Pertussis is an infectious disease affecting infants and children. Its severity is higher in very young infants, as they exhibit whooping cough, staccato, apnea, and choke on their sputum (1,2). Although typical cases are easily recognized, the diagnosis of pertussis in adults or vaccinated children is difficult because they only have atypical mild symptoms such as a prolonged cough without whooping. Therefore, such cases go undiagnosed, and anti-microbial medicines are empirically administered. Additionally there is no reliable diagnostic method for pertussis, when a physician considered the possibility of pertussis. The gold standard is the isolation of *Bordetella pertussis*, however, it requires skillful experience. Bacterial isolation is not always successful because of inadequate sampling, timing, transportation conditions, and other technical issues. Genetic diagnosis by PCR recently became widely available, but this method is less suitable for clinical diagnosis because of the requirement of a thermal cycler or a real-time PCR apparatus (3). However, the most critical issue is the low *B. pertussis* load in the respiratory tract, because most patients visit their physician approximately 2 weeks after the onset of coughing, they have often already taken antibiotics, in addition to having an immunization history.

The incidence of pertussis in adults has been increasing since 2002 in Japan, and several outbreaks in college campuses, high schools, and offices have been reported. In the present study, bacterial isolation and loop-mediated isothermal amplification (LAMP) for the detection of *B. pertussis* genomic DNA were tested, using a primer set reported by Kamachi et al (4). The test subjects consisted of 43 patients aged <15 years who were suspected to have pertussis and were treated at the Saiseikai Central Hospital, Tokyo in 2010 and 2011. Nasopharyngeal swabs (NPSs) were collected at the first visit. *Bordetella* was isolated on a Bordet Gengou agar plate supplemented with 15% sheep defibrinated blood, and the presence of two different *Bordetella* species was confirmed by PCRs targeting IS481 for *B. pertussis* and IS1001 for *B. parapertussis* (1,3). DNA was extracted from NPS samples by using a DNA extraction kit (Toyobo, Osaka, Japan). *Bordetella* genomic DNA was detected by LAMP of the 5′ UTR of pertussis toxin (PT) by using the LAMP amplification kit (LMP 204, Eiken Chemical, Tokyo, Japan). Turbidity was examined using a real-time turbidimeter LA 200 (Teramecs, Kyoto, Japan), and samples with turbidity >0.1 were considered LAMP-positive (4). This study protocol was reviewed and approved by the Ethics Committee of Kitasato Institute Hospital, Tokyo, Japan. Oral informed consent was obtained from all patients.

The results of the isolation of the *Bordetella* species and the detection of its genomic DNA in patients of different age groups are shown in Table 1. Three strains of *B. pertussis* were isolated and 24 out of 43 patients were LAMP-positive for the detection of *B. pertussis* genomic DNA. In addition, 3 strains of *B. parapertussis* were isolated, and their genomic DNAs were detected by PCR in the same patients. There were no cases of dual infection. Approximately 56% of the tested patients were diagnosed with *B. pertussis* infection, and 7% of the patients with clinically suspected pertussis were found to be infected with *B. parapertussis*. There were no observable differences in the severity of clinical symptoms between the two groups.

This study resulted in two important findings: (i) the usefulness of LAMP for diagnosis of pertussis was demonstrated, and (ii) the incidence of *B. parapertussis*
infection was determined. A diagnostic algorithm shows that both PCR and bacterial isolation are useful methods for diagnosis in infants aged 0–3 months, while a serological diagnosis of IgG anti-PT is recommended for individuals aged > 10 years (2). Although antibodies against PT and filamentous hemagglutinin are commonly detected, these antigens are vaccine components. When the patients are older than 1 year, the IgG anti-PT antibody assay becomes meaningless because of vaccine-acquired immunity (1). Since paired samples are required for accurate diagnosis, serological examination was not performed in this study. Instead, bacterial isolation and detection of the genomic DNA were performed for samples obtained from 43 pediatric patients suspected to have pertussis. Because of a regional pertussis outbreak during the study period, the detection rate of *Bordetella* genomic DNA using LAMP was high. Genomic detection of *B. pertussis* in the USA and EU has been carried out using PCR or real-time PCR for IS481 (1,3); however, IS481 is present in other *Bordetella* species, which could lead to false-positive results. LAMP is a very simple and fast procedure that does not require specific equipment. It has a high sensitivity and specificity because the LAMP primers target eight regions in approximately 200 nucleotides (4). Results are obtained within 1 hour, inclusive DNA extraction and genome amplification steps. Another important finding of this study is the incidence of *B. parapertussis* infection. Several reports showed that approximately 2–3% of *Bordetella* isolates were *B. parapertussis*. In the present study, 3 strains (7%) were isolated from 43 patients.

In conclusion, LAMP proved to be a useful method for the detection of *B. pertussis* genomic DNA. The incidence of *B. parapertussis* was 7% among patients suspected to have pertussis.

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**Conflict of interest**  Non to declare.

**REFERENCES**


