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Research Article

Rapid SARS-Cov-2 Infection Spreading within a Family Cluster: The Importance of Gastro-Intestinal Symptoms

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1. Abstract

1.1. Background: In Phase-2 COVID-19 pandemic, timely control of new outbreaks is pivotal to avoid an epidemic rebound. A better knowledge of clinical disease profiles and the development of sensitive diagnostic flow charts will help to take more effectiveactions.

1.2. Patients and Methods: We studied an outbreak occurred in 3 families during a skiing vacation. Index-case was identified by nasopharyngeal swabs nucleic acid test (NAAT) because of typ-ical respiratory syndrome. Serological assays for anti-Spike glycoprotein (anti-S1) or nucleocapsid protein (anti-NP) of SARS-CoV-2 antibodies were performed at 3 time points.

1.3. Results: Index-case was identified 12 days after the primary-case: a young boy with 12 hours incoercible vomit and severe headache. His close contacts had prevalent gastrointestinal/dysgeusia symptoms, whereas typical respiratory syndrome occurred later in older males. Within 10 days 9 of 12 exposed individuals developed symptoms, SARS-CoV-2 infection was confirmed in 10. The most frequent SARS-Cov-2 specific symptoms were associated with the primary virus entry site, namely mouth/head including pathognomonic dysgeusia and smell loss and headache. Respiratory symptoms occurred only in adult males whereas gastrointestinal symptoms prevailed in both children/adults and males/females. NAAT was negative in 3 subjects 13-26 days from mild disease onset, but also at onset in 2 cases, who turned positive 16 days later. Overall, 10/11 (90.9%) patients tested positive for IgG-anti-NP, IgM and total-anti-S1; IgM-anti-S1 became undetectable in 5/10 during follow-up (33-100 days after onset). In 2 cases IgG-anti-NP became negative 3 months after onset, questioning their long lasting duration. Early isolation and strict adherence to preventive measures avoided secondary and tertiary infections.

1.4. Conclusions: This experience suggests the importance of stringent scrutiny for clustered mild-atypical symptoms and dysgeusia, with immediate isolation of suspected cases and NAAT testing complemented by anti-nucleoprotein and Spike-1 for at least 2 weeks, if negative.

2. Keywords: SARS-CoV-2; Gastrointestinal; Atypical symptoms; Antibodies; Dysgeusia; Anti-nucleo- protein; Anti-Spike1; Serology kinetics; Longitudinal analysis

3. Introduction

The corona virus disease 2019 (COVID-19) outbreak has challenged the preparedness of coun-tries facing primary and secondary health care emergency. To reduce the vulnerability to the SARS-

CoV-2 infection spreading a multi task effort is required to deepen the knowledge of the mechanisms of infection and pathogenesis [8]. Differences in mortality might stem from different mechanisms and/or routes of infection or by environmental and/or host factors [16-21]. Their identification will help taking more effective actions for the control and monitoring of SARS-CoV-2 infection to prevent more severe cases of respiratory illness in the elderly and to investigate both relevance and contagiousness of atypical, asymptomatic infections with low respiratory morbidity [13, 14]. With the progressive easing of the lockdown measures in most Countries, the early identification of new cases will be of paramount importance to avoid the epidemic rebounds. In Europe, the first COVID-19 patient was diagnosed in France on January 24; he had travelled history to China (Sante publique France, 2020), as well as the other cases reported on January 28 in Germany (Bayerisches Staatsministerium fur Ge-sundheit und Pflege, 2020). On February 22, the Italian authorities reported a cluster of cases in Lombardy and additional cases from two other Regions, Piedmont and Veneto. Here we describe a very rapid intra/inter familiar SARS-CoV-2 outbreak in 3 families from Tuscany gathered in a ski resort of the Dolomiteson February 29 for one-week vacation.

4. Materials and Methods

4.1. Case Definitions

The identification of the COVID-19 disease in our cluster followed the recommendation provided on 2 March 2020 by the European Centre for Disease Prevention and Control (ECDC, 2020) and World Health Organization (WHO, 2020) for suspected cases requiring di-agnostic testing. In particular, laboratory testing with RT real time PCR assay detecting the SARS-CoV-2 viral RNA was performed in the first patient with acute respiratory tract infection (sudden onset of cough and fever) with no other etiology, because he was coming back from an area reporting community transmission during the 14 days prior to symptom onset (Il Dolomiti, 2020). This case is referred as Index case, whereas the person who first manifested the disease is referred as Primary case [7]. According to the WHO Interim guid-ance published on 19 March2020, testing asymptomatic or mildly symptomatic contacts was considered for all the individuals who have had contact with a COVID-19 case, although subordinated to the rules imposed by the limited capacity of the laboratories during the emergency phase, in Italy testing was allowed only for close con-tacts with typical symptoms (Italian Ministry of Health, 2020).

