

Pertussis in India

Pertussis outbreaks have occurred cyclically since the early 1980s, with large increases in incidence in 1983, 1986, 1990 and 1993 [documented by the Centers for Disease Control and Prevention (CDC, 1995)]. In recent years, outbreaks due to pertussis have been reported from many parts of the world despite the widespread use of a vaccine (CDC, 2006). Non-immunized or partially immunized children are at a greater risk of developing infections, and their presence promotes the maintenance of the infectious agent in the community, especially in developing and underdeveloped countries. There is also a shift in the epidemiology of pertussis, with more cases being reported in older age groups, which is due to waning immunity to *Bordetella pertussis* a number of years after immunization (CDC, 2006).

In the last 30 years, there has been no report of laboratory-confirmed *B. pertussis* from India. Few studies were published (Chavan & Sant, 1972; Ray *et al.*, 1973) before the implementation of the Universal Programme of Immunization by the World Health Organization in India in January 1978 (Park, 2005). In recent years, a decrease in diphtheria–pertussis–tetanus (DPT) vaccine compliance has been reported from different parts of India (Chhabra *et al.*, 2007; Gupta *et al.*, 2006). Although diphtheria is known to be present (Lodha *et al.*, 2000), pertussis has not been documented. We report here laboratory confirmed cases of pertussis in patients presenting to All India Institute of Medical Sciences, the tertiary care hospital of North Delhi, India.

We carried out a preliminary study to look for evidence of respiratory infections due to *B. pertussis* in children presenting with the clinical features of whooping cough, which included fever, rhinorrhoea, spasmodic cough with inspiratory whoop, cyanosis and persistent cough or post-tussive vomiting, attending the outpatient department of the All India Institute of Medical Sciences, India, from October 2006 to May 2007. The study was approved

by the Institutional Human Ethics Committee on 12 January 2005. Two nasopharyngeal swabs were collected from children during their first visit – one for culture and a second for use in a direct fluorescent antibody (DFA) test and PCR using BBL CultureSwab Plus collection and transport system (Becton Dickinson), after obtaining the consent of the parents or guardians. Within 1 h of collection, the first set of swabs were cultured on freshly prepared Bordet–Gengou medium (Difco) supplemented with 5% defibrinated sheep blood and cefalexin (Loeffelholz, 2004). Colonies of *B. pertussis* were identified using standard biochemical reactions and confirmed by the slide agglutination test using *B. pertussis* antiserum (BD Difco) (Loeffelholz, 2004).

The second set of swabs was used for smear examination by the DFA test (Loeffelholz *et al.*, 1999) using BD Difco FA *B. pertussis* antiserum and a standard procedure, and the results were read by two independent observers. The second set of swabs was also tested by PCR after DNA extraction using the QIAamp DNA mini kit (Qiagen). PCR was performed using the protocol described by Loeffelholz *et al.* (1999). To ensure a high specificity for pertussis diagnosis by PCR, we targeted two genes, IS481 and the pertussis toxin-encoding gene, as proposed by Qin *et al.* (2002), as well as the IS1001 gene to rule out *Bordetella parapertussis*, as described by Van der Zee *et al.* (1993). *B. pertussis* strain ATCC 8467 was used as positive control.

Twenty-one patients were included in the analyses based upon the clinical criteria during the period of the study. Two patients were positive by all three methods (DFA, PCR and culture) for *B. pertussis* (Table 1). PCR was carried out for culture DNA as well as swab DNA. Both the IS481 and the pertussis toxin-encoding gene were amplified in the positive strains. None of the patients were positive in the PCR for IS1001. These patients presented within the first 2–3 weeks of experiencing symptoms. While patient 1 was a child

who had a history of only a single dose of DPT vaccine during infancy and was from a low socioeconomic section of the society, patient 5 had complete vaccination including the booster and had educated well-off parents. He was 11 years old and possibly had the infection due to waning immunity during adolescence. Another three patients were positive only by DFA and were culture negative. These patients presented later than 3 weeks after first experiencing the symptoms and gave a history of complete primary immunization during infancy but could not recollect having got their booster doses.

This study, though limited by small sample size, documents the circulation of pertussis in our country and stresses the importance of the vaccination programme in India. We found a total of only two culture-positive cases of pertussis amongst the children presenting with the clinical features of whooping cough, which is because of the patients presenting to the hospital late in the illness (Table 1). Our experience is similar to the earlier studies carried out in India during the 1970s (Chavan & Sant, 1972; Ray *et al.*, 1973) that reported a culture-positive rate of about 8–10% for pertussis. In both those studies it was observed that the frequency of pertussis cases in India was higher during the months of November to June, which is similar to our experience in the present study.

