



## Original Contribution

# Asymptomatic Rotavirus Infections in England: Prevalence, Characteristics, and Risk Factors

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Rotavirus is a major cause of infectious intestinal disease in young children; a substantial prevalence of asymptomatic infection has been reported across all age groups. In this study, the authors determined characteristics of asymptomatic rotavirus infection and potential risk factors for infection. Healthy persons were recruited at random from the general population of England during the Study of Infectious Intestinal Disease in England (1993–1996). Rotavirus infection was identified using reverse-transcription polymerase chain reaction. Multivariable logistic regression was used to compare exposures reported by participants with rotavirus infection with those of participants who tested negative. Multiple imputation was used to account for missing responses in the data set. The age-adjusted prevalence of asymptomatic rotavirus infection was 11%; prevalence was highest in children under age 18 years. Attendance at day care was a risk factor for asymptomatic rotavirus infection in children under age 5 years; living in a household with a baby that was still in diapers was a risk factor in older adults. The results suggest that asymptomatic rotavirus infection is transmitted through the same route as rotavirus infectious intestinal disease: person-to-person contact. More work is needed to understand the role of asymptomatic infections in transmission leading to rotavirus disease.

case-control studies; logistic models; polymerase chain reaction; risk factors; rotavirus infections

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IID, infectious intestinal disease; RT-PCR, reverse-transcription polymerase chain reaction.

Rotavirus is a major cause of diarrhea-associated morbidity and mortality worldwide (1). The majority of children experience rotavirus-associated infectious intestinal disease (IID) by the age of 5 years (2, 3). Immunity developed after the primary and secondary infections is generally protective against disease, although further infections frequently occur, typically without any IID symptoms (4–6). A substantial prevalence of rotavirus infection without IID has been reported in the general population, in both children (7–14) and adults (8, 9, 12, 15), ranging from 3% to 31%, depending on the setting and the age of the study population. These infections are classified as asymptomatic by the absence of diarrhea or vomiting, but infected persons may still display nonspecific symptoms such as fever, headache, nausea, and fatigue (16, 17). These infections are hereafter referred to as “asymptomatic rotavirus infections.”

Risk factors for rotavirus-associated IID have been extensively investigated (12, 18–21), but few researchers have described risk factors for asymptomatic rotavirus infection. Household contact tracing studies have demonstrated asymptomatic infections, in both children and adults, following introduction of a symptomatic child index case into a household (15). A cohort study of day-care centers in North America showed that one-third of children aged 2 years or less experience asymptomatic rotavirus infection each year in this setting (10).

Given that asymptomatic rotavirus infection is so common, it is important to understand the routes of transmission leading to these infections, especially whether there is transmission between asymptotically infected persons or whether they are caused only by contact with symptomatic persons. Such information is essential to an understanding

of the transmission dynamics of rotavirus. In this study, we aimed to describe the prevalence of asymptomatic rotavirus infection in a community-based sample of healthy children and adults, to describe the characteristics of infected persons, and to investigate risk factors for asymptomatic rotavirus infection.

## MATERIALS AND METHODS

### Recruitment

We used data from participants in the Study of Infectious Intestinal Disease in England, conducted between 1993 and 1996 (22). These persons were recruited as controls for a case-control study, either from a prospectively followed cohort in the community or from the registration lists of general practitioners participating in the study (23). Recruitment of controls into the case-control study, from either source, involved individual matching of controls to cases by age and sex. The inclusion criteria for controls specified that they should have no recent history of diarrhea (any loose stools) or vomiting (2 or more vomiting episodes per 24 hours) prior to recruitment (23).

### Specimens and testing

At recruitment, participants submitted fecal specimens for microbiologic testing, for detection of a range of 18 bacterial, viral, and protozoal gastrointestinal pathogens. Group A rotavirus (hereafter referred to as rotavirus) was detected using a commercial enzyme-linked immunosorbent assay (ELISA) (24). Stool specimens were archived and subsequently retested by reverse-transcription polymerase chain reaction (RT-PCR) for rotavirus (8, 24). The RT-PCR assay has a much lower detection limit than ELISA and therefore identifies many more asymptomatic infections (8). For this analysis, participants were classified as rotavirus-infected if they tested positive by either ELISA or RT-PCR or both.

