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Epidemiological study and health management of an imported incident of COVID−19 asymptomatic cases in Haikou, China: A retrospective case series

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ABSTRACT

Background: To control the imported risks brought by all international arrivals, China Customs has implemented strict closed-loop health management policy called "three checks, three screenings and one transfer". This study provides epidemiological evidence for prevention and control measures on imported cases of asymptomatic infections and describes the current COVID-19 prevention and control system on imported risks in China.

Methods: We retrospectively analyzed an imported incident of three asymptomatic carriers. Serum SARS-CoV-2 IgM and IgG antibodies were detected by chemiluminescence and gold immnnochromatography(GICA).

Results: Three cases were reported positive for SARS-CoV-2 nucleic acid on their arrival, total antibodies and IgG, but negative for IgM. The Ct values of cases A, B and C were 34/36/36, 32/33/32 and 25/31/29, respectively. There were 10726434 pair-end reads sequenced for case C, and approximate 80% reads were aligned to the hCoV-19/Wuhan/IVDC-HB-01/2019 genome (EPI_ISL_402119). The viruses of case A and C were homologous and came from the SARS-CoV-2 variant.

Conclusions: Serum antibody IgM and IgG tests are recommended for international travelers from epidemic areas. The "three checks, three screenings and one transfer" policy implemented at custom's entry points was effective in COVID-19 prevention and control.

KEYWORDS: Asymptomatic carriers; "Three checks, Three screenings, One transfer"; COVID-19; SARS-CoV-2

1. Introduction

Since February 5, 2020, the number of newly confirmed cases in China had begun to decline. By March 8, a total of 28 provinces and cities had achieved "zero" growth, of which 15 provinces and cities had realized "zero" growth for more than 10 consecutive days[1]. The increase showed that the epidemic of COVID-19 in China had been effectively curbed. However, in late February, the COVID-19 epidemic status was rapidly spreading worldwide, and the World

Significance

At present, China has been implementing measures to prevent and control the risks of transmission of COVID-19 from overseas, such as restricting international flights, strengthening centralized quarantine and medical observation of overseas immigrants, but the quarantine of asymptomatic carriers has brought great uncertainty. This article reported the management of three imported cases of asymptomatic infection using the "three checks, three screenings and one transfer" policy, and demonstrated how timely experts of cross-sectors, multi-disciplinary and multi-field carried out joint risk assessment and control by adopting this principle.

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Health Organization raised the level of global transmission risk and impact risk of COVID-19 epidemic from "high" to "very high"[2].

By March 2020, the domestic epidemic had been effectively controlled, and overseas imports had become the main source of infected cases. According to China's CDC, from April 16, 2020 to October 12, 2020, a total of 19 398 384 people entered China from abroad, of which 3 103 were infected with the SARS-CoV-2 and 1 612 (51.9%) were considered to have asymptomatic SARS-CoV-2 infection, and they were considered asymptomatic infections because they did not show symptoms until the 13th day of quarantine[3]. More and more studies have shown that both patients with incubation period and asymptomatic infection of COVID-19 are infectious[4–7]. The current management measures for asymptomatic carriers in China was 14 d of centralized quarantine and observation; in principle, continuous two negative nucleic acid tests (at least 24 h apart) can release the quarantine[8–9]. At the beginning of September 2020, the SARS-CoV-2 virus mutated in the United Kingdom. There are different subtypes of the SARS-CoV-2. With the continuous evolution of the strain, its transmission route and transmission power are also constantly changing. Nucleic acid testing and antibody testing are currently the most extensively used screenings and check method[10–14]. Therefore, the Chinese Ministry of Foreign Affairs requires that, starting from the beginning of November 2020, passengers from some countries arriving in China by flight must provide a double-negative test certificate within 48 h before boarding (that is, both the SARS-CoV-2 nucleic acid and IgM antibody are negative, and some countries require twice viral nucleic acid is negative, the second test must be performed within 36 h before boarding). Although China has implemented measures such as restricting international flights and strengthening the centralized quarantine and medical observation of overseas arrivals, asymptomatic carriers brought great uncertainty to entry quarantine[15] which was a normalized prevention method for COVID-19 epidemic. This article reports an epidemiological study of three imported asymptomatic infection cases in Hainan to demonstrate China's COVID-19 prevention and control system deployed for imported risks.

2. Methods

2.1. Study setting

Hainan Province is the southernmost province in China and is located in the tropics. Haikou Customs is one of the gateways for entry into China, but it is no longer a regular entry point after the outbreak of the COVID-19 epidemic worldwide. At 3:43 on September 29, 2020, Haikou Customs detected three cases of asymptomatic infections (cases A, B, and C) with positive nucleic acid of the SARS-CoV-2 among passengers on a chartered flight departed from Malawi (flight number HU480).

