



Outbreak Report

National Outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection associated with lemon and coriander chicken wraps in England & Wales

June-July 2007

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on behalf of the Outbreak Control Committee**

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1. Executive Summary

A national outbreak of Vero cytotoxin-producing *Escherichia coli* (VTEC) O157 infection first identified by Greater Manchester Health Protection Unit (GMHPU) affected 5 English regions and Wales. A joint investigation, led by Local and Regional Services on behalf of the Health Protection Agency (HPA), involved the Centre for Infections (Cfi), Food Standards Agency, Environmental Health Departments in Stockport, Preston, and Milton Keynes, and the National Public Health Services, Wales. Case ascertainment included Scotland and Northern Ireland but no cases were reported and Health Protection Scotland and HPA Northern Ireland were not involved further.

Twelve cases were identified linked with lemon and coriander chicken wrap from a single supermarket chain, consumed between 25th and 29th of June. The wraps were supplied by a single food producer in Milton Keynes. Descriptive epidemiology together with results of phenotypic and genotypic tests on human isolates indicated a point source outbreak; the results of the 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= 0.0002).

The product was promptly removed from sale on 6th July by the supermarket on the identification of the first cases. Extensive testing of raw ingredients and products in the food production unit in Milton Keynes did not yield any positive results and nor did testing of faecal samples from staff in the unit.

The Outbreak Control Committee concludes that one contaminated batch of an ingredient in the chicken wrap probably caused the outbreak. Although VTEC O157 infection resulting from contaminated salad and ready to eat foods has previously been reported, this outbreak was unusual in the potentially large numbers at risk of exposure via a newly released food product.

The swift response to this outbreak was facilitated by the early alert of GMHPU by the Stockport microbiologist, the prompt and thorough investigation of the initial cases, quick sharing of information within the HPA, and effective co-ordination with Environmental Health colleagues, NHS laboratories and the Food Standards Agency.

Lessons learned in this outbreak:

- Local facilities and networks enabled a very prompt response and the detection of a link between 3 geographically unrelated cases.
- The success of the early investigation was due in large part to the availability of an experienced EHO whose main responsibility is food and to the involvement of laboratory colleagues with expertise in the microbiology of food.
- This outbreak showed the benefit of having a Food Water and Environmental (FWE) laboratory network, with the ability to co-ordinate and process large numbers of samples with a fast turn around time in an outbreak situation. Very few commercial laboratories have either the staff trained or the containment facilities necessary to carry out such work and this is a strength of the Health Protection Agency FWE network.
- Involvement of a supermarket representative in the local OCT during the early stage of the investigation was helpful in this instance but may not always be appropriate. It is recommended that involvement of a representative from the industry/company under investigation is considered on a case by case basis early in the investigation and that the OCT decides how communication with the representative is handled.

- 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.
- 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.
- A review of the effectiveness of decontamination of ready to eat salad and herb products is required. It was unclear in this outbreak what best practice is and whether the additional measures introduced would have actually reduced the risk of product contamination. The key to prevention is absence of contamination of such produce in the field and during harvesting.

2. Background to VTEC O157 infection in England and Wales

2.1 Clinical

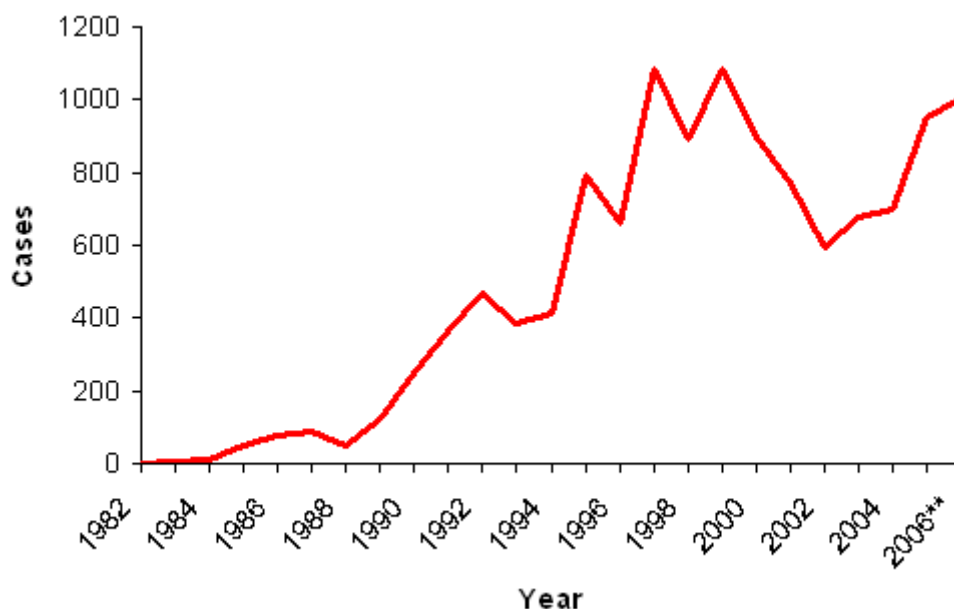
Vero cytotoxin-producing strains of *Escherichia coli* (*E. coli*) of serogroup O157 (VTEC O157) cause a spectrum of illness including diarrhoea and bloody diarrhoea (haemorrhagic colitis), reported in around 50% of cases. The incubation period between exposure and symptom onset is generally between one and six days but the organism may also be carried asymptotically. The most important aspect of infection with VTEC O157 is the ability of the strains to cause serious and potentially fatal disease including haemolytic uraemic syndrome (HUS) (reported in around 5% of cases). Strains produce one or both of two cytotoxins termed VT1 and VT2. Infection with VTEC O157 most often causes apparently sporadic infections, although family outbreaks and general outbreaks of infection can occur.

