

# A Nationwide Surveillance Study on E.coli 0157:H7 and Enterobacteriaceae in Irish Minced Beef Products





A NATIONWIDE SURVEILLANCE STUDY  
ON *E. COLI* O157:H7 AND  
ENTEROBACTERIACEAE  
IN IRISH MINCED BEEF PRODUCTS

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## SUMMARY

A surveillance study on prevalence and numbers of *E. coli* O157: H7 in minced beef (unpackaged or packaged) and beefburgers (frozen, fresh and unpackaged or packaged) was carried out over a period of 12 months in the Republic of Ireland. A total of 1533 products were tested with approximately 15 products collected from each of the 26 counties every 3 months. Mince and beefburgers were collected from both supermarkets and butcher shop outlets. A standard analysis was conducted by sample enrichment, IMS extraction and plating onto SMAC agar with confirmation by PCR. The results showed that 43 retail beef products (2.8 %) contained *E. coli* O157:H7. The number of *E. coli* O157: H7 in 21 of these samples ranged from  $\log_{10}0.51 - 4.03$  cfu g<sup>-1</sup> ( i.e. 3 to 10,700 bacteria per gram) while in the remaining 22 the pathogen was detectable by enrichment only. There was a seasonal effect observed with 33 of 43 positive samples detected in January (n = 8), April /May(n=20) and August (n=5) and the remaining 10 positive samples detected over the other 8 months. Of the beef products testing positive, 32 were purchased from supermarkets and 11 from butcher shops. *E. coli* O157: H7 was recovered from 2.8% (13 / 457) of fresh packaged mince and from 1.88 % (3 / 160) of fresh unpackaged burgers purchased from butcher shops. Of the 43 isolates recovered, 41 contained the virulence genes *vt1*, *vt2*, *EaeA* and *HlyA* while the remaining 2 isolates contained only one of the vt-producing genes (*vt1* or *vt2*).

There was no significant difference between Enterobacteriaceae counts on minced beef purchased from butcher shops or supermarkets. Equally, there was no significant difference between Enterobacteriaceae counts on beefburgers obtained from butcher or supermarket outlets. It was noted that the Enterobacteriaceae counts on fresh unpackaged mince were significantly higher than on fresh unpackaged burgers. Unpackaged fresh mince had the highest Enterobacteriaceae count in each 3 monthly period regardless of whether the product was purchased from supermarkets ( $\log_{10}4.64$  cfu/g) or butcher outlets ( $\log_{10}4.42$  cfu/g).



## INTRODUCTION

Verocytotoxigenic *Escherichia coli*, in particular serogroup O157, have emerged in the last fifteen years as important foodborne pathogens. They are of considerable concern not only because of their increasing incidence worldwide, but also because of the severity of the infection they cause and their low infectious dose. Common complications include severe bloody diarrhoea (haemorrhagic colitis), kidney failure (haemolytic uraemic syndrome) and damage to the central nervous system (thrombocytopenic purpura). This pathogen has caused severe public health and economic problems worldwide with numerous high-profile outbreaks and many sporadic cases. Undercooked beefburgers have caused many food poisoning incidents associated with this pathogen and research has established that the bacterium is present in the faeces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process.

The aim of this study was to determine the incidence and numbers of *E. coli* O157:H7 and Enterobacteriaceae in minced beef (loose and pre-packed) and beefburgers (loose, packed, frozen, branded and unbranded) over a period of 12 months in the 26 counties of the Republic of Ireland.

## METHODS

### Sample plan

- A) 1533 samples from the 26 counties in the Republic of Ireland were tested over a 13 month period for *E. coli* O157:H7 and Enterobacteriaceae
- B) Each county was sampled quarterly:  
Quarter 1 ran from 25<sup>th</sup> March to 21<sup>st</sup> June 2001 and also from the 18<sup>th</sup> to 19<sup>th</sup> of April 2002 to make up sample numbers for Louth/ Meath as problems with sampling were encountered in Quarter 1 of 2001 due to an outbreak of Foot and Mouth disease  
Quarter 2 ran from 28<sup>th</sup> June to 7<sup>th</sup> September 2001



Quarter 3 ran from 11<sup>th</sup> October 2001 to 18<sup>th</sup> January 2002

Quarter 4 ran from 24<sup>th</sup> January to 12<sup>th</sup> April 2002.

