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Determining effectiveness of rotavirus vaccine by immunochromatography and reverse transcriptase polymerase chain reaction: A comparison

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ABSTRACT

Introduction: Because of the large animal reservoirs and reassortment capacity of rotaviruses (RVs) that pose the possibilities of waning the effectiveness of RV-vaccines, it remains essential to monitor vaccine effectiveness (VE) regularly. Although reverse transcription polymerase chain reaction (RT-PCR) remains sensitive for RV detection, physicians, especially in Japan, frequently use immunochromatography (IC)-based kits for RV diagnosis. Recently, IC is being used to calculate VE also. Herein, we investigated the validity of VEs determined by IC compared to that by RT-PCR during an outbreak in Shizuoka Prefecture, Japan.
Methods: RVs in the stool or rectal swabs from children with acute gastroenteritis (AGE) were tested first by IC in the clinic and then by RT-PCR in the laboratory. A test-negative study design was used to examine VE.
Results: Although the specificity of IC assay revealed 100%, its sensitivity remained weaker (67%) than that of RT-PCR that increased up to 88% depending on disease severity. VE assessed by IC remained stronger than that by RT-PCR: 79% (95% CI: 39–93%) by IC, and 58% (95% CI: −20% to 90%) by RT-PCR. However, VEs by IC and RT-PCR appeared almost similar in higher disease severity: 81.5% (95% CI: 40–94%) by IC and 72% (95% CI: 7–92%) by RT-PCR at severity ≥7, while 97.5% (95% CI: 77–99.7%) by IC and 92% (95% CI: 58–98%) by RT-PCR at severity ≥11. We showed that RV-vaccinated children had 80% [OR = 0.192 (95% CI: 0.052–0.709) less chance to be detected by IC.
Conclusion: Although the sensitivity and specificity of IC differ by brand type, generally, IC is not as sensitive as RT-PCR. Despite the VEs remain higher by IC, it looks comparable with that of RT-PCR in severe cases implying that VEs evaluated by IC against severe illness remain useful for VE-monitoring.

1. Introduction

Rotavirus (RV) may cause severe acute gastroenteritis (AGE) and even death mainly among children under 5 years of age [1]. Therefore, RV remains an important pathogen for both clinicians and researchers. To prevent severe RV-illness, two live attenuated oral RV-vaccines, Rotarix (GlaxoSmithKine, Rixensart, Belgium) and RotaTeq (Merck & Co., Whitehouse Station, NJ, USA) were introduced in many countries since 2006 [2]. Rotarix is a monovalent type vaccine that contains an attenuated G1P[8] human strain (89–12) while RotaTeq is a pentavalent type vaccine that bears G1, G2, G3, G4, and P1A[8] human-bovine (WC3) reassortant. Use of these vaccines provides homotypic and heterotypic protections

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against severe RV diarrhea and has been important in reducing RV deaths, globally, among children <5 years old almost by half: from 453,000 in 2008 to 215,000 in 2013 [3–6]. However, outbreaks with RV still remain common in both developing and developed countries [7]. It is because these RV-vaccines are found more effective against developing severe RV-infection rather than protecting from milder ones [8]. In addition, segmented genomic structure and reassortment capabilities of RV always pose considerable threats towards waning vaccine effectiveness (VE) [8]. Continuous monitoring of strain variations and VE is, therefore, crucial for understanding whether the vaccines do also provide enough protection against rare or emerging strains.

Currently, a number of enzyme-linked immunosorbent assay (ELISA)- and immunochromatographic (IC)-based rapid RV-diagnosis kits are commercially available [9]. Of these, the use of IC-based kits is gradually increasing since it is handy, rapid, easy-to-perform and cost-effective and yields high sensitivity and specificity [10,11]. These IC kits usually use colloidal gold-labeled monoclonal antibodies against the capsid protein of gene 6 (VP6) of RVs to detect RV antigens selectively from human feces [12]. Such handy, quick and cost-effective diagnostic kits are useful in clinics and in some settings like refugee camps for timely diagnosis of severe RV infections.

Recently, IC assay has been shown to determine the effectiveness of RV-vaccine in several studies [13–16]. However, the validity of IC assay in determining VE is not yet confirmed. Definitely, utilization of IC kit for assessing VE could be the most useful choice for regular monitoring of VE against RV infections. Therefore, in this study, we intended to examine VEs by IC assay as well as reverse transcription polymerase chain reaction (RT-PCR), and compared these to determine the accuracy of IC assay to determine VE.