2.2 Transmission

The main reservoir for VTEC O157 is the gastrointestinal tract of healthy cattle and sheep and bacteria enter the food chain by faecal contamination of raw food materials. Carcasses may be contaminated during slaughter so that VTEC O157 may be present in raw meat and meat products and bacteria may also be transferred to raw milk. VTEC O157 is killed by adequate cooking of meat and pasteurisation of milk, but consumption of undercooked meat and raw dairy products has resulted in outbreaks of infection. Cross contamination of ready-to-eat foods such as cooked meats and sandwiches can occur and in the United States of America there are also reports of outbreaks linked to vegetables, salads, fruit and juices. Infection can also be acquired through consumption or contact with faecally-contaminated water, including that used for irrigation of crops. Other routes of transmission of VTEC O157 to man include direct contact with animals or their faeces or indirect contact through a contaminated environment. The infectious dose of VTEC O157 is low (<500 bacteria) and infection can spread readily by the faecal-oral route from an affected individual to other members within a family or a close-contact group, making person-to-person spread an important aspect in health protection.

2.3 Epidemiological trends

Infection with VTEC O157 is relatively rare in England and Wales compared with other gastrointestinal pathogens. Laboratory surveillance of VTEC O157 has been carried out since 1982 by the HPA Laboratory of Enteric Pathogens (LEP), the Reference Laboratory for this organism in England and Wales. Numbers of laboratory-confirmed cases in England & Wales increased rapidly from the late 1980s and peaked between 1997 and 1999 (Figure 1). Recent years have shown a further increasing trend with 1002 cases reported in 2006. VTEC O157



fluctuates seasonally with the number of cases usually highest in the 3rd quarter of the year (July to September inclusive) and lowest in the 1st quarter (January to March inclusive)¹.

Figure 1 Number of Vero cytotoxin-producing *E. coli* O157 strains in isolates from humans, (England and Wales) 1982 – 2006¹, examined by Health Protection Agency LEP

2.4 Microbiology

The technique of phage typing is used as a rapid front-line epidemiological tool to differentiate strains of VTEC O157. Since 1999, strains of VTEC O157 phage type (PT) 21/28 with genes for VT2 have been dominant in England and Wales, accounting for 40% of reported isolates in 2006. Strains of PT8 that generally have both VT1 and VT2 genes were the second most numerous type, (22%). Between 2000 and 2006, approximately 57% of outbreaks were attributed to PT21/28 strains and 12% to PT8. DNA-based methods are used to discriminate between strains of the same PT and VT gene type. In the most widely applied technique, large DNA fragments of the genome of the strain are generated and separated by pulsed field gel electrophoresis (PFGE) to give fragment profiles that are analysed by computer software. Strains of different PTs generally do not share PFGE profiles and isolates of the same PT from patients identified epidemiologically in an outbreak usually have indistinguishable profiles that differ from those of strains from contemporary but unlinked cases. Very closely related variant profiles are sometimes found among patients who are strongly linked epidemiologically in an outbreak and conversely, common profiles can be identified in isolates from cases separated geographically and over several years. Therefore PFGE data alone cannot be used to infer that infections are linked in the absence of epidemiological information. Both phenotypic and DNA-based methods of laboratory typing of VTEC O157 isolates are applied to support

epidemiological investigations to link human cases to each other and specific food, animal or environmental sources where appropriate.

3. Recognition of the outbreak

At 16.10 on Thursday 5th July 2007, a Consultant Microbiologist at Stepping Hill Hospital advised the Stockport office of Greater Manchester Health Protection Unit (GMHPU) of two provisional cases of VTEC O157 infection. The cases were geographically unrelated. The Consultant in Communicable Disease Control convened an Outbreak Control Team (OCT) meeting by phone at 16.30, on the same day with the microbiologist, and Environmental Health Officers (EHOs). It was agreed that both cases should be visited as soon as possible and appropriate action (including screening of contacts and advice about occupational risk) be undertaken.

At the next OCT meeting (11.15 on Friday 6th July and involving other GMHPU representatives), the EHO reported that both cases had become ill on 29th June and that the only common link was consumption of a lemon and coriander chicken wrap, purchased from two different branches (Manchester and Stockport) of a major supermarket chain on 26th June. There were no other risk factors for VTEC O157 reported.

Discussion at that meeting considered other cases that GMHPU were currently investigating and it was agreed to obtain additional information about these prior to the third outbreak meeting scheduled for 16.00 that day. It was also decided to contact the supermarket chain and ask for details of the food product. It was also agreed to obtain some lemon and coriander chicken wrap specimens.

