

PCR in CSF and one showed positive antigens in CSF (Table 1). For 5 patients, blood culture was positive for *N. meningitidis*; all showed positive CSF culture. The serogroup distribution was serogroup B for 18 cases (78%), C for 3 cases (13%) and others for 2 cases (9%). Subsets of 11 isolates (48%) were genotyped by using MLST. Five ccs were found, all from serogroup B, with the following distribution: one isolate was not assigned to any known cc (UA) (9%), 2 cc 162 (18%), 2 cc 213 (18%), 3 cc 41/44 (27%) and 3 cc 32 (27%). Phenotypes (serotypes:serosubtypes) were obtained for 16 cases (65%) from the 23 cases; one serogroup C phenotype (2a: P1.5,2) and 15 serogroup B phenotypes: 3 NT:P1.4, 2 1:P1.14 and one each of the following: 14:non-subtypable (NST); 14:P1.7,16; 1:NST; 1:P1.14,16; 1:P1.4; NT:NST; NT:P1.12; NT:P1.16; NT:P1.7,9; NT:P1.9. The minimum inhibitory concentration (MIC) was tested for cefotaxime, amoxicillin and penicillin G for 17 strains. All tested isolates were susceptible to cefotaxime. MICs ranged from 0.002 to 0.032 mg/L. The MIC was ≤ 0.01 mg/L for 94% of isolated strains and ≤ 0.032 mg/L for 100%. For amoxicillin, MICs ranged from 0.047 to 0.38 mg/L. The MIC was < 0.25 mg/L for 88% of isolates. The other isolates (12%) showed intermediate susceptibility to amoxicillin. For penicillin G, MICs ranged from 0.032 to 0.25 mg/L. The MIC was < 0.12 mg/L for 88% of isolates. The other isolates (12%) showed intermediate susceptibility to penicillin G.

Two patients died (9%, girls, cared for at home, who showed late-onset meningitis at days 10 and 23, respectively).

DISCUSSION

To our knowledge, this is the largest published series of NMM. *N. meningitidis* was the third most frequent cause of neonatal bacterial meningitis in our series in France (2.8%), then *L. monocytogenes* (2.4%). All cases occurred in full term newborns, and nearly all were late-onset meningitis (96%), with only one occurring at day 4. These results are similar to those found by Shepard et al.⁴ This specific distribution of *N. meningitidis* as a late-onset infection might be explained by the potential mode of transmission of this bacterium. Most neonatal meningitis agents are fetomaternal or digestive transmitted and are responsible for early- as well as late-onset infections. The predominant late-onset occurrence of NMM might be related to postnatal respiratory transmission (*N. meningitidis* is part of the normal flora of the upper respiratory tract), by direct contact or by droplets.¹⁰

CSF culture was positive for nearly all of our patients (91%); 2 had positive findings on PCR and capsular antigen detection in CSF. As in other studies, the combination of different techniques (CSF culture, CSF Gram staining, and also PCR and capsular antigen detection in CSF) improved diagnostic accuracy.⁵

As in other studies in neonates⁴ or older children in France,^{2,8} serogroup B accounted for most of our NMM cases (78% overall), with rates of serogroup C (13%) and others (9%) also similar. Although limited typing data are available, they suggest phenotypic and genotypic heterogeneity and 45% of isolates did not belong to hyperinvasive ccs.⁸

The epidemiology of bacterial meningitis has changed in the last decades, after the introduction of routine immunization with conjugate vaccines for *Haemophilus influenzae* serotype b, pneumococcus and meningococcus C. Furthermore, implementation of food hygiene measures to prevent *L. monocytogenes* infection divided by 10 the incidence of *L. monocytogenes* neonatal infections between 1984 and 2006 in France.¹¹

The main limitation of our study is some missing data. However, our study was based on a large prospective cohort and included a large number of well-documented cases.

In conclusion, in this large prospective French cohort of neonatal bacterial meningitis, *N. meningitidis* was the third most frequent bacterial species found. This disease occurred only in term neonates, and infections were mainly late onset. Serogroup B was implicated in 78%

of cases, and all strains were susceptible to cefotaxime but not 12% showed reduced susceptibility to aminopenicillin or to penicillin G.

