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Application of pulsed field gel electrophoresis to the 1993 epidemic of whooping cough in the UK

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SUMMARY

The purpose of this study was to DNA fingerprint the majority (64%) of isolates received at the Pertussis Reference Laboratory during the 1993 whooping cough epidemic by pulsed field gel electrophoresis of *Xba* I – generated restriction digests. Two DNA restriction patterns, types 1 and 3, predominated (40% and 23%, respectively, of 180 isolates) but type 2, identified in a previous study was notably absent. Twenty-one new DNA types occurred (24% of isolates), some being atypical as bands 155–230 kb were no longer conserved, but there was no statistically significant difference in their incidence in the upswing (June–September) compared to the downswing (October–December) phase of the epidemic. There was a relatively high proportion of new types, compared to type 1. at the peak (September). About 50% of isolates received were from the North Western Region, where 44% of isolates were DNA type 1. Whereas only 1 out of 10 isolates from Scotland were of this type, suggesting some geographic variation. Statistically significant findings included a higher proportion of isolates from female patients ($P < 0{\cdot}01$), most marked in the 12–24 months age group ($P < 0{\cdot}05$); a higher proportion of infants under 12 months requiring hospital admission compared to older children ($P < 0{\cdot}05$); and a greater number of isolates from unvaccinated children ($P < 0{\cdot}01$). Analysis of serotype according to four age groups (under 3 months, 3–12 months, 12–24 months and above 2 years) showed statistically significant differences ($P < 0{\cdot}05$) with a noticeably lower proportion (38%) of serotype 1,3 in 3–12 months age group and higher prevalence (74%) of serotype 1,3 in the 12–24 months age group. There was no correlation between DNA type and serotype.

INTRODUCTION

Pertussis (whooping cough) is mainly a childhood disease, although it may affect all ages. The disease is most severe, and the incidence of morbidity highest,

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in young children; most fatal cases being in infants in their first year of life. It is still a major cause of death in malnourished populations. It was estimated by the World Health Organisation (WHO) that 600000 deaths due to pertussis occur yearly, mostly in unimmunized infants [1]. The causative agent is *Bordetella pertussis*, a bacterium that causes an endemic disease with periodic epidemics. Although the disease has been well characterized and largely controlled in developed countries, it remains abundant in developing countries [2, 3].

In the UK, the control of whooping cough depends upon its prevention by whole-cell vaccines rather than treatment. After cases of pertussis became statutorily notifiable (in 1940) and national statistics on uptake rates were available (from 1961), a direct inverse correlation between incidence and vaccine uptake rates in different parts of the country was shown [4]. Identifiable peaks signalling an epidemic were observed at intervals of every 4 years [5] even with high vaccine rate uptake. One of the reasons for this 4-year cycle is believed to be the period of time required to increase the number of susceptibles to reach the herd epidemic threshold [6].

The size of epidemics has progressively declined since vaccination was introduced in the 1950s. During the mid-1970s [7, 8], however, a loss of faith in vaccine safety caused a drop in vaccination rate to about 30% which resulted in a dramatic increase in the number of cases. When public confidence returned, the nationwide vaccination rate increased once more, reaching 93% by 1993 [9]. This demonstrates the importance of continued nationwide vaccination programmes in controlling pertussis which still causes death and considerable morbidity [10, 11] in the UK even today.

A whooping cough epidemic was expected in 1993 following the prediction that the disease occurred in epidemic waves at about 4-year intervals. Whooping cough activity re-emerged in 1993, with over 4000 notifications compared to about 2300 in 1992. The 1993 epidemic was considerably smaller than that of 1989–90; 11646 (1989) and 15286 (1990) notified cases were observed (personal communication, Dr E. Miller, PHLS CDSC).

