Dynamic characteristics of Influenza A epidemic in children during the early stage of COVID-19 outbreak

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Research Article

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Abstract

Background: At the beginning of the outbreak of coronavirus infected disease 2019 (COVID-19), children in Wuhan were experiencing an extremely strong influenza A epidemic. This study is aimed to explore the epidemic dynamics characteristics of children with influenza A and its correlation with the early stage spread of COVID-19 in Wuhan.

Methods: This is a retrospective single-center clinical study.From November 28, 2019 to January 23, 2020, a total of 7904 outpatient children with signs of respiratory tract infection were admitted, and a total of 10102 throat swabs were collected. All the detection were performed to the throat swabs of patients, which include the epidemic statues, detection rate, duration of Flu A and B persistence in airway, and the positive rate of COVID-19 nucleic acid.

Results: A total of 10102 throat swabs were obtained from children with respiratory symptoms, including 5450 (53.9%) male and 4652 (46.1%) female. 2899 (28.7%) cases were positive for Influenza A. There were 617 (6.1%) cases of Influenza B . In group of Influenza A , the lowest positive rate was in the infants less than 1 year old (18.4%), and the highest in the group 12 year old (32.1%). During the period of high prevalence of influenza A, there was a low level of infection of influenza B. The detection rate of each age group fluctuated from 3% to 10%. 73.7% of children's influenza A and B virus turned negative within 7 days, and very few children's respiratory influenza virus can last even more than 1 month. Among 35 throat swabs detected with qRT-PCR, 11 (31.4%) were positive for Flu A, and all children were negative for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Conclusions: In the early stage of SARS-CoV-2 transmission, children in Wuhan were experiencing a high intensity of influenza A pandemic, and there was no the mixed infection of SARS-CoV-2 with influenza A. COVID-19 spread caught up the tail of influenza A pandemic.

Introduction

Since the first case of coronavirus disease-2019 (COVID-19) was reported on December 8, 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly in the international wide[1, 2] .As of April 8, 2020, the number of confirmed infections in the world has reached 1 439, 516, with 85, 711 deaths[3]. Children and newborn infants were also involved in[4-6]. The epidemiological characteristics of COVID-19 and the origin of the virus are not clear yet[4-6]. It is speculated that it may be related to wild life trade[8]. However, there is still no final conclusion on the original or intermediate host[2, 4, 9].

Before the outbreak of COVID-19, children in Wuhan were experiencing in a high incidence of influenza A (Flu A) infection. The span of Flu A epidemic just covered the stage from the emergence to rapid transmission of SARS-CoV-2. In order to investigate the correlation of epidemic dynamics between the emergence of SARS-CoV-2 and of Flu A, we carried out a retrospective analysis of the children who had

respiratory infection signs in this period. SARS-CoV-2 nucleic acid was detected from pharyngeal swabs to clarify whether children had been infected with COVID-19 during the pandemic of influenza virus, as well as the role transformation of these virus epidemic intensity during the concurrent phase.

It is urgent to provide more epidemiological data for early identification and prevention of SARS-CoV-2 transmission[7, 10]. In this study, the epidemic dynamics of the role switching of common respiratory virus and the outbreak of a novel emerging virus are analyzed.

Methods

Objects

From November 28, 2019 to January 23, 2020, outpatient children in the Pediatrics Department, Zhongnan Hospital of Wuhan University were included. A total of 7904 children were admitted to the detection for 1 to 5 times, they showed with signs of respiratory tract infection such as fever, nasal congestion, runny nose and headache muscle pain, or accompanied by vomiting, abdominal pain, diarrhea. At last, a total of 10102 pharyngeal swabs were collected, 5934 children were sampled once, 1695 children sampled twice, 303 children sampled 3 times, 32 children sampled 4 times, and 8 children sampled more than 5 times. The longest continuous sampling time was once a week for four weeks. From November 28, 2019 to December 28, 2019, pharyngeal swabs of 35 children were cryopreserved at -18℃ for respiratory pathogenic surveillance in winter. On January 28, 2020, when COVID-19 pandemic in Wuhan, the frozen pharyngeal swabs were taken out for SARS-CoV-2 nucleic acid detection.

Methods

Virus antigen detection

Within 1-3 days after the onset signs of upper respiratory tract occurred, children's pharyngeal swabs were taken from the outpatient department of Pediatrics. The samples were stored in 1 ml DMEM without bovine serum, sealed in refrigerator at 4℃, and then sent to the clinical laboratory for microbial detection on the same day. Influenza virus antigen was detected by immunocolloidal gold method (Wanfu Biotechnology Co., Ltd. China), including influenza A, B and H7 viruses. The test operation was completed according to manufacturer's instruction.

