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COVID-19 in a rural community: outbreak dynamics, contact tracing and environmental RNA

Authors: Isabel G. Fernández-de-Mera[#1], Francisco J Rodríguez del-Río[#2], José de la Fuente[1 3], Marta Pérez-Sancho[4], Dolores Hervás[2], Inmaculada Moreno[5], Mercedes Domínguez[5], Lucas Domínguez[4], Christian Gortázar*[1]

[#]These authors contributed equally to this work.

1.- SaBio Instituto de Investigación en Recursos Cinegéticos IREC (UCLM & CSIC), Ciudad Real, Spain

2.- Local Medical Service Horcajo de los Montes, Ciudad Real, Spain

3.- Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

4.- VISAVET and Department of Animal Health-Faculty of Veterinary Medicine. Universidad Complutense Madrid. Avenida Puerta de Hierro, s/n Madrid, Spain

5.- Servicio de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

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*Corresponding author: Prof. Christian Gortázar. IREC, UCLM-CSIC-JCCM. Ronda de Toledo 12. Ciudad Real 13005, Spain. Tel. 0034 610 444 316 ; Fax 0034 926 295 451. christian.gortázar@uclm.es

Alternate author who can be contacted: PhD. Isabel G. Fernández de Mera. IREC, UCLM-CSIC-JCCM. Ronda de Toledo 12. Ciudad Real 13005, Spain. Tel. 0034617718140 ; Fax 0034926295451. mariaisabel.garcia@uclm.es

Article's main point: Understanding SARS-CoV-2 transmission dynamics is crucial. We recorded and traced all COVID-19 cases in an isolated rural community and sampled households and public sites for environmental RNA. Results indicate maintained virus circulation and call for urgent changes in disease management.

Abstract

Background. Since March 2020, Spain is severely hit by the ongoing pandemic of coronavirus disease 19 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Understanding and disrupting the early transmission dynamics of the infection is crucial for impeding sustained transmission.

Methods. We recorded all COVID-19 cases and traced their contacts in an isolated rural community. We also sampled 10 households, 6 public service sites and the wastewater from the village sewage for environmental SARS-CoV-2 RNA.

Results. The first village patient diagnosed with COVID-19-compatible symptoms occurred on March 3, 2020, twelve days before lockdown. A peak of 39 cases occurred on March 30. By May 15, the accumulated number of symptomatic cases was 53 (6% of the population), of which only 22 (41%) had been tested and confirmed by RT-PCR as SARS-CoV-2 infected, including 16 hospitalized patients. Contacts (n=144) were six times more likely to develop symptoms. Environmental sampling detected SARS-CoV-2 RNA in two households with known active cases and in two public service sites: the petrol station and the pharmacy. Samples from other sites and the wastewater tested negative.

Conclusions. The low proportion of patients tested by RT-PCR calls for urgent changes in disease management. We propose that early testing of all cases and their close contacts would reduce infection spread, reducing the disease burden and fatalities. In a context of restricted testing,

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environmental RNA surveillance might prove useful for early warning and to identify high-risk settings enabling a targeted resource deployment.

Introduction

Corona virus disease 19 (COVID-19) has spread globally. Over 4.6 million COVID-19 cases have been reported from 187 countries, causing more than 311,000 deaths worldwide (https://coronavirus.jhu.edu/map.html; last access 17/05/2020), including 27,650 officially recorded fatalities in Spain as of May 17, 2020. Responses to this unprecedented challenge often include travel bans and social distancing, even with lockdown orders [1], which imply changes in human behavior and determine severe effects on the economy and all kind of activities [2]. The causative agent of COVID-19, SARS-CoV-2, is transmitted by aerosols, but also indirectly through contaminated objects, on which the virus can survive for some time. Even the skin of the hands can eventually act as a means of transmission of the virus [3, 4]. While nucleic acid detection does not imply pathogen viability, this implies that certain surfaces, such as supermarket trolleys, doorknobs, or garbage container handles, as well as the body surfaces of infected people, represent potential sources of contamination [5]. Moreover, SARS-CoV-2 RNA has also been detected in wastewater [6].

