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Biographies

Rachael Lappan completed her PhD on the microbiome of rAOM at the University of Western Australia and Telethon Kids Institute in 2019. She now works as a postdoctoral fellow at Monash University, broadening her horizons with research on enteropathogens and microbial communities in more extreme environments like arid deserts and the atmosphere.

Christopher Peacock, BSc Hons Biological Sciences (1985), Fellow of the Institute of Medical Laboratory Sciences (1988), University of London, PhD Human Genetics on ‘the Susceptibility to Visceral Leishmaniasis’ (1998), University of Cambridge. After running a research and service histology laboratory at the London School of Tropical Medicine and Hygiene, he spent one year on a successful HIV project in Abidjan, West Africa followed by two years in the Amazon region of Brazil working on genetic susceptibility to TB, Leprosy and Leishmaniasis. Having completed his PhD in 1998, he undertook a position as a postdoctoral research associate continuing the work on human susceptibility to infectious diseases followed by a role as a senior computational biologist at the Wellcome Trust Sanger Institute leading to publication of the first Leishmania genome as part a Special edition of Science in 2005 and the publication of a comparative Leishmania genome paper in 2007 published in Nature Genetics. In 2007, he moved to Australia to help set up a Division of Genetics and Health in infectious diseases at the Telethon Institute of Child Health Research and in 2009 took up a senior lecturer position at the University of Western Australia. Shortly afterwards he was awarded one of the inaugural ARC Future Fellowships. In addition to neglected tropical diseases, his research interests now encompass metagenomics, and novel parasitic infections in indigenous wildlife.

Pathogen adaptation to vaccination: the Australian Bordetella pertussis story

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Whooping cough (pertussis) is a highly contagious vaccine preventable respiratory disease caused by the Gram-negative bacterium Bordetella pertussis. Despite high level vaccination coverage over the past 20 years, Australia has one of the highest per capita burdens of pertussis globally. One of the primary factors associated with the re-emergence of pertussis is pathogen adaptation of B. pertussis to the current acellular vaccines used. This article will focus on the genomic and proteomic changes that have occurred in the Australian B. pertussis population, the significance of these adaptive changes on fitness in a vaccinated environment and what we can do to reduce the significant burden of pertussis in the future.

The rising incidence of B. pertussis in Australia

Pertussis vaccinations were first introduced in Australia in 1953 using a whole cell vaccine (WCV), which contained dead B. pertussis cells. This led to a dramatic reduction in the number of pertussis notifications from 767 cases per 100,000 in the 1930s...
to one case per 100 000 by the late 1970s\(^1\). Despite the success of WCVs in reducing pertussis, reports of possible severe side-effects reduced public confidence in the vaccine and led to the replacement of the WCV with a new acellular vaccine (ACV)\(^2\).

ACV was introduced into Australia in 1997, initially as a booster, and by 2000 it was used for all immunisations\(^2\). There are two ACVs used in Australia, the three-component-ACV and the less widely used five-component ACV. The three-component ACV targets three virulence factors; pertussis toxin (Ptx), pertactin (Prn) and filamentous haemagglutinin (Fha), while the five-component vaccine targets two additional fimbriae (Fim2 and Fim3). Since the late 1990s, there has been a steady increase in pertussis notifications observed in Australia and in 2008–2012, Australia experienced a prolonged pertussis epidemic (Figure 1). At the height of the epidemic in 2011, 174 cases per 100 000 were recorded, the highest number documented since the introduction of pertussis vaccination\(^2\). This was followed by a smaller yet still significant epidemic from 2014-2017 with a peak of 95 cases per 100 000 in 2015\(^3\) (Figure 1). Similar trends in increased notification rates have been noted in other countries that switched to the ACV\(^4\)–\(^6\).

### The changing population structure of Australian *B. pertussis* in different vaccine eras

To determine how *B. pertussis* is adapting to vaccine selection pressure, molecular epidemiology typing studies were performed to define the population structure and trace the evolution of *B. pertussis* in Australia from the pre-vaccination and WCV era to the ACV era.

A previous single nucleotide polymorphism (SNP) typing study separated the global pertussis population into 6 SNP clusters (I-VI) based on 65 SNPs\(^7\). Australian *B. pertussis* isolates were mostly found in SNP clusters I-V with SNP cluster V being a minor Australian cluster and SNP cluster VI containing vaccine and pre-vaccine strains. In the WCV period, SNP cluster II was the predominant cluster and was comprised of 33% of strains typed. After the switch to the ACV, the frequency of SNP cluster II isolates decreased to 11% and was replaced with SNP cluster I, which increased to 31% after it emerged during the WCV/ACV transition period\(^7\) (Figure 2). Since the 2008–2012 epidemic, the majority (>90%) of circulating strains in Australia belong to SNP cluster I\(^3\)–\(^8\). Most current circulating strains in other ACV countries typed as *ptxP3* strains are equivalent to SNP cluster I\(^9\).

### Genetic changes in ACV antigen genes of the circulating Australian *B. pertussis* population

The replacement of the WCV with ACV in Australia reduced the number of antigens targeted from hundreds to 3-5 (Figure 2). This has placed greater selection pressure on genes encoding ACV antigens to change and allow for vaccine escape variants to emerge.

