A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce

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SUMMARY

Between 1 August and 15 September 2000, 361 cases of *Salmonella enterica* serotype Typhimurium definitive phage type (DT) 104, resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides, spectinomycin and tetracycline (R-type ACSSuSpT), were identified in England and Wales residents. Molecular typing of 258 isolates of *S.* Typhimurium DT104 R-type ACSSuSpT showed that, although isolates were indistinguishable by pulsed-field gel electrophoresis, 67 % (174/258) were characterized by a particular plasmid profile. A statistically significant association between illness and consumption of lettuce away from home was demonstrated (OR = 7·28; 95 % CI = 2·25–23·57; $P = 0.0006$) in an unmatched case–control study. Environmental investigations revealed that a number of food outlets implicated in the outbreak had common suppliers of salad vegetables. No implicated foods were available for microbiological testing. An environmental audit of three farms that might have supplied salad vegetables to the implicated outlets did not reveal any unsafe agricultural practices. The complexity of the food supply chain and the lack of identifying markers on salad stuffs made tracking salad vegetables back to their origin extremely difficult in most instances. This has implications for public health since food hazard warnings and product withdrawal are contingent on accurate identification of the suspect product.

INTRODUCTION

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is the second most commonly identified *Salmonella* serotype in England and Wales. In 1999, 2424 human isolates of *S.* Typhimurium infections were reported, of which 41 % (990/2424) were definitive phage type 104 (DT104) [1, 2]. Amongst *S.* Typhimurium DT104, resistance to the antimicrobials ampicillin, chloramphenicol, streptomycin,

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sulphonamides, spectinomycin and tetracyclines (R-type ACSSuSpT) is very common [3] and in 1999 71 % (707/990) of isolates displayed this resistance pattern [1]. The organism is widely distributed in food producing animals [4].

The index event was a cluster of seven cases of *S*. Typhimurium DT104 R-type ACSSuSpT linked to a pub in South Cheshire at the beginning of August 2000. Subsequently, during the week ending 18 August 2000 the Laboratory of Enteric Pathogens (LEP) of the Public Health Laboratory Service (PHLS) confirmed *S*. Typhimurium DT104 R-type ACSSuSpT in faecal isolates from 70 individuals with gastrointestinal illness nationally, compared with 34 during the same period of 1999. The cases were distributed throughout England and Wales but were concentrated in the West Midlands and North West NHS Regions. One man, who was part of the South Cheshire cluster, had died.

The PHLS Communicable Disease Surveillance Centre (CDSC) convened a multi-disciplinary Outbreak Control Group (OCG) on 22 August 2000 to identify the source and vehicle of transmission for the outbreak and implement appropriate control measures.

METHODS

Microbiological investigation

For case finding purposes, microbiologists were requested to submit isolates of *S*. Typhimurium (or *Salmonella* O4:i) immediately to LEP for confirmation and further typing. Isolates were phage-typed [5] and screened for antimicrobial resistance using an agar dilution method [6]. Plasmid profile analysis [7] and pulsed-field gel electrophoresis (PFGE) were also performed [8].

Epidemiological investigation

In the descriptive investigation a case was defined as any person resident in England or Wales from whom *S*. Typhimurium DT104 R-type ACSSuSpT had been isolated since 1 August 2000. Infection rates for each District Health Authority were calculated using cases confirmed by LEP as the numerator and Office for National Statistics 1999 mid-year population estimates as the denominator. Local Environmental Health Officers (EHOs) had already investigated many cases as sporadic food poisoning; case-records of their investigations were reviewed. A comprehensive

Box 1. *Null hypothesis generated by trawling questionnaires*

Illness associated with a positive faecal or blood isolate of *S*. Typhimurium DT104 R-type ACSSuSpT with a 2·0 MDa plasmid, confirmed by LEP on or after 29 August 2000 is not associated with the consumption in the 72 h prior to onset of symptoms of:

- food prepared away from home at a take away, snack bar or restaurant
- salad vegetables
- chicken
- cold cooked ham.

