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# A serologically confirmed, case-control study, of a large outbreak of hepatitis A in China, associated with consumption of clams

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## SUMMARY

A matched and serologically confirmed case-control study was carried out to investigate the source of an outbreak of acute hepatitis involving 290000 cases in the suburbs of Shanghai, in January 1988. A total of 132 patients with acute hepatitis from six different hospitals were chosen as cases and the same number of control patients without hepatitis were matched for gender, age, admission date and area of residence. Serum specimens from both case and control patients were detected for specific anti-hepatitis A (HA) IgM antibody and a questionnaire was used to investigate probable risk factors related to the outbreak. The positive rate of anti-HA IgM was 98·48% in the case group and only 0·76% in the control, indicating that the infection was caused by HA virus. The results revealed that the source and mode of transmission were due to the consumption of contaminated and inadequately cooked clams (*Anadara subcrenata lischke*). There was a highly positive dose-response relationship between the odds ratio of contracting HA and the quantity or frequency of clam consumption. The odds ratios of acquiring HA from clams were up to 62·4–63·4 by both group stratification and multiple unconditional logistic regression analyses.

## INTRODUCTION

From the middle of January 1988, an unprecedented epidemic of acute hepatitis occurred in the suburbs of Shanghai, the largest city of the People's Republic of China. According to the Jie Fang Daily newspaper on 23 March 1988, 290000 cases were reported to the local anti-epidemic and health center by the 20 March. More than 90% of patients were 10–39 years old and no gender or occupation differences were detected. *Anadara subcrenata lischke*, a species of clams was considered as the source of this epidemic because a great quantity of the clams was sold in the Shanghai markets 1 month before the outbreak, supplied and transported from Qidong County, Jiangsu Province where high incidence of hepatitis A (HA) was reported. This clam had also been a confirmed source of HA infection in the past

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[1, 2]. Following the serological diagnosis of HA in hospitalized patients with acute hepatitis at different hospitals in Changning District, Shanghai, we carried out a matched case-control study in order to investigate the source and mode of transmission for the epidemic.

## MATERIALS AND METHODS

### *Choice of case and control groups*

One hundred and thirty-two individuals with acute hepatitis who were patients at six different hospitals in Changning District between 25 January and 10 February, 1988 were chosen as case subjects. A same number of patients without hepatitis were chosen as controls. They were from the same hospitals and matched for gender, age, area of residence, and admission date.

### *Data collection*

Using a uniform questionnaire, trained medical students or physicians collected information from case and control groups concerning the probable risk factors relating to the occurrence of hepatitis which they encountered within 1·5 months before admission to the hospital. The 11 features investigated are listed in Table 1.

### *Serological tests*

Serum specimens from all subjects of both case and control groups were collected at the same time as patient data, and tested for the presence of specific anti-HA antibody by an IgM-capture enzyme linked immunosorbent assay (ELISA) as described previously [3, 4]. Briefly, 100 $\mu$l of the serum sample diluted 1:1000 was added to microplates coated with anti-human IgM ($\mu$-chain, DAKO, Denmark) and incubated at 37 °C for 2 h. After washing, HA virus antigen was added and the plate was placed at 4 °C overnight. After washing, biotinylated anti-HA IgG (kindly provided by Dr Harold S. Margolis, Centers for Disease Control, USA) and avidin conjugated with horseradish peroxidase was added sequentially; then substrate was added for reaction after washing. The sensitivity and specificity of the detecting system was consistent with commercial kits (HAVAB-M EIA, Abbott, USA). All samples were tested according to random codes and positive and negative controls were always included.

### *Statistical analysis*

All epidemiological data obtained were entered into an IBM personal computer. A single conditional logistic regression model [5] was used to test the proportional difference for each risk factor between case and control groups. If the model did not converge in the approach of estimating the maximum likelihood function of a factor within 25 loops, the paired $\chi^2$ test was used instead. The trend $\chi^2$ test was adopted to analyse the relationship between the odds ratio of acquiring HA and the quantity or frequency of clam consumption. Later, group stratification analysis [6] was used to estimate the odds ratio with confounding factors controlled. Finally, all independent factors were analysed by the multiple variable unconditional logistic regression model [7].

Table 1. *Variables investigated in connection with the outbreak of acute hepatitis within 1·5 months before admission*

| Identifier | Variable description | Stratification |
|---|---|---|
| X1 | Diarrhoea within 3 days of eating clams | yes or no |
| X2 | Previous hepatitis infection | yes or no |
| X3 | Contact with acute hepatitis patient | yes or no |
| X4 | Degree of contact with acute hepatitis patient | intimate, average or no |
| X5 | History of eating clams | yes or no |
| X6 | Numbers of clams eaten | ⩾ 30, 20–29, 10–19 1–9 or no |
| X7 | Frequencies of eating clams | > 2, 2, 1 or no |
| X8 | Methods of preparing clams | raw, boil or no |
| X9 | History of eating out* | yes or no |
| X10 | History of travel outside metropolitan Shanghai | yes or no |
| X11 | History of drinking unboiled water | yes or no |

* Eating in public places where common utensils (bowls, chopsticks, spoons) are reused without proper cleaning.

