PASS INFORMATION

Title	Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)
Version identifier of the final study report	V260-075/VERSION 1.0
Date of last version of the final study report	Not applicable
EU PAS register number	EUPAS35812
Active substance	G1：2.2×10^6 infectious units；G2：2.8×10^6 infectious units；G3：2.2×10^6 infectious units；G4：2.0×10^6 infectious units；P1A[8]：2.3×10^6 infectious units
Medicinal product	Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell)
Marketing authorisation holder(s)	Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. 1 Merck Drive, P.O. Box 100, Whitehouse Station, NJ 08889, US
Joint PASS	No
Research question and objectives	The primary objective of this post-marketing surveillance is to assess the occurrence of intussusception (IS) among Chinese infants who have been vaccinated with ROTATEQ® during routine clinical practice.
Country(-ies) of study	China
Author	Prof. Si Yan Zhan Peking University Health Science Center, Beijing, 100191, China
Merck Sharp & Dohme Corp Final Repository (REDS) Date	May 20th, 2022

MARKETING AUTHORISATION HOLDER(S)

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1 ABSTRACT

Title

Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)

Keywords

ROTATEQ®, rotavirus vaccine, RV5, intussusception, cohort.

Rationale and background

[redacted]

Research question and objectives

Feasibility objective

•To assess the overall feasibility of conducting the study using NRHIP, by assessing the intussusception (IS) diagnosis validity, the completeness of follow-up and the quality of linkage between immunization register and Electronic Medical Records.

Primary objective

•To assess the incidence of IS (confirmed cases, Brighton Level 1) occurring within 3 months after vaccination with ROTATEQ® in Chinese infants.

Secondary objective

•To describe the occurrence of IS (confirmed cases, Brighton Level 1) in the periods 1 to 7 days, 1 to 14 days, 1 to 21 days, 1 to 42 days and 1 day to 3 months following any dose of ROTATEQ® in Chinese infants.

•To assess the incidence of IS (confirmed cases, Brighton Level 1) among Chinese infants in the same age range as the infants vaccinated with ROTATEQ® but did not receive any rotavirus vaccine.

•To calculate the relative risk (RR) of IS in children vaccinated with ROTATEQ® compared to children from the same birth cohort and within the same age range who did not receive any rotavirus vaccine.

Study design

A cohort study conducted using NRHIP.

Setting

Data for this study was obtained via secondary data collection from NRHIP. NRHIP contains several major databases for Ningbo residents and registers. The immunization register and the EMR database were the data sources for the study.

Subjects and study size, including dropouts

(EU GUIDANCE: 23 JANUARY 2013 EMA/738724/2012)

Subjects

The study population was defined as all infants aged 6 to 45 weeks from December 2018 to June 2021 from the NRHIP immunization register, including infants vaccinated with ROTATEQ® ("vaccinated infants") as well as infants from the same birth cohorts, who received at least one type 1 vaccine but no rotavirus vaccine ("unvaccinated infants").

Study size

Assuming that the annual incidence rate of IS in children under age 1 in Ningbo is 100.6-181.8/100,000, a total of 34,120-61,716 (8,530-15,429 vaccinated and 25,590-46,287 unvaccinated) infants had to be included in the study to detect a RR of 2 with 80% statistical power using a vaccinated to unvaccinated 1:3 ratio in a cohort design.

Before conducting the RR estimation, the study power was recalculated using the actual sample size and the IS incidence rate in the unvaccinated cohort to confirm that 80% power was reached to detect a RR of 2.0 or higher. Poisson regression model was used, without taking covariates into account.

Dropouts

Not applicable as this is a database study.

Variables and data sources

Variables

Exposure: vaccination with ROTATEQ®.

Outcome: Intussusception

Data Sources

Data for this study was obtained via secondary data collection from NRHIP.

Results

There were 190,364 infants in the study population and 108,405 in the analysis population. Among the analysis population, 26,847 infants had received at least one dose of ROTATEQ®; 25,643 (95.52%) of them were fully vaccinated at the time of data cut off (March 24th 2021). The mean age at dose 1, dose 2 and dose 3 was 9.63 weeks, 15.66 weeks and 22.02 weeks, respectively.

A total of 187 infants, aged 6 to 45 weeks, were identified as potential IS cases from the analysis population with adjudication information. Among them, 7 confirmed IS cases occurred within the 3 months risk period after any vaccination in the vaccinated cohort, while 53 confirmed IS cases occurred in the unvaccinated cohort. In both cohorts, the incidence rate of IS reached a peak at age 30 to 37 weeks.

The overall incidence rate of confirmed IS cases that occurred during the 3 months (90 days) risk period following any ROTATEQ® vaccination was 55.59 (95% CI: 22.35, 114.54) per 100,000 person-years. Seven IS cases occurred among the infants vaccinated with

ROTATEQ®; none occurred post dose 1 or dose 2 and all occurred post dose 3. The majority (n=4) of them occurred within 43-90 days post dose 3. Fifty-three confirmed IS cases occurred in the unvaccinated cohort corresponding to an incidence rate of 86.64 (95%CI: 64.90, 113.32) per 100,000 person-years. The crude RR of IS in the vaccinated cohort compared to the unvaccinated cohort was 0.64 (two-sided 90% CI: 0.33, 1.24). The adjusted RR was 0.90 (two-sided 90% CI: 0.46, 1.75), after adjusting for sex, year of birth, location, season of birth and age groups.

Discussion

Most infants in Ningbo included in the study completed the 3-dose vaccination schedule of ROTATEQ®. The 3 months risk period after the first two doses ended before the infants reached the age of natural peak of IS (8 months of age) as reported in the literature. In this study, no cases were observed after vaccination of dose 1 and dose 2, all 7 IS cases occurred after dose 3, majority (n=4) within 43-90 days, coinciding with the expected natural peak of IS.

A Cochrane review of the 4 WHO prequalified rotavirus vaccines showed that in RCTs for each vaccine, no increase was noted in intussusception risks after any dose. However, post-licensure evaluations of rotavirus vaccines have found intussusception risk to vary by vaccine and study location. In several high- and middle-income countries, a low risk of 1–6 excess cases of intussusception per 100,000 vaccinated infants has been documented for both Rotarix and ROTATEQ®. The pathogenic mechanisms involved in intussusception following rotavirus vaccination remain poorly defined.

In this study, the overall point estimate of the incidence rate of IS was lower in the vaccinated cohort (55.59/100,000 person-years) than in the unvaccinated cohort (86.64/100,000 person-years). The 95% CI of the incidence rate in the vaccinated and unvaccinated cohorts overlapped largely, suggesting that the rates of IS were similar between the two groups.

There was insufficient evidence (lower limit of one-sided test for 95%CI 0.46, p-value = 0.7942) to conclude that the RR of IS in the vaccinated compared to unvaccinated infants was greater than 1.0. The hypothesis test had 82.9% power for a one-sided alpha-level of 0.05 or a two-sided alpha-level of 0.10 to detect a 2-fold or greater increased risk of IS in ROTATEQ® vaccinated compared to unvaccinated infants. The estimated RR was 0.90 with a 90% CI of 0.46 to 1.75 indicating the overall RR is between 0.46 and 1.75 with 90% confidence which is consistent with that reported in previous studies.

Conclusion

This was the first post-marketing observational study conducted in China to assess the association between ROTATEQ® vaccination and IS. No increased risk of IS was observed within 3 months (90 days) following ROTATEQ® vaccination in this study.

Marketing Authorisation Holder(s)

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2 LIST OF ABBREVIATIONS

CI	Confidence interval
ADR	Adverse Drug Reaction
AE	Adverse Event
AGE	Acute gastroenteritis
BCG	Bacillus Calmette-Guerin vaccine
CDE	Center for Drug Evaluation
DTaP	Diphtheria, Tetanus, Pertussis vaccine
EPI	Expanded Program on Immunization
FDA	Food and Drug Administration
GPP	Good Pharmacoepidemiology Practices
HepB	Hepatitis B vaccine
HPV	Human papilloma virus
IPV	Inactivated poliovirus vaccine
IRB	Institutional Review Board
IRR	Incidence rate ratio
IS	Intussusception
JEV-I	Japanese encephalitis vaccine, inactivated
JEV-L	Japanese encephalitis vaccine, live attenuated
LLR	Lanzhou Lamb Rotavirus Vaccine
MenA	Group A meningococcal polysaccharide vaccine
MR	Measles-Rubella vaccine
NRHIP	Ningbo Regional Health Information Platform
OPV	Oral Poliovirus vaccine, live attenuated
PKU	Peking University Health Science Center
RR	Relative risk
RV	Rotavirus
RVGE	Rotavirus acute gastroenteritis
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SCCS	Self-controlled cases series
SCRI	Self controlled risk interval
SOP	Standard Operating Procedure
WHO	World Health Organization

3 INVESTIGATORS

Principal investigator	Si Yan Zhan, MD Peking University Health Science Center
Coordinating investigator	NA
Sponsor contacts	PPD MSD R&D (China) Co., Ltd.
Other contacts	PPD MSD R&D (China) Co., Ltd.
Vendor/Collaborator	Si Yan Zhan, Peking University Health Science Center PPD
Investigators	Not applicable

4 OTHER RESPONSIBLE PARTIES

Not applicable.

5 MILESTONES

Milestone	Planned date	Actual date	Comments
Start of data collection	November 27th, 2018 (ROTATEQ® launch in Ningbo)	May 25th, 2021	The planned date was the start of data needed for analysis. The actual date was the starting date for data extraction.
End of data collection	June 30th, 2021	March 19th, 2022	The planned date was the end of data needed for analysis. The actual date was the date for database lock.
Registration in the EU PAS register		July 2nd, 2020	
Final report of study results	March, 2022	May 20th, 2022	

6 RATIONALE AND BACKGROUND

6.1 Background

Rotavirus (RV) is the leading cause of severe gastroenteritis in infants and young children. RV infection was responsible for an estimated 128,500 deaths and 258 million episodes of diarrhea worldwide among children younger than 5 years of age in 2016[1]. In 2009, the World Health Organization (WHO) recommended RV vaccination for all children worldwide, especially in countries with a high number of gastroenteritis-associated deaths[2]. In China, RV caused over 40% of gastroenteritis related hospitalizations and about 30% of gastroenteritis related outpatient visits in children aged < 5 years[3]. Over 50% of RV-related hospitalizations in China occurred by age 1 year and about 90% occurred by age 2 years. Therefore, a vaccine with a schedule that is completed in early infancy has the potential to prevent the majority of the burden of severe RV disease in China[4].

Currently there are 2 types of RV vaccines available on the market in China: the Lanzhou Lamb RV vaccine (LLR, Lanzhou Institute of Biological Products) and ROTATEQ® (Merck & Co., Inc., Kenilworth, NJ, USA). The LLR vaccine has been in use in China since 2000[5] and contains the genotype G10P[15][6]. ROTATEQ® is an oral, live pentavalent (G1, G2, G3, G4, and P1A[8]) human–bovine (WC3) reassortant rotavirus vaccine. The vaccine is indicated for the prevention of RV gastroenteritis in infants and children caused by the serotypes G1, G2, G3, G4, and G9[7].

ROTATEQ® was approved in China on April 12th, 2018. [redacted]

In 1999, the first licensed RV vaccine, Rotashield, was withdrawn from the market less than 1 year after its introduction into the US childhood immunization program due to an association with intussusception (IS)[8].

IS is a rare event in infants and its background incidence in the unvaccinated general population varies in different regions of the world from 9 to 425 cases/100,000 children <1 year per year[9-13]. It has been estimated to range from 80 to 425/100,000 children <1 year of age in China[14-17]. 77.5% of the cases occurring in infants <1 year occurred at age 3 to 8 months[15]. A retrospective observation in Liuzhou, Guangxi province estimated the incidence rate of potential IS (not adjudicated cases) before the introduction of ROTATEQ® to range between 138 (95% confidence interval (CI): 81, 221) and 172 (95% CI: 111, 257) per 100,000 person years (py) (unpublished).

Although clinical trials of the next generation RV vaccines, including ROTATEQ® did not show an increased risk of IS, post-marketing data indicate a potential and small elevated risk with these vaccines, particularly after the first dose.

