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Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties

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SUMMARY

Between November 1992 and February 1993, a large outbreak of *Escherichia coli* O157:H7 infections occurred in the western USA and was associated with eating ground beef patties at restaurants of one fast-food chain. Restaurants that were epidemiologically linked with cases served patties produced on two consecutive dates; cultures of recalled ground beef patties produced on those dates yielded *E. coli* O157:H7 strains indistinguishable from those isolated from patients, confirming the vehicle of illness. Seventy-six ground beef patty samples were cultured quantitatively for *E. coli* O157:H7. The median most probable number of organisms was 1·5 per gram (range, $< 0{\cdot}3$–15) or 67·5 organisms per patty (range, $< 13{\cdot}5$–675). Correlation of the presence of *E. coli* O157:H7 with other bacterial indicators yielded a significant association between coliform count and the presence of *E. coli* O157:H7 ($P = 0{\cdot}04$). A meat traceback to investigate possible sources of contamination revealed cattle were probably initially colonized with *E. coli* O157:H7, and that their slaughter caused surface contamination of meat, which once combined with meat from other sources, resulted in a large number of contaminated ground beef patties. Microbiological testing of meat from lots consumed by persons who became ill was suggestive of an infectious dose for *E. coli* O157:H7 of fewer than 700 organisms. These findings present a strong argument for enforcing zero tolerance for this organism in processed food and for markedly decreasing contamination of raw ground beef. Process controls that incorporate microbiological testing of meat may assist these efforts.

INTRODUCTION

Escherichia coli O157:H7 was first identified as a cause of illness in 1982 during an outbreak of severe bloody diarrhoea that was traced to contaminated hamburgers [1]. It has since emerged as an important pathogen, with its most severe manifestations in young children and the elderly. About 5% of infected persons develop haemolytic uraemic syndrome, characterized by haemolytic anemia, thrombocytopaenia, renal failure and a death rate of 3–5% [2].

Most outbreaks of *E. coli* O157:H7 infections have been the result of transmission through foods of bovine origin, particularly beef, although many other foods have also been implicated [2]. The food handling practices that led to these outbreaks were generally not gross errors but more subtle deficits in cooking

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times or temperatures. This suggests that ingestion of only a small number of bacteria is required for illness. However, no studies have been conducted with human volunteers to determine the infectious dose directly.

E. coli O157:H7 lives in the intestinal tract of healthy cattle and contaminates meat during slaughter and processing [3, 4]. Pathogens on the surface of the meat may be transferred to the interior during grinding, resulting in internal contamination of the ground beef. Despite technological advances in food microbiology, no simple method exists to screen large quantities of raw meat and meat products quickly and reliably for the presence of this organism.

Between November 1992 and February 1993, an outbreak of more than 700 infections with *E. coli* O157:H7 including four deaths occurred in the western United States [5–8]. Infection was associated with eating hamburgers at restaurants of one fast-food chain. In Washington state, where the majority of the cases occurred, all uncooked hamburgers were recalled within a week of recognition of the outbreak. Based on review of hamburger production and distribution records, and discussions with individual restaurant managers, investigators determined that the majority of ground beef patties epidemiologically linked with cases were made and frozen on 19 November 1992 at a meat processing plant in southern California, during the latter half of the hourly production (lots 10–17) for that day. Investigations in Nevada, Idaho, and southern California implicated ground beef patties produced during specific hours of production on 19 November as well as 20 November 1992. Cultures of recalled ground beef patty samples from suspect lots produced on those dates yielded *E. coli* O157:H7 strains indistinguishable from those isolated from patients, confirming that hamburgers were the vehicle of illness. In this report, we provide data from a meat traceback done to investigate the possible sources of contamination. We also present data on numbers of *E. coli* O157:H7 present in ground beef patties from the same production lots consumed by ill persons in the outbreak. Finally, we estimate the dose required for infection and analyse the ability of current standard microbiological tests to predict the presence of *E. coli* O157:H7 in beef.

MATERIALS AND METHODS

Sampling

Suspect lots of ground beef patties were defined as those produced by the implicated plant on 19 and 20 November 1992, that were epidemiologically linked with culture-confirmed *E. coli* O157:H7 infection. A total of 76 ground beef patty samples were collected from 16 of the 17 lots produced on 19 November and 5 of 17 lots produced on 20 November. These were shipped overnight on dry ice to the Center for Food Safety and Quality Enhancement of the University of Georgia for quantitative culture for *E. coli* O157:H7. Samples consisted of either four jumbo (112·5 g) patties or 10 regular (45 g) patties. Lots tested were dependent on availability to the University of Georgia laboratory during the investigation.

