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Influenza A(H7N9) virus emerged and resulted in human infections in Chongqing, southwestern China since 2017

Kun Su[a,b,1], Sheng Ye[b,1], Qin Li[b,1], Weijia Xie[a], Hongyue Yu[a], Li Qi[b], Yu Xiong[b], Han Zhao[b], Baisong Li[b], Hua Ling[b], Yun Tang[b], Bangzhong Xiao[b], Rong Rong[b], Wenge Tang[b,*], Yafei Li[a,**]

[a] *Department of Epidemiology, College of Preventive Medicine, Army Medical University (Third Military Medical University), Chongqing 400038, People's Republic of China*
[b] *Chongqing Municipal Center for Disease Control and Prevention, Chongqing 400042, People's Republic of China*

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A B S T R A C T

Objectives: Influenza A(H7N9) virus has emerged and resulted in human infections in Chongqing, southwestern China since 2017. This study aimed to describe the epidemiological characteristics of the first epidemic in this region.
Methods: The epidemiological data of patients were collected. Live poultry markets (LPMs), commercial poultry farms (CPFs) and backyard poultry farms (BPFs) were monitored, and poultry sources were registered. Samples derived from the patients, their close contacts, and the environments were tested for influenza A(H7N9) virus by real-time reverse transcriptase polymerase chain reaction. Genetic sequencing and phylogenetic analysis were also conducted.
Results: Since the confirmation of the first patient infected with influenza A(H7N9) virus on March 5, 2017, nine patients had been identified within four months in Chongqing. Their mean age was 45 years, 77.8% were male, 66.7% were urban residents and 55.6% were of poultry related occupation. All patients became infected after exposure to live chickens. The median time interval from initial detection of influenza A (H7N9) virus in Chongqing to the patients' onset was 75 days. Since initial detection in February 2017, influenza A(H7N9) virus was detected in 21 (53.8%) counties within four months. The proportion of positive samples was 2.94% (337/11,451) from February 2017 to May 2018, and was higher ($\chi^2 = 75.78$, $P < 0.001$) in LPMs (3.66%, 329/8979) than that in CPFs (0.41%, 5/1229) and BPFs (0.24%, 3/1243). The proportion of positive samples (34.4%, 22/64) at the premises to which the patients were exposed was significantly higher than that (5.7%, 257/4474) in premises with no patients. Phylogenetic analysis indicated that the viruses isolated in Chongqing belonged to the Yangtze River Delta lineage and resembled those circulated in Jiangsu and Anhui provinces between late 2016 and early 2017.
Conclusion: Influenza A(H7N9) virus was newly introduced into Chongqing most likely between late 2016 and early 2017, which swept across half of Chongqing territory and resulted in human infections within months. The most impacted premises and population were LPMs and poultry related workers respectively in the epidemic.

Introduction

Since the outbreak of human infections with a novel avian influenza A(H7N9) virus in February 2013 (Gao et al., 2013), five epidemic waves have been recorded in mainland China (Wang et al., 2017). By September 5, 2018, 1567 human infections had been confirmed, with the fatality rate being approximately 39% (World Health Organization, 2018). During the previous four waves, human infections had mostly been reported from eastern and southern China (Wang et al., 2017; Li et al., 2014). In the fifth

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[*] Corresponding author at: Chongqing Municipal Center for Disease Control and Prevention, Changjiang 2th Road No. 8, Chongqing 400042, People's Republic of China.
[**] Corresponding author at: Department of Epidemiology, College of Preventive Medicine, Army Medical University (Third Military Medical University), Gaotanyan Street No. 30, Chongqing 400038, People's Republic of China.
E-mail addresses: twg@cqcdc.org (W. Tang), liyafei2008@tmmu.edu.cn, liyafei2008@hotmail.com (Y. Li).
[1] These authors contributed equally to this study.

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wave, however, human infections with influenza A(H7N9) virus were identified in Chongqing Municipality (hereinafter called ‘Chongqing’), Sichuan Province, Yunnan Province and Tibet, southwestern China for the first time (Su et al., 2017). The outbreak in these new regions and its large epidemic size immediately alerted the international community that influenza A(H7N9) virus could easily disseminate into more regions under the current prevention and control strategy.

