

## Analysis on the Epidemiological Characteristics of *Escherichia coli* O157:H7 Infection in Xuzhou, Jiangsu, China, 1999<sup>☆</sup>

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Received 8 September 2008

### Abstract

**Objective:** To determine epidemiologic features of an *Escherichia coli* O157:H7 outbreak occurred in Xuzhou, Jiangsu Province, China in 1999, and assess the incidence of *E. coli* O157:H7 in diarrhea patients and host animals and its relationship with disease onset, and provide a scientific basis for establishing prevention and control strategies. **Methods:** Epidemiological, microbiological, and molecular methods were performed to identify risk factors and describe the ecology of *E. coli* O157:H7 in the environment. **Results:** From May to September, in 1999, 99 cases of *E. coli* O157:H7 infection were confirmed. Fifty-six patients were enrolled in the case-control study. Bad personal health habits and poor sanitary conditions in the kitchen were associated with increased risks of infection, whereas hand washing was protective. The household survey indicated that residents in the epidemic region during the outbreak had higher than expected rates of diarrhea. The total *E. coli* O157:H7 carrier rate in the livestock was 12.36%(22/178), specifically 19.15% in cattle, 12.50% in goat, and 11.11% in swine. Numerical analysis of pulsed-field gel electrophoresis(PFGE) profiles divided strains into two clusters with 77.5% homology. One cluster contained 11 strains isolated from diarrheal patients, foods, and animals. The other cluster comprised 10 strains from patients and environment. **Conclusion:** In a large outbreak of *E. coli* O157:H7 infection among predominantly elderly residents in Xuzhou, high rates of carriage of *E. coli* O157:H7 among host animals most likely resulted in contamination of the environment, thereby leading to the outbreak. Effective and preventive control measures should be taken to avoid contamination, including environmental and family health improvement, good personal hygiene, and safe food handling practices.

**Key words:** *Escherichia coli* O157:H7; epidemiological study

### INTRODUCTION

Since it was first recognized as a cause of illness in humans in 1982 in the USA<sup>[1]</sup>, *Escherichia coli* O157:H7 infections have been reported in over 80 countries worldwide, with the highest rate in the USA<sup>[2]</sup>. Healthy cattle are the main recognized animal reservoir and may harbor the organism as part of the bowel flora. Most reported outbreaks are due to contaminated food or

water<sup>[3–5]</sup>. However, direct transmission of *E. coli* O157:H7 from animals and their environment to humans is a growing concern. Few reports have permitted extensive epidemiologic investigations to establish risk factors for infection<sup>[6–9]</sup>. We describe an outbreak in our country that is notable for two reasons: the number of cases permitted extensive characterization of risk factors, and a concurrent environmental study was conducted to define the sources of infection.

### MATERIALS AND METHODS

#### The outbreak

From May to September 1999, an unexpectedly large number of hemolytic uremic syndrome(HUS) cases occurred in Xuzhou city and surrounding counties,

<sup>☆</sup> This work was supported by National High-tech R&D Program (863 Program)(2007AA02Z409).

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Jiangsu province. The first HUS case was reported on May 20. By September 20, 95 suspected *E. coli* O157:H7 infected patients with acute renal failure were reported in 9 counties and districts of 2 municipalities. Of them, 83 died.

### Epidemiologic investigation

Surrounding counties were notified of the outbreak. Physicians and medical microbiology laboratories were asked to look for cases associated with the outbreak, to test stools for *E. coli* O157:H7, to report cases to local CDC, and to forward isolates to provincial CDC laboratory for molecular subtyping.

