Enhanced Viral Etiological Diagnosis of Respiratory System Infection Outbreaks by Use of a Multitarget Nucleic Acid Amplification Assay

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A study was undertaken to assess the utility of the xTAG respiratory viral panel (RVP) for enhanced laboratory investigation of respiratory outbreaks. Specimens (n = 1,108) from 244 suspected respiratory virus outbreaks in 2006 and 2007 in Alberta, Canada, were included in the study. Testing by direct fluorescent antigen detection (DFA) and various in-house nucleic acid amplification tests (NATs) for common respiratory viruses provided an etiological diagnosis in 177 outbreaks (72.5%), with 524 samples testing positive (47.3%) for a respiratory virus. Two hundred samples from 51 unresolved outbreaks were further tested by RVP retrospectively. Fifty-eight samples from 30 unresolved outbreaks had a respiratory virus detected by RVP (47 picornavirus-positive, 9 coronavirus-positive, and 2 influenza virus A-positive samples). Overall, detection of a viral etiological agent was achieved in 90.8% of outbreaks using a combination of DFA, NATs, and RVP. Use of RVP enhances the laboratory investigation of respiratory virus outbreaks and facilitates appropriate patient and outbreak management.

Identification of the etiological agent is important for the management and control of respiratory outbreaks. In the case of viruses where we have options for prophylaxis and therapy (such as influenza virus [IFV]), it is particularly important that sensitive and specific outbreak diagnosis be undertaken in a timely manner (2, 11). Moreover, the identification of circulating respiratory agents in long-term and assisted care centers (LTAC), schools, and day cares provides useful surveillance data for viral epidemiology in the community (6, 12, 18). Respiratory tract infections are responsible for a significant proportion of acute morbidity and physician or emergency room visits, especially where they occur in the very young (36) and the elderly (12). There is considerable clinical overlap between infections caused by different respiratory viruses, and accurate clinical diagnosis cannot be based on symptoms alone (10, 11).

Direct fluorescent antigen detection (DFA) testing and cell culture are the traditional gold standard diagnostic tests for common respiratory viruses (16). Real-time nucleic acid amplification tests (NATs) are more sensitive than DFA and viral culture and can identify a broader range of viruses (13, 14, 22). However, testing for all respiratory viral targets using individual real-time NATs is expensive and laborious. Multiplexing of real-time NATs can reduce the assay sensitivity and is limited compared with antigen detection and culture (27) and in-house real-time NATs (32). The xTAG respiratory viral panel (RVP) from Luminex Molecular Diagnostics (Toronto, Ontario, Canada) allows for multiplex detection of up to 20 different respiratory viral targets using suspension microarray technology. Recent studies show that the RVP assay is sensitive and specific compared with antigen detection and culture (27) and in-house real-time NATs (32). In this study we describe the utility of the RVP assay for detection of respiratory viruses causing outbreaks in Alberta, Canada.

Simultaneous detection of a panel of respiratory viruses using multiplex PCR amplification and detection of products by suspension microarray is one of the most promising approaches for broad detection of respiratory viruses (7, 23, 25, 27, 29, 32). The xTAG respiratory viral panel (RVP) from Luminex Molecular Diagnostics (Toronto, Ontario, Canada) allows for multiplex detection of up to 20 different respiratory viral targets using suspension microarray technology. Recent studies show that the RVP assay is sensitive and specific compared with antigen detection and culture (27) and in-house real-time NATs (32). In this study we describe the utility of the RVP assay for detection of respiratory viruses causing outbreaks in Alberta, Canada.

MATERIALS AND METHODS

Clinical specimens and testing algorithm. Respiratory outbreaks were defined by public health professionals based on the presence of epidemiologically linked symptomatic cases in settings such as LTAC (facilities including lodges for seniors, assisted care, and group homes), schools, day cares, hospitals (acute), and closed communities (e.g., single households). The Provincial Laboratory for Public Health (ProvLab) provides laboratory investigations for all respiratory outbreaks in the province of Alberta (population greater than 3.3 million). The most common specimen types submitted for laboratory diagnosis of respiratory virus outbreaks are nasopharyngeal (NP) swabs/aspirates and throat swabs (TS) in universal transport medium (Copan Diagnostic Inc., Corona, CA).

