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Factors associated with rotavirus diarrhoea in children living in a socially diverse urban centre in Brazil

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ABSTRACT

A case–control study, aimed at identifying factors associated with rotavirus diarrhoea cases presenting to health facilities, was conducted in children from low-income and middle-low-income families in Brazil. Cases were 390 children with diarrhoea and rotavirus in stools; controls were 1674 children without diarrhoea presenting to the same facilities. Data were collected by questionnaire and observations during home visits. Explanatory variables were grouped according to a conceptual model of causation. The ORs by non-conditional logistic regression and population-attributable fractions were calculated. Socioeconomic factors contributed a third of cases, followed by contact with diarrhoea cases and by not being breast fed. In cases aged <1 year, not being breast fed was the main determinant, followed by socioeconomic factors, and crowding and contact outside the home; in older children, socioeconomic factors followed by contact inside and outside the home were the main determinants. Environmental and sanitation variables were not associated with diarrhoea in the final model, and socioeconomic factors were only partly mediated by proximal variables. Transmission of rotavirus appears to be mostly by person-to-person contact, and shows marked social differentials not explained by the biological factors studied. The rotavirus vaccine is unlikely to protect against the full range of circulating genotypes of rotavirus, and understanding rotavirus epidemiology remains essential to the development of control policies.

1. Introduction

Rotaviruses cause approximately 138 million episodes of diarrhoea a year in under-fives worldwide, resulting in 25 million clinic visits, 2 million hospitalizations, and 440 000 deaths.[1] The incidence of rotavirus diarrhoea is similar in developing and developed nations, but mortality by the age of 5 years is 200 times higher in low-income than in high-income countries.[1] Rotaviruses were detected in Brazil for the first time in 1976,[2] not only in patients with diarrhoea[3–5] but also in sewage, rivers and creeks.[6,7] In Brazil, rotaviruses are associated with 12–42% of hospitalized acute cases of diarrhoea in children, and community–based studies indicated an average incidence of 0.25 rotavirus-related diarrhoeal episodes per child-year.[4] In the city of Salvador, where this

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study was conducted, 8% of children with acute diarrhoea in a community-based study,[8] and one in three children hospitalized with acute diarrhoea[9] had rotavirus in stools.

Increased severity of diarrhoea leads to increased levels of medical attention, and where cases are recruited (community, health facility, hospital) will influence which risk factors are identified in research studies. Risk factors for rotavirus diarrhoea were studied in a range of settings.[10–21] The emerging pattern, although the risks were not always statistically significant, is that contact with another case of diarrhoea (in the home or outside the home) increased the risk, and breast feeding decreased the risk, in almost all settings. Day care (and in particular the first month of day care attendance) was found to increase the risk of presenting in studies based in a health facility or a hospital, and low birth weight increased the risk in hospital studies. Proxy variables for poverty such as the education of mother and poor housing carried an increased risk of presentation to a health facility and hospitalization. Food-handling hygiene and sanitation were investigated, with inconsistent results, only in a few studies (Supplementary Table 1).

This study aims to further our understanding of the factors associated with rotavirus diarrhoea presenting to a health facility in a socially variable urban setting in a middle-income country.

2. Methods

2.1. Study design, study population and data collection

The study was carried out between 2002 and 2004 in Salvador, Brazil which has a population of 2.5 million and marked socioeconomic inequalities. This analysis is part of a wider case–control study, investigating determinants of diarrhoea presenting to a health facility, by specific aetiologic agents, in children aged 0–10 years.[22] Cases were children presenting with diarrhoea as a main complaint in five health facilities of Salvador, which are owned by, or sell their services to, the public health system, and controls were selected from children attending the same health facilities, at well-baby consultations or because of other health problems not related to diarrhoea, such as orthopaedic procedures or evaluation before a surgical operation. There was imbalance between health centres in numbers of controls recruited; this reflected the proportion of children attending for diarrhoea compared with other causes suitable for inclusion as controls in each of the units. Rotavirus vaccine was not an issue, because the study was conducted before it was licensed In Brazil (in July 2005) and later introduced in routine vaccinations in 2006. Controls were frequency matched to cases by age and health insurance, as a proxy of socioeconomic status. Potential cases and controls who reported any episode of diarrhoea during the preceding 3 weeks were excluded. The person responsible for the child answered a questionnaire on socioeconomic conditions, characteristics of the dwelling and the domestic and peridomestic environment, child's health and occurrence of exposures to risk factors, defined as transient,[23] in the 10 days preceding the beginning of the diarrhoea episode, or the interview for controls. One week after the interview, a home visit was made, and complementary information about the house and the peridomestic environment was collected by direct observation, together with information, for cases, about the episode itself. Cases gave a stool sample, and were only included in the study when the sample was obtained. One out of approximately five control children, frequency matched by age, was asked for a stool sample; if the child was unable to provide one, the following child in the list would be asked until the sample was obtained. Seventy-three percent of the stool samples were collected at the first contact, and the remainder at the home visit. In this investigation, cases are the children with diarrhoea who tested positive for rotavirus, and controls are all the children without diarrhoea. None of the 384 controls who provided stools had rotavirus infection at the laboratory examination.

