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Honey bee (Apis mellifera L.) colonies as bioindicators of environmental SARS-CoV-2 occurrence

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HIGHLIGHTS

• Atmospheric particulate matter (PM) plays a role in SARS-CoV-2 transmission.
• Apis mellifera colonies are used as bioindicators for environmental sampling.
• SARS-CoV-2 was detected in the PM carried by honey bee foragers.
• A. mellifera colonies can be used in the environmental detection of airborne pathogens.

GRAPHICAL ABSTRACT

ABSTRACT

SARS-CoV-2 is responsible for the COVID-19 pandemic. Airflows sustain the infection spread, and in densely urbanized areas airborne particulate matters (PMs) are deemed to aggravate the viral transmission. Apis mellifera colonies are used as bioindicators as they allow environmental sampling of different nature, PMs included. This experiment demonstrates for the first time the possible use of honey bee colonies in the SARS-CoV-2 monitoring. The trial was conducted in Bologna on 18 March 2021, when the third wave of the Italian pandemic was at its peak and environmental conditions allowed high PM concentrations in the air. Sterile swabs were lined up at the hive entrance to sample the dusty material on the body of returning foragers. All of them resulted positive for the target genes of viral SARS-CoV-2 RNA. Likewise, internal samples were taken, but they resulted in no amplification of the target sequences.

This experiment does not support speculations about the role of honey bees or their products in SARS-CoV-2 transmission. However, it indicates a novel use of A. mellifera colonies in the environmental detection of airborne human pathogens, at least in a densely urbanized area, deserving better understanding and possible integration with data from automatic air samplers.

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1. Introduction

The COVID-19 pandemic originated from the SARS-CoV-2 outbreak reported in China in December 2019 (Lai et al., 2020). The infection reached Italy in February 2020, when the first case was officialised (Grasselli et al., 2020), and quickly spread nationwide. Initially, it affected mainly the North of the country (Distante et al., 2020). Emilia-Romagna lies in that area and eventually turned into the third most...
severely hit Italian administrative region. On a regional population of approximately 4.4 million inhabitants (http://dati.istat.it/Index.aspx), by 31/04/2021 Emilia-Romagna witnessed 346,820 positive cases and 12,380 fatalities (Gamberini et al., 2021; Vignatelli et al., 2021). Official records report 27,790 and 86,856 new cumulative infections, respectively in the course of the first (March 2020) and third (March–April 2021) pandemic wave (Italian Ministry of Health) (Ministero della Salute e Istituto Superiore di Sanità, 2021).

Like other coronaviruses, SARS-CoV-2 is an ssRNA viral strain with a crown-like external layer of spike proteins (Wang et al., 2020a). As the virus misses a lipidic membrane, its stability substantially depends on high relative humidity (Wu et al., 2020a; Wu et al., 2020b). Aerosols generally promote transmission, which may occur through droplets (<5 μm) and droplet nuclei (5-10 μm) (Leung et al., 2020). Before falling, droplets may float in the air for long distances, depending on airflow, temperature, and humidity (Seto, 2015), but droplet nuclei persist airborne only a few hours and tend to disperse within a narrow range (<1 m) (Leung et al., 2020; Li et al., 2020). Thus, aerosols and their size may influence the dispersion of airborne pathogens like SARS-CoV-2 (Zhao et al., 2019).

Atmospheric particulate matter (PM) plays a role in SARS-CoV-2 transmission, as it may bear the virus and foster its spread (Comunian et al., 2020). Conventionally, PM is defined by the particle size. PM_{1.0} and PM_{2.5} are respectively particles and fine particles with a 50% cut-off aerodynamic diameter of 10 and 2.5 μm. Studies indicate a high incidence of new SARS-CoV-2 infections in conditions of remarkable air pollution, especially in dense metropolitan areas connotated by high levels of smog (Liu et al., 2021; Travaglio et al., 2021; Wang et al., 2020b; Wu et al., 2020c; Yao et al., 2021; Zhu et al., 2020). In northern Italy, the SARS-CoV-2 RNA was found in filters used in monitoring the air quality (Belosi et al., 2021; Borro et al., 2020; Chirizzi et al., 2021; Fattorini and Regoli, 2020; Setti et al., 2020). This leads to the hypothesis of PM-mediated vehiculation of the virus.

Emilia-Romagna is one of the four Italian regions encompassing the Po Valley, where the intensity of industrial and urban settlements, road traffic, and agricultural activities act as almost incessant fume generators. On the other hand, the whole area distinguishes for limited air circulation and a relatively stable climate. These conditions hinder the dispersion of air pollutants and promote smog accumulation, especially in the winter (Chirizzi et al., 2021; Contini et al., 2015; Ferrero et al., 2010).

