Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 5 (4): 233-238
October-December, 2005

Routes of Transmission of Cholera in the Border Areas of Zahedan District, Sistan and Baluchestan Province, Summer 2003

[1]Shahrokh Izadi, [2]Seyed-Mehdi Tabatabaei,
[3]Mohammad-Reza Miradi and [4]Khodadad Sheikhzadeh

The objective of this study was to identify the most important routs of transmission of cholera in the rural areas of Zahedan district involved in the outbreak of summer 2003. The outbreak lasted about 50 days. Twenty consecutive cholera cases positive for *Vibrio cholera* O1 in stool culture were compared with 89 controls sampled from the population. The cases have occurred during 28 July to 16 September 2003. In multivariate analysis age (OR=0.84, p=0.004), eating food in parties (OR=34.48, p=0.020), absence of soap in hand-washing place (OR=4.70, p=0.000) and household size (OR=1.30, p=0.002) were significantly associated with cholera. In univariate analysis, getting ice from street vendors also had relationship with catching cholera. Different transmission principles have been mentioned in the literature for cholera, however every outbreak has its own special patterns of transmission. Warning people about these patterns plays key role in controlling cholera outbreaks.

Key words: Case-control, cholera, Iran, Sistan and Baluchestan

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Dr. Shahrokh Izadi
Department of Epidemiology and Biostatistics,
School of Public Health,
Zahedan University of Medical Sciences,
Zahedan, P.O. Box 98135-759, Iran

Tel: +98 541 2442197
Fax: +98 541 2449877

ANSInet
Asian Network for Scientific Information

[1]Department of Epidemiology and Biostatistics, School of Public Health, Zahedan University of Medical Sciences, Zahedan, Iran
[2]Zahedan District Health Center, Iran
[3]Center for Disease Management of Zahedan District Health Center, Iran
[4]Center for Disease Management of Sistan and Baluchestan Province Health Center, Iran

INTRODUCTION

"Asiatic cholera", as it was sometimes called, has been endemic in south Asia, especially the Ganges delta region, from the time of recorded history[1]. 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 then spread rapidly and reached the USSR, Iran and Iraq in 1965-1966[2]. During the years 2001 to 2003 cholera had a decreasing trend in Asia. Within the past few years Iran, Afghanistan and Pakistan report the disease annually, however Iran with about 100 to 150 cases each year has had no important fluctuation in reported cases[3-7]. Every year in Iran the first cases of this disease are form Sistan and Baluchestan province and most cases occur in summer and early fall. In Afghanistan and Pakistan the story is the same i.e. most cases occur in summer[3,4]. The seasonality seems to be related to the ability of vibrios to grow rapidly in warm environmental temperatures[1].

In 2003, 96 confirmed cases were reported from Iran[7], of these 75 were from Zahedan district, which occurred during an outbreak in villages near Iran-Pakistan border. The outbreak began in the last week of July 2003 when a case of cholera was reported in Zahedan city. On 5 August 2003 the first locally transmitted case was reported from Mil-72, a small town near a customs post on Iran-Pakistan border. This outbreak last about 50 days and involved 11 villages near borderline.

Hear we highlight the risk factors for cholera transmission in Zahedan district, during the outbreak of summer 2003, identified by the results of a community based case-control study in the region.

MATERIALS AND METHODS

The source population is about 2054 people who are from 11 villages in the territory of Zahedan district located in the northern part of the Sistan and Baluchestan Province near the borders of Pakistan. The total population of Zahedan district is about 510000 people of this total population about 90% are settled in Zahedan city. In the rural areas of Zahedan district, population is highly scattered and villages are usually located in far distances from each other. This area is located in the neighborhood of Afghanistan and Pakistan to the east and from many years ago Afghan refugees constitute about 10% of the population of Zahedan district. Afghan refugees are mostly settling in the urban regions and some of them are residing transiently while some others are long-term settlers. There are also some nomadic populations that are continuously changing their settling (sometimes between both sides of the border) and their usual occupation is sheep and goat breeding and trading.

The Primary Health Care (PHC) system is well developed and almost all population has filed. The Primary Health Care coverage of Zahedan is about 95% (56 rural Health Houses and 34 urban Health Center). The health network in Iran follows a step-wise referral system. The patients are referred from health houses to urban health centers and if necessary the physician in charge of the rural health center will refer the patients to hospitals. All rural health centers have necessary transport facilities (such as cars and sometimes ambulances). Every year the Zahedan health center performs a local census in the rural areas including the study areas and files all households, that is very useful for health system researches. Zahedan always have had trouble with water. In the rural area, the usual water sources are deep wells and aqueducts.

