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## Effectiveness of rotavirus vaccine against hospitalized rotavirus diarrhea: A case–control study



Maria Y.T. Ichihara<sup>a,\*</sup>, Laura C. Rodrigues<sup>b</sup>, Carlos A.S. Teles Santos<sup>c</sup>,  
 Maria da Gloria L.C. Teixeira<sup>a</sup>, Sandra R. De Jesus<sup>d</sup>, Sheila M. Alvim De Matos<sup>a</sup>,  
 Jose P. Gagliardi Leite<sup>e</sup>, Mauricio L. Barreto<sup>a</sup>

<sup>a</sup> Institute of Collective Health, Federal University of Bahia, Rua Basílio da Gama, s/n, Campus Universitário do Canela, CEP 40110-040, Salvador, Bahia, Brazil

<sup>b</sup> Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, WC1H 7HT London, United Kingdom

<sup>c</sup> Department of Exact Sciences, University of the State of Feira de Santana, Avenida Transnordestina, Novo Horizonte, CEP 44036-900, Feira de Santana, Bahia, Brazil

<sup>d</sup> Institute Multidisciplinar of Health, Campus Anísio Teixeira, Federal University of Bahia, Vitória da Conquista, Bahia, Brazil

<sup>e</sup> Avenida Brasil, 4365–Pav. Hélio & Peggy Pereira, CEP 21040-360, Rio de Janeiro, Manginhos, Brazil

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### ABSTRACT

Rotavirus is one of the leading cause of hospitalization and outpatients visits among children under five years. This study evaluated overall and genotype-specific vaccine effectiveness of oral monovalent rotavirus vaccine (G1P[8] strain) in preventing hospital admission of Brazilian children with rotavirus acute diarrhea.

A hospital based case–control study was conducted in five Regions of Brazil using the National Rotavirus Acute Diarrhea Surveillance System from July 2008 to August 2011. A total of 215 cases (aged 4–24 months) admitted with confirmed rotavirus diarrhea were recruited and 1961 controls hospitalized without diarrhea were frequency matched by sex and age group to cases.

Two-dose adjusted vaccine effectiveness (adjusted by year of birth and the frequency matching variables) was 76% (95%CI: 58–86) lasting for two years. Effectiveness controlled by the available potential confounders was 72% (95%CI: 44–85), suggesting no appreciable confounding by those factors for which adjustment was made. In a half of the cases the rotavirus genotype was G2P[4] and in 15% G1P[8]. Genotype-specific VE (two doses) was 89% (95%CI: 78–95), for G1P[8] and 76% (95%CI: 64–84) for G2P[4]. For all G1, it was 74% (95%CI: 35–90), for all G2, 76% (95%CI: 63–84), and for all non G1/G2 genotypes, 63% (95%CI: –27–99). Effectiveness for one dose was 62% (95%CI: 39–97).

Effectiveness of two-dose monovalent rotavirus vaccine in preventing hospital admission with rotavirus diarrhea was high, lasted for two years and it was similar against both G1P[8] and G2P[4]. Based on the findings of the study we recommend the continued use of rotavirus in the Brazilian National Immunization Program and the monitoring of the early emergence of unusual and novel rotavirus genotypes.

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## 1. Introduction

Acute diarrhea (AD) is a frequent cause of child hospitalization and outpatient visits in children under 5 years [1]. In Brazil, before introduction of the rotavirus vaccine in 2006, about 120,000 hospitalizations a year occurred due to AD in children under five years (DATASUS/Ministry of Health of Brazil, 2006).

Rotavirus is the leading cause of severe acute diarrhea in children in developed and in developing countries and is the major cause of death in poor countries [2,3]. Seven groups of rotavirus have been identified (A to G) and group A (RV-A) is responsible for more than 90% of human rotavirus infections [4]. RV-A has great genetic diversity due almost 60 serotypes (G and P) and the most

\* Corresponding author at: Rua Basílio da Gama, s/n, 4<sup>o</sup> Andar, Campus Universitário do Canela, Canela – CEP 40110-040, Salvador, Bahia, Brazil.  
 Tel.: +55 71 32837445; fax: +55 71 33360695; mobile: +55 71 88453456.

