Epidemiol. Infect. (2015), **143**, 1020–1028. © Cambridge University Press 2014
doi:10.1017/S0950268814001599

Risk factors for sporadic *Vibrio parahaemolyticus* gastroenteritis in east China: a matched case-control study

W. X. YAN[1], Y. DAI[2], Y. J. ZHOU[2], H. LIU[3], S. G. DUAN[3], H. H. HAN[1] AND Y. CHEN[1]*

[1] *Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing, People's Republic of China*
[2] *Institute of Food Safety and Assessment, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, People's Republic of China*
[3] *Department of Food Hygiene and Foodborne Disease Control, Shanghai Municipal Center for Disease Control and Prevention, Shanghai, People's Republic of China*

Received 5 August 2013; Final revision 26 January 2014; Accepted 4 June 2014; first published online 3 July 2014

SUMMARY

To determine risk factors for sporadic *Vibrio parahaemolyticus* gastroenteritis, we conducted a population-based case-control study in sentinel hospital surveillance areas of Shanghai and Jiangsu province, China. Seventy-one patients with diarrhoea and confirmed *V. parahaemolyticus* infections were enrolled, and they were matched with 142 controls for gender, age and residential area. From the multivariable analysis, *V. parahaemolyticus* infections were associated with antibiotics taken during the 4 weeks prior to illness [odds ratio (OR) 8·1, 95% confidence interval (CI) 1·2–56·4)], frequent eating out (OR 3·3, 95% CI 1·1–10·1), and shellfish consumption (OR 3·2, 95% CI 1·0–9·9), with population-attributable fractions of 0·09, 0·25, and 0·14, respectively. Protective factors included keeping the aquatic products refrigerated (OR 0·4, 95% CI 0·1–0·9) and pork consumption (OR 0·2, 95% CI 0·1–0·8). Further study of the association of *V. parahaemolyticus* gastroenteritis with prior antibiotic use and shellfish consumption is needed.

Key words: Case-control studies, China, gastroenteritis, risk factors, *Vibrio parahaemolyticus*.

INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative halophilic and mesophilic bacterium that occurs naturally in estuarine and marine environments worldwide [1, 2]. It is an important human pathogen that causes acute gastroenteritis, generally associated with the consumption of raw or undercooked seafood [3–5]. Thermostable direct haemolysin (TDH), TDH-related haemolysin, and the type III secretion system, contribute to the pathogenicity of *V. parahaemolyticus* [6–8]. This pathogen is the leading cause of seafood-associated gastroenteritis in the USA and a common cause of foodborne illness in many Asian countries, including China, Japan and India [9–11].

Most epidemiological information about *V. parahaemolyticus* infections in China comes from outbreak investigations. Meat and meat products are the most common vehicle of infection in reported outbreaks of *V. parahaemolyticus* infection. Of the 322 outbreaks of *V. parahaemolyticus* infection reported to China National Foodborne Diseases Surveillance Network from 2003 to 2008, meat and meat products were the most frequently implicated food and were associated with 58 (18·0%) outbreaks [12]. However, meat is unlikely to carry *V. parahaemolyticus*.

* Author for correspondence: Dr Y. Chen, Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, 7 Panjiayuan Nanli, Chaoyang District, Beijing 100021, People's Republic of China. (Email: chenyan@cfsa.net.cn)

