Evaluating strategies to improve rotavirus vaccine impact during the second year of life in Malawi

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Rotavirus vaccination has substantially reduced the incidence of rotavirus-associated gastroenteritis (RVGE) in high-income countries, but vaccine impact and estimated effectiveness are lower in low-income countries for reasons that are poorly understood. We used mathematical modeling to quantify rotavirus vaccine impact and investigate reduced vaccine effectiveness, particularly during the second year of life, in Malawi, where vaccination was introduced in October 2012 with doses at 6 and 10 weeks. We fitted models to 12 years of prevaccination data and validated the models against postvaccination data to evaluate the magnitude and duration of vaccine protection. The observed rollout of vaccination in Malawi was predicted to lead to a 26 to 77% decrease in the overall incidence of moderate-to-severe RVGE in 2016, depending on assumptions about waning of vaccine-induced immunity and heterogeneity in vaccine response. Vaccine effectiveness estimates were predicted to be higher among 4- to 11-month-olds than 12- to 23-month-olds, even when vaccine-induced immunity did not wane, due to differences in the rate at which vaccinated and unvaccinated individuals acquire immunity from natural infection. We found that vaccine effectiveness during the first and second years of life could potentially be improved by increasing the proportion of infants who respond to vaccination or by lowering the rotavirus transmission rate. An additional dose of rotavirus vaccine at 9 months of age was predicted to lead to higher estimated vaccine effectiveness but to only modest (5 to 16%) reductions in RVGE incidence over the first 3 years after introduction, regardless of assumptions about waning of vaccine-induced immunity.

INTRODUCTION

Rotavirus is a leading cause of morbidity and death from diarrhea in children worldwide (1, 2). Although the recent introduction of rotavirus vaccines has had a substantial impact on the incidence of rotavirus-associated gastroenteritis (RVGE) in high- and middle-income countries, the impact of vaccination in low-income countries is not as well defined (3). The vast majority of deaths due to rotavirus occurs in low-income countries in Asia and Africa (1, 2), but estimates of vaccine efficacy are lower in these settings. Whereas vaccine efficacy was 85 to 99% in clinical trials conducted in high-income countries (4–7), efficacy was 39 to 67% in trials conducted in low-income countries for all currently available oral rotavirus vaccines (8–12). Nevertheless, the preventable burden of disease in low-income settings is potentially much greater (13).

The overall impact of a vaccination program results from both the direct and indirect effects of vaccination (14). Two key questions regarding the long-term impact of rotavirus vaccines are whether the direct protection conferred by rotavirus vaccination is reduced in low-income countries due to a poorer initial immune response or waning of vaccine-induced immunity and whether vaccination will confer sustained indirect (herd) protection similar to that observed in high-income countries (15).

Concerns have been raised that vaccine-induced immunity to rotavirus wanes among infants from low-income settings. These concerns are based on estimates of the observed individual-level direct effect of vaccination. Rotavirus vaccine efficacy, measured under the idealized conditions of randomized controlled trials, has tended to be lower during the second year of follow-up in low-income settings and correlated with lower titers of serum immunoglobulin A (IgA) mounted in response to vaccination for both the monovalent Rotarix (RV1) and pentavalent RotaTeq (RV5) vaccines across multiple countries (16). Lower vaccine effectiveness during the second year of life, measured under the real-world conditions of postlicensure studies, has also been reported in some, but not all, low-income settings (17). However, commonly used estimates of vaccine efficacy and effectiveness on which inferences about waning immunity are based may be biased, because they fail to take into account immunity from previous infections (18, 19). Nevertheless, it has been suggested that an additional dose of rotavirus vaccine at 9 months of age can improve vaccine effectiveness and thereby vaccine impact, especially in the second year of life (20). Parenteral rotavirus vaccines are also in development and may lead to a more robust and durable immune response particularly in low-income settings (21, 22).

In high-income countries, the introduction of rotavirus vaccination was followed by greater reductions in RVGE incidence than expected from the direct protection of the vaccine alone, including among age groups too old to have received the vaccine, providing evidence of indirect (or herd) protection (23–26). Observed patterns of indirect protection in high-income countries are similar to those predicted by mathematical models (27–29). However, similar evidence of indirect protection in low-income countries has been limited (30, 31).
Here, we use mathematical models to investigate the potential waning of vaccine-induced immunity and to better understand the role of indirect protection in determining the overall impact of vaccination in a low-income setting. We used data from Blantyre, Malawi, where we had more than 10 years of prevaccination surveillance data (32, 33), as well as data from vaccine efficacy trials and recently conducted vaccine effectiveness and impact studies (10, 30, 34–36). We simulated models with and without waning of vaccine-induced immunity and heterogeneity in vaccine response (Table 1) to explore the concordance between model predictions and the observed impact of vaccination. We examined how common vaccine effectiveness estimates vary depending on model assumptions. Validated models were then used to evaluate strategies to improve vaccine effectiveness measures, including increasing the proportion of infants who respond to vaccination, lowering the rotavirus transmission rate, and administering an additional dose of vaccine at 9 months of age.

