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• CLINICAL RESEARCH •

Acute diarrhea during army field exercise in southern China

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Abstract

AIM: During emergency period, infectious diseases can be a major threat to military forces. During field training in southern China, diarrhea is the main cause of nonbattle injury. To evaluate the causes of and risk factors for diarrhea in emergency period, we collected clinical and epidemiological data from the People's Liberation Army (PLA) during field training in southern China.

METHODS: From September 25 to October 2 1997, 2636 military personnel were investigated. Fecal sample cultures for lapactic pathogens were obtained from 103 military personnel with diarrhea. In addition, a questionnaire was administered to 103 cases and 206 controls to evaluate the association between illness and potential risk factors. At the same time, another questionnaire of 1:4 case-case control was administered to 22 severe cases (each severe case paired 4 mild cases).

RESULTS: The training troop's diarrhea incidence rate was significantly higher than that of garrison. The diarrhea incidence rate of officers was significantly lower than that of soldiers. A lapactic pathogen was identified in 63.1% (65/103) of the troops with diarrhea. *Enterotoxigenic Escherichia coli* (35.0%) and *plesiomona shigelloides* (16.5%) were the most common bacterial pathogens. All bacterial isolates were sensitive to norfloxacin and ceftazidine. However, almost all of them were resistant to sulfamethoxazole, trimethoprim-sulfamethoxazole, oxytetracycline, doxycycline, furazolidone, ampicillin and cloromycetin to a different degree. Risk factors associated with diarrhea included drinking raw water, eating outside, contacting diarrhea patients, lacking sanitation, depression, lacking sleep, which were established by multiple-factor logistic regression analysis. In addition, the unit incidence rate was associated with the density of flies and the average daily boiled water available by regression and discriminate analysis.

CONCLUSION: A series of risk factors are associated with the incidence rate of diarrhea. Our results may provide a useful basis for prevention and cure of diarrhea in emergency period of PLA.

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INTRODUCTION

Acute diarrhea (abbreviated diarrhea) is a common disease during peace and war period in the army[1-4]. It has been shown that the year incidence rate of diarrhea varied from 49.5% to 64.0% in the stationed army in southern China and the main pathogens were e*nterotoxigenic Escherichia coli* (ETEC) and *enteropathogenic Escherichia coli* (EPEC)[5]. Diarrhea is also a serious problem for military forces during an emergency period usually including war, military maneuver, dealing with emergency and providing disaster relief, field training, *etc*. In addition, diarrhea is the major cause of nonbattle injury[6,7]. The life style, sanitary system, and appliance of foreign armies are quite different from our army, therefore, the results from their study cannot be applied to our army[8-13]. To probe into the epidemic features, pathogen spectrum and the main risk factors of diarrhea during an emergency period and to provide the basis for taking preventive and therapeutic measures, we carried out an initial study during an army exercising in a coastal training field in southern China.

At the training base in the west of Leizhou Peninsula, weeds and bushes are overgrown and tall trees are rare. There is no other inhabitant except the garrison. The weather is harsh, with constant high temperature, high humidity, and plenty of rain. Insects, such as flies and midges, are present everywhere. Along the coastline, camps, kitchens, reservoirs, simple toilets, garbage disposal facilities, and other life support facilities have been established. At the base, the whole sanitary standard is low and the living condition is hard. During exercises, villagers nearby provide fried dishes, cold dishes, fruits, and other seafood products. None of the food has hygiene certificates. The source of drinking water is from the wells with self-prepared covers. The water supply is limited, and no laboratory test has been carried out to check the quality of the water.

MATERIALS AND METHODS

Concerned definition

As was recommended by WHO, the definition of diarrhea is attacking acutely, having three or more motions with properties changed. Those whose interval between two diarrheas exceeded 7 days were recorded twice. According to the clinical manifestations, cases were divided into group of severe cases and group of mild cases. Severe cases were defined as those with diarrhea of more than 5 stools or accompanied by fever (>=38 ℃).

Surveillance of disease

Our study lasted for 20 days, and was divided into transporting and assembling stage (8 days) and field training stage (12 days). At the first stage, diarrhea patients were investigated retrospectively by battalion medical officers because the army was on the move. We began formal surveillance at the second stage. Battalion medical officers and investigators registered all training soldiers and collected information about diarrhea attack twice a day (morning and evening). They also delivered fecal samples to laboratory for detection of pathogens before antibiotics were used.

