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Respiratory virus surveillance and outbreak investigation

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Abstract

Sensitive, rapid detection of respiratory viruses is needed for surveillance and for investigation of epidemiologically linked cases. The utility of rapid antigen-based methods for detection of common respiratory viruses and to confirm the cause of outbreaks is well established. However, nucleic acid amplification tests (NATs) offer some benefits above antigen or culture-based procedures, with the main advantages being sensitivity and range of pathogens detectable. It is important to understand how changes in our testing methodology alter respiratory virus detection and information for epidemiological studies. For viruses such as influenza A, influenza B and respiratory syncytial virus, NATs offer enhanced sensitivity above antigen assays but still identify the seasonal peaks important for predicting disease and managing time-sensitive prophylaxis. For other viruses, such as rhinoviruses, coronaviruses, human bocavirus and parainfluenza virus type 4, culture and antigen-based procedures are not available and/or lack sensitivity. Thus such targets would be missed if NATs were not included in testing for surveillance and outbreak investigation. As more respiratory viruses are identified there is a need to expand surveillance and further evaluate new technologies and automation beyond currently-available diagnostics to address detection of a broad range of potential pathogens.

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1. Abbreviations:

ELI epidemiological investigation
hMPV human metapneumovirus
hBoV human bocavirus
IFV influenza virus
NAT(s) Nucleic acid amplification test(s)
NP nasopharyngeal
PCR polymerase chain reaction
PIV parainfluenzavirus
RSV respiratory syncytial virus

2. Introduction

Monitoring and surveillance of well-recognized respiratory viruses and potential zoonotic threats is important for management and to minimize community impact (Heeney, 2006). Enhanced surveillance and diagnosis of respiratory illness has the potential to reduce health-care costs enormously (Halasa et al., 2005; Esposito et al., 2006) but there are still significant gaps in our knowledge concerning the range of pathogens which cause respiratory infection and disease, with many cases going undiagnosed. Respiratory virus detection and diagnosis is complex because of the wide range of viruses (and other pathogens) which can present with the same clinical symptoms. Empiric treatment of patients, without a clear diagnosis, may result in implementation of expensive and disruptive public health measures as well as lead to increased spread of the disease.

The seasonality of some respiratory viruses is well recognized, and viral surveillance and laboratory-based diagnostics are important to guide timing of prophylaxis and other interventions. Respiratory syncytial virus (RSV) peaks during winter months each year [although the start and finish of the season varies (Alonso et al., 2007)] and it is important to track seasonality for planning prophylaxis in vulnerable children. For influenza A, adequate surveillance is important for designing appropriate vaccines, planning timing for prophylaxis and for detection of novel viruses. There are many other viral causes for respiratory outbreaks, and use of NATs has enabled us to have a greater understanding of the range and type of viruses responsible.

3. Methods

To provide the broadest possible value, laboratory diagnosis of respiratory tract infections should generate information
Fig. 1. Seasonality of respiratory virus infections. Data are for influenza (IFV) A (1a) and B (1b), respiratory syncytial virus (RSV; 1c) and parainfluenza virus (PIV; 1d). All positive results are shown (unselected) for respiratory samples submitted from 1st January 2004–31st December 2006. In November 2005 a switch was made from use of culture to testing using NATs for DFA-negative NP samples and for all other (non-NP) sample types. Positive results are independent for each test type (DFA positives are not tested by NAT or culture, NAT positives are not subjected to culture etc.).
on viral epidemiology as well as provide clinical information. In many laboratories, diagnosis of respiratory virus infections relies heavily on direct fluorescent antigen (DFA) assays, other rapid antigen detection methods or modified culture procedures. The rapid turn-around of DFA means that this method is still useful for influenza virus (IFV) A, IFVB and RSV, providing a good nasopharyngeal (NP) sample is taken. Positive results are used for cohorting vulnerable individuals, for treatment of the individual and management of a potential outbreak. DFA, however, is not as sensitive as NATs for these targets and additional cases will be identified using this method in DFA-negative NP samples.