To identify specific risk factors for infection, investigators selected and interviewed 56 patients before July. To ascertain the number of cases and for the purpose of performing a case-control study, a confirmed case was defined as acute diarrhea (three or more loose stools in a 24-hour period) occurring in a Xuzhou resident with onset of symptoms from May 20 to September 20, 1999, and accompanied by either HUS or the isolation of *E. coli* O157:H7 from his stool<sup>[9]</sup>. Patients with HUS were defined as Xuzhou residents with the triad of microangiopathic hemolytic anemia (hematocrit < 30%, with evidence of intravascular erythrocyte destruction on a peripheral-blood smear), thrombocytopenia (platelet count, < = 150,000 per cubic millimeter), and evidence of renal involvement (blood urea nitrogen, >=20 mg per deciliter or abnormal urinary sediment). Controls were persons who did not have diarrhea, lived in the same village, with the same gender and age pattern as patients. Two controls were obtained per patient.

Data were collected by interviewing controls, patients or patients' family members using a standardized questionnaire to ascertain their history of diarrheal illness and any potential risk factors (e.g., without tap water, consuming undercooked food, eating leftover food, unsanitary kitchen conditions, and other behaviors). Interviews were conducted before the results of subtyping by PFGE became available, by interviewers who were not aware of the results.

To estimate the rate of illness among residents, a household survey was conducted during the selection of controls for the case-control study. Members of contacted households were queried about any history of acute diarrhea from May to July.

### Environmental investigation

Patients' fecal samples were obtained. Fresh domestic livestock and poultry fecal samples, fly samples, food samples, and water samples were collected in July. Samples were cultured for *E. coli* O157:H7 and subtyped with the use of PFGE.

### Laboratory investigation

Routine testing of patient fecal specimens involved selective culture for *Vibrio. parahaemolyticus* (CLED agar), *Salmonella* spp (DCA agar and XLD agar), *Shigella* spp (DCA agar), *Campylobacter* spp (Campylobacter selective agar), and *E. coli* O157 (cefexime tellurite sorbitol MaConkey agar).

Isolates were confirmed positive for *E. coli* O157:H7 using standard laboratory serological criteria and biochemical analyses. Each isolate was further characterized for the presence of sequences encoding the somatic O157 antigen (rfbO157)<sup>[10]</sup> and the H7 flagellar antigen (fliCH7)<sup>[11]</sup> by PCR. Molecular subtyping of *E. coli* O157:H7 isolates was performed at Jiangsu Provincial CDC using the restriction enzyme *Xba*I as described<sup>[12]</sup>. The PFGE patterns were examined visually and interpreted according to the criteria of Tenover et al<sup>[13]</sup> and Barrett et al<sup>[14]</sup>.

### Statistical analysis

Statistical analyses were conducted with the use of Epi Info, version 6.0. Univariate analyses were performed to screen for statistically significant variables. Then, a stepwise multiple logistics regression analysis was performed to adjust confounders simultaneously and to calculate the multivariate-adjusted odds ratios (OR) for factors<sup>[15]</sup>. The level of model selection was set to be 0.15 for in-and-out models.  $P < 0.05$  was considered to indicate statistical significance. The analysis was stratified according to age to account for differences in exposure that might have been the result of age<sup>[9]</sup>.

## RESULTS

### Epidemiologic and clinical information

By September 20, 1999, 4 diarrheal cases and 95 confirmed HUS cases of *E. coli* O157:H7 infection had been identified. Patients were from five counties and three districts in Xuzhou city and Su Yang county in the adjacent city, Shuqian (**Fig. 1**). Of the 95 HUS patients, 56 (59%) were female. They ranged in age from 5 through 90 years, and 73 (76.84%) were 60 years of age or older (**Table 1**). Dates of onset could be accurately determined for 95 patients and ranged from May 20 through September 20, 1999 (**Fig. 2**).

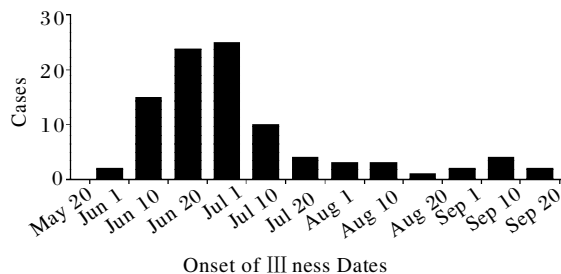
Univariate analysis showed that patients were more likely than controls to have had bad health habit (e.g., eating uncooked and leftover food, and not having tap water) in their daily lives. Bad house sanitary conditions (e.g., unsanitary room and kitchen, and high fly density) was also more common among patients than controls. Drinking bean milk was greater among patients than controls. Residents who washed their hands before eating were less likely to become ill, indicating a protective effect of hand washing (**Table 2**).



