Epidemiol. Infect. (2009), **137**, 375–382. © 2008 Cambridge University Press
doi:10.1017/S0950268808001702 Printed in the United Kingdom

A national outbreak of verotoxin-producing *Escherichia coli* O157 associated with consumption of lemon-and-coriander chicken wraps from a supermarket chain

P. J. WHITTAKER[1]*, W. SOPWITH[1], C. QUIGLEY[1], I. GILLESPIE[2], G. A. WILLSHAW[2], C. LYCETT[3], S. SURMAN-LEE[4], D. BAXTER[1], G. K. ADAK[2] AND Q. SYED[1]

[1] *Health Protection Agency, North West, Liverpool*
[2] *Health Protection Agency, Centre for Infections, London*
[3] *Environmental Health Department Milton Keynes Council*
[4] *Health Protection Agency, RMN London Regional Food Water and Environmental Microbiology Services Laboratory*

(*Accepted 11 November 2008; first published online 23 December 2008*)

SUMMARY

A national outbreak of verotoxin-producing *Escherichia coli* O157 infection affected five English regions and Wales. Twelve cases were associated with lemon-and-coriander chicken wrap from a single supermarket chain consumed over a 5-day period. An outbreak investigation aimed to identify the source of infection. Descriptive epidemiology and phenotypic and genotypic tests on human isolates indicated a point-source outbreak; a case-control study showed a very strong association between consumption of lemon-and-coriander chicken wrap from the single supermarket chain and being a case (OR 46·40, 95% CI 5·39–∞, $P=0{\cdot}0002$). Testing of raw ingredients, products and faecal samples from staff in the food production unit did not yield any positive results. The outbreak was probably caused by one contaminated batch of an ingredient in the chicken wrap. Even when current best practice is in place, ready-to-eat foods can still be a risk for widespread infection.

Key words: *Escherichia coli*, foodborne infections, food safety, gastrointestinal infections, outbreaks.

INTRODUCTION‘

Verotoxin-producing *Escherichia coli* of serogroup O157 (VTEC O157) is an important cause of severe gastrointestinal illness, with clinical manifestations ranging from mild diarrhoea, haemorrhagic colitis, to the potentially fatal haemolytic uraemic syndrome (HUS) [1–3]. The main reservoir of VTEC O157 is the gastrointestinal tract of healthy cattle and sheep, and bacteria enter the food chain by faecal contamination of raw food materials [1]. VTEC O157 is killed by adequate cooking of meat and pasteurization of milk, but consumption of undercooked meat [2, 3] and raw dairy products [4] has resulted in outbreaks of infection. There have also been reports of outbreaks linked to raw salad and vegetable produce [5–11]. Infection with VTEC O157 is relatively rare in England and Wales compared with other gastrointestinal pathogens, with 950 cases reported to the Health Protection Agency (HPA) in 2005, compared with over 4500 cases of *Campylobacter* and over 11 000 cases from *Salmonella* spp. [12]. Laboratory surveillance of

* Author for correspondence: Dr P. Whittaker, Manchester PCT, Floor 2, Mauldeth House, Mauldeth Road West, Manchester M21 9WN, UK.
(Email: paula.whittaker@manchester.nhs.uk)

VTEC O157 has been carried out since 1982 by the HPA [13–19]. For routine surveillance of sporadic presumptive cases of VTEC O157, faecal isolates are sent to HPA Laboratory of Enteric Pathogens (LEP) for confirmation and typing. After confirmation of the diagnosis, an initial urgent enquiry is conducted by the local Health Protection Unit (HPU) using a standard food history questionnaire as a guide to identify the suspected source of exposure and assess the risk of transmission.

On 5 July 2007, a consultant microbiologist in North West England advised the local HPU of two cases of geographically unrelated VTEC O157 infection. Both cases had become ill on 29 June and the only common link was consumption of a lemon-and-coriander chicken wrap, a new product launched on 24 June, purchased from two different branches of a major supermarket chain on 26 June. No other risk factors for VTEC O157 were reported. The production unit where the implicated wraps were prepared was a large facility manufacturing a wide range of ready-to-eat products. There was no cooking of foods on site.

