UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
DEPARTAMENTO DE MEDICINA

DANIELLA PEREIRA MARQUES

EFETIVIDADE DA VACINA CONTRA O ROTAVÍRUS E GENÓTIPOS CIRCULANTES NA AMÉRICA LATINA APÓS A INTRODUÇÃO DA VACINA: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

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Trabalho de Conclusão de Curso apresentado ao Departamento de Medicina da Universidade Federal de Sergipe, como pré-requisito obrigatório para obtenção do título de bacharel em Medicina.

Orientador: Professor Doutor Ricardo Queiroz Gurgel

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LISTA DE SIGLAS

ELISA- Enzyme-Linked Immunosorbent Assay

IgA – Imunoglobulina A

IgG – Imunoglobulina G

PCR – Reação em cadeia polimerase

NSP1 – No structural protein 1

NSP2 – No structural protein 2

NSP3 – No structural protein 3

NSP4 – No structural protein 4

NSP5 – No structural protein 5

NSP6 – No structural protein 6

RNA – Ácido ribonucléico

RNAdf- Ácido ribonucleico fita dupla

RV-A – Rotavírus A

SES – Secretaria de Estado da Saúde

VP – Viral Protein

WHO – World Health Organization

LISTA DE TABELAS

ARTIGO CIENTÍFICO

LISTA DE FIGURAS

ARTIGO CIENTÍFICO

SUMÁRIO

1. REVISÃO DA LITERATURA

1.1. Histórico

O rotavírus é a causa mais comum de doença diarreica grave na infância (GLASS, 2005). Em 1973, Ruth Bishop e colaboradores descreveram partículas virais na mucosa duodenal de crianças com quadro de diarreia aguda não bacteriana. Tal fato foi visualizado pela microscopia eletrônica (BISHOP et al., 1973). Foi inicialmente denominado Orbivírus por sua semelhança morfológica com um gênero anteriormente identificado (KAPIKIAN et al., 2001).

A dupla camada proteica em sua estrutura determinou denominação subsequente de Duovírus. Posteriormente, o termo Rotavírus foi consagrado em graças ao aspecto semelhante ao de uma roda quando vistos à microscopia eletrônica (DAVIDSON et al, 1975; LINHARES et al, 2005). Em 1976, o rotavírus foi identificado no Brasil pelo Dr. Alexandre Linhares, em Belém (LINHARES, 2000).

1.2. Agente etiológico e classificação

O rotavírus pertence à família Reoviridae, gênero Rotavírus. À micrografia eletrônica, a partícula infecciosa apresenta simetria icosaédrica, é desprovido de envelope, e possui aproximadamente 100 nm de diâmetro (ANGEL et al., 2007).

É desprovido de envelope e apresenta capsídeo com três camadas proteicas. O genoma viral possui ácido nucleico de RNA linear, fita dupla, com 11 segmentos e com polaridade positiva. As enzimas requeridas para a transcrição da fita dupla de RNA estão presentes no próprio vírus. Dentre as proteínas estruturais, a VP7 (glicoproteína ou proteína G) e a VP4 (protease clivada ou proteína P) compõem o capsídeo externo e definem o sorotipo viral, além de estarem relacionadas com a indução da reposta imunológica protetora. O capsídeo interno é composto pela VP6, a qual, de acordo com sua especificidade antigênica, permite a classificação dos Rotavírus em sete distintos grupos, designados de A a G (MOLINARO, 2010).

Além das camadas interna e externa, o capsídeo também é formado pelo core, onde estão os 11 segmentos de RNA fita dupla que formam o genoma. Seis

proteínas não estruturais também são codificadas pelos segmentos: NSP1, NSP2, NSP3, NSP4, NSP5 e NSP6 (SANTOS et al., 2008).

A classificação dos rotavírus, a partir da sorologia, é feita em grupos, subgrupos e sorotipos. Sete grupos foram identificados: A, B, C, D, E, F e G, ocorrendo em diversas espécies animais, sendo que os grupos A, B, e C estão associados à doença em humanos (EDUARDO, 2009). O grupo A é o predominante na natureza, está associado à doença no homem e em diversas outras espécies animais (SERRAVALLE, 2007).

Os grupos possuem antígeno comum, que é detectado pela maioria dos testes sorológicos, localizado no componente VP6, na camada média do capsídeo. Esta proteína também determina o subgrupo (I, II, I e II, não I e não II) a qual pertence a cepa (SES, 2008).

Os sorotipos são determinados por duas proteínas (VP4 e VP7) situadas no capsídeo externo (BRAGA et al., 2006).

A VP7 é a glicoproteína que forma a matriz do capsídeo externo, constituindo-se no antígeno dominante na superfície viral, sendo responsável pela determinação dos sorotipos G. Essa glicoproteína também é reconhecida como indutora de anticorpos neutralizantes, incluindo aqueles sorotipo-específicos (WARD et al., 2008).

A VP4 consiste estruturalmente em projeções da matriz proteica, sendo sensível à protease e associada ao genótipo P. Também é indutora de anticorpos neutralizantes, o que confere imunidade protetora em humanos e animais. Uma vez sob clivagem por ação da tripsina pancreática, ela desdobra- se nos componentes antigênicos VP5 e VP8. Estas são associadas à virulência dos rotavírus ao penetrarem na célula (WARD et al., 2008).

Existem 14 sorotipos G (VP7) conhecidos. Destes, dez têm sido descritos como patógenos humanos. Os tipos G1 a G4 constituem a base para o desenvolvimento das vacinas, já que são os mais frequentemente encontrados em todo o mundo. Os tipos G8 e G12 são esporadicamente encontrados. Rotavírus que eram encontrados exclusivamente como patógenos animais, sorotipos G5, G6 e G10, foram isolados em humanos. Nas décadas de 80 e 90 o sorotipo G5 foi encontrado em segmentos da população no Brasil (MORILLO, 2010).

Devido à natureza do genoma dos rotavírus, que possui RNAfd segmentado, este vírus pode evoluir por diferentes mecanismos genéticos. A evolução pode

ocorrer devido ao acúmulo de mutações pontuais ou por mudanças ocasionadas de forma repentina no genoma do vírus. Estas alterações repentinas podem ser determinadas por rearranjos genéticos, reestruturação de segmentos genômicos ou recombinação genética (ESTES et al, 2007).

1.3. Replicação e transmissão viral

A replicação do rotavírus ocorre no citoplasma das microvilosidades do intestino delgado, gerando corpos de inclusão (MOLINARO, 2010). O vírus se localiza principalmente na região proximal do intestino delgado, em particular no jejuno (RAMIG, 2004).

O rotavírus penetra na célula por endocitose, formando vesículas denominadas endossomas. Isto ocorre graças a ativação da VP4, após sua clivagem em duas subunidades a partir da ação de enzimas como elastase, pancreatina e tripsina (GLASS et al, 2006).

Inicialmente, a membrana do endossoma é rompida pelas proteínas da camada externa do capsídeo, variando os níveis de cálcio intracelular, o que provoca a separação das camadas mais externas do capsídeo. As proteínas virais, que são construídas durante a fase de transcrição, são acumuladas em estruturas denomidadas viroplasmas (GRAY et al, 2008).

O vírus apresenta a proteína não estrutural NSP4, que é uma enterotoxina responsável pela descamação das células intestinais na luz do órgão, acarretando uma grande liberação de vírus nas fezes (MOLINARO, 2010).

A excreção viral pode variar entre dois a doze dias. A diarreia causada pelo Rotavírus resulta da alteração na absorção de sódio e glicose, já que as células destruídas são substituídas por células imaturas da cripta, as quais são incapazes de fazer absorção (MOLINARO, 2010).

A lesão do epitélio das vilosidades altera a arquitetura intestinal e está associada às mudanças da estrutura intracelular (edema de mitocôndria, distorção do retículo endoplasmático) e presença de infiltrado inflamatório nas placas de Payer e lâmina própria do intestino. As células mononucleares do infiltrado ativam o sistema nervoso entérico, provocando a motilidade intestinal e o caráter secretório da diarreia (FRANCO et al., 2006)

A transmissão ocorre predominantemente por via fecal-oral. As fezes de crianças infectadas apresentam altas concentrações, mais de 10 milhões de vírus/grama fecal, que são excretados desde dois dias antes até 21 dias após o início dos sinais e sintomas (SOCIEDADE BRASILEIRA DE IMUNIZAÇÕES, 2006). Uma ingestão mínima de 10 a 100 partículas é suficiente para a transmissão entre humanos (GLASS et al, 2006). Outras formas de transmissão citadas na literatura são: brinquedos e superfícies de ambientes como pré-escolas e escolas; água, alimentos e objetos contaminados; e secreções respiratórias (SALVADOR et al., 2011). A presença do vírus em brinquedos e superfícies de ambientes coletivos, como escolas e creches, faz com que surtos nesses locais sejam comuns. A fácil transmissibilidade explica o fato de o rotavírus ser o principal agente da gastroenterite aguda em enfermarias pediátricas (RAY et al, 2007).

1.4. Imunidade

Embora os mecanismos envolvidos na resposta imune à infecção e desenvolvimento de doença por rotavírus não estejam completamente elucidados, admite-se que haja o envolvimento de anticorpos sistêmicos e anticorpos produzidos na mucosa intestinal, além da imunidade mediada por células (DENNEHY 2007; GRAY et al. 2008).

A primeira infecção por rotavírus (natural ou induzida por vacina) resulta em resposta imune de caráter predominantemente homotípico, mediada por anticorpos contra as proteínas VP7 e VP4. Nos processos infecciosos subsequentes (reinfecções), entretanto, o espectro de resposta ampliado assume caráter heterotípico ou de proteção cruzada (VELÁZQUEZ et al., 1996).

Após uma infecção natural, 40% das crianças estão protegidas contra infecção subsequente por rotavírus, 75% contra diarréia, e 88% frente às diarreias graves. Episódios recorrentes de infecção conferem progressivo aumento na proteção (DENNEHY, 2007). Ressalta-se que rotavírus de tipos G distintos reservam entre si proteínas idênticas dos pontos de vista sorológico e genotípico, característica determinante da proteção cruzada (WARD, 2008).

