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Outbreak investigation and case-control study: penta-resistant *Salmonella* Typhimurium DT104 associated with biltong in London in 2008

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SUMMARY

In August 2008 an outbreak of *Salmonella* Typhimurium DT104 occurred in South West London. Sixteen cases were identified with a particular multilocus variable number tandem repeat analysis (MLVA) pattern. In a matched case-control study 14 primary cases were included. These were defined as individuals with gastrointestinal symptoms and *Salmonella* Typhimurium DT104 isolated from a stool specimen, with a characteristic antibiotic resistance profile and MLVA pattern, and diagnosed in a local laboratory. Four controls per case were matched on age, gender and area of residence. Cases were 26 times more likely than controls to have eaten beef biltong, a South African speciality meat product (odds ratio 25·83, 95% confidence interval 4·92–135·59, $P<0·01$). Although environmental investigation failed to identify *Salmonella* in the food product we conclude that beef biltong consumption led to this outbreak. This conclusion has importance in informing the ongoing risk assessment relating to uncontrolled foodstuffs.

Key words: Biltong, food poisoning, gastroenteritis, matched case-control study, *Salmonella*.

INTRODUCTION

Food poisoning caused by *Salmonella* spp. other than typhoid and paratyphoid remains a significant public heath problem in England and Wales, despite a decline in the number of infections over the last two decades from over 28 000 in 1990 to just under 10 000 in 2008 [1]. Food poisoning is notifiable in England and Wales. Microbiological typing is performed on all *Salmonella* isolates referred to the Health Protection Agency's (HPA) Laboratory of Gastrointestinal Pathogens (LGP), HPA Colindale, London. Exceedance reporting is used to detect outbreaks by comparing observed and expected numbers of infections for particular phage types [2]. Widespread national outbreaks detected in this way have included an increase in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) DT191a in 2008 attributed to reptile feed [3].

The most common phage type of *S.* Typhimurium in England and Wales is definitive type (DT) 104 (DT104). *S.* Typhimurium DT104 has been associated historically with porcine and bovine sources, and also with poultry. Outbreaks of *S.* Typhimurium

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DT104 have been reported across the UK, most notably in 1994 [4], 2003 [5] and 2005 [6], with pork sausages, pasteurized milk, and iceberg lettuce implicated as vehicles of infection, respectively.

Within *S*. Typhimurium, certain antimicrobial resistance profiles have been predominant, namely those of ampicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (Su), and tetracyclines (T). Isolates of DT104 with this resistance profile have been referred to as 'penta-resistant' in North America. An unusual property of penta-resistant isolates of DT104 is that the complete resistance spectrum – ACSSuT – is chromosomally encoded and is located on a specific resistance island within the chromosome. Penta-resistant isolates with additional resistance to quinolone antibiotics (nalidixic acid, Nx), and also exhibiting decreased susceptibility to ciprofloxacin (Cp_L), emerged in 1993 following the licensing in the UK of fluoroquinolone-containing products for veterinary usage [7].

We describe an investigation of a local outbreak of penta-resistant *S*. Typhimurium DT104.

Identification of the outbreak

In August 2008, South West London Health Protection Unit's (SWLHPU) *Salmonella* phage-type exceedance reporting system identified an increase in cases of *S*. Typhimurium DT104 in the area. Fourteen cases had been detected over a 6-week period compared to eight over the previous 3 years. Further investigation revealed that the antibiotic resistance profiles of these organisms were identical in ten cases and similar in a further two cases.

METHODS

A multi-agency outbreak control team coordinated the investigation. We defined outbreak cases as individuals who were infected with *S*. Typhimurium DT104 isolated from a stool specimen, with resistance to nalidixic acid and ciprofloxacin, and a matching multilocus variable number of tandem repeat analysis (MLVA) pattern, who were diagnosed in a laboratory in south west (SW) London, or an adjacent area in Surrey, after 16 July 2008.