A third case in North West England, that had also purchased a wrap from the supermarket chain, was reported the following day. All presumptive VTEC O157 isolates were sent for urgent typing. The supermarket withdrew the product on 6 July on the basis of these three presumptive cases. On 9 July, a fourth case was reported in South East England, prompting the formation of a national Outbreak Control Team (OCT). The aim of this study was to identify the source of the outbreak, using a combination of epidemiological, microbiological and environmental investigations.

METHODS

Epidemiological

VTEC O157 is routinely reported through laboratory surveillance and additional epidemiological information may be gathered by HPUs to assist identification and investigation of clusters. HPUs in England & Wales, Health Protection Scotland, and HPA Northern Ireland were asked to report such information on new cases of VTEC O157 to the OCT. Retrospective case ascertainment was pursued for all cases of VTEC O157 reported between 20 June and 1 August through the laboratory surveillance system.

A case-control study was conducted to test the hypothesis that infection was associated with consumption of lemon-and-coriander chicken wraps from supermarket chain P. The study was done as part of the incident investigation and prior to the full number of cases being known. Cases were defined as residents of England & Wales notified since 20 June 2007 (no cases were reported from Scotland or Northern Ireland) infected with a laboratory-confirmed isolate of VTEC O157 phage type (PT) 8 with genes for VT1 + 2 (see Microbiology section below). Asymptomatic controls were recruited through systematic sequential telephone dialling, based on the cases' telephone numbers. Cases and controls with a recent history of foreign travel, or contact with individuals with gastrointestinal infections were excluded. Sample size calculations suggested that 10 and 20 cases and controls, respectively, would be needed to provide sufficient power (80%) to detect a difference at the 95% level. A standard, structured questionnaire was administered to cases and controls by telephone, during evenings and the weekend. This was more detailed than the standard food history questionnaire already administered to cases as part of routine surveillance, and sought demographic, clinical and travel details and exposures to food, water, the environment and animals. Cases were asked about exposures in the 5 days before onset of illness and the 3 weeks prior to interview. Controls were asked about the 5 days prior to interview and the 3 weeks prior to interview. The 3-week time period was included because the main hypothesis under investigation was withdrawn from sale on 6 July and therefore not available for controls to consume in the 5 days before interview.

Data manipulation was undertaken in Stata version 9 (StataCorp, College Station, TX, USA) and statistical analyses were undertaken in Stata version 9 and StatXact version 8. Proportions and medians were compared using the χ^2 test and Student's t test, respectively. The effect of each exposure on being a case was investigated initially using logistic regression whilst controlling for quintiles of age. As the sample size was small, StatXact was used to calculate exact estimates of odds ratios (OR), 95% confidence intervals (CI) and significance tests.

Exposures other than the main hypothesis under investigation, positively associated with being a case and significant at the 90% level, were examined further using multivariate logistic regression analysis. Each variable under consideration was included in a model and its effect on being a case was tested whilst

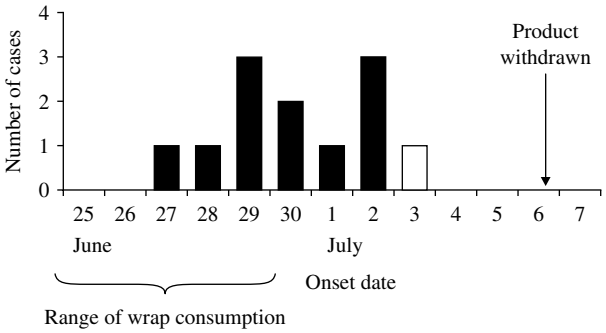

Fig. 1. Bar chart showing date of onset of incident cases with identical molecular type, showing range of hypothesized exposure and date of product withdrawal. [White bar (□) = case with slightly variant PFGE profile.]

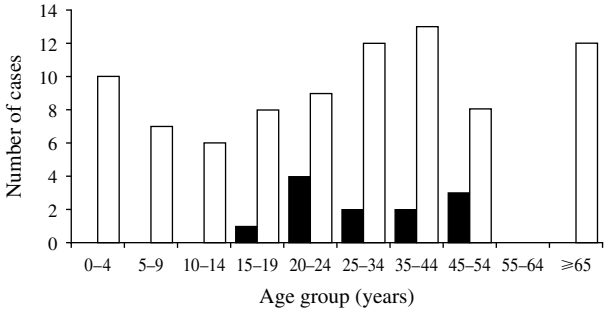

Fig. 2. Bar chart showing number of cases by age group of incident cases of VTEC O157 (■) associated with lemon-and-coriander chicken wrap and background sporadic cases of VTEC O157 (□) reported between 20 June 2007 and 1 August 2007 through routine laboratory surveillance.

controlling for the main hypothesis under investigation and quintiles of age.

