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A meta-analysis to evaluate the effectiveness of real-time PCR for diagnosing novel coronavirus infections

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ABSTRACT. Novel coronavirus (nCoV) belongs to the Coronaviridae family, which includes the virus that causes SARS, or severe acute respiratory syndrome. However, infection source, transmission route, and host of nCoV have not yet been thoroughly characterized. In some cases, nCoV presented a limited person-to-person transmission. Therefore, early diagnosis of nCoV may be of importance for reducing the spread of disease in public. Methods for nCoV diagnosis involve smear dyeing inspection, culture identification, and real-time PCR detection, all of which are proved highly effective. Here, we performed a meta-analysis to evaluate the effectiveness of real-time PCR for diagnosing nCoV infection. Fifteen articles conformed to the inclusion and exclusion criteria for further meta-analysis on the basis of a wide range of publications searched from databases involving PubMed, EMBASE, Web of Science, Medline, ISI. We analyzed the stability and publication bias as well as examined the heterogeneity inspection of real-time PCR detection in contrast to smear staining and culture identification. The fixed-effect model was adopted in our meta-analysis. Our result demonstrated that the combination of real-time PCR and

smear diagnostics yielded an odds ratio (OR) = 1.91, 95% confidence interval (CI) = 1.51-2.41, Z = 5.43, P < 0.05, while the combination of real-time PCR and culture identification yielded OR = 2.44, 95%CI = 1.77-3.37, Z = 5.41, P < 0.05. Therefore, we propose real-time PCR as an efficient method that offers an auxiliary support for future nCoV diagnosis.

Key words: Real-time PCR; Coronavirus; Meta-analysis

INTRODUCTION

Coronavirus belongs to the *Coronavirus* genus of the family Coronaviridae; one of its variants, named SARS virus, can cause severe acute respiratory syndrome (SARS) (Bermingham et al., 2012). Between June 2012 and September 2012, acute lower respiratory infection cases were reported in the Middle East, with pathology similar to that of SARS. Of these, one patient was hospitalized in an intensive care unit and another patient died; they both appeared to have severe acute respiratory infection combined with renal failure (Assiri et al., 2013; Scobey et al., 2013; Martinez et al., 2014). World health organization (WHO) determined that the pathogen is a novel coronavirus with many similarities to SARS virus (Ge et al., 2013). A British scientist released its complete sequence recently (Corman et al., 2012). Three days later, European Union scientists devised the real-time PCR method to detect this novel coronavirus; this test makes rapid diagnosis possible (WHO, 2012). Coronavirus widely exists among humans and animals such as bats, mice, cats, dogs, pigs, and cattle, and these animals are closely related to human beings. The virus can be transmitted between humans and animals (Jadad et al., 1996). Timely and accurate diagnosis is necessary for effective treatment of novel coronavirus (nCoV) infection. Smear dyeing inspection, culture identification, and real-time PCR were reported being used for coronavirus detection, among which, real-time PCR is highly effective (Poon et al., 2003, 2005), but smear dyeing inspection has lower sensitivity and specificity (Pebody et al., 2012), while culture identification is relatively simple and has high specificity but is time consuming (Khuri-Bulos et al., 2014). Methods for early detection and diagnosis of Middle East respiratory syndrome coronavirus, to the same extent, are required, especially in the treatment of early-infected patients (Al-Abdallat et al., 2014). Real-time PCR is rapid with high sensitivity and specificity, so that is preferable for the clinical diagnosis of nCoV infection (Hadjinicolaou et al., 2011). In this study, we validate the effectiveness of real-time PCR for nCoV diagnosis through a systematical meta-analysis on the qualified literature.

MATERIAL AND METHODS

Sources of data

Foreign databases including PubMed, EMBASE, Web of Science, Medline, and ISI were searched, and articles published after August 2003 were collected. The enrolled data did not include unpublished literature and conference papers. The key words were “real-time PCR” and “novel coronavirus”. Manual searching and literature retrospection were also performed.

Inclusion criteria

We included clinical case-control studies or experimental case-control studies, with available line x row information on nCoV as a research object; international (English language) articles published with open access after October 2003; studies with real-time PCR detection as the research subject; and studies using smear dyeing inspection or culture identification for nCoV as a control group.

Exclusion criteria

We excluded duplicated data, with too little information, no control group, without or with wrong data analysis methods, inconsistent data, reviews, and conference papers.