Case A was a 44 years old housewife; case B of seven years old was one of A's daughters. A and B traveled to Malawi from China on August 2, 2019, and have been living in Malawi thereafter. Case A run a hardware store and a total of six people lived in her home in Malawi (A, A's mother, A's husband, B, B's twin younger sister, and a servant). A's mother and B's twin younger sister also returned to China on the same flight with A and B.

Case C was a nine years old male student. At the end of January 2019, C went to Malawi from Guangzhou Baiyun Airport and had been living in Malawi with his parents and brother since then. C's brother and the driver returned to China on the same flight with C.

2.2. Epidemiological study methods

Retrospective case series methods and the CARE guidelines(for case reports) were applied to investigate this case series, an imported case of three asymptomatic carriers. The CARE guidelines were developed by an international group of experts to support an increase in the accuracy, transparency, and usefulness of case reports[16–17]. According to the COVID-19 Prevention and Control Plan (7th Edition) instructed by the Chinese Center for Disease Control and Prevention[18–19], the epidemiological field study method was used to collect basic information of all subjects including diagnosis, treatment, clinical manifestations, laboratory test results, risk factors, history of exposure, contacts, and post disease onset activities. For epidemiological field study, epidemiologists used telephone calls, national standardized living environment and lifestyle questionnaire surveys to collect epidemiological information from patients. Qualified clinic physicians conducted medical history collection, disease treatment and imaging diagnosis, hospital nurses collected biological samples, and laboratory physicians conducted lab testing on research subject's biological specimens.

2.3. Laboratory testing methods

The throat swabs from the subject were collected and tested for SARS-CoV-2 by real-time fluorescence RT-PCR. In the case, 5 mL of fasting venous blood was collected and placed in a yellow-topped vacuum blood collection tube containing separation gel. The serum was prepared after the blood was coagulated and centrifuged for 2 500 r/10 min. Chemiluminescence kit (Shenzhen YHL Biological Technology Co. Ltd.) and GICA kit (Zhuhai Lizhu Reagent Co. Ltd.) were adopted. Strictly followed the offical professional instructions, and serum SARS-CoV-2 IgM and IgG antibodies were detected by chemiluminescence and GICA. Chemiluminescence refers to the usage of i-Flash 3000-C automatic chemiluminescence immunoassay analyzer (Shenzhen YHL Biotechnology Co. Ltd.) and supporting reagents (magnetic particle chemiluminescence).

The virus RNA was provided by the Centre of Disease Control of Hainan Province. Qubit RNA HS kit (Thermo Fisher Q32855) was used to detect RNA concentration of samples. We next performed

PCR by the novel ultra-sensitive coronavirus genome-wide capture amplification kit from microfuture company. The PCR was performed in 30 min polymerase activation at 98℃, followed by 25 cycles at 95℃ for 15 s and 65℃ for 5 min.

Next, the PCR products were purified by VAHTS DNA Clean beads (Vazyme-N411). DNA library was set up based on NEXTflex Rapid DNA-Seq Kit (Bioo Scientific-5144-02) and Dual DNA adapter 96 kit for Illumina (ABcolonal-20287). The PCR was run at 98℃, followed by 10 cycles at 98℃ for 30 s and 65℃ for 30 s, with extension at 72℃ for 1 min. Moreover, the library was purified using VATHS DNA Clean beads. The library was further diluted and denatured with HT1 and NaOH with dilution concentration of 15 pM. High-throughput sequencing was performed in Illumina miSeq.

The sequencing reads were analyzed based on the nCoV tool from Oxford Nanopore Technologies Company. The sequencing reads were first aligned by Bowtie2 to genome and the consensus sequence was constructed by local scripts. The parameters we used were with minimum sequencing depth>=1 and vote=0.3.

Three specimens were received. Due to the capacity limitation of the sequencer, a maximum of two samples were measured. As A and B are mother-daughter, two samples A and C were selected for the computer, but all three samples have been constructed library. Quality monitoring: The Q30 data of the connectors at both ends of the samples of A and C (the accuracy of the sequencing results reached 99.99%) reached 92.32%, 86.51%, 90.67%, and 66.17%. The sequencing results of A were more reliable.

2.4. Diagnostic criteria and related definitions

According to the COVID-19 Diagnosis and Treatment Plan (7th Edition) and COVID-19 Prevention and Control Plan (Seventh Edition)[18–19], the diagnostic criteria for COVID-19 include epidemiological history, clinical manifestations, laboratory testing, and chest imaging. Supplementary Table 1 shows the description of each category.

Once asymptomatic carriers were confirmed, they should be quarantined for 14 d for medical observation. In principle, if the SARS-CoV-2 nucleic acid test of the sample was negative for two consecutive times (at least 24 h apart), the quarantined medical observation can be released; if the SARS-CoV-2 nucleic acid test was still positive then a continuous quarantined medical observation is needed. During the observation period, if the SARS-CoV-2 nucleic acid test was negative for two consecutive times, the quarantined medical observation can be released. During the quarantined medical observation period, routine blood tests, CT imaging examinations and antibody tests should be carried out; when the diagnostic criteria were met, the confirmed cases should be corrected in time. If there was a clinical manifestation, patients should be immediately transferred to a designated medical institution for standardized treatment. Asymptomatic infected people who have been released from quarantined medical observation should continue their 14-day home medical observation and follow-up visits to designated medical institutions in the second and fourth weeks.

