Eur J Pediatr (2007) 166:1189–1191
DOI 10.1007/s00431-006-0378-7

SHORT REPORT

Detection of respiratory pathogens by real-time PCR in children with clinical suspicion of pertussis

Alida M. van Kruijssen · Kate E. Templeton · Roos N. van der Plas · H. Rogier van Doorn · Eric C. J. Claas · Ram N. Sukhai · Ed J. Kuijper

Received: 17 June 2006 / Accepted: 15 November 2006 / Published online: 20 December 2006

Abstract The use of a multiplex respiratory real-time PCR in patients clinically suspected of pertussis increases the number of pathogens detected.

Keywords Real-time PCR · Pertussis · Respiratory virus · Diagnosis

Abbreviations

PCR	polymerase chain reaction
NPAs	nasopharyngeal aspirates
PPV	positive predictive value
NPV	negative predictive value

Bordetella pertussis is a major etiological agent of pertussis (whooping cough). The disease can be atypical and pertussis-like coughing can be caused by other respiratory pathogens [4]. The diagnosis of pertussis is usually suspected clinically, but only proven by specific polymerase chain reaction (PCR) or culture.

PCR methods are more sensitive for *B. pertussis* identification and other respiratory pathogens. Multiplex real-time PCR could be applied as broad-range respiratory PCR.

All patients in whom a diagnostic test for *B. pertussis* was requested from April 2001 to February 2002 were included. The records of the patients were analyzed retrospectively and the patients were assigned to two groups as described in Table 1 [6].

Nasopharyngeal swabs (15 samples), throat swabs (39 samples), sputum (three samples) and nasopharyngeal aspirates (NPAs) (five samples) were received. The PCR assay was performed by a single extraction. The extracted nucleic acid was thereafter added to a four-tube multiplex RNA real-time PCR for 11 RNA respiratory viruses [4] and a four-tube DNA real-time PCR [4, 5]. All PCR products were detected by specific fluorophore-labelled probes that can be distinguished without post-PCR analysis.

Sixty-two patients were seen in the study period from which a diagnostic test for *B. pertussis* was requested. Sufficient clinical information could be obtained on 59/62 (95%) patients and assigned Group I or Group II. The clinical information for 59 patients is shown in Table 2, the symptoms of paroxysmal cough and whoops were significantly associated with Group I.

Analysis of the samples by real-time PCR showed that *B. pertussis* was detected in 17 of the 38 patients in Group I and none of the patients in Group II ($P<0.005$). Using the WHO definition for suspected pertussis used in surveillance as the 'gold standard' for *B. pertussis*, the PCR has a sensitivity, specificity, positive predictive value (PPV) and

Alida M. van Kruijssen and Kate E. Templeton, contributed equally to this work.

A. M. van Kruijssen · R. N. van der Plas · R. N. Sukhai
Department of Pediatrics, Center of Infectious Diseases,
Leiden University Medical Center,
P.O. Box 9600, 2300 RC Leiden, The Netherlands

K. E. Templeton · E. C. J. Claas · E. J. Kuijper
Department of Medical Microbiology,
Center of Infectious Diseases, Leiden University Medical Center,
P.O. Box 9600, 2300 RC Leiden, The Netherlands

H. R. van Doorn
Department of Medical Microbiology, Academic Medical Center,
Meibergrdeef 9,
1005 AZ Amsterdam, The Netherlands

K. E. Templeton (✉)
Specialist Virology Centre, Royal Infirmary Hospital,
51 Little France Crescent,
Edinburgh EH16 5SA, UK
e-mail: kate.templeton@luht.scot.nhs.uk

Table 1 Two groups of rettrospectively analyzed patients.

WHO recommended case definition
Clinical case definition
A case diagnosed as pertussis by a physician **or**
A person with a cough lasting at least 2 weeks with at least one of the following symptoms:
Paroxysms (i.e., fits) of coughing
Inspiratory whooping
Post-tussive vomiting (i.e., vomiting immediately after coughing) without other apparent cause
Criteria for laboratory confirmation
Isolation of *Bordetella pertussis* **or**
Detection of genomic sequences by means of the PCR
Case classification:
Suspected: A case that meets the clinical case definition.
Confirmed: A person with a cough that is laboratory-confirmed or epidemiologically linked to a laboratory confirmed case.
Group I - Case that meets the definition for suspected pertussis
Group II - Case that does not meet the definition for suspected pertussis.