Microbiological investigations

We asked local laboratories to report suspected cases promptly to SWLHPU and to ensure that *Salmonella* isolates were referred to the LGP. All *S*. Typhimurium isolates referred were phage-typed by the LGP using the typing scheme for *S*. Typhimurium described in 1959 [8] and later extended in 1977 [9]. *Salmonella* strains were also screened by the LGP for antimicrobial resistance [10]. Isolates related to this outbreak were further characterized using MLVA [11] and analysed by pulse-field gel electrophoresis (PFGE) [12].

Epidemiological investigations

The cases were described in time, place and person. Environmental health teams routinely request information on all *Salmonella* cases that are reported to them by administering a standard gastrointestinal illness questionnaire which includes general questions on travel and food consumption. Where available, we examined this information for the outbreak cases. We then contacted cases by phone to complete a *Salmonella* trawling questionnaire which included much more detailed questions on their food consumption.

Based on the reported history by several cases of having eaten beef biltong, we designed a case-control study to test the hypothesis that the consumption of biltong, beef products or food from particular premises identified by cases, was associated with being a case.

To be included in the case-control study, cases had to meet the outbreak case definition and also to have had gastrointestinal symptoms. We excluded cases if they had travelled abroad ($\geqslant$3 days overseas in the 7 days before onset). Cases were regarded as secondary and excluded from the study if their onset was between 36 h and 7 days after that of another individual with gastrointestinal illness in their household. Controls were resident in the catchment area for the laboratories where the cases had been diagnosed (SW London and an adjacent area in Surrey) and had the same exclusions regarding travel.

We sought four controls per case, including both case-nominated and general practitioner (GP)-nominated. We considered it likely that diet would vary with age and therefore we asked cases to nominate two controls who were within 10 years of the case's age (i.e. age $\pm$10 years). We asked GPs to identify four individuals on the GP register closest in age to the case. GP-nominated controls were contacted until a maximum of two GP controls for each case completed the telephone questionnaire.

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The study was powered to give 95% confidence and 80% power for detecting an odds ratio (OR) of nine, assuming 50% of cases and 10% of controls were exposed.

We enquired about exposures in the 5 days before onset of illness for cases or, for the controls, in the 5 days from 27 September 2008 to 1 October 2008. Questioning controls about the period during which the cases were exposed aimed to elicit answers about habitual food consumption and a specific recent period was therefore selected. A spell of warm weather about a fortnight before administering the questionnaire was chosen. After piloting the questionnaire and obtaining consent, cases and controls were interviewed by telephone in the 3 weeks following 13 October 2008.

Epidata 3.1 [13] was used for data entry and Stata 9.2 (StataCorp., USA) was used for data analysis. Data were handled according to HPA policy, with safeguards to protect personally identifiable information. We used univariable logistic regression to estimate odds ratios and likelihood ratio P values. To determine which exposures were independently associated with being a case, those with a P value $<0{\cdot}2$ were entered into a multivariable analysis. Exposures which appeared protective on univariable analysis were not entered into the multivariable analysis. We cross-tabulated exposures and noted a high level of collinearity between beef biltong and X outlets. All but one individual who had purchased biltong had also shopped at an X outlet. We therefore did separate analyses for foods and premises. An initial multivariable analysis, using conditional logistic regression to take account of the matched data, failed because of an unacceptable number of inestimable odds ratios given the low degree of discordance between case and controls within the matched sets. We therefore conducted an unmatched backwards stepwise multivariable analysis using logistic regression. We used likelihood ratio tests to calculate P values in the univariable and multivariable models.

Environmental investigations

Environmental health officers from two local boroughs inspected premises, assessed the production methods and took samples from food items that were identified as possible sources during the investigation. In addition, they sought information on relevant suppliers. They also examined their records of previous visits to premises and the results of routine food sampling. The delay in identifying the outbreak (as the outbreak was identified from the results of phage-typing) meant that several weeks had passed between the exposures and the investigation, so no biltong was obtained from the households where it had been consumed.