Microbiological

Faecal specimens from cases and staff at the food production unit were tested by clinical laboratories according to standard operating procedures. All isolates of presumptive VTEC O157 were submitted to the reference laboratory where they were confirmed biochemically as *E. coli* and serotyped [20]. They were tested for the presence of verotoxin and gamma intimin genes by polymerase chain reaction [21, 22] (HPA LEP, unpublished data) and phage-typed [23]. Isolates from the initial cases of VTEC O157 that had consumed chicken wraps were examined by pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with the restriction enzyme *Xba*I [24] in an attempt to identify an outbreak strain. Fragment profiles were analysed, stored and compared using Bionumerics software (Applied Maths, Kortrijk, Belgium). Other restriction enzymes were not used in this investigation. Once the outbreak strain was identified sporadic isolates received between 20 June and 1 August were also screened by PFGE to identify additional potential outbreak cases.

Food and environmental samples from the production unit were tested. Enrichment and separation of presumptive VTEC O157 from background contamination was by immunomagnetic separation with anti-O157 specific immunomagnetic beads and an automated immunomagnetic separator (Dynal, Invitrogen, Paisley, UK). Beads were cultured for VTEC O157 onto cefixime tellurite sorbitol MacConkey agar at 37 °C for 18–24 h. All samples were tested in laboratories with appropriate ACDP 3 containment facilities that held UKAS accreditation to test for the presence of VTEC O157.

Environmental

A detailed investigation of processes and records in the food production unit and traceback of the products used in the wrap was conducted. The investigation focused on the ingredients unique to the wrap and the processes in place for its production, including the staff, equipment, environment and handling practices throughout production.

RESULTS

Epidemiological

During the period of investigation (20 June–1 August 2007), there were 135 cases of VTEC O157 in England & Wales reported through routine laboratory surveillance, of which 81 were 'sporadic' (not linked with travel or known outbreaks) and distributed across all age groups. Twelve of these cases reported consuming lemon-and-coriander chicken wraps between 25 and 29 June (Fig. 1). Onset of illness was from 27 June to 3 July. The median and modal incubation times between consumption of wrap and symptoms were both 3 days (range 2–7 days). The age range of incident cases was restricted to 17- to 50-year-olds (Fig. 2). Cases were widely distributed across England & Wales (Fig. 3).

At the time of the case-control study (conducted 12–14 July), eight cases with the outbreak profile had been identified and were available for interview and 39 controls were recruited (see Table 1). Cases and controls were similar with regard to gender (38%

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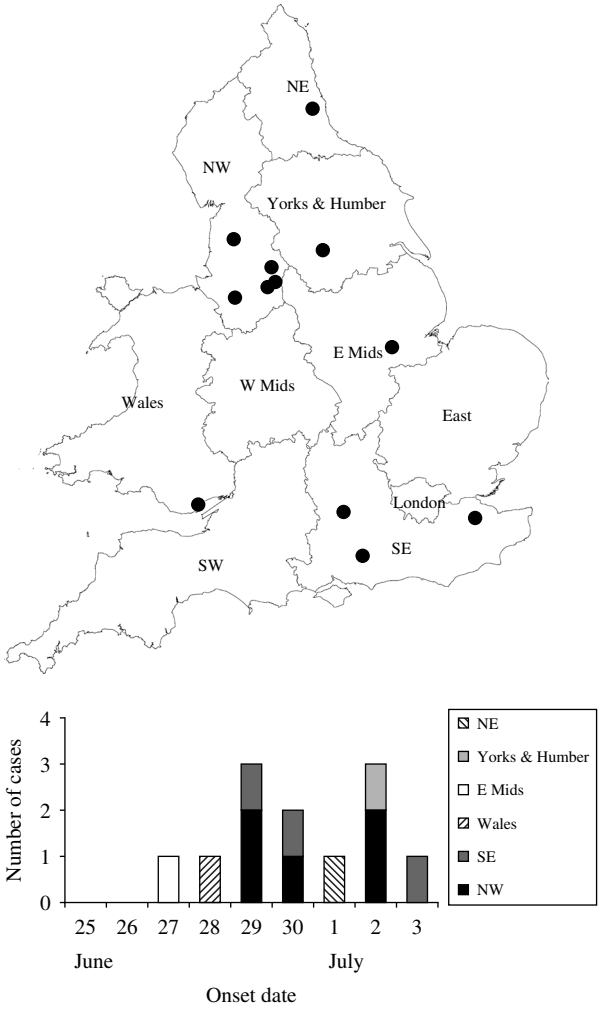

