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Analysis of *Bordetella pertussis* Populations in European Countries with Different Vaccination Policies

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Despite the widespread use of pertussis vaccines during the last decades, pertussis has remained an endemic disease with frequent epidemic outbreaks. Currently two types of vaccines are used: whole-cell vaccines (WCVs) and recently developed acellular vaccines (ACVs). The long-term aim of our studies is to assess the effect of different vaccination policies on the population structure of *Bordetella pertussis* and ultimately on the disease burden in Europe. In the present study, a total of 102 *B. pertussis* isolates from the period 1998 to 2001 from five European countries (Finland, Sweden, Germany, The Netherlands, and France) were characterized. The isolates were analyzed by typing based on variable number of tandem repeats (VNTR); by sequencing of polymorphic genes encoding the surface proteins pertussis toxin S1 and S3 subunits (*ptxA* and *ptxC*), pertactin (*prn*), and tracheal colonization factor (*tcfA*); and by fimbrial serotyping. The results reveal a relationship between geographic location and VNTR types, the frequency of the *ptxC* alleles, and serotypes. We have not observed a relationship between the strain characteristics we studied and vaccination programs. Our results provide a baseline which can be used to reveal changes in the *B. pertussis* population in Europe in the coming years.

The introduction of whole-cell vaccines (WCVs) against pertussis in the 1940s to 1960s has resulted in a dramatic decrease in morbidity and mortality due to pertussis. WCVs were developed in the 1940s, when it was unclear which specific *Bordetella pertussis* antigens contributed to immunity. Further, there are large differences in the ways WCVs are produced by different manufacturers with respect to strains used, culture conditions, and inactivation procedures. This has resulted in WCVs with a wide range of efficacies (1, 25, 38, 40). Very effective WCVs have been used in the United Kingdom and France, whereas less effective WCVs have been used in Canada and The Netherlands (38, 40). In Sweden, vaccination with a WCV was discontinued in 1979 because of its low efficacy. The use of WCVs is associated with side effects such as local reactions, fever, and systemic symptoms. The reactogenicity of WCVs has stimulated the development of less reactogenic and more defined acellular vaccines (ACVs). A number of different ACVs have been developed, all of which contain pertussis toxin (Ptx). In addition to Ptx, the ACVs may contain filamentous hemagglutinin (FHA), pertactin (Prn), and fimbriae (Fim). Although field trials have shown that ACVs are less reactogenic than WCVs (8, 11–14, 34, 35), only a single ACV, comprised of Ptx, Prn, Fim, and FHA, was shown to be as efficacious as a good WCV (19, 26). Because of their lower reactogenicity, ACVs have replaced WCVs in most developed countries.

In Europe there is a high degree of heterogeneity with respect to vaccination history, the type of pertussis vaccines used, and the vaccination schedule. In order to investigate the effect of these differences on the incidence of pertussis and on the population structure of *B. pertussis*, a surveillance program was initiated entitled "European Research Programme for Improved Pertussis Strain Characterization and Surveillance" (acronym, EUpertstrain). One aim of this program is to identify vaccines and vaccination schedules that most effectively reduce pertussis morbidity and mortality, especially in the unvaccinated young. A second aim is to assess the effect of different vaccines on the *B. pertussis* population. Finally, this program allows the evaluation of the switch from WCVs to ACVs. Participating countries include Finland, Sweden, Germany, The Netherlands, and France. The vaccination programs of these countries are compared in Table 1.

In the present study, we compared *B. pertussis* strains circulating in the five countries in the period 1998 to 2001. A collection of 102 *B. pertussis* isolates were analyzed by typing based on variable number of tandem repeats (VNTR); by sequencing of polymorphic genes encoding the surface proteins pertussis toxin S1 and S3 subunits (*ptxA* and *ptxC*), pertactin (*prn*), and tracheal colonization factor (*tcfA*); and by fimbrial serotyping. This study may serve as a baseline to assess

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TABLE 1. Pertussis vaccines, vaccination schedules, and incidences in the five countries studied

