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Rotavirus Genotypes over Time in Vaccine-Introducing and Non-Introducing Countries

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By

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Bachelor of Science
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Abstract

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By Zihao Liu

Background

Rotavirus vaccines have substantially lowered the global rotavirus gastroenteritis burden in children aged <5 years. However, vaccines may offer less protection against some rotavirus genotypes, including G2P[4], and vaccine introduction may also exert vaccine-induced selective pressures on circulating rotavirus strains.

Methods

To examine if strains that vaccines may be less effective against became more dominant over time, we systematically reviewed literature of rotavirus surveillance reporting genotype distributions between 2002 and 2019. We included data from countries with at least 6 years of surveillance data and, if a rotavirus vaccine was introduced into their national immunization program during the time period identified in the data (between 2002 to 2019, specific surveillance period varied by country), at least 2 years of data pre- and post-introduction. We estimated odds of infection by a particular rotavirus genotype in response to G1P[8] strain in different scenario by fitting two multinomial logistic regression. One having vaccine introduction status as main exposure and another having surveillance year as exposure. The final dataset included five vaccine-introducing countries (Ethiopia, Kenya, Italy, South Africa, and Zimbabwe) and five non-introducing countries (Argentina, China, Myanmar, Nepal, and Ukraine).

Results

257 records were used in the analysis, with 83,017 overall positive rotavirus cases included. For vaccine-introducing countries, the odds of infection with G2P[4] increased each year in South Africa [adjusted odds ratio (aOR): 2.47, 95% confidence interval (CI): 2.47-2.48] and Kenya (aOR: 4.24, 95% CI: 4.24-4.25). For non-introducing countries, the odds of infection with G2P[4] increased each year in China (aOR=1.46, 95% CI 1.46-1.48) and Ukraine (aOR=1.93, 95% CI 1.93-1.95). G2P[4] infection odds increased over time in some introducing and non-introducing countries; however, introducing countries had much higher odds of G2P[4] infection as time went on than non-introducing countries.

Conclusion

Both the descriptive analysis and regression analysis indicate an overall decrease of G1P[8] strain and an increase of G2P[4] over time for most countries from both introducing and non-introducing groups. These findings highlight the need for continued surveillance in both vaccine-introducing and non-introducing countries to monitor circulating rotavirus genotypes and assess potential impacts of vaccine introduction.

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INTRODUCTION

Background

Globally, Group A rotaviruses are the most prevalent cause of severe, dehydrating diarrhea in children under the age of five[1]. Rotavirus gastroenteritis (RVGE) claims the lives of over 128,500 children under the age of five on the globe[2] with more than 100 million infections and 2 million hospitalizations[3]. The burden of RVGE has also caused a tremendous amount of pressure on countries' health care, financial sector, and overall population quality of life[4].

Rotavirus is a double stranded RNA virus with genotypes determined by two proteins. G serotypes (often referred to as G types) and P serotypes (referred to as P types) are identified by the outer capsid proteins VP7 and VP4 on the surface of the virus. Because the changes in G-type and P-type may occur independently of one another[5], the G and P dual nomenclature method is often employed to establish the genotype of rotavirus. There are six G types (G1, G2, G3, G4, G9, and G12) and three P types (P[4], P[6], and P[8]) of group A RVs that most often infect people over the globe[6]. G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] are the G and P combinations that account for more than 90% of all human RV strains[7].

A number of effective and safe vaccines have been developed to help decrease the global disease burden caused by rotavirus gastroenteritis and are recommended by the World Health Organization (WHO) for inclusion in national vaccination programs [8]. Currently, four oral rotavirus vaccine, RotaTeq (RV5) (pentavalent, prevent RVGE caused by G1, G2, G3, G4, G9; Merck Vaccines, NJ, USA), Rotarix (RV1) (monovalent G1P[8]; GlaxoSmithKline Biologicals, Belgium), Rotasiil (pentavalent G1, G2, G3, G4, and G9; Serum Institute, Pune, India) and

Rotavac (monovalent G9P[11]; Bharat Biotech, Hyderabad, India) have been licensed and become commercially available in more than 107 countries worldwide by March 2021[9, 10]. The RV5 and RV1 vaccines were effective against the most commonly circulating rotavirus strains, including those that were homotypic (matched all serotypes in the vaccines), partly heterotypic (matched only one of the G-types or the P-type) or fully heterotypic (matched no serotype in the vaccine)[11, 12]. However, potential genetic drift that happens on the VP7 and VP4 protein could result in strains becoming less affected by vaccine antibodies[9], thus, lowering the overall vaccine efficacy. Through these processes, certain previously less-common genotypes may become more predominant after the vaccine introduction, which potentially jeopardizes the impact of the rotavirus immunization campaign.

Objective

Patterns of changes in the genotype distribution of human rotaviruses have been seen through time and across geographic regions, according to surveillance studies[13-15]. In order to determine whether or not vaccine introduction may affect genotype circulation, it is necessary to compare the genotype diversity differences between vaccine-introduced countries and non-introduced countries in order to assess if vaccination affects genotype circulation, Based on previously published data, we report on the frequency of rotavirus G and P genotype combinations across countries and time periods to see whether there has been a shift in genotype distribution and change diversity after the implementation of a vaccination program and whether such change was also observed in vaccine non-introducing countries.

METHOD

We conducted a systematic review to update and expand a prior 2014 literature review done by Leshem et.al[16]. Rotavirus genotype distribution data were collected from different countries around the world. This study compares the temporal change of rotavirus genotype distribution between vaccine introducing and non-introducing countries and aim to evaluate whether such changes were different due to the vaccine introduction.

Search strategy and selection criteria

Using PubMed, Embase, CAB Abstracts, Global Health, and Medline databases, we identified studies published in English or abstracts written in English (no language restriction) between January 24, 2000, and October 16, 2020. The keyword search strategy used in the 2014 literature review for studies reporting genotype distributions was implemented using keywords like “rotavirus”, “genotype”, “surveillance”, “cohort studies”, “genotype”, “strain”, “effectiveness studies” and “case control studies” amongst other topics.

Initial article screening and full article text review were completed using Covidence software (covidence.org) by two of four independent reviewers. Disagreements between reviewers were resolved by consensus. A study was included if it was an original report on the monitoring of acute gastroenteritis in children under the age of five years and included the number of genotyped samples and the percentage or numbers of discovered G-[P] strain combinations in the rotavirus strain data. A study was excluded if it was irrelevant to the review’s objectives, the data involved had already been published in other studies, all data came from samples collected prior to 2000, the study featured a clinical research design, or a

combination of pediatric (children under 5 years old) and adult participants were included and less than 80 percent of samples were from the pediatric population.

Data were extracted in duplicate and recorded using Qualtrics software (Qualtrics, Provo, UT) by two of four reviewers (JC, JP, JW, ZL). Disagreements between reviewers were resolved by consultation with additional reviewers (AA) or consensus. Extracted data included country of study, surveillance period starts and end date (month and year), total number of positive stool samples, number of samples genotyping was attempted for, number of samples with genotype results reported, and either the number of samples or percent of reported samples by genotype when the total number of genotyped samples was known. Genotype was recorded as the specific G and P combination. Mixed-type results (multiple types reported for either G, P, or both) were extracted in one aggregate category. Non-typeable (NT) results (either G, P, or both were NT) were recorded if reported.

