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Seroepidemiology of group A rotavirus in suburban São Paulo, Brazil

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SUMMARY

Age-specific patterns of rotavirus infection were investigated using a randomly selected and representative sample of sera from a suburban community of São Paulo, Brazil screened for class-specific antibodies to group A rotavirus. Age-serology of anti-rotavirus IgG showed primary infection predominant in young infants with a median age of around 18 months consistent with IgM serology suggesting highest rates of recent infection between ages 4 and 48 months. Anti-rotavirus serum IgA prevalence increased gradually with age. Paired samples from infants, collected 1 month apart, indicated high exposure rates with seroconversion occurring in several infants during the reported low transmission season. Between 5 and 10% of adults had elevated IgM levels indicative of recent infection and, potentially, of an important contribution adults may play to rotavirus transmission. Further understanding of the dynamics of rotavirus transmission within populations, at group and serotype level, would benefit the design and monitoring of future immunization programmes.

INTRODUCTION

Worldwide, rotaviral gastroenteritis is responsible for upwards of 1 million deaths and considerable morbidity each year in children under the age of 5 years [1]. The extent of rotavirus infection and associated disease amongst older age groups is less well documented due to milder clinical outcome [2] and thus reduced likelihood for case reporting. Mild disease in infants may also go largely unreported leading to a vast underestimate of the extent of infection and, therefore, of levels of rotavirus transmission within communities. In the UK, for example, a large proportion of rotaviral infection will be treated by community physicians [3].

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Rotaviruses are genetically diverse pathogens. Although many of the 14 VP7 based serotypes have been isolated from humans, four have been shown to be responsible for the majority of clinical disease to date [4]. Licensing of a live oral tetravalent rotavirus vaccine (containing the four common serotypes) is expected in the USA and Europe before the end of 1998 and will be considered as part of the WHO EPI programme [5]. The vaccine is partially effective against severe diarrhoea but apparently does not prevent viral shedding and, therefore, virus transmission. The effects of community-based use of such a vaccine on patterns of infection and disease are largely unknown. In part, this is because of insufficient understanding of the relationship between within host infection dynamics and population level patterns of

immunity and transmission. A better understanding of patterns of transmission, prior to immunization, is required so that optimal immunization programmes can be designed and possible effects on the stability of disease patterns can be predicted.

Detailed cross-sectional seroepidemiological data has proved useful in the past in linking within host patterns of infection with those at the population level [6], including the potential impact of mass vaccination. In particular, this has involved the use of mathematical models of infection transmission [6]. Such methodology applied to rotaviruses would have to be more complex to incorporate the existence of multiple strains and their possible interactions [7] as well as the possible role of animal reservoirs in rural populations. These aims would be a longer term goal of seroepidemiological and theoretical studies. However, basic epidemiological patterns of group A rotavirus remain to be investigated.

Primary infection with rotavirus in infants can occur very rapidly after birth, sometimes within the first few days [8]. Most infants have evidence of infection by 3 years of age as recorded in seroepidemiological studies from several countries [9–13]. However, studies to investigate dynamics of infection, including information on class-specific antibodies, across a broad age range and which are representative of normal populations are few [14]. Most studies of rotavirus epidemiology have concentrated on clinical and, less frequently, on sub-clinical, disease in infants, using (self)-selected samples (e.g. hospital admissions or outpatients) and have largely ignored patterns of infection in older age groups. There is a clear need for well-designed community studies of rotavirus based infection on fully age-stratified samples representative of the study population.

A serum survey conducted in São Paulo in the summer of 1990 [15] has provided the opportunity to investigate patterns of serological markers of rotavirus infection representative of a study population. We report age-specific and class-specific antibody titres to group A rotavirus and the results are interpreted with respect to patterns of viral transmission.