Previously, we developed a means of genetically fingerprinting *B. pertussis* by pulsed field gel electrophoresis (PFGE) of macrorestriction digests [12, 13]. This produced 21 different DNA types from the 130 isolates examined [13]. Fifteen out of the 21 DNA types were first observed during an analysis of 67 (out of 130) isolates from England and Wales [14]. These 67 clinical isolates were randomly selected from a much larger number sent to the Pertussis Reference Laboratory between October 1990 and March 1991. This time period coincided with the tail end of the 1989–90 epidemic and the beginning of the non-epidemic period. That study also showed that predominant strains vary from country to country, with DNA type 1 being the commonest in the UK but totally absent among the German isolates [14].

In the present study, the majority of all *B. pertussis* isolates received at the Pertussis Reference Laboratory from June to December 1993 were typed by PFGE and then compared to the 21 DNA types previously described [13, 14]. It examines the statistical significance of the appearance of new DNA types corresponding to fluctuations in the number of cases and as a possible precipitant of an epidemic. Statistical analysis of the patient's age, sex, vaccination status and

clinical severity, and correlation between epidemiology, genetic heterogeneity and high nation-wide vaccination rate were investigated to address the hypothesis that genetic changes within the organism was one of the precipitating factors of the epidemic.

MATERIALS AND METHODS

Isolates

Clinical isolates used in this study were from throughout Great Britain and had been sent to the Pertussis Reference Laboratory, University of Manchester Medical School, for serotyping. *B. pertussis* isolates from patients with clinically typical or suspected whooping cough were received from the public health laboratories at Bath, Birmingham, Cambridge, Chelmsford, Chester, Gloucester, Hereford, Ipswich, Leeds, Lincoln, Liverpool, London, Manchester, Nottingham, Oxford, Peterborough, Poole, Portsmouth, Preston, Reading, Rhyl, Shrewsbury, Southampton, Stoke-on-Trent, Swansea, and Wolverhampton; and hospital (H) laboratories at Aberdeen Royal Infirmary (RI), Alderhey Children's H (Liverpool), Barking H (Essex), Blackburn RI, Bolton RI, Booth Hall H (Manchester), Burnley General Hospital (GH), Conquest H (East Sussex), Crawley H (West Sussex), Derby RI, Doncaster RI, Dumfries and Galloway RI, Edinburgh City H, Friarage H (Northallerton), Frimley Park H (Surrey), James Paget H (Gt Yarm), Kettering District General Hospital (DGH), Lancaster Moor H, Macclesfield DGH, Maelor GH (Wrexham), Ninewells H (Dundee), North Tyneside GH, Ormskirk DGH, Queen Elizabeth H (Gateshead), Royal Hallamshire H (Sheffield), Royal Manchester Children's H, Sandwell DGH (West Brom), Southern GH (Glasgow), Southport DGH, St. Bartholomew's H (London), Stepping Hill H (Stockport), Tameside DGH, Telford H (Shropshire), University College Hospital (London), Victoria I (Glasgow), West Suffolk H and Whiston H (Liverpool).

Identification and serotyping

The isolates were identified as *B. pertussis* on the basis of colonial morphology on charcoal blood agar (CBA), Gram stain, and serotyping with monospecific agglutinating sera specific for agglutinogens 1, 2 and 3, prepared at the Pertussis Reference Laboratory as previously described [15]. *B. pertussis* first isolated from patients between the months of June and December 1993 were sub-cultured onto CBA slants and stored at 4 °C. Overall, 280 isolates were serotyped, and a random selection of 180 of these (64%) were typed by PFGE. Isolates were then grouped into those received from the North Western (NW) Region (including Liverpool PHL and Merseyside laboratories) and those from other regions, including 10 isolates from Scotland.