For the country-specific analysis, the country's vaccine introduction status was determined using the information provided by International Vaccine Access Center (IVAC)[17] as of November 17th, 2020. Countries with data available for at least 6 consecutive years were considered for analysis. If a country introduced at least one WHO-prequalified rotavirus vaccine into its national or regional immunization program (a vaccine-introducing country) in the years of data extracted, we required they have data available for at least two consecutive surveillance years from both pre and post vaccination era. If one vaccination era had significantly more data available than the other era (more than six years), the country was excluded.

Data Analysis

Descriptive analysis: Data analyses were performed using R version 4.1.1 (R Core Team, 2014). To explore the annual fluctuation of rotavirus strains across different countries, data from different studies were aggregated by country to describe genotype distributions in nations with and without national immunization programs. The distribution of genotype was tabulated by vaccine introduction status (pre and post) for vaccine introducing countries.

Genotype diversity: To better quantify the rotavirus genotype diversity and determine if a change in biodiversity can be observed between pre and post vaccination period, as well as each surveillance year (temporal pattern), two established biodiversity indices were calculated. Simpson's index is a biodiversity indicator measure; it ranges from 0 (lowest diversity) to 1 (maximum diversity). It represents the likelihood that the two strains belong to two separate G and P genotype strain combinations when they were randomly picked and is calculated as $1-\lambda$, where $\lambda = \Sigma_i^{P2}$[18] and pi represents the proportional abundance of a genotype (i). The Shannon Diversity measures the uncertainty in predicting the genotype identification of an individual sample drawn at random and is calculated as $H' = -\sum P_i \ln(P_i)$ [18]. With respect to overall and country specific analysis, the weighted average diversity index score from before and after vaccination introduction were compared using paired t-test. The 'vegan' package in Rstudio was used to calculate both Simpson and Shannon's index[19].

Statistical analysis: We fitted two weighted multinomial logistic regression model for each vaccine-introducing country to examine differences in circulating genotypes before and after vaccination among countries with vaccine introduction and trend over the surveillance year. For both models, G1P[8], G2P[4], G4P[8], G12P[8], G3P[8], G9P[8], mixed type, non-typeable and Other category were used as outcome variables, with G1P[8] used as the reference category.

A strain that is not one of the six main genotypes, mixed or non-typeable, was classified as "other" in the outcome variable. Year since vaccine introduction was calculated by subtracting each surveillance year by the vaccine introduction year. The first model was fitted with vaccine introduction status and the year since the vaccine was introduced. The model was first run as a univariable analysis (crude), including only the vaccine introduction status as a binary variable (pre and post). The adjusted model was then run as we included vaccine introduction status and the year since vaccine introduction as covariates. Only data from vaccine-introducing countries was used, with one “overall” model where all countries’ data were pooled together and then separate models by country.

The second model was applied to both vaccine-introducing and non-introducing nations, with genotype serving as an outcome variable and surveillance year (calendar year) serving as the exposure, with just a few years of surveillance data selected. For the second model, the exposure only involves 7 consecutive year intervals rather than using all recorded calendar years from each country. Depending on data availability, 3 or 4 surveillance years of data were selected from both pre and post vaccination era for vaccine-introducing nations. Data from 2009 to 2015 were picked for non-introducing nations. Model were applied to all vaccine-introducing country and non-introducing countries.

RESULTS

Through electronic search, a total of 5,704 research articles were identified. After reviewing the title and abstracts as initial screening, 67 duplicated articles were excluded, and 4,257 were excluded due to irreverent purpose. The remaining 1380 articles were included for full-text review. 974 were excluded during the full-text review stage due to (Figure 1): having no rotavirus genotyping data reported, unrelated to review’s purpose, did not meet data extraction

requirement, articles have sample recorded from greater than five years old or with less than 80% of samples being under five years old, duplicated data, not from primary data, data reported from hospital acquired infection and data acquired exclusively before 2000.

The remaining 406 articles fulfilled the inclusion criteria for systematic review. An additional 149 papers were excluded for this analysis because they did not report genotypes by calendar year.

The final overall analysis examined 257 articles published between 2006 and 2019 that reported rotavirus genotype distribution in a one-year interval. 86 countries were represented. A total of 83,017 genotyped, rotavirus-positive stool samples were included, including mixed and non-typeable. The reported number of cases and samples typed varied across studies (positive cases: median: 117; range: 9 – 267,415 per study; typed strain: median: 114, range: 9 – 157,762 per study). A specific number of reported G-P combination genotype numbers for all included countries and selected country for country level analysis are presented in Table 2 and Table 3. Five vaccine introducing countries included 22 articles from Ethiopia (4 articles), Italy (7 articles), Kenya (9 articles), South Africa (5 articles), Zimbabwe (1 article). Five non-introducing countries include 33 articles from Argentina (4 articles), China (21 articles), Myanmar (3 articles), Nepal (5 articles), Ukraine (1 article).

Rotavirus strain prevalence and diversity: Vaccine Introduction Countries

The G1P[8] genotype was the most frequent worldwide strain (Table 1). Before vaccine introduction, G1P[8] was dominant in almost all vaccine-introducing countries except for Ethiopia and Zimbabwe. (Table 2). Ethiopia (18.1% to 6.3%, $p<0.001$), Italy (43.7% to 26%, $p<0.001$) and South Africa (27.6% to 20%, $p<0.001$) had all shown a decrease of G1P[8] in the

post vaccine era compared to pre-vaccine. G2P[4] has seen an increase in Ethiopia (8.5% - 11.5%, p=0.42), Kenya (1.5%-19.4%, p<0.001), South Africa (10.8% to 22.1%, p<0.001) and Zimbabwe (11.5% to 12.2%, p=0.84). Some other uncommon genotypes had shown a decrease or minor increase in all vaccine introducing countries, except for Zimbabwe (20.8% to 25.9%).

The overall distribution of strains varied significantly across each surveillance year in vaccine-introducing countries (Figure 2). Between 2002 -2004, the proportion of detected G1P[8] strain in Kenya decreased from 50.6% (90/178) to 30.0% (63/211), and since 2010, the G1P[8] proportion dropped from 25.1% (46/183) to 8.1% (26/321) in 2012. Then a sharp increase leads the proportion of G1P[8] reach 61.7% (137/222) just one year following vaccine introduction in 2014. Since then, G1P[8] has declined from 44.4% (24/54) in 2015 to 19.7% (14/71) in 2016 then to 2.8% (1/36) in 2019. The prevalence of G2P[4] has increased in Kenya since vaccine introduction, with only 1.5% (25/1713) detected in the pre-vaccine period to 19.4% (44/227) in the post-vaccine. G2P[4] was the dominant genotype in 2016, one year after vaccine introduction, with 57.7% (41/71).

