**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 IVB. 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, EUperstrain, was established. The EUperstrain 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 EUperstrain II project was a continuation of EUperstrain 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), IS1002-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 fimbrae (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 EUperstrain I culture collec-
BioNumerics software version 4.61 (Applied Maths, NV, Belgium) and defined was possible to identify separate profiles. The profiles were analyzed by using previously (2). Due to the stability and high resolution of the PFGE method, it recommendations for typing of infectious Disease Control (SMI) for all isolates according to standardized recom-

The 255 isolates of the two EUperstrain collections produced 59 PFGE profiles (Fig. 1). A total of 33 profiles were detected in the 102 isolates belonging to the EUperstrain 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

RESULTS

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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.
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 strain for group IV, BpSR11. 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 EUperstrain 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 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

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

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**Note:** Percentages may not add up to 100% due to rounding.
convenient tool for investigating the lineage of \textit{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 IVb (44.6%) and IVa (22.8%).

A strong association between PFGE profiles/clusters with \textit{pmr} 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 \textit{ptxA, ptcC, pmr, tcfA}, and \textit{fim3} genes was found in a recent study on the \textit{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 (BpSR11, BpSR5, and BpSR12) and 1/2/2/2/A1 (BpSR3, BpSR10, and BpSR13) for the \textit{ptxA, ptcC, pmr, tcfA}, and \textit{fim3} genes, respectively, replaced profiles with allele combinations (BpSR10, and BpSR13) for the \textit{ptxA} and \textit{fim3} genes, respectively, during a period between 1997 and 2004, although this is not reflected in PFGE groups (1). There seems to be a similar trend in the EUpert strain 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 can be 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 \textit{pmr}1 were associated with PFGE group III, and isolates expressing \textit{pmr}3 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 PrnL. From a vaccination point of view, it is important to study the background and possible consequences of \textit{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 \textit{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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