The use of culture versus NATs as an adjunct diagnostic approach, especially for non-NP samples, depends on the laboratory capacity and set-up. Culture methods are, in theory, “catch all” with no need for a pre-conceived idea of the likely cause. In practice, culture is not very sensitive and often negative for many picornaviruses, coronaviruses, human metapneumovirus (hMPV) and human bocavirus (hBoV) which are all recognized causes of respiratory symptomology and disease in the community and in hospitals. The choice of NATs, if appropriate facilities are available, is obvious where maximum sensitivity is required for testing one or a few targets (Lee et al., 2006). Culture can then be reserved for samples which have already been screened and have given positive results by DFA or NAT if an isolate is needed for further analysis.

As detailed elsewhere, however, the broad range of respiratory viruses (and other bacteria) which cause similar symptoms makes set up of NATs complex if the full diagnostic testing repertoire is to be attempted. Multiplex amplification methods with suspension microarray detection may be one diagnostic enhancement which will be useful for sensitive surveillance and outbreak investigation.

4. Results

4.1. Identification and seasonality of respiratory virus infections

Figure 1 provides data on positive results for IFVA (Fig. 1a), IFVB (1b), RSV (1c) and PIV (1d) on unselected samples submitted for respiratory virus investigation. It is important that changes in technology do not skew epidemiological data making it difficult to compare results across different seasons. Despite changes in diagnostic testing methods during 2004–2006 in our laboratory, the seasonality of IFVA, IFVB and RSV is apparent. Over a period of time where a combination of DFA and NATs were utilized for respiratory virus detection and analysis, it is clear that for IFVA, IFVB and RSV whether you monitor positive results by DFA, NAT or any positive test you identify the same seasonal peaks. For PIV the difference in sensitivity between DFA and NAT for PIV1–3 and the added identification of PIV 4 by NAT skews the curves for NATs away from the DFA positive curve. Thus, monitoring PIV by DFA will underestimate the number and significance of PIV infections. With use of NATs the identification of positive PIV cases all year round is more obvious.

Epidemiological data is accumulating for recently-identified respiratory viruses and those not easily identified, except by NAT. Studies over multiple years have demonstrated the seasonality and impact of hMPV (e.g. Bosis et al., 2005; Bouscambert-Duchamp et al., 2005; Sloots et al., 2006; Dare et al., 2007; Manohar et al., 2007; Pabbaraju et al., 2007; Sivaprakasam et al., 2007; van den Hoogen, 2007), picornaviruses (e.g. Jartti et al., 2004; Arden et al., 2006; Jacques et al., 2006; Winther et al., 2006) and coronaviruses (e.g. Vallet et al., 2004; Birch et al., 2005; Chiu et al., 2005; Kaiser et al., 2005; Arden et al., 2006; Esposito et al., 2006; Gerna et al., 2006). hBoV is identified frequently in respiratory samples from young children and is associated with a high co-infection rate (e.g. Arden et al., 2006; Arnold et al., 2006; Bastien et al., 2006; Ma et al., 2006).

Adenoviruses have been recognized as an important cause of respiratory infections in the community and are responsible for some outbreaks [see below, de Mezerville et al. (2006) and Russell et al. (2006)]. Surveillance measures for these viruses have largely relied on culture-based procedures. In fact, culture is relatively sensitive (although slow) for detection of adenoviruses (unlike DFA which lacks sensitivity for this virus). NATs for adenoviruses have the advantage of speed for identification of individual cases and for etiological diagnosis of outbreaks.

