Accuracy of Real-Time Polymerase Chain Reaction (Rt-PCR) As A Supplementary Test For Diagnosing Measles

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INTRODUCTION

Measles is one of the most contagious diseases in the world that affects millions of people globally yearly. It is a vaccine-preventable disease. Malaysia had initiated the measles-containing vaccine immunization programme since the year 1982. Although high vaccination coverage of MCV (above 95%) had been achieved since 2010, Malaysia still had not reach the target for measles elimination status. Diagnosing measles in a country with high vaccination coverage of measles-containing vaccine (MCV) such as Malaysia is difficult especially among suspected measles cases with recent history of MCV immunization that might yield false positive serology IgM results (1). On the other hand, samples that are taken too early (within 72 hours from rash onset) might yield false negative serology IgM result(2). In these cases, Real-time polymerase chain reaction (Rt-PCR) can be used as a supplementary test to confirm or exclude diagnosis.

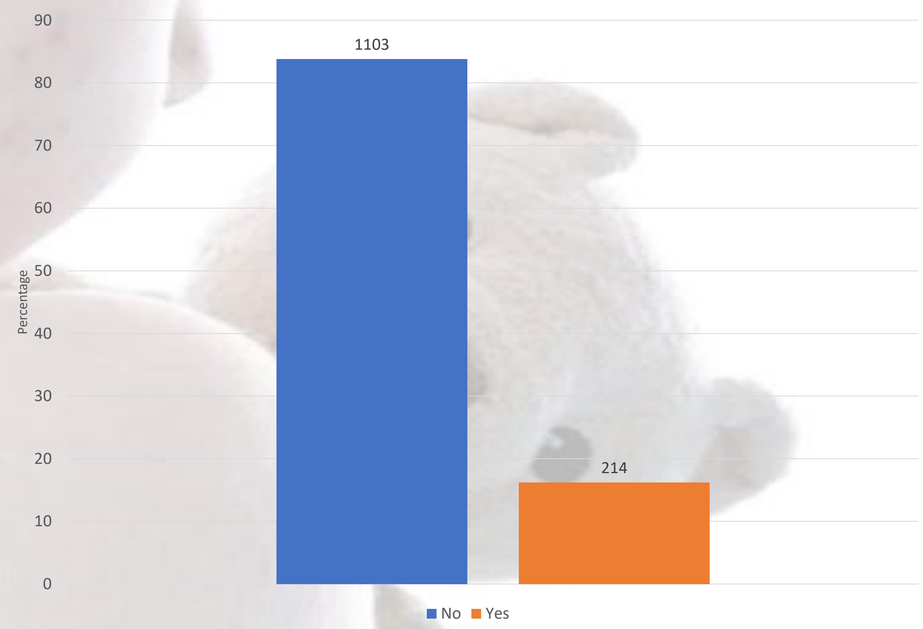
AIM

The aim of this study is to demonstrate the accuracy of the Rt-PCR as a supplementary test for diagnosing measles among suspected measles cases in Malaysia.

METHODOLOGY

A retrospective cross sectional study was conducted using data retrieved from measles laboratory surveillance system. Data were cross-checked with e-measles (electronic measles case-based surveillance system) for data accuracy. Sampling population included all reported suspected measles cases in Malaysia from January 2022 till January 2023 that had both measles serology IgM and Rt-PCR samples. Reported suspected measles cases that did not had enough information for the study variables were excluded. Data was sorted using Microsoft Excel software version 2021, then exported and analysed using the 26th version of IBM SPSS software.

Fiigure 1: Recent MCV (within 56 days before samples collection) status Suspected Measles Cases



RESULTS

A total of 1317 records of reported suspected measles cases were included in the sampling frame. The positivity rate of IgM-,RtPCR+ were highest among those whose samples were collected early, 0-3 and 4-5 days from rash onset, 0.3% and 0.6% respectively (Table 2). Whereas, the positivity rate of IgM+,RtPCR- were highest among those with recent history of MCV immunization, 54.7% (Table 1). There was significant association between dual positivity of serology IgM and Rt-PCR with recent MCV immunization and vaccination status of the suspected measles cases ,($\chi2=565.05$, $p<0.001$) and ($\chi2=298.47$, $p<0.001$).