- C) On each county visit, 15 beef products were collected from 5 retail outlets (3 supermarket type / 2 butcher shop) and the five premises were at 3 different locations {2 large towns (4 premises) and one small town (1 premises)}.
- D) The sample types obtained and the number of each tested in each quarter are outlined in Table 1. It was not possible to sample equal numbers of each product type due to the limited product range available at many stores.
- E) Each premises was visited and sample types selected at random and purchased. The samples were immediately placed into cooler boxes at  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for transportation back to the laboratory where they were stored at  $1^{\circ}\text{C} \pm 1^{\circ}\text{C}$  prior to analysis.

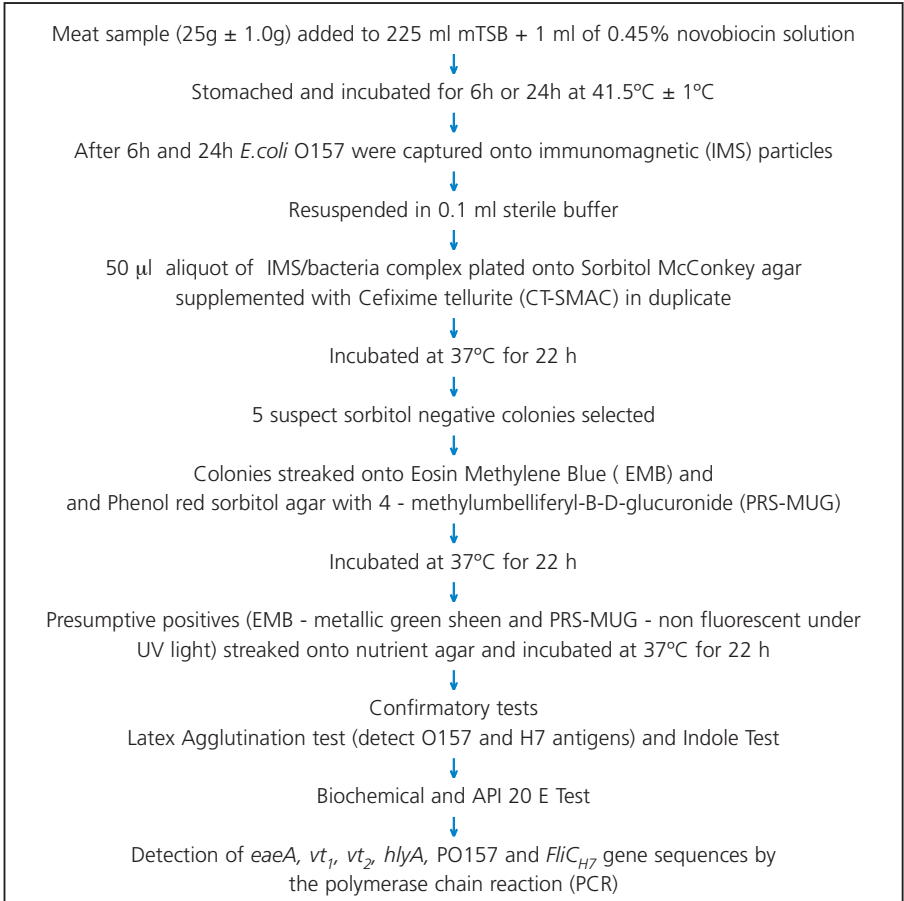
**Table 1:** Numbers and types of beef product sampled in each quarter of the year in the surveillance study.

Product	Product description	Retail Outlet	Number of beef products tested in each quarter				Total
			Q1	Q2	Q3	Q4	
Minced beef	Fresh, unpackaged	Supermarket	79	73	75	72	299
Minced beef	Fresh, packaged	Supermarket	119	117	117	104	457
Minced beef	Fresh, unpackaged	Butcher shop	54	53	52	52	211
Beefburgers	Fresh, packaged	Supermarket	37	45	42	33	157
Beefburgers	Fresh, unpackaged	Butcher shop	36	28	38	38	140
Beefburgers	Fresh, unpackaged	Supermarket	27	16	14	6	63
Beefburgers	Frozen	Supermarket	52	51	52	51	206
<b>Total</b>			<b>404</b>	<b>383</b>	<b>390</b>	<b>356</b>	<b>1533</b>



## Detection of *E. Coli* O157:H7

A flow chart of the method as described in ISO method no. 16654: 2001<sup>1</sup> for the enrichment, isolation and confirmation of *E.coli* O157:H7 in the minced beef products is outlined in Figure 1.



