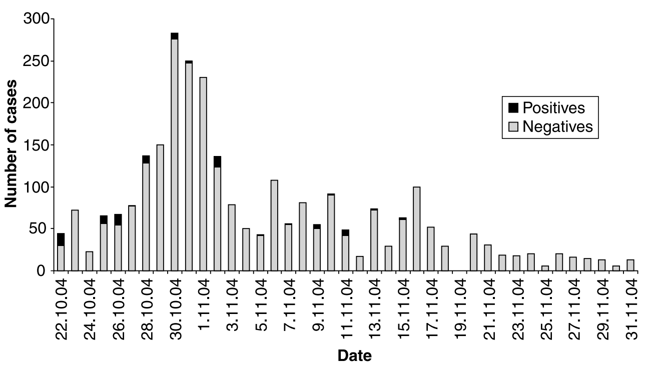

Fig. 2. Number of stool samples for cholera, by day of sampling and result of test, Sarbaz district, Sistan-va-Baluchestan province, October and November 2004.

Table 1. Characteristics of cases and controls, the cholera outbreak of autumn 2004, Sarbaz, Iran

Variables	Case *n* (%)	Control *n* (%)
Age (year)		
1 to 5	32 (37.6)	32 (37.6)
6 to 15	34 (40.4)	30 (35.3)
16 to 25	12 (14.1)	17 (20.0)
> 25	7 (8.2)	6 (7.1)
Education (year)		
Illiterate	43 (50.6)	52 (61.2)
1 to 3	21 (24.7)	11 (12.9)
4 to 6	12 (14.1)	20 (23.5)
> 6	9 (10.6)	2 (2.4)
Family density (person/room)		
2 to 5	26 (30.6)	36 (42.4)
6 to 10	55 (64.7)	46 (54.1)
> 10	4 (4.7)	3 (3.5)
Source of drinking water		
Tap water	58 (68.2)	69 (81.2)
River	3 (3.5)	2 (2.4)
Deep well	9 (10.6)	5 (5.8)
Aqueduct	6 (7.1)	2 (2.4)
Tanker and water containers	9 (10.6)	7 (8.2)
Sanitary condition of lavatory		
Fully sanitary	18 (21.2)	27 (31.8)
Unsanitary	61 (71.7)	53 (62.3)
Without lavatory	6 (7.1)	5 (5.9)
Presence of soap in the hand washing place		
Yes	33 (39.8)	41 (48.8)
No	50 (60.2)	43 (51.2)
Chlorine test of drinking water		
Chlorine positive	79 (96.3)	77 (93.9)
Chlorine negative	3 (3.7)	5 (6.1)

Table 2. Risk factors associated with cholera in the Sarbaz region of Sistan-va-Baluchestan province in southeast Iran, October – November 2004

Variable name	Case *n* (%)	Control *n* (%)	Univariate analysis Matched OR (95% CI)	Multivariate analysis Adjusted OR (95% CI)
Drinking beverages from street vendors			3.28 (1.41 - 7.66)	10.16 (2.55 - 40.50)
Yes	29 (34.1)	13 (15.3)		
No	56 (65.9)	72 (84.7)		
Illiteracy			2.50 (0.97 - 6.44)	5.76 (2.63 - 30.09)
Yes	43 (50.6)	52 (61.2)		
No	42 (49.4)	33 (38.8)		
Hand washing with soap after toilet use			5.67 (1.66 - 19.34)	22.06 (2.91 - 167.11)
No	72 (84.7)	58 (68.2)		
Yes	13 (15.3)	27 (31.8)		
Hand washing before meals			2.11 (0.96 - 4.67)	3.64 (1.03 - 12.82)
No	53 (62.4)	43 (50.6)		
Yes	32 (37.6)	42 (49.4)		
Sex			2.40 (1.15 - 5.02)	3.73 (1.17 - 11.89)
Male	43 (50.6)	29 (34.1)		
Female	42 (49.4)	56 (65.9)		
Eating remaining food from previous meals without reheating			2.71 (1.14 - 6.46)	4.03 (1.23 - 13.18)
Yes	45 (52.9)	34 (40.0)		
No	40 (47.1)	51 (60.0)		
Source of drinking water			2.83 (1.12 - 7.19)	–
Well, river, aqueducts, tankers	27 (31.8)	16 (18.8)		
Tap water	58 (68.2)	69 (81.2)		