Laboratory methods

Selective enrichment

A 25 g sample of each ground beef patty was obtained aseptically under a SterilGARD hood (Baker Company, Sanford, ME), and added to 225 ml of modified Trypticase soy broth [9], which contained 30 g Trypticase soy broth, 10 g casamino acids, 1·5 g bile salt No. 3, 6 g Na_2HPO_4, 1·35 g KH_2PO_4, and 10 mg acriflavin per L. The samples were incubated at 37 °C for 18 h with agitation (100 rpm).

Enzyme-linked immunosorbent assay (ELISA)

A sandwich ELISA was performed in a 96-well polystyrene plate (Gibco, Grand Island, NY). Each well was coated with 1 μg of affinity-purified goat antibody against *E. coli* O157:H7 (Kirkegaard and Perry Laboratories, Gaithersburg, MD) in 100 μl of 50 mM carbonate buffer, pH 9·6, and incubated at room temperature overnight with agitation (150 rpm). The plate was washed in 50 mM Tris, pH 7·4, containing 150 mM NaCl (TBS), and remaining binding sites were blocked with milk diluent-blocking solution (Kirkegaard and Perry) for 1 h at 37 °C. The blocking solution was removed, and 200 μl of enrichment culture was added per well and incubated at 37 °C for 1 h. After the wells were washed four times with TBS containing 0·05% Tween 20 (TBS-T), 100 μl of monoclonal antibody 4E8C12 [10] was added; the plate was then incubated at 37 °C for 1 h. The wells were washed four times with TBS-T, then 100 μl of goat anti-mouse immunoglobulin M+G conjugated to alkaline phosphatase (diluted 1:800 in TBS-T; Kirkegaard and Perry) was added and incubated at 37 °C for 1 h. After washing wells with TBS-T, 100 μl of *p*-nitrophenyl phosphate in 1 M 2-amino-2-methyl-1-propanol (Sigma Chemical Co., St Louis, MO),

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pH 9·9, (1 mg/ml) was added to each well. The plate was incubated at 37 °C for 1 h, and the optical density at 405 nm was determined with an EL 312e microplate reader (Bio-Tek Instruments, Inc., Winnooski, VT). A reading of 0·1 above the negative control was considered as presumptively positive for *E. coli* O157:H7.

Plating following enrichment

After enrichment, culture medium from *E. coli* O157:H7 ELISA-positive samples was serially diluted in 0·85% NaCl, and 0·1 ml of 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} dilutions was inoculated onto a sorbitol–MacConkey agar (Unipath, Oxoid Division, Ogdensburg, NY) plate containing 0·1% 4-methylumbelliferone-β-D-glucoronide (SMA-MUG) in duplicate. Plates were incubated at 37 °C for 18 h.

Selection of E. coli *O157:H7 colonies*

Each colony that was both sorbitol negative and MUG negative was streaked onto a SMA-MUG plate and incubated for 18 h at 37 °C. Sorbitol-negative and MUG-negative colonies were tested for agglutination by the *E. coli* O157 latex agglutination assay (Unipath, Oxoid Division). Presumptive *E. coli* O157 isolates were further identified as *E. coli* O157:H7 by O157 and H7 antisera (Difco Laboratories, Detroit, MI).

Determination of aerobic plate count

One gram of ground beef patty from each sample was serially diluted in 0·1% peptone to 10^{-6}, and 0·1 ml of each dilution was inoculated in duplicate onto plate count agar. The plates were incubated at 37 °C for 48 h.

Determination of most probable number (MPN) of coliforms and E. coli

Ground beef patty (1 g) was added to each of three tubes containing 9 ml of lauryl tryptose (LST, Difco Laboratories) broth. Similarly, 1 ml of 0·1 and 0·01 g dilutions of the patty was added to three tubes of 9 ml of LST for each dilution. The tubes were incubated for 24 h at 37 °C and examined for gas production. A loopful of culture from each tube positive for gas production was transferred to a separate tube containing 9 ml of brilliant green lactose bile broth (Difco Laboratories) broth. The tubes were incubated for 24 h at 37 °C and examined for gas. A loopful of suspension from each tube positive for gas production was transferred to separate tubes containing 9 ml of EC broth. Tubes were incubated or 24 h at 45·5 °C. A loopful of suspension from each tube positive for gas was streaked onto Levine's eosin-methylene blue agar and incubated at 37 °C for 16 h. Colonies typical of *E. coli* (dark-centred with or without metallic sheen) were selected and confirmed as *E. coli* by API 20E miniaturized diagnostic test (Analytab Products, Division of Sherwood Medical, Plainview, NY). MPN was determined according to the protocol described in the FDA Bacteriological Analytical Manual [11].