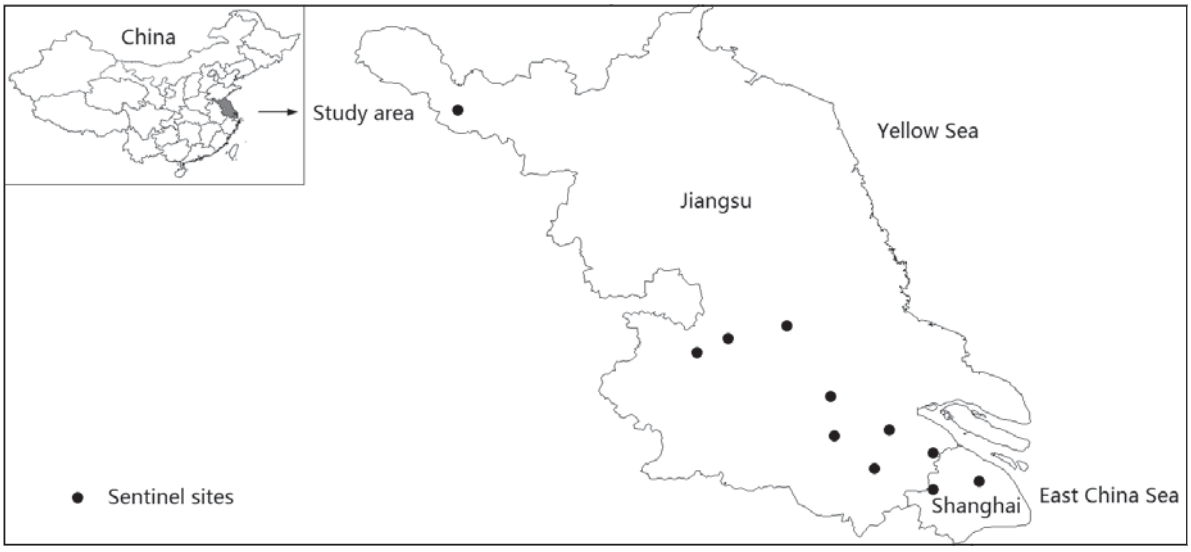

Fig. 1. Map of the sentinel sites in Shanghai and Jiangsu, China.

Whenever meat is implicated in an outbreak, the meat has probably been cross-contaminated with other foods. Although implicated food vehicles were identified in outbreaks, food vehicles in sporadic cases are difficult to detect. However, as shown by data from Japan and the USA, most cases of *V. parahaemolyticus* infection are sporadic, with outbreaks less common [13, 14]. Active surveillance data suggest that in agreement with previous studies, most *V. parahaemolyticus* cases in China are sporadic (data not shown). Epidemiological studies of sporadic *V. parahaemolyticus* infection are limited and there has been only one published case-control study of risk factors for sporadic *V. parahaemolyticus* infection in the USA [15]. Given the increasing prevalence of *V. parahaemolyticus* infection in China, it is important to elucidate the epidemiological characteristics of this pathogen in order to develop appropriate evidence-based prevention and control strategies. Therefore, we conducted a matched case-control study to identify risk factors that could provide leads for preventing sporadic cases of gastroenteritis attributable to *V. parahaemolyticus*.

METHODS

Sentinel hospital surveillance

Sentinel hospital surveillance was conducted at 11 sentinel sites in two coastal provinces, Shanghai (2) and Jiangsu (9), in east China from 1 July 2010 to 30 June 2011 (Fig. 1). The sentinel sites were selected based on their suitability, cooperation of local authorities and feasibility of completing the studies. The sentinel sites were, with the number of participating hospitals in parentheses: (*a*) Shanghai [Luwan (4) and Qingpu (4) district]; (*b*) Jiangsu [urban area (3) and Jiangyin county (4) of Wuxi prefecture; urban area (3) of Xuzhou prefecture; urban area (3), Changshu county (4) and Taicang county (4) of Suzhou prefecture; urban area (3) and Yizheng county (4) of Yangzhou prefecture; and urban area (3) of Taizhou prefecture]. The sentinel hospital surveillance area covered an estimated population of 14 180 386. Of the 39 hospitals participating in the study: 11 (28%) were tertiary hospitals, 10 (26%) were secondary hospitals, and 18 (46%) were primary hospitals.

Cases

Eligible case-patients were individuals aged $\geqslant 1$ year with diarrhoea who had *V. parahaemolyticus* isolated from stool samples, and resided in the surveillance area. Case-patients were excluded if their primary residence was outside the sentinel site, were not accessible (e.g. did not have a home telephone), or if they were identified as secondary cases arising from a single household. Case-patients from recognized outbreaks were not included.

Controls

For each case-patient enrolled, two controls matched for gender, age group and residential area (city block in the urban setting and village in the rural setting) were selected. We chose these factors to

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press

eliminate them as potential confounders. Controls were matched to case-patients using the following age groups: 1–5, 6–17, 18–39, 40–59, and ⩾60 years. Potential controls were excluded if they had reported diarrhoea, nausea, vomiting and abdominal pain in the 30 days prior to interview. Controls were interviewed as soon as possible and within 28 days of the case-patient's interview.