METHODS

Demographic details and methods used in sampling the community have been described in detail previously [15]. The study took place in December and January 1991. Blood samples for serum were collected from the town of Caieiras, in northern São Paulo, chosen as representative of a suburban Brazilian community. Standard theory [16] was applied to achieve a random 2-level cluster sample from families within randomly selected administrative regions. Informed consent was obtained from parents or guardians. Thirty-nine pairs of maternal-cord sera were also collected from Caieiras Regional Hospital. The age-structure of the samples used in this study are presented in Table 1. A repeat blood sample was collected from children under 2 years of age after a period of exactly 1 month. Serum separated from blood samples was stored at -20 °C. Aliquots were prediluted 1:20 in 1:1 phosphate-buffered saline (PBS)/glycerol to preserve antibody during storage and use.

Enzyme-linked immunosorbant assays (EIA) were used to measure group A anti-rotaviral antibodies. Bovine rotavirus strain UKtc was grown in BSC-1 cells and used as antigen, non-infected cells were used as control antigen. Infected and non-infected cells were prepared for use as antigen by freeze-thawing and clarified by low speed centrifugation. A modified indirect EIA was used for the detection of IgG and IgA antibodies. Plates were coated with optimally diluted polyclonal rabbit hyper-immune anti-rotavirus antibody (carbonate buffer, pH 9·6, 4 °C overnight). Antigen or control was added in excess to plates diluted in PBS with 0·05% Tween-20 (Tw) with 2% skimmed-milk powder (SMP) at room temperature (RT) for 2 h followed by washing three times using PBS-Tw, Test sera, diluted in PBS-Tw-SMP, were added at 1/200 to test and control plates for 2 h RT followed by washing. Rabbit anti-human HRP conjugate, IgG or IgA, (Dako) at 1/2000, was added for a further 2 h RT. After final washing TMB substrate in citrate-phosphate buffer was added for 30 min then stopped with 3 M H_2SO_4 and read at 450 nm. Paired samples were screened in adjacent wells on the same plate.

IgM antibodies were detected using a capture-antibody assay with incubation, washing and developing procedures as above. Plates were coated with optimally diluted affinity-purified goat anti-human IgM (Sigma) at 4 °C overnight. Sera was added at 1/100 dilution. Antigen diluted in PBS-Tw-SMP was then added in excess and was subsequently detected using an HRP-linked monoclonal anti-group A rotavirus antibody.

Internal standards made up from a pool of adult sera with high titre class-specific antibody were

Table 1. *Sample sizes, by age, collected in December 1990 (initial survey), including maternal-cord pairs and during a repeat survey (January 1991) exactly 1 month later*

Age in months	(years)	Initial	Repeat
0		39	0
1–2		27	18
3–4		22	18
5–6		27	23
7–8		48	36
9–10		37	22
11–12		27	17
13–18		29	22
19–23		25	17
24	(2)	50	0
36	(3)	44	0
48	(4)	33	0
60	(5)	14	0
–120	(–10)	35	0
–180	(–15)	12	0
–240	(–20)	20	0
–360	(–30)	43	0
–480	(–40)	17	0
Total		549	173

included in all plates. Neat standard was given the arbitrary concentration of 1000 units and double diluted to 1/128 (in PBS/glycerol) to construct a standard curve. Standards were included in each plate against which optical densities of test sera were compared and concentrations calculated in $\log_{10}$ units. Levels of antibody concentrations for antibody classes were not directly comparable. No correlations were seen between IgG, IgM and IgA titres in cord sera showing that the IgM and IgA assays were not detecting non-specifically bound anti-rotaviral IgG. Data were analysed using Quattro® Pro7 (Corel, 1997) and SPSS v.6 (SPSS Inc. 1993).

RESULTS

Frequency distributions of antibody concentrations for all samples for each antibody class can be seen in Figure 1. A clear bimodal distribution for IgG suggested a suitable cut-off for seropositivity at 1·5 log units (Fig. 1*a*). For IgM and IgA negative skewed unimodal distributions indicate considerable overlap between individuals with low titres and seronegatives (Fig. 1*b*, *c*). Class-specific antibody titres for all

