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Colonisation with *Pneumocystis jirovecii* in Australian infants

Sir,

Pneumocystis jirovecii, a fungus with worldwide distribution, causes a severe inflammatory pneumonia in immunocompromised adults and children. The association or causal link between *P. jirovecii* and sudden unexpected infant death is controversial.[1,2] Serological studies have indicated that exposure to *Pneumocystis* occurs at an early age.[3,4] Such colonisation in healthy children may lead to later reactivation and overt disease, although *de novo* infection also occurs.[5] The prevalence of colonisation in children, which appears to be higher than that in adults, is considered clinically relevant since colonised children are postulated to be a reservoir for *Pneumocystis*.[3] Studies in Chile, the US, Zambia and Europe have examined for the presence of *Pneumocystis* using direct detection methods (immunohistochemistry or polymerase chain reaction, PCR) in children and reported prevalence rates ranging between 29% and 100%.[1,3,6–9] However, there are no data on the prevalence of colonisation in Australian infants.

In this pilot study, we sought to determine if detection of *P. jirovecii* in young children without *Pneumocystis* pneumonia was feasible. We examined 50 non-duplicate nasopharyngeal aspirate (NPA) specimens that had been collected for routine virological testing, for the presence of *P. jirovecii* DNA. Nasopharyngeal aspirates from infants aged 2–8 months collected between December 2013 to March 2014 were retrieved from storage at −80°C. None of these patients had a clinical syndrome consistent with *P. jirovecii* pneumonia, nor were they HIV infected, but all had NPA specimens collected because they had respiratory symptoms. These specimens had been tested using the commercial Seeplex RV15 ACE Detection multiplex PCR assay according to manufacturer's instructions (Seegene, Korea). This assay detects influenza A, influenza B, parainfluenza 1, 2, 3 and 4, respiratory syncytial virus A and B, rhinovirus, enterovirus, adenovirus, coronavirus, metapneumovirus, and bocavirus. As part of the study design, we randomly selected 25 specimens from patients in whom no respiratory viruses were identified and 25 specimens from patients who were infected with one or more respiratory viruses.

Detection of *P. jirovecii* DNA by PCR was performed as previously described, with minor modifications.[10,11] DNA was extracted from 500 μL of nasopharyngeal aspirate sample. Briefly, the assay is an 'in-house' real-time TaqMan PCR assay that targets the single copy β-tubulin gene, performed on the LightCycler platform, a closed amplification system (Roche Diagnostics, Germany). In anticipation of lower levels of *Pneumocystis* burden in comparison to patients with overt *P. jirovecii* pneumonia, an additional 10 cycles of amplification were performed in order to increase the assay sensitivity. Therefore, the cycling parameters were 95°C for 10 min, followed by 50 cycles of 95°C for 5 s, 58°C for 20 s and 72°C for 20 s. Analysis of DNA extracts by multi-locus sequence typing (MLST) of four genetic loci: (1) internal transcribed spacer 1 and 2 (ITS1/2) regions of the nuclear rDNA gene cluster; (2) the *P. jirovecii*-specific β-tubulin; (3) mitochondrial large subunit (*mtLSU*); and (4) dihydropteroate synthase genes, was performed as previously described.[10]

Pneumocystis jirovecii DNA was detected in seven NPA specimens (14%). As expected, the burden of *P. jirovecii* was low with PCR cycle threshold (Ct) values ranging between 38 and 50. In contrast, when using this assay to diagnose *Pneumocystis* pneumonia and other disease, samples with a Ct value <37.3 cycles are typically classified as highly suggestive for *P. jirovecii* infection.[10]

The mean age of colonised infants was 190.7 days (range 62–469 days). Four of these patients were co-infected with respiratory viruses including rhinovirus ($n=2$), parainfluenza virus 3 ($n=2$) and adenovirus ($n=1$). In the other three patients, no respiratory viruses were detected. Attempts to perform MLST on the seven DNA extracts that yielded *P. jirovecii* DNA were unsuccessful for some of the loci despite multiple experiments. This was likely due to the low amounts of DNA template present in the extracts and was not further pursued. In contrast, the *mtLSU* gene target that is present in multiple copies in the *P. jirovecii* genome, was able to be amplified and sequenced in three of the DNA extracts.