2. Materials and methods

2.1. Study sample

This study has included children that we reported earlier [8] suffering from AGE during an outbreak of G8P[8] RV strain between February-July 2017, in Shizuoka Prefecture, Japan. The disease severity was clinically evaluated by a professional pediatrician using a Vesikari scale based on 20 points, in which a score of <7 was regarded as mild, 7–10 as moderate, and ≥11 as severe AGE [17]. The study was approved by the ethical committees of Nihon University School of Medicine (25–13–0).

2.2. Diagnosis by IC assay and RT-PCR:

A flow diagram shows the enrollment of children in different stages of the study (Fig. 1). A dual kit of Noro-Rotavirus (IP-Line Duo Noro-Rota immunochromatographic test kit, ImmunoProbe Co., Ltd, Saitama, Japan) was used to detect the infection at the study site in the clinic. The samples were then frozen and transferred to the laboratory of Department of Pathology and Microbiology, Nihon University, where it was processed for viral RNA extraction, cDNA preparation, and PCR analysis as described earlier [8].

2.3. Sensitivity and specificity determination:

Sensitivity and specificity with 95% confidence interval (95% CI) were determined using Stata: version 12. Primarily, sensitivity and specificity of IC assays were examined in all 80 AGE children. Later, these were evaluated independently in vaccinated and unvaccinated population in 65 children aged ≤5 years. Finally, the efficiency of IC kit to detect RV in vaccinated and unvaccinated

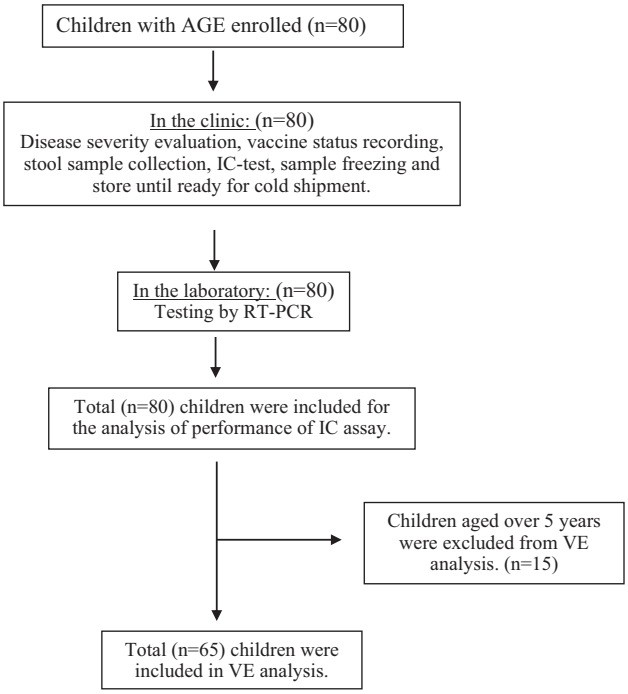

Fig. 1. Flow diagram of children enrolled in different stages of the study.

populations was judged in all 45 RT-PCR confirmed RV-positive children aged ≤5 years.

2.4. VE determination:

The vaccination history (vaccination date, lot number) of a child was confirmed from the record of 'Maternal and Child Health Handbook' which is provided to each mother in Japan during pregnancy. A test-negative study design was used to investigate VE as described previously [18]. VE was calculated as VE = [1-adjusted Odds ratio (OR)] × 100. OR, adjusted for age and sex, was analyzed by logistic regression using SPSS: version 16.

3. Results

3.1. Sensitivity and specificity of IC assay

Stool samples of 80 children suffering from AGE were investigated for RV infection using IC assay and RT-PCR. Of 58 RT-PCR-positive samples, 39 were identified as positive by IC yielding a sensitivity of 67% (95% CI: 55–79%) (Table 1). When the accuracy of IC assay was examined in moderate and severe patients, 34 out of 43 RT-PCR-positive children with moderate or severe infections (severity score ≥7) were diagnosed correctly by IC test yielding a sensitivity of 79% (95% CI: 67–91%) at severity score ≥7 (Table 1). Finally, 22 of 25 RT-PCR-positive severe children were diagnosed accurately by IC kit yielding a sensitivity of 88% (95% CI: 75–100%) at severity score ≥11 (Table 1). These evidence an improved sensitivity of IC assay with increasing disease severity. There was no false-positive result by IC test indicating a specificity of 100% at any stage of severity.

