

**The interaction between respiratory viruses and pathogenic bacteria in the upper respiratory tract of asymptomatic Aboriginal and non-Aboriginal children**

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**Abbreviated title:** Interaction of respiratory viruses and bacteria in healthy children

**Running head:** Viral-bacterial interactions

## ABSTRACT

**Background:** Associations between respiratory viruses and the bacterial pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* may be important in the pathogenesis of otitis media (OM). However, data on asymptomatic identification rates of respiratory viruses are limited, particularly in Indigenous populations, who suffer a high burden of OM.

**Methods:** We describe the identification of respiratory viruses alone and in combination with pathogenic OM bacteria in 1006 nasopharyngeal aspirates collected from asymptomatic Aboriginal and non-Aboriginal children in a longitudinal community-based cohort study in rural Western Australia.

**Results:** Viruses were identified in 42% of samples from Aboriginal and 32% from non-Aboriginal children. Rhinoviruses were the most frequently identified virus with higher identification rates in Aboriginal (23.6%) than non-Aboriginal children (16.5%;  $P = 0.003$ ). Rhinoviruses were associated with *H. influenzae* (OR 2.24, 95%CI 1.24-4.07 for Aboriginal children) and *M. catarrhalis* (OR 1.94, 95%CI 1.05-3.57 for Aboriginal children). Adenoviruses were positively associated with *H. influenzae* in Aboriginal children (OR 3.30, 95%CI 1.19-9.09) and *M. catarrhalis* in non-Aboriginal children (OR 5.75, 95%CI 1.74-19.23), but negatively associated with *S. pneumoniae* in Aboriginal children (OR 0.39, 95%CI 0.18 – 0.84).

**Conclusions:** We found a high identification rate of rhinoviruses and adenoviruses in asymptomatic children. The associations between these viruses and OM bacteria have implications for preventive strategies targeted at specific pathogens.

## INTRODUCTION

Otitis media (OM) is a common childhood illness accounting for a significant proportion of doctor consultations and antibiotic prescriptions.<sup>1-3</sup> In industrialised countries 10-20% of children will suffer more than 3 episodes of OM during the first year of life.<sup>4</sup>

Aboriginal Australian children experience exceptionally high rates of OM and its complications, in particular hearing loss.<sup>5</sup> The peak prevalence of OM in our Kalgoorlie Otitis Media Research Project (KOMRP) was 72% in Aboriginal children aged 5-9 months and 40% in non-Aboriginal children aged 10-14 months.<sup>6</sup> *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* are the 3 most common bacterial pathogens associated with OM in both Indigenous and non-Indigenous populations in Australia and elsewhere<sup>5, 7, 8</sup> and early onset of upper respiratory tract carriage of these pathogens is associated with increased risk of OM.<sup>5, 9</sup> In the KOMRP carriage rates for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were 2-3 times higher in Aboriginal children than in non-Aboriginal children.<sup>8</sup>

Respiratory syncytial virus (RSV), influenza viruses A and B, coronaviruses, adenoviruses, parainfluenza viruses (PIV), rhinoviruses, enteroviruses and mumps viruses have been associated with OM<sup>10-18</sup> and several studies suggest that viruses predispose to acute OM.<sup>10, 19, 20</sup> Furthermore, rhinoviruses have been associated with carriage of *M. catarrhalis* and *S. pneumoniae* in otitis-prone children<sup>21</sup> and synergism has been described between influenza viruses and *S. pneumoniae*.<sup>22</sup>

In order to understand the role of viruses in the etiology and pathogenesis of OM, it is necessary to know the prevalence of viruses in asymptomatic children. This is particularly relevant with increasing use of polymerase chain reaction (PCR) techniques

which have generally resulted in higher detection rates in both symptomatic and asymptomatic subjects.<sup>23, 24</sup> In the only small study to date in Aboriginal Australian infants, viruses were generally identified after bacterial colonisation and onset of OM.<sup>5</sup> However the study was conducted before more sensitive PCR technology became available. There have been no studies reporting on viral identification rates in asymptomatic Australian children.

Using data collected in the KOMRP, we now describe the respiratory viruses identified in asymptomatic Aboriginal and non-Aboriginal children and the relationships between respiratory viruses and bacterial OM pathogens. We hypothesise that Aboriginal children have higher rates of asymptomatic viral identification than non-Aboriginal children and that both rhinoviruses and adenoviruses are associated with increased risk of nasopharyngeal carriage of bacterial OM pathogens in Aboriginal and non-Aboriginal children at the microbe level.

