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Evaluation of the adequacy of measles laboratory diagnostic tests in the era of accelerating measles elimination in Beijing, China

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ABSTRACT

Background: Measles-containing vaccine (MCV) was introduced in 1965 in Beijing and given to the children aged 8m–14y. In population-based surveillance system, real-time polymerase chain reaction (RT-PCR) and immunoglobulin M(IgM) serology tests have been conducted for each suspected case since 2013. We used the surveillance data to evaluate the adequacy of the tests for laboratory confirmation during 2014–2016.

Methods: Informations on IgM tests, RT-PCR, age, vaccination history for confirmed cases were from the surveillance system. Laboratory confirmed cases were defined as cases with positive IgM serology and/or positive RT-PCR. All tests were conducted in the laboratories accredited by Beijing CDC or Beijing CDC laboratory.

Results: Totally 4600 cases were confirmed. Sensitivities of IgM tests within 0–3 days post rash, IgM tests within 4–28 days post rash and RT-PCR within 0–3 days post rash were 56.53%, 82.06% and 94.39%, respectively. The combined sensitivity of IgM tests and RT-PCR decreased by the interval between rash onset and collection of virologic specimen. MCV immunization history lowered sensitivity of IgM tests and RT-PCR. Among the cases aged $\geq$ 15 years, around 95% had no written immunization records. The sensitivity of IgM tests within 0–3 days post rash was less than 60%. Around 60% had unknown immunization histories. Compared with unvaccinated cases based on written records, unvaccinated cases based on recollection had no significantly different sensitivity of laboratory tests. But unknown immunization history significantly lowered sensitivity of RT-PCR within 0–3 days post rash.

Conclusions: Neither IgM tests nor RT-PCR reached 100% sensitive for confirmed cases. Virologic specimen should be collected as early as possible to achieve maxim sensitivity. Cases with unknown immunization history could not be treated as unvaccinated. Combination of the two tests and further laboratory assays were needed especially for vaccinated cases or cases aged $\geq$15 years.

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1. Introduction

In 1965, measles-containing vaccine (MCV) was introduced in China, followed by the Expanded Program on Immunization (EPI) in 1978, which recommended a routine MCV dose to children at 8 months of age. In 1986, a 2nd MCV dose was administered to children aged 7 years. In 1997 the national plan of action for accelerated measles control was developed and called for >90% MCV coverage. In 2005, the recommended age for the 2nd MCV dose was lowered to 18 months. In Beijing, EPI aims to provide $\geq$ 2 doses of MCV vaccination for all children aged 18 months-14 years. As measles incidence has significantly decreased since MCV introduction, China adopted measles elimination goal in 2006, which necessitates well-performed disease surveillance.

Since 1950s, measles has been a notifiable disease in China. And National Notifiable Diseases Reporting System (NNDRS) has been in practice which only covers demographic information, such as age, sex, date of disease onset, and address. In 2005, a case-based measles surveillance system (MSS) was established, which was parallel to NNDRs and used WHO measles case definition [1]. In MSS, information on clinical symptoms, outcome, vaccination status and laboratory tests was collected for each reported case. In 2006, national measles surveillance guideline was developed and included rapid outbreak control measures. For each reported case, contact tracing should be conducted to assure timely reporting of all cases. In 2014, the national guideline was updated. A serum sample of each suspected case (usually collected within 0–3 days after rash onset) has been required to be collected by physicians at first contact. Each hospital should conduct retrospective

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surveillance among all clinical cases in the early, middle and late of each month to ensure appropriate and timely diagnosis of rash illnesses and reporting of each suspected case [2]. In Beijing, a throat swab for real-time polymerase chain reaction (RT-PCR) tests has been required to be collected for each suspected case in addition to serum samples since 2014.

Beijing measles laboratory network (LabNet) was established in 2001 and consists of Beijing Center for Disease Control and Prevention (CDC) and 16 district CDC laboratories. Beijing CDC laboratory is a WHO-accredited laboratory which obtained a perfect score on WHO's proficiency testing (PT) program in 2014. All the network laboratories in Beijing achieved 100% sensitivity and 100% specificity in the external quality assessment for the detection of measles virus by RT-PCR in 2013. That external quality assessment was organized by the National Center for Clinical Laboratories to provide information about the measles diagnosis proficiency of laboratories around mainland China [3].

