Faecal shedding of rotavirus vaccine in Chinese children after vaccination with Lanzhou lamb rotavirus vaccine

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Lanzhou lamb rotavirus vaccine (LLR) is an oral live attenuated vaccine first licensed in China in 2000. To date, > 60 million doses of LLR have been distributed to children. However, very little is known about faecal shedding of LLR in children. Therefore, faecal samples (n = 1,184) were collected from 114 children for 15 days post-vaccination in September–November 2011/2012. Faecal shedding and viral loads were determined by an enzyme immunoassay kit (EIA) and real-time RT-PCR. The complete genome was sequenced and the vaccine strain was isolated by culture in MA104 cells. Approximately 14.0% (16/114) of children had rotavirus-positive samples by EIA for at least 1 day post-vaccination. Viral loads in EIA-positive samples ranged from $<1.0 \times 10^3$ to $1.9 \times 10^8$ copies/g. Faecal shedding occurred as early as post-vaccination day 2 and as late as post-vaccination day 13 and peaked on post-vaccination day 5–10. One LLR strain was isolated by culture in MA104 cells. Sequence analysis showed 99% identity with LLR prototype strain. Faecal shedding of LLR in stool is common within 15 days of LLR vaccination, indicating vaccine strains can replicate in human enteric tissues.

Rotavirus (RV), a member of the Reoviridae family, has a double-stranded RNA genome with 11 segments. RV group A is recognised as the most common pathogen of acute gastroenteritis, especially severe cases, in children < 5 years of age worldwide. The estimated number of RV deaths in children <5 years of age was 215,000 (range, 197,000–233,000), which accounted for 37.3% (95% confidence interval, 34.2–40.5%) of diarrhoea deaths in 2013. In China, RV-associated hospitalisations account for 32–50% of all hospitalisations for diarrhoea among infants and children < 5 years of age.

In 1998, an oral live tetravalent rhesus-human reassortant RV vaccine (RotaShield, Wyeth Laboratories, Marietta, PA, USA) was licensed and subsequently withdrawn from the United States (US) market due to increased risk of intussusception in infants. Recently, a pentavalent bovine-human reassortant RV vaccine (PRV; RotaTeq, Merck, Whitehouse Station, NJ, USA) and a human attenuated monovalent RV vaccine (HRV; Rotarix, GlaxoSmithKline, Brentford, UK) have been licensed in many countries. Studies reported that these three vaccines caused viral shedding in stool samples within a relatively short period (i.e., 30 days) post-vaccination and also confirmed the risk of vaccine strain transmission. Vaccine strain transmission from vaccinated children to unvaccinated contacts may lead to herd immunity. However, it also carries the risk of vaccine-derived disease in immunocompromised patients.

A RV vaccine, Lanzhou lamb rotavirus (LLR) vaccine, has been developed and licensed in China since 2000. LLR is a monovalent ovine attenuated vaccine (serotype (G10P[15]) group A) that is produced in neonatal calf kidney cells. At the end of 2014, a total of 60 million doses of LLR had been distributed to children in China. LLR was shown to confer a certain level of protection against RV gastroenteritis by a population-based active surveillance study, even under a less ideal immunisation schedule. However, very little is known about the post-marketing effectiveness of LLR, especially faecal shedding of LLR. Therefore, in this study, we characterised faecal shedding of LLR in infants post-vaccination.
Results

**General patient information.** A total of 120 infants (54 girls and 66 boys) aged 6–36 months, the median age is 14 months, were enrolled, including 40 children in 2011 and 80 children in 2012. Samples were unavailable in 5.0% (6/120) of children in this study. Of children with available samples, 93.0% (106/114) received their first dose and 7.0% (8/114) just received the second dose of LRR vaccine.