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trawling questionnaire was employed to generate hypotheses for the source of infection.

Geographical mapping

Local public health, environmental health and microbiology colleagues were asked to provide the OCG with the full postcode of the residential address of individuals with confirmed faecal and/or blood isolates of *S*. Typhimurium DT104 R-type ACSSuSpT since 1 August 2000. Where only a partial postcode or address was available a commercial database derived from the electoral register (UK Info®) was used to identify the full postcode of the individual. The residential postcode of cases was entered into geographical mapping software (MapInfo®) to plot the residence of cases on a map of England and Wales.

Case–control study

An unmatched case–control study commenced on 30 August 2000 to test the hypotheses generated by the trawling exercise (Box 1). The population at risk was defined as residents of Birmingham, Wolverhampton, Sandwell, Walsall, Dudley, Shropshire, South Cheshire, North and South Staffordshire and Worcestershire Health Authorities who were aged 18 years and above and who had not travelled outside the United Kingdom in the 7 days prior to the date of onset of illness (cases) or in the 7 days prior to the date of interview (controls).

A case was defined as an individual from the population at risk who had experienced an episode of gastrointestinal illness (i.e. diarrhoea [three or more loose stools in a 24 h period], and/or vomiting, and/or abdominal pain) with a faecal and/or blood isolate of *S*. Typhimurium DT104, R-type ACSSuSpT confirmed

by LEP on or after Tuesday, 29 August 2000 and no other gastrointestinal illness in the household in the 7 days prior to onset of illness in the case. (The case definition was refined subsequently to incorporate the identification of an outbreak strain (*S*. Typhimurium DT104, R-type ACSSuSpT with a 2·0 megadalton (MDa) plasmid).)

Controls were individuals from the population at risk who had not experienced an episode of gastrointestinal illness in the 7 days prior to the interview and who confirmed that there was no other gastrointestinal illness in the household during that time.

Community controls were recruited by postcode from a commercial database (UK Info®) derived from the electoral register. For each case, UK Info® was used to generate a list of names, addresses and telephone numbers of people with the same first four digits of the postcode as the case. Addresses of potential controls where a telephone number was unavailable (because the household was ex-directory) were discarded. The list of remaining potential controls was copied to a spreadsheet. Twenty names were then selected at random from the spreadsheet information. The interviewers telephoned these potential controls in turn, inquiring if the person answering the phone was resident in the household, aged at least 18 years and willing to participate in the investigation. If the control declined to participate, the next potential control on the list was approached. This process was continued until two controls per case were recruited.

Standard, structured questionnaires were administered to cases and controls by telephone interview from Colindale. All interviewers were fully briefed on the questionnaire and interviewing technique. Attempts to contact cases and controls were made up to three times at different times of the day or evening. If unsuccessful at the third attempt a new case or control was contacted. Cases were asked about food exposures in the 3 days prior to the onset of their illness. Controls were asked about food exposures in the 3 days prior to the date of interview.

Statistical analyses

The statistical significance of observed differences in the proportion of isolates that were outbreak related between NHS regions was tested using the χ^2 test.

To assess associations between the consumption of certain food items and illness, single variable risk analysis was performed in EPIINFO [9]. To assess the independence of associations identified in the single

variable analysis, logistic regression was performed in SAS. Variables which in the single variable analysis had a *P*-value of <0·2 were included in the initial logistic regression model. Cases were excluded from the model if there were missing values for the variables included in the model. A final model was obtained by eliminating the least significant variable and then running the model again until only significant variables were included.

Environmental investigations

Environmental Health Officers interviewed early cases to discover the sources of food consumed in the 3 days before onset of symptoms. They visited food retail premises associated with cases in order to establish the origin of implicated foodstuffs served around the time that the cases occurred. The supply chain for any foods associated with more than one case was traced from food retail premises to suppliers and farms. The findings were collated to determine if there was a common supply of implicated foods.