Virus nucleic acid detection

After the outbreak of the novel croronavirus, 35 pharyngeal swabs were thawed on January 28, 2020. The total RNA was extracted and the SARS-CoV-2 nucleic acid was detected by qRT-PCR. Operation was under the instruction of China's novel coronavirus (ORF1ab/N) nucleic acid detection kit (BioGerm Medical Technology Co., Ltd. China) , based on the guidelines of the Center for Disease Control and Prevention in China[11].

Statistical methods

The general information of outpatient children was collected and sorted out by LIS system (Neusoft, China), including gender, age, chief complaint and clinical diagnosis. The data was analyzed by SPSS 26.0 software (IBM, U.S.A.). Data is shown as X±s, and median, t-test is used for comparison between the two groups; chi-square test is used for comparison of measurement data. Person test is used for correlation analysis. $p < 0.05$ is considered statistically significant.

Ethics approval

This study was approved by the Medical Ethical Committee of Zhongnan Hospital of Wuhan University (clinical ethical approval number 2020004) and informed consents of specimen's collection was waived.

Results

1. **Epidemic statues of influenza A and B virus**

During the observation period, 10102 pharyngeal swabs were collected, including 5450 (53.9%) males and 4652 (46.1%) females, with a median age of 6 years (2 months-14 years). The distributions of daily sampling times were showed in Figure 1. Among the samples, 2899 (28.7%) were positive for Flu A, 1634 (30.0%) were male and 1265 (27.2%) were female. There were 617 positive cases of Flu B (6.1%), 336 (6.2%) males and 281 (6.0%) females.

Taking the data of influenza-like symptoms on November 28 as the baseline, there were 47 phryngeal swabs on that day, 3 (6.38%) and 3 (6.38%) were positive for Flu A and Flu B respectively. The first peak of Flu A occurred on December 2, 2019. Outpatient cases for sampling increased by 20% (1.43 folds) compared with the baseline, and the detection rate was 25.37% (3.98 folds). On December 6, 2019, it reached the second peak, with 126 sampling times (2.64 folds), 40 positive people (13.33 folds), and the detection rate of 32.26% (5.06 folds). The highest positive rate of Flu A occurred on December 15, 2020, reaching to 40.12% (6.29 folds). 132 children with Flu A were positive, 44 folds as many as the number of the initial stage. The number of pharygeal swabs was the most on December 22, 2019, with 412 samples per day, and the detection rate was 28.13%. After January 5, 2020, the detection rate of Flu A dropped to below 25%. On January 10, 2020, the number of samples detected was reduced to less than 100. By the end of the observation period, on January 20, 2020, the number of sampling visits and the detection rate of Flu A were reduced to 46 and 4.35% respectively, returning to the level of the initial stage of the epidemic.

During the same time period, the detection rate of Flu B remained a stable proportion, with an median range of daily detection rate of 6.06% (3.29% - 16.67%), while the detection rate was negatively correlated with Flu A (CI 95%, $r = -0.3908$, $P = 0.0026$). In the early and late stage of this Flu A epidemic, the detection rate of Flu B is significantly increase, which can reach to more than 10% of the detected population. Therefore, during the period of intensive incidence of Flu A, there was still a low intensity epidemic of Flu B.

2. **Detection rate of influenza A and B virus in different ages**

According to the age classification, the detection rate of Flu A was lowest in the group less than 1 year old (18.40%), 25% in the group of 3 years old and above, and 32.07% in the group of 12 years old. According to gender classification, the detection rate in boys younger than 1 year old was 19.11%, 2 years old group was 24.30%, 3 years old and above group was more than 25%; The distribution of girls was similar to that of boys, but the detection rate of 2 years old group was less than 20%, and that of 3 years old and above group was more than 25%.These results showed that children over 3 years old were more susceptible to Flu A (Table 1).

There was a low level of infection of Flu B during the high prevalence period of Flu A. the detection rate of each age group fluctuated between 3% and 10% (Table 2). But only 1.9% of the children were positive in the 13 years old group and 0 in the 14 year old group. The epidemic intensity of influenza B was negatively correlated with influenza A ($P < 0.01$). In addition, both Flu A and B antigens (48, 0.48%) were detected in the same pharygeal swab, indicating that there may be presence of mixed infection. Two children were detected with H7 antigen in December 2019.

