Clinical Infectious Diseases Advance Access published March 12, 2012

MAJOR ARTICLE

Changes in Causes of Acute Gastroenteritis in the United Kingdom Over 15 Years: Microbiologic Findings From 2 Prospective, Population-based Studies of Infectious Intestinal Disease

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Background. Large-scale, prospective studies of infectious intestinal disease (IID) in developed countries are uncommon. Two studies of IID incidence and etiology have been conducted in the United Kingdom: the Infectious Intestinal Disease Study in England (IID1) in 1993–1996 and the Second Study of Infectious Intestinal Disease in the Community (IID2) in 2008–2009. We examined changes in etiology and diagnostic yield of IID cases over 15 years.

Methods. Fecal samples submitted by IID cases were examined for a range of bacterial, viral, and protozoal pathogens using traditional and molecular microbiological methods. We calculated the percentage of specimens positive for each organism based on traditional methods and on traditional and molecular methods combined. We compared the distributions of organisms in the 2 studies.

Results. For pathogens investigated in both studies, 40% of fecal samples submitted by cases in IID2 were positive compared with 28% in IID1. Viruses were most frequent among community cases in IID2. *Campylobacter* was the most common bacterial pathogen among cases presenting to healthcare. Major differences between the 2 studies were increases in the detection of norovirus and sapovirus and a decline *Salmonella*.

Conclusions. Most fecal specimens were negative for the pathogens tested in both studies, so new strategies are needed to close the diagnostic gap. Among known pathogens, effective control of norovirus, rotavirus, and *Campylobacter* remain high priorities. The reduction in nontyphoidal salmonellosis demonstrates the success of Europe-wide control strategies, notably an industry-led *Salmonella* control program in poultry in the United Kingdom.

In developed countries, infectious intestinal disease (IID) is often perceived as a minor illness causing substantial morbidity but low mortality. Yet disruption to society and the economy is substantial and is estimated to be €345 million in The Netherlands [1], A$343 million in Australia [2], and Can $3.7 billion in Canada [3]. Food-borne illness costs the UK economy approximately £1.5 billion annually [4]. In New Zealand and the United States, the costs are NZ $216 million [5] and $152 billion [6], respectively, which are enormous sums for preventable diseases.

Few prospective studies of IID in developed countries have included a broad range of pathogens in

Received 4 August 2011; accepted 9 December 2011.

[a]The IID2 Study Executive Committee has been included in the Acknowledgments section.

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DOI: 10.1093/cid/cis028

unselected cases in the community or presenting to healthcare [7, 8, 9, 10, 11, 12]. In the United Kingdom, 2 prospective, population-based studies of IID incidence and etiology in the community have been conducted, the Infectious Intestinal Disease Study in England (IID1) in 1993–1996 [7] and the Second Study of Infectious Intestinal Disease in the Community (IID2) in 2008–2009 [13, 14]. Both used identical case definitions and similar methodologies. They afford a unique opportunity to compare the etiology of IID in community and cases presenting to general practice (GP) 15 years apart.

METHODS

The methodologies of the IID1 and IID2 studies are detailed elsewhere [13, 15]. The main features are summarized in Table 1. Both studies included a community cohort and a healthcare presentation study; the distribution of organisms among community and GP cases was expected to differ because symptom severity influences healthcare seeking [16, 17]. In the cohort studies, we recruited individuals of all ages from the population who were registered with participating GPs. Participants reported weekly whether they had experienced diarrhea and/or vomiting in the previous week. We asked symptomatic individuals to complete a questionnaire and provide a stool specimen, which they mailed directly to the laboratory for microbiologic analysis. In the GP presentation studies, we invited all IID cases who consulted with their GP to complete a symptom questionnaire and provide a stool specimen for microbiologic testing.

Case Definitions

In both studies, IID cases were people with loose stools or clinically significant vomiting lasting <2 weeks in the absence of a known noninfectious cause and preceded by a symptom-free period of 3 weeks. Vomiting was clinically significant if

Table 1. Summary of the Main Features of the Infectious Intestinal Disease Study in England and the Second Study of Infectious Intestinal Disease in the Community

	IID1 [7, 8, 15]	IID2 [13, 14]
Geographical location	England	United Kingdom
Study duration	August 1993–January 1996	April 2008–August 2009
Sampling frame	MRC GPRF	GPRF plus Primary Care Research Networks in England, Wales and Scotland
Prospective cohort study		
Number of practices recruited	70	88
Number of cohort participants recruited	9776	7033
Participation rate (%)	35	9
Method of follow-up	Weekly postcard (100%)	Email (63%) or weekly postcard (37%)
Maximum period of follow-up, weeks	26 (2 cohorts)	52 (1 cohort)
Lost to follow-up (%)	59	9.5
Number of cases reporting symptoms	781	1201
Number of cases submitting specimens	761	782
Percentage of specimens from cases aged <5 years (%)	25.1	15.3
Median time (IQR) from illness onset to specimen submission, days	1 (0–2)	1 (0–3)
General practice presentation study		
Number of practices recruited	34	37
Number of cases meeting the case definition	4026	991
Number of cases submitting specimens	2962	874
Percentage of specimens from cases aged <5 years (%)	31.3	22.0
Median time (IQR) from illness onset to specimen submission	4 (2–7)	6 (4–9)

Abbreviations: GPRF, General Practice Research Framework; IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community; IQR, interquartile range; MRC, Medical Research Council.

it occurred more than once in a 24-hour period and if it incapacitated the case or was accompanied by other symptoms such as cramps or fever. Definite cases were those who met the clinical definition, regardless of whether a causative organism was identified. Probable cases were symptomatic individuals for whom there was insufficient information to classify them as definite cases.

A case of *Clostridium difficile*–associated diarrhea was an individual aged ≥2 years with symptoms of diarrhea not attributable to another cause (ie, the absence of other enteropathogens), occurring at the same time as a positive toxin assay.

Exclusions

In both studies, people were excluded for the following reasons because an infectious etiology and onset date for acute symptoms could not be reliably determined: terminal illness; severe mental incapacity; or recognized, noninfectious causes of diarrhea or vomiting, including Crohn's disease, ulcerative colitis, cystic fibrosis, celiac disease, surgical obstruction, excess alcohol, morning sickness and, in infants, regurgitation. Additional exclusions were non-English speakers without a suitable translator and those traveling outside the United Kingdom in the 10 days before onset of illness.