Literature screening and quality evaluation

Two researchers independently screened the articles in accordance with the abovementioned criteria. QUADAS (Whiting et al., 2003) list was used for the literature evaluation. QUADAS entries include 1) whether the spectrum of cases includes all kinds of cases; 2) whether the selection criteria of the research object are clear; 3) whether the two test methods for evaluation are valid for nCoV infection; 4) whether the test method's time interval is short enough to not be affected by a patient's status change; 5) whether the patients were tested with both of the two methods; 6) whether the same group in each study received the same test method; 7) whether the two test methods are independent (lacking any relationship with each other); 8) whether the test method is clearly described and can be repeated; 9) whether each test was blinded in terms of the results of the other simultaneous tests.

Data analysis

The collected data were checked to establish a database. Review Manager 5.3 was used for statistical analysis. A heterogeneity test of the literature was performed before the meta-analysis to determine the suitable statistic consolidation method. We tested for heterogeneity using the Cochran Q test, which follows a chi-square distribution, with q = 0.10. The I^2 value was used to demonstrate the percentage of interstudy variation in the total variation (including interstudy variation and systematic error). $I^2 < 25\%$ meant low heterogeneity; I^2 between 25 and 50% represented moderate heterogeneity; and $I^2 > 50\%$ indicated high heterogeneity. The random-effect model (the Dersimonian-Laird method) was chosen if heterogeneity was present; the fixed-effect model (the Mantel-Haenszel method) was selected when there was no heterogeneity. An odds ratio (OR) between the observation group and control group was selected as the effect index, and the OR for the combination of methods with its 95% confidence interval (CI) were calculated. The sensitivity of the methods from the literature was analyzed by comparing the combined-effect differences and the conclusion difference after removal of articles with fewer samples when choosing a different statistical model. Funnel diagram analysis and Egger tests were used for detection of a possible publication bias.

RESULTS

General characteristics

In total, 132 articles were found. Thirty-one articles remained after excluding reviews, duplicated publications, and unrelated articles. Of these, 16 articles were eliminated after quality evaluation according to the inclusion and exclusion criteria. Finally, 15 clinical case-control studies on the real-time PCR method for nCoV infection detection were enrolled (Table 1).

Table 1. General information regarding the 15 articles on real-time PCR detection of novel coronavirus infection.

Author	Testing program	Control method	Real-time PCR group		Control group	
			Positive N	Total N	Positive N	Total N
Poon et al., 2003	RCT	Smear dyeing	22	50	11	50
van Elden et al., 2004	RCT	Smear dyeing	28	261	20	243
Gaunt et al., 2010	RCT	Smear dyeing	14	31	1	10
Hemida et al., 2014	RCT	Smear dyeing	37	98	44	170
Poon et al., 2005	RCT	Smear dyeing	14	15	9	15
Shirato et al., 2014	RCT	Smear dyeing	80	105	52	105
Neske et al., 2007	RCT	Smear dyeing	21	49	13	49
Parida et al., 2005	RCT	Smear dyeing	87	100	81	100
Lu et al., 2012	RCT	Smear dyeing	41	239	6	239
Bolotin et al., 2009	RCT	Smear dyeing	40	40	60	61
Guo et al., 2009	RCT	Cultivation	22	49	12	49
van Elden et al., 2004	RCT	Cultivation	20	43	12	49
Templeton et al., 2005	RCT	Cultivation	15	31	1	9
Scheltinga et al., 2005	RCT	Cultivation	38	99	43	170
Bolotin et al., 2009	RCT	Cultivation	78	107	51	105

RCT = Regents competency test. N = number.

Study results

We analyzed the results of real-time PCR detection, smear dyeing inspection, and culture identification. As shown in Figure 1, heterogeneity analysis of the enrolled articles was performed. The heterogeneity between real-time PCR and smear dyeing inspection was $\chi^2 = 10.89$, $P > 0.05$, $I^2 = 0.17\%$; heterogeneity did not exist between the studies. The fixed-effect model was selected for our meta-analysis, $Z = 5.43$, $P < 0.05$. Combination OR was 1.91 (95%CI = 1.51-2.41). It showed that the real-time PCR method is superior to smear dyeing inspection. As shown in Figure 2, heterogeneity analysis of the enrolled articles was performed. The heterogeneity between real-time PCR and smear dyeing inspection was $\chi^2 = 2.41$, $P > 0.05$, $I^2 = 0\%$; heterogeneity did not exist among the studies. The fixed-effect model was selected for our meta-analysis, $Z = 5.41$, $P < 0.05$. The combination OR was 2.44 (95%CI = 1.77-3.37). This result showed that real-time PCR method was superior to culture identification.