At the third OCT meeting, Preston Food Laboratory was also represented and reported the follow up of a local VTEC case (onset 2nd July, not at that time confirmed as VTEC O157). Since the investigating EHO was in attendance on the ward whilst the outbreak meeting was going ahead, it was decided to ask the case directly whether they had consumed the implicated food. The patient reported that they had eaten a "wrap" and that they were almost certain it was a lemon and coriander chicken wrap purchased on 26th June. GMHPU reported that two out of the three cases they were investigating had no link to the putative Stockport outbreak.

Details of the producer of the wrap had been obtained by the EHO and the relevant local authority identified as Milton Keynes. A representative of Milton Keynes Council Environmental Health Department dialed in to the meeting and provided information about the producer's good safety record. It was decided to update the supermarket chain about the third (possible) case and inform the Food Standards Agency (FSA) about the situation.

A fourth OCT meeting was held at 18.30 with the senior microbiologist of the supermarket chain in attendance. At this meeting it was agreed that all presumptive VTEC O157 isolates be sent by courier to the Health Protection Agency Laboratory for Enteric Pathogens (LEP) at Colindale for urgent typing. It was agreed to defer further wrap ingredient sampling until a more systematic investigation could be arranged after the weekend on 9th July. The supermarket reported that they were voluntarily and immediately withdrawing the product on the basis of the two presumptive cases and one possible case identified so far (withdrawal by 7pm on 6th July). No meetings were scheduled for the weekend but it was agreed that one would be organised if any further cases were reported.

The fifth OCT meeting was held at 13.00 on 9th July, 2007, where the situation was reviewed and responsibility for its further management was passed to the NW Regional Epidemiology

Unit. The unit opened its Emergency Operations Centre (NWEOC) and initiated case finding via Local and Regional Services (LaRS).

4. Co-ordination of the National Response

The incident level within the HPA was escalated from 2 (regional) to 3 (divisional) on 9th July when a fourth case was reported from the South East. It was agreed that the Regional Director, HPA NW would co-ordinate the national HPA response and convene a national OCT (NOCT). This first met on 10th July and included representatives from Cfl, LaRS, Regional Microbiology Network (RMN), the FSA and Environmental Health Departments in Stockport, Preston and Milton Keynes. Colleagues from the National Public Health Service (NPHS) Communicable Disease Surveillance Centre (CDSC) Wales were included when a case was reported from Wales (See [National Outbreak Control Committee Core Members](#)). The senior microbiologist from the supermarket chain was a member of the OCT until 12 July. Due to a potential conflict of interest, he was no longer a member after this date but communication was maintained throughout the investigation via Milton Keynes EHOs.

The NOCT met via teleconference regularly until 25 July. During each meeting the following were reviewed and discussed: epidemiological, microbiological and environmental investigation and results, control measures, public health risk and communications.

The Regional Microbiology Network Food Water and Environmental Laboratories co-ordinated by the Health Protection Agency London Regional Food, Water & Environmental Microbiology Services Laboratory (LFWE) examined food, water and environmental samples in support of the investigation.

5. Investigation

5.1 Epidemiological

Health Protection Units (HPUs) were asked to report any recent and new cases of VTEC O157 in their areas that had eaten a chicken wrap from the supermarket chain to the NWEOC and to ensure that isolates from any such cases were sent to the reference laboratory at Cfl. A similar request was made to Health Protection Scotland, the National Public Health Service for Wales and HPA in Northern Ireland. Information sought for these cases included demographic details, age, date of onset of illness and the date the chicken wrap was eaten. Further retrospective case ascertainment was pursued for all cases of VTEC O157 reported since 20th June through the laboratory surveillance system and regional epidemiology units. This was reviewed at the NOCT meeting on the 19th July, at which point case ascertainment was switched to be purely through the laboratory reporting system. A line list of all cases (regardless of wrap consumption) was maintained at the NW Epidemiology Unit and the national epidemiology continuously reviewed.

The primary null hypothesis, that infection was not associated with the consumption of chicken wraps from a single national distributor, was tested using an unmatched case-control study design. Typing data from the Laboratory of Enteric Pathogens, Cfl showed that initial cases were infected with VTEC O157 of phage type 8 (PT8) with genes for VT1 and VT2 (see below). Cases were therefore defined as residents of England & Wales (no cases were reported from Scotland or Northern Ireland) with a laboratory-confirmed VTEC O157 PT 8 VT1+2 infection, notified to HPUs since 20th June 2007. It was noted that this definition was subject to change depending on ongoing phenotypic/molecular characterisation of strains and/or additional epidemiological information. Asymptomatic controls were recruited through

systematic sequential dialing, based on the cases' telephone numbers. Cases and controls with a history of recent foreign travel, contact with individuals with gastrointestinal symptoms or who were part of other known incidents were excluded. Sample size calculations suggested that 10 and 20 eligible cases and controls respectively would need to be interviewed successfully to provide sufficient power (80%) to detect a difference at the 95% level.

In the course of case ascertainment, local HPUs informed the NWEOC of appropriate case contact information to facilitate the interview process. Where contact information was available, cases reported from the North West, North East and Yorkshire and the Humber regions were interviewed by staff from the NWEOC, and cases from other regions were interviewed by staff from Cfl in London. Interviews were conducted by personnel trained in the task and took place at evenings and during the weekend of 13th and 15th July to ensure, as far as was practical, that controls represented the population from which the cases arose.