Pathogen detection

The samples on LB were incubated in alkaline peptone solution

for 24 hours at 37 ℃. Next day 1 ml of culture medium was taken out and put into glycerin and paraffin oil separately. Detection of pathogens was based on the diarrhea detection rules of WHO[14]. ETEC was detected by a DNA probe, which was prepared by our department. The micro-biochemical tubes used to detect the germs of enterabacteriaceae, vibrio and serum of Shigella, Salmonella, EPEC and EIEC were bought from Lanzhou Institute of Biological Products.

The antibiotic susceptibility was performed by K-B method recommended by WHO[15]. The antibiotics included norfloxacin, ceftazidine sulfamethoxazole, trimethoprim-sulfamethoxazole, oxytetracycline, doxycycline, furazolidone, ampicillin and cloromycetin. Standard strain coliform bacillus ATCC25923 and staphulococcus aureus ATCC25923 and pseudomonas ATCC27853 were used for quality control.

Epidemiologic survey

The diarrhea patients identified by surveillance were investigated using "The army acute diarrhea epidemiological case survey form" and "System distress checklist (SCL-90)" in 48 hours by 1:2 case-control study. Controls were chosen from soldiers without diarrhea within 7 days in the same company, with the same sex, the same rank. The differences of age and time of military service were within 1 year. The 129 investigated factors in 15 categories in these two survey forms were analyzed with conditional one-way and multiple logistic regression analysis. To explore the relationship between environmental hygiene and the unit diarrhea incidence, unit environmental hygiene questionnaire of field training troop was filled by surveillance group. In addition, 1:4 case-case control was administered to 22 severe cases (each severe case paired 4 mild cases) to study the main factors of severe clinical manifestations.

Quality control

(1) In order to reduce the failure of report and to increase the collection rate of specimens, we strengthened propaganda and organization and tried to make officers and soldiers fully understand the meaning and purpose of our study. (2) All investigators were trained. (3) To ensure the quality of survey, the screening results from one or two companies were checked randomly and about 10% of the forms in the same day were reinvestigated to identify potential mistakes. (4) The collected fecal specimens were sent in iceboxes and examined by a full-time laboratory technician.

Statistic methods

The data were recorded and checked using SPSS10.0/PC statistical package (SPSS, Chicago). Proportions were compared using the chi-square test with Fisher's exact test. Conditional multiple logistic regression analysis was performed using SAS. Logistic models were established by the maximum-likelihood method. Confidence intervals were calculated by the method of Woolf for univariate analysis and logistic-regression parameter estimates and their standard errors were used for multivariate analysis[16]. A two-tailed P value of 0.05 was considered as statistically significant.

RESULTS

Diarrhea incidence rate

The personnel investigated consisted of 320 officers and 2 316 soldiers in the training troop, and 24 officers and 180 soldiers in the garrison. They were all males, with a mean age of 22 years (SD of 4 years, ranging from 18 to 48 years). There was no difference in constituent ratio in these two troops by statistic analysis (χ^2=0.025, P=0.874). During the whole training period, the diarrhea incidence rate was 7.32% (193/2 636). The diarrhea incidence rate at the second training stage was 4.10% (108/2 636), which was significantly higher than that of the permanent garrison in corresponding time (0.98%, 2/204). The incidence rate of each training company varied from 0.84% to 12.38%. There was also a significant difference among different companies in the same battalion (χ^2=11.105, P<0.001), but no difference was found in the same company. The severe cases who could not work due to diarrhea accounted for 20.4% of the patients with diarrhea.

Pathogen detection

We surveyed 108 diarrhea patients and collected 103 samples (including 37 cotton swabs of anus). The collection rate was 95.4%. Most of the samples were watery and loose stools; few were mucus or pus and bloody stool.

The pathogens found in our study included 72 strains of 6 types of lapactic bacteria (Table 1). The positive detection rate was 63.1% (65/103). The most common pathogen was ETEC, followed by plesiomonas shigelloides. The rate of Shigella and Samonella infection was relatively high. There were 7 samples in which two types of pathogens were detected at the same time. Campylobacter and Yersinia were not found because of the limited conditions on the spot.