In this population-based case-control study, 20 cholera cases were compared with 89 controls sampled from the population. The outbreak began since 28 July 2003 and last up to 16 September 2003. Cases were entered sequentially as they occurred from the beginning of the study up to the end of the outbreak. The beginning of the study was in the last weeks of the outbreak so only the last 20 cases were entered the study. All cases were both, positive for *Vibrio cholera* O1 in stool culture and were symptomatic (moderate to severe diarrhea).

For every case four to five controls were randomly selected using Zahedan health center rolls. Controls were frequency matched to cases by 5-year age group. The controls were selected and interviewed at most within four days of occurrence of cases and were excluded if they or anyone of their households, described any diarrheal episode (more than three loose stools per 24 h) in the preceding 30 days.

Rectal swabs were obtained routinely within the surveillance system, from all patients with diarrhea. Using Cary-Blair transport medium, specimens were sent for culture to the reference laboratory of the district health center. All the specimens were arrived at the laboratory within eight hours of collection and were plated on thiosulfate citrate bile salt sucrose agar (TCBS agar plates) the same day. *V. cholera* was identified by previously published methods[8]. Strains were serotyped by using polyvalent O1 and monospecific Inaba and Ogawa antisera. From all consent controls rectal swabs were obtained and using the same methods as used for cases, were sent for culture in the same laboratory.

An identical questionnaire was filled for cases and controls during an interview by two interviewers adept in

local language. It was not possible to blind interviewers to case-control status of the subjects. Cases were interviewed within at most 24 h of confirmation of their disease by the laboratory.

The following variables were checked: age, sex, nation, household density per room (household size divided by number of living spaces), the source of drinking-water, having refrigerator in house, the way of keeping the rest of the used food, the way of having the rest of the used food, individual hygiene after toilet and before meal, eating and drinking outdoors, getting ice from street vendors, chlorine test of drinking-water, history of traveling of the individual 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 house-toilet and, knowledge about cholera (using multiple choice questions).

Our definition for household is the 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 that were not able to respond many of the questions appropriately. In such situations one of the parents (almost always mother) responded instead of the subject.

Data were analyzed using SPSS version nine and Stata version six. For univariate analysis the odds ratios and their 95% confidence intervals, exact test and Chi-square for linear trend were used. For multivariate analysis, we used logistic regression modeling and calculated adjusted odds ratios and 95% confidence intervals. To select a model the backward elimination procedure was used, 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 it was checked that its parameters equaled zero. Maximum Likelihood (ML) estimation was calculated by using the likelihood ratio test[9]. Asymptotic Standard Errors (AES) were used to find confidence intervals for parameters in the model.

RESULTS

Totally 96 symptomatic diarrhea cases were reported during the outbreak which 75 of them were positive in stool culture (the remaining 21 specimens were reported as non-agglutinating group *Vibrio cholera*). The mean age of these 75 cases was 11.2 years (standard deviation = 12.6 years). Table 1 shows the age distribution of all the cases. As it can be seen more than 78% of the cases were below 15 years of age.

During the study 20 cases and 89 controls were compared with each other. The mean age of cases was 6.15 years (standard deviation = 5.32) and the mean age of controls was 9.29 years (standard deviation = 10.24). In student t-test there was no significant difference between cases and controls with respect to age.

Table 1: Age distribution of all the confirmed cases of the outbreak

Age groups	Frequency	%	Cumulative (%)
0-5 year	36	48.0	48.0
6-10 year	13	17.3	65.3
11-15 year	10	13.3	78.7
16-20 year	6	8.0	86.7
21-40 year	8	10.7	97.3
> 40 year	2	2.7	100.0
Total	75	100.0	

Table 2 shows some of the characteristics of cases and controls. For some of the variables the total does not come to 20 for cases or 89 for controls, which is due to vague recording in some of the questionnaires.

Eighty four percent of the controls (74 people) provided a stool specimen; none of these specimens yielded *V. cholera* O1. All isolates of the cases were biotype El-Tor, serotype Ogawa. Table 3 shows the age distribution of controls by the level of their co-operation in stool sampling. Age has inverse relationship with co-operation in stool sampling (the higher the age, the lower the co-operation in stool sampling) (linear trend, p=0.002). There was no other important difference between consent and non-consent controls.

In univariate analysis (Table 2), a relationship was found between being in the case group and the following variables: getting ice from street vendors, eating food in parties and absence of soap in the hand-washing place.

In multivariate analysis absence of soap in the hand-washing place, eating food in parties, age and household size, were independent risk factors for illness (Table 4). No interactions were significant among independent variables.

Even though in the questionnaire the source of drinking-water was asked as a semi-open multiple-choice question with nine options, the responses were limited to only three choices (Table 2). The water was transmitted from aqueducts or deep wells to residential places, by using 20 L cans or sometimes tankers.

When asking directly about individual hygiene, there is no relationship between washing hands with soap and catching cholera. There is no important difference between cases and controls with regard to the results of chlorine test of drinking-water samples and also with regard to knowledge about cholera (routs of transmission and prevention).

