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SHORT REPORT
Epidemic cholera in urban Zambia: hand soap and dried fish as protective factors

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SUMMARY

Between 28 November 2003 and 23 February 2004, 4343 cases and 154 deaths from cholera (case-fatality rate 3·5%) were reported in Lusaka, Zambia. A case-control study was conducted in February 2004 to assess potential transmission routes and prevention strategies. Consumption of raw vegetables was significantly associated with cholera [adjusted odds ratio (aOR) 4·7, 95% confidence interval (CI) 1·7–13, $P=0{\cdot}003$). Consumption of a local sardine-like fish was protective (aOR 0·3, 95% CI 0·1–0·7, $P=0{\cdot}008$). Hand soap was present in 90% of control homes and 58% of case homes. Observed hand soap was a strongly protective factor (aOR 0·1, 95% CI 0·04–0·4, $P=0{\cdot}001$). No water source or treatment practice was significantly associated with cholera. This study documents the importance of foodborne transmission of cholera, illustrates the protective role of hand washing in an epidemic setting, and identifies a novel possible protective factor, a local fish, which warrants further research.

In the 21st century, epidemic cholera remains a significant public health problem. Of the 575 268 cases reported by the World Health Organization (WHO) since 1 January 2000, over 93% were from sub-Saharan Africa [1]. Zambia has suffered large cholera epidemics in recent years with over 13 000 cases in 1991, 11 659 in 1992, and 11 535 in 1999. A new epidemic emerged in Zambia from November 2003 to January 2004 with 2529 cases and 128 deaths reported; 85% of the cases occurred in Lusaka, the capital city. The Lusaka District Health Management Team (LDHMT) and the United States Centers for Disease Control and Prevention (CDC) collaborated to investigate the epidemic and to help define intervention strategies.

As part of the investigation, clinical procedures and hygiene practices at three of the seven designated Cholera Treatment Centers (CTC) in Lusaka were observed, and a case-control study was conducted to identify exposures associated with cholera and possible points of intervention for cholera prevention. For purposes of the case-control study, a probable case was defined as 3 or more watery stools in 24 h in a person at least 5 years of age. Case-patients were admitted to Chawama or Kanyama CTC in Lusaka between 11 and 22 February 2004. Only the first person in a household with diarrhoea since 30 November 2004 was eligible for the study. All cases had rectal swabs submitted for culture. A confirmed case met the definition for a probable case and had a stool culture positive for *Vibrio cholerae* O1. For the study one

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age-, sex-, and neighbourhood-matched control per case was enrolled. Only persons from households with no history of diarrhoea since the epidemic began were eligible for enrolment. Controls were selected systematically by starting at the case household and, after bypassing the adjacent household, walking right and questioning at every home until a person who met the control definition was identified. If such a person lived in the home but was not present, every effort was made to schedule an appointment for completion of the interview. If no appropriate control was identified after 10 households, the process was repeated walking left, then walking straight ahead from the case home, and then walking from the back of the case home to the next houses in proximity. Sample size was calculated using the methodology set forth by Schlesselman [2]. It was estimated that 66 cases and 66 controls would be required to identify an odds ratio (OR) of 3·0, assuming an exposure prevalence of 40% in cases and 20% in controls with 80% power and 95% significance.

Patients were questioned about their symptoms and any treatment they received before presentation at the CTC. A study instrument based on hypothesis-generating interviews and risk factors for cholera transmission identified in previous investigations was derived. Questions and observations encompassed water, food, sanitation, and hygiene issues, and focused on exposures during the previous week. Interviewers asked about the presence of hand soap and designated hand-washing stations in the home, and then verified answers with direct observation of these items. If the respondent reported use of a locally available sodium hypochlorite solution for household drinking water disinfection, water stored in the home was tested for residual free chlorine, using a field test kit (Aquality™, Cissna Park, IL, USA). All study participants provided verbal consent to participate in the study. One rectal swab from each patient was processed at the LDHMT laboratory. Rectal swabs were streaked directly onto thiosulphate–citrate–bile salts sucrose (TCBS) agar and subsequently placed into alkaline peptone water for enrichment for 8 h. The enriched sample was then plated on TCBS. Serological testing was performed using polyvalent antisera to confirm *V. cholerae* O1 and specific antisera to confirm the serotype. Antimicrobial susceptibility testing was performed at the Tropical Diseases Research Center, Ndola, Zambia, using the disc diffusion method (NCCLS, Wayne, PA, USA).