4.2. Contact Tracing and Data Collection

The definition of contacts for chasing secondary/tertiary cases followed the same indications (ECDC and WHO, 2020). In particular, in our setting, the contacts of a probable or confirmed case were living in the same house, or having had direct physical interaction, or unprotected direct contact with infectious secretions or face-to-face contact within 2 metres and longer than 15 minutes. Risk contacts were isolated at home, and were actively followed-up through daily calls. They were asked to measure their body temperature twice daily for 14 days after their last exposure and, in case of fever or respira-tory symptoms to wear a surgical mask and to contact the dedicated hotline. All cases and contacts were carefully interviewed on expo-sure, clinical history and time course oftheirsymptoms.

4.3. Laboratory Assays

After notification of the study to the Ethical Committee of our Institution all the subjects enrolled provided a written informed consent for reporting their clinical history and performing laboratory tests. Samples from nasopharyngeal swabs were tested for SARS-CoV-2 viral RNA using the Simple xa™ COVID-19 Direct real time RT-PCR Kit according to the manufacturer's instructions (DiaSorin Mo-lecular- US FDA EUA - CE-IVD) at the local reference Laboratory of Virology, University-Hospital of Pisa, Italy. Serological assays to detect antibodies against anti-Spike glycoprotein (S1) or nucleocapsid protein of SARS-CoV2 were performed using Wantai SARS-CoV-2 Ab ELISA, Wantai SARS-CoV-2 IgM ELISA kits (Beijing Wantai Bi-ological Pharmacy Enterprise Co., Ltd, RUO) and SARS-CoV-2 IgG Abbott (Abbott Laboratories Diagnostics Division Abbott Park, IL 60064 USA), respectively at the Laboratory of our Hepatology Unit.

5. Results

5.1. Outbreak Description

Type and time course of the symptoms with the results of molecular assays in the members of the 3 families are shown in (Figure 1).

The three families arrived at the ski resort on Saturday February 29 evening. After the Sunday spent skiing, on Monday March 2 morning (day 0) the 13 years old boy of family 1 (case 1) got incoercible vomit that lasted 12 hours (30 vomiting episodes) and ended with a short lived severe headache. He had a long sleep and recovered completely. On Wednesday morning (day 2) his sister (case 2) developed nausea, dyspeptic syndrome, dysgeusia and loss of smell, soon accompanied by cold symptoms without fever. On Wednesday evening also her fa-ther (case 3) and the lady (case 4) of family 2, who is a medical doctor and frequently visited the room of family 1 during the disease of the boy, developed cold symptoms without fever. On Thursday (day 3) the mother of family 1 (case 5), medical doctor too, experienced di-arrhoea lasting for 4 days and on Saturday (day 5), before leaving for home, she had conjunctivitis, cold symptoms with myalgias and fever on the evening (body temperature up to 38.0° C). Her fever declined after 24 hours, with persistence of cold and occurrence of cough. On Sunday (day 6), the father of family 3 (case 6), who is medi-cal doctor as well and spent the last 2 days skiing with members of family 1, had diarrhoea, myalgias for 3 days followed by mild cough accompanied by intermittent cold for more than one week. Three days after the clinical onset of her husband (day 9), the mother of family 3 (case 7) experienced diarrhoea, myalgia and cold that lasted for few days. After returning home, on Thursday (day 10), the father

of family 2 (case 8) complained of cold and fever (38.5^0 C) that persisted till Saturday (day 12) when, because of the concomitant dry cough, he got a nasopharyngeal swab for SARS-CoV-2 nucleic acid amplification test (NAAT), that turned positive. At this time all the 3 families entered a quarantine period, however the father of family 2 (index case), as medical doctor was already in self-isolation at home from the day when they came back from the mountain (day 5). He had only a close contact on Sunday evening (day 6) with his 52y old brother living in the same house. His brother (case 9) got fever and caught on the next Saturday (day 12) and, on Monday (day 14), had a nasopharyngeal swab positive too: after one week at home with fever and cough, he was admitted to the COVID Centre of the University Hospital of Pisa due to the occurrence of dyspnoea, and discharged 5 days later after rapid clinical improvement. The 12 years old son of family 2 remained asymptomatic and did not undergo any naso-pharyngeal swab; his mother complaining very mild cold symptoms from day 2 to 7, tested negative at the NAAT assays on days 28 and 38, before being readmitted to work at the hospital.