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REFERENCES

- Baud O, Aujard Y. Neonatal bacterial meningitis. *Handb Clin Neurol*. 2013;112:1109–1113.
- Barret A, Deghmane A, Lepoutre A, et al. Invasive meningococcal disease in France in 2012: main epidemiological features. Available at: http://www.winvssantefr/beh/2014/1-2/2014_1-2_4html2014. Accessed February 12, 2016.
- Koplick H. Meningitis in the newborn and in infants under three months of age. *Arch Pediatr*. 1916;481–500.
- Shepard CW, Rosenstein NE, Fischer M; Active Bacterial Core Surveillance Team. Neonatal meningococcal disease in the United States, 1990 to 1999. *Pediatr Infect Dis J*. 2003;22:418–422.
- Wang Y, Guo G, Wang H, et al. Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis. *BMC Pediatr*. 2014;14:224.
- Kiray Baş E, Bülbül A, Cömert S, et al. Neonatal infection with *Neisseria meningitidis*: analysis of a 97-year period plus case study. *J Clin Microbiol*. 2014;52:3478–3482.
- Gaschignard J, Levy C, Romain O, et al. Neonatal bacterial meningitis: 444 cases in 7 years. *Pediatr Infect Dis J*. 2011;30:212–217.
- Levy C, Taha MK, Weil Olivier C, et al; Groupe des pédiatres et microbiologistes de l'Observatoire National des Méningites. Association of meningococcal phenotypes and genotypes with clinical characteristics and mortality of meningitis in children. *Pediatr Infect Dis J*. 2010;29:618–623.
- Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A*. 1998;95:3140–3145.
- Manchanda V, Gupta S, Bhalla P. Meningococcal disease: history, epidemiology, pathogenesis, clinical manifestations, diagnosis, antimicrobial susceptibility and prevention. *Indian J Med Microbiol*. 2006;24:7–19.
- Anonymous. Pregnancy associated and neonatal listeriosis in France: trend from 1984 to 2006. Available at: http://www.winvssantefr/beh/2008/14_15/beh_14_15_2008pdf. Accessed February 12, 2016.

STREPTOCOCCUS GALLOLYTICUS SUBSP. PASTEURIANUS INFECTION IN A NEONATAL INTENSIVE CARE UNIT

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Abstract: We report nosocomial transmission of *Streptococcus gallolyticus* subsp. *pasteurianus* among 3 neonates, 1 of whom died. Genome analysis of the strains showed a specific pattern of metabolic and regulatory functions as well as of expressed antigens and antibiotic resistance genes that might have contributed to their specific virulence.

Key Words: *Streptococcus gallolyticus* subsp. *pasteurianus*, neonatal, transmission, genome sequencing, virulence factors

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Streptococcus gallolyticus subsp. *pasteurianus* is a Lancefield Group D streptococcus, formerly known as *Streptococcus bovis* biotype II.¹ By means of 16S rRNA gene sequencing, the *S. bovis* conglomerate could be differentiated into the genomospecies *S. gallolyticus* subsp. *gallolyticus* (former biotype I), *Streptococcus infantarius* subsp. *coli* (former biotype II/1) and *S. gallolyticus* subsp. *pasteurianus* (former biotype II/2).² *S. gallolyticus* subsp. *pasteurianus* specifically is associated with meningitis,³ whereas *S. gallolyticus* subsp. *gallolyticus* is linked to endocarditis and colon cancer.⁴ We present a cluster of 3 neonatal cases with *S. gallolyticus* subsp. *pasteurianus* and determined the strains' virulence characteristics with genome sequencing.

Patient 1

A preterm male neonate was born at 30 weeks postmenstrual age, after premature rupture of membranes and preterm labor. On day 7, the infant became hemodynamically unstable. Vancomycin and amikacin were initiated for suspected late-onset sepsis. Cerebrospinal fluid showed increased white blood cell count and protein concentration but culture remained sterile. Blood culture grew *S. gallolyticus* subsp. *pasteurianus* susceptible to penicillin. The patient recovered and was discharged at a postnatal age of 57 days.

Patient 2

A second preterm male neonate was born at postmenstrual age of 32 weeks because of maternal HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome and oligohydramnios.