Country	Vaccine	Manufacturer	Start of vaccination (yr)	% Coverage	Vaccination schedule	Incidence/ 100,000[a]	Protein variant expressed in vaccine					
							Vaccine strain	PtxA[f]	Prn	FHA	Fim	TcfA
Finland	WCV[b]	KTL[c]	1952	96	3, 4, 5, 24 mo	6–18	1772	PtxA2	Prn1	ND[h]	Fim2,3	ND
							18530	PtxA4	Prn1	ND	Fim3	ND
Sweden[d]	ACV	Glaxo-SmithKline	1996	~100%	3, 5, 12 mo	11–31	Tohama	PtxA2	Prn1	ND	NP[g]	NP
	ACV	Aventis-Pasteur MSD	1997	~100%	3, 5, 12 mo	11–31		ND	NP	ND	NP	NP
Germany (FRG)	WCV	Behringwerke	1955–1998	variable	3, 4, 5, 12–15 mo			ND	ND	ND	ND	ND
Germany (GDR)	WCV	State Institute	1964–1989	>95				ND	ND	ND	ND	ND
Germany	ACV	Glaxo-SmithKline	1995	>95	3, 4, 5, 11–14 mo, 9–17 yr, health care personnel	6–20	Tohama	PtxA2	Prn1	ND	NP	NP
		Aventis-Pasteur MSD	1995	>95	3, 4, 5, 11–14 mo, 9–17 yr, health care personnel	6–20		ND	NP	ND	NP	NP
		Chiron-Behring	1995	>95	3, 4, 5, 11–14 mo, 9–17 yr, health care professional	6–20		ND	ND	ND	NP	NP
The Netherlands	WCV	NVI[e]	1953	96	3, 4, 5, 11 mo	14–44	134	PtxA2	Prn1	FhaB1	Fim3	TcfA2
							509	PtxA4	Prn7	FhaB2	Fim2 or Fim2,3	TcfA2
France	WCV	Aventis-Pasteur MSD	1959	>90	2, 3, 4, 16–18 mo (until 1998)		IM1414	PtxA4	Prn1	ND	Fim 2 + 3	ND
							IM1416	PtxA2	Prn1	ND	Fim2	ND
	ACV	Glaxo-SmithKline	1998		16–18 mo, 11–13 yr		Tohama	PtxA2	Prn1	ND	NP	NP
		Aventis-Pasteur MSD	1998		16–18 mo, 11–13 yr			ND	NP	ND	NP	NP

[a] Care should be taken when comparing incidences between countries, because of the different surveillance and diagnostic methods used.
[b] Vaccine contains equal amounts of the two strains.
[c] National Public Health Institute, Helsinki, Finland.
[d] In the Northern part of Sweden mainly a three-component ACV is used; in the southern part, a two-component ACV is used.
[e] Netherlands Vaccine Institute, Bilthoven, The Netherlands.
[f] PtxA, pertussis toxin S1 subunit.
[g] NP, not present.
[h] ND, not determined.

the effects of different vaccination policies in Europe on the incidence of pertussis. Ultimately, this may lead to improved vaccines against pertussis.

MATERIALS AND METHODS

Bacterial strains. A total of 102 *B. pertussis* isolates, from the period 1998 to 2001, were characterized in this study. French strains were from 1 year; Swedish, German, and Dutch strains were from 2 years; and the Finnish strains were from 3 years. An attempt was made to obtain an equal number of strains from vaccinated and unvaccinated individuals in the age category 0 to 4 years. However, due to limitations of the strain collections, this was not always possible. For example, in France no cases were observed in vaccinated children in the age category 0 to 4 years. Apart from these conditions, strains were randomly chosen from the collections. Approximately 20 strains per country were analyzed: 20 from Finland, 25 from Sweden, 17 from Germany, 20 from The Netherlands, and 20 from France. Of these isolates, 55 were derived from unvaccinated hosts (ages 0 to 134 months) and 39 were from vaccinated hosts (ages 4 to 318 months). The vaccination status of eight German hosts was unknown.

Vaccination status. Vaccination statuses of hosts from Sweden, Germany, and France were based on documented patient data. Vaccination status in Finland and The Netherlands was deduced from the hosts' age, which is reasonable in view of the high vaccination coverages.