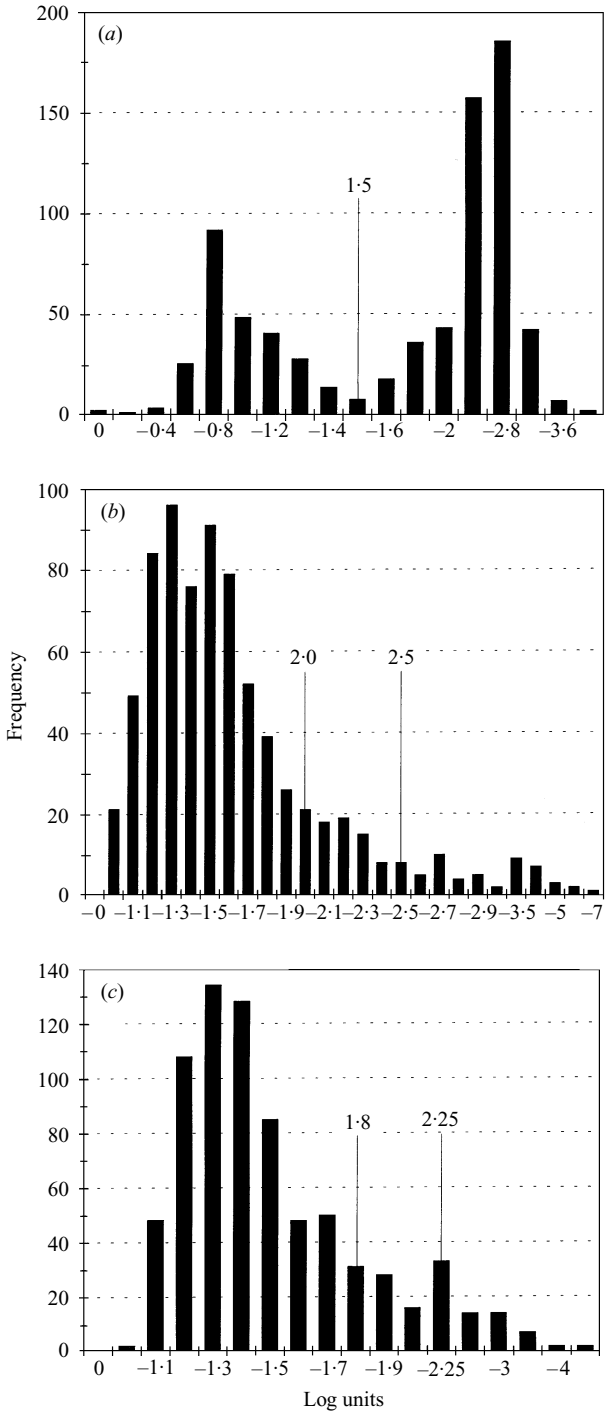

Fig. 1. Frequency distributions of antibody concentrations for total samples ($n = 722$). (*a*) IgG, (*b*) IgM and (*c*) IgA. Graphs show cut-off values ($\log_{10}$ units) used for age-serological profiles.

individuals in the initial survey ($n = 549$), regardless of serostatus are shown in Figure 2(*a*, *b*), for IgG and IgM respectively. Levels of IgG showed a clear decrease over the first 6–9 months of age consistent with decay in maternally derived antibodies. High