Laboratory confirmation is crucial to achieve and verify measles elimination. With increasing immunization coverage of MCV, the proportion of adult cases aged ≥ 15 years increased from 51.99% in 2006 to 67.64% in 2013 in Beijing (unpublished data), some of whom might receive MCV vaccination during their childhood. As MCV vaccination history often leads to false negative immunoglobulin M (IgM) results of Enzyme-Linked Immunosorbent Assay (ELISA) from serum samples collected within 0–3 days after rash [4], IgM tests with serum samples collected within 4–28 days after rash and RT-PCR are necessary. However, few [5] domestic studies have evaluated the adequacy of IgM tests and RT-PCR for measles laboratory confirmation on the basis of population-based surveillance data in the current epidemiological scenario in China. We used MSS data to conduct the evaluation in Beijing from 2014 to 2016.

2. Methods

2.1. Case investigation

Each suspected case in MSS is investigated by Beijing CDC and one of Beijing's 16 district CDCs to collect the required informations by MSS. The vaccination status was determined using written vaccination records (immunization certificates kept by vaccine recipients or immunization cards kept by clinics). For a case without written vaccination records, parental/his recall was based on. One throat swab is collected by CDC staff for RT-PCR during field investigation. In addition to a serum sample routinely collected by physicians at first contact, the 2nd serum sample would be collected within 4–28 days after rash for IgM detection when IgM serology within 0–3 days post rash and RT-PCR within 0–3 days post rash were both negative.

All suspected cases were finally classified as clinical, laboratory confirmed and epidemiologically linked cases based on WHO manual [1]. Since the results of sequence analysis or virus isolation were not required to be reported to MSS during 2014–2016, a laboratory confirmed case was defined as a case with positive IgM serology and/or positive RT-PCR. Among the confirmed cases, cases who had received MCV vaccination within the previous 6–45 days were excluded [4,9], as the positive laboratory results might be explained by vaccination.

2.2. Laboratory testing

2.2.1. Measles IgM detection

Serum specimens collected 0–28 days after rash were tested for measles IgM using commercial ELISA (Virion/SerionGmbH, Würzburg, Germany) according to the manufacturer's instructions.

2.2.2. Viral RNA detection

RNA of measles virus (MeV) was extracted from 200 μl of original specimen using QIAamp Virual RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was eluted with 60 μl of elution buffer and stored at −70 °C until further use.

We used BioPerfectus kits (Jiangsu BioPerfectus Technologies Co., Ltd., Jiangsu, China), which are commercially available fluorescent RT-PCR kits and widely used in China [10], for molecular detection of MeV. RT-PCR tests were performed according to the manufacturer's instructions.

Thermal cycling was performed using the ABI7500 Fast Real-time PCR system (Applied Biosystems, France), under the following conditions: reverse transcription step at 50 °C for 30 min, activation at 95 °C for 5 min, followed by 45 cycles of amplication at 95 °C for 10 s and 55 °C for 40 s. The output was demonstrated as the threshold cycle (Ct) value. Ct value ≤ 36.6 is reported as positive, and Ct value > 36.6 is reported as negative.

All tests were conducted in the laboratories in measles LabNet in Beijing. Clinical specimens were transported to laboratory in 1 day after collection. And results of laboratory tests were reported to MSS within 3 days after the receipt of specimens.

2.3. Statistical analyses

To take into account MCV immunization schedule (the 1st dose at age 8 months; the 2nd dose at age 18 months) in Beijing, measles cases were grouped into 5 groups based on the age of disease onset: 0–7 months, 8–17 months, 18 months-14 years, 15–49 years, ≥50 years. Sensitivity of a laboratory test is defined as the proportion of cases with positive results among the total confirmed cases. Chi-squared test was used to evaluate the distribution of categorical variables. For cases with paired samples, McNemar's test was used to compare the sensitivity of the two laboratory tests. Logistic regressions were used to estimate the impact of annual incidence, case age and immunization status on the sensitivity of tests. We used the year as the alternative of annual incidence. Statistical significance was defined as $P < 0.05$. All analyses were conducted using R version 3.4.4.