VNTR analysis. For VNTR analysis, the number of repeats for each VNTR locus was determined. VNTR analysis was performed using the primers for the five loci VNTR-1 and VNTR-3 to VNTR-6 as described before (32). Each PCR was carried out in a final volume of 20 μl containing 20 pmol of the appropriate primer pair, 10 μl HotStarTaq Mastermix (QIAGEN), 1 M betaine, 1 μM 6-carboxyfluorescein-labeled forward primer, 1 μM reverse primer, and 20 ng of template DNA. Following an initial denaturation at 95°C for 15 min, 28 cycles of 95°C for 20 s, 67°C for 30 s, and 72°C for 1 min were performed. The PCR was completed by a final extension phase of 68°C for 30 min using a GeneAmp 9700PCR system (Perkin-Elmer Applied Biosystems). Two microliters of 100-fold-diluted PCR products was added to a loading buffer containing 10 μl of formamide (Perkin-Elmer Applied Biosystems) and 0.05 μl of MapMarker LOW 70-400Bp (Bioventures Inc.). Before being loaded, the samples were denatured at 95°C for 5 min and then kept on ice. Fluorescently labeled amplicons were subjected to electrophoresis using an ABI PRISM 3700 automatic sequencer. The sizes of the PCR fragments were estimated using the GeneScan software package (Perkin-Elmer Applied Biosystem), and the exact number of complete repeats present was calculated using a derived allele-naming table based on the number of complete repeats which could theoretically be present in a PCR product of a given size, allowing for extra flanking nucleotides and primer size (Bionumerics software package version 3.0; Applied Maths).

Sequencing of genes for polymorphic surface proteins. The four *B. pertussis* loci sequenced were the genes for antigens incorporated in the ACVs that are known to be polymorphic: e.g., genes coding for surface proteins pertussis toxin S1 and S3 subunits (*ptxA* and *ptxC*) and pertactin (*prn*). We also included the gene for tracheal colonization factor (*tcfA*) as it was shown to be one of the most polymorphic *B. pertussis* proteins investigated so far (20, 38). The DNA sequences of these loci are available from GenBank. We have changed the designation of the genes for the pertussis toxin subunits to conform to the published genome sequence of *B. pertussis* (29). The designations ptxS1 and ptxS3 have been changed to ptxA and ptxC, respectively. Numbering of alleles has not been changed (20).

PCR amplification of chromosomal DNA was performed by adding 1 μl of DNA to 19 μl of buffer comprising 50% HotstarTaq Master mix (QIAGEN), 1 μM concentrations of each primer, and 5 or 10% dimethyl sulfoxide. Five percent dimethyl sulfoxide was added to the PCR mix for amplification of *ptxA*, *ptxC*, and *tcfA*. For *prn*, 10% dimethyl sulfoxide was used. Amplification of genes was performed on a GeneAmp PCR 9700 system by using a specific program for each gene (22, 39). The amplified fragments were purified using a PCR purification kit (QIAGEN). Purified fragments were sequenced using the primers that were used in the initial amplification. Sequencing reactions were prepared using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) according to the manufacturer's recommendations for cycle sequencing on the GeneAmp PCR 9700 system. The reactions were analyzed on an ABI PRISM 3700 Genetic Analyzer (PE Applied Biosystems). Sequences were assembled using the SeqMan program of DNASTAR software.

Serotyping. Serotyping of fimbriae was performed by slide agglutination with specific sera. A bacterial colony and a drop of serum that contained antibodies against Fim2 or Fim3 were mixed on a glass slide. After rocking for 1 minute, agglutination was determined.

Statistical analysis. The statistical significances of the frequencies of VNTR, genotypes or serotypes in the different countries, between vaccinated and non-vaccinated hosts or type of vaccine used (WCV or ACV), were calculated using the χ^2 test. In cases in which conditions for χ^2 calculations could not be met (e.g., when all expected values are greater than 1.0 and at least 20% of the expected values are greater than 5), the exact test was performed.