Níveis séricos de anticorpos IgG e IgA contra rotavírus, detectados após infecção natural, têm sido correlacionados com proteção nos estudos conduzidos nos países desenvolvidos e em desenvolvimento, particularmente contra episódios

graves de diarreia. A IgA presente no lúmen intestinal devido ao fenômeno de transudação, reflete os níveis séricos dessa imunoglobulina. Estima-se que os anticorpos intestinais confiram maior proteção contra a doença causada por rotavírus do que aqueles circulantes (WARD, 2008).

Participam desse processo os anticorpos específicos das classes IgA e IgG, dirigidos às proteínas VP7, VP4, VP6 e NSP4. Os linfócitos B atuam na produção dos anticorpos específicos IgA e IgG, induzindo proteção contra doença subsequente. Os linfócitos T CD8+ e CD4+, por sua vez, promoveriam a resolução do quadro infeccioso (DENNEHY, 2007; ANGEL et al., 2007; WARD et al., 2008).

Justifica-se as rotaviroses em lactentes, de forma assintomática, pela passagem de anticorpos maternos passivamente, por via transplacentária ou ingestão de leite materno (LINHARES et al., 1989).

Em pacientes imunocomprometidos, a infecção natural por rotavírus não é sempre associada à acentuação de quadros graves de diarreia, mas a excreção viral pode ser prolongada. Entretanto, indivíduos que têm imunodeficiência congênita, órgãos ou medula óssea transplantados podem experimentam quadros de gastroenterite grave prolongada e por vezes fatal (WHO, 2007).

1.5. Quadro clínico e tratamento da infecção por rotavírus

O quadro clínico das rotaviroses abrange desde quadros assintomáticos até gastroenterites graves que podem levar à desidratação. Os assintomáticos são transitórios, geralmente em neonatos, com diarreia de leve intensidade (WHO, 2007).

Após um período de incubação de 1 a 2 dias, o quadro se instala de forma abrupta com aparecimento de febre e vômitos, seguidos de diarreia aquosa e explosiva, sem sangue, com duração de aproximadamente 3-8 dias (WHO, 2007; GRAY et al., 2008).

Quadros diarreicos podem prolongar-se por 2 a 3 semanas, em geral associados à intolerância à lactose, resultante da redução das dissacaridases, em particular a lactase, advinda da extensa lesão no epitélio intestinal causada pelo rotavirus. Daí advém o comprometimento da absorção de carboidratos na luz

intestinal. A ocorrência de fezes mucopiossanguinolentas suscita a associação a outro patógeno, normalmente de origem bacteriana (GRAY et al., 2008).

A febre normalmente se apresenta durante 24-48 horas, e denota caráter moderado, porém pode alcançar temperaturas superiores a 39°C em 30% das crianças (ACIP/CDC, 2009).

Os vômitos geralmente são intensos e, apesar de terem duração aproximada de 24-48 horas, podem dificultar a terapia de reidratação oral. Além disso, podem ocorrer de forma isolada ou preponderarem em relação ao quadro diarreico.

A febre precede o início da diarreia em 30 a 50% das crianças; os vômitos ocorrem em mais de 80% das infecções; as fezes são aquosas, isotônicas e raramente contêm muco, sangue ou leucócitos em número elevado (SALVADOR et al, 2011).

Além da tríade clássica, ou seja, vômitos, febre e diarreia, eventualmente ainda se observam sintomas como náuseas e cólicas abdominais de intensidade variável. Manifestações extraintestinais associadas aos rotavírus, como otites, quadros respiratórios, hepatite transitória, intussuscepção e enterite necrotizante podem ser explicadas como decorrentes do caráter sistêmico da infecção (BLUTT ET AL., 2006; GRAY et al., 2008).

Segundo Staat et al. (2012), a excreção viral ocorre desde o período de incubação, com pico entre 72- 96 horas após o início dos sintomas, e estende-se até 8-10 dias após a resolução do quadro. Além disso, alega uma hipótese de que há relação direta entre intensidade da excreção viral e a exuberância dos sintomas.

A resolução do quadro infeccioso é auto-limitada e completa. Complicações podem surgir como consequência da desidratação, como distúrbios hidroeletrolíticos, acidose e estado de choque, podendo chegar ao óbito. A duração do quadro clínico é, em média, de quatro a cinco dias, embora o período possa variar de um a dez ou mais dias (SALVADOR et al, 2011; STAAT et al., 2002).

As complicações consequentes ao quadro infeccioso por rotavírus assumem particular magnitude entre crianças nos países em desenvolvimento, devido à dificuldade no acesso aos serviços de pronto atendimento em saúde. Paralelamente, registrem-se condições mórbidas associadas como a desnutrição, baixo peso ao nascer, prematuridade e infecções associadas a outros patógenos. Em adultos, a doença é rara, todavia já ocorreram epidemias nestes indivíduos devido ao grupo B

(MOLINARO, 2010). A doença grave ocorre principalmente em crianças pequenas, mais comumente naquelas com idades entre seis e 24 meses (BERNSTEIN, 2007).

Para o referido diagnóstico, utilizam-se os seguintes métodos: Imunomicroscopia eletrônica, imunodifusão ou ELISA (enzyme-linked immunosorbent assay). Outras técnicas usadas são: a eletroforese do ácido nucléico viral e também a Reação em Cadeia da Polimerase (PCR) (MOLINARO, 2010).

O tratamento da gastroenterite pelo Rotavírus deve ser a reposição hídrica e eletrolítica oral ou parenteral nos casos mais graves. A prevenção da doença pode ser feita através de vacina e também por medidas de saneamento básico. O diagnóstico laboratorial baseia-se na evidenciação dos vírus presente nas fezes de indivíduos infectados recentemente (MOLINARO, 2010).

1.6. Epidemiologia da rotavirose: um problema de saúde pública

A rotavirose constitui-se a principal causa de diarréia grave em crianças, contribuindo para elevada morbidade hospitalar e mortalidade. No Brasil, estudos mostram que, nos menores de cinco anos, entre 20,6%-37,6% dos atendimentos hospitalares estiveram associados à infecção por rotavírus (CARMO, 2006).

Globalmente o rotavírus é responsável a cada ano por 114 milhões de casos de gastroenterite, 24 milhões de consultas, 2,4 milhões de hospitalizações em menores de cinco anos e 611 mil mortes infantis (80% nos países pobres), o que representa cerca de 5% da mortalidade infantil mundial (SALVADOR et al, 2011).

Estima-se no mundo que ao completar 5 anos, quase todas as crianças terão pelo menos um episódio de gastroenterite por Rotavírus, uma em cada cinco crianças terá visitado uma unidade de saúde, uma em cada 65 crianças terá sido hospitalizada por essa causa (NIETO, 2008; PARASHAR et al., 2003).

A incidência da doença por Rotavírus em crianças é similar em países desenvolvidos e em desenvolvimento. No entanto, as crianças em países em desenvolvimento morrem com mais freqüência, devido a vários fatores, entre eles a maior dificuldade em acesso à terapia de hidratação e maior prevalência de desnutrição (PARASHAR et al., 2003).

Além disso, os países em desenvolvimento, principalmente asiáticos e africanos, são os que apresentam maiores taxas de mortalidade devido à diarreia aguda por esse vírus (UNICEF/WHO, 2009). Já nos países desenvolvidos, apesar do número limitado de óbitos, observa-se uma elevada morbidade, revelando o impacto global das infecções por rotavirus A (PARASHAR et al., 2003).

A rotavirose constitui a segunda causa de morte mundial nos menores de cinco anos, sendo precedida apenas pelas infecções respiratórias (NIETO, 2008). No Brasil, dados do DataSUS referentes ao ano de 2006 revelam que ocorreram neste período 2.236 óbitos por doenças diarréicas em menores de 5 anos (SALVADOR et al, 2011).

No Brasil, entre 2006 e 2009, o rotavírus representou 30% do total de casos de diarreia em relação aos outros vírus entéricos. Entre as regiões do país, o rotavírus representou 35% dos casos de diarréia aguda na região norte, 26% dos casos na região nordeste e 21%, 17% e 20% dos casos de diarréia aguda nas regiões centro-oeste, sudeste e sul, respectivamente (MINISTERIO DA SAUDE, 2014).

A sazonalidade do rotavírus no território nacional apresenta-se de forma variável. Ocorre um aumento na incidência do patógeno nos meses mais frios ou no período de seca, entre maio e setembro, nos estados das regiões Centro-Oeste e Sudeste; no Norte e no Nordeste a ocorrência de rotavírus se distribui por todo o ano (SALVADOR et al., 2010).

Diante da importância epidemiológica desse patógeno no panorama mundial, a Organização Mundial da Saúde considera a vacinação como uma das intervenções de saúde pública capaz de gerar o maior impacto na prevenção de doenças infectocontagiosas, como a rotavirose, à semelhança do que ocorre com o consumo de água potável. Dentre os motivos de se vir a consolidar a imunização contra o rotavírus, destacam-se: a ocorrência universal da infecção, sem grandes distinções entre os países desenvolvidos e em desenvolvimento; a capacidade parcial e incipiente da higiene ambiental no controle da rotavirose; a inexistência de um tratamento antiviral efetivo; o fato de a maior mortalidade por diarreia decorre de o rotavírus ocorrer em comunidades pobres; e por ser esta uma enfermidade de alto impacto familiar, social e econômico (SALVADOR et al., 2011)

1.7. Vacinas contra o rotavirus

Em 1998, a vacina Rotashield® (Rhesus Rotavirus Vaccine-Tetravalent RRV-TV) foi licenciada pelo FDA (Food and Drug Administration), nos Estados Unidos. A recomendação de uso são três doses, uma aos dois meses, quarto e sexto mês de idade. A vacina é caracterizada pela mistura de três genótipos reestruturados (G1, G2 e G4) com amostra de macaco Rhesus G3. A obtenção da vacina é feita pela incorporação de material genético codificante VP7 de RV-A humano em RV-A símio (FISHER et al, 2004). Em 1999 a Rotashield® foi retirada do mercado em função da sua associação com a intussuscepção intestinal (GLASS et al., 2005).