3. Results

During 2014–2016, a total of 6962 suspected measles cases were reported. And 4947 (71.06%) were diagnosed as measles infection. Out of the 4947 cases, 347 (7.01%) and 4600 (92.99%) were classified as clinical and laboratory confirmed cases, respectively. Cases who had epidemiological link to laboratory confirmed cases were all confirmed by laboratory tests. Annual measles incidence from 2014 to 2016 was 11.24 per 100,000 population, 6.14 per 100,000 population, and 5.75 per 100,000 population, respectively.

3.1. Samples collection

Among the 6962 suspected cases and 4600 confirmed cases, 6922(99.43%) and 4372 (95.04%) had serum samples collected for IgM tests, respectively. And 78.93% and 81.22% had serum samples collected within 0–3 days post rash, respectively. For the serum samples collected within 4–28 days after rash, the median of sampling time was 5 days post rash.

Out of the 6962 suspected and 4600 confirmed cases, 5626 (80.81%) and 3942 (85.70%) had a throat swab collected, respectively. And 78.45% and 83.65% had throat swabs collected within 0–7 days post rash, respectively (Table 1). For the throat swabs col-

Table 1
Samples collected among cases and sensitivity of IgM serology tests and RT-PCR with throat swabs for laboratory confirmed measles cases in Beijing, 2014–2016.

Days after rash onset	Suspected measles cases (N = 6962)		Confirmed cases (N = 4600)			
	No. of cases	%	No. of cases	%	Cases with positive results	
					No. of cases	%
Blood samples						
0-3d	5495	78.93	3736	81.22	2112	56.53
4-28d	2429	34.89	864	18.78	709	82.06
Throat swabs						
0-3d	4177	59.60	3065	66.63	2893	94.39
4-7d	1312	18.85	783	17.02	683	87.23
8-10d	97	1.39	63	1.37	46	73.02
11-28d	40	0.57	31	0.67	17	54.84

lected within 11–28 days after rash, the median of sampling time was 13 days post rash.

3.2. Sensitivity of IgM serology tests and RT-PCR

Among the 4600 confirmed cases, IgM serology within 0–3 days and 4–28 days after rash had the sensitivity of 56.53% and 82.06%, respectively. The proportion of samples with positive RT-PCR decreased as the interval between rash onset and specimen collection increased ($P < 0.001$). RT-PCR within 0–3 days after rash had the highest sensitivity of 94.39%. RT-PCR sensitivity within 8–10 days after rash was 73.02% (Table 1).

Out of the 4600 confirmed cases, 228 cases had two blood samples, which were collected within 0–3 days and 4–28 days after rash, respectively. None of the 228 cases had positive results of IgM serology within 0–3 days after rash. Among the 228 cases, the sensitivity of IgM serology within 4–28 days after rash was 88.16% (201 cases).

For cases having both serum samples and throat swabs collected, RT-PCR tests within 0–7 days after rash were more sensitive than IgM serology (Table 2).

For the combination of IgM serology within 0–3 days after rash and RT-PCR with throat swabs, the sensitivity decreased by the interval between throat swabs collection and rash onset ($P < 0.001$), from 98.17% within 0–3 days after rash to 75% within 8–10 days after rash onset. Among the cases with the combined results of the above two tests, 3.75% had detectable IgM and undetectable measles RNA when throat swabs were collected within 0–3 days after rash. That percentage rose to 62.50% when throat swabs were collected within 11–28 days after rash (Table 2).

Among the cases with the combined results of IgM serology within 4–28 days after rash and RT-PCR, 10.66% had detectable IgM and undetectable measles RNA when throat swabs were collected within 4–7 days after rash. The percentages rose to 39.13% when throat swabs were collected within 11–28 days after rash (Table 2).