2.2. Laboratory examinations

Approximately 10% (wt/vol) suspensions in Tris-HCl Ca+2 0.01 M (pH 7.2) were prepared from the faecal specimens. Group A rotaviruses were detected by a combined enzyme immunoassay for rotavirus A and adenovirus (EIARA),[24] following the instructions of the manufacturer (Bio-Manguinhos, Oswaldo Cruz Foundation, Ministry of Health, Brasilia, Brazil). Concomitantly, stool suspensions were used for dsRNA extraction by the glass powder method,[25] followed by polyacrylamide gel electrophoresis (PAGE).

2.3. Data analysis

There were 390 cases and 1674 controls. At a significance level of 5%, this study had an 80% power to detect ORs of at least 1.45 for factors with an exposure frequency between 20% and 70% among the controls. The analyses were carried out following a pre-defined conceptual causal model (Figure 1), similar to the framework suggested by Victora et al.,[26] which maps the proposed relationships between social and biological determinants of rotavirus diarrhoea, separating direct biological causes from the social determinants that must act through a biological cause. In our model, socioeconomic factors occupy the most distal level. Three blocks at the intermediate level include the routes of pathogen transmission in diarrhoea (environmental contamination, food handling/preparation and person-to-person contact). The third and most proximal level includes only breast feeding.

Non-conditional logistic regression models were used to estimate the association between diarrhoea and potential determinants; all the models included age, as a continuous (months) variable, and gender as confounding factors, and the health centre of enrolment as a random effect factor. The random effect was used to model the heterogeneity of diarrhoea risk across the health centres that is not explained by the variables in the model (the so-called fixed effects). The Wald test was used to assess the significance of the associations. The covariates that, in these initial models, presented a p-value ≤ 0.20 were admitted to the next step, of intrablock backward selection, and only the covariates with p-values ≤ 0.05 at this second step