After the COVID-19 index case (day 12) diagnosis, the mother of family 1 (medical doctor) underwent her first nasal-pharyngeal swab, two weeks after her first clinical manifestations (day 16) and test-ed positive; on the next week (day 22) all her relatives underwent the NAAT tests and resulted positive. The mother maintained cold symptoms and coughs for one week and experienced a rebound of diarrhea with cold symptoms after an apparent remission from day 20 to 22. The father experienced one episode of severe asthenia with fever (37.8°C) the same day of the nasopharyngeal swab, rapidly im-proving thereafter. Both parents complained of dysgeusia and smell loss for about 3 weeks. The boy was fully asymptomatic since day 2; his sister was fine, but with persisting dysgeusia and smell loss for about 3 weeks. The boy, his sister and father were NAAT negative 37 to 39 days after clinical onset; the mother after 47 days.

All members of family 3 underwent their first NAAT testing on day 21, resulting negative. At that time both parents were already asymp-tomatic, whereas the older daughter (case 10) was complaining of fever and cold since 2 days and developed dysgeusia with smell loss thereafter. The younger daughter (case 11) had the onset of fever and cold on the same day of NAAT testing, with complete recovery after two days. Both of them were positive at the NAAT test repeated on day 37 (19 and 17 days after clinical onset, respectively), whereas their parents remained negative. The 2 girls had a negative swab 53 and 55 days after onset.

All but one adult involved in the outbreak was MD, so they decided to self isolate at home after returning from skiing. Only one of them (case 5) had to spent 3 days at work (from 11th to 13thMarch 2020) and had face-to-face contacts within 2 metres for more than 15 min-utes with 17 individuals (secondary contacts), wearing the surgical mask. In the 2 following weeks, 4contacts developed symptoms (gas-trointestinal in two, cold in one and mixed in one), that were mild and short lasting (less than 3 days) in all cases, none was found positive for SARS-CoV-2 infection by NAAT. The brother of the index case (case 9) had only familiar contacts: his wife remained asymptomatic, whereas the 13 years old twins and the 17 year old son complained of mild gastrointestinal symptoms, none of them were tested by NAAT (Figure 2).

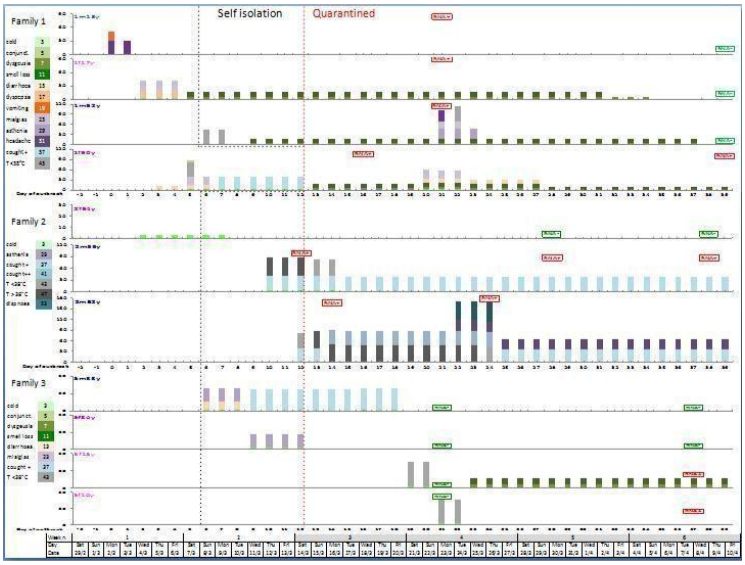

Figure 1: Type and time course of the symptoms during the SARS-CoV-2 outbreak in the 3 families.
Legend: the label reports in sequence: family number, gender and age. To represent overtime the complexity and the severity of the symptoms in the individuals, con-secutive prime numbers from 3 to 53 were associated to each symptom and the sum was plotted in the graph. The values increase with the increasing relevance of the symptoms, being the minimum of 3 attributed to cold and maximum of 53 to dyspnea. Positive results of SARS-CoV-2 by NAAT are shown in red (RNA+) and negative in green (RNA-).