Microbiologic Testing in the Second Study of Infectious Intestinal Disease in the Community

Cases mailed their sample directly to the Health Protection Agency Regional Laboratory (Manchester) [13] in a kit comprising instructions for sample collection, a screw-top plastic universal container with integral plastic spoon, a rigid plastic outer container, a post office–compliant cardboard box, and a strong plastic, postage-paid envelope. We used the following methods:

- culture for *Campylobacter jejuni/coli*, Shiga toxin–producing *Escherichia coli* (STEC) O157, *Listeria monocytogenes*, nontyphoidal *Salmonella* spp., *Shigella* spp. and *Yersinia* spp.;
- enzyme immunoassay (EIA) for *Clostridium perfringens* enterotoxin, *Cryptosporidium parvum*, and *Giardia intestinalis*;
- EIA and polymerase chain reaction (PCR) for *C. difficile* cytotoxins A and B;
- Light microscopy examination of a stained smear for *Cyclospora* and *Cryptosporidium*; and
- EIA for rotavirus and adenovirus 40, 41 (in samples from children <5 years of age).

Any samples that were immunoassay-positive for *C. difficile* toxin or PCR-positive were cultured using National Standard Method BSOP 10 [13]. All *C. difficile* isolates were then ribotyped [18].

All samples were then examined at the Health Protection Agency Centre for Infections (London). We prepared 2 nucleic acid extracts from each sample [19, 20, 21]. We examined each extract using real-time PCR for *C. jejuni*, *C. coli*, *L. monocytogenes*, nontyphoidal *Salmonella* spp., rotavirus, norovirus, sapovirus, adenovirus, astrovirus, *Cryptosporidium* (*C. hominis*, *C. parvum*, *C. meleagridis*, and *C. felis*), *Giardia* spp., and *E. coli* (enteroaggregative and Shiga toxin–producing [genes encoding ST1 and ST2]).

We included DNA (phocine herpesvirus) and RNA (mouse mengovirus) controls in each sample to monitor nucleic acid extraction and reverse transcription. We used positive and negative microbe-specific controls in each assay run to monitor target-specific reagents. Controls were quantitative, enabling use of Westgard rules to determine whether assays were within 3 standard deviations of the expected value and to determine the coefficient of variation [22].

We considered a cycle threshold (CT) value <40 to be a positive result for all organisms except norovirus and rotavirus. A considerable fraction of asymptomatic individuals have low viral loads, yet test positive by reverse-transcription PCR [23] among IID cases with low viral loads; therefore, these organisms are unlikely to be responsible for symptoms [24, 25]. A CT value <30 for both viruses suggests a clinically significant result; that is, disease truly caused by these 2 organisms [24, 25]. For rotavirus, this cutoff point coincides well with positive enzyme-linked immunosorbent assay (ELISA) results [25]. Therefore, a CT value <30 defined clinically significant norovirus and rotavirus infection.

Both laboratories were fully accredited and participated in 2 external quality audits during the IID2 Study.

Data Analysis

For the IID2 cohort and GP presentation components, we computed by study the percentage of specimens positive for each organism among IID cases with a stool sample available for analysis. If a sample was positive for 2 organisms, we counted it as positive in the calculations for both organisms (except for *C. difficile*, as defined above). We computed the percentage of specimens positive for each organism based on routine diagnostic methods and on routine and molecular diagnostic methods combined. We also calculated the percentage of specimens that were negative for all organisms tested. For each percentage, we computed exact binomial 95% confidence intervals.

We compared the frequency of detection of each organism between the IID2 and IID1 studies among cases ascertained in England [8] using only the subset of organisms targeted in both studies (Table 2). Thus, we investigated the additional diagnostic yield achievable using molecular methods. Using PCR methods in IID2 (not widely used in IID1), we expected increased diagnostic yields, particularly for enteric viruses [23, 24, 25]. For organisms tested by PCR in the IID2 study

Table 2. Changes in Microbiological Protocols Between the Infectious Intestinal Disease Study in England and the Second Study of Infectious Intestinal Disease in the Community

Bacteria	Changes From IID1	Reasons for Changes From IID1
Aeromonas spp.	Not tested	No established techniques for identification of small fraction of pathogenic strains; many positives in controls in IID1
Arcobacter spp.	Not tested	Of doubtful pathogenicity and significance
Bacillus spp.	Not tested	Very few cases in IID1; difficult to confirm pathogenicity in individual cases
Campylobacter spp.	2 of 3 selective methods not used in IID2	Very few positives of species other than *C. coli* and *C. jejuni* detected in IID1
Clostridium difficile cytotoxin	Commercial immunoassay to detect toxins A and B replacing in-house cytotoxin test	More practical and backed up by PCR
Clostridium perfringens	Commercial immunoassay to screen for enterotoxin	A more specific and meaningful test than spore counts
Escherichia coli O157	Use CT-SMAC as selective medium	CR-SMAC used in previous study; CT-SMAC now in routine use
Listeria spp.	Include as a new pathogen	*L. monocytogenes* is 1 of the FSA's target organisms
Plesiomonas shigelloides	Not tested	Very low numbers in IID1
Staphylococcus aureus	Not tested	Low numbers in IID1; similar numbers in cases and controls
Vibrio spp.	Not tested except in cases with history of recent foreign travel	Frequency in UK too low for value in this study, so only included for clinical necessity
Yersinia spp.	Change of enrichment protocol	Adopt HPA standard method for enrichment
Protozoa		
Cryptosporidium parvum	Commercial immunoassay and PCR replacing light microscopy	Improve practicality and sensitivity
Giardia intestinalis	Commercial immunoassay and PCR replacing light microscopy	Improve practicality and sensitivity
Viruses		
Adenovirus 40, 41	PCR assays replacing immunoassays and electron microscopy	Improved sensitivity
Astrovirus	PCR assays replacing immunoassays and electron microscopy	Improved sensitivity
Rotavirus A and C	PCR assays replacing immunoassays and electron microscopy	Improved sensitivity
Norovirus	PCR assays replacing immunoassays and electron microscopy	Improved sensitivity
Sapovirus	PCR assays replacing immunoassays and electron microscopy	Improved sensitivity

Abbreviations: CR-SMAC, cefixime rhamnose sorbitol MacConkey agar; CT-SMAC, cefixime tellurite sorbitol MacConkey agar; FSA, Food Standards Agency; HPA, Health Protection Agency; IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community; PCR, polymerase chain reaction.

(*Campylobacter*, *Salmonella*, *Cryptosporidium*, *Giardia*, adenovirus, astrovirus, norovirus, rotavirus, and sapovirus), we compared the percentage positivity using conventional methods in IID1 with that using both conventional and PCR methods in IID2 to establish the added benefit of using molecular diagnostic methods. To account for the differing distribution of pathogens in children and adults, we compared the etiology of IID cases between the 2 studies in children aged <5 years and in those aged ≥5 years.