A key reason for the high transmissibility of Covid-19 is the high level of excretion of SARS-CoV-2 by the upper respiratory tract, even among presymptomatic or fully asymptomatic patients [7]. The percentage of true asymptomatic infected people was calculated at 18% in the well-studied Diamond Princess cruise ship [8]. The average incubation period is 6.4 (range 2-11) days [9]. Consequently, detection of infection based on symptoms is not enough for preventing infection spread in the case of SARS CoV-2 [10]. One way of overcoming this limitation is to trace infected people, testing both symptomatic and asymptomatic contacts in order to identify new infected persons and interrupt the transmission chain. Some models estimate that a combined test and trace strategy would reduce SARS-CoV-2 transmission more effectively than mass testing or isolation [11, 12]. Moreover, contact tracing will be central to control strategies during de-escalation of social distancing. However, models suggest that effective testing and contact tracing strategies require very short testing and tracing delays and an almost 100% tracing coverage [13].

The preliminary results of the ENE-COVID survey show a 5% average antibody prevalence in the Spanish population, with somewhat higher values, around 11%, in the most affected provinces. These include the capital city, Madrid, and several rural provinces around Madrid, including Ciudad Real in Castilla – La Mancha (CLM) region (https://www.ciencia.gob.es/stfls/MICINN/Ministerio/FICHEROS/ENECOVID_Informe_preliminar_cierre_primera_ronda_13Mayo2020.pdf; last access 17 May, 2020). This implies, firstly, that the Spanish population is still far from herd immunity, which in turn means that the COVID-19 epidemy will be prolonged in time. Second, it implies that there are many more cases of infection than those detected by PCR and officially recorded, and this urgently requires a greater diagnostic effort. Hence, contact tracing and testing efforts need to be boosted urgently. Most unfortunately however, testing is often limited to severe symptomatic cases, and contact tracing in not yet in place in some Spanish regions including CLM. As of May 16, 2020, the regional health authority of CLM was still in the process of recruiting and training 400 healthcare workers for contact tracing of known COVID-19 cases (https://www.elheraldodelhenares.com/prov/400-nuevas-enfermeras-se-encargaran-hacer-un-seguimiento-de-casos-y-contactos-de-coronavirus-a-los-nuevos-infectados-en-castilla-la-mancha/; last access 17 May, 2020). This is far away from the massive testing recommendations emanating from the Italian outbreak (May 2020).

In this context, environmental RNA might contribute to improved COVID-19 monitoring in suspected contaminated environments, such as shopping malls, health centers, nursing homes,

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or households of people who have passed COVID-19. Pathogen nucleic acids can be sampled in the environment for detection and monitoring purposes [14]. We hypothesized that nucleic acids of SARS-CoV-2 would be detectable in sites with known recent virus circulation and that environmental RNA sampling could contribute to the early detection and subsequent monitoring of virus circulation, thereby identifying targets for contact tracing and testing for a more efficient COVID-19 control.

Methods

Study site

The village (883 inhabitants in 2019; 4.6/km^2) belongs to Ciudad Real province in Castilla – La Mancha (CLM), southern Spain, about 80 km away from the provincial capital, Ciudad Real, and the Hospital General Universitario Ciudad Real (HGUCR). As most villages in rural Spain, the population is steadily declining (10% loss in the last decade) and ageing (59% >65 years). Before the lockdown, Ciudad Real was among the Spanish provinces with more per capita movement connections with Madrid (180 km from the study village) and had therefore a high risk of SARS-CoV-2 introduction at the onset of the COVID-19 epidemy in Spain [15].

The village municipality and a firm hired by the CLM authorities started street and public service disinfections on March 14 and March 22, 2020, respectively. According to municipal records, disinfections with sprayed 2% hypochlorite took place 1 to 3 times weekly and included the exteriors of the medical center (12 times; occasionally including the inside), pharmacy (3 times, outside only), petrol station (7 times, outside only), and supermarket (8 times, outside only). The community spontaneously organized assistance for home-confined COVID-19 suspects, including food delivery, medicine delivery, cleaning service and medical assistance in order to avoid unnecessary movements, and requested police assistance to enforce home-confinement where needed.