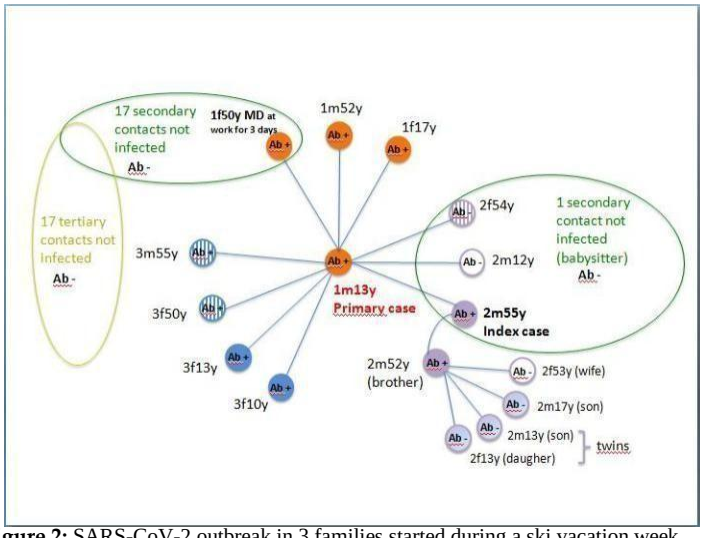

Figure 2: SARS-CoV-2 outbreak in 3 families started during a ski vacation week in the Dolomites
Legend: label indicates family number, gender and age. Void: asymptomatic / no swabs. Shaded: mild symptomatic / no swabs. Bars: symptomatic without viral RNA in the swabs. Full colored: symptomatic with viral RNA in the swabs.Ab + and Ab - indicates presence or absence of antibody response detected in at least one one serum sample.
At the end of March all the 17 secondary contacts of case 5 and their 17 households (10 adults, 5 sons and 2 daughters), who represent tertiary contacts underwent serology testing and all were negative for anti-Spike (S1) antibodies (total and IgM) and anti-nucleocapsid protein (IgG). In family 2 there was only one secondary con-tact (a 31 years old woman who worked as babysitter), who did not develop any symptom and tested negative at serology.

Overall SARS-CoV-2 infection was confirmed by NAAT in 72.7% (8/11) of symptomatic individuals involved in the outbreak (Table 1). Nasopharyngeal swabs, however, were taken at different times from the onset of the symptoms (4-26 days) and 3 subjects with mild disease tested negative at 12, 15 and 26 days after the clinical onset respectively. Only one asymptomatic individual was never tested.

5.2. Serological Assays

During the isolation period a blood sample was obtained on March 24 from the members of family 1 and 3 and on April 4 for family 2. A second blood sample was obtained 2 weeks later in family 1 and 3, and about 4 weeks later in family 2 (after completion of the isolation period). Finally a third sample was obtained within the June 15 (104 days since the outbreak onset) in all the individuals. The results are reported in (Table 2). All samples were tested for anti-Spike glycoprotein (S1) total and IgM antibodies and IgG anti-nucleocapsid protein. All members of family 1 tested positive for the 3 assays at first time point. The girl became IgM anti-Spike glycoprotein (S1) negative at the second time point, all the other family members but the father, at the third time point, when the girl was negative also for IgG anti-nucleocapsid protein.

Table 1: Detection of SARS CoV-2 RNA in swabs.

Fam	Gen	Age (y.)	N.	Symptoms	Date of onset	Date of 1 swab	Days from onset	RNA in swab	Date of 2 swab	Days from onset	RNA in swab	Date of 3 swab	Days from onset	RNA in swab
1	m	13	1	Yes	2-Mar	23-Mar	21	+	10-Apr	39	-			
1	f	17	2	Yes	4-Mar	24-Mar	20	+	10-Apr	37	-			
1	m	52	3	Yes	5-Mar	25-Mar	20	+	10-Apr	36	-			
1	f	50	5	Yes	5-Mar	18-Mar	13	+	10-Apr	37	+	20-Apr	47	-
2	f	54	4	Yes (mild)	4-Mar	30-Mar	26	-	9-Apr	36	-			
2	m	12	-	No	*na*	*nd*			*nd*			*nd*		
2	m	55	8	Yes	10-Mar	14-Mar	4	+	9-Apr	30	+	18-Apr	39	-
2	m	52	9	Yes (severe)	12-Mar	16-Mar	4	+	26-Mar	14	+	15-Apr	34	-
3	m	55	6	Yes	8-Mar	23-Mar	15	-	8-Apr	31	-			
3	f	50	7	Yes	11-Mar	23-Mar	12	-	8-Apr	28	-			
3	f	13	10	Yes	21-Mar	23-Mar	2	-	6-Apr	16	+	13-May	53	-
3	f	10	11	Yes	23-Mar	23-Mar	0	-	8-Apr	16	+	13-May	53	-

Legend:na: not available; nd: not done.

Table 2: Detection of circulating anti-spike 1 total and IgM antibodies, and anti-nucleoprotein IgG antibodies.