DNA typing

Preparation of chromosomal DNA

The isolates were grown on CBA plates from a single colony for 48–52 h at 35 °C. The bacteria were then harvested directly from the plates using a sterile loop and resuspended in 1 ml 50 mM-EDTA (pH 8·0). The cells were pelleted, washed three times with 50 mM-EDTA and the cell concentration adjusted at 600 nm at an

optical density of 1·5–1·9 (1·5 is equivalent to $1{\cdot}2 \times 10^9$ cell/ml). 500 μg/ml lysozyme (Sigma) and 1 mg/ml RNase (Sigma) were added to the bacterial suspension, vortexed and then incubated in a 37 °C water bath for 30 min. An equal volume of 2% low gelling temperature molten agarose was added and the mixture dispensed into a 10-plug mould (Bio-rad). After the plugs were allowed to solidify for 10 min at 4 °C, each plug was divided into four with a clean coverslip. The plugs were then incubated for 48 h at 50 °C in a sterile bijou bottle containing ESP solution (0·5 M-EDTA pH 9·0, 1% w/v *N*-lauryl sarcosine and 3 mg/ml proteinase K) [16].

Restriction digests of chromosomal DNA

The agarose plugs were washed twice with TE buffer pH 8·0 (10 mM-Tris-HCl pH 7·5, 1 mM-EDTA pH 8·0) containing 1 mM phenylmethylsulphonyl fluoride (PMSF) and three times in TE buffer pH 8·0 without PMSF [17]. Three plugs of each strain were placed into an Eppendorf tube containing 300 μl of *Xba* I (Northumbria Biologicals) reaction buffer and allowed to equilibrate for 20 min on ice. Thereafter, 25 units of *Xba* I restriction enzyme were added and the mixture incubated at 37 °C overnight. The reaction was stopped by the addition of 50 mM-EDTA.

Pulsed field gel electrophoresis (PFGE)

Electrophoresis was performed using the CHEF-DR II System (Bio-rad). Gels of 1% agarose in TBE buffer (Tris-HCl 10·3 g/l, boric acid 5·5 g/l, EDTA 0·93 g/l) were made and one plug from each strain was loaded into each well. A lambda DNA ladder (Promega), (oligomers between from 50 and 1000 kb) was included in each gel as a molecular weight marker. A pulse time of 25 sec at the field strength of 4·5 V/cm [18] was applied to the gel for 40 h. Gels were stained with 0·5 mg/l ethidium bromide in distilled water and photographed under a UV transilluminator (254 nm).

The molecular weights of fragments generated by new types were determined by comparison with a lambda DNA ladder and a DNA type 1 control. The reproducibility and the stability of newly observed DNA types was established by repeated DNA preparation and PFGE. Identification of the DNA types was done by visual comparison with DNA types established in the 1992 study [14].

Statistical analysis

Statistical analysis was carried out on 280 isolates and accompanying clinical details received at the Pertussis Reference Laboratory between June and December 1993. This period was further divided into two phases: the upswing phase (June to September) of the epidemic which referred to the rise in both the notifications and the number of isolates received at the Pertussis Reference Laboratory and the downswing phase (October–December), (see Fig. 1). Details regarding age, sex, severity and vaccination history of cases were obtained from the laboratory reports received with the isolates. A total of 180 isolates were genetically typed. Information regarding notifications from England and Wales was provided by the Public Health Laboratory Service, Communicable Disease