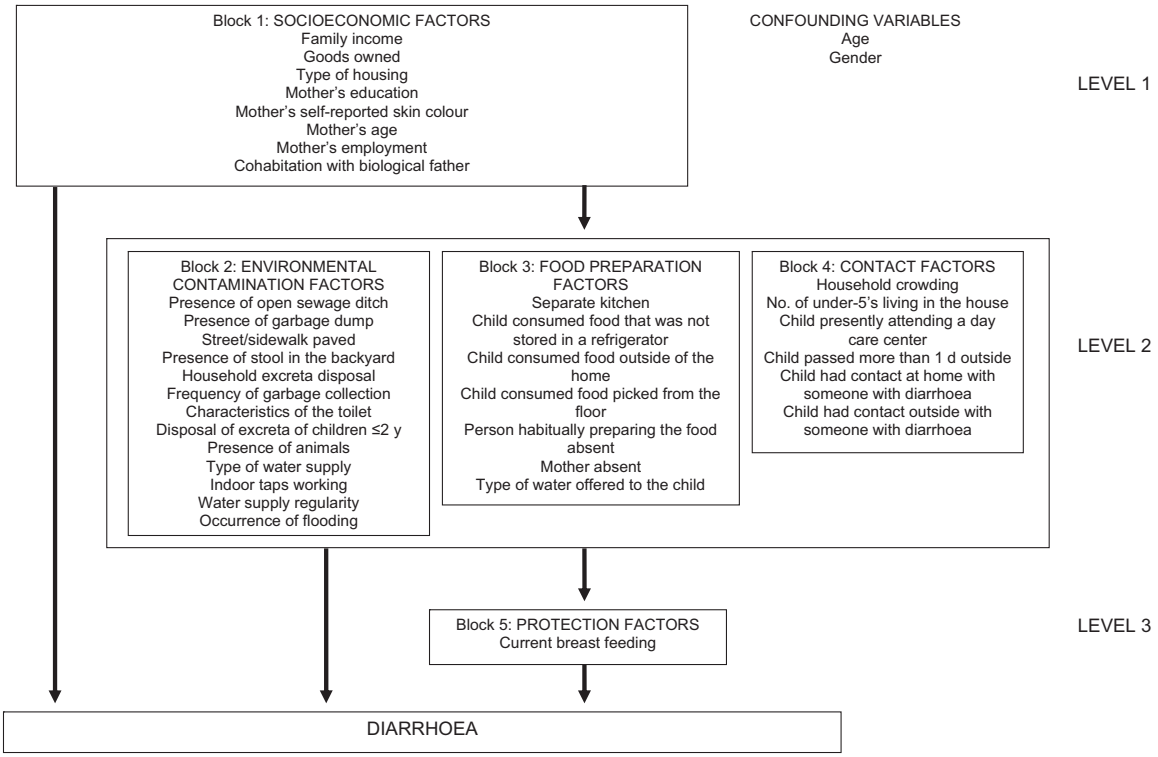

Figure 1. Conceptual model of determinants of diarrhoea with rotavirus.

were included in the hierarchical modelling. This reflects the proposed causal mechanism: Model A, including the variables of block 1, aims to obtain the overall effect of socioeconomic factors; Model B, adding to model A the significant variables of blocks 2–4, estimates the overall effect of the second level, adjusted for confounding in block 1, and the effects of block 1 not mediated through the second level. Model C adds to model B the third level variable, and aims to estimate the overall effect of current breast feeding, adjusted for confounding of blocks 1–4; the effects of blocks 2–4 not prevented by breast feeding; and the effects of block 1 not mediated through blocks 2–5. Models A, B and C were defined by fitting a sequence of multivariate regression models that kept, at the level last added, only the variables selected, again, by backward elimination (p-value $\leq$0.05).

In addition to ORs, individual population-attributable fractions (PAFs) were calculated for each variable, and summary PAFs calculated for each of the three levels and overall. Individual PAFs were calculated as the [(proportion of exposed cases) $\times$ (OR–1)/OR], estimated using the adjusted ORs before the proximal levels were included in the model. Summary PAFs were calculated as the complement of the product of the complements of PAFs of each variable in that level.[27] Summary PAFs for levels used the same ORs as individual PAFs; the overall summary PAF (expressing the proportion of all rotavirus cases explained by all the variables in the model) used the ORs from the model in which all levels were included, as these reflected the risk not mediated by variables in the proximal level.

These analyses were repeated for infants and children aged over 12 months. The analyses were carried out using the STATA statistical package, V 10.0 (STATA Corp., College Station, TX, USA).

3. Results

Three hundred and ninety children with diarrhoea and rotavirus and 1674 controls were included in this study. Overall, 53.3% were boys, 32.7% were <12 months old, 28.7% were 12–23 months old, 26.9% were 24–59 months old, and the rest 60–120 months old. The average duration of the diarrhoea episodes was 7.5 (3.7 SD) days; 89.0% had vomiting, 77.2% had fever and 21.3% underwent hospitalization, for an average of 5.1 (SD 3.6) days. In 78.8% of episodes children received packs of oral rehydration solution, and a further 10.4% received only home-made solutions.

Univariable analysis within each of the five blocks identified significant risk factors (Supplementary Table 2). In the socioeconomic block risk factors included living in a shack, mother's young age, work outside home and low education and owning less than four goods, In the sanitation block risk factors included no indoor piped water and lack of working indoor taps, occurrence of flooding, observed presence of stool in the backyard and inadequate disposal of the excreta of children aged <2 years. In the food preparation block risk factors included children consuming food in non-domestic spaces, mother or habitual food caterer being temporarily absent and children drinking potentially contaminated water. In the contact block risk factors included contact with a person with diarrhoea

outside the home, contact at home with a child <2 years of age with diarrhoea, domestic crowding (three or more persons/room) and presence of two or more children aged <5 years living in the same household as the child in the study. In the last block, not being currently breast fed was significantly associated with diarrhoea.