Records were entered into an Epi-Info database (Version 6; CDC, Atlanta, GA, USA). Data were analysed using STATA 8 (Stata Corporation, College Station, TX, USA) and Epi-info 2002 (CDC). A composite variable for consumption of any of the raw vegetables included in the questionnaire (eggplant, tomato, cabbage, onion, cucumber, and okra) was analysed as a single exposure. Matched ORs were calculated for all exposures using McNemar's test. Multivariate analysis was performed using a conditional logistic regression model. Factors were included in the model based on significance on bivariate analysis or identification as a cholera risk factor in prior studies. Model building included backwards and forwards stepwise selection with an inclusion level of 0·05, and variables were tested for possible effect modification. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were calculated.

Since 1 March 2004, there were 4630 total admissions to CTCs and 153 recorded deaths, excluding 55 persons who were brought in dead (BID) to the CTC. The case-fatality rate (CFR) was 3·5% excluding BID patients, and 4·5% including BID patients. In all 71 cases and 71 controls were enrolled between 11 and 22 February. The median age of patients was 28 years (range 5–75 years); 58% were male. All patients had diarrhoea; other common symptoms were vomiting (86%) and leg cramps (62%). The median duration of illness at the time of interview was 1·3 days (range 0–5 days). Fifty-two percent reported using oral rehydration solution (ORS) before coming to the CTC. Thirty-seven (52%) patients reported use of antibiotics after the onset of symptoms and before presentation at a CTC.

Rectal swabs were obtained from 70 patients. Of those, 52 (74%) yielded *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor at the LDHMT laboratory. Clinical and demographic characteristics of confirmed and probable patients were comparable, except that probable cases were slightly older and more likely to report bloody diarrhoea than confirmed cases. Laboratory-confirmed and probable cases were analysed separately; trends for all associations regarding food, water, sanitation and hygiene were similar (data not shown), and thus results for all cases are reported in aggregate. Resistance to antimicrobial agents was common: chloramphenicol (42%), furazolidone (74%), nalidixic acid (42%), tetracycline (55%), and trimethoprim–sulfamethoxazole (87%). Five percent of isolates were resistant to ciprofloxacin.

Table. *Bivariate analysis of the case and control groups in relation to the various exposures examined*

Exposure	Cases No. (%)	Controls No. (%)	mOR	95% CI	*P* value
Reported chlorination of stored water	47 (66)	48 (68)	1·0	0·5–2·1	0·57
Bottle of chlorine water treatment solution observed	33 (46)	27 (38)	1·4	0·7–2·9	0·2
Free chlorine present in stored water	19 (27)	14 (20)	1·5	0·7–3·5	0·21
Regularly boil drinking water	7 (10)	20 (28)	0·42	0·2–1·0	0·03
Regularly use any water treatment	48 (46)	56 (54)	0·46	0·2–1·2	0·08
Drank any untreated water	48 (67)	37 (52)	1·9	0·9–3·9	0·06
Hand soap observed in home	41 (58)	64 (90)	0·2	0·05–0·4	0·0001
Reports hand washing after defecating	59 (83)	67 (94)	0·3	0·08–1·0	0·02
Designated hand-washing area	5 (7)	8 (11)	0·3	0·2–1·9	0·29
Ate food away from home	44 (54)	37 (46)	1·5	0·8–2·9	0·16
Ate kapenta	34 (48)	49 (69)	0·4	0·2–0·8	0·005
Ate leftover nshima	16 (22)	7 (9)	2·5	1·0–6·4	0·04
Ate raw vegetables	41 (58)	21 (30)	3·9	1·7–9·6	0·0004
Shares latrine with at least one household	60 (85)	67 (94)	2·8	0·9–8·6	0·06

mOR, Matched odds ratio; CI, confidence interval.

