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SARS-CoV-2 detection in patients with influenza-like illness

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Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, Hubei Province, China in late December 2019. We re-analysed 640 throat swabs collected from patients in Wuhan with influenza-like-illness from 6 October 2019 to 21 January 2020 and found that 9 of the 640 throat swabs were positive for SARS-CoV-2 RNA by quantitative PCR, suggesting community transmission of SARS-CoV2 in Wuhan in early January 2020.

The coronavirus disease 2019 (COVID-19) outbreak, which originated from Wuhan, Hubei Province, China, has spread to all of the provinces of China and 28 other countries[1]. As of 23 February 2020, more than 77,000 cases of SARS-CoV-2 infection have been confirmed in China and 60% of these were reported in Wuhan. Its causative pathogen is a phylogenetic sister to the severe acute respiratory syndrome coronavirus (SARS-CoV) and has been designated as SARS-CoV-2 (ref. [2]). However, although SARS-CoV-2 shares 79.6% sequence identity and the same cell receptor with SARS-CoV[3,4], the clinical outcome of SARS-CoV-2 not only includes SARS-like viral pneumonia[5], but also covers milder illness, even asymptomatic infection[6,7]. In fact, analysis by the China CDC indicated that 80.9% of confirmed COVID-19 cases were characterized as the mild or moderate types—that is, without breathing difficulty and hypoxia[7].

Wuhan is the largest city in central China, with a resident population of over fourteen million. The rapid increase in reported cases suggests that community transmission of SARS-CoV-2 had established in the city of Wuhan and nearby regions no later than the end of January[8]. As the rapid molecular diagnostic assay was not available in early January and widespread use was difficult before 23 January 2020, it was difficult to monitor the transmission of SARS-CoV-2 among the community.

To better understand the current epidemic in Wuhan, particularly the status of cases of milder illness, we retrospectively investigated the presence of SARS-CoV-2 among local patients with influenza-like illness (ILI), which were defined as outpatients with a sudden onset of a fever of >38 °C and a cough or sore throat. Sustained sentinel surveillance for ILI cases and their aetiology has been implemented in Wuhan since 2005, based on the National Influenza Surveillance Network of China[9]. Two representative referral hospitals were selected as sentinel hospitals to reflect the trends in ILI in the local population (Supplementary Fig. 1): the Children's Hospital of Wuhan, which is the largest paediatric centre in the province, and Wuhan No. 1 Hospital, a major general hospital with over two million outpatient visits per year. The number of ILI cases and total outpatient numbers were reported weekly by the sentinel hospitals, and

clinical samples were also collected from ILI patients. In this study, 640 throat swabs collected from ILI patients in the 16-week period between 6 October 2019 (week (W) 40 of 2019; 2019W40 hereafter) and 21 January 2020 (2020W03) were re-analysed.

The time period in concern coincided with the winter peak of influenza and other respiratory illnesses. The number of ILI cases in all age groups increased dramatically starting in early December and reached the peak by the New Year (Fig. 1a). In particular, the 5–14 yr group increased over 24-fold during this period (2019W40–W47, 75 cases per week; 2019W52, 1,916 cases). The percentage of ILI patients in all outpatients experienced a similar rise: the average percentage was 1.07% during 2019W40–W47 and soared up to 9.44% in 2020W01. In addition, ILI data for the 2019–2020 winter was significantly higher in comparison to previous years (Fig. 1b,c and Source Data Fig. 1), suggesting the necessity to distinguish between influenza-infected and suspected COVID-19 patients.

The patients with ILI participating in this study comprised 315 males and 325 females, ranging in age from 9 months to 87 yr (median age, 8 yr; mean age, 22.7 yr). SARS-CoV-2 RNA was detected in nine patient specimens (Table 1 and Extended Data Fig. 1), all of which were collected in January 2020 (2020W01–W03) when the seasonal influenza (mainly A/H3 and B/Victoria) remained active; however, no coinfection was detected.

The basic demographic information and illness timeline of the nine patients infected with SARS-CoV-2 is listed in Extended Data Fig. 2. The gender ratio was 1.25 (five males versus four females), and they were all adults (age range: 35–71 yr). These demographic features are consistent with other available reports regarding patients with COVID-19 (refs. [5,7,10]). The onset date of the earliest case was 4 January 2020, one week after the outbreak was reported by hospitals[11]. The average gap between onset and seeking medical help was 1.7 d, which is shorter than a previous report on early diagnosed cases that typically have pneumonia[5,11]. Although the weekly sample size was small, it seems that COVID-19 was gradually expanding among the ILI cases during January. In the last week of observation, the frequency of SARS-CoV-2-positive cases had exceeded that of influenza virus among the group of patients older than 30 yr (Table 1). This finding is consistent with the recent epidemiological estimations about the early transmission dynamics of SARS-CoV-2 (refs. [11,12]). Interestingly, the nine patients with COVID-19 came from six different districts of the Wuhan metropolitan and surrounding areas (Supplementary Fig. 1), which provided additional evidence for community transmission in this region.