3.3. Associated factors of sensitivity of IgM serology and RT-PCR

Out of the 4600 confirmed cases, 539(11.72%), 301(6.54%), 225 (4.89%), 3330(72.39%) and 205(4.46%) cases aged 0–7 months, 8–17 months, 18 months-14 years, 15–49 years and ≥ 50 years, respectively. For the cases aged ≥ 50 years, the median age was 52 years. Among the 4600 cases, the coverage of written immunization records decreased from 97.18% among cases aged < 15 - years to 5.06% among cases aged ≥ 15 years. And the percentage of cases with unknown immunization history decreased from 1.97% to 59.16% (Table 3).

After adjusting for age and MCV immunization history, sensitivity of laboratory tests had no significant association with year. Sensitivity of IgM serology within 0–3 days after rash among cases aged ≥ 15 years was less than 60%, significantly lower than that among cases aged < 15 years. But age of cases had no association with sensitivities of other laboratory tests (Table 4).

Previous MCV vaccination history lowered the sensitivity of IgM serology and RT-PCR within 0–7 days after rash. Among the cases with written immunization records, the sensitivity of RT-PCR within 0–7 days after rash and IgM serology decreased by previous doses of MCV immunization. Among the cases who had no written immunization history but remembered being unvaccinated, all the sensitivities of laboratory tests were not significantly different from those among the cases who were unvaccinated based on written immunization history. Compared with reference group, unknown immunization history was significantly associated

Table 2
Comparison of sensitivity of IgM detection by ELISA and RT-PCR among measles confirmed cases in Beijing, 2014–2016.

IgM detection	MeV- RNA detection							
	0-3d*		4-7d*		8-10d*		11-28d*	
	RT-PCR (-)	RT-PCR (+)	RT-PCR (-)	RT-PCR (+)	RT-PCR (-)	RT-PCR (+)	RT-PCR (-)	RT-PCR (+)
0–3 d*								
IgM(-)	55	1573	16	111	5	5	0	0
IgM(+)	113	1272	35	181	4	6	5	3
McNemar	$P < 0.001$		$P < 0.001$		$P > 0.999$		–	
4-28d*								
IgM(-)	–	–	0	108	0	8	0	4
IgM(+)	–§	–	50§	311	9§	31	9§	10
McNemar	–		$P < 0.001$		$P > 0.999$		$P = 0.267$	

* Time of clinical specimen collection (days after rash onset).
§ Only the cases having the 1st serum sample collected within 4–28 days after rash onset were included.

Table 3
Distribution of MCV immunization history by age of confirmed cases in Beijing, 2014–2016.

	With written immunization records No. (%)				Without written immunization records No. (%)				
	0 dose	1 dose	≥2 doses	Total	0 dose	1 dose	≥2 doses	Unknown	Total
0–7 m	536(1 0 0)	0(0)	0(0)	536(1 0 0)	0(0)	0(0)	0(0)	3(1 0 0)	3(1 0 0)
8m-17 m	238(80.68)	57(19.32)	0(0)	295(1 0 0)	0(0)	3(50)	0(0)	3(50.00)	6(1 0 0)
18m–14y	108(52.94)	18(8.82)	78(38.24)	204(1 0 0)	0(0)	1(4.77)	5(23.81)	15(71.43)	21(1 0 0)
15–49y	4(2.23)	108(60.34)	67(37.43)	179(1 0 0)	1005(31.89)	140(4.44)	36(1.14)	1970(62.52)	3151(1 0 0)
≥50y	0(-)	0(-)	0(-)	0(-)	81(39.51)	5(2.44)	1(0.49)	118(57.56)	205(1 0 0)
Total	886(72.98)	183(15.07)	145(11.94)	1214(1 0 0)	1086(32.07)	149(4.40)	42(1.24)	2109(62.29)	3386(1 0 0)

with lower sensitivity of RT-PCR within 0–3 days after rash (Table 4).

Among the cases who had written immunization records but were unvaccinated, the sensitivity of RT-PCR within 0–3 days after rash was 13.20% higher than that of IgM serology within 4–28 days after rash. Among the cases with the written record of ≥ 2 MCV doses immunization, that rise reached 52.63%.

Among the cases who remembered being unvaccinated, the sensitivity of RT-PCR within 0–3 days after rash was 12.71% higher than that of IgM serology within 4–28 days after rash. Among the cases who remembered receiving 1 dose of MCV previously, that rise was 26.19% (Table 4).

