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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.

Keywords: Respiratory virus; Diagnosis; Nucleic acid amplification; Outbreak; Pandemic; Surveillance

1. Abbreviations:

EI	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

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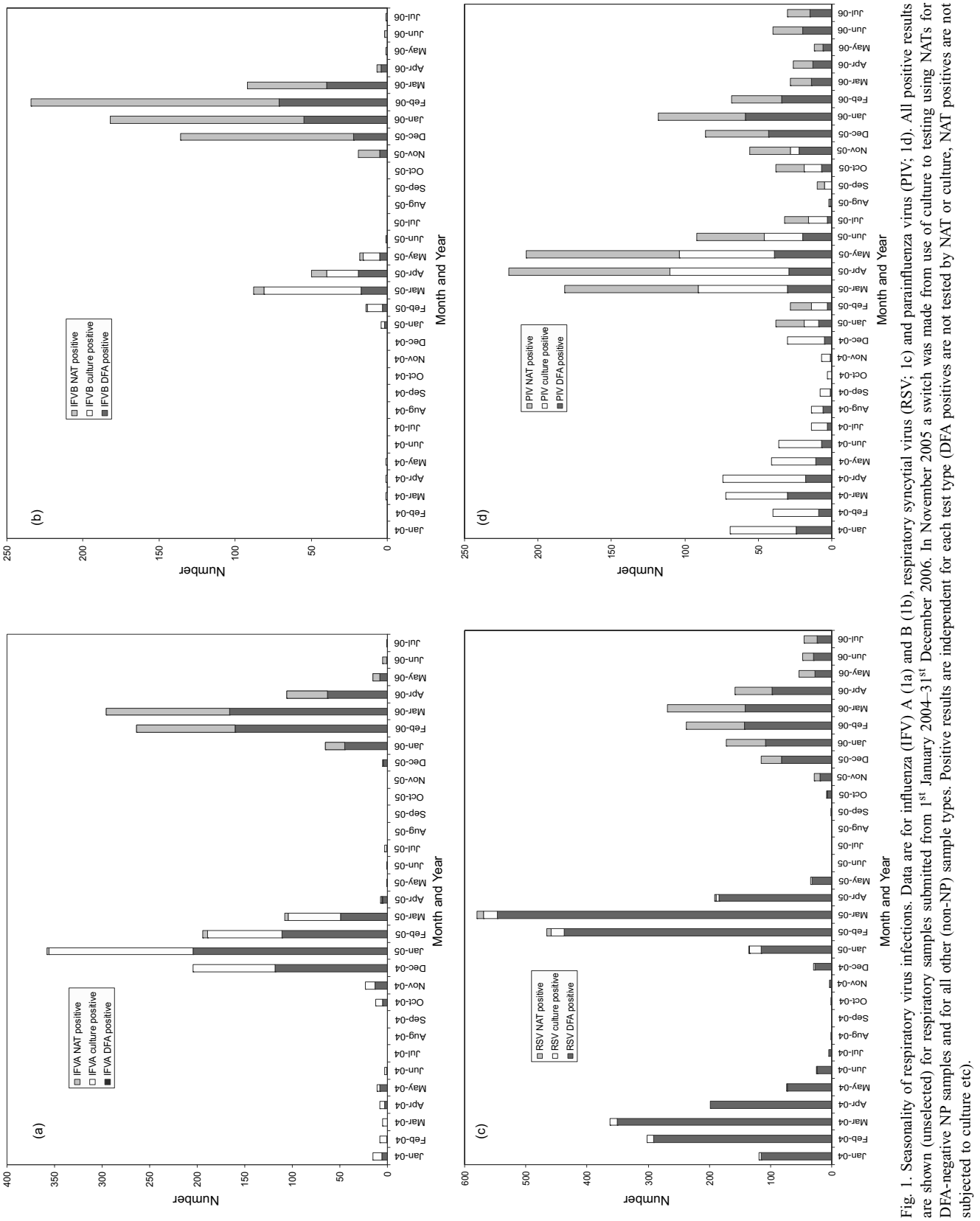

Fig. 1. Seasonality of respiratory virus infections. Data are for influenza (IFV) A (1a) and B (1b), respiratory syncytial virus (RSV; 1c) and parainfluenza virus (PIV; 1d). All positive results are shown (unselected) for respiratory samples submitted from 1st January 2004–31st December 2006. In November 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 on 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 PIV 1–3 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; Manoha 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