Honey bee (Apis mellifera, L. 1758) colonies are frequently used as bioindicators in environmental monitoring plans (Bargańska et al., 2016; Rissato et al., 2007; van der Steen et al., 2012; van der Steen, 2016). Individual morphology and behaviour make honey bees suitable for this purpose for their: i) high mobility within the explored area; ii) large populations of flying colony members; iii) high sensitivity to chemical pollution; iv) frequent flights of forager bees; v) ubiquity; vi) flight radius approximately 1.5 km wide, corresponding to 7 km²; vii) body covered by hairs and bristles, capturing pollen and other particles during the flight (Celli and Maccagnani, 2003; Herrero-Latorre et al., 2017; Porrini et al., 2002), PMs included (Capitani et al., 2021; Negri et al., 2015; Pellecchia and Negri, 2018). That makes possible the detection of environmental contaminants: radioactive fallouts (Bari et al., 2021; Borro et al., 2020; Chirizzi et al., 2021; Fattorini and Regoli, 2020; Setti et al., 2020). This is recognized by the Centers for Disease Control and Prevention (CDC), intended for the detection of two conserved regions of N gene (N1 and N2) [54]. Accordingly, a total reaction volume of...

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15 μL was prepared as follows: 5.0 μL TaqPath™ 1-Step RT-qPCR Master Mix2x (ThermoFisher), 1.5 μL combined primers probes mentioned above, 6.5 μL DNase/RNase-Free distilled water, 2 μL RNA extract from samples. The same analytical procedure was used for all samples, irrespective of their nature.

The reference honey bee gene β-actin was selected to control the successful amplification and confirm sample integrity throughout the analytical procedure (Chen et al., 2005). The 2019-nCoV_N_Positive Control (Integrated DNA Technologies) was used as the positive control containing the complete SARS-CoV-2 nucleocapsid gene. All RT-Real Time assays were made in two technical replicates on an Applied Biosystems 7300 Real-Time PCR System (ThermoFisher).

2.3. Environmental and epidemiological characterization

The landscape of the study area was described with the open-source geographic information system Q-GIS v 3.10. A circular buffer with a radius of 1.5 km around the apiary site was deemed a representative range for the forager bee activity (Porrini et al., 2014, 2002).

The minimal spatial resolution was consistent with the regional cartography data “2017-Coperture vettoriali uso del suolo in dettaglio”, which relies on a surface details of 0.16 Ha for polygonal areas and a length of 7 m for linear components (https://geoportale.regione.emilia-romagna.it/catalogo/dati-cartografici/pianificazione-e-catasto/uso-del-suolo/layer-9). The map legend bases on the third CORINE Land Cover level, which was further detailed according to the fourth level. Those data describe the landscape of the area of interest with remarkably better details than the newer CORINE Land Cover Map 2018, that applies at national level (http://groupware.sinanet.isprambiente.it/uso-copertura-e-consumo-di-suolo/library/copertura-del-suolo/corine-land-cover/clc2018_shapefile).

The open-source system Dext3r (https://simc.arpae.it/dext3r/), implemented by the Agency for Prevention, Environment and Energy of the Emilia-Romagna region (Arpae), was used to retrieve environmental data for the period 7-23 March 2021. In detail, meteorological data (precipitation, air temperature, scalar wind speed, relative humidity) and pollen grain concentration refer to the automatic weather monitoring station located in Dozza (44°32′02.3″N 11°22′05.4″E). The closest automatic environmental monitoring station to obtain concentration data of PM10 and PM2.5 was the one located in San Felice (44°30′00.1″N 11°19′42.6″E) (Fig. 3).

The searchable dashboard based on the open-source system ArcGIS provided by the Italian Ministry of Health (https://opendatadpc.maps.arcgis.com/apps/dashboards/b0c68bce2ccee478eaac82fe38d4138b1).
was accessed to download epidemiological data of new daily cases, total positives and deceased at national level and for the metropolitan city of Bologna.

3. Results

The colonies selected for the trial measured 4.2 ± 0.4 combs with adult bees and 3.4 ± 0.4 combs containing brood (average ± standard error).

The target genes of viral SARS-CoV-2 RNA were amplified from all extracts from the swabs collecting dusty material from returning foragers. None of the internal samples, either swabs or bee bread, scored positive for the N gene of SARS-CoV-2.

The leftmost column of Table 1 reports the surface coverage characterization according to the four CORINE levels that were present within the 1.5 km buffer from the apiary site. Consistently with the highly populated nature of the territory, artificial surfaces occupied almost 90% of the buffer, whereas agriculture, forest/seminal natural coverage, and water bodies were restricted to minor areas. The other columns and Fig. 4 provide better details of land use. Categories corresponding to limited surfaces (≤2.5%) in II-IV CORINE Land Cover levels are not shown. Urban, industrial, commercial, and transport elements covered altogether more than 70% of the whole buffer surface, and transport infrastructures alone almost 20%.

Figs. 5 and 6 show respectively the SARS-CoV-2 incidence at national level and the local environmental and epidemiologic situation occurred in the period of the trial.