In the Rotavirus Efficacy and Safety Trial (REST)[18], 34,837 vaccine recipients and 34,788 placebo recipients were monitored by active surveillance to identify potential cases of IS within 7, 14, and 42 days after each dose. Overall, there were 6 cases among ROTATEQ® recipients and 5 cases among placebo recipients.

The results did not suggest an increased risk of IS relative to placebo with a relative risk (RR) of 1.6 (95% CI: 0.4; 6.4). In addition, among vaccine recipients, there were no confirmed cases of IS within the 42-day period after the first dose. A systematic review and meta-analysis including 38,339 vaccine recipients and 38,363 placebo recipients from 4 clinical trials in 2006 to 2017 did not show an association between ROTATEQ® vaccination and an elevated risk of IS among neonates or infants[19].

Large cohort studies, conducted in the US between 2006 and 2010 found no increased risk of IS after vaccination with ROTATEQ® as compared to pre-vaccination background incidence and/or unvaccinated infants[20-22].

Subsequent studies using a self-controlled case series (SCCS) or a self-controlled risk interval (SCRI) design, performed in several countries, indicated that RV vaccines carry an increased risk of IS, with up to 6 additional cases per 100,000 infants within 7 days of vaccination. It remains unclear whether RV vaccines affect the overall incidence of IS based

on longer periods of follow-up[23]. In most studies, IS is considered potentially related to RV vaccination if it occurs within 1 month after vaccination.

A meta-analysis on the risk of IS after ROTATEQ® vaccination was performed including 4 post-marketing studies[24-27] using the self-controlled cases series (SCCS) design[28]. It indicated that the RR during 1 to 7 days after the first, second and third dose of ROTATEQ® ranged from 3.5 to 9.9, 1.4 to 2.8 and 0.7 to 1.7, respectively. The pooled estimates of the RR (95% CI) after first, second and third doses were 4.6 (3.1, 6.9), 1.6 (1.2, 2.3) and 1.1 (0.7, 1.7). A retrospective study at a single center in Korea evaluated the relationship between ROTATEQ® vaccination and the risk of IS by dividing the number of observed excess cases by the number of expected cases[29]. It indicated that the RR (95% CI) for all doses and dose 2 were respectively 1.0 (0.1, 5.1) and 3.2 (0.4, 15.6) within 4 weeks after vaccination. No IS cases were observed after dose 1 and dose 3.

To our knowledge, no data has been published as of today, evaluating the IS risk following vaccination with the local RV vaccine, LLR since its licensure in 2000[5].

6.2 Rationale

Following initial licensure of ROTATEQ® in 2005, IS is a recognized and well-characterized safety concern that is adequately addressed in the product label and is currently monitored by the Sponsor as an important identified risk. In the Chinese label, it is indicated that "In a post-marketing observational study in the US, cases of IS were observed in temporal association within 21 days following the first dose of ROTATEQ®, with a clustering of cases in the first 7 days. "[7]

To minimize the risk of IS, ROTATEQ® has a restrictive age-indication with a first dose to be administered at age 6 to 12 weeks, subsequent doses with a 4- to 10- weeks interval and the third dose at no later than 32 weeks of age.[7]

In the phase 3 clinical trial in China, a total of 4,040 healthy infants aged 6-12 weeks were enrolled and randomly assigned in a 1:1 ratio to receive ROTATEQ® or placebo. Two IS cases were reported during the entire study period, both in the ROTATEQ® group. One case

occurred on Day 32 post dose 1 and the other case on Day 53 post dose 3. Both patients recovered after appropriate treatment[30].

The aim of this study is to provide information on the incidence of IS after a large-scale vaccination with ROTATEQ® in Chinese infants.

7 RESEARCH QUESTION AND OBJECTIVES

7.1 Feasibility objective

- To assess the overall feasibility for conducting the study using NRHIP by assessing the IS diagnosis validity, the completeness of follow-up and the quality of linkage between immunization register and Electronic Medical Record.

7.2 Primary Objective

- To assess the incidence of IS (confirmed cases, Brighton Level 1) occurring within 3 months after vaccination with ROTATEQ® in Chinese infants.

7.3 Secondary Objective

- To describe the occurrence of IS (confirmed cases, Brighton Level 1) in the periods 1 to 7 days, 1 to 14 days, 1 to 21 days, 1 to 42 days and 1 day to 3 months following any dose of ROTATEQ® in Chinese infants.

- To assess the incidence of IS (confirmed cases, Brighton Level 1) among Chinese infants in the same age range as the infants vaccinated with ROTATEQ® but did not receive any rotavirus vaccine.

- To evaluate the RR of IS in children vaccinated with ROTATEQ® compared to children from the same birth cohort and within the same age range who did not receive any rotavirus vaccine.

8 AMENDMENTS AND UPDATES

Number	Date	Section of study protocol	Amendment or update	Reason
None				

9 RESEARCH METHODS

9.1 Study design

This study did not involve active administration of ROTATEQ®. All recipients of ROTATEQ® studied received ROTATEQ® in the course of usual clinical practice. The design was a cohort study using secondary data from NRHIP to monitor IS incidence within 3 months following ROTATEQ® vaccination. A comparison group included infants vaccinated with at least one type 1 childhood vaccine before 6 weeks of age, but no rotavirus vaccine, from the same data source.

All infants were identified from the NRHIP immunization register, linked to the NRHIP Electronic Medical Records (EMR) and followed to detect a potential occurrence of IS within 3 months of receipt of the last dose (vaccinated infants) or from age 6 weeks to 45 weeks (unvaccinated children). All suspected IS cases were reviewed by an adjudication committee to confirm IS cases according to the Brighton Collaboration criteria. Number of vaccinated infants, number of IS cases occurring after vaccination with ROTATEQ® overall and by dose and time interval after vaccination, and detailed case description as well as the IS incidence rate in vaccinated infants were summarized. The RR was calculated by comparing the incidence of IS that occurred in infants up to 3 months after vaccination with the last dose of ROTATEQ® that they received with the incidence of IS in “unvaccinated infants” during the age 6 to 45 weeks. An up to 1:3 vaccinated: unvaccinated ratio was used.

China Expanded Program on Immunization (EPI) covers children from birth to 7 years, including at least 9 type 1 and 5 type 2 vaccines. Type 1 vaccines for Chinese infants under 1 year of age include Bacillus Calmette-Guerin vaccine (BCG) and hepatitis B vaccine (HepB) at birth, HepB at 1 month age, Inactivated poliovirus vaccine (IPV) at 2 months age, Oral

Poliovirus vaccine, live attenuated (OPV) at 3 and 4 months age, Diphtheria-tetanus-pertussis acellular vaccine (DTaP) at 3, 4 and 5 months age, HepB at 6 months age, Measles-rubella vaccine, live attenuated (MR), Japanese encephalitis vaccine, live attenuated (JEV-L), and Japanese encephalitis vaccine, inactivated (JEV-I) at 8 months age, as well as Group A meningococcal polysaccharide vaccine (MenA) from 6 months to 18 months age with 2 doses[31]. As the schedule of those type 1 vaccines overlapped with ROTATEQ® schedule plus 3 months risk period, and had almost 100% coverage in China[32], their vaccination date was considered as an indicator for the completeness of follow-up.

Type 1 vaccines in this study are defined as the vaccines that are recommended by the national or local immunization program, including monovalent vaccines, combined vaccines or poly-valent vaccines that contain the antigens of the monovalent vaccine, regardless of whether they are free of charge or paid for out of pocket.

9.2 Setting

The city of Ningbo is located in the east of the Zhejiang Province, in the south wing of the Yangtze River Delta, in the middle of China's Southeastern coastline. In 2017, the number of Ningbo city's resident population was about 8.0 million. 61,258 children were born, and the crude birth rate was 10.31‰. A total of 31,873 newborns were male, with a male-to-female ratio of 108:100. In 2017, the disposable per capita income of Ningbo residents was 48,233 yuan (and therefore much higher than the national per capita income in 2017 of 25,973 yuan), with an increase of 8.0% over the previous year.

The study was conducted retrospectively with data collection starting at the time when ROTATEQ® became available in Ningbo on November 27th, 2018, and ending on June 30th, 2021.

NRHIP was the only regional health data platform we could access when this study was initiated. The NRHIP was established in 2013 and certified as the "top-level regional platform" by the former China Ministry of Health in 2016. The NRHIP covers the population of Ningbo, which counted approximately 7.9 million inhabitants in 2016.

9.3 Subjects

The study population was defined as all infants aged 6 to 45 weeks from December 2018 to June 2021 from the NRHIP immunization register, including infants vaccinated with ROTATEQ® ("vaccinated infants") as well as infants from the same birth cohorts, who received at least one type 1 vaccine but no rotavirus vaccine ("unvaccinated infants").

Inclusion criteria

- All infants aged 6 to 45 weeks from December 2018 to June 2021 from the NRHIP immunization register.
- Infants who received at least one dose of ROTATEQ® at age 6 to 12 weeks as recorded in the NRHIP immunization register will be considered vaccinated infants.
- Infant from the same birth cohort as the "vaccinated infants" who received at least one dose of a type 1 childhood vaccines before 6 weeks of age, but no rotavirus vaccine as recorded in the NRHIP immunization register will be considered unvaccinated infants.

Exclusion criteria

- Vaccinated infants:
    - Infants with IS before they received the first dose of ROTATEQ®;
    - Infants who were vaccinated out of the indicated age schedule, i.e., who received a first vaccine dose before 6 weeks or after 12 weeks of age and/or any dose after 32 weeks of age;
    - Infants who received one or more doses of LLR or any other RV vaccines in addition to ROTATEQ® during the study period;
    - Infants without any recorded type 1 vaccination at age 8 to 9 months or until their individual end of follow-up 3 months after their last ROTATEQ® dose

(whatever came first) and no IS diagnosis during follow-up, as those infants are considered to be lost to follow-up.

- Unvaccinated infants
  - Infants with IS prior to 6 weeks of age;
  - Infants without any recorded type 1 vaccination at age 8 to 9 months and no IS diagnosis during follow-up, are considered to be lost to follow-up.

Additional eligibility criteria

For infants who are born outside of Ningbo, the EMR data, including information on IS that occurred outside Ningbo, may not be fully captured in the NRHIP. It would therefore be impossible to assure that infants had not experienced IS before they received the first dose of ROTATEQ® or prior to 6 weeks of age. For this reason, only infants born in Ningbo were included in the study. The following process was followed to determine whether an infant was born in Ningbo. Children who met any of the 3 criteria in the process below were considered born in Ningbo:

First, the hospital of birth was checked. If the hospital of birth was located in Ningbo, then the infant was considered locally born.

Second, the vaccination place of the first dose of HepB or BCG was checked. The adherence to dosing schedule (given within 24 hours after birth) for HepB or BCG is very high in China. Infants receive the first dose before hospital discharge (usually within 3 days in Ningbo). Therefore, if the first dose of HepB or BCG was given ≤ 3 days after birth in Ningbo, the infant was considered locally born.

Third, the household address was checked in the register. An infant with a household address registered in Ningbo is very likely to be locally born. Children who were born outside Ningbo are generally not included in the household register in Ningbo.

9.4 Variables

This was a cohort study. All exposure, outcome and covariate variables were extracted at the end of the study period and linked to the EMR records for detection of potential IS occurrence.

9.4.1Exposure

The study product of interest is ROTATEQ®, however this study did not involve active administration of ROTATEQ®. The study included infants exposed to ROTATEQ® from routine practice and included in the NRHIP immunization registry.

9.4.2Outcome

The study outcome was IS occurrence. The case definition for IS for this study conformed to the level 1 criteria from the Brighton collaboration case definition.

9.4.3Covariates

The following covariates were abstracted from the NRHIP immunization register and EMR.

From NRHIP immunization register

- Infant's gender;
- Infant's location (urban/ rural);
- Dose number (dose 1, 2, and 3);
- Infant's age in weeks at dose 1, 2 and 3.

From NRHIP EMR

- Infant's age in weeks when IS occurred;
- Interval between IS onset and most recent dose prior to the IS onset (1-7 days, 1-14 days, 1-21 days, 1-42 days, 1 -90 days (3 months)).