Determination of MPN of E. coli *O157:H7*

Ground beef patty (1 g) was added to each of three tubes containing 9 ml of mTSB [9] and incubated at 37 °C for 24 h. Similarly, 0·1 and 0·01 g dilutions of the patty were added to three tubes of 9 ml of mTSB for each dilution. After incubation, 1 ml from each tube was serially diluted in 0·85% NaCl, and 0·1 ml from 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} dilutions was inoculated onto SMA–MUG plates in duplicate. The plates were incubated at 37 °C for 18 h. Colonies typical of *E. coli* O157:H7 were selected and confirmed as *E. coli* O157:H7 according to the procedures described above. Calculation of MPN was based on the sample dilutions and number of tubes with confirmed *E. coli* O157:H7, according to the protocol described in the FDA Bacteriological Analytical Manual [11].

Analysis

The associations between aerobic plate count, MPN of coliforms, and MPN of faecal coliforms and presence of *E. coli* O157:H7 were examined using the Kruskal–Wallis non-parametric test. Values for the MPN of coliforms and MPN of fecal coliforms that were recorded by the laboratory as >110 were considered equal to 110 for the analysis.

The Pearson correlation coefficient was used to determine the relationship between sets of quantitative microbiologic results.

The number of organisms in a patty was determined by multiplying the MPN per gram of *E. coli* O157:H7 by the patty weight.

Meat traceback

Meat used in the production of ground beef patties from those lots epidemiologically linked with illness

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Table 1. *Aerobic plate counts, most probable number of coliforms, most probable number of* E. coli, *most probable number of* E. coli *O157:H7, and number of samples that yielded* E. coli *O157:H7 over the number tested per lot. Western US* E. coli *O157:H7 outbreak, Nov 1992–Feb 1993*

Lot no.	Aerobic plate count ($\times 10^3$)	Coliforms (MPN/g)	*E. coli* (MPN/g)	*E. coli* O157:H7 (MPN/g)	*E. coli* O157:H7 (MPN/patty)	Samples +/ samples tested*
Regular (45 g) patties produced 19 November 1992						
4	75	> 110	> 110	< 0·3	< 13·5	1/5
9	0·5	> 110	46	1·5	67·5	1/5
10	13·5	110	1·1	2·8	126·0	1/3
12	23·5	50	2·1	15	675·0	3/4
12	15	> 110	> 110	ND	ND	
12	5	> 110	15	ND	ND	
16	74·5	50	4	0·3	13·5	1/1
17	30	9	2·1	1·5	67·5	1/7
Jumbo (112·5 g) patties produced 20 November 1992						
7	260	> 110	110	ND	ND	1/1

N.D., not determined.

* Lots sampled from 19 November 1992 which did not yield *E. coli* O157:H7 were: lot 1 (3 samples), 2(4), 5(3), 6(6), 7(6), 8(3), 11(1), 13(1), 14(1), 15(1); from November 20, 1992; 6(1), 8(2), 12(1), 16(1).

was traced as far back to its source as possible. Facilities were inspected and records reviewed at a meat processing plant, a boning plant, and five slaughter plants. Animals were traced whenever possible to their farm of origin.

RESULTS

Thirty-four lots of 281 000 lbs of ground beef patties were manufactured in the implicated plant on 19 and 20 November 1992. Of the 21 lots tested by the University of Georgia laboratory, 7 yielded *E. coli* O157:H7 (Table 1). The median MPN of *E. coli* O157:H7 was 1·5 organisms/g (range, < 0·3–15) or 67·5 organisms/patty (range, < 13·5–675) (Table 1).

The values for aerobic plate count, coliforms, and *E. coli* varied widely among lots, although the MPN of faecal coliforms was determined to be equivalent to the MPN of *E. coli* for the samples assayed. However lots that yielded *E. coli* O157:H7 had higher aerobic plate counts, coliform counts, and *E. coli* counts than those that did not yield *E. coli* O157:H7 (Table 2). The association between the presence of *E. coli* O157:H7 and the coliform count was significant at the $P < 0{\cdot}05$ level, although substantial overlap in coliform counts between positive and negative samples occurred. The association between the presence of *E. coli* O157:H7 and the aerobic plate count was of borderline significance.

No correlation between trends of results for aerobic plate count, MPN of coliforms, and MPN of *E. coli* was found.