The days before the sampling, the concentration of airborne particulate showed peaks consisting in i) increased concentration (8–11 March) of airborne pollen from plants belonging to the botanical families of Cupressaceae, Taxaceae, and Salicaceae (especially poplar), and ii) PM$_{2.5}$ and PM$_{10}$ exceeding the legal thresholds of respectively 25 μg/m$^3$ and 40 μg/m$^3$ (8–12 March) eliciting local restrictions to vehicle circulation (Fig. 6A). Subsequently, the relatively steady air temperature and relative humidity, moderate winds and insignificant rainfalls (14 March: 0.5 kg/m$^2$) (Fig. 6B–C) were not compatible with substantial particulate renovation and/or washing away.

Official data show that the province of Bologna was hit by increasing COVID-19 positive cases in the second and third week of March 2021, with new daily infected people approximately ranging between 400 and 1000 individuals (Fig. 6D).

4. Discussion

This study was not intended to assess a relationship between the SARS-CoV-2 infection and the presence of honey bees, the contact with them, or the human consumption of honey bee products. Nothing in it supports speculations on this articulate subject.

The trial was conducted during a COVID-19 pandemic peak in a densely urbanized area of northern Italy to investigate the possibility to spot out the viral RNA in the particulate left by returning forager
bees at the entrance of their hive. The study was conducted on one single day of sampling. Nonetheless, it clearly showed the possible use of honey bee colonies in the monitoring of outbreaks of airborne pathogens.

Indeed, honey bees are often used as bioindicators to monitor the environmental occurrence of pesticides and other harmful compounds (Barišić et al., 1994; Celli and Maccagnani, 2003; Skorbilowicz et al., 2018; van der Steen et al., 2012), but rarely their strict interaction with natural and artificial elements of their habitat has been exploited in pathogen detection. To the best of the Authors’ knowledge, only few such cases have been reported so far (Ghini et al., 2002; van der Steen, 2016), when the 1990s witnessed an epidemic of fire blight caused by the Gram-negative bacterium Erwinia amylovora and mainly hitting pomaceous fruit trees in several countries (Norelli et al., 2003; Oh and Beer, 2005; Vanneste, 2009). At that time, the bees were suspected as potential vectors of the infection, but one of the Authors (SG) contributed to assess the positive aspects of honey bee-based monitoring plans in the early prediction of new outbreaks (Ghini et al., 2002; Girotti et al., 2005; Sabatini et al., 2006). The present study is well in line with those previous outcomes and represents the first known attempt to extend the honey bee monitoring to the detection of human infections sustained by airborne viruses.

This study was inspired by the previous detection of measurable concentrations of SARS-CoV-2 in airborne PMs recovered from artificial air samplers (Belosi et al., 2021; Borro et al., 2020; Chirizzi et al., 2021; Fattorini and Regoli, 2020) and by the peculiar morphology and behaviour of honey bees. External traits of the bee body bestow them the ability to intercept small particles, namely pollen grains, making them effective in capturing PMs. Besides, in one colony, the forager bees can systematically inspect wide areas of the territory around the apiary, which may be roughly estimated at around 7 km² (Porrini et al., 2014, 2002). This all made honey bees promising indicators of airborne SARS-CoV-2 during a COVID-19 outbreak in an area characterized by high PM concentration.

In the specific case here considered, the putative flight range of forager bees included busy streets and motorways, residential districts, shopping areas, factories, etc., as shown by the GIS analysis of the area surrounding the apiary site. Those are regular sources of PMs, which may be aggravated by the heating systems that, at the time of the study, were still functioning.

All swab arrays fixed in front of the hive entrances were found positive to SARS-CoV-2. As in principle direct contact between bees and infective sources can be excluded, the environmental contamination of the foragers must be assumed. This is compatible with

Fig. 4. Land use within the buffer with radius of 1.5 km from the apiary site according to the third level of CORINE Land Cover. Legend - red: continuous urban fabric; orange: discontinuous urban fabric; grey: industrial and commercial units; black: road and rail networks; brown: construction sites; dark green: green and urban areas; light green: sport and leisure facilities; yellow: permanently irrigated areas.

Fig. 5. Rolling 7-day average of new infected and fatalities during the three waves of the Italian pandemic. The sampling date is highlighted with a red mark on each curve.
both the high viral circulation and the elevated airborne PM concentration in the period of the trial. Besides, the combination between mild climate and relatively high humidity that are typical of the early bee season in the study area may have played a role in the effective sampling of viral RNA, as air temperature of 5–15 °C and RH% of 44–84% have been reported as conditions promoting the virus survival (Casanova et al., 2010; Chan et al., 2011; Marzoli et al., 2021; Mecenas et al., 2020; Riddell et al., 2020; Sajadi et al.,...
Undoubtedly, instrumental air samplers provide essential data, but research efforts should be made also to synergize them with the information coming from networks of monitoring hives.

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Credit authorship contribution statement

Giovanni Cilia: Conceptualization, Investigation, Writing – original draft, Drafting – review & editing. Laura Bortolotti: Investigation, Funding acquisition, Supervision, Writing – review & editing. Sergio Albertazzi: Investigation, Writing – original draft, Writing – review & editing. Severino Chinì: Conceptualization, Investigation, Writing – review & editing. Antonio Nanetti: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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