Bivariate analysis of the case and control groups in relation to the various exposures examined is shown in the Table. No individual fruit, vegetable, or meat food item was significantly associated with cholera. However, consumption of any of the raw vegetables included in the composite variable was significantly associated with cholera [matched odds ratio (mOR) 3·9, 95% CI 1·7–8·9, $P=0{\cdot}0004$]. Eating leftover nshima, the local staple maize porridge, was also associated with cholera (mOR 2·5, 95% CI 1·0–6·4, $P=0{\cdot}04$). Consumption of kapenta, a local sardine-like fish was protective (mOR 0·35, 95% CI 0·2–0·80, $P=0{\cdot}005$).

Cases and controls were similar in water-related exposures; boiling was protective (mOR 0·42, 95% CI 0·2–1·0, $P=0{\cdot}03$), but only seven (10%) cases and 20 (28%) controls reported using this treatment. Although 93% of both cases and controls had access to the municipal water system, 41% of all respondents reported interruptions at least once per week, and 13% reported daily interruptions. Municipal water was tested in the subject's home when requested by the study participant; no samples showed detectable free chlorine. Sixty-six percent of patients and 68% of controls reported use of in-home chlorination, but at inspection, among households reporting regular chlorine use, only 46% of case households and 38% of control households had a bottle of chlorine present. Among households reporting chlorine use, testing detected free chlorine residuals in stored water in 27% of case homes and 20% of control homes (mOR 1·5, 95% CI 0·7–3·3, $P=0{\cdot}21$).

Hand soap was observed in 58% of case homes and 90% of control homes. The presence of hand soap was significantly protective against cholera (mOR 0·14, 95% CI 0·05–0·4, $P=0{\cdot}0001$). A designated hand-washing area was noted in 7% of case homes and 11% of control homes.

Variables in the final multivariate model included consumption of raw vegetables, presence of hand soap in the home, and consumption of kapenta. Consumption of raw vegetables was significantly associated with case status (aOR 4·7, 95% CI 1·7–13, $P=0{\cdot}003$). The presence of hand soap in the home (aOR 0·1, 95% CI 0·04–0·4, $P=0{\cdot}001$) and consumption of kapenta (aOR 0·3, 95% CI 0·1–0·7, $P=0{\cdot}008$) were identified as independent protective factors. Water treatment, either by boiling or home chlorination did not emerge as a significant protective factor when added to the model after adjusting for hand soap and consumption of raw vegetables and kapenta.

This outbreak of cholera in Lusaka, Zambia, in which over 4000 cases were reported, was strongly related to foodborne exposures. The case-control study identified significant independent associations between food items and cholera. The protective effect for hand hygiene based on the indicator of observed hand soap in the home was also consistent with foodborne transmission. As evidenced by the lack of significant associations between cholera and water-related exposures, direct waterborne transmission probably played a minimal role in this setting.

Consumption of raw vegetables was strongly associated with cholera in Lusaka. Raw vegetables have been associated with cholera in previous outbreaks. In Israel, the use of raw sewage for fertilizer on vegetable farms was implicated in cholera transmission [3]. Consumption of raw produce was strongly associated with cholera in a Peruvian city where fruits and vegetables were splashed with sewage-contaminated river water during transport to markets [4]. *Vibrio cholerae* can survive on raw produce for 2–5 days [5] and produce can be exposed to these organisms at any time between the farm and the consumer's mouth. Exposure could occur if the vegetables were handled in markets which lack facilities for maintaining adequate hygiene, particularly during an urban epidemic when environmental *V. cholerae* counts are high. Contamination of produce in this Zambian outbreak may have occurred due to the absence of basic sanitary conditions at the main market, Soweto, where thousands of people work and shop daily on a site that lacks latrines and running water and has chronic flooding due to blocked drains. The market was temporarily closed by local officials as part of the epidemic response. Clean-up efforts thus far have included refuse collection and removing blockages from the main drainage sewer. Provision of running water, sufficient latrines, and hygiene interventions for food vendors should be considered a key part of future cholera prevention strategies.