## RESULTS

### *Detection of anti-HA IgM*

IgM antibodies to HA were detected in 130 of 132 (98·48%) serum specimens of acute hepatitis patients and in only 1 of 132 controls (0·76%) (Table 2). The presence of anti-HA IgM in the patients with acute hepatitis strongly supports the view that the large epidemic was caused by HA, particularly as no other causative agents were implicated (J. X. Wang, unpublished data).

One matched pair of sera gave a positive result for the case and control patient; two pairs gave a negative result in both patients. These three pairs were not considered further. Thus, a total of 258 samples (129 matched pairs) were included in the analysis.

### *Single variable analysis*

Statistical analysis was carried out on 129 matched pairs from which clinical false positive and negative diagnoses had been excluded. Of the 11 variables which may have been associated with the outbreak, only one method of preparing clams (X8) was used (scalding washed clams in boiling water for several seconds = 'raw') and no one drank unboiled water (X11); consequently these two features were not analysed further. The following variables were significantly different between case and control groups (Table 3): diarrhoea within 3 days of eating clams (X1), history of eating clams (X5), numbers of clams eaten (X6), frequencies of eating clams (X7) and history of eating out (X8). Table 4 shows that the odds ratio of acquiring HA increased with the quantity or frequency of clam consumption.

### *Group stratification analysis*

In the above single variable analysis, diarrhoea within 3 days (X1) was an outcome of eating clams, so the only significant variables which could have been related to the cause and transmission were a history of eating clams (X5) and a

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Table 2. *Anti-HA IgM antibody positive rate in case and control groups*

| Group | No. tested | No. positive | Positive rate (%) |
|---|---|---|---|
| Case | 132 | 130 | 98·48 |
| Control | 132 | 1 | 0·76 |

Table 3. *Single conditional logistic regression analysis of variables for contracting HA*

| Variable | Exposure condition* ++ | +− | −+ | −− | O.R. | 95% C.L. |
|---|---|---|---|---|---|---|
| Diarrhoea within 3 days of eating clams | 4 | 32 | 9 | 84 | 3·56 | 1·70–7·45 |
| Previous hepatitis infection | 8 | 3 | 9 | 109 | 0·33 | 0·09–1·23 |
| Contact with acute hepatitis patient | 37 | 17 | 15 | 60 | 1·13 | 0·57–2·27 |
| Contact degree with acute hepatitis patient† | | | | | 0·93 | 0·61–1·42 |
| History of eating clams‡ | 40 | 85 | 0 | 4 | | |
| Numbers of clams eaten† | | | | | 3·96 | 2·35–6·68 |
| Frequencies of eating clams† | | | | | 3·73 | 2·44–5·69 |
| History of eat out | 12 | 69 | 28 | 20 | 2·46 | 1·69–3·60 |
| History of travel outside metropolitan Shanghai | 5 | 16 | 7 | 101 | 2·29 | 0·94–5·56 |

* Both case and control exposed to the variable (+ +), case exposed but control not, (+ −), control exposed but case not, (− +), and both case and control not (− −).

† Crude data not presented because of more than two strata. For the data about the quantity and frequency of clam consumption, see Table 4.

‡ Paired $\chi^2$ test: $\chi^2 = 83{\cdot}01$, $P < 10^{-4}$.

Table 4. *Dose-response relationship between odds ratio of HA and the quantity or frequency of clam consumption*

| Group | Numbers of clams eaten* 0 | 1–9 | 10–19 | 20–29 | ⩾ 30 | Frequencies of eating clams† 0 | 1 | 2 | > 2 |
|---|---|---|---|---|---|---|---|---|---|
| Case | 4 | 24 | 34 | 43 | 24 | 4 | 19 | 73 | 33 |
| Control | 89 | 10 | 10 | 14 | 6 | 89 | 7 | 23 | 10 |
| O.R. | 1·0 | 53·4 | 75·7 | 68·3 | 89·0 | 1·0 | 60·4 | 70·6 | 73·4 |

* Numbers of clams eaten: $\chi^2_{\text{trend}} = 89{\cdot}78$, $P < 10^{-5}$.

† Frequencies of eating claims: $\chi^2_{\text{trend}} = 98{\cdot}80$, $P < 10^{-8}$.

history of eating out (X9). In order to assess the independent association of these two variables, we classified the data as two strata according to the presence and absence of a history of eating out (Table 5). The result showed that eating clams leading to the disease was independent of a history of eating out and the odds ratio was up to 62·4. A small but significant independent association between eating out and contracting the disease was also demonstrated (Table 5).