4.2. Identification and etiological diagnosis of outbreaks

As shown in Figure 2, most respiratory outbreaks (epidemiologically linked cases) occur in the winter months (October–March) in Alberta. Using our current diagnostic testing algorithm, which identifies IFVA, IFVB, PIV 1–4, RSV, hMPV and adenoviruses, we are now able to make an etiological diagnosis in more than 80% of outbreaks investigated (Table 1, data for 2006). The use of NATs for analysis of DFA-negative NP samples and for all other (non-NP) samples has increased the number of samples with a detectable virus as part of an outbreak investigation from between 17.9% and 30.8% of samples positive in 2003–2005 to 53.7% of samples positive in 2006 (Table 1). Since the introduction of NATs to our testing algorithm the number of samples with more than one virus-positive result has increased. For samples tested from possible outbreaks in 2003–2005, only 3 (of 1958; 0.2%) had a mixed infection identified compared with 15 samples (of 712; 2.1%) for outbreaks in 2006.

Despite changes in testing methodology, IFVA is still the most commonly recognized cause of respiratory virus outbreaks (Figure 3 and Table 2), although IFVB, RSV, PIV, hMPV and adenovirus are also associated (as also shown by Dollner et al., 2004; Faden et al., 2005; Honda et al., 2006; Russell et al., 2006; Boivin et al., 2007). Additionally, in Alberta, a considerable number of outbreaks are associated...
Fig. 2. Seasonality of reported respiratory virus outbreaks. Data are from outbreaks reported because of epidemiologically linked cases of respiratory symptoms in acute care hospitals, schools and long-term and assisted care centres in Alberta, Canada. All outbreaks are included where samples were submitted for respiratory viral investigation. Total number of outbreaks was 496 over this time period (2003–2006). EI = epidemiological investigation.

Table 1
Analysis of results for respiratory samples submitted for viral diagnosis as part of an outbreak investigationa

| Year | Number of outbreaks with virus identified/number of outbreak investigations (% positive) | Number of positive samples/number submitted as part of outbreak investigation (% positive) |
|------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| 2003 | 98/152 (64.5)                                                                           | 280/908 (30.8)                                                                        |
| 2004 | 22/57 (38.6)                                                                             | 59/330 (17.9)                                                                          |
| 2005b| 94/126 (74.6)                                                                            | 267/720 (37.1)                                                                         |
| 2006 | 134/161 (83.2)                                                                           | 382/712 (53.7)                                                                         |
| 2003–2006 | 348/496 (70.2)                                                                      | 988/2670 (37.0)                                                                        |

a Samples were a mix of respiratory specimens collected and tested (all methods) 2003–2006.
b In November 2005 a change in testing algorithm was implemented to incorporate use of NATs (see methods).

4.3. Expanded testing and outbreak investigation using NATs

Although using a combination of antigen and NATs has enabled a viral etiological diagnosis to be made in the majority of outbreaks in Alberta there are still epidemiologically linked cases for which a virus is not identified using our current testing algorithm. Undiagnosed outbreaks probably involve viruses (or bacteria) that are not part of our routine testing panel, such as rhinoviruses (Hicks et al., 2006; Kiang et al., 2007), coronaviruses (Birch et al., 2005) and IFVC (Matsuzaki et al., 2007).

5. Discussion

It is important that we use the best available diagnostic tools to identify common and unusual respiratory viruses as a cause of individual symptomatic cases in the community as well as for outbreak investigation, particularly as they cannot necessarily be predicted year on year. The economic costs of respiratory virus outbreaks are apparent and have been modeled (Achonu et al., 2005; Halasa et al., 2005; Russell et al., 2006).
The use of NATs has enhanced detection of IFVA, IFVB and RSV. However, antigen-based tests may still be useful for these viruses and, whichever approach is routine for the diagnostic laboratory, the same seasonal peaks of virus activity are identified.

