Spatial epidemiology of hemorrhagic fever with renal syndrome and rodent hantavirus infection in Guangzhou city, China: lessons learned from a study with long-term surveillance data

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Abstract

Background: Though Guangzhou city is not a region with a high burden of hemorrhagic fever with renal syndrome (HFRS) comparing with other areas in China, outbreaks still occur in the city due to the existence of urban rodents and a large urban population at risk. In this study, descriptive temporal analysis and geographic information system (GIS)-based spatial analysis were performed on reported HFRS cases and rodent surveys with the goals of identifying risk areas for public health interventions and presenting the methods and outcomes of the surveillance that can be studied by similar urban settings.

Methods: Period prevalence and the number of positive cases at different geographical scales of the city during 2008-2016 were calculated and summarized based on HFRS and rodent-borne disease surveillance data. Spatial point pattern analyses were conducted to describe global and local clustering effects of different measurements of human HFRS and rodent hantavirus at old-town and whole-city levels. Results: Geographical distributions of HFRS cases and traps with hantavirus antibody-carrying rodents from 2008 to 2016 were mapped at the city and old-district levels in the aspect of the crude number of positive cases and period prevalence. The weighted K-function suggested that no global clustering effect existed for both the rodent and human cases in different scales of the city. Using Gi*(d) statistic, 22 significant local clusters of high infection (P<0.05) with different radius were discovered for the human and rodent surveillance data, and a zoonotic link was built based on the overlay of clusters of the two species. Also, the seasonal occurrence of HFRS presented a summer peak in the city.

Conclusion: The applications of descriptive temporal analysis, GIS and spatial point pattern analysis, offer ways to quantify zoonotic hantavirus risks across different times and spatial scales and to further recognize putative environmental determinants potentially influential in increased disease risks. The study also fills information gaps about hantavirus surveillance in the non-endemic region, and
the methods used for rodent survey in China for controlling HFRS, which is not well documented.

Background

Hemorrhagic fever with renal syndrome (HFRS) is a zoonotic infectious disease transmitted by rodents; it is caused by various species of hantaviruses and widely distributed in Afro-Eurasia (1). There is a wide distribution of HFRS in eastern Asia, especially in Russia, Korea, and China (1). In the Americas, hantavirus leads to hantavirus pulmonary syndrome (HPS) rather than HFRS. In Australia, no human case of hantavirus infection was reported.

Several risk factors contribute to the transmission of hantavirus. Direct exposures to rodents and their excrements is the most predominant risk factor (2). Humans are indirectly exposed to the pathogens when inhaling aerosolized urine, saliva or droppings of the infected animals. Human to human transmission of hantaviruses is rare.

Recreational and occupational outdoor activities are included as another related set of risk factors globally (3). Meteorological changes are environmental determinants affecting the population of rodents, which changes the disease burden in humans (4).

HFRS in China accounts for nearly 90% of total HFRS cases worldwide, and the country has the most serious endemic situation around the globe (5). In spite of combined intervention measures including environmental regulation, control of rodents, and vaccination, 20,000 - 50,000 human cases were reported annually in China. The disease burden of HFRS has high heterogeneities across the country – regions in Shandong, Hebei, Henan provinces, and cities in the northeastern part of the country had the most likely clusters of HFRS, the Lingnan region in southern China is not a traditional “hotspot” of HFRS (6).

Guangzhou city, located in the humid subtropical climate zone of Lingnan region in southern China, is an urbanized metropolitan with a high population. In 2019, the city has
a population of around 12-million, and the number keeps increasing. Urban villages, a byproduct of urbanization in China, were built to offer an affordable location for migrant workers to reside in. These villages were widely distributed in the city. Within the villages, the population density is high, making it difficult to recycle garbage and creating a good habitat for hantavirus-carrying rodents. According to Guangzhou Center for Disease and Control (GZCDC), occurrence of HFRS can be observed each year in the urban villages. The rodent can not only spread hantavirus to residents in the villages but relocate to other neighborhood and pose public health threats.