Chongqing is one of the economic centers of southwestern China and the largest municipality under direct control of the national government, which has a population of 31 million in 2016 and covers approximately 82,400 square kilometers. It is about 1700 km away from Yangtze River Delta in eastern China and 1300 km from Pearl River Delta in southern China, which are the two established outbreak sources of influenza A(H7N9) virus (Wang et al., 2016). There had been no report of human infection with avian influenza virus including H7N9, H5N1, H9N2 and H10N8 before 2017, and seasonal influenza A(H3N2) and influenza A(H1N1) pdm09 were the dominant influenza A viruses circulating in Chongqing in recent years (Qi et al., 2016).

Influenza A(H7N9) virus emerged and resulted in human infections in Chongqing, southwestern China since 2017. The present study aims to describe the epidemiological characteristics of the first epidemic in this new region regarding human infection, environment contamination by the virus and genetic evolution of the virus.

Methods

Patient identification and epidemiological investigation

Suspected patients with influenza A(H7N9) virus infection were identified through the Chinese surveillance system for pneumonia of unknown origin and the Chinese sentinel surveillance system for influenza-like illness. Respiratory and blood samples of suspected patients were collected. Consistent with previous studies (Gao et al., 2013; Li et al., 2014), a patient with influenza A(H7N9) virus infection was verified by means of real-time reverse transcriptase polymerase chain reaction (rRT-PCR), viral isolation, or serologic testing. A structured questionnaire was employed to collect demographic, epidemiologic and clinical data on each patient, through a review of medical records and interviews with patients and their relatives, contacts and health care workers. Patients’ poultry exposure history was collected in detail including information on date, location, manner, and duration of the exposure, and breed, living status (live, sick or dead), source, flock size and transportation mode of the poultry to which the patients were exposed were documented using a semi-structured questionnaire. We collected the information on the poultry to which the patients were exposed based on interviews with poultry dealers, the dealers’ purchasing and shipping records, and our field observations.

A close contact was defined as a person known to have been within 2 m of, or to have had direct contact with the respiratory secretions or fecal material of, a patient with laboratory-confirmed influenza A(H7N9) infection any time from the day before the onset of illness to when the patient was isolated in the hospital or died. The nasopharyngeal swabs were collected from all close contacts within 24 h after the patient was virologically confirmed. Each close contact was registered and observed medically for 10 days for respiratory symptoms after their last exposure. The collection of nasopharyngeal swabs was repeated if the close contact developed influenza-like illness during the 10 days of medical observation. All swabs were tested for influenza A(H7N9) virus using rRT-PCR in influenza network laboratories.

Environmental surveillance on influenza A(H7N9) virus

A surveillance program for influenza A(H7N9) virus in poultry-related premises had been conducted in 8 counties or districts (hereinafter called ‘counties’) from March 2013 to December 2016 in Chongqing. A sampling premise was defined as a live poultry market, a commercial poultry farm or a backyard poultry farm. A multi-stage sampling strategy was performed to select sampling counties and premises. At stage 1, two districts (Nanan and Jiulongpo) were randomly selected out of 9 downtown districts, and six counties (Jiangjin, Tongliang, Changshou, Liangping, Youyang, Dianjiang) were randomly selected out of 30 suburb counties. At stage 2, the two largest LPMs, one CPF and one village were selected in each selected county of stage 1. At stage 3, the largest BPF was selected in the selected village of stage 2. Therefore, there were four premises (two largest LPMs, one CPF and one BPF) that could be selected as sampling places in each selected county. Every month, two or three premises were randomly selected out of the four premises to collect at least 6 environment samples prior to disinfection. Since January 2017, the surveillance area was expanded to all 39 counties.

Environment sample types included live poultry feces, poultry cage swabs, drinking water, sewage from cleaning poultry, and swabs of tables used for slaughtering or processing poultry. Poultry information such as breed, living status (live, sick or dead), source, flock size and transportation mode were documented using a semi-structured questionnaire by the staff from the county center for disease control and prevention (CDC). This information was collected based on the interviews of poultry dealers, the dealers’ purchasing and shipping records, and the field observations. The environment samples were tested for influenza A virus, and positive samples were further tested for influenza A(H7N9) virus using rRT-PCR in influenza network laboratories. The detailed collection methods of environment samples and rRT-PCR assay for influenza A(H7N9) virus have been described in a previous study (He et al., 2015).