### Epidemiologic data

Participants were asked to complete a risk factor questionnaire providing information on age, sex, gastrointestinal and nonspecific symptoms during the previous 3 weeks, pet ownership, household composition, school and day-care attendance (for children aged less than 16 years), social class (based on the Office of Population Censuses and Surveys Standard Occupational Classification (25)), and hand hygiene. For the previous 10 days before questionnaire completion, participants provided information on infectious contacts inside and outside the home, foreign travel, consumption of food and drink, and participation in water sports. Adults completed the questionnaire themselves; a parent or guardian completed the questionnaire on behalf of children aged less than 16 years (23).

### Inclusion criteria and case definition

The outcome of interest in this analysis was asymptomatic rotavirus infection. Participants who had been free of diarrhea and vomiting for at least 3 weeks prior to recruit-

ment were considered asymptomatic with respect to IID, although they may have experienced other symptoms during that period. Asymptomatic participants infected with rotavirus were classified as having asymptomatic rotavirus infections; asymptomatic participants who tested negative for rotavirus were used as the control group for this analysis (rotavirus-negative controls).

### Conceptual framework

We used a hierarchical conceptual framework (26) to investigate potential risk factors for asymptomatic rotavirus infection by comparing exposures reported by persons with asymptomatic rotavirus infections with exposures reported by rotavirus-negative controls. We investigated exposures which are recognized to be associated with rotavirus-associated IID or which may be involved in these transmission routes. The conceptual framework had 3 levels: 1) distal factors, which are general characteristics and long-term behaviors (e.g., socioeconomic and demographic information); 2) intermediate factors, which are specific behaviors that may increase the risk of exposure for a short time but are not necessarily always a direct source of infection; and 3) proximal factors which are a direct source of infection. The conceptual framework is shown in Table 1. For the intermediate and proximal risk factors, participants were asked to report exposures that had occurred during the 10 days before questionnaire completion only.

Additionally, we investigated consumption of foods that had previously been associated with reduced risk of rotavirus disease in this study population. These foods were fresh and dried fruit, nonoily fish, and salad prepared and consumed at home (20).

The intermediate and proximal risk-factor models were adjusted for higher-level variables in the conceptual framework. Indicator variables for general practice and month since the beginning of the study were added to the proximal risk-factor model that included infectious contacts, to account for both geographic and temporal variation in rotavirus transmission.

### Dealing with missing values

We carried out 2 separate analyses. The first analysis included all participants, with creation of a categorical indicator for missing responses (missing indicator), and the second analysis included all participants with missing responses imputed (multiple imputation).

Any missing responses in the explanatory variables included in the conceptual framework were imputed using imputation by chained equations, in Stata 10.1 (27, 28). The multiple imputation prediction model, which is used to select the most likely value for a missing response, included all variables in the conceptual framework. In addition, indicator variables for the following characteristics were included in the prediction model: general practice; month since the beginning of the study; route of recruitment into the study (community cohort or general practitioner registration list); rotavirus infection status; and rotavirus season in England and Wales during the study period

**Table 1.** Conceptual Framework Used in an Analysis of Risk Factors for Asymptomatic Rotavirus Infection, Study of Infectious Intestinal Disease in England, 1993–1996

Category and Variable	Reference No.
Distal factors (general characteristics and long-term behaviors)	
Age	
Sex	
Social class <sup>a</sup>	
Accommodation ownership	20
Accommodation size (no. of rooms)	20
Household age structure (presence of children aged <5 years)	55
Household crowding (no. of people per room)	
Baby in diapers living in household	59
Pet ownership	
Sharing a bathroom or toilet with another household	17, 43
Nursery/day-care attendance (children aged <5 years only)	43, 46
Breastfeeding (infants aged <1 year only)	52–54
Hand hygiene <sup>b</sup>	
Intermediate factors <sup>c</sup> (time-limited behaviors)	
Participation in water sports	67, 68
Foreign travel	20
Contact with animals	59, 60
Food (raw fruit/nonoily fish/salad prepared and consumed at home) <sup>d</sup>	20
Proximal factors <sup>c</sup> (direct sources of infection)	
Household infectious contact	12, 15, 17, 18, 20, 49, 51, 69
Infectious contact outside household	19, 70

<sup>a</sup> Social class was based on the occupation of the main wage earner in the household (25).

