Burden of disease and circulating serotypes of rotavirus infection in sub-Saharan Africa: systematic review and meta-analysis

Elisabeth Sanchez-Padilla, Rebecca F Grais, Philippe J Guerin, Andrew D Steele, Marie-Eve Burny, Francisco J Luquero

Two new rotavirus vaccines have recently been licensed in many countries. However, their efficacy has only been shown against certain serotypes commonly circulating in Europe, North America, and Latin America, but thought to be globally important. To assess the potential impact of these vaccines in sub-Saharan Africa, where rotavirus mortality is high, knowledge of prevalent types is essential because an effective rotavirus vaccine is needed to protect against prevailing serotypes in the community. We did two systematic reviews and two meta-analyses of the most recent published data on the burden of rotavirus disease in children aged under 5 years and rotavirus serotypes circulating in countries in sub-Saharan Africa. Eligible studies were selected from PubMed/Medline, Cochrane Library, EmBase, LILACS, Academic Search Premier, Biological Abstracts, ISI Web of Science, and the African Index Medicus. Depending on the heterogeneity, DerSimonian–Laird random-effects or fixed-effects models were used for meta-analyses. Geographical variability in rotavirus burden within countries in sub-Saharan Africa is substantial, and most countries lack information on rotavirus epidemiology. We estimated that annual mortality for this region was 243·3 (95% CI 187·6–301·7) deaths per 100 000 under 5 years (ie, a total of 300 000 children die of rotavirus infection in this region each year). The most common G type detected was G1 (34·9%), followed by G2 (9·1%), and G3 (8·6%). The most common P types detected were P[8] (35·5%) and P[6] (27·5%). Accurate information should be collected from surveillance based on standardised methods in these countries to obtain comparable data on the burden of disease and the circulating strains to assess the potential impact of vaccine introduction.

Epicentre, Paris, France
(E Sanchez-Padilla MD, R F Grais PhD, P J Guerin MD, F J Luquero MD); **PATH, Seattle, WA, USA** (A D Steele PhD); **Médecins Sans Frontières, Brussels, Belgium** (M-E Burny MD); **and European Programme for Intervention Epidemiology Training, Stockholm, Sweden** (F J Luquero)

Correspondence to:
Elisabeth Sanchez-Padilla, Epicentre, Research Unit, 8 rue Saint-Sabin, Paris, 75011, France
elisabeth.sanchez@epicentre.msf.org

Introduction

Two large clinical trials, published in January, 2006, assessed the efficacy and safety of two rotavirus vaccines in infants.[1,2] Both vaccines are currently prequalified by WHO and their introduction into national immunisation programmes has recently been recommended.[3] Rotaviruses are the leading cause of severe diarrhoeal disease and dehydration in infants and young children in high-income and low-income countries.[4] The virus is composed of three protein shells: an outer and an inner capsid, and an internal shell that encases the 11-segment double-stranded RNA genome. The inner capsid protein (VP6) allows the classification into various groups, of which three groups (A–C) are known to include human rotaviruses. Nearly all of the commonly detected rotaviruses that affect human beings belong to group A.[5] The two structural outer capsid proteins, VP7 (G glycoprotein) and VP4 (P protein) define the G and P serotypes of the virus, respectively. These major antigens are involved in virus neutralisation, and hence elicit the production of neutralising antibodies in the host and are thought to be important for vaccine development. These antigens allow the classification of rotaviruses into a dual nomenclature system, depending on the G–P antigen combination (eg, G1P[8] or G2P[4]).

The new rotavirus vaccines have proven efficacy against some human rotavirus serotypes. Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) has been shown to be effective in the prevention of severe rotavirus gastroenteritis caused by G1P[8], G3P[8], G4P[8], and G9P[8].[1] Rotateq (Merck Co, Whitehouse Station, NJ, USA) has also proven to be effective in the prevention of hospital admissions and visits to the emergency department due to rotavirus strains G1, G3, G4, and G9.[2] Limited data also exists on efficacy against G2 strains for both vaccines. To assess the expected impact of the vaccine introduction, knowledge of prevalent types is essential because an effective rotavirus vaccine should protect against prevailing serotypes in the community.

Recent estimates show that serotypes G1, G3, G4, and G9 account for 90% of all rotavirus infections in North America and Europe; however, these serotypes are responsible for less than 70% of cases in Africa.[6] Whereas P[8] and P[4] account for over 90% of P types circulating worldwide, the relative frequency of these two serotypes seems to be lower in Africa, where P[6] accounts for almost a third of all P types detected.[6]

Countries in sub-Saharan Africa have a higher burden of diarrhoeal diseases and rotavirus-related deaths than the rest of the world.[7,8] Although rotavirus is recognised as the most important causal agent implicated in severe diarrhoea in young children, summarised information available on its epidemiology in sub-Saharan Africa is limited to 19 studies from nine countries, mostly from eastern and southern Africa.[6,9]

We did two systematic reviews and two meta-analyses of the most recent published data on the burden of rotavirus disease in children aged under 5 years and on rotavirus serotypes circulating in countries in sub-Saharan Africa.
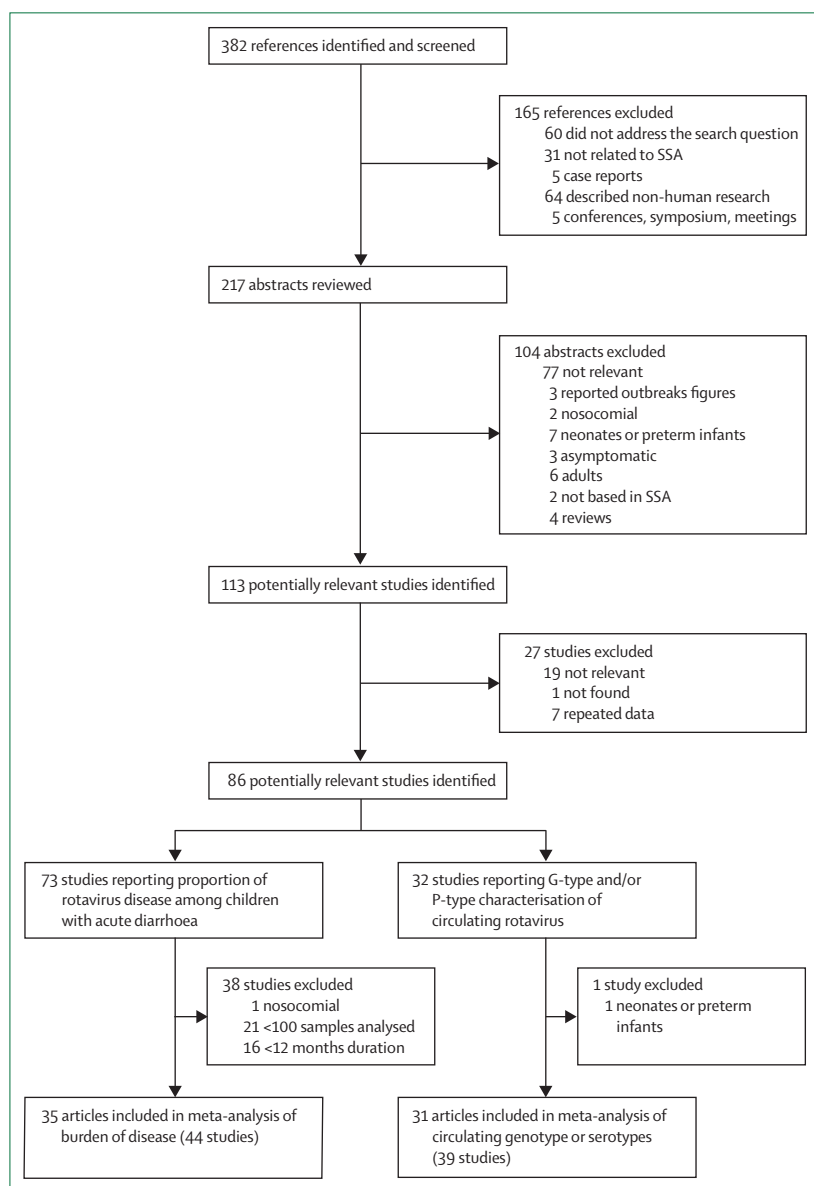
Figure 1: **Flow diagram of articles and studies included in both parts of the analysis**
SSA=sub-Saharan Africa.

