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# *Bordetella pertussis* isolation in general practice: 1977–79 whooping cough epidemic in West Glamorgan

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## SUMMARY

Some of the factors influencing the isolation rate of *Bordetella pertussis* during a whooping cough epidemic in West Glamorgan, Wales, are reported. The organism was isolated from 39% of patients with clinical whooping cough, pernasal swabbing being much more successful than cough plates. Isolation rates were increased in the non-immunized, particularly in the first year of life. Erythromycin and co-trimoxazole significantly reduced the isolation rate of *B. pertussis* but this did not occur with penicillin. In this study 20% of patients were culture positive 6 weeks after the onset of their infection. It is suggested that the Department of Health and Social Security recommendation of a minimum period of three weeks exclusion of children from school is inadequate. During the epidemic, the proportion of strains of *B. pertussis* containing antigen 2 more than doubled.

## INTRODUCTION

During 1974 adverse publicity resulted in a dramatic drop in the acceptance rate for whooping cough vaccination in England and Wales. While 79·5% of children born in 1971 were vaccinated, only 38% of children born in 1974 received the vaccine. The position was much worse in West Glamorgan where the acceptance rate was 38% during 1971, less than half of the corresponding figure for England and Wales, and dropped to the low level of 9·5% in 1974 (Swansea Research Unit, 1981). Consequently, it was feared that the next outbreak of whooping cough around 1977–8, would be very much bigger than any in recent years. In fact, it started in early autumn 1977, and showed two peaks of incidence, one during the spring and the other in the autumn of 1978. It did not wane until the spring of 1979. It became possible to study most of this outbreak, at first with the help of

the health visitors in the area, and later with the research nurses who were appointed. At the peak of the epidemic the research staff reached a total of five full-time nurses and two full-time secretaries. Recording for the study started in November 1977 and continued until early March 1979. This paper describes the bacteriological aspects of the study.

## MATERIALS AND METHODS

All 212 general practitioners who had patients in West Glamorgan (population approximately 360000) agreed to co-operate in the project. The whooping cough notifications were made by telephone either to the Swansea Public Health Laboratory or to the Neath Pathology Laboratory. The nurses visited one of the two laboratories daily to collect whooping cough investigation outfits, questionnaire forms and the names and addresses of notified cases. If possible the household was visited that day, and after filling in the initial part of the questionnaire, a pernasal swab was taken from the notified case as well as from any other occupant with symptoms.

A second visit was made 2 weeks later to make further observations and take swabs from any secondary cases. After approximately 3 months, the nurses made a final call in order to study the outcome and complete the questionnaire.

### *Collection of specimens*

The nurses were instructed on how to take swabs by the medical members of the research team. In addition, the nurses practised swabbing on each other and on other members of the team until they had become confident and proficient. At the beginning of the study they went into some of the houses in pairs in order to standardize the technique of swabbing, and the method by which they completed the questionnaire.

The technique of swabbing consisted of passing a pernasal swab (Medical Wire and Equipment Co. Ltd, Corsham, Wilts) gently along the floor of the nose for about 5 cm or more until it met the resistance of the posterior wall of the nasopharynx. For young infants it was found helpful to bend the swab slightly into the shape of an arc, and direct the tip downwards. The swab was rotated gently before being withdrawn.

The whooping cough outfits, consisting of plastic bags each containing a pernasal swab, two charcoal agar plates and a bijou bottle of virus transport medium, were stored at 4 °C in the refrigerator. The nurses helped themselves to outfits when they visited the laboratory. Immediately after being taken, the swab was rubbed over at least three-quarters of the surface of a charcoal agar plate; the plates were not spread with a loop subsequently. The swab was then put into the bijou bottle of virus transport medium and the end cut off with scissors. Often the swabbing precipitated a spasm of coughing and when this occurred a cough plate was taken.

### *Microbiological investigations*

The majority of plates and swabs were taken to the laboratory on the day of swabbing but a few collected in the evening were kept in a domestic refrigerator overnight and brought in the following day.

The plates were incubated at 36 °C in an atmosphere kept moist with a container of water. They were examined after 2 days and daily for a further five days. Identification was made by slide agglutination of suspicious colonies using pertussis antiserum.

Although the majority of colonies were typically glistening and dome shaped, there were a few strains whose colonies had a similar size and hue but had a depressed centre. Subcultures were made onto charcoal agar slopes, without blood or antibiotics, in bijoux bottles and sent to Dr N. W. Preston at Manchester for serotyping.