Three farms were also visited, environmental samples taken and an audit of agricultural practices conducted according to a protocol provided by the Environmental Surveillance Unit of CDSC. Eight environmental samples from these three farms were examined for the presence of *Salmonella* spp.

RESULTS

Microbiological

Between 1 August and 15 September 2000, 361 human isolates of *S*. Typhimurium DT104 R-type ACSSuSpT were confirmed by LEP. Figure 1 shows the number of laboratory confirmed cases of *S*. Typhimurium DT104 R-type ACSSuSpT by week of confirmation by LEP.

Over a third (134/361, 37%) of the cases reported between 1 August and 15 September 2000 were from laboratories in the West Midlands, which is where the largest increase over the same time period in 1999 was also seen (2·51/100000 compared with 0·26/100000). Smaller increases were seen in a number of other regions.

Plasmid analysis was performed on 258 of the 263 (98%) confirmed human isolates of *S*. Typhimurium DT104 R-type ACSSuSpT. Of these, 174 (67%) were characterized by the possession of a 2·0 MDa plasmid, in addition to the 60 MDa plasmid that is common to many strains of *S*. Typhimurium. Forty-seven isolates

Table 1. *Plasmid profile of* S. *Typhimurium DT104 R-type ACSSuSpT isolates identified in August 2000 by NHS region, England and Wales*

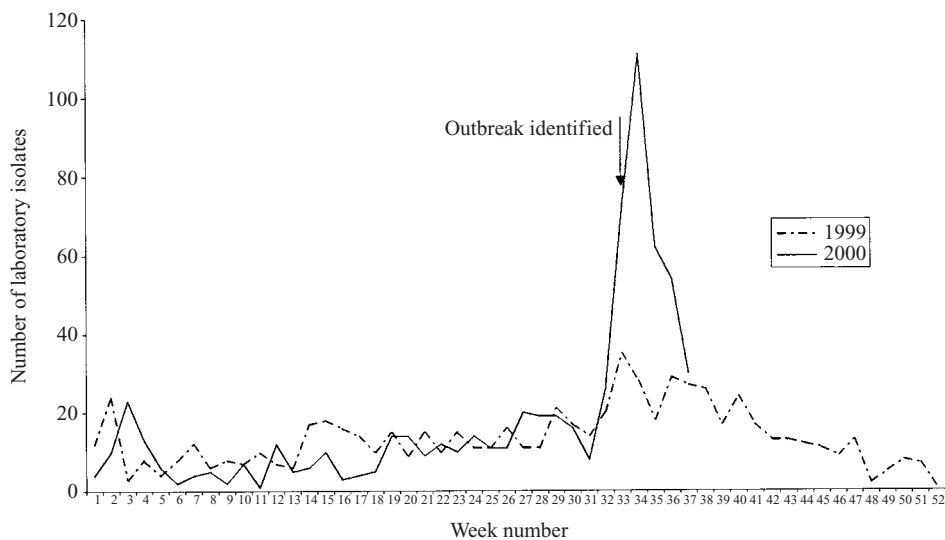| Plasmids (MDa) | NHS region | | | | | | | | | |
	Eastern	London	North West	Northern and Yorkshire	South East	South West	Trent	Wales	West Midlands	Total
60	4	19	8	12	7	6	1	4	6	67
60, 2·0	3	5	22	21	4	7	17	6	89	174
Others	2	2	3	0	1	5	1	1	2	17
Total	9	26	33	33	12	18	19	11	97	258

Fig. 1. Number of laboratory confirmed cases of *S.* Typhimurium DT104 R-type ACSSuSpT by week of isolation. England and Wales, 1999–2000.

of *S.* Typhimurium DT104 R-type ACSSuSpT were also studied by PFGE. Of these, 39 possessed the distinctive outbreak plasmid profile but all 47 were indistinguishable when studied by PFGE.