**Figure 1:** Method (ISO 16654) employed for detection of *E. coli* O157:H7 in beef products.

<sup>1</sup> International Organisation for Standardisation (ISO) no. 16654: 2001 - Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157.

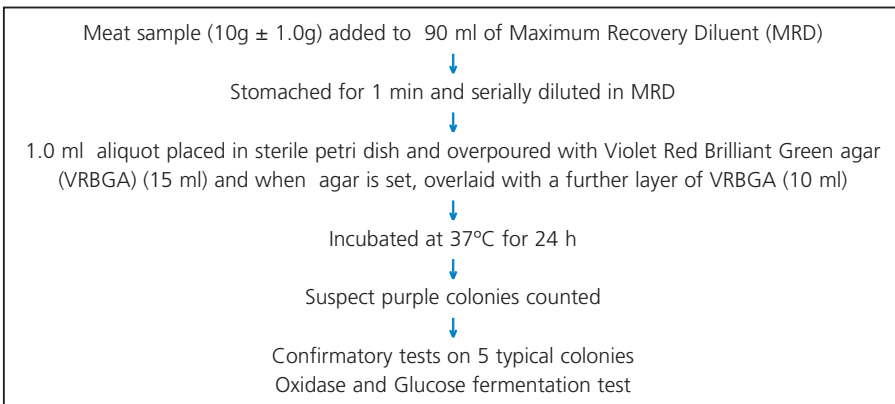


## Enumeration of *E. Coli* O157:H7

A direct plate count was also carried out to determine the initial number of *E. coli* O157:H7 present in the meat products. A meat sample (10 g) was placed in a stomacher bag with 0.1% peptone water (90 ml) and homogenised for 2 min in a stomacher. A 1.0ml aliquot was serially diluted in 9 ml volumes of 0.1% peptone water and plated in triplicate onto CT-SMAC agar. The confirmatory procedures are described in Figure 1.

## Enumeration of Enterobacteriaceae

Beef products were tested for the presence of Enterobacteriaceae using the BS method 5763<sup>2</sup>. A flow diagram of the method is shown in Figure 2.



**Figure 2:** Method used to enumerate and confirm the presence of Enterobacteriaceae (BS 5763) in beef products.

## RESULTS

### *E. coli* O157:H7

Of the 1533 minced beef products collected over a 13 month period, a total of 43 (2.8%) were positive for *E. coli* O157:H7. Tables 2-5 give details of the

<sup>2</sup> British Standards method no. 5763 for Enterobacteriaceae.





**Table 2:** Summary of location, month, premises, product type and *E. coli* O157: H7 count ( $\log_{10}$ cfu g<sup>-1</sup>) for positive samples in Quarter 1 (April to June).

County	Town	Month	Premises type	Product type	Count	Verotoxin genes		Verotoxin production	
						vt1	vt2	vt1	vt2
Wicklow	Greystones	March	Butcher	mince fresh (u)	1.22	+	+	-	-
Tipperary	Thurles	March	Supermarket	mince fresh (map)	1.18	+	+	-	-
Meath	Navan	April	Supermarket	mince fresh (u)	E	+	+	+++	+++
Meath	Kells	April	Butcher	mince fresh (u)	E	+	+	-	-
Meath	Navan	April	Butcher	mince fresh (u)	E	+	+	-	-
Meath	Navan	April	Supermarket	mince fresh (map)	1.18	+	+	-	-
Meath	Navan	April	Supermarket	mince fresh (map)	1.00	+	+	-	-
Louth	Dundalk	April	Supermarket	burgers frozen (p)	E	+	+	+++	+++
Dublin	Ballyfermot	April	Butcher	mince fresh (u)	1.89	+	+	-	-
Sligo	Sligo	May	Supermarket	burgers fresh (map)	3.04	+	+	+++	-
Sligo	Tubbercurry	May	Butcher	mince fresh (u)	2.40	-	+	-	-
Mayo	Westport	May	Butcher	burgers fresh (u)	0.90	+	+	-	-
Mayo	Castlebar	May	Supermarket	mince fresh (u)	2.69	+	+	+++	-