Methods

Search strategy and selection criteria

We systematically searched PubMed/Medline, Cochrane Library, EmBase, LILACS, Academic Search Premier, Biological Abstracts, ISI Web of Science, and the African Index Medicus for articles published from January, 1990, to April, 2009, in English, French, Italian, Portuguese, or Spanish. We searched using the terms "rotavirus" and the name of the country. All countries in sub-Saharan Africa, as defined by the United Nations Educational, Scientific and Cultural Organisation were searched individually.[10]

The list of publications obtained through this search was narrowed to studies thought to be relevant to disease burden and serotype circulation. Retrieved studies had to report either the proportion of rotavirus disease among children with acute diarrhoea, or G type, P type, or both characterisation of circulating rotavirus. Studies meeting both criteria were included in both reviews.

Studies based only on preterm infants or neonates, on asymptomatic or immunocompromised children, on children aged older than 5 years, or limited to outbreaks or to hospital-acquired infections were excluded. In addition, to quantify the burden of rotavirus disease, studies lasting less than 12 months or including fewer than 100 children were excluded.

Data abstraction and quality assessment

Two reviewers (ESP and FJL) independently extracted data from the detected studies. We gathered the following information from every study: country, study duration, definition of diarrhoea, inclusion criteria, representativeness and precision of the estimates, age-group, setting (community, outpatient department, or hospital based), number of samples tested, number of rotavirus-positive samples, typing method (if any), number of samples typed, and number of positive samples for each G and P type. Data were entered into a Microsoft Office Excel database by the two reviewers (ESP and FJL). Differences in the data extracted were resolved by discussion with a third reviewer (RFG). We included cross-sectional and cohort studies. Studies reported from the same country were cross-referenced by location and time period to avoid data duplication.

Burden of rotavirus disease

We did a meta-analysis to obtain a point proportion of diarrhoea due to rotavirus for each country for which data was available and for all sub-Saharan Africa by age-group. We used fixed-effects models or the DerSimonian–Laird random-effects methods,[11] depending on the heterogeneity of the studies, on the basis of Cochran's Q-test. The variances of the raw proportions given in each study were stabilised using a Freeman–Tukey-type arcsine square-root transformation (y=arcsine[$\sqrt{(r/(n+1))}$]+arcsine[$\sqrt{(r/(n+1)/(n+1))}$]), with a variance of $1/(n+1)$, where n is the population size.[12] We calculated the I^2 statistic as a measure of the proportion of the overall variation in the proportion that was attributable to between-study heterogeneity. We also did a stratified analysis to assess the weight of rotavirus infection on diarrhoeal disease according to age and setting of the study (community, outpatient department, or hospital based). If there was only one study in the country, we calculated the 95% CI by use of the exact test of Clopper–Pearson.[13]

To calculate the mortality rate due to rotavirus, we followed the same methods published previously for obtaining global estimates of rotavirus deaths,[8,14] and recommended by WHO.[15] By country and for all sub-Saharan Africa, we multiplied the population aged under 5 years by the mortality in this age-group to obtain the
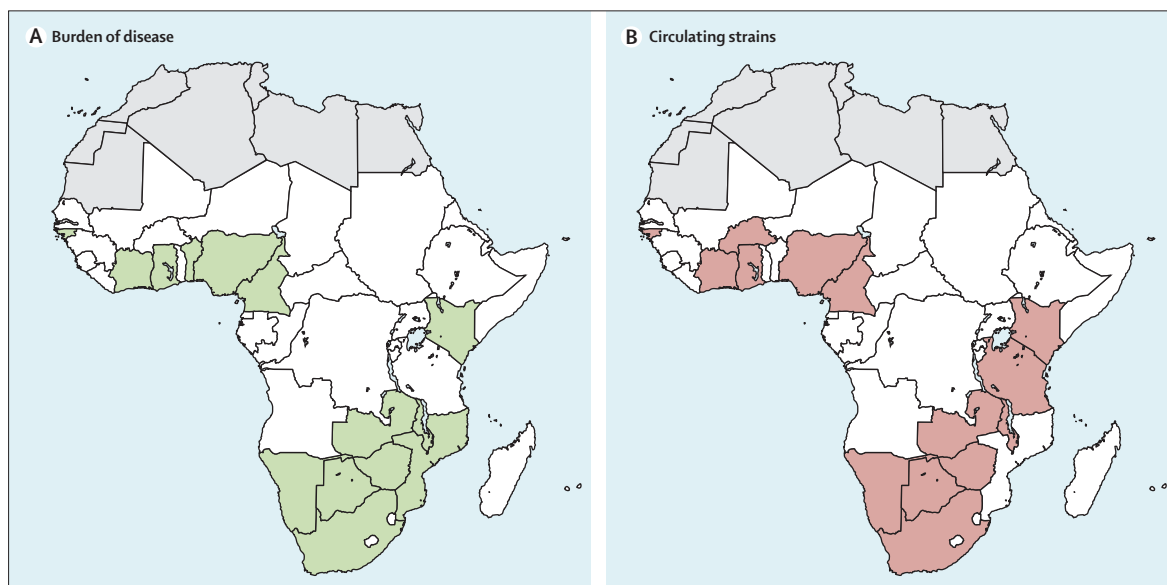
Figure 2: **Countries with published information on burden of rotavirus disease (A) and circulating rotavirus strains (B)**
Sub-Saharan Africa, 1990–2009. The following countries provided data on both burden and circulating strains: Botswana, Cameroon, Côte d'Ivoire, Ghana, Guinea Bissau, Kenya, Malawi, Namibia, Nigeria, South Africa, Zambia, and Zimbabwe. Benin and Mozambique provided data on burden only; Burkina Faso and Tanzania provided data on circulating strains only.

total number of deaths.[16] We then multiplied this number by the proportion of deaths due to diarrhoea (WHO country profiles) and by the combined proportion of diarrhoea hospital admissions due to rotavirus infection in children aged under 5 years (obtained through meta-analysis).

Circulating strains

The proportion of P genotypes and G serotypes or genotypes, non-typeable strains, and mixed infections were analysed independently. Again, the combined proportion of the different genotypes or serotypes for each country and for all sub-Saharan Africa was calculated using fixed-effects models or the DerSimonian–Laird random-effects method, depending on the heterogeneity of the studies, as explained above. All analyses were done with the statistical software R (version 2.7.2).

Results

Our search identified 382 articles that were relevant to the objectives of this review. We excluded 335 articles according to the exclusion criteria. One article reported the results of 15 different studies done in 12 countries in sub-Saharan Africa.[17] No additional studies were identified through citations in articles. Figure 1 summarises the selection process and shows the number of articles included in both parts of the review. 35 articles (44 studies) were analysed for the burden of rotavirus disease and 31 articles (39 studies) for the circulating strains.[17–70]

Characteristics of the patient population, study design, setting, and severe diarrhoea definition differed among the included studies, and typing method varied widely (webappendix).[17–70] The studies were done between 1984 and 2007, and the median starting date was 1997 (IQR 1991–99). Definition of acute diarrhoea was missing for 14 of 35 articles reporting data on burden of disease, and the selection criteria was absent in 22 of these studies. The age range used as inclusion criteria varied among studies, and the categories used to report the proportion of rotavirus diarrhoea by age-group also varied substantially. Samples were screened by EIA or ELISA in 35 studies, and with latex agglutination in four studies.[17,18,31,46] Polyacrylamide gel electrophoresis was used in two studies, either combined with ELISA and electron microscopy,[30] or only with ELISA.[47] One study used both ELISA and electron microscopy,[25] another ELISA and latex agglutination,[61] and one used electropherotyping.[53] Rotavirus type-G characterisation was done in 35 studies, in five studies by ELISA,[25,47,57,59,64] in 27 studies by reverse-transcription PCR (RT-PCR), and in three studies by both techniques.[17,38,58] P-type genotyping was done in 33 studies, all of which used RT-PCR (webappendix).