9.5 Data sources and measurement

Data for this study were obtained via secondary data collection from NRHIP. NRHIP contains several major databases for Ningbo residents and registers. The immunization registers and the EMR database were the data sources for the present study.

Table 1 List of variables in the NRHIP immunization register and EMR to be used in the study

Data source		Time range	Key variable
Immunization register	Children	2000-present	ID, vaccination record No., name, date of birth, gender, vaccine name, date of vaccination, age at vaccination, vaccination clinic, dosage, dose No., vaccine batch No., and vaccine manufacturer.
EMR	Outpatient	2015-present	ID, name, date of birth, gender, medical institution No., visit No., time of visit, time of onset, ICD-10*, prescription No., drug name, dosage, unit, date of prescription, days of dispensing, lab test and its result.
	Inpatient	2015-present	ID, name, date of birth, gender, medical institution No., inpatient No., time of onset, ICD-10, date of discharge.

* The ICD code was from 1 to 10 per visit, depending on the medical condition(s) of patient. All ICD codes were available in the platform.

NRHIP immunization register

NRHIP covers 11 Centers for Disease Control and Prevention (CDCs). In 2005, the vaccination information system of the Ningbo CDC was completed and fully implemented covering all vaccination clinics in Ningbo city. There are 167 points of vaccination in Ningbo city, including 10 hospitals and 157 community health service centers.

The vaccination information system collects personal basic information and vaccination records in real time. Personal IDs are registered together with the vaccination records. The records contain personal basic information, vaccination information including vaccine name and lot number. Vaccination is required to be recorded in the CDC's immunization register for each vaccine recipient, including recipient's age, gender, personal ID, date of vaccination, vaccine name, injection site, vaccine batch number, dose number, manufacturer, name of the vaccination clinic, etc. (Table 1).

NRHIP EMR database

Among more than 4,000 medical facilities that are located in Ningbo, NRHIP EMR covers the most important 249 medical institutions (46 general hospitals, 21 specialized hospitals, 11 maternity and pediatric hospitals, 6 Chinese hospitals for traditional medicine, and 160 community health centers, and 5 other institutes). Twenty-one of the hospitals are Grade A hospitals, 26 are tier 2 hospitals. Two hundred twenty-one of these hospitals are public hospitals and 28 are large private hospitals. The NRHIP EMR system contains the information that is listed in Table 1. As all pediatric and all community health centers of Ningbo are covered by HRHIP EMR, it is very likely that all IS cases that are treated in Ningbo are included in the database.

A feasibility assessment was conducted to assess if NRHIP could be used for this study. The results of this assessment is presented in section 10.1.

9.5.1 Study Procedures

This study was based on secondary data collection and didn't involve active administration of ROTATEQ®. The study protocol was submitted for approval to the ethnic committees at PKU and NRHIP. Permission of waiver of informed consent for this study was granted. The study was also approved by the Human Genetic Resources Administration of China (HGRAC) for International Cooperation Study. The study conduct encompasses the following steps:

9.5.2 Feasibility assessment

Prior to initiating the study to monitor the incidence of IS after large scale vaccination of ROTATEQ® in NRHIP, a feasibility assessment was conducted

- To assess if IS cases could be reliably extracted from the NRHIP by evaluating if EMR data were sufficiently recorded to adjudicate the IS cases according to the Brighton criteria;

- To assess the rate of follow-up in the target population over the entire observation period (6-45 weeks of age);

- To evaluate the linkage rate (between NRHIP EMR and immunization registry) in the target population.

IS diagnosis validity

The process of assessing the IS diagnosis validity included 50 IS cases regardless their ROTATEQ® vaccination status using the following procedures:

- Explore ICD codes and/or other key codes and/or text of diagnosis to identify all potential IS cases;

- Evaluate the NRHIP EMR database accessibility by extracting IS cases from infants age-eligible to be vaccinated with ROTATEQ® (born after 2018) from EMR/database, including ICD codes, clinical manifestation, imaging and demographic data, like birth date, gender, address;

- Evaluate the completeness of data for each selected IS case to adjudicate the case according to the Brighton criteria;

Rate of follow-up

- To assess the percentage of infants in the immunization register who have follow-up from the time point of first childhood vaccination until any type 1 vaccination at age 8 to 9 months based on a sample of at least 50 infants.

Linkage rate

- To assess the rate of linkage for IS cases identified from NRHIP EMR to their records in the immunization register, using demographic data (such as infants' ID, name, gender and birth date) based on a random sample of 50 IS cases.

Completeness of NRHIP EMR

- To understand the work mechanism and the timelag in the NRHIP for the data transmission from each registered hospital to the platform.

It was pre-defined in the protocol that NRHIP would be considered not appropriate, if

- the linkage rate between the immunization register and EMR <50%; OR
- it was impossible to identify IS cases in the database; OR
- no longitudinal data (immunization records and/or health encounter data) were available to assess the completeness of follow-up; OR
- it was impossible to differentiate the LLR from ROTATEQ® in the immunization register.

If the feasibility assessment had concluded that NRHIP was not appropriate for the study conduct, other data sources had to be explored for the main study.

9.5.3 Main study procedures

Linking data from the immunization register and the EMR within the NRHIP

Infants' IDs were used for the linkage between immunization register and EMR database for enrolled children between age 6 to 45 weeks (unvaccinated infants) or 3 months after they had received the last dose of ROTATEQ® (vaccinated infants).

For those infants without complete IDs in the two databases, infants' name (or parent's name), gender, birth date and resident's address were combined into an algorithm for deterministic record linkage. Infants' IDs were used to validate linkage results by comparing this information both from immunization register and EMR database. Vaccine names and lot numbers of each dose were used to validate whether those infants were vaccinated with ROTATEQ®.

Identification of infants vaccinated with ROTATEQ®

There are 2 types of RV vaccines available in Ningbo city. In the immunization register, the LLR was recorded as "Rotavirus" (in Chinese), while ROTATEQ® was recorded as "PENTAVALENT Rotavirus" (in Chinese). To register the vaccine in the immunization register, the bar code of the RV vaccine was scanned, therefore the probability that a wrong vaccine name was entered in the NRHIP immunization register was very low. The infants who received at least one dose of ROTATEQ® (identified through the Chinese word "PENTAVALENT Rotavirus" = vaccine name) were identified as the vaccinated infants.

Identification of the cohort of unvaccinated infants

Infants from the same birth cohort as the infants vaccinated with ROTATEQ® are identified, if they had received at least one dose of a type 1 vaccine before age 6 weeks and no rotavirus vaccine (any brand).

Infants with follow-up from 6 weeks to 45 weeks of age over the study period were identified from the NRHIP immunization register. Only infants born after October 16th, 2018 (who were at least 42 days [6 weeks] old on November 27, 2018 when ROTATEQ® was launched in Ningbo) and before August 12th, 2020 (322 days [45 weeks] old on June 30th, 2021) were included in the study.

For both infants who received at least one dose of ROTATEQ® (identified through the Chinese word "PENTAVALENT Rotavirus" = vaccine name) and infants from the unvaccinated cohort, the following information were extracted from the NRHIP immunization register:

- Infant's ID;
- Infant's name;
- Infant's gender;
- Infant's birth date;

- Parent's name and resident address;
- Vaccine name;
- Dose number;
- Date of of vaccination for each dose;
- Lot number for each dose;
- Other rotavirus vaccines, including dose and date of vaccination at any time during the observation period
- Date of type 1 vaccines including, but not limited to DTaP (Diphtheria, Tetanus, Pertussis), HepB (Hepatitis B), MR (Measles-Rubella), MPSV-A (Meningococcal polysaccharide), at age 8 to 9 months in both cohorts to identify infants that are lost to follow-up.

The individual age of each infant at dose 1, 2 and 3 vaccination was computed using the date of vaccination of each dose and infant's birth date.

Exclusion of infants who did not receive any type 1 vaccine at age 8 to 9 months or until their individual end of follow-up 3 months after their last ROTATEQ® dose (whatever came first), and no IS diagnosis during follow-up

Vaccinated infants had to be followed until 3 months after administration of the last ROTATEQ® dose. For infants who had received three doses, the follow-up time ended between the ages ~26 weeks in case of early schedule and ~44 weeks in case of late schedule. (Early schedule: dose 1 at 6 weeks, dose 2 at 10 weeks, dose 3 at 14 weeks + 3 months follow-up =~26 weeks; late schedule: dose 3 at 32 weeks + 3 months of follow-up time = ~44 weeks). Unvaccinated infants were followed from age 6 weeks to 45 weeks.

To ensure that infants who moved out of the Ningbo region before the end of the follow-up time were excluded from the study, all infants without diagnosed IS and who did not receive any type 1 vaccine at age 8 to 9 months or until their individual end of follow-up 3 months

after their last ROTATEQ® dose (whatever came first) were excluded from the study. Vaccine coverage with type 1 vaccines in Yinzhou (the most developed district in Ningbo city), is very high, almost 100%[32].

Identification of potential IS cases

All eligible infants were identified in the EMR using the infant's ID or the substitute ID. The ICD-10 code and hospital discharge diagnosis of these children were searched to identify potential IS either through ICD-10 code K56.1 or Chinese term for intussusception through the hospital discharge (including outpatient/ emergency/ inpatient/ radiology) diagnosis.

For vaccinated infants, any potential IS that occurred from Dose 1 up to 3 months following the last dose of ROTATEQ® was captured. The risk period ended at the end of data collection. For unvaccinated infants, any potential IS that occurred during their age from 6-45 weeks was extracted.

For all identified potential IS cases, the following information was extracted:

- Infant's ID;
- Infant's name;
- Infant's gender;
- Infant's birth date;
- Parent's name and resident address;
- IS diagnoses code (ICD-10 code: K56.1)
- Hospital discharge diagnosis (diagnosis term in Chinese: Chang Tao);
- Date of IS onset;
- Description and conclusion of radiographic examination, including Barium and air contrast enema ultrasound;

- Description and surgical findings for each IS cases;

- Pathology reports, including an anatomical lead point, such as intestinal polyps, lymphosarcoma, Meckel's diverticulum, and lymphoid hyperplasia (Peyer Patches);

- Plain abdominal radiographic report, describing as the target sign and the meniscus sign.

Infant's age at IS occurrence was computed by infants' birth date and the date of IS onset. Date of first and last dose of ROTATEQ® vaccinations from immunization register and date of IS onset was used to exclude IS cases which occurred before vaccination and/or more than 3 months following the last dose, as well as to calculate the interval between IS onset and preceding dose.

Adjudication of potential IS cases

Suspected IS cases in infants aged 6-45 weeks in the analysis population were adjudicated. Level 1 of diagnostic certainty for IS requires surgical, radiological, or autopsy criteria. Surgical criteria: The demonstration of invagination of the intestine at surgery; Radiologic criteria: The demonstration of intestinal invagination by either gas or liquid contrast enema, the demonstration of an intra-abdominal mass with specific features by ultrasound that is proven to be reduced by hydrostatic enema on post-reduction ultrasound. Autopsy criteria: The demonstration of invagination of the intestine.

The detailed description of IS from radiographic findings and surgical intervention was collected from the NRHIP EMR database. Suspected cases were adjudicated by the Adjudication Committee (AC) based on the Brighton Collaboration criteria described in the "Standard Operating Procedure (SOP) for the Adjudication of IS cases". Three pediatricians, independent of study sponsor, were invited as AC members. Two members of the AC individually reviewed each IS case. If the committee members disagreed on whether a case met the criteria defined in the SOP, a meeting was held with the committee chairman (Chief physician, Beijing Children's Hospital) for the final adjudication. The AC members evaluated each case with respect to the pre-specified Criteria for Adjudication as defined by

the Brighton collaboration and didn't have access to the vaccination status of the cases. The adjudicator determined, based on the Brighton criteria, his/her clinical judgment and the evidence at hand, if more information was needed to confirm or refute the case.

Only cases that were confirmed as Brighton level 1 by AC, in accordance with the standard operating procedure, were considered for the primary and secondary analyses. Cases that were unconfirmed or for which medical records were not available for adjudication as well as negatively adjudicated cases were not considered for any analysis.

