

SHORT REPORT

Bordetella parapertussis outbreak in Bisham, Pakistan in 2009–2010: fallout of the 9/11 syndrome

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SUMMARY

Pertussis or whooping cough is a highly contagious community disease mainly caused by *Bordetella pertussis* and *B. parapertussis*. We report a minor outbreak of whooping cough (2009–2010) in symptomatic subjects from Bisham, near Swat, Khyber Pukhtoonkhawa province, Pakistan. Interestingly, our results show that all the culture-positive isolates ($n = 21$) collected from children (average age 3·46 years), were identified as *B. parapertussis* after routine identification tests and PCR IS481, IS1001 and IS1002. Furthermore, in the affected patients, none had received immunization with diphtheria-pertussis-tetanus (DTP_w) vaccine. Therefore, the possibility of the re-emergence of the disease due to limitation of basic health services as a result of the political unrest due to the 9/11 situation is also examined. Moreover, we discuss the importance of vaccinating both adults and children with DTP_wP_{aw} vaccine containing both organisms for better protection.

Key words: *B. parapertussis*, DTP_w, immunization policy, outbreak, whooping cough.

The existing high child mortality rate (85·5/1000 in children aged <5 years) has become a great challenge bearing in mind Pakistan's high population growth rate (>1·8% per year) [1]. This problem becomes even more acute in the absence of appropriate health-care considering the fact that children are highly vulnerable in parts of Khyber Pukhtoonkhawa (KPK) province of Pakistan to vaccine-preventable diseases, due particularly to the political unrest after the 9/11 sequence of events.

Although the incidence of whooping cough (pertussis) remains highest in neonates who have not received any of their scheduled pertussis-containing vaccinations, which are generally associated with

Bordetella pertussis, it is known that another *Bordetella* species, *B. parapertussis* also contributes to the disease burden worldwide [2]. Previous researches have already shown up to 35% of pertussis-like cases in highly vaccinated populations of some European countries as well as in Pakistan to be caused by *B. parapertussis* [3, 4]. The exact prevalence of *B. parapertussis* infections due to the mild or sub-clinical course of the disease probably go unnoticed and may be underestimated [5]. For that reason community-based data on the occurrence of *B. parapertussis* infection is scarce [5, 6].

Pakistan is a predominantly rural developing country with its ≥ 190 million inhabitants facing political turmoil since the 9/11 incident. This has resulted in inaccessibility of vaccinators to susceptible populations in many parts of the country [7]. Therefore, it is a major concern for health sector workers to have some understanding of disease outbreak and its aetiological agent for better risk assessment. In this regard

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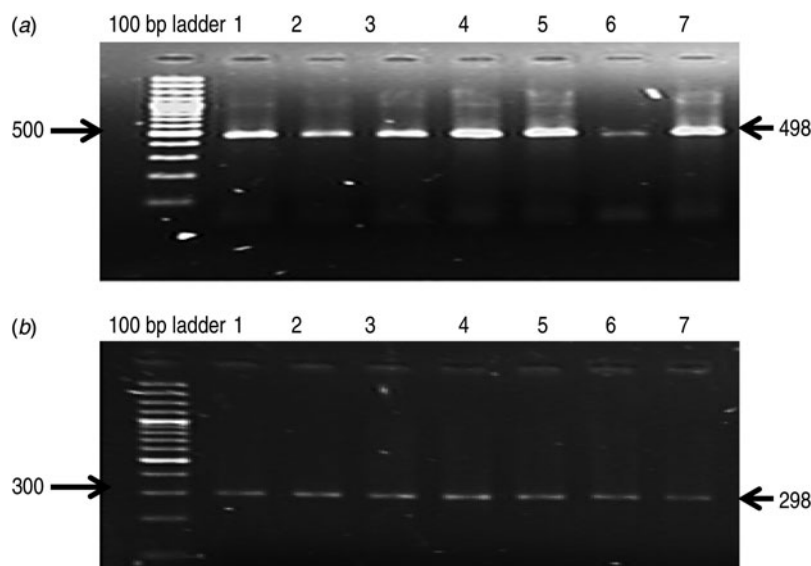


Fig. 1. Agarose gel electrophoresis showing PCR amplification products (a) IS1001, (b) IS1002, respectively. The first lane represents the positive control, *B. paraptentussis* B0024. Lanes 2–7 are some of the representative *B. paraptentussis* isolates collected during December 2009–January 2010.

we report a case study from the geographically well isolated small town of Bisham in Shangla district near Swat in KPK province where a local epidemic due to *B. paraptentussis* occurred during 2009–2010.