Meat used to produce ground beef patties on 19 and 20 November came from both domestic and foreign sources. Fresh meat from multiple suppliers and frozen imported meat was ground separately and then combined into a 'batch' according to a specific formulation and reground. Up to five batches were formulated in an hour, each containing meat from a variety of suppliers. The lot number indicated the production hour. One lot could contain meat from as many as five batches and from many different suppliers. Meat was traced back to a boning plant common to several lots epidemiologically linked with illness. The meat from this boning plant came from six slaughter plants, five within the United States and one in Canada. Four hundred and forty-three carcasses from the five domestic slaughter plants plus an unknown number from the Canadian slaughter plant could have been used to produce the meat provided by the common boning plant. Animals supplied by the US slaughter plants were traced to saleyards, farms, and dealers in six states.

Observation of procedures at the five US slaughter plants and one boning plant revealed some possible mechanisms for contamination. Two slaughter plants used bed slaughter, a technique in which the carcass is laid supine, hide intact, on a bed of rails 8–10 inches above the floor. At one location, during skinning of

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Table 2. *Correlation of the presence of* E. coli *O157:H7 with other bacterial indicators in 76 samples from 21 suspect lots produced on the dates linked with illness, Western US* E. coli *O157:H7 outbreak, Nov 1992–Feb 1993*

Indicator	*E. coli* O157:H7 positive ($n = 9$)	*E. coli* O157:H7 negative ($n = 67$)	*P*-value
Aerobic plate count (median number $\times 10^3$)	24 [0·5–260]*	7·5 [0·01–360]	0·11
Coliform count (median MPN/g)	110 [9–> 110]†	46 [0·4–> 110]‡	0·04
E. coli (median MPN/g)	15 [1·1–> 110]	9·3 [0–> 110]	0·5

* Range is given in square brackets.
† 6 of 9 positive samples $\geqslant 110$.
‡ 25 of 67 (37%) negative samples $\leqslant 46$.

the chest and abdomen, the inside surfaces of loose hide touched the floor then swung back and touched the meat. At another plant that used this method, investigators observed that a broom used to sweep faeces from the floor touched the inside portion of the hide more than once. At both locations, investigators observed a carcass suspended by the hindlegs while the front end remained on the floor; these carcasses were dragged on loose flaps of hide to the next station for evisceration. At one plant, investigators observed employees skinning the tail and tying off the rectum by hand, then immediately handling exposed meat on carcasses. Investigators also observed employees handling both the hide and skinned areas of the carcasses without intervening handwashing. At these two slaughter plants, skinning and carcass movement were done manually. The three other slaughter plants used a more mechanized slaughtering method during which the carcass remained suspended off the floor at all times and required less direct handling.

At the boning plant, a 25 m long table, which consisted of a hard plastic conveyer belt with interlocking joints, carried several tons of boned meat daily to one end for packaging. This surface was sanitized only once every 24 h.

DISCUSSION

The microbiological results from this investigation provide some suggestive evidence that the infectious dose of *E. coli* O157:H7 may be fewer than 700 organisms, the concentration present in raw ground beef from lot 12, the most contaminated lot. It may be substantially fewer, because the meat was cooked before consumption, which undoubtedly further reduced the number of organisms, and because other lots that also may have caused illness had fewer organisms per raw patty. It is unlikely that the infectious dose will be determined accurately in studies with healthy volunteers because the lack of specific treatment and potential severity of complications of infection make these studies unethical. The possibility of such a low infectious dose is consistent with the patterns of transmission demonstrated by *E. coli* O157:H7 in past outbreaks [2], which include an ability to spread easily directly from one person to another [12], and transmission by water [13] and apple cider [14]. A subsequent outbreak implicating dry fermented salami in the US [15] demonstrated that even lower doses of the bacteria can cause disease. The fact that the meat samples tested were obtained and shipped frozen, thereby preventing bacterial growth, adds further validity to the low counts of *E. coli* O157:H7 we obtained. However, the data only provide suggestive evidence of the range of concentrations that may have been responsible for illness limited by the fact that although the ground beef tested was from the same lots as those consumed by patients, lots were large and patties not tested may have had even higher counts than those recorded here. Those lots with low counts were less likely to have caused illness. The sharp decline in the number of new cases after the ground beef patty recall [5], confirmed that the ground beef patties recalled and tested caused the illness. Lot 12 was the most predominant lot in Washington restaurants during the outbreak, and most ground beef patties consumed by patients were probably from this lot. Production and distribution records verified that lot 12 had been shipped to and was most likely in use by restaurants during the week before cases occurred. Three of four samples from lot 12 tested positive by the University of Georgia laboratory for *E. coli* O157:H7, a higher percentage than any other lot (Table 1).