### RESULTS

**Model fit**

We modified a previously developed mathematical model of the transmission dynamics of rotavirus (27, 28, 37) to explicitly test hypotheses about the nature of vaccine-induced immunity (Table 1 and fig. S1). We fitted our models to prevaccination data on the number of RVGE cases among children <5 years of age at Queen Elizabeth Central Hospital (QECH), Blantyre, between July 1997 and December 2009 (Fig. 1 and table S1). The prevaccination model captured the age distribution of RVGE cases, although it slightly underestimated cases among 5- and 12-month-olds and slightly overestimated cases among 8- to 10-month-olds. In Blantyre, the basic reproductive number ($R_0$) of rotavirus, defined as the expected number of secondary infections caused by a primary case in a fully susceptible population, was estimated to be 78.8 [95% credible interval (CrI), 70.5 to 96.2] (table S1), but this estimate depended on assumptions about the relative infectiousness of subclinical infections and duration of immunity after infection (table S2).

**Predicted impact of vaccination**

Malawi introduced rotavirus vaccination on 28 October 2012, with two doses of RV1 administered orally at 6 and 10 weeks of age (36). Vaccine coverage among age-eligible children in Blantyre increased rapidly, with ≥96% of infants receiving at least one dose by June 2014 (fig. S2).

The observed rollout of rotavirus vaccination was predicted to lead to a 12.2 to 27.0% reduction in the overall incidence of RVGE cases at QECH during the first year after vaccine introduction, depending on our assumptions about waning of vaccine-induced immunity and the probability of responding to each dose (Table 2 and Fig. 2). By 2016, the predicted reduction in overall incidence was 25.6 to 76.7%, whereas the observed incidence of RVGE was 50.8% lower than the prevaccination period (Table 2 and fig. S3). Models 3 and 4, which assumed that vaccination provides a temporary complete immunity that wanes over time, predicted the smallest decrease in the overall incidence. In contrast, model 1, which assumed an equal probability of responding to each dose and vaccine-induced immunity that provides partial but long-lasting protection (comparable to natural immunity), predicted the largest decrease (Fig. 2 and fig. S3). The greatest reduction in incidence was predicted to occur among infants 4 to 11 months of age, with more modest reductions in incidence predicted among 0- to 3-month-olds and 12- to 23-month-olds in all models; a slight increase in cases among infants 12 to 23 months old was predicted when we assumed waning of vaccine-induced immunity (Table 2).

The range of model predictions was generally consistent with the observed number of cases (Table 2) and with the 33% decline in

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Description</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waning of vaccine-induced immunity</td>
<td>No Vaccine-induced immunity is comparable to natural immunity, lowering the risk of reinfection and probability of RVGE given infection; this immunity does not wane</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes Vaccine-induced immunity provides complete protection against infection with rotavirus; this immunity wanes over time, leaving the infant with the same susceptibility to rotavirus infection and RVGE as before vaccination</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heterogeneity in vaccine response</td>
<td>No The probability of responding to each vaccine dose is independent; infants who responded to the first vaccine dose have the same probability of responding to the second vaccine dose as those who failed to respond</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes Infants who responded to the first dose of the vaccine are more likely to respond to subsequent doses, whereas infants who failed to respond to the first dose are more likely to be nonresponders (they would fail to respond to rotavirus vaccination regardless of the number of doses administered)</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
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</table>

*Models 1 and 2 were not fitted to the postvaccination data.*
rotavirus positivity previously reported (30). However, none of our models captured the observed increase in RVGE cases among 12- to 23-month-olds in the first year after vaccine introduction (Fig. 2 and Table 2). Our models also predicted smaller declines in incidence among 0- to 3-month-olds than observed (Table 2 and fig. S3), although there may have been less effort to test infants who were too young to have been vaccinated. The model assuming waning of vaccine-induced immunity and homogeneity in vaccine response (model 3) provided the best fit to the postvaccination data, whereas model 1 provided the worst fit (Fig. 2 and table S3). However, models 3 and 4 tended to slightly overpredict cases among vaccinated individuals, whereas model 1 and, to a lesser extent, model 2 tended to underpredict cases among vaccinated individuals (fig. S4).

Vaccine effectiveness estimates
To demonstrate how commonly used estimates of vaccine effectiveness based on estimates of relative odds or risk (19) may be biased and not reflect “true” underlying vaccine-conferring protection (as assumed in the models), we compared the predicted vaccine effectiveness estimates from the four models to one another and to those calculated from an ongoing case-control study. The model-predicted vaccine effectiveness estimates from the four models to one another and to those assumed in the models), we compared the predicted vaccine effectiveness estimates from the four models to one another and to those calculated from an ongoing case-control study. The model-predicted vaccine effectiveness estimates varied depending on assumptions about the probability of responding to each dose and the waning of vaccine-induced immunity (Table S4). The overall incidence of RVGE among <5 years old predicted by models 1 to 4 was similar to that predicted by the corresponding models with direct protection only, but incidence was predicted to be higher among 0- to 3-month-olds and higher among 12- to 23-month-olds when accounting for both direct and indirect protection (Table S5).

Strategies to improve vaccine effectiveness and impact
We modeled three different scenarios for improving vaccine performance, as measured by estimated vaccine effectiveness and overall impact: (i) increasing the proportion of infants who seroconvert to the first and second dose of vaccine, (ii) reducing the transmission rate of rotavirus through nonvaccine interventions, and (iii) administering an additional vaccine dose at 9 months of age.

The predicted vaccine effectiveness estimates depended strongly on the proportion of infants who respond to each dose (Fig. 4, A and B). Estimates of effectiveness among 12- to 23-month-olds varied from 81.2% when only 40% of individuals responded to each dose to 12.2% when 100% of infants responded to each dose, assuming waning of vaccine-induced immunity (model 3). Results were similar for the other models (figs. S5 and S6).