Data sources and field sampling

Starting on March 1, 2020, the local physician (FR) recorded all suspect COVID-19 cases along with the official testing results and hospital stay records. Case definition included bilateral pneumonia, often with anosmia and dysgeusia. Pausymptomatic patients without bilateral pneumonia were not listed as suspect cases. Contacts were listed for each case and included household members and close relatives. Contacts without symptoms after 21 days were later deleted from the list. All patient testing was performed at HGUCR under the coordination of the CLM regional health authorities.

On May 13, 2020, we sampled 10 households (2 with PCR-confirmed active cases; 6 with PCR-confirmed older cases; 2 with non-tested older cases), 6 public service sites (Table 1) and the wastewater from the village sewage for environmental SARS-CoV-2 RNA. Dry sponges (3M™ Dry-Sponge; 3M-España, Madrid) were pre-hydrated with 15 ml of an isotonic surfactant and virus-inactivating liquid (patent pending) able to collect nucleic acids on surfaces and other substrates [14]. On each site visited, one to four sponges were smoothly rubbed over surfaces in likely contact with people's hands or gloves (Environment, E) or over the hands (with or without gloves) and clothing of the persons present (Person, P). Environment sampling in public service sites included surfaces such as keyboards, tables, chairs, refrigerators and entry door handles. Environment sampling in households always included the toothpaste tube(s), fridge and oven handles, and the main door handle. For wastewater sampling, 5 ml of liquid collected from the village's main sewage drain were mixed with an equivalent volume of the liquid used in the sponges. The collected samples were refrigerated until processed in the laboratory.

Laboratory procedures

Once in the laboratory, a volume of 2 ml was extracted from each sample, collected in a screw cap tube and centrifuged at 12.000g for 10 minutes. Viral RNA was extracted from 200 µl of solution taken from the bottom of the tube, using the NucleoSpin RNA Virus kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

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Detection of SARS-CoV-2 RNA was then performed by real-time RT-PCR assays, targeting the envelop protein (E)-encoding gene and two targets (IP2 and IP4) of RNA-dependent RNA polymerase gene (RdRp), according to protocols included in the WHO guidelines (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance) [16, 17]. Primer sets used are detailed in Table 2. The positive control for real-time RT-PCR is an in vitro transcribed RNA derived from the strain BetaCoV_Wuhan_WIV04_2019 (EPI_ISL_402124), loaned by the Pasteur Institute (Paris, France). Nuclease free water was used as negative control.

Real-time RT-PCR was carried out using the SuperScript III Platinum One-Step qRT-PCR Kit (ThermoFisher, Massachusetts, USA), according to manufacturer's protocol. A CFX96 Touch Real-Time PCR Detection System Thermal Cycler (BioRad, Berkeley, USA) was used to carry out the reactions.

Role of the funding source

This study had no specific funding. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Outbreak timeline and patient testing

The first village patient with COVID-19-compatible symptoms was diagnosed on March 3, 2020, 12 days before lockdown was in place in Spain (March 15, 2020). This first case occurred 4 days after a funeral that had been celebrated in the village attracting visitors from the capital, Madrid. Interventions carried out for COVID-19 control in the village included the national lockdown since March 15; hypochlorite disinfections of public spaces since March 22; personal hygiene measures such as frequent handwashing, hand and household disinfection, and facemask use; as well as home confinement or hospitalization of all known symptomatic cases. A peak of 39 symptomatic COVID-19 cases occurred on March 30, including 3 ICU cases, 9 hospital cases and 27 home confinement ones. The number of cases and contacts started to decline since March 30, 15 days after lockdown. By May 16, 2020, the accumulated number of symptomatic cases was 53 (6%), of which 22 (41%) had been confirmed by PCR as SARS-CoV-2 infected, including 16 patients (30%) which required hospitalization at HGUCR (Figure 1). Three fatalities occurred on March 29, April 3 and May 4, respectively, representing a case fatality rate of 13.6% among the PCR-confirmed cases and of 5.7% among the total cases recorded in the village.