Our use of DFA and NAT identifies PIV throughout the year without any particular seasonality. Previous studies have identified some PIV seasonality with PIV 1 and PIV 3 tending to exclude each other in a particular year (Fry et al., 2006). Use of NATs and active surveillance in vulnerable groups will identify PIV 1−3 in more cases than will be seen by antigen or culture-based procedures. Information on carriage and infection with PIVs is useful but cross-transmission may be difficult to prevent and not all PIV-infected immunocompromised patients require therapy (Dignan et al., 2006). PIV 4 may be an important cause of outbreaks (Lau et al., 2005) and would not be identified efficiently by antigen or culture methods.

Despite our lack of association of adenoviruses with a large number of outbreaks in Alberta, this group of viruses can cause significant disease in vulnerable individuals (Faden et al., 2005) as well as outbreaks with economic impact in military and naval training centres (Russell et al., 2006). Like PIV, adenoviruses are increasingly recognized in immunocompromised individuals with the advent of more detailed surveillance and sensitive laboratory tests (such as NATs). As treatment for such infections may be considered in immunocompromised patients, rapid diagnostic turn-around may become more important.

Rhinoviruses are not routinely identified without the use of NATs. Symptoms, exacerbations and association with outbreaks are much greater than previously recognized for
this group of viruses (Hicks et al., 2006; Kusel et al., 2006; Khetasuriani et al., 2007; Kiang et al., 2007; Miller et al., 2007). They have a distinct seasonality when detailed surveillance using NATs is undertaken (Winther et al., 2006).

Coronaviruses are under-diagnosed unless NATs are utilized, and these viruses have been linked with outbreaks where expanded testing has been undertaken (Vallet et al., 2004; Birch et al., 2005; Chiu et al., 2005; Kaiser et al., 2005; Arden et al., 2006; Esposito et al., 2006; Gerna et al., 2006; Khetasuriani et al., 2007).

To date, the study of hBoV infections has revealed a predominance of infection in young children with, or without, a co-infecting pathogen (Arden et al., 2006; Arnold et al., 2006; Bastien et al., 2006; Ma et al., 2006). Only detailed surveillance (probably using NATs) will allow us to assess the full clinical impact of this virus.

6. Conclusion

Detection, surveillance and analysis of respiratory viruses are well established using antigen, culture and nucleic acid-based tests. The identification of novel respiratory viruses and the need to enhance etiological diagnosis in individual cases and epidemiologically linked outbreaks has led to re-evaluation of current testing methods. DFA and culture are still very limited in terms of sensitivity and range of viral pathogens which can be identified. While individual (target-specific) NATs enhance sensitivity, further technological enhancements are needed for broad virus amplification and detection. Multiplex amplification procedures with microarray detection of products may be one way to undertake broad-spectrum viral surveillance and outbreak investigation but more studies are needed to confirm the suitability of this technology for this particular purpose.

Conflict of interest statement

None declared.

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References

Achonu C, Laporte A, Gardam MA. The financial impact of controlling a respiratory virus outbreak in a teaching hospital: lessons learned from SARS: Can J Public Health 2005;96:52−4.

Alonso A, Andres JM, Garmendia JR, Diez I, Gil JM, Ardua, J. Bronchiolitis due to respiratory syncytial virus in hospitalized children: a study of seasonal rhythm: Acta Pediatr 2007;96:731−5.

Arden KE, McErlan P, Nissen MD, Sloots TP, Mackay IM. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections: J Med Virol 2006; 78:1232−40.

Arnold JC, Singh KK, Spector SA, Sawyer MH. Human bocavirus: prevalence and clinical spectrum at a children's hospital: Clin Infect Dis 2006;43:283−8.

Bastien N, Brandt K, Dust K, Ward D, Li, Y. Human Bocavirus infection, Canada: Emerg Infect Dis 2006;12:848−50.

Birch CJ, Clothier HJ, Secull A, Tran T, Catton MC, Lambert SB, Druce JD. Human coronavirus OC43 causes influenza-like illness in residents and staff of aged-care facilities in Melbourne, Australia: Epidemiol Infect 2005;133:273−7.