Fig. 3. Map of England & Wales showing location of incident cases of VTEC O157 (●) associated with lemon-and-coriander chicken wrap and bar chart showing number and location of incident cases by date of symptom onset.

male in each, χ^2, $P=0{\cdot}99$) but controls were older than cases (mean 51·5 *vs.* 30·9 years, *t* test, $P=0{\cdot}003$). Cases and controls were distributed throughout the country and were reflective of the overall geographical distribution of all outbreak cases.

In single-risk variable logistic regression analysis (see Table 1) comparing cases' exposures in the 5 days before onset of illness with controls' exposures in the 5 days before interview, cases were more likely to report eating food away from home from supermarket chain P (OR 10·75, 95% CI 1·14–∞, $P=0{\cdot}04$) than controls. They reported eating chicken sandwiches (including rolls, baguettes or wraps) from outside the home more frequently (OR 17·02, 95% CI 1·86–∞, $P=0{\cdot}01$) and were more likely to shop in supermarket chain P (OR 19·97, 95% CI 1·70–1237·00, $P=0{\cdot}01$). When asked about specific exposures, cases were more likely to report the consumption of a lemon-and-coriander chicken wrap from supermarket chain P (OR 46·40, 95% CI 5·39–∞, $P=0{\cdot}0002$) than controls.

Further investigation of the nested effect of different fillings in chicken sandwiches found that in most instances, the individual fillings had no effect on the likelihood of being a case compared to no exposure to chicken sandwiches. However, individuals who reported coriander in chicken sandwiches were more likely to be a case than those who did not (OR 15·46, 95% CI 1·28–846·00, $P=0{\cdot}03$).

The effect of the above variables was considered further using multivariate logistic regression analysis. When the consumption of lemon-and-coriander chicken wrap from supermarket chain P was controlled for, none of the other variables tested remained significant. The consumption of lemon-and-coriander chicken wrap from supermarket chain P remained significant when the variables described above were controlled for, except when usually conducting shopping in supermarket chain P was controlled for.

In single-risk variable logistic regression analysis comparing cases' exposures in the 5 days before onset of illness with controls' exposures in the 3 weeks before interview, only the consumption of a lemon-and-coriander chicken wrap from supermarket chain P remained significant (OR 46·40, 95% CI 5·39–∞, $P=0{\cdot}0002$). When the nested effect of different fillings in chicken sandwiches from outside the home was investigated, the inclusion of coriander increased the likelihood of being a case as above (OR 15·46, 95% CI 1·28–846, $P=0{\cdot}03$). The results of multivariate logistic regression were the same as above.

Microbiological

The three initial isolates from the North West were VTEC O157 belonging to PT8 that had genes for both VT1 and VT2 and had the same PFGE profile. This outbreak profile was used to define cases for the case-control study (see above).

Isolates from 11 cases identified from interviews as having consumed lemon-and-coriander chicken wraps were confirmed as VTEC O157 PT8 VT1+2. Ten of the 11 isolates had indistinguishable PFGE profiles of *Xba*I fragments and one isolate lacked one fragment of the profile. Screening of 62 apparently sporadic isolates by PFGE identified four cases infected with PT8 strains having the outbreak profile. Follow-up confirmed that one of these had eaten lemon-and-coriander chicken wrap from supermarket chain P

https://doi.org/10.1017/S0950268808001702 Published online by Cambridge University Press

Table 1. *Results of case-control study showing odds ratios for single-risk variable logistic regression analysis comparing case exposure 5 days before illness and control exposure 5 days before interview*