Information was available on the proportion of rotavirus diarrhoea for 14 countries of 48 in the region (figure 2). The proportion of diarrhoea caused by rotavirus ranged between 0·6% for children aged under 2 years who were admitted to hospital in Mozambique[46] and 55·9% for children aged under 5 years who attended a hospital outpatient department in Nigeria.[53]

In a stratified analysis, by age and setting, meta-analysis showed that the highest proportion of diarrhoea attributable to rotavirus was in hospital-admitted children aged under 1 year (table 1). The lowest proportion was observed in community-based studies that included

	Studies (n)	Samples (n)	Proportion of diarrhoea due to rotavirus infection (95% CI)	Test for heterogeneity (I^2)
Community based*				
<3 years[35,36]	2	2400	3·4% (2·6–4·1)†	55·8%
<5 years[31]	1	196	6·6% (3·6–11·1)‡	..
Outpatient department§				
<1 year[42,47,53,54,55,70]	6	1195	32·8% (21·0–45·7)¶	95·1%
<2 years[17‖,22,24,26,30,39,42,47,50,53–56,70]	15	8413	27·1% (20·9–33·8)¶	97·6%
<3 years[22,25,37,39,42,47,54,55,61]	9	4366	23·1% (18·4–28·0)¶	92·6%
<5 years[17**,19,21,22,30,37,39,42,45,47,48,50,53–56,59,60]	20	9572	21·8% (18·5–25·2)¶	93·8%
Hospital based				
<1 year[41,63,65,66,69]	5	2444	39·4% (31·8–47·3)¶	93·0%
<2 years[17‖,41,46,63,65,66,69]	8	7549	28·0%†† (17·5–39·8)¶	99·1%
<3 years[18,41,63,65,69]	5	4309	37·5% (25·7–50·2)¶	98·4%
<5 years[17‖,18,41,45,52,53,63,69]	9	6584	35·4% (27·3–43·9)¶	97·9%

*No studies provided data for children in the age-groups <1 year and <2 years. †Fixed-effects model. ‡Exact (Clopper–Pearson) estimate. §Studies may have included children who required subsequent hospital admission. ¶Random-effects model. ‖Two studies included. **Three studies included. ††In a sensitivity analysis, excluding the study with an outlier (0·6%; Mandomando et al[46]), the summary proportion obtained was 34·1% (26·1–42·6), I^2=98·1%.

Table 1: Summary of diarrhoeal episodes attributable to rotavirus infection by age and setting in sub-Saharan Africa, 1990–2009

children aged under 3 years. Figure 3 shows a forest plot of the proportion of diarrhoea attributable to rotavirus by setting.

In sub-Saharan Africa, the global estimate of the annual rotavirus mortality rate was 243·3 (95% CI 187·6–301·7) deaths per 100 000 children aged under 5 years. This figure corresponds to 308 579 deaths per year due to rotavirus in this age-group. Mortality estimates could be made for seven countries from which the proportion of hospital admissions due to rotavirus diarrhoea in children aged under 5 years was available. The rotavirus mortality rate ranged from 6·2 (South Africa) to 301·0 (Nigeria) per 100 000 children-years (table 2). The median annual rotavirus mortality rate for the included countries was 193·3 (IQR 161·55–228·45) cases per 100 000 children younger than 5 years. The annual number of deaths due to rotavirus infection ranged from 323 in South Africa to 71 144 in Nigeria.

Information on the circulating rotavirus strains was available for 14 countries (figure 2). Summary proportions for each genotype are shown in tables 3 and 4. The most frequent G type detected was G1 (34·9%), followed by G2 (9·1%), and G3 (8·6%). G8 accounted for 3·3% of the infections, and G4 and G9 represented 1·9% and 2·6% each. The combined summary proportion (random-effects) for genotypes G1–G4 and G9 was 68·4% (95% CI 62·3–74·3; I^2=94%; p<0·001). G12 strains were found in two studies, one done in Ghana (3·5%; 5 of 142)[27] and the other in Malawi (5·5%; 30 of 546).[45] 7·8% of the samples contained more than one G-type strain, and in 15·4% the G type remained unknown (table 3).

The most frequent P types detected were P[8] (35·5%) and P[6] (27·5%). P[4] accounted for 7·3% of the samples, and other P types for less than 0·5% each. Mixed genotypes accounted for 5·6% of the P-typed strains. Genotypes P[1], P[2], P[12], and P[14] were detected in one study each: P[1] in Nigeria (0·9%; 1 of 110),[51] P[2] in Guinea Bissau (1·2%; 2 of 167),[34] P[12] in South Africa (2·6%; 6 of 227),[62] and P[14] also in South Africa (2·1%; 11 of 525).[17] Untyped P types accounted for 15·5% of the samples (table 4). The most common genotypes that presented with mixed infections were G2 and G8, and P[6], P[4], and P[8].

Discussion

This study gathers the most recent published data on the burden of disease and the circulating strains of rotavirus in sub-Saharan Africa, and underscores the lack of information about rotavirus epidemiology in most of these countries. Rotavirus mortality rates vary widely within and between countries in sub-Saharan Africa from which information is available, as do the genotypes of circulating rotavirus strains. Our estimate that approximately 300 000 children aged under 5 years die of rotavirus infection in sub-Saharan Africa each year is higher than the most recent data provided by WHO (251 783 deaths per year).[71] The difference between these estimates is mainly due to the assumed percentage of diarrhoeal deaths attributable to rotavirus. We applied the summary proportion of rotavirus infection in hospital-admitted children aged under 5 years who were suffering from watery diarrhoea. However, the number of available studies to calculate this estimate was low, which means that imprecise estimates were used and substantial variations exist due to extreme values, as in the study by Mandomando and colleagues.[46] Therefore, even slight variations in the studies included can significantly modify the global figures. Moreover, the reporting methods used are not standardised, which makes difficult the classification of studies by setting.