Table 1 shows the number of isolates of *S.* Typhimurium DT104 R-type ACSSuSpT by Region and plasmid profile. There was a statistically significant difference ($P < 0.001$) between Regions in the proportion of isolates that possessed the additional 2·0 MDa plasmid. In the West Midlands, 92 % (89/97) of isolates tested possessed the additional 2 MDa plasmid. The outbreak strain was therefore defined as *S.* Typhimurium DT104, R-type ACSSuSpT with a 2·0 MDa plasmid.

Of the 258 isolates that underwent plasmid profile analysis, the postcode of residence of the case was identified for 243. The referring laboratory postcode alone was available for a further 14 and for one isolate no postcode could be identified. The 257 cases with an

associated postcode were plotted on a map of England and Wales (Fig. 2).

Case–control study

In the hypothesis-generating interviews the exposures reported by more than 70 % of cases were milk (91 %), fruit (91 %), salad (85 %), poultry (82 %), sandwiches (76 %), eggs (73 %) and pasta or rice (70 %). No national catering or retail outlet was mentioned more than might have been expected based on market share. No major branded food product was identified. Three clusters based on premises were evident – a pub, a sandwich bar and a kebab shop.

Between 30 August and 6 September 2000, 34 cases with *S.* Typhimurium DT104 R-type ACSSuSpT were telephoned, 27 responded and 26 were willing to be interviewed. Subsequent microbiological examination showed that four cases did not fulfil the refined case
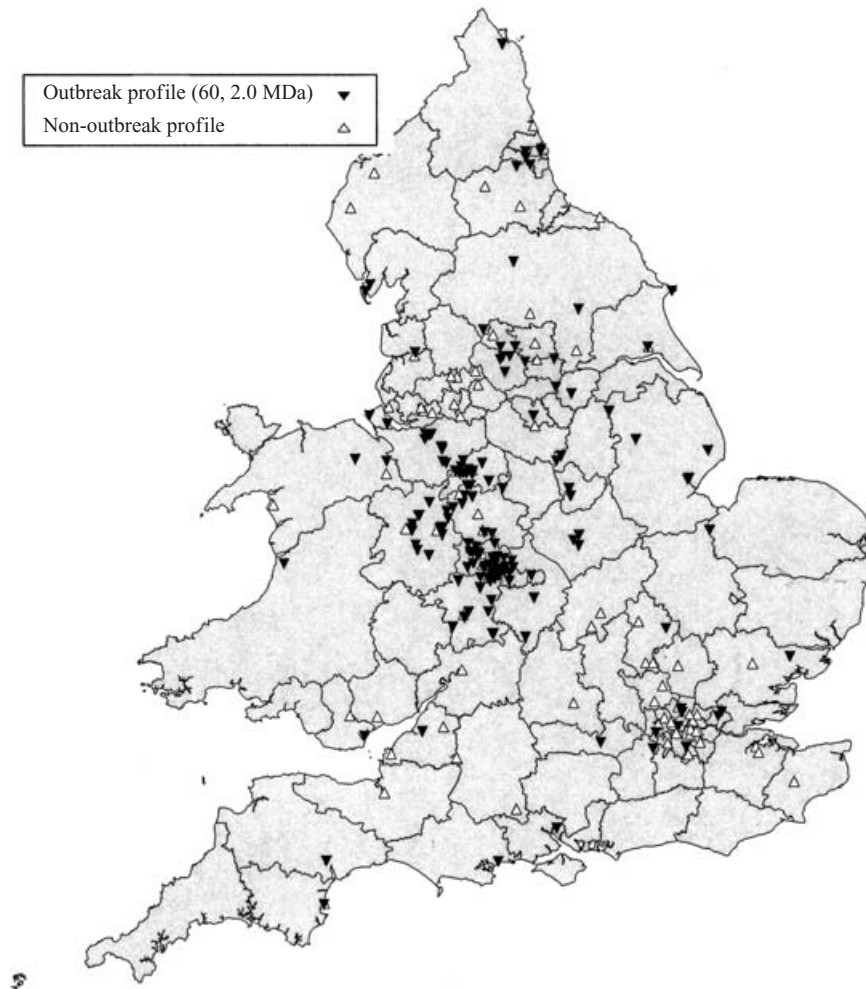
Fig. 2. Geographical plot of 257 cases of *S*. typhimurium DT104 R-type ACSSuSpT identified in August 2000 and investigated by plasmid profile analysis. England and Wales.

definition (isolates did not posses the additional 2·0 MDa plasmid), so these cases were excluded from the analysis. In all 255 potential controls were telephoned; 99 answered, 58 were willing to participate in the study but 6 did not fulfil the control definition. This left information from 22 cases and 52 controls for analysis. Cases were younger than controls (median age of cases = 33 years; median age of controls = 57 years; $P < 0.001$).

