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## Research articles

# CLINICAL LABORATORY PRACTICES FOR THE DETECTION OF ROTAVIRUS IN ENGLAND AND WALES: CAN SURVEILLANCE BASED ON ROUTINE LABORATORY TESTING DATA BE USED TO EVALUATE THE IMPACT OF VACCINATION?

C J Atchison (Christina.Atchison@lshtm.ac.uk)<sup>1</sup>, B A Lopman<sup>1,2</sup>, C J Harris<sup>3</sup>, C C Tam<sup>1</sup>, M Iturriza Gómara<sup>4</sup>, J J Gray<sup>4</sup>

1. Infectious Diseases Epidemiology Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

2. Gastrointestinal, Emerging and Zoonotic Infections Department, Centre for Infections, Health Protection Agency, London, United Kingdom

3. Standards Unit, Department of Evaluations and Standards, Centre for Infections, Health Protection Agency, London, United Kingdom

4. Enteric Virus Unit, Virus Reference Department, Centre for Infections, Health Protection Agency, London, United Kingdom

Two rotavirus vaccines have recently been licensed in Europe. Rotavirus surveillance data in many European countries are based on reports of laboratory-confirmed rotavirus infections. If surveillance data based on routine laboratory testing data are to be used to evaluate the impact of vaccination programmes, it is important to determine how the data are influenced by differences in testing practices, and how these practices are likely to affect the ability of the surveillance data to represent trends in rotavirus disease in the community. We conducted a survey of laboratory testing policies for rotavirus gastroenteritis in England and Wales in 2008. 60% (94/156) of laboratories responded to the survey. 91% of reporting laboratories offered routine testing for rotavirus all year round and 89% of laboratories offered routine rotavirus testing of all stool specimens from children under the age of five years. In 96% of laboratories, rotavirus detection was presently done either by rapid immunochromatographic tests or by enzyme-linked immunosorbent assay. Currently, rotavirus testing policies among laboratories in England and Wales are relatively homogenous. Therefore, surveillance based on laboratory testing data is likely to be representative of rotavirus disease trends in the community in the most frequently affected age groups (children under the age of five years) and could be used to help determine the impact of a rotavirus vaccine.

### Introduction

Two rotavirus vaccines with comparably good safety and efficacy profiles are now licensed for use [1,2]. In England and Wales the introduction of rotavirus vaccination is currently under consideration. However, some countries have already introduced them into routine childhood immunisation schedules with good effect [3,4]. In the United States, in February 2006, the Advisory Committee on Immunization Practices recommended "RotaTeq®", a live, oral, human-bovine reassortant rotavirus vaccine for routine use in infants [5]. Preliminary analysis of the national surveillance data for 2007-8 indicated that during the rotavirus season (July 2007 to May 2008) there were fewer cases and that the timing of

the peak in incidence was delayed by two to four months compared to previous seasons [3]. This provides the first indication, post-licensure, that rotavirus vaccination reduces the burden of rotavirus disease in a large population and is consistent with the effects of vaccination seen for other childhood diseases [6].

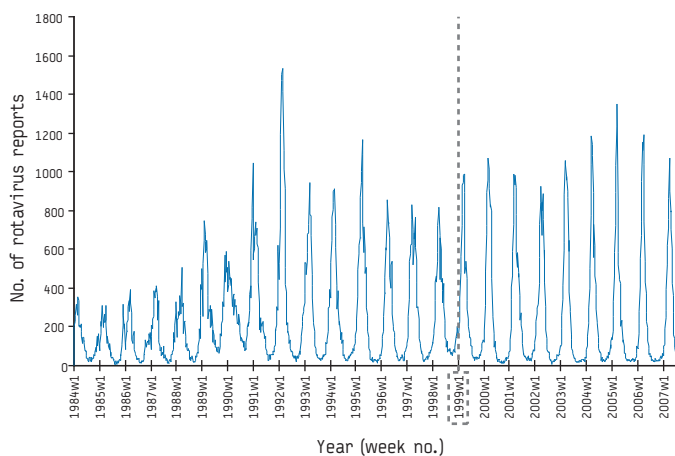
In England the estimated rate of rotavirus gastroenteritis in the community is 7.1 cases per 1,000 persons per year [7]. Though mortality is rare [8], rotavirus is recognised as a major burden on health services. The annual incidence of rotavirus hospitalisations in England is approximately 4.5 per 1,000 children under the age of five years [9,10]. Each year rotavirus is estimated to be responsible for 14,300 hospitalisations, 29,700 accident and emergency consultations and 90,600-133,400 general practice consultations in children under the age of five years in England and Wales [10]. The cost to the National Health Service is estimated to be GBP 14.2 million per year [10].