A total of 1,184 stool samples were collected from 114 children, including 343 samples collected in 2011 and 841 samples collected in 2012. Approximately 3.5% (4/114) of children were sampled for 4 days, 11.4% (13/114) were sampled for 5–7 days, 4.39% (5/114) were sampled for 8–9 days, and 80.7% (92/114) were sampled for 10–15 days. Concerning number of samples, 5.5% (7/114) of children had <5 samples, 29.8% (34/114) had 6–10 samples, and 64.0% (73/114) had 11–15 samples. Two or three samples per day were collected from nine children who had diarrhoea.

**RV antigen testing.** Approximately 14.0% (16/114) of children had RV-positive stool samples by EIA for at least 1 day within the 15-day post-vaccination period. Faecal shedding of LRR occurred as early as post-vaccination day 2 and as late as post-vaccination day 13 and peaked on post-vaccination day 5–10 (Table 1). The mean duration (± standard deviation) of faecal shedding of LRR was 3.7 ± 1.6 days.

Fifty-five samples were RV-positive by EIA. Of these samples, 58.2% (32/55) had OD values ranging 0.2–0.5, 30.9% (17/55) had OD values ranging 0.5–1.0, 7.3% (4/55) had OD values ranging 1.0–1.5, and 3.6% (2/55) had OD values >1.5. Approximately 13.2% (14/106) of children who received the first dose and 25.0% (2/8) of children who received the two doses of LRR exhibited faecal shedding of LRR, but there was no difference in vaccine viral faecal shedding between the two groups (p > 0.05, Chi-square test with continuity correction). 75% (12/16) faecal shedding of LRR were most detected in 6 to 15 months, but there was no difference in vaccine viral faecal shedding between the three age groups (1–12 months, 13–24 months and 25–36 months) (p > 0.05, Wilcoxon Scores test). The details of faecal shedding of LRR are presented in Fig. 1 and Table 2.

**Viral load evaluation.** No RV-positive samples were detected by real-time RT-PCR from the samples of the three children who were RV-negative by EIA. Viral loads in EIA-positive samples ranged from <1.0 × 10^3 to 1.9 × 10^8 copies/g by real-time RT-PCR. Approximately 3.6% (2/55) of RV-positive samples by EIA had > 1.0 × 10^8 copies/g, 65.5% (36/55) had < 1.0 × 10^5–1.0 × 10^8 copies/g, 23.6% (13/55) had 1 × 10^5–1 × 10^6 copies/g, and 7.3% (4/55) were negative for LRR (Table 2). Total viral loads of the two bottles LRR with each of 3 ml as positive control were 1.1 × 10^8 and 2.7 × 10^6 copies/g, respectively.

**RV cultivation and genome analysis.** Cytopathology was observed in one sample on post-vaccination day 8 from a 1-year-old boy who received 1 dose of LRR. LRR strain was also detected from the culture supernatant by...
Table 2. LLR shedding patterns and viral loads (copies per gram of stool) of infants with rotavirus positive stool specimens *Is real-time PCR, # samples were cultured. The data of post vaccine day 1, day 14, day 15 were not showing for they are all negative.