E-mail addresses: [my.ichihara@gmail.com](mailto:my.ichihara@gmail.com) (M.Y.T. Ichihara),  
[laura.rodrigues@lshtm.ac.uk](mailto:laura.rodrigues@lshtm.ac.uk) (L.C. Rodrigues), [carlosateles@yahoo.com.br](mailto:carlosateles@yahoo.com.br)  
 (C.A.S. Teles Santos), [magloria@ufba.br](mailto:magloria@ufba.br) (M.d.G.L.C. Teixeira), [sandreg@hotmmail.com](mailto:sandreg@hotmmail.com)  
 (S.R. De Jesus), [sheilaalvim@hotmail.com](mailto:sheilaalvim@hotmail.com) (S.M. Alvim De Matos),  
[jpgleite@gmail.com](mailto:jpgleite@gmail.com) (J.P. Gagliardi Leite), [mauricio@ufba.br](mailto:mauricio@ufba.br) (M.L. Barreto).

common strains are: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] [5]. In Brazil, between 12% and 42% of children under 5 years with diarrhea had positive stool samples for RV-A before the introduction of the RV-A vaccine. This increased from 22% to 38% in children hospitalized for AD [6,7]. More than 51 genotype combinations were reported and the most common genotypes described were G1P[8], G9P[8] and G2P[4] [8].

Vaccination is the better measure to prevent rotavirus [1,2,9] and its adoption has been recommended by World Health Organization [10]. An attenuated monovalent human RV-A (G1P[8] strain; Rotarix®) and a pentavalent bovine-human reassortant (G1,G2,G3,G4 and P[8] strains; RotaTeq®) are licensed worldwide. Rotarix® was introduced in the Brazilian National Immunization Program (BNIP) in 2006 in a two-dose schedule at 2 and 4 months of age and co-administered with tetravalent, pneumococcal and poliovirus vaccines.

RV-A vaccine efficacy against severe RV-A AD varied between more than 90% Europe and Asia, 85% in Latin America, 72% in South Africa to 49% in Malawi [11–14]. Three case-control studies carried out in a high income country (Belgium) [15] and in low to middle-income countries (El Salvador and Bolivia) [16,17] found a two-dose vaccine effectiveness of 90%; 76% and 77% respectively and a one-dose effectiveness of 91%; 51% and 56% respectively against hospitalization by RV-A AD. In Brazil, two small case control studies showed a range of 40–85% effectiveness in preventing hospitalization caused by G2P[4] [18,19]. The reason for variation in vaccine protection is not clear and has been attributed to antigen diversity, malnutrition and higher incidence of other enteric pathogens [20]. There is strong suggestion of cross protection among genotypes [11–14].

The introduction of RV-A vaccination was followed by a reduction in child hospitalization due to all causes of AD in Brazil, El Salvador and Mexico ranging from 17 to 51% [21–23] and a reduction in mortality from AD in children under 5 years in Brazil of 22% and in Mexico of 41% [24].

This study will evaluate the overall effectiveness of the oral monovalent vaccine, used in routine health services, in preventing Brazilian child hospitalization with RV-A AD. It will also evaluate overall and genotype-specific VE by time since second dose vaccination (up to two years), and genotype-specific VE.

## 2. Methods

### 2.1. Study design

This was a hospital based case-control study, frequency-matched by sex and age group. Hospitals were general hospitals which received children with a large range of diseases coming from a similar geographical catchment area. Seventeen of the hospitals enrolled in the RV-A AD National Surveillance System were invited to participate in the study, based on having had a large number of RV-A positive samples in 2007, adequate level of organization of the unit and data accessibility. After consultation and agreement on logistical arrangements with the Federal Health Surveillance (SVS/MS), the epidemiological surveillance of the hospitals and of the states, the Central Public Health and National Reference Laboratories, 10 hospitals located in five macro-regions of Brazil (6 state capital cities and 4 municipalities) were selected.

## 3. Participants

### 3.1. Eligible children

Children were eligible to participate in the study if they were admitted in the study hospitals, were aged 4 to 24 months (and therefore old enough to have received their second dose of rotavirus vaccine) and did not have diarrhea up to three weeks before admission or during hospitalization. All eligible children

were listed and screened to exclude children who had any health condition presumed to reduce vaccine effectiveness (immunodeficiency, gastrointestinal disease (e.g. diverticulitis), malformations or neoplasm conditions related to vaccine effectiveness, general signs and symptoms, infectious and parasitic diseases), those who had received the second dose of vaccine in the 15 days before hospitalization, or whose vaccination did not follow the BNIP schedule. All that fulfilled the specific criteria for either effective's case or control were included. This aimed to select controls from the population that produced the cases, as cases hospitalized by AD or by other diseases were likely to come from the same population given the universal health care system in Brazil.

### 3.2. Potential cases and controls

Inclusion criteria for potential cases were: admission with AD (defined as three or more liquid stools in 24 h, up to 14 days before admission), stool sample was collected until 48 h after admission and positive for RV-A and stay in hospital for at least 24 h. Children were included in the study in the first hospitalization only and had no associate disease.