Identification of publication bias

Because meta-analysis is a type of observational study, errors can occur in the process of study inclusion and analysis, resulting in incorrect results. The funnel plot can be used to evaluate the bias of the literature. As shown in Figures 3 and 4, the graphs between real-time PCR and smear dyeing inspection, as well as those between real-time PCR and culture identification, were almost symmetrical. Most studies were located at the top of the funnel figure,

which means that the research publication bias was small. The Egger test showed that the publication bias between real-time PCR and smear dyeing inspection, as well as that between real-time PCR and culture identification was P = 0.103 and P = 0.043, respectively. This finding suggested that the publication bias existed between real-time PCR and cultivation identification but not between real-time PCR and smear dyeing inspection.

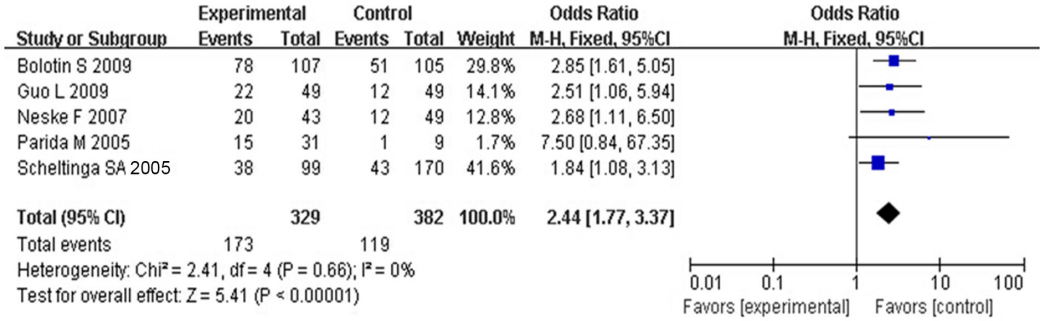

Figure 1. Forest map between real-time PCR and culture identification.

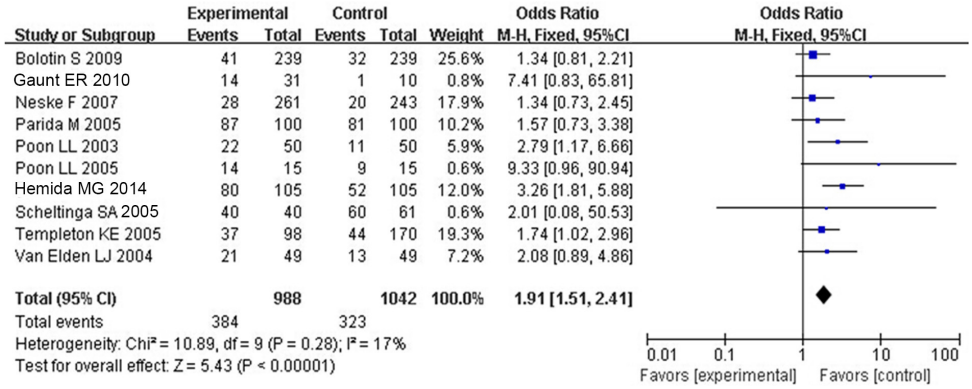

Figure 2. Forest map between real-time PCR and smear dyeing inspection.

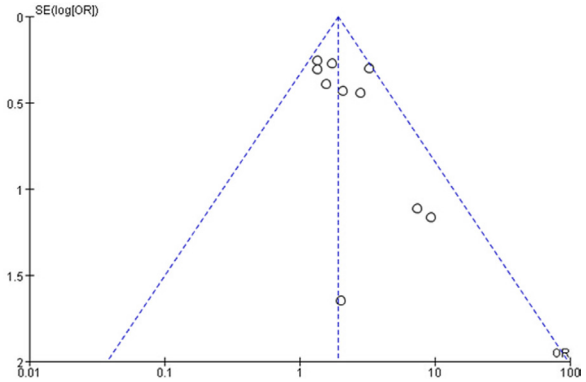

Figure 3. Funnel diagram between real-time PCR and smear dyeing inspection on novel coronavirus infection.