| Infant | day 2 | day 3 | day 4 | day 5 | day 6 | day 7 | day 8 | day 9 | day 10 | day 11 | day 12 | day 13 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| EIA    | PCR*  | EIA   | PCR*  | EIA   | PCR*  | EIA   | PCR*  | EIA   | PCR*  | EIA   | PCR*  | EIA   |
| 1      | —     | —     | —     | —     | ++    | +     | 2.1 10^6 | N     | —     | —     | —     | —     |
| 2      | —     | —     | —     | —     | ++    | +     | 1.7 10^6 | +     | +     | 1.7 10^6 | +     | +     | N     |
| 3      | —     | —     | —     | —     | —     | +     | 3.7 10^6 | —     | —     | N     | —     | N     |
| 4      | —     | N     | —     | —     | —     | +     | 4.9 10^6 | —     | —     | —     | —     | —     |
| 5      | N     | —     | —     | —     | —     | +     | 1.0 10^6 | N     | ++    | 4.7 10^6 | N     | —     | N     |
| 6      | —     | —     | N     | —     | +     | < 1.0 10^6 | N     | N     | ++    | 2.3 10^6 | N     | +     | < 1.0 10^6 | N     |
| 7      | N     | —     | —     | —     | +     | < 1.0 10^6 | +     | +     | 1.9 10^6 | +     | +     | 1.8 10^6 | +     | +     | 1.7 10^6 | +     | —     | —     |
| 8      | —     | N     | —     | N     | +     | < 1.0 10^6 | ++    | 3.9 10^6 | N     | N     | ++    | < 1.0 10^6 | N     | —     | N     |
| 9      | —     | N     | —     | N     | +     | < 1.0 10^6 | ++    | 3.1 10^6 | —     | N     | ++    | —     | —     | N     |
| 10     | N     | —     | —     | —     | +     | < 1.0 10^6 | +     | +     | 1.0 10^6 | +     | +     | 4.3 10^6 | +     | +     | < 1.0 10^6 | N     | 0     | —     | —     |
| 11     | —     | —     | N     | +     | 2.1 10^6 | +     | < 1.0 10^6 | +     | 6.6 10^6 | +     | +     | 1.9 10^6 | +     | ++    | < 1.0 10^6 | N     | 0     | —     | —     |
| 12     | —     | —     | —     | —     | +     | 1.6 10^6 | +     | +     | 1.5 10^6 | —     | —     | —     | —     | —     | —     | —     |
| 13     | +     | < 1.0 10^6 | +     | 2.7 10^6 | +     | 1.1 10^6 | —     | —     | —     | —     | —     | —     | —     | —     |
| 14     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     | —     |
| 15     | —     | —     | +     | < 1.0 10^6 | +     | +     | 1.3 10^6 | +     | 5.0 10^6 | —     | —     | —     | —     | +     | < 1.0 10^6 | +     | 3.7 10^6 | —     | —     | —     |
| 16     | N     | N     | —     | —     | —     | +     | < 1.0 10^6 | +     | < 1.0 10^6 | +     | 2.6 10^6 | +     | 2.7 10^6 | +     | 1.3 10^6 | +     | —     | N     |

Table 3. The mutation of the nucleids and amino sequence of the near complete genome. *The numbers show the mutation position of nucleids and amino sequences.

| segments | 2012034 mutation | 2014059 mutation |
|----------|------------------|------------------|
| NS       | AA               | AA               |
| VP1      | 1485T>A to G 1865C to G 3012C TO T | 616C TO N |
| VP2      | no               | no               |
| VP3      | no               | no               |
| VP4      | 152T to C 1172A to G 1328C TO T | 47 5 to A 440S to L 338Q to R |
| VP6      | no               | no               |
| VP7      | no               | no               |
| NSP1     | no               | no               |
| NSP2     | no               | no               |
| NSP3     | no               | no               |
| NSP4     | no               | no               |
| NSP5     | no               | no               |

RT-PCR and EIA. Furthermore, 10 partial NSP3 fragments were sequenced from 10 children and two near complete genomes of LLR strains were acquired from 2 other children, however, no predominant RV or LLR NSP3 fragments was detected from samples with the four lowest viral load subjects by RT-PCR. In each sample, gene segments were 99--100% identical to corresponding gene fragments of LLR parental strains by BLASTn and rare mutations in amino acids were also found (Table 3). Genome classification analysis showed that the LLR strain is G10-P[15]-I10-R2-C2-M2-A11-N2-T3-E2-H3.

