Evolution of *Bordetella pertussis*

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Bordetella pertussis, a Gram-negative bacterium, causes whooping cough (pertussis) in humans. Vaccination against pertussis was first introduced in the 1950s. The coverage of primary vaccination has been high in industrialized countries where acellular vaccines (ACVs) are commonly used. In many developing countries, the vaccination coverage is increasing and whole-cell vaccines (WCVs) are mainly in use. WCVs include inactivated whole bacteria, whereas ACVs comprise purified antigens including pertussis toxin (Ptx), filamentous hemagglutinin, pertactin (Prn), fimbriae (Fim) 2 and Fim3 in different combinations and concentrations. Compared with WCVs, ACVs are less reactogenic and are also used for booster vaccinations in older children and adults. Despite extensive vaccinations, pertussis has resurged in industrialized countries since the beginning of the 21st century.[1–3] Major explanations for the resurgence are waning immunity associated with ACVs and bacterial adaptation to vaccine-induced immunity. Compared with the vaccine strains, continuous changes have been observed in genomes of *B. pertussis* circulating in immunized populations. Common methods used for surveillance of *B. pertussis* isolates include serotyping, genotyping for vaccine antigens and genomic analyses by multilocus variable number of tandem repeat analysis (MLVA) and pulsed-field gel electrophoresis (PFGE).[4] More recently, whole genome sequencing has been applied.[5,6] Many studies demonstrate that variation of *B. pertussis* occurs in both phenotypes and genotypes.

CHARACTERISTICS OF *B. PERTUSSIS* IN INDUSTRIALIZED COUNTRIES WHERE WCVS HAVE BEEN REPLACED BY ACVS

B. pertussis produces 3 serotypes: Fim2, Fim3 or Fim2 and 3. Fim2 isolates predominate in unvaccinated populations, whereas they are largely displaced by Fim3 strains, when vaccination is introduced with WCVs containing both Fim2 and Fim3. Today, isolates with Fim3 predominate in most countries. In Finland, ACV has been used since 2005, and the ACV does not contain purified Fim2/3 antigens. The frequency of Fim3 was about 50% in 2005, increased to 100% in 2007 and peaked until 2009. In 2013 to 2015, frequencies of Fim3 and Fim2 isolates were 20% and 80%, respectively. It remains to be demonstrated what were the determinants of these changes, that is, natural infection, vaccination or both.

B. pertussis variation with respect to Ptx and Prn was first reported in 1998 in the Netherlands, where WCV has been used for more than 40 years. Polymorphisms in Ptx are caused by point mutation and are mostly found in the enzymatic subunit S1 (*ptxA*). So far, 8 alleles (*ptxA1* to *ptxA8*) have been reported. Of them, 5 (*ptxA1*, *ptxA2*, *ptxA4*, *ptxA5* and *ptxA8*) code for polymorphic proteins and the other 3 produce the same protein as *ptxA1*. Thirteen *prn* alleles have been described, and these polymorphisms are mainly caused by insertions or deletions of the repeating unit of 5 amino acids GGXXP. Almost all vaccine strains used for production of WCVs and ACVs carry *ptxA2* or *ptxA4* and *prn1* or *prn7*, whereas the circulating isolates harbor *ptxA1* and *prn2* or *prn3* (Table 1).

Polymorphisms in the promoter region of the *Ptx* gene arise from point mutations. Currently, 17 *ptxP* alleles have been documented, and in the prevaccine era, isolates with the *ptxP1* and *ptxP2* alleles predominated. After the introduction of vaccination, mainly *ptxP1* alleles were detected. The vaccine strains also harbor *ptxP1*. Although isolates with *ptxP3* were first detected in the late 1980s,[1] their rapid expansion in many industrial countries started in the late 1990s at a time when ACVs were being introduced. Today, the *ptxP3* isolates are common in Europe, Australia and the United States (Table 1). The point mutation in *ptxP3* isolates occurs in a binding site for the transcriptional regulator of *B. pertussis* and therefore it could increase the binding affinity of the transcriptional regulator and result in increased production of Ptx protein. Indeed, the *ptxP3* isolates tested appeared to produce more Ptx than *ptxP1* strains.[1] The emergence of *ptxP3* strains was also found to