A RotaTeq® foi inicialmente licenciada pela Merck (Merck Research Laboratories) em 2006 nos Estados Unidos (VESIKARI et al, 2006). É uma vacina pentavalente baseada em uma linhagem de rotavírus que infecta bovinos, a WC3, além de recombinantes de humanos e bovinos. A linhagem WC3 é naturalmente atenuada por humanos, mas não confere ampla proteção cruzada. Desse modo, cada um dos vírus recombinantes contém um gene que codifica a proteína do capsídeo externo dos sorotipos mais comuns em humanos, são eles: G1, G2, G3 e G4 além do genótipo P[8] (GLASS E PARASHAR, 2006). A vacina deve ser administrada em três doses, aos dois, quatro e seis meses de idade (VESIKARI et al, 2006).

A Rotarix® é uma vacina desenvolvida pela GlaxoSmithKline Biologicals. Contém 10 unidades formadoras de foco da amostra de RV-A humano RIX4414 do genótipo G1P[8] (RUIZ-PALACIOS et al., 2006). Essa linhagem vacinal se replica bem no intestino e fornece proteção cruzada contra a maioria dos outros sorotipos, principalmente aos genótipos G3, G4 e G9, que são normalmente associados ao genótipo P[8]. Fornece uma eficácia de 41% associada ao genótipo P[4] (GLASS E PARASHAR, 2006; RUIZ-PALACIOS et al, 2006). Esta vacina integra o calendário básico de vacinação atual do Sistema Único de Saúde brasileiro. É denominada "vacina oral de rotavírus humano (VORH)", a qual, no mercado internacional, foi licenciada com o nome comercial de "Rotarix” (SALVADOR et al., 2011).

O Ministério da Saúde preconiza o esquema vacinal correspondente a duas doses, administradas aos dois e quatro meses de idade. A primeira dose pode ser administrada a partir de 1 mês e 15 dias até 3 meses e 15 dias. A segunda dose

pode ser administrada a partir de 3 meses e 15 dias até 7 meses e 29 dias (BRASIL, 2014).

O esquema com duas doses de vacina foi considerado imunogênico em lactentes previamente soronegativos, havendo tendência de soroconversão com concentrações virais elevadas. A segunda dose da vacina, por sua vez, aumentou significativamente as taxas de soropositividade (SALVADOR et al., 2011).

A VORH deverá ser adiada nos seguintes casos: na presença de doenças agudas febris graves ou de diarreia que necessita de hospitalização. A vacina é contraindicada caso o paciente tenha imunodeficiência congênita ou adquirida, faça uso de corticosteroides em doses imunossupressoras, seja portador de doença gastrointestinal crônica, malformação congênita do trato digestivo, história prévia de intussuscepção ou histórico de reação alérgica grave a um dos componentes da vacina, em dose anterior até duas horas após a aplicação desta (BRASIL, 2014).

As duas últimas vacinas demonstraram grande eficácia em relação à doença causada por rotavírus. A RotaTeq® foi testada nos EUA e na Finlândia, em 34.035 crianças no grupo vacinal e 34.003 no grupo placebo. A intussuscepção ocorreu em 12 crianças que receberam a vacina e em 15 do grupo tratado com placebo. A eficácia contra os rotavírus dos sorogrupos G1 a G4 foi de 74% e a eficácia contra a gastrenterite grave foi de 98% (VESIKARI et al, 2006).

A Rotarix® foi testada em 31.673 crianças que receberam a vacina e 31.673 tratadas com placebo. Os ensaios foram realizados na Finlândia e em 11 países Latino Americanos: Argentina, Brasil, Chile, Colômbia, República Dominicana, Honduras, México, Nicarágua, Panamá, Peru e Venezuela. A eficácia da vacina contra a gastrenterite grave causada por rotavírus foi de 85%. Seis crianças do grupo vacinal e sete do grupo controle apresentaram quadro de intussuscepção (RUIZ-PALACIOS et al., 2006).

Segundo Glass e Parashar (2006), um dos aspectos de maior importância em termos de saúde pública foi a magnitude da redução das hospitalizações por diarréia associada a qualquer causa. Na América Latina, a vacinação com Rotarix® reduziu as hospitalizações por diarréia em crianças com idade inferior a um ano em 42%. Nos EUA e na Finlândia, a RotaTeq® reduziu internações em 63% também no

primeiro ano de vida. Na América Latina, essa redução pode implicar também na diminuição da mortalidade.

A vacina Rotarix® apresenta 84,7% de eficácia contra a diarréia severa causada pelo rotavírus e 85% contra as hospitalizações causadas pela diarréia da rotavirose (RUIZ-PALACIOS et al, 2006).

1.8. Introdução da vacina contra o rotavírus na América Latina

A partir de 2006, 14 países e um território da América Latina introduziram a vacina contra o rotavírus em seus programas nacionais de imunização. A vacina Rotarix® foi introduzida no Brasil (2006), Equador (2007), El Salvador (2006), Panamá (2006), México (2007), Venezuela (2006), Honduras (2009), Paraguai (2010) e Guatemala (2009) (LINHARES et al, 2011).

Já a Rotateq® foi introduzida na Nicarágua (2006), Ilhas Cayman (2009) e Guiana (2010) (LINHARES et al, 2011).

Alguns países como Cuba, República Dominicana, Panama, Costa Rica até então não introduziram a vacinação contra o rotavírus em seu calendário nacional de imunizações (BOURDETT-STANZIOLA, 2008; RIBAS, 2011).

1.9. Genótipos no Brasil e no Mundo

Estudos demonstram a proporção dos genótipos ao longo dos anos, e sua variação de acordo com a cobertura vacinal e localidade. Segundo Leite (2008), a percepção dos genótipos circulantes no Brasil é dividida em período pré-vacinação (1982-2005) e pós-vacinação (2006-2007).

Durante o período da pré- vacinação, 43% dos genótipos identificados foram G1P[8]/G1P[não tipificado], 20% foram G9P[8]/G9P[não tipificado], 9% foram G2P[4]/G2P[não tipificado], 6% foram G3P[8]/G3P[não tipificado], 4% foram G4P[8]/G4P[não tipificado] e 4% G5P[8]/G5P[não tipificado]. As combinações atípicas ou outros genótipos foram vistos em 6% das amostras e as infecções mistas foram 7% das amostras positivas. Entre 1980-1995, com 9% de prevalência, identificou-se o genótipo G5P[8] nos estados de Alagoas, Bahia, Goiás, Pará,

Paraná, Pernambuco, Piauí, Rio de Janeiro, São Paulo e Distrito Federal (LEITE, 2008).

Resultado semelhante foi visto por Gurgel et al.(2008) em análise dos genótipos circulantes no Brasil antes da vacinação (entre 1986-2006). Observou-se que P[8]G1 foi o genótipo mais frequente (43%), seguido por P[8]G9 (22%), P[4]G2 (7%), P[não tipificado]G1 (5%) e P[8]G4 (4%). Infecções mistas foram 2% das amostras positivas.

Após a introdução da vacina no Brasil em 2006, o vírus foi identificado em 74% das amostras positivas, seguido pelos genótipos G1 (3%), G3 (3%) e G9 (11%). Os genótipos mistos e atípicos corresponderam a 8% das amostras (Ministério da Saúde, 2014). Além disso, houve predominância do genótipo G2P[4] e G2P[não tipificado] nos estados de Minas Gerais, Rio de Janeiro, Pernambuco, Piauí e Sergipe (LOPMAN et al., 2011).

Amostras analisadas no Nordeste brasileiro sugeriram que a vacina estaria selecionando o genótipo G2, já que ele teve maior incidência após 2006 (GURGEL et al., 2007). Outros autores ratificaram a alteração na epidemiologia deste genótipo. No Nordeste, o genótipo G2 foi encontrado em 1,4% das amostras em 2005, 44% em 2006 e 95% em 2007 (GURGEL et al., 2009).

Em 2008, na cidade de Goiânia, o genótipo G2 foi responsável por 62,5% das infecções por rotavírus (BORGES, 2011). O genótipo G2P[4] foi predominante na mesma época em Salvador e São Paulo (MUNFORD, 2009) Também no Paraná, entre 2005 e 2009, o genótipo G2 foi predominante (NOZAWA, 2010). Já no Pará, este genótipo foi prevalente (90%) nos anos de 2006 a 2008, seguido por G1P[8] (6,67%) e G9P[8] (3,33%) (MASCARENHAS, 2010). E no Rio de Janeiro, o mesmo genótipo predominou no ano pós vacina, comparado aos anos anteriores: 1,4% em 2005, 44% em 2006 e 96% em 2007 (CARVALHO-COSTA et al., 2009).

Estudo feito na Amazônia, durante 27 anos (1981-2008), com 993 amostras de rotavírus com sorotipo definido, constatou que o G1 predominou em 43% do total. O tipo G2 exibiu caráter cíclico quanto à ocorrência, enquanto o G9 emergiu no início da década de 1990. Em termos de combinação, prevaleceram as amostras do tipo G1P[8] e G2P[4]. A vigilância sistemática da circulação de cepas de rotavírus após a introdução da vacina em larga escala no Brasil denota a expressiva ocorrência do tipo G2P[4], em 61-91% das amostras circulantes. Tal achado pode estar associado ao seu padrão cíclico de circulação ou à uma pressão seletiva

exercida pela introdução da vacina no Programa Nacional de Imunizações (DE OLIVEIRA et al, 2008). Achados semelhantes em relação ao tipo G2 foram encontrados em Recife, Pernambuco (NAKAGOMI et al, 2008).

Em Aracaju, um estudo feito com amostras coletadas de 1841 crianças entre 2006 e 2012, após a introdução da vacina, demonstrou os genótipos identificados no hospital de referência em urgência do estado de Sergipe. Em 2006, os genótipos identificados foram G2P[4] (89%), G[não tipificado]P[4] (6%) e G2T[não tipificado] (6%). Em 2007, G2P[4] (93%), G[não tipificado]P[não tipificado] (7%). Em 2008, G2P[4] foi encontrado em 100% das amostras. Em 2009, G1P[8] foi visto em 68%, seguido por G2P[4] (22%), G2P[não tipificado] (5%), G2P[4]P[8] e G[não tipificado]P[não tipificado] com 2% cada. Em 2010, G2P[4] correspondeu a 65%, seguido por G1P[6] (10%), G1P[4] (8%), G2P[6] (5%), e com 3% cada estão G2P[4]P[8], G1P[8], G8P[4], G12P[8], G12P[4]. Em 2011, G2P[4], G3P[8] e G8P[4] corresponderam a 25% cada, seguidos por G2G8P[4] e G2P[não tipificado] com 13% cada. Por fim, em 2012 G8P[4] correspondeu a 54%, seguido por G8P[6] (15%), G1G2P[4] (15%), G3P[8] (8%) e G8G2P[4] (8%) (GURGEL et al., 2014).