3.2. Vaccine effectiveness (VE)

Among 65 children with AGE under 5 years of age, 36 were vaccinated: 32 received Rotarix while 4 received RotaTeq. All of them completed their full doses (either 2 doses for Rotarix or 3 doses for

Table 1
Sensitivity and specificity of IC assay determined at different disease severity.

IC Results	RT-PCR results					
	Severity score ≥ 1		Severity score ≥ 7		Severity score ≥ 11	
	RV-positive	RV-negative	RV-positive	RV-negative	RV-positive	RV-negative
Positive	39	0	34	0	22	0
Negative	19	22	9	11	3	2
Sensitivity (95% CI)	67% (55–79%)		79% (67–91%)		88% (75–100%)	
Specificity	100%		100%		100%	

RotaTeq) of vaccination. We did not include 15 children over 5 years of age in VE analysis because RV-vaccine was not that much available in Japan during the first 6 months of their life (only 1 child over 5 years was found vaccinated). Since the sensitivity of IC test was increased with the advancement of disease severity, we determined VEs by IC assays at different severity levels and compared these to that of RT-PCR performed earlier [18].

Among 65 AGE Children ≤5 years, IC assay detected 27 RV-positive and 38 RV-negative children that included 9 and 27 vaccinated children, respectively (Table 2). VE was determined 79% (95% CI: 39–93%) by IC (P = 0.004), while it was only 58% (−20% to 90%) by RT-PCR (P = 0.128) as we reported earlier [18]. For moderate and severe infections (severity score ≥7), VE was estimated 81.5% (95% CI: 40–94%) by IC (P = 0.005) compared to 72% (95% CI: 7–92%) by RT-PCR (P = 0.038). Finally, in severe children (severity score ≥11), VE was scored 97.5% (95% CI: 77–99.7%) by IC (P = 0.001) compared to 92% (95% CI: 58–98%) by RT-PCR (P = 0.001). Thus, with the increase of disease severity, VEs measured by IC were found to be more accurate and similar to that of RT-PCR.

3.3. Lower detection of RV by IC in vaccinated population

To investigate the reason of higher VE by IC assay, we examined whether RV-detection-rate by IC was similar in both vaccinated and unvaccinated population. We found that the sensitivity of IC in vaccinated population was only 41% (95% CI: 20–61%) while it was as high as 78% (95% CI: 61–91%) in unvaccinated population (Table 3). As shown in Table 4, among 45 RT-PCR confirmed RV cases, IC could detect 27 (60%) of which only 9 were vaccinated. Thus, IC was evidenced nearly 80% less efficient (OR = 0.192, 95% CI: 0.052–0.709) in detecting RV infection in vaccinated population which remained statistically significant (P = 0.013) (Table 4). Finally, to find out the reason of less detection of RV by IC in vaccinated population, we checked the distribution of disease severity and age groups according to children's vaccination status and RV-infection status as determined by RT-PCR and IC assay (Table 5). As shown in Table 5, most vaccinated children experienced mild or moderate infections. Yet, among the children with mild infections, 3 out of 5 RT-PCR-positive children were accurately detected by IC kit in unvaccinated children, whereas only 2 out of 10 RT-PCR-positive children were detected by IC test in vaccinated group, suggesting that RV is more poorly detected by IC kit among vaccinated

Table 4
Detection of RV-infection by IC in vaccinated and unvaccinated population.

	RT-PCR-positive all cases (n = 45)	
	IC-positive	IC-negative
Vaccinated	9	13
Unvaccinated	18	5
OR (95% CI)	0.192 (0.052–0.709)	
P-value	0.013*	

Asterisks indicate statistically significant values.

Table 2
Vaccine effectiveness (VE) determined at different disease severity.