Current burden of disease estimates are, in part, generated using the national rotavirus surveillance data. Evaluating the need for and the impact of a rotavirus vaccine in the United Kingdom (UK) will rely partly on these surveillance data. At present, surveillance in England and Wales is based on reports of laboratory-confirmed rotavirus infections from over 150 clinical microbiology laboratories. Rotavirus reports show marked seasonality, currently peaking between February and March each year [9]. The majority of reported laboratory-confirmed rotavirus infections occur in children under the age of five years (94% of all reports in which the patient's age is recorded) [9].

However, only a fraction of community cases are reported to national surveillance. It has been estimated that for every rotavirus case reported to national surveillance in England there are 1.5 positive laboratory investigations, 11.3 cases who present to general practice, and overall 35 cases in the community [7]. Using the rotavirus national surveillance data to investigate

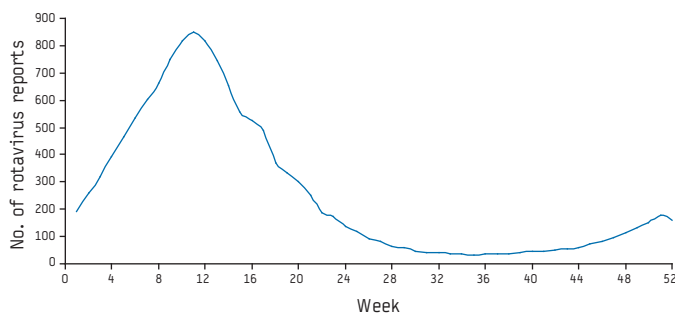
population disease patterns or potentially, to evaluate the impact of vaccination, requires that trends in laboratory-confirmed rotavirus infections are representative of trends in rotavirus gastroenteritis in the population. Variations in reporting practices, criteria for rotavirus testing and the diagnostic methods used, either between laboratories or from year to year, may create biases when using laboratory testing for surveillance data. If testing is only offered at certain times of year or in certain age groups, seasonal patterns of rotavirus disease in the population will be distorted in the national surveillance data. Understanding the effect of biases in laboratory testing and reporting practices on national data is fundamental to understanding the extent to which patterns observed in the surveillance data reflect underlying community trends. This study aims to examine how laboratory policies for rotavirus testing and reporting have affected rotavirus surveillance data since 1984.

**FIGURE 1**  
Weekly number of laboratory-confirmed rotavirus reports in England and Wales, 1984-2007



The dashed line indicates the start of 1999, the year in which most laboratories switched to using ELISAs or rapid immunochromatographic tests for rotavirus testing.  
Source: Health Protection Agency rotavirus national surveillance data.

