On the trail of the novel coronavirus at the start of the pandemic in the Lazio region: a retrospective research of SARS-CoV-2 in nasopharyngeal swabs.

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#### Abstract

SARS-CoV-2 RNA was not detected in 219 nasal/oro-pharyngeal swabs, analyzed for respiratory disease diagnosis at the National Institute for Infectious Diseases "Lazzaro Spallanzani", from November 2019 to January 2020; these results suggest lack of unrecognized SARS-CoV-2 circulation before the identification of the first two cases in Italy.

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### Abstract

SARS-CoV-2 RNA was not detected in 219 nasal/oro-pharyngeal swabs, analyzed for respiratory disease diagnosis at the National Institute for Infectious Diseases "Lazzaro Spallanzani", from November 2019 to January 2020; these results suggest lack of unrecognized SARS-CoV-2 circulation before the identification of the first two cases in Italy.

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The first cases of COVID-19 diagnosed in Italy, at the National Institute for Infectious Diseases "Lazzaro Spallanzani" (INMI) in Rome, were two Chinese travelers (on January 29, 2020) as reported to the WHO European Region surveillance system [1, 2]. However, these cases did not ignite local transmission of the virus; in fact, the first case on SARS-CoV-2 infection acquired by local transmission in Italy dates back to February 21, 2020 in Codogno (Lodi province), while in Lazio region the first autochthonous case was diagnosed on March 1, 2020.

An interesting paper recently published [3] showed an oropharyngeal swabs (OPS) sample resulted PCR positive for the SARS-CoV-2 RNA, among samples collected for rubella and measles surveillance purpose, from young patients in Milan (September 2019 - February 2020). The Authors reported the earliest evidence of the presence of SARSCoV-2 RNA in December 2019, dating back to about 3 months before the first COVID-19 case in Italy, anticipating the beginning of the outbreak in Lombardy region to late autumn 2019 [3], in agreement with other evidence of early SARSCoV-2 spread in northern Italy [4].

We conducted a similar investigation in Lazio region, by analysing 219 naso/oro-pharyngeal swabs (NPS) obtained from patients hospitalize at INMI or in other hospitals of Lazio region for the presence of respiratory symptoms. The demographic data of the study patients are reported in Table 1. These specimens were collected from November 19, 2019 to January 27, 2020 and analyzed immediately after arrival in the laboratory for the presence of non-SARS-CoV-2 respiratory pathogens, using QIAstat-Dx Respiratory Panel (Qiagen s.r.l, USA). Hence, they were frozen at -80°C and never defrosted, until the SARS-CoV-2 RNA assay, that was recently carried out for the purpose of this study, using Aptima<sup>TM</sup> SARS-CoV-2 (Hologic, Inc., CA) or Simplexa COVID-19 Direct (DiaSorin Molecular LLC, CA) molecular assays. Out of 219 NPS, 71 resulted positive for non-SARS-CoV-2 respiratory pathogens and no one was positive for the SARS-CoV-2 RNA (Table 1).

Although limited to a small number of samples, these data are well representative of epidemiological situation in Lazio region during the considered period, as they reflect the frequency of respiratory pathogens observed in the same period of the previous year, with the exception of Influenza A cases, accordingly to the epidemic seasonal trend (https://w3.iss.it/site/RMI/influnet/pagine/stagioni.aspx) (Table 1).

In addition, it is worth to note that our results are perfectly aligned with the data of wastewater-based epidemiology, reported by Italian Istituto Superiore di Sanita, which highlight the presence of SARS-CoV-2 RNA in Rome sewage since March 31, 2020 [5].

Therefore, the results obtained with the molecular test for SARS-CoV-2 performed retrospectively on the samples collected in the previous three months before the first case diagnosed at INMI, support the start the SARS-CoV-2 circulation in Lazio region shortly before March 2020, confirming the epidemiological data so far available.

**Table 1.** Description of tested naso/oro-pharyngeal swabs.

Period	Period	November 2018 - January 2019	November 2019 - Januar
N. Samples	N. Samples	250	219
Age [mean (min – max) years]	Age [mean (min – max) years]	55 (1 - 90)	54 (0 - 99)
Gender [%F - $\%$ M]	Gender [%F - $\%$ M]	37.2 - 62.8	42.5 - 57.5
Pathogens [N.Positive (%)]	Pathogens [N.Positive (%)]		
Influenza A viruses:	Influenza A viruses:	67 (26.8)	26 (11.9)
	H1N1  pdm09	55 (22.0)	22 (10.0)
	H3	8 (3.2)	4 (1.8)
Rhino/Enterovirus	Rhino/Enterovirus	21 (8.4)	14 (6.4)
Respiratory Syncytial Virus	Respiratory Syncytial Virus	23 (9.2)	12 (5.5)
Coronavirus non-SARS-CoV-2	Coronavirus non-SARS-CoV-2	8 (3.2)	4 (1.8)
$Mycoplasma\ Pneumoniae$	$Mycoplasma\ Pneumoniae$	1(0.4)	9 (4.1)
Others	Others	13 (5.2)	12 (5.5)

Period	Period	November 2018 - January 2019	November 2019 - Januar
Coinfections	Coinfections	20 (8.0)	6 (2.7)

<sup>\*</sup>statistical evaluation based on Fisher exact test (https://www.scistat.com/statisticaltests/fisher.php).

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### Author's contribution

Amendola A, Sberna G: analysed data, performed laboratory testing, wrote manuscript; Lalle E, Bordi L: designed the study, read the manuscript, performed laboratory testing; Capobianchi MR: discussed results, read and revised manuscript; Ippolito G, Nicastri E: discussed results.

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## Competing interests

None declared.

# Ethical approval

Ethical approval was not required as residual samples were used and patients' data remained anonymous.

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