During 2006 to 2007 at the ProvLab, NP samples were first subjected to DFA using monoclonal antibodies from Imagen (Lenexa, KS) for IFVA, IFVB, respiratory syncytial virus (RSV), and parainfluenza viruses (PIV) 1 to 3. DFA-negative NP specimens were tested by a panel of in-house real-time NATs for IFVA and IFVB, RSV (A and B), PIV 1 to 4, human metapneumovirus (hMPV), and respiratory adenoviruses (AdVs) (13, 15). All other specimen types (mainly TS in the case of outbreaks) were subjected to the above-mentioned NATs without DFA testing (13, 15). In this study, samples from outbreaks without any etiological diagnosis after DFA and NAT testing were tested using the RVP assay.

Extraction of specimens, NATs, and RVP. Nucleic acid extraction from respiratory specimens was performed using the easyMAG and associated reagents (bioMérieux, St. Laurent, Quebec, Canada) as described previously (32, 38).
TABLE 1. Location and specimen type for respiratory virus outbreaks during 2006 and 2007 in Alberta, Canada

<table>
<thead>
<tr>
<th>Yr</th>
<th>Total No. (%) of outbreaks in:</th>
<th>No. (%) of tested specimens</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Long-term care</td>
</tr>
<tr>
<td>2006</td>
<td>160</td>
<td>82 (51.2%)</td>
</tr>
<tr>
<td>2007</td>
<td>84</td>
<td>66 (78.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>148 (60.6%)</td>
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</table>

* Others: nose swabs, ocular swabs, sputum, tracheal aspirates, and bronchoalveolar lavage fluid.

Real-time NATs were performed according to previously published methods for IFV A and B, RSV (A and B), PIV 1 to 4, hMPV, and AdV using the easyQ platform (bioMérieux) for real-time nucleic acid sequence-based amplification, and 7900 SDS for other real-time assays (Applied Biosystems, Foster City, CA) (13, 20, 22, 31, 34, 38).

As described above, specimens from outbreaks from 2006 to 2007 that did not have an identifiable respiratory virus were tested by the RVP assay (n = 200). Targets included in the RVP were IFV A, H1 and H3 subtyping; IFV B; RSV (A and B); PIV 1 to 4; hMPV; AdV; human coronaviruses (hCoVs) 229E, OC43, NL63, and HKU1; and picornaviruses including enteroviruses and human rhinoviruses (HRVs). RVP was performed according to the manufacturer’s instructions with the exception that specimens included in this study were primarily extracted for in-house NATs; thus, bacteriophage MS2 was not spiked into the samples as an internal control prior to extraction. MS2 RNA was added into the master mix for use as an amplification and detection control only. Although the RVP has been cleared by the FDA for the majority of targets, the hCoV and PIV 4 components included in this study are not included in the FDA-cleared version of the assay.

Confirmation of RVP-positive samples. Samples which gave a positive result by RVP for targets not included in the DFA/NAT screen such as hCoVs and picornaviruses were subjected to in-house real-time NATs for the same targets as confirmation (22) (our unpublished data).

Further characterization of picornavirus-positive samples was undertaken by reverse transcription-PCR of the 5' noncoding region (as detailed below) (24). A total of 47 samples containing detectable picornavirus sequences by the RVP assay were subjected to a first round of amplification directly from the sample extract using primers P1-1 (CAAGCACTTCTGTYWCCCC) and P2-1 (AAGCAACCAATTCAGGAGCC), and seminested amplification was performed using forward primer P1-1 and three reversed primers, P2-1 (CAAGCACTTCTGTYWCCCC), P2-2 (TTAGCCACATTCAGGAGCC), and P2-3 (TTAGCCGACATTAGGGG) as described previously.

Statistical analysis. The SPSS software, version 16.0 (SPSS Inc., Chicago, IL) was utilized for statistical analysis of the data. The McNemar test was used to assess the impact of RVP on outbreak resolution (compared with DFA and in-house NATs). Association of outbreaks with particular seasons was assessed using Pearson chi-square analysis. In all cases, a P of <0.05 was utilized to denote a statistically significant difference between parameters compared. Variation in patients’ ages was expressed as a standard deviation, and differences in results between age groups was assessed by χ² analyses.