Vaccine adverse events. Approximately 15.8% (19/120) of children had adverse events, however, no family members with close contact with the vaccinated child demonstrated discomfort during the 15-day post-vaccination period. All symptoms began on day 1–5 post-vaccination. No child required outpatient treatment. There were 4.2% (5/120) of children who had low-grade fever of 37.5–38.5°C and 1.7% (2/120) of children...
who had fever of 39.0 °C for 1–3 days. Approximately 2.5% (3/120) and 0.83% (1/120) of children developed nausea and vomiting for 1–3 days and 5 days, respectively. Additionally, 7.5% (9/120) of children reported diarrhoea for 1–3 days with 3–5 episodes/day. One 26-month-old girl who had an allergy to tetanus toxoid vaccine developed erythema on her body for 2 days from day 4 post-vaccination. One 7-month-old boy had irritability for 1 day on day 4 post-vaccination. 94.74% (18/19) adverse events were found in the first dose in the vaccine age between 6 to 17 months (Fig. 1). There were no differences of the adverse events within the three age groups ($\chi^2 = 0.359, p > 0.05$). One or more samples were collected when the child reported adverse event, however, none of the samples was positive for RV by EIA or real-time RT-PCR.

**Discussion**

In China, LLR is currently the only approved vaccine for RV in children. A 3-ml dose of LLR contains > 5.5 lg CCID$_{50}$ live virus/ml. LLR is mainly used for children aged 2 months to 3 years at a schedule of 1 dose annually before the RV epidemic season. More than 60 million doses have been administered to children <5 years of age. However, few preclinical studies, clinical trials, and post-marketing surveillance studies of the effectiveness and adverse events of LLR are available.

In the present study, LLR was detected from day 2–13 post-vaccination and faecal shedding of LLR vaccine lasted from 1–7 day. One LLR strain was also isolated from samples by cultivation in MA104 cells. Faecal shedding peaked at day 5–8 post-vaccination. The faecal shedding LLR most detected in the first dose between 6 to 15 months. However, additional investigation in a greater number of cases is necessary to determine whether the number of doses has an effect on viral shedding post-vaccination.

Based on a Jennerian approach, animal strains that are naturally attenuated in humans exploited for candidate rotavirus vaccines for humans, which were shown to replicate to a lower extent in humans than in their homologous2. In our study, Viral loads exceeded 1.0 × 10$^6$ copies/dose in two samples, which is greater than 1 dose of LLR (mean viral load, 5.7 × 10$^6$ copies/dose), it’s also proved that LLR can replicate in human. Compared to the rotavirus vaccines on market, after the first dose of PRV or HRV, the vaccine shedding rate of infant during 28-day post-vaccination period were 43–56% tested by EIA and 94% tested by real-time RT-PCR, and the vaccine shedding duration were > 14 days in 53.3% and > 30 days in 30.0% of vaccination individuals for PRV and HRV, respectively3,14. LLR showed a lower vaccine shedding rate and a shorter duration of shedding. Viral replication of LLR in the human intestinal tract may be limited and less than that of PRV and HRV. The ability of replication of LLR in human is lower or rotavirus antibody can inhibit the replication of LLR virus for the first dose vaccine when children had infected wild rotavirus. Further study is necessary to evaluate potential reassortant with wild type RV strains or transmission in human.

Rare mutations were found, however, it is unknown if these mutations occurred during replication in the human intestinal tract. Additionally, whether the slightly higher viral loads observed are related to these mutations is unknown. Therefore, verification of LLR parental strains should be improved and viral shedding in stool post-vaccination must be continuously monitored.

Few reports on the protective effect and safety of LLR have been published. Du et al. confirmed that LLR can induce CD4+$^+$ memory T cells, which is a potential indicator of immunogenicity and protection, in mice13. Another study demonstrated LLR effectiveness against RV-associated hospitalisation was 73.3% in Guangzhou, China16 while PRV and HRV vaccine effectiveness against RV-associated hospitalisation was >89% in Hong Kong, a neighbouring region17,18. Additionally, LLR provided a certain level of protection against RV gastroenteritis in villages located in five townships of Hebei, China19. The limited replication of LLR in the human intestinal tract may attribute to its effectiveness to some extent.