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During the outbreak, ground beef patty samples were tested for the presence of *E. coli* O157:H7 by several other laboratories, including Silliker Laboratories, California Department of Health Services, Washington Department of Health, Nevada State Health Division, and the US Department of Agriculture. All lots produced on 19 November 1992 were tested as well as all lots produced on 20 November 1992 that were available (lots 4–8, 12, 16 and 17). No lot tested positive by any of these laboratories that did not also test positive by the Food Safety and Quality Enhancement Laboratory of the University of Georgia. In this report we include only the results from the University of Georgia laboratory because extensive quantitative culturing for *E. coli* O157:H7 was done only by this laboratory.

The meat traceback provided insight into the enormous complexity of the farm-to-table continuum for meat, and revealed possible mechanisms for the contamination that led to the outbreak. It is most likely that this outbreak originated from gastrointestinal colonization of one or more cattle with *E. coli* O157:H7. Current slaughtering methods then likely contributed to surface contamination of meat which occurred from contact with faecally soiled hides or intestinal contents of colonized animals. The contaminated meat, through central processing, was then mixed and ground with meat from numerous other sources, resulting in a large number of contaminated ground beef patties. The restaurant chain's cooking method using suboptimal temperatures was insufficient to kill the *E. coli* O157:H7 in the patties [5, 7].

The use of microbiological testing of raw meat as a measure of sanitary quality has been a controversial issue in recent years for the industry. An array of microbiological standards have been both proposed and established in the United States and Canada with variable success [16–18] and have centred around bacterial limits for aerobic plate counts and *E. coli* (MPN) counts. Issues raised have included high aerobic plate counts which do not coincide with meat quality [19], the effect of incubation temperature on aerobic plate counts (with higher counts at 20 °C than at 35 °C) [19, 20], and heterogeneous distribution of *E. coli* in ground beef, making sampling poorly representative of any given batch [19, 21] and contributing to the imprecision of an already inefficient 3-tube MPN assay [19, 22–24]. Many in the industry feel that microbiological criteria can be used more effectively as guidelines in conjunction with better control of temperatures, equipment cleanliness, sanitation, and timeliness in the handling and distribution of ground beef [25, 26].

Although our results show that coliform counts were significantly associated with the presence of *E. coli* O157:H7, the actual value of this test as a screen for the presence of *E. coli* O157:H7 in ground beef is questionable because of the considerable overlap of values between positive and negative specimens. Interestingly, the MPN of *E. coli* did not show any correlation with the presence of *E. coli* O157:H7. Perhaps this reflects the heterogeneity of distribution of *E. coli* in ground beef [19, 21]. Because *E. coli* also thrives well outside the intestinal tract, its presence is not necessarily an indicator of faecal contamination [20]. An elevated aerobic plate count was also associated with the presence of *E. coli* O157:H7, although this finding did not reach statistical significance. Other studies have found an opposing trend or lack of correlation between APC and *E. coli* MPN and have attributed this finding to the effect of meat flora on the viability or detectability of *E. coli* in the sample [19, 22]. We found no correlation between trends for any of the results.

This outbreak provides an example of the need for both process controls for meat to reduce the occurrence and numbers of pathogenic organisms, and microbiological testing of meat and meat products. Although past US Department of Agriculture regulations required only gross inspection of carcasses, more comprehensive regulations under the Hazard Analysis Critical Control Point (HACCP) programme, including process controls that incorporate guidelines for microbiological testing of meat, have recently been passed [27] and have already been implemented successfully by some producers who have reported a marked decrease in pathogen counts in raw meat [28]. To assure the safety of meat and meat products, microbiological testing is necessary both as an indication of process control and as the basis for a pathogen-reduction performance standard. How useful microbiological testing for pathogens or indicator bacteria will be has yet to be determined. Although our data did not clearly identify a microbiological indicator for the detection of *E. coli* O157:H7 in ground beef, we did find a correlation between coliform count and the presence of *E. coli* O157:H7. Considerably more samples from a variety of sources must be tested in a larger study before broad conclusions can be drawn regarding this finding. It may serve to be useful as a screening test in

a production plant where numerous samples can be taken, to determine which lots need to be examined more closely.

The fact that the dose required for infection with *E. coli* O157:H7 is so low it necessitates the need for enforcing zero tolerance of this organism in processed food and for markedly decreasing contamination of raw ground beef. The question is then, how does one rapidly and accurately test for its presence in an industry where microbial guidelines remain an unsatisfactory method for determining overall sanitary quality? Our data suggest the coliform count may serve as a useful adjunct along with other measures in screening lots for the presence of *E. coli* O157:H7. The correlation between coliform count and *E. coli* O157:H7 is worth re-examining and may be useful in a HACCP programme for ground beef.

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