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## Short communication

## Etiology of bronchiolitis in a hospitalized pediatric population: Prospective multicenter study

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## ABSTRACT

**Background:** In 2006, bronchiolitis due to adenovirus nosocomial infections resulted in the closure of a pediatric department in northern Portugal.

**Objectives:** To determine the etiology of bronchiolitis in northern Portugal.

**Study design:** It was a prospective multicenter study on the etiology of bronchiolitis during the respiratory syncytial virus (RSV) season (November–April). Children  $\leq 24$  months of age admitted for a first wheezing episode were included. Nasopharyngeal specimens were analyzed by an indirect immunofluorescent-antibody assay (IFA) for RSV, adenovirus (HAdV), parainfluenza (PIV) 1–3 and influenza (IV) A and B and by polymerase chain reaction (PCR) or reverse transcription-PCR for the same viruses and for human metapneumovirus (hMPV), bocavirus (HBoV), rhinovirus (HRV), coronaviruses (229/E; NL63; OC43; HKU1) and enterovirus.

**Results:** During this period, 253 children were included, 249 IFA analyses and 207 PCRs were performed. IFA detected RSV in 58.1%; PCR increased it to 66.7%. IFA detected HAdV in 3.2%, PCR 10.0%. PCR detected IV A in 5; IV B in 2; PIV 1 in 6, PIV 2 in 4 and PIV 3 in 11 cases. HBoV, as single agent in 2 cases, and HRV were positive in 8 samples and hMPV in 11. With this virus panel, 19.7% remained without etiology.

**Conclusions:** The most frequent agent was RSV, followed by HAdV. PCR can be cost-effective and more accurate than IFA, which is crucial for HAdV that may be associated with significant mortality (IFA alone did not detect 2/3 of the cases).

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## 1. Background

Bronchiolitis is a viral lower respiratory tract infection (VRI) in a child <24 months of age, with signs/symptoms such as rhinitis, tachypnea, wheezing, cough, crackles, use of accessory muscles, and/or nasal flaring.<sup>1</sup>

An American Academy of Pediatrics' (AAP) guideline recommends that diagnosis should be based on history and physical examination, and discourages virologic testing because it rarely impact management. It states that it is useful when patient cohorting is possible<sup>2</sup> and for investigation.

## 2. Objective

To determine the relative viral frequency in children with bronchiolitis.

## 3. Study design

We conducted a prospective multicenter study in a temperate northwest region of Portugal, bounded to the north by Galiza and to the west by the Atlantic Ocean.

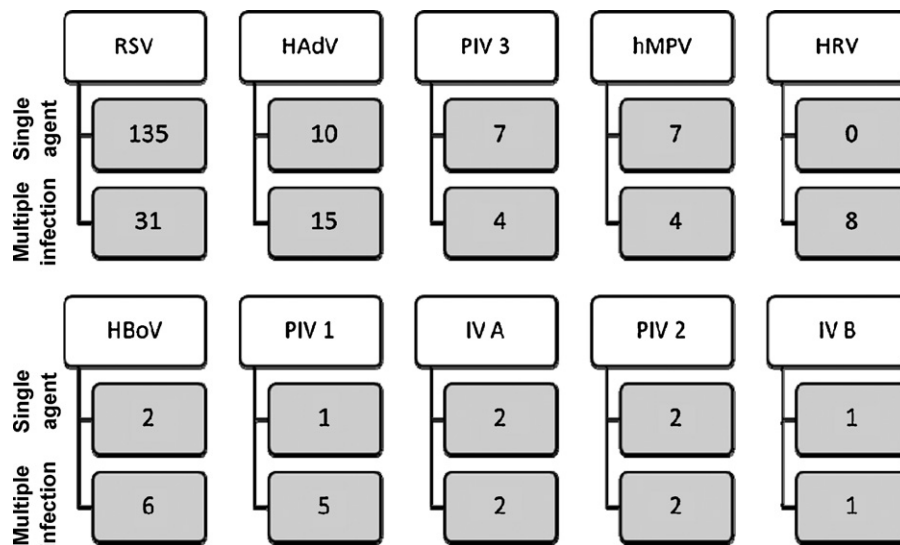
This study involved the 5 hospitals of the region. All children <24 months admitted with bronchiolitis were included. The same AAP definition was adopted by all hospitals. A history of any previous wheezing episode excluded the child.

The study started with the first respiratory syncytial virus (RSV) detection and was conducted from November 1, 2007 to April 30, 2008.

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**Fig. 1.** Number of cases detected for each virus. These are the final values and include the sum of the IFA and the PCR. RSV, respiratory syncytial virus; HAdV, adenovirus; PIV, parainfluenza 3; hMPV, human metapneumovirus; HRV, rhinovirus; HBoV, bocavirus; PIV 1, parainfluenza 1; IV A, influenza A; PIV 2, parainfluenza 2; IV B, influenza B.

The parents signed a written informed consent and filled a questionnaire, and a child's nasopharyngeal aspirate (NA) was collected. Each patient contributed only with one sample. When a single child had multiple samples, the earliest was selected.

Rapid viral testing was conducted in the origin hospital using an indirect immunofluorescent-antibody assay (IFA) for RSV, adenovirus, parainfluenza viruses (PIV) 1–3 and influenza virus (IV) A and B.<sup>3</sup> All hospitals followed a common reagent's protocol.

The remaining samples were frozen at  $-20^{\circ}\text{C}$  and transported to Braga Hospital (BH).

Nucleic acids extraction was performed on 0.25 mL of each sample using the Magna Pure Total Nucleic Acid Isolation Kit (Roche Applied Science®) with the external lysis protocol, following the manufacturer's instructions (FMI).