Gene sequencing of influenza A(H7N9) virus

In Chongqing Municipal CDC, the H7N9 positive samples were inoculated in cells of MDCK and lysed in lysis buffer. At the Chinese National Influenza Center, RNA of influenza A(H7N9) virus was extracted using an RNeasy minikit (Qiagen, Hilden, Germany). The extracted RNA was subjected to reverse transcription and amplification using a SuperScript III One-Step RT-PCR system (Thermo Fisher, Waltham, MA, USA). Genome sequencing was implemented on a MiSeq high-throughput sequencing platform (Illumina, Inc., San Diego, CA, USA) with a paired read length of 300 bp.

The reference sequences of influenza A(H7N9) viruses were downloaded from the Global Initiative on Sharing Avian Influenza Data (GISAID) (https://www.gisaid.org/) and Genbank (https://www.ncbi.nlm.nih.gov//genbank/).

Phylogenetic analysis

We aligned the HA and NA gene sequences of influenza A (H7N9) virus using Molecular Evolutionary Genetic Analysis software (MEGA, version 7.0) (Kumar et al., 2016). Reference influenza A(H7N9) viruses were selected earlier in China from 2013 to 2017. A phylogenetic tree was constructed by a neighbour-joining method with 1,000 bootstrap replicates, and we compared the genetic identity of isolates of influenza A(H7N9) viruses in Chongqing to reference influenza A(H7N9) viruses selected based on phylogenetic tree by using BLAST (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Ethics statement

The National Health and Family Planning Commission ruled that data collection from humans infected with avian influenza A (H7N9) virus and their close contacts was part of the continuing public health investigation of an emerging outbreak and was exempt from institutional review board assessment.

Results

Human infections with influenza A(H7N9) virus

There were no human infections with influenza A(H7N9) virus reported in Chongqing until 5 March, 2017, when a 65-year-old male who had developed acute fever, cough and pneumonia, was virologically confirmed in Fengjie county, Chongqing. This was unexpected because it was only 25 days after the first positive environment sample for influenza A(H7N9) virus was confirmed in February 2017 in a sample of chicken feces from a CPF in Chongqing. From March 2017, a total of nine patients had been confirmed continuously within four months (Figure 1), including 8 patients identified from the surveillance system for pneumonia of unknown origin and 1 from the sentinel surveillance system for influenza-like illness. From July 2017 to May 2018, no more patients were confirmed. The patients identified were found in 8 distributed counties and none of the patients had been in contact with another patient (Figure 2**B**). No cluster of human infection was found in the epidemic. The median time interval from influenza A(H7N9) virus initially detected in a poultry related environment of Chongqing to the patients' onset was 75 days (Table 1).

Among the nine patients, both the mean and median ages were 45 years (interquartile range: 41–49, range: 25–66), 77.8% were male, 66.7% were urban residents and 55.6% were of poultry related occupation (poultry dealers, poultry butchers and farmers raising poultry on a backyard farm). The patients had a higher proportion of poultry dealers and/or butchers (33.3%, 3/9) than that of the overall Chinese epidemic (Fisher's exact test P value = 0.042).

The initial symptoms were influenza-like symptoms of fever (100%), cough (88.9%), muscle pain (55.6%) and sore throat (33.3%) etc. Severe pneumonia (100%) and acute respiratory distress syndrome (100%) developed 4–7 days after onset. The median days from illness onset to initiation of antiviral treatment and diagnosis were 7 and 8 respectively in the epidemic. All patients were treated with anti-viral, mechanical ventilation and other intense supportive therapies. However, only 4 survived with the other 5 patients resulting in death, including that of a pregnant woman.

Epidemiological investigation

All patients had a history of contact with live chickens 10 days before disease onset, but did not have contact with ducks, geese, wild birds, pigs, known febrile people or anyone who had respiratory symptoms, and no travel out of Chongqing. Six patients (66.7%) were exposed to live chickens in LPMs, two (22.2%) in BPFs and one (11.1%) in a restaurant kitchen. There were live chickens originally transported from other provinces in the premises to which the patients were exposed. It was also found that live chickens were transported from closed LPMs in the urban areas of a county to its countryside, where influenza A(H7N9) virus was detected and a second patient was confirmed. The reasons why the patients came to the premises to which they were exposed included occupational exposure (33.3%), passing by live chicken storage (33.3%), raising poultry on BPFs (22.2%), and buying chickens in the LPM (11.1%) (Table 2).