Most of the statistically significant socioeconomic factors were still significantly associated with diarrhoea in the intrablock analysis (Table 1; Supplementary Table 3, Model A), and kept similar effect magnitudes when each of the more proximal levels (postulated to mediate their effect) were introduced to the model (Supplementary Table 3, Models B and C). By contrast, of the eight factors of the second level that were significant in the intrablock analyses (Supplementary Table 3), only three (outside contact, home contact with cases of diarrhoea, and children consuming food in non-domestic spaces) remained significant when the first level was added (Table 1; Supplementary Table 3, Model B); the magnitude of their effects did not change extensively after adjusting for breast feeding (Supplementary Table 3, Model C). Finally, lack of current breast feeding was still associated, after adjusting for the first and second level factors, with a 38% higher chance of diarrhoea (Table 1; Supplementary Table 3, Model C).

The socioeconomic block contributed the highest proportion of cases (PAF 41%), due to mother's employment (25%) and limited schooling (13%); living in a shack contributed only 3% of the cases, despite the very high OR, because of the very low proportion (4%) of exposed cases. The contact block contributed the second highest proportion of cases (PAF 19%), equally due to outside and home contact. Lack of breast feeding had the second highest individual PAF (17%), followed by consumption of food outside the home (PAF 7%). The model explained two-thirds of all cases (PAF 64%) (Table 1).

A different picture appears when we consider infants separately. Fewer factors were significantly associated in the initial analysis (Supplementary Table 2): in the socioeconomic block, mother's low education and young age, and in the environmental block, lack of indoor taps working and lack of flush toilet. Consumption of food in non-domestic spaces and outside contact with a person with diarrhoea were associated with remarkably high ORs; home contact with a person with diarrhoea and domestic crowding were also associated. Lack of current breast feeding was significantly associated with a 149% higher chance of diarrhoea. In the hierarchical analysis, the effect of mother's young age was similar to that revealed by the initial analysis (Table 1; Supplementary Table 4, Model A); mother's education appeared to be partly mediated by the second level factors (Supplementary Table 4, Models B and C). At the second level, only household crowding and home contact with someone with diarrhoea remained significantly associated with diarrhoea (Table 1; Supplementary Table 4, Model B), and the effect of the former variable appeared slightly mediated by breast feeding (Supplementary Table 4, Model C). After adjusting for the first and second level factors, lack of current breast feeding was associated, if compared to the initial analysis, with an even higher chance of diarrhoea (Table 1; Supplementary Table 4, Model C). The highest PAF, 34%, was contributed to by lack of current breast feeding, given its high risk and high frequency. The socioeconomic block presented the second highest summary PAF (21%), mostly due to mother's education (16%), followed by contact factors (PAF 19%). The total PAF explained by the model was 56% (Table 1).

For the older children (Table 1; Supplementary Table 5), breast feeding was not significant; of the social variables, education of mother was not significant but ownership of goods was; and contact with another case of diarrhoea had a significant effect both outside and inside the house (with children both under and over 2). Among these children, the highest PAF, 32%, was contributed by the socioeconomic factors, followed by contact factors (PAF 24%) and consumption of food outside the home (PAF 10%), and the total PAF explained by the model was 51%.

4. Discussion

In our study no controls had rotavirus which is similar to the very low positive rate of rotavirus in children without diarrhoea found in other studies.[10,28]

In children aged <10 years, socioeconomic factors explained a large proportion of the cases (PAF 41%). Interpersonal contacts with diarrhoea cases were the second group of factors (PAF 19%), followed by child not being breast fed (PAF 17%) and consumption of foods outside the household (PAF 7%). A similar predominance of socioeconomic factors and interpersonal contacts was found in the main study of all diarrhoeas,[22] suggesting a profile of pathogenic agents in Salvador probably dominated by virus, and maybe shared with other agents also spreading via interpersonal routes. In infants, the most important factor was the child not being breast fed (PAF 34%), followed by socioeconomic factors (PAF 21%) and by crowding and interpersonal contact with other diarrhoea cases (PAF 19%). In older children, contact inside and outside the house and ownership of goods, but not mother's education, increased the risk. Environmental and sanitation variables were not associated with rotavirus diarrhoea once the other variables were controlled for in the final model at any age. The use of a hierarchical modelling made it clear that most of the socioeconomic effect was not mediated by the factors directly related to transmission that were investigated here.

This study was conducted in the main primary health care units of the city of Salvador, and we feel confident that cases were a good representation of diarrhoea cases seeking medical care at the primary level. A strength of our study is that all cases were requested to provide a stool sample, even those that under routine practice would not have their stools tested, which is expected to provide a more complete picture of factors associated with cases of rotavirus diarrhoea presenting to health services. A potential limitation of the study is recall bias, which is always a possible issue in case–control design.