A standard, structured questionnaire was administered to cases and controls by telephone. Demographic and clinical information were sought, in addition to travel history and exposures to food, water, the environment and animals. Cases were asked about exposures in the five days before onset of illness whereas controls were asked about the five days prior to interview. Both cases and controls were also asked about exposures in the three weeks before interview in order to control for the fact that the main hypothesis under investigation was withdrawn from sale on the 6th July and therefore would not necessarily have been available for controls to consume in the five days before interview.

Completed case and control questionnaires were double entered in a bespoke EpiData 3.0 database held on a secure network drive. The resulting datasets were compared and any discrepancies checked and corrected on the dataset taken forward for analysis. Data manipulation was undertaken in Stata version 9 and statistical analyses were undertaken in Stata version 9 and StatXact version 8. Proportions and medians were compared using the chi-squared test and the Students 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, 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 controlling for the main hypothesis under investigation and quintiles of age. A score test with exact variance was generated to test the Null hypothesis that the regression co-efficient corresponding to that variable was zero.

5.2 Microbiological

5.2.1 Human Samples

All isolates of presumptive VTEC O157 submitted to LEP were confirmed biochemically as *E. coli* and tested for the presence of Vero cytotoxin (VT) genes by polymerase chain reaction (PCR). Cultures were phage typed according to the scheme for VTEC O157 and serotyped. Isolates of presumptive VTEC O157 from the three initially identified cases in the North West were received in the LEP on 9th and 10th July. In addition to the above routine tests, these isolates were compared by pulsed field gel electrophoresis (PFGE) of genomic DNA digested with the restriction enzyme *Xba*I. Fragment profiles were analysed, stored and compared using Bionumerics software. Other restriction enzymes were not used in this investigation.

Results on the first cases linked to consumption of suspect sandwiches showed that all isolates were VTEC O157 belonging to PT 8 that had genes for both VT1 and VT2 and had the

same PFGE profile. This profile was therefore used to define presumptive outbreak cases for epidemiological investigation (see above). Isolates from all other cases identified from interviews as potentially linked to sandwiches were examined by the routine methods described above and by PFGE. No presumptive isolates were identified amongst food, environment and food handler specimens during the investigation.

In order to investigate how widespread were strains of PT8, VT1+2 that possessed the outbreak PFGE profile, screening of sporadic PT8 isolates was instigated. Isolates were excluded from testing if information on the sender's form indicated recent foreign travel or if they were part of known local outbreaks; a single isolate was tested from household outbreaks. On these criteria, 62 isolates were tested out of 81 PT8 cultures received in LEP from the start of week 25 (first receipt date 20th June) up to and including 1st August. The identities of patients infected with a strain that appeared to be indistinguishable from the outbreak strain were notified to the OCT for epidemiological investigation.

Faecal samples were collected from 131 staff at the production unit that produced the wrap and examined in the Microbiology Laboratory, Luton & Dunstable NHS Foundation Trust.

5.2.2 Food and Environmental Samples

The majority of samples had been previously examined by an independent laboratory for the food retailer; however the methodology they employed was of low sensitivity. As VTEC O157 has a low infective dose; methods used to detect them from food sources must be both sensitive and allow the recovery of damaged/stressed organisms from a potential source. The methods employed by the commercial testing laboratory did not use immuno-magnetic separation and may have missed low numbers of VTEC O157 if present. In response to a request from the OCT these samples were forwarded to London Regional Food, Water & Environmental Microbiology Services Laboratory (LFWE) for testing using a sensitive method which included both enrichment and separation of target organisms from background contamination by a technique called immuno-magnetic separation which is selective for VTEC O157.

LFWE received a total of 402 samples related to the outbreak. Of these 28 samples were received directly from Milton Keynes Borough Council Environmental Health Department on 11th July comprising: 14 foods, 9 of raw ingredients and 5 wraps, 3 water samples from the washing process and 11 swab samples taken from the production company that produced the wrap. On the 16th July a further 372 food samples were received by LFWE from Alcontrol which had been submitted from the production company. These included 218 sandwiches (inc. 12 lemon and coriander wraps); 36 salads and 115 samples of raw ingredients including coriander, salad vegetables, and meats.

These samples had previously been frozen and when they arrived at LFWE in open crates they were in the process of defrosting; the samples were wet and leaking and labels were sliding off some samples. The potential for cross contamination was deemed to be high by the LFWE food examiners and it took two senior staff a day to make these samples safe to handle by decontaminating the outside of samples and repackaging them where appropriate. Testing for VTEC O157 is a very resource consuming examination, because of the need for an enrichment step to recover damaged / stressed bacteria and the examination after both 6 and 24 hours. These two intervals are set to both optimise the potential for recovery whilst reducing the impact of background contamination but means that staff had to work an extended day to complete the examination. Because of the resource implications, the need for a fast turnaround time and the requirement for category 3 facilities other Health Protection Agency food and water and NHS collaborating laboratories within the Regional Microbiology Network with appropriate accreditation and facilities were contacted for assistance. All the laboratories

contacted responded positively and following the above safety precautions and documentation 254 samples were sent to five other UKAS accredited Food, Water and Environmental laboratories including: West Midlands Food, Water and Environmental Service (Birmingham laboratory; 42 samples; Shrewsbury laboratory 31 and Stoke Laboratory 30 samples respectively) Food, Water and Environmental Laboratory Norwich samples 76; Food and Environmental Services North West 75 samples. The remaining 118 food samples were tested by the LFWE.