After gently agitating the swabs in the virus transport medium, 0·1 ml volumes of the fluid were inoculated into one tube each of monkey kidney and HEp2 tissue culture cells. These were incubated at 34 °C and examined every other day for cytopathic effect. The monkey kidney cells were tested for the presence of a haemadsorbing agent at 5, 10 and 15 days.

Identification of virus isolates was made by neutralization or immunofluorescent tests. The influenza strains were typed at the Virus Reference Laboratory, Colindale, London.

*Culture medium*

Initially 'Oxoid' charcoal agar with added 'Difco' Proteose Peptone No. 3 and cephalexin, as recommended by Sutcliffe & Abbott (1972) was used but did not give very good growth. The medium was therefore changed to one made according to the following formula: Proteose Peptone No. 3 (Difco) 10·0 g, Lab Lemco powder (Oxoid) 10·0 g, peptone bacteriological (Evans Medical) 10·0 g, soluble starch AR (BDH) 10·0 g, sodium chloride AR 5·0 g, nicotinic acid 100 $\mu$g, Agar No. 3 (Oxoid) 12·0 g and bacteriological charcoal (Oxoid) 4·0 g.

All the ingredients, except the charcoal, were added to 1 l of distilled or deionized water and heated until dissolved. The pH was adjusted to 7·5–7·6. The charcoal was added, mixed thoroughly and distributed in 400 ml amounts. These were autoclaved at 121 °C for 15 min. For use, 40 ml defibrinated horse blood and 20 ml of cephalexin 800 $\mu$g/ml were added to the molten base, previously cooled to 50 °C, mixed well and thick plates (30 ml per 9 cm diameter Petri dish) poured at as low a temperature as possible to prevent sedimentation of the charcoal whilst the agar solidified. The surface of the plates was dried but overdrying avoided. The plates were stored in sealed plastic bags at 4 °C and used for up to 10 days. This medium gave larger colonies of *Bordetella pertussis* on primary isolation and there appeared to be an improvement in the isolation rate. All media were made and plates poured at the Swansea Public Health Laboratory.

## RESULTS

Analysis of 3148 cases notified as whooping cough revealed 2321 cases of clinical whooping cough and 905 were confirmed by the isolation of *B. pertussis*. The clinical cases showed a slight preponderance of girls (51·7 %). One thousand five hundred and five cases (65 %) were under the age of 5 years and there were 235 (10 %) adults over 20 years of age.

Table 1. *Isolation of* B. pertussis *according to month*

| Month | No. of isolations | Notified cases | | Clinical whooping cough | |
|---|---|---|---|---|---|
| | | Total | Positive (%) | Total | Positive (%) |
| Nov./Dec. 1977 | 18 | 71 | 25·4 | 60 | 30·0 |
| Jan. 1978 | 33 | 166 | 21·1 | 107 | 32·7 |
| Feb. | 26 | 197 | 13·2 | 109 | 23·8 |
| Mar. | 35 | 147 | 23·8 | 107 | 32·7 |
| Apr. | 28 | 99 | 28·3 | 77 | 36·4 |
| May | 7 | 35 | 20·0 | 31 | 22·6 |
| June | 26 | 77 | 33·8 | 57 | 45·6 |
| July | 36 | 145 | 24·8 | 122 | 29·6 |
| Aug. | 37 | 80 | 46·3 | 62 | 59·7 |
| Sept. | 67 | 194 | 34·6 | 160 | 41·9 |
| Oct. | 135 | 335 | 40·3 | 259 | 52·1 |
| Nov. | 162 | 534 | 30·3 | 405 | 40·0 |
| Dec. | 121 | 490 | 24·7 | 344 | 35·2 |
| Jan. 1979 | 98 | 305 | 32·1 | 236 | 41·5 |
| Feb./Mar. 1979 | 74 | 273 | 27·1 | 185 | 40·0 |
| Total | 905 | 3148 | 28·7 | 2321 | 39·0 |

*Isolations rates*

The monthly isolation rate of *B. pertussis* from notified cases from the middle of November 1977 to the middle of March 1979 is given in Table 1. This varied between 13·2 % for February 1978 and 46·3 % during August 1978 with an average rate of 28·7 % for the whole period. This rate was influenced by the number of notified cases who on clinical grounds did not satisfy the diagnostic criteria of whooping cough. From the questionnaire these were either found to have some other infection, such as early measles or a common cold, or to have an illness of short duration which did not resemble whooping cough either in severity, length of illness or in clinical pattern.