p = packaged; u = unpackaged; map =modified atmosphere packaging; E= detectable only by enrichment; +++ = strong agglutination reaction; ++ = very strong agglutination reaction; all isolates contained HlyA, EaeA, PO157and fliC<sub>H</sub> genes.

**Table 3:** Summary of location, month, premises, product type and *E.coli* O157:H7 count ( $\log_{10}$ cfu g<sup>-1</sup>) for positive samples in Quarter 2 (July to September).

County	Town	Month	Premises type	Product type	Count	Verotoxin genes				Verotoxin production	
						vt1	vt2	+	+	vt1	vt2
Wicklow	Greystones	March	Butcher	mince fresh (u)	1.22	+	+	+	+	-	-
Kilkenny	Kilkenny	July	Supermarket	mince fresh (map)	E	+	+	+	+	-	-
Kilkenny	Kilkenny	July	Supermarket	mince fresh (map)	3.04	+	+	+	+	-	-
Kildare	Kildare	July	Butcher shop	mince fresh (u)	2.30	+	+	+	+	-	-
Dublin	Ballyfermot	July	Supermarket	burgers fresh (map)	2.07	+	+	+	+	-	-
Leitrim	Carrick -on-Shannon	August	Supermarket	mince fresh (map)	0.60	+	+	+	+	-	++
Leitrim	Ballinamore	August	Butcher shop	mince fresh (u)	1.65	+	+	+	+	+++	-
Cavan	Cavan	August	Supermarket	burgers fresh (map)	0.95	+	+	+	+	+++	-
Cavan	Cavan	August	Supermarket	mince fresh (map)	E	+	+	+	+	+++	-
Cavan	Cavan	August	Supermarket	mince fresh (map)	E	+	+	+	+	+++	-

p = packaged; u = unpackaged; map = modified atmosphere packaging; E= detectable only by enrichment; ++ = strong agglutination reaction; +++ = very strong agglutination reaction; all isolates contained HlyA, EaeA, PO157 and FliC<sub>H</sub> genes.





**Table 4:** Summary of location, month, premises, product type and *E.coli* O157: H7 count ( $\log_{10}$ cfu g<sup>-1</sup>) for positive samples in Quarter 3 (October to January).

County	Town	Month	Premises type	Product type	Count	Verotoxin genes		Verotoxin production	
						vt1	vt2	vt1	vt2
Sligo	Sligo	January	supermarket	mince fresh (map)	E	+	+	-	+++
Sligo	Sligo	January	supermarket	mince fresh (map)	0.81	+	+	-	-
Limerick	Limerick	January	supermarket	burger frozen (p)	E	+	+	+++	++
Cork	Cork	December	supermarket	burger fresh (map)	E	+	+	-	-

p = packaged; map = modified atmosphere packaging; E= detectable only by enrichment;

++ = strong agglutination reaction; +++ = very strong agglutination reaction; all isolates contained HlyA, EaeA, PO157 and FliC H genes.

**Table 5:** Summary of location, month, premises, product type and *E.coli* O157: H7 count ( $\log_{10}$ cfu g<sup>-1</sup>) for positive samples in Quarter 4 (February to April).