In Ethiopia, the proportion G2P[4] fluctuated frequently in both pre- and post-vaccine periods. 17.3% (9/52) was detected in 2008 and it dropped to 8.6% (7/81) in 2012. In the post-vaccine era, the proportion increased from 6.8% in 2015, two years after introduction, to 18.9% in 2016 then dropped to 5.3% in 2017. Such variability of G2P[4] was also observed in South Africa. The proportion was low in both 2004 (6.1%, 13/214) and 2006 (2.6%, 5/191). Since vaccine introduction in South Africa in 2009, the proportion of G2P[4] has maintained high, often higher than 20% in each surveillance year among all other genotypes.

Rotavirus strain prevalence and diversity: Vaccine Non-Introducing Countries

The overall distribution of genotype varies across non-introducing country (Table 3). The change in distribution of genotype over each calendar year indicates that (Figure 3), China has seen a continue and steady decline of G1P[8] strain, from 61.3% (185/302) in 2008 to 2.4% (9/381) in 2017. Nepal showed an opposite trend compared to China in terms of the distribution of G1P[8] genotype. Since 2009, Nepal experienced a steady increase of G1P[8], from 2.7% (12/442) detected in 2009 to 27.3% (15/55) in 2015.

At the same time, increase of G2P[4] has been observed in Argentina, from 7.1% (3/42) and 7.7% (1/13) in 2005 and 2006 to 33% (137/415) in 2012 and 42.9% (224/522) in 2013, Myanmar (1.9% in 2009 to 40% in 2013), China (6.6% in 2008 to 18.2% in 2016) and Ukraine (14.9% in 2008 to 22.1% in 2015). Also, an upward trend of G9P[8] strain was also detected during the same period, increasing from 6% (18/302) in 2008 and 5.5% (36/653) in 2009 to 56.9% (203/357) in 2016 and 82.2% (313/381) in 2017. Similar trend was observed in Myanmar where G9P[8] was not often detected but contributed a high proportion later. Only 0.34% (1/291) was detected in 2011 and later increased to 54% (27/50) in 2015.

Genotype Diversity Index and vaccination introduction status

For the country specific analysis in the vaccine introducing countries, Ethiopia has the highest average Simpson Index and Shannon Index across all surveillance years (H'=2.00; D=0.85). When compared between pre- and post-vaccination time (Table 4), Ethiopia, Italy, and Zimbabwe were shown to have a higher Shannon's index and Simpson's Index in the post vaccination time. When comparing the temporal relationship between genotype diversity and the year since vaccination was introduced (Figure 4), both the Simpson's index and Shannon's index

displayed fluctuation between each year since the vaccine introduction. Among the five countries in the country-specific analysis, only Ethiopia had shown an upward trend in both indexes after the vaccination introduction.

For non-introduction countries, China experienced an increase in both indexes from 2008 to 2012 then a graduate decline until 2017. On the contrary, Nepal had seen a gradual increase in both indexes from 2009 till 2015.

Multinomial Logistic Regression

For all vaccine-introducing countries overall, comparing post- to pre-vaccine era (Figure 5), G12P[8] (adjusted multinomial odds ratio (aMOR)= 2.65; 95% CI: 2.49–2.82), G2P[4] (aMOR= 2.93; 95% CI: 2.79-3.07), G3P[8] (aMOR= 3.81; 95% CI: 3.58–4.06) and G9P[8] (aMOR= 1.11; 95% CI: 1.05-1.17) were more likely to be the infecting genotype relative to G1P[8], after adjusting for years since introduction. Infections caused by G4P[8] (aMOR= 0.30; 95% CI: 0.29–0.3), mixed genotype (aMOR= 0.6; 95% CI: 0.56–0.63) un-typable strain (aMOR= 0.79; 95% CI: 0.74–0.84) and other uncommon genotype strain (aMOR= 0.79; 95% CI: 0.75–0.83), were less common in the post-vaccination era as compared to pre-vaccination time.

The distribution of rotavirus genotypes differed according to the country for the specific country-level analysis. The trend of certain genotypes between the two eras differs by country. Three of the five selected countries had seen an increase in the G2P[4] strain in the post-vaccination era as compared to pre-vaccination while adjusting for year since vaccine introduction, with the aMOR observed in Ethiopia (aMOR= 21.38; 95% CI: 16.72-27.32,

p<0.001), Italy (aMOR= 8.48; 95% CI: 7.06-11.00, p<0.001) and Kenya (aMOR= 5.41; 95% CI: 5.08-5.76, p<0.001)

For the multinomial regression model including only surveillance year, the odds of infection caused by G2P[4] strain increased in South Africa (aMOR=2.47, 95% CI 2.473-2.476, p<0.001), Kenya (aMOR= 4.24, 95% CI 4.241-4.249, p<0.001) relative to G1P[8] as surveillance year increased by one year. For non-introduction counties, both China (aMOR=1.46, 95% CI 1.458-1.476, p<0.001) and Ukraine (aMOR=1.93, 95% CI 1.934-1.949, p<0.001) has seen an increase in the later year of surveillance period, but relatively lower compared to vaccine-introducing countries.

DISCUSSION

In our systematic review and meta-analysis, we found some shifts in the distribution and diversity of rotavirus genotypes in the vaccine introducing countries following the introduction of the rotavirus vaccine. However, the changes in the overall diversity and most detected strains did not differ significantly between vaccine-introducing countries and non-introducing countries. A decrease in G1P[8] strain proportion and an increase in G2P[4] strain proportion has been a common trend observed among some vaccine introducing and non-introducing countries over the surveillance period.

Prior to the introduction of rotavirus vaccinations, circulating strains differed across all vaccine introducing countries and non-introducing countries over a particular time, and the predominant stain remained constant for several years until the vaccine was introduced. Some countries have seen a high predominance of G1P[8] strain prior introduction and seen a decline after vaccine introduction. These findings are consistent with the findings of prior research[20-

23], which have shown that the live-attenuated monovalent Rotarix vaccine, which all five vaccine introducing countries in this study adopted, provides considerable protection against this completely homotypic strain [9, 24, 25].

Following the introduction of the rotavirus vaccine, genotype surveillance data revealed that there were temporal changes in both countries with and without rotavirus vaccination programs, illustrating annual fluctuations of rotavirus strains within countries after vaccine introduction. G2P[4] and other major genotypes have emerged as the most prevalent circulating rotavirus genotypes in the post-vaccination period. The predominance of G2P[4] field strains has been documented in some vaccine introducing countries[26]. However, this has also been observed in countries that have not yet implemented the rotavirus vaccine in the national immunization program, such as Argentina, China, South Korea[27], Bangladesh[28], Paraguay[29] and Honduras[30]. A study done by Esteban et.al[31] focused on genotype distribution in Latin America countries suggested that such observation may be due to a geographical phenomenon, or a cyclic pattern of rotavirus strains rather than the selection pressure caused by the introduction of rotavirus monovalent vaccination. The study revealed that even in countries where Rotarix vaccine has not been introduced, G2 strains have become more frequently detected in Argentina (2004 to 2007) and it showed a similar fluctuating pattern in an earlier surveillance study done in the same city in Argentina (1996 to 1998) and Brazil, with prevalence of 34% in Rio de Janeiro [32] and 43% in Buenos Aires [33] and a similar reemergence of G2 strains in both countries. Interestingly, such observation was also observed in the neighboring country Paraguay[29]. Moreover, according to VP7 gene sequence analysis conducted in Vizzi et.al [34], the rotavirus from Caracas, Venezuela was more closely related to global strains of the G2-II lineage identified in the earlier time from another Venezuelan city

before the vaccine period, which did not indicate an evolutionary change of rotavirus isolated between pre and post vaccine period. This finding indicate insufficient evidence proving that the change in genotype distribution and emergence of new genotype resulted from vaccine introduction.