Viable virus from faecal shedding of PRV and HRV can result in herd immunity against RV disease in unvaccinated young children, older children, and adults, which has been confirmed in developed regions such as Europe, the US, Latin America, and Australasia12,19,20. However, these effects remain unclear in developing regions3. PRV and HRV can also cause infection in immunodeficient and immunosuppressed individuals who have close contact with vaccinated individuals via faecal-oral transmission21–23. Thus, LLR may also induce herd immunity in nonvaccinated children and infection risk in immunodeficient individuals who have close contact with vaccinated individuals. LLR has been in use in China since 2000, however, LLR is not included in the National Immunization Program in China. Thus, LLR vaccination coverage rates remain very low in Guangzhou and Hebei10,16. The rate of LLR faecal shedding and viral loads were decreased, therefore, herd immunity induced by LLR may be lower than that of the other two RV vaccines. Herd immunity and vaccine cost-effectiveness may increase in China if vaccination coverage improves. Therefore, strategies to improve LLR coverage rates, including vaccination completion rates, should be developed.

Approximately 12.7–14.9% of HRV vaccinated individuals and 27% of PRV vaccinated individuals experienced a vaccine-related adverse event of mild or moderate intensity within 30 days16. In this study, 15.8% (19/120) of vaccinated individuals reported adverse events, such as fever, nausea, vomiting, and diarrhoea. All adverse events were noted during the first dose of LLR with an adjusted adverse events rate of 17.0% (19/112). Adverse events of LLR are similar to or slightly more severe than those of HRV and PRV. Although no RV was detected in the stool samples of children with adverse events, we cannot rule out other potential infections by other pathogens that can cause an acute gastroenteritis, such as norovirus. For instance, development of Kawasaki disease was reported in a girl who received the second dose of LLR and the first dose of a freeze-dried live attenuated hepatitis A vaccine24. Few serious adverse events have been reported in > 60 million doses during the 16-year period since LLR was first administered in China, and the relatively weak replication capacity in the human enteric tract suggests the vaccine is safe for use in children. However, further post-vaccination surveillance of adverse events is necessary.

There are some potential limitations associated with the present study. The study population was restricted to one community with a relatively homogeneous population. Additionally, the post-vaccination period was short.
and may not have been sufficient to record viral shedding and adverse events. Continued post-vaccination surveillance including additional regions and longer study duration are needed to fully evaluate LLR efficacy.

The present study described faecal shedding post-vaccination with LLR, which provides a foundation to expand its use and further research.

**Nucleotide sequence accession numbers.** The two complete genomes have been deposited in the GenBank database under accession numbers KY113326-KY113347. The accession numbers of other NSP3 fragments are MF125696-MF125705.

**Materials and Methods**

**Study participant enrolment.** Enrollment began in September and ended in November of 2011 and 2012, when the vaccine was distributed to children in a community of Beijing, China. We enrolled infants aged 2–36 months who had been deemed eligible to receive their first to third doses of LLR by community doctors.

**Sample and data collection.** After providing verbal consent, primary caretakers were given a bag including five sample collection bottles and instructions on how to collect stool samples during the 15-day post-vaccination period, other collection bottles were sent to the primary caretakers when samples were took to lab. Primary caretakers were also trained to swab the stool into the sample bottle at the same time. All samples were assigned a unique study number consisting of the patient number, sample number, post-vaccination day, and collection day. All samples and clinical symptoms related to vaccine adverse events within 15 days post-vaccination were collected from primary caretakers and sent to labs on the same day by IVDC staff. All samples were stored at −20 °C at IVDC. Furthermore, study team members followed up with caretakers by telephone within 15 days post-vaccination to answer any questions about LLR. All methods described in this article followed the protocol which has been approved by the IRB committee of National Institute for Viral Disease Control and Prevention, China CDC.