According to its definition, the clinical manifestation of ILI overlaps with the mild/moderate type of COVID-19, which is defined as

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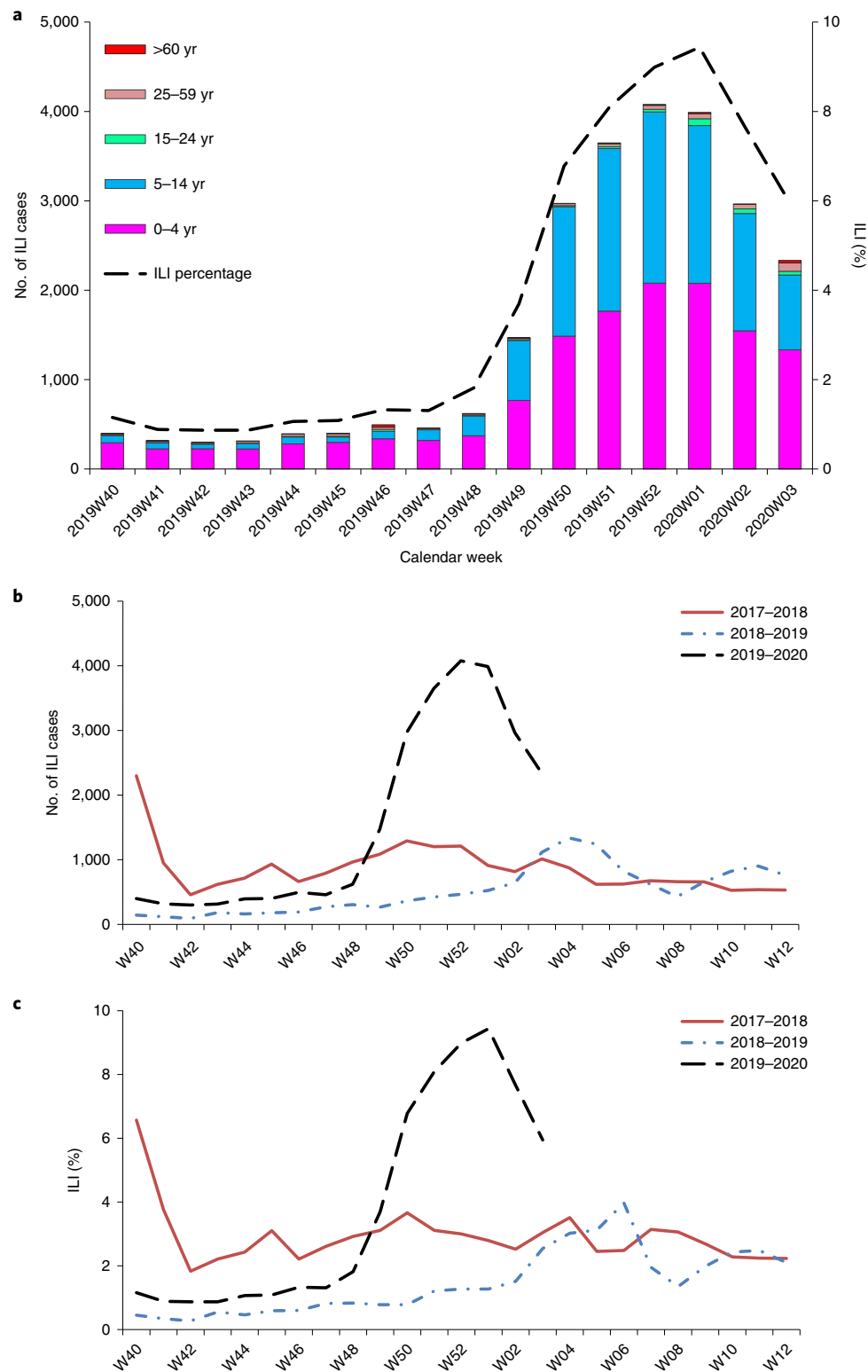✉e-mail: liumq33@hotmail.com

Fig. 1 | ILI surveillance data from two sentinel hospitals in Wuhan. a, Number of ILI cases and percentage of outpatients with ILI between 6 October 2019 and 21 January 2020 (2019W40 to 2020W03). The vertical columns scaled on the left *y* axis report the weekly ILI case numbers and the colour blocks in the columns represent the different age groups of the patients with ILI. The dashed line shows the percentage of outpatients with ILI and is scaled on the right *y* axis. **b**, Number of ILI cases that occurred during the winter influenza season of the past three years. **c**, Percentage of ILI during the winter influenza season of the past three years. **b,c**, Data from week 40 to week 12 of the following year are presented, except for 2019–2020 as the ILI surveillance was suspended to handle the COVID-19 epidemic.

both non-pneumonia and pneumonia cases without dyspnoea and hypoxia[7]. The ILI surveillance samples thus provided us a unique opportunity to investigate the early expansion of SARS-CoV-2 in

the local population. A main concern was the representativeness of these samples. The two sentinel hospitals in Wuhan were both highly reputable public hospitals with large catchment populations.