**Fig. 1** Outlined sites of *E. coli* O157:H7 infections in Jiangsu Province, China, in 1999

**Table 1** Number of reported cases and age-specific incidence rates for cases of HUS with onset of diarrhea

Age (YR)	No. of cases	Constituent ratio(%)	No. of died cases	Case fatality rate(%)
<5	0	0.00	0	/
5-9	1	1.05	0	0.00
10-19	0	0.00	0	/
20-29	1	1.05	0	0.00
30-39	6	6.32	5	83.33
40-49	3	3.16	3	100.00
50-59	11	11.58	8	72.73
60-69	20	21.05	18	90.00
70-79	39	41.05	34	87.18
80-90	14	14.74	14	100.00
Total	95	100.00	82	86.32



**Fig. 2** Outbreak-associated HUS cases in Jiangsu Province, May to Sep 1999

**Table 2** Univariate analysis of risk factors for HUA cases among residents

Category	Summary Odds Ratio	95% confidence interval	P Value
Personal health habit			
Eating uncooked food	3.493	(1.56–7.80)	0.002 3
Eating leftover food	2.918	(1.29–6.62)	0.010 4
Not washing hands before eating	4.869	(2.18–10.89)	0.000 1
Not having tap water	4.626	(1.24–17.27)	0.022 7
House sanitary status			
Unsanitary kitchen status	4.180	(1.74–10.05)	0.001 4
Unsanitary room status	3.091	(1.39–6.88)	0.005 5
High fly density	3.333	(1.32–8.41)	0.010 8
No fly-proof facility	2.529	(1.09–5.87)	0.030 7

On multivariate logistic-regression analysis, eating uncooked food(odds ratio[OR] 4.679; 95% confidence interval[CI], 1.58 to 13.88;  $P=0.005$  4), eating leftover

food(OR 5.404; 95% CI, 1.74 to 16.78;  $P=0.003$  5), not washing hands before eating(OR 3.984; 95% CI, 1.44 to 11.00;  $P=0.007$  6), not having tap water(OR 19.108; 95% CI, 2.38 to 153.63;  $P=0.005$  5), and poor sanitary conditions in the kitchen(OR 5.293; 95% CI, 1.33 to 21.00;  $P=0.017$  8) remained significant risk factors for infection(**Table 3**).

**Table 3** Multivariate analysis of risk factors among patients and controls

Category	Adjusted Odds Ratio	95% confidence interval	P Value
Eating uncooked food	4.679	(1.58-13.88)	0.005 4
Eating leftover food	5.404	(1.74-16.78)	0.003 5
Not washing hands before eating	3.984	(1.44-11.00)	0.007 6
Not having tap water	19.108	(2.38-153.63)	0.005 5
Unsanitary kitchen status	5.293	(1.33-21.00)	0.017 8

In the process of obtaining controls, we visited 5060 households and reviewed 20209 persons in the epidemic villages. Of these people, 719(3.56 %) had diarrheal manifestation. At the same time, 51603 persons in 13057 households in the non-epidemic villages in the same counties were also interviewed. Of them, 884(1.73%) had diarrheal manifestation. The diarrheal occurrence in the outbreak region was significantly higher than that in the non-outbreak region( $\chi^2=219.73$ ,  $P<0.01$ )(**Table 4**).

**Table 4** The attack rate of diarrhea for residents living in the outbreak and non-outbreak region

Area	Ill	Total	Attack rate%	$\chi^2=219.73$
Persons in the outbreak region	719	20209	3.56	$P<0.01$
Persons in the non-outbreak region	884	51063	1.73	

## Environmental investigation

In epidemic area, the primary source of income of residents was from agriculture, followed by raising domestic livestock. Human, livestock, and poultry manure was not separated from residence by a minimum distance and there was no manure management.