The episode date was the date of the first recorded (inpatient or outpatient) diagnostic, radiographic or surgical procedure used to determine whether the suspected case fulfills the criteria for a confirmed (level 1) case. In situations where records did not indicate the date of the procedure, the hospital admission date was considered as episode date. For cases that were not hospitalized, the date of the diagnosis was considered as episode date.

The individual IS episode was defined as one single episode if the medical records of IS were reported within 7 days. The available diagnosis information within 3 days before and after the episode date was extracted into the data extraction form for adjudication. For analyses in which subjects had more than one IS, each subject contributed only the first episode of Brighton level 1 throughout the risk period (i.e. a maximum of one IS occurrence was counted for each study subject).

9.6 Bias

For each subject, the ICD-10 code and hospital discharge diagnosis, including the Chinese term for intussusception, were searched to identify all potential IS cases from the EMR.

The case definition for IS for this study conformed to the level 1 criteria from the Brighton collaboration case definition. There was a risk of capturing IS cases that were not true cases if diagnosis was based on ICD-10 codes or diagnosis terms in the platform only, affecting the observed association between ROTATEQ® vaccination and IS. In order to reduce the misclassification bias, a case adjudication committee was formed to adjudicate each potential

IS case based on the available information for diagnosis, following the pre-specified adjudication SOP.

In order to reduce the potential confounding bias, several covariates that might have had a potential impact on ROTATEQ® vaccination or the outcomes of IS were extracted from the NRHIP and analyzed in this study. These covariates included sex, year of birth, location, season of birth, age groups. The Poisson regression model was used to adjust for these covariates.

9.7 Study size

Assuming that the annual incidence rate of IS in children under age 1 in Ningbo was 100.6-181.8/100,000 ,[15, 16] a total of 29,445-53,259 (9,815-17,753 vaccinated and 19,630-35,506 unvaccinated) infants had to be included in the study to detect a RR of 2 with 80% statistical power using a vaccinated to unvaccinated 1:2 ratio cohort design. If the ratio was 1:3, the sample size would be 34,120-61,716 (8,530-15,429 vaccinated and 25,590-46,287 unvaccinated) (Table 2).

Table 2 Number of vaccinated infants needed to detect a 1.5- to 3.0-fold increase in the occurrence of IS * in the concurrent cohort design

Ratio of vaccinated: control	IS background incidence (1/100,000)	Detectable relative risk			
		1.5	2.0	2.5	3.0
1:2	100.6	60,181	17,753	9,088	5,784
	181.8	33,277	9,815	5,024	3,197
1:3	100.6	52,837	15,429	7,832	4,950
	181.8	29,216	8,530	4,330	2,736

*80% statistical power

Before conducting the RR estimation, the study power was recalculated using the actual data and the following statistical parameters: IS incidence rate of 86.64/100,000 person years in the unvaccinated cohort, RR of 2.0, mean exposure time of 0.69 years, ROTATEQ® vaccination rate of 0.247 in binomial distribution. Poisson regression model was used with

no covariates. The statistical power is 0.829 for one-sided test with α=0.05, or two-sided test with α=0.10.

9.8 Data transformation

Only Brighton level 1 confirmed cases were included in the analysis. The age of 6-45 weeks was divided into five equal age groups.

Exposed person-time was defined as person-time 1 to 90 days after any dose of ROTATEQ® vaccination (including 1st, 2nd, 3rd dose). If the time interval between two successive ROTATEQ® doses was equal or less than (≤) 90 days, then the follow-up duration was calculated as follows: If no IS episode occurred, the follow-up duration= the date of the last dose – the date of the first dose + 90 days; if an IS episode occurred within 90 days after the last dose, the follow-up duration= the date of IS episode – the date of the first dose. If the interval time between the two doses was more than (>) 90 days, the exposure time for the previous dose was truncated at 90 days. If the end of follow-up time was more than (>) 90 days after the last dose, the exposure time for the last dose was truncated at 90 days.

Unexposed person-time included the time from 6 to 45 weeks of age among unvaccinated infants.

9.8.1Data management

In this study, data management procedures included the following steps: data extraction, data cleaning, data linkage, de-identification, case adjudication, database lock, and data analysis. Ningbo CDC was responsible for coding, data extraction, data cleaning, data linkage and de-identification. PKU was responsible for providing technical support and quality control. PKU was also responsible for developing and executing programming codes after de-identification, such as case adjudication, database lock and data analysis.

All data management activities were undertaken by the Peking University and Ningbo CDC, and followed all procedures detailed in a separate “Data Management Plan”.

9.9 Statistical methods

9.9.1Main summary measures

Descriptive data analysis of the aggregated information was performed and the results were displayed in tabulated form. Descriptive statistics were used to describe baseline characteristics of vaccinated infants that were displayed in summary tables. Categorical variables were described using frequencies and percentages, while continuous variables were presented using medians and interquartile ranges. All analyses were performed using the Statistical Analysis System (SAS version 9.4 or higher).

Statistical tests whenever required: For continuous variables, Kruskal-Wallis test were performed. For categorical variables, Chi-square test were performed. The incidence rate and 95% confidence interval of IS was calculated using the exact poisson distribution. P-values were rounded to 3 decimal places. '<.001' were displayed when the P-value was less than 0.001, and '>.999' were displayed when the P-value was greater than 0.999.

9.9.2Main statistical methods

Primary objective

This study estimated the incidence of IS occurring within 3 months after vaccination with ROTATEQ®. For each subject, the incidence of IS in the given risk period was defined as the first occurrence of IS in the EMR, identified either through ICD-10 code or through hospital discharge diagnosis, within 3 months following the ROTATEQ® vaccination. Infants were followed until 3 months after the last dose they received, i.e. 3 months after dose three for fully vaccinated infants, 3 months after dose two for those who only received two doses and 3 months after dose one for infants who only received one dose.

For analyses in which subjects had more than one IS, each subject contributed only once throughout the whole risk period (i.e. a maximum of one IS occurrence was taken into account for each study subject). The denominator was all infants vaccinated with at least one dose of ROTATEQ® who were still living in Ningbo or later as documented by IS diagnosis up to 3 months after vaccination or vaccinated with any type 1 vaccine at age 8 to 9 months

or before individual end of follow-up 3 months after last ROTATEQ® dose (whatever came first).

When sample size allowed the results were stratified by age.

Secondary objective

The occurrence of IS cases has been described with respect to the time-interval post-vaccination and in relation to the dose number.

The risk period after a vaccine dose was censored when a subsequent dose was administered and the risk period of the subsequent dose started; i.e. an IS case that occurred 10 days following the second dose of ROTATEQ® and therefore 40 days after the first dose (for a 30 days interval between dose 1 and dose 2), was only counted in the 1-14 days range of dose 2 and NOT in the 1-42 days range of dose 1.

To evaluate RR of IS in infants vaccinated with ROTATEQ® compared to infants from the same birth cohort and within the same age range who did not receive any rotavirus vaccine, the hypothesis to be tested was:

H0: RR=1.0

H1: RR>1.0 (the study was powered for RR≥2.0)

H0 represented the null hypothesis and H1 represented the alternative hypothesis.

The analysis should only be done once the necessary sample size was sufficient to reach 80% statistical power (one-sided test, alpha= 0.05) to detect a RR of the incidence of IS of 2.0 or higher in vaccinated infants compared to unvaccinated infants.

The RR and its one-sided 95% confidence interval (alpha=0.05), two-sided 90% confidence interval (alpha=0.10) were calculated by comparing the incidence rate (per 100,000 person-years) of IS in infants who had received ROTATEQ® with the incidence rate (per 100,000 person-years) of IS in infants from the same birth cohort who did not receive any rotavirus

vaccine. The incidence rate ratio (IRR) estimate was adjusted for sex, year of birth, location, season of birth, age groups in the Poisson regression model

9.9.3Missing values

All analyses were carried out using all available data. A participant with missing data on one variable was used only in calculations that did not involve that variable. This allowed analysis with larger sample sizes than when using complete case report forms.

9.9.4Sensitivity analyses

Not applicable.

9.9.5Amendments to the statistical analysis plan

Not applicable.

9.10 Quality control

By signing the protocol, all parties followed applicable standard operating procedures (SOPs) for non-interventional study. All parties also agreed to ensuring all existing and new study personnel were appropriately trained to ensure the study was conducted and data were generated, documented, and reported in compliance with the protocol, Good Pharmacoepidemiology Practice (GPP), and all applicable national, and local laws, rules and regulations. All parties maintained transparency and open communication in order to effectively manage the study and proactively mitigate any risks.

The Sponsor met with Peking University on a bi-weekly basis, reviewed the data management plan and statistical analysis plan, conducted audit visits to ensure oversight and conduct of the study were completed in accordance with the protocol, quality standards (e.g. GPP), and applicable laws and regulations. There was no significant quality issue (SQI) identified during the conduct of the study. An SQI was any issue with the potential to negatively impact, either directly or indirectly, the rights, safety and well-being of patients or study participants and/or the integrity of the data.

Quality of data linkage

The data from CDC (immunization registry), the hospital (electronic medical records), and information systems are integrated, verified, stored, exchanged, and shared in the NRHIP.

Vaccination（including type 1 vaccines and rotavirus vaccines) and outcome information (including IS diagnoses) were collected from the NRHIP, and the data were linked at individual level in the NRHIP in Ningbo. Personal identification variables and database-specific unique index variables were used for the linkage of the subjects across different datasets in the NRHIP to allow for each person's vaccination status to be combined with outcomes.

In the NRHIP, ID variables were missing for a high proportion of infants in the EMR. Multiple linkage steps were applied to minimize the loss of data and to ensure accurate linkage of subjects in the platform. The dataset linkage steps are described in the study data management plan.

Standardization of IS diagnoses

1) Data extraction form: A deidentified and standardized data extraction form was developed according to the international general standards for vaccine safety monitoring (Brighton Criteria), to extract the diagnosis and treatment information of potential IS cases.

2) Identification of cases: All patients from the analysis population were identified through fuzzy matching algorithm using ICD-10 (K56.1) or diagnosis term in Chinese ("Chang Tao") from outpatient (including emergency treatment), hospital records, and radiology records in NRHIP EMR. In the feasibility assessment, there were some cases with radiology diagnosis but no hospital discharge diagnosis. Therefore, radiology diagnosis was added to the identification process for potential IS cases.

3) Extraction of medical records of IS from NRHIP EMR: The medical institution code, medical department visits, reception time/admission time, episode date, basic information of patients (ID number, name, gender, age, and region.), surgery name, surgery date, X-ray number, MRI number, and CT number were extracted from the NRHIP platform. In addition,

X-ray, ultrasound, CT, MRI and other image information, were extracted if available in the platform.

4) De-identification: The name and ID number were de-identified. The history of rotavirus vaccination was blinded before the diagnostic information was provided to experts.

5) Adjudication: Two external physicians, who were trained to independently to review the data extraction forms of IS, adjudicated the IS cases following the pre-specified adjudication SOP, and if there was disagreement, a senior expert(Chief physician, Beijing Children's Hospital) was involved to reach alignment.

Information integrity

The vaccination registry and EMR data in the NRHIP are set up and managed strictly following the local health authority's requirements and regulations. Data integrity had previously been assessed in a preliminary feasibility assessment using data from 2017 in the NRHIP. All vaccination clinics in Ningbo city have been included in the system. The key variables associated with vaccination are all collected in the system.

The data analyses were conducted according to the study protocol and the statistical analysis plan. Programming for this project was conducted by a primary analyst and validated by a separate analyst (validation analyst). For all data processing steps, the validation analyst reviewed the program along with input and output datasets.

10 RESULTS

10.1 Feasibility assessment

Ninety eight percent of the IS cases had at least one diagnostic variable captured in the EMR. Ninety-two percent of the target population were followed over the entire observation period from 6 to 45 weeks of age and the linkage rate between the NRHIP EMR and immunization registry in the target population was 96%. Vaccine codes and vaccine product codes that were needed to differentiate between LLR (local rotavirus vaccine) and ROTATEQ® were

available for all vaccinated infants in the platform. The completeness of ROTATEQ® vaccination records, including vaccination date was also 100%.