Table 1 Pathogen detection rate

Pathogen	Number of patients	Detection rate (%)
ETEC	36	35%
Plesiomonas shigelloides	17	16.5%
Salmonella	6	5.8%
Shigella	5	4.9%
EIEC	3	2.9%
Aeromonas schubertii	2	1.9%
Aeromonas hydrophila	1	1%
Vibrio metschnikovi	1	1%
Vibrio vulnificus	1	1%

The susceptibility of seventy-two strains of lapactic bacteria to nine types of antibiotics was examined. It showed that all these bacteria were sensitive to norfloxacin (95.8%) and ceftazidine (100%). All the lapactic bacteria were resistant to sulfamethoxazole, trimethoprim-sulfamethoxazole, oxytetracycline, doxycycline, furazolidone, ampicillin and cloromycetin to a different degree (Table 2)[17,18].

Table 2 Resistance rate of pathogens to antibiotics

Antibiotics	ETEC *n**=36	Vibrio *n*=22	Salmonnlla *n*=6	Shigella *n*=5	EIEC *n*=3	Overall *n*=72
Sulfamethoxazole	91.7	68.2	83.3	100.0	66.7	83.3
Trimethoprim-Sulfamethoxazole	91.7	59.1	83.3	80.0	66.7	79.2
Oxytetracycline	69.4	77.3	100.0	100.0	100.0	75.0
Doxycycline	61.1	72.7	100.0	100.0	100.0	69.4
Furazolidone	16.7	90.9	50.5	100.0	33.3	52.0
Ampicillin	30.6	36.4	50.0	40.0	33.3	34.7
Cloromycetin	25.0	4.5	0.0	40.0	33.3	18.1
Norfloxacin	5.6	0.0	0.0	20.0	0.0	4.2
Ceftazidine	0.0	0.0	0.0	0.0	0.0	0.0

**n* was the number of strains.

Sixty-eight environmental specimens (drinking water, flies, seafood, cooking utensils of the training troops and local vendors) were examined for lapactic pathogens[19-22]. Forty-two strains of 10 types of lapactic pathogens were detected, including 14 strains of lapactic vibrio, 12 strains of aerobacter

cloacae, 8 strains of plesiomonas shigelloides, 8 strains of aeromonas, 7 strains of ETEC, 3 strains of shigella, 2 strains of salmonella and 1 strain of EIEC (Table 3). All the environmental specimens had lapactic pathogens to some extent, especially flies, seafood, and cooking utensils of the local vendors, which reached 87.5%, 80.0%, and 73.3%, respectively[23].

Table 3 Detection rate of lapactic pathogens from environmental specimens

Source of specimen	Number	Detection rate (%)
Flies	8	87.5
Seafood	10	80.0
Cooking utensils of vendors	15	73.3
Drinking water	15	66.7
Cooking utensils of troops	10	40.0
Camping appliances	10	20.0
Total	68	61.8

Risk factors analysis

Analysis of individual risk factors of diarrhea Unconditional single factor logistic regression analysis was performed, and the result showed that 25 factors were in association with individual diarrhea. In order to control interactions and confounding factors, and to make the studied factors more actually significant in theory, the above-mentioned 25 factors were entered to the equation of conditional multivariate logistic regression following the significant level <0.05, seven statistical significant variables were screened in the end (Table 4).

Analysis of unit risk factors of diarrhea Thirty-seven environmental sanitary questionnaires, which were checked to satisfy statistic requirements, were analyzed. Linear stepping regression was performed to determine the relationship between the 23 environmental factors with the incidence of field training troops. The result indicated that the density of flies in toilets, garbage disposal methods and daily average boiled water supply per person were the main factors. The regression equation was incidence=-3.107+2.051*density of flies in toilets + 1.601* garbage disposal methods - 0.743* daily average boiled water supply per person. The standard regression coefficient was 0.544, 0.264, and 0.201, respectively. The R square was 0.784. The variance analysis showed that F value of the regression equation was 40.34 (P<0.001).

To simplify the equation and improve the goodness of fit, ten curves estimation (quadratic, compound, growth, logarithmic, cubic, s, exponential, power, inverse and logistic) was used to fit the relation between the density of flies in toilets and the diarrhea incidence. R square of 5 curves estimation (power, compound, growth, exponential and logistic) was larger than that of linear regression equation, of which power' s R square reached 0.816 and the left four' s R square was 0.798.

The diarrhea incidence rates were further divided into 3 groups: ≤3%, 3-6%, ≥6%. According to the field environmental sanitary questionnaire and the incidence, stepping discriminate function was used to establish the discriminate function. The discriminate function was composed of six variables (density of flies in toilets, toilet disinfection, density of flies in garbage dump, disposal method of garbage, density of flies near waste water and use of anti-fly cover) (Table 5). The false discriminate rate was 0%, 13.3% and 11.1%, respectively. The total false discriminate rate was 91.9%.