Santos (2005) apresentou a distribuição global dos genótipos de rotavírus após análise de 124 estudos de 52 países, nos cinco continentes, entre 1989 e 2004. Foi evidenciado que os genótipos P[8]G1, P[4]G2, P[8]G3 e P[8]G4 foram responsáveis por 88,5% da diarréia causada pelo rotavírus mundialmente.

Estes quatro genótipos respondem por 90% das infecções na América do Norte, Europa e Australia. Já na América do Sul e Ásia, representaram 68%, enquanto na África, 50%. O genótipo P[8]G1 isolado representou mais de 70% das infecções na América do Norte, Australia e Europa. Já na América do Sul e Ásia, representaram apenas 30%, enquanto na África, 23%. As infecções mistas foram detectadas na América do Sul (15%), Ásia (12%), África (4,8%), América do Norte (2,3%), Austrália e Europa (1,8%) (SANTOS, 2005).

Em todos os continentes, o genótipo G1 foi predominou entre o tipo G. Na Ásia, América do Norte e Europa, as cepas G1-G4 responderam por 97,5% das infecções por rotavírus analisadas. Na América do Sul, África e Austrália a frequência de G1-G4 foi 89%, 83,5% e 90,4% respectivamente.

Gouvea e colaboradores (1999) afirmaram que o genótipo G5 já circulava entre as crianças brasileiras com diarréia desde 1982. Este padrão é semelhante ao reportado na Argentina e Paraguai, o que indica uma disseminação do vírus pela

América do Sul. Ao longo dos anos a incidência de G5 reduziu; no Rio de Janeiro apresentava 57% em 1996, já entre 1998 e 2003, 0% (SANTOS, 2005).

O sorotipo G8 geralmente é encontrado associado ao P[10] na África. Já o G9 é comumente associado ao P[8] ou P[6] e representa 4,1% das infecções globais. É reportado como o sorotipo mais prevalente em Tokyo e Sapporo, Japão, entre 1998-1999, com taxas de prevalência de 52,9% e 71,4% respectivamente (ZHOU, 2000).

Entre 2001-2002, o sorotipo G9 isoladamente foi o mais infectante na Austrália (40,4%). O P[6] foi detectado em neonatos assintomáticos na Austrália, Venezuela e Inglaterra. O P[9] geralmente carrega consigo G1 ou G3, mas foram encontradas exceções em amostras na Itália, Hungria e Estados Unidos, associado ao G6, e na Tailândia, Japão e Argentina associado ao G12 (SANTOS, 2005).

O sorotipo G12, detectado na Índia, tem ocorrido de forma crescente em escala global, com especificidade P[8] ou P[6], podendo representar o próximo genótipo emergente e talvez determinar uma mudança nas estratégias voltadas à vacinação contra rotavírus (ANGEL et al., 2007). Neves et al. (2015) também evidenciou a crescente prevalência do sorotipo G12 em crianças infectadas no estado do Acre. A combinação identificada por este estudo foi G12P[8], vista em 27,3% das amostras estudadas (NEVES et al., 2015).

A distribuição epidemiológica dos sorotipos de rotavírus sugere que a imunidade sorotipo-específica é fundamental, mas não exclusiva, na proteção, como um claro indicador de que proteção heterotípica também ocorre e é clinicamente significativa (FRANCO et al, 2006).

Desde que o rotavírus foi descoberto como um importante patógeno entérico em crianças, há mais de 40, os pesquisadores têm feito esforços no desenvolvimento de uma vacina contra o rotavírus. Os presentes estudos em modelos animais e humanos revelam que a doença de rotavírus pode ser controlada e prevenida pela vacinação, aliada a outras medidas de saúde pública, como o saneamento básico (WANG, 2015).

A vacinação contra o rotavírus também responde pela redução da mortalidade em crianças com rotavirose na América Latina. A eficácia da vacina Rotarix contra doença severa pelo rotavírus na América Latina foi 85% (RUIZ-PALACIOS et al, 2006). Assim, é perceptível a redução do quadro diarréico e número de internações em países onde a vacina contra o rotavírus está no programa de imunizações (WANG, 2015).

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3. ARTIGO

Rotavirus vaccines effectiveness and genotypes circulating in Latin America after rotavirus vaccine introduction: a systematic review and meta-analysis

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Abstract

Background: We aimed to describe the effectiveness of the vaccines and the frequency of rotavirus strains experienced by Latin America (LA) countries after vaccine introduction.

Methods: We performed a systematic review and meta-analysis of published studies.

Findings: We found a rotavirus incidence of 16.1% (95%CI 13.2-19.3) in LA. G2 was the most prevalent G genotype (51.6%, 95%CI 37.8-65.3), followed by G9 (14.5%, 95%CI 7.4-2.3) and G1 (14.2%, 95%CI 6.9-23.3). Among P genotype, P[4] (54.1%, 95%CI 41.3-66.5), P[8] (33.2%, 95%CI 21.9-45.5), and P[6] (3.9%, 95%CI 1.7-6.7) were the most common. G2P[4] was the most frequently found genotype in most studies. The estimated overall odds ratio was 0.50 (95% CI, 0.43-0.59), indicating a protective efficacy of 50% against infection among vaccinated children after exposure compared with unvaccinated children.

Interpretation: Rotavirus vaccines are effective in preventing rotavirus-diarrhoea in children in LA countries. Surveillance studies after vaccine introduction with detail to rotavirus incidence and rotavirus genotype are essential to assess rotavirus incidence and rotavirus genotype circulation in various settings.

Introduction

Diarrhoea is the second most important cause of childhood death worldwide and rotavirus is the pathogen most frequently associated with severe diarrhoea episodes [1]. More than 90% of the deaths caused by rotavirus occur in low and middle income countries [2] and in Latin America (LA) alone, rotavirus diarrhoea causes >70,000 annual hospitalisations and 15,000 deaths [3].

In 2006, two live-attenuated rotavirus vaccines were licensed [4,5] and in 2009, the World Health Organization (WHO) recommended that rotavirus vaccines be included in the national immunization programmes of countries with high diarrhoea-related child mortality.[6] The two vaccines currently licensed are a pentavalent (G1, G2, G3, G4, P[8]) human–bovine reassortant vaccine (RotaTeq (RV5); Merck, Whitehouse Station, NJ, USA); and a monovalent (G1P[8]) vaccine derived from an attenuated human strain (Rotarix (RV1); GlaxoSmithKline Biologicals, Rixensart, Belgium).

The LA region was among the early adopters of the vaccines. More than 16 countries and one territory in the region have introduced at least one of the vaccines among their national immunization programs and national programs have since reported significant reductions in severe rotavirus-diarrhoea episodes, all-cause diarrhoea-related hospitalizations and ambulatory consultations [7–9]. Early reports also described that a large proportion of rotavirus-diarrhoea episodes were associated with the heterotypic G2P[4] genotype [10,11]. This occurrence could have been a temporal coincidence, as the genotype was circulating in the region in countries with and without rotavirus vaccinations [3]. However its prominence could have been the result of the immunological pressure exerted by the vaccine, which facilitated the selection of genotypes for which the vaccines have lower efficacy [12]. Although similar changes have been reported from Belgium [13], Austria [14] and Australia [15]; a systematic

review concluded that its selection was due to the vaccine selective pressure and that further evidence is needed [16].

The vaccines are being introduced in a much larger number of countries and the initial cohorts of vaccinated children are approaching 10 years of age. This large scale regional experience has resulted in multiple reports to assess their efficacy to reduce severe diarrhoea. However there is a paucity of studies to describe the efficacy of the vaccine to prevent rotavirus infection.

We conducted a systematic review and meta-analysis to describe the effectiveness of the vaccines against rotavirus infection in LA and the frequency of rotavirus genotypes reported after vaccine introduction.

Methods

Search strategy and selection criteria

We conducted a systematic review using PubMed, the Latin American and Caribbean Health Sciences Literature (LILACS) and SCOPUS databases to identify studies published between January 2006 and September 2014 in Portuguese, Spanish and English. Publications were identified using the search terms “rotavirus”, “rotavirus infection” and “rotavirus vaccine” and related terms. The full search strategy is shown in the appendix. Two independent reviewers (VSS and DPM) screened the title and abstract for relevance. Articles considered to have original material were obtained and assessed in detail.

To assess the incidence and rotavirus vaccine effectiveness, we included all observational studies conducted in LA that included children under 12 year-old with symptoms of acute gastroenteritis that had used Enzyme Immune-Assay (EIA) or Enzyme Linked Immune-Assays (ELISA) for the identification of rotavirus. The same

papers were included for the description of genotypes is they had used reverse-transcription polymerase chain reaction (RT-PCR). For the description of strain distribution, we included studies than reported the number of samples tested and the proportions of G and P combinations.

We excluded clinical trials, articles without frequencies or percentages of rotavirus-positive children, those including children with persistent diarrhoea (>2 weeks duration), nosocomial infections, rotavirus B and C infections or limited to outbreaks. Studies conducted before rotavirus vaccine implementation in a country were excluded.

Data extraction

Pre-defined tables for data extraction were developed and piloted with 10 papers. The information extracted from the studies included author, title, journal, publication year, country, start and end dates, study design, sample size, number of rotavirus-positive and negative samples (overall and by vaccination status), age range of participants, study setting (hospital, ambulatory clinics or the community), vaccine type, rotavirus vaccine coverage, rotavirus incidence, genotypes identified and frequency. Stool samples with rotavirus and other pathogens were considered to be rotavirus-positive. Not all studies reported all variables and percentages were calculated using the number of studies reporting each variable as the denominator. The country of the study was classified using the World Bank's classification for economic development [17] to describe the epidemiological context. To assess vaccine effectiveness, we extracted the number of vaccinated and unvaccinated children who had rotavirus.