<sup>b</sup> Measured as the response of the person in the household responsible for food shopping and preparation to the statement, “It doesn’t matter whether you wash your hands or not before handling food.” Response options were agree, disagree, and don’t know.

<sup>c</sup> Reporting of intermediate and proximal risk factors was limited to the 10 days before completion of the questionnaire.

<sup>d</sup> These foods were previously found to be associated with lower odds of rotavirus-associated infectious intestinal disease.

(1993–1996), defined from reports of laboratory-confirmed rotavirus diagnoses made to the Health Protection Agency (see the Web Appendix, which is posted on the *Journal’s* Web site (<http://aje.oxfordjournals.org/>)). There were no missing data in these indicator variables; they only informed the imputation of missing responses in the explanatory variables from the conceptual framework. Missing responses for children aged less than 5 years were imputed separately from those for older children and adults. Fifty imputed data sets were created and analyzed together.

For older children and adults, a second round of imputation was completed, structuring the prediction model to allow for potential interaction between age and other variables selected for the final model. The prediction model for this second round of imputation included variables selected for the final model, sex, age, and the additional indicator variables.

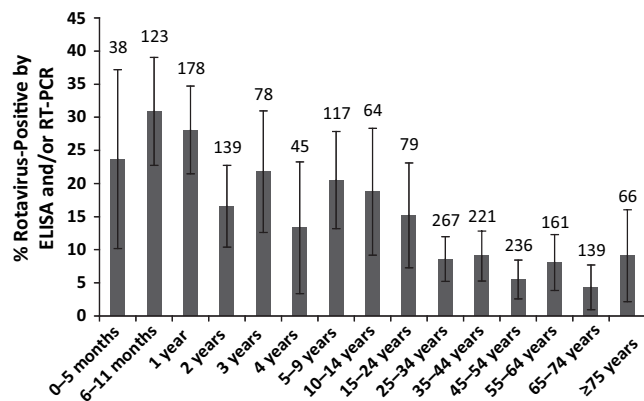
### Regression modeling

Standard logistic regression models were fitted using Stata 10.1 (28). The imputed data sets were analyzed in

Stata 10.1 using the *ice* suite of commands (27, 28); within this program, the logistic regression model is fitted separately to each of the 50 imputed data sets. The results are then combined to create 1 point estimate for each odds ratio and standard errors that take into account uncertainty in both the multiple imputation process and the standard regression (27).

For each analysis, the distal risk-factor model was fitted first, and any variables with a *P* value below 0.1 were selected for inclusion in the final model, for further investigation of their effects. This variable selection process was repeated for the intermediate and proximal risk-factor models. The results presented include variables with a *P* value less than 0.1 in the final model.

Interactions between age and exposures related to contact with an infectious person were investigated in the final model for children aged 5 years or older and adults, because of potential variation in exposure levels across this large age range and because of age-related variation in immunity. The likelihood ratio test was used to evaluate the importance of the interaction term in the standard regression model



**Figure 1.** Age-specific prevalence of asymptomatic rotavirus infection among healthy persons, by age group, Study of Infectious Intestinal Disease in England, 1993–1996. Rotavirus positivity was determined by means of enzyme-linked immunosorbent assay (ELISA) and/or reverse-transcription polymerase chain reaction (RT-PCR). Numbers above the columns show the sample size for each age group. Bars, 95% confidence interval.

(missing indicator), and a combined Wald test of the interaction terms was used in analysis of the multiply imputed data set.

### Statistical power

Minimum detectable univariate odds ratios were calculated using PS software, version 2.1.30 (29), with a type I error probability of 0.05 and 90% statistical power. The minimum detectable odds ratios were used to qualitatively assess whether an absence of association might be due to lack of statistical power.

## RESULTS

A total of 272 persons with asymptomatic rotavirus infections and 1,679 rotavirus-negative controls were included in the analysis.