There were no patients with human-to-human transmitted infections. A total of 813 close contacts were identified, and 22 developed respiratory symptoms during the 10-day follow-up. None of the nasopharyngeal swabs, collected at the start of their medical observation and on the onset of influenza-like symptoms, were positive for influenza A(H7N9) virus by rRT-PCR, indicating that no human-to-human transmission occurred in the epidemic.

Environmental influenza A(H7N9) virus detection

From March 2013 through May 2018, we collected 15,731 environment samples from poultry-related premises in Chongqing. None of the 4,280 samples were tested positive for influenza A(H7N9) virus between March 2013 and January 2017. The first positive environment sample for influenza A(H7N9) virus was confirmed in February 2017 in a sample of chicken feces from a CPF where the chickens were transported from the neighbouring Sichuan province. Since the initial detection in February 2017, influenza A(H7N9) virus was detected in 21 (53.8%) counties within four months. The proportion of positive samples was 2.94% (337/11,451) from February 2017 to May 2018, and was higher ($\chi^2 = 75.78$, $P < 0.001$) in LPMs (3.66%, 329/8979) than that in CPFs (0.41%, 5/1229) and BPFs (0.24%, 3/1243).

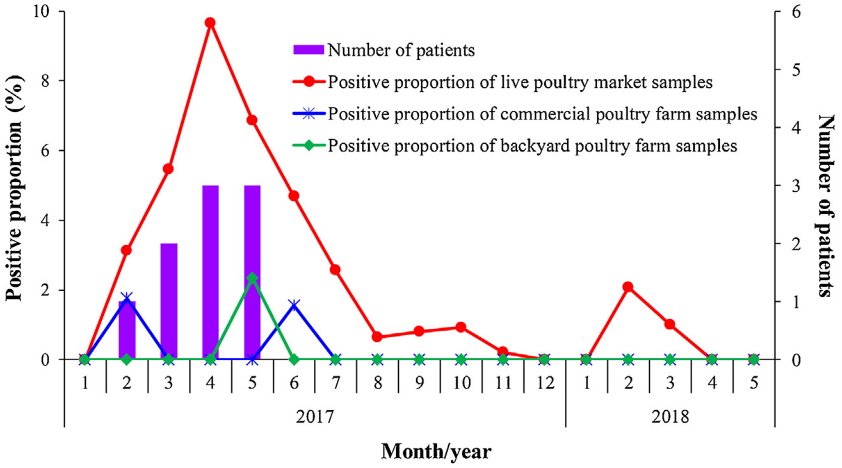

Figure 1. Positive proportion of environmental samples for influenza A(H7N9) virus and number of patients with influenza A(H7N9) virus infection in Chongqing, China, January 2017–May 2018. The purple bars represent the number of patients with influenza A(H7N9) virus infection in Chongqing. The red, blue and green lines represent the positive proportions of samples collected from live poultry markets, commercial poultry farms and backyard poultry farms respectively.