**FIGURE 2**  
Average weekly number of laboratory-confirmed rotavirus reports in England and Wales, 1984-2007

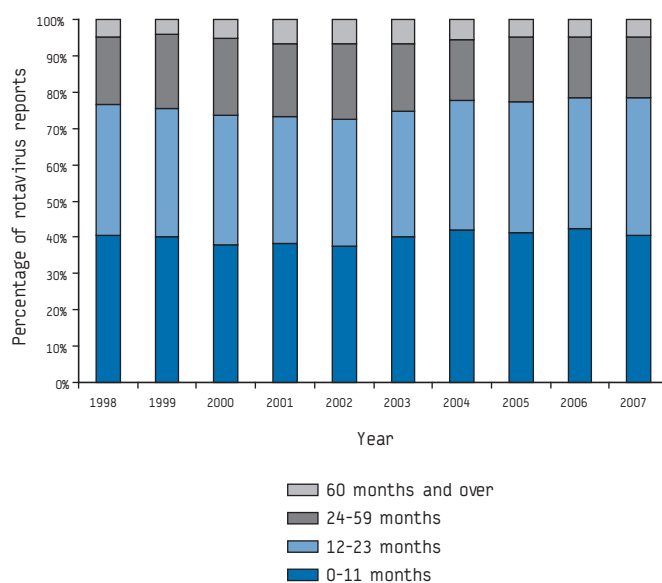


Source: Health Protection Agency rotavirus national surveillance data.

## Methods

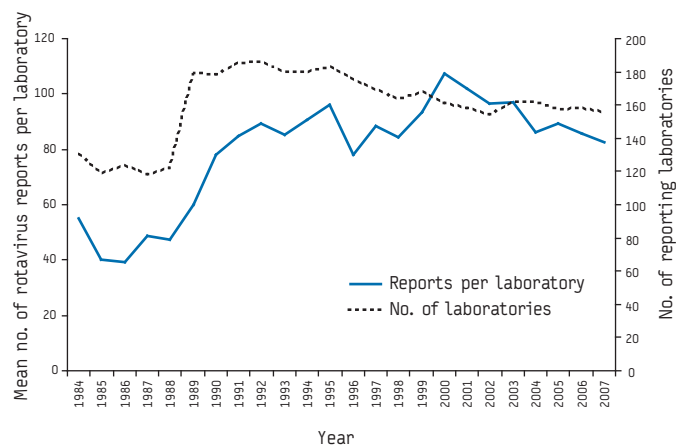
The Health Protection Agency (HPA) Centre for Infections receives reports of laboratory-confirmed rotavirus infections for England and Wales. Reporting is on a voluntary basis but is strongly encouraged. All reports have mandatory data fields for reporting laboratory, patient identifier, age, sex, pathogen, specimen type and specimen date. Laboratories feed reports into a set of database modules (some still send printed reports or paper report forms) and these are electronically transferred to regional HPA units which

**FIGURE 3**  
Age distribution of laboratory-confirmed rotavirus reports, England and Wales, 1998-2007



Source: Health Protection Agency rotavirus national surveillance data.

**FIGURE 4**  
Mean number of reported rotavirus infections per reporting laboratory and number of reporting laboratories in England and Wales, 1984-2007



Source: Health Protection Agency rotavirus national surveillance data.

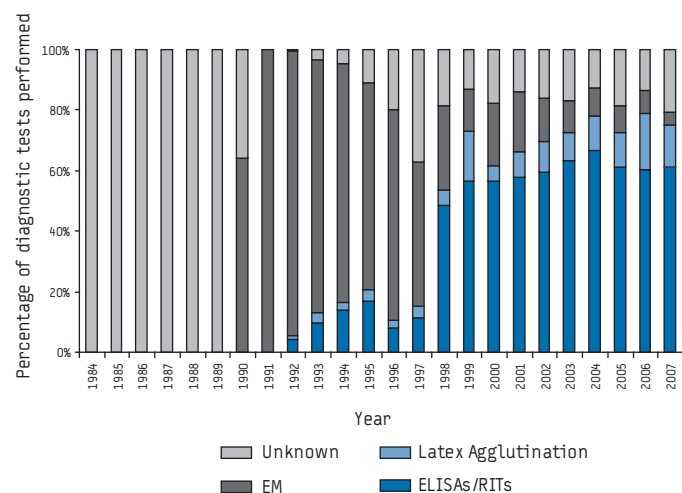
collect the reports before transferring them to 'LabBase', the national laboratory reporting database at the Centre for Infections [11].