Questionnaire

Interviews were performed by trained health workers using a standard questionnaire. The questionnaire was validated and developed specifically for the purposes of this study. It was at the discretion of the parent or guardian as to whether an individual aged 12–18 years was interviewed directly. Information from individuals aged <12 years was obtained from the parent or guardian who was most familiar with their diet and behaviour. Verbal informed consent was obtained from all respondents before the interview. Surveys were conducted in Chinese. The questionnaire and study protocol were reviewed and approved by the Committee on Human Experimentation of the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention (CDC).

For case-patients, all questions related to the 5-day period preceding their onset of diarrhoea, except for prior use of antibiotics or antidiarrhoeals, which were based on the preceding 4 weeks. Controls were asked about the 5 days or 4 weeks prior to interview. Data collected included demographics; pre-existing illness; previous medication use; travel history (travel to other city/province); animal contact; refrigeration of certain aquatic products (i.e. fish, shrimp, crab and shellfish, whether of freshwater or saltwater origin) at home; other family kitchen practices; food consumption; meals eaten outside of the home; and drinking water source. Case-patients were also asked questions about their illness.

Laboratory methods

Hospitals were responsible for collecting stool specimens from patients with diarrhoea, and CDC laboratories for testing stool specimens for *V. parahaemolyticus*. Rectal swabs or stool specimens were enriched in alkaline peptone water or sodium chloride Violet Purple enrichment broth (6/11 local CDC laboratories used the enrichment broth to increase the sensitivity of stool culture), followed by subcultivation onto thiosulfate citrate bile salts sucrose agar, sodium chloride sucrose agar, or CHROMagar Vibrio agar (CHROMagar, France) for the isolation of *V. parahaemolyticus*. The plates were incubated at 37 °C for 18–24 h.

Sample size

The sample size of the study was calculated using shellfish consumption as the most important exposure to be assessed. The expected number of cases in the study region for a 12-month period was about 68. Based on a case-control ratio of 1:2, this number would allow the detection of an association with an odds ratio (OR) of 3, at the 5% significance level with 80% power (assuming 10% of controls are exposed). Power calculations were performed using PS 3·0 [16].

Data analysis

Interview data were entered into EpiData v. 3.1 (EpiData Association, Denmark). All the data analyses were performed using the software package SPSS v. 16.0 (SPSS Inc., USA).

The data were analysed using conditional logistic regression for matched case-control studies [17]. ORs and 95% confidence intervals (CIs) of individual factors were obtained. Statistical significance was assessed using the likelihood ratio test. Variables significantly associated with infection at a P value <0·10 by univariable analysis were included in a multivariable conditional model. If two or more independent variables were significantly associated with each other ($P<0·05$), the more biologically plausible variable was included in the model and the other was discarded. In multivariable analysis, to determine the variables in the model we used forward elimination method. Variables with $P<0·05$ were included in the final model. Interactions among the factors were examined in the logistic models.

Population-attributable fraction (PAF)

The PAF is the proportion of all cases attributable to a particular risk factor. We estimated the PAF for the factors from the results of the multivariable logistic regression models, using the formula: p(OR – 1)/OR, where p is the proportion exposed among cases [18].

RESULTS

Sentinel hospital surveillance

From July 2010 to June 2011, the isolation of *V. parahaemolyticus* from stool specimens was: Shanghai,

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press

Table 1. *Socioeconomic characteristics of case-patients and controls enrolled in a population-based case-control study to identify risk factors for* V. parahaemolyticus *gastroenteritis, China, July 2010–June 2011*

Socioeconomic characteristics	Cases ($n=71$) No. (%)	Controls ($n=142$) No. (%)	*P* value
Education			0·493
Primary school and lower	7 (9·9)	12 (8·5)	
Secondary school	16 (22·5)	29 (20·4)	
High school and above	48 (67·6)	101 (71·1)	
Total family income per year			0·731
0–29 999 yuan*	8 (11·3)	20 (14·1)	
30 000–79 999 yuan	42 (59·2)	80 (56·3)	
⩾80 000 yuan	17 (23·9)	40 (28·2)	
Refused to answer	4 (5·6)	2 (1·4)	

* 10 yuan = 0·95 British pound sterling.