The transmission rate of rotavirus, quantified by $R_0$, also influenced the model-predicted vaccine effectiveness estimates (Fig. 4, C and D). For any given value of the proportion of infants who respond to
An additional vaccine dose at 9 months of age was predicted to lead to a slightly higher estimated vaccine effectiveness in infants 4 to 11 months of age (model 1: VE1, 82.9%; 95% PI, 75.0 to 88.6%) and considerably higher values during the second year of life (VE2, 88.3%; 95% PI, 82.0 to 92.8%) (Fig. 5A). However, estimated effectiveness was predicted to increase over time in all models even without the additional vaccine dose, as vaccine coverage stabilized and the force of rotavirus infection declined (figs. S8 to S10). Compared to the modeled impact of the current two-dose schedule, the model-predicted incidence of RVGE was reduced by 32.5% (95% PI, 24.6 to 39.2%) among 12- to 23-month-olds over the first 3 years, but the overall incidence of RVGE predicted by model 1 was only 16.2% (95% PI, 11.8 to 20.3%) lower than predicted without the additional dose (Fig. 5, B and F). The predicted overall impact of an additional 9-month dose of rotavirus vaccine was similar (11.5%; 95% PI, 8.4 to 14.6%) if we assumed that vaccine-induced immunity waned over time (model 3) but was lower (5.2 to 8.6%) if we assumed heterogeneity in vaccine response (models 2 and 4) (Fig. 5, C to F). A similar reduction in incidence could be achieved by increasing the proportion of infants who respond to each dose of the vaccine by 7 to 12% (equivalent to a 54 to 57% seroconversion rate) when assuming homogeneity in vaccine response (models 1 and 3) or by increasing the proportion of infants who respond to the first dose of vaccine by 3 to 11% when assuming heterogeneity in vaccine response (models 2 and 4), or a 20 to 45% reduction in \( R_0 \) (fig. S11).

**DISCUSSION**

Using mathematical models fitted to prevaccination data on RVGE cases in Blantyre, Malawi, we quantified the overall impact of vaccination resulting from both the direct and indirect effects. We demonstrated that commonly used measures of vaccine effectiveness are expected to be lower for the second year of life even if vaccine-induced immunity does not wane. The observed rotavirus vaccine impact and estimated vaccine effectiveness in Malawi are consistent with either waning of vaccine-induced immunity or heterogeneity in vaccine response. We predicted that an additional dose of rotavirus vaccine at 9 months of age would lead to modest improvements in overall vaccine impact regardless of whether vaccine-induced immunity wanes. Similar reductions in RVGE incidence could be achieved with relatively small improvements in the vaccine response rate, because the majority of rotavirus cases still occur during the first year of life.

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**Table 2. Observed and model-predicted vaccine impact.** The observed reduction in annual incidence of RVGE cases compared to the incidence predicted in the absence of vaccination is shown. Overall incidence is reported as the number of cases per 100,000 children <5 years of age in Blantyre city, and age-specific incidence is reported as the observed number of cases per year.  

<table>
<thead>
<tr>
<th>Age group</th>
<th>Overall</th>
<th>0–3 months</th>
<th>4–11 months</th>
<th>12–23 months</th>
<th>Overall</th>
<th>0–3 months</th>
<th>4–11 months</th>
<th>12–23 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>156.1 (−6.1%)</td>
<td>15 (46.3%)</td>
<td>144 (−63.6%)</td>
<td>46 (−44.0%)</td>
<td>147.6 (&lt;1%)</td>
<td>28.7 (&lt;1%)</td>
<td>136.3 (&lt;1%)</td>
<td>31.0 (&lt;1%)</td>
</tr>
<tr>
<td>2013</td>
<td>136.1 (14.6%)</td>
<td>8 (73.7%)</td>
<td>101 (31.7%)</td>
<td>75 (−72.6%)</td>
<td>116.5–140.2 (12.2–27.0%)</td>
<td>20.4–27.9 (10.8–34.9%)</td>
<td>103.2–126.7 (14.9–30.7%)</td>
<td>38.2–40.8 (3.3–9.4%)</td>
</tr>
<tr>
<td>2014</td>
<td>87.5 (44.2%)</td>
<td>5 (83.3%)</td>
<td>70 (52.1%)</td>
<td>46 (4.0%)</td>
<td>61.2–119.6 (23.8–61.0%)</td>
<td>13.7–24.2 (21.3–55.7%)</td>
<td>48.8–99.4 (32.4–66.8%)</td>
<td>23.6–47.6 (−2.3–49.2%)</td>
</tr>
<tr>
<td>2015</td>
<td>106.2 (26.0%)</td>
<td>6 (78.3%)</td>
<td>82 (39.3%)</td>
<td>55 (−31.9%)</td>
<td>36.4–105.7 (26.6–74.7%)</td>
<td>10.3–21.2 (25.4–63.8%)</td>
<td>27.5–81.7 (40.0–79.8%)</td>
<td>13.0–43.7 (−7.7–67.9%)</td>
</tr>
<tr>
<td>2016</td>
<td>56.9 (50.8%)</td>
<td>6 (73.3%)</td>
<td>48 (56.5%)</td>
<td>23 (28.2%)</td>
<td>27.0–86.1 (25.6–76.7%)</td>
<td>8.3–17.4 (24.7–64.2%)</td>
<td>21.3–66.2 (40.4–80.8%)</td>
<td>8.4–33.9 (−9.0–73.1%)</td>
</tr>
<tr>
<td>2013–2016</td>
<td>95.8 (32.9%)</td>
<td>25 (77.4%)</td>
<td>301 (44.2%)</td>
<td>199 (−20.5%)</td>
<td>77.7–119.8 (21.7–58.3%)</td>
<td>52.7–90.9 (20.1–53.7%)</td>
<td>200.8–374.0 (31.1–63.0%)</td>
<td>83.3–165.9 (−3.4–48.1%)</td>
</tr>
</tbody>
</table>