Such patients comprised over a quarter of the total and if they are excluded the isolation rate of *B. pertussis* was 905 out of 2321 (39·0 %).

The vast majority (887) of the 905 patients from whom *B. pertussis* was isolated had an illness lasting more than 3 weeks. There were 10 in whom the illness lasted between 2 and 3 weeks, seven in whom it lasted between 1 and 2 weeks and only one who had a short illness lasting less than 1 week.

*Comparison of cough plates and pernasal swabs*

There were 507 patients from whom *B. pertussis* was isolated who had a spasm of coughing which occurred either as a result of irritation following the taking of the pernasal swab or while the nurse was carrying out the interview. Cough plates were taken on these occasions. In 153 instances *B. pertussis* was isolated from both the pernasal swab and the cough plate, in 336 instances the pernasal swab was positive and the cough plate negative and in only 18 instances was the cough plate positive and the pernasal swab negative.

Table 2. *Effect of antibiotic therapy on isolation rate in patients with clinical whooping cough*

| Antibiotics | Total | Positive (%) | Positive (%) |
|---|---|---|---|
| None | 1439 | 575 | 40·0 |
| Erythromycin | 368 | 114 | 31·0 |
| Penicillins | 443 | 199 | 44·9 |
| Cotrimoxazole | 63 | 16 | 25·4 |
| Tetracycline | 8 | 1 | 12·5 |
| Total | 2321 | 905 | |

Table 3. *Isolation of* B. pertussis *in children according to age and vaccination state*

| | Fully vaccinated | | | Unvaccinated | | |
|---|---|---|---|---|---|---|
| Age (years) | Total | No. | Positive (%) | Total | No. | Positive (%) |
| 0–1 | 4 | 1 | (25·0) | 218 | 115 | (52·8) |
| 1–2 | 22 | 7 | (31·8) | 237 | 121 | (51·1) |
| 2–3 | 26 | 9 | (34·6) | 230 | 110 | (47·8) |
| 3–4 | 38 | 8 | (21·1) | 328 | 152 | (46·3) |
| 4–5 | 36 | 16 | (44·4) | 279 | 121 | (43·4) |
| 5–6 | 81 | 10 | (12·3) | 75 | 27 | (36·0) |
| 6–7 | 69 | 17 | (24·6) | 23 | 5 | (21·7) |
| 7–8 | 72 | 28 | (38·9) | 22 | 7 | (31·8) |
| 8–9 | 47 | 12 | (25·5) | 7 | 3 | (42·9) |
| 9–10 | 29 | 10 | (34·5) | 7 | 1 | (14·3) |
| Total | 424 | 118 | 27·8 | 1426 | 662 | 46·4 |

*Effect of antibiotic therapy on isolation rate*

The number of patients who had been on an antibiotic for at least 2 days before swabbing was recorded together with the name of the therapeutic agent. Table 2 shows that both erythromycin and co-trimoxazole therapy significantly reduced the chances of isolating *B. pertussis* ($P$ values $< 0{\cdot}005$ and $< 0{\cdot}05$ respectively) but penicillins did not affect the isolation rate. There was only one isolation from the eight patients treated with tetracycline. *In vitro* a selection of strains were all found to be sensitive to the four antibiotics.

*Isolation according to age and vaccination state*

The isolation rate in unvaccinated children under the age of 10 years with clinical whooping cough is given in Table 3. This shows a gradual reduction in isolation rate as the age increases.

Amongst those with clinical whooping cough under 10 years of age there were 1426 patients who had not been vaccinated and 424 who had been fully vaccinated (Table 3). The isolation rate was 46·4 % in those who had not been vaccinated as against 27·8 % in those who had been fully vaccinated.