DISCUSSION

One of the important risk factors in this outbreak was eating food in parties. Usually cholera is considered as a

Table 2: Characteristics of cases and controls, the cholera outbreak of summer 2003, Zahedan, Iran

	Cases (n = 20)		Controls (n = 89)			
	Number	%	Number	%	Crude OR[a]	95% CI[b]
Sex						
Male	12.0	60.0	37	41.6	1.00	
Female	8	40.0	52	58.4	2.10	0.78-5.67
Age						
1 to 5 years	15	75.0	38	43.2	1.00	
6 to 10 years	2	10.0	19	21.6	3.75	0.77-18.11
11 to 15 years	1	5.0	19	21.6	7.50	0.92-61.11
16 to 20 years	2	10.0	8	9.1	1.57	0.29-8.31
> 20 years	0	0.0	4	4.5		
Nation						
Iran	11	55.0	50	58.1	1.00	
Afghan	7	35.0	24	27.9	0.75	0.26-2.19
Pakistan	2	10.0	12	14.0	1.32	0.26-6.76
Household size						
≤ 4	16	18.0	1	5.0	1.00	
5 to 7	32	36.0	5	25.0	2.50	0.27-23.23
8 to 10	33	37.1	11	55.0	5.33	0.63-44.98
11 to 12	5	5.6	2	10.0	6.40	0.47-86.34
≥ 13	3	3.4	1	5.0	5.33	0.25-110.79
Source of drinking-water						
Tap water	3	15.0	7	7.9	1.00	
Deep well	8	40.0	35	39.3	1.87	0.39-8.88
Aqueducts	8	40.0	33	37.1	1.76	0.37-8.39
Tap water and deep well	1	5.0	14	15.7	6.00	0.52-68.7
Use of remaining food						
Use it warm	4	22.2	18	20.7	1.00	
Use it hot	11	61.1	59	67.8	1.19	0.33-4.20
Discard it	3	16.7	10	11.5	0.74	0.13-3.99
Eating food in parties						
Negative	5	30.0	3	3.4	1.00	
Positive	14	70.0	86	3.4	10.23	2.19-47.69
Sanitary condition of toilet						
Fully sanitary	2	10.0	13	14.8	1.00	
Not fully sanitary	3	15.0	11	12.5	0.56	0.08-4.00
Insanitary	8	40.0	29	33.0	0.55	0.10-2.99
Without toilet	7	35.0	35	39.8	0.76	0.14-4.19
Soap in hand-washing place						
Absent	8	40.0	16	18.0	1.00	
Present	12	60.0	73	82.0	3.04	1.07-8.65
Ice from street vendors						
Positive	2	10.0	0	0.0	p-value = 0.032	
Negative	18	90.0	89	100.0	(Exact test, 2 sided)	

[a] odds ratio, [b]confidence interval

Table 3: Age distribution of controls by the level of their co-operation in stool sampling, the cholera outbreak of summer 2003, Zahedan, Iran

	Consent	Not consent
Age groups	Number (%)	Number (%)
1 to 5 years	35 (47.3)	3 (21.3)
6 to 10 years	17 (23.0)	2 (14.3)
11 to 15 years	16 (21.6)	3 (21.4)
16 to 20 years	4 (5.4)	4 (28.6)
> 20 years	2 (2.7)	2 (14.3)

Table 4: The results of logistic regression analysis, the cholera outbreak of summer 2003, Zahedan, Iran

Variable	Odds ratio	95% CI	p-value (Likelihood ratio test)
Age[a]	0.84	0.72- 0.97	0.004
Eating food in parties[b]	34.48	4.76-250.00	0.020
Soap in hand-washing place[c]	4.70	1.28-17.30	0.000
Household size[d]	1.30	1.07-1.57	0.002

[a]Year, [b]Eating/not eating, [c]Absent/present , [d] Person

water-born disease, however, food also has an important role in cholera transmission and in fact food and water each play their own part[1,13]. In the questionnaire eating outdoor was considered as semi-open multiple-choice question but all the positive responses were limited to eating food in parties and weddings. The point is that in other studies there is mostly stress on eating food in the funerals[13,14] or getting food from street vendors[11]. This point shows the importance of following sanitary regulations in weddings and parties in addition to funerals.

As it was mentioned the controls were frequency matched to cases by five-years age group and even tough in t-test there was no statistically significant difference between cases and controls however in

multivariate analysis age had relationship with the disease. This is due to the remaining effect of this important variable, in other words our matching has not been so effective in removing the effect of age completely. The effect of age is not under predictable if we think of the study area as an endemic area. In cholera endemic areas, the highest attack rates are in children aged two to four years[1].