### Prevalence and characteristics of asymptomatic rotavirus infection

The highest prevalence of asymptomatic rotavirus infection was in children aged less than 2 years, with almost one-third of children in this age group being infected (Figure 1). Asymptomatic rotavirus infection remained common up to age 24 years (at least 10%), with lower prevalence in older age groups, but it was always between 4% and 9% (Figure 1). The prevalences of asymptomatic infection were similar in males and females, both among children aged less than 5 years (both sexes: 24% (95% confidence interval (CI): 19, 29)) and among older children and adults (males: 11% (95% CI: 8, 13); females: 9% (95% CI: 7, 11)).

There was no marked seasonality in the prevalence of asymptomatic rotavirus infection in children aged less than 5 years. Prevalence ranged from 15% to 34% across the year, and the 95% confidence intervals for all monthly prevalences overlapped substantially (data not shown). In older children and adults, the highest prevalence occurred in February (17%) and June (15%); prevalence was lowest in December (2%) and fluctuated around 10% throughout the rest of the year.

Of the persons with asymptomatic rotavirus infection, 33% reported having had rhinitis, cough, or a sore throat during the 3 weeks before completion of the questionnaire, as compared with 21% of rotavirus-negative controls (prevalence ratio (adjusted for month of the year) = 1.6, 95% CI: 1.3, 2.0).

### Risk factors for asymptomatic rotavirus infection

Results from the multiple imputation models are presented in Table 2 and Table 3. The final model for the missing indicator analysis was identical to that from the multiple imputation analysis, with very similar effect estimates. Results from the missing indicator analysis are provided in Web Table 1 and Web Table 2 (<http://aje.oxfordjournals.org/>).

Children aged less than 5 years who were being cared for by a family member or child-minder (e.g., nanny) had lower odds of asymptomatic rotavirus infection than children who attended a nursery school, crèche, or play group or were

**Table 2.** Risk Factors for Asymptomatic Rotavirus Infection in Children Aged Less Than 5 Years, Study of Infectious Intestinal Disease in England, 1993–1996

Nursery Attendance	Exposure Prevalence, %		Odds Ratio <sup>a</sup>	95% Confidence Interval	P Value <sup>b</sup>
	Rotavirus-Negative (n = 458)	Rotavirus-Positive (n = 143)			
Nursery group/school, crèche, or play group	54.4	58.7	1.0		
Child-minder (e.g., nanny)	5.7	2.1	0.3	0.1, 1.0	0.06
Family care	2.2	1.4	0.7	0.2, 2.2	0.5
School	3.5	2.8	0.8	0.3, 2.6	0.7
Missing data	34.3	35.0			

<sup>a</sup> Odds ratios were adjusted for age (0–5 months, 6–11 months, 1 year, 2 years, 3 years, or 4 years).

<sup>b</sup> P value from a Wald test of regression coefficients.

**Table 3.** Risk Factors for Asymptomatic Rotavirus Infection in Older Children (Aged 5 Years or More) and Adults, Study of Infectious Intestinal Disease in England, 1993–1996

	Exposure Prevalence, %		Odds Ratio	95% Confidence Interval	P Value <sup>a</sup>
	Rotavirus-Negative (n = 1,221)	Rotavirus-Positive (n = 129)			
Baby in diapers in household					
Age-adjusted odds ratio <sup>b</sup>					
No	88.3	83.0	1.0		
Yes	8.7	14.0	1.7	0.9, 3.0	0.08
Missing data	3.0	3.0			
Age-group-specific odds ratio <sup>c</sup> , years					
5–24	9.9	8.3	0.8	0.3, 2.6	0.8
25–44	18.7	25.6	1.5	0.7, 3.0	0.3
≥45	0.5	7.9	15.0	2.9, 76.9	0.001
Salad consumed at home in previous 10 days <sup>b</sup>					
No	29.9	41.1	1.0		
Yes	70.1	58.9	0.6	0.4, 0.9	0.02

<sup>a</sup> P value from a Wald test of regression coefficients.

<sup>b</sup> Odds ratio was adjusted for age group (5–18, 19–24, 25–34, 35–44, or ≥45 years).