The most accepted estimates of global rotavirus disease burden are based on the proportion of rotavirus cases seen at each health-care setting.[4] Good knowledge of the health-seeking behaviour of patients with diarrhoea is needed to extrapolate the observed proportion in different settings to obtain the global burden of this disease. This information is missing in several regions of the world, including sub-Saharan Africa. The most accepted estimates of global rotavirus burden in low-income countries[8,14] are based on the health-seeking behaviour seen in a Chilean cohort study,[72] but health-seeking behaviour is likely to vary between geographical regions, and the distribution of cases in the different health-care settings varies substantially between Latin America and Africa. These limitations underscore the importance of not only gathering more information on rotavirus disease burden in sub-Saharan Africa, but ensuring that a
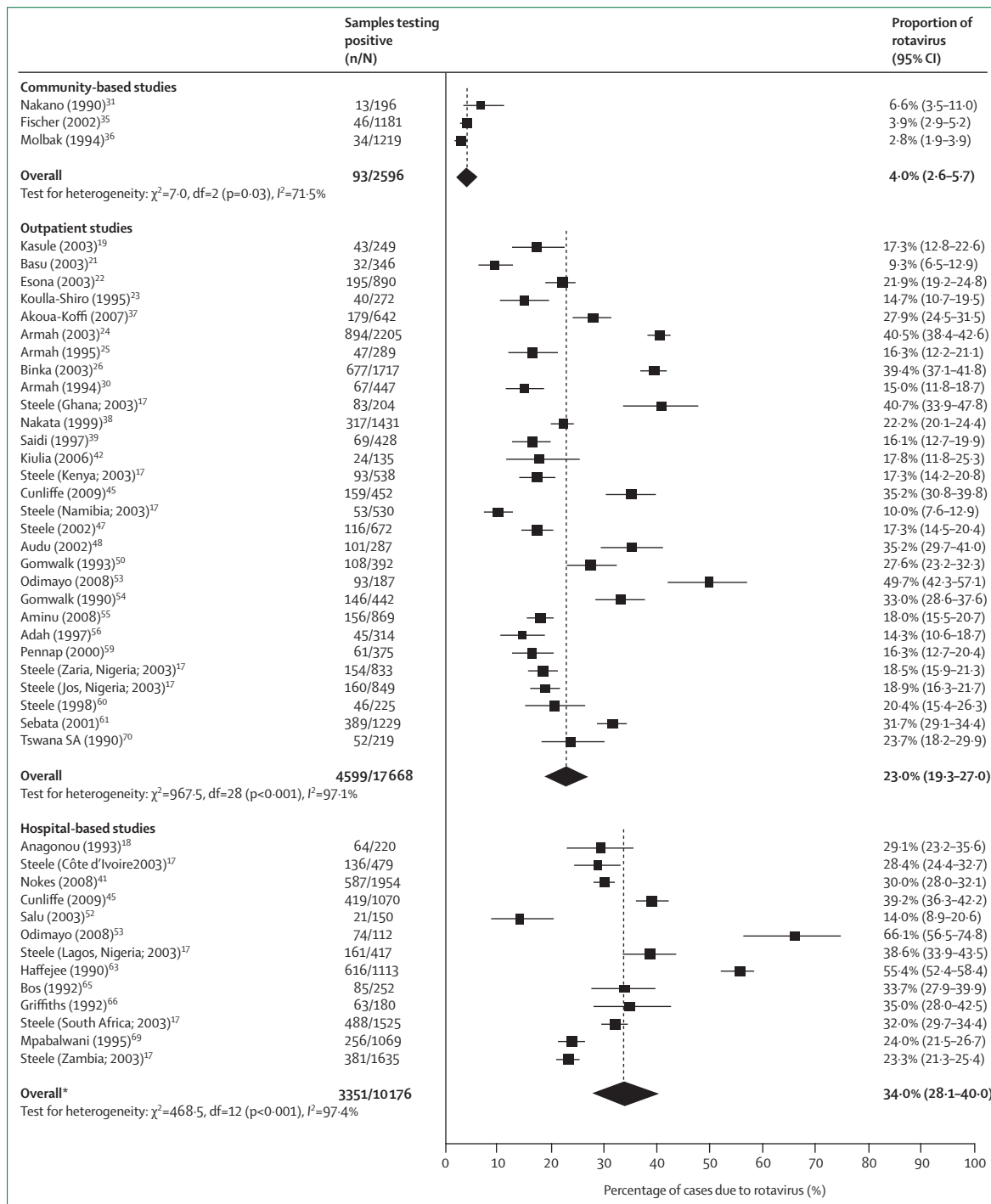
	Samples testing positive (n/N)		Proportion of rotavirus (95% CI)
Community-based studies			
Nakano (1990)[31]	13/196		6·6% (3·5–11·0)
Fischer (2002)[35]	46/1181		3·9% (2·9–5·2)
Molbak (1994)[36]	34/1219		2·8% (1·9–3·9)
Overall	**93/2596**		**4·0% (2·6–5·7)**
Test for heterogeneity: χ^2=7·0, df=2 (p=0·03), I^2=71·5%			
Outpatient studies			
Kasule (2003)[19]	43/249		17·3% (12·8–22·6)
Basu (2003)[21]	32/346		9·3% (6·5–12·9)
Esona (2003)[22]	195/890		21·9% (19·2–24·8)
Koulla-Shiro (1995)[23]	40/272		14·7% (10·7–19·5)
Akoua-Koffi (2007)[37]	179/642		27·9% (24·5–31·5)
Armah (2003)[24]	894/2205		40·5% (38·4–42·6)
Armah (1995)[25]	47/289		16·3% (12·2–21·1)
Binka (2003)[26]	677/1717		39·4% (37·1–41·8)
Armah (1994)[30]	67/447		15·0% (11·8–18·7)
Steele (Ghana; 2003)[17]	83/204		40·7% (33·9–47·8)
Nakata (1999)[38]	317/1431		22·2% (20·1–24·4)
Saidi (1997)[39]	69/428		16·1% (12·7–19·9)
Kiulia (2006)[42]	24/135		17·8% (11·8–25·3)
Steele (Kenya; 2003)[17]	93/538		17·3% (14·2–20·8)
Cunliffe (2009)[45]	159/452		35·2% (30·8–39·8)
Steele (Namibia; 2003)[17]	53/530		10·0% (7·6–12·9)
Steele (2002)[47]	116/672		17·3% (14·5–20·4)
Audu (2002)[48]	101/287		35·2% (29·7–41·0)
Gomwalk (1993)[50]	108/392		27·6% (23·2–32·3)
Odimayo (2008)[53]	93/187		49·7% (42·3–57·1)
Gomwalk (1990)[54]	146/442		33·0% (28·6–37·6)
Aminu (2008)[55]	156/869		18·0% (15·5–20·7)
Adah (1997)[56]	45/314		14·3% (10·6–18·7)
Pennap (2000)[59]	61/375		16·3% (12·7–20·4)
Steele (Zaria, Nigeria; 2003)[17]	154/833		18·5% (15·9–21·3)
Steele (Jos, Nigeria; 2003)[17]	160/849		18·9% (16·3–21·7)
Steele (1998)[60]	46/225		20·4% (15·4–26·3)
Sebata (2001)[61]	389/1229		31·7% (29·1–34·4)
Tswana SA (1990)[70]	52/219		23·7% (18·2–29·9)
Overall	**4599/17668**		**23·0% (19·3–27·0)**
Test for heterogeneity: χ^2=967·5, df=28 (p<0·001), I^2=97·1%			
Hospital-based studies			
Anagonou (1993)[18]	64/220		29·1% (23·2–35·6)
Steele (Côte d'Ivoire2003)[17]	136/479		28·4% (24·4–32·7)
Nokes (2008)[41]	587/1954		30·0% (28·0–32·1)
Cunliffe (2009)[45]	419/1070		39·2% (36·3–42·2)
Salu (2003)[52]	21/150		14·0% (8·9–20·6)
Odimayo (2008)[53]	74/112		66·1% (56·5–74·8)
Steele (Lagos, Nigeria; 2003)[17]	161/417		38·6% (33·9–43·5)
Haffejee (1990)[63]	616/1113		55·4% (52·4–58·4)
Bos (1992)[65]	85/252		33·7% (27·9–39·9)
Griffiths (1992)[66]	63/180		35·0% (28·0–42·5)
Steele (South Africa; 2003)[17]	488/1525		32·0% (29·7–34·4)
Mpabalwani (1995)[69]	256/1069		24·0% (21·5–26·7)
Steele (Zambia; 2003)[17]	381/1635		23·3% (21·3–25·4)
Overall*	**3351/10176**		**34·0% (28·1–40·0)**
Test for heterogeneity: χ^2=468·5, df=12 (p<0·001), I^2=97·4%			

0 10 20 30 40 50 60 70 80 90 100

Percentage of cases due to rotavirus (%)

Figure 3: **Forest plots of the proportion of diarrhoea cases due to rotavirus stratified by setting**
Sub-Saharan Africa, 1990–2008. *Total estimate excludes Mandomando et al[46] (study considered an outlier). Combined estimates were obtained by use of DerSimonian–Laird random-effects models.

standard method is used in data collection and reporting to produce robust estimates of public-health relevance.