Since Lingnan is not a region of high risk for HFRS, the epidemiology of the disease is understudied. However, GZCDC owns a robust rodent-borne disease surveillance project, and hantavirus monitoring is included its objective. For hantavirus surveillance from the human side, Guangzhou not only has passive surveillance for HFRS, which belongs to Chinese notifiable infectious disease reporting system, a city-level active surveillance system targeting high risk local areas also exist. Hence, these surveillances offer a good opportunity for studying HFRS in Guangzhou city so as to strengthen the system and provide lessons of surveillance for other cities (i.e., Macau, Hong Kong) in the Lingnan region.

Spatial analysis is a useful tool for infectious disease surveillance as it notifies public health official areas with high risk, which is necessary for an effective intervention. However, the spatial patterns of HFRS and rodent hantavirus infection remain unclear in Guangzhou city. Using human and rodent surveillance data, our study implements spatial analysis to describe the distribution pattern of HFRS in Guangzhou city in different spatial scales, and to study the geographical associations between the disease burden of HFRS in humans and hantavirus infection in rodents. As mentioned before, although a lot of studies have been conducted to understand the epidemiology of HFRS in China, little
research was conducted to understand the epidemic pattern of the disease in Lingnan and Guangzhou, where is a highly dense population center with high influx of migrant workers. Also, in spite of the fact that Guangzhou is not a region with a high disease burden of HFRS, it has its particular characteristics of HFRS transmission dynamics due to its urban setting. It is worth to have an understanding of HFRS and rodent hantavirus in this region where related research is neglected.

Methods

Ethics statement

The study was reviewed and approved by the Ethics Committee of Guangzhou Center for Disease Control and Prevention (GZCDC – ER (A) 2016006).

Study area

The study site is located in Guangzhou city (112°57'E - 114°3'E, 22°26'N - 23°56'N), which is the capital of Guangdong province in southern China (S1 Fig). The city has a permanent population of 13,501,100 distributed in 11 districts with a total land area of 7434.40 km². The districts of Liwan, Yuexiu and Haizhu are considered to be old areas with a population of 3,857,643 and with the sum area of 58,357 km². The other districts (Tianhe, Baiyun, Huangpu, Panyu, Huadu, Nansha, Zengcheng, Conghua) are newly developed after the 1980s.

Data collection and management

De-identified Records on HFRS cases between 2008 and 2016 were obtained from the active and passive HFRS surveillance systems at GZCDC, which included 2,154 human HFRS cases reported in Guangzhou. The data were collected from hospitals, clinics, community-level hygiene centers and CDCs in the 11 districts. In our study, these agencies will be considered as health care provider collectively. The records on human HFRS cases were further reported to and arranged by the division of parasitic and endemic
diseases control in GZCDC, which provided the data. To calculate period prevalence, the total number of people receiving diagnostic tests in each healthcare center was recorded, this group of people is from the following sources: individuals visited the center as they suspected themselves were infected by hantavirus based on comparing their personal feelings with symptoms described in the media campaign against HFRS, or the ones thought they were at risk of the infection due to the environments they were exposed to; another source is the at risk personals contacted by clinical doctors since they were found to be susceptible of infection based on epidemiological history. These three groups of people were defined as the population at risk for HFRS in this study. Human prevalence of HFRS was calculated as the number of individuals tested positive divided by the population at risk in the same location. Also, the number of cases in each location was summarized.

Data of rodents (covering the year 2008 to 2016) in the city were previously collected by local rodent control companies and CDCs in each district. This active trapping activity was led by GZCDC’s rodent-borne disease surveillance project. rodents were gathered by placing Sherman traps at trapping sites during nightfall, and the traps were recalled in the morning of the second day post-deployment. Trapping locations were selected by district-level CDCs; the traps were set in both indoor environments of residential areas and outdoor wild areas. For the indoor trapping at the district level, 20 to 30 households were selected each month, and three to five traps were placed in each household. For the outdoor trapping at the same administrative level, the monthly trapping activities were held in farms, grasslands, ponds areas, brush woods, forests and other rodent habitats. Although sampling bias may exist due to the subjected selection of the trapping locations, this has been reduced as much as possible based on a priori knowledge of the field workers. Blood and lung samples were collected from the captured animals;
immunofluorescent antibody reaction (IFA) and reverse transcriptase – polymerase chain reaction (RT-PCR) tests were used to determine whether rodents were infected with hantavirus. The number of rodents with positive results in a trap divided by the number of rodents caught in the same trap is the period prevalence of rodents in the trap. The number of positive rodents in each trap was also recorded.