4. Discussion

Measles case-based surveillance with laboratory support is critical to guide measles elimination especially in the countries, such as China, with low-prevalence. In our study, the high proportion of suspected cases with clinical specimens collected and the high proportion of laboratory confirmed infections among cases demonstrated well-performed measles surveillance in Beijing.

In our results, the sensitivity of IgM serology within 0–3 days after rash was lower than that within 4–28 days after rash. The sensitivity of RT-PCR within 0–7 days after rash was higher than that of IgM tests. Detection rate of MeV-RNA by RT-PCR decreased by the interval between specimen collection and rash onset. The positive rate of IgM tests within 0–3 days after rash among unvaccinated cases (cases aged 0–7 months or without MCV immunization history based on written immunization record) was around 80%. The above results were consistent with previous studies [4,7,8]. The detection rate of MeV-RNA by RT-PCR within 0–3 days post rash was 93% in our results, which was also lower than in some studies [7,9]. The difference might result from variations in PCR reagents, gene targets, PCR platform, extraction methods, and primer/probe sequences. In our study, sensitivity of RT-PCR within 8–10 days after rash reached above 70%, which suggested throat swabs collected up to 10 days after rash might still be worthwhile [4].

Diagnostic criteria for measles issued by National Health and Family Planning Commission (NHFPC) of China[10] support the rejection of measles diagnosis in the two following situation: (1) suspected cases with negative IgM serology within 4–28 days post rash; (2) suspected cases with negative results of both IgM tests within 0–3 days post rash and RT-PCR tests within 0–5 days post rash. In the 1st situation, around 20% confirmed cases (Table 1) in our study would be missed if they had no specimen collected for RT-PCR. Table 4 showed that RT-PCR within 0–3 days after rash was especially needed when the cases with MCV vaccination histories had negative IgM serology within 4–28 days after rash. In the 2nd situation, 1.83% of the confirmed cases would be missed, since the combined sensitivity of IgM tests within 0–3 days after rash and RT-PCR within 0–3 days after rash was 98.17%. And throat swabs for RT-PCR needed to be collected as early as possible to achieve maxim sensitivity (Table 2).

Table 2 also showed that when throat swabs for RT-PCR was collected long after rash onset, IgM serology tests would be much necessary.

In our study, 76.85% cases were aged ≥ 15 years, which was higher than that in 2013. Although people aged 15–49 years might have got vaccinated by MCV, some of them were still vulnerable to measles virus due to waning vaccine-induced immunity or low vaccination coverage. Meanwhile, people aged ≥ 15 years were not targeted by routine immunization, the majority of them had no written records in our study, which made it difficult to evaluate immune status. Table 4 showed that among the cases aged ≥ 15 years, positive rate of IgM tests within 0–3 days post rash was much lower than that among younger cases. The association was statistically significant even after adjusting for immunization history. The most possible reason might be that some of those cases might have vaccine-induced immunity and the accurate number of MCV doses they had got might be underestimated by their recollection [11]. However, age was not significantly associated with the sensitivities of IgM serology within 4–28 days after rash and RT-PCR within 0–7 days after rash. Both of the sensitivities among the cases aged ≥ 15 years reached above 80%. So these two tests might be of great importance for laboratory confirmation of adult cases [12].

In our results, previous MCV immunization histories lowered the sensitivity of IgM tests. The reason might be that cases with MCV immunization history mounted no or a more rapid IgM response, which could not be detected [7]. Previous MCV immunization histories also lowered the sensitivity of RT-PCR within 0–7 days after rash, which was different from most studies [6–8]. A possible reason might be that previous MCV immunization provided partial prevention against measles virus infection which might decrease viral load in cases or mounted a rapid viral throat infection. Therefore virus RNA might be undetectable by RT-PCR. That reason could also explain the less severe symptom of a case with MCV immunization history. Another possible reason might be that our RT-PCR tests were less sensitive than in those studies.

The sensitivities of all laboratory tests among unvaccinated cases based on recollection were not significantly different from those among unvaccinated cases based on written immunization records. These results suggested that recollection of immunization history could be referred to when evaluating MCV immunization status of cases.