We conducted all analyses in Stata 11.0 (StataCorp) and Excel 2007 software.

Ethics

We obtained a favorable ethical opinion from the National Health Service North West Research Ethics Committee (07/MRE08/5).

RESULTS

Results of the IID2 Study

Among community cases, 75% of stool samples were submitted within 3 days of illness onset, compared with 9 days among GP cases. In a logistic regression analysis (not shown), only specimens submitted ≥10 days after onset were more

Table 3. Microbiological Findings in Cohort Cases in the Second Study of Infectious Intestinal Disease in the Community

Pathogen	Test	No. Identified	Tested	% Identified	95% CI
Bacteria					
Clostridium difficile[a]	All	1	715	0.1	(0–0.8)
	EIA	0	715	0.0	(0–0.5)
	PCR	1	693	0.1	(0–0.8)
Clostridium perfringens	Toxin	6	772	0.8	(0.3–1.7)
Campylobacter	All	36	782	4.6	(3.2–6.3)
	All culture	28	767	3.7	(2.4–5.2)
	Direct culture	18	766	2.3	(1.4–3.7)
	Enrichment	27	766	3.5	(2.3–5.1)
	PCR	31	782	4.0	(2.7–5.6)
Escherichia coli O157 STEC	Culture	1	768	0.1	(0–0.7)
E. coli non–O157 STEC	PCR	6	781	0.8	(0.3–1.7)
Enteroaggregative *E. coli*	PCR	15	782	1.9	(1.1–3.1)
Listeria	Culture and/or PCR	0	769	0.0	(0–0.5)
Salmonella	All	2	782	0.3	(0–0.9)
	Culture	2	768	0.3	(0–0.9)
	PCR	1	782	0.1	(0–0.7)
Shigella	Culture	0	768	0.0	(0–0.5)
Yersinia	All culture	0	769	0.0	(0–0.5)
	Direct culture	0	769	0.0	(0–0.5)
	Enrichment	0	769	0.0	(0–0.5)
Protozoa					
Cryptosporidium	All	3	782	0.4	(0.1–1.1)
	EIA	2	768	0.3	(0–.9)
	PCR	3	782	0.4	(0.1–1.1)
Cyclospora	Microscopy	0	768	0.0	(0–0.5)
Giardia	All	6	782	0.8	(0.3–1.7)
	EIA	3	768	0.4	(0.1–1.1)
	PCR	6	782	0.8	(0.3–1.7)
Viruses					
Adenovirus	ELISA and/or PCR[b]	28	782	3.6	(2.4–5.1)
Astrovirus	PCR	14	782	1.8	(1–3)
Norovirus	PCR	129	782	16.5	(14–19.3)
Rotavirus	ELISA and/or PCR[b]	32	782	4.1	(2.8–5.7)
Sapovirus	PCR	72	782	9.2	(7.3–11.5)
No pathogen identified		471	782	60.2	(56.7–63.7)

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; STEC, Shiga toxin-producing *Escherichia coli*.

[a] Only specimens from cases aged ≥2 years were tested for *Clostridium difficile*.

[b] ELISA for adenovirus and rotavirus was conducted on specimens from cases aged <5 years.

likely to test negative for all pathogens tested after adjusting for other factors.

Community Cases

Viruses were the most common organisms among community cases; norovirus, sapovirus, and rotavirus infection were identified in 16.5%, 9.2%, and 4.1% of specimens, respectively (Table 3). *Campylobacter* was the most common bacterial agent; 4.6% of specimens tested positive for *Campylobacter* by culture or PCR (3.7% by culture alone). Enteroaggregative *E. coli* was found by PCR in 1.9% of specimens. Other bacterial pathogens were identified with a frequency of <1%. No *C. difficile*–positive specimens were identified using immunoassay methods. Overall, 60.2% of specimens were negative for all pathogens tested.

Cases Presenting to the General Practitioner

Campylobacter (13.0%) and norovirus (12.4%) were the most common agents among GP cases (Table 4). By contrast, *Salmonella* was less common than *C. perfringens*, enteroaggregative *E. coli*, *Cryptosporidium* spp., or *Giardia intestinalis*. Only

Table 4. Microbiological Findings in General Practice Presentation Cases in the Second Study of Infectious Intestinal Disease in the Community

Pathogen	Test	No. Identified	Tested	% Identified	95% CI
Bacteria					
Clostridium difficile[a]	All	10	738	1.4	(0.7–2.5)
	EIA	1	736	0.1	(0–0.8)
	PCR	9	719	1.3	(0.6–2.4)
Clostridium perfringens	Toxin	19	868	2.2	(1.3–3.4)
Campylobacter	All	114	874	13.0	(10.9–15.5)
	All culture	69	866	8.0	(6.3–10)
	Direct culture	48	866	5.5	(4.1–7.3)
	Enrichment	65	863	7.5	(5.9–9.5)
	PCR	105	874	12.0	(9.9–14.4)
E. coli O157 STEC	Culture	1	866	0.1	(0–0.6)
E. coli non-O157 STEC	PCR	7	866	0.8	(0.3–1.6)
Enteroaggregative *Escherichia coli*	PCR	12	874	1.4	(0.7–2.4)
Listeria	Culture and/or PCR	0	865	0.0	(0–0.4)
Salmonella	All	7	874	0.8	(0.3–1.6)
	Culture	7	866	0.8	(0.3–1.7)
	PCR	6	874	0.7	(0.3–1.5)
Shigella	Culture	0	866	0.0	(0–0.4)
Yersinia	All	1	866	0.1	(0–0.6)
	Direct culture	0	865	0.0	(0–0.4)
	Enrichment	1	866	0.1	(0–0.6)
Protozoa					
Cryptosporidium	All	12	874	1.4	(0.7–2.4)
	EIA	9	863	1.0	(0.5–2)
	PCR	12	874	1.4	(0.7–2.4)
Cyclospora	Microscopy	0	861	0.0	(0–0.4)
Giardia	All	9	874	1.0	(0.5–1.9)
	EIA	6	863	0.7	(0.3–1.5)
	PCR	9	874	1.0	(0.5–1.9)
Viruses					
Adenovirus	ELISA and/or PCR[b]	30	874	3.4	(2.3–4.9)
Astrovirus	PCR	22	874	2.5	(1.6–3.8)
Norovirus	PCR	108	874	12.4	(10.2–14.7)
Rotavirus	ELISA and/or PCR[b]	64	874	7.3	(5.7–9.3)
Sapovirus	PCR	77	874	8.8	(7–10.9)
No pathogen identified		425	874	48.6	(45.3–52)

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; STEC, Shiga toxin-producing *Escherichia coli*

[a] Only specimens from cases aged 2 years and above were tested for *C. difficile*.