*Percent reduction is relative to the model-predicted incidence (per 100,000 children <5 years old) and annual number of cases in the corresponding years in the absence of vaccination. | Range in best-fit model predictions is given for the four models of response to vaccination. | Cases per 100,000 children <5 years old per year, based on population census estimates for Blantyre city. Population estimates by age were only available through 2014; estimates for 2015 and 2016 (and for the postvaccination period from 2013 to 2016) are based on the most recent (2014) data for the size of the population <5 years old. | Mean annual overall incidence and cases by age for 1998–2006 and 2008–2009. | Rotavirus vaccination (RV1) was introduced 28 October 2012, with routine doses administered at 6 and 10 weeks of age.
Estimates of vaccine efficacy and effectiveness during the second year of life will be biased and not reflect the true protection conferred by vaccination, even in clinical trial settings, if they fail to take into account heterogeneity in transmission and the protection conferred by previous infections (19, 39). Vaccinated and unvaccinated infants can no longer be considered randomized after a 1 year of follow-up due to their differential risk of natural infections during the first year of life. Unvaccinated individuals are able to “catch up” to vaccinated individuals because they gain immunity through natural infections, making them more comparable during the second year of life. This will lead to lower estimates of vaccine effectiveness even in the absence of waning immunity, particularly when the rate of (re)infection is high, as it is for rotavirus in Malawi. Therefore, we cannot conclusively determine from the vaccine effectiveness estimates whether vaccine-induced immunity is waning. This has been noted previously for other imperfectly immunizing infections, such as malaria (40). It has been previously shown that it is possible to partially correct for this bias by controlling for previous episodes of RVGE in the analysis (18). However, infants can also gain partial protection from asymptomatic rotavirus infections (41, 42). Such infections have not been tested for in rotavirus vaccine trials or postlicensure effectiveness studies and therefore cannot be taken into account using traditional analytical frameworks.

Fig. 2. Observed and model-predicted vaccine impact by age. The observed (thin black lines) and model-predicted (colored lines) number of RVGE cases per week at QECH are plotted for cases 0 to 11 months old (n = 539; left, blue), 12 to 23 months old (n = 269; middle, green), and 24 to 59 months old (n = 35; right, red) for (A) model 1, (B) model 2, (C) model 3, and (D) model 4. The light gray line is the 13-week moving average of the observed number of cases, and the shaded colored regions represent the prediction intervals of the models accounting for parameter uncertainty and observation error. The vertical dashed line represents the time of vaccine introduction.

Fig. 3. Observed and predicted vaccine effectiveness estimates by age. Observed (red lines) and model-predicted (gray bars) vaccine effectiveness estimates are plotted for children 4 to 11 months (n = 278 cases, 655 controls) and 12 to 23 months of age (n = 113 cases, 291 controls) under the four different model assumptions. The red circles represent the estimated mean vaccine effectiveness, and the red lines show the 95% confidence intervals. The black error bars represent the 95% prediction intervals associated with parameter uncertainty.
Indirect protection (herd immunity) is defined as the reduction in incidence among unvaccinated individuals in a population where the vaccine is available compared to the incidence in a completely unvaccinated population (43) and is an important component of overall vaccine impact. Indirect protection has been evident in a number of high-income countries after rotavirus vaccine introduction (23–26, 31) and has played a substantial role in reducing the incidence of RVGE among unvaccinated and age-ineligible children. Estimates of indirect protection in the United States varied from 14 to 82% during the first 4 years after vaccine introduction (26). Declines in incidence among age-ineligible children have been noted in Rwanda after vaccine introduction (44). However, no indirect protection was observed during a cluster-randomized trial of rotavirus vaccine in Bangladesh (45). In Malawi, some evidence of indirect protection was observed among infants <1 year of age, as noted previously (30), but the number of cases among children 1 year of age and older actually increased relative to the prevaccination period, particularly during the first year after vaccine introduction. This may be due in part to variation in reporting effort, because we also observed an increase in the number of rotavirus-negative acute gastroenteritis cases. Although our models predict substantial indirect protection among all children <5 years old compared to the incidence rate expected in the absence of vaccination, this was not apparent when comparing the overall impact to that expected from the direct effects of vaccination alone. The latter should not be interpreted as a failure of the vaccine to provide indirect protection, because vaccination is expected to increase the average age of infection (46) and the majority of cases among >1-year-olds have occurred among vaccinated individuals. Model-predicted estimates of vaccine effectiveness varied depending on both the proportion of infants who respond to each vaccine dose and the rotavirus transmission rate. When the proportion of infants who respond to each dose is high and when $R_0$ is low, as is the case in most high-income countries (27, 28, 38), the estimated vaccine effectiveness was predicted to remain high during the second year of life even when vaccine-induced immunity wanes. This suggests that other interventions aimed at lowering the rotavirus transmission rate, such as improvements in sanitation and hygiene, could have synergistic benefits for improving vaccine impact. However, when not all infants respond to vaccination and when $R_0$ is high, estimated vaccine effectiveness is predicted to be lower particularly during the second year of life. Rates of seroconversion to rotavirus vaccination tended to be lower in settings with high under-five mortality rates (16). The reasons underlying these lower rates of seroconversion are not fully understood but are likely multifactorial and common to other live oral vaccines (47). Recent studies have suggested that interference with oral poliovirus vaccines (48), enterovirus infection (49), and the microbiome (50) may all play a role in explaining the poor response to live oral rotavirus vaccines, which may lead to the development of new strategies to improve vaccine response. Neonatal oral rotavirus vaccines may offer one solution and demonstrated higher vaccine efficacy than an infant vaccine schedule in one trial (51). Parenteral inactivated rotavirus vaccines are also currently being developed and may help to overcome the problems associated with live oral vaccines (21, 22).