2.5. Literature research methods

The search strategy was designed to be inclusive and focused on three key elements: COVID-19 asymptomatic infection, imported cases, and prevention and control. The retrieval of academically published relevant research or studies was based on the major Chinese and English electronic databases: Chinese National Knowledge Infrastructure (CNKI), Chinese Science Citation Database (CSCD), CBMdisc and PubMed, published before 3 February 2021. The retrieval of published relevant news reports and national legislation was based on the Chinese online search engine Baidu or the homepage websites of the issuing ministries. To identify the relevant studies, the reviewers reviewed all titles, abstracts and full texts generated from the searches.

2.6. Ethics approval

Human research ethics approval was obtained from the Institutional Review Board of the Hainan Women and Children's Medical Center on March 18, 2021. Informed consents were obtained from the participants and/or their guardians.

2.7. Patient and public involvement

This was a retrospective case series study and no patients were involved in the study design, setting the research questions or the outcome measures directly. No patients were asked to advise interpretation or writing up of results.

3. Results

3.1. Epidemiological study results

3.1.1. Investigation of three imported asymptomatic carriers

Cases A and B: A stated: "since the end of February 2020, my family had stayed at home in Malawi without going out, and the self-owned store was only managed by my husband". A's husband was diagnosed COVID-19 infection locally on July 9, 2020 and he was the first confirmed case in this family. A's husband was treated at home for self-quarantined after being confirmed. On August 5, he tested negative for nucleic acid. All family members were tested for nucleic acid locally and only A's result was positive on

July 16. A was self-quarantined at home for 20 d, and took Chinese medicine for self-treatment. Her two daughters and mother were self-quarantined in her friend's hotel, and they returned home after the couple were both tested negative. They family tested for SARS-CoV-2 nucleic acid at the agency company contracted with the Chinese Embassy in Malawi on September 22 and 26, and the results were all negative, and they declared healthy before boarding. On September 27, the family of four people (A, B, A's mother and B's twin sister) took the direct international flight HU480 from Lilongwe, Malawi to Haikou, China at 18:30 pm (Malawi time). They kept wearing face masks throughout the journey (without dining on plane). A's seat was 45K and B's seat was 35C. The flight arrived at Haikou Meilan International Airport at 13:40 pm on September 28 (Beijing time). Nasopharyngeal swabs and blood samples of all passengers were collected for SARS-CoV-2 nucleic acid, IgM and IgG tests on their arrival at Haikou Customs. Meanwhile, all passengers were transferred to a designated hotel for quarantine and observation. At 3:43 am on September 29, the testing results of A and B were reported positive for SARS-CoV-2 nucleic acid, total antibodies (chemiluminescence method) and IgG (colloidal gold method) but negative for IgM. On the morning of September 29, local CDC collected throat swab specimens from A and B again for SARS-CoV-2 nucleic acid retest, and the results were negative.

Case C stated: "During the epidemic period in Malawi, I rarely went out, and I always wore face mask when I was out". C reported no history of contact with confirmed cases or suspected cases. C tested for nucleic acid twice in Malawi on September 18 and 25, and the results were all negative. On September 27, C boarded flight HU480 with his brother and driver from Lilongwe, Malawi to Haikou directly. C wore a face mask throughout the journey without dinning. He was transferred to the designated quarantine hotel after being collected nasopharyngeal swab samples by Haikou Customs. At 3:43 am on September 29, C's testing results were reported as SARS-CoV-2 nucleic acid (+), total antibody (+), IgM (-), and IgG (+). C declared no fever or other discomfort. He was sent to the designated hospital for observation at 13:00, and the nucleic acid retest results were negative on September 30, October 2, 4, 10, and 11. He was discharged and transferred to the centralized quarantine hotel on October 12 for centralized quarantine and medical observation. On October 19 and 20, C's SARS-CoV-2 nucleic acid tests were positive again, and C declared no fever or discomfort during this period. C was hospitalized again and multiple nucleic acid tests were negative on the next day and thereafter. There were no abnormal laboratory or imaging results during his hospitalization periods. He was discharged on December 3 and returned to his hometown Zhejiang Province on December 17.