RESULTS

Microbiological findings

Sixteen cases met the outbreak case definition. Isolates from these cases had the same MLVA pattern with pattern reference 02-07-12-28-03. These isolates exhibited a common antimicrobial resistance profile which included resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulphonamides (Su), tetracyclines (T), nalidixic acid (Nx), and reduced susceptibility to ciprofloxacin (Cp_L). They had the same PFGE profile defined as STYMXB.0264 in the PulseNet Europe database.

Epidemiological findings

The onset dates of these 16 cases were between 17 July 2008 and 18 August 2008 (Fig. 1). The majority of cases lived in two adjacent south London boroughs, with two cases living in Surrey. Nine were born in the UK, four were born in South Africa, two were from other countries and the country of birth was not known for one case. Age varied from <1 year to 56 years (median 28 years), and half were male. Four of the 16 were admitted to hospital, one attended the emergency room and no deaths were reported.

The trawling questionnaires completed for ten cases revealed that five had purchased food from a specific small chain of South African food outlets (X outlets), and five had eaten biltong. One infant had been given biltong to chew on while teething. Piri piri was included in the epidemiological study because spices were another common exposure in the trawling questionnaire. Although several cases had eaten chicken, the trawling questionnaire did not suggest a common source for it. There was an anecdotal report that a staff member at the outlet also had gastrointestinal symptoms, although as this individual had left the UK in the interim it was not possible to verify this information.

Fourteen cases met the case-control study definition after two secondary cases were excluded. In the case-control study there were 48 participants

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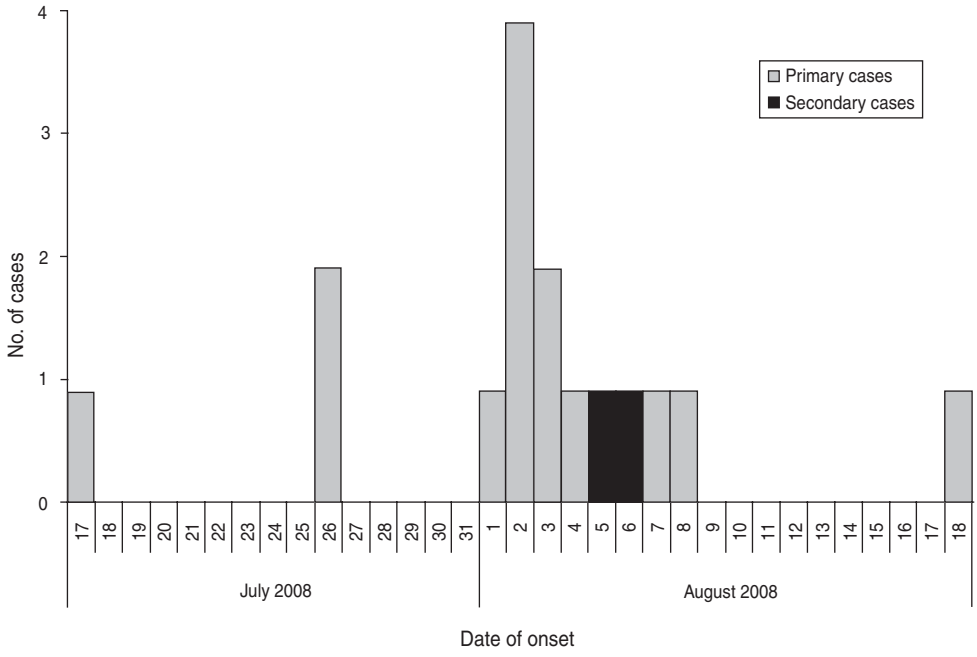

Fig. 1. Date of onset of cases in the outbreak of penta-resistant *Salmonella* Typhimurium DT104 with the same MLVA pattern with pattern reference 02-07-12-28-03.

(13 cases and 35 controls) (13 case-nominated and 22 GP-nominated). The age of cases ranged from <1 year to 56 years (median 27 years). The age of controls ranged from <1 year to 61 years (median 26 years). GP-nominated controls tended to be younger (median age 22·5 years), compared to case-nominated controls (median age 32 years), although the difference was not statistically significant.