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TABLE 1. Vaccination Programs and Circulating *Bordetella pertussis* Isolates in Selected Countries*

Country	Vaccination With			Allele Frequency After 2000 (%)			Isolates Without Prn Production	
	WCV	ACV	ACV for Boosters	*ptxA1*	*prn2/3*	*ptxP3*	First Appearance	% in 2012 or After
United States	1949	1997	2005	100	>95	>95	2010	60
Australia	1953	1999	1997	100	>95	>95	2008	80
United Kingdom	1950s	2004	2001	100	>95	Not applicable	0	0
Finland	1952	2005	2003	100	>95	>95	2011	5
Netherlands	1953	2005	2001	100	>95	>95	2010 to 2012	3
France	1959	2002	1998	94	94	90	2003	<14
Poland	1960	—	2004	98	50	18	0	0
China	1960s	2013	—	100	8	8	0	0

*Partly based on refs. [1–10].

be associated with resurgence of pertussis in Australia and the Netherlands.

The allele profiles of vaccine strains are mainly *ptxA2/prn1/ptxP1*. However, the current isolates circulating in the United States, Australia and most European countries contain *ptxA1/prn2/ptxP3* (Table 1). For *ptxA*, the change to nonvaccine types occurred in 1960 to 1980, for *prn* in 1980 to 1990 and for *ptxP* in 1990 to 2000. It should be kept in mind that in industrial countries, vaccinations with WCVs were started more than 50 years ago, and the switch from WCVs to ACVs took place in last 10 to 20 years. Furthermore, in these countries, the vaccination coverage has been generally high.

About 300 distinct MLVA types have been reported (http://www.mlva.net/), and currently, the predominant type is MT27 in the United States, Australia and most of European countries. A linkage between *ptxP3* and MT27 has been documented, and almost all *ptxP3* isolates had MLVA type MT27.[4]

For genomic typing of *B. pertussis*, PFGE technique has high resolution compared with MLVA. By using PFGE, the EUpertstrain group has recently compared 396 clinical isolates collected in 3 periods: 1998 to 2001 (N = 102), 2004 to 2005 (N = 154) and 2007 to 2009 (N = 140) from 9 countries including Denmark, Finland, France, Germany, the Netherlands, Norway, Poland, Sweden and the United Kingdom.[4] Altogether, 81 distinct profiles were identified, 5 of which (BpSR3, BpSR5, BpSR10, BpSR11 and BpSR12) were found in over 60% of the testing isolates and shown to be predominant in all countries except Poland. Although emergence of new PFGE profiles was not detected, frequencies of the 5 common profiles varied during the 3 study periods. For example, there was a decrease in frequency of BpSR11 and increase in frequency of BpSR3 and BpSR10 with time.

Whole genome sequencing has proven to be most informative for surveillance of bacterial pathogens. Recently, a comparative study based on a global collection of 343 clinical isolates revealed that the worldwide *B. pertussis* population underwent major changes during last 60 years.[5] The phylogeographic analyses of these isolates suggest that adaptive evolution of this pathogen is closely associated with the introduction of vaccines. When complete genomes of 40 clinical isolates from Finland (high vaccination coverage) and China (relatively low coverage) were compared, the study revealed that the molecular clock moved at different rates in the 2 countries and in distinct periods, further showing that evolution of *B. pertussis* was closely associated with the country vaccination coverage.[6]