<sup>c</sup> P for interaction = 0.01.

already attending school, after we accounted for age-related differences in these exposures (Table 2).

In older children and adults, the presence of a baby in diapers in the household slightly increased the odds of asymptomatic rotavirus infection across all age groups (Table 3). However, there was strong evidence that age modified the effect of the presence of a baby in the household, with a great increase in the odds of infection among adults aged 45 years or older (Table 3). Eating salad prepared at home was associated with lower odds of asymptomatic rotavirus infection in older children and adults (Table 3).

We found no evidence of an association between asymptomatic rotavirus infection and contact with persons who had IID symptoms, nor was there an association with accommodation characteristics. Breastfeeding was not protective against asymptomatic rotavirus infection in infants. Web Table 3 and Web Table 4 (<http://aje.oxfordjournals.org/>) show the minimum detectable univariate odds ratios and univariate and multivariate odds ratios from the multiple imputation analysis for all variables that were not associated with asymptomatic rotavirus infection in the analysis.

## DISCUSSION

In this study, we have shown that the highest prevalence of asymptomatic rotavirus infection in England is found among young children in the general population; therefore, the age distribution of asymptomatic rotavirus infection is similar to the age distribution of rotavirus-associated IID (24). However, at least 5% of persons in older age groups were also infected. We found evidence that attendance at day care increased the risk of asymptomatic rotavirus infection in young children and that the presence of a baby in

diapers in the household substantially increased the risk of asymptomatic infection in adults aged 45 years or older. Preparing and eating salad at home was associated with a lower risk of rotavirus infection in older children and adults.

The collection of specimens from healthy persons in the Study of Infectious Intestinal Disease in England has allowed us to evaluate risk factors for asymptomatic rotavirus infection, which has not been done before. ELISA correlates well with rotavirus-associated IID because of the relatively high detection limit in comparison with RT-PCR (30, 31); in this study, we used the more sensitive RT-PCR assay to identify a large number of persons with asymptomatic rotavirus infection, who tend to have lower viral loads (31, 32). We used multiple imputation to account for missing responses in the data set, rather than assuming that those participants who completed all questionnaire items would be representative of all participants eligible for inclusion in the study (33, 34). There was a substantial proportion of missing responses for attendance at day care in children aged less than 5 years, which may have increased the variance in the multiple imputation model; we used 50 imputations to address this problem (34).

The asymptomatic rotavirus infections detected in this study were prevalent infections, not incident infections. The study participants were recruited at random from the general population, prior to determination of their rotavirus infection status. The duration of asymptomatic rotavirus infection was not measured, and it is likely that it varied substantially between individuals, as does the duration of virus shedding in symptomatic infection (35–39). Therefore, it is possible that for some persons, rotavirus infection occurred prior to the 10-day exposure period that was used for the

proximal risk factors in the questionnaire. Similarly, for those participants who were infected during the questionnaire exposure period, if their fecal specimen was not collected at the time of questionnaire completion, their rotavirus infection status may not have corresponded to the exposures reported. Only studies with frequent and regular stool testing in healthy persons could avoid this misclassification of exposure or outcome. However, such studies are resource-intensive and may be difficult to justify in terms of the benefits to patients or epidemiologic knowledge, without first demonstrating that asymptomatic infections are important for continuing transmission.

The lack of association between asymptomatic rotavirus infection and recent infectious contacts may be due to the inclusion of prevalent asymptomatic infections in the study. This is reflected in the increased risk associated with more distal factors that influence the probability of contact with infectious persons (day-care attendance and having a baby in the household); the highest incidence of rotavirus-associated IID is in infants and young children, so contact with this group increases the probability of rotavirus infection. However, the prevalence of asymptomatic infection is also high among infants and young children, so it is possible that transmission from asymptotically infected persons is an important source of further asymptomatic infections. While rotavirus shedding from asymptomatic persons may contribute to ongoing transmission, it is unlikely that asymptomatic rotavirus infections arise exclusively from other asymptomatic infections—that is, that asymptomatic infections arise from ingestion of smaller quantities of rotavirus—because the probability of illness after experimental rotavirus infection in adult volunteers is not related to the infecting dose (39). Furthermore, longitudinal studies in infants show that exposure history (i.e., preexisting immunity) is a strong predictor of the occurrence of symptoms after rotavirus infection (4, 40), indicating that host-level factors are likely to be the important determinants of symptom development after rotavirus infection.

