



## A Case of Pediatric Pertussis Unhesitatingly Diagnosed with the Insight of Microbiology Laboratory

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### Authors' contributions

This work was carried out in collaboration between all the authors. Authors AH and YK wrote the first draft of the manuscript. Authors NK and EH managed the literature searches. Authors TN and KO reviewed the manuscript. Author YK supervised the study. All authors read and approved the final manuscript.

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Case Study

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### ABSTRACT

We describe a pediatric pertussis case occurred in an unvaccinated six-month-old male infant. The newborn patient was rapidly diagnosed by means of the insight of the clinical microbiology laboratory, despite the absence of comments on the suspicion of pertussis. The keen insight of the

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laboratory lay in noticing the discrepancies between the usual culture results and the gram-stain findings on the respiratory secretion submitted for bacteriological examination with the clinical diagnosis of aspiration pneumonitis. It should be highly stressed that the careful consideration of findings from gram-stained preparations surely guide correct diagnosis.

**Keywords:** *Bordetella pertussis*; pediatric; gram's stain; isolation; LAMP; loop-mediated isothermal amplification.

## 1. INTRODUCTION

Currently, the pertussis acellular vaccination has been carried out in Japan using the pertussis toxin and filamentous hemagglutinin as the major antigens, and the number of pediatric pertussis patients were significantly decreased, with the wide spread of its vaccination. In fact, according to the review article concerning the pertussis epidemiology in Japan by Kanai, M. [1], the number of reported pertussis patients in Japan was dramatically reduced from 122,796 cases in 1950 to only 206 cases in 1971. However, there have remained many difficult cases in the definitive diagnosis of pertussis. Moreover, since April in 1999 when the new law in Japan for infectious diseases was enforced, clinically pertussis-like illnesses have been classified as pertussis. Indeed, the 5,208 pediatric pertussis cases were reported in 2009 from fixed pediatric facilities in Japan [2]. However, due to the difficulty in its definitive diagnosis, the pseudo pertussis patients were possibly considered to be included in the 5,208 cases along with the true pertussis patients [2]. In fact, most infants suffering from pertussis is easy to be clinically diagnosed by observing the characteristic inspiratory whistle sound, and/or prolonged bout of coughing bouts. Because not a few pertussis patients would represent atypical clinical symptoms, it is often difficult to distinguish them from other respiratory infections. In recent years, clinical microbiology laboratories have been diagnosing pertussis by means of serological and molecular biological examinations in addition to the traditional bacterial culture test. In these days, genetic examinations for the rapid diagnosis [2] in particular have made remarkable progress in the past 10 years.

We have succeeded in diagnosing a *Bordetella pertussis* infection from the respiratory secretion of a pediatric patient by the careful reading of the gram-stained preparations, followed by observing positive loop-mediated isothermal amplification (LAMP) test against pertussis. This paper is to report a case of pediatric patient with pertussis rapidly confirmed by the insight of the clinical

microbiology laboratory, despite the absence of comment on the suspicion of pertussis.

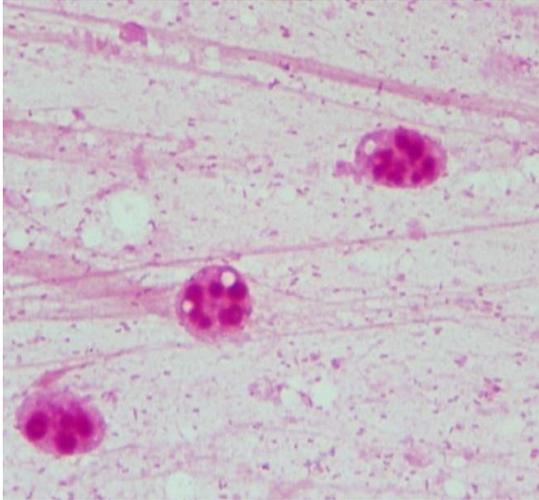
## 2. CASE STORY

The patient was a six-week-old male infant with no appreciable medical profiles. He first was admitted to a nearby medical facility for the 2-week-long cough on November 4 in 2014. However, on the day of his admission, he developed bradycardia accompanied both by reduction in arterial oxygen saturation and by slight fever (36.9°C). Indeed, his saturation values of hemoglobin with oxygen as measured by pulse oximetry (SpO<sub>2</sub>) was reduced to 50% accompanied by the development of bradycardia. He was therefore required to have an emergency hospitalization, and was transferred to the pediatric intensive care unit (PICU) in Nagano Children's Hospital, Azumino 399-8288, Japan, immediately after his endotracheal intubation. Biochemical blood analysis revealed that the CRP value is in the normal range (Table 1), which is a characteristic feature in children with pertussis [3]. Chest X-ray examination showed atelectatic raising the question of foreign body or pneumonia.

Respiratory secretion was submitted to the Department of Laboratory Medicine, Nagano Children's Hospital, for bacteriological examination with the clinical diagnosis of aspiration pneumonitis without any additional comments concerning the suspicion of pertussis. Routine microbiological culture procedures were carried out using Chocolate agar, Sheep blood agar (Trypticase soy agar II with 5% sheep blood) and modified Drigalski agar media (all Nippon Becton Dickinson Co., Ltd., Tokyo, Japan), followed by the microscopic examinations of Gram-stained preparations of the respiratory secretion.