[b] ELISA for adenovirus and rotavirus was conducted in specimens from cases aged <5 years.

0.1% had *C. difficile* toxin detected in the stool (by EIA). Viruses were common among cases presenting to the GP. No pathogen was identified in 48.6% of specimens.

Etiology of IID Cases in the Community and Presenting to the General Practitioner in the IID2 Study

Campylobacter and rotavirus were more common among GP cases than community cases (Figure 1). Norovirus was more common among community cases, but was found in >12% of GP cases. Smaller differences were seen for other organisms, although the number of positive specimens was low.

An additional 4.2% of community cases and 2.5% of GP cases had norovirus CT values between 30 and 40, indicating low-level subclinical viral shedding. For rotavirus the percentages were 5.6% and 5.2%, respectively.

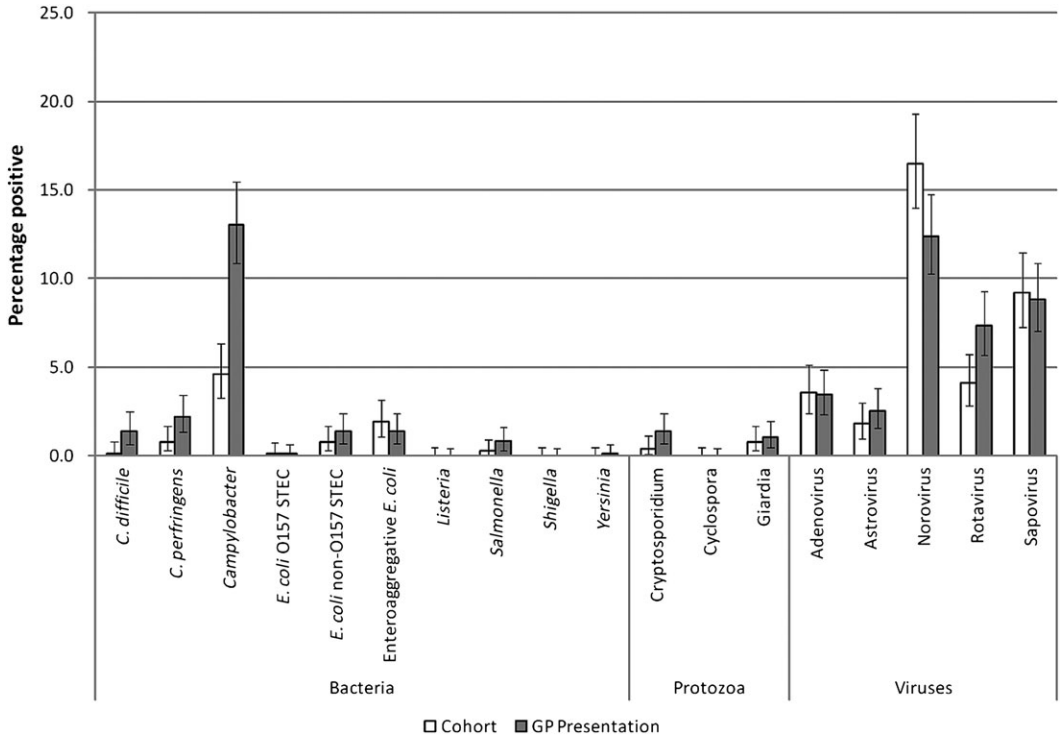

Figure 1. Microbiological findings in cohort and general practice presentation cases in the second Study of Infectious Intestinal Disease in the Community. Abbreviations: GP, general practice; STEC, Shiga toxin-producing *Escherichia coli*.

Mixed Infections

Infections with 2 or more organisms were identified in 37 (4.7%) community cases. These mixed infections primarily involved adenovirus, norovirus, or sapovirus. Among GP cases, 40 (4.6%) had mixed infections, the majority involving adenovirus, norovirus, sapovirus, or *Campylobacter*.

Comparison of Organism Distributions in the IID1 and IID2 Studies in England

Norovirus and sapovirus were identified more frequently in IID2 than in IID1, both among community cases (Figure 2) and GP cases (Figure 3). Certain pathogens were less common among GP cases presenting to the GP in IID2, most notably enteroaggregative *E. coli* and *Salmonella*, but there were few positive specimens for these organisms. There was also a notable decrease in detection of *Y. enterocolitica* in IID2 relative to IID1.

Figure 4 compares the detection of different organisms among children <5 years (panel A) and those aged ≥5 years (panel B) in community (blue circles) and GP cases (orange circles) between IID1 and IID2. Organisms on the diagonal were identified with similar frequency in both studies. Among children <5 years, the major differences between the 2 studies were the greater detection of viral agents in IID2, particularly norovirus and sapovirus, and the greater detection of bacterial agents among community cases in IID1. In addition, *E. coli* non–O157 STEC was identified more frequently among community cases in IID2. Similarly, among cases aged ≥5 years, norovirus, sapovirus, adenovirus, and *E. coli* non–O157 STEC were identified more frequently in IID2, both among community and GP cases. However, *Salmonella*, *C. perfringens*, *E. coli* O157 STEC, and enteroaggregative *E. coli* occurred more frequently among both community and GP cases in IID1. *Clostridium difficile* and astrovirus were also identified more frequently among community cases in IID1.

In comparing the same set of organisms between the 2 studies, 40% of specimens from community cases had at least 1 organism detected in IID2 compared with 28% in IID1. For cases aged <5 years, the corresponding percentages were 60% and 48%, respectively. The difference between the 2 studies is primarily due to greater virus detection among community cases in IID2 (Figure 5). Among GP cases, the differences were less marked because the relative increase in detection of viruses in IID2 was offset by the greater frequency of bacterial agents identified in IID1.

