The number of travellers continues to increase in Spain and Europe, which can increase the incidence of these mixed infections. The recent travel history should be recognized as an epidemiological data to highlight not only the clinical diagnosis but also the microbiological one.

## Ethical approval

Not necessary.

## Informed consent

Not necessary.

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# Differential diagnosis by RT-PCR of *Bordetella bronchiseptica* in a child without previous pathologic antecedents suffering whooping cough☆

## *Diagnóstico diferencial de* Bordetella bronchiseptica *por RT-PCR en un niño con tos paroxística sin antecedentes patológicos previos*

The genus *Bordetella* includes various species that can affect human beings.[1] Among them, *B. pertussis* stands out for its clinical-epidemiological relevance.[2] However, other species such as *B. parapertussis*, *B. holmesii*[1] and, occasionally, *B. bronchiseptica*[3,4] can cause similar whooping cough symptoms.

The objective of this study was to describe a case of *B. bronchiseptica* infection and the strategy used for its microbiological diagnosis. A 21-month-old male, with no clinical history of chronic respiratory diseases or immunological abnormalities, who attended the emergency department with a paroxysmal cough accompanied by vomiting, which had started nine days previously, with no apnoea, but accompanied by stridor. The patient was correctly immunised for his age against whooping cough, with three doses of diphtheria vaccine, tetanus vaccine and acellular pertussis vaccine administered at 2, 4 and 11 months. The family lived with a dog that had not shown signs of disease.

In light of these symptoms compatible with whooping cough, nasopharyngeal lavage samples were obtained, and an initial dose of azithromycin at 10 mg/kg/day followed by home treatment with 5 mg/kg/day for four days was prescribed. The patient progressed favourably, with no complications. The sample of nasopharyngeal lavage was studied using a commercial multiplex real-time polymerase chain reaction (RT-PCR) technique (Smart Bp/Bpp, Cepheid AB, Sweden), based on the detection of the insertion sequences IS*481* and IS*1001*. According to the manufacturer's instructions, the results were classified presumptively as positive for both *B. pertussis* and *B. parapertussis*. The sample was subsequently processed using five independent RT-PCR assays against different markers: IS*481*, IS*1001*, promoter region of the pertussis toxin gene (BPTP), *B. pertussis* porin protein gene (BPTD_0837) and the insertion sequence similar to IS*1001* of *B. holmesii* (hIS*1001*). The results obtained (IS*481* confirmed as positive, IS*1001* confirmed as positive, BPTP positive, BPTD_0837 negative and hIS*1001* negative) contributed to an identification as *B. bronchiseptica* in accordance with the algorithm described in Table 1.

While *B. bronchiseptica* grows in common media such as MacConkey agar, the culture of *B. pertussis* is problematic and lacks sensitivity. For this reason, the diagnosis of whooping cough is currently based on PCR techniques on nasopharyngeal samples, using several targets such as the insertion segments IS*481* and IS*1001*, used to identify *B. pertussis* and *B. parapertussis*, respectively, as probable.[5,6] Nevertheless, these sequences are not absolutely specific to these species.[6] The sequence IS*481* can be found along with *B. pertussis* in *B. holmesii* and in some strains of *B. bronchiseptica*.[6]

**Table 1**
Algorithm of multimarker RT-PCR assays for the identification of the main species of *Bordetella* spp.

| Interpretation | Result of the assays | | | | |
|---|---|---|---|---|---|
| | IS*482* | BPTP | BPTD_0837 | IS*1001* | hIS*1001* |
| *B. pertussis* | + | + | + | – | – |
| *B. parapertussis* | – | – | – | + | – |
| *B. holmesii* | + | – | – | – | + |
| *B. bronchiseptica* | ± | + | – | ± | – |

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Likewise, the sequence IS*1001* can be present, in addition to *B. parapertussis*, in *B. bronchiseptica*.[6] This sequence also shows great similarity with hIS*1001*, present in *B. holmesii*.[5] A simultaneously positive PCR result for IS*481* and S*1001* could initially be considered a co-infection by different species of *Bordetella*.[6] In these situations, the use of other amplification targets may clarify the diagnosis. Both *B. pertussis* and *B. bronchiseptica* are positive for BPTP.[7] However, the gene BPTD_0837 is specific to *B. pertussis* and does not show cross-reactivity with other *Bordetella* species.[8] Other authors have used two structural genes of flagellin as markers, one common for *B. bronchiseptica*/*B. parapertussis* (Bb/Bpp-Fla) and another distinctive one for *B. parapertussis* (Bpp2-Fla).[9]

Among the limitations of this study, it should be noted that a bacterial culture was not carried out, a differential virological diagnosis was not carried out, nor was the sequencing of amplification products carried out to confirm the result definitively.

*B. bronchiseptica* is considered an opportunistic microorganism which can, in particular, infect the respiratory tract of patients with immunosuppression or cystic fibrosis.[3,4] *B. bronchiseptica* causes infections in different mammals[1] and its transmission has been related to living with pets.[4] The prevalence of colonisation in healthy dogs seems to be important.[10] Although in this case we assumed that *B. bronchiseptica* was the probable cause of the symptoms of whooping cough according to its molecular detection, it is not possible to ensure that it was not merely a commensal bacterium. Nevertheless, this microorganism should be taken into account in the differential diagnosis of *B. pertussis* and *B parapertussis* when commercial RT-PCR techniques are used.

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## Non-toxigenic *Corynebacterium diphtheriae* biotype *belfanti* in a diabetic patient with upper tract respiratory infection☆

## Corynebacterium diphtheriae *biotipo* belfanti *no toxigénico en una paciente diabética con infección del tracto respiratorio superior*

This case discusses a 77-year-old woman who went to her primary care doctor with a sore throat, cough, drowsiness, greenish sputum and afebrile (36.7 °C). She presented as history of interest diabetes mellitus secondary to steroid treatment and bronchiectasis. Her recent vaccination record included the flu vaccine and tetanus–diphtheria vaccine, which was administered in 2003. She had no history of contact with animals or recent travel. A sample of sputum was collected for culture and abundant leukocytes and Gram-positive bacilli were observed in the Gram stain (Fig. 1). After 24 h of incubation, in Columbia CNA agar with 5% sheep blood and chocolate agar, very small pure colonies were isolated in culture (~1 mm diameter), catalase-

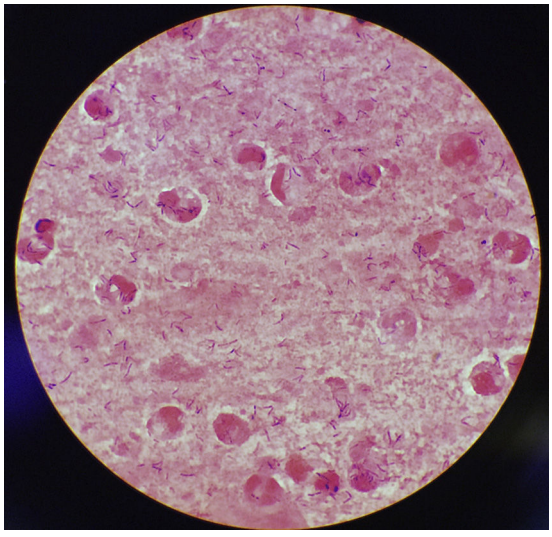

**Fig. 1.** Gram stain of sputum (1000×) in which abundant leukocytes and Gram-positive bacilli of *Corynebacteriaceae* morphology can be observed.

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