Genotypic diversity (GD) based on DNA typing was calculated by the equation $GD = (n/[n - 1]) \times (1 - \Sigma x_i^2)$, where x_i is the frequency of the DNA type, and n is the number of strains (37). Statistical significance of the difference in genotypic diversity between countries was calculated using the theory of U statistics (33). Statistical significance of the difference in genotypic diversity between vaccinated and unvaccinated was calculated using the method of jackknife (7).

RESULTS

Two approaches were used to characterize isolates: sequencing of virulence genes for polymorphic surface proteins and VNTR typing. *B. pertussis* isolates ($n = 102$) were selected from the period 1998 to 2001 from five different European countries (Finland, Sweden, Germany, The Netherlands, and France). From each country, 17 to 25 clinical isolates were analyzed which were derived from unvaccinated hosts ($n = 55$; age, 0 to 134 months; average age, 24 months) or vaccinated hosts ($n = 39$; age, 4 to 318 months; average age, 48 months). The vaccination status of eight German hosts was unknown.

VNTR analysis. A total of 16 different VNTR types were identified in the 102 isolates. The two most common VNTR types identified (VT27 and VT29) accounted for 54% (55 of 102) and 22% (22 of 102) of all the isolates analyzed, respectively (Table 2). These two profiles were found in all five countries, ranging in frequency from 40% in Finland to 76% to 90% in the other countries. Most of the remaining VNTR types were country specific, and 8% of the isolates generated a unique VNTR profile. The frequencies of the VNTR types in the five countries were significantly different ($P = 0.0066$). Differences in frequencies of VNTR types between vaccinated and unvaccinated hosts were not significant ($P = 0.60$). Based on VNTR types, the lowest GD in the *B. pertussis* population was found in the French bacterial population (GD = 0.51), whereas the highest diversity was found in the Finnish bacterial population (GD = 0.80). The GDs in Finland and Germany were significantly different from those in Sweden, The Netherlands, and France ($P < 0.05$). Isolates from vaccinated hosts showed a higher GD compared to those from unvaccinated hosts (respectively, 0.715 and 0.586). The difference was not significant, however ($P > 0.05$).

Sequencing of genes for polymorphic surface proteins. Previous work revealed polymorphism in genes for the S1 and S3 subunits of pertussis toxin (*ptxA* and *ptxC*, respectively), pertactin (*prn*), and tracheal colonization factor (*tcfA*) (5, 38). The frequencies of the alleles for these genes in the five countries were compared.

***ptxC* alleles.** Two *ptxC* alleles, *ptxC1* and *ptxC2* (previously designated *ptxS3-1* and *ptxS3-2*, respectively), have been described which differ at a single nucleotide resulting in a silent mutation (38). The *ptxC1* allele was found to predominate in Finland and Sweden (respectively, 80% and 68% of the isolates), whereas *ptxC2* predominated in Germany, The Netherlands, and France (76%, 70%, and 75% respectively) (Table 3).

TABLE 2. VNTR type frequencies and genotypic diversities of *B. pertussis* in five European countries[a]

VNTR type	% (no.) of VNTR type in:					Grand total (% [n = 102])
	Finland (n = 20; GD = 0.80)	Sweden (n = 25; GD = 0.56)	Germany (n = 17; GD = 0.62)	The Netherlands (n = 20; GD = 0.52)	France (n = 20; GD = 0.51)	
16					5 (1)	1
19	5 (1)					1
22				5 (1)		1
25			12 (2)			2
27	30 (6)	60 (15)	53 (9)	60 (12)	65 (13)	54
28	25 (5)					5
29	10 (2)	24 (6)	24 (4)	30 (6)	20 (4)	22
30		4 (1)				1
43			6 (1)			1
55	10 (2)					2
71	5 (1)	8 (2)		5 (1)		4
72	10 (2)					2
73	5 (1)					1
74					5 (1)	1
75					5 (1)	1
76		4 (1)	6 (1)			2

[a] The number of strains analyzed is in parentheses. The distribution of the VNTR types was analyzed with the exact test. The frequencies of the VNTR types were significantly different between the five countries ($P = 0.0066$). n, number of strains in each country investigated. GDs in Finland and Germany were significantly different from GDs in Sweden, The Netherlands, and France ($P < 0.05$).