County	Town	Month	Premises type	Product type	Count	Verotoxin genes		Verotoxin production	
						vt1	vt2	vt1	vt2
Wexford	Wexford	January	supermarket	mince fresh (u)	3.43	+	+	-	-
Waterford	Waterford	January	supermarket	mince fresh (map)	4.03	+	+	-	-
Waterford	Waterford	January	supermarket	mince fresh (map)	2.17	+	+	-	-
Kilkenny	Thomastown	January	supermarket	burger frozen (p)	0.51	+	+	-	-
Kilkenny	Thomastown	January	supermarket	mince fresh (u)	1.69	+	+	-	-
Clare	Shannon	February	supermarket	burger frozen (p)	E	+	+	+++	+++
Meath	Ashbourne	February	supermarket	burger frozen (p)	E	+	+	-	-
Roscommon	Bellaghadereen	February	supermarket	mince fresh (u)	E	+	+	-	-
Donegal	Letterkenny	March	supermarket	burger fresh (map)	E	+	+	+++	+++
Donegal	Letterkenny	March	supermarket	mince fresh (map)	E	+	+	+++	+++
Donegal	Letterkenny	March	supermarket	burger fresh (map)	E	+	+	+++	+++
Donegal	Bundoran	March	butcher	burger fresh (u)	E	+	+	+++	+++
Tipperary	Thurles	March	supermarket	mince fresh (u)	E	+	+	-	-
Tipperary	Thurles	March	supermarket	burger fresh (map)	E	+	+	+++	++
Cork	Cork	March	supermarket	burger frozen (p)	E	+	+	+++	++
Cavan	Baillieborough	April	butcher	mince fresh (u)	E	+	+	+++	++
Offaly	Birr	April	butcher	mince fresh (u)	E	+	+	+++	++

p = packaged; u = unpackaged; map =modified atmosphere packaging; E= detectable only by enrichment; +++ = strong agglutination reaction; ++ = very strong agglutination reaction; all isolates contained HylA, EaeA, PO157and fliC<sub>H</sub> genes.





types of products in which *E. coli* O157:H7 was detected together with the towns and premises type from which these products were purchased in each quarter of the year. The numbers ranged from  $\log_{10}0.51$  to  $4.03 \text{ cfu g}^{-1}$ . In 22 of the 43 products that tested positive for *E. coli* O157:H7, the number of bacteria was below  $\log_{10}0.51 \text{ cfu g}^{-1}$  and required an enrichment procedure for their detection.

#### *Quarterly differences*

There was a seasonal effect in the study with higher numbers of positive samples in quarter 4 (4.78%) (January to April 2002). Quarters 1, 2 and 3 yielded 3.22%, 2.35% and 1.03% samples positive respectively.

#### *Establishment*

32 of the positive products were purchased from supermarkets and 11 from butcher shops.

#### *Product type*

The product type yielding the highest incidence of *E. coli* O157:H7 was fresh packaged mince with 13 of 457 samples positive (2.8%). The product with the lowest occurrence of *E. coli* O157:H7 was fresh unpackaged burgers purchased from butcher shops with 3 of 140 samples positive (2.1%). *E. coli* O157:H7 was not found in fresh unpackaged burgers from supermarkets.

#### *Virulence factors*

All 43 positive beef products contained virulence factors. Forty one of the products contained the genes encoded for verotoxins (vt1 and vt2) and the attachment and effacement (*eae*) genes. Two of the strains contained the *eae* gene and only one of the verotoxin producing genes. In quarter 1, one unpackaged fresh mince sample contained only vt2 and in quarter 2, a packaged fresh mince sample contained only vt1.

#### *Relation to enterobacteriaceae*

There was no correlation between high enterobacteriaceae counts and the presence of *E. coli* O157:H7 with *E. coli* O157:H7 being recovered from beef products with both low and high enterobacteriaceae counts.



## Enterobacteriaceae

All products collected were analysed to determine the numbers of Enterobacteriaceae present. The mean Enterobacteriaceae counts on each product type are shown in Table 6.

The Enterobacteriaceae counts on fresh unpackaged mince were significantly higher ( $P < 0.001$ ) than on fresh unpackaged burgers. There was no significant difference between the mean Enterobacteriaceae counts on mince beef from butcher shops or supermarkets. Equally, there was also no significant difference between Enterobacteriaceae counts on beefburgers obtained from butcher or supermarket outlets. Over all four quarters, the product type with the highest Enterobacteriaceae count was unpackaged fresh mince ( $\log_{10}4.64$  cfu g<sup>-1</sup>); frozen burgers had the lowest counts ( $\log_{10}2.42$  cfu g<sup>-1</sup>)