All cases lived in SW London except the Surrey case who mentioned when interviewed that he habitually stopped off on his way home from central London to purchase biltong from a retail branch of an X outlet. A separate analysis by 'branch' of X outlets was not possible because ten of the 12 participants who used X outlets had purchased from the producing branch so the outlets were grouped together for analysis.

Table 1 displays the results of the univariable logistic regression analysis. Very similar results were obtained using univariable χ^2 (or Fisher's exact) test, as appropriate, and conditional logistic regression. Cases were 26 times more likely to have eaten beef biltong [OR 25·83, 95% confidence interval (CI) 4·92–135·59, $P<0·01$]. Purchasing of food from the chain of X outlets or of food from any local butcher was also significantly more likely in cases. The only apparently protective effect was the consumption of minced beef, which was not included in the multivariable analysis.

Multivariable analyses were conducted, but the models that best fitted the data for both food exposures and food venues were univariable, with no evidence of confounding. For food exposures, the only variable in the final model was beef biltong while for the premises only, the final model contained only X outlets (OR 32·00, 95% CI 5·49–186·54, $P<0·01$).

Environmental findings

X outlet is a small chain with one premises manufacturing biltong to be sold at all outlets. There were no specific regulatory standards for biltong production but the outlet complied with general regulatory standards and had good food handling practices. Meat was dried in ovens at a temperature of 30–35 °C. Investigation of the spices added and the meat provenance revealed no concerns. We noted that the biltong was then frozen after production and subsequently defrosted, with the possibility of adding water to the product which could permit growth of pathogens. Enquiries with the outlets revealed there is no seasonality associated with biltong sale or consumption; it is a product eaten all year round.

Samples of different speciality meats from X outlets including *droewors* (dried spiced sausage), and beef and ostrich biltong, were examined microbiologically and all samples were found to be negative. Records also revealed that routine sampling of ostrich and beef

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Table 1. *Association between infection and food items and food outlets in a case-control study of an outbreak of penta-resistant* Salmonella *Typhimurium DT104, London 2008*

	Cases ($n=13$)		Controls ($n=35$)				
Exposure factor	Exposed	Unexposed	Exposed	Unexposed	OR	(95% CI)	*P* value
Meat products							
Beef	13	0	25	10	Inestimable		**0·03**
Burgers	5	8	5	30	3·75	(0·87–16·22)	0·08
Minced beef	2	11	18	17	0·18	(0·03–0·95)	**0·02**
Steak	4	9	4	31	3·44	(0·72–16·59)	0·13
Roast beef	1	12	4	31	0·65	(0·07–6·38)	0·70
Beef stew	2	11	6	29	0·88	(0·15–5·03)	0·88
Beef in pies	1	12	2	33	1·38	(0·11–16·58)	0·80
Beef sausages	1	12	1	34	2·83	(0·16–48·93)	0·48
Cold beef	1	12	4	31	0·65	(0·07–6·38)	0·70
Boerewors	2	11	6	29	0·88	(0·15–5·03)	0·88
Beef biltong	10	3	4	31	25·83	(4·92–135·59)	**<0·01**
Other beef	3	10	4	31	2·33	(0·44–12·20)	0·33
Piri piri sauce	3	10	3	30	3·00	(0·52–17·32)	0·23
Outlets							
Outlet W	8	5	18	17	1·51	(0·41–5·54)	0·53
Outlet Y	11	2	21	14	3·67	(0·70–19·12)	0·09
Outlet U	2	11	7	28	0·73	(0·13–4·06)	0·71
Outlet V	1	12	7	28	0·33	(0·04–3·01)	0·28
Other supermarket	5	8	17	18	0·66	(0·18–2·43)	0·53
Outlet X	9	3	3	32	32·00	(5·49–186·54)	**<0·01**
Outlet Z	0	12	2	33	0·00	Inestimable	0·27
Any local butcher (including X outlet)	6	7	5	30	5·14	(1·21–21·8)	**0·03**
Any local sandwich shop	1	12	4	31	0·65	(0·07–6·38)	0·70

OR, Odds ratio; CI, confidence interval.
Factors in the questionnaire to which none of the study participants were exposed have been omitted.

biltong undertaken at one of the X outlets in September 2008 as part of Local Authorities Co-ordinators of Regulatory Services (LACORS) sampling programmes, was negative.