In order to determine the prevalence of pertussis in Bisham we collected a total of 50 samples from 12 houses in adjacent streets during the months of December 2009 and January 2010, from patients with a short history of cough, i.e. up to 4 or 5 days. Household contacts where two or more siblings were available were recruited for the study. Prior approval by the Ethics Committee of the Department of Biosciences, COMSATS Institute of Information Technology, Islamabad as well as parental consent was obtained before sample acquisition. Information on age, gender and vaccination history pertaining to each patient was recorded. The age of patients recruited in this case study ranged from 6 months to 5 years. Nasopharyngeal swabs were obtained carefully as described in standard CDC protocols in order to minimize cross-contamination and transferred in transport medium. Samples were cultured on Bordet–Gengou (BG) agar containing 10% horse blood and 40 µg/ml cefalexin to minimize growth of other bacteria and routine biochemical tests were performed before DNA extraction and polymerase chain reaction (PCR). Bacterial culture, identification, DNA extraction and PCR were performed as described previously [8, 9]. Routine identification tests included nitrate reduction, urease, citrate, indole, oxidase, and

catalase. Growth on MacConkey agar was used for initial identification. Further confirmation based on the formation of β -haemolytic colonies as seen on BG agar supplemented with 5% defibrinated sheep blood and a positive reaction of these colonies with anti-serum to *B. paraptentussis* ZM11 (Remel, The Netherlands) was achieved. Total genomic DNA templates isolated were screened for the presence of insertional sequences IS481, IS1001 and IS1002 [8, 9]. The presence of IS1001 was first detected in these to confirm *B. paraptentussis* positivity, next amplification of IS1002 was used to confirm the origin of the isolate, i.e. human *B. paraptentussis* (Fig. 1). *B. paraptentussis* (B0024) was used as a positive control.

During the course of this study we observed a minor outbreak of *B. paraptentussis* in Bisham (2009–2010), in patients with cough-like illness, in particular children aged ≤ 5 years (December–January, 2009–2010) (Table 1). *B. paraptentussis* was isolated from 21 patients (mean age 3.46 years). During the first peak of the outbreak (December 2009), 12 children proved to be culture positive for *B. paraptentussis* whereas during the second peak (January 2010), nine children tested positive for *B. paraptentussis*. One hundred percent *B. paraptentussis* cases were detected during the surveillance period.

The investigation was performed with the objective of evaluating the species of *Bordetella* circulating in the population that had not been vaccinated during the past 3–4 years. Vaccination history (dates of

Table 1. Summary of *B. parapertussis* isolates and affected clinical cases

Sample no.	PCR results for IS1001	PCR results for IS1002	Culture results	Age (years)/sex	Vaccination status
1	+ve	+ve	+ve	5·0/M	–ve
2	+ve	+ve	+ve	4·6/F	–ve
3	+ve	+ve	+ve	3·6/M	–ve
4	+ve	+ve	+ve	2·6/M	–ve
5	+ve	+ve	+ve	3·0/M	–ve
6	+ve	+ve	+ve	3·8/M	–ve
7	+ve	+ve	+ve	4·6/F	–ve
8	+ve	+ve	+ve	2·6/F	–ve
9	+ve	+ve	+ve	1·6/M	–ve
10	+ve	+ve	+ve	3·6/F	–ve
11	+ve	+ve	+ve	1·6/M	–ve
12	+ve	+ve	+ve	2·6/F	–ve
13	+ve	+ve	+ve	0·6/F	–ve
14	+ve	+ve	+ve	3·6/F	–ve
15	+ve	+ve	+ve	4·0/M	–ve
16	+ve	+ve	+ve	4·6/F	–ve
17	+ve	+ve	+ve	3·6/M	–ve
18	+ve	+ve	+ve	2·6/M	–ve
19	+ve	+ve	+ve	4·6/M	–ve
20	+ve	+ve	+ve	5·0/M	–ve
21	+ve	+ve	+ve	5·0/M	–ve

PCR, Polymerase chain reaction.

vaccinations) for all subjects was obtained in order to evaluate vaccine coverage and efficacy. Coincidentally, all isolates were recovered from non-vaccinated patients.

Additional data from adjacent towns would have provided a more comprehensive overview of the situation in the region. However, further follow-up could not be carried out due to concerns regarding prevailing political unrest in the region at that particular time. Since we detected *B. parapertussis* in a large number of clinical samples from patients that had not been vaccinated, we speculate that this was due to the deteriorating political events and lack of security, compromising outreach of vaccinators in the region [7].

In order to evaluate vaccination status in the region, vaccine coverage in KPK with reference to the rest of the country was analysed. Vaccine coverage data obtained from the Extended Immunization Programme (EPI) show about 44–55% pertussis vaccine coverage in the relevant major district, Swat, during the years 2008–2010 compared to overall coverage in Pakistan (Supplementary Figs S1 and S2). This could be a reflection of the vaccinators being unable to reach the susceptible population in that area due to the prevailing political unrest in the KPK region during the study period. Furthermore, with the

existing health system in Pakistan, like many other countries in the region, effective control of disease is difficult due to lack of trained health experts, poorly equipped laboratories and facilities lacking the capacity to distinguish *B. pertussis* and *B. parapertussis*. This situation is further exacerbated due to the poor outreach of vaccinators in times of political turmoil.