Cases number		Vaccinated (%) in cases	Control number	Vaccinated (%) in controls	Adjusted VE (95% CI)	P-value
All RV infections (severity score ≥1)						
IC	27	9 (33.3%)	38	27 (71%)	79% (39% to 93%)	0.004*
RT-PCR	45	22 (48.8%)	20	14 (70%)	58% (-30% to 86%)	0.135
Moderate-severe infections (severity score ≥7)						
IC	23	7 (30.4%)	38	27 (71%)	81.5% (40% to 94%)	0.005*
RT-PCR	31	12 (38.7%)	20	14 (70%)	72% (7% to 92%)	0.038*
Severe infections (severity score ≥11)						
IC	15	1 (6.6%)	38	27 (71%)	97.5% (77% to 99.7%)	0.001*
RT-PCR	18	3 (16.6%)	20	14 (70%)	92% (58% to 98%)	0.001*

VE was determined against RV infection (Severity Score ≥1, ≥7 and ≥11) by (1-adjusted OR) X100. OR adjusted for age with 95% CI and P-value was determined by binary logistic regression. Asterisks indicate statistically significant values.

Table 3
Sensitivity and specificity of IC determined in unvaccinated and vaccinated population.

IC Results	RT-PCR results			
	Unvaccinated (n = 29)		Vaccinated (n = 36)	
	RV-positive	RV-negative	RV-positive	RV-negative
Positive	18	0	9	0
Negative	5	6	13	14
Sensitivity (95% CI)	78% (61–91%)		41% (20–61%)	
Specificity	100%		100%	

Table 5
Disease severity and age distribution among unvaccinated and vaccinated children with RV infection status determined by RT-PCR and IC assay.

Characteristics	Unvaccinated (n = 43)				Vaccinated (n = 37)			
	RT-PCR-positive	RT-PCR-negative	IC-positive	IC-negative	RT-PCR-positive	RT-PCR-negative	IC-positive	IC-negative
Disease severity (n)								
Mild (26)	5	3	3	5	10	8	2	16
Moderate (27)	8	4	5	7	10	5	7	8
Severe (27)	22	1	21	2	3	1	2	2
Age group (n)								
0–12 m (7)	4	1	4	1	1	1	1	1
13–24 m (27)	8	1	6	3	10	8	1	17
25–36 m (17)	5	2	3	4	7	3	5	5
37–48 m (8)	3	1	3	1	3	1	3	1
49–60 m (6)	3	1	2	2	1	1	0	2
>60 m (15)	12	2	11	3	1	0	1	0

children than unvaccinated ones. Again, a large number of misdiagnosis (only 1 out of 10 RT-PCR-positives was detected by IC-assay) occurred in 13–24 m age group in vaccinated population but not in unvaccinated ones, suggesting that children within 1.5 years after receiving of RV-vaccine remain less sensitive for IC detection.

4. Discussion

This study was originally obtained during an outbreak of G8P[8] RV strain in Shizuoka prefecture, Japan, which has been reported earlier [8,18]. Unlike VE determination by a sensitive RT-PCR method, the aim of the present study was to examine how VEs determined by IC differ from that determined by RT-PCR. It is because the use of IC assay to evaluate VE is increasing. We took RT-PCR as standard for comparison because conventional RT-PCR is more common in laboratories than quantitative RT-PCR (qRT-PCR), and usually more sensitive and specific than other detection techniques like ELISA [19–21]. In addition, RT-PCR is preferred more for laboratory detection since these PCR products can be used further for molecular analysis in sequencing.

In the present study, the sensitivity of IC kit was determined 67% (95% CI: 55–79%) for all RV infection, which was increased up to 88% (95% CI: 75–100%) to detect severe RV infections, while the specificity remained 100% for any disease severity (Table 1). Previously, the sensitivity and specificity of this particular commercial kit were shown 100% in the children admitted to hospitals with AGE in Thailand [22]. The study included only 29 RT-PCR-positive children, mostly infected with common G3P[8] RV strain, and disease severity and vaccine status of the children were not considered. It is expected that the stools of children suffering from severe infections contain a large number of RV-antigens that have boosted the sensitivity of the kit in severe cases.