All samples were tested in laboratories with appropriate category 3 containment facilities which hold UKAS accreditation for the examination for the presence of VTEC O157. The methodology includes enrichment in modified tryptone soya broth (mTSB) followed by an automated immunomagnetic separation technique after 6 h and 24h incubation to increase sensitivity and optimise the potential for isolation of the target organism. The technique used allows the separation of the target organism from background contaminating flora by use of O157 specific immunomagnetic beads and an automated immunomagnetic separator (DynaL, Invitrogen Paisley UK), followed by selective culture for VTEC O157 onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) at 37°C for 18 to 24 hours.

The sample tested by LFWE by culture were also analysed for the presence of O157 DNA using BAX[®] PCR System *E.coli* O157 (Dupont Qualicon, Wilmington, US). For detection of VTEC O157 using the BAX[®] System the manufacturer's procedure was followed and the manufacturer's reagents were used as specified.

From the initial samples taken by the production company Enterobacteriaceae from the raw ingredients were also screened to ensure that they were not VTEC O157.

5.3 Environmental

The lemon and coriander chicken wrap had 15 ingredients, 10 of which were used in other products and included roast chicken chunks, starch, soft cheese, 50% mayonnaise, 30% mayonnaise, lemon zest, red peppers, iceberg lettuce, rocket & coriander. The five ingredients unique to the product were frozen milled lime leaf, green peppers, coconut milk, green Thai mayonnaise and thick chili wrap. The ingredients were mixed into four composites and these were 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.

The product was launched on 24th June 2007 with the production of 2,760 wraps on the first day and 2,850 on the following day. There were therefore 5,610 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 24th June has a use by date of 26th June). Wraps were distributed through eight of the national supermarket chain depots and the number of wraps sent to each of these varied greatly.

Milton Keynes Environmental Health Department conducted detailed investigation of processes and records in the food production unit and traced back the products used in the wrap. Their focus of investigation was on the five ingredients unique to the wrap and the processes in place for its production, including the staff, equipment, environment and handling practices throughout production. Food and environmental samples taken were described in 5.2.2.

6. Communications

The Food Standards Agency led on communications during this incident, working closely with communications teams for HPA North West and Milton Keynes Local Authority. It was agreed that all media calls would be routed through to the FSA press office. All statement lines and releases were agreed through the OCT and communications representatives were involved in the OCT teleconferences, giving media advice as the incident developed.

Local print media in the Milton Keynes area reported the outbreak (while the source was still being confirmed), as a result of which calls were received from local television and the national BBC health team. Coverage was limited to the Milton Keynes area.

Lines taken with the media were shared in advance with officials at the supermarket chain and with the production unit that produced the wrap.

Communications representatives on the OCT briefed the Strategic Health Authority at the outset of the incident and kept them briefed throughout.

7. Results

7.1 Epidemiological

Active case ascertainment by HPU identified 11 cases of VTEC O157 reporting consumption of lemon and coriander chicken wrap purchased from the supermarket chain and all of these were considered to be infected with the 'outbreak strain' of *E. coli* O157 (see Microbiology below). A twelfth potential case with the outbreak strain was initially identified by LEP, and epidemiologically confirmed as consuming a chicken wrap from the supermarket by the local HPU, using a follow-up case questionnaire. The 12 incident cases reported onset of illness between 27th June 2007 – 3rd July 2007 and all had consumed lemon and coriander chicken wraps between Monday 25th - Friday 29th June (Figure 2). The median and modal incubation times between consumption of wrap and symptoms were both three days (range 2-7 days).

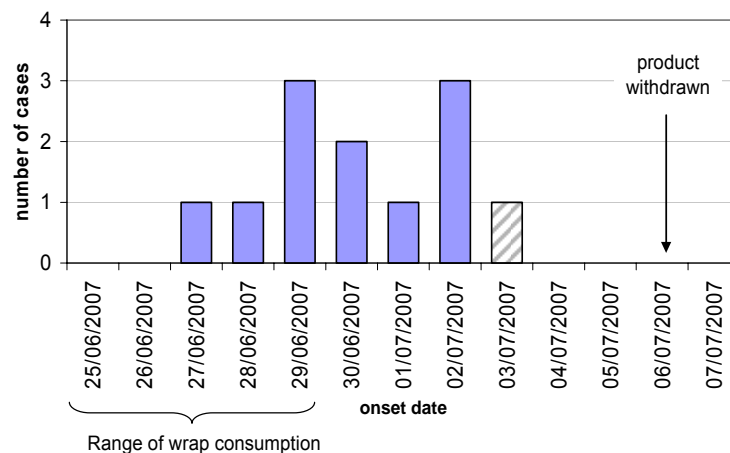


Figure 2 Onset of incident cases with identical molecular type, showing range of hypothesised exposure and date of product withdrawal as a result of the investigation. (Hatched bar case represents the single case with slightly variant PFGE profile.)

