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Recovery of *Bordetella pertussis* from PCR-Positive Nasopharyngeal Samples Is Dependent on Bacterial Load

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Viable *Bordetella pertussis* isolates are essential for surveillance purposes. We performed culture of 223 PCR-positive nasopharyngeal samples. *B. pertussis* was recovered from 45 (20.2%) of the samples. Growth was associated with a high bacterial load, as determined by PCR. Culture from PCR-positive samples is a feasible approach to recover *B. pertussis* isolates, and culture can be limited to samples with a high bacterial load.

Despite a continuous immunization program against pertussis (whooping cough) in Norway from 1952, the incidence of pertussis has increased in the past 2 decades (3). Mechanisms proposed as explanation for this include waning of immunity and vaccine-driven virulence evolution (7).

Real-time PCR has become the method of choice for detection of *Bordetella pertussis* in nasopharyngeal (NP) samples, making culture redundant (9). However, isolation of *B. pertussis* is of importance for epidemiologic purposes. Bacterial evolution under vaccine-induced immune pressure can be studied by characterization of *B. pertussis* isolates (4), and culture is essential for antimicrobial susceptibility testing and for understanding the public health implications of changes in the molecular epidemiology of *B. pertussis*.

Isolation of *B. pertussis* from NP swabs obtained for analysis by PCR is possible, and direct streaking onto charcoal agar plates in the diagnostic laboratory has been tried with success (Kees J. Heuvelman, personal communication). The aim of the present study was to obtain *B. pertussis* isolates from NP swabs at the reference laboratory for *B. pertussis* at the Norwegian Institute of Public Health (NIPH) with minimal extra labor imposed on the primary diagnostic laboratory; only samples that scored positive for *B. pertussis* by real-time PCR were forwarded to NIPH for plating and culture.

Two medical microbiology laboratories participated in the study: Fürst Medical Laboratory, Oslo (Lab 1), and the Department of Microbiology, Vestfold Hospital Trust (Lab 2). NP samples were obtained using a nylon flocked swab and transported in 1 ml of modified liquid Amies transport medium (ESwab; Copan Diagnostics, Inc., Corona, CA). DNA was extracted directly from 200 μl of transport medium using MagnaPure LC (Roche, Indianapolis, IN). In-house protocols for real-time PCR targeting insertion sequences (IS) were used—either IS*481* alone (Lab 1) (2) or both IS*481* and IS*1001* (Lab 2) (6). Each laboratory used its own criteria to score a sample as positive. Among other criteria, Lab 1 scored samples with a threshold cycle (C_T) value of <36 as positive, and samples with a C_T value of 36 to 40 were retested for reproducibility before scoring as positive. In Lab II, the total number of cycles was 35. The assays were not further compared for the purpose of the present study. Samples scored as positive in the IS*481* assay between 18 November 2011 and 13 February 2012 at Lab 1 and between 7 January 2012 and 7 February 2012 at Lab 2 were consecutively included in the study. The samples were kept refrigerated at 4°C and forwarded to NIPH once a week. All specimens were plated onto charcoal agar supplemented with defibrinated horse blood and cephalexin (8). Agar plates were incubated in humid air at 36°C for 7 days. Small, round colonies with a gray, pearly, and smooth appearance, suspected of being *B. pertussis*, were subcultivated and Gram stained. *B. pertussis* was confirmed by agglutination with specific antisera against Fim 2 and Fim 3 and a negative reaction with *Bordetella parapertussis* antiserum (in-house monoclonal antibodies).

The association of *B. pertussis* recovery with delay from sampling to plating and bacterial load (C_T value) was studied by chi-square tests (SPSS 17.0). *P* values of <0.05 were considered statistically significant.

Overall, 223 specimens positive in the IS*481* assay were received at NIPH: 169 from Lab I and 54 from Lab II. There was no significant difference between isolation rates from the two laboratories (data not shown). The age of the patients included in the study ranged from 0 to 83 years; the median age was 17 years. *B. pertussis* was successfully isolated from 45 (20.2%) of the samples: 20 isolates (44.4%) belonged to serotype 3, and 25 (55.6%) belonged to serotype 2. No agglutination was observed with *B. parapertussis* antiserum. The bacterial load, measured by C_T value, was significantly associated with recovery of viable *B. pertussis* cells ($P < 0.001$) (Table 1). With an arbitrary cutoff set at a C_T value of <30, the sensitivity of culture increased to 41 (50.1%) of 81 samples, while viable isolates were recovered from only 4 (2.8%) of 142 samples with a C_T value of ≥ 30. The median delay from sampling to plating at NIPH was 3 days for both culture-negative and culture-positive samples, ranging from 1 to 17 days and 2 to 15 days, respectively. Ten (22.2%) of the 45 positive samples were plated more than 5 days after sampling. The recovery rate de-

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TABLE 1 Growth of *Bordetella pertussis* from PCR-positive samples by C_T value and delay from sampling to plating

C_T value[a]	No. of samples culture positive/total (%)		
		Delay to culture[b]:	
	Overall	<5 days	≥5 days
<15	6/7 (86)	5/5 (100)	1/2 (50)
15–19	13/14 (93)	10/10 (100)	3/4 (75)
20–24	13/23 (56.5)	11/18 (61)	2/5 (40)
25–29	9/37 (24)	8/27 (30)	1/10 (10)
30–34	4/48 (8.3)	1/30 (3.3)	3/18 (17)
≥35	0/94 (0)	0/67 (0)	0/27 (0)
Total	45/223 (20)	35/157 (22)	10/66 (15)

[a] C_T, threshold cycle.
[b] Shown is the number of days from nasopharyngeal sampling to plating onto charcoal agar.

creased after more than 5 days (Table 1), although not statistically significantly.

The present study demonstrates that *B. pertussis* can successfully be isolated from NP samples obtained for PCR analysis. Viable isolates were recovered after transport of the original sample vial to the reference laboratory. Recovery was strongly associated with bacterial load, while delay from sampling to plating appeared to be less important. This is in accordance with a study by Halperin et al. (1) demonstrating prolonged survival of *B. pertussis* in phosphate-buffered saline.

The aim of the study was neither to determine the sensitivity of culture relative to real-time PCR nor to optimize a method for culture from NP swabs. More careful and rapid handling of NP samples and the use of more suitable transport media, such as Regan-Lowe, might have yielded more isolates. Furthermore, as DNA from nonviable bacteria might be detected in the PCR assay, isolation of viable bacteria cannot be expected from all PCR-positive samples. IS*481* is a multicopy target, and false-positive reactions may occur due to the presence of IS*481* in other *Bordetella* species, such as *B. holmesii* (5). Recent analysis has shown a low presence of *B. holmesii* and *B. bronchiseptica* species in this patient population (data not shown). The specificity of the diagnostic real-time PCR assays should be addressed in further studies.

In conclusion, we have demonstrated that *B. pertussis* can survive for several days in modified liquid Amies transport medium following preimplemented sample collection procedures and that culture secondary to PCR is a feasible approach for obtaining *B. pertussis* isolates. The positivity rate appears to be sufficient for surveillance purposes when *B. pertussis* is endemic, and culture of *B. pertussis* for epidemiological purposes can be limited to selected PCR-positive samples.

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