Assessment of the risk of bias and study quality

The risks of bias and study quality were assessed by two independent reviewers using the Newcastle-Ottawa Scale (NOS) for observational studies. NOS scores ranges from 0

to 9. Scores >6 were considered high quality and ≤6 points were considered low quality. Disagreements were resolved by a third reviewer.

Statistical analyses

Prevalence of rotavirus diarrhoea and genotype distribution

The overall prevalence of laboratory-confirmed rotavirus diarrhoea and the proportion of P and G genotypes were calculated using the variance-stabilizing Freeman-Tukey double-arcsine transformation with an inverse-variance random-effects model [18,19]. For genotypes reported as 0%, a Bayesian estimation was used to make all proportions non-zero by adding 0.5 isolates to the numerator and 1.0 isolates to the denominator in the calculation of all genotype-specific proportions. A Pareto chart was designed to display the strains and cumulative genotype distribution.

The country prevalence was calculated using the arcsine transformation in a random-effects model. For countries with only one study, the prevalence and 95% confidence intervals (95%CI) were calculated according to Newcombe's method [20]. Meta-analysis of single proportions were conducted in RStudio using the "*metafor"* package. The "*rworldmap*", "*maps*", "*mapdata*", "*sp*", and "*maptools*" packages were used for mapping and visualization of rotavirus distribution.

Vaccine effectiveness

The odds ratio (OR) and 95% confidence intervals (CI) were calculated for all studies. The OR was defined as the odds of laboratory-confirmed rotavirus infection in vaccinated patients divided by the odds of laboratory-confirmed rotavirus infection in unvaccinated controls. We expressed the protective effect of the vaccines as the relative odds reduction using the formula [100% x (1-OR)]. The OR was considered to be

adequate in this analysis as the baseline level of rotavirus infection was low and the difference between the OR and the risk ratio is unlikely to be important when the baseline risk is less than 30% [21].

Analysis was performed using the DerSimonian and Laird random effects model [22] and used Forest plots to present the pooled OR and 95%CI. Each study was represented by a square in the plot, proportional to the study's weight in the meta-analysis. Two-sided p-values <0.05 were considered statistically significant. Heterogeneity was investigated by the Cochran Q test using a cut-off of 10% for significance [23] and quantified using the I^2 index [100% x (Q-df)/Q] [24]. L'Abbé's plot was used to demonstrate the dispersion of the individual study results [25] by plotting the observed estimates in the rotavirus vaccine group against the observed estimates in the control group. A Baujat plot was drawn to identify studies as potential sources of heterogeneity and to quantify the contribution of these studies to the pooled OR [26]. Other potential sources of heterogeneity were explored by comparing results grouped according to study-level characteristics and by using meta-regression to assess the significance of the differences. The characteristics explored were the study quality (high *vs* low quality), income (lower- *vs* upper-middle income countries), vaccine (RV1 *vs* RV5), latitude (degrees), and vaccination coverage (percentage). R^2 index was used to quantify the proportion of variance explained by the covariates [24]. The assumptions of normality, independence, and homogeneity of residuals were verified using diagnostic plots.

Publication bias was assessed using funnel plots of the individual estimates in log units against the standard error and regression tests were performed to analyse the plot asymmetry.

Results

The search strategy identified 4,614 records. After screening titles and abstracts, 159 full-text articles were assessed for eligibility, resulting in 46 studies for the full analysis (Figure 1). Of these, 32 (69.6%) were cross-sectional, nine (19.5%) case-control and five (10.9%) cohort studies. Twenty-eight (63.6%) studies were hospital-based, six (13.6%) community-based, five (11.4%) hospital and community-based, three (6.8%) from ambulatory clinics and two (4.5%) did not report the setting. Most studies (93.2%) included only children <5 years old. The full-characteristics of the studies are shown in Appendix 1.

Incidence of rotavirus diarrhoea and genotype distribution

Forty-six studies provided data on the proportion of laboratory-confirmed rotavirus cases (Table S1). Overall, 9,948 (16.1%; 95%CI 13.2-19.3) of 67,048 children were rotavirus-positive with the lowest and highest proportion of rotavirus-positive cases reported in Nicaragua (10.5%, 95%CI 6.3-15.6) and Peru (35.9%, 95%CI 27.8-44.9), respectively. There was high-level heterogeneity across the studies (I^2 = 99.1%, p <0.001). Figure 2 describes the proportion of children with rotavirus-positive diarrhoea by country.

The G and P genotype distributions were available for 5,920 and 5,845 isolates from 39 studies, as shown in Table 1. Most isolates were reported from Brazil, Nicaragua, and Colombia. G2 was the most prevalent G genotype (51.6%, 95%CI 37.8-65.3), followed by G9 (14.5%, 95%CI 17.4-23) and G1 (14.2%, 95%CI 6.9-23.3). The most common P genotypes were P[4] (54.1%, 95%CI 41.3-66.5), P[8] (33.2%, 95%CI 21.9-45.5), and P[6] (3.9%, 95%CI 1.7-6.7).

G2P[4] was the most prevalent G/P combination in Brazil (54.2%, 95%CI 32.8-74.9), Argentina (46.6%, 95%CI 38.9-54.4), Ecuador (50.0%, 95%CI 33.6-66.4) and Colombia (57.3%, 95%CI 27.1-84.8) and the second most common combination in Nicaragua (20.3%, 95%CI 0.2-54.6), Chile (6.8%, 95%CI 4.0-11.3) and Bolivia (28.9%, 95%CI 23.7-34.7). G9P[8] was most frequent in Chile (81.7%, 95%CI 75.6-86.5) and Bolivia (41.8%, 95%CI 35.9-47.9); and the second most frequent combination in Argentina (16.4%, 95%CI 1.3-41.8%), Ecuador (37.5%, 95%CI 22.9-54.8), and Colombia (7.8%, 95%CI 3.0-14.4). G1P[8] (32.9%, 95%CI 6.2-66.7), G9P[4] (100%, 95%CI 80.6-100), and G12P[6] (33.3%, 95%CI 19.2-51.2) were the most predominant genotypes in Nicaragua, Mexico, and Peru, respectively (Figure 3).

Vaccine effectiveness against rotavirus infection

Twenty studies involving 15,980 children were included for the analysis of vaccine effectiveness against infection. These included 2,166 (17.4%) rotavirus-positive cases among 12,465 vaccinated children and 944 (26.9%) rotavirus-positive cases among 3,515 unvaccinated children. The overall OR was 0.50 (95% CI, 0.43-0.59), resulting in an overall vaccine effectiveness against diarrhoea infection of 50%. The effectiveness was similar for RV1 (49%, 95%CI 37.0-58.0; 14 studies) and RV5 (52%, 95%CI 37.0-64.0; 6 studies) (p = 0.71) (Figure 4). There was however significant heterogeneity across studies (p = 0.041) and the inconsistency was moderately large (I^2 = 38.6%) (Suppl Figure S2). The omission of any of the studies did not make significant differences in vaccine effectiveness, suggesting a high stability of the meta-analysis. One study (Justino *et al*) [27] accounted for most of the between-study heterogeneity and its exclusion reduced heterogeneity to I^2 = 22.3% (p = 0.185).

The type of vaccine used, country income, and vaccination coverage did not explain the heterogeneity observed (adjusted R^2 = 0%), while latitude (adjusted R^2 = 14.6%) and study quality (adjusted R^2 = 48.2%) were the major sources of heterogeneity.

All studies were in middle income countries. Lower- and upper-middle income countries had no difference in vaccine effectiveness (p = 0.614). Vaccine effectiveness in upper-middle income countries (Argentina[28], Brazil[8,10,27,29–34], Mexico [35], and Venezuela [36]) was 47% (95%CI 32.0-59.0) and in lower-middle income countries 52% (95%CI 40.0-61.0) (Bolivia [37], El Salvador [38], and Nicaragua [39–44]) .

Protection against rotavirus infection was associated with latitude (18 studies). Studies below 10º latitude reported no evidence of protection (OR 0.91, 95% CI 0.45-1.86) and at 20º and 30º latitude, vaccine effectiveness increased to 64.3% (95% CI 42.9-77.7) and 77.7% (95% CI 38-92), respectively. However the associations were not statistically significant. The vaccination coverage reported ranged from 65.0% to 98.0%. The vaccines protective effect ranged from 47.5% (95% CI 8.1-70.0) to 50.8% (95% CI 33.2-63.6) increasing by approximately 1% for every 10% increase in vaccination coverage, but this correlation was not statistically significant (p = 0.871).

Vaccine effectiveness in low quality studies (55.4%, 95% CI 47.6-62.1; 8 studies) was higher than in the high quality studies (38%, 95% CI 20.3-51.7; 12 studies) (p = 0.03), but the funnel plot and regression test for asymmetry suggested that there was no evidence of publication bias (p = 0.553) (Suppl Figure S3).

Discussion

Rotavirus-related diarrhoea is still an important public health problem in low- and middle-income countries and the early and widespread use of the vaccines in LA has resulted in a large number of studies and samples analysed, providing an excellent

opportunity for their post-licensure evaluation. This meta-analysis estimated that rotavirus vaccines prevent about 50% of rotavirus-diarrhoea infections in LA, and that both vaccines (RV1 and RV5) had similar effectiveness.

The clinical trials conducted for the registration of the vaccines included a large number of children from middle- and high-income countries from Europe, North America and LA and their main end-points focused on severe diarrhoea episodes and hospitalization. Their efficacy against severe rotavirus-diarrhoea ranged from 85% to 98% and rotavirus-associated hospitalization ranged from 85% to 94% [4,5]. In the same trials, vaccine efficacy for hospital admission for diarrhoea of any cause was 39%, but data on the efficacy of the vaccines to reduce infections was not reported [45].

The efficacy of the RV1 vaccine in low-income African countries is lower [46,47], suggesting that the efficacy may vary by setting or geographical location, although the causes for these variation are currently unexplained. The vaccine effectiveness against rotavirus infection of 50% may explain the large reduction in clinic consultations other than in hospitals reported by several studies and provides a measure of the potential impact of the vaccine on rotavirus infection when implemented under routine conditions.