## **MATERIALS AND METHODS**

### **Study population**

Kalgoorlie-Boulder is the largest town in the Goldfields region of Western Australia, located 600km east of Perth in a semi-arid zone. The KOMRP has been described in detail elsewhere.<sup>6, 8</sup> In brief, between April 1999 and January 2003, 100 Aboriginal and 180 non-Aboriginal children living within a one-hour drive of Kalgoorlie-Boulder were enrolled at birth and followed up regularly to age 2 years. Multiple births and children with severe congenital abnormalities or birthweight <2000g were excluded. A total of 1559 nasopharyngeal aspirates (NPAs) were collected during routine follow-up visits at 1-3 weeks, 6-8 weeks and again at 4, 6, 12, 18 and 24 months. To identify viruses in the

nasopharynx, we selected all NPAs from children who had at least four specimens collected during the study. Thus, 1006 NPAs from asymptomatic Aboriginal and non-Aboriginal children were available for virology testing. We tested the first 396 specimens that we collected for rhinoviruses, adenoviruses, RSV, influenza viruses A and B, coronaviruses, PIV and human metapneumoviruses (HMPV). Because of financial constraints we restricted virology testing of the remaining 610 specimens to rhinoviruses, adenoviruses, RSV and influenza A and B viruses.

### **Laboratory methods**

Nasopharyngeal aspirates (NPAs) were collected at each visit. One mL of saline was then added to the specimen which was stored for viral identification. For bacterial culture, a 0.5 mL volume of mucus plug, or if no visible plug, the gently mixed specimen, was pipetted into 1 mL of skim milk-tryptone-glucose-glycerol broth. All samples were stored at -20°C until sent to the study laboratory in Perth on dry ice, usually within 72 hours, for long-term storage at -70°C. Methods used for primary isolation and identification of bacterial pathogens in NPA specimens have been described previously.<sup>8</sup> Primary inocula were made on selective media and organisms of interest were subcultured and subjected to confirmatory tests using standard methods.<sup>25</sup> To identify viruses, nucleic acid was extracted from samples using the QIAmp Viral RNA kit (QIAGEN Sciences, Maryland, USA) in accordance with the manufacturer's protocol. In-house modified nested or semi-nested PCR amplification was performed for rhinoviruses, adenoviruses, RSV, HMPV, influenza viruses A and B, coronaviruses and PIV types 1–3. Amplicons were detected by ethidium bromide agarose gel electrophoresis.

## Statistical analysis

NPA specimens were grouped into seven age categories (<1, 1-2, 3-4, 5-9, 10-14, 15-19 and  $\geq 20$  months), based on the timing of scheduled follow-up visits. The viral identification rate was defined as the proportion of specimens positive for a particular virus. Chi-square tests were used to compare viral identification rates between Aboriginal and non-Aboriginal children. Adenoviruses and rhinoviruses were the only viruses identified in sufficient numbers for further analysis. We used logistic regression models incorporating generalized estimating equations (GEE) adjusted for age, gender and the proportion of virus-positive samples for the virus under investigation for each child to examine associations between rhinoviruses or adenoviruses and simultaneous carriage of *S. pneumoniae*, *M. catarrhalis* or *H. influenzae*. This method was used as our study consisted of correlated longitudinal data, with children having multiple specimens collected over the duration of the study. A GEE model accounts for correlated observations and therefore produces more accurate standard errors.<sup>26</sup> Separate models were used for Aboriginal and non-Aboriginal children. These semi-adjusted models were investigating the overall interactions between viruses and bacteria at the microbe level in Aboriginal and non-Aboriginal children. To investigate independent effects between rhinoviruses or adenoviruses and pathogenic bacteria, further models were developed adjusting for the same factors as the semi-adjusted models as well as for the presence of other bacteria and rhinoviruses or adenoviruses. In both semi-adjusted and fully-adjusted models we determined odds ratios (OR) with 95% confidence intervals to indicate the strength of the associations. An OR >1 represents a positive association between the identification of the virus and bacteria under investigation and an OR <1 represents a negative association. All analyses were performed using Stata version 9.0 and SPSS version 15.0.

## **Ethical approval**

The study design and protocol for the KOMRP were approved by the Western Australian Aboriginal Health and Information Ethics Committee, the Northern Goldfields Health Service and Nursing Education Ethics Committee in Kalgoorlie, Princess Margaret Hospital for Children Ethics Committee and the Confidentiality of Health Information Committee of the Health Department of Western Australia.

## **RESULTS**

### **Nasopharyngeal specimens**

The 1006 specimens tested for the presence of respiratory viruses were from 79 Aboriginal children (436 specimens, average 5.5 per child) and 88 non-Aboriginal children (570 specimens, average 6.5 per child). In Aboriginal children, 262 (60.1%) specimens were from boys and in non-Aboriginal children 305 (53.5%) were from boys. Generally, specimens were equally distributed across the seven age groups, but there were fewer specimens from Aboriginal children aged  $\geq 20$  months (10.8%,  $n=47$ ) than younger Aboriginal children (eg. 5-9 months 16.3%,  $n=71$ ;  $P = 0.56$ ).