**Description of temporal and spatial patterns**

With a focus on exploring seasonality, we explored the time-series characteristics of monthly HFRS occurrence (“forecast” package in R, version 3.5.1) in Guangzhou city. Temporal plots of annual cases and prevalence were also plotted to display the temporal distributions of HFRS. A district-level polygon based map at the scale of 1:50,000 were retrieved from Trimble data marketplace (7). Locations of health care providers and all traps were georeferenced by retrieving coordinates for each location, and dot maps with district-level point layers containing these addresses were created. The number of reported human cases and positive rodents and the number of total at-risk humans/captured rodents at the same location were summarized in terms of latitude and longitude. To adjust for small number problem (causing high variation in measures of disease frequency) in reporting of disease prevalence for areas with small population size, maps of both the prevalence and the number (i.e., case count) of positive human HFRS or rodent hantavirus in different years were created so that the two measurements could cross-reference each other. Compared with the other parts of the city, the old districts (i.e., Yuexiu, Liwan, Haizhu) had a higher population of permanent residents and more healthcare providers and higher frequencies of rodent trapping activity; the geographical distributions of health care providers and rodent traps were more homogeneous, and the number of them was more abundant. Due to these facts, a particular emphasis was placed on these three districts using the same analyses for the whole city. For the human HFRS
surveillance data, we performed the temporal and spatial analysis based on the assumption that the people visited the healthcare providers were all lived in Guangzhou city, and the individuals would choose the closest provider to receive tests or seek treatment for HFRS. This assumption is based on the fact that the early stage of HFRS is acute and painful, so a susceptible patient of HFRS will select the most proximal healthcare provider to seek diagnosis/treatment. The assumption is also eligible for the healthy at-risk personnel contacted by clinicians for HFRS tests; this part of work belongs to the active surveillance which is conducted by community-level hygiene centers, an individual would visit the closest provider to receive test (rather than a distant one) for convenience. Hence, the spatial and temporal patterns identified based on the medical institutions can closely represent the real distribution of HFRS in Guangzhou city. Since the ranges of the two disease measurements (i.e., prevalence and number of positive case) varied in different geographical scales and times, they were categorized in different ways by equal interval mode. The variance of prevalence/the number of cases were indicated by different colors and circle sizes in maps. The disease mapping was conducted using QGIS 2.18.15 software (8), and the projection of the maps in this study was Asian Lambert Conformal Conic.

**Point pattern analysis**

Point pattern analysis is a study of spatial arrangement of points in a geographical area (9). In this study, the analysis was used to evaluate if clustering patterns of human HFRS cases and rodent hantavirus existed in the two scales of the city, and clustering tests based on the software program Point Pattern Analysis (PPA) (10) was used to calculate a global spatial statistic - the weighted-K function, and the local spatial statistic - the Gi*(d). Spatial statistics is computed by giving differential weight to the distances between studied events (11). The weighted-K function utilizes a Euclidean distance matrix of all
distances among points for analyzing the spatial distribution patterns of measurements of values (case count and prevalence) among all places (rodent traps and healthcare providers). The analysis is conducted in rectangular areas, which are created based on the magnitude (size) of the study area, the number of points (i.e., health care providers, traps), distances among points, and the value of each point (e.g., case count, prevalence). Compared with a confidence interval generated based on independent and random allocations of observed values to the defined locations for an identified number of Monte Carlo Simulations, the P value for whether the observed pattern distributes randomly is determined. In this study, the weighted-K function was used to determine whether global clustering existed in the city and old-district levels. The Gi*(d) local test is used to quantify clustering of weighted events at the local level, and it is able to identify hot spots via comparing the value of a given point to all other values within specified searching distances without including the point of focus. Due to the existence of multiple comparisons in the test, an adjusted significant level is determined (12). In this study, Gi*(d) was used to assess local clustering of the two measurements of infection of hantavirus in the two species and different geographical scales of the city.