Boivin G, De Serres G, Hamelin ME, Cote S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, Couture C. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility: Clin Infect Dis 2007;44:1152−8.

Bosis S, Esposito S, Nieters HG, Crowari P, Osterhaus AD, Principi N. Impact of human metapneumovirus in childhood: Comparison with respiratory syncytial virus and influenza viruses: J Med Virol 2005;75:101−4.

Bouscambert-Duchamp M, Lina B, Trompette A, Moret H, Motte J, Andreoletti L. Detection of human metapneumovirus RNA sequences in nasopharyngeal aspirates of young French children with acute bronchiolitis by real-time reverse transcriptase PCR and phylogenetic analysis: J Clin Microbiol 2005;43:1411−4.

Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LL, Peiris JS. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China: Clin Infect Dis 2005;40:1721−9.

Dare R, Sanghavi S, Bullotta A, Keightley MC, George KS, Wadowsky RM, Paterson DL, McCurry KR, Reinhart TA, Husain S, Rinaldo CR. Diagnosis of human metapneumovirus infection in immunosuppressed lung transplant recipients and children evaluated for pertussis: J Clin Microbiol 2007;45:548−52.

de Mezerville MH, Tellier R, Richardson S, Hebert D, Doyle J, Allen U. Adenoviral infections in pediatric transplant recipients: a hospital-based study: Pediatr Infect Dis J 2006;25:815−8.

Dignan F, Alvares C, Riley U, Ethell M, Cunningham D, Treleaven J, Ashley S, Bendig J, Morgan G, Potter M. Parainfluenza type 3 infection post stem cell transplant: high prevalence but low mortality: J Hosp Infect 2006;63:452−8.

Dollner H, Risnes K, Radtke A, Nordbo SA. Outbreak of human metapneumovirus infection in Norwegian children: Pediatr Infect Dis J 2004;23:436−40.

Esposito S, Bousi S, Niesters HG, Tremolati E, Begliatti E, Rognoni A, Tagliabue C, Principi N, Osterhaus AD. Impact of human coronavirus infections in otherwise healthy children who attended an emergency department: J Med Virol 2006;78:1609−15.

Faden H, Wynn RJ, Campagna L, Ryan RM. Outbreak of adenovirus type 30 in a neonatal intensive care unit: J Pediatr 2005;146:523−7.

Fry AM, Curns AT, Harbour K, Hutt Wagner L, Holman RC, Anderson LJ. Seasonal trends of human parainfluenza viral infections: United States, 1990−2004: Clin Infect Dis 2006;43:1016−22.
Gerna G, Campanini G, Rovida F, Percivalle E, Sarasin A, Marchi A, Baldanti F. Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients: J Med Virol 2006;78:938–49.

Halasa NB, Williams JV, Wilson GJ, Walsh WF, Schaffner W, Wright PF. Medical and economic impact of a respiratory syncytial virus outbreak in a neonatal intensive care unit: Pediatr Infect Dis J 2005;24:1040–4.

Heeney JL. Zoonotic viral diseases and the frontier of early diagnosis, control and prevention: J Intern Med 2006;260:399–408.

Hicks LA, Shepard CW, Britz PH, Erdman DD, Fischer M, Flannery BL, Peck AJ, Lu X, Thacker WL, Benson RF, Tondella ML, Moll ME, Whitney CG, Anderson LJ, Feikin DR. Two outbreaks of severe respiratory disease in nursing homes associated with rhinovirus: J Am Geriatr Soc 2006;54:284–9.

Honda H, Iwahashi J, Kashiwagi T, Imamura Y, Hamada N, Anraku T, Ueda S, Kanda T, Takahashi T, Morimoto S. Outbreak of human metapneumovirus infection in elderly inpatients in Japan: J Am Geriatr Soc 2006;54:177–80.