Our results showed that unknown immunization status could lower the sensitivity of RT-PCR within 0–3 days after rash onset even adjusting for other factors, which suggested that cases with unknown immunization status might not be treated as unvaccinated.

There were several limitations to our study: (1) false positive results could occur in IgM tests especially in measles elimination settings [9]. That would result in overestimation of case numbers and underestimation of sensitivity of RT-PCR in our study. (2) The positive rate of IgM tests within 4–28 days after rash among unvaccinated cases was around 80%, inconsistent with the theoretically rate of 100% [4,13]. One possible reason might be that IgM

Table 4
Distribution of sensitivity of IgM serology tests and RT-PCR by year, age and MCV immunization history among measles confirmed cases in Beijing, 2014–2016.

	0-3d* IgM(+)		4-28d* IgM(+)		0-3d* RT-PCR(+)		4-7d* RT-PCR(+)		8-10d* RT-PCR(+)		≥11d* RT-PCR(+)	
	No(%)	OR_{adj}(95% CI)	No(%)	OR_{adj}(95% CI)	No(%)	OR_{adj}(95% CI)	No(%)	OR_{adj}(95% CI)	No(%)	OR_{adj}(95% CI)	No(%)	OR_{adj}(95% CI)
Year												
2014	1019 (56.17)	Ref	377 (80.73)	Ref	1473 (94.79)	Ref	435 (85.29)	Ref	32 (74.42)	Ref	13 (56.52)	Ref
2015	575 (53.84)	0.898 (0.764–1.055)	133 (82.10)	1.058 (0.658–1.702)	671 (93.07)	0.735 (0.508–1.065)	129 (95.56)	1.857 (0.917–2.197)	11 (91.67)	4.043 (0.348–46.983)	3 (75.00)	1.715 (0.127–23.132)
2016	518 (48.55)	0.922 (0.786–1.081)	199 (84.68)	1.274 (0.821–1.978)	749 (94.81)	0.954 (0.641–1.421)	119 (86.23)	1.012 (0.572–1.788)	3 (37.50)	0.297 (0.035–2.491)	1 (25.00)	0.279 (0.019–4.145)
Age												
0–7m	339 (76.87)	Ref	86 (80.37)	Ref	274 (94.81)	Ref	109 (90.83)	Ref	9 (100.00)	Ref	5 (62.50)	Ref
8m–17m	205 (82.00)	1.108 (0.757–1.898)	43 (84.31)	1.998 (0.722–5.533)	139 (89.10)	0.771 (0.327–1.820)	57 (89.06)	1.093 (0.365–3.274)	4 (66.67)	–	2 (66.67)	–
18m–14y	137 (74.05)	1.970 (0.675–2.360)	34 (72.34)	2.031 (0.589–6.995)	99 (91.67)	2.537 (0.699–9.202)	38 (76.00)	0.720 (0.216–2.399)	4 (80.00)	–	2 (66.67)	0.639 (0.028–14.670)
15–49y	1331 (45.90)	0.316 (0.173–0.577)	515 (82.93)	0.840 (0.646–1.636)	2254 (94.91)	8.086 (0.736–27.997)	453 (87.12)	3.381 (0.641–22.894)	25 (69.44)	–	8 (47.06)	–
>50y	100 (57.80)	0.469 (0.238–0.922)	31 (81.58)	3.182 (0.652–15.533)	127 (92.70)	5.557 (0.463–22.654)	26 (89.66)	4.434 (0.506–38.875)	4 (57.14)	–	0(–)	–
Immunization history												
With written immunization records												
0 dose	582 (80.28)	Ref	141 (83.93)	Ref	438 (95.01)	Ref	186 (90.73)	Ref	15 (93.75)	Ref	7 (63.64)	Ref
1 dose	70 (43.21)	0.343 (0.202–0.584)	21 (70.00)	0.181 (0.052–0.626)	115 (89.84)	0.194 (0.076–0.493)	19 (79.17)	0.220 (0.058–0.841)	2 (100.00)	–	1 (50.00)	–
≥2 doses	51 (40.48)	0.239 (0.131–0.437)	18 (60.00)	0.135 (0.036–0.503)	87 (91.58)	0.124 (0.033–0.461)	15 (68.18)	0.189 (0.047–0.766)	2 (50.00)	–	0(–)	–
Without written immunization records												
0 dose	525 (57.31)	1.243 (0.698–2.216)	184 (84.79)	0.338 (0.091–1.263)	690 (95.57)	1.145 (0.783–1.484)	190 (90.05)	0.248 (0.044–1.407)	15 (83.33)	–	4 (50.00)	–
1 dose	50 (38.76)	0.572 (0.295–1.108)	26 (74.29)	0.174 (0.041–0.736)	90 (93.75)	0.104 (0.026–0.418)	18 (69.23)	0.063 (0.010–0.413)	2 (50.00)	–	1 (50.00)	–
≥2 doses	9(22.50)	0.205 (0.081–0.521)	8 (100.00)	– –	29 (90.62)	0.083 (0.016–0.424)	2(66.67)	0.063 (0.003–1.182)	0(–)	–	0(–)	–
Unknown	825 (44.57)	0.741 (0.420–1.308)	311 (82.71)	0.288 (0.081–1.031)	1444 (94.32)	0.112 (0.035–0.362)	253 (86.64)	0.185 (0.034–1.004)	12 (54.55)	–	4 (50.00)	–