Despite the vaccine effectiveness of 50%, the use of these vaccines were considered as important interventions for the prevention of severe rotavirus episodes and rotavirus deaths in children aged <5 years. In perspective, it is estimated that from 2007 to 2025, universal vaccination could result in >16,000 fewer deaths due to rotavirus and 141 medical visits averted for every 1000 children vaccinated in LA countries [48].

Overall, our findings showed a decrease in the proportion of diarrhoea infections caused by rotavirus in the post-vaccine era and highlights the large impact that rotavirus vaccinations have on rotavirus-diarrhoea disease, with the proportion of children with

rotavirus infections being 16% (range, 11.2% to 35.9%), which was lower than the proportion of children with rotavirus reported before vaccination introduction (range 24%-47%).[3,49,50] However, the proportion of diarrhoea episodes caused by rotavirus varied considerably among countries. Such finding may be explained by some reasons. First, the setting of the studies and the case definitions of acute diarrhoea varied across the studies; second, some countries were represented by only one study or were conducted very early after vaccine introduction, which complicates the interpretation of the data.

It is well established that rotavirus genotypes before introduction of the vaccines varied over time and the peak frequency of a strain was often followed by a trough and replacement by a different genotype mixture [51]. Circulating strains also differed across regions during a given period. The strains most commonly found in LA were G1P[8], G9P[8] and in a lower proportion G2P[4], which are similar to the most frequent genotypes reported worldwide [3,51,52]. After the introduction of the vaccines, a high proportion of studies reported that the highest number of cases was due to the G2P[4] strain, especially in countries that adopted the RV1 vaccine. Similar changes were observed in Oceania[15] and Europe[13,14,53]. In contrast, Latin American countries, that had not adopted the rotavirus vaccination into their national immunization programs up to 2012 (e.g. as Cuba, Panama, Costa Rica and Dominican Republic) reported that the G9P[8] (>75%) strain was the most frequently genotype circulating among their children with diarrhoea [54,55].

A recent meta-analysis reported that the protection against G2P[4] strain was lower in LA (39%) and Europe (58%) compared with homotypic and partly-heterotypic strains (>80%) [16], suggesting the possibility that the RV1 vaccine exerted immunological pressure [56]. However, a more recent study in Brazil reported a decrease of G2P[4]

incidence from 2011 onwards and that others genotypes, such as G8P[4], G8P[6] and G3P[8] had become more frequent, while G2P[4] has decreased significantly therefore suggesting that whatever the mechanism underlying these changes, it is likely that genotype variation is likely to continue after vaccine introduction [57].

Our results should be treated with caution. The methods used to obtain data may not have been uniform across studies, while in some places rotavirus incidence and genotype distribution were based in a single study for six countries. Furthermore, countries who have not adopted the vaccine on a large scale, such as Chile and Argentine, allow their use by private practitioners, which may provide services to a substantial proportion of their middle income populations. We were also unable to demonstrate vaccine effectiveness by age categories or diarrhoea severity, because information on age was not available. Finally, few studies reported data per year, restricting our ability to describe strain trends over time.

In conclusion, post-licensure studies have reported that rotavirus vaccines are effective in preventing rotavirus infection in substantial numbers of under 5 years-old children in LA. This evidence strengthens the importance of these vaccines as an effective intervention for reducing the burden of diarrhoea and on rotavirus-specific diarrhoea. Despite these findings, continued surveillance after vaccine introduction is needed to describe the long term rotavirus incidence and rotavirus genotype distribution to monitor the impact of the vaccines and the potential emergence of heterotypic strains.

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Table 1. Rotavirus G and P genotype distribution in Latin America, 2006-2014.

Genotype	Isolates (n)	Proportion (%)	95%CI
G1	1501	14.2	6.9-23.3
G2	3170	51.6	37.8-65.3
G3	335	3.6	1.7-6.0
G4	97	0.3	0.0-0.8
G5	33	0.0	0.0-0.2
G8	29	0.0	0.0-0.04
G9	703	14.5	7.4-23.0
G10	1	0.0	0.0-0.2
G12	50	0.8	0.1-1.9
G un-typeable	90	1.2	0.2-2.6
P[4]	3208	54.1	41.3-66.5
P[6]	327	3.9	1.7-6.7
P[8]	2265	33.2	21.9-45.5
P[9]	1	0.0	0.0-0.1
P[10]	5	0.0	0.0-0.2
P un-typeable	111	2.2	0.8-4.2

The proportions of genotypes were calculated by using random-effects model.

Figure 1. Flow diagram of study selection.

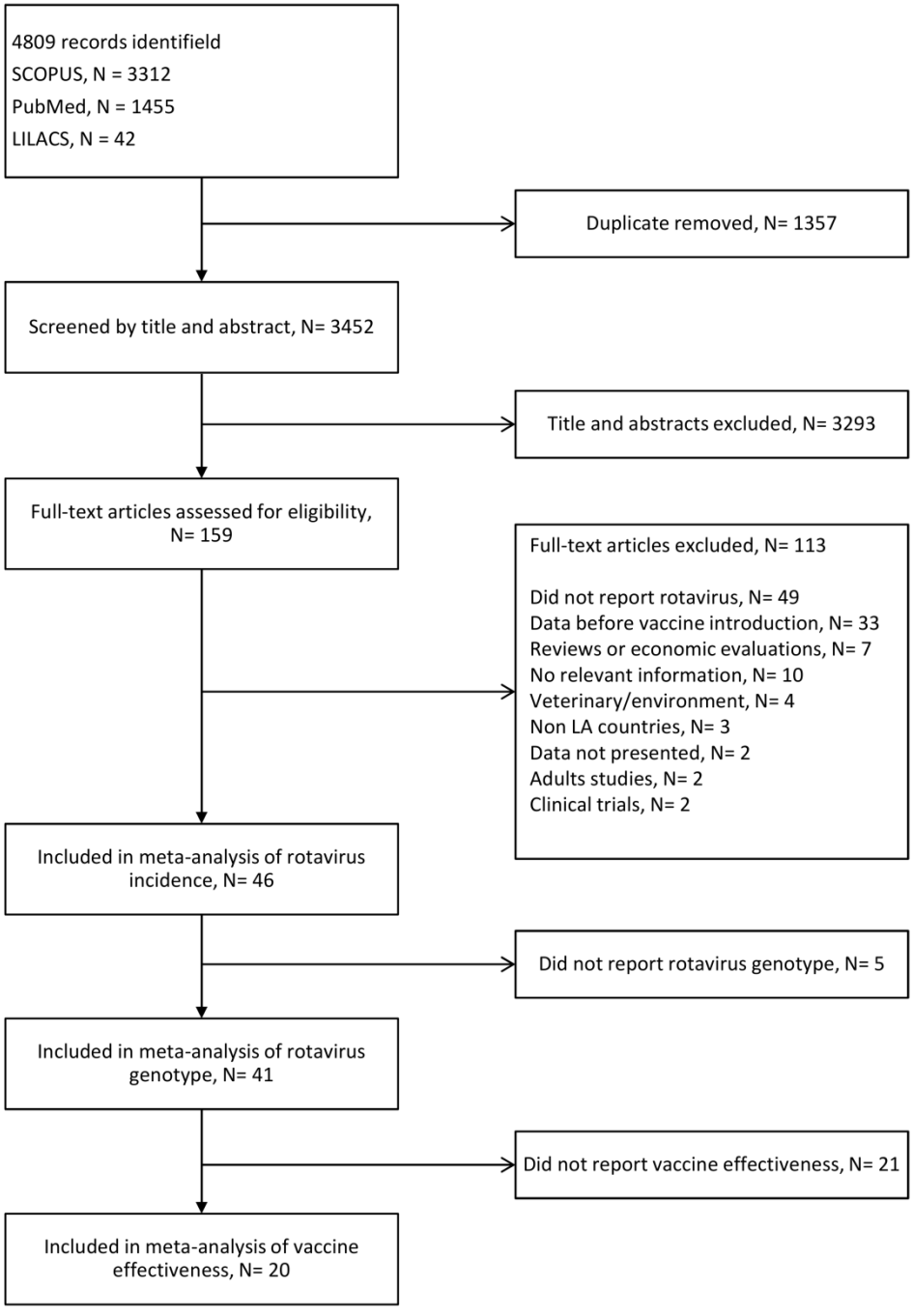


Figure 2. Proportion of children with rotavirus diarrhoea in Latin America, 2006-2014