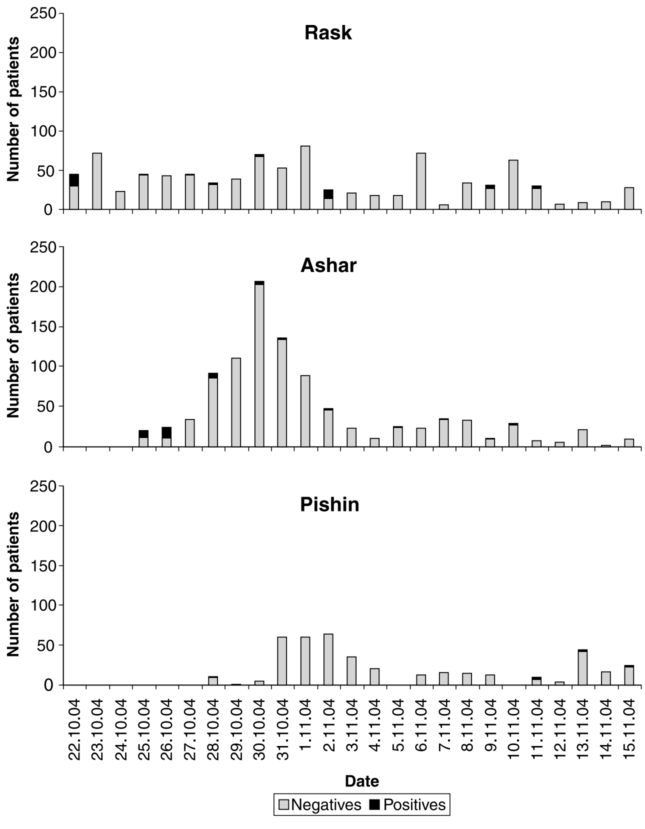

Fig. 3. Time sequence of clinic admission for cholera, by result of stool culture and geographic focus of occurrence, Sarbaz district, Sistan-va-Baluchestan province, Iran.

the Rask region, and the third cluster with 8 (8.9%) cases was located in the Pishin region near the Pakistan border.

DISCUSSION

As mentioned previously, the case definition for taking stool samples from suspected diarrheal patients was wider than that recommended by World Health Organization (WHO), and all age groups including those below 5 years old were sampled for stool culture (13). The result was interesting, as 34 (37.6%) of all 90 culture-confirmed patients were less than 5 years old. Considering the fact that Sistan-va-Baluchestan is endemic for cholera, this finding is not surprising; however, it appears to indicate strongly that the WHO recommendations with regard to age range in case definition of cholera in endemic areas needs reconsideration.

The outbreak of Sarbaz district in 2004 continued for less than 1 month and did not spread to other districts. This was the first time within the past 4 years that only part of a district was involved in an outbreak of cholera. In the years 2001 and 2002, six districts and in 2003, two districts were involved in cholera outbreaks. This may be proof of the effectiveness of control measures currently in place. The epidemic curves of outbreak in different regions show the sequence of spread of the disease in the area (Figure 3). A marked peak can be seen only in the Ashar area, where most of the villages do not have a water pipe system and so chlorination of water is more dependent on the hygienic practices of people and on the house to house visits of health workers. In some of these regions that have been suffering from drought for the past few years, there is no distinct place for people to go to get water, and chlorination of these scattered water sources is not so easy. In this situation and especially in regions that do not have a water pipe system the main approach for decontamination of drinking water and prevention of water-born diseases has been community education and the distribution of chlorination solution among households. However, in statistical analysis the source of drinking water (tap water versus other sources such as well, aqueducts and tankers) was not of particular importance and a statistically significant association between the disease and the source of drinking water was meaningful only in univariate analysis (OR = 2.83; 95% CI: 1.12 - 7.19). An examination of water samples from the houses of both cases and controls showed that almost all samples had a sufficient amount of chlorine (96.3 and 93.9%, respectively) (Table 1). Even though this finding validates the effectiveness of decontamination of drinking water by different methods, it also shows that water has not been the main source of transmission of cholera and increases the importance of examining other sources of cholera transmission, such as contaminated food and poor hygienic practices (Table 2).

A look at the list of the risk factors shows that some of the most important sanitary practices such as 'hand washing with soap after toilet use' have the highest association with the disease (OR = 22.06; 95% CI: 2.91-167.11). Previous studies in other societies have shown that washing hands with soap can decrease the risk of diarrheal disease by 47% (95% CI: 24 - 63%), and the promotion of hand washing with soap in homes in developing countries should become a public health intervention of choice (14,15).

The other important risk factor in this outbreak was 'drinking beverages from street vendors' (OR = 10.16; 95% CI: 2.55 - 40.50). This finding is not too surprising, and in many other outbreaks drinking and eating from street vendors have been mentioned as important risk factors (16-18). As can be seen in Table 2, 'eating remaining food from previous meals without reheating' had a strong relationship with catching cholera (OR = 4.03; 95% CI: 1.23 - 13.18). Such a relationship has been mentioned in outbreaks in other countries, and in fact a delay between cooking and eating provides *V. cholerae* with the required time for replication to reach a large inoculum which is needed to produce disease (15,19).

All the above findings point to the fact that despite suitable coverage of PHC, the hygienic standards of Sarbaz society at the individual level are poor and it can be seen clearly that even though the development of PHC improves the conditions that control the spread of an epidemic, this is not enough to overcome poor social sanitary standards when people do not follow basic personal hygiene regulations.

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