On day 34, he suddenly deteriorated, developed septic shock, diffuse intravascular coagulation, progressive metabolic acidosis, respiratory failure and pulmonary hemorrhage requiring mechanical ventilation. He died within 2 hours. From 2 blood cultures *S. gallolyticus* subsp. *pasteurianus* was grown, susceptible to penicillin, amoxicillin, gentamicin and vancomycin and resistant to erythromycin.

Because patients 1 and 2 were in close proximity on the Neonatal Intensive Care Unit, nosocomial transmission was suspected. Therefore, contact isolation was initiated for patient 1, the patients' rooms were additionally cleaned and disinfected, extra focus on hand hygiene compliance and rectal screening on the ward were initiated. As a result, patient 3 was detected.

Patient 3

A male with intrauterine growth restriction in monozygotic twins was born at 30-week postmenstrual age. During his stay in the Neonatal Intensive Care Unit, he developed 2 episodes of necrotizing enterocolitis. On day 64, rectal screening culture was positive for *S. gallolyticus* subsp. *pasteurianus*. At that time, the boy was asymptomatic and was nursed in isolation up to discharge.

METHODS

The *S. gallolyticus* subsp. *pasteurianus* strains were identified by means of matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany).

TABLE 1. Major Functions Encoded by Genes* Specific to *S. pasteurianus* Strains ATCC 43144 and the Strain from the Second Patient (HC-2909-2)

Functional Categories	Strain ATCC 43144	HC-2909-2
Carbon metabolism	Allulose PTS permease and metabolism Keto desoxygluconate permease Dihydroxyacetone operon Alcohol dehydrogenase	Putative sugar ABC transporter system Oligo-1,6-glucosidase Alpha galactosidase and sucrose-specific PTS system PTS system, ascorbate family L-Fucose and L-fucosyl oligosaccharides utilization operon
DNA metabolism and regulators	CRISPR-cas (type 2-B) DNA cytosine methyltransferase DNA-binding protein	CRISPR-cas (type 2-A) TCS (2x) CovR CovS, virulence TCS Transcription regulator coupled with an ABC transporter Type 2 restriction modification system DNA-cytosine methyltransferase
Surface polysaccharides	Capsular polysaccharide† Glycosyl transferase	Capsular polysaccharide† D-Alanyl-D-alanine carboxypeptidase
Antibiotics	L-Antibiotic, Nisin U biosynthesis L-Antibiotic ABC transporter	rRNA (Adenine-N(6))-methyltransferase (EryR) Aminoglycoside phosphotransferase Aminoglycoside 6-adenylyltransferase Adenine phosphoribosyltransferase Spectinomycin 9-O-adenylyltransferase tet(M), tetracycline resistance gene‡ L-Antibiotic ABC transporter Bacteriocin locus and competence, including comDE TCS
Surface proteins	CnaB motif LPXTG protein Sortase C, cell wall ribonuclease	Aggregation substance LPXTG protein S-layer homology domain protein LPXTG surface proteins of unknown function (2x) Sortase A (paralogous gene) Collagen adhesion protein
Ion transport	trk K+ uptake	

*Phages, remnant phages and mobile genomic elements are not reported.

†Corresponds to 2 different capsular operons.

‡Present but inactivated by a frameshift mutation in the ATCC 43144 strain.

PTS indicates phosphotransferase sugar transport system; TCS, 2-component regulatory system.

Bold indicates the main genes associated with virulence potency and antimicrobial resistance that are discussed in the text.

They were typed with pulsed-field gel electrophoresis (PFGE) adapted after Descheemaeker et al⁵ at the Belgian National Reference Laboratory for *Enterococcus* spp., UZA, Antwerp.

