CLINICAL CASE REPORT: PERTUSSIS INFECTION FOLLOWED BY A PARAPERTUSSIS INFECTION IN THE SAME CHILD

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ABSTRACT
Whooping cough is a vaccine-preventable, acute respiratory disease caused by the gram-negative bacterium Bordetella pertussis. In recent years there has been a worldwide recurrence of pertussis infection. The most vulnerable group in society are infants under one year of age, they are at the greatest risk of severe complications or even death. Whooping cough is usually associated with infection caused by B. pertussis, but Bordetella parapertussis can also cause pertussis-like symptoms. The disease is known as parapertussis. Clinical data alone are not sufficient to differentiate between the two infections. Modern, fast and reliable diagnostic is needed. Bordetella holmesii, viral infections caused by RSV, adenovirus, etc. can present with pertussis-like symptoms and should be diagnosed and treated accordingly.

The aim of this study is to present a clinical case of a child with pertussis infection followed by parapertussis infection. To diagnose and differentiate the two infections, a real-time PCR molecular genetic method was used to detect the genes specific for the causative agents. Pertussis vaccination does not protect against B. parapertussis infections, and cross-immunity between the two bacteria has not been observed. Therefore, in the presence of pertussis-like symptoms (paroxysms of persistent cough, vomiting after coughing), it is advisable to differentiate between B. pertussis and B. parapertussis infection, especially in populations with high pertussis vaccination coverage.

Key words: PCR, Bordetella pertussis, Bordetella parapertussis, vaccination

INTRODUCTION
Whooping cough is primarily a vaccine-preventable, acute respiratory disease caused by the gram-negative bacterium Bordetella pertussis. Infants under one year of age are the most vulnerable age group in society, with the highest risk of severe illness, hospitalization or even death (1). Since the introduction of pertussis vaccinations (with whole-cell and acellular pertussis vaccines), pathogen prevalence, morbidity and mortality have been significantly reduced worldwide (2). During the last two decades a resurgence of the disease and an increasing incidence of cases have been observed, even in countries with high vaccination coverage (3, 4).

Pertussis infection continues to be widespread, with a proven cyclicity of outbreaks (5). The main reasons for these trends are several: the established genetic differences between vaccine and community-circulating strains, proven antigenic changes leading to stronger immune suppression, improved disease surveillance, improved laboratory diagnostic methods (4).

According to ECDC data for 2018 the reported cases of pertussis in 30 EU/EEA countries were 35636 with 10 deaths. For 2019 there were 38992 cases of the disease and 11 deaths, and for 2020 - 12113 cases and four deaths, respectively (6). The significant reduction in reported cases of whooping cough for

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2020 (as for other respiratory diseases) is probably due to the measures limiting the Covid 19 pandemic (lockdown, social distancing measures, imposed isolation, work from home, closure of childcare facilities - nurseries, kindergartens and schools). Whooping cough affects all age groups in society, but infants under one year of age are the most vulnerable, with the highest risk of severe illness, hospitalization or even death. ECDC reported an incidence rate of 44.4 per 100,000 and three deaths in this age group for 2018 (1). According to CDC data, the pertussis hospitalization rate for patients under 6 months of age is the highest - 30.8% (7).

Whooping cough is usually associated with an infection caused by *B. pertussis*, but *B. parapertussis* can also cause pertussis-like symptoms. Parapertussis is a highly contagious respiratory disease affecting all age groups of the population. The causative agent is the Gram-negative bacterium *B. parapertussis*, which is related to *B. pertussis*, but does not produce pertussis toxin, due to mutations in the promoter region of the genes encoding this toxin (10). Clinical findings in patients infected with parapertussis vary. Some patients are asymptomatic, others show typical pertussis symptoms in terms of severity and duration of the cough, nocturnal attacks and post-cough vomiting, but most patients with *B. parapertussis* have milder symptoms with shorter duration (8, 9). Infants (under 6 months of age) may experience a more severe course of parapertussis infection. A pertussis and parapertussis co-infection is possible in all age groups. After an infection with parapertussis, immunity is established, but there is no cross-immunity between pertussis and parapertussis. Therefore, pertussis vaccination does not protect against parapertussis infection (11, 12).