High heterogeneity was also seen in the reporting of circulating strains, and noteworthy geographic and time-dependent differences between countries were observed. RT-PCR was used by most of the studies, and although it is much more sensitive than serological methods, many strains remained untyped. Reasons for untyping could be because the correct primers were not used or because new or unusual genotypes were

	Population in 2005 of children <5 years*	Annual mortality rate in children <5 years (per 1000)†	Proportion of deaths caused by diarrhoea†	Proportion (95% CI) of diarrhoea due to rotavirus (hospital based)‡	Annual mortality rate (95% CI) due to rotavirus (per 100 000)	Annual number of deaths due to rotavirus (95% CI)
Benin[18]	1 332 000	38·6	17·1%	29·1% (23·5–35·4)	191·9 (154·9–233·6)	2556 (2064 –3111)
Côte d'Ivoire[17]	3 004 000	194·0	14·8%	28·4% (24·5–32·6)	194·6 (168·2–223·3)	5845 (5051–6709)
Kenya[41]	5 948 000	30·2	16·5%	30·0% (28·0–32·1)	149·6 (139·7–159·9)	8898 (8308–9512)
Malawi[45]	2 467 000	42·1	18·1%	39·2% (36·3–42·1)	298·7 (276·7–321·2)	7368 (6826–7924)
Nigeria[17,52,53]	23 635 000	50·0	15·7%	38·3% (15·3–64·6)	301·0 (119·8–507·6)	71 144 (28 327–119 965)
South Africa[63]	5 239 000	13·9	0·8%	55·3% (52·4–58·2)	6·2 (5·8–6·5)	323 (306–340)
Zambia[69]	2 141 000	45·8	17·5%	23·9% (21·5–26·6)	192·0 (172·3–213·3)	4111 (3688–4566)
Total	126 836 000	41·4	16·6%	35·4% (27·3–43·9)	243·3 (187·6–301·7)	308 579 (237 972–382 672)

*United Nations population estimates.[16] †Data from WHO country profiles (http://www.who.int/countries/en/). ‡All estimates were calculated using Clopper–Pearson exact CIs, except for Nigeria where a random-effect model was used.

Table 2: Rotavirus mortality in children aged under 5 years in sub-Saharan African countries, 1990–2009

	Studies (n)	Summary proportion by G type							
		G1	G2	G3	G4	G8	G9	Mixed	Untyped
		n, % (95% CI)	n, % (95% CI)	n, % (95% CI)	n, % (95% CI)	n, % (95% CI)	n, % (95% CI)	n, % (95% CI)	n, % (95% CI)
Botswana[17,19,20]	3	45, 42·9%* (1·3 to 93·6)	4, 5·7%* (0·2 to 18·1)	7, 9·1%† (2·8 to 15·4)	0, ··	0, ··	0, ··	13, 16·0%* (0·0 to 55·9)	11, 14·9%† (7·1 to 22·7)
Burkina Faso[17]	1	6, 16·2%‡ (6·2 to 32·0)	28, 75·7%‡ (58·8 to 88·2)	0, ··	0, ··	0, ··	0, ··	0, ··	3, 8·1%‡ (1·7 to 21·9)
Cameroon[17]§	2	113, 47·0%* (28·7 to 65·7)	14, 3·0%* (0·1 to 14·0)	69, 26·3%† (21·0 to 31·7)	12, 2·7%* (0·1 to 12·0)	4, 1·6%† (0·1 to 3·2)	0, ··	14, 3·0%* (0·1 to 14·0)	37, 16·0%* (7·0 to 27·9)
Côte d'Ivoire[17]	1	64, 48·1%‡ (39·4 to 56·9)	4, 3·0%‡ (0·8 to 7·5)	0, ··	0, ··	8, 6·0%‡ (2·6 to 11·5)	0, ··	22, 16·5%‡ (10·7 to 24·0)	35, 26·3%‡ (19·1 to 34·7)
Ghana[25-29]	5	154, 22·4%* (0·1 to 67·0)	91, 18·3%* (3·7 to 40·6)	79, 17·6%* (1·8 to 44·5)	7, 1·6%* (0·0 to 5·6)	11, 1·2%* (0·0 to 4·2)	71, 5·0%* (0·0 to 20·6)	22, 6·6%* (2·0 to 13·7)	62, 11·8%* (3·6 to 23·8)
Guinea Bissau[32-34]	3	29, 8·5%† (5·6 to 11·4)	112, 30·3%* (19·6 to 42·3)	3, 0·9%† (0·0 to 1·9)¶	2, 0·7%† (0·0 to 1·6)¶	28, 8·3%* (0·8 to 22·6)	0, ··	101, 32·7%* (2·9 to 75·0)	72, 11·9%* (0·0 to 42·6)
Kenya[38,40]	2	89, 45·4%* (10·3 to 83·4)	53, 10·0%* (0·2 to 31·9)	3, 1·0%† (0·0 to 2·1)¶	134, 21·4%* (0·1 to 70·3)	9, 2·8%† (1·0 to 4·6)	3, 7·3%* (0·9 to 36·0)	0, ··	46, 8·9%* (0·3 to 27·9)
Malawi[44,45]	2	442, 43·9%* (18·3 to 71·4)	6, 0·5%* (0·0 to 2·3)	113, 7·9%* (5·3 to 51·9)	26, 2·3%* (0·2 to 11·5)	300, 31·6%* (25·7 to 37·8)	16, 1·6%* (0·4 to 3·6)	8, 0·9%† (0·3 to 1·5)	19, 1·2%* (0·3 to 7·1)
Namibia[17]	1	38, 71·7%‡ (57·7 to 83·2)	5, 9·4%‡ (3·1 to 20·7)	0, ··	0, ··	0, ··	0, ··	0, ··	10, 18·9%‡ (9·4 to 32·0)
Nigeria[17,47,48,51,56-59]	8	307, 30·9%* (19·8 to 43·2)	25, 1·9%* (0·2 to 5·5)	161, 12·6%* (5·4 to 22·3)	1, 0·2%† (0·0 to 0·5)¶	43, 3·0%* (0·3 to 8·6)	48, 2·2%* (0·2 to 6·3)	126, 13·4%* (8·3 to 19·5)	215, 27·6%* (11·7 to 47·1)
South Africa[17,64]	2	392, 55·2%* (32·6 to 76·7)	49, 8·0%† (5·9 to 10·1)	21, 2·2%* (0·0 to 8·7)	30, 4·9%† (3·2 to 6·6)	10, 1·7%† (0·6 to 2·7)	21, 2·2%* (0·0 to 8·7)	21, 2·2%* (0·0 to 8·7)	75, 21·9%* (2·7 to 52·5)
Tanzania[17,67]	2	58, 36·5%* (0·2 to 90·4)	6, 3·4%* (0·1 to 15·0)	5, 4·5%† (0·9 to 8·1)	0, ··	0, ··	43, 38·0%* (5·1 to 100·0)	5, 3·0%* (0·0 to 12·6)	11, 9·1%† (4·1 to 14·1)
Zambia[17,68]	2	167, 60·5%* (27·8 to 88·6)	84, 21·5%* (10·5 to 35·0)	28, 4·0%* (0·3 to 19·3)	4, 1·4%† (0·2 to 2·7)	0, ··	0, ··	14, 2·5%* (0·0 to 9·7)	40, 12·0%† (8·5 to 15·5)
Zimbabwe[17]	1	4, 20·0%‡ (5·7 to 43·7)	0, ··	10, 50·0%‡ (27·2 to 72·8)	0, ··	0, ··	0, ··	1, 5·0%‡ (0·1 to 24·9)	5, 25·0%‡ (8·7 to 49·1)
Total	35	1908, 34·9%* (26·6 to 43·6)	481, 9·1%* (5·4 to 13·7)	499, 8·6%* (4·9 to 13·3)	216, 1·9%* (0·6 to 3·9)	413, 3·3%* (1·2 to 6·3)	202, 2·6%* (1·0 to 4·7)	347, 7·8%* (4·8 to 11·5)	641, 15·4%* (10·8 to 20·6)

*Random-effects model. †Fixed-effects model. ‡Exact (Clopper–Pearson) estimate. §Two studies from the same reference were included. ¶Left-truncated estimate.