3.1.2. Investigation and management of close contacts

There were 254 travelers on flight HU480 on September 27, 2020, including 26 crew members and 228 passengers. According to the criteria of close contacts, a total of 174 travelers were identified, including 25 crew members and 149 passengers. All close contacts were strictly controlled in accordance with the "COVID-19 Prevention and Control Plan (Seventh Edition)", for quarantine and testing. Five of 174 close contacts declared discomfort or being detected fever were transferred to designated hospital for further diagnosis and treatment. Throat swab samples were collected for nucleic acid tests on September 28 and 29 for them and were all negative. They were kept follow-up health management in hospital. The remaining 169 close contacts were centralized quarantined in a designated hotel. The second nucleic acid test was conducted on September 30, and the third nucleic acid test was conducted after 14-day quarantine (October 11) for them.

Family members of A and C: A's mother and her younger daughter (the younger sister of the twins), and C's 14-year-old brother were identified as close contacts because they were all in the same flight and had close contact with A or C before boarding. Figures 1 and 2 show the epidemiological investigation results and health management procedures for these two families.

3.2. Clinical examination and laboratory test results

Cases A, B and C were admitted to designated hospital in Haikou on September 29 when they were detected positive for SARS-CoV-2 by Haikou Customs. The clinical and laboratory results after admission were as follow(Figure 1 and 2):

Case A: Body temperature was 36.8℃, blood pressure was 118/80 mmHg, heart rate was 84 beats/min, and breathing was 20 breaths/min. The physical examination was normal. Laboratory test showed: white blood cells count 5.19×10^9/L, lymphocytes 22.6%, neutrophils 72.1%, platelets 212×10^9/L, and C-reactive protein (CRP) 0.24 mg/L. Chest CT: a few fibrous foci in the medial segment of the right middle lobe, spotty calcification in the left lobe of the liver. A was diagnosed as asymptomatic infection.

Case B: Body temperature was 36.8℃, blood pressure was 104/72 mmHg, heart rate was 108 beats/min, and breathing was 20 breaths/min. Her physical examination was normal. Laboratory test showed: white blood cells count 4.83×10^9/L, lymphocytes 54.6%, neutrophils 36.3%, platelets 313×10^9/L, and CRP 0.65 mg/L. Chest CT: there were no abnormalities on bilateral CT scans. B was diagnosed as asymptomatic infection.

Case C: Body temperature was 36.5℃, blood pressure was 106/60 mmHg, heart rate was 102 beats/min, and breathing was 19 breaths/min. His physical examination was normal. Laboratory test showed: white blood cells count 9.13×10^9/L, lymphocytes 30.8%, neutrophils 61.8%,

platelets 414×10^9/L, CRP 1.09 mg/L; blood biochemical liver function: alanine aminotransferase 123.9 U/L, aspartate aminotransferase 55.9 U/L. Chest CT: There was no abnormalities on bilateral CT scan. C was diagnosed as asymptomatic infection.

SARS-CoV-2 nucleic acid tests were taken for A, B and C four times in hospital on September 30, October 2, October 4, and October 11, and the results were all negative. They were discharged on October 12 and transferred to a designated hotel for 14-day centralized quarantine and medical observation.

Case C was re-admitted to hospital on October 21 for repositive of SARS-CoV-2 in daily health check. His body temperature was 37.4 ℃ after re-admission, heart rate was 126 beats/min, bilateral tonsils were swollen with degree I to II, and there were no other abnormalities in the physical examination. Laboratory tests showed: white blood cells count 9.43×10^9/L, lymphocytes 31.9%, neutrophils 58.4%, platelets 397×10^9/L, CRP 1.26 mg/L; blood biochemical liver function: alanine aminotransferase 268 U/L, aspartate aminotransferase 314 U/L. CT: plain scan showed no abnormalities on chest but fatty liver. The admission diagnosis were: 1. COVID-19 infection; 2. Liver function insufficiency; 3. Fatty liver. Recombinant human interferon α-2b spray was used for antiviral treatment, compound glycyrrhizin injection + deursoxycholic acid capsules were used for liver protection and symptomatic treatment. On December 3, C was fully recovered and discharged, and then he was immediately transferred to a designated hotel for further 14-day centralized quarantine and medical observation.

3.3. The gene sequencing results

Table 1 shows the gene sequencing results and indicates that the viruses of cases A and C were homologous, and both were from American strain BA.1.

We tested the samples collected by Haikou Customs on September 28, the Ct values of cases A, B and C were 34/36/36, 32/33/32, and 25/31/29 respectively. The viral load of B's sample was very low and the metagenomic sequencing was difficult to capture. On October 21, the re-positive sample of Case A was enriched by PCR to obtain sufficient viral nucleic acid and sequenced again.

In total, there were 10 726 434 pair-end reads sequenced in Case A's re-positive sample. Approximate 80% reads were aligned to the hCoV-19/Wuhan/IVDC-HB-01/2019 genome (EPI_ISL_402119). Based on the nCoV tool and Artic pipeline, we obtained the consensus sequence with 17 235 bp in length. These results demonstrated that Case C was SARS-CoV-2 positive. Next, we compared this sequence with 702 published sequences of SARS-CoV-2, and we found that the consensus sequence was similar with the sequence from Kenya/P2601/2020. It suggested that the strain was the American strain.