RESULTS

Outbreak investigation analysis. During the course of this study, 244 outbreaks related to respiratory illness in LTAC, schools/day cares, hospitals, and the community (within a household environment) were declared, with 160 (65.6%) from 2006 and 84 (34.4%) from 2007 (Table 1). The median numbers of samples per outbreak in 2006 and 2007 were 7.0 (range: 1 to 21) and 7.5 (range: 1 to 19), respectively. The winter season in Alberta is considered to be from October to March of each year. The number of respiratory outbreaks was significantly higher in the winter months than in the rest of the year in 2006 and 2007 (χ²; P = 0.0035). Numbers used for computation of these statistics are provided in Fig. 1.

A total of 1,108 specimens were submitted from the 244 outbreaks, with 711 from 2006 and 397 from 2007. The majority of specimens received from these outbreaks were NP aspirates or swabs (n = 939; 84.7%) or TS (n = 164; 14.8%). Other specimen types comprised nose swabs, ocular swabs, sputum, tracheal samples, and bronchoalveolar lavage fluid (n = 5; 0.5%). Similar distributions of specimen types were seen in 2006 (569 [80.0%] NP swabs/aspirates and 139 [19.6%] TS) and 2007 (370 [93.2%] NP swabs/aspirates and 25 [6.3%] TS) The distributions of sample types tested in 2006 and 2007 are shown in Table 1.

In 2006, the outbreak settings were distributed equally among LTAC and schools/day cares: 51.2% and 46.2%, respectively. In 2007, 78.6% of the outbreaks were in LTAC and 9.5% in schools/day cares, with a small number of outbreaks in hospital acute care and community settings, as shown in Table 1. The median age of patients tested in 2006 was 71.5 years (range: 23 days to 105 years), and it was 81.1 years (range: 2 months to 101 years) in 2007.

DFA and in-house NAT results. Table 2 provides a summary of the number of outbreaks and specimens tested; positive results obtained by the different methods are also shown. A total of 711 samples from 160 outbreaks were submitted in 2006 for diagnosis of respiratory viruses. Of these, 382 (53.7%) samples from 134 (83.8%) outbreaks gave a positive result for one or more respiratory viral targets by DFA and/or NATs. In 2007, 397 samples were tested from 84 outbreaks. Of these, 142 (35.8%) samples from 43 (51.2%) respiratory outbreaks gave positive results for one or more respiratory viral pathogens using our DFA and/or NAT algorithm.

Distribution of respiratory viruses identified by DFA/NATs. Respiratory viral targets were identified in 177 outbreaks by DFA and/or NATs in 2006 and 2007. IFVs were a major cause of respiratory viral outbreaks, as shown in Fig. 2, and IFVA and IFVB were detected as the only etiological agent in 36.9% (n = 90) and 45.8% (n = 11) of total outbreaks (n = 244), respectively. IFV was identified with other non-IFV respiratory viruses in 16.4% of outbreaks (n = 40). Viruses other than IFVA or -B detected as the etiological agents in outbreaks were RSV (3.7%; n = 9), PIV (4.9%; n = 12), and hMPV (3.7%; n = 9); in addition, there were six outbreaks (2.5%) due to non-IFV mixed viruses. As a result, in 27.5% (n = 67) of the total outbreaks (n = 244), no etiological agent was found. Total diagnosis rate for respiratory viruses by DFA and NATs in the 2 years combined was 72.5% (n = 177) (Table 2 and Fig. 2).

The ages of patients with a positive DFA or NAT result ranged from 3 months to 106 years, (median age = 70.0 years) in 2006. DFA- or NAT-positive outbreaks in 2006 (n = 134)
occurred in all settings tested, including 64 (47.8%) in LTAC, 69 (51.5%) in schools/day cares, and 1 (0.7%) in the community. The ages of patients with a virus identified for their symptoms as part of an outbreak in 2007 ranged from 18 months to 100 years (median age = 81.2 years) in 2007. The distribution of outbreaks due to viruses detected by DFA or NAT (n = 43) was 32 (74.4%) in LTAC, 4 (9.3%) in schools/day cares and 7 (16.3%) in hospitals.