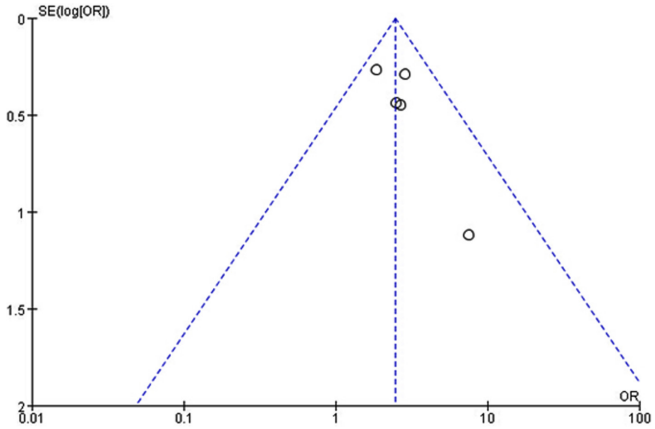

Figure 4. Funnel diagram between real-time PCR and culture identification on novel coronavirus infection.

DISCUSSION

We analyzed the effectiveness of real-time PCR at diagnosing nCoV infection by means of evidence-based medicine. Fifteen articles on real-time PCR method for nCoV infection detection were identified as candidates among 132 studies, according to the inclusion and exclusion criteria of quality evaluation.

Meta-analysis was conducted to evaluate the usefulness of real-time PCR for diagnosing nCoV infection. The results suggest that the real-time PCR method is superior to both smear dyeing inspection and culture identification. The funnel diagram is generally considered a reference index for bias in meta-analysis. Our research showed that the two funnel diagrams are symmetrical. The Egger test showed that a publication bias existed between real-time PCR and culture identification, which might be caused by the information bias.

Our meta-analysis results indicate that the real-time PCR method is effective at detection of nCoV infection. The combination OR value revealed that the real-time PCR method is 2.44-fold more sensitive than culture identification, and 1.91-fold more sensitive than smear dyeing inspection.The real-time PCR therefore shows favorable profile of nCoV infection detection compared to smear dyeing inspection and culture identification and has been widely used.

Despite effective for diagnosing nCoV, real-time PCR has not yet been satisfactory with some limitations of false-positive and false-negative results. A false-positive result is mainly due to contamination that occurs at some point in the entire operating procedure. Thus, each step must strictly abide by the relevant operation specifications in the process of PCR detection. A false-negative result is mainly caused by irregularities in the procedure that can result in DNA damage. The DNA extraction procedure will affect genomic DNA amount, quality, and integrity and thus will affect the process of DNA amplification. A high concentration of genomic DNA can lead to a considerable amount of nonspecific products, whereas too little genomic DNA might result in failure to detect some sequences (ECDC, 2012).

In the present study, we formulated a comprehensive search strategy according to the requirements of systematic evaluation, and two researchers participated in the searching and extraction process at the same time. Nevertheless, there are still some limitations to this study: 1) The number of the enrolled articles is relatively small; this situation may cause a

bias. 2) There are no limitations on age, race, or present illness information, and a stratified meta-analysis was not performed. 3) The retrieval language was limited to English; therefore, a language bias cannot be ruled out. In conclusion, real-time PCR is an efficient method for detecting nCoV infection and can be disseminated as an auxiliary assay for detecting nCoV infection.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Al-Abdallat MM, Payne DC, Alqasrawi S, Rha B, et al. (2014). Hospital-associated outbreak of Middle East respiratory syndrome coronavirus: a serologic, epidemiologic, and clinical description. *Clin. Infect. Dis.* 59: 1225-1233.

Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, et al. (2013). Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect. Dis.* 13: 752-761.

Bermingham A, Chand MA, Brown CS, Aarons E, et al. (2012). Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. *Euro Surveill.* 17: 20290.

Bolotin S, Robertson AV, Eshaghi A, De Lima C, et al. (2009). Development of a novel real-time reverse-transcriptase PCR method for the detection of H275Y positive influenza A H1N1 isolates. *J. Virol. Methods* 158: 190-194.

Corman VM, Eckerle I, Bleicker T, Zaki A, et al. (2012). Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Euro Surveill.* 17: 20285.

ECDC (2012). Rapid risk assessment: severe respiratory disease associated with a novel coronavirus. European Centers for Disease Control Stockholm. Available at [http://ecdc.europa.eu/en/publications/Publications/20121207-Novel-coronavirus-rapid-risk-assessment.pdf].