Fam	Gen	Age (y.)	N.	Symptoms	Date of onset	Date 1st blood test	Days from onset	Anti Sp-1 Tot	Anti Sp-1 IgM	Anti NcIgG	Date 2nd blood test	Days from onset	Anti Sp-1 Tot	Anti Sp-1 IgM	Anti NcIgG	Date 3nd blood test	Days from onset	Anti Sp-1 Tot	Anti Sp-1 IgM	Anti NcIgG
1	m	13	1	Yes	2-Mar	24-Mar	22	+	+	+	6-Apr	35	+	+	+	10-Jun	100	+	-	+
1	f	17	2	Yes	4-Mar	24-Mar	20	+	+	+	6-Apr	33	+	-	+	9-Jun	97	+	-	-
1	m	52	3	Yes	5-Mar	24-Mar	19	+	+	+	6-Apr	32	+	+	+	9-Jun	96	+	+	+
1	f	50	5	Yes	5-Mar	24-Mar	19	+	+	+	6-Apr	32	+	+	+	5-Jun	92	+	-	+
2	f	54	4	Yes (mild)	4-Mar	3-Apr	30	-	-	-	28-Apr	55	-	-	-	12-Jun	100	-	-	-
2	m	12	-	No	*na*	3-Apr	*na*	-	-	-	28-Apr	*na*	-	-	-	na				
2	m	55	8	Yes	10-Mar	3-Apr	24	+	+	+	28-Apr	49	+	-	+	16-Jun	98	+	-	+
2	m	52	9	Yes (severe)	12-Mar	3-Apr	22	+	+	+	28-Apr	47	+	+	+	16-Jun	96	+	+	+
3	m	55	6	Yes	8-Mar	24-Mar	16	+	+	+	7-Apr	30	+	+	+	12-Jun	96	+	+	+
3	f	50	7	Yes	11-Mar	24-Mar	13	-	+	+	7-Apr	27	-	+	+	12-Jun	93	+	-	-
3	f	13	10	Yes	21-Mar	24-Mar	3	-	-	-	7-Apr	17	+	+	+	12-Jun	83	+	-	+
3	f	10	11	Yes	23-Mar	24-Mar	1	-	-	-	7-Apr	15	+	+	+	12-Jun	81	+	-	+

Legend: Anti Sp-1: anti-spike S1 total and IgM antibodies (Wantai); Anti NP: anti-nucleoprotein IgG antibodies (Abbott).

In family 2, the 12 years old son (the only asymptomatic subject) and her mother (case 4), who complained of a mild cold from day 2 to 7, tested negative at the 3 assays. The father (index case) and his brother (cases 8 and 9) were total anti-Spike (S1) and anti-nucleocapsid pro-tein positive when they were re-admitted to work, 49 and 47 days af-ter their disease onset. At the same time point IgM anti-SARS-CoV-2 was positive only in case 9, not anymore in the index case. The wife and the three children of case 9 were negative for the 3 assays after the end of the quarantine.

The father of family 3 (case 6), symptomatic from day 6 to 18, tested positive for all the 3 assays at the 3 time points. His wife (case 6), who had only mild symptoms from day 9 to 12, tested positive for IgM anti-Spike (S1) at the first 2 time points, but negative at the third (56 days after disease onset). By converse, she was positive for IgG an-ti-nucleocapsid protein at all the 3 time points. Their daughters (cases 10 and11), tested negative for all the anti SARS-CoV-2 Ab at the on-set of their clinical manifestations, but total and IgM anti-Spike (S1) and IgG anti-nucleocapsid proteins turned positive on day 15 and 17.

Assuming that all the 11 symptomatic individuals in the family clusters got SARS-CoV-2 infection, NAAT in swabs taken according to ECDC and WHO guidelines during the epidemic was able to reveal the infection in 8 (72.7%) patients. The combination of total and IgM anti Spike (S1) and IgG anti-nucleocapsid protein tests repeated 2-4 weeks apart allowed diagnosis of 2 more cases (10/11), reaching 90.9% sensitivity. The only asymptomatic subject, never tested by NAAT, was serologically negative.

6. Discussion

The history of this rapid SARS-CoV-2 outbreak, where 11 of 12 individuals within the family cluster were exposed to the same virus strain and developed symptoms in a very short time and 10 had a confirmed infection, suggests that an early identification of the infected individuals and their timely isolation are the most important action to limit the spread of the infection. The rapid control of new outbreaks is pivotal in the pandemic Phase-2 when, with the progressive eas- ing Phase-1 lockdown, such a measure remains the only action to block the epidemic rebound. Actually, the 12 days lag time from the onset of the symptoms in the primary case and the identification of the index case appears too long for an appropriate management and effective containment of the infection. Luckily, in our outbreak 5 of 6 adults underwent a voluntary self-isolation at home, whereas due to the concomitant lockdown imposed by the Italian Government, also the 5 young students remained at home. Altogether these mea-sures avoided a larger spreading of the infection in spite of a delayed diagnosis and the long lasting viral shedding indicated by the results of the repeated nasopharyngeal swabs. Furthermore, it is noteworthy that the strict adherence by the Family 1 mother to preventive mea-sures of transmission (physical distancing and surgical mask wearing) during her 3 working days in the Hospital avoided the spread of the infection to her secondary/tertiary contacts (Figure 2).