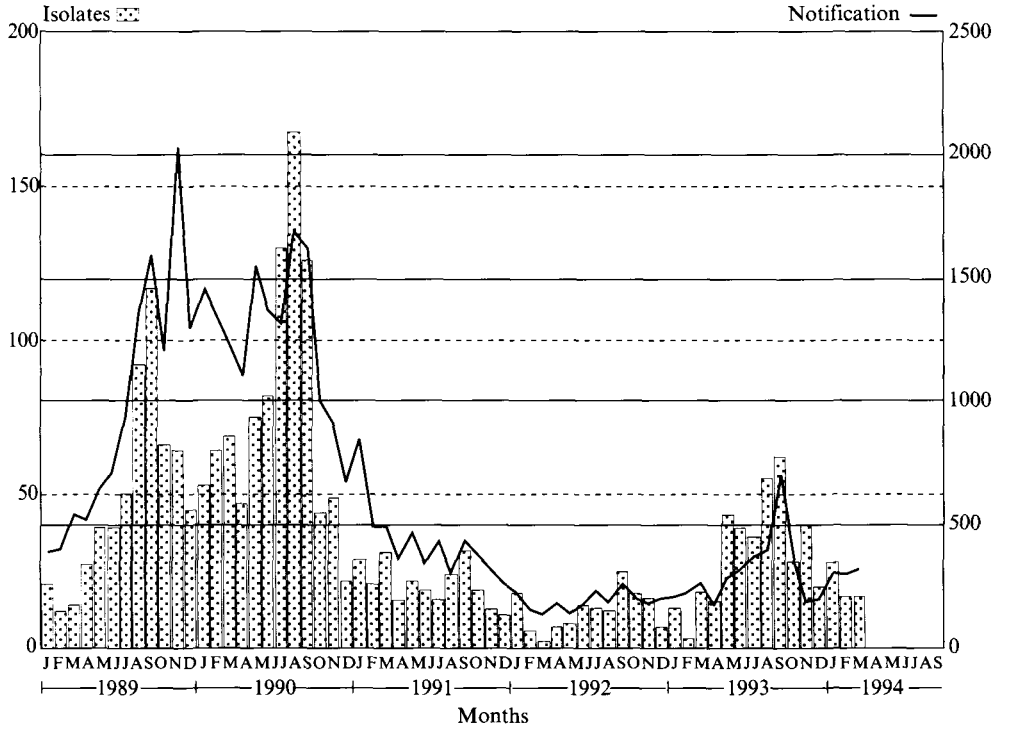

Fig. 1. Notifications to the PHL [——] and the number of isolates received at the Pertussis Reference Laboratory [▨].

Surveillance Centre (PHLS CDSC). For the purpose of statistical analysis, the only marker of severity was whether the patient required hospital admission although we appreciated that children could be admitted for other, such as social, reasons. Vaccination status of the patients was categorized as either fully vaccinated (three doses), partially vaccinated or unvaccinated. Age grouping was as follows: under 3 months old, 3–12 months, 12–24 months and above 2 years. The patients' vaccination status, severity of illness, age and sex were not always available. The DNA types were categorized into multiply occurring new types (23–32), new unique types (33–43), and previously recognized types [13] (1–21) (see Table 1–3).

The statistical analyses comprised cross-tabulation and the construction of contingency tables using standard software; Statistical Package for the Social Sciences (SPSS, SPSS Inc. Chicago, Illinois, USA). To determine the probability of an association between any two specified variables in the population from which the sample was derived, the chi-squared (chi^2) test was used in conjunction with the contingency tables. χ^2 was calculated for each of the contingency tables [19]. Unless stated otherwise, the number of degrees of freedom was 1 and Yates correction was applied.

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Table 1. *Characterization of isolates with previously recognized types*

DNA type	Size of DNA fragments (kb)														Number of isolates		
	412	375	340	315	297	280	271	256	240	224	215	200	155	130	Upswing (Jun–Sept)	Downswing (Oct–Dec)	Total (Jun–Dec)
1	+	−	−	+	−	+	+	+	−	+	−	+	+	+	48	24	72
3	−	−	−	+	−	+	+	+	+	+	−	DB*	+	+	24	18	42
4	−	+	−	+	−	+	+	−	+	+	−	DB	+	+	3	1	4
5	−	−	+	+	−	+	+	−	+	+	−	+	+	+	3	1	4
6	+	−	+	+	−	+	+	+	−	+	−	+	+	+	1	0	1
9	+	−	−	+	−	+	−	+	−	−	−	DB	+	+	2	2	4
11	−	+	−	+	−	+	−	+	+	+	−	+	+	+	0	1	1
12	+	−	−	+	−	−	+	+	−	−	−	DB	+	+	5	3	8
																	Total = 136

* DB, Double band.

