Atypical DS-1–like G1P[8] rotaviruses emerged in 2013 in Malawi after rotavirus vaccine introduction. Vaccine effectiveness among infants hospitalized with acute DS-1–like G1P[8] rotavirus gastroenteritis was 85.6% (95% CI 34.4%–96.8%). These findings suggest that vaccine provides protection against these strains despite their emergence coinciding with vaccine introduction.

**The Study**

We used enzyme immunoassay (EIA) to detect rotaviruses in stool samples collected from children <5 years of age with acute gastroenteritis at Queen Elisabeth Central Hospital (QECH; Blantyre, Malawi) (2). We used reverse transcription PCR to assign G and P genotypes to rotavirus-positive samples (10,11). Samples with sufficient volume and containing G1 (n = 110), G2 (n = 64), or other (G4, G9, or G12, n = 42) rotavirus strains were selected at random during January 2013–December 2015.

We generated rotavirus whole-genome sequences (WGS) using the HiSeq 2000 platform (Illumina Inc., https://www.illumina.com) as described previously (10). We derived consensus sequences using Geneious (https://www.geneious.com) and genotyped them using Ro taC (http://rotac.regatools.be). All complete nucleotide
sequences generated in this study were deposited into Gen-Bank (12) (accession nos. MG181227–941).

We calculated rotavirus VE using logistic regression to compare 2-dose versus 0-dose vaccination status among hospitalized strain-specific rotavirus diarrhea case-patients and concurrently hospitalized control patients with non–rotavirus-caused diarrhea, matched by age at admission. We defined concurrence of controls for each endpoint (Table) as any patient hospitalized for diarrhea who tested negative for rotavirus occurring in the same date range (between the first and last hospitalization strain-specific case) in which cases of strain-specific rotavirus were detected. We limited VE analysis to infants <12 months of age because previous analysis did not demonstrate statistically significant protection in the second year of life (VE 31.7%, 95% CI –140.6% to 80.6%) (2). We obtained ethics approval from the National Health Sciences Research Committee, Malawi (867), and the Research Ethics Committee of the University of Liverpool, Liverpool, UK (000490).

Of 216 rotavirus strains sequenced, 114 (53%) had a Wa-like and 88 (44%) a DS-1–like genotype constellation. Among Wa-like strains, 72% were G1, <1% were G2, and 25% were G12. Of the DS-1–like strains, 31% were G1 and 69% were G2. Of the 110 G1 strains analyzed by WGS, 75% were Wa-like and 25% were DS-1–like. We detected atypical G1 rotaviruses with DS-1–like genotype constellation in Malawi in 2013; their circulation peaked in 2014 and subsequently decreased in 2015 (<1%, 1/72) (Figure).

In logistic regression analysis adjusted for year of presentation, Rotarix effectiveness against DS-1–like G1P[8] rotavirus was 85.6% (95% CI 34.4%–96.8%; p = 0.01). Effectiveness estimates against Wa-like G1 (VE 76.7%, 95% CI –153.8% to 97.9%) and DS-1–like G2 (VE 48.5%, 95% CI –154.3% to 89.6%) rotaviruses included wide bounds and the null value (Table).

Conclusions

Atypical DS-1–like G1 rotavirus strains emerged in Malawi shortly after Rotarix vaccine introduction (10). Although strain oscillation and emergence of novel types have been reported globally in the absence of vaccination, the mechanisms driving this phenomenon are not well understood. It is possible that the emergence of these DS-1–like G1P[8] strains was coincidental with vaccine introduction. The high VE strongly suggests that escape from vaccine-induced immunity is not the driver for emergence. The swift decline in prevalence of these strains is in contrast with more sustained changes in strain circulation described in other settings in the context of high VE (13). The decline could have been precipitated by the observed high VE or may represent a natural phenomenon related to viral fitness and associated periodic nature of the circulation of the DS-1–like strains, which has been observed historically and globally in the absence of vaccine. These findings support continued use of rotavirus vaccine in this population as an intervention to reduce severe diarrhea caused by rotavirus strains possessing either Wa-like or DS-1–like genetic backbones. The observed decline in rotavirus hospitalizations in children after vaccine introduction (2), together with reduction in infant diarrhea deaths in Malawi (14), are public health benefits that could be sustained through rotavirus vaccination in this region, which has one of the highest burdens of rotavirus disease.

The VE against DS-1–like G1P[8] strains in this study resembles our previous findings of VE of 82% (95% CI 42%–95%) against all G1P[8] strains 3 years after vaccine introduction (2013–2015) (2). In contrast, we were unable to demonstrate statistically significant VE against DS-1–like G2 rotaviruses despite a comparable number of such strains, consistent with our earlier study (VE 45.9%, 95% CI –47.0% to 80.1%; p = 0.228) (2). The apparently lower VE against rotavirus disease caused by DS-1–like strains associated with G2, but not with G1P[8], lends support to the proposed dominant role of the outer capsid proteins VP7 and VP4 as drivers of homotypic protection. Although increasing evidence suggests that Rotarix vaccine does not provide the same degree of protection against G2 strains as G1 strains, this difference in protection appears to have little effect on total VE among populations in which vaccination performs optimally and high VE is maintained. However, the difference in protection between the strains may exacerbate underperformance of rotavirus vaccines in low-resource settings such as Malawi, where overall VE is generally lower for reasons that remain poorly understood (2,15).

Table. Point estimates of vaccine effectiveness by rotavirus genotype constellation based on the complete genetic composition of rotavirus strains, Blantyre, Malawi*

<table>
<thead>
<tr>
<th>Rotavirus strain type</th>
<th>Sequenced strains from test-positive case-patients†</th>
<th>Rotavirus test-negative controls</th>
<th>Adjusted logistic regression for year of presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested positive</td>
<td>No. known vaccine status</td>
<td>No. (%) 2-dose vaccine</td>
</tr>
<tr>
<td>DS-1–like G1P[8]</td>
<td>13</td>
<td>13</td>
<td>10 (76.9)</td>
</tr>
<tr>
<td>DS-1–like G2</td>
<td>30</td>
<td>28</td>
<td>24 (85.7)</td>
</tr>
<tr>
<td>Wa-like G1</td>
<td>38</td>
<td>38</td>
<td>34 (89.5)</td>
</tr>
</tbody>
</table>

*Rotavirus strains detected at Queen Elizabeth Central Hospital during January 2013–December 2015. Case-patients were fully vaccinated infants <12 mo of age.
†Complete whole-genome sequences were generated.
We could not demonstrate statistically significant effectiveness against Wa-like G1P[8] rotaviruses (p = 0.23). Wa-like G1P[8] cases became dominant and replaced DS-1-like G1P[8] once vaccine coverage had reached high and stable levels (Figure). At high population vaccine coverage, case–control analysis of VE became challenging and difficult to power sufficiently.

Our data demonstrate that Rotarix provides a high degree of protection against severe disease caused by homotypic G1P[8] rotaviruses in Malawi regardless of genomic backbone. VE for patients <1 year of age is comparable to that seen in middle-income countries. The lower VE against heterotypic G2P[4] strains previously described (15) suggests that more detailed immune response studies, clarification of the correlates of protection for rotavirus disease, and strain surveillance are needed to monitor the impact of sustained, high vaccine coverage on rotavirus strain distribution.

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Dr. Jere is a Wellcome International Training Fellow who conducted this research as part of his postdoctoral studies. His research interests are in enteric viral pathogens.

References