DISCUSSION

In the IID2 study, norovirus was the most common viral agent among community IID cases, and *Campylobacter* spp. was the most common bacterial cause. A major difference between the

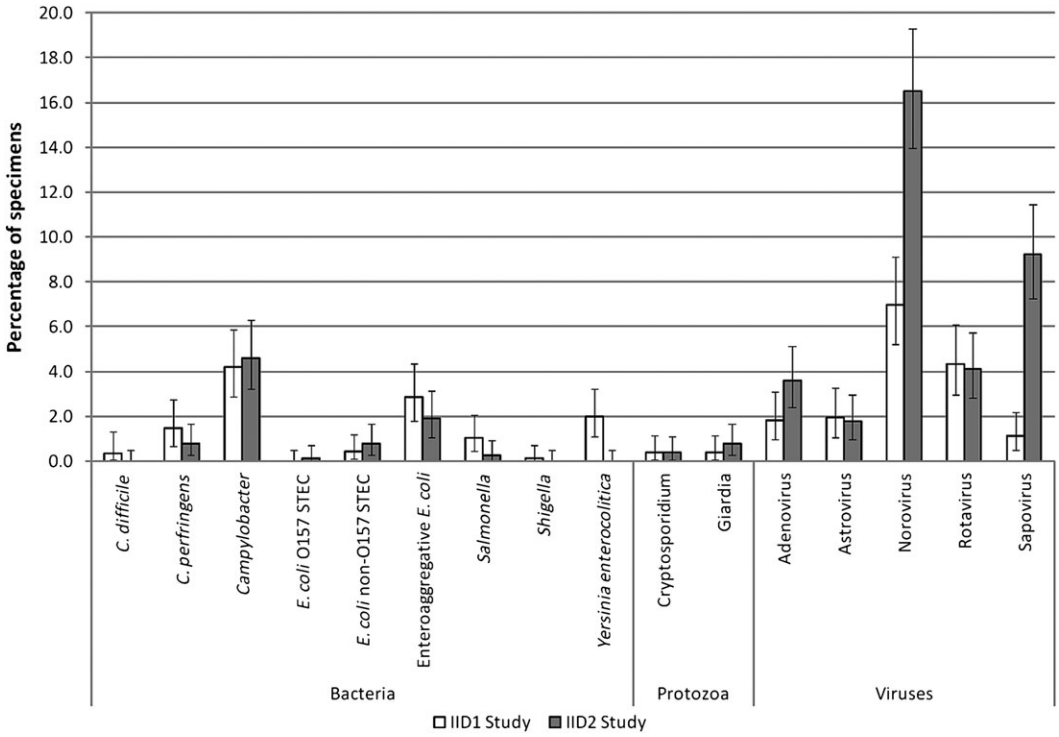

Figure 2. Microbiological findings among community cases of infectious intestinal disease in the Infectious Intestinal Disease Study in England and the second Study of Infectious Intestinal Disease in the Community. Abbreviations: IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community; STEC, Shiga toxin-producing *Escherichia coli*.

2 studies is the greater detection of viral agents, particularly among cases for patients aged ≥5 years. In the IID2 study, there is some evidence that adenovirus viral loads are higher among children aged <5 years (data not shown); use of PCR enabled detection among older individuals with intermediate viral loads that would likely have been missed in IID1. For sapovirus, there is little evidence in our data for age-specific differences in viral load or age-related differences in timing of specimen submission. Increased detection of sapovirus in older individuals is thus likely to reflect both greater sensitivity of PCR and a change in the epidemiology of this organism; the high frequency of sapovirus coincided with the introduction of a new genotype in the United Kingdom [26].

In general, bacterial agents were found less frequently in IID2 than IID1. There might have been general decreases in the incidence of certain pathogens or increases in other pathogens (such as sapovirus) that have driven down frequency of detection of other agents.

Norovirus, sapovirus, and *Campylobacter* were common among GP cases. The higher detection rate for norovirus and sapovirus means that, even if small percentages result in GP consultations, the absolute number of consultations is higher than for other pathogens because both viruses are very common in the community. *Campylobacter* might result in more severe illness, which also influences the likelihood of GP consultation [16, 17], and could explain why detection was more common among cases aged ≥5 years. Nevertheless, norovirus and sapovirus were also common among GP cases in this age group. Among community and GP cases, *C. perfringens*, *Salmonella* spp., *L. monocytogenes*, *E. coli* O157 STEC, and *C. difficile* were uncommon. The drop in salmonellosis between IID1 and IID2 further reflects the success of the UK industry–led *Salmonella* control program in broiler-breeder and laying poultry flocks [27]. The fall in *Yersinia* cases might reflect improved slaughterhouse hygiene and/or decreased pork consumption following the foot-and-mouth disease outbreaks in 2001 and 2007 [28].

Strengths and Limitations

Participation in the IID2 cohort was low (but comparable to major concurrent UK cohort studies) and less than IID1 [14]. However, the low participation in IID2 was offset by good compliance with weekly follow-up. Drop-outs among participants were far fewer than in IID1.

The inclusion of molecular methods in IID2 enabled investigation of low-volume samples and increased the diagnostic yield among community cases by around 10% compared with IID1. The PCR methods particularly increased the

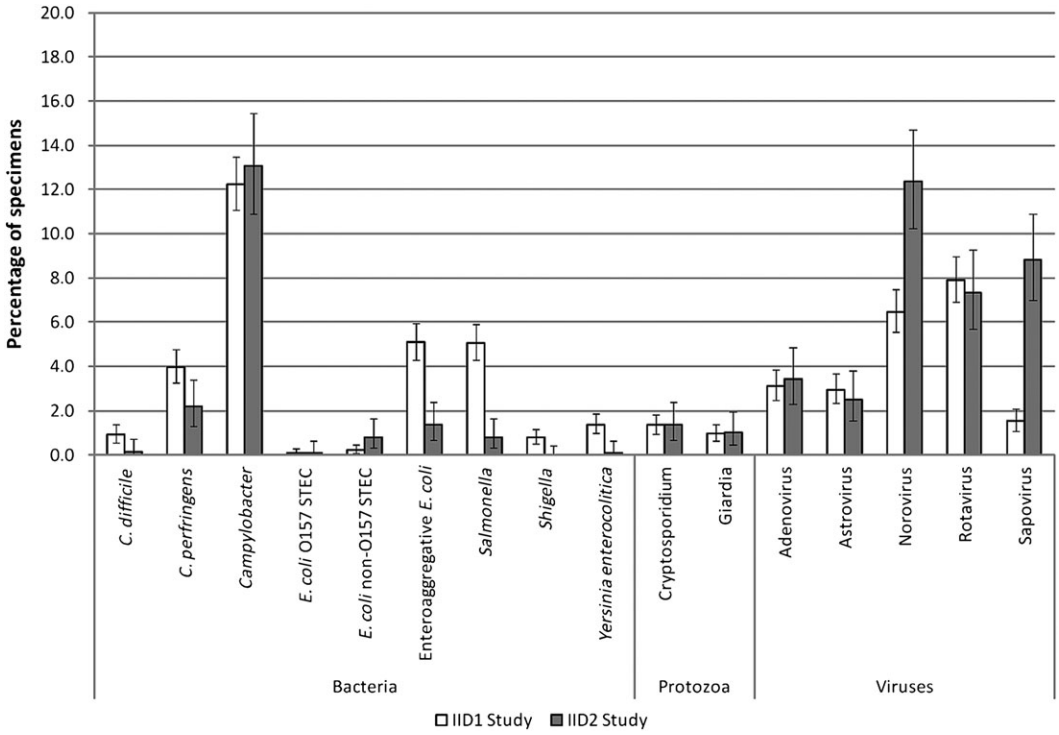

Figure 3. Microbiological findings among infectious intestinal disease cases presenting to general practice in the Infectious Intestinal Disease Study in England and the second Study of Infectious Intestinal Disease in the Community. Abbreviations: IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community; STEC, Shiga toxin-producing *Escherichia coli*.

