

Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta

M. E. LOCKING¹, S. J. O'BRIEN^{1*}, W. J. REILLY¹, E. M. WRIGHT^{1,2},
D. M. CAMPBELL¹, J. E. COIA³, L. M. BROWNING¹ AND C. N. RAMSAY⁴

¹ Scottish Centre for Infection and Environmental Health, Clifton House, Clifton Place, Glasgow G3 7LN

² Department of Statistics and Modelling Science, University of Strathclyde, Livingstone Tower, Richmond Street, Glasgow G1 1XH

³ Department of Clinical Microbiology, Western General Hospital NHS Trust, Crewe Road, Edinburgh EH4 2XU

⁴ Department of Public Health, Lothian Health, Deaconess House, 148 Pleasance, Edinburgh EH8 9RS

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SUMMARY

To determine environmental risk factors for sporadic *E. coli* O157 infection in Scotland we undertook a prospective, matched case-control study between 1 October 1996 and 31 March 1999. One hundred and eighty-three cases and 545 matched controls were recruited. Contact with animal faeces (OR = 3.65; 95% CI 1.81, 7.34; $P < 0.0005$) and likely contact with animal faeces (OR = 4.8; 95% CI 2.42, 9.48; $P < 0.0005$) emerged as strong risk factors for infection. Certain exposures (mainly food-related) were inversely associated with infection i.e. were statistically protective. Most striking was the consumption of bottled water (OR = 0.28; 95% CI 0.15, 0.52; $P < 0.0005$). Transmission of *E. coli* O157 does not occur simply through contaminated food. Members of the public need to be aware of the potential for acquiring *E. coli* O157 through contamination of the environment with animal faeces so that they may take measures to mitigate their risk.

INTRODUCTION

The spectrum of clinical illness caused by infection with *Escherichia coli* O157 ranges from mild diarrhoea, through bloody diarrhoea and haemorrhagic colitis to severe complications with haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) [1]. Infection with *E. coli* O157 is a leading cause of acute renal failure in children and a considerable proportion of those who survive develop long term renal impairment [2].

Highly publicised outbreaks worldwide, including those in Japan [3] and Central Scotland in 1996 [4], have tended to focus attention on aspects of food

safety. Cases occurring as part of outbreaks account for only a minority of all cases of *E. coli* O157 identified [5, 6] yet relatively few studies of sporadic *E. coli* O157 infection have been undertaken [6–8]. A case-control study performed in Wales identified visiting private farms, or having a household member whose occupation involved contact with farm animals, as risk factors for sporadic *E. coli* O157 infection [8].

The incidence of *E. coli* O157 infection in Scotland is substantially higher than elsewhere in the United Kingdom [1]. In a previous descriptive study an unexpectedly high proportion of cases who had been exposed to environmental factors prior to the onset of their illness was found: gardening or garden play (36%); contact with farms (20%) or with farm animal by-products (17%); and recent drinking water supply

* Author for correspondence: PHLS Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ.

failures (12%) [9]. Therefore we performed a prospective, matched case-control study to determine the relative importance of these environmental exposures as risk factors for sporadic cases of *E. coli* O157 infection in Scotland.

METHODS

Recruitment of cases and controls

A case was defined as a person with abdominal pain or diarrhoea, from whom *E. coli* O157 had been isolated on faecal culture and/or from whom serological evidence of infection with *E. coli* O157 had been demonstrated; or as a clinical case of HUS with accompanying evidence of *E. coli* O157 infection. Cases were identified from three sources. These were routine laboratory reports to the Scottish Centre for Infection and Environmental Health (SCIEH), laboratory forms sent with strains to the Scottish *E. coli* O157 Reference Laboratory in Aberdeen and cases of HUS reported to the British Paediatric Surveillance Unit via SCIEH. Cases diagnosed between October 1996 and March 1999 were eligible for inclusion in the study. They were excluded if they were secondary cases, or if their medical histories revealed any of the following: travel abroad during the incubation period (14 days prior to onset of illness); normal residence outside Scotland; evidence of mixed infection; or association with a known outbreak. General Practitioners (GPs) were contacted in order to allow them to approach the case regarding the study if they so wished.