Inclusion criteria for controls were: admission from the same hospitals of the cases with respiratory, genitourinary, musculoskeletal, nervous systems, skin and subcutaneous tissue, ear and mastoid processes, eye and adnexa diseases, and external causes. Controls were not included if they had a previous history of RV-A diarrhea or had a vaccine-preventable disease (as children who did not receive one vaccine are more likely to not receive other vaccines).

All potential controls fulfilling the criteria above undergone a further selection for frequency matching, so that the all effective controls had the same distribution of the main confounding variables (sex and age group on admission: 4–6 months; 7–11 months and 12–24 months) as the cases. This approach aimed to select from the pool of potential controls, an effective control group with the same distribution of confounders as the effective cases; in the situation in which more controls than needed were available in the frequency matched groups they were selected at random. Random selection of frequency matched effective controls from the pool of potential controls was done using the “sample” command of the Stata version 11.0

### 3.3. Effective cases and controls

*Cases:* All potential cases fulfilling the criteria above and had stools positive for rotavirus confirmed by the reference laboratory were included.

*Controls:* All potential controls fulfilling the criteria above and random selected for frequency matching were included.

One stool sample was collected up to 48 h after admission as part of the RV-A AD Surveillance System. Samples were stored and transported to the LACENs of each State where the hospital was located, according to the guidelines of the General Coordination of Public Health Laboratories/Ministry of Health of Brazil (CGLAB/SVS/MS). RV-A investigation was done by Enzyme Immune Assay (EIA), using commercial kits, following the manufacture' recommendation (Dako® or Oxoide®).

### 3.4. Laboratory investigation of potential cases

All positive samples for RV-A and 25% of negative samples were sent to a reference laboratory. According to the LACEN localization, this was either the National Reference Laboratory (Evandro Chagas Institute [Belém, PA], or a Regional Reference Laboratory (Adolfo Lutz Institute [São Paulo, SP], and Oswaldo Cruz Institute [Rio de Janeiro, RJ]). Results were confirmed by EIA and polyacrylamide gel electrophoresis (PAGE) according to Leite et al. [25].

Fecal suspensions and nucleic acids extraction were carried out according to Leite et al. [25] and Boom et al. [26], respectively. The RV-Genotyping was conducted using RT-PCR as described by Das et al. [27] (“G” genotype) and Gentsch et al. [28] (“P” genotypes). RV-A genotypes were e-mailed to CGLAB/SVS/MS and sent to the Institute of Collective Health, Federal University of Bahia (ISC/UFBa).

#### 4. Data collection

Information from cases and controls was collected by interviewers who visited all hospitals daily, from July 2008 to August 2011. Medical records were reviewed and the child’s carer answered a standard questionnaire on identification, clinical history and evolution, socio-economic status, sanitation, feeding and nutritional status of the child, and maternal reproductive aspects. The vaccination status of the child was assessed through the vaccination card, asked for during hospitalization. Also, data were obtained by home visits, telephone or the family health team of the area of residence of the child. Vaccination status was classified according to the presence and number of doses and time between last dose and hospitalization. Weight at admission was taken from hospital records and its deficit evaluated according to the weight-age standards of the National Centre for Health Statistics (NCHS) for boys and girls [29]. Mother’s skin color was self reported.

Questionnaires for all potential cases and controls were sent to ISC/UFBa and reviewers confirmed the classification of cases and controls by assessing the inclusion and exclusion criteria.

To complement data on maternal reproductive period and child birth we consulted live births routine data (SINASC) from 7 cities. This system covers 80–90% of births in Brazil. The child age on admission and on administration of first and second doses and breastfeeding duration were calculated in days at the date of admission. Cases and controls were classified into three age-groups, according to age on admission: 4–6 months, 7–11 months and 12–24 months.

#### 5. Sample size

The minimum sample size required (using EPI-INFO 6.0) was 88 cases and 88 controls (for vaccine coverage of 70%, VE of 65%, 95% confidence interval and 90% power. The achieved sample size of 215 cases and 1961 controls enabled estimation of genotype-specific vaccine effectiveness.

