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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 and 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...
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>
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<tr>
<th>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</th>
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<tr>
<td><strong>Geographical location</strong></td>
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<td>Study duration</td>
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<td>Prospective cohort study</td>
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<td>Number of practices recruited</td>
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<tr>
<td>Number of cohort participants recruited</td>
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<tr>
<td>Participation rate (%)</td>
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<td>Method of follow-up</td>
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<td>Maximum period of follow-up, weeks</td>
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<td>Lost to follow-up (%)</td>
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<td>Number of cases reporting symptoms</td>
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<tr>
<td>Number of cases submitting specimens</td>
</tr>
<tr>
<td>Percentage of specimens from cases aged $&lt;5$ years (%)</td>
</tr>
<tr>
<td>Median time (IQR) from illness onset to specimen submission, days</td>
</tr>
<tr>
<td>General practice presentation study</td>
</tr>
<tr>
<td>Number of practices recruited</td>
</tr>
<tr>
<td>Number of cases meeting the case definition</td>
</tr>
<tr>
<td>Number of cases submitting specimens</td>
</tr>
<tr>
<td>Percentage of specimens from cases aged $&lt;5$ years (%)</td>
</tr>
<tr>
<td>Median time (IQR) from illness onset to specimen submission</td>
</tr>
</tbody>
</table>

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 (i.e., 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...
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...
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). Enteroinvasive *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>
<thead>
<tr>
<th>Pathogen</th>
<th>Test</th>
<th>No. Identified</th>
<th>Tested</th>
<th>% Identified</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>All</td>
<td>1</td>
<td>715</td>
<td>0.1</td>
<td>(0–0.9)</td>
</tr>
<tr>
<td></td>
<td>EIA</td>
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<td>715</td>
<td>0.0</td>
<td>(0–0.5)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>1</td>
<td>693</td>
<td>0.1</td>
<td>(0–0.8)</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
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<td>6</td>
<td>772</td>
<td>0.8</td>
<td>(0.3–1.7)</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
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<td>782</td>
<td>4.6</td>
<td>(2.2–6.3)</td>
</tr>
<tr>
<td></td>
<td>All culture</td>
<td>28</td>
<td>767</td>
<td>3.7</td>
<td>(2.4–5.2)</td>
</tr>
<tr>
<td></td>
<td>Direct culture</td>
<td>18</td>
<td>766</td>
<td>2.3</td>
<td>(1.4–3.7)</td>
</tr>
<tr>
<td></td>
<td>Enrichment</td>
<td>27</td>
<td>766</td>
<td>3.5</td>
<td>(2.3–5.1)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>31</td>
<td>782</td>
<td>4.0</td>
<td>(2.7–5.6)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Culture</td>
<td>1</td>
<td>768</td>
<td>0.1</td>
<td>(0–0.7)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>6</td>
<td>781</td>
<td>0.8</td>
<td>(0.3–1.7)</td>
</tr>
<tr>
<td><em>Enteroaggregative E. coli</em></td>
<td>PCR</td>
<td>15</td>
<td>782</td>
<td>1.9</td>
<td>(1.1–3.1)</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>Culture and/or PCR</td>
<td>0</td>
<td>769</td>
<td>0.0</td>
<td>(0–0.5)</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>All</td>
<td>2</td>
<td>782</td>
<td>0.3</td>
<td>(0–0.9)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>2</td>
<td>768</td>
<td>0.3</td>
<td>(0–0.9)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>1</td>
<td>782</td>
<td>0.1</td>
<td>(0–0.7)</td>
</tr>
<tr>
<td><em>Shigella</em></td>
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<td>768</td>
<td>0.0</td>
<td>(0–0.5)</td>
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<tr>
<td><em>Yersinia</em></td>
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<td>769</td>
<td>0.0</td>
<td>(0–0.5)</td>
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<tr>
<td></td>
<td>Direct culture</td>
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<td>769</td>
<td>0.0</td>
<td>(0–0.5)</td>
</tr>
<tr>
<td></td>
<td>Enrichment</td>
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<td>769</td>
<td>0.0</td>
<td>(0–0.5)</td>
</tr>
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<td>Protozoa</td>
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<td>782</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>EIA</td>
<td>2</td>
<td>768</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR</td>
<td>3</td>
<td>782</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td>Microscopy</td>
<td>0</td>
<td>768</td>
<td>0.0</td>
<td>(0–0.5)</td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>All</td>
<td>6</td>
<td>782</td>
<td>0.8</td>
<td>(0.3–1.7)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>3</td>
<td>782</td>
<td>0.8</td>
<td>(0.3–1.7)</td>
</tr>
<tr>
<td>Viruses</td>
<td><em>Adenovirus</em></td>
<td>ELISA and/or PCR</td>
<td>28</td>
<td>782</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td><em>Astrovirus</em></td>
<td>PCR</td>
<td>14</td>
<td>782</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td><em>Norovirus</em></td>
<td>PCR</td>
<td>129</td>
<td>782</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td><em>Rotavirus</em></td>
<td>ELISA and/or PCR</td>
<td>32</td>
<td>782</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td><em>Sapovirus</em></td>
<td>PCR</td>
<td>72</td>
<td>782</td>
<td>9.2</td>
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<tr>
<td></td>
<td>No pathogen identified</td>
<td>471</td>
<td>782</td>
<td>60.2</td>
<td>(56.7–63.7)</td>
</tr>
</tbody>
</table>

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.
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
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 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

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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.
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

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**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.
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.
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 paroviruses. 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**

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*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. 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.
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