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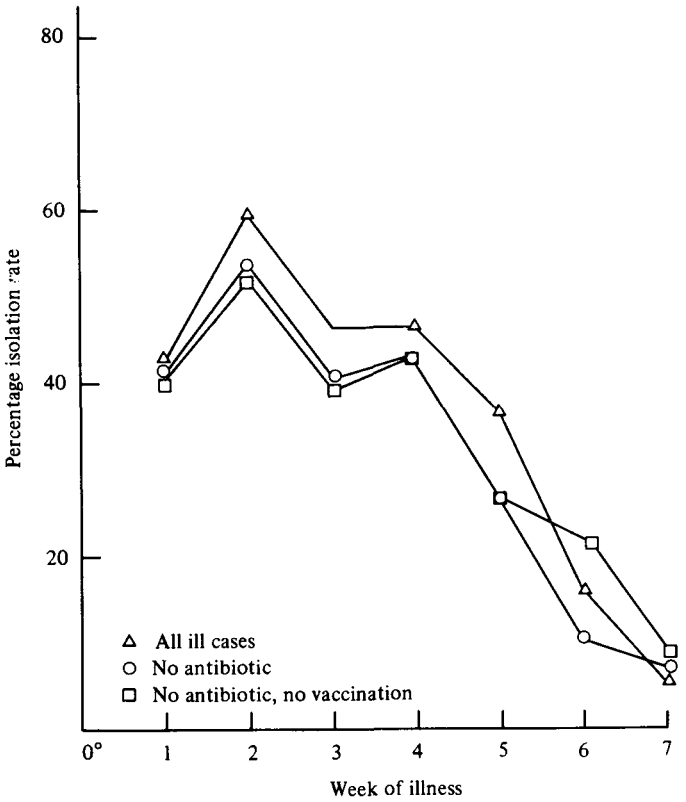


Fig. 1. Isolation rate according to the week of illness. △, All ill cases; ○, those not given an antibiotic and, □, those who had not been vaccinated and were not given an antibiotic.

*Isolation according to length of illness before swabbing*

The percentage isolation rate according to the number of weeks after onset is given Fig. 1 and this was best during the second week of illness. There was still a 40 % isolation rate at 4 weeks and between 15 % and 20 % at 6 weeks. This rate was not influenced either by the vaccination state or by antibiotic therapy.

*Isolation according to nurse taking swab*

There were changes of nurses on two occasions during the early part of the survey and it was only found possible to determine the isolation rates of *B. pertussis* from swabs taken by five nurses over the same 5 months period from late October 1978 to the beginning of March 1979. There was a variation from 20 % to 32 %. The results however are not strictly comparable because the nurses collected specimens from different areas.

*Isolation according to day of incubation of plates*

The charcoal agar plates were normally brought to the laboratory on the same day as the specimen was collected. Those from 135 patients were put into a domestic refrigerator overnight because they were collected late or at a week-end. There were 32 isolations of *B. pertussis*, making an isolation rate of 23·7 %. This is slightly lower than the average of 28·7 % shown in Table 1.

Table 4. *Change in proportion of* B. pertussis *serotypes over epidemic period*

| Period | No. of isolates of type 1.3 | 1.2.3 | 1.2 | Types 1.2.3 (%) |
|---|---|---|---|---|
| Dec. 77 to Mar. 78 | 53 | 11 | 0 | 17·1 |
| Apr. 78 to June 78 | 31 | 4 | 0 | 11·4 |
| July 78 to Sept. 78 | 89 | 19 | 2 | 17·3 |
| Oct. 78 to Dec. 78 | 261 | 52 | 9 | 16·1 |
| Jan. 79 to Mar. 79 | 71 | 50 | 5 | 39·7 |

*Typing results*

Six hundred and fifty seven strains of *B. pertussis* were serotyped. The predominant serotype was 1.3 and there was no significant difference in the distribution between the age groups 0–4 and 5–9 years. There were four families from whom two different serotypes were obtained. There was a significant change in the ratio of the two main serotypes between the beginning and end of the epidemic (Table 4), the percentage isolation of serotype 1.2.3 having more than doubled.

*Virus isolation*

Pernasal swabs in virus transport media received at the Swansea laboratory were examined in monkey kidney and Hep 2 tissue-culture tubes. There were 18 isolations from 1762 swabs. These comprised seven influenza A, two influenza B, three parainfluenza 1, three parainfluenza 3 and three adenovirus. Both *B. pertussis* and a virus were isolated from four patients suggesting that in these persons the finding of the virus was coincidental and unrelated to whooping cough which is known to be caused by *B. pertussis*.

Ten of these 18 patients from whom a virus was isolated had clinical whooping cough. These included the four from whom *B. pertussis* was isolated and there were a further two patients from households where this organism was isolated from a home contact. The average length of illness was 10½ weeks in the 10 patients with clinical whooping cough, but only 3 weeks in the other eight patients.

## DISCUSSION

This was a large outbreak of whooping cough which followed a period of a very low rate of immunization with pertussis vaccine in West Glamorgan. (Swansea Research Unit, 1981). The experience gained from this study showed the importance of a close collaboration between the laboratory and those working in the field.

The rate of isolation of *B. pertussis* varied between the nurses but it was noticed that they improved with experience. The rate would also depend on the accuracy of diagnosis by the doctor who made the notification. If a nurse worked for a period in an area where the diagnosis was poor she would have a correspondingly low isolation rate. If she worked in an area with a large number of notified cases of whooping cough, for example in a school, she would have a high isolation rate.