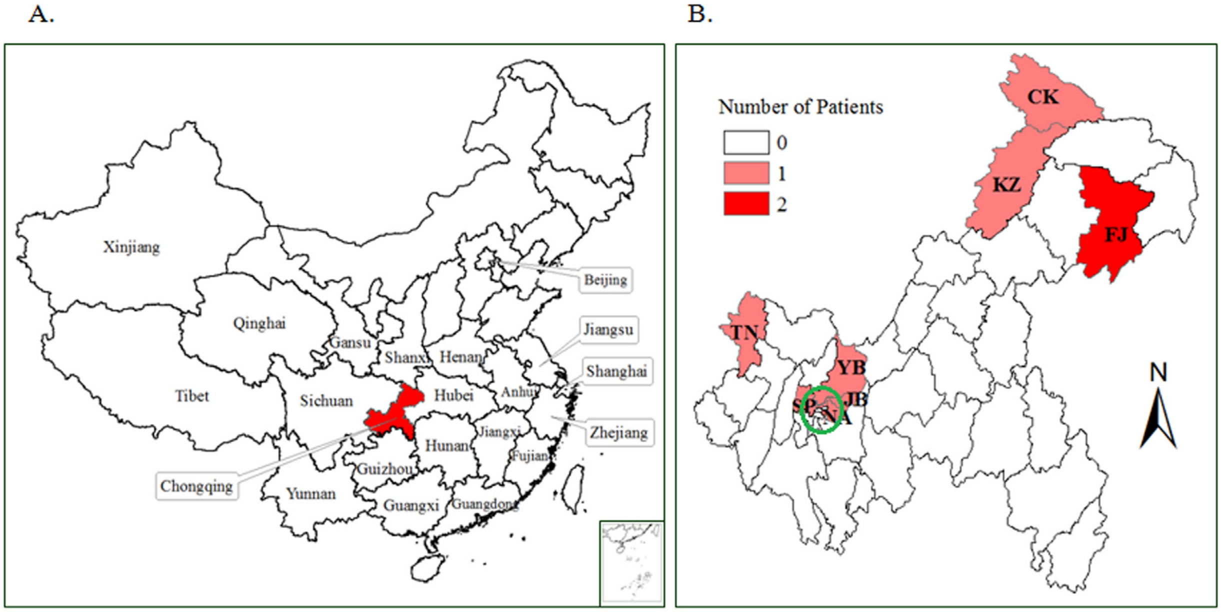

Figure 2. Location of Chongqing in southwestern China, with neighboring provinces (A) and geographical distribution of patients with influenza A(H7N9) virus infection in Chongqing (B), China, January 2017–May 2018. In map A, the red area indicates the region of Chongqing. In Map B, dark red, pale red and white indicate the county or district with two, one or no patient with influenza A(H7N9) virus infection respectively; and the green circle indicates the location of downtown area of Chongqing.

Table 1
Time interval from initial detection of influenza A(H7N9) virus in poultry-related environment to patients' onset in Chongqing, China, January 2017–May 2018.

Patient number	Date of onset	Time interval (days)
1	Feb. 23, 2017	15
2	Mar. 6, 2017	26
3	Mar. 19, 2017	39
4	Apr. 20, 2017	71
5	Apr. 25, 2017	76
6	Apr. 24, 2017	75
7	May. 8, 2017	89
8	May. 29, 2017	110
9	May. 28, 2017	109
Mean/median	–	68/75

Table 2
Poultry exposure history of patients with influenza A(H7N9) virus infection in Chongqing, China, January 2017–May 2018.

Variable	No.	Proportion (%)
Premises exposed by the patients		
Live poultry market	6	66.7
Backyard poultry farm	2	22.2
Restaurant kitchen	1	11.1
Why the patients came to the premises exposed by the patients		
Occupational exposure to poultry	3	33.3
Passing-by live chicken storage	3	33.3
Raising poultry on backyard farm	2	22.2
Buying poultry in the live poultry market	1	11.1
Poultry breed exposed by the patients		
Chicken	9	100.0
Other	0	0.0
Condition of the poultry exposed by the patients		
Live	9	100.0
Dead	0	0.0
Live poultry from other provinces in the premises exposed by the patients		
Yes	9	100.0
No	0	0.0

Table 3
Comparison of influenza A(H7N9) virus detection of two periods in Chongqing, China, February 2017–May 2018.

Variable	Feb. 2017–Jun. 2017	Jul. 2017–May 2018	Sum	χ^2	P
Samples					
Total No.	5348	6103	11451		
Positive No.	275	62	337		
Positive proportion (%)	5.14	1.02	2.94	169.9	<0.001
Premises					
Total times of premises monitored	523	861	1384		
Total times of premises with positive samples detected	88	13	101		
Positive proportion (%)	16.83	1.51	7.30	112.82	<0.001

The proportion of positive samples in LPMs had a high monthly peak in April 2017 (9.67%, 49/507) and another low monthly peak in February 2018 (2.07%, 11/531) (Figure 1).