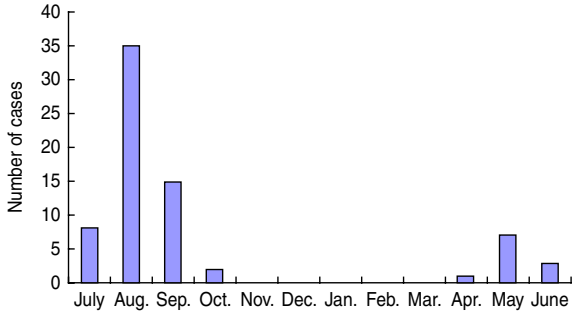

Fig. 2. Date of onset of *V. parahaemolyticus* gastroenteritis by month, China, July 2010–June 2011.

1·1% (48/4548); Jiangsu, 2·2% (52/2328). Of the 100 *V. parahaemolyticus* gastroenteritis cases reported from the catchment area, 29 were excluded from the study because the subjects were not accessible (22 from Shanghai and seven from Jiangsu). The case-control study recruited 71 case-patients of *V. parahaemolyticus* gastroenteritis and 142 matched controls over the 12-month period. The education level and income of cases and controls were similar (Table 1).

Clinical characteristics

Case-patients were interviewed with a mean delay of 7 days (range 3–18 days) from the onset of symptoms. Controls were interviewed with a mean delay of 5 days after case interview (range −1 to 24 days). One case-patient was interviewed 1 day later than the matched controls. Forty-one (57·7%) case-patients were female; median age was 34 years (range 2–77 years). Only one case-patient was aged <5 years. Seven case-patients had taken an antibiotic in the 4 weeks before onset of illness. Seven reported underlying medical conditions: chronic gastrointestinal illness (3), hypertension (3), haemorrhoids (1). Dates of onset of illness of case-patients were distributed from April to October, with the peak in August (Fig. 2).

Table 2. *Clinical characteristics of case-patients infected with* V. parahaemolyticus*, China, July 2010–June 2011*

Characteristics	No.	%
Diarrhoea	71	100·0
Abdominal cramps	61	85·9
Nausea	40	56·3
Vomiting	36	50·7
Tenesmus	26	36·6
Fever	24	33·8
Chills	18	25·4
Joint pain	8	11·3
Constipation	3	4·2
Bloody stool	2	2·8
Hospitalized	4	5·6
Received antimicrobials	38	53·5
Received antidiarrhoeal agent	37	52·1

The most frequently reported symptoms besides diarrhoea were abdominal cramps and nausea (Table 2). Among case-patients, 14% reported having had ⩾10 stools per 24-h period. The median duration of illness was 2 days (range 1–7 days). Hospitalization was reported by 6% of the case-patients; the median duration of hospital stay in this group was 2 days. None of the case-patients died. Use of antimicrobial medications and antidiarrhoeal agents was common; 54% (38/71) of case-patients reported taking an antimicrobial medication as a result of their illness, and

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press

Table 3. *Univariable analysis of risk factors associated with* V. parahaemolyticus *gastroenteritis, China, July 2010–June 2011*

Risk factor	Cases ($n=71$) No. (%)	Controls ($n=142$) No. (%)	OR (95% CI)
Took antibiotics in the 4 weeks prior to illness	7 (9·9)	2 (1·4)	7·0 (1·5–33·7)
Frequency of eating out in the past 5 days			
1–2 times	25 (35·2)	37 (26·1)	2·7 (1·3–5·5)
⩾3 times	25 (35·2)	36 (25·4)	3·8 (1·5–9·8)
Ate shellfish in the past 5 days	14 (19·7)	13 (9·2)	3·1 (1·2–8·1)
Keep aquatic products refrigerated in the past 5 days	25 (35·2)	73 (51·4)	0·3 (0·1–0·7)
Ate poultry other than chicken in the past 5 days	20 (28·2)	62 (43·7)	0·4 (0·2–0·9)
Consumed pasteurized milk in the past 5 days	23 (32·4)	69 (48·6)	0·4 (0·2–0·8)
Ate pork in the past 5 days	63 (88·7)	135 (95·1)	0·3 (0·1–1·1)
Ate eggs in the past 5 days	47 (66·2)	131 (92·3)	0·1 (0·1–0·4)
Ate nuts in the past 5 days	10 (14·1)	65 (45·8)	0·1 (0·0–0·2)

OR, Odds ratio; CI, confidence interval.