We present both OR and PAF: the OR as a measure of the strength of the association, and the PAF as a measure of the relative contribution of the risk factors to the burden of the disease presenting to heath services, therefore indicating the importance of the risk factor from a public health perspective. Because Salvador is a city in transition, the relative

Table 1
ORs and 95% CIs for the best models[a] for the three hierarchical levels by total and by age group

Determinant		Total		Children aged <12 m		Children aged 12–<60 m	
		OR (95% CI)	PAF	OR (95% CI)	PAF	OR (95% CI)	PAF
Level 1							
Block 1: Socioeconomic factors							
Goods owned	>4					1.00	
	1–4					1.50 (1.02–2.19)	0.26
Type of housing	House/apartment	1.00				1.00	
	Shack	4.20 (1.97–8.95)	0.03			3.64 (1.44–9.18)	0.04
Mother's education	High school or higher education	1.00		1.00			
	4th to 8th grade	1.23 (0.95–1.60)	0.08	1.17 (0.76–1.82)	0.07		
	<4th grade or without schooling	1.58 (1.07–2.33)	0.05	2.27 (1.18–4.38)	0.09		
Mother's age (y)	>18	1.00		1.00		1.00	
	≤18	1.96 (1.07–2.33)	0.05	2.26 (1.18–4.34)	0.08	1.99 (1.01–3.91)	0.04
Mother's employment	Work at home	1.00					
	No work	1.26 (0.78–2.03)	0.14				
	Work outside home	1.84 (1.10–3.08)	0.11				
Socioeconomic factors PAF			0.41		0.21		0.32
Level 2							
Block 2: Environmental contamination factors							
Environmental factors PAF			0.00		0.00		0.00
Block 3: Food preparation factors							
Child consumed food outside of the home	No/yes, in another house	1.00				1.00	
	Yes, in other places	2.36 (1.50–3.71)	0.07			2.84 (1.61–5.00)	0.10
Food factors PAF			0.07		0.00		0.10
Block 4: Contact factors							
Household crowding	1–2 persons/room			1.00			
	≥3 persons/room			1.80 (1.09–2.96)	0.13		
Child had contact at home with someone with diarrhoea	No	1.00				1.00	
	Yes, with person(s) aged ≥2 y	1.74 (1.24–2.46)	0.08			2.11 (1.33–3.33)	0.11
	Yes, with person(s) aged <2 y	2.80 (1.16–6.76)	0.02			4.13 (1.49–11.47)	0.03
Child had contact outside with someone with diarrhoea	No	1.00		1.00		1.00	
	Yes	7.51 (4.29–13.14)	0.10	8.58 (2.75–26.78)	0.07	9.64 (4.39–21.14)	0.11
Contact factors PAF			0.19		0.19		0.24
Level 3							
Block 5: Protection factors							
Child on breast feeding	Yes	1.00		1.00			
	No	1.38 (1.03–1.84)	0.17	2.61 (1.67–4.09)	0.34		
Protection factors PAF			0.17		0.34		0.00
Total PAF			0.64		0.56		0.51

PAF: population-attributable fraction.
[a] Overall effect of the factors at each level.

contribution of the risk factors for rotavirus diarrhoea is changing, and the PAFs registered the importance of different variables at specific moments in this evolving process of change. A city-wide sanitation programme started in 1996 and by 2004 increased the sewerage coverage at household level from 26% to >60%, reducing by 22% the overall longitudinal diarrhoea prevalence in preschool children.[29]

Contact with a known case of diarrhoea (both outside and inside the house, particularly with children aged <2 years) was associated, overall and in older children, with rotavirus presenting to a health facility; in younger children only contact outside the house was a risk. It is biologically more plausible that contact increases the risk of acquiring infection rather than the risk of presenting to a health facility. Contact with another case of diarrhoea increasing the risk of transmission is consistent with the literature,[11–13,15,16,18,20,21] and the increase in risk associated with young contacts is consistent with rotavirus being a frequent cause of diarrhoea in the young. Measures to reduce the opportunity for children with diarrhoea to transmit infection include reduction of crowding, increase in hygiene, and keeping ill children at home. Although inadequate disposal of young children faeces has been postulated as a source of environment contamination,[30] we did not confirm this in our study. So our results support the hypothesis of person to person, rather than faecal–oral, being the main mode of rotavirus transmission.