10.1.1 IS diagnosis validity

10.1.1.1 Completeness of IS diagnostic variables

Potential IS cases were identified using the ICD-10 code (K56.1) or the diagnosis term in Chinese ("Chang Tao") from the hospital discharge records (including outpatient/ emergency/ inpatient/ radiology) of the study population in the NRHIP.

IS cases were extracted from the EMR using IS diagnosis records. However, as only a small proportion of the infants had ID numbers in the EMR, it was not possible during the feasibility assessment to identify if there were infants with more than one record. Therefore, one IS case might have been counted multiple times.

Overall, potential IS cases with personal unique identification from infants born between 16th Oct 2018 and 12th Aug 2020 were extracted. Then, 50 records of potential IS cases were randomly selected to evaluate the completeness of IS diagnostic variables.

The diagnostic variables were defined as surgical, radiological and autopsy records that were needed for the case adjudication of IS cases level 1 according to the Brighton criteria. Among 50 potential IS cases with personal unique identification, one (2%) had surgical records, 47 (94%) had at least one radiological record (ultrasound/X-ray/computerized tomography scan), and 17 (34%) had enema. No autopsy records were identified. In total, 49 records from potential IS cases (98%) had at least one of the above diagnostic information.

Table 3 Completeness of IS diagnostic variables in the sample of 50 IS cases

	Number of IS case, n	Percentage, %
Surgical records	1	2
Radiological records		
CT/Ultrasound/X ray	47	94
Enema	17	34
Autopsy records	0	0
Any records of surgical, radiological, autopsy	49	98

10.1.1.2 Follow-up rate

Among infants born in Ningbo between 16 Oct 2018 and 12 Aug 2020 who were registered in the NRHIP, we randomly selected 50 infants. Forty-six of these infants (92%) had received at least one dose of any type 1 vaccine at 8-9 months of age.

Table 4 Rate of follow-up in a sample of 50 infants

	Number of infants, n	Percentage, %
Any type 1 vaccine in infants aged 8- 9 months	46	92

10.1.2 Linkage rate

10.1.2.1 Completeness of ROTATEQ® vaccination data

A sample of 50 randomly selected infants who had received at least one dose of ROTATEQ®, had received a total of 145 doses. The completeness of ROTATEQ® vaccination records, including vaccine code (code for each specific vaccine in the platform), vaccination date, and vaccine product code (vaccine manufacturer code) was assessed. All vaccination records had a completion rate of 100%.

Table 5 Completeness of ROTATEQ® vaccination records in a sample of 145 doses of ROTATEQ® that were administered to 50 infants

	Number of doses, n	Percentage, %
Vaccine code	145	100%
Vaccination date	145	100%
Vaccine product code	145	100%
ROTATEQ® vaccination records*	145	100%

*Including all items of vaccine code, vaccination date, vaccine product code.

10.1.2.2 Proportion of any dose of type 1 vaccine received from birth to 6 weeks of age

In a randomly selected sample of 50 infants born in Ningbo, the proportion of any dose of type 1 vaccine received from birth to 6 weeks of age was 100%.

Table 6 Proportion of any dose of type 1 vaccine received from birth to 6 weeks of age in a sample of 50 infants

	Number of infants, n	Percentage, %
Any type 1 vaccine for infants received from birth to 6 weeks of age	50	100%

10.1.2.3 The linkage rate between potential IS case and immunization register in a sample of 50 IS cases

The linkage rate between IS cases from the EMR and immunization register was 96% in a sample of 50 randomly selected IS cases with personal unique identification.

Table 7 The linkage rate between potential IS cases and immunization register in a sample of 50 IS cases

	Number of cases, n	Percentage, %
Successful linkage cases	48	96

10.1.2.4 Proportion of vaccine-specific information to differentiate between LLR and ROTATEQ® in a sample of 50 infants vaccinated with any RV vaccine

A sample of 50 infants who were randomly selected from infants born in Ningbo and vaccinated with ROTATEQ® or LLR, received a total of 114 doses of rotavirus vaccine, of which 114 doses (100%) had a vaccine code, 113 doses (99%) had a vaccine product code, and 114 doses (100%) had any vaccination information (vaccine code or vaccine product code).

Table 8 Proportion of vaccine-specific information to differentiate between LLR and ROTATEQ® in a sample of 114 RV vaccine doses that were administered to 50 infants

	Number of doses, n	Percentage, %
Vaccine code	114	100
Vaccine product code	113	99
Any vaccination information*	114	100

*Including any of the following: vaccine code, vaccine product code.

10.1.2.5 Data Capture in NRHIP EMR

The process of data transmission from the hospitals to the NRHIP: Ningbo health authority sets up the regional health information system, which centralizes the databases of the hospital information system (HIS) of each hospital in Ningbo prefecture. Following the prefectural standard for data transformation, the dataset in the HIS was cleaned and transformed into structured and semi-structured data. One copy of the transformed dataset is archived in the platform for health research/ surveillance, the other copy is stored offsite.

The Ningbo health authority requires the data to be uploaded in real time or daily. The platform has a quality check tool to monitor the number of uploaded health records. The data was uploaded automatically.

10.2 Participants

10.2.1 Protection of Human Subjects

In this study, an analysis of routinely collected data that were entered into the NRHIP database was performed. No intervention was applied in this study. Therefore, subject recruitment and informed consent were not applicable. All participants' privacy was well-protected and data management followed GPP, laws, regulations, local regulations, and institutional requirements. The investigators' roles and responsibilities and data permission were clearly defined in the data management plan.

This study was approved by the Institutional Review Board (IRB) of Peking University and Ningbo CDC with a waiver for informed consent. The study application was also approved by the Human Genetic Resources Administration of China (HGRAC) for International Cooperation Study. Only investigators of PKU and Ningbo CDC had access to the data in this study. All study related data access and processing activities were initiated after IRB and HGRAC's approval.

Ningbo CDC removed the identification information of all patients and transferred the de-identified database to PKU study team to protect personal privacy. PKU prepared the final analysis data set using the de-identified database. All the staff involved in this study and their main responsibilities and rights were clearly determined to ensure data security. All documents, codes and materials in the study process were recorded and stored, and any database and materials could not be transmitted or copied to the network outside Ningbo CDC for analysis.

10.2.2 Selection of the analysis population

The study population comprised all infants from the NRHIP immunization register born between Oct 16 2018 and Aug 12 2020 (n=190,364). After exclusion of infants who were not born in Ningbo (n=54,364), 136,000 infants were assigned to the unvaccinated infants (n=106,914) or ROTATEQ® vaccinated infants (n=29,086) according to their vaccination status with ROTATEQ®.

A total of 81,558 unvaccinated infants were finally included after exclusion of 0.26% infants who had not received any dose of a type 1 vaccine before 6 weeks of age, 15.39% infants who had received any rotavirus vaccine other than ROTATEQ®, 0.02% infants with IS prior to 6 weeks of age, and 8.05% infants who were lost to follow-up.

A total of 26,847 vaccinated infants were finally included after exclusion of 6.03% infants who were vaccinated out of the indicated age schedule, i.e., infants who had received a first vaccine dose before 6 weeks or after 12 weeks of age and/or any dose after 32 weeks of age, 0.65% infants who had received any other rotavirus vaccine in addition to ROTATEQ®, 0.02% infants with IS before they received the first dose of ROTATEQ®, and 1% infants who were lost to follow-up.

Finally, the unvaccinated and vaccinated infants who met the inclusion and exclusion criteria constituted the analysis population.

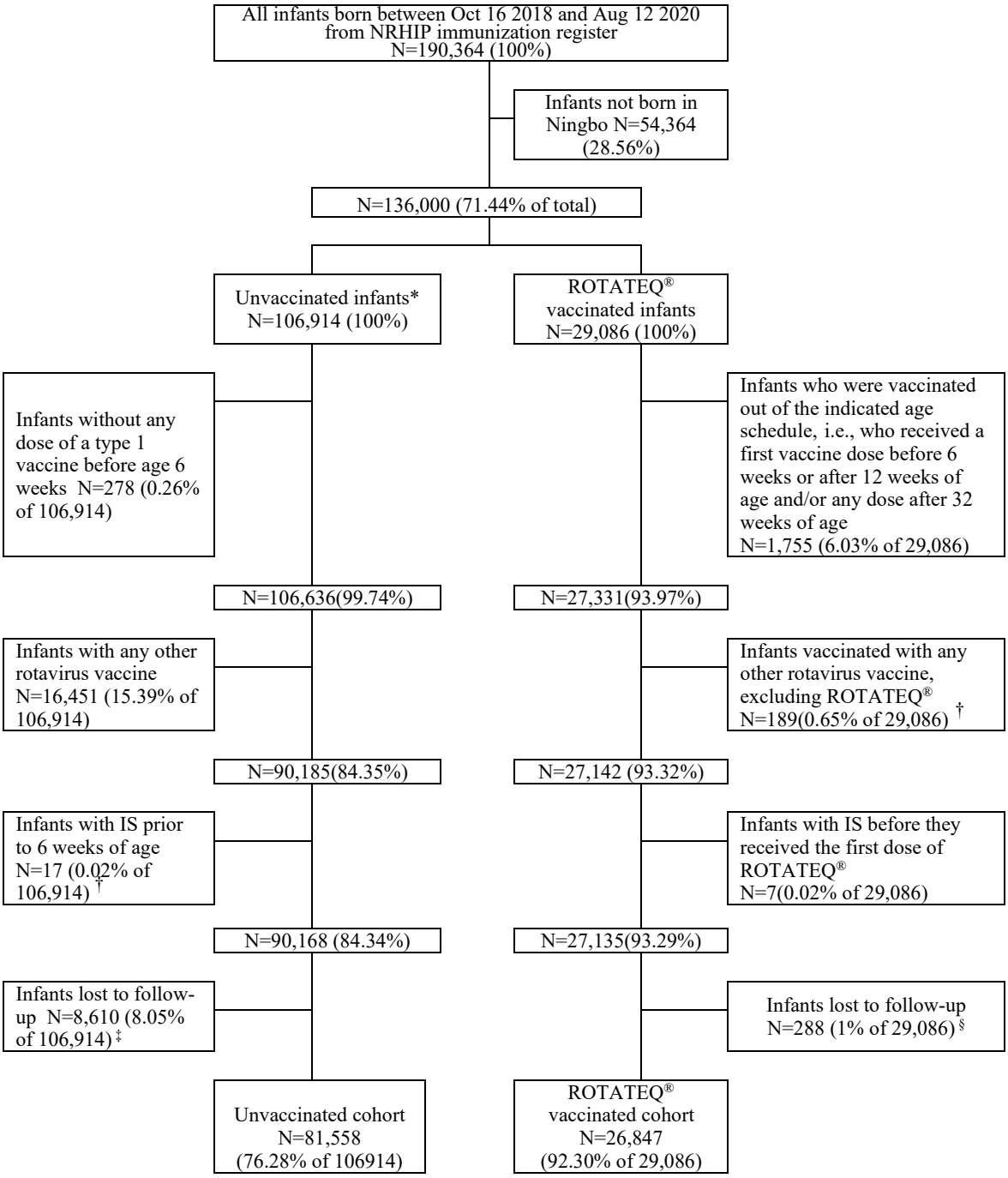

Figure 1 Flowchart from study population to analysis population

*Infants unvaccinated with ROTATEQ®.
†If any mention of potential IS in the records of hospital discharge.
‡Definition of "lost to follow-up" among unvaccinated infants: Infants without any recorded type 1 vaccination at age 8 to 9 months and no IS diagnosis during follow-up are considered to be lost to follow-up.
§Definition of "lost to follow-up" among vaccinated infants: Infants without any recorded type 1 vaccination at age 8 to 9 months or until their individual end of follow-up three months after their last ROTATEQ® dose (whatever comes first) , and no IS diagnosis during follow-up.