For weighted-K and Gi*(d) statistics used in this study, different parameters were used in different scales. As the analysis in the whole-city level, the maximum search radius was defined as 20,000 m and 50 was chosen as the number of increments. 5,000 m and 50 times were selected as the maximum search radius and increments respectively for the analysis in the old-district level. The above searching distances and increments were set as parameters for both the weight-K and Gi*(d) statistics. The selection of the above parameters was based on the geographical shape of the city and allocations of healthcare resources and rodent traps (S2 and S3 Figs). For weight-K function, 999 was set as the number of Monte Carlo permutations for creating confidence intervals.
Results

Distributions of traps and healthcare resources

From 2008 to 2016, the geographic distribution of rodent traps indicated that among the 123 traps placed throughout the city, 70 (56.91%) of the traps were located in the three old districts (S3 Fig). The placements of traps in the other parts of the city were relatively sparse. In the peripheral areas of the city such as Conghua and Nansha districts, there were only two traps being placed. The distribution of healthcare providers was similar to the allocation of traps (S2 and S3 Figs) - most of the healthcare facilities were located in the old districts and southwest part of Tianhe district. These districts were located in the Midwestern part of the city. The farther the distance from this part of the city, the less the healthcare facilities that conducted HFRS examinations existed.

Descriptive temporal and spatial analyses

At the city level, there were 17,314 patients surveyed by CDCs and received the HFRS diagnostic tests from 2008 to 2016; this group of people was considered as the population at risk in this study. Among the individuals who received the examinations of HFRS, 2,154 persons tested positive (12.44%). The period prevalence during the whole study duration was 12.44%. From 2008 to 2011, the annual prevalence increased from 9.33% to 17.92% (S4 Fig). After peaking in 2011, the annual prevalence presented a decreasing trend until the end of the period of analysis (2016) (S4 Fig). For the change of cases across different years, the unimodal trend peaked in 2014 and decreased thereafter (S5 Fig). In case of weekly occurrences, a clear seasonality trend could be observed - the number of cases in each January followed the decreasing trend of the previous year, the case occurrences kept decreasing until late February, when is the ending time of winter in the region. An increasing trend began in early spring (early March) and continued until late summer (late September), after that it changed to a decreasing pattern which continued till the
February of the next year (Fig. 1).

Fig. 1 Temporal patterns of weekly HFRS occurrences in Guangzhou city, 2008-2016.

At the old-district level, 11,229 persons were tested for HFRS infection during the entire study period and 1,322 (11.77%) of them tested positive. The population received the tests in the three old districts accounted for 64.86% of the population received the tests in the whole city. Due to the fact that the sample size in the old districts was larger, and the areas owned a significant public health value; same descriptive temporal analyses were conducted for this region. The changing trends of annual prevalence and cases of human HFRS in the old towns (S6 and S7 Figs) were similar to the ones in the whole city (S4 and S5 Figs).

Human HFRS distribution based on the location of health care providers indicated that cases were mainly located in the Midwestern part of the city, and fewer cases were located in the outer and more rural parts of the city (S8 and S9 Figs). All districts in Guangzhou city reported human HFRS cases over the nine-year study (S8 and S9 Figs). Four districts located in the metro area had a higher prevalence across different years (S8 Fig). The distributions and prevalence of cases in the peripheral part of the city varied by year. The four districts with the highest prevalence during the 9-year period were Yuexiu, Liwan and Haizhu, which were analyzed together as old districts due to the similarity of urban structure among them, and the fourth district is Tianhe district, which was newly developed after the 1990s. The old districts had a total prevalence of 11.78% and Tianhe district had a prevalence of 14.06% across the nine-year study period. For the measurement of disease burden using the number of cases in each location (S10 and S11 Figs), the results were identical to the ones using prevalence (S8 and S9 Figs).

A total of 6,090 rodents were caught during the entire study period in 123 traps across the city, with 643 of them testing positive for hantavirus antibodies (10.56%); Hantavirus
infection prevalence in rodents was similar to the aggregated prevalence in humans for the same period. Because the locations of rodent surveys were selected by various rodent control companies under the supervisions of district-level CDCs, the spatial distributions of traps in different years were different, since different companies had different preferences of selecting trapping locations. For this reason, the analysis on rodent surveillance data was treated in a cross-sectional way to yield a more widespread distribution of traps and larger sample size. Similar to the case in human surveys, a number of surveyed rodents with positive testing results were located in the mid-western part of the city (S12 and S13 Figs). All districts except Huadu detected rodents carrying hantavirus antibodies over the whole study period. In the off-lying districts, it should be noticed that Zengcheng and Baiyun districts also detected the existence of hantavirus antibody in six and five traps, respectively (S12 and S13 Figs).