**RV antigen detection and Viral load evaluation.** A 10% suspension was made by dissolving stool samples in phosphate-buffered saline for enzyme immunoassay (EIA) (Oxoid Prospect ELISA Kit, Basingstoke, United Kingdom) according to the manufacturer’s protocol. The optical density (OD) of the positive control was 2.5 while OD of the negative control was 0.1. Thus, the positive threshold for EIA was determined as 0.21.

RV double-stranded RNA (dsRNA) from 10% stool suspensions was extracted using an RNA kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. All samples from random selected three children with RV-negative samples by EIA were sent for viral load determination by real-time RT-PCR targeting the NSP3 gene of the majority of genotype of rotavirus to confirm the sensitivity of EIA. Viral loads of RV-positive samples by EIA and two bottles of LLR were also determined by the real-time RT-PCR assay. Samples when the children were uncomfortable were also detected by the real-time RT-PCR assay. Serial dilutions of purified RNA transcribed from plasmids containing the synthesised NSP3 gene were used as a quantification standard. Samples with the five highest viral loads were used for virus isolation and samples with the two highest viral loads were sent for genotyping and genome sequencing. Samples with the highest viral loads from one subject were amplified by RT-PCR to confirm the virus strains with specific primers NSP3r1 and LLR NSP3R. Specific primers NSP3-1F/NSP3-1R were used to amplify a short fragment of the NSP3 region of LLR from the highest viral load samples by real-time PCR but negative by NSP3r1 and LLR NSP3R primers, then G typing for the dominant rotavirus in China, including G1,G2,G3,G4,G8 and G9 genotype rotavirus, was performed using a semi-nested PCR to genotype the samples by primers NSP3-1F/NSP3-1R.

**Nucleotide sequencing and analysis.** Fragments amplified by primer pairs NSP3-1F/NSP3-1R and aBT1G1,Act2G2, G3, aDT4G4,G9/VP7R were sequenced. Two complete genomes from samples with the highest viral loads were amplified and sequenced. All primers used are listed in Supplementary Table S1. Virus genotype and nucleotide sequence similarities were analysed by the RotaC v2.0 (http://rotac.regatools.be/) automated genotyping tool and BLASTn (http://www.ncbi.nlm.nih.gov/).

**Cultivation of LLR in MA104 cells.** Five samples were also culture-adapted to MA104 cells as previously described with some modification. Briefly, 1 ml of a 10% stool suspension was filtered through a 0.45-μm sterile filter (Merck Millipore, Billerica, USA) and activated in the presence of 15 μg of trypsin (Sigma-Aldrich Cat.No. 85450 C, St. Louis, USA) for 1 h at 37 °C. Confluent monolayers of MA104 cells in roller tubes were then inoculated with stool suspensions for 1 h, unabsorbed virus was removed, and 2 ml of DMEM containing neomycin (Gibco Laboratories, California, USA) for 1 h at 37 °C to room temperature) for three times before the next passage, freeze-thawed cell lysates treated with trypsin as described above for preparation of stool supernatants and was performed subsequent passages as described above. After four passages, lysates were tested for RV by real-time RT-PCR and EIA.