Table 3: G-type characterisation studies in sub-Saharan African countries, 1990–2009

present.[6] Information on the circulating strains is important to enable assessment of the potential impact of rotavirus vaccination; to be effective, the vaccine must protect against the most prevalent serotypes. As other studies have shown, the most common genotypes (G1–G4, P[8]) worldwide are underrepresented in countries in sub-Saharan Africa, and, by contrast, uncommon serotypes are more prevalent in this

	Studies (n)	Summary proportion by P type													
		P[4]		P[6]		P[8]		P[9]		P[10]		Mixed		Untyped	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Botswana[17,19,20]	3	1	1.8%* (0.0–4.8)†	13	18.2%‡ (2.9–42.5)	38	32.8%‡ (0.0–88.5)	0	..	0	..	5	5.2%‡ (0.1–22.8)	23	32.2%‡ (6.1–66.8)
Burkina Faso[17]	1	0	..	28	75.7%§ (58.8–88.2)	5	13.5%§ (4.5–28.8)	0	..	0	..	2	5.4%§ (0.7–18.2)	2	5.4%§ (0.7–18.2)
Cameroon[17]	2	21	6.0%‡ (0.1–20.1)	10	15.4%‡ (10.6–82.4)	147	50.5%‡ (9.7–90.9)	4	2.2%* (0.2–4.1)	4	2.2%* (0.2–4.1)	0	..	29	13.6%* (9.1–18.2)
Côte d'Ivoire[17,37]	2	5	1.8%* (0.3–3.3)	87	28.8%‡ (14.0–46.4)	108	31.8%‡ (8.3–61.9)	1	0.4%* (0.0–1.2)†	0	..	37	11.9%* (8.3–15.5)	74	23.8%* (19.1–28.6)
Ghana[17,26–29]	5	101	15.9%‡ (3.7–34.5)	193	33.4%‡ (15.2–54.6)	272	28.3%‡ (6.0–58.9)	0	..	4	0.8%* (0.1–1.5)	27	4.1%* (2.6–5.7)	61	9.2%* (4.5–15.5)
Guinea Bissau[32–34]	3	65	18.8%* (14.7–22.9)	131	37.8%* (32.7–43.0)	32	7.8%‡ (2.7–15.2)	3	0.9%* (0.0–1.9)†	0	..	65	20.7%‡ (3.1–48.1)	49	10.4%‡ (0.8–28.8)
Kenya[17,40]	2	19	9.1%* (5.3–12.9)	56	25.6%* (19.8–31.3)	109	49.6%* (43.0–56.3)	0	..	4	2.2%* (0.3–4.1)	0	..	32	15.0%* (10.3–19.7)
Malawi[44,45]	2	72	7.6%* (5.9–9.3)	262	27.8%‡ (18.5–38.3)	574	59.3%‡ (50.7–67.6)	0	..	0	..	12	1.3%‡ (0.0–4.1)	40	3.7%‡ (0.6–9.1)
Namibia[17]	1	5	9.4%§ (3.1–20.7)	0	..	38	71.7%§ (57.7–83.2)	0	..	0	..	0	..	10	18.9%§ (9.4–32.0)
Nigeria[17,48,49,51,58]	5	4	1.4%* (0.1–2.7)	121	38.6%‡ (26.6–51.4)	59	18.5%‡ (9.0–30.4)	1	0.8%* (0.0–1.8)†	0	..	38	18.5%‡ (8.2–31.9)	98	26.6%‡ (8.0–51.1)
South Africa[17,62]	2	61	10.4%‡ (2.4–23.0)	107	12.3%‡ (3.0–26.5)	420	52.0%‡ (33.4–70.4)	3	0.5%* (0.0–0.9)†	3	0.5%* (0.0–0.9)†	29	4.0%* (2.6–5.4)	112	19.9%‡ (3.2–46.0)
Tanzania[17,67]	2	1	1.0%* (0.0–2.7)†	27	16.2%‡ (0.2–49.7)	82	68.3%‡ (35.4–93.2)	0	..	0	..	0	..	18	14.5%* (8.4–20.6)
Zambia[17,68]	2	20	13.2%* (7.9–18.5)	62	21.9%‡ (10.9–93.3)	65	49.1%‡ (2.8–96.6)	0	..	0	..	0	..	10	6.9%* (2.9–10.9)
Zimbabwe[17]	1	0	..	10	50.0%§ (27.2–72.8)	4	20.0%§ (5.7–43.7)	0	..	0	..	1	5.0%§ (0.1–24.9)	5	25.0%§ (8.7–49.1)
Total	33	375	7.3%‡ (4.9–10.1)	1107	27.5%‡ (21.2–34.3)	1953	35.5%‡ (27.1–44.4)	12	0.4%* (0.2–0.6)	15	0.4%* (0.2–0.6)	216	5.6%‡ (3.5–8.2)	563	15.5%‡ (11.4–20.0)

*Fixed-effects model. †Left-truncated estimate. ‡Random-effects model. §Exact (Clopper–Pearson) estimate.

Table 4: P-type characterisation studies in sub-Saharan African countries, 1990–2009

continent.[6,9] The G8 serotype has been frequently detected in Africa since the mid-1990s, and represents the fourth most common genotype, whereas outside Africa it has seldom been detected.[6,9]

The two new licensed vaccines have proven their efficacy mainly against the G1–G4 and G9, the most common serotypes in high-income countries, but our analysis shows that these serotypes only represent 68% of the circulating strains in sub-Saharan Africa. If the current vaccine formulation does not produce cross-protection against other genotypes more prevalent in countries in sub-Saharan Africa, their effectiveness could be reduced in this setting. Therefore, these genotypes may need to be included in future vaccines in order for them to be effective in these regions.[73] New P–G strains could emerge as the result of mixed rotavirus infections that account for up to 8% of the samples (summary proportion). When mixed infections with distinct rotavirus strains occur, the gene segments may reassort independently, producing reassortant strains, which is an important source of viral diversity.[74] Investigation of future rotavirus vaccines should consider the geographical variation in the distribution of genotypes. In India, a vaccine that potentially protects against local strains is under development.[6]

The key limitation of this review is that the results may not be representative of all countries in sub-Saharan Africa. Data on disease burden and strain information was not available for many countries or did not meet the inclusion criteria for this analysis. Moreover, the included studies may not be representative of the country as a whole; none of the studies covered the whole country, and information on the representativeness of the sample was generally not given. Nevertheless, we tried to ensure that the most valid studies concerning the two objectives of this review were included through restrictive inclusion criteria. Another limitation is that mortality rate estimates are based on prevalence and not on incidence studies. Incidence studies are rare and are clearly needed for future research.

WHO has recently recommended the inclusion of rotavirus vaccine worldwide, based principally on the additional preliminary results of vaccine efficacy studies in two African countries (Malawi and South Africa).[75] Concerns about the safety and feasibility of the introduction of the rotavirus vaccine in low-income countries have been commented on elsewhere,[24,76,77] and include the previous poor responses of live oral vaccines observed in these settings,[78,79] problems delivering the recommended number of doses, timing of first dose administration, and the risk of intussusception if given to children older than recommended by manufacturers.[80]

Documentation on the burden of disease and the circulating strains is essential to assess the effectiveness of the vaccines and the impact of their future introduction. To assess the burden of disease and the circulating strains, regional surveillance by standardised methods, which enable comparison between countries, should be either reinforced or developed. WHO strongly recommends sentinel surveillance before the introduction of the vaccines to monitor vaccine impact (although absence of surveillance should not stop vaccine introduction).[75] These sentinel surveillance systems should ideally measure mortality and morbidity, both hospital and community based, and the strains circulating, by RT-PCR based on new primers and standardised techniques. The African Regional Network–Rotavirus Surveillance Network is currently doing hospital-based surveillance of rotavirus disease in children aged under 5 years in 14 African countries with the support from WHO,[81] US Centers for Disease Control and Prevention,[82] and PATH's rotavirus vaccine programme. This critical effort should be expanded to other countries. After the implementation of the vaccines, adapted surveillance will ideally allow the detection of the impact of rotavirus vaccines on the burden of disease, possible adverse events, and the emergence of new strains as rotaviruses evolve.