#### 6. Statistical analysis

Vaccine effectiveness was obtained by multivariable unconditional logistic regression, which is appropriate when frequency matching is used. The odds ratio was adjusted for: a) sex and age both used for frequency-matching, b) year of birth, to control coverage of vaccine by year and c) robust variance estimation of Jackknife, with clusters being hospitals. Potential confounders were included in the final logistic model when the *p*-value of association was <0.20 (bivariate analysis). We used the backward method to analyze the presence of confounding. The best adjustment was given by the Akaike information criterion (AIC) [30]. Given the absence of confounding by measured variables apparent in the analysis by number of doses, the subsequent analysis by time since second dose vaccination, genotype-specific was conducted without controlling for confounders other than age, sex, year of birth, and robust variance estimation of Jackknife. The frequency of missing values for any confounding variable was very low (less than 1%), and they were attributed to the category of reference (considered not exposed) to keep all cases in the analysis. We repeated the

analysis stratified by year of admission to control for increasing vaccine coverage with time. A sensitivity analysis (for two doses only) was done, in which cases and controls with missing vaccination cards were treated as vaccinated or unvaccinated, so assuming non differential missingness. The VE was calculated by the following formula:  $VE = (1 - \text{odds ratio of vaccination}) \times 100$ . Statistical analysis was performed with Stata version 12.1 (Copyright 1985–2011 StataCorp).

**Ethics:** This study was approved by the Committee of ISC/UFBa (Protocol 017-08/CEP/ISC-2008). Carers of participating children signed a written informed consent form.

## 7. Results

### 7.1. Study population

A total of 4955 eligible children aged between 4 and 24 months were recruited into the study from July 2008 to August 2011. Of these, 697 children did not fulfill the criteria of inclusion related to information on vaccination: 268 did not have a vaccine card; 299 had received vaccination in a different schedule from that recommended by the BNIP; and 130 had received the second dose fewer than 15 days before admission. (Fig. 1 shows the breakdown of exclusions for effective cases and controls). In addition, 298 eligible children with AD did not fulfill the criteria of inclusion related to the stool sample collection: in 202 a stool sample was not collected; in 33 the samples were lost, and in 63 the sample was collected too long after admission. Samples of 965 potential cases were tested for RV-A with the following results: 722 were negative (of which 142 had another virus identified and 28 were positive on the first test but negative in the reference laboratory) and 215 were positive for RV-A confirmed by EIA and/or PAGE and RT-PCR. Of all eligible children for controls, 191 had developed diarrhea during hospitalization and were not selected to the study and 843 were not needed given the frequency match. A total of 215 effective cases and 1961 effective controls were recruited.

Characteristics of the study population are presented in the Supplementary tables (1a,1b,1c). The mean age of the cases and controls was 14 months. Compared to controls, cases had lower socio-economic status and sanitary level, their mothers had fewer years of schooling and their families lived in smaller houses with many family members and more than one child under 5 years. Smoking and alcohol consumption during pregnancy and delayed start of prenatal care were significantly higher among cases. Also, one or more visits to health services or hospitalizations due to diarrhea before the current admission were more frequent in cases than controls. There was a higher proportion of controls who were never exclusively breastfed (12.1%) compared with cases (7.4%).

The use of vaccine between cases and controls was significantly different: 31.2% (67) cases were not vaccinated compared with 10.3% (201) of controls, whereas 53.5% (115) of the cases and 75.5% (1481) of the controls had received two doses of vaccine.

Of the children up to two years admitted to hospital with AD, 22.3% were RV-A positive and 156 (73%) were genotyped. The distribution of RV-A G and P genotypes is presented in Fig. 2 and Supplementary Table 2 G and P genotypes were identified in 135 (63.3%) of all RV-A positive samples (*n* = 215), and only “G” or “P” types in 21 samples. There was a predominance of the G2P [4] genotype (51.3%, *n* = 80) followed by G1P [8] (15.4%, *n* = 24). Of all observed genotypes, G2 was found in 57% (*n* = 89) and G1 in 23% (*n* = 36). The other genotypes characterized were: mixed groups (*n* = 14), G9 (*n* = 6), G3 (*n* = 3), and unusual strains such as G12 (*n* = 2) and Group C (*n* = 1). Mixed infections and unusual genotypes were identified in 10.9% of the RV-A positive samples.