Table 2. *Characterization of isolates with multiply occurring new types*

DNA type	Size of DNA fragments (kb)														Number of isolates		
	412	375	340	315	297	280	271	256	240	224	215	200	155	130	Upswing (Jun–Sept)	Downswing (Oct–Dec)	Total (Jun–Dec)
23	−	−	−	+	+	+	+	−	+	+	−	DB*	+	+	5	2	7
24	+	−	+	+	−	+	+	−	−	+	−	+	+	+	7	0	7
25	−	−	−	+	+	+	+	+	−	−	−	DB	3 bands†		2	0	2
26	+	−	−	DB	−	−	+	DB	−	−	−	−	+	+	1	2	3
27	−	+	−	+	−	+	+	−	+	+	−	+	+	−	1	2	3
28	+	−	+	+	−	+	−	−	−	+	−	+	+	+	1	2	3
29	−	−	DB	−	+	+	+	−	−	−	−	−‡	3 bands†		0	2	2
30	+	−	−	−	−	+	+	+	−	+	−	+	3 bands†		2	0	2
31	+	−	+	−	−	+	+	−	−	+	−	−	+	+	2	0	2
32	+	−	−	+	−	+	+	+	+	+	−	+	+	+	2	0	2
																	Total = 33

* DB = Double band. † Extra band between 155 and 130 kb. ‡ Extra band at 178 kb.

Table 3. *Characterization of isolates with new unique types*

DNA type	Size of DNA fragments (kb)														Number of isolates	
	412	375	340	315	297	280	271	256	240	224	215	200	155	130	Upswing (Jun–Sept)	Downswing (Oct–Dec)
33	−	+	−	+	+	+	−	−	+	+	−	+	+	+	1	0
34	+	−	−	+	−	−	+	+	+	+	−	DB*	+	+	1	0
35	−	−	−	+	−	+	+	+	−	+	−	+	+	+	1	0
36	−	−	−	+	−	+	+	+	+	−	−	+	+	+	1	0
37	−	+	+	−	+	+	+	−	+	+	−	+	+	+	1	0
38	+	+	−	+	−	+	+	+	−	+	−	+	+	+	1	0
39	−	+	+	+	−	+	−	−	+	+	−	+	+	+	1	0
40	+	−	−	+	+	+	+	+	+	+	−	DB	+	+	1	0
41	+	−	−	+	−	−	+	+	−	+	−	+†	−	+	0	1
42	+	−	+	−	−	+	+	−	+	+	+	+	+	+	0	1
43	+	−	−	+	−	+	+	+	−	+	−	+†	+	+	0	1

* DB, Double band.
† Extra band at 178 kb.

RESULTS

DNA typing by PFGE

All 180 isolates that were tested were typable. Altogther, 29 different DNA types were observed. DNA type 1 (Fig. 2, lane 1) predominated and encompassed 72 of the 180 isolates (40%). DNA type 3 (Fig. 2, lane 3) accounted for 42 isolates (23%). Previously described DNA types 4, 5, 6, 9, 11 and 12 accounted for 22 (12%) of the isolates. The remaining 44 isolates (24%) gave rise to new DNA types (23–43). DNA types 33, 41 and 42 were seen alone among the 10 isolates from Scotland. DNA type 1 predominated in isolates of serotype 1,2 (60%) whereas DNA type 3 was commonest among serotype 1,3 isolates (31%). The new DNA types 23–43 were predominantly of serotype 1,3 (60%).

The 21 new types were identified on the basis of the variations in 14 intense bands that ranged from 130–412 kb (see Tables 1–3). Seventy-six percent of the isolates belonged to established DNA types (1–12, Table 1), 18% were new multiply-occurring DNA types (designated types 23–32; see Table 2) and 6% (designated types 33–43; see Table 3) were new unique types, each found in only one isolate. DNA types 25, 29 and 30 displayed a unique additional fragment between 155 and 130 kb. DNA type 27 (Fig. 2, lane 7) and type 41 lacked 130 and 155 kb fragments respectively.