3. **Time of influenza A and B virus antigens turning negative**

The time from positive to negative was recorded in 1191 patients. The median time of Flu A antigen turning negative was 6 days (1-30 days). 73.6% of pharygeal swabs turned negative within 7 days, and 3.7% of pharygeal swabs remained positive for more than 14 days. Among them, 4 children aged 2-4 years old with upper respiratory tract infection were persistent positive over 30 days after symptoms disappeared. 192 cases Flu B completed twice tests. The median time of antigen turning negative was 6 days (2-19 days). These results showed that the turning negative time of Flu A and B virus in pharygeal swabs of most children was less than 7 days, but the survival time of respiratory influenza virus in a few children can last for more than 1 month.

4. **Alternation of influenza A epidemic and COVID-19 outbreak**

The prevalence of Flu A in children in 2019 is different from that in the past, with highly overlaps the early spread of SARS-CoV-2 in terms of epidemic time. Since COVID-19 is a sudden outbreak, we conducted a retrospective study on the pharygeal swab samples of children kept in our laboratory at the same time (Table 3).There were 19 boys and 16 girls, the median age was 7.7 years (2 months-14y). Fever or cough was the chief complaint in most cases, accounting for 91.4% (32 / 35) of fever and 31.4% (11 / 35) of cough. Among the 35 pharygeal swabs, 11 were positive for Flu A (31.42%). No Flu B was detected. All pharygeal swabs of children were negative for SARS-CoV-2 nucleic acid screening. According to the date of first confirmed COVID-19, the cases were divided into two groups. There was no significant difference between the two groups in clinical characteristics, absolute values, percentages of peripheral blood leukocytes and lymphocytes. The results showed that there was no co infection of SARS-CoV-2 and influenza A in children with respiratory symptoms in Wuhan at the beginning of COVID-19 outbreak.

Discussion

Because the epidemic of COVID-19 has the characteristics of concealment, abruptness and high infectivity, it is essential to study the epidemic dynamics of other respiratory viruses before, during and after the epidemic, especially the characteristics that the high epidemic period of influenza virus was parallel to COVID-19 beginning, so as to understand the law of the outbreak of SARS-CoV-2[12, 13].

Our study suggests that there was an extremely active Flu A epidemic before the COVID-19 outbreak. The prevalence of Flu A has a clear time interval, from the beginning to the end, lasting about 50 days. The first generation of COVID-19 patients has appeared in Wuhan in the early period of Flu A prevalence, both of which are in a concurrent epidemic situation. With the weakening of the epidemic intensity of Flu A, the infectivity and pathogenicity of SARS-CoV-2 explosive enhanced (Figure 2).

Because the SARS-CoV-2 genome sequence is different from the known coronavirus, little is known about its epidemiological characteristics and pathogenic pathological basis[14]. It is well known that the prevalence of common respiratory viral diseases in children, such as influenza A, influenza B, parainfluenza virus and syncytial virus, is often accompanied by obvious seasonality[15-17], and the initial stage of COVID-19 outbreak was also the peak period of influenza virus in children in winter 2019. Whether this phenomenon happened by chance, or whether the epidemic of Flu A provided the susceptible population with immune adaptation for the outbreak of COVID-19, or increased the susceptibility to SARS-CoV-2, these are factors worthy of attention. COVID-19 infected child, the first case in China, was diagnosed in Shenzhen on January 20, 2020, with a history of Wuhan tourism. The first child case in Wuhan was confirmed 8 days later than the boy in Shenzhen, which meant that the early infection may have occurred in Wuhan children[18]. However, due to the limitations of the over strict criteria, no confirmed cases could be made in time. It is speculated that when the first case of COVID-19 occurred, the virus may has already prevalent in Wuhan. According to our study, children with respiratory symptoms did not have mixed infection of SARS-CoV-2 and influenza A before December 28, 2019.

The retention time of respiratory virus in vivo is very important for the prevention of infectious disease transmitting by airborne or droplet-nuclei. It is very difficult right now to make the isolation policy for patients and close contacts with COVID-19. One of the reasons is that the time of SARS-CoV-2 stay in vivo, especially in patients' airway, is not accurately understood, which causes trouble for the work of prevention[6]. In this study, we observed the time when the antigen of airway virus turned negative in children infected with influenza. The median time of pharyngeal swab turning negative was 6 days for Flu A and Flu B. About 3.7% of children can be detected influenza virus stay in airway more than 14 days. It is noteworthy that 4 children aged in 2-4 years old, who have no underlying diseases, have been detected the persistent positive of Flu A for more than 30 days. Therefore, it is necessary to pay special attention to these children with persistent viral status as potential infectious sources.