In the single variable risk analysis, eating any food prepared away from home was statistically associated with illness (Table 2). Several food items eaten away from home were statistically associated with illness. No foods eaten at home were statistically associated with illness.

With the exception of 'chicken kebabs' and 'other chicken dishes', all food variables that had a *P*-value of < 0·2 in the single variable analysis were entered into

the logistic regression model. 'Chicken kebabs' and 'other chicken dishes' were not included as only two cases and zero controls ate these items. The variables included in the multivariable model were; age, chicken sandwich, chicken salad, ham salad, Indian chicken, lettuce, tomato and cucumber. Lettuce and tomato were the combined responses of the two questions asked to determine if lettuce or tomatoes were eaten in any form away from home. This model included information from 17 cases and 52 controls. The final multivariable model included 22 cases and 52 controls (Table 3).

Environmental investigations

Initial case-investigations of apparently sporadic cases by EHOs revealed a common wholesale supplier of salad vegetables for cases in one district. Four clusters

Table 2. *Single variable risk analysis of foods eaten away from home in the outbreak of* S. Typhimurium *DT104 R-type ACSSuSpT*

Variable	Cases exposed (not exposed)	Controls exposed (not exposed)	Odds ratio	95 % CI	P-value
Eating out and from take-aways					
Eat any food prepared away from home?	18 (4)	24 (28)	5·25	1·38, 21·67	0·01
Sandwich bar	5 (16)	4 (47)	3·67	0·72, 19·39	0·11*
Mobile caterer	2 (20)	1 (50)	5·00	0·32, 150·88	0·21*
Fast food restaurant	6 (16)	8 (43)	2·02	0·51, 7·92	0·33*
Pub	8 (13)	7 (45)	3·96	1·03, 15·51	0·03*
Cafe	0 (22)	1 (51)	0·00	0·00, 43·35	1·00
Restaurant	4 (18)	6 (46)	1·70	0·35, 8·12	0·47
Hotel	1 (21)	1 (51)	2·43	0·00, 95·87	0·51*
Canteen	1 (21)	1 (51)	2·43	0·00, 95·87	0·51*
Any other venue	5 (17)	3 (49)	4·80	0·85, 29·46	0·05*
Foods eaten away from home					
Chicken sandwich	4 (17)	2 (50)	5·88	0·80, 52·39	0·05*
Ham sandwich	4 (17)	4 (48)	2·82	0·51, 15·82	0·22*
Corned beef sandwich	0 (21)	0 (52)	n.a.c.		
Roast beef sandwich	1 (20)	1 (51)	2·55	0·00, 100·89	0·50*
Cheese sandwich	1 (20)	1 (51)	2·55	0·00, 100·89	0·50*
Chicken salad	3 (19)	0 (52)	—	1·02, —	0·02*
Ham salad	3 (19)	0 (52)	—	1·02, —	0·02*
Cold beef salad	1 (21)	0 (52)	—	0·06, —	0·30*
Cheese salad	1 (21)	0 (52)	—	0·06, —	0·30*
Hamburger	1 (20)	3 (49)	0·82	0·00, 9·94	1·00*
Hot dog	1 (20)	0 (52)	—	0·06, —	0·29*
Chicken kebab	2 (19)	0 (52)	—	0·47, —	0·08*
Meat kebab	1 (20)	0 (52)	—	0·06, —	0·29*
Fried chicken	0 (21)	0 (52)	n.a.c.		
Indian chicken	5 (17)	1 (51)	15·00	1·48, 370·98	0·01*
Indian beef	0 (22)	0 (52)	n.a.c.		
Chinese chicken	0 (22)	0 (51)	n.a.c.		
Chinese beef	1 (21)	0 (51)	—	0·06, —	0·30*
Other chicken dish	2 (17)	0 (48)	—	0·49, —	0·08*
Other beef dish	0 (20)	1 (47)	0·00	0·00, 44·20	1·00*
Fresh fruit	3 (17)	4 (48)	2·12	0·33, 13·15	0·39*
Lettuce	15 (7)	12 (40)	7·14	2·07, 25·64	<0·001
Tomatoes	11 (10)	8 (44)	6·05	1·67, 22·66	0·003
Cucumber	9 (9)	6 (46)	7·67	1·85, 33·42	0·002
Peppers	1 (20)	0 (52)	—	0·06, —	0·29*
Onions	1 (20)	4 (48)	0·60	0·02, 6·49	1·00*
Carrots	2 (19)	2 (49)	2·58	0·23, 28·70	0·57*
Mayonnaise	6 (15)	9 (43)	1·91	0·49, 7·36	0·34*
Lettuce†	12 (8)	8 (44)	8·25	2·21, 32·31	<0·001
Tomatoes†	6 (13)	4 (48)	5·54	1·13, 28·73	0·02*
Carrots†	0 (18)	1 (51)	0·00	0·00, 53·49	1·00*
Lettuce‡	16 (6)	12 (40)	8·89	2·49, 33·38	<0·001
Tomato‡	11 (10)	8 (44)	6·05	1·67, 22·66	0·003