3.4. Prevention and control process for overseas entry personnel

To prevent and control the imported risks brought by overseas entry personnel, the General Administration of Customs of the People's Republic of China implemented 'three checks, three screenings and one transfer' policy and it worked well so far. The 'three checks' referred to health declaration, temperature monitoring and medical check; 'three screenings' referred to further epidemiological investigation, medical examination and observation, and laboratory test for suspected travelers; 'one transfer' was implemented directly from customs to medical institutes once the suspected travelers need further medical observation or treatment[20]. Figure 3 shows the flowchart of Case C managed by the 'three checks, three screenings and one transfer' policy.

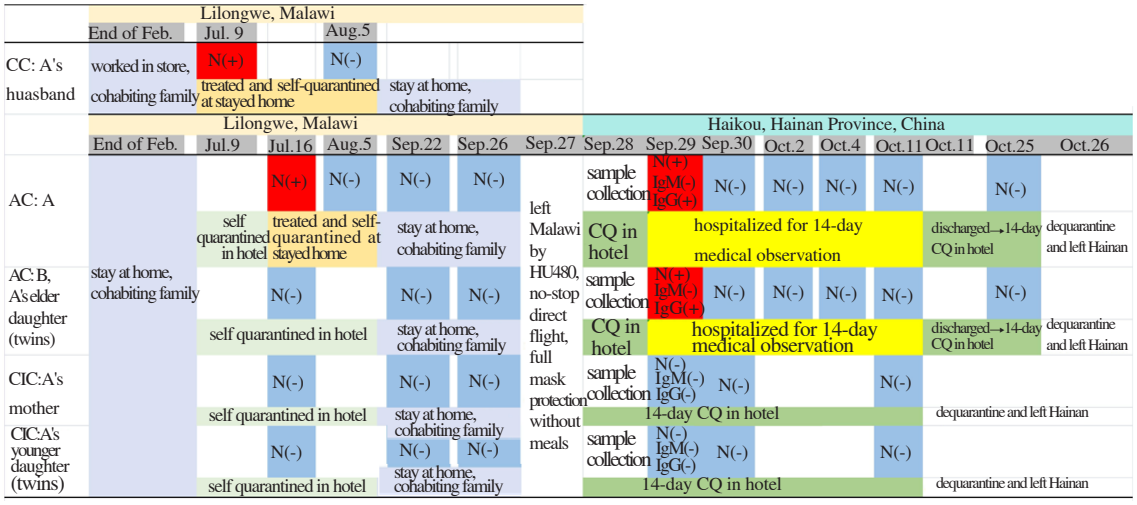

Figure 1. Epidemiological investigation and health management for case A and her family in 2020. CC: confirmed case; ClC: close contact; AC: asymptomatic carrier; N: SARS-CoV-2 nucleic acid detection; CQ: centralized quarantined medical observation

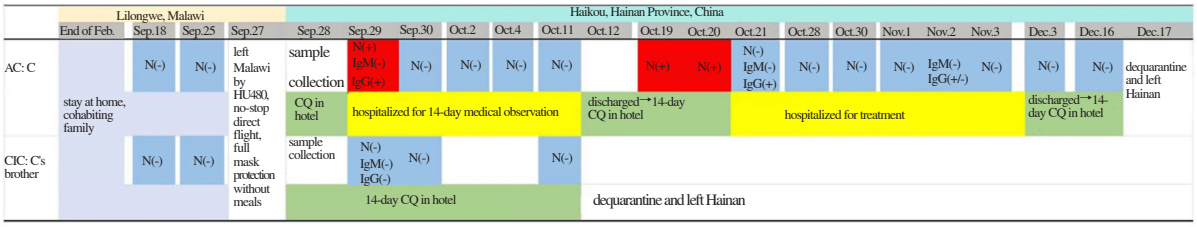

Figure 2. Epidemiological investigation and health management for case B and his brother in 2020. CC: confirmed case; ClC: close contact; AC: asymptomatic carrier; N: SARS-CoV-2 nucleic acid detection; CQ: centralized quarantined medical observation

Table 1. Comparison result of human reference genome and virus SARS-CoV-2.

	human reference genome				SARS-CoV-2		
sample	reads after quality control	consistent comparison (1 time)	consistent comparison (>1 time)	inconsistent comparison	consistent comparison (1 time)	consistent comparison (>1 time)	inconsistent comparison
Case A	1 711 753	41 687	14 057	6 059	22	145	0
Case C	5 819 493	295 586	80 382	83 754	0	8	0

Note: SARS-CoV-2 was compared with Wuhan Virus Reference Genome (Serial no. MN908947).