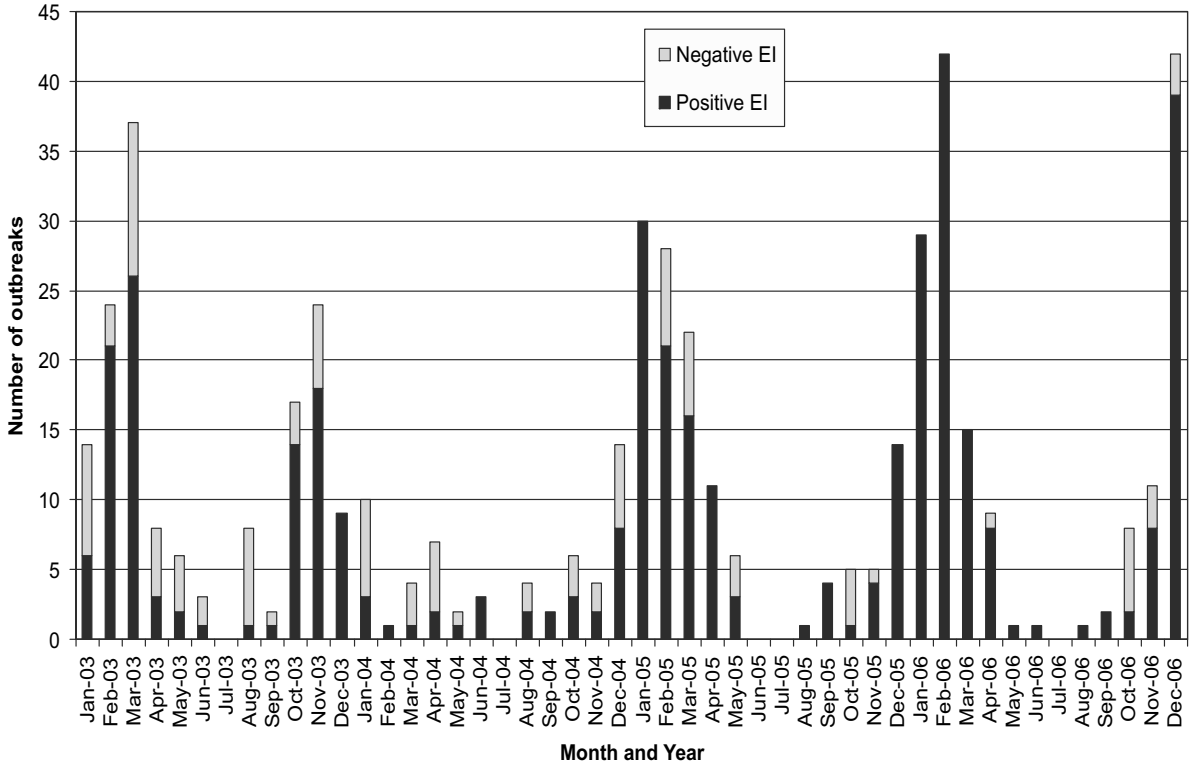

Fig. 2. Seasonality of reported respiratory virus outbreaks. Data are from outbreaks reported because of epidemiologically linked cases of respiratory symptoms in acute care hospitals, schools and long-term and assisted care centres in Alberta, Canada. All outbreaks are included where samples were submitted 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 investigation[a]

2005[b]	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)
2005[b]	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).

with mixed infections. It is clear that enhanced testing, and particularly use of NATs, allows identification of more mixed respiratory virus outbreaks (and samples containing more than one detectable target). A breakdown of results for respiratory outbreaks is given in Table 2.

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).

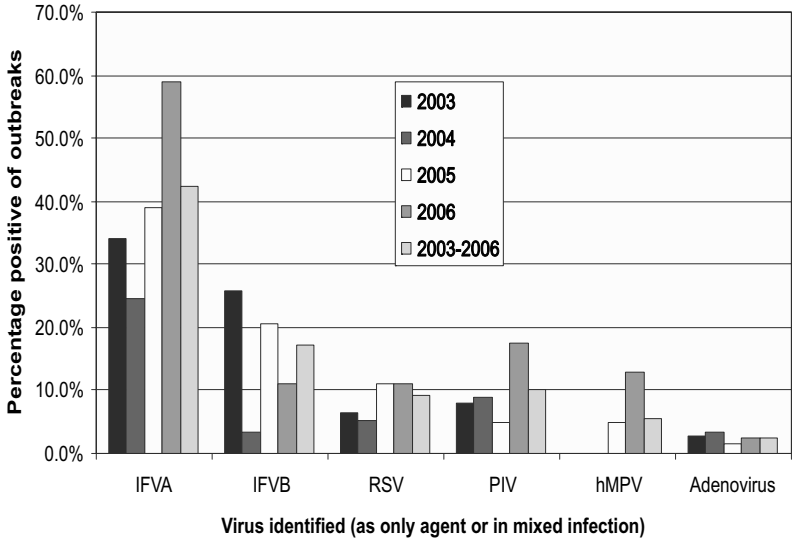

Fig. 3. Analysis of viral causes of outbreaks. Data are from outbreaks investigated for a possible respiratory virus etiology. A change in testing algorithm was implemented in November 2005 which included NAT for DFA-negative NP samples (and for all non-NP samples). Human metapneumovirus (hMPV) testing was incorporated into outbreak investigations in November 2005. Total number of outbreaks was 496 over this time period. IFV = influenza virus, PIV = parainfluenzavirus, RSV = respiratory syncytial virus, hMPV = human metapneumovirus.

Table 2
Analysis of outbreaks where a viral etiology was confirmed[a]

Virus/mix identified	Number (%) of positive outbreaks	
	2003–2005	2006
Single etiology	185 (86.4)	93 (69.4)
Mixed IFVA and IFVB	9 (4.2)	3 (2.2)
IFVA or IFVB with other virus(es)	16 (7.5)	32 (23.9)
Non-IFV mixed virus	4 (1.9)	6 (4.5)
Total outbreaks	214 (100)	134 (100)

[a] Samples were a mix of respiratory specimens collected and tested (all methods) 2003–2006.
Prior to November 2005 outbreak investigation utilized DFA/culture for IFVA, IFVB, PIV 1–3, RSV and adenoviruses. After November 2005 DFA-negative NP samples (and all non-NP samples) were subjected to NAT panel for IFVA, IFVB, PIV 1–4, RSV, hMPV and adenoviruses.
IFV = influenza virus, PIV = parainfluenzavirus, RSV = respiratory syncytial virus, hMPV = human metapneumovirus, DFA = direct fluorescent antigen, NP = nasopharyngeal.

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; Khetsuriani 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; Khetsuriani 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 mciroarray 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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