Very recently, *B. pertussis* not producing Prn has been increasingly isolated in countries experiencing epidemics of pertussis.[2,3] During the outbreak in Washington, United States, in 2012, over 60% of isolates did not produce Prn (Table 1).[2] Even higher frequency of Prn-negative isolates was observed in Australia during the epidemic period of 2008 to 2012.[3] The Prn-deficient isolates were first detected in France in 2003.[7] However, no dramatic rise was found in this country, and in 2013, its frequency was around 15%. Although Prn-deficient isolates were also reported in other European countries such as Finland, the Netherlands, Norway and Sweden, their frequencies were generally low (Table 1).[8] Several mechanisms such as gene deletion, internal stop codon created by mutation and promoter inversion can cause Prn deficiency. However, the most common one is due to the mutation mediated by IS*481* insertion. Interestingly, Prn deficiency was not observed among the global collection of 343 isolates predominantly from 2008 or earlier.[5] It is generally believed that emergence of Prn-deficient isolates is driven by ACVs. Notably, the United States and Australia are 2 of the countries in the world where ACVs were first used for primary vaccinations. In Europe, Sweden and Italy started with ACVs for primary vaccinations in the mid-1990s. However, the vaccination strategies in the 2 countries differ from the United States and Australia. In Sweden, vaccination with WCVs was stopped between 1979 and 1995, whereas in Italy, the vaccination coverage of WCVs was quite low before the 1990s. Therefore, it seems that the immunity and the extent of selective pressure induced by ACVs in whole populations in Sweden and Italy may differ from those in the United States and Australia where the pertussis vaccinations have been continuously used and the coverage has been high. As ACVs have now been used in Sweden and Italy for almost 20 years, it will be determined whether there will be an emergence of Prn-deficient isolates in these countries.

The emergence of Prn-deficient isolates raises questions about its role in pertussis. An analysis of clinical symptoms caused by Prn-positive and Prn-negative isolates in French infants did not show major differences between the 2 groups, suggesting that the lack of Prn does not seem to affect bacterial virulence or transmission.[7] When a large number of patients from whom Prn-positive and Prn-negative strains were isolated were compared in the United States, a significant association between vaccination and Prn status was found, suggesting that vaccinated persons have greater susceptibility to be infected by Prn-negative strains compared with Prn-positive ones.[3] Another important question that needs to be addressed is the impact of Prn-deficient isolates on effectiveness/efficacy of ACVs. Further, it remains to be shown whether there will be emergence of Ptx- or filamentous hemagglutinin-deficient isolates in ACV-immunized populations since the 2 antigens are also included in ACVs.

CHARACTERISTICS OF *B. PERTUSSIS* IN COUNTRIES WHERE WCVS HAVE BEEN CONTINUOUSLY USED

Our current knowledge about *B. pertussis* evolution is mostly obtained from industrial countries where ACVs have been used. To better understand bacterial adaptation to ACV-induced selection pressure, we

need to look at *B. pertussis* circulating in countries where WCVs have been continuously used. Poland is 1 European country in which WCV is still in use for primary vaccination. In Poland, the currently dominant allele profiles are *ptxA1/prn1/ptxP1* and *ptxA1/prn2/ptxP1* (Table 1).[4] The difference in allele profiles observed between Poland and most European countries was also confirmed by genomic analyses of PFGE and MLVA. In China, WCVs have been continuously used since 1960s. About 100 isolates collected during 2013 to 2014 from several different regions of this country were studied, and the prevalent allele profile was found to be *ptxA1/prn1/ptxP1* (Table 1).[9] Furthermore, the common MLVA type and PFGE profiles identified in Europe were seldom detected in China.[10] These findings from Poland and China indicate that *B. pertussis* circulating in countries where WCVs have been continuously used differ from those in industrialized countries where ACVs have been in use since 1990s, suggesting that ACVs induce accelerated selection pressure for bacterial population compared with WCVs.

CONCLUSIONS

Evolution of *B. pertussis* after the start of vaccination occurs toward nonvaccine-type strains, although the timing and speed of changes in bacterial populations may differ in different countries. Bacterial adaptation to vaccine-induced immunity started with alteration in the structural genes coding for vaccine antigens and was followed by functional changes with respect to production of vaccine antigens such as Prn. Ongoing surveillance of *B. pertussis* populations and evaluation of the impact of these changes on effectiveness of vaccines and disease incidence are critical to inform the optimal vaccination programs to prevent pertussis.

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