4. Discussion

4.1. "Nucleic acid amplification+IgM+IgG" screening technique can improve the diagnostic efficiency for asymptomatic carriers

Currently, the epidemiological investigations on imported cases in China mostly rely on the interviews and laboratory testing results, lacking of a cross-national COVID-19 information sharing mechanism or tracing database system. The longer period of recall, the more bias presents of interview. Therefore, lab testing results prone to be more reliable and sensitive. However, sample collection, handling, and transport directly impact a nucleic acid amplification assay's analytical sensitivity, and a high-sensitivity assay may result in an increased risk of false positive reporting due to contaminated work areas (from previously processed positive samples)[21]. Immunoassays detecting anti-SARS-CoV-2 IgM and/or IgG indicate exposure to the virus. A comprehensive literature review reported the antibody tests for COVID-19 with variable performance (sensitivity varying from 45% to 100%, specificity from 96% to 100%)[22]. Haikou Customs and CDC applied "Nucleic acid amplification+IgM+IgG" screening technique to all overseas travelers when they entering China, which was intended to improve the diagnostic efficiency, especially for detection of asymptomatic infected persons.

Hong K's study observed most patients with prolonged positive RT-PCR were clinically stable, 78.4% denied any symptoms, the median time of PCR positivity up to last follow-up was 78 d, and the longest duration of positive viral RNA was 120 d. This study confirmed prolonged presence of viral nucleic acid in clinically recovered COVID-19 patients, and it was not associated with effective infectiousness[9]. A recent Chinese study has observed a typical asymptomatic transmission to the cohabiting family members, which even caused severe COVID-19 pneumonia, and he concluded the asymptomatic carriers identified from close contacts were prone to be mildly ill during hospitalization[23]. In our study, we observed Case A was the close contact of a confirmed case (her husband) in Malawi. She was confirmed infection and conducted self quarantine and self medication by taking traditional Chinese medicine at home in Malawi. Her mother and Case B (her daughter) were both tested for IgG positive on their arrival, which suggested past infection among family members even case A and her husband both were tested for negative after treatment, and it may indicate the prolonged presence of virus in them.

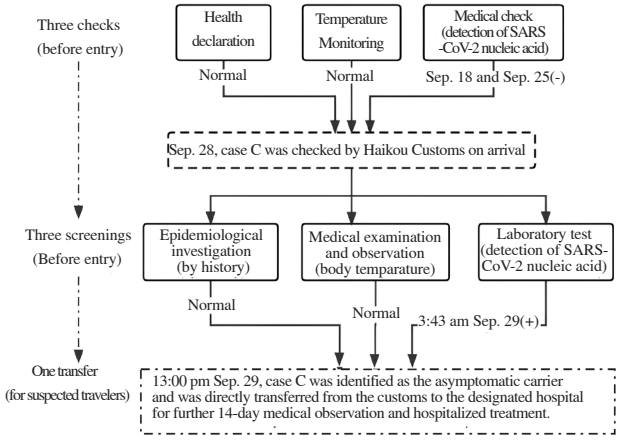

Figure 3. Flowchart of Case C as an example going through the risk prevention and control process for overseas entry personnel.

Chemiluminescence was one of the most commonly used detection methods for detecting coronavirus-specific antibodies in clinical

practice. Zhengzhou Antu Biological Co., Ltd. SARS-CoV-2 IgM detection kit of chemiluminescence method showed that its clinical sensitivity was 87.68%, specificity 99.69%, IgG sensitivity 87.19%, specificity 99.69%, the sensitivity of the two combined detection 90.15%, the specificity 99.37%, and the chemiluminescence method is also featured by simple operation and short detection time, which was suitable for the clinical detection of large-scale specimens. Studies have shown that of the 25 671 specimens of subjects, all 2019-nCoV nucleic acid tests were negative, 214 cases were positive for 2019-nCoV IgM antibodies by chemiluminescence, 193 cases (0.01%) had previously suffered from COVID-193 among IgM-positive subjects, 171 cases (0.67%) were vaccinated against COVID-19, and 40 cases (0.16%) were false positive. There were many interfering factors for the detection of 2019-nCoV-specific IgM and IgG antibodies by chemiluminescence, and the case of false positive should be fully considered clinically[24]. The patient's epidemiological history, vaccination history, clinical manifestations, imaging tests, nucleic acid detection results, age, underlying disease, antibody titers and other factors should be comprehensively considered, and IgM and IgG antibodies should be detected at the same time to avoid misleading clinical diagnosis by false positive results. Age was the influencing factor of the detection of false positives by the SARS-CoV-2 antibody chemiluminescence method, and the possibility of false positives in elderly patients is higher.