Contributors
ESP, RFG, PJG, and FJL had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ESP, RFG, PJG, ADS, MEB and FJL took part in the creation and design of the study, interpretation of the data, drafting of the paper, and critical revision.

Conflicts of interest
The authors declare no conflicts of interest.

References
1   Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006; **354**: 11–22.
2   Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006; **354**: 23–33.
3   WHO. United Nations prequalified vaccines. WHO list of vaccines for purchase by UN agencies as of July 2009. http://www.who.int/immunization_standards/vaccine_quality/pq_suppliers/en/ (accessed July 16, 2009).
4   Parashar UD, Bresee JS, Glass RI. The global burden of diarrhoeal disease in children. *Bull World Health Organ* 2003; **81**: 236.
5   Estes MK. Rotaviruses and their replication. In: Knipe DM, Howley PM, eds. Fields virology, vol 2, 4th edn. Philadelphia: Lippincott, Williams & Wilkins; 2001: 1747–86.
6   Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* 2005; **15**: 29–56.
7   Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; **81**: 197–204.
8   Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis* 2006; **12**: 304–06.
9   Gentsch JR, Laird AR, Bielfelt B, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *J Infect Dis* 2005; **192** (suppl 1): S146–59.
10   UNESCO. List of countries: sub-Saharan Africa. http://www.uis.unesco.org/profiles/en/edu/countries40350.html (accessed July 17, 2009).
11   DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177–88.
12   Freeman MF, Tukey JW. Transformations related to the angular and the square root. *Ann Math Stat* 1950; **21**: 607–11.
13   Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934; **26**: 404–13.
14   Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003; **9**: 565–72.
15   WHO. External review of burden of disease attributable to rotavirus: 30 November to 1 December 2005. http://www.who.int/immunization_monitoring/burden/Rota_virus_Q5_mortality_estimates_external_review_report_2006_may.pdf (accessed July 16, 2009).
16   United Nations Populations Information Network. World population prospects: the 2008 revision population database. http://esa.un.org/unpp/index.asp?panel=2 (accessed July 17, 2009).
17   Steele AD, Ivanoff B. Rotavirus strains circulating in Africa during 1996–1999: emergence of G9 strains and P[6] strains. *Vaccine* 2003; **21**: 361–67.
18   Anagonou SY, Koumakpai S, Josse R, Massougbodji A, Sadeler BC, Martet G. Rotavirus gastroenteritis in a pediatric service at the National University Hospital Center of Cotonou (Benin). *Med Trop (Mars)* 1993; **53**: 105–07.
19   Kasule M, Sebunya TK, Gashe BA, Armah G, Steele AD. Detection and characterization of human rotavirus among children with diarrhoea in Botswana. *Trop Med Int Health* 2003; **8**: 1137–42.
20   Kebaabetswe LP, Sebunya TK, Matsheka MI, Ndung'u T. Detection and molecular characterisation of group a rotavirus from children in northern Botswana. *East Afr Med J* 2005; **82**: 203–08.
21   Basu G, Rossouw J, Sebunya TK, et al. Prevalence of rotavirus, adenovirus and astrovirus infection in young children with gastroenteritis in Gaborone, Botswana. *East Afr Med J* 2003; **80**: 652–55.
22   Esona MD, Armah GE, Steele AD. Molecular epidemiology of rotavirus infection in Western Cameroon. *J Trop Pediatr* 2003; **49**: 160–63.
23   Koulla-Shiro S, Loe C, Ekoe T. Prevalence of *Campylobacter enteritis* in children from Yaounde (Cameroon). *Cent Afr J Med* 1995; **41**: 91–94.
24   Armah GE, Steele AD, Binka FN, et al. Changing patterns of rotavirus genotypes in Ghana: emergence of human rotavirus G9 as a major cause of diarrhea in children. *J Clin Microbiol* 2003; **41**: 2317–22.

25 Armah GE, Hori H, Anyanful A, et al. Human rotavirus subgroups and severity of associated diarrhoea in Ghana. *Afr J Health Sci* 1995; **2:** 388–91.

26 Binka FN, Anto FK, Oduro AR, et al. Incidence and risk factors of paediatric rotavirus diarrhoea in northern Ghana. *Trop Med Int Health* 2003; **8:** 840–46.

27 Silva PA, Stark K, Mockenhaupt FP, et al. Molecular characterization of enteric viral agents from children in northern region of Ghana. *J Med Virol* 2008; **80:** 1790–98.

28 Armah GE, Pager CT, Asmah RH, et al. Prevalence of unusual human rotavirus strains in Ghanaian children. *J Med Virol* 2001; **63:** 67–71.

29 Asmah RH, Green J, Armah GE, et al. Rotavirus G and P genotypes in rural Ghana. *J Clin Microbiol* 2001; **39:** 1981–84.

30 Armah GE, Mingle JA, Dodoo AK, et al. Seasonality of rotavirus infection in Ghana. *Ann Trop Paediatr* 1994; **14:** 223–29.

31 Nakano T, Binka FN, Afari EA, et al. Survey of enteropathogenic agents in children with and without diarrhoea in Ghana. *J Trop Med Hyg* 1990; **93:** 408–12.

32 Nielsen NM, Eugen-Olsen J, Aaby P, Molbak K, Rodrigues A, Fischer TK. Characterisation of rotavirus strains among hospitalised and non-hospitalised children in Guinea-Bissau, 2002: a high frequency of mixed infections with serotype G8. *J Clin Virol* 2005; **34:** 13–21.

33 Fischer TK, Page NA, Griffin DD, et al. Characterization of incompletely typed rotavirus strains from Guinea-Bissau: identification of G8 and G9 types and a high frequency of mixed infections. *Virology* 2003; **311:** 125–33.

34 Fischer TK, Steinsland H, Molbak K, et al. Genotype profiles of rotavirus strains from children in a suburban community in Guinea-Bissau, western Africa. *J Clin Microbiol* 2000; **38:** 264–67.

35 Fischer TK, Valentiner-Branth P, Steinsland H, et al. Protective immunity after natural rotavirus infection: a community cohort study of newborn children in Guinea-Bissau, west Africa. *J Infect Dis* 2002; **186:** 593–97.

36 Molbak K, Wested N, Hojlyng N, et al. The etiology of early childhood diarrhea: a community study from Guinea-Bissau. *J Infect Dis* 1994; **169:** 581–87.

37 Akoua-Koffi C, Akran V, Peenze I, et al. Epidemiological and virological aspects rotavirus diarrhoea in Abidjan, Côte d'Ivoire (1997–2000). *Bull Soc Pathol Exot* 2007; **100:** 246–49.

38 Nakata S, Gatheru Z, Ukae S, et al. Epidemiological study of the G serotype distribution of group A rotaviruses in Kenya from 1991 to 1994. *J Med Virol* 1999; **58:** 296–303.

39 Saidi SM, Iijima Y, Sang WK, et al. Epidemiological study on infectious diarrheal diseases in children in a coastal rural area of Kenya. *Microbiol Immunol* 1997; **41:** 773–78.

40 Cunliffe NA, Dove W, Bunn JE, et al. Expanding global distribution of rotavirus serotype G9: detection in Libya, Kenya, and Cuba. *Emerg Infect Dis* 2001; **7:** 890–92.

41 Nokes DJ, Abwao J, Pamba A, et al. Incidence and clinical characteristics of group A rotavirus infections among children admitted to hospital in Kilifi, Kenya. *PLoS Med* 2008; **5:** e153.

42 Kiulia NM, Peenze I, Dewar J, et al. Molecular characterisation of the rotavirus strains prevalent in Maua, Meru North, Kenya. *East Afr Med J* 2006; **83:** 360–65.

43 Cunliffe NA, Gondwe JS, Broadhead RL, et al. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. *J Med Virol* 1999; **57:** 308–12.