52% (37/71) used an antidiarrhoeal agent. Fluoroquinolones were the most commonly used class of antibiotics; 78% of case-patients who took an antibiotic for their illness reported receiving a fluoroquinolone. Of 38 case-patients who took antibiotics for their *V. parahaemolyticus* infection, three (8%) also reported taking antibiotics during the 4 weeks prior to illness.

Of 31 case-patients who were followed in the 3 months after *V. parahaemolyticus* gastroenteritis, none developed symptoms of irritable bowel syndrome or reactive arthritis as diagnosed by their doctor.

Univariable analysis

In univariable analysis, prior antibiotic use was highly associated with illness (OR 7·0, 95% CI 1·5–33·7). Case-patients were more likely than controls to have reported eating outside the home. Overall, shellfish consumption was associated with increased risk (Table 3). In August, case-patients were four times more likely to eat shellfish compared to controls (OR 4·0, 95% CI 1·2–13·3). However, no association was observed between illness and shellfish consumption in other months (OR 2·0, 95% CI 0·4–9·9). Consumption of shellfish was reported by 14 (20%) of 71 patients and 13 (9·2%) of 142 controls. Only one case-patient (1%) reported eating raw shellfish. Factors associated with decreased risk were keeping the aquatic products refrigerated; and consumption of poultry other than chicken, pasteurized milk, pork, eggs, and nuts.

Infection was not statistically associated with having an underlying medical condition; prior antidiarrhoeal use; travel; animal contact; other family kitchen practices; consumption of fish, shrimp, crabs, chicken, beef, delicatessen meat, raw milk, dairy products, fruits, vegetables; or drinking-water source.

We found that cleaning kitchen counters with dishwashing liquid or detergent was more frequently reported by controls than by cases, although it did not reach statistical significance (64·8% *vs.* 56·3%; OR 0·6, 95% CI 0·3–1·1).

Assessment of the association between independent variables

When analysed separately, consumption of pasteurized milk and eggs were highly negative associated with consumption of shellfish, and consumption of nuts was highly associated with eggs. However, shellfish exposure is more biologically plausible than exposure to pasteurized milk, eggs, and nuts. Therefore, these variables were not included in the model.

Multivariable analysis

In the final multivariable model, antibiotics taken in the 4 weeks prior to illness (OR 8·1, 95% CI 1·2–56·4), eating out ⩾3 times (OR 3·3, 95% CI

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press

Table 4. *Multivariable analysis of risk factors associated with* V. parahaemolyticus *gastroenteritis, China, July 2010–June 2011*

Risk factor	OR (95% CI)	PAF (%)
Took antibiotics in the 4 weeks prior to illness	8·1 (1·2–56·4)	8·6
Frequency of eating out in the past 5 days		
1–2 times	1·7 (0·7–4·0)	
⩾3 times	3·3 (1·1–10·1)	24·6
Ate shellfish in the past 5 days	3·2 (1·0–9·9)	13·6
Ate poultry other than chicken in the past 5 days	0·5 (0·2–1·0)	
Keep aquatic products refrigerated in the past 5 days	0·4 (0·1–0·9)	
Ate pork in the past 5 days	0·2 (0·1–0·8)	

OR, Odds ratio; CI, confidence interval; PAF, population-attributable fraction.

1·1–10·1), and shellfish consumption (OR 3·2, 95% CI 1·0–9·9) were independent factors associated with an increased risk for illness (Table 4). Keeping the aquatic products refrigerated (OR 0·4, 95% CI 0·1–0·9) and pork consumption (OR 0·2, 95% CI 0·1–0·8) were independent factors associated with a reduced risk for infection. There was no association between eating poultry other than chicken in the 5 days before illness or interview and *V. parahaemolyticus* infection (OR 0·5, 95% CI 0·2–1·0) after adjusting for other risk factors. No significant first-order interactions were detected among the factors included in the analysis.

PAF

Prior antibiotic use was reported by 9·9% of cases, frequent eating out by 35·2%, and shellfish consumption by 19·7%. The percentage of cases attributable to each of these risk factors was as follows: antibiotics taken in the 4 weeks prior to illness, 9%; frequent eating out, 25%; and shellfish consumption, 14% (Table 3).

DISCUSSION

This is the first reported study of the risk factors associated with sporadic *V. parahaemolyticus* gastroenteritis in China. Our study demonstrates that antibiotics taken in the 4 weeks prior to illness, frequent eating out and shellfish consumption were significant risk factors for sporadic *V. parahaemolyticus* gastroenteritis.