Up to four controls, matched for sex and age-group and with no clinical or microbiological evidence of *E. coli* O157 infection, were sought for each case. They were identified at random by either the patient's GP or the laboratory where the case's clinical specimen had been processed. The aim was to recruit two GP nominated and two laboratory controls for each case. As with cases, a phased process of approaching the GP prior to the subject was employed for controls.

Ethical approval was obtained from all 15 local research ethics' committees in Scotland.

Data collection and validation

Written consent to participate in the study was obtained from cases and controls (or a parent or guardian for children recruited to the study). A single researcher conducted telephone interviews with cases

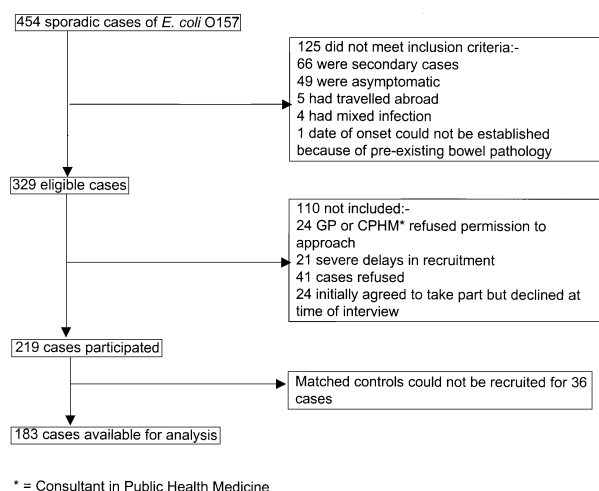


Fig. 1. Case-control study profile.

and controls (or their parent or guardian) using a standard, structured questionnaire. We recorded exposures for 14 days prior to the onset of illness in the case. The questionnaire covered demographic and clinical details, food, water, animal and environmental exposures. A handful of cases and controls could not be contacted by telephone. Questionnaires with reply paid envelopes were posted to them and a reminder letter sent a fortnight later to those who had not replied. Data were entered onto an EPIINFO (version 6.04c) database [10] and validated by means of double data entry.

Statistical analysis

The difference in median ages between males and females was tested using a Mann-Whitney test. Conditional logistic regression was employed to assess possible risk factors for *E. coli* O157 infection. Since some of these factors will not be independent, conditional multiple logistic regression models were fitted. A backwards stepwise regression algorithm was applied to factors with *P* values less than 0.2 in the univariate analysis. Certain questions were only relevant for subsets of participants, resulting in small sample sizes for these factors. For such variables, logistic regression was performed due to insufficient data to apply matched analyses. All analyses were performed using Stata [11].

RESULTS

Between October 1996 and March 1999 454 sporadic cases of *E. coli* O157 infection, including 7 deaths,

Table 1. Risk factors for sporadic *E. coli* O157 infection; results of univariate analysis