In our study, being currently breast fed (whether or not other foods were consumed) was significantly protective in children under 1 year. This is consistent with previous findings of good protection in the early years of life and no protection afterwards.[10,14]

Day care was not significantly associated in multivariate analysis, although it has been identified as a risk in other health facility and hospital based studies, particularly in the first month attending day care.[15,16] The absence of an association in our study may be due to the fact that in Brazil is usual for children to accompany their parents in everyday social occasions, so opportunities for rotavirus transmission are not restricted to the crèche environment. This is consistent with the increasing risk in children aged <2 years with contact with diarrhoea outside home.

Finally, socioeconomic variables—living in a shack, mother's young age, low education and work outside home—contributed to 30–40% of all cases, and this did not decrease when we controlled for proximal variables (postulated to mediate the effect of socioeconomic variables). How can we interpret this fact? There is a limited number of possible explanations: that socioeconomic variables act through behaviours or environmental exposures facilitating transmission of rotavirus that we did not investigate, or were not able to measure accurately; that socioeconomic variables are associated with increased vulnerability of the children to the virus (for example via a nutritional deficiency) or that they increase not the risk of disease but the risk of presentation to health services given disease (this would require presentation of controls for well-baby consultations or other causes not to be influenced by those social variables in the same degree). Tam et al. have shown that in England, mothers with low education are more likely to present to health services, for the same age of the child and severity of disease.[31] Data is scarce in Salvador, but preliminary analysis of a cohort study described elsewhere[32] suggest that in Salvador the reverse is true, and the higher the educational level of the mother the higher the likelihood of presentation to health services given diarrhoea (A. Strina, personal observation).

So if this is confirmed, and there is a major impact on rotavirus incidence of living in a shack, mothers' young age, low education and work outside home, a large proportion of cases attributable to socioeconomic factors remains with no known mediating mechanism. The possibility of prevention for this group of cases (other than by vaccination) rests into two complementary options: to continue the efforts to identify the mechanisms enabling infection or disease, and to address them directly; and to improve social conditions, for example through actions targeted to supply better housing or to improve maternal education.

The results of this study confirm that diarrhoea with rotavirus requiring medical attention is not restricted to a narrowly defined group and confirm that vaccination should be universal rather than targeted. Our results also identified social differential in risk of rotavirus disease severe enough to require medical attention that are not clearly mediated by the biological variables studied; thus providing a baseline for post vaccination studies to explore, among other questions, whether vaccination reduced these social differentials.

This study was conducted before the routine vaccination, with an anti-G1P[8] RV-A vaccine, was introduced in Brazil in March 2006, reaching in 2009 the coverage of 81.5% for the 2nd dose.[33] Protection against severe rotavirus diarrhoea assessed in a multicentre trial, carried out mostly in Latin America, was 92% for the G1P[8] genotype, 87% for pooled G3P[8], G4P[8] and G9P[8] genotypes, but only 41% for the fully heterotopic G2P[4] genotype.[34] The lower protection for this genotype was confirmed in a second trial.[35] Following introduction of rotavirus vaccine, it was observed that the proportion of rotavirus diarrhoeas in Brazil caused by G2P[4]/G2P[non typed] genotypes was 74%,[36] suggesting that vaccine is unlikely to protect against the full range of circulating genotypes of rotavirus. Early evidence suggesting that vaccination might not remove concern with rotavirus as a cause of diarrhoea comes from a 5-year long study of hospitalizations (before and after introduction of vaccine) showing only a marginal reduction of hospitalization rate for childhood gastroenteritis, following the vaccine introduction.[37]

Understanding the rotavirus epidemiology remains, therefore, necessary to define control policies other than vaccination.

Authors' contributions: AS, LCR, SC, HCR and MLB designed the study; AS and SRF carried out the data collection; SRF, AMF and JPL performed the laboratory analyses; AS and SRF analysed the data; AS, LCR, SC, SRF, JPL, HCR and MLB interpreted the data; AS drafted the manuscript. All authors contributed to the revision of the manuscript and read and approved the final version. AS and MLB are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: The protocol for this study was granted approval by the Research Ethics Committee of the Instituto de Saúde Coletiva, Federal University of Bahia. The children were only included in the study after the adults responsible for them had read and signed a statement of free and informed consent.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2012.02.001.

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