**Data analysis.** Cumulative and daily proportions of faecal shedding of LLR for 15 days post-vaccination were calculated. Duration of shedding and peak shedding days within the 15-day post-vaccination period was determined. Adverse events related to LLR were also evaluated. The difference in vaccine viral faecal shedding between children grouped 0–12 months, 13–24 months and 25–36 months was tested by wilcoxon and the difference in the adverse events was tested chi-square by SPSS16.0.
References
1. Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A. & Glass, R. I. Global illness and deaths caused by rotavirus disease in children. *Emerging infectious diseases* **9**, 565–572 (2003).
2. Lopman, B. A., Curns, A. T., Yen, C. & Parashar, U. D. Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States. *The Journal of infectious diseases* **204**, 980–986, https://doi.org/10.1093/infdis/jir492 (2011).
3. Zhang, J. et al. Active, population-based surveillance for rotavirus gastroenteritis in Chinese children: Beijing Municipality and Gansu Province, China. *The Pediatric infectious disease journal* **34**, 40–46, https://doi.org/10.1097/INF.0000000000000505 (2015).
4. Murphy, T. V. et al. Intussusception among infants given an oral rotavirus vaccine. *The New England journal of medicine* **344**, 564–572, https://doi.org/10.1056/NEJM200102223440804 (2001).
5. Bravo, L. et al. Reactogenicity and safety of the human rotavirus vaccine, Rotarix, in The Philippines, Sri Lanka, and India: a post-marketing surveillance study. *Human vaccines & immunotherapeutics* **10**, 2276–2283, https://doi.org/10.4161/hv.29280 (2014).
6. Lawrence, J. et al. Safety and immunogenicity of pentavalent rotavirus vaccine in a randomized, double-blind, placebo-controlled study in healthy elderly subjects. *Human vaccines & immunotherapeutics* **10**, 2247–2254, https://doi.org/10.4161/hv.29107 (2014).
7. Yen, C. et al. Detection of fecal shedding of rotavirus vaccine in infants following their first dose of pentavalent rotavirus vaccine. *Vaccine* **29**, 4151–4155, https://doi.org/10.1016/j.vaccine.2011.03.074 (2011).
8. Medical Advisory Committee of the Immune Deficiency, F. Recommendations for live viral and bacterial vaccines in immunodeficient patients and their close contacts. *The Journal of allergy and clinical immunology* **133**, 961–966, https://doi.org/10.1016/j.jaci.2013.11.043 (2014).
9. Anderson, E. J. Rotavirus vaccines: viral shedding and risk of transmission. *The Lancet Infectious Diseases* **8**, 642–649, https://doi.org/10.1016/s1473-3099(08)70231-7 (2008).
10. Zhen, S. S. et al. Effectiveness of the live attenuated rotavirus vaccine produced by a domestic manufacturer in China studied using a population-based case-control design. *Emerging microbes & infections* **4**, e64, https://doi.org/10.1038/emmi.2015.64 (2015).
11. Li, D. et al. Genetic type of Rotavirus Vaccine Strain LLR in China is G1P[14]. *Bing du xue bao—Chinese journal of virology* **31**, 170–173 (2015).
12. Matthijssens, J. et al. Full Genomic Analysis of Human Rotavirus Strain B4106 and Lapine Rotavirus Strain 30/96 Provides Evidence for Interspecies Transmission. *Journal of Virology* **80**, 3801–3810, https://doi.org/10.1128/jvi.00380-3810.2006 (2006).
13. Hsieh, Y. C. et al. Comparison of virus shedding after live attenuated and pentavalent reassortant rotavirus vaccine. *Vaccine* **32**, 1199–1204, https://doi.org/10.1016/j.vaccine.2013.08.041 (2014).
14. Markkula, J., Hemming, M. & Vesikari, T. Detection of Vaccine-derived Rotavirus Strains in Nonimmunocompromised Children up to 3–6 Months After RotaTeq® Vaccination. *The Pediatric infectious disease journal* **34**, 296–298, https://doi.org/10.1097/inf.0000000000000579 (2015).