We acknowledge that there are many shortcomings and limitations in this paper. Due to the retrospective analysis, the starting time of the study was determined according to the time of retained samples, which

failed to fully reflect the epidemic trend of influenza A and SARS-CoV-2; Secondly, the sample size for COVID-19 detection is relatively small because of the reason that Wuhan started level response for the prevention of COVID-19 and pediatric outpatient service failed to operate normally. Thirdly, this is a single center study, the number of pharygeal swabs preserved was limited and the number of children infected with SARS-CoV-2 could not be screened more widely.

To sum up, at the beginning of SARS-CoV-2 epidemic, the children in Wuhan were experienced the influenza A epidemic, and the time of influenza overlapped with the early stage of SARS-CoV-2 epidemic. The time of influenza virus turned negative in airway can be as long as 30 days.

Declarations

Author Contributions: Dr. Zhao had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Drs Wang and Liu are co-first authors, contributed equally to the work.

Concept and design: Drs.Wang, Liu, Zheng and Zhao.

Acquisition, analysis, or interpretation of data: Wang, Feng, Liu, Zheng, Wu and Pan.

Drafting of the manuscript: Wang, Liu, Zheng and Zhao.

Critical revision of the manuscript for important intellectual content: Wang, Liu and Zhao.

Statistical analysis: Wang, Liu and Zhao.

Supervision: Dr. Zhao Dongchi

Conflict of Interest Disclosures: We declare that there is no conflict of interest.

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Disclaimer: The opinions expressed herein reflect the collective views of the coauthors.

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Tables

Table 1 Positive rate of influenza A in different ages

Age(y)	Total(n)	Flu A(n)	Positive rate(%)	Male(n)	Flu A(n)	Positive rate(%)	Female(n)	Flu A(n)	Positive rate(%)
1	582	107	18.38	325	62	19.1%	257	45	17.51
2	727	177	24.35	417	120	28.8%	310	57	18.39
3	1112	288	25.90	621	165	26.6%	491	123	25.05
4	1057	310	29.33	556	163	29.3%	501	147	29.34
5	906	282	31.13	478	161	33.7%	428	121	28.27
6	1044	302	28.93	568	171	30.1%	476	131	27.52
7	1055	311	29.48	546	169	31.0%	509	142	27.90
8	969	297	30.65	517	173	33.5%	452	124	27.43
9	706	217	30.74	390	125	32.1%	316	92	29.11
10	542	171	31.55	275	91	33.1%	267	80	29.96
11	460	141	30.65	243	73	30.0%	217	68	31.34
12	535	175	32.71	288	89	30.9%	247	86	34.82
13	351	105	29.91	195	63	32.3%	156	42	26.92
14	56	16	28.57	31	9	29.0%	25	7	28.00
Total	10102	2899	28.70	5450	1634	30.0%	4652	1265	27.19

Table 2 Positive rate of influenza B in different ages

Age(y)	Total（n）	Flu B(n)	Positive rate(%)	Male(n)	Flu B(n)	Positive rate(%)	Female(n)	Flu B(n)	Positive rate(%)
1	582	23	3.95	325	14	4.31	257	9	3.50
2	727	22	3.03	417	11	2.64	310	11	3.55
3	1112	42	3.78	621	27	4.35	491	15	3.05
4	1057	72	6.81	556	35	6.29	501	37	7.39
5	906	82	9.05	478	49	10.25	428	33	7.71
6	1044	99	9.48	568	50	8.80	476	49	10.29
7	1055	84	7.96	546	45	8.24	509	39	7.66
8	969	69	7.12	517	37	7.16	452	32	7.08
9	706	33	4.67	390	12	3.08	316	21	6.65
10	542	29	5.35	275	21	7.64	267	8	3.00
11	460	25	5.43	243	13	5.35	217	12	5.53
12	535	25	4.67	288	13	4.51	247	12	4.86
13	351	12	3.42	195	9	4.62	156	3	1.92
14	56	0	0.00	31	0	0.00	25	0	0.00
Total	10102	617	6.11	5450	336	6.17	4652	281	6.04