In this study, we have demonstrated colonisation with *P. jirovecii* in Australian infants with a point prevalence of 14%, and as such, have opened up discussion on methodological issues that are relevant for future studies. Although reports from elsewhere have demonstrated a higher prevalence, there were a number of methodological differences in our study. Here, we used a single copy target for real-time PCR whereas other studies using PCR have employed multi-copy PCR amplification targets such as the *mtLSU* or the major surface glycoprotein (*MSG*) genes.[3,6,9] In addition, previous studies reporting a significantly higher prevalence examined autopsy lung specimens.[1,6] Because of the retrospective nature of the present pilot study, we were limited to using a small volume of specimen (500 μL) from the upper respiratory tract. Other studies using this specimen type have used between 200 and 3000 μL of sample for DNA extraction.[3,12] The relative sensitivities of *P. jirovecii* PCR using different types of respiratory tract specimens is not well defined in children, but in adults lower respiratory tract specimens are preferred.[13] However, in practice, when assessing for colonisation in infants, sampling the upper respiratory tract by the least invasive means is of high importance.

Previous studies have indicated that colonisation appears to be common in children during upper respiratory tract symptoms or infection.[3] Our pilot study was not designed to determine any associations between *P. jirovecii* colonisation and co-infection with respiratory viruses, but this is an important consideration for future studies. *Pneumocystis jirovecii* may provide an alternative aetiological diagnosis for upper respiratory tract symptoms,[3] and pathogens may co-exist and act synergistically in infection and disease. For instance, co-infection has been demonstrated to result in more severe disease caused by other

respiratory tract pathogens, influenza A and *Staphylococcus aureus* and *Streptococcus pneumoniae*.[14]

We have previously described an outbreak of *P. jirovecii* pneumonia in renal transplant recipients caused by two closely related genotypes of *P. jirovecii,*[10] which subsequently spread to other institutions.[15] Thus, we attempted MLST typing in this study to determine whether similar genotypes were present in infants. Unfortunately, we were unable to amplify all loci for all PCR positive specimens due to the assumed low fungal burden as also described by others.[16] This indicates the need for larger volume sampling in future prospective studies examining for *P. jirovecii* prevalence in children.

In conclusion, although limited by a small sample size and the retrospective nature of the study, the results suggest that *Pneumocystis* colonisation does occur in infants, and provides a basis for further investigation into the prevalence and molecular epidemiology of *P. jirovecii* in the paediatric population, including longitudinal studies to determine the natural history and consequence, if any, of colonisation.

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Sternoclavicular septic arthritis caused by *Neisseria elongata* subspecies *nitroreducens*

Sir,

Neisseria elongata subspecies *nitroreducens* is a rare pathogen, most commonly described as a cause of septicaemia and endocarditis.[1–5] It has also been isolated in the context of osteomyelitis and spondylodiscitis.[1,5] We report a case of sternoclavicular septic arthritis due to this agent, which occurred following an intra-articular injection of cortisone.

A previously well 49-year-old man was admitted to hospital with worsening pain and warmth over the region of his right sternoclavicular joint. The pain had started 9 weeks earlier after a musculoskeletal injury he sustained lifting weights at the gym. Shortly after the injury his local doctor referred him for an intra-articular cortisone injection, which was performed aseptically under ultrasound guidance. Five weeks prior to hospital admission he visited the dentist and had cleaning carried out. No procedure breaching the oral mucosa was performed. Four days prior to hospital admission the pain intensified, redness developed over the joint and he experienced chills. He had no history of intravenous drug use (IVDU).

Initial examination revealed redness, swelling and tenderness over the right sternoclavicular joint, but was otherwise unremarkable. The white cell count was 11.6×10^9/L (normal range 4.0–11.0×10^9/L), with a predominant neutrophilia, and the CRP was 113 mg/L (normal range <5 mg/L). Blood cultures were drawn but were ultimately negative. He was taken to theatre for aspiration and washout of the joint. Two tissue samples and a swab were taken intraoperatively for microbiological testing. He was initially treated with flucloxacillin 2 g intravenously, 6 hourly.

After 3 days of culture at 37°C, both tissue specimens and the swab grew small (1 mm diameter) colonies of a non-motile,