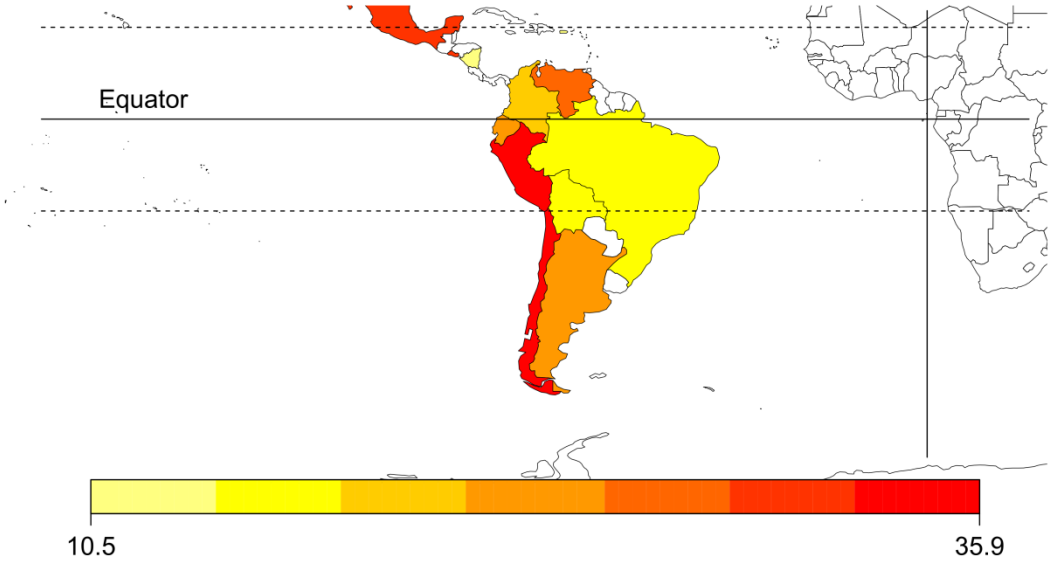


Figure 3. Geographical areas in which rotavirus genotypes are prevalent

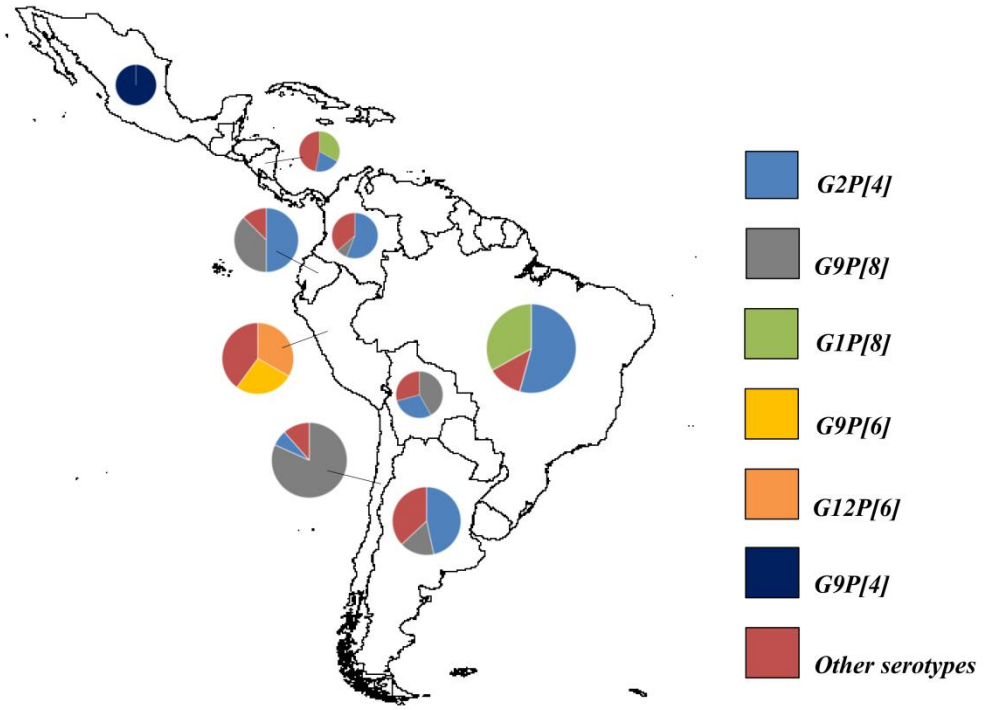
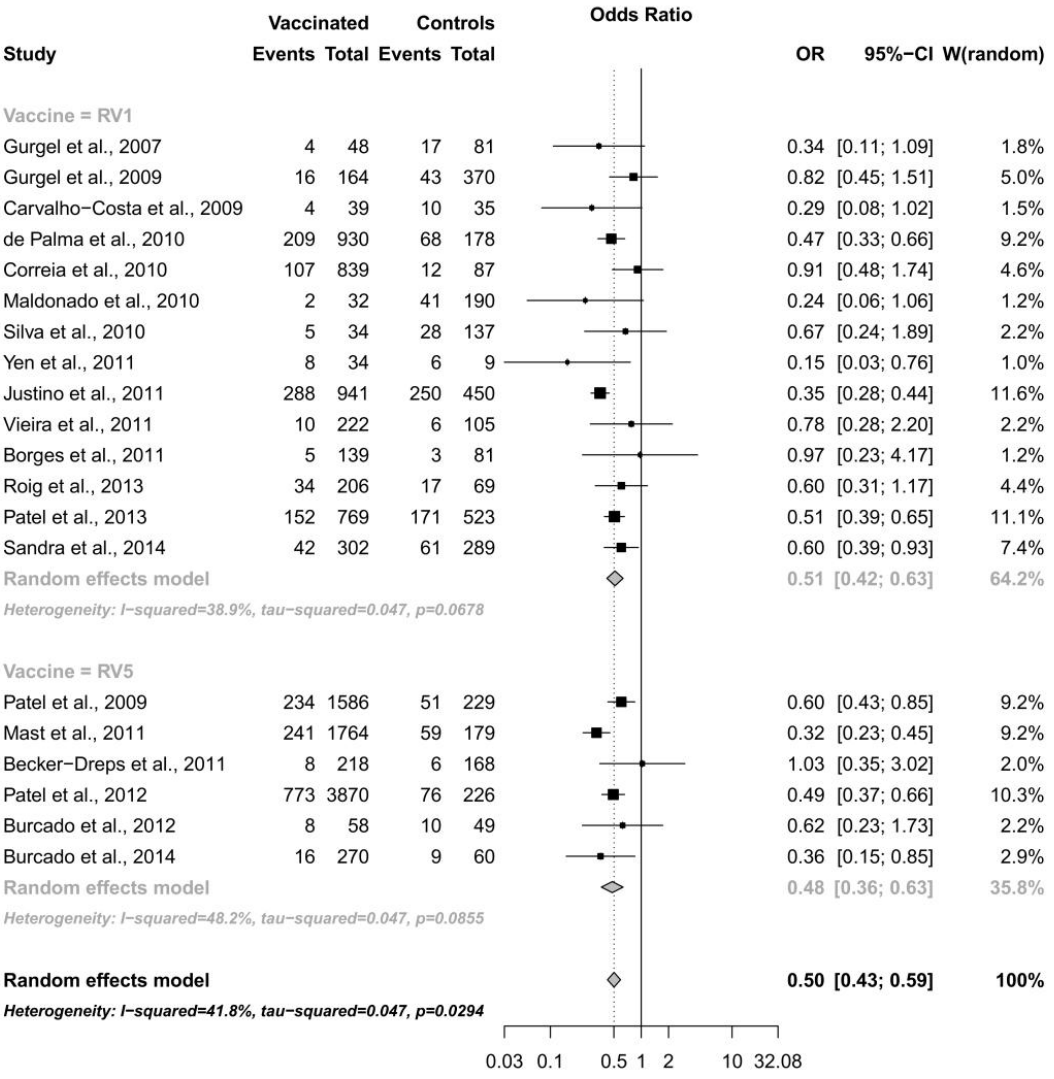


Figure 4. Forest plot of rotavirus vaccine effectiveness against rotavirus infection



Supplementary appendix

Search strategy PubMed

Search	Query	Itens found
#1	Search "Rotavirus"[MeSH] OR (rotaviruses) OR (neonatal calf diarrhoea virus) OR "Rotavirus Infections"[MeSH] (infection, rotavirus) OR (infections, rotavirus) OR (rotavirus infection) AND "Rotavirus Vaccines"[MeSH] OR (vaccines, rotavirus)	2,762
#2	Search "Rotavirus"[MeSH] OR (rotaviruses) OR (neonatal calf diarrhoea virus) OR "Rotavirus Infections"[MeSH] (infection, rotavirus) OR (infections, rotavirus) OR (rotavirus infection) AND "Rotavirus Vaccines"[MeSH] OR (vaccines, rotavirus) Filters: Humans	2,232
#3	Search "Rotavirus"[MeSH] OR (rotaviruses) OR (neonatal calf diarrhoea virus) OR "Rotavirus Infections"[MeSH] (infection, rotavirus) OR (infections, rotavirus) OR (rotavirus infection) AND "Rotavirus Vaccines"[MeSH] OR (vaccines, rotavirus) Filters: Publication date from 2006/01/01 to 2014/09/13; Humans	**1,455**

Search strategy SCOPUS

Search	Query	Itens found
#1	(title-abs-key (rotavirus) or title-abs-key (rotavirus infections) and title-abs-key (rotavirus vaccines)) and pubyear > 2004 and pubyear < 2015	3,494
#2	title-abs-key (rotavirus) and pubyear > 2005 and pubyear < 2015	6,935
#3	title-abs-key (rotavirus) or title-abs-key (rotavirus infections) and title-abs-key (rotavirus vaccines)) and pubyear > 2005 and pubyear < 2015	**3,312**

Search strategy LILACS

Search	Query	Itens

		found
#1	(mh:"Rotavirus") OR (mh: B04.820.630.790$) OR (mh: B04.909.777.714.790$) OR (mh: SP4.011.107.268.444.755$) OR (mh:“Infecções por Rotavirus”) OR (mh:” Rotavirus Infections”) OR (mh:“Infecciones por Rotavirus”) OR (mh:C02.782.791.814$)) AND ((mh:“Vacinas contra Rotavirus”) OR (vacinas) OR (vacinas candidatas usadas para prevenir infecção com Rotavirus) OR (mh:“Rotavirus Vaccines”) OR (mh:“Vacunas contra Rotavirus”) OR (mh:D20.215.894.899.760$))	**42**

Table S1. Study categorization

Study	Year of publication	Period	Country	Type of study	Setting	Sample size	Rotavirus-positive	NOS	Rotavirus incidence analysis	Rotavirus genotype analysis	Rotavirus vaccine effectiveness analysis
Upper middle-income, RV1											
Gurgel, 2007	2007	2006-07	Brazil	CS	H	129	21	8	Y	Y	Y
Nakagomi, 2008	2008	2006-07	Brazil	CS	H	470	70	8	Y	Y	N
Carvalho-Costa, 2009	2009	2006-07	Brazil	CS	H	197	46	8	Y	Y	Y
Gurgel, 2009	2009	2006-08	Brazil	CS	H	534	59	9	Y	Y	Y
Munford, 2009	2009	2006	Brazil	CS	H	402	191	9	Y	N	N
Correia, 2010	2010	2006-08	Brazil	CS	H	926	119	8	Y	Y	Y
Esteban, 2010	2010	2006-07	Argentina	CS	H	292	49	9	Y	Y	N
Maldonado, 2010	2010	2006-07	Venezuela	CS	H	241	47	6	Y	N	Y
Marillo, 2010	2010	2006-08	Brazil	CS	-	62	13	4	Y	Y	N
Mascarenhas, 2010	2010	2006-08	Brazil	CS	H	241	16	6	Y	Y	N
Nunes, 2010	2010	2006-07	Brazil	CS	C	124	31	8	Y	Y	N
Sáfadi, 2010	2010	2006-08	Brazil	CS	H	204	36	8	Y	Y	N
Silva, 2010	2010	2007-08	Brazil	CS	H	171	33	4	Y	Y	Y
Borges, 2011	2011	2008	Brazil	CS	C	220	8	9	Y	Y	Y
Carvalho-Costa, 2011	2011	2006-09	Brazil	CS	H	4817	908	9	Y	Y	N
Cilli, 2011	2011	2006-09	Brazil	CS	A	320	80	7	Y	Y	N
Gómez, 2011	2011	2006-09	Brazil	CS	H	75	75	8	Y	Y	N
Justino, 2011	2011	2008-09	Brazil	CC	H	1391	538	7	Y	Y	Y
Vieira, 2011	2011	2006-08	Brazil	CH	C	444	16	7	Y	Y	Y
Yen, 2011	2011	2010	Mexico	CC	H	56	16	8	Y	Y	Y
Dulgheroff, 2012	2012	2007-10	Brazil	CS	H+A	630	76	7	Y	Y	N