An additional dose of rotavirus vaccine at 9 months of age has been suggested as a strategy to improve vaccine effectiveness and impact in developing countries. Immunogenicity trials conducted in Bangladesh and Mali have demonstrated that an additional dose of either RV1 (20) or RV5 (52) administered at 9 months of age increased geometric mean titers and rates of IgA and IgG seroconversion. Another recent trial demonstrated higher rates of seroconversion after three doses of RV1 administered at 6, 10, and 14 weeks of age compared to the current two-dose schedule (53). A third dose of vaccine at 14 weeks of age also led to modest improvements in immunogenicity and efficacy in other RV1 trials, including one

![Fig. 4. Relationship between the vaccine response, the basic reproductive number, and model-predicted vaccine effectiveness estimates.](http://stm.sciencemag.org/)

The model-predicted vaccine effectiveness estimates among (A) infants 4 to 11 months old and (B) children 12 to 23 months old is indicated by the color bar for different values of the proportion of infants who respond to the first and second dose for model 3 (the model assuming waning of vaccine-induced immunity and no heterogeneity in vaccine response). The model-predicted vaccine effectiveness estimates among (C) infants 4 to 11 months old and (D) children 12 to 23 months old is indicated by the color bar for different values of the basic reproductive number ($R_0$) and the proportion of infants who respond to each dose in model 3. The black bars represent the 95% CrI of the estimated parameters for our model fitted to the prevaccination data for Blantyre, Malawi.
conducted in Malawi (10, 34, 54), although these trials were underpowered to detect such a difference. However, the potential impact of an additional dose of rotavirus vaccine has yet to be evaluated in the context of larger, programmatic use.

Although an additional dose of rotavirus vaccine at 9 months of age in Malawi was predicted to result in a higher estimated vaccine effectiveness and was associated with a 32% decline in incidence among 12- to 23-month-olds, the predicted reductions in overall incidence were modest (5 to 16%), because the majority of RVGE cases still occurs during the first year of life. This reduction in overall incidence is similar to that predicted by a static model (2 to 24%) (55). We found that the overall impact of an additional dose was lowest when we assumed heterogeneity in vaccine response, because under this assumption, individuals who failed to respond to the first vaccine dose would also be less likely to respond to subsequent vaccine doses. Thus, it will be critical to determine whether an additional dose of rotavirus vaccine at 9 months of age will benefit those who failed to respond to earlier vaccine doses.

There are several limitations of our study that should be considered. As with all modeling studies, we needed to make a number of simplifying assumptions to model the transmission of rotavirus and protection conferred by vaccination. The four models of vaccine protection that we considered here (Table 1) are by no means exhaustive and represent different extremes with regard to the response to vaccination and waning of vaccine-induced immunity; the reality is likely somewhere in between. We also did not consider potential differences in natural and vaccine-induced immunity against the various rotavirus genotypes. Previous analyses have suggested that the estimated vaccine effectiveness in Malawi is higher against RVGE caused by G1P[8] strains compared to that estimated for G2P[4] and other fully heterotypic strains, but the overall distribution of genotypes causing RVGE in Malawi remains relatively unchanged since vaccine introduction (35).

Last, we did not have data on previous rotavirus infections and episodes of RVGE among the cases and controls in our vaccine effectiveness study. Therefore, it was not possible to obtain unbiased estimates of vaccine effectiveness by controlling for previous infections (18).

We believe that our findings are generalizable to other settings with high rates of rotavirus transmission and low rates of seroconversion after vaccination with oral rotavirus vaccines. Model-predicted
vaccine effectiveness estimates varied considerably depending on the basic reproductive number \((R_0)\) and proportion of infants who responded to each dose of vaccine. Thus, differences in the rate of transmission of rotavirus may help to explain why estimates of effectiveness have remained high during the second year of life in some low-income settings but not others (17).

Mathematical modeling provides a valuable tool for understanding the observed impacts of vaccination resulting from both direct and indirect protection and for evaluating future strategies to improve vaccine impact. Our analysis suggests that future efforts should focus on improving the immune response to vaccination, possibly through the development of parenteral rotavirus vaccines or new strategies for the delivery of oral vaccines (for example, a neonatal dose). An additional vaccine dose at 9 months of age was predicted to lead to only modest gains in vaccine impact. However, more studies are needed to gain empirical, unbiased estimates of efficacy and to evaluate the cost effectiveness of such strategies.