During the period of investigation (20th June 2007 – 1st August 2007), there were 135 cases of VTEC O157 reported through routine laboratory surveillance, of which 81 were 'sporadic' (not linked with travel or known outbreaks) and distributed across all age groups. In contrast, the

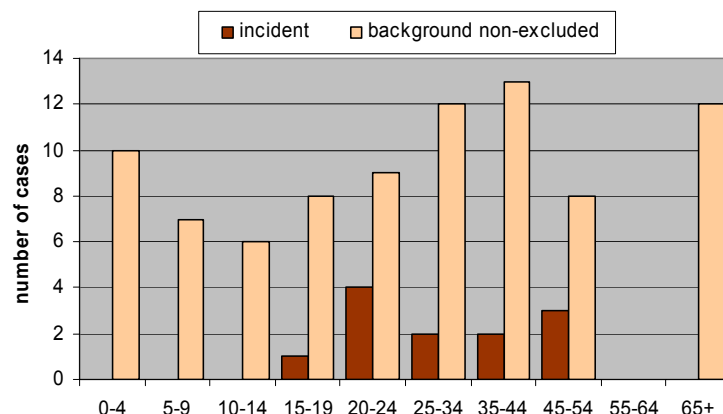
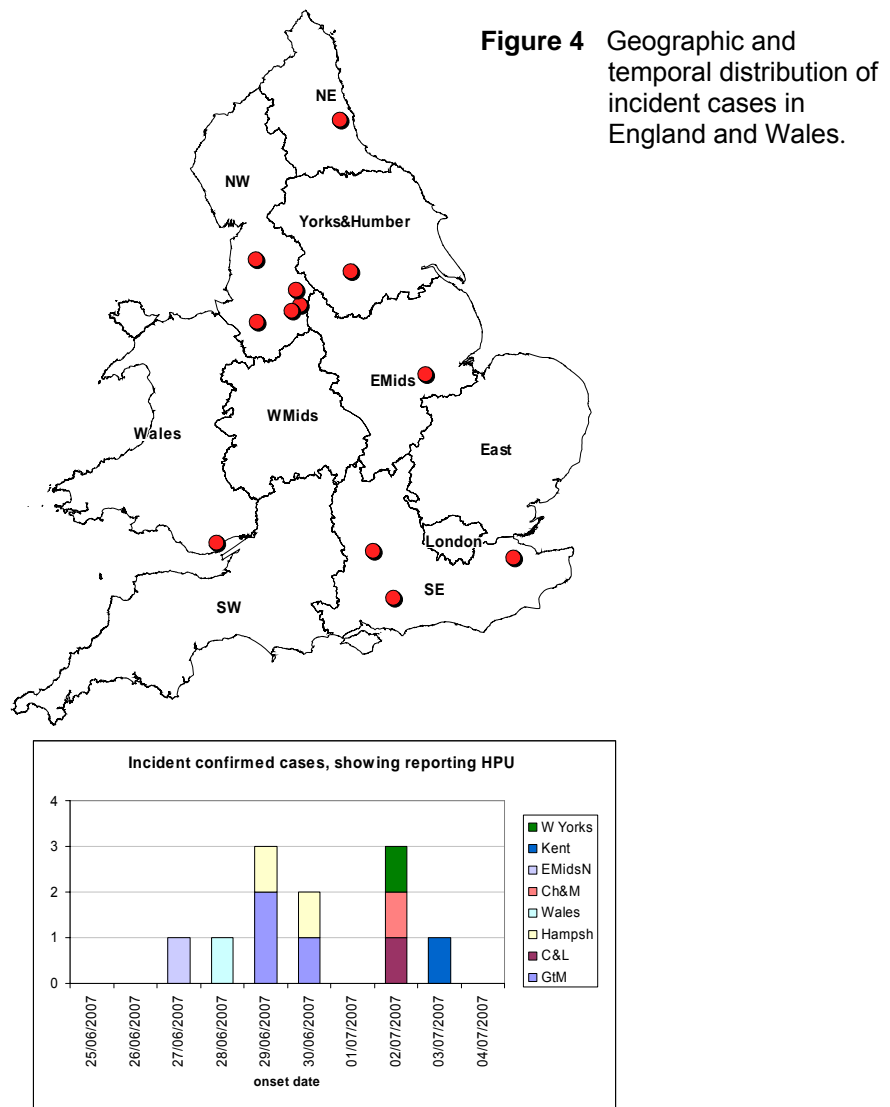


Figure 3 Age distribution of incident cases compared with background cases of VTEC O157 reported between 20th June 2007 and 1st August 2007 through routine laboratory surveillance.

age range of incident cases was restricted to 17-50 year olds (Figure 3). Cases were reported

from across England: North West (5), South East (3), Wales, Yorkshire & Humber, North East and E Midlands (1 each) (Figure 4).



For the case control study, 19 cases and 40 controls were interviewed between 12th and 14th July. After exclusions on the basis of subsequently available typing, eight cases with the 'outbreak strain' were analysed with 39 controls (one control was excluded on the basis of recent foreign travel). Cases and controls were similar with regard to gender (38% male in each, χ^2 P=0.99) but controls were older than cases in terms of age (mean 51.5 vs. 30.9 years, T test P=0.003). 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 comparing cases' exposures in the five days before onset of illness with controls' exposures in the five days before interview, cases were more likely to report consuming food eaten out of the home from supermarket chain P (OR 10.75; 95% CI 1.14-∞; P=0.04) than controls. They reported the consumption of chicken sandwiches, rolls, baguettes or wraps from outside the home more frequently (OR 17.02; 95% CI 1.86-∞; P=0.01) and were more likely to usually shop in supermarket chain P (OR 19.97; 95% CI 1.70-1237.00; P=0.01). 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=0.0002) than controls.