Medical microbiology laboratories reporting to the HPA include the National Health Service (NHS) and regional or collaborating HPA laboratories. These laboratories are mostly based within hospitals and all provide a clinical diagnostic microbiology service to both primary and secondary healthcare providers. Regional and collaborating HPA laboratories, in addition, provide specialist advice and support to other laboratories and microbiology services for health protection purposes. From a total of 208 NHS and HPA laboratories in England and Wales in 2007 [12,13], 156 were responsible for reporting cases of laboratory-confirmed rotavirus infections to national surveillance.

In May 2008 we distributed, by email, a structured questionnaire to the manager and consultant microbiologist (usually a medically-qualified doctor specialised in the diagnostics and management of infections) in each of these 156 laboratories. These laboratories were contacted directly using details available from the Department of Health [13], or via the regional consultant microbiologist who distributed the questionnaire to laboratories in their region. Two email reminders were sent if laboratories had not responded by August 2008. The survey included questions on the following (see Table):

1. The number of stools tested and positive for rotavirus in 2007,
2. Diagnostic tests used for rotavirus detection,
3. Policies on screening by age,
4. Months of the year in which routine rotavirus testing was performed,
5. Other indications for testing,
6. Dates and details of changes to testing policies over the period 1990-2007.

**FIGURE 5**  
**Diagnostic tests used for rotavirus detection in reported laboratory-confirmed rotavirus infections in England and Wales, 1984-2007**



Source: survey answers.

'Routine' rotavirus testing was defined as rotavirus testing carried out on all stool specimens from gastroenteritis cases fitting a policy's inclusion criteria.

We used analysis of variance [14] to investigate whether certain testing policies were associated with higher positivity rates for rotavirus detection in stool specimens tested, and whether certain characteristics of a laboratory were associated with higher reporting efficiencies. Reporting efficiency was defined as the percentage of laboratory-confirmed rotavirus infections detected by a laboratory that were reported to LabBase. This was determined by dividing the number of rotavirus reports from a laboratory in LabBase in 2007 by the number of positive rotavirus specimens from that laboratory in the same year (survey question). This gives an indication of how efficient a laboratory was at reporting rotavirus diagnoses to national surveillance. For example, a reporting efficiency for a laboratory of 20% would mean that one in five rotavirus infections detected by that laboratory were reported or transferred to national surveillance.

To determine the effects of changes in diagnostic testing methods on long-term trends in national surveillance data, linear regression models were fitted to estimate whether the number of reports in a year were associated with the proportion of cases in that year diagnosed by a particular diagnostic test.

## Results

### The England and Wales rotavirus surveillance data (LabBase)

A total of 290,708 laboratory-confirmed rotavirus infections were reported in England and Wales between 1984 and 2007. Rotavirus reports showed marked seasonality that was regular and consistent over the surveillance period (Figure 1).

The rise in the number of rotavirus reports typically began in November and fell back to baseline in June. The peak in reported rotavirus infections was between February and April when 65%-70% of all reports occurred each year (Figure 2).

56% of laboratory-confirmed rotavirus infections were in male patients and 94% of all reports were in children under the age of five years. Information on age or date of birth of rotavirus cases was consistently recorded in LabBase from 1998 onwards. The age distribution of cases did not change over the surveillance period 1998-2007 (Figure 3) and cases in all age groups showed a similar seasonal pattern.

The number of rotavirus reports in England and Wales increased dramatically from the early 1990s (Figure 1). This sudden increase coincided with a rise in the average annual number of reports per reporting laboratory from 1989 (Figure 4) and an increase in the number of laboratories reporting each year from 1989 (Figure 4). While similar numbers of total annual reports have been received over the last 15 years, the number of contributing laboratories has declined slightly in the present decade compared with the 1990s (Figure 4).

In the surveillance data, basic information was also available on which type of diagnostic test was used in each reported laboratory-confirmed rotavirus infection. Prior to 1990 most laboratories did not report the method of rotavirus detection. Between 1990 and 1997 electron microscopy (EM) was the most frequent diagnostic test used. In 1998, there was a dramatic shift to enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic tests (RITs), which subsequently predominated (Figure 5).