* Fisher's Exact Test.

† Eaten in sandwiches, burgers or kebabs.

‡ Combination of the two questions asking if lettuce or tomato was eaten, to give the result as to whether or not that food was eaten away from home in any form.

n.a.c., not able to calculate.

Table 3. *Final multi-variable model*

Explanatory variable	Odds ratio	95% confidence interval	*P*-value
Chicken eaten away from home in Indian Restaurant	8·3	0·77, 89·88	0·05
Lettuce eaten away from home	7·28	2·25, 23·57	0·0006

in other districts were identified where three or more cases were linked to particular food outlets. Investigation of these outlets identified a number of common suppliers of salad vegetables. Due to the short shelf life of salad vegetables, it proved impossible to acquire any suspect foods for microbiological analysis. It was possible to trace back the salad vegetable supply chain from the clusters, via retailers, caterers, wholesalers and other middlemen to two wholesale markets in the West Midlands and one wholesale supplier in the North West Region (Fig. 3). The chain of supply further back from these common sources was complex and varied. The outlets did not have common suppliers of other foods, including meat.

Three tentative sources common to two or more clusters were identified. One was a large commercial grower of salad crops and it was not surprising that this company supplied more than one wholesaler. Three farms, two that might have supplied salad vegetables to two of the wholesale markets and one that might have supplied a wholesaler, were identified. An audit of agricultural practices on these farms did not identify any procedures that might have contributed to the outbreak. No salmonellas were isolated from any of the eight environmental samples examined from these three farms.

DISCUSSION

S. Typhimurium DT104 R-type ACSSuSpT is a common cause of gastrointestinal illness in the United Kingdom. One major hurdle in investigating an outbreak caused by a common organism is differentiating those cases associated with the outbreak from background cases. If background cases are included in the epidemiological investigation the chances of detecting the exposure responsible for the outbreak is reduced. In this investigation, molecular typing techniques were applied in conjunction with real-time geographical mapping to identify suitable microbiological and epidemiological parameters with which to identify cases and controls. The use of plasmid analysis identified

successfully a characteristic outbreak strain. In previous studies only around 5% of *S*. Typhimurium DT104 R-type ACSSuSpT isolates posses a 2·0 MDa plasmid in addition to the 60 MDa plasmid that is common to many strains of *S*. Typhimurium [10].