Control measures

Advice was given to cases on how to avoid onward transmission within the household to reduce the occurrence of secondary cases. Although there was no product withdrawal the implicated outlets were thoroughly investigated, the processes and procedures of biltong production were reviewed and advice given on compliance with current legislation. Surveillance was maintained. Case-finding focused on ensuring microbiologists were aware of the outbreak and sent any relevant isolates to the reference laboratory for typing. The outbreak control team also engaged with the regulator to explore issues relating to the manufacture of biltong in the UK.

DISCUSSION

This is the first outbreak of infectious disease in the UK to be epidemiologically linked to the consumption of biltong [14]. Biltong consumption accounted for the majority of illness. Seventy-seven percent of cases ate beef biltong and 75% ate a product from an X outlet. The association of illness with X outlets and beef biltong was also strong. Further evidence is provided by two unexpected links to the product – the baby who was given it for teething and the geographical outliers who had visited an X outlet to buy biltong on their way home.

The outbreak was detected after a triggering of the routine exceedance monitoring of *Salmonella* phage types, indicating unusually high numbers of a *S.* Typhimurium DT104. It is unlikely that this outbreak would have been detected without the additional phage-typing information being available for routine exceedance analysis. This is because there

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is a high background rate of notifications of apparent sporadic non-typhoidal *Salmonella* which makes identifying links between relatively small numbers of cases outside the household setting difficult.

Case-finding was based on laboratory-identified *Salmonella* isolates. We asked microbiologists to refer samples whose provisional typing was consistent with this cluster forthwith for definitive typing at the national laboratory and to inform the Health Protection Unit promptly. This cluster could not have been distinguished clinically from other causes of diarrhoea so no attempt was made to ask clinical staff to identify cases.

The epidemiological evidence stemmed from a case-control study. As there were relatively few cases for inclusion in an analytical study, the study aimed to obtain information from four controls per case to maximize power. We selected controls from two sources to aid cooperation and to balance the risk of selecting controls who are too similar to cases with the need to sample from the population from which the cases were drawn. Obtaining the GP-nominated controls was difficult but was eventually achieved with repeated reminders. In contrast, the cases were generally willing to nominate controls and to inform them that we would be contacting them. We noted from their accents that many case-nominated controls were from the same southern African communities but did not specifically ask their ethnicity or country of origin.

With the aim of reducing recall bias we assessed exposures in the controls during a recent time period, rather than that during which the cases were exposed. This could have resulted in an inaccurate estimation of exposure in controls if there was seasonal variation in biltong availability or consumption, but the outlet reported consistent year round consumption, and therefore we do not expect differential misclassification to result.

There are, however, two possible issues that could affect our results. First, if patient-nominated controls were very similar to cases in terms of exposure then we might have overestimated exposure among them. Indeed there was such low discordance between cases and controls that a matched analysis was not feasible. Second, by the time the controls were interviewed the outbreak was over and, if the putative contamination was no longer present by the time of the investigation, then controls who ate biltong would not be expected to become ill. Either of these would tend to bias the result towards the null. Therefore we believe that the finding of a strong and highly significant association between illness and eating beef biltong is even more marked [15].

There was no evidence from the microbiological food examination of contamination of the beef biltong with *S.* Typhimurium DT104. The investigation was delayed because the outbreak was only detected as a result of phage-typing, so this finding would be consistent with contamination of a single batch which was no longer present when the sampling was conducted. We had no further information about a food handler, allegedly with gastrointestinal symptoms, who had left the country by the time of the investigation.