The results from multinomial logistic regression using surveillance year as exposure indicated greater odds of G2P[4] infection relative to G1P[8] over the surveillance period among countries with vaccine programs (South Africa and Kenya) than non-introducing countries (China and Ukraine). Such observation is the result of reduction in the number of G1 genotypes with respect to the same or increased number of G2P[4] strains of each corresponding period. The drop in G1[8] has been observed in many vaccine-introducing countries in the study. However, such reduction of G1P[8] was also observed in countries like China and Ukraine. The logistic regression results indicated that countries with vaccine introduction, experience a greater disparity between G1P[8] and G2P[4] genotype over time. Despite having no vaccine introduced into national immunization programs, countries like China and Ukraine still made it available in the private sector[35, 36]. However, due to the lack of data on exact vaccine coverage among these non-introducing countries, it makes it difficult to conclude whether such changes in genotype distribution have been influenced by the vaccine introduction in the private market, nor can this be completely ruled out.

There are several limitations that need to be considered. First, there were only 10 countries with annualized genotype distribution data included in country specific analysis. Other nations have only had limited and discontinuous records of genotype diversity. Because the evolutionary dynamic of rotavirus reflects a continuously changing condition in year-to-year fluctuations, it is critical to conduct such analysis that includes a larger number of countries.

Despite our efforts to minimize the issue of country representation in both vaccine introducing and non-introducing countries, we still encountered an unbalanced situation in which the majority of vaccine introducing countries in our study are African countries and the majority of non-introducing countries are Asian countries. Many of the countries between introducing and non-introducing groups could be fundamentally different in overall vaccine roll-out strategy and fundamental genotype distributions, which could both determine the overall impact of vaccination. Known geographic differences in genotype distributions [15, 37, 38] precluded our ability to compare the actual outcomes of genotype distribution with those that would have been expected if the vaccination had not been introduced. In another way, the countries used in both vaccine introducing and non-introducing group, might not be the most appropriate counterfactual to each other, due to a number of uncontrollable factors. Furthermore, the research did not account for the possible variability of certain vaccination implementation strategies. Such heterogeneity in vaccine implementation strategy within the same country was not uncommon; some countries implemented vaccine programs on a different timeline (regional & global), and some introduced different vaccine brands for each region, which may result in different genotype distribution across regions due to different vaccine coverage and immunity composition. Italy was regarded as a vaccine-introducing nation, yet the rotavirus vaccine was only accessible in the Sicily area in 2013[39], four years before it was formally included in the country's universal immunization program. Due to this regional introduction strategy, Italy has a distinct vaccination coverage pattern, which may be a factor in the year-to-year variation in genotype distribution. Moreover, although vaccine was introduced universally in countries like Kenya, coverage of rotavirus vaccination still varies between socio-economically different sub-counties[40]. A similar finding of the impact of different vaccine introduction in the same country was also

reported in Australia. Study done by Roczo-Farkas et.al[15] reported the post-vaccination monitoring data from Australia revealed that the different genotype evolution pattern was happening as a consequence of selection pressure from the different vaccination program Australia observed. This result in the predominance of G12P[8] strains in RotaTeq-implementing states and a predominance of G2P[4] and equine-like G3P[8] genotypes in Rotarix-implementing states, which were hypothesized as due to the antigenic differences between the rotavirus vaccines and circulating strains in the VP4 and VP7 antigenic region. For the purpose of future studies, apart from more comprehensive global genotype distribution, analysis focusing on regional genotype distribution is also needed to better understand the potential impact of mass rotavirus immunization strategy on the genotype dynamic, while also removing potential heterogeneity introduced by a diverse vaccination roll-out plan.

In conclusion, a significant decline in G1P[8] genotype proportion and an increase in G2P[4] genotype proportion was identified across numerous vaccines introducing and non-introducing countries during the monitoring period. However, there is insufficient data to establish a causal relationship between temporal changes in rotavirus genotype strains and the vaccination program. While it is true that a shift in strain prevalence as a result of vaccine use cannot be completely ruled out, the natural cycling of genotypes, potential interspecies reassortment[25, 41, 42], and regional differences in strain distributions are likely to make attribution of short-term changes in genotype patterns and the degree of such changes from vaccination particularly difficult to ascertain. Even though it is surprising to see that there has been no obvious temporal pattern of persistent vaccine-related shift in genotype where mass immunization has occurred, it is critical to continue strain monitoring in order to detect any changes in strain prevalence associated with increased vaccine usage around the world.

TABLES AND FIGURES

Table 1. Overall Rotavirus genotype distribution (G and P types)

	Overall	
Genotype	**n**	**%**
G1P[8]	25872	31.2
G2P[4]	10780	13.0
G3P[8]	7839	9.4
G4P[8]	3457	4.2
G9P[8]	12144	14.6
G12P[8]	4310	5.2
Mixed	3974	4.8
Non-Typeable	4381	5.3
Others	10260	12.4
Total	83017	100

a. 'Other' refers to genotypes that are uncommon and contributed less than 5 percent of total samples from studies.
b. Mixed: more than one genotype found in an individual sample.

Table 2. Rotavirus genotype distribution (G and P types) in vaccine introducing countries

	Ethiopia (%)		Italy(%)		Kenya(%)		South Africa(%)		Zimbabwe(%)	
Genotype	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
G1P[8]	34 (18.1)	12 (6.3)	1496 (43.7)	109 (26)	556 (32.5)	76 (33.4)	204 (27.6)	422 (20.0)	19 (6.1)	349 (32.9)
G2P[4]	16 (8.5)	22 (11.5)	287 (8.4)	16 (3.8)	25 (1.5)	44 (19.4)	80 (10.8)	466 (22.1)	36 (11.5)	129 (12.2)
G3P[8]	2 (1.1)	23 (12.0)	123 (3.6)	17 (4.1)	14 (0.8)	50 (22.0)	141 (19.2)	58 (2.8)	0 (0)	0 (0)
G4P[8]	0 (0)	0 (0)	353 (10.3)	46 (11.0)	22 (1.3)	1 (0.4)	0 (0)	1 (0.004)	0 (0)	0 (0)
G9P[8]	7 (3.7)	25 (13.1)	739 (21.6)	58 (13.8)	255 (14.9)	0 (0)	21 (2.8)	276 (13.1)	67 (21.5)	127 (12.0)
G12P[8]	46 (24.5)	19 (10.0)	2 (0.05)	125 (29.8)	2 (0.01)	0 (0)	0 (0)	384 (18.2)	5 (1.6)	6 (0.6)
Mixed	29 (15.4)	18 (9.4)	257 (7.5)	6 (1.4)	154 (9.0)	4 (1.8)	57 (7.7)	48 (2.3)	63 (20.2)	111 (10.5)
Non-Typeable	14 (7.4)	31 (16.2)	80 (2.3)	36 (8.6)	260 (15.1)	23 (10.1)	50 (6.8)	13 (0.6)	57 (18.3)	63 (5.9)
Others	40 (21.3)	41 (21.5)	80 (2.3)	6 (1.4)	425 (24.8)	29 (12.8)	185 (25.1)	437 (20.1)	65 (20.8)	275 (25.9)
Total	188	191	3417	419	1713	227	738	2105	312	1060

a. 'Other' refers to genotypes that are uncommon and contributed less than 5 percent of samples from studies.
b. Mixed: more than one genotype found in an individual sample.