Serotyping

In this small 1993 epidemic, 159 of the 280 isolates were of serotype 1,3 (57%), 116 were of serotype 1,2 (41%) and 5 were of serotype 1,2,3 (2%). There was no apparent correlation between DNA type and serotype.

Analysis of data

Out of the 280 referrals received at the Pertussis Reference Laboratory, a significantly ($P < 0{\cdot}01$) larger number of isolates were from unvaccinated children (131) than vaccinated children (21). No details of the vaccination status of the remaining 128 cases were available.

A slightly higher proportion of the new (multiply occurring and unique) DNA types 23–43 appeared in the upswing phase (17 DNA types) compared to the downswing phase (8 DNA types) but this was not statistically significant. There were 31 isolates, out of 117, with unusually occurring DNA types (types 23–43) in the upswing phase of the epidemic (June–September; Table 2 and 3) compared to 13 of 63 during the downswing phase (October–December; Table 2 and 3). At the peak of the epidemic in September, there were almost equal numbers of isolates in the type 1 group (9 isolates) compared to those making up new (multiply occurring and unique) types (8 in all). In contrast, 2 months later, there were more than twice as many type 1 isolates (13) compared to those with unusual types (five isolates).

There were 15 DNA typed isolates out of 21 serotyped isolates from fully-vaccinated children, of which 9 (60%) were DNA type 1, 3 (20%) were of DNA type 3 (all of serotype 1,3), 2 (13%) were of DNA type 9 and 1 (7%) was of DNA type 23. Seven of these nine DNA type 1 isolates were of serotype 1,2 and the rest

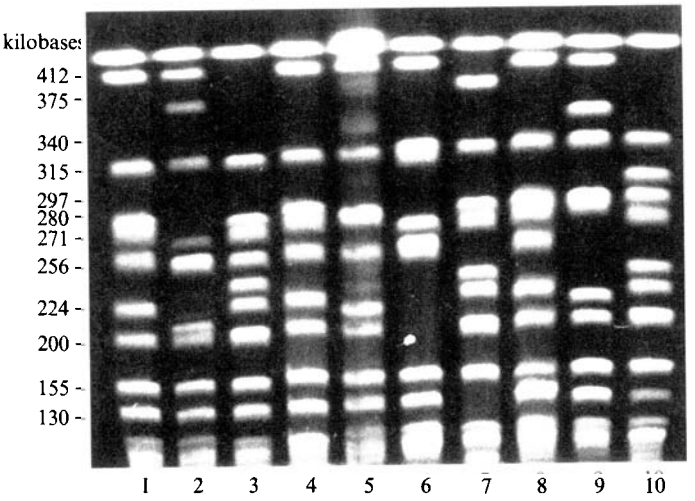

Fig. 2. Pulsed field gel electrophoresis of *B. pertussis* isolates: Lane 1, Control DNA type 1; Lane 2, DNA type 44; Lane 3, DNA type 3; Lane 4, DNA type 1; Lane 5, DNA type 1; Lane 6, DNA type 26; Lane 7, DNA type 27; Lane 8, DNA type 1; Lane 9, DNA type 28; Lane 10, DNA type 23.

were of serotype 1, 3. Overall, 10 out of 21 isolates from fully-vaccinated children were of serotype 1, 2 and the rest were of serotype 1, 3.

Analysis of serotype according to the four age groups showed significant differences ($P < 0{\cdot}05$, 3 D.F.) with a noticeably lower proportion (38%) of serotype 1, 3 in the 3–12 months age group (16 out of 42), whereas in the 12–24 months age group, this occurred with a much higher prevalence of 74% (17 out of 23). Overall, the prevalence of serotype 1, 3 was 56% (141 out of 250). Significant differences were not apparent in serotype 1, 2. Information regarding the age of the patient was unavailable for 30 out of the 280 isolates serotyped.