Table 2 Clinical characteristics of patients in study group

	Pertussis[a] (*n*=38) Group I	Non-pertussis[b] (*n*=21) Group II	Whole group (*n*=59) (%)	*P*[c]
Male	16	9	27	NS
Age				
<1	18 (13)	10 (3)	28	NS
1–12	16	8	26	NS
>12	4	3	8	NS
Pertussis vaccination	25	18	42	0.018
Immunocompromised	3	2	5	NS
Stage of disease[d]				
Catarrhal	17	9	26	NS
Paroxysmal	12	5	17	NS
Convalescent	3	4	7	NS
Clinical details[d]				
Cough	32	18	50	NS
Paroxysmal	26	8	34	<0.005
Whoop	5	0	5	0.045
Vomiting	16	9	25	NS
Fever	5	5	11	0.014
GP	16	12	28	NS
Out-patient clinic	8	4	12	NS
Hospital admission	14	8	22	NS

[a] Patients that meet the clinical definition for suspected pertussis
[b] Patients that did not meet the clinical definition for suspected pertussis
[c] Statistical differences between Group I and Group II
[d] Incomplete clinical details for six cases in the pertussis group and three cases in the non-pertussis group
Numbers in parentheses indicate no pertussis vaccination
No clinical information was available for three cases and these were not assigned to either group

negative predictive value (NPV) of 45, 100, 100 and 48%, respectively. Other pathogens were detected in group I as shown in Table 3. Some respiratory viruses were detected in Group II. All other respiratory pathogens had sensitivity and specificity lower than *B. pertussis.* Pathogens were detected by PCR in 31 out of 38 (82%) cases and 10 out of 21 (48%) cases in Group I and Group II, respectively. This difference was significant (p=0.01).

Detection of *B. pertussis* by PCR was indicated on the basis of clinical presentation and PPV of 100% was obtained. However, 21 out of 38 cases that met the clinical definition of pertussis were negative for *B. pertussis*. Therefore, either the *B. pertussis* PCR gave false-negative results or the definition gave a false-positive result. Different sample types have different sensitivities, with NPAs being the most sensitive. PCR can also give false-negative results and fewer diagnoses in those patients with pertussis could be due to the quality of the sample.

Other pathogens were detected in 31 out of 38 patients with clinical diagnosis of pertussis. Other pathogens that cause a pertussis-like disease have previously been described using serological assays and Cherry et al. describe that pertussis can be misdiagnosed, as there are a number of viral and bacterial pathogens other than *B. pertussis* that can cause a paroxysmal cough [1, 2]. It has been well described that PCR is more sensitive than culture at detecting *B. pertussis* in the acute phase [3]. The use of PCR for diagnosis would help improve microbiological diagnosis, improve the speed of results, and reduce the need for the reliance on imperfect clinical criteria for diagnosis. This would prompt more appropriate treatment of *B. pertussis* and other atypical bacteria, differentiating them

Table 3 Pertussis-related symptoms in relation to pathogen detected

Pathogens	Group I[a] (*n*=38)	Group II[b] (*n*=21)	*P*[c]
Bordetella Pertussis	17	0	<0.005
Other atypical bacteria[d]	2	0	NS
Human rhinoviruses	16	4	NS
Respiratory syncytial virus	1	5	0.02
Parainfluenza viruses	2	3	NS
Adenovirus	6	1	NS
Human coronavirus –229E	1	0	NS
Human metapneumovirus	0	1	NS
Mixed infections	14	4	NS
No infection	7	11	0.01

[a] Patients that meet the clinical definition for suspected pertussis
[b] Patients that did not meet the clinical definition for suspected pertussis
[c] Statistical differences between Group I and Group II
[d] *Mycoplasma pneumoniae* (1) and *Chlamydophila pneumoniae* (1)
No influenza A&B virus, human coronavirus OC43, *B. parapertussis* or *B. holmesii* were detected

from viral infections as well as use of vaccination to prevent spread of pertussis.

In conclusion, multiplex PCR in a combined approach identifies *B. pertussis* and other pathogens causing pertussis-like symptoms and use of this form of diagnosis might help in treatment and improve management of patients.

References

1. Cherry JD, Grimprel E, Guiso N, Heininger U, Mertsola J (2005) Defining pertussis epidemiology: clinical, microbiologic and serologic perspectives. Pediatr Infect Dis J 24(suppl):S25–S34
2. Jackson LA, Cherry JD, Wang SP, Grayston JT (2000) Frequency of serological evidence of *Bordetella* infections and mixed infections with other respiratory pathogens in university students with cough illnesses. Clin Infect Dis 31:3–6
3. Loeffelholz MJ, Thompson CJ, Long KS, Gilchrist MJ (1999) Comparison of PCR, culture, and direct fluorescent-antibody testing for detection of *Bordetella pertussis*. J Clin Microbiol 37:2872–2876
4. Templeton KE, Scheltinga SA, van den Eden WCJFM, Graffelman AW, van den Broek PJ, Claas EC (2005) Improved diagnosis of etiology of community-acquired pneumonia using real-time PCR. Clin Infect Dis 41:345–351
5. Templeton KE, Scheltinga SA, van der Zee, Diederen BM, van Kuijssen AM, Goossens H, Kuijper EJ, Claas EC (2003) Evaluation of real-time PCR for detection of and discrimination between *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella holmesii* for clinical diagnosis. J Clin Microbiol 41:4121–4126
6. World Health Organisation (2005) Vaccines and biologicals. WHO-recommended standards for surveillance of selected vaccine-preventable diseases. WHO/V&B/03.01 (http://www.who.int/vaccines-documents/DocsPDF03/www742.pdf)