The number of isolates of *E. coli* O157:H7 recovered from patients and environment is summarized in **Table 5**. Six of 330 human fecal samples were colonized with *E. coli* O157:H7. Among of them, 2 strains were isolated from 30 fecal samples collected from cases with diarrhea and HUS, and 4 from 300 fecal samples of diarrheal patients. Twenty-two of 178 animals (12.36%) were colonized with *E. coli* O157:H7. Overall, cattle and goat were more often colonized than other animals. Two of 57 fly samples were colonized with *E. coli* O157:H7. Eight strains of *E. coli* O157:H7 were isolated from 252 food samples(3.2%). None of the 36 vegetables and fruits samples and 37 water

samples yielded *E. coli* O157:H7.

**Table 5** Carrier rate of *E. coli* O157:H7 in patient and environment

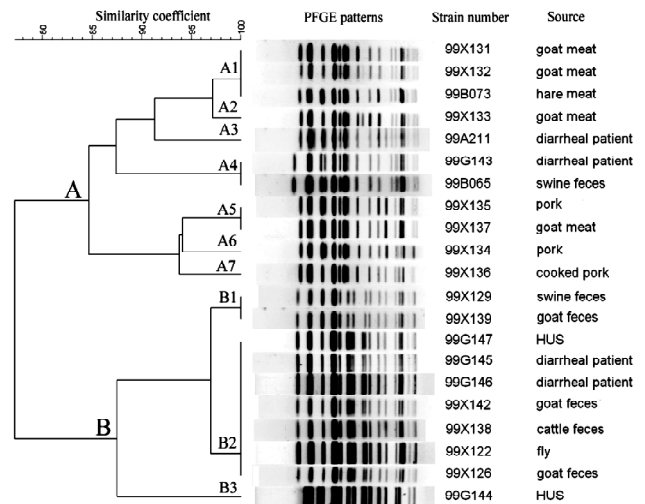
Source	No. of isolates/ No. of samples	Rate(%)
Patients	6/330	1.82
Patients with diarrhea	4/300	1.33
Patients with diarrhea and HUS	2/30	6.67
Animals	22/178	12.36
Cattle	9/47	19.15
Goat	6/48	12.50
Swine	4/36	11.11
Chicken	3/47	6.38
Flies	2/57	3.51
Food	8/252	3.17
Raw meat	4/76	5.26
Cooked meat	3/92	3.26
Pickles	1/48	2.08
Vegetables and fruits	0/38	0
Water	0/37	0

### Laboratory results

*Xba*I was used in the molecular typing of the 37 test strains of *E. coli* O157:H7 isolated from patients and environmental sources. There were 15-18 bands detected by PFGE among the 37 tested strains (full data not shown). The comparison of the PFGE patterns of 21 strains isolated from patients (99G143-99G147, 99A211) and environment (99X131-99X137, 99B073, 99B065, 99X122, 99X126, 99X129, 99X138, 99X139, 99X142) is shown in Fig. 3. Cluster analysis of the resulting dendrogram revealed two major clusters (Fig. 3A, B) defined at the 77.5% homological level. Three strains isolated from food (Fig. 3A1) with the same PFGE pattern, was closely related to two strains from food (Fig. 3A2) and one diarrheal patient (Fig. 3A3). One strain isolated from swine feces was colonized with *E. coli* O157:H7 that had the same pattern as isolates recovered from diarrheal patients (Fig. 3A4). Some strains from food had closely related PFGE patterns (Fig. 3A5-7). Six isolates from swine, goat, cattle, and fly colonized with *E. coli* O157:H7 had the same PFGE pattern as the isolates from diarrheal and HUS patients in the outbreak (Fig. 3B2), which was closely related to the pattern of two strains from swine and goat (Fig. 3B1).