**Luminex xTAG RVP results.** RVP testing was performed on available samples from outbreaks which were not resolved after testing by DFA/NATs. A total of 200 specimens (2006, 72; 2007, 128) from 51 outbreaks (2006, 22; 2007, 29) that were tested previously by DFA or NATs without positive results were available for further testing by RVP retrospectively (Table 2). The total number of outbreaks used for analysis by a combination of all testing methods was 228.

**Analysis of RVP results for 2006.** For samples from 2006, RVP gave positive results for 20 samples from 12 outbreaks (LTAC, n = 10; schools/day cares, n = 1; hospital, n = 1) without an identified cause by DFA/NAT. RVP testing was not performed for four unresolved outbreaks for which samples were not available. Of these 20 positives detected by the RVP

![FIG. 1. Monthly distribution of outbreaks analyzed by a combination of testing methods. The numbers of resolved outbreaks reported by DFA and NATs, additional positive results by RVP, and unresolved outbreaks not tested by RVP in 2006 and 2007 are included. October to March was considered the winter season in Alberta, Canada, as denoted by the double-headed arrows.](http://jcm.asm.org/)

**TABLE 2. Summary of outbreak investigations by DFA, NATs, and RVP assays**

<table>
<thead>
<tr>
<th>Yr</th>
<th>Outbreaks positive by DFA/NATs</th>
<th>Outbreaks negative by DFA/NATs and positive by RVP</th>
<th>Outbreaks positive by DFA/NATs/RVP&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Outbreak samples positive by DFA/NATs</th>
<th>Outbreak samples negative by DFA/NATs and positive by RVP</th>
<th>Outbreak samples positive by DFA/NATs/RVP&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>2006</td>
<td>134/160 (83.8)</td>
<td>12/22 (54.5)</td>
<td>146/156 (93.6)</td>
<td>382/711 (53.7)</td>
<td>20/72 (27.8)</td>
<td>402/677 (59.4)</td>
</tr>
<tr>
<td>2007</td>
<td>43/84 (51.2)</td>
<td>18/29 (62.1)</td>
<td>61/72 (84.7)</td>
<td>142/397 (35.8)</td>
<td>38/128 (29.7)</td>
<td>180/355 (50.7)</td>
</tr>
<tr>
<td>Total</td>
<td>177/244 (72.5)</td>
<td>30/51 (58.8)</td>
<td>207/228 (90.8)</td>
<td>524/1,108 (47.3)</td>
<td>58/200 (29.0)</td>
<td>582/1,032 (56.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only samples and outbreaks available for analysis by RVP are included in these columns.

<sup>b</sup> Samples from 4 outbreaks in 2006 and 12 in 2007 were not available for RVP testing as indicated in Results.
assay, 17 samples from 9 outbreaks were positive for picornaviruses. Other respiratory viruses identified in individual samples by RVP were hCoV OC43 \((n = 1)\), hCoV HKU1 \((n = 1)\), and IFVA \((n = 1)\). Thus, use of the RVP assay increased the proportion of resolved outbreaks from 83.8% to 93.6% in 2006 (Table 2). The monthly distribution of resolved and unresolved outbreaks is shown in Fig. 1. The resolved outbreaks \((n = 12)\) were in all settings tested, including nine (75.0%) in LTAC, one (8.3%) in a school, and two (16.7%) in acute care hospitals. The breakdown of positive results for outbreaks by month is given in Fig. 2. The ages of patients with a positive RVP result ranged from 2 months to 100 years (median age 83.8 years) in 2006. The ages of patients giving a positive result by RVP were not significantly different from those of patients giving a negative result \((\chi^2; P = 1.000)\).

**Analysis of RVP results for 2007.** Of the 128 samples from 29 unresolved outbreaks in 2007, 38 samples (from 18 outbreaks) gave a positive result by the RVP assay. Samples from 12 unresolved outbreaks were unavailable for RVP testing. The targets detected in samples from outbreaks without a causative agent identified by DFA/NAT were picornavirus \((n = 30)\), hCoV NL63 \((n = 5)\), hCoV OC43 \((n = 2)\), and IFVA \((n = 1)\). This resulted in an etiological diagnosis for 18 outbreaks which were not resolved by DFA/NAT, 17 from LTAC and 1 from the community (household). The outbreaks resolved by RVP were distributed throughout the year as shown in Fig. 1. Thus, use of the RVP assay increased the proportion of resolved outbreaks from 51.2% to 84.7% in 2007 (Table 2). The monthly distribution of resolved and unresolved outbreaks is shown in Fig. 1. The breakdown of positive results for outbreaks by month is given in Fig. 2.