* Time of clinical specimen collection (days after rash onset).

antibody usually peaks within 7–10 days post rash [4]. That is later than the timing of collection of the most serum within 4–28 days post rash in our study, which led to false negative results. Another reason might be the unsatisfying sensitivity of IgM assay in our study. That further supported the necessity of RT-PCR. And more sensitive IgM assays might be needed for highly suspicious cases. (3) Symptoms of measles among vaccinated cases are usually mild. Therefore, IgG detection on both acute and convalescent phase sera was necessary. And IgG avidity assays should be conducted among the cases that had MCV immunization history or the cases in young adults with an unknown vaccination history in China. (4) Measles virus genotype was not determined in our results, which could be used to distinguish between wild type virus infection and symptoms caused by recent MCV vaccination. Although confirmed cases

who had received MCV vaccination within the previous 6–45 days were excluded, some cases might still be caused by recent MCV immunization due to the lack of written immunization records in our study. However, lack of written immunization records mostly occurred among the cases aged ≥ 15 years, so our conclusion might not be significantly altered. (5) Around 20% of suspected cases had throat swab collected > 3 days after rash onset, which could lead to false negative results of RT-PCR. So case number might be underestimated and sensitivity of IgM assays might be overestimated. (6) a large proportion of cases with no written immunization history in our study could lead to overestimation or underestimation of association estimate between sensitivity of laboratory tests and immunization history. However, among these cases, immunization history did significantly lower the sensitivity of laboratory tests.

In conclusion, neither IgM tests nor RT-PCR could achieve 100% sensitive for confirmed cases. Virologic specimen should be collected as early as possible to achieve maxim sensitivity. Combination of two laboratory tests and further laboratory tests are greatly needed especially for cases with vaccination history or cases aged ≥ 15 years in the era of measles elimination.

Notes

For the type of our study formal consent is not required.

Contributor's statement

Rui Ma: Dr. Ma conceptualized and designed the study, conducted data analyses, interpreted results, drafted the manuscript, and approved the final manuscript as submitted.

Li Lu: Dr. Lu conceptualized the study, supervised data processing, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Luodan Suo: Dr. Suo conducted data preprocessing and initial analyses, reviewed the manuscript, and approved the final manuscript as submitted.

Jiazi Zhangzhu: Dr. Zhangzhu conducted initial analyses, reviewed the manuscript, and approved the final manuscript as submitted.

Meng Chen: Dr. Chen supervised laboratory tests, reviewed the manuscript, and approved the final manuscript as submitted.

Juan Li: Dr. Li interpreted the results, reviewed the manuscript, and approved the final manuscript as submitted.

Xinghuo Pang: Dr. Pang reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Declaration of Competing Interest

All authors report no potential conflict of interest.

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