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Bordetella pertussis Strains Circulating in Europe in 1999 to 2004 as Determined by Pulsed-Field Gel Electrophoresis▽

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Clinical isolates of *Bordetella pertussis* collected during the year 2004 ($n = 153$) in eight European countries, Denmark, Finland, France, Germany, The Netherlands, Poland, Sweden, and United Kingdom, were analyzed by pulsed-field gel electrophoresis (PFGE), and their PFGE profiles were compared with those of isolates collected in 1999 ($n = 102$). The 255 isolates produced 59 distinct PFGE profiles. Among the 153 isolates from 2004, 36 profiles were found, while within the 102 isolates from 1999, 33 profiles were detected. One PFGE profile, BpSR11, was dominant (30% to 50%) in all countries except Denmark (10%) and Poland (0%). In comparison with 1999, there was an increase in BpSR11 prevalence in Finland in 2004 from 5% to 40%, coinciding with a major incidence peak. Some other PFGE profiles seemed to be associated with limited dissemination. Poland was the only country in which the most common actual European PFGE profiles were not found. In a dendrogram analysis, all common PFGE profiles were identified within PFGE group IV, and BpSR11 clustered together with PFGE subgroup IVβ. Compared to the 1999 isolates, PFGE group V representative for pertactin variant *prn3* strains had disappeared, and a new cluster was seen. In conclusion, some PFGE profiles, such as BpSR11, evidently have a higher capacity to spread, suggesting increased fitness to the present immunological environment. It is therefore of major interest to continue with surveillance programs of *B. pertussis* isolates, as both waning vaccine-derived immunity and strain variation may play a role in the persistence of pertussis.

Whooping cough or pertussis is still a significant disease with regular outbreaks despite the introduction of mass vaccination and good coverage of the programs. The resurgence of pertussis has been observed in the United States, Europe, Canada, Asia, and Australia (8, 13, 15, 19, 26, 27). Insight into the polymorphism of *Bordetella pertussis*, the causative agent of pertussis, and its capacity to adapt to population immunity is important to understand pertussis epidemiology (17).

To investigate *B. pertussis* strains circulating in the European countries with different vaccination programs and to elucidate possible emergence of bacterial variants with increased fitness, a European research program for strain characterization and surveillance, EUpertstrain, was established. The EUpertstrain I project was initiated in 2001 and supported by the European Commission. Initially, the members participating were the pertussis reference groups from Finland, France, Germany, The Netherlands, and Sweden. The EUpertstrain II project was a continuation of EUpertstrain I and was supported by GlaxoSmithKline (Rixensart, Belgium) and Sanofi Pasteur and Sanofi Pasteur MSD (Lyon, France). In addition to the above-mentioned five countries, Denmark, Poland, and United Kingdom joined the project. All eight participating countries use different vaccination schedules and use at least partly different vaccines (Table 1).

For epidemiological characterization of *B. pertussis* isolates, various DNA-based techniques are available, including pulsed-field gel electrophoresis (PFGE) (2, 4, 12, 16), IS*1002*-based fingerprinting (18, 23), multilocus sequence typing (24), multilocus variable-number tandem repeat analysis (MLVA) (20, 22), and recently whole-genome DNA microarray (6, 9). Multilocus sequence typing has been used successfully to assess variation in a number of *B. pertussis* surface protein-encoding genes, including genes encoding pertussis toxin (Ptx), pertactin (Prn), tracheal colonization factor (TcfA), and serotype 3 fimbriae (Fim3) (1, 16, 21).

For epidemiological studies, PFGE has the best discriminatory power, and a standard protocol was chosen as the reference method by a group of experts meeting in Paris in 1999 (16). Reference strains were defined and made available. These strains represented five major PFGE groups (I to V) as well as three subgroups in PFGE group IV (7, 25).

The methods to be chosen for epidemiological typing depend on the objective of the study. In a previous study by Caro et al. (7) the PFGE patterns of EUpertstrain I culture collec-

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TABLE 1. Vaccination programs up to 2004 in eight countries participating in the two EUpertstrain projects