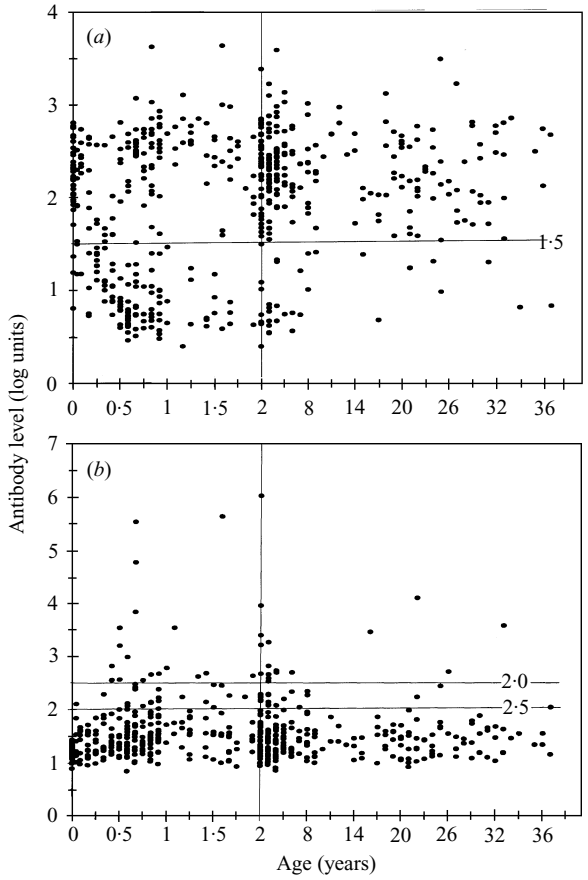

Fig. 2. Scatter plots showing antibody levels (log_{10} units) for all samples ($n = 549$). (*a*) IgG and (*b*) IgM. Note that age (*x* axis) is presented on two separate linear scales; 0–2 years and 3–38 years.

Table 2. *Summary statistics for IgG antibody levels for seropositive individuals (designated as > 1·5 log_{10} units, see Fig. 1a) aged between 6 months and 40 years showing separate details for maternal and cord samples*

Age group (months)	No.	Mean (log_{10} units)	Variance
6–15	71	2·51	0·118
16–35	69	2·35	0·174
36–60	78	2·36	0·143
72–120	27	2·25	0·116
132–240	30	2·34	0·149
252–480	53	2·23	0·190
Maternal	39	2·07	0·214
Cord	39	2·20	0·169

titres were recorded in some individuals in the first few months after birth suggesting recent infection. From 6 months of age two populations of individuals are

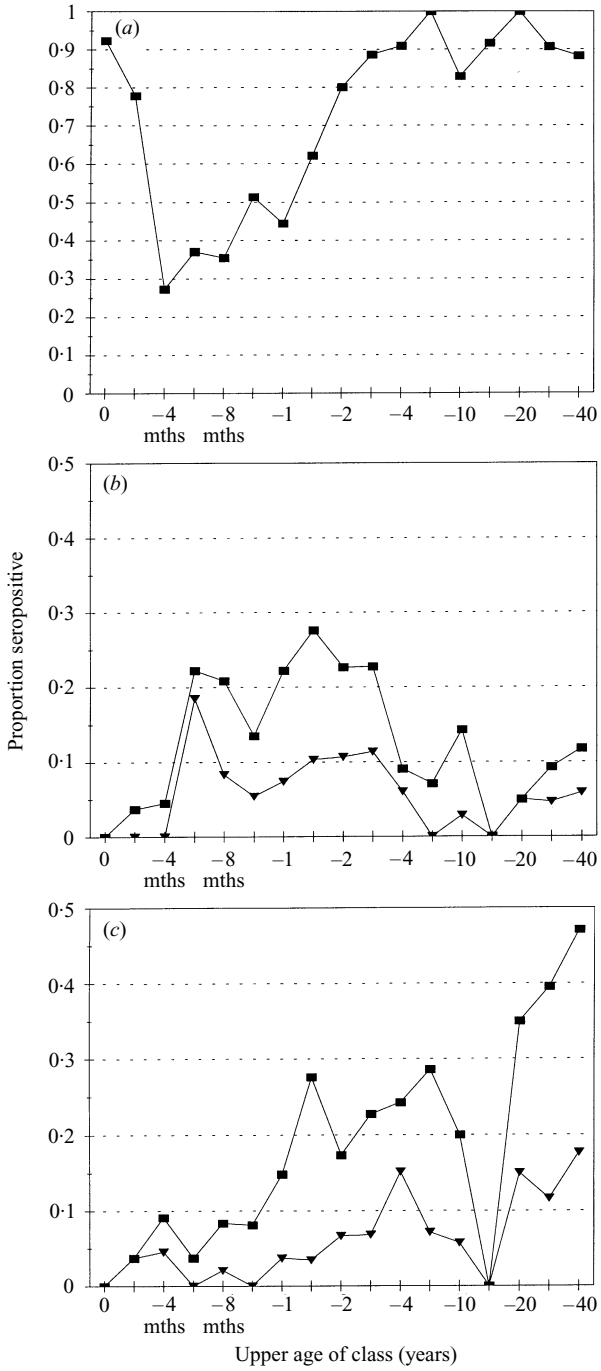

Fig. 3. The change, with age, in the proportion seropositive using arbitrary cut-off values (log_{10} units) based on frequency distributions in Figure 1. (*a*) IgG cut-off at 1·5 (■); (*b*) IgM at 2·0 (■) and 2·5 (▼); (*c*) IgA at 1·8 (■) and 2·25 (▼). Samples sizes for each age class as in Table 1.