Only 23 of the 883 village inhabitants (2.6%) have been RT-PCR tested for SARS-CoV-19 since the onset of the local outbreak in early March 2020. Of these 23, only 9 (39%) had a second negative RT-PCR after recovery. The remaining 30 symptomatic cases have not been tested. Each case had on average 2.7 ±1.8 close contacts (range 0-9). The total number of known close contacts of the 53 recorded cases was 144, and the daily number of contacts reached a peak of 77 on March 30, 2020. Cases were six times more likely to occur among close contacts (28 of 144) than in the general population (25 of 739; Fisher's test, $P<0.0001$). Two of three fatalities were close contacts of cases. However, despite repeated requests from the local physician, neither the remaining household members nor other close contacts of these 53 cases were tested.

Environmental RNA sampling

Environmental sampling took place on May 13, 2020, 71 days after onset of the local outbreak. We detected SARS-CoV-2 RNA in the two sampled households with known active cases. Additionally, environmental SARS-CoV-2 RNA was also found in one of six households with an older PCR-confirmed case, as well as in two public service sites: the petrol station and the pharmacy. Samples from other sites and the wastewater samples tested negative (Table 1). These sites were positive for at least two of the three RT-PCR reactions performed, and in all cases these samples were positive for the SARS-CoV-2-specific RdRP-IP4 and RdRP-IP2 PCRs targeting the coronavirus RNA-dependent RNA polymerase. Hence, medical records and environmental RNA sampling coincide in signaling ongoing SARS-CoV-2 circulation in the study site at the end of the study period, on May 13, 2020.

Discussion

By combining medical records and environmental RNA detection, this descriptive epidemiological survey provides valuable insights into COVID-19 dynamics, intervention strategies and future tracing and testing needs in a rural village from a severely affected region. The results evidence that this local and relatively isolated population suffered the first COVID-19 outbreak with a peak of cases between March 15 and April 15, 2020, and both medical records and environmental RNA sampling coincide in signaling that SARS-CoV-2 was still circulating 2,5 months after the first case. Surprisingly, only less than half of the symptomatic cases were PCR tested by the CLM health services and, despite spontaneous contact tracing, no testing of contacts was performed in a setting where even blanket testing would have been advisable (May 2020).

Interventions carried out for COVID-19 control in the village, including the national lockdown, increased hygiene and disinfection, as well as home confinement (with community-provided assistance) or hospitalization of all known symptomatic cases, managed to reduce the incidence and drive the number of known active cases to a minimum of 3 as of May 15, 2020. We speculate that early testing of all cases and their close contacts (less than 80 RT-PCRs at the peak) would have reduced the disease burden and possibly avoided fatalities. Moreover, not testing recovered patients may lead to additional psychical distress [18] and economical losses [19] due to unnecessarily prolonged confinement.

There is a need to balance the interventions to reduce human-to-human transmission with the need to minimize social disruption and economic impact due to COVID-19 [20]. The future course of COVID-19 will depend on testing and tracing, and both are currently not enough in CLM, as evidenced in this survey. In face of the ongoing easing of the Spanish lockdown, starting on May 18 for Ciudad Real province, we propose three actions to improve disease management in order to avoid a new peak and possible additional fatalities. Actions applicable to this village are probably also valid for many similar settings in the rural regions of Europe.

First, PCR testing is urgently needed for all patients with COVID-19 compatible symptoms, as well as for their household members and other close contacts. Increased testing is feasible at HGUCR and can be expanded to additional accredited laboratories already available in Ciudad Real and elsewhere in CLM. The high number of contacts identified in this survey (144; Figure 1) suggests that knowledge of the local community and social networking can serve as an efficient substitute of contact-tracing apps, at least in small villages. During the ongoing de-escalation process, a highly effective contact tracing followed by testing and case isolation should serve to control further outbreaks of COVID-19 [11].

Second, the rapid spread of Covid-19, the clear evidence of the transmission of SARS-CoV-2 from asymptomatic people, and the need to relax the current practices of confinement and social distancing, advocate the expansion of tests of SARS-CoV-2 to the surveillance of priority environments due to their special risk [10]. In the study village there is both medical and environmental RNA evidence suggesting ongoing virus circulation in households and in public sites such as the pharmacy and the petrol station. Thus, disinfection activities should be expanded and need to include the inside of the main public spaces as already done in the medical center (which tested negative despite of being a high-risk site). Households should receive additional information on good disinfection practices. Persons at risk and close contacts of cases should avoid public sites and strengthen all preventive measures.