There was a statistically significant higher proportion of infants (under 12 months) hospitalized (49 out of 120, 41%) compared to older children (32 out of 160, 20%) ($P < 0{\cdot}05$). Moreover, the proportion of children admitted to hospital decreased with successive age groups ($P < 0{\cdot}05$, 3 D.F.). They were as follows: under 3 months age group (48%), 3–12 months age group (36%), 12–24 months age group (30%) and above 2 years age group (21%). A higher percentage of unvaccinated and partially vaccinated versus fully vaccinated cases was observed among children below 12 months old (72%) compared to patients above 12 months (36%). This difference, however, was not statistically significant. Although relatively few isolates (21) of *B. pertussis* were received from fully vaccinated children between the ages of 6 months to 12 years, the infection in 6 of these was severe enough to require hospital admission. Four of these 6 isolates were of serotype 1, 2. Also, 3 out of 4 of the severe infections (requiring hospital admission) among the fully vaccinated children (aged 1–12 years) were the result of infection by pertussis with DNA type 1. Information regarding the age of the patient was available for 169 out of 180 isolates fingerprinted.

There was a statistically significant higher proportion ($P < 0{\cdot}01$) of whooping cough infections in females overall, with a ratio of 7 females to 5 males. In all age groups, there were more females than males, with 39 females to 28 males within

the under 3 months age group, 23 females to 18 males in the 3–12 months age group, 19 females to 3 males in the 12–24 months age group and 60 females to 51 males within the over the 2 years age group. The lower incidence among males in the 12–24 months age group was significantly lower ($P < 0{\cdot}05$, 3 D.F.) than in the other age groups. Information regarding the age and sex of the patient was unavailable for 39 out of 280 isolates serotyped.

The number of isolates from the NW Region accounted for about 50% of the total number of isolates between June and December. Over the study period only 3 out of the 11 unique DNA types (37, 38, 39) came from this region. In contrast, out of the 10 isolates from Scotland, only one DNA type 1 and one DNA type 3 was observed but three new unique DNA types (33, 41 and 42) were recognized.

DISCUSSION

This study differed from the earlier work in that the great majority (180 out of 266, 64%) of all isolates received at the Pertussis Reference Laboratory were DNA fingerprinted. An attempt was made to correlate DNA type with vaccination status, severity, age, sex and phase of the 1993 epidemic.

The *Xba* I-generated macrorestriction digests analysed by PFGE provided reproducible DNA fingerprints of *B. pertussis* and the restriction fragments showed a high degree of polymorphism. Bands at 130 and 155 kb were previously thought to be too conserved to be of discriminatory value [13, 14] because they were present in all of the 21 DNA types observed, whereas, in this study, the absence or inclusion of bands at 155 and 130 kb or the inclusion of an intermediate band were important criteria for differentiation of the 21 new DNA types observed (Tables 2 and 3).

The increase in the number of isolates received at the reference laboratory was also reflected in a rise in the number of notifications of whooping cough in England and Wales (Fig. 1). The high proportion of isolates from the North Western Region in the epidemic of 1993 was mirrored by the increased number of notified cases from this region [20]. Whether this was because the NW Region actually had more cases of pertussis during this epidemic or because the Pertussis Reference Laboratory is located here is not known.

Certain DNA types appear to be unique to particular geographic locations. DNA type 22 comprised 20% of isolates from a Canadian outbreak sent to the Pertussis Reference Laboratory for typing in 1992 [21]. It was not observed among UK isolates, in this or the previous study, or in isolates from Germany [14] and was notable for the appearance of a band at 230 kb and the lack of the 155 kb fragment previously thought to be conserved. Although only 10 isolates were available from Scotland, the distribution of DNA types suggests a high proportion of types less commonly seen elsewhere in the UK.