Variable	Odds ratio	95% confidence interval	P-value	% cases exposed*	% controls exposed*
Environmental and animal exposures					
Contact with animal faeces (excluding pets)	4.02	2.34, 6.93	< 0.0005	21.0	8.3
Likely contact with animal faeces (excl. pets)	3.36	2.07, 5.44	< 0.0005	28.4	15.6
Contact with animals (excluding pets)	3.34	2.07, 5.38	< 0.0005	27.1	11.4
Visited farm, smallholding, or zoo	2.34	1.56, 3.69	< 0.0005	33.5	19.0
Ate at farm, smallholding, or zoo	1.26	0.39, 4.06	0.69	71.2	66.7
Does home have garden space	0.87	0.41, 1.83	0.71	94.0	94.3
Is the garden next to field/farm	2.23	1.44, 3.45	< 0.0005	28.7	17.6
Worked or played in garden, field etc	0.85	0.56, 1.28	0.44	60.9	64.4
Contact with soil during above	2.80	1.31, 5.99	0.008	83.8	73.0
Contact with manure during above	2.56	0.94, 6.93	0.065	12.1	5.0
Contact with pet animal	0.81	0.53, 1.22	0.31	71.8	76.5
Contact with unwell pet animal	0.72	0.31, 1.69	0.44	8.5	10.1
Water exposures					
Drank untreated water	1.16	0.35, 3.60	0.85	2.3	2.4
Recreational contact with water	0.98	0.62, 1.53	0.92	20.2	20.8
Drank tap water (unheated)	0.73	0.41, 1.29	0.28	89.0	91.2
Drank bottled water	0.44	0.29, 0.69	< 0.0005	20.6	33.6
Food exposures					
Contact with raw fish	1.04	0.64, 1.70	0.86	22.3	22.1
Contact with raw other meat	0.75	0.46, 1.20	0.22	34.5	37.6
Contact with raw poultry/game	0.61	0.38, 0.98	0.04	25.4	32.6
Contact with raw salad/vegetables	0.56	0.37, 0.85	0.007	68.1	78.5
Ate red meat	1.68	0.82, 3.47	0.16	94.4	90.4
Ate pre-cooked sliced cold meat	1.06	0.71, 1.57	0.78	74.4	72.4
Ate burgers	1.05	0.69, 1.60	0.83	26.6	25.0
Ate other meat products	0.98	0.64, 1.56	0.92	76.5	76.0
Ate fish/seafood	0.79	0.54, 1.16	0.22	63.5	68.3
Consumed milk or milk products	0.79	0.33, 1.87	0.59	95.6	96.7
Ate salad or fresh vegetables	0.66	0.29, 1.52	0.33	94.4	95.8
Ate eggs	0.60	0.40, 0.91	0.016	60.7	69.5
Ate cheese or cheese products	0.59	0.38, 0.93	0.024	80.7	86.4
Ate poultry/game	0.48	0.30, 0.76	0.002	78.2	87.8
Ate food from a barbecue	1.33	0.64, 2.78	0.44	6.7	5.5
Ate food from a takeaway	0.65	0.44, 0.98	0.037	57.2	65.3
Ate in premises outside the home	0.60	0.36, 1.00	0.05	84.4	89.9
Ate any food prepared outside home	0.58	0.29, 1.15	0.12	91.1	94.3
Travel and contact exposures					
Contact with person with diarrhoea	1.17	0.70, 1.94	0.56	14.4	13.2
Subject away from home daytime	0.81	0.50, 1.33	0.40	79.1	82.4
Subject had nights away from home	0.66	0.44, 0.99	0.04	22.5	30.0

* Percentages of cases and controls exposed to each variable ignoring matching.

were reported to SCIEH, of which 329 met the inclusion criteria. Following refusals by patients or their GPs, 219 cases agreed to participate (Fig. 1). We were unable to recruit controls for 36 of the cases, leaving 183 cases and their 545 matched controls (256 recruited from GPs and 289 from laboratories) available for analysis.

One hundred and six cases (58%) were female and 77 (42%) were male. The cases ranged in age from

3 months to 90 years (mean age 28.4 years (median age 22 years)). Forty-four percent of cases (80/183) were under the age of 10 years (31% (57/183) under the age of 5 years). Male cases tended to be younger than females (Mann–Whitney test: $P = 0.001$). Non-participants were similar to the study cases in terms of age and gender distribution. Diarrhoea was reported by 178 (97%) cases, bloody diarrhoea by 141 (77%) and 104 (57%) were admitted to hospital. Fifteen

Table 2. Risk factors for sporadic cases of *E. coli* O157 infection: results of multivariate analysis

Variable	Odds ratio	95% CI	P-value
Likely contact with animal faeces (excl. pets)	4.80	2.42, 9.48	< 0.0005
Contact with animal faeces (excluding pets)	3.65	1.81, 7.34	< 0.0005
Ate poultry/game	0.53	0.28, 0.97	0.041
Contact with raw salad/vegetables	0.48	0.27, 0.85	0.012
Drank bottled water	0.28	0.15, 0.52	< 0.0005

(8%) cases developed HUS, all but one of whom were children.

Single variable risk analysis

Various types of animal and/or environmental exposure were strongly associated with risk of *E. coli* O157 infection, including visiting a farm, small-holding or zoo, contact with animals (other than pets) or their faeces, contact with the soil and having a garden next to a field or farm (Table 1). We explored the relationship with individual animal species and found that, in unmatched univariate analysis, an increased risk of *E. coli* O157 infection was only associated with contact with cattle (OR = 3.59; 95% confidence interval (CI) 1.52, 8.47; $P = 0.004$). People whose employment brought them into contact with the farming environment also had a greatly increased risk of *E. coli* O157 infection (OR = 13.48; 95% CI 3.54, 51.34; $P < 0.0005$) although the numbers involved were small.

