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## BRIEF REPORT

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# Absence of human papillomavirus in nasopharyngeal swabs from infants in a population at high risk of human papillomavirus infection

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## ABSTRACT

Maternal urogenital human papillomavirus (HPV) infection may place neonates at risk of HPV acquisition and subsequently lower respiratory infections as HPV can influence development of immunity. The respiratory HPV prevalence is not known in remote-dwelling Aboriginal infants, who are at high risk of respiratory infection and where the population prevalence of urogenital HPV in women is high. These data are necessary to inform HPV vaccination regimens. A retrospective analysis using PCR specific for HPV was performed on 64 stored nasopharyngeal swabs from remote-dwelling Aboriginal infants < 6 months of age, with and without hospitalised pneumonia. HPV DNA was not detected in any specimen. Despite the negative result, we cannot exclude a role for HPV in respiratory infections affecting infants in this population; however, our data do not support HPV as an important contributor to acute respiratory infection in remote-dwelling Aboriginal children.

## KEYWORDS

Human papillomavirus, Nasopharyngeal swabs, Infants

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## INTRODUCTION

Human papillomavirus (HPV) disease has a wide range of clinical manifestations. HPV genotypes associated with oncology diseases are involved in the development of genitourinary and oropharyngeal cancers, while other genotypes can cause genital warts and serious respiratory disorders in both adults and children.<sup>1</sup> Respiratory papillomatosis is a rare condition, but is the most common benign neoplasm of the larynx among children.<sup>2</sup> This condition is primarily caused by types HPV-6 and HPV-11<sup>2</sup> which are targeted by the quadrivalent and nonavalent HPV vaccines (Gardasil<sup>®</sup> and Gardasil9<sup>®</sup>).

In infants, the most commonly known manifestation of HPV is respiratory papillomatosis where stridor is often the presenting symptom. However, HPV infection is also associated with impaired humoral and innate immune responses that predispose the child to increased risk of infection.<sup>3</sup> Children may thus also present with recurrent pneumonia, bronchiectasis, and declining pulmonary status.<sup>2</sup> In Australia between 2000 and 2013, the estimated prevalence of respiratory papillomatosis was 0.81 per 100 000 among children aged < 15 years, peaking at age 5 to 9 years (1.1 per 100 000),<sup>4</sup> and is expected to decline with broader HPV vaccine coverage.

Children may acquire HPV *in utero*, perinatally, or subsequently through horizontal transmission via saliva or other contacts.<sup>5</sup> Ten international studies examining HPV DNA in nasopharyngeal aspirates taken from neonates after delivery, reported prevalences varying from 1.5% to 37%.<sup>5</sup> In addition, seven studies that examined HPV DNA in buccal swabs collected from infants aged 1–4 days reported prevalence of 0.9% to 56%.<sup>5</sup> Subsequently, HPV DNA can persist in some children; the Finnish Family Study found that HPV DNA persisted for at least 36 months in 10% of infants positive at delivery for high-risk HPV genotypes (12 HPV types examined of > 206 identified types).<sup>6</sup>

Currently, there are no known data on HPV DNA prevalence in respiratory specimens in Australian Aboriginal children. This is despite a high prevalence of urogenital HPV infection in Aboriginal women in northern Australia (42% [95% CI, 37%–48%] in one community-based study)<sup>7</sup> prior to vaccine introduction and known vertical transmission of the virus.<sup>5</sup> Further, Australian children living in remote Northern Territory communities experience high rates of acute and chronic lower respiratory infection (ALRI), including pneumonia, with one fifth hospitalised with an ALRI in the first year of life,<sup>8</sup> and 1 in 68 diagnosed with bronchiectasis.<sup>9</sup> It is unknown whether airway infection with HPV contributes to the development of acute and/or chronic lower respiratory tract infections in Aboriginal children in remote communities. Should HPV in the upper airways prove

to be prevalent in children at risk of lower respiratory infection, expansion of HPV vaccination regimens to at-risk infants may be warranted.

Our aim was to determine the prevalence of HPV DNA in nasopharyngeal swabs (NPS) from infants up to six months of age with and without pneumonia. We hypothesised that HPV DNA would be detected in NPS specimens from Aboriginal infants from remote communities, with higher positivity in those who had pneumonia.

## METHODS

### Ethical approval

Retrospective analysis of NPS was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (2011-1668) and conforms to the provisions of the Declaration of Helsinki. The NPS were sourced from children with and without pneumonia enrolled in three studies (1996–2004)<sup>10-12</sup> with written informed consent for further laboratory analyses. Patient anonymity was preserved.