Our study shows that people infected with *V. parahaemolyticus* were eight times more likely to have been on antibiotics in the 4 weeks prior to becoming ill compared to controls for the same time period. Previous studies have identified prior antibiotic use as a risk factor for *Salmonella* and *Campylobacter* infections [19–22]. Prior antibiotic use may increase susceptibility to ingested pathogens through several mechanisms, including prolonged alterations of the gut bacterial flora which would decrease individuals' resistance to infection when they became exposed. It is also possible that sub-clinical infection already exists and antibiotic use might be enough to induce a clinical case [23].

In the present study, 53·5% of the case-patients were treated with an antimicrobial agent. However, gastroenteritis caused by *V. parahaemolyticus* is self-limiting in most patients; antimicrobial therapy does not decrease the duration of symptoms. Inappropriate usage of antimicrobial agents to treat humans is a major problem in China, as many Chinese take antibiotics before diagnostic tests are performed [24, 25]. It is reported that nearly half of acute gastroenteritis cases have taken antibiotics in China, which is much higher than in other countries [26].

It is unlikely that patients who reported antibiotic use prior to illness were actually reporting antibiotics taken after diagnosis. In the present study, only 8% patients took both antibiotics prior to illness and after diagnosis. If antibiotic use in the controls is underestimated, this may result in a false association of antibiotic use and *V. parahaemolyticus* infection. In this study, 1·4% of controls took antibiotics which is consistent with previous data on antibiotic use in the Chinese population (1·5%) [26].

Given the evidence implicating prior antibiotic use as a risk factor for human *V. parahaemolyticus* gastroenteritis, it is important to investigate the factors influencing the use of antibiotics, and to promote prudent usage of antibiotics by healthcare providers and the public.

In the multivariable analysis, there is an association between foodborne infections and frequent eating out. Outbreaks of *V. parahaemolyticus* are also frequently traced to food served commercially. During 2003–2008, a total of 248 (77%) of 322 outbreaks of *V. parahaemolyticus* reported to CDC occurred in restaurants or other commercial food settings [12]. This may represent cross-contamination of a variety of foods with *V. parahaemolyticus* pathogens present initially in seafood. Such cross-contamination is difficult to assess by case-control methodology because it is not obvious to the consumers. Other studies also

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press

highlight the association between sporadic foodborne bacterial infections and eating out [27, 28].

In the present study, consumption of shellfish increased the odds for disease by more than threefold. We estimated that almost 14% of *V. parahaemolyticus* cases occurring in the population are attributable to shellfish consumption. Although shellfish have been suspected as a potential vehicle for *V. parahaemolyticus* infections, this is the first time that eating shellfish was demonstrated to be associated with sporadic *V. parahaemolyticus* infections in China. Infections of *V. parahaemolyticus* due to the consumption of raw oysters are more common during the warmer months [29]. High levels of *V. parahaemolyticus* have been detected in retail oysters collected during spring and summer months in southeast China [30]. *V. parahaemolyticus* would be of little public health importance if shellfish were always consumed after they were cooked. This study suggests that greater public health education should be focused on the proper handling of cooked shellfish products.

Keeping the aquatic products refrigerated was independently associated with a decreased risk of infection. The minimum temperatures required for the growth of *V. parahaemolyticus* are 8·3 °C [31]. Poor temperature control of storage favours bacterial growth. The shellfish were generally stored at ambient temperatures without cooling devices at the markets in China, which would lead to significant increases in the *V. parahaemolyticus* level. Continued prevention efforts to educate the food handler and consumer about the risks from keeping the aquatic products unrefrigerated are needed.

Our study identified that consumption of pork and poultry other than chicken might protect against infection, nevertheless we believe the observed effect was probably due to controls being more likely to eat pork and poultry other than chicken than cases, as their frequency of shellfish consumption was lower, thereby creating spurious protective effects.