15. Du, J. et al. Evaluation of oral Lanzhou lamb rotavirus vaccine via passive transmission with CD4(+)/CD8(+) T lymphocytes. *Virus research* **116**, 101–106, https://doi.org/10.1016/j.virusres.2006.05.006 (2016).
16. Yu, C. et al. Effectiveness of Lanzhou lamb rotavirus vaccine against rotavirus gastroenteritis requiring hospitalization: A matched case-control study. *Vaccine* **25**, 8765–8761, https://doi.org/10.1016/j.vaccine.2007.10.036 (2007).
17. El Khoury, A., Mast, T. C., Carlet, M., Markson, L. & Goveia, M. Projecting the effectiveness of RotaTeq® against rotavirus-related hospitalizations and deaths in six Asian countries. *Human Vaccines 7*, 500–510, https://doi.org/10.4161/hv.7.5.14620 (2014).
18. Yeung, K. H. T. et al. Rotavirus vaccine effectiveness in Hong Kong children. *Vaccine* **34**, 4935–4942, https://doi.org/10.1016/j.vaccine.2016.08.047 (2016).
19. Lopman, B. A., Curns, A. T., Yen, C. & Parashar, U. D. Infant Rotavirus Vaccination May Provide Indirect Protection to Older Children and Adults in the United States. *The Journal of infectious diseases* **204**, 980–986, https://doi.org/10.1093/infdis/jir492 (2011).
20. Curns, A. T. et al. Reduction in Acute Gastroenteritis Hospitalizations among US Children After Introduction of Rotavirus Vaccine: Analysis of Hospital Discharge Data from 18 US States. *The Journal of infectious diseases* **201**, 1617–1624, https://doi.org/10.1098/rsif.2014.0203 (2010).
21. Bennett, A., Bar-Zeev, N. & Curlliffe, N. A. Measuring indirect effects of rotavirus vaccine in low income countries. *Vaccine* **34**, 4331–4333, https://doi.org/10.1016/j.vaccine.2016.07.001 (2016).
22. Shaghaghi, M. et al. Vaccine-Derived Polioviruses and Children with Primary Immunodeficiency, Iran, 1995–2014. *Emerging infectious diseases* **22**, 1712–1719, https://doi.org/10.3201/eid2210.151071 (2016).
23. Prelog, M. et al. Universal Mass Vaccination Against Rotavirus: Indirect Effects on Rotavirus Infections in Neonates and Unvaccinated Young Infants Not Eligible for Vaccination. *J Infect Dis* **214**, 546–555, https://doi.org/10.1093/infdis/jiw186 [pii] (2016).
24. Yin, S., Liubao, P., Chongqing, T. & Xiaomin, W. The first case of Kawasaki disease in a 20-month old baby following immunization with rotavirus vaccine and hepatitis A vaccine in China: A case report. *Human vaccines & immunotherapeutics* **11**, 2740–2743, https://doi.org/10.1080/15548590.2015.1050571 (2015).
25. Alkali, B. R., Danjei, A. I., Magaji, A. A. & Bilbis, L. S. Clinical Symptoms of Human Rotavirus Infection Observed in Children in Sokoto, Nigeria. *Advances in Virology* **2015**, 1–6, https://doi.org/10.1155/2015/890957 (2015).
26. Asmah, R. H. et al. Rotavirus G and P Genotypes in Rural Ghana. *Journal of Clinical Microbiology* **39**, 1981–1984, https://doi.org/10.1128/jcm.39.5.1981-1984.2001 (2001).
27. Matthijssens, J., et al. Full Genome-Based Classification of Rotaviruses Reveals a Common Origin between Human Wa-Like and Porcine Rotavirus Strains and Human DS-1-Like and Bovine Rotavirus Strains. *Journal of Virology* **82**, 3204–3219, https://doi.org/10.1128/jvi.02257-07 (2008).
28. Sato, K., Inaba, Y., Shinozaki, T., Fujii, R. & Matumoto, M. Isolation of human rotavirus in cell cultures: brief report. *Arch Virolog* **69**, 155–160 (1981).
29. Ward, R. L., Knowlton, D. R. & Pierce, M. J. Efficiency of human rotavirus propagation in cell culture. *J Clin Microbiol* **19**, 748–753 (1984).

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Author Contributions
Z.J.D., N.L. and J.S.L designed research; J.S.L., B.C., H.C.G., L.L.L., and L.L. performed research; J.S.L. and D.D.L. analyzed data; B.C. collected the sample; J.S.L. wrote the paper. All authors reviewed the manuscript.

Additional Information
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