4.2. Overseas entry personnel and goods are the critical control points in China

China has been implementing a very strict and meticulous prevention, control and management process and measures for overseas entry personnel since the domestic risks were under well controlled, and the strategy can effectively track down and achieve good prevention and control outcomes. For instance, Chinese government established a set of risk prevention and control system and dynamic adjustable strategies for imported COVID-19 risks. Shaohua Sun, the deputy director of the Operations Monitoring Center of the Civil Aviation Administration of China, introduced at the press conference of the National Joint Prevention and Control Mechanism on March 23, 2020, that the choice of China's aviation entry point must meet three conditions: Firstly, it must be an international airport. It can accept the landing and take-off of international flights, and has the conditions for customs clearance. Secondly, the airport's security capabilities must meet safety operation regulations and standards such as E-class aircraft (Airbus A330 and Boeing 777) can land and operate there, and have a good safety operation record. The professional capabilities of the security facilities and airport agencies must meet the requirements. Thirdly, the airport must have major domestic airline subsidiaries or sales offices, and have certain security resources for flight stops[25]. According to the above conditions, from 0:00 on March 23, 2020 Beijing time, all international passenger flights destined for Beijing must depart from one of the 12 designated first entry points, which were Tianjin, Shijiazhuang, Taiyuan, Hohhot, Shanghai Pudong, Jinan, Qingdao, Nanjing, Shenyang, Dalian, Zhengzhou, and Xi'an international airports in mainland China. Starting at 0:00 on June 8, the designated first entry point for international passenger flights destined for Beijing was added from 12 to 16[26].

However, in addition to the overseas entry personnel, many environmental transmission cases in China have been reported, especially the imported cold chain food. The packaging, logistics and sales workers had close contacts with the suspected imported cold chain food and they have become key occupational exposure groups. Therefore, in addition to closely tracking and controlling the overseas entry personnel and goods, the environmental sterilization and routine detection of every critical point in the transmission link are very important. The case we reported warns Chinese public health workers that they should pay more attention to the unique status of imported asymptomatic carriers when the local cases are under well-controlled and keep them closely observed and tracked to prevent future community transmission based on a confidential information sharing system when dealing with the global public health crisis of pandemic of COVID-19 and other emerging infectious diseases.

4.3. Implement the "three checks, three screenings and one transfer" and strengthen the joint prevention and control mechanism

Since the outbreak of the COVID-2019 epidemic, under the unified leadership of the CPC Central Committee and the State Council, the General Administration of Customs has taken strict port prevention and control measures in accordance with the requirements of a series of laws and regulations such as the Border Health quarantine law, the law on the prevention and control of infectious diseases, and the International Health Regulations formulated by the World Health Organization, to prevent internal exports and external imports, and resolutely curb the spread of the epidemic through ports. The General Administration of Customs of the People's Republic of China issued and implemented the policy of "three checks, three screenings and one transfer", which is conform to the anti-epidemic philosophy of achieve early detection, early reporting, early isolation, and early treatment.

The "three checks" refers to the three screening links of health declaration card verification, body temperature monitoring and screening and medical inspection. Among them, the health declaration verification of inbound personnel should be carried

out "double guarantee" to ensure that the information can be traced and the responsibility can be pursued. Implement two or more temperature screenings, and retest key personnel with mercury thermometers on the basis of "infrared temperature measurement and spot temperature gun thermometer measurement". At each travel inspection and customs clearance site, professionals are arranged to carry out medical inspections, focusing on checking whether the entry and exit personnel have symptoms such as fever, fatigue, and cough.

The "three screenings" refers to the strict implementation of the three screening links of epidemiological investigation, medical investigation and laboratory testing and investigation. Among them, the key personnel are strictly implemented epidemiological investigation, so that suspected can be located and confirmed and traceable. In the strict implementation of medical screening, according to the results of epidemiological surveys, medical examinations such as body temperature re-testing are carried out. In the strict implementation of sampling and testing, all respiratory samples such as nasopharyngeal swabs are collected from all inbound personnel and sent to the laboratory for nucleic acid testing of the new coronavirus, and blood samples are collected for testing of those with symptoms.

The quarantine disposal link implements "one transfer". Confirmed cases, suspected cases, close contacts, and persons with fever and respiratory symptoms entering the country would be transferred to designated hospitals or local joint prevention and control mechanisms for isolation observation. Concurrently, handover records would be made. For other personnel, all of them are transferred to the local joint prevention and control mechanism for unified centralized isolation, and the relevant information is promptly reported to the relevant local departments.

By applying this "three checks, three screenings and one transfer" strategy, all entry and exit personnel are required to make a health declaration, and the customs will review the declaration at the port, strictly check whether there are symptoms, or a history of travelling to the area or country where the epidemic occurred within 14 d or other close contact. We can closely track the development of the epidemic at home and abroad, and collect various epidemic information in an all-round way through multiple channels. According to the different stages of the development and change of the epidemic, timely organize cross-sectors, multi-disciplinary and multi-field experts to carry out joint risk assessment work, and then a series of effective countermeasures have been adopted at ports across the country in a targeted manner to achieve scientific prevention and control and precise policies.

