

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 1970s¹. 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 immunisations². 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 vaccination². This was followed by a smaller yet still significant epidemic from 2014–2017 with a peak of 95 cases per 100 000 in 2015³ (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 SNPs⁷. Australian *B. pertussis* isolates were mostly found in SNP clusters I–IV 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 period⁷ (Figure 2). Since the 2008–2012 epidemic, the majority (>90%) of circulating strains in Australia belong to SNP cluster I^{3,8}. Most current

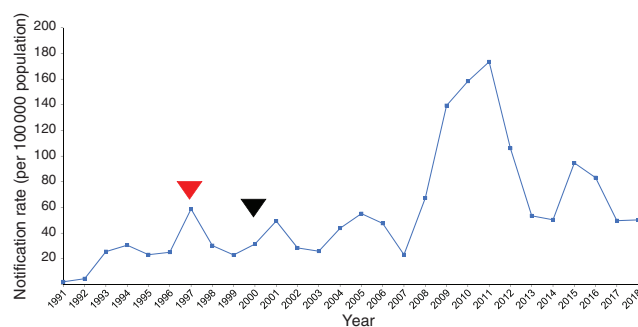


Figure 1. Number of pertussis notifications in Australia from 1991–2018. Introduction of the ACV as a booster in 1997 (▼) and exclusive use of ACV for all vaccinations after 2000 (▼) are indicated. Data from the Department of Health, 2019.

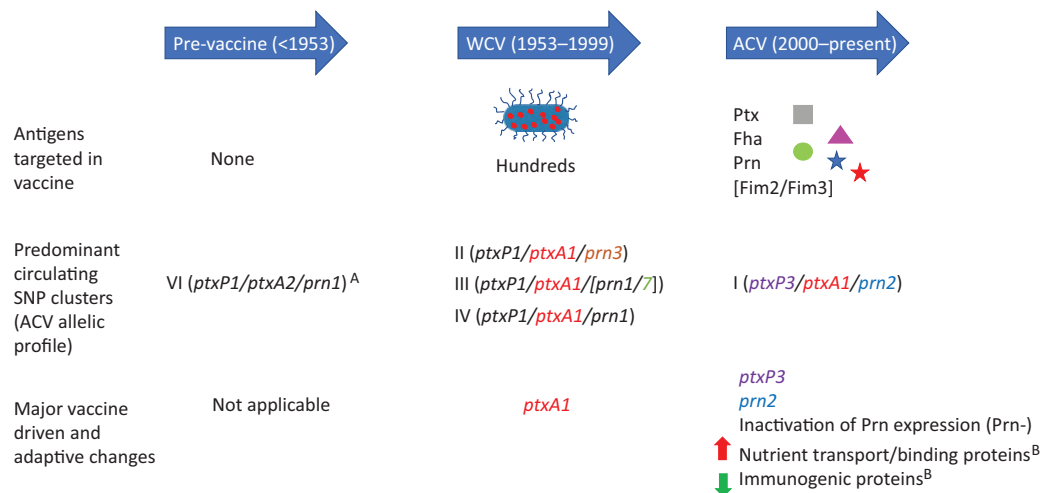


Figure 2. The changing population structure and adaptation of *B. pertussis* in Australia over three different vaccine periods. The circulating SNP clusters are displayed from the most predominant (at the top) to the least (bottom). ^AIncludes the vaccine strain Tohama I used to manufacture the WCV and ACV. ^BRelative to SNP cluster II.

circulating strains in other ACV countries typed as *ptxP3* strains are equivalent to SNP cluster I⁹.

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 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 used⁴ 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 ACV⁴. These mutations in *ptxA1* and *prn2* alter amino acid residues in known B/T immune epitopes thereby altering recognition from ACV-generated immunity^{4,10}. 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 environment¹¹.

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 promoter¹². This new promoter is

associated with increased pertussis toxin production, and possibly virulence and disease severity compared with strains carrying the original *ptxP1* allele^{13,14}. Increased pertussis toxin production is thought to provide a selective advantage by delaying neutrophil recruitment and modulating the immune response¹⁵. Using a mixed-infection mouse model, SNP cluster I strains outcompeted SNP cluster II strains (carrying *ptxP1*, *prn3* and *ptxA1*) in a vaccinated environment¹⁶. 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^{5,6,17}. 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 isolated¹⁸. However, within a decade this increased to 90% by 2017, the highest proportion of Prn deficient strains in the world³. Over 20 independent mechanisms for Prn inactivation have been documented^{5,6,18}. This diversity of inactivation suggests a beneficial selection pressure for convergent evolution and that Prn deficient strains emerged independently from multiple different clones¹⁹. 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^{20,21}. 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^{14,22}. 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 Australia³ while Ptx inactivation has been reported in other countries¹⁷. 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 environment¹⁶. 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^{23,24} (Figure 2). Some of these proteomic differences were associated with genetic mutations which are also found in other global pertussis strains²³. Additionally, several other transcriptomic studies have reported gene expression differences associated with the current epidemic pertussis strains^{25,26}. 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 countries²⁷. Although *B. pertussis* is adapting to the ACV, the current ACV remains effective in preventing pertussis disease in fully immunised individuals²⁸. Additionally, maternal immunisation and cocooning are also effective strategies at protecting the most vulnerable newborns from pertussis^{29,30}. 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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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*.