### **Viruses identified in nasopharyngeal specimens**

In the 396 samples that were tested for all 7 viruses, one or more viruses were identified in 42.1% of samples from Aboriginal children and 31.5% of samples from non-Aboriginal children. Overall, rhinoviruses were the most frequently identified viruses (19.6%), followed by adenoviruses, coronaviruses, PIV, HMPV, RSV and influenza A and B viruses (Table 1). Rhinoviruses and adenoviruses were identified more often in NPAs collected from Aboriginal children than from non-Aboriginal children (23.6% vs

16.5% for rhinoviruses and 8.5% vs 3.5% for adenoviruses, Table 1). The proportion of samples positive for rhinoviruses increased to 35.2% in Aboriginal children and 22.1% in non-Aboriginal children by age 5-9 months and declined thereafter (Fig. 1). Adenoviruses were most frequently identified at age 10-14 months (18.6% in Aboriginal and 8.6% in non-Aboriginal children, Fig. 1).

### **Associations between viruses and bacterial OM pathogens**

Table 2 (online only) shows the co-occurrence of *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* and adenoviruses or rhinoviruses in Aboriginal and non-Aboriginal children. Overall, one or more of the bacterial OM pathogens co-occurred with one or more viruses in 70% (307/436) of specimens from Aboriginal children and 45% (259/570) of specimens from non-Aboriginal children. When rhinoviruses or adenoviruses were not identified, a higher proportion of specimens also had no OM bacteria identified compared with rhinovirus- and adenovirus-positive specimens. For example, in Aboriginal children, 37% of rhinovirus-negative specimens had no bacterial pathogens compared with 7% of rhinovirus-positive specimens. In Aboriginal children, all 3 bacterial OM pathogens were isolated from 42% of rhinovirus-positive specimens and from 49% of adenovirus-positive specimens (Table 2, online only). In non-Aboriginal children, co-occurrence of bacteria and viruses was less frequent than among Aboriginal children. In 44% of rhinovirus-positive specimens from non-Aboriginal children and in 20% of adenovirus-positive specimens, none of the 3 bacterial OM pathogens were isolated. All 3 bacterial OM pathogens were isolated from 6% (n=6) of rhinovirus-positive specimens and 10% (n=2) of adenovirus-positive specimens (Table 2, online only). Two-thirds (n=4) of coronavirus-positive specimens in Aboriginal children also grew all 3 bacterial OM

pathogens, but none of the coronavirus-positive specimens in non-Aboriginal children grew all 3; rather 62.5% (n=5) of coronavirus-positive specimens grew no OM bacteria.

In regression models adjusting for age, gender and the proportion of rhinovirus-positive specimens per child, the presence of rhinoviruses significantly increased the odds of identifying each of the 3 bacterial OM pathogens in Aboriginal children (Table 3). The strongest association was between rhinoviruses and *H. influenzae* (OR 2.91, 95% CI 1.76-4.83). When these models were further adjusted for the presence of other bacteria and adenoviruses, all positive associations remained significant except for that with *S. pneumoniae*. While there was a positive association between rhinoviruses and the 3 bacterial OM pathogens in non-Aboriginal children, none of the associations reached statistical significance (Table 3).

Isolation of *H. influenzae* was also strongly associated with adenoviruses in Aboriginal children (OR 3.29, 95% CI 2.19-8.40) and this remained significant after adjusting for the presence of rhinoviruses and the other bacterial OM pathogens (Table 4). In the semi-adjusted model, no significant association was seen between adenoviruses and *S. pneumoniae* in Aboriginal children but in the model adjusting for the presence of other bacteria and rhinoviruses there was a significant negative association between these pathogens. The identification of adenoviruses resulted in a 61% reduction in the odds of *S. pneumoniae* isolation (OR 0.39, 95% CI 0.18-0.84, Table 4). There was a strong positive association between adenoviruses and *M. catarrhalis* in non-Aboriginal children, both in the semi-adjusted (OR 5.71, 95%CI 1.67-19.61) and the fully-adjusted models (OR 5.75, 95% CI 1.74-19.23, Table 4).

### **Simultaneous identification of viruses**

More than one virus was simultaneously identified in 32 specimens (12.5% of all virus-positive specimens), 22 (16.5% of all virus-positive specimens) from Aboriginal children and 10 (8.2% of all virus-positive specimens) from non-Aboriginal children.

Adenoviruses and rhinoviruses were identified simultaneously in 23 of these 32 specimens, 15 from Aboriginal children and 8 from non-Aboriginal children. In 1 specimen from an Aboriginal child, 3 viruses were identified simultaneously (rhinovirus, adenovirus and coronavirus). The remaining 9 specimens with multiple viruses (7 of which were from Aboriginal children) involved either a rhinovirus or an adenovirus identified simultaneously with a coronavirus on 4 occasions, HMPV on 2 occasions, RSV on 2 occasions and PIV on 1 occasion.

## **DISCUSSION**

This is the first report to describe nasopharyngeal carriage of respiratory viruses and their associations with respiratory bacteria in asymptomatic Indigenous and non-Indigenous children. In our study in rural Western Australia, Aboriginal children have a higher rate of respiratory viruses identified in the nasopharynx than non-Aboriginal children and are more likely to have viruses identified in conjunction with bacterial OM pathogens. We found positive associations between identification of rhinoviruses and each of the 3 bacterial OM pathogens in all children, between adenoviruses and *M. catarrhalis* in non-Aboriginal children, between adenoviruses and *H. influenzae* in Aboriginal children, but a negative association between adenoviruses and *S. pneumoniae* in Aboriginal children.