Table 3. Rotavirus genotype distribution (G and P types) in vaccine non-introducing countries

	Argentina		China		Myanmar		Nepal		Ukraine	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
G1P[8]	640	26.0	1506	16.8	173	14.8	304	14.4	173	19.3
G2P[4]	645	27.2	671	7.5	125	10.7	229	10.8	111	12.4
G3P[8]	194	8.2	1351	15.1	28	2.4	1	0.1	87	9.7
G4P[8]	75	3.2	15	0.2	0	0	0	0	460	51.2
G9P[8]	209	8.8	3639	40.7	135	11.6	35	1.7	28	3.1
G12P[8]	394	16.6	1	0.01	413	35.4	59	2.8	5	0.6
Mixed	59	2.5	719	8.0	59	5.1	265	12.5	4	0.5
Non-Typeable	52	2.2	259	2.9	100	8.6	234	11.1	0	0
Others	106	4.5	783	8.8	133	11.4	986	46.7	30	3.34
Total	2374	100	8944	100	1166	100	2113	100	898	100

a. 'Other' refers to genotypes that are uncommon and contributed less than 5 percent of samples from studies.
b. Mixed: more than one genotype found in an individual sample.

Table 4. Rotavirus Genotype Diversity by Pre- and Postvaccine Periods

	Simpson's Index		Shannon's Index	
Country	**Pre-Vaccine**	**Post-Vaccine**	**Pre-Vaccine**	**Post-Vaccine**
Overall	0.68	0.62	1.49	1.39
Italy	0.65	0.71	1.39	1.50
Ethiopia	0.82	0.85	1.89	2.00
Kenya	0.77	0.53	1.88	1.21
South Africa	0.76	0.75	1.85	1.74
Zimbabwe	0.76	0.76	1.79	1.80

Changes in the Shannon diversity index and the Simpson diversity index were evaluated before and after the introduction of the vaccination vaccine for five vaccine introducing countries and overall distribution that include all studies.

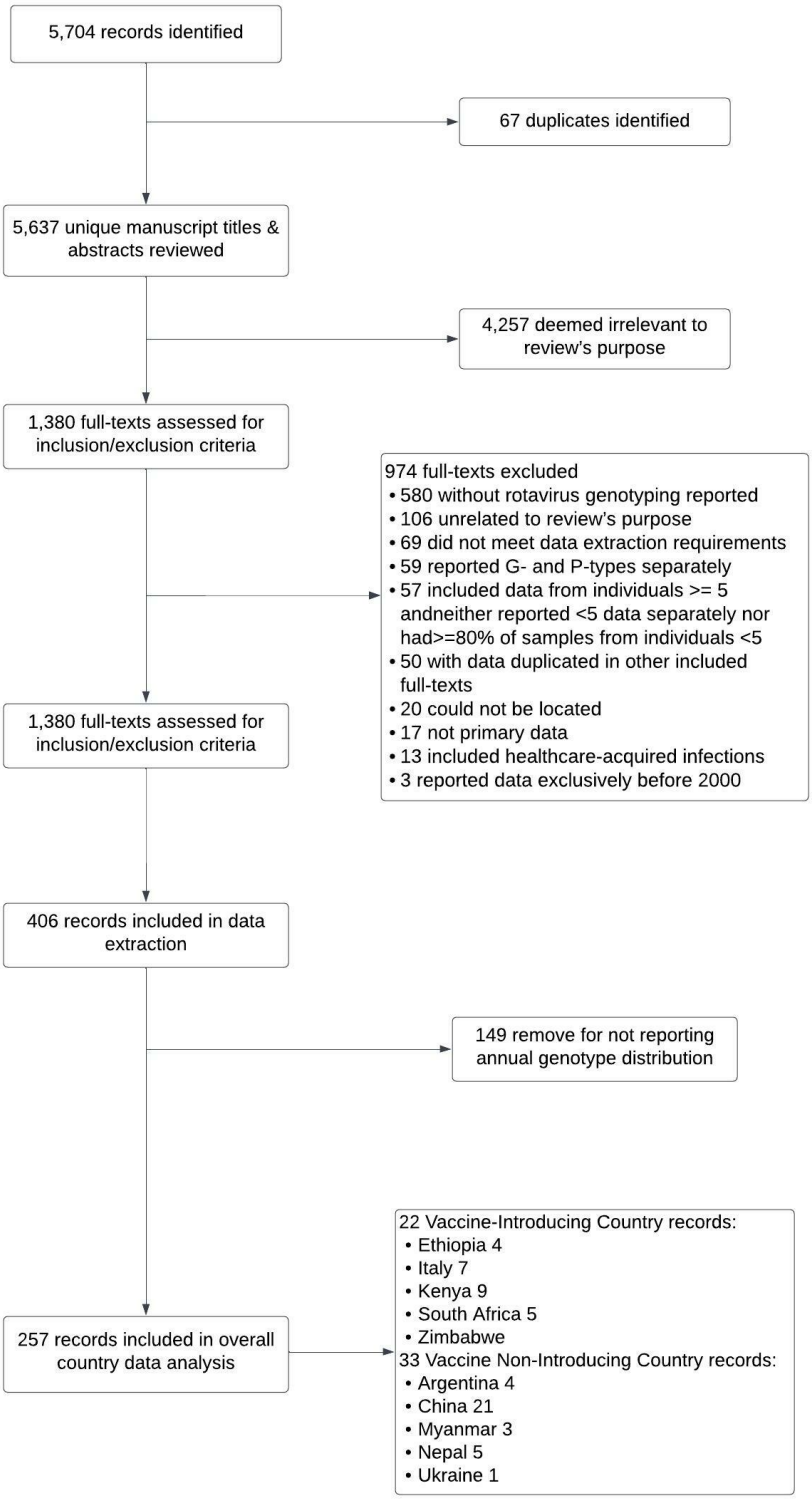

Figure 1. PRISMA flow chart: Flow chart for the systematic review to evaluate the rotavirus distribution between vaccine introducing and non-introducing countries.