	Recent History of MCV (within 56 days)	
	Yes	**No**
IgM+,RtPCR+	0.5%	0.5%
IgM+,RtPCR-	54.7%	2.9%
IgM-,RtPCR+	1.9%	0%
Total Positivity Rate	64%	3.4%

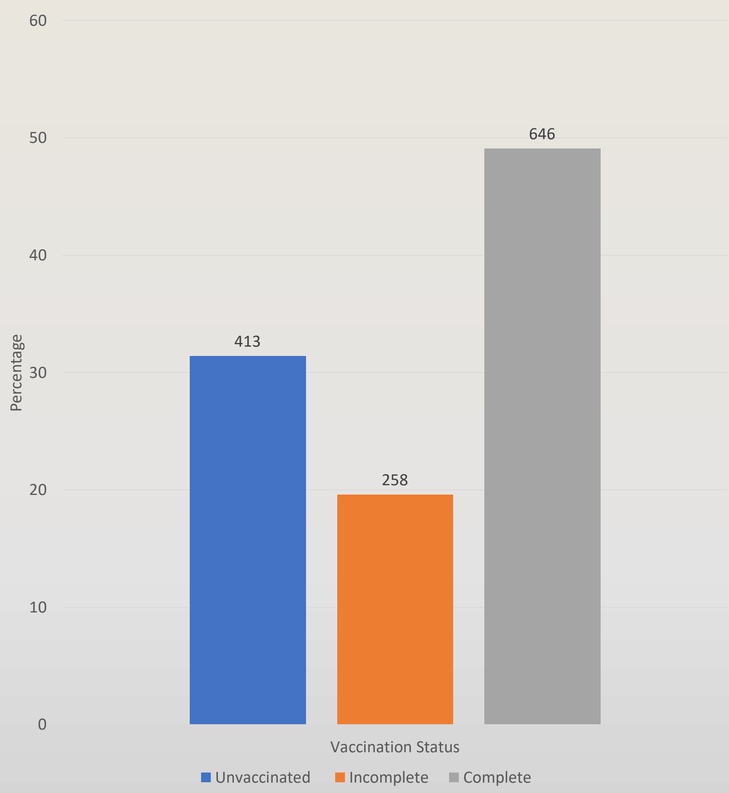
Table 1: Positivity Rate of Serology IgM & Rt-PCR According to Recent MCV

	Timing of Samples Collection from Rash Onset			
	0-3 days	**4-5 days**	**6-10 days**	**>10 days**
IgM+,RtPCR+	1.6%	1.2%	2.6%	0%
IgM+,RtPCR-	10.2%	15.3%	18.4%	14.3%
IgM-,RtPCR+	0.3%	0.6%	0%	0%
Total positivity rate	9.6%	2%	1.2%	0.2%

Table 2: Positivity Rate of Serology IgM & Rt-PCR According to Timing of Samples Collection

Multivariate analysis (Table 3) revealed that unvaccinated and incompletely vaccinated suspected measles cases were 8 times more likely to yield IgM+,RtPCR+ results (true measles infection) compared to those who had completed two doses of MCVs. On the other hand, unvaccinated individuals were five time less likely to yield IgM+,RtPCR- results compared to those who had received MCV immunization. This was consistent with the other finding of the study, that individuals with recent MCV immunization were 20 times more likely to yield IgM+,RtPCR results, which indicated that these results were false positive IgM evidenced by the negativity of the Rt-PCR.

Figure 2: Vaccination Status of Suspected Measles Cases



Dual Positivity	Factors	aOR	95% CI Lower	95% CI Upper	*p*-value
IgM+,RtPCR+	**Vaccination status**				
	0	7.565	1.17	48.76	0.033*
	1	8.076	1.59	41.00	0.012*
	2	1.000			
IgM+,RtPCR-	**Recent MCV**				
	No	0.049	0.03	0.084	<0.001*
	Yes	1.000			
	Vaccination status				
	0	0.196	0.06	0.583	0.003*
	1	2.035	1.19	3.455	0.009*
	2	1.000			

Table 3: Predictors of Dual Positivity Serology IgM & Rt-PCR

RECOMMENDATION

Out of 5020 suspected measles cases samples, only 1852 Rt-PCR samples were taken as a supplementary test for the year 2022. The study had demonstrated the accuracy of Rt-PCR as a supplementary test to exclude false negative IgM results among samples that were taken too early from rash onset, and false positive IgM results among suspected cases with recent MCV. The Rt-PCR test should be made compulsory for every suspected measles cases.

REFERENCES

1. Hubschen et. al, (2017) Challenges of measles and rubella laboratory diagnostic in the era of elimination
2. Michel et. al (2013) Rapid molecular diagnosis of measles virus infection in an epidemic settings.