The transmission chain in our outbreak is shown by the link between contacts and onset of symptoms, including atypical ones (Figure 1) and confirmed by NAAT (obtained 1-26 days after clinical onset) and serological assays (obtained 1-55 days after the clinical onset). During the emergency phase of the epidemic, however, a major diagnostic limitation was the NAAT performed only after "typical respiratory symptoms" (fever >38°C and dry cough with/without dyspnoea) for at least 3 days. This unique opportunity of studying the natural his-tory of symptoms at presentation in different individuals exposed to the same virus strain underlines the importance of gastrointestinal symptoms like diarrhoea, dyspepsia and vomit which were not con-sidered hallmarks of SARS-Cov-2 infection. The most common (> 90% of cases, Table 3) symptoms at presentation were un-specif- ic, namely cold and/or fever associated with asthenia and/or myal- gia particularly in adults. Among SARS-Cov-2 specific symptoms the most frequent were related with the primary virus entry site, namely mouth/head and included the pathognomonic dysgeusia and smell loss and headache (Table 3). The second specific symptom series was shared by both gastro-intestinal and respiratory symptoms overall, but whereas respiratory symptoms (cough and dyspnea) occurred only in adult males whereas gastrointestinal symptoms prevailed in both children/adults and males/females (Table 3). Fortunately, some of our patients, who were medical doctors, underwent a late swab test once the index case was identified, but they were already quar-antined according to the procedures activated to minimize the in-fection spreading within our Hospital. All Family 1 members got a NAAT (19 to 21 days from the clinical onset) to better understand the infection spreading, given the clinical heterogeneity. Consequent-ly we could demonstrate that the primary case, the Family 1 youngest member who suffered of severe vomit and headache, but recovered rapidly without other clinical manifestation, was still NAAT positive 21 days after the clinical onset symptoms in spite of being asymp-tomatic since almost 3 weeks. Gastrointestinal symptoms (vomiting, dyspepsia and diarrhoea) were reported in SARS-CoV-2 infected patients, however their occurrence at presentation is rather unusu-al, although more frequent in children than in adults (10% vs 3%) [9, 15, 20]. Thus, children who have higher probability of not being diagnosed may contribute significantly to the spread of the infection because of their long-lasting virus carrier ship proven in our and Chi-nese cases (2-4weeks) [3]. The NAAT was positive 19-21 days after clinical onset not only in the primary case but also in his sister, who complained of dyspepsia, dysgeusia, smell loss and minimal cold symptoms. All Family 1 members developed symptoms within 3 days one from the other; both parents showed a relapsing course of their symptoms (dysgeusia, smell loss, cold and diarrhoea) without acute respiratory symptoms (mild cough in the mother only). Thus for an earlier diagnosis and isolation of primary cases, gastrointestinal symptoms should be taken in much higher consideration. Moreover, pathognomonic manifestations, like dysgeusia and smell loss should prompt NAAT testing, particularly when occurring in temporal relationship among close contacts.

Table 3: Prevalence and Duration of Symptoms in Infected Individuals.

	Asthenia	Myalgia	Cold	Fever	Dysgeusia	Smell Loss	Headache	Cough	Dyspnea	Vomit	Dyspepsia	Diarrhea
Subjects												
1M13y							2			1		
1F17y		3	3		30	27						3
1M52y	3	2		4	29	29	1					
1F50y				5	15	27		6			3	9
2F54y			6									
2M55y				5				30				
2M52y	18			13				28	3			
3M55y		3	3					10				3
3F50y		4	4									
3F13y			2	2	17	17						
3F10y			2	2								
Total	2	4	6 54.5%	5	4	4	3	4	1	1	1	3
%	18,2%	36,4%	-20	45,5%	36,4%	36,4%	27,3%	36,4%	9%	9%	9%	27,3%
(days)	-21	-12		-31	-91	-100	-3	-74	-3	-1	-3	-14
Overall	**5 (45,5%)**		**10 (90,9%)**		**5 (45,5%)**			4 (36,4%)		4 (36.4%)		
children	1 (25%)		**3 (75%)**		**3(75%)**			0		2 (50%)		
adults	**4 (57,1%)**		**7 (100%)**		2 (28.6%)			**4 (57,1%)**		2 (50%)		
females	2 (33,3%)		**6 (100%)**		3 (50%)			0		2 (33.3%)		
males	**3 (60%)**		**4 (80%)**		2 (40.%)			**4 (100%)**		2 (40%)		

Legend: Sars-CoV-2 as a common corona virus caused cold and fever and associated symptoms (asthenia and myalgia) in > 90% of infected individuals, particularly in adults. Among Sars-CoV-2 specific symptoms the most frequent were related with the primary virus entry site, namely mouth/head and included the pathognomonic dysgeusia and smell loss and headache. The second specific symptomseries was shared by both gastro-intestinal and respiratory symptoms overall, but whereas respiratory symptoms occurred only in adult males gastrointestinal ones had comparable prevalence in children/adults and males/females.