10.3 Descriptive data

The baseline characteristics of the study population and the analysis population are shown in Table 9. A slightly higher proportion of the study population were males (52.15%), more infants were born in 2019 (57.48%), and from urban areas (54.62%). There were 33,078 infants vaccinated with at least one dose of ROTATEQ®, and 30,667 (92.71%) of them had received three doses. More infants (57.40%) were vaccinated with at least one dose of ROTATEQ® in 2020, and the majority of doses (59.45%) were administered in the calendar year 2020.

Consistent with the study population, a slightly higher proportion of infants from the analysis population were male (52.30%), born in 2019 (58.10%) and from urban areas (55.06%). A total of 26,847 infants were vaccinated with at least one dose of ROTATEQ®, and 25,643 (95.52%) of them had received three doses. More infants (57.29%) were vaccinated with at least one dose of ROTATEQ® in 2020, and the majority of doses (59.28%) were administered in the calender year 2020.

Table 9 Baseline characteristics of the study population and analysis population

	Study population (N=190,364)	Analysis population (N=108,405)
Total		
Sex, n (%)		
Male	99,279(52.15)	56,694(52.30)
Female	91,083(47.85)	51,710(47.70)
Missing	2(0.00)	1(0.00)
Year of birth, n (%)		
2018*	26,677(14.01)	12,761(11.77)
2019	109,429(57.48)	62,978(58.10)
2020*	54,258(28.50)	32,666(30.13)
Missing	0(0.00)	0(0.00)
Location, n (%)		
Urban	103,983(54.62)	59,684(55.06)
Rural	85,123(44.72)	48,483(44.72)
Missing	1,258(0.66)	238(0.22)
Total number of infants vaccinated with ROTATEQ® (at least one dose), n	33,078	26,847
No. of infants vaccinated with at least 1 dose in calendar year, n (%)		
2018†	27(0.07)	8(0.03)
2019	15,708(40.39)	12,901(40.82)
2020	22,325(57.40)	18,102(57.29)
2021†	831(2.14)	588(1.86)
Number of infants per doses received[$]		
1 dose, n (%)	1,219(3.69)	644(2.40)
2 doses, n (%)	1,189(3.59)	558(2.08)
3 doses, n (%)	30,667(92.71)	25,643(95.52)
≥4 doses, n (%)	1(0.00)	1(0.00)
Missing	2(0.01)	1(0.00)
Total number of doses administered		
2018†	27(0.03)	8(0.01)
2019	37,867(39.61)	31,426(39.95)
2020	56,840(59.45)	46,635(59.28)
2021†	871(0.91)	601(0.76)

*2018 and 2020 data were not available for the entire year. (Only infants born between October 16th, 2018 and August 12th, 2020 were included in the study)

†2018 and 2021 data were not available for the entire year. (Only infants vaccinated with ROTATEQ® between November 27th 2018 and March 24th 2021 were included in the study)

As shown in Table 10, 649 (1.96%) and 19,386 (12.33%) vaccinated and unvaccinated infants, respectively, were lost to follow-up.

Table 10 Number and percent of loss to follow-up in the study population*

	Vaccinated population†	Unvaccinated population‡
Total	33,078	157,286
Loss to follow-up		
No.	649	19,386
Percent (%)	1.96	12.33

* The study population in this table included the infants born out of Ningbo.
†Lost to follow-up among unvaccinated infants: No recorded type 1 vaccination at age 8 to 9 months and no IS diagnosis during follow-up.
‡Lost to follow-up among vaccinated infants: No recorded type 1 vaccination at age 8 to 9 months or until their individual end of follow-up 3 months after their last ROTATEQ® dose (whatever came first), and no IS diagnosis during follow-up.

The baseline characteristics of the ROTATEQ® vaccinated and unvaccinated cohorts in the analysis population are presented in Table 11. The proportion of males was very similar in the vaccinated (52.60%) and the unvaccinated cohort (52.20%) (p-value =0.44). The majority of infants from the vaccinated cohort lived in urban areas (75.20%), compared to 48.43% from the unvaccinated cohort (p-value <0.001). The proportion of infants born in 2018, 2019 and 2020 was 1.90%, 54.31%, and 43.80% in the vaccinated cohort compared to 15.02%, 59.34% and 25.64% in the unvaccinated cohort (p-value <0.001). The proportion of infants born from February to April, May to July, August to October and November to January was 28.30%, 33.96%, 20.63% and 17.10% in the vaccinated cohort, compared to 24.29%, 23.84%, 20.11% and 31.76% in the unvaccinated cohort (p-value <0.001).

The baseline ROTATEQ® relevant characteristics in the vaccinated cohort are presented in Table 12. The median age (interquartile range) at vaccination of dose 1, dose 2, and dose 3 was 10 (9,11) weeks, 15 (14,17) weeks, and 21 (19,24) weeks, respectively. The mean age (SD) at vaccination of dose 1, dose 2, and dose 3 was 9.63 (1.48) weeks, 15.66 (2.46) weeks, and 22.02 (3.43) weeks. The median age (in weeks) at dose 1 in 2018, 2019 and 2020 was 11 weeks, 10 weeks and 10 weeks.

Table 11 Baseline characteristics of the ROTATEQ® vaccinated and unvaccinated cohorts in the analysis population

	Vaccinated cohort	Unvaccinated cohort	Chi-square	p-value
Total	26,847	81,558		
Sex, n (%)			1.64	0.440
Male	14,122(52.60)	42,572(52.20)		
Female	12725(47.40)	38,985(47.80)		
Missing	0(0.00)	1(0.00)		
Location, n (%)			5,892.58	<0.001
Urban	20,189(75.20)	39,495(48.43)		
Rural	6,583(24.52)	41,900(51.37)		
Missing	75(0.28)	163(0.20)		
Year of birth*			5,255.27	<0.001
1=2018	509(1.90)	12,252(15.02)		
2=2019	14,580(54.31)	48,398(59.34)		
3=2020	11,758(43.80)	20,908(25.64)		
Season of birth			2,459.94	<0.001
1=February-April	7,599(28.30)	19,807(24.29)		
2=May-July	9,117(33.96)	19,441(23.84)		
3=August-October	5,539(20.63)	16,405(20.11)		
4=November-January	4,592(17.10)	25,905(31.76)		

*2018 and 2020 data were not available for the entire year. Only infants born between October 16th, 2018 and August 12th, 2020 were included in the study.

Table 12 Baseline characteristics related to ROTATEQ® vaccination for ROTATEQ® vaccinated cohort

	Vaccinated infants
Age at vaccination by dose, weeks	
Dose 1, median (Interquartile range)	10(9,11)
Dose 2, median (Interquartile range)	15(14,17)
Dose 3, median (Interquartile range)	21(19,24)
Missing, n (%)	17(7,27)
Age at vaccination by dose, weeks	
Dose 1, mean (SD)	9.63(1.48)
Dose 2, mean (SD)	15.66(2.46)
Dose 3, mean (SD)	22.02(3.43)
Missing, n (%)	25.00(NA)
Age at dose 1 in birth year, weeks	
2018* median (Interquartile range)	11(10,11)
2019 median (Interquartile range)	10(9,11)
2020* median (Interquartile range)	10(8,11)

* Calendar year of birth. 2018 and 2020 data were not available for the entire year. Only infants born between October 16th, 2018 and August 12th, 2020 were included in the study.

10.4 Outcome data

A total of 187 infants aged 6 to 45 weeks with potential IS (cases) and available adjudication information were identified in the analysis population. A total of 191 IS episodes occurred among the 187 infants as some of them had more than 1 episode. However, only the first episode of Brighton level 1 was counted. Seventy-four cases were confirmed as level 1 (75 episodes), while 90 were non-cases (93 episodes) and 23 were unconfirmed cases (23 episodes). Among the 23 unconfirmed cases, 9 were in the vaccinated cohort and 14 were in the unvaccinated cohort. Among the 74 confirmed level 1 cases, 53 cases occurred in the unvaccinated cohort and 21 cases occurred in the vaccinated cohort. Among the cases in the vaccinated cohort, 7 occurred within the 3 months risk period and 14 occurred out of the 3 months risk period.

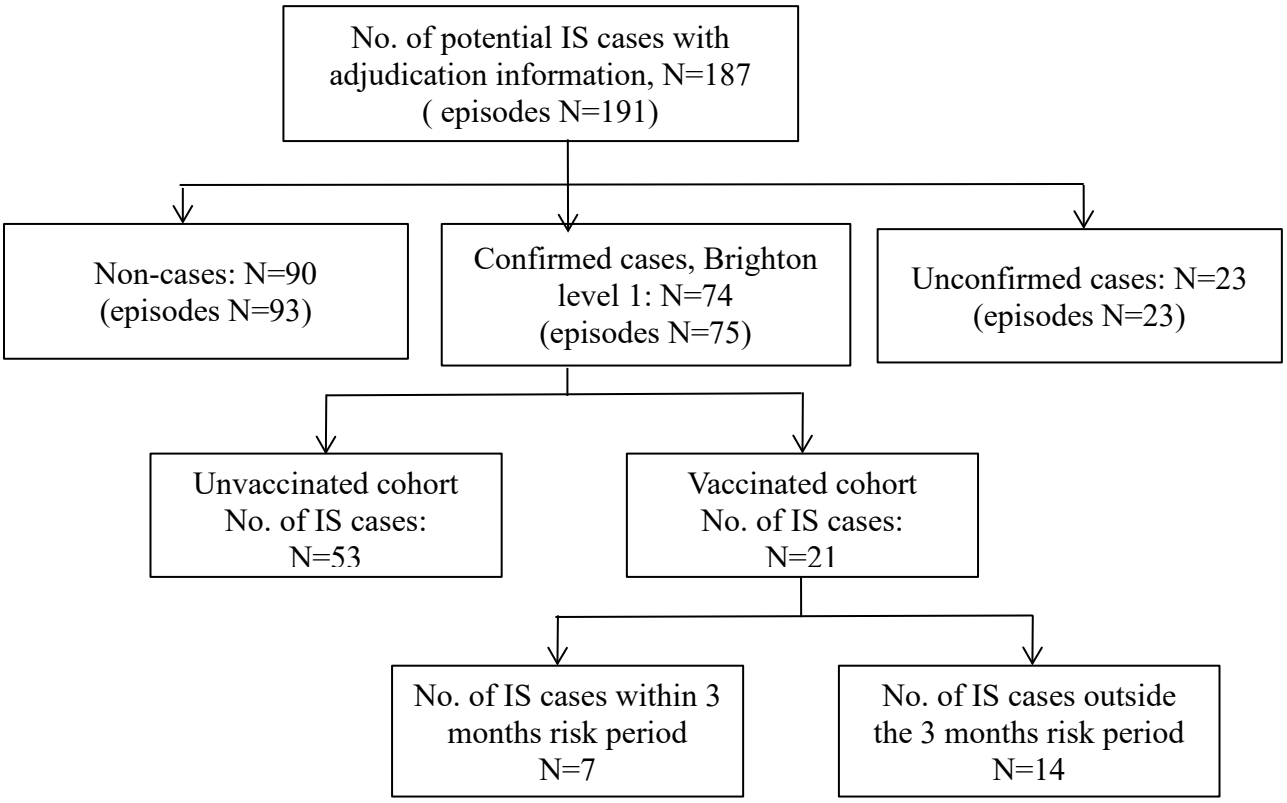

Figure 2 Flowchart of IS case adjudication

Note: Confirmed case (Brighton level 1) - A suspected case that went through the adjudication process and was considered to meet the criteria of Brighton level 1 case definition.
Non-case - A suspected case that went through the adjudication process and did not meet the criteria of Brighton level 1 case definition.
Unconfirmed cases - The case could not be adjudicated as the available information was not sufficient.

(EU GUIDANCE: 23 JANUARY 2013 EMA/738724/2012)

Baseline characteristics of infants with confirmed IS cases that occurred within 3 months following ROTATEQ® vaccination are presented in Table 13. A total of 7 IS cases were identified within the 3 months risk period. The mean age when an IS episode occurred was 30.57 weeks. There were 2 IS cases in each age groups 22-29, 30-37 and 38-45 weeks. More cases occurred in infants from urban areas (5, 71.43%) .