Numerous gram-negative short rods reminiscent of *Haemophilus* species accompanied by many polymorphonuclear leukocytes, as shown in Fig. 1, were demonstrated on the gram-stained respiratory secretion specimen, strongly

suspicious of developing respiratory infection. Actually, we first regarded these dust-like short rods as *Haemophilus influenzae*, the causal agent of the aspiration pneumonia.



**Fig. 1. Numerous gram-negative dust-like small to tiny coccobacilli reminiscent of *Haemophilus influenzae* observed on gram-stained respiratory secretions by Bartholomew & Mittwer method**

**Table 1. Laboratory findings**

Hematology		Biochemistry	
White blood cells	5.53×10 <sup>9</sup> /L	Total protein	4.0×10 <sup>1</sup> g/L
Stab cell	0%	Albumin	2.9×10 <sup>1</sup> g/L
Segmented cell	52.0%	Na	142mEq/L
Eosinophil	0%	K	3.7mEq/L
Basophil	0%	Cl	110mEq/L
Lymphocyte	42.0%	C-reactive protein	1.0×10 <sup>-3</sup> g/L
Monocyte	6.0%		
Red blood cells	3.10×10 <sup>12</sup> /L		
Hemoglobin	9.6×10 <sup>1</sup> g/L		
Hematocrit	28.2%		
Platelet counts	3.65×10 <sup>4</sup> /L		

After incubation at 35°C for 24-hrs, small quantities of *Haemophilus*-like colonies appeared on the Chocolate agar plate (Nippon Becton Dickinson), which was finally identified as *H. influenzae* by ID-Test HN20 system (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) together with X/V-factor-requirement and hemolytic activity tests using *Haemophilus* ID Quad with Growth Factors (Nippon Becton Dickinson). However, no other microorganisms suggestive of possible respiratory pathogens were detected. Notwithstanding the fact that a large number of

small rod-shaped dust-like-cells were observed on the gram-stained preparation slide glasses, only a small quantities of *H. influenzae* had been appeared on the Chocolate agar plate (Nippon Becton Dickinson). We took notice of this conflicting phenomenon between the gram-stain and the culture findings, and this discrepancies should be suggestive of the presence of any organisms which failed to grow on chocolate and sheep blood agar media (Nippon Becton Dickinson) other than *H. influenzae* strains. In consequence, we strongly suspected that the uncultured organisms should be *B. pertussis* organisms. Therefore, loop-mediated isothermal amplification (LAMP) method was soon applied to the respiratory secretion sample to detect *B. pertussis* and positive reaction was obtained for the presence of *B. pertussis*. This LAMP assay has been demonstrated to sensitively detect *B. pertussis* with high levels of agreement with results of conventional PCR [4-5].

He was soon definitively diagnosed as pertussis, and was treated with azithromycin for 5 days and was additionally administered with cefotaxime for 7 days, which gradually eased his breathing. He was transferred from PICU to the general ward on the 5th day of hospitalization, despite of his occasionally persisting cough.

### 3. DISCUSSION

*B. pertussis* organisms are extremely infectious agents. Moreover, in cases when they infect unvaccinated infants [6-7], obtaining definitive diagnosis with rapidity provide appropriate remedies to pediatric patients, like this case.

Gram stains in cases of clinically suspected pulmonary infections sometimes bring favorable predicted diagnosis.

In this case, insight of the clinical microbiology laboratory led to the diagnosis of pertussis. However, without any comments on the suspicion of pertussis, most of the hospital is likely to overlook the whooping cough. Of course, this was the first case encountered in our hospital.

Pertussis vaccination in Japan has become a regular vaccination to infants between 3 months of age up to 12 months with the interval at least from 3 to 8 weeks. Therefore, almost all the infants are vaccinated. Unfortunately the infant in this case had unvaccinated because he had not yet turned 3 months old.

Increased awareness, new diagnostic tools, and better registration of pertussis patients might most likely associated with the increase in reporting pertussis infections. Although the most probable source of pertussis in infants, particularly in unvaccinated newborns should be ascribed to the exposure within pertussis-infected family units. As it turned out afterwards, two routes, described below, emerged as possible infection. One route is that on October 19 he was taken out by his parents for approximately two hours to the nearby Shinto shrine for celebrating his birth and for wishing his happiness and good fortune, where he was taken photographs with his parents, which is called "Omiyamairi", the Japanese traditional custom for children at around 1 month old. Another route is that his elder brother at the age of three, a nursery school toddler, had sometimes been having slight fits of coughing without making any medical attention.

He had already received the pertussis vaccination three times up to 12 months of age, however, the assessment of the level of immunity was unadministered. We should reaffirm both the efficacy of immunization and the risk of still acquiring pertussis even in those who had received full immunization. Either of these two routes might be indeed the possible route of the transmitting pertussis, however, both were devoid of any conclusive evidence for confirming sources of the infection.

#### 4. CONCLUSION

This was an infant pertussis case occurred in an unvaccinated infant, diagnosed with the insight of clinical microbiology laboratory.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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