



# Enfermedades Infecciosas y Microbiología Clínica

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## Letter to the Editor

### Retrospective search of SARS-CoV-2 in respiratory samples in Vallès Occidental (Barcelona, Spain) before the first case was reported



#### *Búsqueda retrospectiva del SARS-CoV-2 en muestras respiratorias en el Vallès Occidental (Barcelona, España) antes de que se notificara el primer caso*

Dear Editor:

SARS-CoV-2 was first reported in Wuhan (China) in December 2019<sup>1</sup> and detected in Europe for the first time on January 24th 2020.<sup>2</sup>

The first imported case in Spain was detected on January 31st<sup>3</sup> and, in Catalonia, on February 25th in Barcelona.<sup>4</sup> On February 26th Andalucía confirmed the first case of SARS-CoV-2 without travel history to any affected country. This is thought to be the first case of community transmission in Spain. Up to May 1st, the country has notified 215,216 confirmed cases.<sup>5</sup>

Vallès Occidental region is an area near Barcelona (Spain) with 925,237 inhabitants in 2019 (12% of Catalonia's total population).<sup>6</sup> It is estimated that there are 580,000 daily trips from and to Barcelona, 43% of which are made on public transport. The first case in this area was reported on February 25th.

According to a Spanish phylogenetic study of SARS-CoV-2, there are two clusters that originated in Spain around February 14th–18th 2020 and generated local transmission in the country.<sup>7</sup> That is, 10 days before the first community transmitted case was detected in Spain.

Regarding these data we hypothesize that SARS-CoV-2 might have been circulating in Vallès Occidental before the first case was detected.

A retrospective study was performed re-analyzing –80 °C frozen nucleic acid extracts from respiratory samples previously analyzed by Allplex™ Respiratory Panel Assays (Seegene, Korea). These samples belonged to hospitalized, ICU and emergency room patients with suspected respiratory tract infection and had been collected from January 1st to February 25th 2020. The sanitary area of our institution comprises 800,000 inhabitants from Vallès Occidental (86% of the total area population).

Automated nucleic acids extraction was performed using MagCore® (RBCBioscience, Taiwan) system. A RNA internal control was included in the samples before freezing and was used as RNA integrity control as well as internal control to Allplex™ 2019-nCoV RT-qPCR. Commercial multiplex real time RT-PCR Allplex™ 2019-nCoV (Seegene, Korea) was performed for simultaneous detection of RdRP and N genes specific for SARS-CoV-2 and E gene, present in all Sarbecovirus.

A total of 170 samples from the respiratory tract were analyzed (41 nasopharyngeal aspirates, 27 bronchoalveolar lavages, 6 tracheal aspirates, and 96 nasopharyngeal swabs): 30% of the samples belonged to pediatric patients (median age: 2 years. ICR: 0–6) and 53% of adult samples belonged to women (median age: 70 years. ICR: 51.5–82). A total of 87% (149/170) of the samples were positive for winter season common respiratory virus (36.2% influenza virus, 16.8% rhinovirus, 8.8% coronavirus, 6.1% respiratory syncytial virus, 12% of other respiratory virus and 20.1% co-infection or two or more respiratory virus). In none of the samples SARS-CoV-2 was detected.

In early March, Spain suffered an explosion of COVID-19 that led to a national lockdown, but it is believed that the virus was already present in the country days before the first case was confirmed.<sup>7</sup> Moreover, the virus has been detected in retrospective wastewater analysis of samples dated before the first case was declared in some regions of Spain,<sup>8</sup> but similar studies have not been performed yet in our geographic area. Similar findings have been reported in other countries<sup>9</sup> and it would be of great interest to perform this analysis widely. In a Chinese study similar to ours, it was described that SARS-CoV-2 virus was undetectably circulating camouflaged among flu cases.<sup>10</sup> In our case, the virus was not detected in any of the tested samples. This may be related to different possibilities, such as patients showing mild symptoms that did not require medical attention, patients having medical care in another institution or an explosive spreading scenario starting with a very low number of cases and a sample size not big enough.

To our knowledge this is the first study of these characteristics in Spain. The fact that an area with such proximity to a big focus of transmission and such a high number of displacements per day may have had no cases before the first confirmed detection may help to understand the dynamics of SARS-CoV-2. We encourage our colleagues from other institutions to perform similar researches.

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