The positive proportions of the samples and premises were higher from February to June 2017 than from July 2017 to May 2018 (Table 3). In the premises to which the patients were exposed, the proportion of positive samples was 34.4% (22/64), which was significantly higher ($\chi^2 = 89.6$, $P < 0.001$) than that (5.7%, 257/4474) in premises with no patients during the same period. Field investigation on poultry sources demonstrated that there were live chickens transported from other provinces in 100% (59/59) of H7N9-virus-positive premises between February and June 2017, compared to 11.1% (1/9) between July 2017 and May 2018.

Viral analysis

We performed sequencing analysis on HA and NA genes of influenza A(H7N9) viruses isolates in Chongqing. These viruses had no polybasic amino acid sequence at the HA cleavage site, and were of low pathogenicity avian influenza ones to poultry. The phylogenetic trees of the HA (Figure 3**A**) and NA (Figure 3**B**)

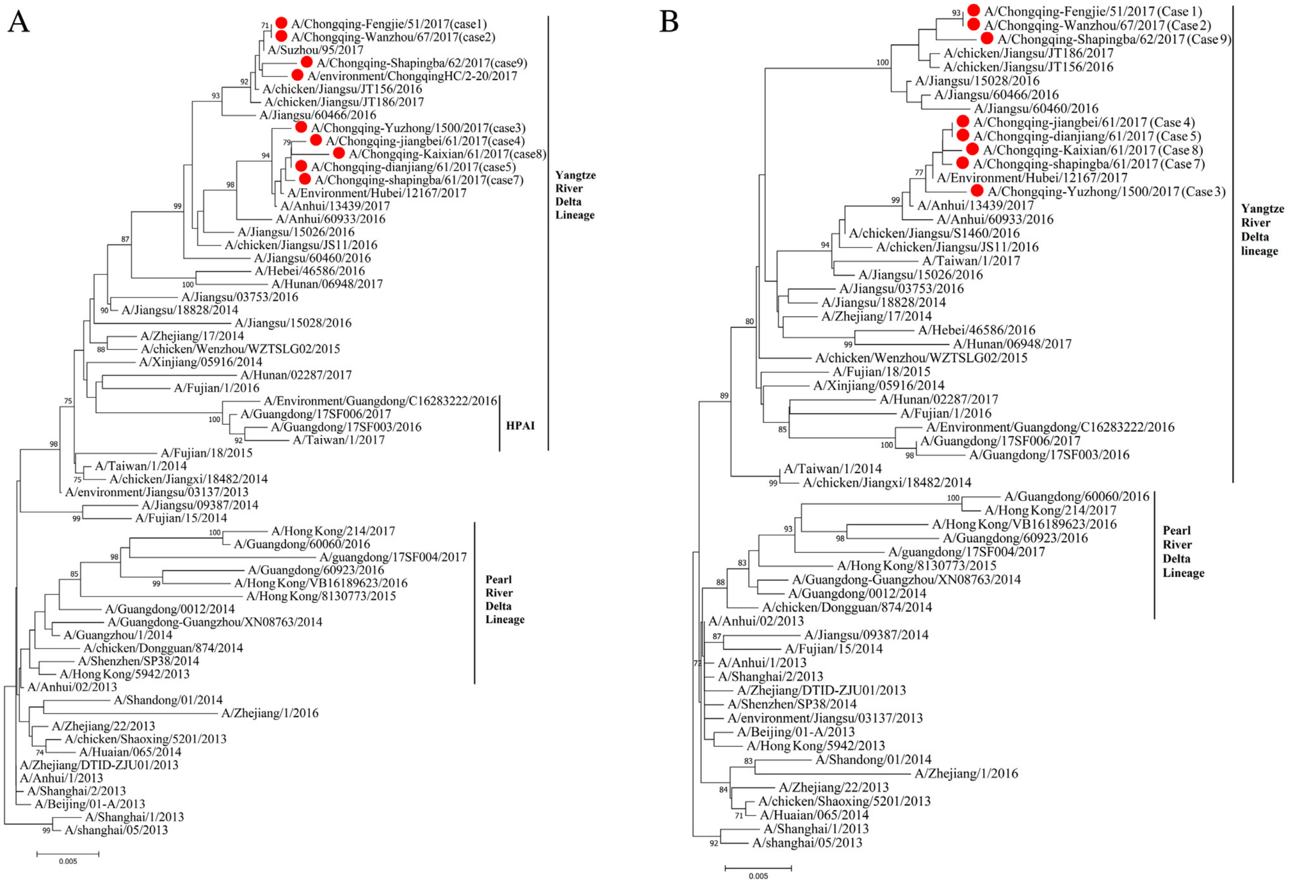

Figure 3. Phylogenetic analysis of HA (A) and NA (B) sequences of influenza A(H7N9) viruses isolated in Chongqing, China and that of reference influenza A(H7N9) viruses identified earlier in China, 2013–2018. Red dots indicate the viruses isolated in Chongqing and the note in the bracket explains from which patient the virus was isolated. The evolutionary history was inferred using the Neighbor-Joining method. The scale bar represents the number of substitutions per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches.

showed that the viruses isolated in Chongqing belonged to the Yangtze River Delta branch, sharing the same ancestor with those circulated in Jiangsu, Anhui and Hubei provinces from late 2016 to early 2017 during the fifth wave of H7N9 epidemic. And all these viruses were descended from influenza A virus (A/Shanghai/1/2013 (H7N9)) and (A/shanghai/05/2013(H7N9)).

Although the influenza A(H7N9) viruses isolated in Chongqing had the same origin, they were not identical and clustered in two clades of the phylogenetic tree, which suggested that these influenza A(H7N9) viruses might be of different sources. Four viruses isolated in Chongqing were nearly identical to those found in chickens and infected patients in Jiangsu province, and the other five viruses were nearly identical to those found in infected patients in Anhui province. For influenza A(H7N9) viruses isolated in Chongqing, the integrated identity of HA and NA genes ranges from 99.19% to 99.90% compared to that of the neighboring or ancestor viruses in the phylogenetic tree (Table S1, S2 and S3 in the Supplemental material).

Discussion

In this investigation, we identified the first epidemic of human infection with influenza A(H7N9) virus in Chongqing, southwestern China, and presented epidemiological characteristics of this epidemic regarding human infection, environment contamination by the virus, and genetic evolution of the virus.