![Figure 1. Number of pertussis notifications in Australia from 1991-2018. Introduction of the ACV as a booster in 1997 (•) and exclusive use of ACV for all vaccinations after 2000 (▼) are indicated. Data from the Department of Health, 2019.](image)

![Figure 2. The changing population structure and adaptation of *B. pertussis* in Australia over three different vaccine periods. The circulating SNP clusters are displayed from the most predominant (at the top) to the least (bottom). \(^4\)Includes the vaccine strain Tohama I used to manufacture the WCV and ACV. \(^6\)Relative to SNP cluster II.](image)
in Australia. There are two major evolutionary changes observed due to increased vaccine selection pressure: ACV allelic divergence and inactivation of ACV antigen expression (Figure 2).

The greatest ACV allelic divergence is seen in ptxA and prn alleles. SNP cluster I carries the prn2 and ptxA1 alleles which encode antigenic variants that differ from the prn1 and ptxA2 alleles in Tohama I, the strain used to produce the WCV and ACV. The ptxA1 allele emerged prior to the introduction of WCV but only expanded after WCVs were used and is present in all cluster I-IV strains. Similarly the prn2 allele emerged during the WCV period in SNP cluster I but expanded after the introduction of ACV. These mutations in ptxA1 and prn2 alter amino acid residues in known B/T immune epitopes thereby altering recognition from ACV-generated immunity. A double allelic exchange mutant of ACV alleles prn1 and ptxA2 with non-ACV alleles prn2 and ptxA1 conferred a greater survival rate in ACV immunised mice than the wild type strain demonstrating the selective advantage of antigen mismatch in a highly vaccinated environment.

Besides mismatches in ACV antigens, the current predominance of SNP cluster I is also associated with carrying the ptxP3 allele which encodes a variant pertussis toxin promoter. This new promoter is associated with increased pertussis toxin production, and possibly virulence and disease severity compared with strains carrying the original ptxP1 allele. Increased pertussis toxin production is thought to provide a selective advantage by delaying neutrophil recruitment and modulating the immune response. Using a mixed-infection mouse model, SNP cluster I strains outcompeted SNP cluster II strains (carrying ptxP1, prn3 and ptxA1) in a vaccinated environment. This demonstrates that these genomic changes have increased the overall fitness of the circulating population of B. pertussis and contribute to the predominance of SNP cluster I in an ACV environment.

Recently, strains which do not express ACV antigens have emerged in many developed countries, most notably the Prn deficient strains. In Australia, Prn deficient strains are primarily found in SNP cluster I and were first detected at the start of the 2008 epidemic, making up 5% of strains isolated. However, within a decade this increased to 90% by 2017, the highest proportion of Prn deficient strains in the world. Over 20 independent mechanisms for Prn inactivation have been documented. This diversity of inactivation suggests a beneficial selection pressure for convergent evolution and that Prn deficient strains emerged independently from multiple different clones. Findings from multiple mouse studies support increased fitness for losing pertactin in an ACV environment with higher survival of Prn deficient strains compared to Prn producing strains in ACV-vaccinated mouse. Prn deficient strains also displayed no differences in disease severity compared to Prn producing strains but have a higher likelihood to cause disease and persist longer in ACV vaccinated individuals. Furthermore, in a mixed infection mouse model, Prn deficient strains had poorer survival in unvaccinated mice compared to Prn producing strains therefore providing evidence that Prn inactivation is an ACV-driven phenomenon. Finally, inactivation of Fha has also been detected in Australia while Ptx inactivation has been reported in other countries. However, these mutant strains are rare, and it remains to be seen whether the loss of these virulence factors increases the fitness of B. pertussis in an ACV environment.

Proteomic changes and broadening the view of pathogen adaptation to vaccination

In addition to increased fitness in an ACV environment, it was shown that SNP cluster I also outcompetes SNP cluster II in an unvaccinated environment. This suggests that there are other changes between the two clusters that contribute to the current predominance of SNP cluster I in Australia. In proteomic studies, our laboratory identified increased expression of previously unknown adaptations in SNP cluster I of reduced expression of immunogenic proteins such as the type III secretion system and upregulation of amino acid and metal ion transport proteins and adhesins together with broadening the view of pathogen adaptation to vaccination. Some of these proteomic differences were associated with genetic mutations which are also found in other global pertussis strains. Additionally, several other transcriptomic studies have reported gene expression differences associated with the current epidemic pertussis strains. Together, these studies have broadened our understanding of how B. pertussis is evolving and identified additional pathogen factors important for the re-emergence of pertussis.

Future strategies to reduce the burden of pertussis

The ongoing evolution of B. pertussis in response to vaccine selection requires continued long-term epidemiological surveillance in Australia to monitor vaccine escape strains and the possible introduction of emerging antibiotic resistant B. pertussis strains from other countries. Although B. pertussis is adapting to the ACV, the current ACV remains effective in preventing pertussis disease in fully immunised individuals. Maternal immunisation and cocooning are also effective strategies at protecting the most vulnerable newborns from pertussis. However, for long term prevention and protection, an improved vaccine is required in the future. Current proposed strategies to alter
vaccine alleles to better match SNP cluster I will improve our ability to target these strains but will not be sufficient to combat Prn deficient strains. Therefore, a future pertussis vaccine should broaden the number of antigens targeted and ensure that these antigens are essential to *B. pertussis* survival so as to limit further pathogen adaptation. To do this, further research is required to better understand fundamental aspects of pertussis biology and adaptation as well as the processes behind vaccine and host-induced immunity.

**Conflicts of interest**
The author declares no conflicts of interest.

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**References**


**Biography**

Laurence Don Wai Luu is a postdoctoral research associate in Professor Ruiting Lan’s lab at the University of New South Wales. His research interest involves the application of genomics and proteomics to better understand the biology and evolution of *Bordetella pertussis*. 