The advantages of "three checks, three screenings and one transfer" strategy can be listed as below. Firstly, this strategy can be applied to not only COVID-19 but also other major diseases epidemic abroad with high imported risk, such as Ebola, Lassa fever, Monkeypox and other infectious diseases, can be detected and prevented from the frontier. Secondly, this strategy can strengthen the joint prevention and control mechanism, and it is a good example of implementing the One Health concept[27–29]. All member units are working under the unified leadership of the joint prevention and control mechanism of the State Council, the customs have more opportunities to work closely with other member units, and the National Health Commission can timely revise the port prevention and control technical plan in accordance with the updated situation. Thirdly, this strategy can further improve information sharing mechanisms, open up channels for information exchange, and share data resources. However, to implement this strategy successfully needs a very strong leadership to smooth each step of the whole process. To built a multi-level, full-chain, three-dimensional prevention and control system need to break down the technical and human resource barriers for better communication and coordination.

4.4. Hainan, the biggest free trade port, is facing potential global health challenges

On June 1, 2020, the Central Committee of the Communist Party of China(CPC) and the State Council issued the Overall Plan for the Construction of Hainan Free Trade Port. According to the Item 38 Prevention and control of public health risks from Part two Framework design, it was described as 'We must accelerate the construction of public health prevention, control and treatment systems, establish monitoring and early warning, emergency response platforms and decision-making command systems for infectious diseases and public health emergencies, and improve early prevention, risk analysis and timely handling capabilities[30]. On the background of building the largest free trade port in China before 2025, Hainan has the mission of ensuring public health safety. In Hainan, the construction of an effective and national and international rapid response network is more urgently needed than ever.

4.5. Limitations of this study

Haikou was not on the list of designated air entry port by Civil Aviation Administration of China, and the international airport was closed from March, 2020[24–25]. Haikou only accepted few international charter flights with special entry permission. Due to the limited number of overseas entry personnel and reported COVID-19 cases, the sample size was limited. Moreover, these three reported asymptomatic carriers and their family members were not local Hainanese and they all returned to hometowns when dequarantine ended in Haikou, which caused the problems on case tracking and follow-up study.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

Zhang F and Lin GT completed the literature review, analyzed the data and wrote the manuscrpit equally. Jin YM collected the epidemiological study data, Wu T and Fan LC collected the clinical data from two hospitals. Chen JC contributed to literature review and data collection. Lv ZY collected the gene sequencing data and developed the research idea with Xiang W. Xiang W provided funding and coordinated all sectors to provide great supports to this research.

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Original Article

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Epidemiological study and health management of an imported incident of COVID−19 asymptomatic cases in Haikou, China: A retrospective case series

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Supplementary Table

Supplementary Table 1. Diagnostic criteria for COVID-19

Category	Items
Evidence	
Epidemiological history	(1)history of travelling or residence of where cases were reported to the community within 14 d before the onset; (2)in contact contact with a confirmed case or asymptomatic infected person within 14 d prior to the onset of the disease; (3)in contact with who have fever and/or with respiratory symptoms from the community where cases or asymptomatic infections were reported; (4)definition of cluster disease: within 14 d in a small area (such as homes, offices, school classes, etc.), 2 cases and The above cases of fever and/or respiratory symptoms.
Clinical manifestations	(1)fever and/or respiratory symptoms and other clinical manifestations related to COVID-19; (2)with imaging characteristics of COVID-19; (3) total number of WBC is normal or decreased in the early stage of onset, and the lymphocyte count is normal or decreased.
Pathogenic or serological evidence	(1)real-time fluorescent RT-PCR detection of SARS-CoV-2 nucleic acid positive; (2)viral gene sequencing, which is highly homologous to known SARS-CoV-2; (3)SARS-CoV-2 specific IgM antibodies and IgG antibodies are both positive; (4)SARS-CoV-2 IgG antibody changes from negative to positive or the IgG antibody titration in the recovery phase is 4 times or more higher than that in the acute phase.
Diagnosis criteria of cases	
Suspect cases	(1)any one of the epidemiological history and any two of the clinical manifestations; (2)if there is no clear epidemiological history, any two of the clinical manifestations are met, and SARS-CoV-2 IgM antibody is positive; or any three of the clinical manifestations are met.
Confirmed cases	suspected cases have either etiological or serological evidence.
Asymptomatic carriers/ Asymptomatic infected persons	Respiratory and other specimens tested positive for the etiological of COVID-19, without relevant clinical manifestations, such as fever, dry cough, sore throat and other self-perceived or clinically recognizable symptoms and signs, and CT imaging without imaging features of COVID-19.
Close contacts	(1)were defined as those in proximity to cases within 2d before the onset of symptoms of suspected and confirmed cases or 2d before the sampling of asymptomatic infected persons when effective protection or distancing measures were not in effect.
	(2)those in proximity to cases in aggregated epidemic settings within 2 weeks before diagnosis such as homes, offices, school classes, and so forth with 2 or more cases of fever and/or respiratory symptoms.

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