**Point pattern analysis**

Within the two scales, weighted K-function revealed the two measurements at locations of both healthcare providers and rodent traps were randomly distributed within the pattern of points, which indicated that global spatial clustering didn’t exist (Fig. 2).

**Fig. 2** Example of weighted K-function result. For all of the global weighted-K tests analyzed, all observed L(d)s fell within the intervals of Minimum L(d)s and Maximum L(d)s like this graph. This indicated all of the points being tested showed a complete spatial random pattern.

For the analyses of local clustering, Gi*(d) statistics identified 22 clusters in different scales of the city for humans and rodents. For the analysis of the healthcare providers in the whole city using the number of positive cases in each location as measurement, there were nine significant local clusters located in the eastern part of Yuexiu district, the southwestern part of Tianhe district and the eastern part of Haizhu district (S14 Fig).
six clusters in Yuexiu district were so closed that they dissolved together. In these hot spots of Yuexiu district, five of them had a radius of 800 m and one of them had a radius of 100 m (S14 Fig). In Tianhe district, the two clusters with the same radius of 400 m were also proximal to each other (S14 Fig). The cluster detected in Haizhu districts had a radius of 3,200 m, which also covered nearby Tianhe, Huangpu and Panyu districts (S14 Fig). In the whole city scale, for the analysis of rodent traps performed using the same measurement, two overlaid clusters with the same radius of 2,000 m were discovered in the southern part of Baiyun district which shared borders with Yuexiu district (S15 Fig). Another cluster with a radius of 4,000 m was detected in southern Tianhe district (S15 Fig). Interestingly, this cluster intersected the cluster of the healthcare provider in Haizhu district, which built up a zoonotic link of rodent hantavirus infection and human HFRS (Fig. 3).

Fig. 3 Overlay of significant human HFRS and rodent hantavirus clustering (Gi*[d]> 3.71, P<0.05) of high positive case count in Guangzhou city between 2008 to 2016. The map was generated using free open source software QGIS 2.18.15 (http://www.qgis.org/en/site/about/index.html) and publicly available shapefiles from Trimble Data Marketplace (https://data.trimble.com/).

In the city-wide scale, two significant clusters of high human infection prevalence with the same radius of 400 m were located in southwestern Tianhe district (S16 Fig). No cluster existed for the rodent prevalence data. In Tianhe district, the location of the clusters intersected with the ones calculated for the number of positive cases (S14 Fig and S16 Fig).

When the same local analysis was conducted for the old districts using the number of positive cases, two clusters with radiuses of 400 m and 5,000 m were found in the mid-eastern part of Yuexiu district and eastern part of Haizhu district, respectively (S17 Fig).
For this two clusters, it should be noticed that they overlaid with the result in the scale of the whole city using the same measurement (S14 Fig and S17 Fig). As the analysis for the positive case count of rodent trap data in these three districts, three dissolved clusters located in the central part of Liwan district were found proximal to each other, they had radiuses of 3,600 m, 3,100 m, and 2,900 m (S18 Fig). Compared with the area of Liwan district, the total area of the three clusters was relatively large in this old town, this suggested that Liwan district had a higher number of rodents carrying hantavirus comparing with other two old towns.

In the scale of old districts, the clusters generated using prevalence data from healthcare providers and rodent traps were generally smaller in area than the other clusters mentioned before (Fig. 3). Two clusters existed for the spatial clustering in healthcare resources, one was located in the mid-western part of Yuexiu district, and it had a radius of 100 m; the other one was positioned in the mid-eastern part of Liwan district, which also covered the western part of Haizhu district (S19 Fig). Only one cluster was found for the prevalence of rodent traps, it was located in the mid-northern part of Yuexiu district and had a radius of 400 m (S20 Fig).

Discussion

In this study, descriptive spatial and temporal analyses and statistical point pattern analyses of human HFRS and rodent hantavirus were conducted at the district level of Guangzhou city at different spatial scales. Our analyses pinpointed geographical areas with significant high number of human HFRS by medical institutions and rodent hantavirus in Guangzhou; making possible a geographical zoonotic connection between the disease hotspots in humans and rodents.