The ages of patients with a positive target detected by RVP ranged from 28 to 100 years (median age 85.7 years) in 2007. The ages of patients giving a positive result by RVP were not significantly different from those of patients with a negative result \((\chi^2; P = 0.1753)\).

**Analysis of results for all outbreaks 2006 and 2007.** Viral etiological agents detected in outbreaks \((n = 244)\) by a combination of all three methodologies (DFA or NAT and RVP) are summarized in Fig. 3. Thirty samples from 51 outbreaks unresolved by DFA/NAT gave positive results for picornavirus \((n = 22)\), hCoV \((n = 6)\), and IFVA \((n = 2)\) by RVP.

By combining all three methodologies, 207 of 228 (90.8%) outbreaks were resolved (Table 2 and Fig. 1). A significant difference was observed in a comparison of resolved outbreaks with and without RVP testing in both 2006 and 2007 \((P \leq 0.0257;\) McNemar analysis).

**Seasonality of respiratory outbreaks.** Figure 2 shows the distribution of viral targets over the different months in 2006 and 2007 identified by a combination of all three methods. IFVA was detected in outbreaks during the months of November to May; IFVB-related outbreaks were not as prevalent as
IFVA-related outbreaks during the study period and were identified from November to February and also in May. hMPV was associated with outbreaks from November to March, and RSV was associated with outbreaks from October to March. PIV was detected in all months tested except the summer months of July, August, and September. In 2006, AdVs were identified and found as the etiological agents in the months of February, October, and December. Coronaviruses were restricted to the winter months of January, February, and March, and picornaviruses were distributed throughout the 2 years. AdVs were not responsible for any outbreak in 2007.

There was a significant difference in the numbers of outbreaks resolved by DFA/NAT in winter months (October to March) and nonwinter months ($\chi^2; P < 0.0001$), presumably due to the predominance of IFV, RSV, and PIV infections in winter. After the additional RVP results were included, there was a trend toward enhanced resolution of outbreaks in nonwinter months (April to September) compared with winter months, but this did not reach statistical significance ($\chi^2; P = 0.0940$). It is likely that, because of the enhanced detection of hCoVs and picornaviruses, the RVP had more impact on identification of a viral etiology for nonresolved outbreaks in the nonwinter months although fewer respiratory outbreaks are identified during this time.

**Confirmation of RVP-positive samples.** Samples that gave a positive result by the RVP assay for IFVA and hCoVs (OC43, NL63, and HKU1) were confirmed by in-house NATs (13, 22, 31; our unpublished data). Confirmation of a picornavirus-positive result by amplification and sequencing of the 5' non-coding region of HRV was successful in 44 of 47 positive samples using primers reported previously (24). No amplified product was obtained for three picornavirus-positive samples identified by RVP, likely due to a low viral load in the sample. Interestingly, many of the rhinoviruses associated with outbreaks belonged to the divergent group reported to be associated with childhood infection (24). Further detailed analysis of these HRVs will be undertaken in future studies.

**DISCUSSION**

Respiratory virus outbreaks are associated with a considerable impact on health care resources (3, 19, 35). There is a limitation in using DFA and culture for detection of respiratory viruses because of low sensitivity and the range of viral pathogens which can be identified. Probe-based NATs, in a real-time detection format, are sensitive and specific but can be expensive and laborious when a broad range of respiratory viruses need to be identified. The use of a panel of singleplex or small multiplex real-time assays for detection of the main causes of respiratory virus outbreaks would not be practical in many diagnostic laboratories, nor would such an approach be cost-effective where a full range of possible viral causes needs to be investigated.