**MATERIALS AND METHODS**

**Study design**

This was a mathematical modeling study with three primary objectives: (i) to evaluate the evidence for waning of vaccine-induced immunity against RVGE, (ii) to estimate the level of indirect protection conferred by rotavirus vaccination, and (iii) to evaluate strategies to improve rotavirus vaccine effectiveness and impact in a low-income setting. We fitted our model to pre- and postvaccination data on the weekly number of RVGE cases at QECH in Blantyre, Malawi and compared to data from a case-control study of vaccine effectiveness in Blantyre. Additional methods and details of the model can be found in the Supplementary Materials and Methods.

**Data**

The surveillance platform and data have been described previously (32, 33). Briefly, inpatients and outpatients <5 years of age who presented to QECH with acute gastroenteritis (defined as the passage of three or more watery stools in a 24-hour period for <14 days) were enrolled. Written informed consent was obtained from a parent or guardian, and a single stool sample was collected from each child as soon as possible and tested for rotavirus by enzyme immunoassay (Rotaclone, Meridian Diagnostics); positive samples were further characterized by G- and P-type using reverse transcription polymerase chain reaction. During the periods of prevaccination surveillance (1 July 1997 to 30 June 2007 and 1 January 2008 to 31 December 2009), enrollment was restricted to weekdays (Monday to Friday) from 8:00 a.m. to 17:00 p.m. Diagnostic stool testing is not a part of routine clinical care at QECH; therefore, data were not available outside of the study periods (1 July to 31 December 2007 and 1 January 2010 to 31 December 2011).

Enhanced surveillance was initiated at QECH in January 2012 in anticipation of the introduction of rotavirus vaccination (on 28 October 2012) (35, 36); we evaluated the data through August 2017. Enrollment of all children meeting the case definition of acute gastroenteritis occurred Monday to Saturday. A case-control study was initiated on 29 October 2012 to evaluate vaccine effectiveness. Cases were defined as vaccine age-eligible children presenting with acute gastroenteritis who tested positive for rotavirus by enzyme immunoassay, whereas controls were unmatched vaccine age-eligible children presenting with acute gastroenteritis who tested negative for rotavirus (35, 36). Rotavirus vaccination status was obtained for both rotavirus-positive and -negative children from government-issued patient-held “health passports”; those with a missing record (18%) were excluded from estimates of vaccine effectiveness. Ethical approval for the data collection was obtained from the National Health Sciences Research Committee, Lilongwe, Malawi (867) and from the University of Liverpool Research Ethics Committee (000490).

**Statistical analysis**

We estimated the observed vaccine effectiveness \((VE_{obs})\) and corresponding 95% CI as one minus the odds ratio of receiving two doses of rotavirus vaccine among rotavirus-positive acute gastroenteritis cases versus rotavirus-negative diarrheal controls using logistic regression, adjusting for age, and year and month of presentation, as in previous analyses (30, 35).

**Model description**

We modified a previously developed mathematical model used to describe patterns of RVGE incidence in high-income countries (27, 28, 37) and adapted this model to the low-income country context. Briefly, the model assumes that individuals are born with maternal immunity that provides protection against rotavirus infection and disease (fig. S1A). After the waning of maternal immunity, individuals are assumed to be fully susceptible to rotavirus infection and disease. Susceptible individuals are infected at a rate \(\lambda(t)\), also known as the force of infection. Only a fraction of individuals with their first infection will experience moderate-to-severe RVGE. After infection, we assume that individuals gain temporary immunity and then become susceptible to reinfection at a reduced rate. Subsequent infections are assumed to have lower infectiousness, shorter average duration, and lower probability of symptoms. We differentiate among \(i = 1, 2, 3\), or more previous infections and assume that all subsequent infections \((i \geq 3)\) do not lead to observed cases of moderate-to-severe RVGE. We further divide each state into 1-month age classes for individuals <2 years of age, 1-year age classes for children 2 to 4 years old, and 5-year age classes for children and adults 5 to \(\geq 75\) years old. The number of individuals in each state is simulated using a series of differential equations.

**Modeling vaccination**

We simulated the impact of vaccination in two ways to explore assumptions about the potential waning of vaccine-induced immunity (Table 1). First, we assumed that each vaccine dose mimics one natural infection among those who respond to the vaccine—thus providing partial immunity against reinfection and full protection against moderate-to-severe RVGE after two “successful” vaccine doses and/or natural infections (models 1 and 2). We include separate compartments for vaccinated individuals and assumed that those who failed to respond to a vaccine dose would stay in their same compartment in the corresponding vaccinated state, whereas those who responded moved to the next recovered (and vaccinated) compartment (fig. S1A). We also explored the alternative assumption that a single dose of vaccine provided temporary but complete immunity against reinfection with rotavirus among those who responded (models 3 and 4) (fig. S1B). After the waning of vaccine-induced immunity, vaccinated infants returned to their previous level of susceptibility, whereas those who responded to both vaccine doses moved to the next vaccinated (and protected) compartment.
Vaccine coverage in our model was parameterized on the basis of the observed proportion of age-eligible children with rotavirus-negative acute gastroenteritis who had received at least one or two doses of the vaccine collected as part of the case-control study (fig. S2). The numerator consisted of the weekly number of rotavirus-negative controls who were age eligible and reported having received at least one or two doses of rotavirus vaccine, whereas the denominator consisted of the weekly number of all age-eligible diarrheal controls who tested negative for rotavirus. We then smoothed the one- and two-dose coverage estimates by calculating the 27-week moving average.