Gaunt ER, Hardie A, Claas EC, Simmonds P, et al. (2010). Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J. Clin. Microbiol.* 48: 2940-2947.

Ge XY, Li JL, Yang XL, Chmura AA, et al. (2013). Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 503: 535-538.

Guo L, Garten RJ, Foust AS, Sessions WM, et al. (2009). Rapid identification of oseltamivir-resistant influenza A (H1N1) viruses with H274Y mutation by RT-PCR/restriction fragment length polymorphism assay. *Antiviral Res.* 82: 29-33.

Hadjinicolaou AV, Farcas GA, Demetriou VL, Mazzulli T, et al. (2011). Development of a molecular-beacon-based multi-allelic real-time RT-PCR assay for the detection of human coronavirus causing severe acute respiratory syndrome (SARS-CoV): a general methodology for detecting rapidly mutating viruses. *Arch. Virol.* 156: 671-680.

Hemida MG, Chu DK, Poon LL, Perera RA, et al. (2014). MERS coronavirus in dromedary camel herd, Saudi Arabia. *Emerg. Infect. Dis.* 20: 1231-1234.

Jadad AR, Moore RA, Carroll D, Jenkinson C, et al. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control. Clin. Trials* 17: 1-12.

Khuri-Bulos N, Payne DC, Lu X, Erdman D, et al. (2014). Middle East respiratory syndrome coronavirus not detected in children hospitalized with acute respiratory illness in Amman, Jordan, March 2010 to September 2012. *Clin. Microbiol. Infect.* 20: 678-682.

Lu R, Yu X, Wang W, Duan X, et al. (2012). Characterization of human coronavirus etiology in Chinese adults with acute upper respiratory tract infection by real-time RT-PCR assays. *PLOS One* 7: e38638.

Martinez MJ, Marcos MA, Gonzalo V, Zboromyrska Y, et al. (2014). Lack of detection of Middle East respiratory syndrome coronavirus in mild and severe respiratory infections in Catalonia, northeastern Spain. *New Microbes New Infect.* 2: 27-28

Neske F, Blessing K, Tollmann F, Schubert J, et al. (2007). Real-time PCR for diagnosis of human bocavirus infections

and phylogenetic analysis. *J. Clin. Microbiol.* 45: 2116-2122.
Parida M, Horioke K, Ishida H, Dash PK, et al. (2005). Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay. *J. Clin. Microbiol.* 43: 2895-2903.
Pebody RG, Chand MA, Thomas HL, Green HK, et al. (2012). The United Kingdom public health response to an imported laboratory confirmed case of a novel coronavirus in September 2012. *Euro Surveill.* 17: 20292.
Poon LL, Chan KH, Wong OK, Yam WC, et al. (2003). Early diagnosis of SARS coronavirus infection by real time RT-PCR. *J. Clin. Virol.* 28: 233-238.
Poon LL, Wong BW, Chan KH, Ng SS, et al. (2005). Evaluation of real-time reverse transcriptase PCR and real-time loop-mediated amplification assays for severe acute respiratory syndrome coronavirus detection. *J. Clin. Microbiol.* 43: 3457-3459.
Scheltinga SA, Templeton KE, Beersma MF and Claas EC (2005). Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. *J. Clin. Virol.* 33: 306-311.
Scobey T, Yount BL, Sims AC, Donaldson EF, et al. (2013). Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. *Proc. Natl. Acad. Sci. U.S.A.* 110: 16157-16162.
Shirato K, Yano T, Senba S, Akachi S, et al. (2014). Detection of Middle East respiratory syndrome coronavirus using reverse transcription loop-mediated isothermal amplification (RT-LAMP). *Virol. J.* 11: 139.
Templeton KE, Scheltinga SA, van den Eeden WC, Graffelman AW, et al. (2005). Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. *Clin. Infect. Dis.* 41: 345-351.
vanElden LJ, van Loon AM, van Alphen F, Hendriksen KA, et al. (2004). Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J. Infect. Dis.* 189: 652-657.
Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, et al. (2003). The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med. Res. Methodol.* 3: 25.
WHO (2012). Global alert and response (GAR): revised interim case definition-novel coronavirus. World Health Organization, Geneva. Available at [http://www.who.int/csr/disease/coronavirus_infections/case_definition_29092012/en/].