Table 1 | Virological test results of ILI surveillance samples collected between 6 October 2019 (2019W40) and 21 January 2020 (2020W03) in Wuhan, China

ILI surveillance week	SARS-CoV-2 no. / total no. (%)		Influenza virus no. / total no. (%)		
	All age groups	≥30 yr	All age groups	0–29 yr	≥30 yr
2019W40	0/40	0/15	0/40	0/25	0/15
2019W41	0/40	0/17	0/40	0/23	0/17
2019W42	0/40	0/14	0/40	0/26	0/14
2019W43	0/40	0/14	0/40	0/26	0/14
2019W44	0/40	0/13	0/40	0/27	0/13
2019W45	0/40	0/17	0/40	0/23	0/17
2019W46	0/40	0/17	0/40	0/23	0/17
2019W47	0/40	0/17	6/40 (15.0)	6/23 (26.1)	0/17
2019W48	0/40	0/15	11/40 (27.5)	11/25 (44.0)	0/15
2019W49	0/40	0/19	11/40 (27.5)	10/21 (47.6)	1/19 (5.3)
2019W50	0/40	NA	17/40 (42.5)	17/40 (42.5)	NA
2019W51	0/40	NA	26/40 (65.0)	26/40 (65.0)	NA
2019W52	0/40	NA	19/40 (47.5)	19/40 (47.5)	NA
2020W01	1/40 (2.5)	1/12 (8.3)	18/40 (45.0)	15/28 (53.6)	3/12 (25.0)
2020W02	3/40 (7.5)	3/14 (21.4)	19/40 (47.5)	15/26 (57.7)	4/14 (28.6)
2020W03	5/40 (12.5)	5/19 (26.3)	15/40 (30.0)	15/21 (71.4)	0/19

NA, not applicable.

They were located downtown and could be easily accessed from the whole metropolitan area through public transportation (Supplementary Fig. 1). In addition, China has achieved universal basic health insurance in the lack of hierarchical medical system[13], making large public hospitals the priority choice of patients, which minimized the selection of symptom severity in this study.

There is some discussion regarding the reliability of the SARS-CoV-2 quantitative PCR test and the applicability of upper respiratory tract specimens, including throat swab[14,15]; however, in this study, the possibility of false negative results will not weaken the main finding that SARS-CoV-2 was present in January in patients with ILI. As the epidemic developed rapidly, the ILI surveillance in Wuhan was suspended in 2020W04, because both the CDC virology laboratory and the sentinel hospitals were reformed to focus on handling the explosive medical needs of COVID-19. By the time this manuscript was revised, the number of cases confirmed daily in Wuhan had begun to decline following one month of traffic restrictions. There is an urgent need for a systematic population serological investigation to reveal the full status as well as the history of COVID-19.

In summary, our work adds information to the understanding of the early stage of the current epidemic. The detection of SARS-CoV-2 cases among local patients with ILI suggests that community transmission was established in Wuhan early in January. Concerning the recent outbreaks happening on the Diamond Princess Cruise ship and Daequ, South Korea, we suggest strengthening the pathogen surveillance of ILI cases in all regions facing the danger of SARS-CoV-2 community transmission.

Methods

Sample and information collection. The ILI statistics and throat swab samples for this study were collected during the routine ILI surveillance of two national influenza sentinel hospitals in Wuhan. A patient was identified as an ILI case in the outpatient department of both hospitals if they had a sudden onset of a fever >38 °C as well as a cough or sore throat, according to the latest National Influenza Surveillance Plan[16]. The number of ILI cases and total outpatient numbers were collected weekly. Clinical samples of patients with ILI were also collected if the patient had a fever for less than 3 d and had not been treated with antiviral drugs.

After verbal informed consent was obtained from parents or caretakers of underage patients, throat swabs were collected from patients with ILI in 3.5 ml viral transport medium and delivered to the Wuhan Center for Disease Prevention and Control for laboratory diagnosis of the influenza virus. Each sentinel hospital was required to collect 20 ILI samples every calendar week.