	Cases	Controls
Number interviewed	19	40
Excluded	11 did not match case definition*	1 foreign travel
Included in case-control analysis	8	39
Number of men	3	15
Number of women	5	24
Mean age (years)	30·9 (*t* test, $P=0{\cdot}003$)	51·5
Odds ratio for eating food away from home from supermarket P (95% CI)	10·75 (1·14–∞, $P=0{\cdot}04$)	1
Odds ratio for eating chicken sandwiches from outside the home (95% CI)	17·02 (1·86–∞, $P=0{\cdot}01$)	1
Odds ratio for usually shopping at supermarket P (95% CI)	19·97 (1·70–1237·00, $P=0{\cdot}01$)	1
Odds ratio for eating a lemon-and-coriander chicken wrap from supermarket P (95% CI)	46·40 (5·39–∞, $P=0{\cdot}0002$)	1

CI, Confidence interval.

* Resident of England & Wales notified since 20 June 2007 with laboratory-confirmed isolate of VTEC O157 PT8 VT1 + 2.

and was considered an epidemiologically linked case in the outbreak.

A total of 402 food and environmental samples were tested, including 223 sandwiches, 36 salads, 124 raw ingredients (including coriander, salad vegetables and meats), water samples from the washing process and swabs. VTEC O157 was not detected in any of the food and environmental samples, or faecal samples from screening of 131 staff at the food production unit.

Environmental

The product was launched on 24 June 2007 with the production of 2760 wraps on the first day and 2850 on the following day. There were therefore 5610 wraps in circulation before the first reported consumption by a case. These had a shelf life of 'production day' plus 2 days (i.e. production 24 June had a 'use by date' of 26 June). Wraps were distributed across the country via the national supermarket chain. No leftover wraps were available for testing from the cases, but leftover wraps for testing were collected from the production unit.

The lemon-and-coriander chicken wrap had 15 ingredients, 10 of which were used in other products (roast chicken chunks, starch, soft cheese, 50% mayonnaise, 30% mayonnaise, lemon zest, red peppers, iceberg lettuce, rocket and coriander) with the remainder unique to the wrap (frozen milled lime leaf, green peppers, coconut milk, green Thai mayonnaise and thick chilli wrap). The filling ingredients were mixed into four composites and then added to the wrap by members of staff using hands or a scoop; an intensive handling process for which members of staff wore gloves.

Quality control at the production unit was dependent upon detailed ingredient traceability systems and use of accredited suppliers who provided certification for the quality of products. Very few minor gaps in traceability and certification were identified. Controls in place to prevent cross-contamination from adjacent production lines and directly from infected staff were considered good, but there remained some risk of cross-contamination between lines. There was no evidence that contaminated product had actually been used or that cross-contamination with the wraps had taken place.

Despite negative microbiological findings, the main suspicion rested on the fresh salad produce used in the wrap. After a review of herb washing procedures, the supermarket chain reported that they, in association with the production company, had instigated additional control measures in the manufacturing process for fresh herbs such as coriander: a 15-min chlorine soak was introduced prior to the existing 2-min wash in chlorine.

DISCUSSION

Twelve cases of VTEC O157 PT8 VT1 + 2 infections in England & Wales were associated with lemon-and-coriander chicken wrap from a single supermarket

https://doi.org/10.1017/S0950268808001702 Published online by Cambridge University Press

chain. The results of the descriptive and analytical epidemiological studies suggest that one contaminated batch of an ingredient in the wrap was the probable source of the outbreak. This outbreak highlights the potential of fresh ready-to-eat products to act as vehicles for widespread infection.

The age distribution of cases (17–50 years) was different from that of background sporadic cases, with no children or elderly cases reported as part of the outbreak. The results of the analytical study showed a very strong association between consumption of lemon-and-coriander chicken wrap from a single supermarket chain and being a case. These results could have been affected by recall bias, a particular issue in case-control studies; however, there was no publicity regarding the outbreak at the time of the epidemiological studies.

Microbiological results also support a point-source outbreak where infection resulted from a shared risk. Isolates from all but one of the cases were found to have indistinguishable PFGE profiles. Combining phenotypic and genotypic laboratory typing results with the clinical and epidemiological data was vital to differentiate 'sporadic' infection from outbreak cases in this incident. Some strains of VTEC O157 are widely distributed in the community and animal reservoirs, and PFGE data alone cannot be used to infer that infections are linked in the absence of epidemiological information.