The control of the virus spread within family members in the household appears quite difficult: in family 3 the results of NAAT and Ab sequential assays (Table 1 and 2) suggest that the parents transmit-ted the infection to their daughters. Similarly, the only close contact (without preventive measures) outside the persons present in the ski resort, was the brother of the index case (case 8), who spent one evening with him, still asymptomatic and developed COVID symptoms thereafter (case 9). Nevertheless, the family 2 son who had very close contacts for several days with the primary case and with his sister in the ski resort, and with his father (the index case) until he became symptomatic, did not develop any symptom/marker of infection. Furthermore, also the 3 children of case 9 did not develop detectable antibodies in spite of minor gastroenterological symptoms. These findings raise the question whether patients with asymptomatic infection or mild disease limited to the mucosal virus entry, but without systemic involvement, might lack circulating antibodies detectable by currently available assays. Accordingly, the Family 2 mother, who developed mild cold without fever, timely related with the exposure to the primary case, not only resulted negative at the nasopharyngeal swab, unfortunately obtained only 26 days after her symptoms, but also at the antibody tests. In addition, the Family 3 mother with mild and short lasting disease was IgM anti-Spike (S1) and IgG anti-nu-cleocapsid protein positive in the first time points (13 and 27 days after onset), then weakly positive for total anti-Spike (S1) antibody (92 days from onset), but negative for both IgM anti-Spike (S1) and IgG anti-nucleocapsid protein.

A bias of our study of both NAAT and antibodies kinetics is the sampling limited by the criteria of patient's selection for nasopharyngeal swabs and by the constraints of the home-quarantine. The nasopharyngeal swabs were performed within a large range of 1 to 26 days from the onset of symptoms. Even if serological assays were missing before day 13 (with exclusion of the Family 3 sisters who were tested at the very beginning of their disease), we monitored the antibody fluctuations up to 3 months. Combined NAAT and antibodies testing prompted interesting observations: the detection of viral nucleic acid, the SARS-CoV-2 gold standard test [5, 17], was positive in 73% of cases and negative in 3 patients when performed 13-26 days after a mild disease onset. Noteworthy, the first nasopha-ryngeal swab was negative at disease onset in the Family 3 daughters, who turned positive 16 days later. These findings confirm that the nasopharyngeal swab in spite of very high specificity has asensitivity hampered by the swab sampling errors [25, 26]. Its complementation with the serological assays proved that the infection occurred in 2 more cases. Overall, 10 of the 11 (90.9%) individuals who developed symptoms during the outbreak tested positive for IgG anti-nucleo-protein, IgM anti-Spike1 and total anti-Spike1 antibodies. IgM anti-bodies against anti-Spike (S1) were detected in all cases during the early phase of the infection and became undetectable 33 to100 days after disease onset in 50% of cases. Our findings agree with previous reports, showing significant sensitivity of serological assays after 10-12 days from clinical onset [4] and earlier IgM antibody response that declines within few weeks after disease resolution. The serologic test-ing, combining both the anti-nucleocapsid protein and anti-Spike1 antibodies appears to be the more consistent approach, although the evidence that 2 cases (2 and 7) became IgG anti-nucleocapsid-pro-tein negative 3 months after disease onset raises the question about the persistence of these antibodies and prompts future studies to monitor overtime the kinetics of the antibody response to clinical severity of COVID-19. Moreover, it would be important to improve the sensitivity of NAAT assays, maybe testing also saliva and stool

specimens, in particular in cases with gastrointestinal symptoms [1, 23-24].

In conclusion, an early identification of primary cases is mandatory to properly contain the spread of the infection and the mostimport-ant action is the stringent scrutiny of clusters of mild gastrointes-tinal symptoms combined with pathognomonic signs of dysgeusia and/or loss of smell in the household contacts. The identification of these symptoms cluster should prompt the immediate isolation of the suspected cases and their NAAT testing to be repeated and com-plemented by serological antibody assays for both anti-nucleoprotein and anti-Spike1 antigens every week for at least 2 weeks, if negative.