The mapping of traps and healthcare resources revealed that the mid-western part of the city had the highest burden of HFRS and hantavirus. It is known that the distributions of
Hantaviruses in humans and rodents are associated with the geographical location of socio-economic status (SES) and human population density. This can be explained by the fact that individuals with lower SES tend to live in the areas with poor hygiene, which increase their potential exposure to rodents and their excreta [23]. The places with high population density yield more food and produce more waste, which promote the population growth of rodents (13-15). Urban Guangzhou owns highly heterogeneous distribution of these two drivers (i.e., food and waste) (16). Hence, the two anthropological factors should be considered when interpreting the distributions of human HFRS and rodent carrying hantavirus. Furthermore, the array of non-anthropological factors that led to disease clusters is multifaceted. In the metro areas that rodent and human clusters are located (Fig. 4), it can be seen that the clustering effects are overlaid with multiple environmental factors such as high density of building, close proximity to water, variance of greenspace, elevation, land use and the other factors they may explain the occurrence of human HFRS and rodent hantavirus. Similar determinants were also observed in other studies understanding the epidemiology of zoonotic hantavirus. For instance, artificial area, cropland, elevation were found associated with the increase risk of HFRS in Shanxi province, China (17). Landscape elements like normalized difference vegetation index (NDVI), elevation, and coverage of forests were remarkably associated with HFRS in China (18). Besides, meteorological factors also determine the occurrence of HFRS cases. There was a summer peak for the weekly case occurrence of HFRS annually (Fig. 1). The causes for the summer peak and the variation of the number of cases in different years may be explained by the warm temperature, high humidity and high precipitation in the sub-tropical Lingnan region, where Guangzhou is located (19). These elements create a better environment for the survival of hantavirus and increase the probability of human exposure to the pathogen. Except for the above factors associated
with the occurrence of HFRS, the spatial distributions and temporal changes of the disease can also be influenced by the reproduction and activities of rodents, and viral types (20, 21).

Fig. 4 Zoonotic clustering (Gi*[d] > 3.71, P < 0.05) in the context of urban environment in Guangzhou city from 2008 to 2016. The map was generated using free open source software QGIS 2.18.15 (http://www.qgis.org/en/site/about/index.html), publicly available shapefiles from Trimble Data Marketplace (https://data.trimble.com/), and open source Google satellite image was retrieved via QGIS.

Except for discovering the overlaying pattern of human HFRS clusters and rodent hantavirus clusters by occurrence, this study summarized the long-term surveillance outcomes of rodent hantavirus in Guangzhou city, which has not been well documented. We found the distributions of traps were similar to the locations of healthcare resources. This implied the implementation of the surveillance policy varied by districts - the districts in the midwestern urban area implemented the rodent survey better than the outlying districts due to the outlying areas owned fewer funding resources. The maps developed in this work provided a basis for strengthening the of rodent trapping surveys and offered insights for public health planning and allocation of healthcare resources.

In our research, global and local spatial statistics were implemented to qualify whether clustering existed in the weighted locations and quantify the extent of clusters by prevalence and case counts. While global weighted-K statistics do not identify particular clusters, local Gi*(d) statistic allowed detection of specific locations of clusters for high values of measurement (22). The spatial distribution of rodent hantavirus and HFRS in Guangzhou city were nonrandom and clustered locally with different radiuses, up to 5,000 m. The areas and locations of the zoonotic clustering patterns varied by the scales of the study. Also, the way that PPA was implemented in our analyses offers a case study for
other similar urban settings in visualizing disease hotspot for their surveillances.

Limitation of this study should be noted. The spatial differences in area between the hotspots generated by the two measurements (i.e., prevalence, case count) were more likely caused by the “small number problem”. In this study, prevalence was calculated by dividing the number of humans or rodents detected with positive hantavirus by the number of humans or rodents received the test. The variance of prevalence relied heavily on the magnitude of the denominator, and the measurement may become problematic when the number of observations is small. Hence, we also reported the case count to adjust for this issue, allowing a comparison between the outcomes by different measurements. Due to a lack of human movement data in our surveillance, the assumption was made that the population at risk by definition would seek the closest institutions to receive tests of HFRS, making this the other limitation in our study. In the data collection stages, potential selection bias existed since the local-level healthcare workers were more likely to provide examinations for people presented the clinical syndromes and trap rodents from higher risk areas based on a priori knowledge. The variance of prevalence was high and sometimes the prevalence was equaled to one due to no negative result was collected, and the unbiased underlying prevalence might be totally unremarkable. This is also a common problem for epidemiological researches based on infectious disease surveillance data (23) - unlike classical epidemiological studies which usually include control groups, infectious disease surveillance usually gathers information from individuals with diseases so that sometimes it is not proper to calculate measurements of disease frequency such as incidence and prevalence.