Country	Yrs of vaccination program	Vaccination schedule[a]	Booster schedule[a]
Finland[b]	1952	3, 4, and 5 mo DTwP	20 to 24 mo DTwP
The Netherlands	1953	2, 3, 4, and 11 mo DTwP-IPV-Hib	
France	1959	3, 4, and 5 mo DTwP-IPV	16 to 8 mo DTwP-IPV
	1995	2, 3, and 4 mo DTwP-IPV-Hib	16 to 18 mo DTwP-IPV-Hib
	1998	2, 3, and 4 mo DTwP-IPV-Hib	16 to 18 mo DTaP-IPV-Hib, 11 to 13 yr DtaP-IPV
Germany	1995	2, 3, and 4 mo DTaP-IPV-Hib-HB	11 to 14 mo DTaP-IPV-Hib-HB
	2000	2, 3, and 4 mo DTaP-IPV-Hib-HB	11 to 14 mo DTaP-IPV-Hib-HB, 9 to 17 yr dTaP or dTaP-IPV
Sweden	1953	3, 5, and 12 mo DTwP	
	1979–1996	No vaccination	
	1996	3, 5, and 12 mo DTPa	
	1998	3, 5, and 12 mo DTaP-IPV-Hib	
Denmark	1961	5, 6, 7, and 15 mo DTwP	
	1969	5 and 9 wk and 10 mo wP (nonadsorbed)	
	1997	3, 5, and 12 mo DTaP-IPV	
	2002	3, 5, and 12 mo DTaP-IPV/HIB	
	2003		5 yr TdaP
	2004		5 yr Tdap-IPV
Poland	1960	2, 3 to 4, 5, and 16 to 17 mo DTwP	
	2004		6 yr DtaP
United Kingdom	1950	3, 5, and 10 mo DTwP	
	1990	2, 3, and 4 mo DTwP	
	2000/2001	DtaP-Hib[c]	
	October 2001		aP added to preschool DT

[a] The age of the children (in weeks, months, or years) when the vaccine or booster was given and the specific vaccine are shown in the vaccination and booster schedules. Vaccine abbreviations: DTwP, diphtheria–tetanus–whole-cell pertussis; IPV, inactivated poliovirus; Hib, *Haemophilus influenzae* type b; DTaP, diphtheria-tetanus-acellular pertussis; HB, hepatitis B; wP, whole-cell pertussis; aP, acellular pertussis.

[b] For Finland, acellular pertussis vaccine was given to children at 6 years of age starting January 2003.

[c] In the United Kingdom, three-component aP was used temporarily in the primary DTaP-Hib vaccine from 2000 to 2001.

tion strains were studied by means of cluster analysis, adequate for establishing the genetic relationships between the strains.

In this paper we used the discriminatory power of PFGE to identify and trace PFGE profiles represented in material collected from the eight countries participating in EUpertstrain II project. For comparison, EUpertstrain I culture collection strains were also included.

MATERIALS AND METHODS

Collection of strains. (i) EUpertstrain I. In the EUpertstrain I project, which is also referred to as the 1999 period in this paper, sets of approximately 20 isolates from each country, altogether 102 strains, were collected from children less than 5 years of age in Finland, France, Germany, The Netherlands, and Sweden during 1999 to 2001.

(ii) EUpertstrain II. In the EUpertstrain II project, which is also referred to as the 2004 period in this paper, a collection of 153 isolates from children in the five countries participating in EUpertstrain I project plus Denmark, Poland (13 isolates only), and United Kingdom during the year 2004 was established. The selection criteria were the same as for EUpertstrain I. All isolates in EUpertstrain I and II were lyophilized at the Swedish Institute for Infectious Disease Control (SMI) and redistributed to each partner.

PFGE methodology. PFGE was performed at the Swedish Institute for Infectious Disease Control (SMI) for all isolates according to standardized recommendations for typing of *B. pertussis* (16), with the modifications described previously (2). Due to the stability and high resolution of the PFGE method, it was possible to identify separate profiles. The profiles were analyzed by using BioNumerics software version 4.61 (Applied Maths, NV, Belgium) and defined as distinct DNA band patterns that differed by at least one band and designated BpSR1, BpSR2, BpSR3, and so on for those strains first detected in Sweden. If the strain was first identified in a country other than Sweden, such as Poland, it was designated BpPLR1 and so on. A cluster analysis was also performed using BioNumerics software with the unweighted-pair group method using arithmetic average algorithm with 2% band tolerance and 1.5% optimization settings. For the purpose of comparability, the same band tolerance and optimization settings used in the previous EUpertstrain paper (7) were used. Reference strains 18323 (PFGE group I), Tohama I (PFGE group II), Bp134 (PFGE group III), B902 (PFGE group IVα), FR743 (PFGE group IVβ), FIN12 (PFGE group IVγ), and FR287 (PFGE group V) were included in the dendrogram for traceability.

RESULTS

The 255 isolates of the two EUpertstrain collections produced 59 PFGE profiles (Fig. 1). A total of 33 profiles were detected in the 102 isolates belonging to the EUpertstrain collection of the 1999 period, while 36 profiles were found in the 153 isolates from the 2004 period. Ten PFGE profiles were common in the two periods of study.