References

1. Azzi L, Carcano G, Gianfagna F. Saliva is a reliable tool to detect SARS-CoV-2, Journal of Infection.
2. Bayerisches Staatsministeriumfür Gesundheit und Pflege. Bestatigter Coronavirus-Fall in Bayern- Infektionsschutzmaßnahmenlaufen. Mu-nich: BSfGuP; 2020.
3. Cai J, Xu J, Lin D, Yang z, Xu L, Qu Z et al. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features. Clinical Infectious Diseases. 2020.
4. Cheng MP, Yansouni CP, Basta NE, et al. Sero diagnostics for Severe Acute Respiratory Syndrome-Related Coronavirus-2: A Narrative Re-view [published online ahead of print, 2020 Jun 4]. Ann Intern Med. 2020; M20-2854.
5. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020.
6. https://www.ecdc.europa.eu/en/covid-19ECDC.%20An%20agen-cy%20of%20the%20European%20Union.%20Available%20at%20 https:/www.ecdc.europa.eu/en/case-definition-and-european-sur-veillance-human-infection-novel-coronavirus-2019-ncov.%20Ac-cessed%20April%2028,%202020.
7. Giesecke J. Primary and index cases. The Lancet. 384 (9959): 2024. 2014.
8. Gilbert M, Pullano G, Pinotti F, Valdano E, Poletto C, Boelle PY et al. Preparedness and vulnerability of African countries against impor-tations of COVID-19: a modelling study. Lancet. 2020 Feb 20. pii: S0140-6736: 30411-6.
9. Gu J, Han B, Wang J. COVID-19: Gastrointestinal manifestations and potential fecal-oral transmission.Gastroenterology. 2020 Mar 3. pii: S0016-5085: 30281-X.
10. Hoffmann M, Kleine-Weber H, Schroeder S, Muller MA, Drosten C, Pohlmann SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.
11. https://www.ildolomiti.it/cronaIl%20Dolomiti.%20https:/www.ildo-lomiti.it/cronaca/2020/coronavirus-la-mappa-dei-positivi-comune-per-comune-36-casi-a-bolzano-ma-in-val-gardena-dove-ci-sono-gli-impianti-ce-ne-sono-a-decine.%20%20Accessed%20April%2028,%20 2020.
12. Italian Ministry of Health.Circular0005443-22/02/2020-DG-PRE-DGPRE-P at: http://www.salute.gov.it/portale/temi/ p2_6.jsp?lingua=italiano&id=813&area=Malattie infettive& menu=vuoto.
13. Lai C-C, Liu YH, Wang C-Y, Wang Y-H, Hsueh S-C, Yen M-Y et al. Asymptomatic Carrier State, Acute Respiratory Disease, and Pneumonia Due to Severe Acute Respiratory Syndrome Coro-navirus 2 (SARS-CoV-2): Facts and Myths. J Microbiol Immunol Infect. 2020: S1684-1182(20)30040-2.
14. Li Y-C, Bai W-Z, Hashikawa T. The Neuroinvasive Potential of SARS-CoV2 May Be at Least Partially Responsible for the Respi-ratory Failure of COVID-19 Patients J Med Virol 2020.
15. Pan L, Mi M, Gang Ren H et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study.
16. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarityin Case Fatality Rates (CFR) of COVID-19/SARS-COV-2 in Italy and China. J Infect Dev Ctries J Infect Dev Ctries. 2020; 14: 125-8.
17. Remuzzi A and Remuzzi G. COVID-19 and Italy: what next? The Lancet, March12, 2019.
18. Sante publique France. Epidémie de coronavirus Covid-19 au départ de Wuhan, Chine. Paris: Santé publique France; 2020.
19. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z et al. Character-istics of COVID-19 infection in Beijing J Infect. 2020 Feb 27. pii: S0163-4453(20)30101-8.
20. Tian Y, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. Aliment PharmacolTher. 2020; 51: 843-51.
21. Wang Y, Wang Y, Chen Y. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. J Med Virol. 2020.
22. WHO. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. Available at https://www.who.int/ publications-detail/laboratory-testing-for-2019-novel-corona-virus-in-suspected-human-cases-20200117. Accessed April 28, 2020.
23. Williams E, Bond K, Zhang B, Putland M, Williamson DA, Saliva as a non-invasive specimen for detection of SARS-CoV-2. JCM Accepted Manuscript Posted Online 21 April 2020. J. Clin. Mi-crobiol.
24. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M et al. Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs.
25. Xia N, Wang G, Gong W Serological test is an efficient supple-ment of RNA detection for confirmation of SARS-CoV-2 infec-tion. Preprints posted 2020.
26. Xie C, Jiang L, Huang G, Pu H, Gong B, Lin H et al. Comparison of different samples for 2019 novel coronavirus detection by nu-cleic acid amplification tests. Int J Infect Dis; 2020.