Abstract

**Background/Aim**: Analysis of the characteristics of influenza virus in imported cases in Guangxi Province of China.

**Materials and methods**: Throat swabs of imported cases with influenza-like symptoms were detected by real-time PCR from July 2016 to 2019.

**Results**: 1292 laboratory detections of influenza were reported in 3974 influenza-like cases, of which 71.67% (926) were influenza A. The percentage of test positive was 32.82%. The proportion of detections of influenza B was 28.33% (366). 70.51% of the cases mostly came from Vietnam (911). 86.76% (1121) of the cases were imported from Dongxing port, Nanning Airport and Pingxiang port. There was no statistical difference in all age groups. At the same time, 3 of the untyped A-type specimens were sequenced by next-generation sequencing. Among them, the sequences of 2 specimens from Vietnam had high homology with the influenza strain H3N2 in Hong Kong in 2017. The specimen sequence from Thailand is highly homologous to the influenza pandemic strain H1N1 in Brisbane, Australia in 2018.

**Conclusion**: Imported influenza cases in Guangxi have occurred throughout the year, when Winter and spring were the higher. The cases mostly came from Vietnam with influenza A. Relevant measures should be taken to control the further spread of the virus.
Key words: Epidemic, flu-imported situation, influenza, virus, travelers.
1. Introduction

Influenza viruses are associated with a substantial global burden of morbidity and mortality every year [1, 2]. Influenza is a costly disease for pig producers and understanding its epidemiology is critical to control it [3]. Human influenza viruses can be divided into three types A, B and C according to the antigenicity of nuclear proteins [4]. The antigens of influenza A virus are prone to mutation, which often leads to influenza pandemics worldwide[5]. Influenza B virus has less antigenic variation with common sporadic epidemic, mainly causing small and medium epidemics in local areas[6]. Influenza C virus mainly appears in scattered form, mainly affecting infants and young children, and generally do not cause epidemics [7]. Influenza A and B viruses are the main pathogens causing human influenza [8, 9]. In recent years, the influenza A epidemic has shown an increasing trend, and the local epidemic frequency caused by influenza A and B viruses has increased around the world [10, 11].

In February 2017, Guangxi introduced the "Cross-Border Labor Cooperation Pilot Work Plan", which fully leveraged the siphon effect of cheap labor in Vietnam's border provinces and regions, met the growing border economic and trade needs, and further enhanced the competitiveness of Guangxi border areas. However, with the influx of Vietnamese labor force, and the development of tourism, travel in Southeast Asian countries with strong exoticism has become a hot spot. The transmission risk of imported sexually transmitted diseases in the border areas between China and Vietnam will also increase. Influenza A and B viruses are generally susceptible to the population, the number of high-risk groups is large, and there are many hidden
infections carrying influenza viruses around the world. There is a large input and risk of epidemic.

The aim of this study was to identify and compare the seasonality and epidemiological feature of seasonal influenza subtypes after the 2009A/H1N1 pandemic and to lay a foundation for further investigation into the social and environmental factors affecting seasonal influenza transmission, providing guidance to develop a more specific strategy and to strengthen the surveillance of infectious diseases at Guangxi ports.

2. Materials and methods

2.1 Subjects

Guangxi is located in the southwest of China. Guangxi is contiguous to Vietnam. The boundary line is about 637 km. It is the only province which has have 25 ports, including seaport, airport and land port. From July 2016 to December 2019, from the immigration personnel at Guangxi Port, epidemiological investigations were conducted. Inclusion criteria: fever axillary temperature (37.5 ℃), runny nose, stuffy nose, cough, headache, myalgia, fatigue, vomiting and / or diarrhea. Exclusion criteria: self-declaration without cold symptoms or other discomfort symptoms. The pharyngeal swab samples were collected and saved at -40 ℃ to be tested.

2.2 Extraction and detection of nucleic acid

The nucleic acid extraction adopts the automatic nucleic acid extraction instrument and matching reagents of American PerkinElmer Company, and the nucleic acid was
extracted strictly in accordance with the instructions of the kit. Influenza virus detection kit (Guangzhou Daan Gene Biotechnology Co., Ltd.) was used to detect and type influenza virus by using the ABI7500 real-time fluorescent PCR instrument and real-time fluorescent RT-PCR method for port samples. It was strictly followed the reagent instructions. It had no Ct value or Ct≥38 and no obvious amplification curve, which was judged as negative; Ct <35, and with obvious amplification curve, was judged as positive; Ct = 35-38, re-examination was performed once; if the result had obvious expansion curve, it was judged as positive, otherwise it was negative.