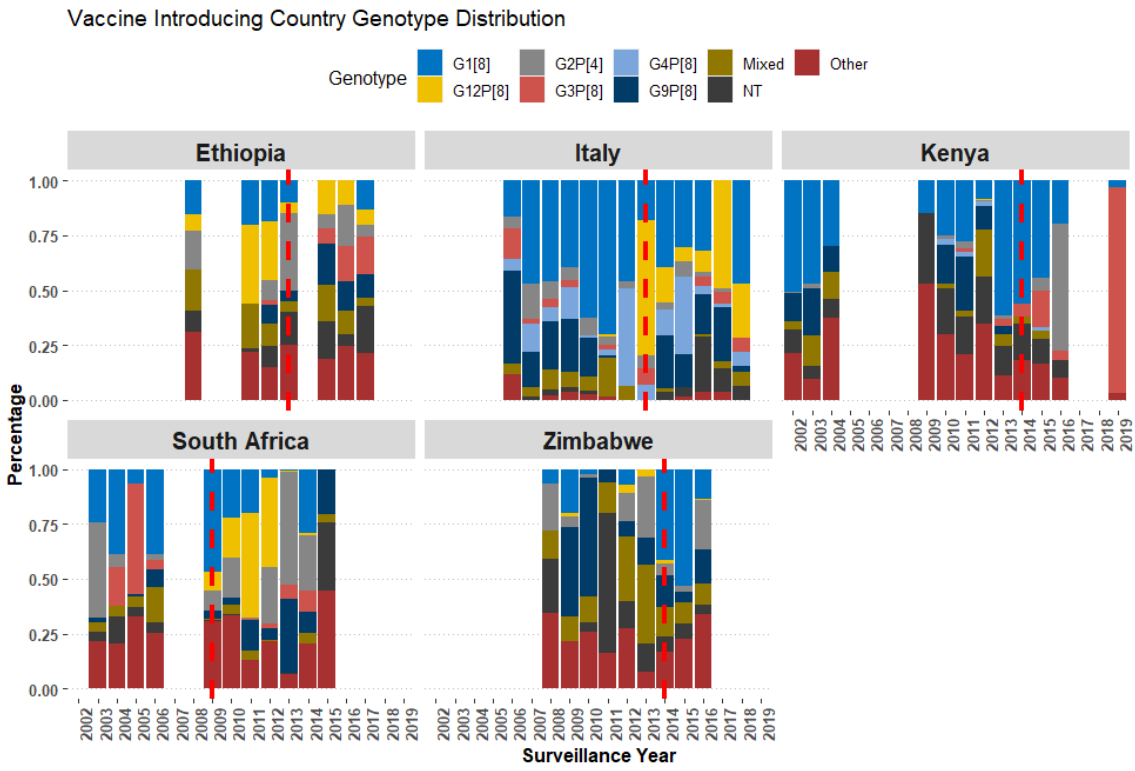

Figure 2. Vaccine Introducing Countries Genotype Distribution by Surveillance year, the red line indicate vaccine introducing year

a. Red dotted line indicates vaccine introduction year.
b. 'Other' refers to genotypes that are uncommon and contributed less than 5 percent of samples from studies.
c. Mixed: more than one genotype found in an individual sample.

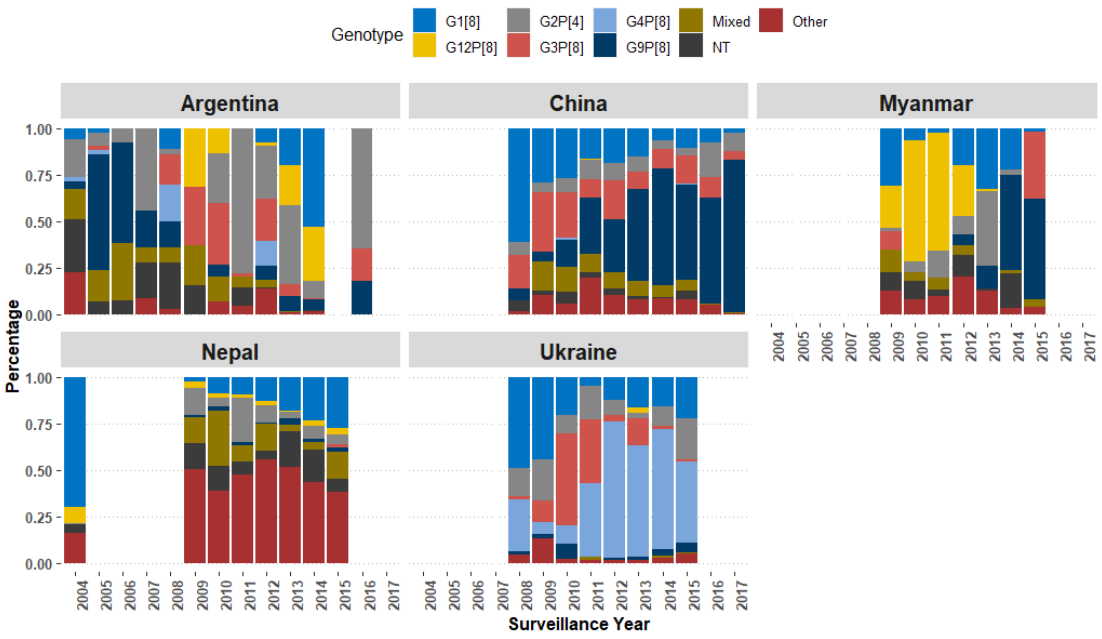

Figure 3. Vaccine Non-Introducing Countries Genotype Distribution by Surveillance Year

a. Genotype proportions of typed rotavirus cases by surveillance year and stratified by vaccine non-introducing countries.
b. 'Other' refers to genotypes that are uncommon and contributed less than 5 percent of samples from studies.
c. Mixed: more than one genotype found in an individual sample.