To our knowledge this was the first outbreak investigation in which commercial software was used to identify potential controls. One major practical advantage of this approach was the speed with which potential controls could be identified, a huge benefit over a weekend. This is despite the fact that large numbers of telephone calls were made. A disadvantage is that the list does not contain households with ex-directory telephone numbers. However, since all the cases' telephone numbers were listed in the telephone book this should not have introduced a major source of bias with respect to the two comparison groups.

In the case–control study, cases were around seven times more likely to have consumed lettuce prepared away from home than controls. Unfortunately, cases were significantly younger than the controls. Older people might have been at home a greater proportion of the day and therefore be more likely to be selected as controls through a telephone recruitment process. This selection bias occurred despite pre-emptive attempts to reduce it by undertaking interviews both in the daytime and the evening. If age were an independent predictor of illness and dietary behaviour varied with age, spurious associations between certain food exposures and illness might be identified. However, age was included in the multivariable analysis, which showed that, independent of age, consumption of lettuce prepared away from home was associated with illness.

One question raised by this investigation is why consumption of lettuce from fast food outlets was associated with illness whilst consumption of lettuce at home was not? The size and distribution of the outbreak suggests that the contaminated food was widely distributed. If lettuce were the vehicle for the infection, it seems improbable that a widely distributed batch of contaminated lettuce would only be distributed to fast
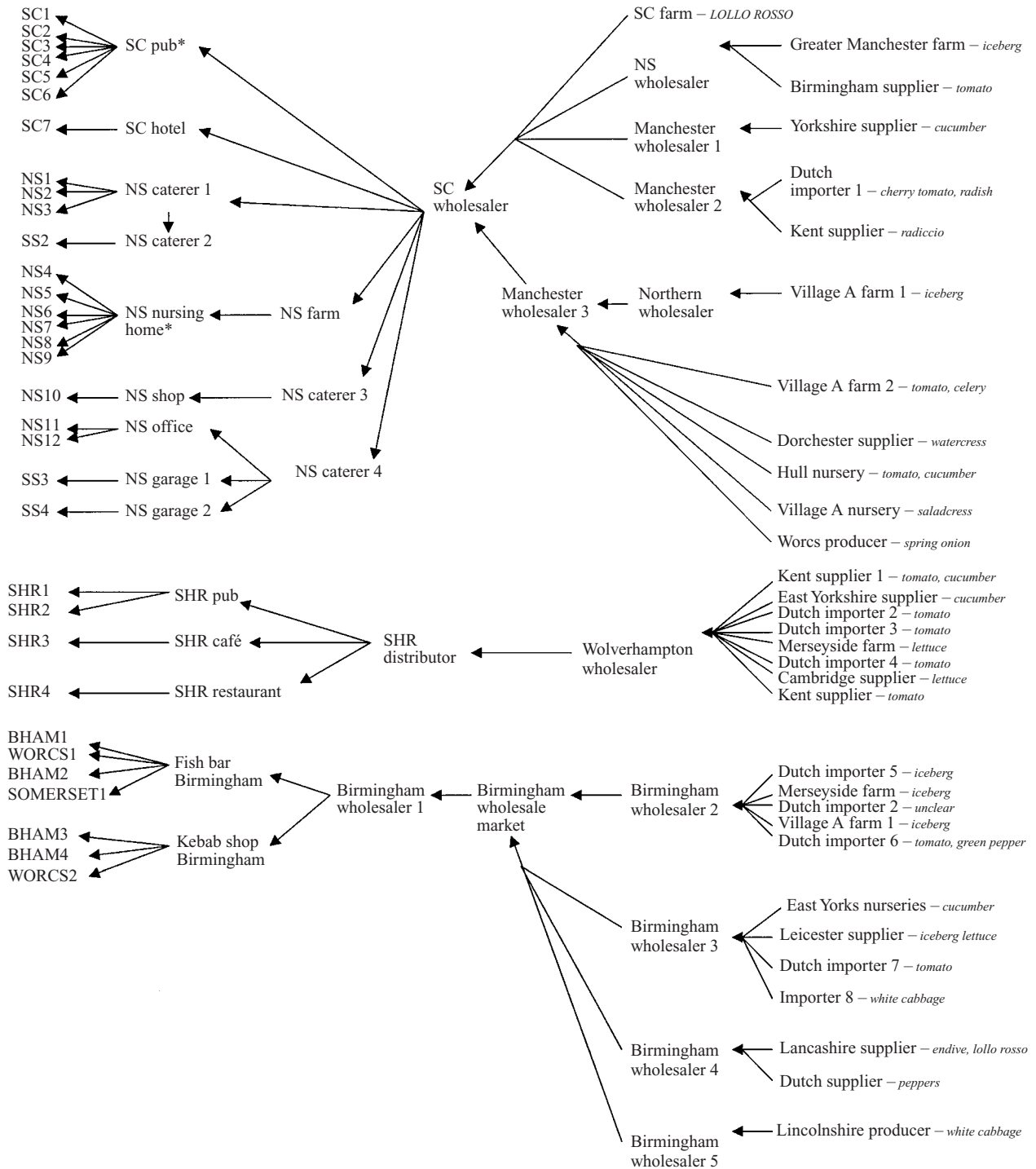
Fig. 3. Salad supply chain. NS, North staffs; SS, south staffs; SHR, Shropshire; BHAM, Birmingham; WORCS, Worcestershire; SC, South Cheshire.