### DISCUSSION

We describe a large outbreak of *E. coli* O157:H7 infections that were directly transmitted from animals and their environment to people. Coincident with the pattern described in other studies<sup>[6]</sup>, 97.89% of cases of the HUS presented with bloody diarrhea between June and September. But the occurrence and the fatality rate from HUS during the outbreak was very much higher than described elsewhere<sup>[17]</sup>. It was likely that physicians in the epidemic area were not aware of this



**Fig. 3** PFGE profiles of the genomic DNAs of *E. coli* O157:H7 strains isolated from patients and environment digested with *Xba* I and schematic diagram of dendrogram analysis of the strains

newly recognized syndrome and using antibiotics just after the onset of illness. An association exists between the use of antibiotics and increased mortality with *E. coli* O157:H7 infection<sup>[17]</sup>.

*E. coli* O157:H7 was isolated from 6 (1.82%) of the 330 patient stool specimens. None of the 6 specimens were positive for the usual enteric pathogens, including *Vibrio parahaemolyticus*, *Salmonella*, *Shigella*, and *Campylobacter*. All 6 patients had grossly bloody diarrhea with clinical manifestations typical of hemorrhagic colitis; two of them died of HUS. Our rate of isolation of *E. coli* O157:H7 in patients with bloody diarrheal illness, was much lower than that observed in other similar studies<sup>[18]</sup>. This may have resulted from the infected person with onset of non-bloody diarrhea unwilling to seek medical attention. As a result, the opportunity to isolate the patient and collect a stool sample was lost. The rate of isolation decreases with delay in collection of stool samples. In addition, cultures obtained after the administration of antibiotics often produce negative results<sup>[18]</sup>. Food-borne transmission of *E. coli* O157:H7 is the most important means of infection. But in our study, no common food or water exposure was identified during early patient interviews. The outbreak may not be an isolated event. A case-control study conducted by the CDC found that bad personal health habit and bad house sanitary conditions were associated with an increased risk of *E. coli* O157:H7 infection.

The primary animal reservoir for *E. coli* O157:H7 is ruminant livestock, which are asymptotically colonized. The proportion of animals colonized with *E. coli* O157:H7 in the epidemic area (12.36%) was higher than in other studies (rates are typically in the

range of less than 0.5 % to 2 %) [19]. High rates of colonization were identified among cattle (19.15%), goat (12.50%), and swine (11.11%). During the outbreak the high prevalence of *E. coli* O157:H7 colonization in the herd most likely contributed to increased contamination of animal hides and the environment, as well as cross-contamination during food preparation. This was confirmed by the high rate of *E. coli* O157:H7 isolation from meat products (7 of 166). This increased contamination in turn created greater risk to residents.

Evidence from a household survey suggested that higher than expected rates of diarrhea occurred among local residents, indicating excess illness that was not identified by routine case finding. This highlights the potential scale of *E. coli* O157:H7 infection and suggests that the importance of this organism in epidemic area may previously have been underestimated.

By using *Xba*I PFGE typing, *E. coli* O157:H7 isolated from patient, animals, and food demonstrated more clonal diversity than expected. These results indicated that *E. coli* O157:H7 strains were widespread in Xuzhou in infected animals and humans, as well as contaminated food products. Cattle, goat, and swine appeared to be important animal reservoir of these strains. These microorganisms can enter food during meat and dairy product processing if hygienic conditions are inadequate. At the same time, the fly could be a carrier of transmission associated with the outbreak.

Based on our findings, *E. coli* O157:H7 can be passed to humans indirectly via feces and manure spread on land. The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and the domestic environment. It is important to make it clear to residents that appropriate management of slurry and manure is necessary to avoid contamination and reduce the risk of infection. An effective method of eliminating *E. coli* O157:H7 from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking thoroughly or pasteurization) and simple control measures such as washing hands before eating and after contact with animals or animal feces. Fly killing is another effective way to protect food from *E. coli* O157:H7 contamination. Important public health measures include increasing physician awareness of *E. coli* O157:H7 infection, and mandating case reporting.

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