In spite of the limitations, we identified clusters of hantaviruses in rodents and humans and discovered the geographical zoonotic overlay of these two types of hotspots located in the eastern border of Tianhe and Haizhu districts (Fig. 3 and Fig. 4). In the hotspots
which HFRS and rodent hantavirus burden are high, focusing on prevention strategies at these regions of high risk can optimize the effectiveness of health maintenance program of the city and break the zoonotic link of hantavirus transmission. Individuals living in the hotspot areas should be informed of the potential of infection and be given advise on prevention methods. Our study is the first in the region that utilized both human surveillance and rodent survey information to conduct point pattern analysis and discovered the spatial zoonotic overlay of the two forms of hantavirus infection across species.

Conclusions
This study described the temporal pattern and investigated the spatial distribution of human HFRS and rodent hantavirus in Guangzhou city, China, during 2008 to 2016 using existing surveillance data, GIS, and spatial statistics. PPA identified clusters in central and central-west parts of the city and found the zoonotic connection between the rodent and human clusters, which have not been reported previously. The data also provided evidence that the occurrence of HFRS followed a seasonal trend peaking in summer. These findings offered information for the allocation of medical and public health resources in preventing the disease in human and eliminate the pathogen in urban rodents, and also lay a foundation to conduct further research in the environmental determinants in the urban settings responsible for the increase of zoonotic transmission risk.

Abbreviations
HFRS: hemorrhagic fever with renal syndrome
GIS: geographic information system
HPS: hantavirus pulmonary syndrome
GZCDC: Guangzhou Center for Disease and Control
IFA: immunofluorescent antibody reaction  
RT-PCR: reverse transcriptase - polymerase chain reaction  
PPA: Point Pattern Analysis  

Declarations  

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Availability of data and material  
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.  

Authors’ contribution  
DC, SC and YW originally designed the study. DC, SL, and YW analyzed and interpreted the data, YW, SC, ZY acquired the data and supervised study. SL, XL substantially revised the manuscript. All authors approved the final version of the manuscript.  

Ethics approval and consent to participate  
The ethics committee of GZCDC approved the study, which was in line with the declaration
of Helsinki and the “Involved biomedical research ethics review approach” from the Order No. 11 of the National Health and Family Planning Commission of the People’s Republic of China.

**Consent for publication**

The study used de-identified data so does not require a consent to publish.

**Competing interests**

The authors declare that they have no competing interests

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Figures
Figure 1

Temporal patterns of weekly HFRS occurrences in Guangzhou city, 2008-2016.
Example of weighted K-function result. For all of the global weighted-K tests analyzed, all observed $L(d)$s fell within the intervals of Minimum $L(d)$s and Maximum $L(d)$s like this graph. This indicated all of the points being tested showed a complete spatial random pattern.
Overlay of significant human HFRS and rodent hantavirus clustering ($\text{Gi}^*[d] > 3.71$, $P<0.05$) of high positive case count in Guangzhou city between 2008 to 2016. The map was generated using free open source software QGIS 2.18.15 (http://www.qgis.org/en/site/about/index.html) and publicly available shapefiles from Trimble Data Marketplace (https://data.trimble.com/). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Zoonotic clustering (Gi* [d] > 3.71, P < 0.05) in the context of urban environment in Guangzhou city from 2008 to 2016. The map was generated using free open source software QGIS 2.18.15 (http://www.qgis.org/en/site/about/index.html), publicly available shapefiles from Trimble Data Marketplace (https://data.trimble.com/), and open source Google satellite image was retrieved via QGIS.

Supplementary Files

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S14_Fig.png
S17_Fig.png
S18_Fig.png
S16_Fig.png