Outbreak investigations of *V. parahaemolyticus* infections in China have implicated meat and meat products as the major food with cross-contamination as the major contributing factor [12]. The natural habitat of *V. parahaemolyticus* is estuarine and marine environments. If cross-contamination is the main cause of *V. parahaemolyticus* infections in China, and the source of the *V. parahaemolyticus* contamination is expected to be seafood, it would be expected that serving seafood in the same place would be linked to outbreaks as well. The infectious dose of *V. parahaemolyticus* is generally believed to be very high. To confirm *V. parahaemolyticus* outbreaks, isolating 10^5 Kanagawa-positive organisms/g from epidemiologically implicated food is a sufficient condition [32]. Food poisoning of *V. parahaemolyticus* infection in China is defined as *V. parahaemolyticus* strains with the same serotype or other biological properties isolated from the implicated food, equipment or utensils used, and faecal samples or vomitus from patients [33]. There is no requirement regarding the level of *V. parahaemolyticus* in the implicated food, which may lead to misjudgement of the implicated food. Further investigation and research on this issue is required.

Due to the limitations inherent in the case-control study design, bias cannot be excluded as an alternative explanation for our findings. We did not enrol every diagnosed patient during the study period, and differences in the enrolment procedure could have introduced bias. Because of the design limitation, we did not collect the detailed information for the excluded cases, so we cannot compare the difference in demographics between excluded cases and enrolled cases. In our study, we attempted to reduce recall bias by limiting the recall period to 5 days prior to illness onset (cases) and interview (controls). To minimize potential information bias, all interviewers were trained thoroughly and a standardized questionnaire and protocol for case and control interviews were used.

In summary, our study illustrates that prior antibiotic use, frequent eating out, and shellfish consumption are risk factors for *V. parahaemolyticus* gastroenteritis in east China. Therefore, efforts to prevent *V. parahaemolyticus* gastroenteritis should be focused on implementation of farm-to-table measures aimed at shellfish. Additionally, public health, physicians and consumers should promote activities associated with these risk factors that decrease the opportunity for disease. These findings are based on relatively limited data and should be considered preliminary. Further studies are needed to determine more fully the risk factors for *V. parahaemolyticus* infection, such as prior antibiotic use and shellfish consumption, in order to develop and implement appropriate intervention strategies.

ACKNOWLEDGEMENTS

We thank all local CDCs and hospitals for their considerable efforts. We also thank the study participants, parents, and guardians for their cooperation. The