Biltong is a traditional South African food product and its consumption in the UK has increased following the migration of South Africans, although it is not clear what quantities are consumed [14].

A recent literature review for the Food Standards Agency (FSA) in the UK has reported on the microbiological hazards associated with eating biltong, including a detailed description of its preparation [14]. Biltong is an uncooked, marinated (with salt and acid), low-temperature air-dried product made from strips of meat (usually beef or game) [14]. It is typically marinated for 18–24 h at 4 °C and dried at 35 °C at 30% humidity for 6 days.

Reduction in pathogens is dependent on a combination of the drying and marinating process. In experimental work, reductions of *Salmonella* counts of up to three logs have been found in making biltong. Pathogen reductions increase as acidic marination takes place and water activity is reduced [14], and a key issue is the speed with which the water content can be reduced. The faster this occurs the greater the reduction in pathogen count [14].

The US Department of Agriculture (USDA) Food Safety and Inspection Service notes that commercially manufactured jerky (a similar meat product which may be produced by air or oven drying) is safe, with the process monitored in federally inspected plants by inspectors of the USDA Food Safety and Inspection Service [16]. However, one group of researchers tested biltong during its manufacture and found that it would not meet USDA requirements for cooked or fermented products, in terms of the reduction in *E. coli* O157:H7 colony-forming units [17]. The authors found that it might meet less stringent standards if the 'starting material' tested negative and if biltong could be classified as a fermented product.

Two studies from South Africa have reported *Salmonella* contamination in biltong, ranging from 0·8% to 16% of samples [18, 19].

Outbreaks of *Salmonella* linked to the consumption of biltong have been reported. The review for the FSA identified four biltong-related outbreaks since 1949. *Salmonella* was implicated in all, including *S*. Lanita, *S*. Newport and *S*. Anatum [14]. A further outbreak of *Salmonella* related to biltong was reported in Los Angeles County, USA in 2001 affecting six people [20]. With regard to biltong and jerky, the review for the FSA concludes that 'the most frequent and significant outbreaks have arisen from enteric bacteria coming from the raw meat and from cross-contamination and poor handling. The use of contaminated spices may also pose a risk' [14].

The FSA-commissioned review has made suggestions about all steps of the biltong preparation process and notes that this provides 'outline requirements for a hazard analysis and critical control points (HACCP) approach to the small scale manufacture of biltong and jerky' and suggests that this could be used as 'the basis for developing detailed guidance to assist manufacturers of biltong and similar products' [14]. In this review it is suggested that the next steps would be to collate existing guidance on preparing fermented meat products to develop risk-based guidance for small companies considering making such products [14].

Development of robust and detailed guidance would ensure that the preparation of such speciality items is standardized and made as safe as possible; however, no UK guidelines are currently available. The Advisory Committee on the Microbiological Safety of Food has stated that there is currently 'insufficient evidence for the FSA to provide advice to food producers and local authorities on the production of biltong' [21]. More experimental evidence was required on the effect of processing techniques before risks could be assessed. There was also a need for clarification on outbreaks said to be associated with biltong to ensure that the epidemiology about the source of infection was accurately modelled and the source identified.

Our small outbreak provides some evidence of risk to human health at least in this local situation. It has been estimated that for every case of *Salmonella* infection reported to national surveillance there are 4·7 in the community [22]. On this basis an estimated number of around 75 cases might have been expected in this outbreak. Further investigation would be needed to establish how frequently biltong is eaten in the UK as well as whether it constitutes a risk factor for foodborne illness.

CONCLUSIONS

On the basis of the epidemiological evidence we attributed this outbreak to contamination of beef biltong manufactured over a limited period by X outlets. There are no specific UK guidelines for the manufacture of biltong; however, in response to enquiries from local environmental health teams, the FSA has commissioned a review of the microbiological hazards associated with biltong and other dried meats [14]. This outbreak adds to the evidence to inform an assessment on whether guidance for the manufacture of biltong is needed.

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DECLARATION OF INTEREST

None.

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