The use of technology involving multiplex amplification of targets with detection of products using suspension microarrays is now well established and has been applied to the diagnosis of individual cases of respiratory virus infection with great success (7, 23, 25, 27, 29, 32). In this study, we investigated the utility of the xTAG RVP assay for detection of respiratory viruses associated with outbreaks for which a viral cause could not be found using DFA/NAT combination. The RVP assay provides for the additional identification of coronaviruses (229E, OC43, NL63, and HKU1) and picornaviruses and the subtyping of IFVA hemagglutinin, which are not included in our routine diagnostic algorithm using DFA and in-house NATs.

Despite the use of a range of NATs, we were able to find a viral cause for respiratory disease outbreaks in only 50% of outbreaks in 2007 using our then-routine algorithm of DFA/NAT combination. As expected, RVP had the most impact on identification of viruses not included in our testing algorithm (especially rhinoviruses), but it also identified two IFVA-positive samples which had been missed by DFA/NAT in 2006 and 2007.

Although it has been reported that HRVs cause a significant
proportion of acute infections in children and may be associated with lower respiratory tract infections and severe presentations (4, 30), the impact of such infections in the elderly has not been reported widely. In Alberta in 2007, outbreaks found to be associated with HRV infection after RVP testing caused considerable concern and resulted in severe symptoms in elderly individuals as part of outbreaks at LTAC. We found that rhinoviruses were an important cause of outbreaks outside of the winter months and especially impacted LTAC. The presentation of HRV infection is clearly not always mild, and the cause of “off season” outbreaks could not have been predicted easily based on clinical presentation. Interestingly, many of the HRVs found associated with outbreaks in 2006 and 2007 were closely related to a novel group of viruses identified recently (24, 30).

Although we did not find many outbreaks caused by hCoVs in Alberta in 2006 to 2007, such infections can be quite variable from year to year. In one study, hCoV OC43 was shown to be associated with outbreaks in elderly care facilities with “IFV-like” clinical presentation (5). Despite early data indicating that hMPV may not be associated with significant disease in adults, we (34) and others (6, 9, 21, 26, 31, 37) have shown that hMPV is also associated with respiratory disease outbreaks, with the elderly being particularly vulnerable to severe presentations. Thus, the broad-spectrum detection of viruses which cannot be identified easily by DFA/culture (for example, hCoVs and hMPV) using assays such as RVP will be very important in enhancing our understanding of respiratory virus morbidity and mortality.

In this study, we investigated the utility of RVP for providing enhanced etiological diagnosis of respiratory outbreaks compared to DFA/NAT testing. Our previous studies confirmed the excellent sensitivity and specificity of RVP for detection of the main causes of respiratory virus outbreaks in Alberta (IFVA, IFVB, hMPV, RSV, and PIV 1 to 3) (32). We were also able to show that, in the majority of cases, we were able to obtain subtyping information for seasonal IFVA simultaneously with a positive result by RVP. This provides critical information to guide prophylaxis and therapy, as IFVA H3 viruses resistant to adamantanes and IFVA H1 viruses resistant to neuraminidase inhibitors are increasingly identified (1, 8, 33). Based on these data, the Luminex xTAG RVP assay has been implemented in our laboratory for investigation of acute respiratory infection outbreaks that have no etiological diagnosis by DFA. The RVP has thus replaced in-house NATs in our routine testing algorithm, providing an efficient and cost-effective means of sensitive, broad respiratory virus testing.

A recent analysis of the use and clinical impact of RVP confirmed that this test is very cost-effective compared with DFA and culture when the prevalence of infection is >11% (28). Using a combination of DFA and RVP had an intermediate cost and provides the best combination of rapid turnaround and sensitivity for many laboratories. Although clearly this approach provides expanded detection of respiratory viruses, there is still scope for increasing the number of viruses detected. In a recent study, the RVP test demonstrated superior sensitivity for the detection of all IFV strains, including the novel swine origin H1N1 (17). Further evaluation and appropriate enhancement of the assay will be needed to ensure that novel viruses (including potential pandemic IFVs) are detected by RVP and other multiplexed NATs. Inclusion of viruses such as human bocavirus and respiratory polyomaviruses and bacteria causing overlapping symptoms will enhance our ability to identify causes for respiratory infection and disease.

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REFERENCES

18. Halasa, N. B., J. V. Williams, G. J. Wilson, W. F. Walsh, W. Schaffner, and...