Eluates were subjected to the Seeplex RV/PB18 ASE (Seegene, USA) Multiplex real-time PCR (RT-PCR) detection of RSV A and B, adenovirus, PIV types 1–3, IV A and B, bocavirus, rhinovirus, coronavirus 229E/NL63 and OC43/HKU1 and enterovirus was performed without prior knowledge of the IFA result. Human metapneumovirus (hMPV) was analyzed by RT-PCR.<sup>4</sup>

If IFA and PCR gave discrepant results, the latter was confirmed by RT-PCR for RSV, PCR for bocavirus and nested-PCR for adenovirus using primers, probes and conditions described in the literature.<sup>5–7</sup>

This study was approved by the BH Ethics Committee.

#### 4. Results

During this period, 253 children were hospitalized with bronchiolitis; 249 NA were analyzed by IFA and 207 by PCR. This loss was due to collection of insufficient material or errors in the freezing and transport ( $n=42$ ).

RSV was detected by IFA in 147 cases, 8 false-positives and 27 false-negatives were identified by PCR ( $n=207$ ). The rate of detection increased from 58.1% to 66.7% with PCR. The RSV A was responsible for 36.7% and the RSV B for 32.9%.

Adenovirus was detected in 8 cases by IFA alone, by PCR, 3 were false-positives and 20 false-negatives. The detection rate raised from 3.2% to 10.0% ( $n=197$ ).

The IFA alone detected 4 cases of IV A and none of IV B. By PCR, we detected 1 IV A and 2 IV B false-negatives and no false-positives ( $n=197$ ). The total positivity for IV A was 5 cases and for IV B 2 cases.

PIV 1 was detected in 1 sample with IFA and in 6 after testing with PCR ( $n=197$ ). PIV 2 was present in 2 samples by IFA. After PCR there was 1 false-positive and 3 false-negatives ( $n=197$ ); the detection increased from 2 to 4 cases. PIV 3 was detected in 16 samples with the IFA alone, 6 of which were false-positives and 1 false-negative ( $n=197$ ). The number of cases decline from 16 to 11.

We found 8 cases positive for bocavirus, as single agent in 2 cases ( $n=197$ ), 8 for rhinovirus ( $n=197$ ) and 11 for hMPV ( $n=196$ ).

All samples analyzed for coronavirus 229E/NL63 and OC43/HKU1 and for enterovirus were negative ( $n=197$ ).

The positive cases for IFA and negative by PCR were double checked by RT-PCR and remained negative.

Fig. 1 describes the relative frequency of each virus.

The infections caused by more than one virus were present in 13.3% and after the evaluation by this extensive panel of viruses, 49 cases (19.7%) remained without an etiology.

#### 5. Discussion

We demonstrated, using sensitive molecular-based assays, a high rate of respiratory virus detection among a large sample of children evaluated for bronchiolitis in a hospital setting.

RSV was the most frequent agent, but it was detected in only two thirds of children.

A previous study analysing the viral detection capacities of culture, IFA and multiplex PCR in children with VRI found PCR to be the most sensitive method with 91.5% of children having a virus detected in their NA.<sup>8</sup> Another study in infants with lower VRI performed PCR on NA and detected viruses in 77% of patients,<sup>9,10</sup> a value quite similar to ours. The majority of the studies report RSV as the most common agent,<sup>8,9,11</sup> but the prevalence of rhinovirus is much higher than ours in some studies, mainly those designed for ambulatory care, probably because it mainly causes upper airway disease, with no need for admission.

We found a high rate of false-negatives when using the IFA alone, mainly with adenovirus,<sup>3,7</sup> PCR has tripled the rate of detection. This is important, since adenovirus is associated with significant morbidity and mortality.

Sequence variation in the primer/probe sequences is not uncommon in virus which may result in a false negative PCR and a second PCR assay directed against another part of the genome using RT-PCR was necessary.

Only for PIV 3, the rate of detection decreased with the use of PCR.

We want to emphasize the presence of bocavirus as a causative agent, because it was detected in 8 cases (2 single agent). In one child diarrhoea was associated and may suggest that bocavirus can cause acute gastroenteritis and/or bronchiolitis. Since bocavirus may be associated with asymptomatic shedding or an etiological agent of bronchiolitis, determining the viral load may help to distinguish between it.

The absence of coronavirus leads us to speculate that it can cause a more benign illness, which did not lead to hospital admission. Enterovirus are usually associated with infection in the summer months and are not normally associated with VRI.

Limitations of our study include the sample loss between the collection and the performance of PCR and the use of residual clinical samples. We did not extend the study into the summer months, potentially decreasing the detection of PIV or EV infections, but included the bronchiolitis' "season". Results confirm RSV as the most frequent agent, but also highlight the potential significance of other viruses such as adenovirus, hMPV and PIV 3 in children hospitalized with bronchiolitis. Given the presence of viral co-infections in RSV bronchiolitis, it is important to recognize the potential limitations of rapid RSV testing alone. Being so, we consider the strategy of sequential testing for respiratory virus a little dangerous, because it will certainly miss the detection in some cases. In the market there are RT-PCR kits that perform the tests rapidly using only one sample.

Another limitation is that we only included admitted children, which changes the rate of detection of some virus. However, these were the most severe cases and the ones that need investigation.

The AAP states that virologic testing is useful when patient cohorting is possible and we think that PCR is a more accurate method, with a higher detection rate than IFA.

They are also cost-effective, when there are enough cases.<sup>12</sup>

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The authors disclose any financial or personal relationship with other people or organisations that could inappropriately influence their work.

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