visible, those with IgG levels < log 1·5 units (seronegative) and those with levels > log 1·5 units (seropositive) indicative of experience of primary infection. Beyond 2–3 years of age these two populations are less distinct with most individuals ap-

pearing seropositive. Means and variance of titres in different age groups were calculated for individuals with titres greater than 1·5 log units (Table 2.). Linear regression analysis revealed a marginally significant increase in titre between 6 and 15 months of age inclusive ($n = 71$, $F = 3{\cdot}98$, $P = 0{\cdot}0499$). Overall there was a significant decline in titre through to adulthood (age $> = 6$ months, $n = 328$, $F = 6{\cdot}74$, $P = 0{\cdot}0098$). Only IgG antibodies were maternally-derived. There was significant correlation in IgG titres between maternal and cord sera (correlation coefficient = 0·949) and mean titres (Table 2) in cord sera were significantly higher (*t*-test for paired samples, $t = -5{\cdot}41$, D.F. = 38, 95% CI $-0{\cdot}179$, $-0{\cdot}082$).

Age-specific trends in IgM antibody levels, Figure 2*b*, revealed that, whilst most individuals have low level antibody, some of individuals have significantly higher concentrations. Highest IgM titres were seen in infants aged between 6 months and 2 years of age. Nevertheless, all age groups, including adults, had individuals with high IgM titres.

Arbitrary cut-offs were defined from frequency distributions of antibody titres (Fig. 1) to illustrate age-trends more clearly. The age-serological profiles in Figure 3 give a clearer indication of age patterns of apparent seropositivity. There was a rapid rise of IgG prevalence, from 3 months to 5 years stabilizing around 90–95% in adults. IgM seroprevalence appears highest in the age range 6 months to 3 years and IgA prevalence appears to rise throughout the age profile. A prevalence of 0% in the 11 to 15-year-old age group was odd but possibly an artifact of small sample size ($n = 12$).

A comparison of IgG antibody levels in paired samples revealed only two samples with clear increases in antibody titre (data not shown). A greater proportion showed a decrease in antibody which, when stratified by age (Fig. 4*a*) could be seen mostly in infants less than 5 months of age reflecting decay in maternally-derived antibody. In most samples there was little variation. There was clearly more variation in IgM levels between the two time points (Fig. 4*b*). Several samples showed a large increase in titre with those initially high showing a decrease over the one month period. Increases were evenly distributed across age groups and decreases in slightly older infants (data not shown). IgA titres (Fig. 4*c*) were also seen to increase as well as decrease during the 1 month period in a number of individuals but were less pronounced than IgM.

Correlations between class-specific antibody titres within individuals in the cross-sectional survey and in paired samples were investigated but with small sample sizes of high titre individuals no obvious patterns emerged.