Influenza A(H7N9) virus was newly introduced into Chongqing most likely between late 2016 and early 2017. The basis of the epidemiological finding includes the following aspects. First, based on a robust surveillance system, there was no influenza A(H7N9) virus detected in LPMs, CPFs and BPFs until February 2017 in Chongqing. Second, nine patients who lived in Chongqing had been confirmed to be infected with influenza A(H7N9) virus continuously within four months since March 2017, through the surveillance of pneumonia of unknown origin and influenza-like illness, while there was no confirmation prior to February 2017. Third, phylogenetic analysis showed that the viruses isolated in Chongqing belonged to the Yangtze River Delta lineage and resembled those circulated in Jiangsu and Anhui provinces between late 2016 and early 2017.

Influenza A(H7N9) virus spread quickly across Chongqing since its initial detection, and the most impacted premises were LPMs. Although the role of wild birds cannot be ruled out, outbreak investigations suggested that live poultry transportation most likely played an important role in the process. Potentially infected poultry from other provinces entered the markets of Chongqing. In eastern and southern China, the fifth wave had more human infections and more serious contamination in poultry-related environments than the previous four waves (Wang et al., 2017; Zhou et al., 2017; Huo et al., 2017). Many affected counties closed LPMs and suspended live poultry trade, which would have contributed to the transportation of live poultry to remote epidemic-free regions (Huo et al., 2017; Yu et al., 2014; Fournie and Pfeiffer, 2014; Zhou et al., 2015). On the other hand, potentially infected poultry were transported from one place to another within Chongqing. In Chongqing, all LPMs in one county would be closed if influenza A(H7N9) virus were detected in one of its LPMs or human infection occurred in the county, which could promote the transportation of potentially infected poultry to other places.

The introduction into and spread within Chongqing of influenza A(H7N9) virus soon led to human infection, whereas there were no reported human influenza A(H5 or H9) virus infections despite its more sustainable and serious contamination in the territory for over 5 years. The results added more epidemiological evidence that influenza A(H7N9) virus is more capable of breaking through a species barrier to infect humans than influenza A(H5 or H9) virus due to different binding receptors (Xiong et al., 2013; Zhou et al., 2013). All H7N9 human patients in Chongqing were infected with the virus after being exposed to live chickens, the patients were from both urban and rural areas, and no close contact was confirmed to be infected. These findings are similar with previous studies (Bao et al., 2013; Han et al., 2013; Chen et al., 2013; Yang et al., 2017).