Table 5 Linear discriminant function coefficients

Factor	Linear discriminant	Function coefficient	
	<3%	3%-6%	≥6%
Disinfection of toilets	29.354	46.645	45.882
Garbage disposal methods	-14.564	-34.839	-25.324
Density of flies in toilets	9.127	7.143	19.911
Density of flies in garbage dumps	8.753	16.595	16.056
Density of flies near waste-water	15.855	38.361	27.858
Use of anti-fly cover	21.243	34.128	32.386
Constant	-46.378	-117.197	-133.264

Analysis of case-case control study Twenty-two case-case control study questionnaires were collected, 20 of them had no missing data and were analyzed by single factor conditional logistic regression. The result showed that 30 possible risk factors had a relationship with severe symptoms. Drinking raw water within seven days, eating outside, not washing hand before eating, were then analyzed by conditional logistic multiple-regression (Table 6).

DISCUSSION

The diarrhea incidence rate during the training stage was 4.10%, which was significantly higher than that of the garrison at the corresponding time (0.98%) and much higher than the 10 day incidence rate (1.35%) of an stationed army in the same season[5]. Because of the differences in duration, location and diagnostic standard in an emergency period, the rates could not be directly compared, however, the increase of diarrhea incidence rate was a common phenomenon during emergency. There must be some factors that contribute to the increased diarrhea incidence, and

Table 4 Multiple-factor conditional logistic- regression results of case-control study

Factor	Regression coefficient	Standard error	Odds ratio	95%Confidence interval	P value
Drinking raw water	3.1460	0.4638	23.2148	9.3641-57.6862	0.0000
Eating outside	3.1365	0.6055	23.0229	7.0273-75.4280	0.0000
Contacting with patients	2.9447	0.5707	19.0055	6.2104-58.1625	0.0000
Lack of hygiene knowledge	1.7776	0.5071	5.9155	2.1893-15.9833	0.0005
Depression	11654	0.3633	3.2071	1.5735-6.5364	0.0013
Not frequently cutting fingernails	1.0504	0.3888	2.8588	1.3341-6.1258	0.0069
Lack of sleep	0.6555	0.21264	1.9261	1.2360-3.0016	0.0038

Table 6 Multiple-factor conditional logistic regression results of case-case control study

Factors	Regression coefficient	Standard error	Odd ratio (OR)	95% confidence interval	P value
Drinking raw water	3.8610	1.1724	47.5119	4.7738-472.8718	0.0010
Eating outside	2.5711	0.7506	13.0802	3.0040-56.9537	0.0006
Not washing hand before eating	1.3445	0.6142	2.7487	1.1519-12.7877	0.0286

if we can find these factors and control them, we should be able to strengthen the fighting capacity of the army.

The incidence rate of officers was clearly lower than that of soldiers, which was not in accordance with the results from stationed army and other related researches[24]. For example, a research of Hyams in "desert shield action" suggested the incidence rate was not affected by age and rank, while another study showed that the risk of officers was a little higher than that of soldiers. More data are still needed to confirm the real features of our army[25]. According to our study, the possible explanation was that the training and working of soldiers were more intensive, meanwhile their hygiene habits were worse than officers. In addition, they drank raw water and eating outside more often than officers[26].

From our research, the time distribution of diarrhea can be concluded as follow. The number of patients increased obviously in the early days of an action, then became stable after three or four days, and sustained at a certain rate[27]. During this period the incidence rate in each unit of the army was different and small outbreak could be seen occasionally, which was similar to some conclusions from foreign armies. The possible reasons of the increased rate during the early time were as follows. Normal living pattern of soldiers was disrupted and physically weakened. During training, soldiers increased significantly their contact with environments and the chances of infection were also increased. Failure to adapt to a new environment and lack of immunity, and no sanitary and anti-epidemic measures were taken. These possible reasons still need to be further explored. To control the incidence peak at early stages, we should strengthen propaganda on hygiene and enhance soldiers' abilities to self-guard against diarrhea. In addition, antibiotics should be used to prevent special diarrheas (such as tourist diarrhea, soldier diarrhea with special task_), but attention should be paid to selection and time limit of antibiotics[28,29]. With the army' s adapting to the environment and the perfection of preventive measures, the incidence rate maintained at a lower degree. However, because of the existence of many unhygienic temporary food stalls outside the camps and soldiers often eating outside, outbreak of different scales did take place[30,31]. Apart from propaganda, we should strengthen the discipline and forbid soldiers to eat outside the camps.