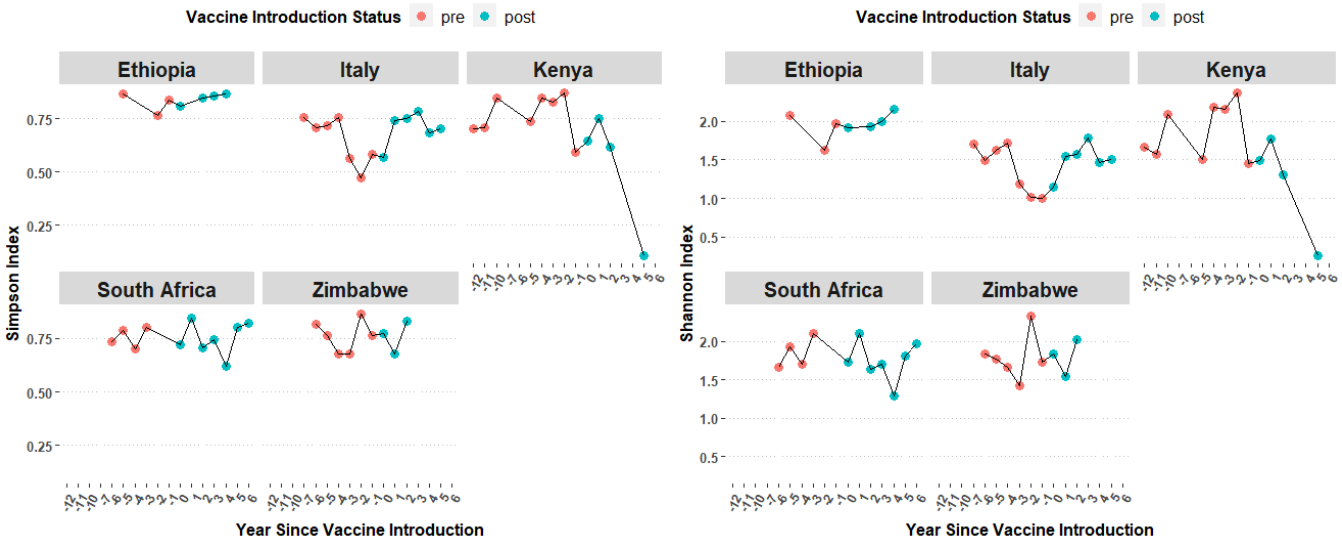

Figure 4. Simpson and Shannon Index of Vaccine Introducing Countries

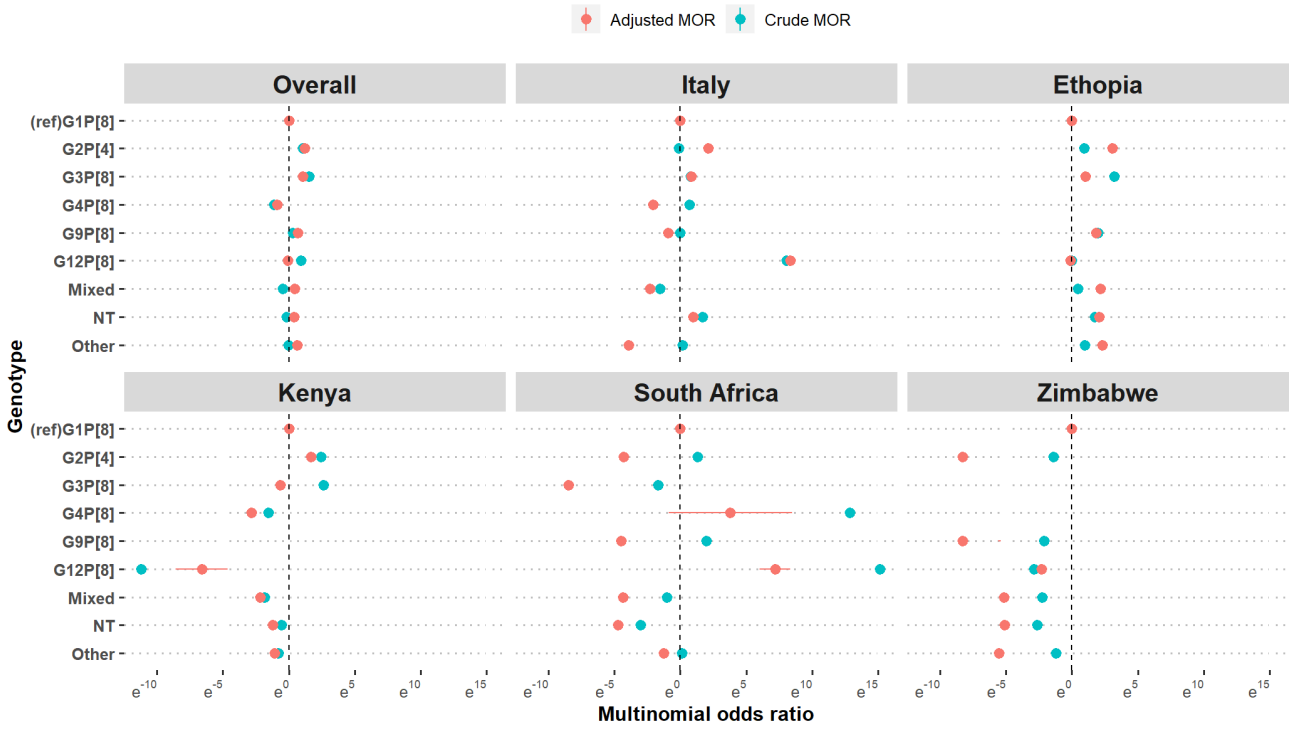

Figure 5. Multinomial Logistic Regression Output.

MORs for vaccination period incidence were computed using weighted multinomial logistic regression with the outcome variable genotype (G1P[8] as the reference group). Models were also adjusted for years since vaccine introduction (range from -12 to 12 years).