However, it is very interesting that the patients of Chongqing had a higher proportion of poultry dealers and/or butchers than that of the overall Chinese epidemic (Wang et al., 2017). This may be due to the fact that the epidemic was newly introduced and the most impacted premises and population were LPMs and poultry related workers. It is also interesting that the proportion of positive samples was much higher in the premises to which the patients were exposed than that in other premises, and the positive proportions of the samples and premises were higher from February to June 2017 (with human infections confirmed) than from July 2017 to May 2018 (with no human infection confirmed). These evidences may suggest that a more serious environment contamination by the virus was the risk factor for human infection.

After its introduction, influenza A(H7N9) virus has probably established in Chongqing. The virus has been detected continuously in LPMs after July 2017 with a low monthly peak of proportion of positive samples in February 2018. If the virus becomes enzootic among poultry in rural areas of Chongqing, it would pose a long-term threat to public health and the poultry industry. Influenza A virus mutated frequently (Wang et al., 2014), and several studies have already reported mutations of influenza A (H7N9) virus related to high pathogenicity to poultry (Quan et al., 2018; Ke et al., 2017), increasing binding affinity to facilitate viral transmission (Wang et al., 2018), reducing sensitivity to oseltamivir (Gao et al., 2014), and the increased virulence and expanded host range to ducks (Shi et al., 2018). Furthermore, influenza A (H7N9) virus might re-assort with other subtype influenza A virus (Zhu et al., 2013; Chen et al., 2016) with human and some animals acting as mixing vessels. If influenza A(H7N9) virus obtains genes associated with sustainable human-to-human transmissibility, it would be a disaster (Su et al., 2017; Alcorn, 2013).

Therefore, the prevention and control of influenza A(H7N9) virus should be held under high provision and priority. Regular surveillance on influenza A(H7N9) virus in poultry related environments could be the first important step. Our practice of surveillance enables us to assess the risk of human infection in a timely manner, and track and eliminate the source of the virus under the joint prevention and control mechanisms. LPMs are the key places to control influenza A(H7N9) virus epidemic. More rigorous management should be implemented in live poultry markets to decrease the chance of exposure to poultry for human beings. In the long term, more radically effective control measures should be put into place, such as prohibition of inter-regional live poultry transportation, permanent closure of LPMs, and centralized slaughtering (Wang et al., 2017; Su et al., 2017; Lam et al., 2015).

Our study had several limitations. First, environment surveillance on influenza A(H7N9) virus had not been conducted in all counties before 2017. Although we tried our best to make the surveillance premises be representative, there was a still possibility that the virus circulated in counties without surveillance before 2017. Second, environment surveillance in rural areas was insufficient due to limited financial support, which may lead to an under-estimation of environment contamination by the virus in rural areas. Third, we could not pin down the exact location where live poultry were from due to the lack of a poultry tracing system in mainland China. Forth, some cases of human infections of influenza A(H7N9) virus were probably missed due to surveillance limitations.

Conclusions

In conclusion, we identified the first epidemic of human infection with influenza A(H7N9) virus in Chongqing, southwestern China, and presented the epidemiological characteristics of this epidemic. Our findings imply that: 1. Influenza A(H7N9) virus was newly introduced into Chongqing most likely between late 2016 and early 2017, and swept across half of Chongqing territory and resulted in human infections within months. 2. The most impacted premises and population in the epidemic were LPMs and poultry related workers, respectively. 3. Influenza A(H7N9) virus has probably established in Chongqing, posing a long-term threat to public health and the local poultry industry, and surveillance should be strengthened in both urban and rural areas. 4. Preventive measures should focus on rigorous management for LPMs and decreasing the chance of exposure to poultry for human beings.

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The opinions in this article are the authors' own and do not reflect the view of Chongqing Municipal CDC or Chongqing Municipal Commission of Health and Family Planning.

Conflicts of interest

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.011.

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