The high potential for rotavirus transmission in day-care settings has been documented in many previous studies (41–47), as has the transmission of infection from children to adults within households (15, 17, 48–51). We found evidence of an increased risk of infection to adults from infants but not from sharing a household with children aged 1–5 years; this may reflect the lower incidence of symptomatic infection in children over 2 or 3 years of age in comparison with younger children (24).

In accordance with other studies, we found no evidence that breastfeeding was protective against asymptomatic rotavirus infection (52–54). Preparing and eating salad at home has previously been associated with lower risk of rotavirus-associated IID in this study population (20). It has been suggested that any protective effect of salad consumption is likely to operate via a long-term mechanism, such as boosting of intestinal immunity, or that salad consumption is correlated with other general lifestyle or dietary factors that are protective against IID (20, 22).

In contrast to studies of rotavirus-associated IID, we found no association between rotavirus infection and accommodation size (20), household crowding (55), or social

class. While investigators in several studies reported an increased risk of rotavirus-associated IID in children from lower social classes, many recruited rotavirus cases at general practices or hospitals (20, 56, 57), so it is possible that the frequently reported association between higher levels of social deprivation and rotavirus-associated IID is confounded by a greater tendency to seek medical care in lower social classes (58). Accordingly, no increased risk in lower social classes would be expected for asymptomatic infection.

We found no increased risk of rotavirus infection from contact with pets or other animals (59). While there is substantial evidence that some rotavirus strains circulating in human populations may have originated from animal viruses, particularly in low-income countries, it is likely that zoonotic transmission is rare, with subsequent spread of these viruses in humans occurring only after reassortment with a human rotavirus strain (60).

Investigators in other studies have reported typical symptoms of viral infection, such as fever, headache, and nausea, during rotavirus infections in which no diarrhea or vomiting occurs (16, 17). We found an excess of cold-like symptoms among asymptomatic rotavirus cases during the 3 weeks prior to completion of the epidemiologic questionnaire, although details on fever in healthy persons were not collected in the Study of Infectious Intestinal Disease in England. While it is possible that the cold-like symptoms may have been due to rotavirus infection, viruses causing colds and influenza are transmitted through similar routes as rotavirus (61), so coinfection with a respiratory virus may actually have been responsible for these cold-like symptoms.

The prevalence of asymptomatic rotavirus infection was relatively constant throughout the year, across all age groups, which has been observed in previous studies of rotavirus infection (62) but is in contrast to the distinct winter or spring seasonality of pediatric rotavirus-associated IID in temperate countries (63, 64). Therefore, the epidemiologic relation between symptomatic and asymptomatic rotavirus infection remains unclear. A recent mathematical modeling study suggested that variation in the seasonality of rotavirus-associated IID across the United States may be attributable to regional differences in birth rates; the majority of newborn, susceptible children are infected during each annual epidemic, and the timing of the next epidemic depends on the rate of accumulation of further susceptible persons from new births (64). If the availability of susceptible persons is the major factor driving rotavirus-associated IID seasonality, similar seasonality may not be expected in asymptomatic infection, because people remain susceptible to asymptomatic infection throughout life.

The findings from this study suggest that asymptomatic rotavirus infections are transmitted through the same routes as rotavirus-associated IID, predominantly person-to-person. It is therefore likely that host immunity, rather than infection route or dose, determines whether disease develops after infection. This conclusion is supported by evidence from experimental adult inoculation studies and longitudinal studies of natural pediatric rotavirus infection (4, 39, 65, 66). Previous studies have demonstrated that smaller quantities of rotavirus are shed during

asymptomatic rotavirus infections than during symptomatic rotavirus infections (31, 32); therefore, asymptotically infected persons may be less infectious, but further work is needed to determine their role in the transmission and persistence of rotavirus in human populations.

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