Despite this apparent heterogeneity, some of the profiles appeared to predominate. Table 2 shows the 11 most common profiles found in five or more isolates, covering 53% to 90% of the materials from each country with the exception of Poland. Moreover, 70% of all isolates studied were found to belong to these 11 common PFGE profiles. None of the profiles common

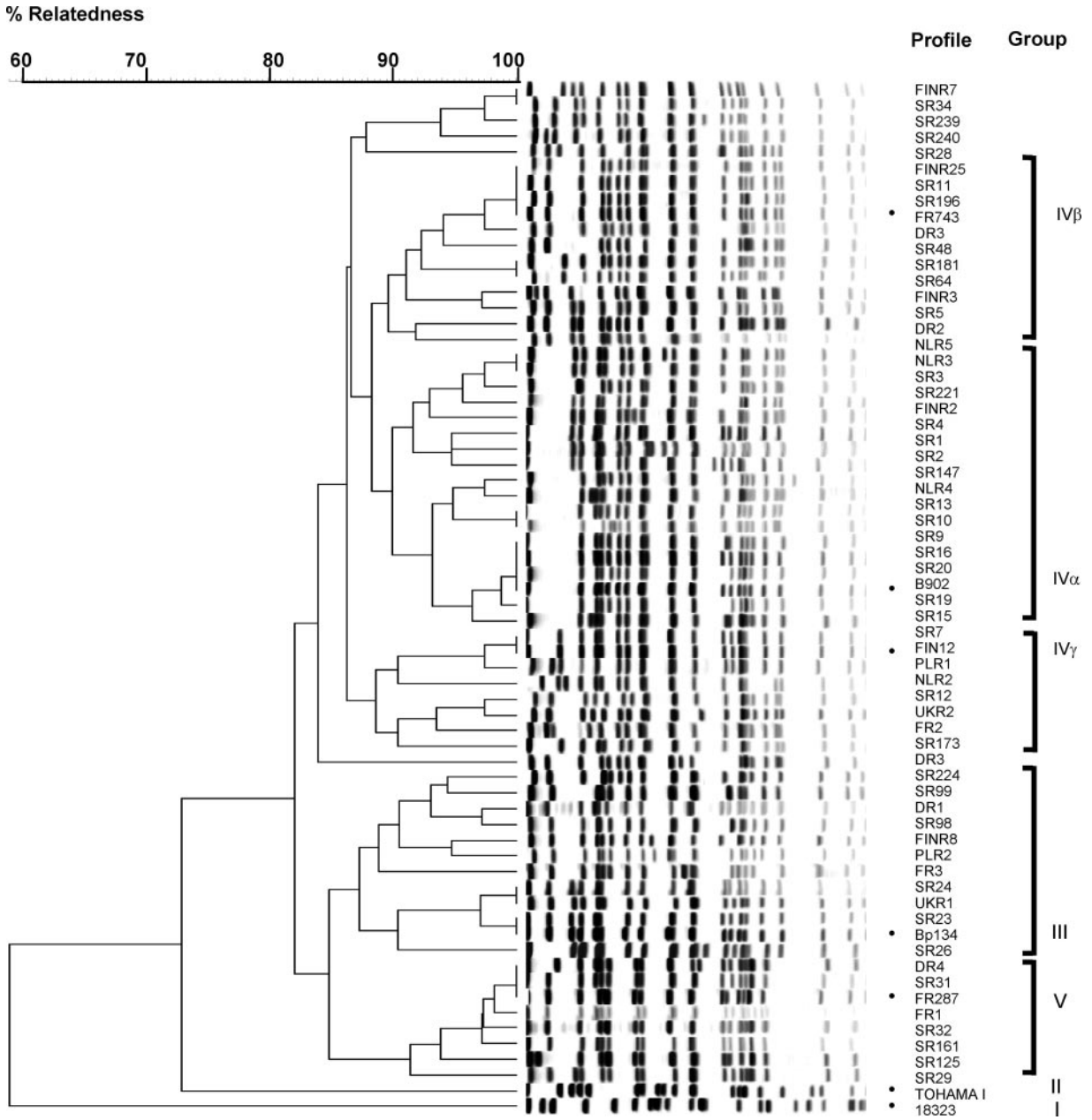

FIG. 1. Dendrogram of 59 PFGE profiles of *B. pertussis* circulating in eight European countries in 1999 to 2004. Reference strains for different PFGE groups (7, 25) are indicated (●). The PFGE groups were identified to have an overall relatedness of approximately 83%.

in other European countries was found in Poland. In particular, one profile, BpSR11, was predominant in this study. This profile was found in 5% to 45% of the isolates in the collection from the 1999 period and 10% to 50% of the isolates in the collection of the 2004 period, excluding Poland. In comparison with the 1999 period, there was an increase of BpSR11 in Finland from 5% to 40%. BpSR11 was characterized by the following allele combination: *ptxA1*, *ptxC2*, *prn2*, *tcfA2*, and *fim3B* (1).