44 Cunliffe NA, Gondwe JS, Graham SM, et al. Rotavirus strain diversity in Blantyre, Malawi, from 1997 to 1999. *J Clin Microbiol* 2001; **39:** 836–43.

45 Cunliffe NA, Ngwira BM, Dove W, et al. Serotype g12 rotaviruses, Lilongwe, Malawi. *Emerg Infect Dis* 2009; **15:** 87–90.

46 Mandomando IM, Macete EV, Ruiz J, et al. Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. *Am J Trop Med Hyg* 2007; **76:** 522–27.

47 Steele AD, Nimzing L, Peenze I, et al. Circulation of the novel G9 and G8 rotavirus strains in Nigeria in 1998/1999. *J Med Virol* 2002; **67:** 608–12.

48 Audu R, Omilabu SA, De Beer M, Peenze I, Steele AD. Diversity of human rotavirus VP6, VP7, and VP4 in Lagos State, Nigeria. *J Health Popul Nutr* 2002; **20:** 59–64.

49 Adah MI, Rohwedder A, Olaleye OD, Durojaiye OA, Werchau H. Further characterization of field strains of rotavirus from Nigeria VP4 genotype P6 most frequently identified among symptomatically infected children. *J Trop Pediatr* 1997; **43:** 267–74.

50 Gomwalk NE, Umoh UJ, Gosham LT, Ahmad AA. Influence of climatic factors on rotavirus infection among children with acute gastroenteritis in Zaria, northern Nigeria. *J Trop Pediatr* 1993; **39:** 293–97.

51 Adah MI, Wade A, Taniguchi K. Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. *J Clin Microbiol* 2001; **39:** 3969–75.

52 Salu OB, Audu R, Geyer A, Steele AD, Oyefolu AO. Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. *J Clin Microbiol* 2003; **41:** 913–14.

53 Odimayo MS, Olanrewaju WI, Omilabu SA, Adegboro B. Prevalence of rotavirus-induced diarrhea among children under 5 years in Ilorin, Nigeria. *J Trop Pediatr* 2008; **54:** 343–46.

54 Gomwalk NE, Gosham LT, Umoh UJ. Rotavirus gastroenteritis in pediatric diarrhoea in Jos, Nigeria. *J Trop Pediatr* 1990; **36:** 52–55.

55 Aminu M, Ahmad AA, Umoh JU. Rotavirus infection in four states in north-western Nigeria. *Niger J Med* 2008; **17:** 285–90.

56 Adah MI, Rohwedder A, Olaleye OD, Durojaiye OA, Werchau H. Serotype of Nigerian rotavirus strains. *Trop Med Int Health* 1997; **2:** 363–70.

57 Avery RM, Shelton AP, Beards GM, Omotade OO, Oyejide OC, Olaleye DO. Viral agents associated with infantile gastroenteritis in Nigeria: relative prevalence of adenovirus serotypes 40 and 41, astrovirus, and rotavirus serotypes 1 to 4. *J Diarrhoeal Dis Res* 1992; **10:** 105–08.

58 Audu R, Omilabu SA, Peenze I, Steele D. Viral diarrhoea in young children in two districts in Nigeria. *Cent Afr J Med* 2002; **48:** 59–63.

59 Pennap G, Peenze, De Beer M, et al. VP6 subgroup and VP7 serotype of human rotavirus in Zaria, northern Nigeria. *J Trop Pediatr* 2000; **46:** 344–47.

60 Steele AD, Basetse HR, Blacklow NR, Herrmann JE. Astrovirus infection in South Africa: a pilot study. *Ann Trop Paediatr* 1998; **18:** 315–19.

61 Sebata T, Steele AD. Atypical rotavirus identified from young children with diarrhoea in South Africa. *J Health Popul Nutr* 2001; **19:** 199–203.

62 Mphahlele MJ, Steele AD. Relative frequency of human rotavirus VP4 (P) genotypes recovered over a ten-year period from South African children with diarrhea. *J Med Virol* 1995; **47:** 1–5.

63 Haffejee IE, Moosa A. Rotavirus studies in Indian (Asian) South African infants with acute gastro-enteritis, I: microbiological and epidemiological aspects. *Ann Trop Paediatr* 1990; **10:** 165–72.

64 Mnisi YN, Williams MM, Steele AD. Subgroup and serotype epidemiology of human rotaviruses recovered at Ga-Rankuwa, southern Africa. *Cent Afr J Med* 1992; **38:** 221–25.

65 Bos P, Mnisi YN, Steele AD. The molecular epidemiology of rotavirus infection in Ga-Rankuwa, southern Africa. *Cent Afr J Med* 1992; **38:** 286–90.

66 Griffiths FH, Steele AD, Alexander JJ. The molecular epidemiology of rotavirus-associated gastro-enteritis in the Transkei, southern Africa. *Ann Trop Paediatr* 1992; **12:** 259–64.

67 Moyo SJ, Gro N, Kirsti V, et al. Prevalence of enteropathogenic viruses and molecular characterization of group A rotavirus among children with diarrhea in Dar es Salaam Tanzania. *BMC Public Health* 2007; **7:** 359.

68 Steele AD, Kasolo FC, Bos P, Peenze I, Oshitani H, Mpabalwani E. Characterization of VP6 subgroup, VP7 and VP4 genotype of rotavirus strains in Lusaka, Zambia. *Ann Trop Paediatr* 1998; **18:** 111–16.

69 Mpabalwani M, Oshitani H, Kasolo F, et al. Rotavirus gastro-enteritis in hospitalized children with acute diarrhoea in Zambia. *Ann Trop Paediatr* 1995; **15:** 39–43.

70 Tswana SA, Jorgensen PH, Halliwell RW, Kapaata R, Moyo SR. The incidence of rotavirus infection in children from two selected study areas in Zimbabwe. *Cent Afr J Med* 1990; **36:** 241–46.

71   WHO. Global and national estimates of deaths under age five attributable to rotavirus infection: 2004. As of 31 March 2006. http://www.who.int/immunization_monitoring/burden/Global_national_estimates_2004_deaths_under_age_five_attributable_to_rotavirus_infection_2004.pdf (accessed July 16, 2009).

72   Ferreccio C, Prado V, Ojeda A, et al. Epidemiologic patterns of acute diarrhea and endemic *Shigella* infections in children in a poor periurban setting in Santiago, Chile. *Am J Epidemiol* 1991; **134:** 614–27.

73   Kapikian AZ, Hoshino Y. To serotype or not to serotype: that is still the question. *J Infect Dis* 2007; **195:** 611–14.

74   Iturriza-Gomara M, Isherwood B, Desselberger U, Gray J. Reassortment in vivo: driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *J Virol* 2001; **75:** 3696–705.

75   WHO. Meeting of the immunization Strategic Advisory Group of Experts, April 2009—conclusions and recommendations. *Wkly Epidemiol Rec* 2009; **84:** 220–36.

76   Glass RI, Parashar UD, Bresee JS, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet* 2006; **368:** 323–32.

77   Parez N. Rotavirus gastroenteritis: why to back up the development of new vaccines? *Comp Immunol Microbiol Infect Dis* 2008; **31:** 253–69.

78   Su-Arehawaratana P, Singharaj P, Taylor DN, et al. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. *J Infect Dis* 1992; **165:** 1042–48.

79   Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. *Rev Infect Dis* 1991; **13:** 926–39.

80   de Oliveira LH, Danovaro-Holliday MC, Matus CR, Andrus JK. Rotavirus vaccine introduction in the Americas: progress and lessons learned. *Expert Rev Vaccines* 2008; **7:** 345–53.

81   WHO. New and under-utilized vaccines implementation (NUVI): rotavirus. http://www.who.int/nuvi/rotavirus/en/ (accessed July 17, 2009).

82   Centers for Disease Control and Prevention. Global rotavirus surveillance: AFRO region. http://www.cdc.gov/rotavirus/global_surveillance/afro.htm (accessed July 17, 2009).