There are no similar Australian studies apart from one small study with Aboriginal infants conducted before availability of PCR.<sup>5</sup> In addition, few studies elsewhere have

investigated the presence of viruses in asymptomatic children and hence comparisons between our study and others are difficult. In a review comparing the identification of viruses by PCR and conventional diagnostic methods in asymptomatic subjects, Jarti and colleagues<sup>24</sup> reported similar PCR viral detection rates to those seen in non-Aboriginal children in our study for rhinoviruses (15.1% vs our 16.5%), adenoviruses (5.3% vs 3.5%), coronaviruses (2.5% vs 3.6%) and PIV (0.5% vs 1.8%). A Dutch study identified rhinoviruses in 28% of specimens at age 12 months and in 14% of specimens at age 24 months from asymptomatic children at routine health checks<sup>27</sup> with results similar to ours, although influenza viruses, coronaviruses and PIV were identified less often than in our study. Johnston and colleagues<sup>28</sup> reported a lower rate of detection of rhinoviruses (12%) from asymptomatic samples than our study. Another Dutch study identified viruses in 68% of asymptomatic children aged under 4 years attending general practitioners for non-respiratory illnesses,<sup>29</sup> with rhinoviruses being the most commonly identified pathogen, although unlike our study, no adenoviruses were identified. There are no data available in Indigenous populations from other parts of the world to compare with our findings. Australian Aboriginal children could be considered “otitis-prone” since they have a high burden of OM.<sup>6, 8</sup> A prospective Finnish study of otitis-prone children<sup>21</sup> identified rhinoviruses in 39% of specimens collected from asymptomatic children compared with our 24% in Aboriginal children, but did not identify enough adenoviruses to warrant analysis. From the limited comparisons that can be made, our study suggests a high prevalence of rhinoviruses and adenoviruses in the community.

There is a growing awareness of the need to characterize interactions between respiratory bacteria and viruses.<sup>30, 31</sup> Asymptomatic carriage of viruses may occur shortly before symptoms develop or represent prolonged viral shedding after an illness and may increase

the risk of secondary bacterial infections and disease including OM, especially in Aboriginal children. Additionally, it is important to know the relative contributions of viruses and bacteria to the burden of OM in different populations to ensure appropriate case management and the development of preventive strategies. Viral vaccines, in particular influenza vaccines,<sup>32, 33</sup> might play a role in preventing secondary bacterial infection and subsequent diseases such as OM.

Previously, we used multivariate random effects models<sup>19</sup> with the KOMRP data to differentiate between host-level (which takes into account impaired immunity and environmental factors such as crowding) and microbe-level correlations between bacterial and viral pathogens. We found associations primarily at the microbe-level for rhinoviruses, though in Aboriginal children there was also an association at the host-level for rhinoviruses and *S. pneumoniae*.<sup>19</sup> The viral-bacterial interactions at the microbe-level support the hypothesis that viruses predispose to bacterial adherence and colonization. In our current analysis, by adjusting for the proportion of rhinovirus- or adenovirus-positive specimens in the appropriate models, we are looking at associations only at the microbe-level. In our semi-adjusted models, our results are similar to those of the earlier modelling analysis. By further adjusting for the presence of other bacteria and either rhinoviruses or adenoviruses as appropriate, we have extended this analysis to investigate independent effects between a single virus and a single bacterium.

We have now found independent associations between rhinoviruses and both *M. catarrhalis* and *H. influenzae* in Aboriginal children, adenoviruses and *H. influenzae* in Aboriginal children and adenoviruses and *M. catarrhalis* in non-Aboriginal children. Our findings suggest synergism between both rhinoviruses and adenoviruses and these two

bacterial OM pathogens regardless of other pathogens that may be involved on a causal pathway. A Finnish study found that rhinoviruses were positively associated with *M. catarrhalis* and a trend towards a positive association was seen with *S. pneumoniae* but no association with *H. influenzae*,<sup>21</sup> which is partly consistent with our results. Previous studies in humans have not identified adenoviruses in sufficient numbers to conduct analyses for rates of co-occurrence with bacterial pathogens;<sup>21</sup> however in the chinchilla model, some synergism has been found as OM was most severe when animals were inoculated with adenovirus 7 days prior to inoculation of non-typeable *H. influenzae*.<sup>34</sup>