The frequencies of the two alleles in the five countries were significantly different ($P = 0.0001$). The frequencies of the two alleles in vaccinated and unvaccinated individuals were not significantly different ($P = 0.53$; not shown).

***ptxA* alleles.** Six *ptxA* alleles, which contain nonsilent mutations, have been found in *B. pertussis* populations (5, 18, 21, 22). All pertussis vaccines analyzed to date are derived from strains carrying the *ptxA2* and *ptxA4* alleles (previously designated *ptxS1-2* and ptx*S1-4*, respectively), coding for the subunit variants PtxA2 and PtxA4, respectively (Table 1). A single *ptxA* allele, *ptxA1* (previously designated *ptxS1-1*), was observed in this study (Table 3).

***prn* alleles.** Twelve *prn* alleles have been observed in *B. pertussis* populations, of which 3 (*prn1*, *prn2*, and *prn3*) were found in this study (5, 18, 21, 22). The three alleles differ in nonsilent mutations. Vaccines used in Finland, Sweden, Germany, The Netherlands, and France are derived from strains producing the Prn1 variant (Table 1). This variant was found in low frequencies in all five countries (0% to 6%) (Table 3). Interestingly, all Prn1 strains ($n = 3$) were isolated from nonvaccinated hosts. Strains with the *prn2* allele predominated in all countries, ranging in frequency from 75% in France to 95% in The Netherlands. The frequency of *prn3* strains ranged from 0 to 20%. No *prn3* strains were observed in Finland. The frequencies of the three *prn* alleles in the five countries and in vaccinated and unvaccinated individuals (not shown) were not significantly different ($P = 0.22$ and 0.66, respectively, exact test).

***tcfA* alleles.** Five *tcfA* alleles have been described (38), of which two (*tcfA*2 and *tcfA*3) were found in this study. The two alleles differ in nonsilent mutations.The *tcfA2* allele is found in the vaccines used to produce the French and Dutch WCVs. It is not known which *tcfA* alleles are present in the Finnish vaccine strains. TcfA is not a component of ACVs used in Sweden and Germany (Table 1). The *tcfA2* allele predominated in all countries, ranging from 80% in France to 100% in Finland (Table 2). The frequencies of the two *tcfA* alleles in the five countries and in vaccinated and unvaccinated individuals

TABLE 3. Distribution of alleles in *B. pertussis* strains from different countries

Allele[a]	% (no. of strains) in[b]:					
	Finland (n = 20)	Sweden (n = 25)	Germany (n = 17)	The Netherlands (n = 20)	France (n = 20)	Total (n = 102)
ptxC1	80 (16)	68 (17)	24 (4)	30 (6)	25 (5)	47
ptxC2	20 (4)	32 (8)	76 (13)	70 (14)	75 (15)	53
ptxA1	100 (20)	100 (25)	100 (17)	100 (20)	100 (20)	100
prn1	5 (1)	0 (0)	6 (1)	0 (0)	5 (1)	3
prn2	95 (19)	88 (22)	76 (13)	95 (20)	75 (15)	87
prn3	0 (0)	12 (3)	18 (3)	5 (1)	20 (4)	10
tcfA2	100 (20)	88 (22)	82 (14)	95 (19)	80 (16)	89
tcfA3	0 (0)	12 (3)	18 (3)	5 (1)	20 (4)	11
St-2	90 (18)	64 (16)	6 (1)	15 (3)	0 (0)	37
St-3	10 (2)	20 (5)	94 (16)	85 (17)	90 (18)	57
St-2 + 3	0 (0)	16 (4)	0 (0)	0 (0)	10 (2)	6

[a] *ptxC*, pertussis toxin S3 subunit; *ptxA*, pertussis toxin S1 subunit; St, serotype.
[b] The distributions of the alleles and serotypes were analyzed with the exact test. The frequencies of *ptxC* and the serotypes were significantly different in the five countries ($P \leq 0.0001$). The frequencies of the other alleles did not differ significantly between the countries.