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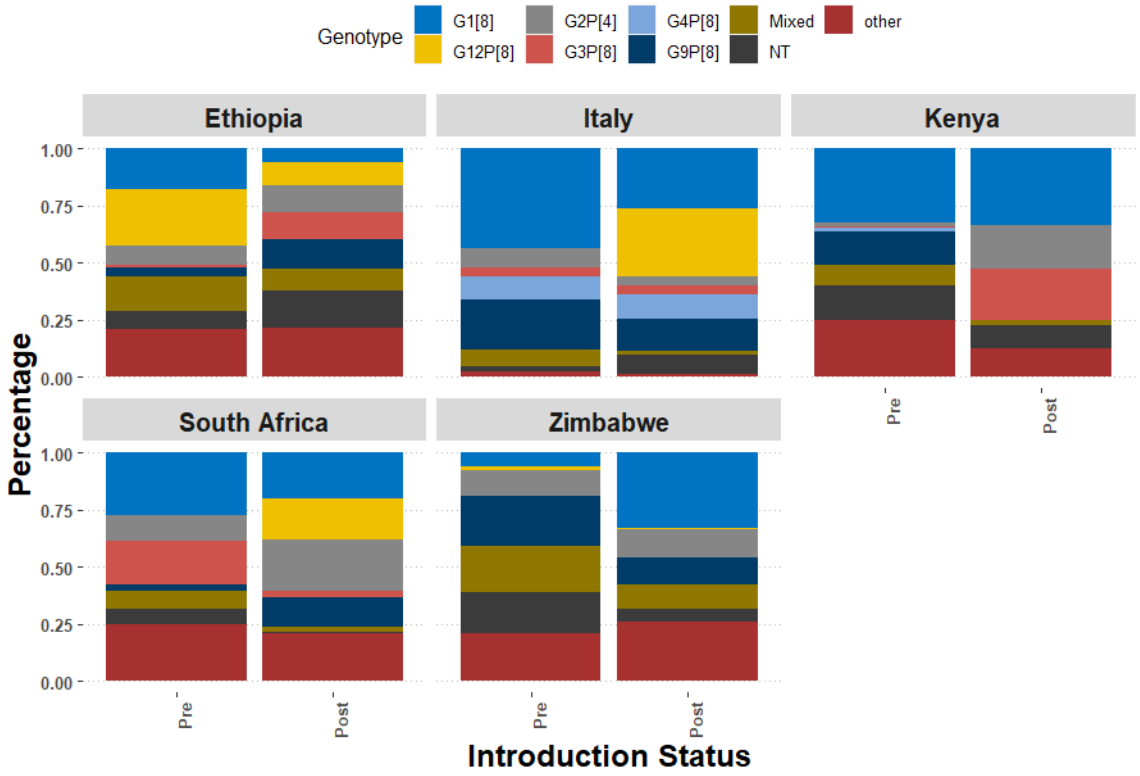
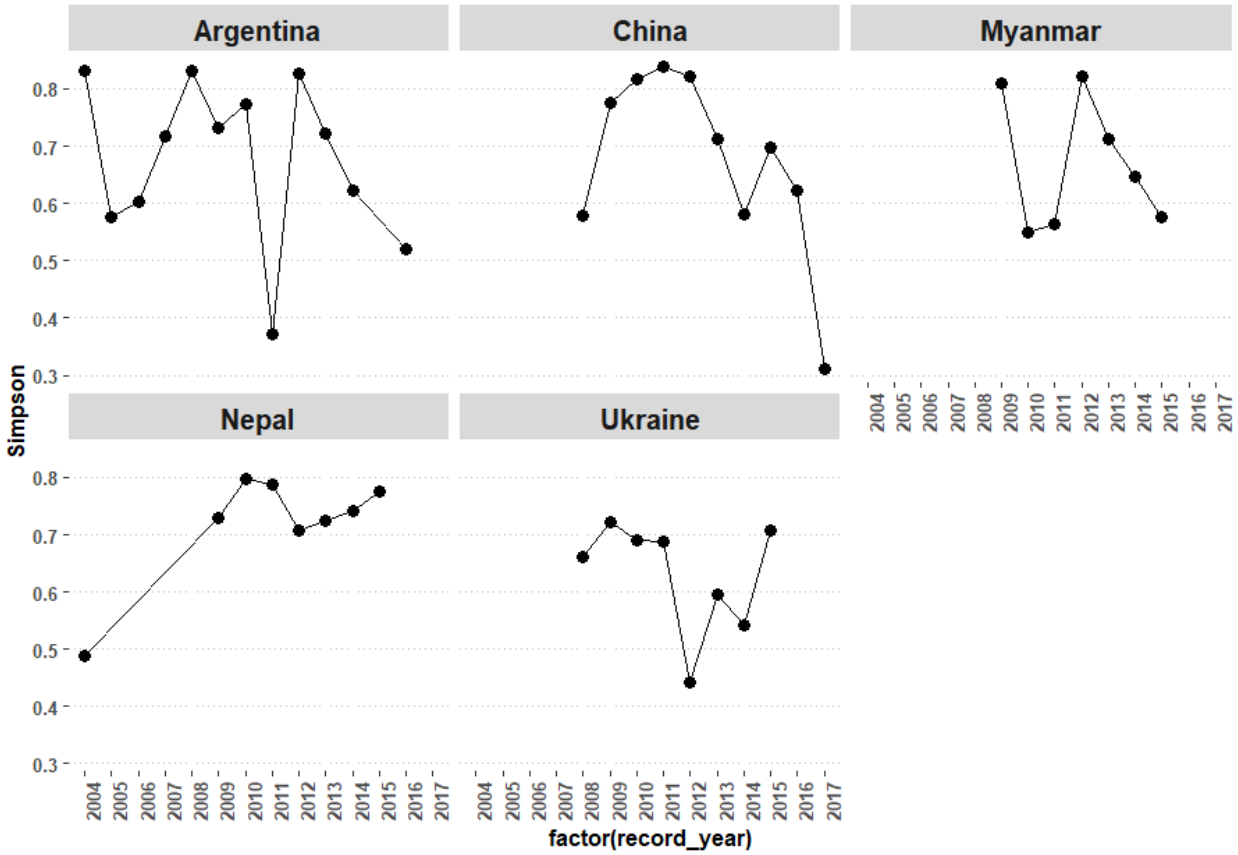
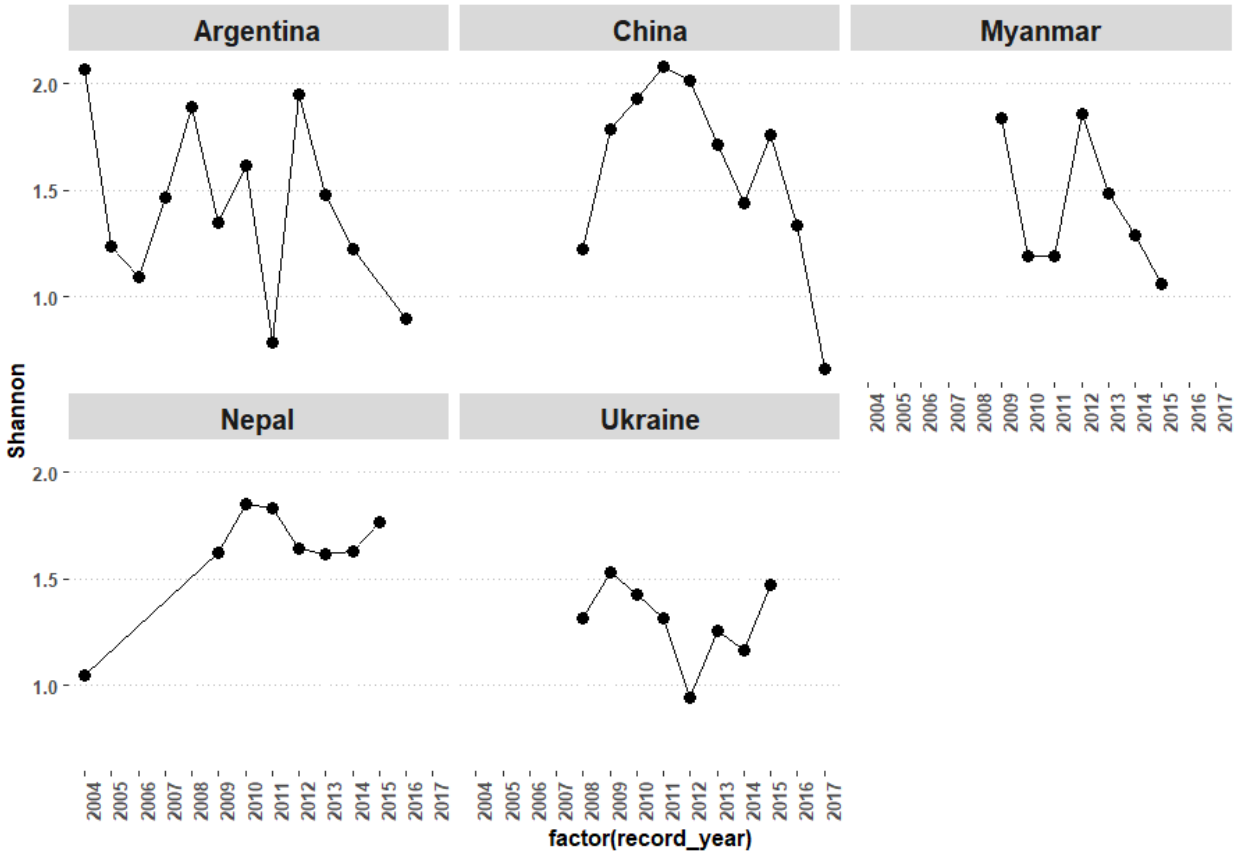
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Genotype distribution between pre and post vaccine introduction



Supplementary Figure 2. Simpson diversity index of vaccine non-introducing countries across surveillance year



Supplementary Figure 3. Shannon diversity index of vaccine non-introducing countries across surveillance year