To confirm the identification, to definitely demonstrate the clonality of the 3 isolates and to search for possible virulence factors explaining the invasiveness of the strains in patients 1 and 2, the genome sequences of the 3 strains were determined by using the Illumina technology. Libraries were constructed by using the Illumina Nextera XT kit. Sequencing was performed on a Illumina MiSeq, 300-base single read run. Sequences were assembled with Velvet.⁶ The complete 16S rRNA gene sequences were extracted from the genome sequence assembly of the 3 clinical isolates. The genomic data of the second patient's strain (HC-2909-2) were deposited at EMBL (genome project ERS578714). Illumina reads alignments were conducted with Burrows-Wheeler aligner (BWA).⁷ Single-nucleotide polymorphism (SNP) calling was done using genome analysis toolkit.⁸ To identify specific features of these strains, we compared their genomes with that of strain American Type Culture Collection (ATCC) 43144 (genbank accession number AP012054).⁹ Regions specific to the 3 strains were extracted by assembly of the BWA unmapped reads. Contigs larger than 300bp were considered and automatically annotated by using the rapid annotation using subsystem technology (RAST) server.¹⁰ The 266 protein-encoding genes annotated in 46 contigs were searched for genes of interest: antibiotic resistance and putative virulence-associated functions. SNPs with strain ATCC 43144 were visualized by using SyntView.¹¹ Differences between the 3 isolates were determined by aligning reads on the contigs of strain HC-2909-2 using BRESEQ.¹²

RESULTS

All 3 strains belonged to the same PFGE type A, indicating clonality knowing that PFGE is the most discriminative technique for this species. Moreover, clustering of cases with this uncommon invasive pathogen on the same hospital ward in this short 3-week period is a strong indication of nosocomial transmission.

Genome sequencing showed that the 3 strains were virtually identical, confirming PFGE data: the patients' strains differed by maximum 4 SNPs from each other. The sequence of the 16S rRNA genes was 100% identical over the whole length (1462bp) to that of the published ATCC strain *S. gallolyticus* subsp. *pasteurianus* 43144.⁹ Whole-genome comparisons with strain ATCC 43144 confirmed the species identification and showed that the patients' strains are rather distantly related to the ATCC strain with 0.4% of polymorphism. Interestingly, analysis of the SNP distribution by SyntView (http://genopole.pasteur.fr/SynTView/flash/Streptococcus_pasteurianus/SynWeb.html) along the genome alignment shows an uneven distribution of SNP density with alternate pattern of regions of high and low SNP density, suggesting a high recombination rate involving large genomic regions as previously described in *Streptococcus agalactiae*.¹³

Both the ATCC 43144 and the patients' strains possessed specific genes. However, the 3 patient strains analyzed here showed unique features possibly associated with their fitness and virulence (Table 1).

DISCUSSION

We analyzed the specific genes of the 3 patients' *S. pasteurianus* strains in search for their virulence potency. These strains had a complete operon for the utilization of L-fucose and L-fucosyl oligosaccharides. Fucose, a mucin component is both a carbon source and a signal molecule. Blast search revealed that this locus is missing in sequenced *S. pasteurianus* and *S. gallolyticus* strains but that it is present in *Streptococcus suis* pointing at a recent acquisition of the locus by lateral gene transfer. This locus encodes a protein highly similar to the α -L-fucosidase from *Bifidobacterium*. Likewise, Stahl

et al¹⁴ showed enhanced survival in the piglet gut of *Campylobacter jejuni* strains that use L-fucose as a substrate of growth. The CovRS 2-component system regulating the transition from commensalism to invasiveness in group A and group B streptococci is missing in the ATCC 43144 strain but is present in these *S. gallolyticus* isolates. Furthermore, the ATCC 43144 strain expresses a different capsule operon and a different locus for the synthesis of bacteriocin and competence compared with the sequenced strains. How the specific gene content of these 3 strains might contribute to their virulence remains to be analyzed. Probably more significant is the identification of antibiotic resistance genes in all 3 strains: *tet(M)*: tetracycline resistance determinant, *ermB*: an adenine N-6-methyltransferase conferring resistance to erythromycin, *aad(E)*: a streptomycin aminoglycoside 6-adenyltransferase, and *aphA3*: a putative spectinomycin adenyltransferase. All these genotypic resistance determinants were associated with minimum inhibitory concentrations of at least 64 μ g/mL, suggesting a phenotypic resistance to these antibiotics for all 3 strains.

In conclusion, nosocomial transmission of *S. gallolyticus* subsp. *pasteurianus* in a Neonatal Intensive Care Unit resulted in screening and isolation measures. No further infections/colonizations with this species arose. This shows the importance of awareness of possible transmission of microorganisms other than the familiar multidrug-resistant organisms.

Genome analysis of the 3 strains showed a specific pattern of metabolic and regulatory functions as well as of expressed antigens that might have contributed to the specific virulence of these strains. In addition, the number of antibiotic resistance genes present in its genome possibly witnesses a long history of interaction with humans in a disease or hospital context, which also might be associated with an increased capacity for dissemination.