In both collections, the profiles BpSR10, BpSR5, and BpSR12 were found in six of the eight participating countries at frequencies of 5 to 25%, also indicating a capacity to spread to other countries. The BpSR13 profile was found only in Sweden and Denmark (frequencies of 25% and 10%, respectively), and BpSR16 and BpSR7 were found only in Sweden and Finland (frequencies of 10% to 16%). BpSR173 and BpSR3 were found in France, The Netherlands, and Germany, indicating a limited dissemination of these PFGE types (frequencies of 4% to 26%).

The BpSR7 and BpSR147 profiles were found only during the 1999 period (frequencies of 12% to 15%), and other profiles, like BpSR3 (frequencies of 10% to 26%) and BpSR13 (frequencies of 10% to 25%), were found only during the 2004 period.

TABLE 2. Proportions of 11 predominant PFGE profiles identified in eight European countries in the period from 1999 to 2004

PFGE profile	Cluster/ group	Proportion (%) of PFGE profile in the following country and yr:													Total no. of isolates	Prevalence (%)
		Finland		France		Germany		The Netherlands		Sweden		Denmark, 2004	Poland, 2004	United Kingdom, 2004		
		1999	2004	1999	2004	1999	2004	1999	2004	1999	2004					
BpSR11	IVβ	5	40	45	50	35	17	30	30	20	35	10	0	45	73	29
BpSR10	IVα	5	5	10	20	6	6	25	17	0	10	20	0	0	25	10
BpSR5	IVβ	5	0	0	0	6	22	15	4	4	5	15	0	15	18	7
BpSR12	IVγ	0	0	10	5	6	0	0	4	4	5	25	0	15	15	6
BpSR3	IVα	0	0	0	10	0	11	0	26	0	0	10	0	0	12	5
BpSR13	IVα	0	0	0	0	0	0	0	0	0	25	10	0	0	7	3
BpSR16	IVα	0	10	0	0	0	0	0	0	16	0	0	0	0	6	2
BpSR19	IVα	0	5	0	0	0	0	20	0	4	0	0	0	0	6	2
BpSR7	IVγ	15	0	0	0	0	0	0	0	12	0	0	0	0	6	2
BpSR147	IVα	25	0	0	0	0	0	0	0	0	0	0	0	0	5	2
BpSR173	IVγ	0	0	5	0	0	17	0	4	0	0	0	0	0	5	2
Total		55	60	70	85	53	73	90	85	60	80	90	0	75		

There were seven different profiles identified among the Polish isolates. The profiles BpPLR1 and BpPLR2, together representing four isolates, were found exclusively in Polish isolates in the present study. The profile BpSR98, representing three isolates, was also found in Germany. The other Polish profiles, BpSR64 and BpSR98, were found frequently in previous Swedish and French strain collections, while BpSR34, BpSR239, and BpSR240 were found sporadically.

To investigate the genetic relationship between the strains, a cluster analysis was performed (Fig. 1) on strains, including the reference strains for groups I to V, and the genetic relationships between the strains were identified as described elsewhere (16, 25).

The BpSR11 profile clustered with BpSR5 and the group IVβ reference strain FR743. BpSR3, BpSR10, and BpSR13 clustered with the reference strain for group IVα, B902. BpSR12 and BpSR173 clustered with the reference strain FIN12 for group IVγ.

A new cluster was discovered; this cluster included profiles mainly from Poland (BpSR34, BpSR239, and BpSR240) and Finland (BPFINR7) as well as one isolate from Denmark (BpSR28). The remaining Polish isolates clustered with the reference strains for group III (six isolates), group IVβ (three isolates), and group IVγ (one isolate). Group IVβ strains, which were the most common in the other European countries, were represented in Poland by BpSR64. In Finland, strains belonging to groups IVα and IVγ were common during the 1999 period; however, they seemed to be replaced by group IVβ strains more recently (5).

Isolates that clustered with the group V reference strain FR287 were seen sporadically during the 1999 period but not found in the 2004 period.