Table 13 Baseline characteristics of confirmed IS cases within 3 months following ROTATEQ® vaccination among the ROTATEQ® vaccinated cohort

	IS cases (n=7) *
Age at IS episode	
Mean, weeks	30.57
6-13 weeks, n (%)	0(0.00)
14-21 weeks, n (%)	1(14.29)
22-29 weeks, n (%)	2(28.57)
30-37 weeks, n (%)	2(28.57)
38-45 weeks, n (%)	2(28.57)
Sex, n (%)	
Male	4(57.14)
Female	3(42.86)
Missing	0(0.00)
Calendar year of IS episode, n (%)	
2018†	0(0.00)
2019	1(14.29)
2020	4(57.14)
2021†	2(28.57)
Location, n (%)	
Urban	5(71.43)
Rural	2(28.57)
Missing	0(0.00)

* The denominator is the total number of IS cases within 3 months following ROTATEQ® vaccination among the ROTATEQ® vaccinated cohort.
† IS cases within the risk period between November 27th 2018 and June 30th 2021 were extracted for adjudication.

Baseline characteristics of confirmed IS cases among the unvaccinated cohort are presented in Table 14. A total of 53 IS cases were identified in the surveillance period. The mean age when an IS episode occurred was 35.23 weeks. Most cases occurred at age 30-37 weeks (45.28%) and 38-45 weeks (39.62%). Most cases (40, 75.47%) occurred in males.

Table 14 Baseline characteristics of confirmed IS cases among the unvaccinated cohort

	IS cases (n=53) *
Age at IS episode	
Mean, weeks	35.23
6-13 weeks, n (%)	1(1.89)
14-21 weeks, n (%)	2(3.77)
22-29 weeks, n (%)	5(9.43)
30-37 weeks, n (%)	24(45.28)
38-45 weeks, n (%)	21(39.62)
Sex, n (%)	
Male	40(75.47)
Female	13(24.53)
Missing	0(0.00)
Calendar year of IS episode, n (%)	
2018 †	0(0.00)
2019	21(39.62)
2020	27(50.94)
2021 †	5(9.43)
Location, n (%)	
Urban	26(49.06)
Rural	27(50.94)
Missing	0(0.00)

Note：*The denominator is the total number of IS cases among the unvaccinated cohort.
† IS cases between November 27th 2018 and June 30th 2021 were extracted for adjudication.

10.5 Main results

10.5.1 Incidence rate of confirmed IS within 3 months following ROTATEQ® vaccination

As shown in Table 15, a total of 7 confirmed IS cases occurred within 3 months following any dose of ROTATEQ® vaccination, all of which occurred post dose 3. The vaccinated cohort contributed 12,592.14 person-years and the incidence of IS was 55.59 (95%CI: 22.35, 114.54) per 100,000 person-years. The incidence of IS was 0 (95%CI: 0, 157.10), 24.9 (95%CI: 0.63, 138.72), 50.83 (95%CI: 6.16, 183.62), 95.6 (95%CI: 11.58, 345.33), and 995.22 (95%CI: 120.53, 3595.09) per 100,000 person-years in the sequential age-groups of 6-13, 14-21, 22-29, 30-37 and 38-45 weeks, respectively.

Table 15 Incidence rate of confirmed IS within 3 months following ROTATEQ® vaccination

	Number of IS cases, n	Person-years	Incidence rate of IS (per100,000 person-years)	95% CI
Total	7	12,592.14	55.59	22.35, 114.54
Age group				
6-13 weeks, n (%)*	0	2,348.08	0	0, 157.10
14-21 weeks, n (%)	1	4,016.35	24.9	0.63, 138.72
22-29 weeks, n (%)	2	3,934.63	50.83	6.16, 183.62
30-37 weeks, n (%)	2	2,092.12	95.6	11.58, 345.33
38-45 weeks, n (%)†	2	200.96	995.22	120.53, 3,595.09

* The number of person-years in the age group of 6-13 weeks is low, as start follow was depending on the date of vaccination with dose 1: 25% of infants started follow-up at 9 weeks, 50% of infants at 10 weeks and75% of infantsat 11 weeks.
†The number of person-years in the age group38-45 weeks is low as it includes only infants who received dose 3 late. The follow-up in infants with an early schedule of 6, 10, 14 weeks ended at age 26 weeks. 50% of infants were followed up to 33 weeks and 75% up to 36 weeks.

10.5.2 Number of confirmed IS cases by risk period in relation to ROTATEQ® dose

As shown in Table 16, no IS cases occurred in any of the risk periods 1-7, 1-14, 1-21, 1-42 and 1-90 days after dose 1 and dose 2. Seven cases occurred after dose 3 in the whole risk period of 1-90 days. The cumulative number of cases that occurred in the risk periods 1-7, 1-14, 1-21 and 1-42 days was 0, 1, 1 and 3, respectively.

Table 16 Number of confirmed IS cases by risk period in relation to ROTATEQ® dose

Risk period	Dose 1, n	Dose 2, n	Dose 3, n	Any Dose, n
1-7 d	0	0	0	0
1-14 d	0	0	1	1
1-21 d	0	0	1	1
1-42 d	0	0	3	3
1-90 d	0	0	7	7

10.5.3 Incidence rate of confirmed IS cases in the unvaccinated cohort

As shown in Table 17, a total number of 53 confirmed IS cases occurred in the unvaccinated cohort. The unvaccinated cohort contributed 61,172.09 person-years and the incidence of IS was 86.64 (95%CI: 64.90, 113.32) per 100,000 person-years. The incidence of IS increased with age from 8 per 100,000 person-years (95%CI: 0.20, 44.56) in the 6-13-week age group to 191.98 per 100,000 person-years (95%CI: 123.00, 285.65) in the 30-37 week-age group, and did not further increase in the 38-45-week age group with an incidence of 188.2 (95%CI: 116.50, 287.70) per 100,000 person-years.

Table 17 Incidence rate of confirmed IS cases in the unvaccinated cohort

	Number of IS cases, n	Person-years	Incidence rate of IS (per100,000 person-years)	95% CI
Unvaccinated cohort	53	61,172.09	86.64	64.90, 113.32
Age groups				
6-13 weeks	1	12,504.30	8.00	0.20, 44.56
14-21 weeks	2	12,504.24	15.99	1.94, 57.78
22-29 weeks	5	12,503.64	39.99	12.98, 93.32
30-37 weeks	24	12,501.42	191.98	123.00, 285.65
38-45 weeks	21	11,158.50	188.20	116.50, 287.70

10.5.4 The adjusted relative risk of confirmed IS in the ROTATEQ® vaccinated cohort compared to the unvaccinated cohort

The crude RR of IS was 0.64 (two-sided 90% CI:0.33, 1.24). The RR estimate was adjusted for potential confounders including sex, year of birth, location, season of birth, age groups and ROTATEQ® vaccination status in the Poisson regression model. The adjusted RR of IS in the vaccinated cohort compared to the unvaccinated cohort was 0.90 (two-sided 90% CI: 0.46, 1.75). Male and older age were associated with an increased risk of IS, while other covariates were not associated with a statistically significant increased risk.

Table 18 The adjusted relative risk of confirmed IS in the ROTATEQ® vaccinated cohort compared to the unvaccinated cohort

Factors	p Value	RR	90% CI of RR
ROTATEQ® vaccination status			
No		reference	
Yes	0.7942	0.90	0.46, 1.75
Sex			
Male		reference	
Female	<0.001	0.49	0.40,0.61
Year of birth			
2018		reference	
2019	0.638	0.84	0.45,1.55
2020	0.642	1.23	0.59,2.58
Location			
Urban		reference	
Rural	0.949	1.02	0.66,1.57
Season of birth			
Feb-Apr		reference	
May-Jul	0.797	0.89	0.44,1.83
Aug-Oct	0.132	1.94	0.94,3.98
Nov-Jan	0.144	1.81	0.93,3.52
Age groups			
6-13 weeks		reference	
14-21 weeks	0.383	2.73	0.41,18.16
22-29 weeks	0.034	9.14	1.63,51.16
30-37 weeks	<0.001	30.35	5.71,161.33
38-45 weeks	0.003	20.45	3.73,112.19

All variables including sex, year of birth, location, season of birth, age groups, ROTATEQ® vaccination status were forced into the Poisson regression model. With no covariates in poisson regression model, the study power is 0.829 for one-sided test with α=0.05, or two-sided test with α=0.10.

(EU GUIDANCE: 23 JANUARY 2013 EMA/738724/2012)

10.6 Other analyses

Not applicable in this report.

10.7 Adverse events/adverse reactions

This is a surveillance study based on a secondary analysis of data that were routinely collected in Ningbo and preserved in the NRHIP. Specific case identification methods were applied to identify IS cases in NRHIP. Medical records of IS cases in NRHIP, including surgical, radiological and autopsy records that were needed for the case adjudication of IS cases, were extracted and reviewed based on the pre-specified methods by AC members. Adverse events (AEs) and product quality complaints (PQCs) were not actively solicited in this study according to the study design, however, during review of medical records, the SAR/NSAR and other events which meet criteria were to be reported. For AEs observed in secondary data collection, only cases with an explicit and definitive notation (by a healthcare provider) of a causal relationship with a product in the medical records or other secondary data being reviewed should be reported as NSAR/SARs. During review of secondary data, causality should never be assigned retrospectively.

During the study period, 21 IS cases were observed in the ROTATEQ® vaccinated cohort, among whom 7 were within 3 months risk period after any dose. These 21 cases of IS did not have explicit and definitive notation by a healthcare provider of a causal relationship in the medical records, which means they didn't meet the criteria for AE reporting in secondary data collection study. Thus the 21 cases of IS were not reported to the sponsor's safety database. There were no PQCs (with or without AE), special situations (regardless of causality), or spontaneously reported AEs/PQCs for MSD product observed in this study.

11 DISCUSSION

11.1 Key results

In the feasibility assessment, EMR data were sufficiently recorded to adjudicate the IS cases according to the Brighton Level 1 criteria; 98% of the IS cases had at least one diagnostic

variable available. Ninety two percent of the target population were followed over the entire observation period from 6 to 45 weeks of age and the linkage rate between the NRHIP EMR and immunization registry in the target population was 96%. The information of vaccine code and vaccine product (manufacturer) code in the platform that could differentiate between LLR (local rotavirus vaccine) and ROTATEQ® was available for all vaccinated infants. The completeness of ROTATEQ® vaccination records, including vaccination date was also 100%.

Among the analysis population, 26,847 infants had received at least one dose of ROTATEQ® and 25,643 (95.52%) of them were fully vaccinated at the time of data cut off (March 24th, 2021). The total number of doses administrated increased from 12,901 doses in 2019 to 18,102 doses in 2020. The mean age at dose 1, dose 2 and dose 3 was 9.63 weeks, 15.66 weeks and 22.02 weeks. The median age at dose 1 was 11 weeks in 2018 and 10 weeks in 2019 and 2020.

There was no statistically significant difference in the sex distribution among the vaccinated cohort and the unvaccinated cohort. Most infants (75.20%) from the vaccinated cohort were from urban areas while most infants from the unvaccinated cohort (51.37%) were from rural areas. As ROTATEQ® only became available in Ningbo at the end of 2018, the proportion of infants from the vaccinated cohort who were born in the year 2018 was very low (1.90%) while 15.02% of the infants from the unvaccinated cohort were born in 2018.

Seven confirmed IS cases occurred within the 3 months risk period after any ROTATEQ® vaccination in the vaccinated cohort, and 53 confirmed IS cases occurred in the unvaccinated cohort. In both cohorts, the cases reached a peak at age 30 to 37 weeks. Most of the cases occurred in male infants: 75.47% of cases in the unvaccinated cohort and 57.14% of cases in the vaccinated cohort. The proportion of infants with IS from urban areas (71.43%) was higher in the vaccinated cohort than in the unvaccinated cohort (49.06%), which was consistent with the fact that most infants (75.20%) from the vaccinated cohort were from urban areas and most infants from the unvaccinated cohort (51.37%) were from rural areas.