sensitivity of virus detection. Similar increases in virus detection were seen among GP cases, but the overall benefit was offset by the greater detection of bacterial agents in IID1.

Specimens from asymptomatic individuals were not available in IID2. Identifying an appropriate cutoff value to define a positive PCR result is difficult without information on CT value distributions among controls. We have shown previously that a CT value of <30 is a good indicator of IID genuinely caused by norovirus and rotavirus [23, 24] and therefore these definitions were used for clinically significant norovirus and rotavirus infection here. Although viral loads might be affected by late specimen collection, we found no differences in CT values between specimens collected within and after 3 days of illness onset (data available but not shown) and made no adjustments for timing of specimen collection. In the absence of similar data on CT value cutoffs for other organisms, we used a more sensitive cutoff value of <40 for other pathogens. We found good agreement between PCR and culture results for both *Campylobacter* and *Salmonella*, but might have overestimated incidence of other pathogens if disease in IID cases with high CT values (low pathogen loads) was not actually due to infection with those organisms.

Mixed infections were identified in less than 5% of specimens. Without controls, it is difficult to determine whether coinfection is coincidental, whether it is due to shared routes of infection, or whether certain pairs of pathogens are more often associated with disease (as opposed to asymptomatic infection). The frequency of mixed infections in our study might be lower than in other studies because we considered as negative those norovirus and rotavirus specimens with low viral loads that are unlikely to be responsible for clinical disease.

We detected only *L. monocytogenes* and *C. difficile* that was diarrhea associated and so underestimated the clinical impact of these infections. Furthermore, we did not collect data on hospital stays or antibiotic usage that might aid interpretation of *C. difficile* results.

Negative Stool Specimens

Using identical case definitions and a comparable set of organisms, an etiologic agent was detected in 40% of community cases in IID2 compared with <30% in IID1. Among GP cases, the diagnostic yield was around 50% in both studies. A number of possible reasons exist for the high percentage of cases with unknown etiology. First, we did not define the term "diarrhea" to participants; it is possible that individuals reported transient changes in bowel habit not caused by IID.

Alternatively, these cases could be due to organisms not included in our diagnostic algorithms, such as enteropathogenic

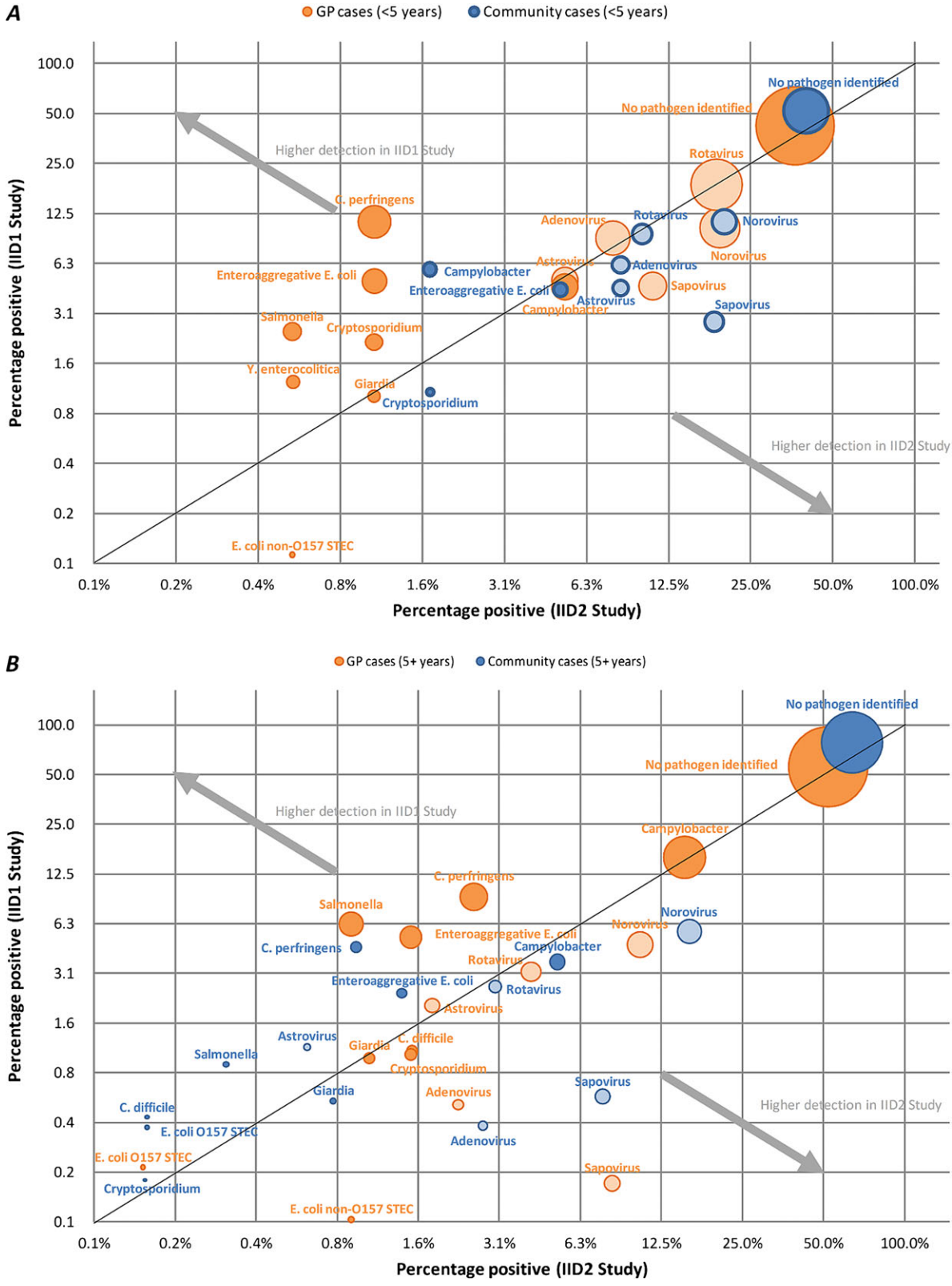

Figure 4. *A*, Percentage of specimens testing positive for different organisms among community and general practice cases for patients aged <5 years in the Infectious Intestinal Disease Study in England (IID1) and the second Study of Infectious Intestinal Disease in the Community (IID2). *B*, Percentage of specimens positive for different organisms among community and general practice cases for patients aged >5 years in the IID1 and IID2 studies, England. Abbreviations: GP, general practice; IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community; STEC, Shiga toxin-producing *Escherichia coli*.