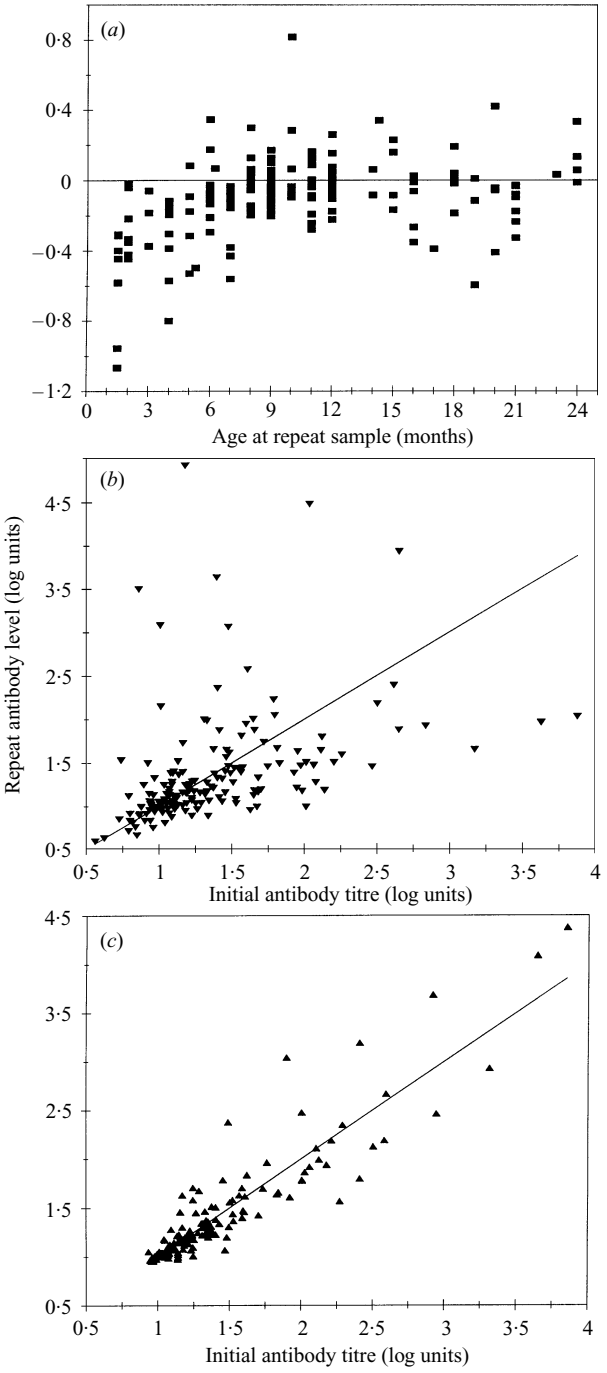

Fig. 4. The change in antibody titre after 1 month in paired samples from individuals aged between 1 and 24 months ($n = 173$) for (*a*) IgG, (*b*) IgM, (*c*) IgA. Graphs show (*a*) the change in titre for each monthly age group (IgG) or (*b*, *c*) the initial titre plotted against the titre in the repeat sample (IgM and IgA).

DISCUSSION

Studies in São Paulo, Brazil have identified rotavirus as an important cause of morbidity [17, 18]. Transmission has been reported to occur throughout the year but to show seasonality peaking in the winter months (July–September) [19]. The aim of this study was to use basic seroepidemiology as a means of increasing our understanding of infection dynamics within this population, provide data to support future mathematical modelling of rotavirus infection and form a basis for more detailed serotype-specific epidemiological studies. Samples used in this study were not biased towards individuals who may have gastroenteritis, or self-selected through hospital admissions or outpatients although individuals who may have had recent disease were not actively excluded. They are, therefore, representative of the normal population and the prevalence of any recent or current infection or seroconversion that occurs within the individuals due to rotavirus can be used as an indicator of transmission within the wider community at that time. Although transmission of rotavirus occurs throughout the year the study was carried out when transmission is reportedly lower. It is possible, therefore, that different serological patterns would be seen for a study conducted during the winter months. However, cross-sectional age-serological profiles not only measure age-dependent effects but time-dependent processes as well [6]. Seasonality in transmission patterns could be reflected in cross-sectional seroepidemiological surveys given large enough sample sizes to enhance sensitivity.