The nested effect of different fillings in chicken sandwiches, rolls, baguettes or wraps from outside the home was investigated further. In most instances, the individual fillings had no effect on the likelihood of being a case or a control compared to no exposure to chicken sandwiches. However, individuals who reported coriander in chicken sandwiches, rolls, baguettes or wraps from outside the home were more likely to be a case compared with individuals who did not report this exposure (OR 15.46; 95% CI 1.28-846.00; P=0.03).

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. Conversely, the consumption of lemon and coriander chicken wrap from supermarket chain P remained significant when variables described above were controlled for. The only exception to this was when usually conducting shopping in supermarket chain P was controlled for.

The significant exposure under test (chicken wrap) had been removed from sale at least 5 days before controls were interviewed. To take account of this, analysis was also done comparing cases' exposures in the five days before onset of illness with controls' exposures in the *three weeks* before interview. In single risk variable logistic regression analysis, only the consumption of lemon, coriander & chicken wraps from supermarket chain P remained significant (OR 46.40; 95% CI 5.39-∞; P= 0.0002). When the nested effect of different fillings in chicken sandwiches, rolls, baguettes or wraps 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=0.03). The results of multivariate logistic regression were the same as above.

7.2 Microbiological

7.2.1 Human Samples

Isolates from 11 cases identified from interviews as having consumed lemon and coriander chicken wraps were confirmed as VTEC O157 that belonged to PT8 and possessed genes for both VT1 and VT2. All were received in LEP between 6th July and 13th July. Ten of the 11 isolates had indistinguishable PFGE profiles of *Xba*I fragments and one isolate (from Kent) lacked one fragment of the profile. This minor variation in profile has been found in many of the outbreaks of VTEC O157 infection investigated in LEP and is not considered significant providing there are strong epidemiological data linking the patient to an outbreak.

Screening of 62 apparently sporadic isolates by PFGE identified four cases infected with PT8 strains having the outbreak profile. Follow-up indicated that one of the cases had eaten lemon and coriander chicken wrap from the supermarket chain under investigation. The isolate was received on 11th July from the North East and this individual was designated as a twelfth outbreak case.

Of the other three cases, two isolates with the outbreak profile were received on 20th and 25th June and it was subsequently found that one of these may have been infected in Austria whilst the other had an onset outside the period of investigation and a food history that did not include any sandwich consumption. The remaining case, from whom an isolate was received on 13th July, did not report any sandwich consumption either. None of the PT8 strains received between 14th July and when screening ceased after 1st August possessed the same profile as those from outbreak cases.

Faecal samples from the 131 staff at the production unit were all negative.

7.2.2 Food and Environmental Samples

VTEC O157 was not detected in any of the 402 sample food, water or swab samples tested by the network after either 6 or 24 hours incubation by cultural analysis. VTEC O157 specific DNA was not detected by PCR analysis using the BAX and VTEC O157 kit. Screening of Enterobacteriaceae also revealed no positive isolates for VTEC O157.

7.3 Environmental

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 and quality control was largely dependent upon detailed ingredient traceability systems and use of accredited suppliers who provided certification for the quality of products. These were thoroughly reviewed and very few (minor) gaps in traceability and certification were identified as a result. Apart from the wrap ingredients, attention was also focused on the risks of cross-contamination from adjacent production lines and directly from infected staff. Controls in place were considered good but there remained some risk of cross-contamination between lines. One particular batch of mozzarella pearls used on an adjacent line was under suspicion following a previously positive identification of *E. coli* (non-VTEC) and a gap in the sampling records. However, there was no evidence to suggest that contaminated product had actually been used or that cross-contamination with the wraps had taken place.

Of the unique ingredients, only the traceability of the lime leaf (also an ingredient of the Thai mayonnaise) was in question. However, despite there being two international suppliers used for this product, it was milled and frozen in the UK and the same batch used in the wrap was available for testing. As described, these tests were negative and the risk from lime leaf was considered minimal. The coconut milk used was pasteurized and tinned. Certification for the other unique ingredients was intact.

Despite negative microbiological findings, the main suspicion rested on the fresh salad products used in the wrap. There was a review of herb washing procedures and corrective actions taken on low chlorine levels and temperature assessments of product cleaning water. The supermarket chain also 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 minute chlorine soak was introduced prior to the existing two minute wash in chlorine.

As a result of the investigation, the production unit put in place several further enhancements of their quality assurance systems:

- a specific post was created to better co-ordinate the traceability and certification of incoming ingredients and oversee all aspects of quality control within the unit
- new risk assessments were introduced, specifically based on enteric pathogen control, and applied to all ingredients used in the unit
- closer review of new suppliers and new ingredients from existing suppliers to gain a fuller understanding of how incoming ingredients were being processed
- issue of a best practice document to all suppliers detailing what is expected in terms of sourcing and product testing prior to dispatch to the sandwich manufacturer
- hosting a shared learning day with technical personnel from other companies within the group, which included presentations by a microbiologist and a Milton Keynes Council EHO.