O'Ryan, 2012	2012	2009-10	Chile	CH	H	967	296	8	Y	Y	N
Assis, 2013	2013	2006-11	Brazil	CS	A	529	54	7	Y	Y	N
Gómez, 2013	2013	2009-10	Brazil	CS	H	6	6	6	Y	Y	N
Lopman, 2013	2013	2011-12	Ecuador	CC	C	404	76	7	Y	Y	N
Pereira, 2013	2013		Brazil			198	30	5	Y	Y	N
Roig, 2013	2013	2009-10	Argentina	CS	H	275	51	9	Y	Y	Y
Cotes-Cantillo, 2014	2014	2011-13	Colombia	CC	A	1051	193	6	Y	Y	N
Espejo, 2014	2014	2012-12	Peru	CH	H	117	42	8	Y	Y	N
Ichiara, 2014	2014	2008-11	Brazil	CC	H	2176	215	7	Y	Y	N
Mandile, 2014	2014	2008-11	Argentina	CS	H	663	139	9	Y	Y	N
Peláez-Carvajal, 2014	2014	2008-12	Colombia	CS	H	467	467	6	Y	Y	N
Sandra, 2014	2014	2006-08	Brazil	CS	-	591	103	7	Y	Y	Y
Soares, 2014	2014	2011-12	Brazil	CH	H	764	263	4	Y	Y	N
Lower middle-income, RV1											
de Palma, 2010	2010	2007-09	El Salvador	CC	H+C	2061	323	6	Y	Y	Y
Patel, 2011	2011	2006-09	El Salvador	CS	H	8287	1635	7	Y	N	N
Patel, 2013	2013	2010-11	Bolivia	CS	H+C	2318	400	7	Y	Y	Y
Lower middle-income, RV5											
Patel, 2009	2009	2007-08	Nicaragua	CC	H	1615	285	6	Y	Y	Y
Becker-Dreps, 2011	2011	2008-09	Nicaragua	CS	C	392	14	9	Y	Y	Y
Mast, 2011	2011	2007-09	Nicaragua	CC	H+C	6174	1082	7	Y	Y	Y
Bucardo, 2012	2012	2010-10	Nicaragua	CS	H	107	18	9	Y	Y	Y
García-Puebla, 2012	2012	2007-08	Puerto Rico	CS	H	7686	1199	3	Y	N	N
Patel, 2012	2012	2007-10	Nicaragua	CC	H	11573	1016	6	Y	Y	Y
Becker-Dreps, 2014	2014	2010-11	Nicaragua	CH	C	826	18	8	Y	Y	N

Bucardo, 2014	2014	2009-10	Nicaragua	CS	H+C	330	25	8	Y	Y	Y
Khawaja, 2014	2014	2007-09	Nicaragua	CS	H	6064	1082	6	Y	Y	N

RV1= Rotarix vaccine; RV5= Rotateq vaccine; CS= cross-sectional study; CC= case-control study; CH= cohort study; NOS= New Castle-Ottawa scale; H= hospital; C= community; A= ambulatory.

Figures

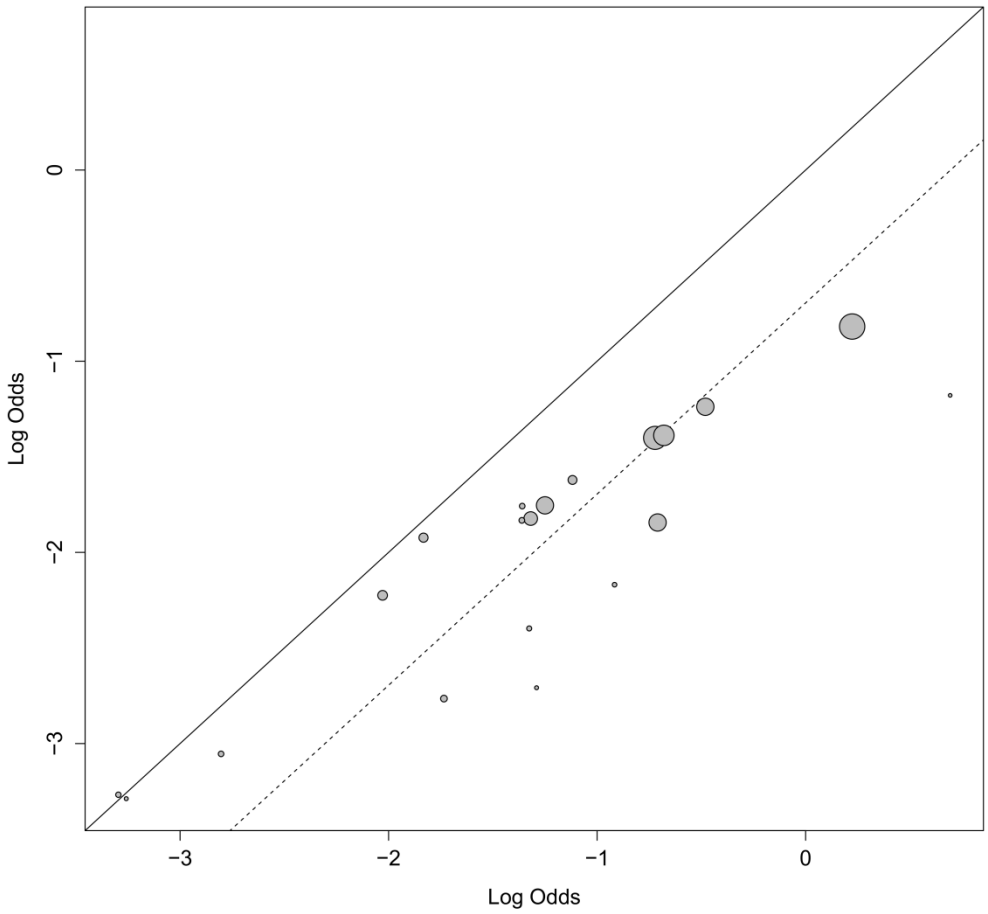


Figure S1. L'Abbé plot for estimates of rotavirus infection in the vaccine (x axis) and control (y axis) group. Circles on the solid diagonal line represent studies where the odds of infection did not differ between the two groups. Circles below the solid line represent studies where the effect measure was lower in the vaccine group. The dashed line indicates the pooled estimate based on the fitted model. The size of the circles is drawn proportional to the study size.

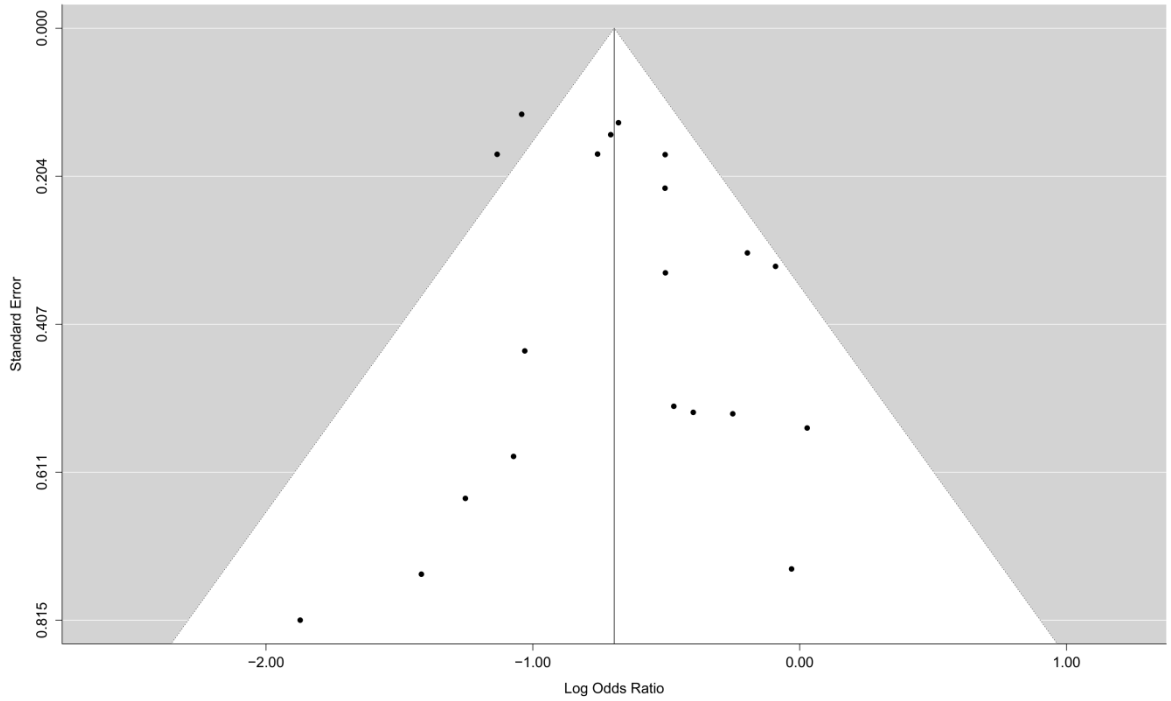


Figure S2. Funnel plot of natural logarithm of OR against the standard error in each study.