## The Laboratory Survey of Rotavirus Testing Policies

### Response

Ninety-four of 156 (60%) microbiology laboratories in England and Wales returned completed questionnaires.

### Current diagnostic methods used

Most laboratories used RITs as their first line diagnostic method for rotavirus detection, either dual adenovirus/rotavirus RIT or single rotavirus RIT (Table). ELISAs were the second most common test used. Only 4% of laboratories currently used EM or latex agglutination to detect rotavirus in stool specimens.

TABLE

### Routine laboratory testing policies for rotavirus in England and Wales in 2007 (survey of 94 laboratories)

Testing Policy	No. of Laboratories (%)
First line diagnostic method (n=94)	
ELISA	22 (23%)
Electron microscopy	2 (2.1%)
Latex agglutination	2 (2.1%)
Dual adenovirus/rotavirus RIT	34 (36%)
Single rotavirus RIT	34 (36%)
Seasonal policies for testing (n=94)	
All year	86 (91%)
All months except July	1 (1.1%)
October to May	4 (4.3%)
January to April	2 (2.1%)
July to December	1 (1.1%)
Age policies for testing, in years (n=94)	
< 3	6 (6.4%)
< 5	58 (62%)
< 6	4 (4.3%)
< 8	1 (1.1%)
< 10	1 (1.1%)
< 12	3 (3.2%)
< 16	8 (8.5%)
< 2 and ≥ 65	2 (2.1%)
< 5 and ≥ 60	1 (1.1%)
< 5 and ≥ 65	8 (8.5%)
≥ 65	2 (2.1%)
Other indications for testing (n=94)	
Clinician's request	94 (100%)
Diarrhoeal outbreak in ≥ 65 year-olds	35 (37%)
Diarrhoeal outbreak in paediatric ward	11 (12%)
Adult diarrhoeal outbreak when norovirus PCR-negative	4 (4.3%)
All liquid stools	1 (1.1%)
Stool specimens from immunocompromised patients	12 (13%)
Stool specimens from nursery workers	2 (2.1%)

ELISA: enzyme-linked immunosorbent assay; RIT: rapid immunochromatographic tests.

### Seasonal policies for testing

91% (86/94) of laboratories routinely tested for rotavirus all year round. The exceptions were one laboratory which routinely tested in all months except July, four laboratories which routinely tested only from October to May, two laboratories which routinely tested only from January to April and one laboratory which routinely tested only from July to December (Table).

### Age policies for testing

There was some variation in the age policies currently used for testing (Table). Complete testing for rotavirus in stool specimens from gastroenteritis cases in children under the age of five years was routinely performed in most laboratories (89%, 84/94). The two laboratories that routinely tested only in ≥65 year-olds served hospitals that did not have a paediatric department. Of the laboratories that routinely tested for rotavirus in children only (all age policies up to and including <16 year-olds), 43% had a policy whereby an institutional or hospital outbreak of diarrhoea in ≥65 year-olds would be an additional indication for rotavirus testing.

### Other testing policies

Other indications for rotavirus testing included stool specimens sent from immunocompromised patients, nursery workers, outbreaks in paediatric wards, adult outbreaks when testing for norovirus was PCR-negative and all liquid stool specimens (Table). All laboratories tested for rotavirus in response to a specific clinical request, but 38% stated that the request would be referred to a Consultant Microbiologist if the patient from whom the stool specimen was collected did not meet any of the routine testing criteria.

### Testing policies associated with higher positivity rates

No associations were found between the mean rotavirus positivity rates and the diagnostic method, seasonal or age policy currently used by laboratories (p values ≥ 0.1 for all testing policies investigated). The sample size for this analysis was small, as 38% of laboratories did not provide positivity rates. This resulted in wide confidence intervals for our estimates.