The proportion of individuals who responded after each dose of the vaccine was informed by seroconversion data from the RV1 vaccine trial conducted in Malawi (34). We assumed that the proportion of individuals who seroconverted in the trial (defined as an antirotavirus IgA antibody concentration of ≥20 U/ml) represents the proportion of individuals who received protection equivalent to two natural infections, which, in our models, would mean that the infant was fully protected against moderate-to-severe RVGE. Although there is no established correlate of protection for RVGE, a serum IgA concentration of ≥20 U/ml has been shown to at least partially predict vaccine efficacy at both the individual and population scale (56). Among those in the two-dose arm of the immunogenicity trial in Malawi, 17 of 36 (47.2%; 95% CI, 30 to 64%) infants seroconverted, whereas in the three-dose arm, 28 of 49 (57.1%; 95% CI, 42 to 72%) infants seroconverted (34).

We explored two different assumptions for the proportion of infants who responded to each dose (Table 1). First, we assumed that the proportion of infants responding to the first and second dose was equal and independent of one another. The probability of infants responding to each dose (and thus receiving partial protection against RVGE) could thus be estimated by taking the square root of the rate of seroconversion in the two-dose group. We assumed that the probability of responding to a third dose of the vaccine at 9 months of age was the same as the probability of responding to previous doses.

Alternatively, we might assume that there is heterogeneity in vaccine response, such that individuals who failed to respond to the first dose may be less likely to respond to subsequent doses. Thus, we assumed some fraction of infants would fail to seroconvert regardless of the number of doses (“nonresponders”) and estimated the proportion of nonresponders, and the probability of responding among the remaining segment of the population based on the proportion of infants who seroconverted after two versus three doses during the vaccine trial in Malawi. Uncertainty in the probability of responding to each dose of the vaccine was characterized using beta distributions.

Model-predicted vaccine effects

We calculated three different vaccine effects from our model: (i) the direct effect (that is, vaccine effectiveness), (ii) the indirect effect (also called herd protection), and (iii) the overall effect (vaccine impact). The estimated vaccine effectiveness was an output of our model, as determined by the proportion of vaccinated individuals who responded after each dose of the vaccine and the reduction in risk of infection and of RVGE given infection. In line with conventional methods used to estimate vaccine efficacy for rotavirus vaccines (for example, (10)), we estimated the vaccine effectiveness predicted by the model as one minus the relative incidence of moderate-to-severe RVGE cases among vaccinated versus unvaccinated by year of age:

\[ \text{VE}_{RR,A} = 1 - \frac{\sum_{t=1}^{t_i} H_v, A(t) \sum_{t=1}^{t_i} V_u(t)}{\sum_{t=1}^{t_i} H_v, A(t) \sum_{t=1}^{t_i} U_u(t)} \]

where \( t_i \) is the week of vaccine introduction, \( t_f \) is the last week of follow-up, \( H_v, A(t) \) and \( V_u(t) \) are the model-predicted number of reported RVGE cases among vaccinated and unvaccinated individuals \( A \) years of age in week \( t \), respectively, and \( V_v(t) \) and \( U_u(t) \) are the total number of vaccinated and unvaccinated individuals \( A \) years of age in week \( t \) in the model (and, hence, the sum is the person-time contribution to the vaccinated and unvaccinated states, respectively).

We calculated the model-predicted indirect effect in two ways. First, we estimated the indirect effect in each age group as one minus the relative risk of reported RVGE among unvaccinated children compared to the model-predicted risk in the absence of vaccination:

\[ \text{IE}_A = 1 - \frac{\sum_{t=1}^{t_f} H_v, A(t) \sum_{t=1}^{t_f} U_u(t)}{\sum_{t=1}^{t_f} H_{novacc}, A(t) \sum_{t=1}^{t_f} N_u(t)} \]

where \( H_{novacc}, A(t) \) is the model-predicted number of reported RVGE cases among children in age group \( A \) in week \( t \) in the absence of vaccination (that is, when vaccine coverage is set to zero), and \( N_u(t) \) is the total number of individuals of in age group \( A \) in the population during week \( t \) in the model. Next, we simulated the model while fixing \( \lambda(t) \) at the force of infection predicted in the absence of vaccination (that is, assuming no reduction in transmission after vaccine introduction). We then compared the predicted overall vaccine impact for the full dynamic models to the impact for the corresponding models with direct protection only.

Last, we estimated the vaccine impact (overall effect) as one minus the model-predicted incidence of RVGE cases with vaccination (accounting for both the direct and indirect effects) divided by the incidence predicted by the model in the absence of vaccination. We estimated the vaccine impact for each year after vaccine introduction.

Model fitting and external validation

To relate the model-predicted incidence of rotavirus infection to the observed incidence of RVGE, we assumed that only a fraction \( h \) of individuals with moderate-to-severe RVGE would present to QECH, have a stool sample collected, and test positive for rotavirus. We then assumed that the number of cases of age \( a \) in week \( t \) was Poisson-distributed with a mean equal to the age-specific number of model-predicted incident cases of moderate-to-severe RVGE times the “reporting fraction” \( h \). Because the rotavirus testing effort varied through time, we allowed \( h \) to vary proportional to the 2-year moving average of the number of rotavirus-negative cases at QECH (fig. S12) and estimated the mean reporting fraction for the prevaccination period. We initially fit the model to the prevaccination incidence of RVGE cases via maximum a posterior estimation and then used a Markov chain Monte Carlo to obtain posterior distributions for our model parameters (fig. S13).