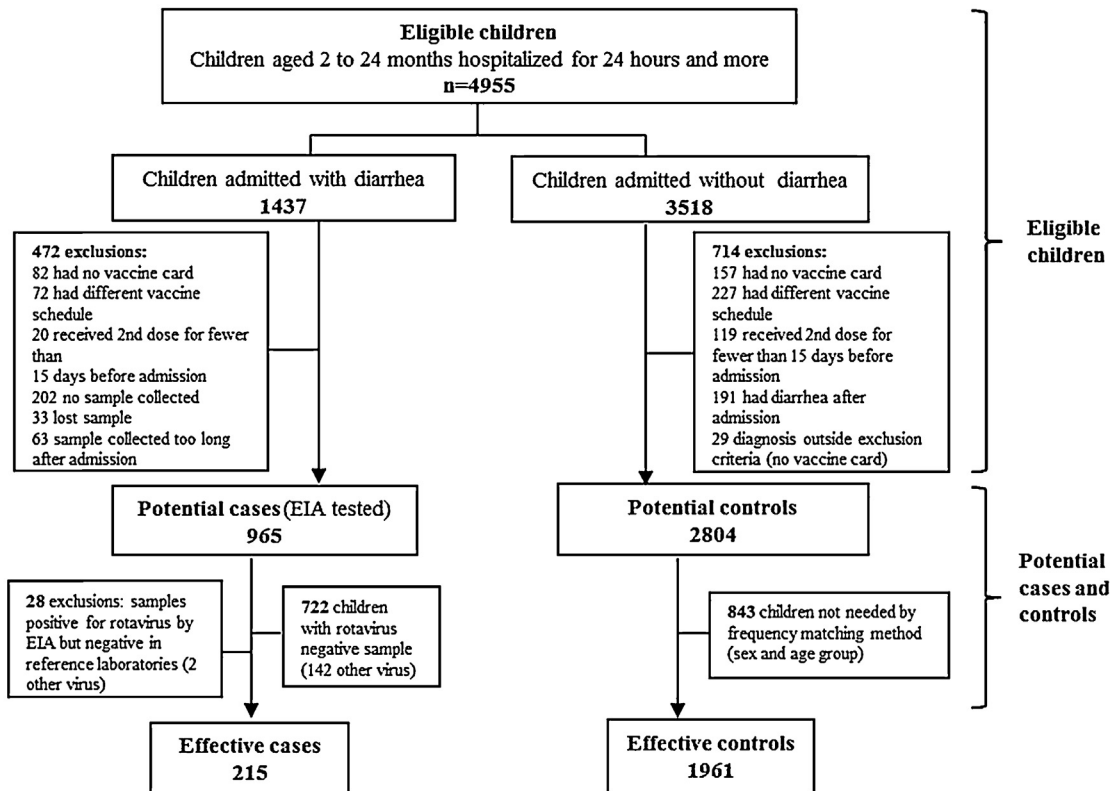


Fig. 1. Study population.

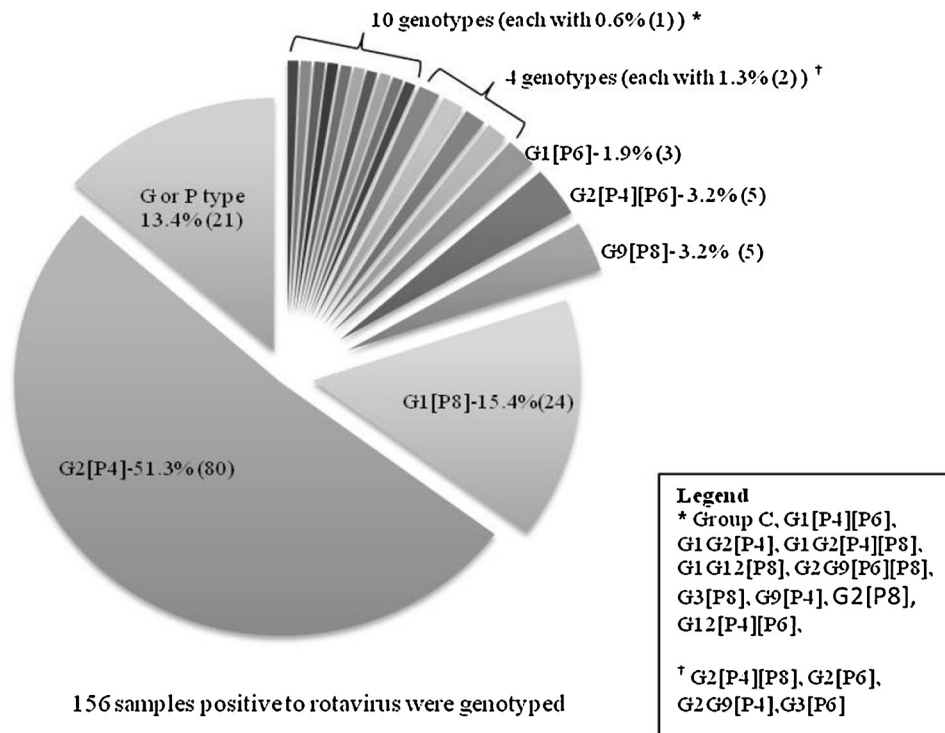


Fig. 2. Genotypes circulating in Brazil from 2008 to 2011.