Of particular interest is the competitive interaction we found between *S. pneumoniae* and adenoviruses in Aboriginal children. This suggests that the presence of adenoviruses inhibits the growth of *S. pneumoniae* independent of whether *M. catarrhalis* and *H. influenzae* are present or not. This has not been previously reported and warrants further investigation. *In-vitro* studies have shown that while there is a complex effect of respiratory viruses on bacterial adhesion to respiratory epithelia cells, these have generally shown an enhancing effect of viral infection,<sup>35</sup> including adenoviruses and *S. pneumoniae*.<sup>36</sup> In contrast, an *in vivo* study in the chinchilla model adenoviruses did not enhance colonization by *S. pneumoniae*, contrary to the enhancing effect of influenza A virus,<sup>37</sup> and our earlier modelling analysis found a negative association, albeit insignificant, between adenoviruses and *S. pneumoniae*<sup>19</sup> giving further weight to our finding. From our study, we cannot determine whether viral infection preceded, followed or coincided with the bacterial infection, but our results support the hypothesis that rhinoviruses and adenoviruses are independently associated with increased, or in the case of adenoviruses and *S. pneumoniae*, decreased bacterial carriage in the nasopharynx of Aboriginal and non-Aboriginal children.

Our study does have some other limitations. We were only able to test for the 7 respiratory viruses under investigation on a restricted set of specimens. While specimens were collected during routine follow-up visits, it is possible that some children may have been experiencing mild upper respiratory symptoms at the time, but not severe enough to be classified as an illness episode. This could have resulted in falsely high viral identification rates. However, specimens from children who were ill at the time of collection were excluded from our analysis.

Despite these small limitations, our data provide a platform on which to determine the role of rhinoviruses and adenoviruses and bacteria in the etiology and severity of OM and acute lower respiratory infections. The high identification rate of adenoviruses and rhinoviruses and concurrent pathogenic OM bacteria in asymptomatic Aboriginal children may relate to larger family sizes, more crowded living conditions than in non-Aboriginal households and higher transmission rates<sup>6</sup>, P. Jacoby, unpublished data). Improved housing and promotion of frequent handwashing for Aboriginal people are needed to reduce carriage and transmission of respiratory pathogens. Our findings have implications for prevention strategies targeting individual pathogens and there is now a need to characterise these associations between viruses and bacteria in times of active acute respiratory infection. Such investigations are possible through our state-wide population-based data linkage system and we are in the process of linking respiratory pathogen data with demographic, hospitalisation, emergency department presentation data in a cohort of 245,000 births.

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## REFERENCES

1. Freid VM, Makuc DM, Rooks RN. Ambulatory health care visits by children: principal diagnosis and place of visit. *Vital and Health Statistics Series*. 1998;13:1-23.
2. Otters HB, van der Wouden JC, Schellevis FG, van Suijlekom-Smit LWA, Koes BW. Trends in prescribing antibiotics for children in Dutch general practice. *J Antimicrob Chemother*. 2004;53:361-366.