Furthermore, data pertaining to the overall prevalence of *Bordetella* infections in the Pakistani population, whether immunized or not, is lacking. Despite overall high vaccine coverage in children, clinical cases have been reported consistently throughout the country (Supplementary Figs S3 and S4).

Previous reports depict a relatively smaller percentage of *B. parapertussis* existence in countries like France and Iran where pertussis vaccine coverage has been high [10, 11]. While in countries like Finland where diphtheria-pertussis-tetanus (DTP_w) vaccine is used, a large percentage of pertussis cases are caused by *B. parapertussis*. Therefore, it can be suggested that factors other than vaccination such as geographical elements may also contribute to the higher incidence of *B. parapertussis* disease [12]. This fact is further supported by surveillance data suggesting decreased effectiveness of typical pertussis vaccines against *B. parapertussis* [13]. A recent study by Mughal *et al.*,

revealed the presence of pertussis in vaccinated children in the Khairpur district of Sindh [14]. Moreover, pertussis outbreak data from the National Institute of Health (NIH), Pakistan show a total of 158 suspected pertussis cases during the years 2011–2013 in KPK alone, which may be possibly due to *B. parapertussis* (Supplementary Fig. S4). This study, as well as pertussis outbreak data from NIH, further affirms the inefficiency of current vaccine protocols in elimination of the disease. Another study comparing the prevalence of *B. pertussis* vs. *B. parapertussis* surprisingly showed all isolates to be *B. parapertussis* [15]. It is known that efficacy and protection against *B. parapertussis* is significantly higher with the *B. pertussis* whole-cell vaccine DTwp rather than the acellular vaccine DTap. Furthermore, data obtained from studies on mice suggest that the level of protection and course of the infection are strain specific [16]. The available acellular vaccines target *B. pertussis* antigens and show little or no effectiveness against *B. parapertussis*. Furthermore, these *in vivo* infection studies reveal that DTap vaccination against *B. pertussis* can interfere with the normal host response leading to clearance of *B. parapertussis*, increasing susceptibility to infection by this bacterium [16]. Consequently, there is a possibility of improving pertussis vaccines by including *B. parapertussis* antigens as adopted by some East European countries [5, 13]. The likelihood of *B. parapertussis* emerging in populations where *B. pertussis* is endemic and DTP_w is the routinely used vaccine is high. This is also supported by the fact that *B. parapertussis*-induced immunity protects against both species in contrast to *B. pertussis*-induced immunity which protects only against *B. pertussis* infection [17].

By contrast, considering that routine whole-cell vaccines may offer protection against *B. parapertussis*; we believe that the local outbreak of *B. parapertussis* in Bisham, near Swat, may actually have been a result of non-compliance with the vaccine schedule in this area due to ‘war-on-terror’ before 2009–2010. This is supported by the observation that increased vaccine coverage in certain districts in KPK limited disease incidence as seen in a decrease of reported cases in the following years (Supplementary Fig. S2). Vaccination in the KPK region according to a recent survey conducted by the National Institute of Population Studies during the period 2012–2013 shows increased coverage of the DTP3 vaccine compared to other vaccines. However, overall coverage still remains about 10% lower than that reported in

Punjab with less than 40% of children having vaccination cards. The authors also report threats received during the survey in KPK and Balochistan, where security issues run deeper and hence vaccine coverage is even lower [18]. Several other studies from war-affected and conflict-ridden regions show that security threat within the country is an important factor affecting health services and overall immunization coverage [19, 20]. Providing increased security to vaccinators in the region as well as maintaining the vaccines in provincial rather federal headquarters will enable a more efficient response.

This high percentage of *B. parapertussis* cases reported from Bisham creates a disturbing situation for healthcare professionals as well as policy makers. Moreover, *B. parapertussis* infections may also be associated with their atypical manifestation in the adult population of Pakistan; therefore, we propose adult vaccination with whole-cell vaccines containing both *B. pertussis* and *B. parapertussis* to reduce the risk of infection in infants and children through adult reservoirs.

A convincing explanation of this puzzle is difficult because both *B. pertussis* and *B. parapertussis* infections are not systematically recorded in Pakistan. However, a preschool booster to increase herd immunity and to reduce transmission to susceptible subjects is a useful strategy, a practice that has been adopted in many countries [21]. Additionally, adult vaccination is also an important approach to control pertussis infection [22]. We strongly recommend ensuring that infants are vaccinated at the correct age and that all scheduled doses comprising of both *B. pertussis* and *B. parapertussis* (DTP_wP_{aw}) are administered on time. Here the role of primary healthcare units should be emphasized, especially in rural areas of the country. Quick diagnosis and treatment of pertussis cases in children and adults having contact with infants would limit the infection and should be provided in primary healthcare units.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268814003732>.

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DECLARATION OF INTEREST

None.

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