### Laboratory reporting

All laboratories had a policy to report all rotavirus-positive specimens to the HPA Centre for Infections. On average, 71% (range 22-111%) of rotavirus infections detected by a given laboratory corresponded to a case report from that laboratory in LabBase in 2007. Reporting efficiencies over 100% could have resulted from errors during data input or delayed reporting. No associations were found between reporting efficiencies and rotavirus testing policies, affiliation of the laboratory to the HPA, whether a laboratory received specimens from more than one hospital or whether these hospitals were paediatric hospitals or had paediatric departments (p values ≥ 0.1 for all laboratory characteristics investigated).

### Changes to laboratory practices

Thirty-nine of 94 (41%) laboratories provided data on whether testing policies changed over the last 15 years. Of the 32 laboratories (34% of all laboratories in the survey) reporting a change, 14 changed only the brand of the commercial assay they used and 18 changed the type of diagnostic method used, although only 11 of the 32 laboratories reporting changes could give the dates of when these changes occurred. Laboratories tended to switch from using ELISA, latex agglutination or EM to RITs from about 2000. These observations were consistent with information from the national database described above, which demonstrated

a national shift in diagnostic testing practices from using EM to ELISA or RITs after 1998 (Figure 4). If the surveillance data had been affected by this shift in diagnostic practice, one might have expected an artificial rise in the overall numbers of reported cases after the late 1990s as ELISA and RITs are more sensitive and less specific than EM for rotavirus detection [15]. However, we found no association between annual number of laboratory reports and the proportion of cases diagnosed by each diagnostic method ( $p$  values  $\geq 0.1$  for all diagnostic methods). Using LabBase and our survey results, we identified 59 laboratories that, from 1999 onwards, tested more than 90% of stool specimens for rotavirus each year by ELISAs or RITs.

### Discussion

This study demonstrated that rotavirus testing policies in laboratories contributing to surveillance in England and Wales were reasonably consistent in 2007-8. The majority of laboratories were using RITs to detect rotavirus in stool specimens and were offering routine rotavirus testing all year round in children under the age of five years. These testing criteria for rotavirus are in accordance to those recommended in the National Standard Methods [16]. These are a set of standard operating procedures and guidance notes developed by the Standards Unit at the HPA to establish minimum best practice quality and efficiency in clinical microbiology laboratories in the UK.

No particular testing policy was found to be associated with higher positivity rates for rotavirus detection. This was unexpected, since laboratories testing only children under the age of five years might be expected to have higher positivity rates than those also testing older age groups. However, 38% of laboratories did not provide positivity rates. The resulting small sample size and wide confidence intervals may explain our failure to detect any associations. We reported that in 2007, on average, one in 1.4 (71%) rotavirus infections detected by a laboratory resulted in a case report from that laboratory to the national surveillance database "LabBase". This estimate is consistent with a previous study which reported that for one rotavirus case reported to national surveillance in England there were 1.5 laboratory-positive investigations [7].

In addition, we demonstrated how the number of rotavirus reports can be dramatically influenced by sudden changes in the number of laboratories reporting, and therefore why long term trends in the England and Wales rotavirus surveillance data must be interpreted with caution. Changes in the number of laboratories reporting and in the mean number of reports per laboratory both occurred around 1989. These changes coincided with a doubling of the number of rotavirus reports in England and Wales during the same period. During the late 1980s, developments in rotavirus vaccine research took place and there was a renewed interest in rotavirus epidemiology [17]. This could account for the changes in laboratory reporting practices seen at this time. A slight reduction in the number of reporting laboratories was observed towards the end of the study period. We attribute this decline to recent changes in the delivery of microbiology services in the UK that have resulted in the closing and merging of microbiology laboratories as well as the sharing of services between laboratories. This would also explain why the fall in number of reporting laboratories did not coincide with a fall in the overall number of laboratory-confirmed rotavirus infections reported.