The overall incidence rate of confirmed IS that occurred within 3 months following ROTATEQ® vaccination was 55.59 (95%CI: 22.35, 114.54) per 100,000 person-years. Seven IS cases occurred among the infants vaccinated with ROTATEQ®, none occurred post dose 1 or post dose 2, and all occurred post dose 3. The majority (n=4) of them occurred within 43-90 days post dose 3. The incidence rate of confirmed IS that occurred in the unvaccinated cohort was 86.64 (95%CI: 64.90, 113.32) per 100,000 person-years, and reached a peak at age 30 to 37 weeks.

The crude RR of IS in the vaccinated cohort compared to the unvaccinated cohort was 0.64 (two-sided 90% CI:0.33, 1.24). The RR was 0.90 (two-sided 90% CI: 0.46, 1.75) after adjusting for sex, year of birth, location, season of birth and age group. The risk of IS increased with age and was higher in males compared to females. No other covariates were associated with a statistically significant higher or lower risk.

11.2 Limitations

Our study has several limitations:

- Non-differential misclassification of outcome: A small percentage (estimated at about 4%) of potential IS cases could not be linked to the immunization register, and this is concordant with the high linkage rate of 96% in the feasibility assessment. Furthermore, IS cases might not have been captured if medical care was sought in hospitals that were not covered by NRHIP, but the possibility that IS cases were treated outside of Ningbo is expected to be low. IS is an acute disease which requires urgent medical attention. There were 23 unconfirmed cases with limited information that could not be adjudicated, with the proportion of unconfirmed cases comparable between the vaccinated cohort (within 3 month risk period) and the unvaccinated cohort; therefore, if cases of IS were not captured, this would result in an underestimate of the incidence rate in both vaccinated and unvaccinated cohorts. However, it is expected that IS cases that were not captured were very limited, with similar rate of misclassification in the vaccinated and unvaccinated cohorts. Non-differential misclassification could incline the results towards the null hypothesis and would not change the direction of the association.

- As specific symptoms of IS and their onset date were not registered in the NRHIP, the diagnosis date was used as a proxy for IS onset. However, symptoms might have started before a case was diagnosed. However, considering the acute nature and severe presentation of IS disease, which requires urgent medical treatment with reduction (nonoperative or operative), the onset date should be very close to or on the same day as the diagnosis date. The episode date was pre-specified in Data Management Plan for all IS cases. Thus, using the diagnosis date of IS cases instead of the onset date was expected to have a very limited impact on the study results.

- The proportion of infants who were lost to follow-up was lower in the ROTATEQ® vaccinated cohort compared to the unvaccinated cohort. Among the unvaccinated cohort, infants were considered lost to follow-up when no type 1 vaccination was recorded for them at age 8 to 9 months and no IS case had occurred during the risk period. However, as ROTATEQ® vaccinated infants were followed for 3 months after the last dose received, their follow-up time might have ended before the age of 8 to 9 months, independent of whether or not they had received type 1 vaccines at that time. Since the number of type 1 vaccines administered before 8 months of age is higher than the number administered at age 8 to 9 months, the loss to follow-up rate was lower in the vaccinated cohort. Also, the ROTATEQ® vaccinated infants generally have higher tendency to receive routine childhood vaccines than unvaccinated infants. Considering that the same definition and capture method of IS cases was used in the two cohorts, the loss to follow-up and the outcome of IS could be considered independent. Therefore, the differential loss to follow-up (7%) between these two cohorts might have slightly overestimated the incidence of IS in the unvaccinated cohort, and underestimated the RR.

- The follow-up time for the unvaccinated cohort started at 6 weeks of age. For the vaccinated cohort, although the inclusion/exclusion criteria allowed enrollment of infants from 6 weeks of age on, 75% of infants received the 1st dose at age 9-11 weeks. Therefore, the follow-up of the majority of the infants in the ROTATEQ® vaccinated cohort started at 9 weeks of age or older. Given that IS is a contraindication for ROTATEQ® vaccination, only infants without previous IS were included in the vaccinated cohort. However, the

incidence of IS was extremely low in this very young age group. There was only one case that occurred in the unvaccinated arm before 10 weeks.

11.3 Interpretation

Based on the criteria of the feasibility assessment pre-specified in Statistical Analysis Plan (SAP), NRHIP was considered appropriate for the study. IS cases could be identified and sufficient information could be extracted for case adjudication. The vaccination information was complete and reliable, and it was possible to differentiate between the various rotavirus vaccines. Also, the dates of vaccination could be captured. As the EMR entries could be linked with the immunization registry in the NRHIP, a retrospective cohort study could be conducted. Prior to this study, two other studies demonstrated the appropriateness of NRHIP for the safety monitoring of a recently licensed vaccine[33,34].

The proportion of males and females was similar in the vaccinated and unvaccinated cohorts. However, the proportion of infants from urban areas was higher in the vaccinated cohort (75.20%). This is probably due to the higher socio-economic status of residents in urban areas since ROTATEQ® is not included in the national immunization program nor local immunization program, but must be paid out of pocket in China. Previous studies showed that vaccinated infants tended to have better socio-economic status and were more likely to be residents of urban areas[35]. The lower proportion (1.90%) of infants vaccinated with ROTATEQ® who were born in the year 2018 compared to unvaccinated infants (15.02%) in the study was due to the late availability of ROTATEQ® on 27th November 2018.

Among the analysis population, the number of infants who had received 3 doses of ROTATEQ® at the data cutoff date (March 24th 2021) was almost as high as the number of those who had received one dose, indicating that most infants completed the 3-dose schedule of ROTATEQ®. The mean age at dose 1, dose 2 and dose 3 was 9.63 weeks, 15.66 weeks and 22.02 weeks, respectively, indicating that infants in Ningbo generally completed the 3 doses earlier, before 32 weeks of age. The 3 months risk period after the first two doses ended before the infants reached the age of the natural peak of IS (8 months of age)[36]. All 7

IS cases occurred after dose 3, mostly within 43-90 days post vaccination. No IS case occurred post dose 1 and 2 after vaccination with ROTATEQ®. The apparently high incidence in infants in the age group 38 to 45 weeks in the vaccinated cohort can be explained by the small sample size in that age group and the natural peak age of IS. Consistent with other studies, more IS cases were observed in males compared to females[37,38].

The overall incidence rate of IS occurring within 3 months after vaccination with ROTATEQ® in this study was similar to the unvaccinated cohort. The incidence rate of IS generally increased with age, though this was more appreciable in the unvaccinated cohort. The same increasing trend with age was observed in studies that assessed the background incidence in mainland China[16,17] and in other Asian countries or regions, such as South Korea[38], and Chinese Taiwan[37]. The IS incidence rate was lower in this study, compared to other studies[16,17,37,38] conducted in Asia. A possible explanation is that the definition of what constitutes a case differs between studies. Only confirmed IS cases (Brighton level 1) were analysed in this study, while other studies may report all potential IS cases, including those who were not adjudicated. The average annual background incidence of potential IS cases in children aged <2 years reported between 2016-2018 in Ningbo[36] was 523.62 cases per 100,000 population, which is higher than the incidence reported in other studies from China or other Asian countries[37,38]. However, these estimates were based on potential IS cases (without adjudication), that occurred in infants and children up to 2 years of age, covering the natural peak age for IS. Also, recurrent IS cases may have been counted in these studies, making a direct comparison to the results of this study not possible.

The wide 95% CI (120.53, 3595.09 per 100,000 person-years) for the reported incidence rate in the age group 38-45 weeks in the vaccinated cohort could be explained by the limited follow-up time in this age group . The number of person-years in other age groups ranged between 2,092.12 and 4,016.35, while only 200.96 person-years were accrued in the age group of infants from 38-45 weeks. The reason for the limited number of person-years in this age group was that only infants who received the 3rd dose of ROTATEQ® at the upper age limit were included. The follow-up in infants with an early schedule of 6, 10, 14 weeks ended at age 26 weeks, and therefore these infants did not contribute to the follow-up in the

age groups of 30-37 and 38-45 weeks old. Fifty percent of infants were followed up to 33 weeks and 75% were followed up to 36 weeks. The mean age at vaccination of the third dose of ROTATEQ® was low among the infants in the analysis population and most of them ended the follow-up before the natural peak age for IS. Therefore, the number of infants in the age group 38-45 weeks was low and this is the reason for the imprecise risk estimate and wide confidence interval. However, children from the unvaccinated cohort, were all followed until age 45 weeks (unless they had an IS episode before the end of follow-up). The overall point estimate of the incidence rate of IS was lower in the vaccinated cohort (55.59/100,000 person-years) compared to the unvaccinated cohort (86.64/100,000 person-years). The 95%CI of the incidence rate in the vaccinated and unvaccinated cohorts overlapped, suggesting that the incidence rate of IS was similar between the two groups.

There was insufficient evidence (lower limit of one-sided test for 95%CI 0.46, p-value = 0.7942) to conclude that the RR of IS in the vaccinated compared to unvaccinated infants was greater than 1.0. The hypothesis test had 82.9% power for a one-sided alpha-level of 0.05 or a two-sided alpha-level of 0.10 to detect a 2-fold or greater increased risk of IS in ROTATEQ® vaccinated compared to unvaccinated infants. The estimated RR was 0.90 with a 90% CI of 0.46 to 1.75, indicating the overall RR is between 0.46 and 1.75 with 90% confidence which is consistent with that reported in previous studies[21,22,26].

No statistically significant difference was observed for the overall RR of IS in the vaccinated and unvaccinated cohorts in other studies using the same cohort study design [21,22,26]. A Cochrane review of the 4 WHO prequalified rotavirus vaccines showed that in RCTs for each vaccine, no increase was noted in intussusception risks after any dose. However, post-licensure evaluations of rotavirus vaccines have found intussusception risk to vary by vaccine and study location. In several high- and middle-income countries, a low risk of 1–6 excess cases of intussusception per 100,000 vaccinated infants has been documented for both Rotarix and ROTATEQ®[39]. The pathogenic mechanisms involved in intussusception following rotavirus vaccination remain poorly defined[39].

11.4 Generalisability

This was the first post-marketing observational study conducted in China to assess the association between ROTATEQ® vaccination and IS. The design is a cohort study conducted using the best available regional platform (NRHIP) in China. All infants aged 6 to 45 weeks from December 2018 to June 2021 from the NRHIP immunization register that met the inclusion and exclusion criteria were included in the analysis. All potential IS cases from the analysis population were adjudicated according to the pre-specified adjudication criteria and only cases that met the Brighton level 1 criteria were included in the analysis.

The Ningbo region is located in the eastern part of China and has a higher socio-economic status compared to other regions in China. As ROTATEQ® is not reimbursed by public insurance in China, infants with higher socioeconomic status may have more access to the vaccine and as such vaccine coverage with ROTATEQ® is expected to be higher in Ningbo compared to other parts of China where the socio-economic level is lower. Because infants are widely covered by public health insurance in China, and given that IS is an acute disease requiring urgent medical attention, access to medical care at nearby hospitals does not seem to be impacted irrespective of where the infants reside. However, the background incidence of IS among infants may vary from region to region, which may also be due to study designs and definition of an IS case, including the application of case adjudication methods.

In conclusion, these regional differences are not likely to impact the generalizability of the study results. In addition, the quality of NRHIP, the large sample size included and the coverage(17.38% in the study population) of ROTATEQ® in Ningbo were factors that contributed to a robust study.

12 OTHER INFORMATION

Not applicable in this report.

13 CONCLUSION

This was the first post-marketing observational study conducted in China to assess the association between ROTATEQ® vaccination and IS. No increased risk of IS was observed following 3 months(90 days) of ROTATEQ® vaccination in this study.

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Annex 1 List of stand-alone documents

Number	Document reference number	Date	Title
1	V260-075/Protocol Version 3.0	13-October-2020	Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)
2	V260-075/SAP Version 1.0	28-January-2022	Statistical Analysis Plan for Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)

Annex 2 Study Protocol

Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)

V260-075 study report-Annex 2-St

Annex 3 Study Statistical Analysis Plan

Statistical Analysis Plan for Post-marketing surveillance to monitor the incidence of intussusception after large-scale vaccination with Reassortant Rotavirus Vaccine, Live, Oral, Pentavalent (Vero Cell) (ROTATEQ®) in Chinese infants using the Ningbo Regional Health Information Platform (NRHIP)

V260-075 study report-Annex 3-St