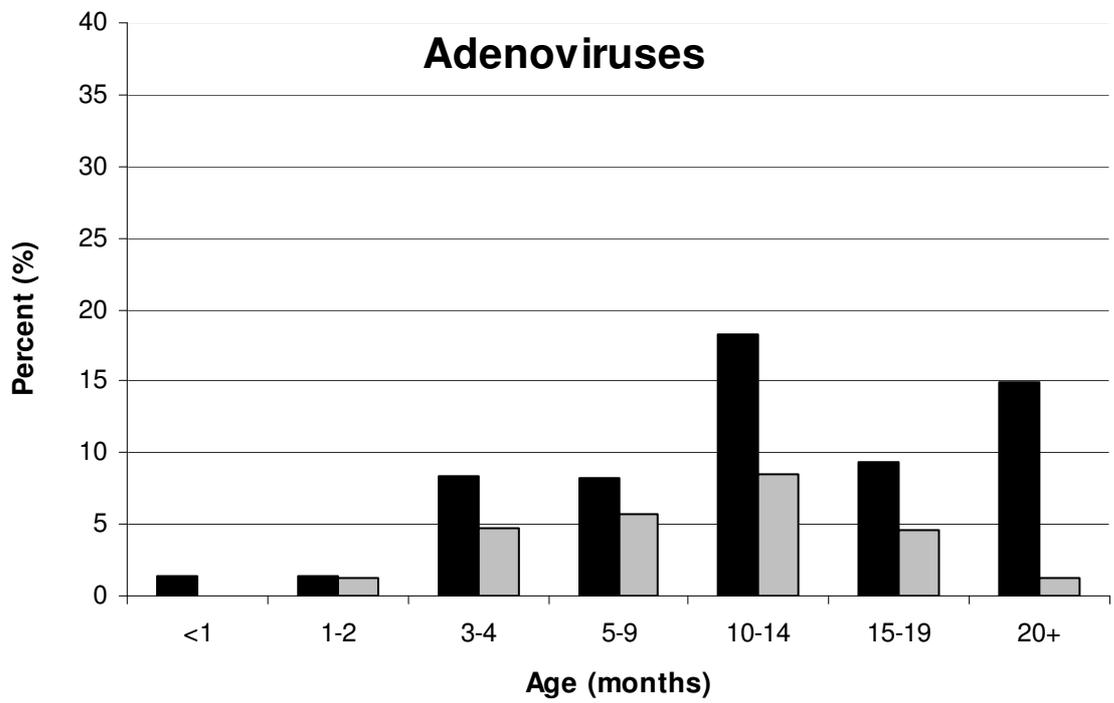
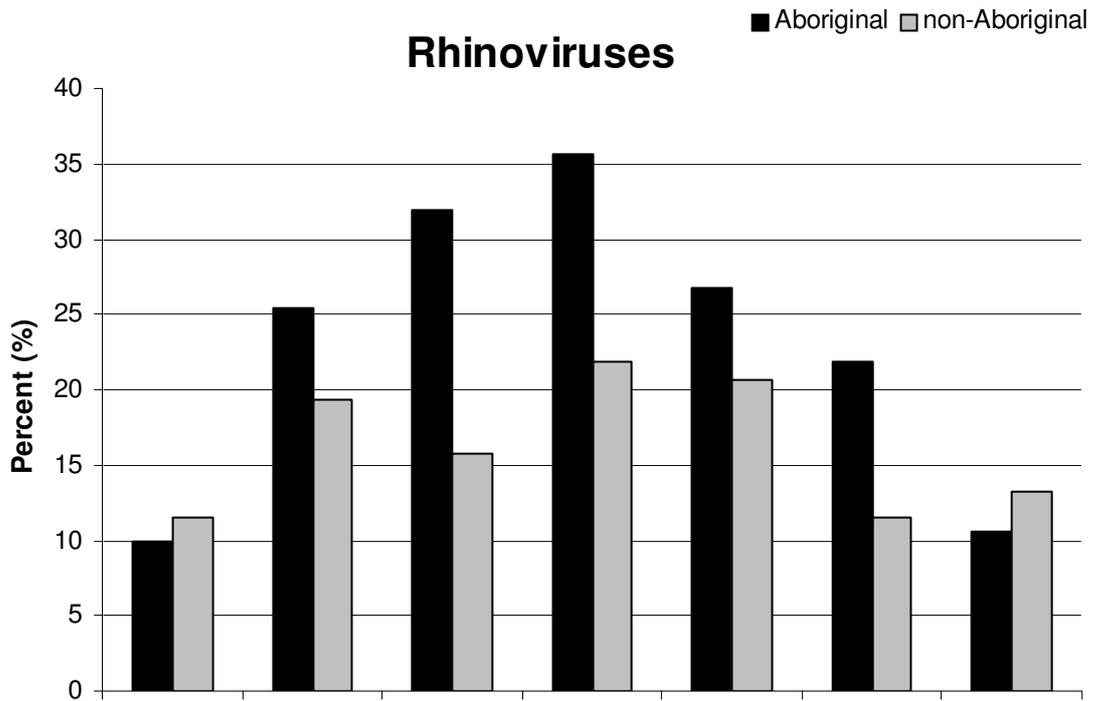
3. Pan Y, Henderson J, Britt H. Antibiotic prescribing in Australian general practice: how has it changed from 1990-91 to 2002-03? *Respir Med.* 2006;100:2004-2011.
4. Rovers MM, Schilder AG, Zielhuis GA, Rosenfeld RM. Otitis media. *Lancet.* 2004;363:465-473.
5. Leach AJ, Boswell JB, Asche V, Nienhuys TG, Mathews JD. Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian Aboriginal infants. *Pediatr Infect Dis J.* 1994;13:983-989.
6. Lehmann D, Arumugaswamy A, Elsbury D et al. The Kalgoorlie Otitis Media Research Project: rationale, methods, population characteristics and ethical considerations. *Paediatr Perinat Epidemiol.* 2008;22:60-71.
7. Kilpi T, Herva E, Kajjalainen T, Syrjanen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J.* 2001;20:654-662.
8. Watson K, Carville K, Bowman J et al. Upper respiratory tract bacterial carriage in Aboriginal and non-Aboriginal children in a semi-arid area of Western Australia. *Pediatr Infect Dis J.* 2006;25:782-790.
9. Faden H, Duffy L, Wasielewski R et al. Relationship between nasopharyngeal colonization and the development of otitis media in children. *J Infect Dis.* 1997;175:1440-1445.
10. Buchman CA, Brinson GM. Viral otitis media. *Curr Allergy Asthma Rep.* 2003;3:335-340.
11. Chantzi FM, Papadopoulos NG, Bairamis T et al. Human rhinoviruses in otitis media with effusion. *Pediatr Allergy Immunol.* 2006;17:514-518.
12. Chonmaitree T. Viral and bacterial interaction in acute otitis media. *Pediatr Infect Dis J.* 2000;19:S24-30.