**Sequence analysis**

At the same time, parts of the samples were sequenced with the Illumin II NextSeq 2000 sequencer, and the sequenced sequence was spliced, cleaned and edited using cluster X software 3.0. MEGA 5 software was used to carry out the Neighbor-Joining tree, and repeat the construction of the evolution tree 1000 times through the Interior Branch Test method to judge the reliability of its internal branches.

### 3. Results

#### 3.1 Laboratory testing

A total of 3974 pharyngeal swab samples of influenza-like cases were collected, and the positive rate of influenza detection was 32.51% (1292 cases). Influenza A accounted for 71.67% (926 cases), and influenza B virus accounted for 28.33% (366 cases). Among them, H3N2 accounted for 38.00% (394 cases), H1N1 accounted for 30.50% (394 cases), and untyped A accounted for 3.17% (41 cases) (Table 1). A total of 4 imported influenza peaks occurred during the study period; the first influenza peak occurred in July 2017, mainly H3N2; influenza peak entered the second peak period in January 2018, mainly H1N1 and type B; influenza peak entered the third
high peak period in 2019 January, mainly H1N1; and influenza peak entered the
fourth peak period, mainly type B in May 2019 (Figure 1).

3.4 Age distribution

Among the 1292 cases, there were 819 males and 473 females, with more males than
females. Influenza cases were detected in five age groups, including \( \leq 15, 16-30, \)
31-45, 46-60 and > 60 years old, indicating that different age have similar chance to
have influenza (Figure 2).

3.2 Origin of the case

Among the 1292 cases of influenza, in addition to the lack of information in 41 cases,
the rest were mainly from 20 countries and regions including Association of Southeast
Asian Nations (ASEAN) countries, Taiwan, Hong Kong and South Korea. Among
them, Vietnam had a maximum of 911 cases, accounting for 70.51%; followed by
Thailand 133 cases, accounting for 10.29%. ASEAN countries imported 1,203 cases
of influenza, accounting for 93.11%; non-ASEAN countries and regions imported 61
cases of influenza, accounting for 4.72%. There were 33 cases mainly from in Taiwan,
accounting for 2.55%. The main influenza virus subtypes were H3N2 and H1N1
(Table 2 and Figure 3).

3.3 Case entry port

Influenza cases were mainly imported from Dongxing Port, a total of 562 cases,
accounting for 43.50%; followed by Nanning Airport Port with 331 cases, accounting
for 25.62%; followed by Pingxiang Port with 228 cases, accounting for 17.65%. The
influenza cases detected at these three ports accounted for 86.76% of the total
imported influenza cases at Guangxi Port (Table 3 and Figure 4).

3.5 Influenza virus gene sequence analysis and homology comparison

In this study, three of the untyped influenza A virus specimens were sequenced by whole genome. Among them, the sequences of two specimens from Vietnam had high homology with the 2017 influenza pandemic strain H3N2 in Hong Kong (Figure 5A). The sequence of a specimen from Thailand was highly homologous to the influenza pandemic strain H1N1 in Brisbane, Australia in 2018 (Figure 5B).

4. Discussion

The zoonotic viruses can emerge unexpectedly in nature resulting in the establishment of viral infections with important economic and health burden [12]. Influenza viruses infect many vertebrates, with Influenza A, B and C viruses infecting humans, and their high mutation rates allow the evasion of immunity [13]. While the early start and higher intensity of the 2012/13 influenza A virus epidemic was not unprecedented, it was the first influenza A virus epidemic season since the 2009 H1N1 influenza pandemic where the H3N2 subtype predominated [14]. Influenza A and B fluctuated simultaneously or alternately. This was consistent with the global influenza trend in recent years and the local influenza situation [15-17]. Influenza A and B viruses can infect people of different ages, and children and adolescents were high-risk people with influenza A or B virus infection [18]. In this study, 70.51% (911) of the confirmed cases of influenza were mainly from Vietnam, so Vietnamese entry personnel were the key surveillance objects of influenza. The imported influenza cases were mainly influenza A virus infection in winter and spring, and influenza B
virus was distributed. The influenza cases were detected in all age groups, which was consistent with previous studies.