**Table 6:** Mean Enterobacteriaceae counts on beef products collected in the surveillance study.

Product type	Retail outlet	Number of products tested	Mean count ( $\log_{10}$ cfu g <sup>-1</sup> )
Fresh unpackaged mince	Butcher	211	4.42
Fresh unpackaged mince	Supermarket	299	4.64
Fresh packaged mince	Supermarket	457	3.62
Fresh unpackaged burgers	Butcher	140	3.40
Fresh unpackaged burgers	Supermarket	63	3.36
Fresh packaged burgers	Supermarket	157	2.95
Frozen branded burgers	Supermarket	206	2.42



## CONCLUSIONS

*E. coli* O157:H7 has an estimated infectious dose of 10 organisms. Therefore, a combination of relatively high prevalence (2.8%) and occasional high contamination levels presents a potential risk to public health. It is likely that this risk is controlled only by the cooking process to which the meat is subjected in consumers' homes. In the case of beef products with gross contamination, the risk of cross-contamination in the home is increased.

However, analysis of human infection data from the National Disease Surveillance Centre showed no correlation between the incidence of *E. coli* O157:H7 positive samples of minced beef and beefburgers and reported incidences of *E. coli* O157:H7 infection in the same geographical area over the same time period. Therefore we must assume that either cases of foodborne disease are unreported, which is unlikely, or that the final cooking and handling of the products by the consumer is preventing live micro-organisms from being ingested. This puts an unacceptable burden on the final control point in the food chain and clearly further action is required within farms and abattoirs to reduce the prevalence and numbers of *E. coli* O157:H7 in beef.

The survey found no correlation between this organism and Enterobacteriaceae counts. *E. coli* O157:H7 was recovered from products with both low and high Enterobacteriaceae counts. These findings are in line with other studies that show that Enterobacteriaceae may not be used as indicator organisms for the presence of *E. coli* O157:H7. Interestingly, there was no significant difference between the Enterobacteriaceae counts on products from butcher shops and supermarkets which indicates that hygienic practices, while different, are equally effective within their respective retail setting. This had no bearing upon the prevalence of *E. coli* O157:H7 in beef products from the two types of establishment.



## RECOMMENDATIONS TO INDUSTRY

The fact that *E. coli* O157:H7 prevalence in minced beef and beef burgers was independent of retail outlet, packaging format and mincing environment (factory *vs* shop) suggests that the prevalence is unaffected by the retail distribution chain and may be solely a function of the contamination of meat used in the manufacture of minced beef. This in turn suggests that apart from maintaining temperature control and hygienic handling practices, there is little more that retailers can do to reduce the prevalence of *E. coli* O157:H7. This puts the onus for control on the manufacturer and the slaughter facility.

Unfortunately, the prevalence rates are so low that it is unlikely that an economically viable end-product testing regime could control *E. coli* O157:H7 in minced beef and beef burgers. There is no evidence from this study that practices in the various retail environments are increasing the presence of *E. coli* O157:H7 as the figures are similar for beef products taken in butcher shops and supermarkets whether sold loose or pre-packed. Therefore, the emphasis has to be on controlling the organism on the farm and in the slaughter plant and cutting hall. This can be done to a certain extent by a combination of Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) systems along with an increased awareness of the risks by food handlers. It is only when everything that can be done is being done that it is reasonable to expect consumers to eliminate any residual risk by cooking in the domestic environment.





## PUBLICATIONS FROM THIS PROJECT

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**Cagney, C., Crowley, H., Duffy, G., Sheridan, J.J., O' Brien, S., Carney, E., Anderson, A., McDowell, D.A. and Blair, I.S.** 2003. Prevalence and numbers of *Escherichia coli* O157: H7 in minced beef and beefburgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology (submitted)*

**Cagney, C., Duffy, G., Crowley, H., Sheridan, J.J., O' Brien, S., Carney, E., Anderson, A., McDowell, D.A. and Blair, I.S.** 2003. Enterobacteriaceae in minced beef and beefburgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology (for submission)*

## USEFUL REFERENCE

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**Bolton, D.J., Sheridan, J.J. and Doherty A.M.** 2000. HACCP for Irish beef slaughter. The National Food Centre, Dublin 15. ISBN 1841 701211, 1-58.

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