Contact with animal faeces (other than pet faeces) did not necessarily result from an organized visit to farm or zoo premises or direct contact with a farm animal. For example they touched manure whilst retrieving a ball from a field, on cars, bicycles, other vehicles or on dogs which had been on farmland. Animal faeces were also brought into cases' homes on clothing or footwear.

Certain exposures (mainly food-related) were inversely associated with infection i.e. were statistically protective. The most striking of these was the consumption of bottled water.

There was no evidence of an increased risk for any of the following exposures: any specific raw or cooked food including burgers or barbecued items; contact with a person with diarrhoea; interruptions or changes to domestic water supplies and exposure to recreational water. For those who drank tap water, the risk associated with drinking water from a private supply was borderline (OR = 2.29; 95% CI = 0.97,

5.40; $P = 0.059$), but the numbers of such cases and controls were very small.

Logistic regression analysis

In the logistic regression analysis the risk of developing *E. coli* O157 infection remained strongly associated with contact with animal faeces and likely contact with animal faeces (Table 2). The association with visits to farms, small-holdings or zoos, was no longer significant. Drinking bottled water remained strongly inversely associated with infection.

DISCUSSION

In our study contact, or likely contact, with animal faeces (other than pet faeces), regardless of occupation, emerged as a strong risk factor for *E. coli* O157 infection. Contact with animal faeces did not occur solely during organized visits to farms. The findings are biologically plausible and accord with what is known about survival of *E. coli* O157 in the environment in both cattle faeces and in soil [12, 13]. The fact that the organism can persist for long periods in the environment in a viable state means that pastureland can remain contaminated even after cattle have been moved off. People walking, or driving, through farmland may then pick up the organism on clothing, shoes or vehicles. Evidence from outbreak investigations also supports the fact that infection with *E. coli* O157 can result from contact with an environment contaminated with cattle faeces, and that the cattle do not have to be present [14].

This study was not primarily designed to test hypotheses concerning food exposures although some food groups were included as potential confounders. It is, perhaps, reassuring that we found no increased risk of infection associated with beef products, suggesting that, perhaps, the Chief Medical Officer's advice to the public [15, 16] about cooking beef products properly and the appeal not to accept

undercooked (pink) beef, particularly ground beef, has had an effect. The fact that the consumption of bottled water appeared to be so strongly protective was intriguing and merits further investigation. This might reflect the fact that people who drink bottled water choose not to drink tap water, have different lifestyles and thus exposure experiences, or have acquired immunity to *E. coli* O157.

Our study had certain limitations. During the study only 183 of the 329 eligible cases were included. The greatest loss of potential cases was due to patients declining to take part or failing to respond once they had agreed. The Central Scotland outbreak, which occurred 2 months after the commencement of the study, had a negative effect on case recruitment. The intense media spotlight discouraged cases from participating. We chose 14 days as the incubation period for *E. coli* O157 infection, the upper limit recorded in the literature [17, 18]. This might have led to difficulties with recall although our experience was that questions were answered fully. We had to employ two types of control selected from different populations and hence subject to different sampling biases. The two types of control were similar to each other in terms of exposure characteristics but were not similar to the cases, making a convincing argument that the associations found in the study were real. Interviewer bias was minimized by using a single researcher to collect information from cases and controls.

Implications

Our expanding knowledge of the ecology and epidemiology of *E. coli* O157 demonstrates that transmission to man may occur via a variety of routes. This study has highlighted the importance of contact with animal faeces as a risk factor for sporadic cases of *E. coli* O157 infection in Scotland. Guidance already exists for farmers and teachers in mitigating the risk of *E. coli* O157 infection associated with visits to open farms [19]. Our study has shown, however, that exposure to animal faeces may occur in situations beyond the direct control of the farmer making the task of preventing this exposure more difficult.

There is no effective treatment for human infection, other than supportive measures. To date there appear to be no effective control measures in the bovine reservoir. Prevention of infection is therefore critical in reducing the morbidity and mortality associated with *E. coli* O157. It is important that members of the public are aware of the potential danger posed by

exposure to animal faeces so that they may take simple measures to mitigate their risk. Simple measures such as attention to personal hygiene as well as food hygiene are an important element of any prevention strategy for *E. coli* O157 infection.

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