A universal influenza vaccine could considerably alleviate the public health burden of both seasonal and pandemic influenza [19]. The effect of influenza vaccine was closely related to the degree of matching of vaccine strains and epidemic strains [20, 21]. Whole-genome sequencing could provide a faster and more reliable method for outbreak monitoring and supplement routine infection prevention and control team work to allow the prevention of transmission [22]. Therefore, strengthening the clinical and epidemiological monitoring of influenza virus infection at ports was conducive to the early detection of mutant strains, scientific assessment of their occurrence risks, epidemic trends, and potential risk areas to better prevent and control the international spread of influenza, the prevention and control of influenza in Guangxi ports provided a theoretical basis for molecular epidemiology. At the same time, we should strengthen the port health and quarantine line of defense to give full play to the frontier health and quarantine responsibilities of preventing the infectious diseases from entering and leaving.

5. Conclusion

This study highlights the complexity of imported influenza virus activity in a subtropical region of southwest China and suggests that the epidemic features of influenza A and B vary by subtype and different years. The current one-season vaccination in Guangxi, China should be carefully reconsidered.
References


Table 1. Imported influenza cases at Guangxi Ports from 2016 to 2019

<table>
<thead>
<tr>
<th>Influenza-a-like cases</th>
<th>Influenza cases</th>
<th>Proportion of influenza virus subtypes (cases) / influenza cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive rate (%)</td>
<td>H1N1</td>
</tr>
<tr>
<td>Total</td>
<td>32.51%</td>
<td>394/30.50%</td>
</tr>
</tbody>
</table>
Table 2. Sources of imported influenza cases at Guangxi Ports from 2016 to 2019

<table>
<thead>
<tr>
<th>Sources</th>
<th>Vietnam</th>
<th>Thailand</th>
<th>Malaysia</th>
<th>Cambodia</th>
<th>Singapore</th>
<th>Indonesia</th>
<th>Myanmar</th>
<th>Laos</th>
<th>Philipines</th>
<th>Brunei</th>
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<th>Hong Kong</th>
<th>South Korea</th>
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<td>15</td>
<td>13</td>
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<td>20</td>
<td>17</td>
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<td>34</td>
<td>29</td>
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<td>H1N1</td>
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<td>43</td>
<td>16</td>
<td>16</td>
<td>11</td>
<td>10</td>
<td>5</td>
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<td>16</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>18</td>
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<td>46</td>
<td>23</td>
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<td>12</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>5</td>
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<tr>
<td>Unclassified influenza A</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Influenza B</td>
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<td>11</td>
<td>4</td>
<td>5</td>
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Table 3. Distribution of imported influenza cases at Guangxi Ports from 2016 to 2019

<table>
<thead>
<tr>
<th>Input port</th>
<th>Pingxiang</th>
<th>Dongxing</th>
<th>Nanning Airport</th>
<th>Shuikou Airport</th>
<th>Beihai</th>
<th>Fangchenggang</th>
<th>Long Bang</th>
<th>Qinzhou</th>
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<td>216</td>
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<td>81</td>
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<td>15</td>
</tr>
<tr>
<td>Influenza cases</td>
<td>228</td>
<td>562</td>
<td>331</td>
<td>62</td>
<td>55</td>
<td>23</td>
<td>17</td>
<td>13</td>
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</tbody>
</table>
Figure 1. Time distribution of imported influenza positive cases at Guangxi Port from 2016 to 2019.

Figure 2. Number of cases and percentage of positive cases of imported influenza in various age groups at Guangxi Port from July 2016 to December 2019.
Figure 3. Sources of imported influenza cases at Guangxi Ports

Figure 4. Distribution of imported influenza cases at Guangxi Ports
Figure 5 Phylogenetic tree analysis of influenza virus sequences from Vietnamese patient specimens (2 copies) (A). Phylogenetic tree analysis of influenza virus sequence of patients entering Thailand (B).