In spite of the failure to detect Campylobacter and Yersinia due to limited condition on spot, 61.3% detection rate indicated that bacterial diarrhea was a main cause in Summer and Autumn during field training in coastal area in southern China. It was reported ETEC was the most common pathogen in stationed army[32] and so was in our study, which was in accordance with some reports from American army[8]. It suggested that ETEC was one of the focal points in diarrhea prevention in army, no matter Chinese or foreign[8,33-35], stationed or training in field. We should enhance the basic clinical and preventive researches on diarrhea to guarantee fighting capacity. Moreover, we detected a relatively high rate of vibrio, especially that of plesiomonas shigelloides which was 16.5%, at the second place[36]. This may be correlated with training at coastal area as water, food, and articles for daily use contain high counts of such pathogens (results not shown). Many studies showed that this germ had a relatively high detection rate in southeast coastal area.

It is important to evaluate hygiene standards of our army and develop related education to analyze individual risk factors of diarrhea. The incidence rate will decrease markedly if we can efficiently control these risk factors. As shown in Table 4, after multi variable analysis seven variables emerged as significant risk factors, which were drinking raw water, eating outside, lack of hygiene knowledge, not frequently trimming nails, contacting with diarrhea patients, having no enough sleep and depression. Of these factors the first two were consistent with the results from stationed army, which indicates that the two risk factors are common in our military officers and soldiers. So it is necessary to strengthen sanitary education, to enforce administration, and to change unhealthy habits. During the survey, we found that none of the patients was separated from others and all the patients ate, slept and trained with the healthy personnel, which made contact transmission more easily.

For a long time, mental factors of diarrhea in an emergency period have been ignored. To explore the relationship between mental factors and diarrhea in an emergency period, we used "SCL-90" widely used in foreign countries, especially in mental health field. In a study, the mental condition of new recruits and the influence of each mental factor on injury in military training were analyzed by "SCL-90" in our army, and evaluation of the results was excellent. It indicated that using "SCL-90" to analyze the mental factors was feasible[37-40]. In an emergency period, especially in modern warfare, intense danger and cruelty would cause a great pressure on serviceman' s mentality. Therefore, mental factor may be a risk factor of diarrhea in an emergency period. In our study, 1:2 case-control study was performed to evaluate the relation between mental factor and diarrhea. The result of single-factor analysis suggested that the gross score and 9 factors (except paranoiac factor) were associated with diarrhea. In addition, the result of multiple-factor analysis suggested that depression was a risk factor of diarrhea, as only depression was entered to the aggressive equation. Whether this relation exists or not is still a problem worth further studying. The possible explanation is that mental factor, acting through the neuroendocrinic system, can lead to enterocinesia and gastroenteric secretion disorder or weaken the immune functions, so the body is more susceptible to pathogens, resulting in diarrhea[41,42].

Case-case control study has been used to analyze the risk factors of chronic infant diarrhea[40] but not used to analyze that of diarrhea in a military emergency period. For the first time, we used it to analyze the risk factors of diarrhea in an emergency period. During the emergency period, it was critical to deal with the problems which influenced the fighting capacity due to limited human and material resources. Our study demonstrated that 20.4% of diarrhea patients could not work, because of the severe clinical manifestations. If these patients can be prevented from diarrhea, the fighting capacity will be greatly increased. In our study, the result indicated that three risk factors (drinking raw water 7 days before diarrhea, eating outside, not washing hand before eating) had a relation with severe clinical manifestations. Overall, the result indicated that preventive measures should be taken to control the three risk factors.

No report has been published on using unit incidence as risk factors to prevent diarrhea. In our study, the unit diarrhea incidence rate was different. To maintain the fighting capacity, decreasing the unit incidence rate is important. If we can find out the unit risk factors, measures can be taken to control them. The result of linear stepping regression showed that three (density of flies in toilets, garbage disposal methods and daily average boiled-water supply) out of 23 potential factors might be the cause of the increased unit incidence. From the standard regression coefficient, the density of flies in toilets had the major influence on diarrhea incidence rate. The higher the density of flies in toilets, the higher the diarrhea incidence rate. Fly is an important route of transmission, which has been confirmed before. The study of the Gulf War also demonstrated that persistent existence of flies was an important reason of low-epidemic diarrhea. Meanwhile, we thought the garbage disposal methods have a relation with the density of flies. Some troops buried garbage by the sanitary unit strictly, thus preventing the reproduction of flies.

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