REFERENCES

- Klatte JM, Clarridge JE 3rd, Bratcher D, et al. A longitudinal case series description of meningitis due to *Streptococcus gallolyticus* subsp. *pasteurianus* in infants. *J Clin Microbiol*. 2012;50:57–60.
- Beck M, Frodl R, Funke G. Comprehensive study of strains previously designated *Streptococcus bovis* consecutively isolated from human blood cultures and emended description of *Streptococcus gallolyticus* and *Streptococcus infantarius* subsp. *coli*. *J Clin Microbiol*. 2008;46:2966–2972.
- Floret N, Bailly P, Thouverez M, et al. A cluster of bloodstream infections caused by *Streptococcus gallolyticus* subspecies *pasteurianus* that involved 5 preterm neonates in a university hospital during a 2-month period. *Infect Control Hosp Epidemiol*. 2010;31:194–196.
- Romero B, Morosini MI, Loza E, et al. Reidentification of *Streptococcus bovis* isolates causing bacteremia according to the new taxonomy criteria: still an issue? *J Clin Microbiol*. 2011;49:3228–3233.
- Descheemaeker P, Lammens C, Pot B, et al. Evaluation of arbitrarily primed PCR analysis and pulsed-field gel electrophoresis of large genomic DNA fragments for identification of enterococci important in human medicine. *Int J Syst Bacteriol*. 1997;47:555–561.
- Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res*. 2008;18:821–829.
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25:1754–1760.
- McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20:1297–1303.
- Lin IH, Liu TT, Teng YT, et al. Sequencing and comparative genome analysis of two pathogenic *Streptococcus gallolyticus* subspecies: genome plasticity, adaptation and virulence. *PLoS One*. 2011;6:e20519.
- Aziz RK, Bartels D, Best AA, et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics*. 2008;9:75.
- Lechat P, Souche E, Moszer I. SyntView—an interactive multi-view genome browser for next-generation comparative microorganism genomics. *BMC Bioinformatics*. 2013;14:277.
- Deatherage DE, Barrick JE. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. *Methods Mol Biol*. 2014;1151:165–188.

13. Brochet M, Rusniok C, Couvé E, et al. Shaping a bacterial genome by large chromosomal replacements, the evolutionary history of *Streptococcus agalactiae*. *Proc Natl Acad Sci U S A*. 2008;105:15961–15966.
14. Stahl M, Friis LM, Nothhaft H, et al. L-fucose utilization provides *Campylobacter jejuni* with a competitive advantage. *Proc Natl Acad Sci U S A*. 2011;108:7194–7199.

TWO DISTINCT EPISODES OF WHOOPING COUGH CAUSED BY CONSECUTIVE *BORDETELLA PERTUSSIS* AND *BORDETELLA PARAPERTUSSIS* INFECTIONS IN A FULLY IMMUNIZED HEALTHY BOY

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Abstract: We describe a 5-year-old, fully immunized boy with polymerase chain reaction-proven consecutive *Bordetella pertussis* and *Bordetella parapertussis* infections causing typical whooping cough at the age of 2 and 5 years, respectively. Neither pertussis immunization nor disease provides reliable immunity against further episodes of whooping cough.

Key Words: whooping cough, pertussis, *Bordetella pertussis*, *Bordetella parapertussis*

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The authors have no conflicts of interest to disclose.