The reasons for low culture positivity – patients seeking health care late in the disease and a low index of clinical suspicion leading to a delay in sample collection for cultures – emphasize the need to collect samples within 4 weeks of coughing. However, prior antibiotic treatment by the general physician in the community, a common practice in our set up, can also be responsible for a low culture yield. In light of these observations it is important to strengthen the laboratory infrastructure to enable confirmation of the clinical diagnosis of pertussis. In addition, the culture would help to

Table 1. Characteristics of the patients with positive results in laboratory tests for *B. pertussis* infection

Patient no.	ID no.	Age	Date of sample collection	Duration of symptoms (days)	DFA	PCR	Culture
1	PR-4/20.01.07	4 years	20.01.07	20	Positive	Positive	Positive
2	PR-6/7.02.07	7 years	7.02.07	35	Positive	Negative	Negative
3	PR-8/21.02.07	9 months	21.02.07	80	Positive	Negative	Negative
4	PR-6/21.03.07	5 years	21.03.07	30	Positive	Negative	Negative
5	PR-1/16.05.07	11 years	16.05.07	15	Positive	Positive	Positive

characterize the strains further. We feel that the main reason for the presence of pertussis in a developing country like ours could be the lack of education in many sections of society, which could lead to poor vaccination compliance. Even amongst the higher socioeconomic classes the fear of side effects due to pertussis whole cell vaccine (used in India at present) can be a reason for avoiding the vaccination. In a study conducted in India to assess the child immunization coverage in Alwar district of Rajasthan (west of Delhi) it was observed that 82% children were fully immunized in urban areas as compared to 45% in rural areas (Gupta *et al.*, 2006). A high drop-out rate was found for DPT vaccination (25.3%) in rural areas as compared to urban areas (7.7%). Similarly, in another study to assess the immunization coverage in two urbanized villages of East Delhi it was observed that although the coverage for the first three doses of DPT vaccine was between 70 and 80%, it was only 40% for the DPT booster (Chhabra *et al.*, 2007). These surveys have shown a wide variation in vaccination rates across regions, states and different strata of society. The presence of inadequately immunized children helps maintain the circulation of the infectious agent within the community. We already have reported the occurrence of diphtheria in the population, which is indirect evidence of inadequate DPT vaccine coverage (Lodha *et al.*, 2000).

The main limitation of our study is the small number of patients analysed, which is not sufficient to allow any conclusions to be drawn; but our work does demonstrate

the ongoing circulation of *B. pertussis* in India. It reiterates the need for consolidating the ongoing surveillance, vaccination coverage and adolescent immunization against pertussis.

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CDC (1995). Pertussis – United States, January 1992 – June 1995. *MMWR Morb Mortal Wkly Rep* **44**, 525–529.

CDC (2006). Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine. *MMWR Morb Mortal Wkly Rep* **55**, 1–33.

Chavan, A. S. & Sant, M. V. (1972). Cultural diagnosis of *Bordetella pertussis* infection. *J Postgrad Med* **18**, 68–73.

Chhabra, P., Nair, P., Gupta, A., Sandhir, M. & Kannan, A. T. (2007). Immunization in urbanized villages of Delhi. *Indian J Pediatr* **74**, 131–134.

Gupta, R. S., Gupta, A., Gupta, H. O., Venkatesh, S. & Lal, S. (2006). Mother and child service coverage: reproductive and child health programme in Alwar district, Rajasthan state. *J Commun Dis* **38**, 79–87.

Lodha, R., Dash, N., Kapil, A. & Kabra, S. K. (2000). Diphtheria in urban slums in north India. *Lancet* **355**, 204.

Loeffelholz, M. J. (2004). *Bordetella*. In *Manual of Clinical Microbiology*, 8th edn, vol. 1, pp. 780–788. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller & R. H. Tenover. Washington, DC: American Society for Microbiology.

Loeffelholz, M. J., Thompson, C. J., Long, K. S. & Gilchrist, M. J. R. (1999). Comparison of PCR, culture, and direct fluorescent-antibody testing for detection of *Bordetella pertussis*. *J Clin Microbiol* **37**, 2872–2876.

Park, K. (2005). Principles of epidemiology and epidemiological methods. In *Park's Textbook of Preventive and Social Medicine*, 18th edn, pp. 100–105. Jabalpur: Banarsidas Bhanot.

Qin, X., Turgeon, D. K., Ingersoll, B. P., Monsaas, P. W., Lemoine, C. J., Tsosie, T., Stapp, L. O. & Abe, P. M. (2002). *Bordetella pertussis* PCR: simultaneous targeting of signature sequences. *Diagn Microbiol Infect Dis* **43**, 269–275.

Ray, M., Agarwal, K. C. & Chitkara, N. L. (1973). *Bordetella pertussis* infection in Chandigarh. *Indian J Med Res* **61**, 645–648.

Van der Zee, A., Agterberg, C., Van Agterveld, M., Peeters, M. & Mooi, F. R. (1993). Characterization of IS1001, an insertion sequence element of *Bordetella parapertussis*. *J Bacteriol* **175**, 141–147.