At the peak of the epidemic (September), the number of new DNA types almost equalled the number of DNA type 1 isolates, but as the epidemic subsided, DNA type 1 predominated once more and persisted into the later part of the epidemic. During the upswing phase, there were more new DNA types than in the downswing phase, suggesting greater genetic plasticity, but this did not reach statistical significance. It might have been more apparent had we investigated a

population with a low vaccination rate, in whom the host selection pressures on *B. pertussis* would have been different.

Whole cell pertussis vaccines used in Britain contained all three agglutinogens recommended by the WHO. Although effective against infection with all serotypes, the vaccine has had a higher efficacy against serotypes 1,2 and 1,2,3 than against serotype 1,3 [22]. In the vaccinated child, type 1,3 is much more common than serotype 1,2 because the child responds better to agglutinogen 2 compared to agglutinogen 3 and is therefore, less well protected against type 1,3 [23]. The high proportion of fully vaccinated children with type 1,2 infection in this study was therefore unexpected. Whether this was due to sampling error or a poor response to vaccination is uncertain. Also, the type 1,2 organism is highly fimbriated and hence has a colonising advantage. Partially vaccinated patients were categorized with unvaccinated individuals because partial vaccination is known to be not fully protective [23, 25].

Over the years, it was felt that the morbidity and mortality rates were rather higher in females than in males in contrast to most other specific childhood fevers [26]. The statistically significant higher proportion of whooping cough infections in female patients ($P < 0{\cdot}01$) confirms this observation.

Between 1980 and 1991, the case fatality rate was overall 1:4850 and 74% of deaths were in infants under 1 year [10]; the present study showed a statistically significant ($P < 0{\cdot}05$) higher proportion in this age group were admitted to hospital. This emphasises the importance of complete immunization as early as possible but without sacrificing the need for a good antibody response to the vaccine. Infants up to the age of 2 months have an even high fatality rate of 1:200 but at this age, they are too young for immunization to be effective. Protection of the very young is best effected through strong herd immunity achieved by high levels of vaccine uptake among older siblings because there is little passive protection from the mother. In order to try to reduce the incidence and severity of whooping cough in very young children, an accelerated schedule was introduced in June 1990, in which children as young as 2 months were given their first dose of pertussis vaccine [24].

DNA type 2, which was absent from the 180 isolates fingerprinted in this study, accounted for 15% (10 out of 67) of the selected isolates typed between September 1990 to March 1991 [14]. DNA types 1 and 3 accounted for a large proportion (18% and 22% respectively) of the isolates typed previously, a trend that has been confirmed (40% and 23% respectively) in this study in which the majority of isolates were typed.

The nature of the precipitant(s) responsible for the occurrence of epidemics of whooping cough every 4 years remains uncertain. Despite the very high vaccination rate in 1993 (93%), an epidemic did occur but on a much smaller scale than in previous years when the vaccination rate was poor [9]. Current whole-cell vaccines are based on a few strains of *B. pertussis* and possibly the 'failure' of the vaccine to completely prevent an epidemic could in part be due to the emergence of genetic variants. These could be the result of small changes in genotype, as suggested by the increased number of new types observed at the peak of the epidemic and, to some extent, during the upswing phase. This situation would be analogous to the genetic drift which precipitates epidemics of influenza due to

breakdown in herd immunity. DNA type 1 dominated this epidemic, accounting for 40% of isolates, though at its peak (September), there was an almost equal number of new types as type 1. Were the investigation to be repeated during an epidemic in a less highly vaccinated community, new genetic types might have been more evident because the size of the circulating population of *B. pertussis* would be greater, facilitating the emergence of new genetic types through random mutation. Further investigation into future epidemics in less highly vaccinated populations will be required to ascertain whether this is indeed a contributory factor.

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