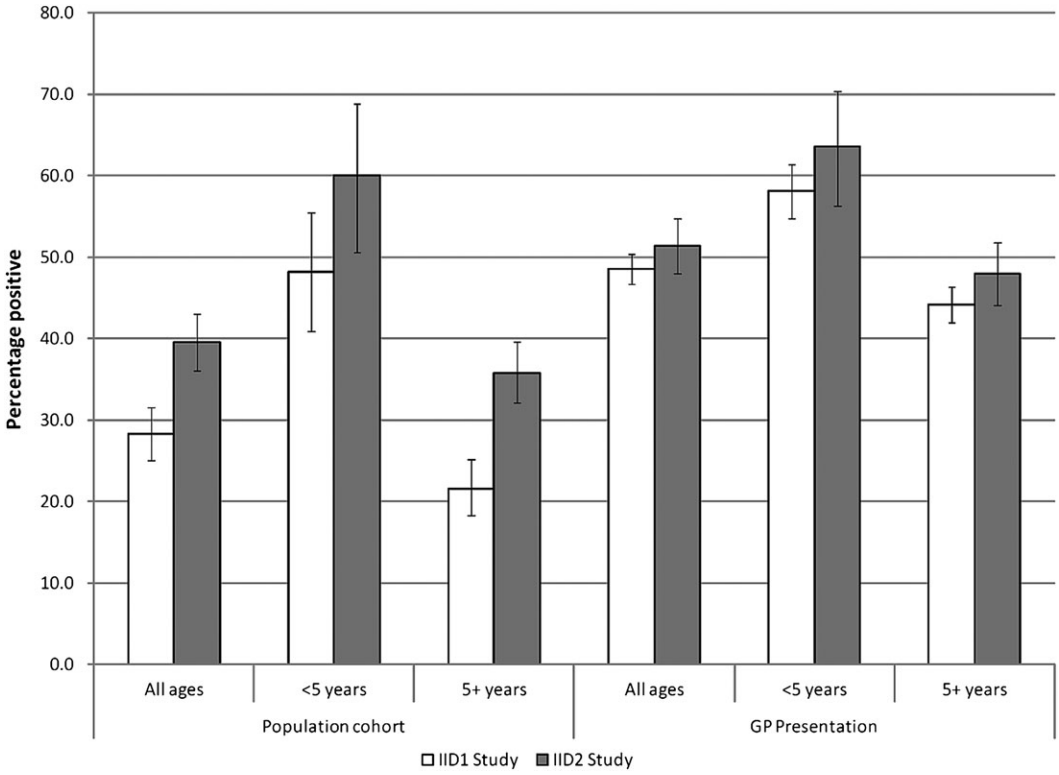

Figure 5. Detection of bacteria, viruses, and protozoa among infectious intestinal disease cases for patients <5 years and ≥5 years of age in Infectious Intestinal Disease Study in England and the second Study of Infectious Intestinal Disease in the Community, England. Abbreviations: GP, general practice; IID1, Infectious Intestinal Disease Study in England; IID2, second Study of Infectious Intestinal Disease in the Community.

or enterotoxigenic *E. coli*. Three "new" bacterial etiologic agents of diarrhea have been described recently [29]: *Klebsiella oxytoca*, which appears to cause disease following perturbation of the gut flora by antibiotic treatment, like *C. difficile* [30]; enterotoxigenic *Bacteroides fragilis* in children [31]; and *Laribacter hongkongensis* [32]. Similarly, several viruses have been proposed as causes of IID (particularly in children), including coronaviruses, picobirnaviruses, pestiviruses, and toroviruses [33]. Recently, interest has been focused on the parvoviruses. At least 3 human bocaviruses (HBoV) have been associated with IID, and 1 Australian study identified human bocavirus 2 in 17.2% of children with IID compared with 8.1% of controls [34].

CONCLUSIONS

The major change in pathogen distribution between IID1 and IID2 was a drop in *Salmonella* cases, indicating the success of European-wide interventions and, notably, an industry-led *Salmonella* control program in chickens in the United Kingdom. However, the majority of stool specimens submitted in both studies by cases were negative for pathogens included in our diagnostic panel. New-generation sequencing techniques [35, 36], though not yet adapted for widespread use [37], afford immense opportunities to identify novel pathogens to close the diagnostic gap. Among the known pathogens, effective control of norovirus, rotavirus, and *Campylobacter* infections remains a high priority in the meantime.

Notes

Acknowledgments. We wish to thank all the participants, study nurses, general practitioners, practice staff, laboratory, research, and administrative staff who took part in the IID2 Study. We are grateful to the Medical Research Council General Practice Research Framework, the Primary Care Research Networks in England and Northern Ireland, and the Scottish Primary Care Research Network for assistance with the recruitment of General Practices.

Author contributions. S. J. O. B., C. C. T., L. C. R., F. J. B., J. Mc. L., D. S. T., G. R., and J. J. G. conceived and designed the IID2 study, and S. J. O. B. led it. G. R. and J. D. led the studies in primary care. F. J. B., J. J. G., M. I.-G., B. W., and J. W. led the microbiological analyses in the IID2 study, which were conducted by L. B., D. C., F. H., K. M., and A. R. D. S. T. led the microbiological analysis of the IID1 study. C. C. T. and L. C. R. undertook the statistical analyses. C. C. T., D. S. T., and S. J. O. B. drafted the manuscript. All authors have read and approved the final manuscript. All authors had full access to the whole dataset (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. S. J. O. B. is the guarantor of the study.

Members of the IID2 Study Executive Committee are Bob Adak, Eric Bolton, Paul Cook (chair), John Cowden, Meirion Evans, Jim Gray, Paul Hunter, Louise Letley, Jim McLauchlin, Keith Neal, Sarah O'Brien, Greta Rait, Laura Rodrigues, Gillian Smith, Brian Smyth, and David Tompkins.