We found that the sensitivity of IC kit was particularly low in vaccinated children (41%) compared to unvaccinated children (78%) (Table 3). Vaccinated children had 80% less chance to be detected by IC assay [OR 0.192 (95% CI: 0.052–0.709)] which remained statistically significant (P = 0.013) (Table 4). This could be a joint affect of two possible reasons. Firstly, since vaccinated children experienced less severe infection (Table 5), consequently, IC-kits showed less sensitivity to weaker infections, which remains consistent with our findings of Table 1. Another possible explanation is that since IC kit bears colloidal gold-labeled monoclonal antibodies that detect viral antigens in the stool samples, the antigens in the stools of vaccinated children may remain neutralized largely by sIgA; therefore, not detected well by IC assay in vaccinated children. This notion remains compatible with our data of Table 5, where RV detection by IC kit in children in first 1.5 years after receiving RV-vaccine remained less sensitive.

The VE estimated by IC assay was 79% (95% CI: 39–93%) differing from 58% (95% CI: −20 to 90%) determined by RT-PCR (Table 2). VEs that were found increasing by both RT-PCR and IC assays in case of higher disease severity (Table 2) remain quite consistent with our earlier report demonstrating that existing RV-vaccines remain effective to prevent disease progression towards severe illness [8]. Previously, Tate et al. [23] demonstrated 84% (95% CI: 71–91%) VE when determined by enzyme immunoassays (EIAs) while it was only 14% (95% CI: −105% to 64%) as estimated by qRT-PCR. Usually, the sensitivity of EIAs and IC assay remain comparable and VE determined by EIA remained similar to that we found here by IC assay. On the other hand, qRT-PCR remained more sensitive than the conventional RT-PCR, and detected very low levels of RV circulating in the healthy population that was not associated with illness for which the VE determined by qRT-PCR remained low [23].

Notably, we have shown here that as the sensitivity of IC assay was improved with the advancement of disease severity, VEs determined by IC assay at greater illness became closer to VEs determined by RT-PCR (Table 2). Therefore, VEs calculated from IC assay at higher severity looked more accurate.

In this study as well as in our earlier report [22], this particular IC kit did not exhibit any false-positive result; therefore, the specificity of IC kits remained 100% (Table 1). However, specificity was not reported 100% in many other studies using different brands of IC kits, emphasizing that the sensitivity and specificity of IC kits may differ with brand types [24]. Similarly, the acceptance of VE also may vary for different commercial brands. However, detection of RV infection using IC assay usually demonstrates better performance than that for other viruses like adenovirus and norovirus [22,25]. Importantly, these performances of IC kits were investigated during an outbreak of an emerging G8P[8] strain. Nearly, 90% RT-PCR confirmed RV-positive children were infected with G8P[8] strain [8]. Our data, thus, suggest that these IC-based kits are competent to detect uncommon strains also.

Small sample size remained an important limitation of this study. Nevertheless, the study conveys that VE determined by IC assay remains higher than that determined by RT-PCR, particularly when all the three stages (mild, moderate, severe) of infection are considered. Several studies [13–16], are consistent with our data, found similarly high VEs by IC assay. Our data suggest that if the VEs determined by RT-PCR may not remain as high as found by IC assay. However, the risk of contamination remains high during RT-PCR. To minimize the risk, we took several precautions like use of separate room and/or chamber for sample processing, master mix preparation and sample loading. In addition, 10% of these samples were reassessed by both IC assay and RT-PCR to ensure the quality of the result.

RV-illness still represents a major public health problem in children that cause a considerable economic burden for any country [26]. Furthermore, the efficacy and/or effectiveness of RV-vaccines has been found much lower (40–60%) in low- and middle-income countries [26] that poses an additional demand

of regular monitoring of VE in these resource constraint countries. Assessing VEs against severe RV-infection through appropriate use of IC-based kits could be the most effective choice in these countries where RT-PCR deem not feasible.

5. Conclusions

Recently, the application of IC assay to determine VE against RV has been increased. Our data imply that IC assay is less sensitive to detect RV in vaccinated children particularly within 1.5 years after receiving vaccine. Although VEs determined by IC assay remains a little higher than that determined by RT-PCR, it remains comparable in severe cases. Noteworthy, these vaccines were mainly designed to provide protection against severe RV infections. Therefore, regular monitoring of VE against severe infections is particularly important. Despite that VE evaluated by IC is not fully accurate, it can give us an approximate idea of VE useful for continuous monitoring.

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Declaration of Competing Interest

We do not have any association either directly or indirectly that might pose a conflict of interest.

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