Our survey results are in contrast to the findings of a previous study which looked at policies for rotavirus testing in eight

laboratories in the East of England region between 1990 and 1998 [18]. That study reported marked differences in age and seasonal testing policies between laboratories. Due to the small sample size, their results are less likely to be representative of laboratories across England and Wales than ours. Our national survey may have failed to detect those earlier findings from 10 years ago because the laboratories previously studied may have closed or merged with other laboratories since then. It is also possible that changes in practices from 10 years ago or more were not reported because staff responsible for testing in the past and able to recall such a change may no longer work in the laboratory.

Our survey is subject to limitations. There was a poor response (41% of surveyed laboratories answered) to survey questions regarding changes to testing policies over the last 15 years. However, given the regularity of the seasonal pattern of laboratory-confirmed rotavirus reports, it is reasonable to assume that either few changes in policy took place or that the changes had little effect on the surveillance data. Our conclusions cannot be extended to laboratories that do not report cases of rotavirus to the HPA as we only surveyed reporting laboratories. Non-reporting laboratories will not influence surveillance data as they do not contribute any reports. Sixty-two of 156 (40%) laboratories did not respond to the survey. Differences between responders and non-responders might have resulted in bias. Non-responders may be laboratories that have little interest and testing experience in rotavirus disease. They may also be the laboratories with poor reporting efficiencies or inconsistent rotavirus testing policies, and therefore did not respond because they were unwilling to disclose this information.

Our survey of clinical laboratory practices for rotavirus testing in England and Wales suggests that it may be reasonable to assume that seasonal patterns in rotavirus surveillance data based on reports of laboratory-confirmed rotavirus infections are representative of patterns of rotavirus disease in children under the age of five years. Specifically, surveillance data are representative of cases for which a specimen is tested, not necessarily all rotavirus cases. As most laboratories do not test routinely in adults, the patterns of disease in this age group are less likely to be represented in the surveillance data. This is not likely to be a problem as vaccine policy questions relate primarily to children. If clinical testing policies remain as they are at present, the surveillance data could be used to assess the impact of rotavirus vaccination on the seasonality of rotavirus infections in England and Wales.

However, laboratory testing practices are not the only factor influencing how accurately the surveillance data reflect the epidemiological trends of rotavirus disease. Surveillance data represent only a fraction of cases occurring in the community as only a minority seek medical attention, and of these, stool specimens are investigated for only a fraction [7]. Therefore, surveillance data also reflect healthcare-seeking behaviour of parents of young children suffering from diarrhoea, and clinical practices regarding stool sampling of those children. If care seeking or stool sampling practices change with the advent of vaccination, there would be temporal biases in the laboratory-based data. This would limit its value in evaluating the impact of a vaccination programme, even if laboratory testing practices remain unchanged. In this respect, key additional data to be collected would be the number of negative tests, so that the proportion positive for rotavirus can be assessed. We recommend that this is collected nationally in the period following licensure of a new vaccine. It may also be possible

to introduce national guidelines for the sampling of children with diarrhoea to standardise practice.

Most laboratories in England and Wales started using ELISA and RITs for rotavirus testing after 1999. These tests have higher sensitivity but lower specificity than previously used diagnostics. Therefore, using data subsequent to 1999 would provide the most appropriate baseline information against which post-licensure trends can be assessed (see Figure 1). We have identified 59 laboratories that predominantly used ELISAs or RITs after 1999. Data from these laboratories would yield the clearest baseline information (i.e. secular trends independent of diagnostic testing issues). Assuming they continue to use these methods post-licensure, evaluations using data from these laboratories would minimise biases.

In order to assess the effectiveness of a rotavirus vaccine it will be crucial to link the surveillance data to vaccination history in child health records. If vaccination is introduced, those responsible for monitoring its effects should consider encouraging laboratories to broaden their age-based testing policies. Vaccination is likely to increase the age of infection [6] and this may be missed by the surveillance data if age policies remain restricted to the youngest age groups. Other national surveillance centres in Europe may benefit from performing a similar survey of laboratory practices for rotavirus testing to aid in the interpretation of their surveillance data and in anticipation of vaccination.

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