(not shown) were not significantly different ($P = 0.22$ and 1.0, respectively).

Fimbrial serotypes. *B. pertussis* produces two serologically distinct fimbriae, designated serotype 2 and serotype 3 fimbriae, respectively (17, 23). As strains may express a single serotype or both serotypes, three combinations are possible: serotype 2, serotype 3, and serotype 2 plus 3. WCVs used in the five countries harbor both fimbrial serotypes (Table 1). Fimbrial antigens are absent from ACVs used in Sweden, Germany, and France. Serotype 2 strains predominated in Finland and Sweden (frequencies of 90% and 56%, respectively), whereas serotype 3 strains predominated in Germany, The Netherlands, and France (frequencies of 94%, 85%, and 90%, respectively) (Table 3). Serotype 2-plus-3 strains were only found in Sweden and France (frequencies of 8% and 10%, respectively). The frequencies of the fimbrial serotypes in the five countries were significantly different ($P < 0.0001$). The frequencies of the serotypes in vaccinated and unvaccinated individuals (not shown) were not significantly different ($P = 0.65$).

DISCUSSION

As part of a European research program for improved *B. pertussis* strain characterization and surveillance, we compared the population structures of *B. pertussis* in five European countries: Finland, Sweden, Germany, The Netherlands, and France. VNTR typing of the 102 clinical isolates revealed the presence of 16 different VNTR types. The frequencies of the VNTR types in the five countries were significantly different ($P = 0.0066$). This may reflect geographic isolation of the populations or local factors such as differences in populations and vaccines used. However, the frequencies of VNTR types did not differ significantly in vaccinated and unvaccinated hosts or in populations vaccinated with ACVs and WCVs ($P = 0.95$). It should be noted that the vaccination status of the Finnish and Dutch patients was deduced from their age, which is reasonable in view of the high vaccination coverage. However, this may overestimate the fraction of vaccinated individuals, as unvaccinated hosts will be more susceptible to infection than vaccinated hosts.

The majority of isolates in Sweden, Germany, The Netherlands, and France were represented by the VNTR types VT27 and VT29 (75 to 90%). Among the Finnish isolates, the VNTR types VT27 and VT28 dominated (frequencies of 30% and 25%, respectively). With respect to VNTR types, the Finnish *B. pertussis* population was quite distinct compared to the other four countries. The highest and lowest GD based on VNTR typing were found in Finland (GD, 0.80) and France (GD, 0.51), respectively. A low genetic diversity was also observed in The Netherlands (GD, 0.52). The differences in genotypic diversity may have several causes. The high genotypic diversity in Finland may be due to a low human population density resulting in isolated *B. pertussis* populations. The low genotypic diversity observed in the Dutch *B. pertussis* population may be due the expansion of a distinct strain in recent years (38).

The frequencies of a number of alleles for virulence factors were also studied. Previous studies have revealed very little polymorphism in *B. pertussis* surface proteins. (5, 18, 21, 22, 28, 38). We focused on genes for proteins incorporated in the ACVs and which were shown to be polymorphic in previous studies: *ptxC*, *ptxA*, and *prn*. Further, although *tcfA* is not part of ACVs, it was also included as it was found to be highly polymorphic in a previous study (38). Finally, isolates were serotyped to determine the type of fimbriae produced. Fimbriae are part of a number of ACVs. However, the ACVs used in some of the five countries did not contain fimbriae at the time the isolates were collected (Table 1). The largest difference between countries was observed with respect to the *ptxC* allele ($P = 0.0001$). We found that the *ptxC1* allele occurred more frequently in Finland (80%) and Sweden (68%) than in the more southern countries investigated (24% to 30%). In these latter countries, the *ptxC2* allele predominated. Interestingly, the two alleles observed differ in a silent mutation. In The Netherlands, a recent expansion of the *ptxC2 allele* was observed, suggesting this allele is linked to an as yet unidentified locus which confers a selective advantage (38) The frequencies of the *ptxC* alleles did not differ significantly in vaccinated and unvaccinated hosts or in countries using ACVs and WCVs ($P = 0.53$ and 0.69, respectively).