We validated our models by comparing model predictions for the estimated overall vaccine impact, vaccine effectiveness, and...
indirect effect to the observed vaccine effects. To predict the incidence of RVGE cases presenting to QECH in the postvaccination period, we assumed that the rotavirus transmission rate, seasonality, and mean reporting fraction would not change between the 15-year prevaccination period and the 5-year postvaccination period. Thus, in our models, the only thing that should explain changes to RVGE incidence is the introduction of vaccination. To account for changes in surveillance effort over time, we multiplied the model-predicted number of RVGE cases by a scaling factor equal to the moving average of the number of rotavirus-negative cases in each age group divided by average number of rotavirus-negative cases per week during the prevaccination period.

For models 1 and 2, we did not use the postvaccination data for model fitting. However, for models 3 and 4, we needed to estimate the duration of vaccine-induced immunity by fitting the model-predicted incidence of RVGE cases to the observed data on rotavirus-positive acute gastroenteritis cases at QECH for the period from January 2012 to August 2017. We also estimated the proportion of infants who responded to each dose, using informative priors for the latter (fig. S14). The fit of the four models to the postvaccination data is compared in table S3.

**Scenarios to improve vaccine performance**
We assessed the potential impact of measures aimed at increasing the proportion of infants who seroconvert to the first and second dose of rotavirus vaccine by calculating the estimated vaccine effectiveness for children 4 to 11 months versus 12 to 23 months of age while varying the proportion of infants who respond to each dose between 0.4 and 1. To evaluate the potential impact of nonvaccine interventions that could reduce the transmission rate of rotavirus and to compare to vaccine effectiveness estimates from higher-income countries, we estimated the vaccine effectiveness for children 4 to 11 months versus 12 to 23 months of age for baseline values of \( R_0 \) varying from 25 to 100 [consistent with the range of \( R_0 \) values estimated for different countries (37)], while also varying the proportion of infants who respond to each dose. Last, we evaluated the impact of an additional vaccine dose administered at 9 months of age by assuming that one- and two-dose vaccine coverage would remain at its most recently observed level and that coverage with the booster dose would be 81% beginning in January 2018, based on the latest available estimate of measles vaccine coverage in Malawi (57). We compared the predicted impact of an additional dose to the other two strategies to determine the level of improvement in immunogenicity or reduction in \( R_0 \) that would be needed to achieve similar reductions in the incidence of RVGE over the next 3 years, assuming that each intervention was implemented as a step function in January 2018.

**REFERENCES AND NOTES**


**Funding:** This work was supported by grant R01AI112970 from the U.S. NIH/National Institute of Allergy and Infectious Diseases to V.E.P. Rotavirus surveillance in Blantyre, Malawi, was supported by a Wellcome Trust Programme Grant to N.A.C. (number 091909/Z/10/Z). A.B. was the recipient of a Wellcome Trust Clinical PhD Fellowship (number 102466/Z/13/A). K.C.J. was supported by a Wellcome Trust Public Health and Tropical Medicine Training Fellowship (number 201945/Z/16/Z). **Author contributions:** V.E.P. and N.A.C. conceived and designed the study. V.E.P. developed models. A.B., N.B.-Z., K.C.J., and N.A.C. collected data. V.E.P., A.B., N.B.-Z., and N.A.C. analyzed data. B.A.L., J.A.L., and U.D.P. helped to interpret the results. All authors wrote the manuscript. **Competing interests:** V.E.P. is a member of the WHO Immunization and Vaccine-related Implementation Research Advisory Committee (WIR-AC) and has received reimbursement from Merck for travel expenses to attend a Scientific Input Engagement unrelated to rotavirus vaccines, N.B.-Z. and K.C.J. have received research grant support from GlaxoSmithKline Biologicals for work on rotavirus vaccines. N.B.Z. also reports income from Takeda Pharmaceuticals. B.A.L. reports personal fees from Takeda Pharmaceuticals, personal fees from the CDC Foundation, and personal fees from Hall Booth Smith PC, all unrelated to rotavirus vaccines. J.A.L. has received research grants and consulting fees from Merck and Pfizer, unrelated to the current work. N.A.C. has received research grant support and honoraria for participation in rotavirus vaccine Independent Data Monitoring Committee meetings from GlaxoSmithKline Biologicals. **Data and materials availability:** All data associated with this manuscript are present in the paper or the Supplementary Materials. Model code and data necessary to reproduce the results of the study are available at DOI: 10.5281/zenodo.3336499.

Submitted 5 October 2018
Resubmitted 8 February 2019
Accepted 25 July 2019
Published 14 August 2019
10.1126/scitranslmed.aav6419

Evaluating strategies to improve rotavirus vaccine impact during the second year of life in Malawi

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Sci Transl Med 11, eaav6419.
DOI: 10.1126/scitranslmed.aav6419

Examining disparity in protection against rotavirus
The rotavirus vaccine is less effective in low-income countries compared to high-income countries, although the reasons for this are unknown. Pitzer et al. used mathematical modeling to analyze infection data in Malawian children before and after the introduction of a rotavirus vaccine to examine the nature of vaccine-induced immunity. They found that endemic infection increased the protection observed in the clinical study control arm, reducing the apparent efficacy of the vaccine arm. The authors also determined that a booster dose at 9 months is unlikely to lead to substantially improved protection and suggest that efforts may be better placed into developing a more immunogenic vaccine.