Table 3 Clinical characteristics of 35 children tested for SARS-co2 nucleic acid

		Before COVID-19	After COVID-19
	Number of cases	19	16
	Average age (years)	5.36±2.60	10.20±3.62
Performances	Fever(%)	89.47%（17/19）	93.75%（15/16）
	Cough(%)	57.89%（11/19）	0
	Nausea(%)	0.05%(1/19)	0
	Dizziness(%)	0	6.25%(1/16)
	Headache(%)	0	6.25%(1/16)
Laboratory tests	WBC count (×10 9 / l)	9.17±4.60	8.08±2.70
	Neu percentage (%)	65.30±14.09	72.34±10.91
	Lym percentage (%)	22.62±13.51	15.95±7.86
	Declined lym percentage (%)	57.89%(11/19)	73.33%(11/15)
	Mon percentage (%)	10.83±3.32	10.72±3.26
	Increased mon percentage (%)	68.42%(13/19)	93.33%(14/15)
	hCRP (mg / L)	8.65±9.62	8.02±10.13
	Increased hCRP (%)	57.89%(11/19)	60.00%(9/15)
Virus antigen detection	Influenza A virus (%)	21.05%(4/19)	43.75%(7/16)
	Influenza B virus (%)	0	0
	H7 subtype avian influenza virus(%)	0	0

COVID-19, Corona virus disease-19

WBC, white blood cell Neu, neutrophil Lym, lymphocyte Mon, monocyte hsCRP, high sensitivity C-reactive protein