Table 5. *The odds ratios of eating clams with eating out controlled for contracting HA. Changning, Shanghai, 1988*

| | Ate out | | No eating out | | Total | |
|---|---|---|---|---|---|---|
| Group | Ate clams | No clams eaten | Ate clams | No clams eaten | Ate clams | No clams eaten |
| Case | 79 | 2 | 46 | 2 | 125 | 4 |
| Control | 14 | 26 | 26 | 63 | 40 | 89 |
| Total | 93 | 28 | 72 | 65 | 165 | 89 |

* Eating clams: $\chi^2_{M-H} = 108{\cdot}46$, $P < 10^{-6}$, $O.R. = 62{\cdot}4$.
Eating out: $\chi^2_{M-H} = 9{\cdot}40$, $P = 0{\cdot}002$, $O.R. = 3.1$.

Table 6. *Multiple unconditional logistic regression analysis of five independent variables for contracting HA*

| Variable | O.R. | 95% C.L. | p value |
|---|---|---|---|
| Previous hepatitis infection | 0·74 | 0·09–6·05 | 0·779 |
| Contact with acute hepatitis patient | 0·66 | 0·27–1·60 | 0·358 |
| History of eating clams | 63·43 | 21·44–187·65 | < 0·001 |
| History of eating out | 1·97 | 1·26–3·09 | 0·003 |
| History of travel outside metropolitan Shanghai | 1·01 | 0·31–3·26 | 0·976 |

*Multiple logistic analysis*

Of 11 variables listed in Table 1, we studied the following 5 in a multiple variable unconditional logistic regression model to determine their independent association with the HA outbreak: previous hepatitis infection (X2), contact with acute hepatitis patients (X3), history of eating clams (X5), history of eating out (X9), and history of travel out metropolitan Shanghai (X10). Results are shown in Table 6 and confirmed that the strongest association was with eating clams. An additional significant but smaller independent risk factor associated with the disease was that of eating out. The odds ratio of contracting HA by eating clams, estimated by the regression model, was 63·4 (Table 6), which was quite close to the one calculated by group stratification analysis.

## DISCUSSION

Because IgM antibody against HA virus develops early in infection and disappears quickly, the detection of HA specific IgM antibody in a single serum specimen taken at an appropriate time is considered as a reliable method for laboratory diagnosis of a recent infection. During the hepatitis epidemic investigated, specific anti-HA IgM antibody was found in 130/132 (98·48%) of patients with acute hepatitis but in only 1/132 matched control specimens. The very high frequency in the clinically ill group suggests that the epidemic was in fact caused by HA. The results were substantiated by later detection of the HA virus particles in the stool samples from acute patients [8].

A very close association was found between having eaten clams and occurrence of the disease during the outbreak. From the middle of December 1987 to the

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beginning of January 1988, a large quantity of clams (*Anadara subcrenata lischke*) was supplied to Shanghai markets. In order to preserve the flavour, people prepared clams for consumption by washing followed by scalding in boiling water for several seconds. After eating the inadequately cooked clams, a diarrhoea retrospectively confirmed as a bacillary dysentery caused by *Shigella flexneri* occurred within 3 days (S. L. Hu, unpublished data). About 1 month after, an unprecedented outbreak of hepatitis occurred. This time interval was a little less than the average incubation period of hepatitis A [10]. The odds ratio for an association between HA and eating clams was estimated to be more than 60 by both group stratification and multiple variable logistic regression. In addition, the odds ratios of inquiring HA was found positively associated with the quantity and frequency of clam consumption. Thus, the HA infection was acquired by the consumption of inadequately cooled clams. The odds ratio of HA associated with consumption of clams could have been underestimated by the fact that we did not use tests to detect anti-HA IgG antibody.

Besides a history of eating clams, a history of eating out was also evaluated by both stratification and multiple analysis and found to be an independent risk factor associated with contracting HA. It is possible that improperly cleaned eating utensils provided a probable transmission vehicle for the infection. Eating out had been demonstrated as one of important reasons why a constant incidence of HA remains in these areas all the year round [1].

Common epidemics of HA due to the consumption of contaminated clams have been reported on numerous occasions [1, 2, 10–12], however, the outbreak reported here is a particularly large one which involved some 290000 cases. Clams are likely to transmit HA because they filter large quantities of water in order to obtain food and oxygen. During this process they also concentrate any bacteria and viruses present in the water. In addition, they may retain the viruses even after 100 h of elimination [13]. Because HA virus is more heat stable than other enteroviruses, the brief steaming or scalding of contaminated clams used to maintain flavour and consistency is probably inadequate to inactivate the virus [14, 15]; this outbreak serves to confirm this opinion.

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