authors also gratefully thank Dr Yukiko Yamada, Japan, for her advice on the manuscript. This project was financially supported by the National Health and Family Planning Commission of the People's Republic of China (no. 201302005) and the Universities Innovation Ability Promotion Plan – Collaborative Innovation Centre for Green Processing and Safety Control of Food in Beijing and Tianjin Area.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Joseph SW, Colwell RR, Kaper JB.** *Vibrio parahaemolyticus* and related halophilic vibrios. *Critical Reviews in Microbiology* 1982; **10**: 77–124.
2. **Kaneko T, Colwell RR.** Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Journal of Bacteriology* 1973; **113**: 24–32.
3. **Daniels NA, *et al.*** *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *Journal of Infectious Diseases* 2000; **181**: 1661–1666.
4. **Su YC, Liu C.** *Vibrio parahaemolyticus*: a concern of seafood safety. *Food Microbiology* 2007; **24**: 549–558.
5. **Iwamoto M, *et al.*** Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews* 2010; **23**: 399–411.
6. **Shirai H, *et al.*** Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of *Vibrio parahaemolyticus* with gastroenteritis. *Infection and Immunity* 1990; **58**: 3568–3573.
7. **Bej AK, *et al.*** Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tl, tdh* and *trh*. *Journal of Microbiological Methods* 1999; **36**: 215–225.
8. **Jones JL, *et al.*** Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. *Journal of Clinical Microbiology* 2012; **50**: 2343–2352.
9. **Alam MJ, *et al.*** Environmental investigation of potentially pathogenic *Vibrio parahaemolyticus* in the Seto-Inland Sea, Japan. *FEMS Microbiology Letters* 2002; **208**: 83–87.
10. **Chen Y, *et al.*** Foodborne disease outbreaks in 2006 report of the National Foodborne Disease Surveillance Network, China. *Wei Sheng Yan Jiu* 2010; **39**: 331–334.
11. **Raghunath P, *et al.*** Detection and molecular characterization of *Vibrio parahaemolyticus* isolated from seafood harvested along the southwest coast of India. *Food Microbiology* 2008; **25**: 824–830.
12. **Wu Y, *et al.*** Epidemiology of foodborne disease outbreaks caused by *Vibrio parahaemolyticus*, China, 2003–2008. *Food Control* 2014; **46**: 197–202.
13. **Kubota K, *et al.*** Estimating the burden of acute gastroenteritis and foodborne illness caused by *Campylobacter, Salmonella*, and *Vibrio parahaemolyticus* by using population-based telephone survey data, Miyagi Prefecture, Japan, 2005 to 2006. *Journal of Food Protection* 2011; **74**: 1592–1598.
14. **Scallan E, *et al.*** Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* 2011; **17**: 7–15.
15. **Bean NH, *et al.*** Crayfish: a newly recognized vehicle for vibrio infections. *Epidemiology and Infection* 1998; **121**: 269–273.
16. **Dupont WD, Plummer WD. Jr.** PS: Power and sample size calculation (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Accessed 5 August 2013.
17. **Rothman K, Greenland S, Lash T.** *Modern Epidemiology*, 3rd edn. Philadelphia, Pennsylvania: Lippincott-Raven, 2008.
18. **Rockhill B, Newman B, Weinberg C.** Use and misuse of population attributable fractions. *American Journal of Public Health* 1998; **88**: 15–19.
19. **Delarocque-Astagneau E, *et al.*** Risk factors for the occurrence of sporadic *Salmonella enterica* serotype *typhimurium* infections in children in France: a national case-control study. *Clinical Infectious Diseases* 2000; **31**: 488–492.
20. **Dore K, *et al.*** Risk factors for *Salmonella* typhimurium DT104 and non-DT104 infection: a Canadian multi-provincial case-control study. *Epidemiology and Infection* 2004; **132**: 485–493.
21. **Effler P, *et al.*** Sporadic *Campylobacter jejuni* infections in Hawaii: associations with prior antibiotic use and commercially prepared chicken. *Journal of Infectious Diseases* 2001; **183**: 1152–1155.
22. **Glynn MK, *et al.*** Prior antimicrobial agent use increases the risk of sporadic infections with multidrug-resistant *Salmonella enterica* serotype Typhimurium: a FoodNet case-control study, 1996–1997. *Clinical Infectious Diseases* 2004; **38** (Suppl. 3): S227–236.
23. **Cohen ML, Tauxe RV.** Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. *Science* 1986; **234**: 964–969.
24. **Ran L, *et al.*** Laboratory-based surveillance of non-typhoidal *Salmonella* infections in China. *Foodborne Pathogens and Disease* 2011; **8**: 921–927.
25. **Zhou YJ, *et al.*** Population-based estimate of the burden of acute gastrointestinal illness in Jiangsu province, China, 2010–2011. *Epidemiology and Infection* 2013; **141**: 944–952.
26. **Chen Y, *et al.*** Burden of self-reported acute gastrointestinal illness in China: a population-based survey. *BMC Public Health* 2013; **13**: 456.
27. **Sobel J, *et al.*** The pandemic of *Salmonella* enteritidis phage type 4 reaches Utah: a complex investigation confirms the need for continuing rigorous control measures. *Epidemiology and Infection* 2000; **125**: 1–8.
28. **Kimura AC, *et al.*** Chicken consumption is a newly identified risk factor for sporadic *Salmonella enterica* serotype Enteritidis infections in the United States: a case-control study in FoodNet sites. *Clinical Infectious Diseases* 2004; **38** (Suppl. 3): S244–252.

29. **Hlady WG, Klontz KC.** The epidemiology of *Vibrio* infections in Florida, 1981–1993. *Journal of Infectious Diseases* 1996; **173**: 1176–1183.
30. **Chen Y, *et al.*** Foodborne pathogens in retail oysters in south China. *Biomedical and Environmental Sciences* 2010; **23**: 32–36.
31. **Miles DW, *et al.*** Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus*. *International Journal of Food Microbiology* 1997; **38**: 133–142.
32. **Todd ECD, *et al.*** *Procedures to Investigate Foodborne Illness*, 6th edn. New York: International Association for Food Protection, 2011.
33. **Ministry of Health of the People's Republic of China.** *WS/T 81–1996 Diagnostic Criteria and Principles of Management for Food Poisoning of* Vibrio parahaemolyticus. Beijing: China Standards Publishing House, 1996.

https://doi.org/10.1017/S0950268814001599 Published online by Cambridge University Press