13. Heikkinen T. The role of respiratory viruses in otitis media. *Vaccine*. 2001;19:51-55.
14. Koivunen P, Kontiokari T, Niemela M, Pokka T, Uhari M. Time to development of acute otitis media during an upper respiratory tract infection in children. *Pediatr Infect Dis J*. 1999;18:303-305.
15. Peltola VT, Boyd KL, McAuley JL, Rehg JE, McCullers JA. Bacterial sinusitis and otitis media following influenza virus infection in ferrets. *Infect Immun*. 2006;74:2562-2567.
16. Vesa S, Kleemola M, Blomqvist S et al. Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. *Pediatr Infect Dis J*. 2001;20:574-581.
17. Alper CM, Winther B, Mandel EM, Hendley JO, Doyle WJ. Rate of concurrent otitis media in upper respiratory tract infections with specific viruses. *Arch Otolaryngol Head Neck Surg*. 2009;135:17-21.
18. Yano H, Okitsu N, Hori T et al. Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media. *Acta Otolaryngol (Stockh)*. 2009;129:19-24.
19. Jacoby P, Watson K, Bowman J et al. Modelling the co-occurrence of *Streptococcus pneumoniae* with other bacterial and viral pathogens in the upper respiratory tract. *Vaccine*. 2007;25:2458-2464.
20. Nokso-Koivisto J, Hovi T, Pitkaranta A. Viral upper respiratory tract infections in young children with emphasis on acute otitis media. *Int J Pediatr Otorhinolaryngol*. 2006;70:1333-1342.

21. Pitkaranta A, Roivainen M, Blomgren K et al. Presence of viral and bacterial pathogens in the nasopharynx of otitis-prone children: A prospective study. *Int J Pediatr Otorhinolaryngol.* 2006;70:647-654.
22. McCullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev.* 2006;19:571-582.
23. Leven M, Goossens H. Relevance of nucleic acid amplification techniques for diagnosis of respiratory tract infections in the clinical laboratory. *Clin Microbiol Rev.* 1997;10:242-256.
24. Jartti T, Jartti L, Peltola V, Waris M, Ruuskanen O. Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatr Infect Dis J.* 2008;27:1103-1107.
25. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC, editors. *Manual of Clinical Microbiology.* 8th ed. Washington: ASM Press; 2003.
26. Hanley JA, Negassa A, Edwards MDd, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation.[see comment]. *Am J Epidemiol.* 2003;157:364-375.
27. van Bente I, Koopman L, Niesters B et al. Predominance of rhinovirus in the nose of symptomatic and asymptomatic infants. *Pediatr Allergy Immunol.* 2003;14:363-370.
28. Johnston SL, Sanderson G, Pattemore PK et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol.* 1993;31:111-117.
29. van Gageldonk-Lafeber AB, Heijnen MLA, Bartelds AIM et al. A case-control study of acute respiratory tract infection in general practice patients in the Netherlands. *Clin Infect Dis.* 2005;41:490-497.

30. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect Dis*. 2006;6:303-312.
31. Peltola VT, McCullers JA. Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *Pediatr Infect Dis J*. 2004;23.
32. Hambidge SJ, Glanz JM, France EK et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. *JAMA*. 2006;296:1990-1997.
33. Harper S, Fukuda K, Uyeki T, Cox N, Bridges C. Prevention and Control of Influenza. *MMWR Recomm Rep*. 2005;54:1-40.
34. Suzuki K, Bakaletz LO. Synergistic effect of adenovirus type 1 and nontypeable *Haemophilus influenzae* in a chinchilla model of experimental otitis media. *Infect Immun*. 1994;62:1710-1718.
35. Avadhanula V, Rodriguez CA, Devincenzo JP et al. Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner. *J Virol*. 2006;80:1629-1636.
36. Hakansson A, Kidd A, Wadell G, Sabharwal H, Svanborg C. Adenovirus infection enhances in vitro adherence of *Streptococcus pneumoniae*. *Infect Immun*. 1994;62:2707-2714.
37. Tong HH, Fisher LM, Kosunick GM, DeMaria TF. Effect of adenovirus type 1 and influenza A virus on *Streptococcus pneumoniae* nasopharyngeal colonization and otitis media in the chinchilla. *Ann Otol Rhinol Laryngol*. 2000;109:1021-1027.

**FIGURE 1.** Proportion of rhinoviruses and adenoviruses identified in nasopharyngeal specimens of asymptomatic Aboriginal and non-Aboriginal children by age group.



**TABLE 1.** Respiratory viruses identified in nasopharyngeal samples collected from asymptomatic Aboriginal and non-Aboriginal children.

Virus	Aboriginal		Non-Aboriginal		Total	
	no.	no. (%)	no.	no. (%)	no.	no. (%)
	collected	positive	collected	positive	collected	positive
Rhinovirus	436	103 (23.6)	570	94 (16.5)	1006	197 (19.6)
Adenovirus	435*	37 (8.5)	570	20 (3.5)	1005*	57 (5.7)
RSV	436	2 (0.5)	570	3 (0.5)	1006	5 (0.5)
Influenza A	436	2 (0.5)	570	2 (0.4)	1006	4 (0.4)
Influenza B	436	0 (-)	570	1 (0.2)	1006	1 (0.1)
Coronavirus	171	6 (3.5)	225	8 (3.6)	396	14 (3.5)
PIV	171	3 (1.8)	225	4 (1.8)	396	7 (1.8)
HMPV	171	3 (1.8)	225	0 (-)	396	3 (0.8)
Any virus in total dataset <sup>†</sup>	436	133 (30.5)	570	122 (21.4)	1006	255 (25.3)

RSV, respiratory syncytial virus; PIV, parainfluenza virus; HMPV, human metapneumovirus.