food outlets. One possible explanation is that contaminated lettuce did find its way into both commercial food outlets and private homes but washing of raw vegetables in particular fast food outlets was less thorough or effective than in the home.

Environmental investigations of local clusters supported the hypothesis that lettuce prepared in fast food restaurants was the likely source of infection. Contaminated lettuce has been identified as a source of infection with *Escherichia coli* O157 and *Shigella*

sonnei (11–13) but is an unusual vehicle for salmonella infection. The infective dose for salmonella infection is generally much larger than for *Escherichia coli* O157 or *Shigella sonnei*, suggesting that either the infective dose for salmonella in this outbreak was unusually small, or the level of contamination was considerable. Experimental inoculation of shredded lettuce with *S*. Baildon has shown that, during storage at 4 °C for 12 days initial populations of $3 \cdot 28^{10}$ c.f.u./g of lettuce fell by about 2^{10} c.f.u./g but were not reduced to undetectable levels [14].

Possible mechanisms by which a large batch of salad vegetables could have been contaminated include:

- Use of contaminated water to irrigate the crops.
- Use of contaminated water to apply pesticides or other dressings.
- Use of human or animal sewage as a crop fertiliser.
- Use of contaminated water to wash the crop once harvested.
- Transport of the harvested crop in a contaminated vehicle/storage system, e.g. trucks previously used for transporting waste.

Unfortunately, the complexity of the food supply chain and the lack of identifying markers on salad stuffs made it extremely difficult to track salad vegetables back to the original grower. In some cases, the supply chain was traced through five stages before reaching a firm that imported the salad items from a wholesale market on the European mainland. These long supply chains not only cause problems in tracing food, but may also have implications for the assumptions that arise from the use of the word 'fresh' to describe them. Labelling of fresh salad produce is not sufficient to allow proper tracing of products. This has implications for public health since food hazard warnings and product withdrawal are contingent on accurate identification of the suspect product.

Salad vegetables have been implicated in a number of outbreaks of gastrointestinal disease [11–13, 15–17]. One key factor in successful transmission of infection from salad vegetables might be the perception that these food products are ready-to-eat [15]. This perception must be eroded. Salad vegetables and fruit are not ready-to-eat and must be washed before eating.

Finally, whilst technical expertise was crucial to this investigation it would not have succeeded without the extensive fieldwork of local public health, microbiology and environmental health professionals. Identifying patient details such as postcodes and investigating the local food supply chain is the legitimate work of local investigators. This investigation demonstrates the power and utility of local networks supported by central expertise and capacity.

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