The results show that pernasal swabs taken in the home were superior to cough plates for the isolation of *B. pertussis*. This is in agreement with the findings of

Bradford & Slavin (1940). There were, however, a few isolations from cough plates where the pernasal swab was negative.

The overall isolation rate for notified cases was 28·7 % which is similar to that found by U.K. Public Health Laboratories (personal communications). The rate, however, varied according to the prevalence of other diseases resembling whooping cough in the area. This was particularly noticeable at the peak of the outbreak (October–December 1978) when the overall isolation rate fell from about 40 % to 25 %. This is the time of year when an increase in common-cold like illness can be expected and some of them may be mistaken for whooping cough.

The ability to isolate *B. pertussis* was significantly decreased by the administration of antibiotics particularly erythromycin and co-trimoxazole and possibly tetracycline but this drug is not to be recommended for children. Islur, Anglin & Middleton (1975) showed that erythromycin was rapidly effective in reducing the length of time positive cultures could be obtained and failed to isolate the organism after five days of treatment. The penicillins, of which ampicillin was the most frequently prescribed, had no effect on the isolation rate.

Previous pertussis vaccination appeared to lower the overall isolation rate from 46·4 % to 27·8 % in children under the age of 10. However, these figures are not strictly comparable because the majority of children in the unvaccinated group were under 5 years of age whereas most of the vaccinated children were over this age. In the under 5-year-old age group, comprising 126 vaccinated and 1292 unvaccinated, the isolation rates were 32·5 % and 47·8 %. In the 5- to under 10-year age group, comprising 298 vaccinated and 134 unvaccinated, the rates were 25·8 % and 32·0 % respectively. However, a comparison of the individual age groups suggests that vaccination does have a slight effect in reducing the isolation rate.

The Public Health Laboratory Service Working Party Report (1973) showed only slightly lower rates of isolation of *B. pertussis* in those vaccinated, but there has been a change in the vaccines since this study was carried out.

Table 3 shows that in non-immunized children, isolation is most frequent during the first year of life and that there is an inverse correlation between isolation rate and age. This agrees with the findings of a Combined Scottish study (1970) on the diagnosis of whooping cough and also a study of whooping cough in Glasgow (Walker, 1979). The reason for this may be that the illness is more severe in the younger age groups and more organisms are coughed up, or possibly that the diagnosis is made more easily in younger children.

Figure 1 demonstrates that the isolation rate of *B. pertussis* in unimmunized patients with clinical whooping cough rises from about 40 % at onset to about 60 % during the second week, after which there is a fall. Previous vaccination or antibiotic therapy had no effect on the pattern. The highest isolation rates were from swabs taken between 10 and 13 days. This could be the stage at which increased desquamation of epithelial cells killed by adhering *B. pertussis* organisms might be coughed up during a paroxysm of coughing.

The Department of Health and Social Security (1977) in their memorandum on the control of infectious diseases in schools, recommends a minimum period of exclusion from school of 21 days from onset of paroxysmal cough. In the present study 15–20 % of patients were still found to be carrying *B. pertussis* at 6 weeks and it is therefore questionable whether exclusion from school for 3 weeks is likely

to have any significant effect in controlling an outbreak. Donald (1938) carried out serial isolations in hospital and found an appreciable drop in isolation rate from 44% during the fourth week to 7% in the fifth week but he was using only cough plates for the diagnosis.

The results show a change in the percentage of strains containing antigen 2 during the course of the outbreak. Serotype 1.2 appeared during the period July–September 1978 and the percentage of serotype 1.2.3 doubled from about 17% during 1978 to 39% at the beginning to 1979. Subsequent figures for isolation at the Swansea Public Health Laboratory showed that this trend continued over the next 18 months. From April 1979 to December 1980, 55% of isolates were serotype 1.2.3.

The increase in percentage of serotype 1.2.3 during and after the outbreak is probably dependent on the low vaccination uptake in the community. Preston (1976) showed that there was a predominance of serotype 1.3 in vaccinated communities whereas serotype 1.2.3. was predominant before the introduction of mass vaccination. This change of predominant serotype has also taken place in the rest of the U.K. (Public Health Laboratory Service, 1978 and 1980). There was a change from 12% serotype 1.2.3 during January–March 1978 to 23% during July–September 1980. It will be interesting to see if serotype 1.2.3 predominates during the next epidemic peak and if there is a change in mortality or morbidity.

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