Differences in the vaccination programs (Table 1) did not seem to have a direct influence on the distribution of profiles. A possible exception is Poland with its very unique pattern of PFGE profiles and the use of a national whole-cell pertussis (Pw) vaccine.

DISCUSSION

In this present work, we studied separate PFGE profiles, as it was shown that these were reproducible and stable, thereby taking advantage of the great discriminatory power of PFGE (2). This type of analysis provides detailed data useful for epidemiology and for the selection of certain predominant profiles, which are of interest for further investigation.

Despite the limited number of isolates from each country, analysis of the EUpertstrain culture collections reveals a gradual expansion of certain PFGE profiles within the *B. pertussis* population of the participating European countries. BpSR11 represents an example of this, expanding to 30% to 50% in most of the participating countries (Table 2). Strain FR743, the reference strain for group IVβ, was also typed as BpSR11. It was first isolated in France around 1996. Isolates sharing the profile of group IVβ increased in frequency in 1996 to 1997 in the country and has since become the most frequent group in France (7, 25). Some profiles, such as such those in group V, also seem to have disappeared.

A more detailed analysis of temporal changes in the PFGE profiles of Swedish *B. pertussis* isolates revealed that the BpSR11 profile was first isolated in Uddevalla on the western coast of Sweden in 1997. From 1999, coinciding with a major incidence peak, it was widely spread in Sweden and has been predominant since then (1). The same profile was observed in Finland for the first time in 1999 and was then the most prevalent profile, up to 56% in the incidence peak of 2003 (10). Interestingly, in a Swedish follow-up study, it was shown that BpSR11 was statistically more frequent among pertussis cases with long duration of hospitalization (3).

Weber et al. (25) used a cluster analysis based on the same algorithm for the investigation of relationships between strains. Their French isolates could be classified in five groups. The historical isolates belonged to clusters I and II and were not represented in the circulating strains at all. The other three clusters represented the circulating isolates with a clear trend of shifts from groups III and V to group IV. Grouping is a

convenient tool for investigating the lineage of *B. pertussis* strains over time. In the paper by Caro et al. (7), it was concluded that the isolates belonging to the collection of the 1999 period were found to be very similar and fell into the same major PFGE groups, with a predominance of groups IVβ (44.6%) and IVα (22.8%).

A strong association between PFGE profiles/clusters with *prn* type and to a lesser extent with fimbrial serotype has been demonstrated in the previous studies (11, 14, 25). Furthermore, a strong association between PFGE profiles and different combinations of the alleles for the *ptxA*, *ptxC*, *prn*, *tcfA*, and *fim3* genes was found in a recent study on the *B. pertussis* population in Sweden during an acellular pertussis vaccine period between 1997 and 2004, although this is not reflected in PFGE groups (1). Isolates with the allele combinations *1/2/2/2/B* (BpSR11, BpSR5, and BpSR12) and *1/2/2/2/A1* (BpSR3, BpSR10, and BpSR13) for the *ptxA*, *ptxC*, *prn*, *tcfA*, and *fim3* genes, respectively, replaced profiles with allele combinations *1/1/2/2/A1* (BpSR16) and *1/1/3/3/A1* (BpSR31) during a period of 7 years after the introduction of acellular pertussis vaccines (1). There seems to be a similar trend in the EUpertstrain results.

Interestingly, Poland was the only country in this study in which the most common European PFGE profiles were not found. This may be partially explained by the very limited number of strains ($n = 13$) included in this study. Further study with a large number of isolates is needed to ensure that they are representative. It was noted that several changes of vaccine strains had occurred in the nationally produced DTwP (diphtheria–tetanus–whole-cell pertussis) vaccine. It is also important to make these strains available for extended analyses of properties.

In conclusion, some PFGE profiles, such as BpSR11, evidently have a higher capacity to spread, suggesting they show increased fitness in the present immunological environment. Some other profiles are seen mainly in local outbreaks and seem to persist for shorter periods of time. Isolates expressing the genetic marker *prn1* were associated with PFGE group III, and isolates expressing *prn3* were associated with group V, a group that probably represents "older" types. In this context, it is interesting to notice the fact that both the whole-cell and acellular vaccines currently used in Europe today most often are derived from strains producing Prn1. From a vaccination point of view, it is important to study the background and possible consequences of *B. pertussis* polymorphism and the mechanisms that might influence the bacterial adaptation to population immunity. It is therefore of major interest to continue with surveillance programs of *B. pertussis* isolates, as both waning vaccine-derived immunity and strain variation may play a role in the persistence of pertussis.

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