Jacques J, Bouscambert-Duchamp M, Moret H, Carquin J, Brodard V, Lina B, Motte J, Andreoletti L. Association of respiratory picornaviruses with acute bronchiolitis in French infants: J Clin Virol 2006;35:463–6.

Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children: Emerg Infect Dis 2004;10:1095–101.

Kaiser L, Regamey N, Roita H, Deffner C, Frey U. Human coronavirus NL63 associated with lower respiratory tract symptoms in early life: Pediatr Infect Dis J 2005;24:1015–7.

Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, Teague WG. Prevalence of viral respiratory tract infections in children with asthma: J Allergy Clin Immunol 2007;119:314–21.

Kuang D, Yagi S, Kantardjieff KA, Kim EJ, Louie JK, Schnurr DP. Molecular characterization of a variant rhinovirus from an outbreak associated with uncommonly high mortality: J Clin Virol 2007;38:227–37.

Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study: Pediatr Infect Dis J 2006;25:680–6.

Lau SK, To WK, Tse PW, Chan AK, Woo PC, Tsui HW, Leung AF, Li KS, Chan PK, Lim WW, Yung RW, Chan KH, Yuen KY. Human parainfluenza virus 4 outbreak and the role of diagnostic tests: J Clin Microbiol 2005;43:4515–21.

Lee BE, Robinson JL, Khurana V, Pang XL, Preiksaitis JK, Fox JD. Enhanced identification of viral and atypical bacterial pathogens in lower respiratory tract samples with nucleic acid amplification tests: J Med Virol 2006;78:702–10.

Ma X, Endo R, Ishiguro N, Ebihara T, Ishiko H, Ariga T, Kikuta H. Detection of human bocavirus in Japanese children with lower respiratory tract infections: J Clin Microbiol 2006;44:1132–4.

Manohal C, Espinosa S, Aho SL, Huet F, Pothier P. Epidemiological and clinical features of hMPV, RSV and RV infections in young children: J Clin Virol 2007;38:221–6.

Matsuzaki Y, Abiko C, Mizuta K, Sugawara K, Takashita E, Muraki Y, Suzuki H, Mikawa M, Shimada S, Sato K, Kuzuya M, Takao S, Wakatsuki K, Itagaki T, Hongu S, Nishimura, H. A nationwide epidemic of influenza C virus infection in Japan in 2004: J Clin Microbiol 2007;45:783–8.

Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, Hartert TV, Anderson LJ, Weinberg GA, Hall CB, Iwane MK, Edwards KM. Rhinovirus-associated hospitalizations in young children: J Infect Dis 2007;195:773–81.

Pabbaraju K, Wong S, McMillan T, Lee BE, Fox JD. Diagnosis and epidemiological studies of human metapneumovirus using real-time PCR. J Clin Virol 2007. In press.

Russell KL, Hawksworth AW, Ryan MA, Strickler J, Irvine M, Hansen CJ, Gray GC, Gaydos JC. Vaccine-preventable adenoviral respiratory illness in US military recruits, 1999–2004: Vaccine 2006;24:2835–42.

Sivaprakasam V, Collins TC, Atikien C, Carman WF. Life-threatening human metapneumovirus infections in West of Scotland: J Clin Virol 2007;39:234–7.

Sloots TP, Mackay IM, BiaLastiewicz S, Jacob KC, McQueen E, Harnett GB, Siebert DJ, Masters BL, Young PR, Nissen MD. Human metapneumovirus, Australia, 2001–2004: Emerg Infect Dis 2006;12:1263–6.

Vallet S, Gagneur A, Talbot PJ, Legrand MC, Sizun J, Picard B. Detection of human Coronavirus 229E in nasal specimens in large scale studies using an RT-PCR hybridization assay: Mol Cell Probes 2004;18:75–80.

van den Hoogen BG. Respiratory tract infection due to human metapneumovirus among elderly patients: Clin Infect Dis 2005;41:1159–60.

Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season: J Med Virol 2006;78:644–50.