Within the population tested, we found no polymorphism in the *ptxA* gene: all strains were of the nonvaccine type *ptxA1*. These results are consistent with previous studies which revealed that, in Finland, The Netherlands, and France, vaccine-type alleles were replaced by nonvaccine types (21, 22, 40).

The pertactin gene (*prn*) is one of the most polymorphic *B. pertussis* genes investigated. The vaccine-type allele (*prn1*) was found in very low frequency (0 to 6%) in all countries, whereas the nonvaccine-type alleles (*prn2* and *prn3*) were found in frequencies of 75 to 95% and 0 to 20%, respectively. Previous work showed that, in Finland and The Netherlands, the frequency of *prn1* decreased from approximately 100% to the present level after introduction of nationwide vaccination (21, 22). Stratifying the *B. pertussis* strains according to country of origin, vaccination status of host, or type of vaccine used (WCV or ACV) did not reveal significant differences in *prn* allele frequencies. However, the three isolates expressing the vaccine-type allele were found in unvaccinated hosts only (ages 2, 4, and 51 months, respectively).

Of the two *tcfA* alleles detected, *tcfA2* predominated in all five countries (frequencies, 80% to 100%). TcfA is not part of ACVs; however, it is found in WCVs. The Dutch WCV isolates contain the *tcfA2* allele, and it is perhaps somewhat unexpected that this allele is predominant in The Netherlands. We presume that this may be due to its linkage with the *prn2* allele (38). Consistent with a previous study using Dutch strains, we found that 97% of the *tcfA2* strains carried *prn2*, and 91% of the *tcfA3* strains carried *prn3*. If the *prn2* allele confers a larger degree of fitness on strains than the *prn3* allele, *tcfA2* may hitchhike to predominance. Stratifying the *B. pertussis* isolates according to country of origin, vaccination status of host, or type of vaccine used (WCV or ACV) did not reveal significant differences in *tcfA* allele frequencies.

Serotype 2 isolates predominated in Finland and Sweden (frequencies of 90% and 56%, respectively), whereas serotype 3 isolates were found to predominate in Germany, The Netherlands, and France (frequencies of 94%, 85%, and 90%, respectively). The serotype frequencies were significantly different when isolates were stratified according to country of origin ($P < 0.0001$). Interestingly, serotype 3 isolates normally predominate in populations vaccinated with WCVs (3, 4, 9, 30,

31). In Sweden, serotype 2 fimbriae predominated in 1979 to 1996, the vaccine-free period; in 2003, serotype 3 fimbriae replaced serotype 2 fimbriae as the most prevalent serotype, reaching a frequency of more than 90%. The 25 Swedish isolates analyzed in this study are part of a total of 589 isolates that were collected in the period 1999 to 2000 and which comprised 35% serotype 2 fimbria isolates. It is unclear if there is a causal relationship between the introduction of ACVs and the change in serotypes in Sweden. It has been suggested that some ACVs are contaminated with fimbriae (2). Stratifying the *B. pertussis* isolates according to vaccination status of host or type of vaccine used (WCV or ACV) did not reveal significant differences in serotype frequencies.

The present study reveals a relationship between geographic location and VNTR types, the frequency of the *ptxC* alleles, and serotypes. Further studies are required to elucidate the underlying causes. We have not observed a relationship between the strain characteristics we studied and vaccination programs. This may be due to the fact that some vaccines have been introduced recently or to the limited number of strains analyzed. The relative contribution of geography and vaccination in the genesis of distinct *B. pertussis* populations in Europe requires further study. This work may serve as a baseline for future studies and may ultimately lead to a more efficient vaccination against pertussis. PCR is replacing culture in the diagnosis of pertussis, and this has hampered the collection of strains for epidemiological studies. We recommend the implementation of a system to actively collect *Bordetella* strains in Europe. Strains can be characterized according to standard procedures (20, 38). Further, data should be collected on the date and place of isolation, patient age and vaccination status, and the vaccine used. Although antibiotic resistance in *B. pertussis* is rare, future studies may include this aspect also (41).

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