### 8. Vaccine effectiveness

The two-dose adjusted VE (adjusted for year of birth and the frequency matching variables) was 76% (95%CI: 58–86) (Table 1).

Effectiveness controlled by the available potential confounders was very similar (72%, 95%CI: 44–85), suggesting no appreciable confounding by those factors for which adjustment was made.

**Table 1**  
Effectiveness of oral monovalent rotavirus vaccine in preventing hospital admission in Brazilian children with rotavirus acute diarrhea by number of doses received.

Vaccination by Number of doses	Case n	Control n	OR <sup>a</sup> (95%CI)	VE <sup>a</sup> (95%CI)	ORadj <sup>b</sup> (95%CI)	VE <sup>b</sup> (95%CI)
Unvaccinated	67	201	1	–	1	–
Fully vaccinated (two doses)	115	1481	0.24 (0.14–0.42)	76 (58–86)	0.28 (0.15–0.56)	72 (44–85)
Partially vaccinated (one dose)	33	279	0.38 (0.23–0.61)	62 (39–77)	0.40 (0.25–0.63)	60 (37–75)
AIC <sup>c</sup>			1318.253		1194.454	

<sup>a</sup> Odds ratio adjusted by year of birth and variables (sex and age group) used for frequency matching, and robust variance estimation of Jackknife, where the clusters were the hospitals. The vaccine effectiveness (VE) was calculated by  $(1 - OR) \times 100\%$ .

<sup>b</sup> Odds ratio adjusted by year of birth and variables (sex and age group) of frequency matching and confounders (mother's schooling, mother's absence from home, smoking during pregnancy, type and regularity of water supply, number of AD hospitalizations before current admission and exclusive breastfeeding) and robust variance estimation of Jackknife, where the clusters were the hospitals. <sup>c</sup> Akaike information criteria for measuring the goodness of fit of the statistic model.

We excluded a similar proportion of cases (5.7%) and controls (5.3%) because they did not have cards. Sensitivity analysis showed that if they were included as vaccinated, VE (two doses) would be 66% (95%CI: 42–80) and if included as unvaccinated VE would be 74% (95%CI: 53–86).

The VE (adjusted for year of birth and the frequency matching variables) for one dose was 62% (95%CI: 39–97) and one dose VE adjusted for other potential confounders was 60% (95%CI: 37–75). Table 2 shows that VE was similar in those with time since second dose vaccination until hospitalization stratified by one year (71%; 95%CI: 54–82) and two years (78%; 95%CI: 52–90). The VE for G1P[8] and G2P[4] by time since second dose vaccination was marginally higher for G1P[8] (90%; 95%CI: -0.92–100 for one year and 89%; 95%CI: 0.01–99 for two years) than G2P[4] (77%; 95%CI: 57–88 for one year and 75%; 95%CI: 56–86 for two years) significant.

Table 3 presents genotype-specific VE by number of doses. VE (two doses) was 89% (95%CI: 78–95) for G1P[8]; 76% (95%CI: 64–84) for G2P [4]; 74% (95%CI: 35–90) for all G1; 76% (95%CI: 63–84) for all G2 and 63% (95%CI: -27–99) for all the non G1/G2 genotypes.

Estimated VE remained very similar when analysis was stratified by year of admission suggesting that VE did not change with increasing vaccine coverage (data not presented).

## 9. Discussion

Two-dose VE was 76% (95%CI: 44–85), in spite of the great diversity of rotavirus genotypes circulating in Brazil and a predominance of G2P[4] genotype (51.3%). We found a 10.9% mixed and unusual genotypes as expected in developing countries [31,32]. The VE

lasted for two years after second dose vaccination and it was higher for G1P[8] than G2P[4].

Variation of RV-A vaccine efficacy and effectiveness have been reported in the literature: efficacy was higher in Europe (96.4% against RV-A severe AD) [11] than in a low income country (Malawi, 49.2% against all diarrhea and 57.5% against hospitalized diarrhea) [13] and in countries with high mortality (63%) [33]. In the middle income countries of Latin America [12], efficacy was 84.8% against severe AD; in South Africa it was 72.2% against all diarrhea [13]. This study showed similar effectiveness to that found in El Salvador [16] and Bolivia [17] (73% and 76% for severe diarrhea) and in a smaller study in Brazil [18] (75.8% against hospitalized diarrhea), but lower than in Belgium (90%) [15].