Figures

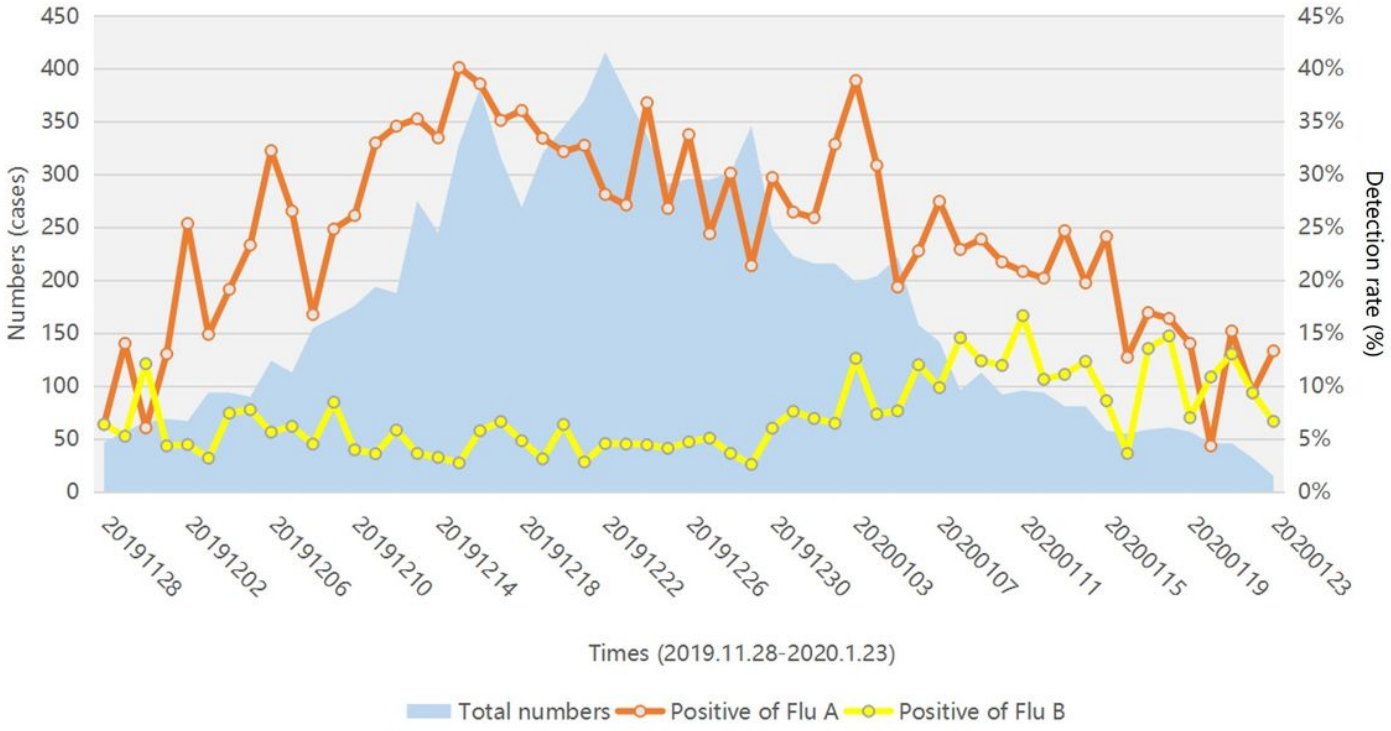

Figure 1

Epidemic of influenza A and B during the early stage of COVID-19 emerging. In the early stage of SARS-CoV-2 epidemic, children in Wuhan had a high intensity epidemic of influenza A, the epidemic peak of influenza A appeared on 2nd, 6th, 15th December 2019 and 1th January 2020, and the trough on 5th, 15th, 19th January 2020. Influenza B was also accompanied at low levels spread.

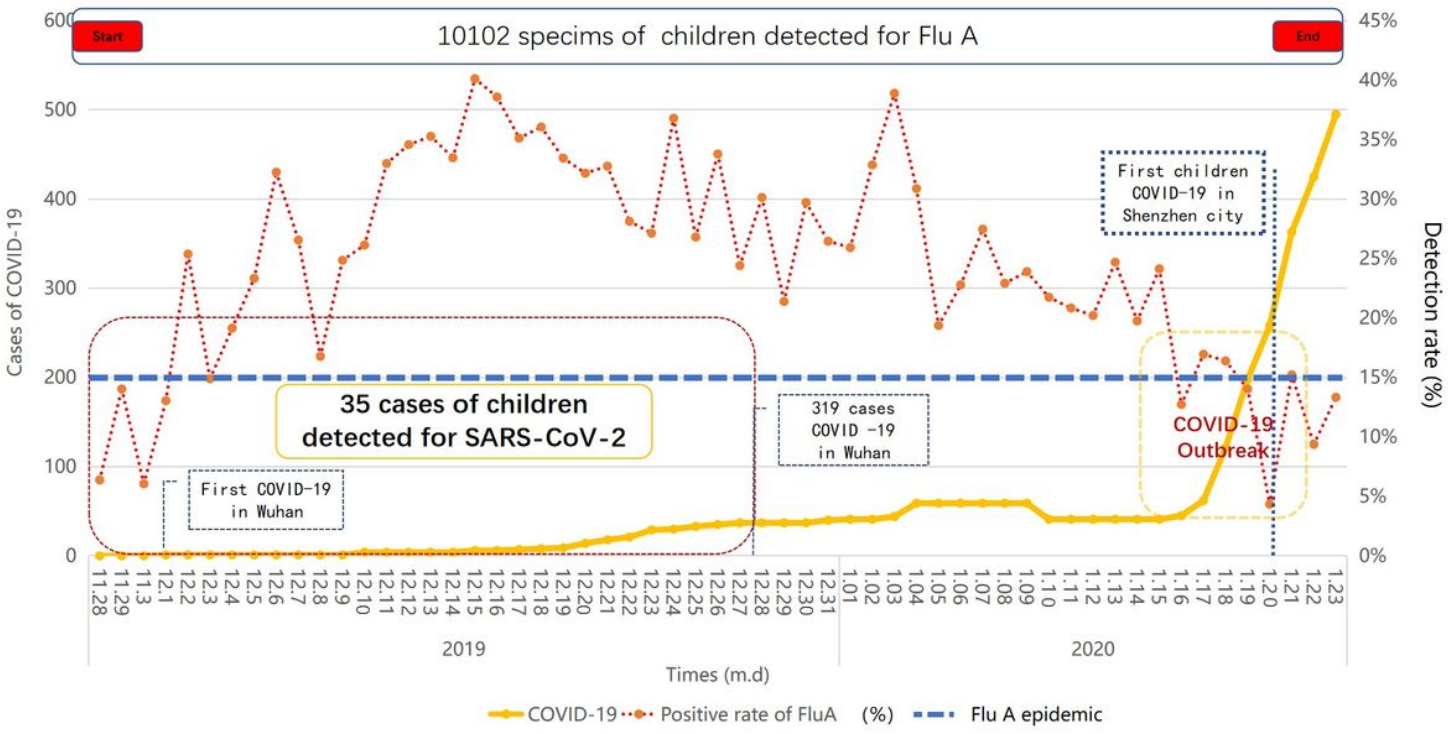

Figure 2

Alternation of influenza A epidemic and COVID-19 outbreak. The epidemic time of influenza A highly overlaps with the early spread of SARS-CoV-2. And the outbreak of COVID-19 comes after the end of influenza A epidemic.