A strong protective association with the presence of hand soap in the home was identified. Hand washing may have been particularly important in this largely foodborne outbreak by interrupting transmission of organisms during food preparation, service and consumption in households where hand soap was available. An asymptomatic but infected food preparer could inoculate *V. cholerae* into cooked food. Food can also be contaminated via kitchen utensils, where *V. cholerae* has been shown to persist for 1–2 days [6]. Hand washing with soap would minimize risk by preventing inoculation of food items during food preparation or during consumption from a communal bowl. Several studies have documented the importance of hygiene as a preventive intervention against cholera [7, 8]. As illustrated in this outbreak where contaminated food was a major vehicle of transmission, hand washing with soap remains a simple, affordable intervention that can be practised in the home while more resource-intensive public interventions, like infrastructure at markets, are planned and implemented. While the presence of hand soap is a proxy measure for actual hand-washing behaviour, a proxy measure based on direct observation may be more valid than self-reported behaviour. One evaluation of indicators for hygiene behaviour performed in Bangladesh documented significant overreporting of desirable behaviours in a hygiene-practices questionnaire in comparison with observations [9]. In that study, both knowledge, attitudes, and practice surveys and 24-h recall surveys were unreliable when checked against direct observations. It is possible that the presence of hand soap was a surrogate for disposable income and thus only a marker for higher economic status; however, the use of close neighbours of cases as controls should have accounted for potential confounding due to socio-economic status.

The apparent protective effect of consumption of kapenta in this study is not readily explained. Kapenta is a sardine-like, freshwater fish that has been a dietary staple among poorer Zambian households since its introduction in the late 1960s to alleviate severe protein malnutrition. It is usually sun-dried and sautéed whole, sometimes with a vegetable relish. If kapenta were simply a marker for improved nutritional status, one would expect similar findings for other animal protein sources, but that was not the case in this analysis. Perhaps the apparent protective association simply reflects a choice of kapenta over other foods that may have been vehicles for cholera. Alternatively, it may be a marker for some other exposure or an indicator of relatively higher economic status rather than an inherent protective substance in the kapenta itself. However, a recent cholera vaccine trial in Mozambique included a case-control study that also documented a statistically significant protective effect for consumption of dried fish [10]. While this finding may simply be a statistical aberration, further investigation into possible mechanisms of protection is warranted.

The study had certain limitations. Controls were not tested for serological evidence of recent cholera infection. Because up to 75% of infections with *V. cholerae* O1 El Tor are asymptomatic, controls could have been misclassified despite the fact that there was no diarrhoea in their households during the epidemic. This type of misclassification would bias findings towards the null hypothesis. Finally, although neighbourhood-matching should have effectively controlled for confounding due to economic status, we did not obtain direct measures of economic status in this analysis and subtle differences may still have existed between patients and their controls. These differences could have influenced the results, as kapenta and hand soap are both potentially associated with economic status.

An effective cholera prevention strategy addresses individual behaviour and public health practices. Based on this study, we can define interventions from both of these perspectives. Steps to prevent illness due to raw produce include prohibiting agricultural irrigation with sewage, improving facilities for hygiene and sanitation at markets, and educating the public and vendors on safe food handling. Individuals should thoroughly wash produce in treated water prior to consumption; during epidemics, when produce may be particularly prone to contamination due to high levels of *V. cholerae* in the environment, all produce should be cooked and eaten hot to minimize the risk of cholera. Educational campaigns should emphasize hand washing with soap as primary prevention against cholera in the outbreak setting. The unexpected finding of a protective effect for consumption of dried kapenta should be investigated in more depth to determine the mechanism of this association and any possible implications for cholera prevention and control.

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DECLARATION OF INTEREST

None.

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