Detection of SARS-CoV-2 and influenza virus by real-time PCR with reverse transcription. We performed a commercial quantitative PCR assay (BioGerm) approved by the China CDC and National Medical Products Administration to detect the presence of SARS-CoV-2 in the throat swab specimens of patients with ILI. The targets of this assay were both the ORF1ab and N gene of SARS-CoV-2, and the detection limit was 1,000 copies ml⁻¹. The nucleic acids used in the SARS-CoV-2 detection were initially extracted from 200 µl of throat swabs medium using a PANA9600E automated nucleic acid extraction system (Tianlong). They had being employed for the detection and subtyping of influenza virus[17], and were well kept at −70 °C before the SARS-CoV-2 experiments. All assays have been established in our laboratory and the procedures were performed following the manufacturers' instructions. For the specific primers and probes used in the influenza virus detection, refer to Supplementary Table 1.

Approval for patient recruitment. This study was reviewed and approved by the Ethics Committee of the Wuhan Center for Disease Prevention and Control. Verbal informed consent was obtained from parents or caretakers of underage patients.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Source data for Fig. 1 are provided with the paper.

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Author contributions

W.-H.K. analysed the data and prepared the manuscript. Y.L. and M.-W.P. conducted the experiments for the detection of influenza and SARS-CoV-2. D.-G.K. and X.-B.Y. collected the specimens and epidemic information. L.W. analysed the data and critically revised the manuscript. M.-Q.L. conducted the SARS-CoV-2 detection, analysed the data and critically revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-020-0713-1.

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		Tested ILI-patients		
		Total	Influenza virus positive	SARS-CoV-2
n		640 (100%)	142 (22.19%)	9 (1.41%)
Sex				
	Female	325 (50.78%)	69 (21.23%)	4 (1.23%)
	Male	315 (49.22%)	73 (23.17%)	5 (1.59%)
Age, years				
	Mean (SD)	22.7 (24.6)	10.0 (11.9)	49.6 (11.2)
	Range	1-87	1-71	35-71
	<5	199	31	0
	5-9	136	76	0
	10-17	51	21	0
	18-29	51	6	0
	30-39	43	3	2
	40-49	25	0	3
	50-60	44	2	3
	>60	90	3	1
Days from illness onset to visiting hospital				
	0 day	82	32	0
	1 day	305	78	6
	2 days	181	27	0
	3 days	72	5	3

Extended Data Fig. 1 | Demographic information of tested ILI patients collected in Wuhan between 6 October 2019 and 21 January 2020 (2019W40 to 2020W03).

Sample ID	Gender	Age	Date of onset	Date visiting hospital	Influenza virus	SARS-Cov-2 ORF1ab/N gene
200077	F	46	2020-1-4	2020-1-7	-	+/+
200101	M	36	2020-1-10	2020-1-13	-	+/+
200110	F	35	2020-1-12	2020-1-13	-	+/+
200115	F	48	2020-1-10	2020-1-13	-	+/+
200144	M	53	2020-1-20	2020-1-21	-	+/+
200145	M	59	2020-1-20	2020-1-21	-	+/+
200149	M	71	2020-1-20	2020-1-21	-	+/+
200153	M	53	2020-1-20	2020-1-21	-	+/+
200158	F	45	2020-1-20	2020-1-21	-	+/+

Extended Data Fig. 2 | Demographic information and illness timeline of nine SARS-CoV-2 infected ILI patients in Wuhan.

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Data collection	No commercial software was used in this manuscript.
Data analysis	No commercial software was used in this manuscript.

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	SARS-CoV-2 was detected from the ILI patients in Wuhan, China
Research sample	640 throat swabs collected from ILI patients between October 6, 2019 and January 21, 2020 were analyzed.
Sampling strategy	This is a retrospective study, all specimens collected were analyzed.
Data collection	Clinical samples were collected according to the latest National Influenza Surveillance Plan, and the information was collected by doctors.
Timing and spatial scale	From October 6, 2019 to January 21, 2020.
Data exclusions	No data was excluded from the analysis.
Reproducibility	The results were double checked by authors.
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☑ No

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☑	☐ Eukaryotic cell lines
☑	☐ Palaeontology
☑	☐ Animals and other organisms
☐	☑ Human research participants
☑	☐ Clinical data

Methods

n/a	Involved in the study
☑	☐ ChIP-seq
☑	☐ Flow cytometry
☑	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	See above
Recruitment	All specimens collected from ILI patients in the 16-week period between October 6, 2019 and January 21, 2020 were recruited.
Ethics oversight	This study was reviewed and approved by the Ethics Committee of Wuhan Centers for Disease Prevention & Control. Verbal informed consent was obtained from parents or caretakers.

Note that full information on the approval of the study protocol must also be provided in the manuscript.