Two-dose VE remained high for two years. This is similar to other countries with low mortality; but different from some countries with high mortality where VE decreases in the second year after vaccination [5]. A recent study in Nicaragua also found no waning for the pentavalent vaccine in children aged 12 months or more with very severe AD [34]. Other reasons for the finding that effectiveness did not decrease in the second year in our study are: we explored VE from time since second dose vaccine while most countries estimated VE by time since birth; and we estimated VE against severe cases only. Besides, declines observed in other studies could be related to the small numbers to estimate effectiveness in the second year of life [35]. There is no agreement as to the reasons for the variation in VE and in duration of VE in the literature. The fact that effectiveness in Brazil was similar to other middle income countries in terms of overall protection against hospitalized AD and similar to European countries in relation to waning might help to advance in this exploration.

**Table 2**  
Effectiveness of oral monovalent rotavirus vaccine in preventing hospital admission in Brazilian children with rotavirus acute diarrhea (overall and genotype-specific) stratified by time from receiving the second dose of vaccine until hospital admission.

Time since the Second dose	Case n	Control n	OR <sup>a</sup> (95%CI)	VE <sup>a</sup> (95%CI)
Overall				
Unvaccinated	67	201	1	–
<1 year	71	938	0.26 (0.16–0.42)	74 (58–84)
1 to 2 years	44	543	0.22 (0.10–0.46)	78 (54–90)
			AIC <sup>b</sup> (1114.384)	
G1P[8]				
Unvaccinated	9	201	1	–
<1 year	4	938	0.10 (0.005–1.92)	90 (-0.92–100)
1 to 2 years	3	543	0.11 (0.01–99)	89 (0.01–99)
			AIC <sup>b</sup> (168.4906)	
G2P[4]				
Unvaccinated	25	201	1	–
<1 year	22	938	0.23 (0.12–0.43)	77 (57–88)
1 to 2 years	19	543	0.25 (0.14–0.44)	75 (56–86)
			AIC <sup>b</sup> (533.5553)	

<sup>a</sup> Odds Ratio adjusted by year of birth and variables (sex and age group) used for frequency matching, and robust variance estimation of Jackknife, where the clusters were the hospitals. The vaccine effectiveness (VE) was calculated by  $(1 - OR) \times 100\%$ .

<sup>b</sup> Akaike information criteria for measuring the goodness of fit of the statistic model.

**Table 3**  
Genotype-specific vaccine effectiveness of oral monovalent rotavirus vaccine by number of doses received.

Vaccination by Rotavirus genotype	Case n	Control n	OR <sup>a</sup> (95%CI)	VE <sup>a</sup> (95%CI)
<b>G1P[8]</b>				
Unvaccinated	9	201	1	–
Fully vaccinated (two doses)	7	1481	0.11 (0.05–0.22)	89 (78–95)
Partially vaccinated (one dose)	8	279	0.69 (0.30–1.57) <i>AIC</i> <sup>b</sup> (236.815)	31 (–57–70)
<b>G2P[4]</b>				
Unvaccinated	25	201	1	–
Fully vaccinated (two doses)	41	1481	0.24 (0.16–0.36)	76 (64–84)
Partially vaccinated (one dose)	14	279	0.43 (0.22–0.85) <i>AIC</i> <sup>b</sup> (641.170)	57 (15–78)
<b>G1</b>				
Unvaccinated	10	201	1	–
Fully vaccinated (two doses)	18	1481	0.26 (0.10–0.65)	74 (35–90)
Partially vaccinated (one dose)	8	279	0.62 (0.22–1.79) <i>AIC</i> <sup>b</sup> (350.510)	38 (–79–78)
<b>G2</b>				
Unvaccinated	29	201	1	–
Fully vaccinated (two doses)	50	1481	0.24 (0.16–0.37)	76 (63–84)
Partially vaccinated (one dose)	17	279	0.44 (0.26–0.74) <i>AIC</i> <sup>b</sup> (743.862)	56 (26–74)
<b>Non G1/G2</b>				
Unvaccinated	3	201	1	–
Fully vaccinated (two doses)	6	1481	0.37 (0.11–1.27)	63 (–27–99)
Partially vaccinated (one dose)	2	279	0.47 (0.72–3.09) <i>AIC</i> <sup>b</sup> (137.232)	53 (–2.09–28)

<sup>a</sup> Odds ratio adjusted by year of birth and variables (sex and age group) used for frequency matching, and robust variance estimation of Jackknife, where the clusters were the hospitals. The vaccine effectiveness (VE) was calculated by  $(1 - OR) \times 100\%$ .

<sup>b</sup> Akaike information criteria for measuring the goodness of fit of the statistic model.