\* Insufficient volume in one specimen to conduct virological testing.

<sup>†</sup>Only 396 specimens were tested for coronavirus, PIV and HMPV.

**TABLE 2.** The co-occurrence of bacterial otitis media pathogens with rhinoviruses and adenoviruses in nasopharyngeal specimens from asymptomatic Aboriginal and non-Aboriginal children.

Subjects	Total	No Bacteria	Pnc only	MC only	HI only	Pnc and MC	Pnc and HI	MC and HI	Pnc, MC and HI
	N	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)
Aboriginal children									
Rhinovirus positive	103	7 (6.8)	4 (3.9)	7 (6.8)	9 (8.7)	17 (16.5)	7 (6.8)	9 (8.7)	43 (41.7)
Rhinovirus negative	333	122 (36.6)	30 (9.0)	27 (8.1)	17 (5.1)	39 (11.7)	14 (4.2)	19 (5.7)	65 (19.5)
Adenovirus positive	37	4 (10.8)	0 (-)	2 (5.4)	3 (8.1)	3 (8.1)	2 (5.4)	5 (13.5)	18 (48.6)
Adenovirus negative	398	125 (31.4)	34 (8.5)	32 (8.0)	23 (5.8)	53 (13.3)	19 (4.8)	22 (5.5)	90 (22.6)
Non-Aboriginal children									
Rhinovirus positive	94	41 (43.6)	10 (10.6)	14 (14.9)	2 (2.1)	13 (13.8)	4 (4.3)	4 (4.3)	6 (6.4)
Rhinovirus negative	476	270 (56.7)	52 (10.9)	53 (11.1)	14 (2.9)	44 (9.2)	6 (1.3)	14 (2.9)	23 (4.8)
Adenovirus positive	20	4 (20.0)	1 (5.0)	4 (20.0)	0 (-)	6 (30.0)	0 (-)	3 (15.0)	2 (10.0)
Adenovirus negative	550	307 (55.8)	61 (11.1)	63 (11.5)	16 (2.9)	51 (9.3)	10 (1.8)	15 (2.7)	27 (4.9)

Pnc, *S. pneumoniae*; MC, *M. catarrhalis*; HI, *H. influenzae*

**TABLE 3.** Associations between isolation of bacterial OM pathogens and rhinoviruses in asymptomatic Aboriginal and non-Aboriginal children.

Subjects	OR*	95%CI	OR <sup>†</sup>	95% CI
Aboriginal children				
<i>M. catarrhalis</i>	2.67	1.60 – 4.44	1.94	1.05 – 3.57
<i>S. pneumoniae</i>	1.91	1.15 – 3.17	1.29	0.75 – 2.23
<i>H. influenzae</i>	2.91	1.76 – 4.83	2.24	1.24 – 4.07
Non-Aboriginal children				
<i>M. catarrhalis</i>	1.43	0.86 – 2.38	1.15	0.64 – 2.08
<i>S. pneumoniae</i>	1.49	0.90 – 2.46	1.37	0.80 – 2.34
<i>H. influenzae</i>	1.64	0.89 – 3.04	1.44	0.74 – 2.79

OR, odds ratio; CI, confidence interval.

\*Adjusted for age, age<sup>2</sup>, gender, proportion of rhinovirus-positive specimens per child

<sup>†</sup>Adjusted for age, age<sup>2</sup>, gender, proportion of rhinovirus-positive specimens per child, identification of adenovirus, isolation of the 2 other bacterial OM pathogens

**TABLE 4.** Associations between isolation of bacterial OM pathogens and adenoviruses in asymptomatic Aboriginal and non-Aboriginal children.

Subjects	OR <sup>*</sup>	95%CI	OR <sup>†</sup>	95% CI
Aboriginal children				
<i>M. catarrhalis</i>	1.96	0.84 – 4.52	1.83	0.65 – 5.18
<i>S. pneumoniae</i>	0.75	0.41 – 1.36	0.39	0.18 – 0.84
<i>H. influenzae</i>	3.29	2.19 – 8.40	3.30	1.19 – 9.09
Non-Aboriginal children				
<i>M. catarrhalis</i>	5.71	1.67 – 19.61	5.75	1.74 – 19.23
<i>S. pneumoniae</i>	1.81	0.88 – 3.68	1.17	0.51 – 2.68
<i>H. influenzae</i>	0.87	0.36 – 2.11	0.44	0.16 – 1.24

OR, odds ratio; CI, confidence interval.

\* Adjusted for age, age<sup>2</sup>, gender, proportion of adenovirus-positive specimens per child

† Adjusted for age, age<sup>2</sup>, gender, proportion of adenovirus-positive specimens per child, identification of rhinovirus, isolation of the 2 other bacterial OM pathogens