Even though the relationship between getting ice from street vendors was statistically significant in univariate analysis in multivariate analysis this variable was eliminated from the model. However this variable deserves more attention. Regardless of sanitary regulations of the production process in the factory, the distribution system and selling condition of these ices is not sanitary at all and control of this situation is not so easy. The relationship between getting ice from street vendors and cholera transmission has been insisted upon in another studies too[11,12].

Doing usual sanitary practices and hand washing after toilet and before meal, were checked by means of 4 multiple-choice semi-open questions and as it was mentioned before, there was no statistically significant difference between cases and controls. But when the presence of soap in the hand-washing place was checked by interviewers (an objective proof), it was found out that there is a difference. This may be regarded as an obvious difference between knowledge and practice and in addition this finding also stresses on the importance of the objective data collections in comparison with subjective claims. On the other hand other studies have also shown similar relationships between washing hands and diarrheal diseases[10].

In chlorination test of drinking-water there was no significant difference between cases and controls. This can be the result of interventions of the Zahedan district health center that had been especially strengthened since the beginning of the outbreak. For several years these areas has been suffering from drought. In these areas there is not distinct places for people to go to get water and chlorination of these scattered water sources is not so easy. The main approach for disinfection of water and prevention of water-born diseases has been teaching of the community and distribution of chlorination solution between households.

In multivariate analysis household size had direct relationship with being in the case group. This may be attributed to the effect of overcrowding, even though close contact has not been mentioned as a typical way of transmission of cholera.

Endemic cholera is a highly preventable disease, requiring only proper sanitation and safe drinking water to reduce its transmission within a community[1]. However regardless of identical transmission principles, every community depending upon ecological, socioeconomic and cultural condition has its own patterns of transmission. An understanding of these conditions should help to limit and control outbreaks of cholera.

ACKNOWLEDGMENTS

This study was financially supported by Zahedan University of Medical Sciences. Our thanks are due to Dr. Aziz-Allah Jahantigh (Head of Sistan and Baluchestan Province health center), Dr. Jamshid Ordoni-Aval, Dr. Alireza Amirabadi and the other staffs of Sistan and Baluchestan Province Health Center and all the staffs of Zahedan Health Center for their special cooperation and help throughout the study.

In addition we are going to thank Mr. Gol-Mohammad Rigi, Mr. Mohammad Hosein Rigi and Mr. Mohammad-Jan Shahbakhsh for providing fieldwork for this study.

REFERENCES

1. Sack, D.A., R.B. Sack, G.B. Nair and A.K. Siddique, 2004. Cholera. Lancet, 17: 223-233.
2. Cholera, Fact Sheet N107; Revised March 2000. WHO/OMS, http://www.who.int/
3. Alam, M., Y.N. Akhtar and S.S. Ali *et al*., 2003. Seasonal variation in bacterial pathogens isolated from stool samples in Karachi, Pakistan. J. Pak. Med. Assoc., 53:125-129.
4. Abbas, Z., S.M. Jafri, S. Abid and H. Khan, 1995. Cholera admissions in adults 1989-1994: A hospital based study. J. Pak. Med. Assoc., 45: 91-93.
5. World Health Organization, 2002. Cholera 2001. Wkly Epidemiol Rec., 31: 257-265.
6. World Health Organization, 2003. Cholera 2002. Wkly Epidemiol Rec., 31: 269-276.
7. World Health Organization, 2004. Cholera 2003. Wkly Epidemiol Rec., 31: 281-288.
8. World Health Organization, 1993. Guidelines for cholera control. World Health Organization, Geneva, Switzerland.
9. Agresti, A., 1996. An Introduction to Categorical Data Analysis. New York, John Wiley and Sons Inc. pp: 127-129.
10. Curtis, V. and S. Cairncross, 2003. Effect of washing hands with soap on diarrhoea risk in the community: A systematic review. Lancet Infect. Dis., 3: 275-281.
11. Guthmann, J.P., 1995. Epidemic cholera in Latin America: Spread and routes of transmission. J. Trop. Med. Hyg., 98: 419-427.

12. Agressi, T.K., F. Cess, S. Claon and L. Kouadio, 2001. Salubrity of ice sold in blocks: From production to sales. Bull. Soc. Pathol. Exot., 94: 401-402.
13. Shapiro, R.L., M.R. Otieno and P.M. Adcock *et al.,* 1999. Transmission of epidemic *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from lake Victoria: An environmental reservoir for cholera? Am. J. Trop. Med. Hyg., 60: 271-276.
14. Gunnlaugsson, G., J. Einarsdottir, F.J. Angulo, S.A. Mentambanar, A. Passa and R.V. Tauxe, 1998. Funerals during the 1994 cholera epidemic in Guinea-Bissau, West Africa: The need for disinfection of bodies of perso ns dying of cholera. Epidemiol. Infect., 120: 7-15.