A single dose offered some protection, consistent with the literature (although the VE was higher than in El Salvador [16] and Bolivia [17] and lower than in Belgium (91%)) [15].

The good effectiveness identified is consistent with the reduction in the rate of child hospitalization and mortality by AD in Brazil following the introduction of vaccine in Brazil [21].

Genotype-specific VE was high for G1P[8] (89%) and slightly lower for G2P[4] (76%) indicating a degree of cross protection. Animal models shown that immunity to group A rotavirus (RVA) present homotypic and heterotypic components. Repeat RVA infections acquired naturally or by vaccination, increase protective immunity to include multiple serotypes, as indicated by development of cross-neutralizing antibodies and cross-reactive epitope-blocking antibodies specific for VP7 and VP4 antigens. In the human vaccine clinical trials (monovalent, Rotarix®; pentavalent, RotaTeq®) as well as in the follow-up studies, both vaccines presented homotypic as well as heterotypic protection against different RVA genotypes, including G2P[4] and G9P[8] genotypes [12,19,36,37].

Genotype specific VE also remained high in the second year, in contrast with the findings for middle income countries. VE was 74% for all G1 types, 76% for all G2 types and lower for the non G1/G2 type (63%), although numbers were small. The result of VE against G2P[4] is similar to the two small studies carried out in Brazil (75.4% to 77% to G2P[4]) but unlike them, effectiveness against both G1P[8] and G2P[4] did not fall in the second year [18,19].

There is a discussion as to whether vaccine use leads to serotype replacement [19]. The high effectiveness against both G1P[8] and G2P[4] suggests that the predominance of G2P[4] is most likely a cyclical pattern of rotavirus strains occurrence in Brazil as previously reported [38,39].

This study avoided the possibility of artificially reducing effectiveness by using controls without diarrhea rather than controls with diarrhea and (potential false) no rotavirus in stool. Using EIA, PAGE and RT-PCR we confirmed that all cases were true cases of RV-A.

The data collection strategy allowed us to obtain individual data, to control for possible confounding and verify interactions in overall VE. After controlling for seven variables, no confounding was identified.

We were unable to investigate either if effectiveness declines after two years of second dose vaccine or whether there is an interaction with oral poliovirus vaccine as the two vaccines are given at the same time. We assumed non differential missingness in the sensitivity analysis. Although this was a case control study recall bias is not relevant because we did not rely on recall of vaccination; we used a record (vaccine card) for establishment of the main exposure.

Only 73% of genotypes of the RV-A positive sample were identified. This could hide the circulation of other genotypes, although, we were able to estimate genotype-specific VE for the most common circulating strains.

In conclusion, we showed consistent effectiveness of two-dose oral monovalent vaccine in preventing hospital admissions of Brazilian children with RV-A AD, closer to European than Africa VE. Protection lasted for two years and it was similar against G1P[8] and G2P[4] and slightly lower against non G1/G2. The first dose already conferred some protection.

The findings of the study supports the continued use of rotavirus in the Brazilian National Immunization Program and the monitoring for early detection of emergence of unusual and novel rotavirus genotypes.

Since this vaccine (which requires only two doses and is co-administered with other vaccines) provides adequate protection, the benefits of a change to a multivalent vaccine requiring three doses might be questionable: this may not increase protection and lead to incomplete vaccination schemes.

It might be useful to conduct cost-effectiveness studies to inform national immunization policy. In addition, other effectiveness studies should investigate what is behind the observed variation in monovalent rotavirus vaccine VE.

Finally, it is important to identify early emergence of unusual and novel rotavirus genotypes so that the vaccine effectiveness can be verified.

### Conflict of interest statement

All authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could influence its outcome.

### Contributors

MYTI designed the study, managed the field work, analyzed and interpreted the data and wrote the paper. LCR contributed to the analysis and the interpretation of the data and wrote up. CASTS contributed to analysis and interpretation of the data; MdaGLCT contributed to interpretation of the data; SR did the initial analysis of the data; SMAM contributed to prepare the data to analysis; JPGL contributed with the design of the study and interpretation of the data; MLB contributed with the design of the study, analysis and interpretation of the data. All the authors contributed to edit the paper. The manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. The order of authors listed in the manuscript has been approved by all of us. All authors have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing the authors confirm that they have followed the regulations of their institutions concerning intellectual property.

### Ethical approval

This study was approved by the Committee of Institute of Collective Health, Federal University of Bahia (Protocol 017-08/CEP/ISC-2008), by four local ethics committees. Consent to participate was obtained from all the hospitals. Carers of participating children signed written an informed consent form.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2014.01.007>.

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