Third, we suggest that environmental RNA surveillance can improve early detection and effective contact tracing, as well as make SARS-CoV-2 monitoring more cost-efficient. This tool facilitates identifying places, objects or substrates at risk due to the increased presence of SARS-CoV-2 RNA, thereby serving as an early warning system. It also allows monitoring the presence of SARS-CoV-2 RNA over time and at different spatial scales, from individual households to entire municipalities. This could aid in decision-making in relation to the de-escalation phases.

These results support the use of environmental RNA surveillance for the effective, noninvasive and cost-effective monitoring of COVID-19 disease spread. In a context of restricted testing, the identification of high-risk locations and settings would contribute to disease control by early case detection to reduce virus transmission and clinical symptoms, and the evaluation of possible indirect transmission routes.

Contributors

FR, IG and CG planned the study. Field data and samples were collected by FR, DH and CG. MD, LD, MP and IM performed laboratory procedures for environmental sampling. IG and JF performed and interpreted the RT-PCR testing. Data analysis was led by CG, IG and FR. All authors interpreted the study findings, contributed to the manuscript, and approved the final version for publication.

Declaration of interests

We declare no competing interests.

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Table 1.- Environmental RNA detection. Columns present the RT-PCR results for 17 sites or substrates where the environment (E) or gloves and clothing (P) were sampled for SARS-CoV-2 RNA in a rural village in Ciudad Real province, Spain, during the first COVID-19 outbreak. (***) indicates households with active cases on May 13, 2020; (*) indicates households with confirmed older cases.

Sampling site	Samples taken	RT-PCR results				Remarks
		RdRP-IP4	RdRP-IP2	Egene	Interpretation	
Medical center	E, 2P	-	-	-	Negative	
Pharmacy	E	+	+	-	Positive	E positive
Postal office	E	-	-	-	Negative	
Petrol station	E	+	+	-	Positive	E positive
Supermarket	E	-	-	-	Negative	
Police	2P	-	-	-	Negative	
Household 1 (***)	E, P	+	+	+	Positive	E and P positive
Household 2 (*)	E, 2P	-	-	-	Negative	
Household 3 (*)	P	-	-	-	Negative	
Household 4	P	-	-	-	Negative	
Household 5 (*)	E, P	-	-	-	Negative	
Household 6 (*)	E, 3P	+	+	+	Negative	E positive
Household 7 (*)	E, 2P	-	-	+	Negative	
Household 8 (*)	E, P	-	-	-	Negative	
Household 9	P	-	-	-	Negative	
Household 10 (***)	E, P	+	+	+	Positive	P positive
Wastewater	2x5ml	-	-	-	Negative	
Total 17 sites	32 samples				6 positive	(5 positive sites)

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Table 2.- Primer sequences and amplified fragment sizes in base pairs.

Primer target	Sequence 5'-3'	PCR fragment size
Gene RdRp / nCoV_IP2		
nCoV_IP2-12669Fw	ATGAGCTTAGTCCTGTTG	108 bp
nCoV_IP2-12759Rv	CTCCCTTTGTTGTGTTGT	
nCoV_IP2-12696b Probe(+)	AGATGTCTTGTGCTGCCGGTA [5']Hex [3']BHQ-1	
Gene RdRp / nCoV_IP4		
nCoV_IP4-14059Fw	GGTAACTGGTATGATTTCG	107 bp
nCoV_IP4-14146Rv	CTGGTCAAGGTTAATATAGG	
nCoV_IP4-14084 Probe(+)	TCATACAAACCACGCCAGG [5']Fam [3']BHQ-1	
Gene E / E_Sarbeco		
E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	125 bp
E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	
E_Sarbeco_P1	ACACTAGCCATCCTTACTGCGCTTCG [5']Fam [3']BHQ-1	

Figure 1.- Timeline of the COVID-19 outbreak in a Spanish village, from March 1 to May 15, 2020. Active cases are divided in home confinement, hospital and ICU. Contacts include household members and close relatives. Contacts without clinical signs after 21 days are deleted from the contacts list.