**CLINICAL CASE**

The clinical case describes a 5-year-old male patient who was diagnosed with pertussis and ten months later - with parapertussis. A parallel molecular genetic assay for the species *B. pertussis* and *B. parapertussis* was conducted.

The clinical signs during the first infection were a prolonged dry cough for 1 month with severe coughing bouts. The child was afebrile. Treatment with azithromycin was carried out for 6 days before taking the material for genetic testing. The patient had received all doses of pertussis vaccines according to the Immunization Calendar of the Republic of Bulgaria (13). Clinical symptoms when proving *B. parapertussis* during the second examination were a dry cough for two weeks without severe coughing bouts or fever. Antibiotics had not been applied before taking a sample for molecular genetic testing.

**MATERIALS AND METHODS**

Two clinical samples (nasopharyngeal secretions) from the patient were obtained in the NRL "Molecular Microbiology" in NCIPD, from which DNA was extracted for the diagnostic of pertussis and parapertussis.

To detect *B. pertussis* and *B. parapertussis*, a Real-Time PCR test was used that detects genes specific for the two causative agents. To prove *B. pertussis*, an analysis was performed to detect the pertussis toxin gene promoter, and to prove *B. parapertussis*, gene IS1001 was analyzed. The assays were performed using an RT-PCR analyzer Gentier96, TIANLONG, China. The simplex PCR assay was performed in 20µl of reaction mixture containing 10µl 1x real-time PCR buffer Takara Premix exTaq, 1,2µl of each primer (F/R), 1,2µl of 5 µM probe, 1,4µl dH2O and 5µl of extracted sample DNA. The cycling condition included: 95°C for 30s, 45 cycles: 95°C for 5s, 62°C for 30s. Data acquisition of a signal was set at 62°C during each cycle. Primers and probes used in the RT PCR were:

- ptxP-F 5-GCGTGCAGATTCTGCGTAC-3,
- ptxP-R 5-TGATGGTGCCTATTTTACGG-3,
- ptxP-Probe 5-FAM-ACACGGCATGAACCTCTTCGGC-3 BHQ1;
- IS1001-F 5-AGGGGCTTGGCTGGCTGGCGATA-3,
- IS1001-R 5-CGCGGCGCTGCTCCTGAGCGGTGCT-3,
- IS1001-Probe 5-FAM-AGGCTCGGCTGCGTGCAGGTGCGCG-3 BHQ1 (14).

**RESULTS AND DISCUSSION**

A parallel analysis for the presence of *B. pertussis* and *B. parapertussis* DNA was performed with both nasopharyngeal secretions from the same child. Analysis of the first material detected the
pertussis toxin gene promoter (a marker for pertussis infection) and negative amplification for IS1001 (for detection of B. parapertussis) After the first antibiotic treatment, the follow-up result for B.pertussis was negative. The second test ten months later was negative for B. pertussis amplification and positive for B. parapertussis. The second test, after applied antibiotic treatment, was negative for B.parapertussis.

Infections caused by B. parapertussis are common but usually overlooked and underdiagnosed. Data indicate that 1% to 35% of Bordetella infections are caused by B. parapertussis (8).

CONCLUSION
It has been shown that clinical symptoms alone are insufficient to distinguish pertussis from parapertussis infections (15). Some viral infections caused by RSV, adenovirus, etc. may present with similar pertussis-like symptoms. In patients with pertussis-like symptoms, especially in populations with high immunization pertussis coverage, highly specific molecular diagnostic tests are needed. RT-PCR permits to establish a rapid and accurate etiological diagnosis, and in the case of a proven viral infection - to prevent the unnecessary use of antibiotics.

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