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Acellular pertussis vaccines (APV) are known to be of suboptimal efficacy against typical pertussis or whooping cough (defined by the World Health Organization as ≥ 21 days of cough with ≥ 1 typical sign such as paroxysms, whoop, or post-tussive vomiting) caused by *Bordetella pertussis* with point estimates in the range of 70% to 92% after a complete primary immunization series of 3 or 4 doses in the first 2 years of life.¹ Like immunization, natural infection with *B. pertussis* also does not lead to persistent and reliable immunity against reinfection with the same organism and the time intervals between repeated episodes published in the literature range from 4 to 20 years.² Similarly, whooping cough caused by *Bordetella parapertussis* followed by whooping cough caused by *B. pertussis* infection was reported in 4 unimmunized Swedish children in 1994.³ Approximately 40 years earlier, as part of a large Danish whooping cough surveillance study from 1950 to 1957, Lautrop⁴ identified 78 children and 1 adult with either simultaneous *B. pertussis* and *B. parapertussis* infections in “about one-third” of cases, *B. pertussis* infection that was followed by *B. parapertussis* infections in 4 children and in the remaining great majority of cases, *B. parapertussis* infection was followed by *B. pertussis* infection. In this Danish case series, apparently only a few of these children had a history of 3 immunizations at weekly intervals with an unspecified whole-cell pertussis component vaccine (WPV) at that time, however, no vaccine effectiveness analyses were performed. With regards to prevention of whooping cough caused by *B. parapertussis* infection, one WPV (manufactured by Lederle [Pearl River, NY], at that time) was less efficacious than the APV comparator (Lederle/Takeda 4 component) in a study performed by members of our group⁵: the point estimates for efficacy against typical (ie, WHO defined) whooping cough were 58% (95% confidence interval: 14–80) and 25% (–45 to 61), respectively. In an efficacy trial of different manufacturers' WPV and APV vac-

cines, no evidence of efficacy against whooping cough caused by *B. parapertussis* infection was found.⁶

It should be noted that these were post hoc analyses as none of the trials was designed to assess vaccine efficacy against *B. parapertussis* infections.

We report on a boy with polymerase chain reaction-proven consecutive *B. pertussis* and *B. parapertussis* infections leading to typical whooping cough disease at the age of 2 years and 1 month and 5 years and 2 months, that is, 4 and 41 months, respectively, after the 4th dose of a complete primary pertussis immunization series. To our knowledge, this is the first such report in an age-appropriately immunized child in the era of APVs and it adds to the enigma of *B. pertussis* and *B. parapertussis* infections.

CASE

This 5-year-old boy presented to one of us (D.S.) with a history of 14 days of cough. Since he had a very similar cough disease 37 months before, which had been diagnosed by PCR as pertussis caused by *B. pertussis* infection, the child's parents and the pediatrician suspected a reinfection. Therefore, a nasopharyngeal swab was obtained for PCR that was negative for *B. pertussis* but positive for *B. parapertussis*. He received clarithromycin orally and continued to cough for 6 weeks. No other family members developed a cough and the source of infection remained unclear. At the age of 2, 3, 5 and 21 months, the boy had received a series of 4 doses of acellular pertussis combination vaccine according to the recommended 3 + 1 schedule in Germany. The vaccine used was Infanrix hexa, manufactured and distributed in many European countries by GlaxoSmith-Kline (Rixensart, Belgium), which contains defined amounts of diphtheria and tetanus toxoid, 3 *B. pertussis* antigens (pertussis toxoid, filamentous hemagglutinin and pertactin), inactivated poliomyelitis virus types 1–3, *Haemophilus influenzae* type B polyribosyl ribitol phosphate and hepatitis B surface antigen.

DISCUSSION

In comparative cohort studies, signs and symptoms of cough illness caused by *B. parapertussis* infection are on average milder and of shorter duration when compared with those caused by *B. pertussis* infection.^{7,8} However, as demonstrated in our case, when confronted with an individual patient, illness caused by *B. parapertussis* infection is clinically indistinguishable from illness caused by *B. pertussis*. Importantly, *B. pertussis* infection or vaccination with a whole-cell pertussis vaccine does not protect against whooping cough caused by *B. parapertussis* and infection with *B. parapertussis* does not appear to induce protection against disease caused by *B. pertussis*.^{3,5,6,8} Moreover, there is lack of evidence for APV in use today to protect against *B. parapertussis* disease. Therefore, there is a need for application of specific diagnostic tests to discriminate between *B. pertussis* and *B. parapertussis* infections as part of pertussis surveillance accompanying immunization programs.

Physicians confronted with children with cough illness must consider the possibility of *B. parapertussis* infection irrespective of the patient's immunization history and/or previously diagnosed whooping cough by *B. pertussis* infection. In the absence of a specific serologic test to confirm *B. parapertussis* infection, a reliable diagnosis relies on demonstration of the organism by culture or PCR.⁹

REFERENCES

1. Cherry JD, Heininger U. Pertussis and other *Bordetella* infections. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ, eds. *Textbook of Pediatric Infectious Diseases*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2014:1616–1639.