Financial support. This work was supported by the United Kingdom Food Standards Agency and the Department of Health [grant number B18021]; the Department of Health; the Scottish Primary Care Research Network; National Health Service (NHS) Greater Glasgow and Clyde; NHS Grampian; NHS Tayside; the Welsh Assembly Government (Wales Office of Research and Development) and, in Northern Ireland, the Health and Social Care (HSC) Public Health Agency (HSC Research and Development). The IID1 study was supported by the Medical Research Council and the Department of Health in England.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. van den Brandhof WE, De Wit GA, de Wit MA, van Duynhoven YT. Costs of gastroenteritis in The Netherlands. Epidemiol Infect **2004**; 132:211–21.
2. Hellard ME, Sinclair MI, Harris AH, Kirk M, Fairley CK. Cost of community gastroenteritis. J Gastroenterol Hepatol **2003**; 18:322–8.
3. Thomas MK, Majowicz SE, Pollari F, Sockett PN. Burden of acute gastrointestinal illness in Canada, 1999–2007: interim summary of NSAGI activities. Can Commun Dis Rep **2008**; 34:8–15.
4. Food Standards Agency. Annual report of the chief scientist 2009/10. Available at <http://www.food.gov.uk/multimedia/pdfs/csr0910a.pdf>. Accessed 27 July 2011.
5. Scott WG, Scott HM, Lake RJ, Baker MG. Economic cost to New Zealand of foodborne infectious disease. N Z Med J **2000**; 113:281–4.
6. The Lancet. The price of foodborne illness in the USA. Lancet **2010**; 375:866.
7. Wheeler JG, Sethi D, Cowden JM, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ **1999**; 318:1046–50.
8. Tompkins DS, Hudson MJ, Smith HR, et al. A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health **1999**; 2:108–13.
9. de Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Vinjé J, van Duynhoven YT. Etiology of gastroenteritis in sentinel general practices in the Netherlands. Clin Infect Dis **2001**; 33:280–8.
10. de Wit MA, Koopmans MP, Kortbeek LM, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol **2001**; 154:666–74.
11. Nataro JP, Mai V, Johnson J, et al. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis **2006**; 43:402–7.
12. Monto AS, Koopman JS, Longini IM, Isaacson RE. The Tecumseh study. XII. Enteric agents in the community, 1976–1981. J Infect Dis **1983**; 148:284–91.
13. O'Brien SJ, Rait G, Hunter PR, et al. Methods for determining disease burden and calibrating national surveillance data in the United Kingdom: the second study of infectious intestinal disease in the community (IID2 study). BMC Med Res Methodol **2010**; 10:39.
14. Tam CC, Rodrigues LC, Viviani L, et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut **2011**; 61:69–77.
15. Sethi D, Wheeler JG, Cowden JM, et al. A study of infectious intestinal disease in England: plan and methods of data collection. Commun Dis Public Health **1999**; 2:101–7.
16. Tam CC, Rodrigues LC, O'Brien SJ. The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int J Epidemiol **2003**; 32:99–105.
17. Scallan E, Jones TF, Cronquist A, et al. Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. Foodborne Pathog Dis **2006**; 3:432–8.
18. Brazier JS, Raybould R, Patel B, et al. Distribution and antimicrobial susceptibility patterns of *Clostridium difficile* PCR ribotypes in English hospitals, 2007–08. Euro Surveill **2008**; 13:pii=19000.
19. Boom R, Sol CJ, Salimans MM, et al. Rapid and simple method for purification of nucleic acids. J Clin Microbiol **1990**; 28:495–503.
20. Green J, Norcott JP, Lewis D, Arnold C, Brown DW. Norwalk-like viruses: demonstration of genomic diversity by polymerase chain reaction. J Clin Microbiol **1993**; 31:3007–12.
21. McLauchlin J, Pedraza-Diaz S, Amar-Hoetzeneder C, Nichols GL. Genetic characterization of Cryptosporidium strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. J Clin Microbiol **1999**; 37:3153–8.
22. Westgard JO, Groth T, Aronsson T, Falk H, de Verdier CH. Performance characteristics of rules for internal quality control: probabilities for false rejection and error detection. Clin Chem **1977**; 23:1857–67.
23. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993–1996). Eur J Clin Microbiol Infect Dis **2007**; 26:311–23.
24. Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J. Diagnosing norovirus associated infectious intestinal disease using viral load. BMC Infect Dis **2009**; 9:63.
25. Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J. Diagnosing rotavirus A associated IID: using ELISA to identify a cut-off for real time RT-PCR. J Clin Virol **2009**; 44:242–5.
26. Svraka S, Vennema H, van der Veer B, et al. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. J Clin Microbiol **2010**; 48:2191–8.
27. Gillespie IA, O'Brien SJ, Adak GK, Ward LR, Smith HR. Foodborne general outbreaks of *Salmonella* enteritidis phage type 4 infection, England and Wales, 1992–2002: where are the risks? Epidemiol Infect **2005**; 133:795–801.
28. Hutber AM, Kitching RP, Fishwick JC, Bires J. Foot-and-mouth disease: the question of implementing vaccinal control during an epidemic. Vet J **2011**; 188:18–23.
29. Marcos LA, DuPont HL. Advances in defining etiology and new therapeutic approaches in acute diarrhea. J Infect **2007**; 55:385–93.
30. Gorkiewicz G. Nosocomial and antibiotic-associated diarrhoea caused by organisms other than *Clostridium difficile*. Int J Antimicrob Agents **2009**; 33(Suppl 1):S37–41.
31. Sears CL. Enterotoxigenic *Bacteroides fragilis*: a rogue among symbiotes. Clin Microbiol Rev **2009**; 22:349–69.
32. Woo PC, Lau SK, Teng JL, et al. Association of *Laribacter hongkongensis* in community-acquired gastroenteritis with travel and eating fish: a multicentre case-control study. Lancet **2004**; 363:1941–7.
33. Wilhelmi I, Roman E, Sánchez-Fauquier A. Viruses causing gastroenteritis. Clin Microbiol Infect **2003**; 9:247–62.
34. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog **2009**; 5:e1000391.
35. Finkbeiner SR, Allred AF, Tarr PI, Klein EJ, Kirkwood CD, Wang D. Metagenomic analysis of human diarrhea: viral detection and discovery. PLoS Pathog **2008**; 4:e1000011.
36. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature **2010**; 464:59–65.
37. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect **2008**; 14:908–34.