Although cut-off points were used to differentiate seropositive from seronegative individuals, data presented as titres from all individuals can give a more detailed impression of the dynamics of infection within communities. Other than for IgG no precise distinction between seropositive and negatives could be presumed, however arbitrary cut-offs gave indications of changes with age in the proportion of individuals with relatively high antibody levels. We have used IgM, shown to be short-lived after clinical rotaviral infection [20], as a marker of current or recent infection. The dynamics of an IgM response upon repeated infection are not well understood and may vary between individuals dependant on age, time since last exposure and perhaps more importantly on the infecting rotavirus serotype. It may be that repeated infection with a homologous serotype induces a lower or negligible IgM response. IgG seroconversion is certainly not always associated with an IgM response [21] suggesting that using IgM may underestimate reinfection. Furthermore, loss of IgM activity due to long term storage was likely to add to an underestimate of prevalence. Age-specific prevalence of high IgM antibody titres illustrates that most infection occurs in infants aged 4–24 months of age corresponding to the rapid rise in IgG seropositivity at this time. Significant changes in IgG antibody titres were observed and reflect age-specific dynamics of infection. An increase in mean titre during the second 6 months after birth, when most primary infections occur could simply result from the time required to develop a full IgG response following infection. Repeated exposure to infection boosting IgG responses or an increased immunocompetence with age, has been suggested for other viral infection, e.g. respiratory syncytial virus [22]. The decline in titre thereafter implies reduced exposure to reinfection or that reinfection does not subsequently affect IgG antibody responses. Mean titres continue to decline through adulthood as has been seen for other viral infections [23, 24] but remain seropositive indicating a long duration and/or constant re-exposure. The IgG profile does not reflect the amount of infection that may be occurring in older age groups after primary infection which can be seen from the IgM profile. High IgM levels seen in adults clearly implies a high re-exposure frequency. Although adults are becoming reinfected their role in transmission through viral shedding has not been studied extensively. Adults may act as an important source of infection and may contribute to maintenance of virus within communities [25]. The role of adult transmission in different epidemiological and socioeconomic settings, as well as the potential role of animals (livestock) in rural communities, needs further investigation.

The proportion IgA seropositive, which clearly rose constantly with age, is less easy to interpret. Low serum IgA titres within the community may not reflect its immunological importance during infection nor be sensitive enough to reflect the dynamics of secretory IgA in the intestine, likely to be the most important humoral response in protection, albeit short-lived [21, 26]. The increase in IgA seroprevalence with age may simply reflect cumulative exposure to the virus, or the increase in total IgA known to develop gradually with age [27], rather than dynamics of transmission and antibody titre may be more informative in population-based studies.

A lack of association between titres of different

classes of antibody was perhaps not surprising given the complex dynamics of humoral responses likely to exist. With the paired samples, although individuals appeared to become infected or show evidence of recent infection (IgM), the numbers were low and corresponding changes in IgA and IgG may have been missed within the 1 month period. Several other factors may influence antibody titre changes, e.g. infants are likely to be breast fed, the protective effect of which has caused debate [28] and may influence development of serum antibody. Additionally, as a strictly intestinal pathogen, antibody changes in the serum may not be as sensitive or immediate as those in the intestine, particularly those due to sub-clinical, repeated infection. The role of cell-mediated immunity (CMI) in rotaviral infection has been examined in animal models [29, 30] and clearly has an important role, although studies in humans have been limited [28]. It is not known how CMI may influence the age-specific dynamics of infection within populations.

This study has not taken into consideration the extensive genetic diversity of rotaviruses. Recent molecular epidemiological studies have now shown that although four serotypes are responsible for the majority of human disease globally, and it is these four to which a vaccine is being developed, other serotypes are not uncommon in many areas [31, 32] including São Paulo [33]. Seroepidemiological studies which examine age-specific rates of infection with different serotypes [34, 35] will give more insight into rates of transmission and interactions between these four, and other serotypes less prevalent in humans, especially now that EIA assays, more appropriate for population-based studies as opposed to plaque neutralization tests, have been developed [12, 36].

The rationale behind immunization programmes where the focus is on interruption of transmission, such as for measles and rubella, is to increase population immunity, by reducing the proportion susceptible, beyond a threshold which reduces the effective reproductive rate (R) below one such that chains of transmission cannot be perpetuated [6]. Those vaccinated or infected are considered immune for life and play no further role in transmission. This would not be the case with rotavirus where repeated infection, and, therefore, transmission occurs in all age classes. A rotaviral vaccine which neither provides complete protective immunity nor interrupts transmission will have, as yet, unexplored effects on patterns of infection and disease, particularly with the underlying complexity of genetic diversity. Furthermore, heterologous protection in natural disease may be less marked with vaccine protection. Studies on the population level effects of interacting rotavirus strains, each conferring partial cross-immunity accompanied by relevant immuno-epidemiological studies are required.

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