### NPS testing

NPS specimens collected from 40 Aboriginal children from remote Northern Territory communities with no diagnosis of any ALRI and aged 3–25 weeks, and from 24 Aboriginal children hospitalised with pneumonia and aged 1 to 23 weeks (Table 1) were tested in 2013. Where longitudinal specimens were taken from an individual infant, only the first NPS was tested. These NPS represented all eligible specimens in the three studies where there was written informed consent for further laboratory analyses. The NPS had been collected into 1 mL skim milk tryptone glucose glycerol broth and stored at –80 °C. HPV DNA was previously shown to be detectable in vaginal swabs stored in media at –20 °C for at least 12 years.<sup>13</sup> An aliquot of 200 µL of the NPS was extracted by the automated MagNA Pure 96 isolation and purification system (Roche Molecular Systems) using DNA and the Viral NA Small Volume kit. Each extracted sample was assessed for: i) Sample adequacy using a quantitative PCR for a 260-bp fragment of the human beta-globin gene<sup>14</sup>; and ii) HPV DNA using L1 consensus primers PGMY09/PGMY11<sup>15,16</sup> combined with PCR-ELISA detection (with established sensitivity of 10 copies per reaction for the mucosal HPV genotypes).<sup>17</sup>

## RESULTS

All NPS were positive for human beta-globin with an average of  $2.49 \times 10^3$  and  $2.24 \times 10^3$  copies per reaction detected for samples collected from children with and without pneumonia ( $P = 0.366$ ). However, all (64/64) NPS were negative for HPV DNA (one-sided 97.5% CI, 0.94–1.0).

**TABLE 1** Description of original studies

Study description	Years	Number of specimens	Median age (range), weeks	% HPV DNA positive
<b>Children without pneumonia</b>				
Placebo-controlled RCT of long-term amoxicillin (up to 6m) for treatment of OME in 3 communities. <sup>10</sup> Total enrolled; <i>n</i> = 103	1996–2001	40	7.64 (1.00–23.42)	0
Longitudinal carriage study of infants receiving PCV7 and 23PPV in 3 communities. <sup>11</sup> Total enrolled; <i>n</i> = 148	2001–2004			
<b>Children with pneumonia</b>				
RCT of zinc and vitamin A supplementation in pneumonia. <sup>12</sup> Total enrolled; <i>n</i> = 187	2001–2002	24	15.17 (2.99–25.35)	0

RCT, randomized controlled trial; OME, otitis media with effusion; PCV7, 7-valent pneumococcal conjugate vaccine; 23PPV, 23-valent pneumococcal polysaccharide vaccine; NPS, nasopharyngeal swab; m, months.

## DISCUSSION

In this study we did not detect HPV DNA in NPS from a cohort of 64 Aboriginal infants up to six months of age with and without pneumonia. This absence of HPV is unlikely to be due to swab storage or inadequate sampling, as we successfully detected DNA from other virus species in these specimens,<sup>18</sup> and human beta-globin gene levels indicated sufficient mucosal sampling.

The absence of HPV DNA needs to be considered in relation to the study design. This was a retrospective analysis of archived specimens collected for studies that were not designed to assess the prevalence of HPV.<sup>10–12</sup> Therefore, the NPS under analysis varied in child age (between 1 and 25 weeks), and we did not know the HPV status of the mother and/or the primary carer. Furthermore, the cohort of 64 infants was small for this type of study but suggests a prevalence of <1.6% (ie. 1 in 64). The eligible sample number also represented a small proportion (13%–16%) of the original study enrolments and the annual Aboriginal birth cohort in the Northern Territory (approximately 1550). Given these limitations, we cannot exclude a role for HPV in respiratory infections (or infections at other body sites) affecting infants; however, our data do not support HPV as an important contributor to acute respiratory infection in the population tested.

Our study was performed on NPS collected during 1996–2004 prior to introduction of HPV vaccines. Prevalence data from well women's health checks for Aboriginal women in northern Australia in 1999 found HPV infection in 42% in 1090 women of all ages (and up to 62% in 16–20 year olds).<sup>7</sup> Since introduction of HPV vaccination (Gardasil®) in Australia in 2007, there has been a marked reduction in clinic presentations for genital warts by Aboriginal and Torres Strait Islander Australians aged 21–30 years,<sup>19</sup> but not in older age groups. Surveillance studies continue to monitor new cases of HPV disease and associated types; however, HPV-related disease remains a concern as does its role in humoral and innate immune

impairment.<sup>3</sup> The question of whether HPV plays a role in the very high rates of respiratory infection with immune impairment<sup>20</sup> in Aboriginal infants remains incompletely addressed; however, our results suggest that vertical HPV transmission is an unlikely contributor. Whilst these data do not demonstrate a need for further research on prevalence of HPV in remote-dwelling Aboriginal infants, the broader implications of HPV vaccination strategies in groups with high disease rates remain important.

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## CONFLICT OF INTEREST

None.

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