8. Discussion

Prompt investigation of three cases of VTEC O157 reported over a two day period in North West England resulted in early identification of this outbreak. The only exposure that was common to the three cases was consumption of lemon and coriander chicken wrap from a single supermarket chain. The results of the descriptive and analytical epidemiological studies provide evidence for this food being the source of the outbreak. The age distribution of cases (17 to 50 years) was different from that of background sporadic cases, with no children or elderly cases reported as part of the outbreak. Most cases were able to provide specific dates, between 25th to 29th June, on which the lemon and coriander chicken wrap was consumed. The epidemic curve shows that the last date of onset was 3rd July and that there were no further cases following the withdrawal of the product on 6th July. 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 from the cases provide further evidence that this was a point source outbreak where cases resulted from a shared risk. Isolates from all but one of the cases were found to have indistinguishable PFGE profiles. The importance of combining phenotypic and genotypic laboratory typing results with the clinical and epidemiological data in order to differentiate 'sporadic' infection from linked cases is well illustrated by this investigation. In addition, the wide distribution of some strains of VTEC O157 in the community and animal reservoir, illustrates that PFGE data alone cannot be used to infer that infections are linked in the absence of epidemiological information.

The lemon and chicken coriander 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 spinach², lettuce^{3,4}, alfalfa sprouts⁵, radish sprouts⁶ and cucumber.⁷ Investigations have often found a link between the implicated food vehicle and cattle or cattle faeces (directly or via contaminated compost or irrigation water).³ Contamination of a parsley crop has been found to persist for more than 5 months after treatment of soil with O157 contaminated compost.⁸ There are no published outbreaks linked with consumption of chicken, although a case control study in rural Italy has shown an association of illness with live backyard poultry contact.⁹ Other uncooked non-bovine products have been implicated in cases of VTEC infection, including small scale production of pork salami¹⁰, unpasteurised goats cheese¹¹ and unpasteurised apple cider.¹²

It is not possible to conclude how contamination of wraps occurred in this outbreak or to pinpoint a single ingredient source. Extensive testing of raw ingredients and products from the food production unit in Milton Keynes did not yield any positive results and this was also the case with faecal samples from staff at the unit. 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. All the ingredients were extensively traced back to suppliers and there were no significant gaps in the necessary certification of products entering the site. The controls already in place at the production unit were considered to be good and these have been further improved as a result of the incident. There is some uncertainty about the effectiveness of washing herb and salad ingredients with chlorine in the context of VTEC; it was felt that the control measures with regard to washing instigated by the supermarket in this respect superseded current best practice (personal communication).

The number of cases associated with this outbreak was small and this, together with negative microbiological results, suggests that one contaminated batch of an ingredient/product or a limited cross contamination event caused the outbreak during the last week in June. The infectious dose for VTEC O157 is very low and even 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, exacerbated perhaps by the 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.

It is sobering that even in the context of what is considered a very well maintained production unit, the risk of product contamination with VTEC O157 remains and can still result in widespread cases of illness.

The swift response to this outbreak was facilitated by the early alert of GMHPU by the Stockport microbiologist, the prompt and thorough investigation of the initial cases, quick sharing of information throughout the HPA, and co-ordination with environmental health colleagues and the FSA. The co-ordinating laboratory, LFWE, ensured that all the laboratories taking part in the examination of food and environmental samples had the consumable and kit resources required and arranged for urgent supplies to be forwarded. The representative from manufacturer of the immunomagnetic beads was also very helpful in arranging for urgent supplies to be sent to enable the FWE laboratories to cope with the large number of samples at very short notice. The dedication of FWE staff committed to providing outbreak support and who worked long hours to turn around such a large number of samples in a short time frame is commendable.

9. Conclusions

There is strong epidemiological and microbiological evidence that this outbreak of VTEC O157 was due to a common source of infection. The case control study strongly implicated a nationally distributed chicken wrap as the vehicle of infection. However, extensive investigation of manufacturing processes and microbiological testing of constituent ingredients and unsold wraps resulted in no detection of VTEC O157 and it was not possible to identify the precise point at which the eaten wraps may have become contaminated. This outbreak demonstrates that there is a risk of infection with VTEC associated with consumption of fresh ready to eat products even where strict production controls and processes are in place.

10. Lessons Learned

- Local facilities and networks enabled a very prompt response and the detection of a link between 3 geographically unrelated cases.
- The success of the early investigation was due in large part to the availability of an experienced EHO whose main responsibility is food and to the involvement of laboratory colleagues with expertise in the microbiology of food.
- This outbreak showed the benefit of having an FWE laboratory network, with the ability to co-ordinate and process large numbers of samples with a fast turn around time in an outbreak situation. Very few commercial laboratories have either the staff trained or the containment facilities necessary to carry out such work and this is a strength of the Health Protection Agency FWE laboratory network.

- Involvement of a supermarket representative in the local OCT during the early stage of the investigation was helpful in this instance but may not always be appropriate. It is recommended that involvement of a representative from the industry/company under investigation is considered on a case by case basis early in the investigation and that the OCT decides how communication with the representative is handled.
- 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 (eg web based) to all relevant colleagues within the HPA.
- 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.
- A review of the evidence on most effective decontamination of ready to eat salad and herb products is required. It was unclear in this outbreak what best practice is and whether the additional measures introduced would have actually lessened the risk of product contamination.

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