The lemon-and-coriander chicken wraps that were implicated in this outbreak included chicken, processed flavourings and fresh salad ingredients. Several previous foodborne outbreaks of VTEC O157 have been associated with contaminated vegetables and salad ingredients including spinach [9], lettuce [6, 7], alfalfa sprouts [8], radish sprouts [10] and cucumber [11]. Investigators have often found a link between the implicated food vehicle and cattle or cattle faeces (directly or via contaminated compost or irrigation water) [6]. Contamination of a parsley crop has been found to persist for >5 months after treatment of soil with VTEC O157-contaminated compost [25]. Studies on lettuce suggest that pre-harvest crop contamination via contaminated irrigation water can occur through plant roots [26]. There are no published outbreaks linked with consumption of chicken, although a case-control study in rural Italy has shown an association between illness and live backyard poultry contact [27]. Other uncooked non-bovine products have been implicated in cases of VTEC O157 infection, including small-scale production of pork salami [28], unpasteurized goats' cheese [29] and unpasteurized apple cider [30]. Previous reported outbreaks of VTEC O157 associated with ready-to-eat sandwiches have involved cold cooked meats [31, 32]. Cross-contamination between raw and cooked meats at delicatessen counters in supermarket stores has caused two reported outbreaks in the United Kingdom [33, 34].

The small numbers of cases together with the negative microbiological results suggest that one contaminated batch of an ingredient or a limited cross-contamination event caused the outbreak. The infectious dose for VTEC O157 is very low and contamination of a small amount of any of the ingredients could be implicated, even if they were not unique to the wrap. Plausible scenarios might include contamination of a batch of salad crops or herbs arising as a result of heavy rainfall and flooding occurring in the weeks before the incident, or a limited lapse in infection control procedures within the production unit leading to cross-contamination from another product line or from a member of staff. Epidemiological analysis in the case-control study suggests that coriander might have a specific role in this outbreak, but this might only reflect the fact that individuals are not always aware of specific ingredients in sandwiches, or that cases were more assiduous in their responses than controls. Given the epidemiological findings that the likelihood of being a case was increased by the inclusion of coriander in the wraps, the focus of the environmental investigation on ingredients unique to the wrap may have weakened the findings of the outbreak investigation.

This outbreak highlights the fact that even where strict production controls and processes are in place, the risk of contamination of ready-to-eat foods with VTEC O157 remains and can result in widespread infections. Consumption of mass-produced ready-to-eat foods is increasing; in this outbreak, over 2500 of the implicated sandwiches were distributed nationally each day. Further outbreaks of VTEC O157 associated with contaminated fresh ready-to-eat foods, such as salads and sandwiches are likely in the future. Public health professionals need to be aware of the risks posed by ready-to-eat foods and should consider them as potential vehicles of VTEC O157 infection.

The swift response to this outbreak was facilitated by the prompt and thorough investigation of the initial cases, quick sharing of information throughout the HPA, and coordination with environmental health colleagues and the Food Standards Agency

(FSA). This outbreak shows the benefit of having a Food, Water and Environmental (FWE) laboratory network, with the ability to coordinate and process large numbers of samples with a fast turnaround time in an outbreak situation. The attack rate of 4·2/1000 sandwiches, with over 2500 sandwiches produced and distributed across the country each day, means that even the slightest delay might have resulted in a huge number of cases. Very few commercial laboratories have either the staff trained or the containment facilities necessary to carry out such work; this is a strength of the HPA FWE laboratory network. Investigation of national VTEC O157 outbreaks would be facilitated by an agreed consistent minimum dataset across the HPA; the dataset should include both epidemiological and microbiological data and should be widely accessible (e.g. web based) to all relevant colleagues within the HPA.

ACKNOWLEDGEMENTS

The authors are grateful for the help of the environmental health officers who investigated the cases and the Regional Food, Water & Environmental Microbiology Service Laboratories in London, West Midlands, Norwich and North West England, the National Public Health Service for Wales and the Food Standards Agency. This work was funded by the Health Protection Agency.

DECLARATION OF INTEREST

None.

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