Preplanned Studies

Hemorrhagic Fever with Renal Syndrome — Liaoning Province, China, 1999–2018

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Summary

What is already known on this topic?

Hemorrhagic fever with renal syndrome (HFRS) is endemic in Liaoning Province. Both Seoul and Hantaan virus are circulating in rodents, and epidemic outbreaks and sporadic cases have been recorded every year since the disease was recognized.

What is added by this report?

The epidemic trend of HFRS over the past 20 years (1999–2018) in Liaoning was analyzed, which showed both regional complexity and consistence with the epidemic in China. Genetic and antigenic stability of the circulating hantavirus were demonstrated, which suggested the effectiveness of the approved inactivated vaccine currently used in China.

What are the implications for public health practice?

Precise risk-based strategic practices that are integrated and regional are required for further improvement of the prevention and control of HFRS.

Hemorrhagic fever with renal syndrome (HFRS) is an important public health threat in China, and serious epidemic outbreaks and sporadic cases were recorded in the 1980s and 1990s. The incidence and mortality have declined in the past 20 years in China, but the epidemic characteristics of HFRS are highly variable at both the provincial and county levels. Liaoning Province is typically a natural focus of HFRS, and endemic HFRS in Liaoning might reflect the challenges facing China. A total of 39,352 HFRS cases with 285 deaths were reported to the Chinese National Notifiable Disease Surveillance System (NNDSS) from 1999 to 2018, and the incidence and case fatality rate demonstrated region-specific characteristics and spatiotemporal variation of the HFRS epidemic. Hantaviruses circulating in rodents were detected that showed the Seoul virus was the major pathogen causing HFRS in the province. In addition, sub-genotype III is the major genotype, and sub-genotype I and IV of Seoul virus and sub-genotype VI and VII of Hantaan virus were also detected in Liaoning. The dynamic changing of hotspots, the scale of the outbreaks, and sporadic cases required precise risk-based strategies that are integrated and regional precise to target the affected people for further improvement of HFRS prevention and control.

Data for HFRS patients were obtained from the Chinese National Notifiable Disease Surveillance System (NNDSS), and the data were analyzed with Excel (version 2013, Microsoft, USA) and SPSS software (version 16.0, SPSS Inc., USA). Geographic maps were created using ArcGIS Software (ESRI, Redlands, CA, USA).

Rodents were trapped with snap-traps in residential areas and the fields of national HFRS surveillance sites. The rodents serum antibodies against hantavirus were detected using double antigen sandwich ELISA (*1*). The rodents' lungs were tested for hantavirus antigen with fluorescence labelled monoclonal antibody by direct immunofluorescence assay (*2*) and for viral genomic RNA by real-time RT-PCR (*3*). Positive test samples were loaded to Vero E6 cells for virus isolation. The antigenicity of new viral isolates was evaluated with 30 reference sera collected from recovered patients of HFRS using the plaque reduction neutralization test (PRNT) assay. The complete L, M, and S segments of the viral genome were amplified by overlapping PCR and sequenced with the amplification primers using BIG DYE terminator and a capillary sequencer. The hantavirus genomic sequence deposits in GenBank were collected for comparative analysis. All the sequences were aligned with the MAFFT program, version 7 (EMBL-EBI, UK). Comparative analyses of nucleotide sequences and amino acid sequences were operated by DNASTAR (Madison, Wisconsin, USA). Phylogenetic trees were generated using the neighbor-joining (NJ) method implemented in MEGA6.0 (*4*). Topologies were evaluated by bootstrap analysis of 1000 iterations.

A total of 39,352 HFRS cases with 285 deaths were

reported from 1999 to 2018 in Liaoning. The incidence rate varied from 1.71 to 13.05 per 100,000 persons annually, reaching a peak in 2004, decreasing significantly from 2006, reaching a plateau in 2007, then fluctuating between 1.71–3.0 per 100,000 persons (Figure 1). The national incidence rate, meanwhile, varied from 0.65 to 3.93 per 100,000 persons between 1999 and 2018, started to decrease dramatically since 1999 reaching a plateau in 2007, then fluctuated at a level of 0.65–0.99 per 100,000 (Figure 1).

In Liaoning, reported HFRS cases peak twice annually. The spring peak, occurring between March and May, contributed 35% of cases, and the fall-winter peak, occurring between October and December, contributed 26.5% of cases overall. In most prefecture-level cities, the spring peak was greater than the fall-winter peak, however, the monthly distribution of HFRS varied in different years and places, and the fall/winter peak was greater in Fushun City and Dandong City.

The reported cases involved all the 14 cities in the province, but 7 cities contributed 84% of reported cases. These cities are Huludao (7,114 cases, 18.08%), Jinzhou (5,367 cases, 13.64%), Dandong (5,038 cases, 12.80%), Benxi (4,631 cases, 11.77%), Shenyang (4,194 cases, 10.77%), Anshan (3,524 cases, 8.96%), and Fushun (3,191 cases, 8.11%). The fluctuations in incidence for each city were not synchronous, and the difference was statistically significant (Figure 2).

The ages of the 23,554 HFRS cases reported in 2004–2018, ranging from infancy to 85 years (median: 43 years). The majority of the cases (85.27%) occurred between 15–60 years, while 1.78% were in children <14 years and 12.94% were in persons >60 years. In the past 20 years, the proportion of reported cases in

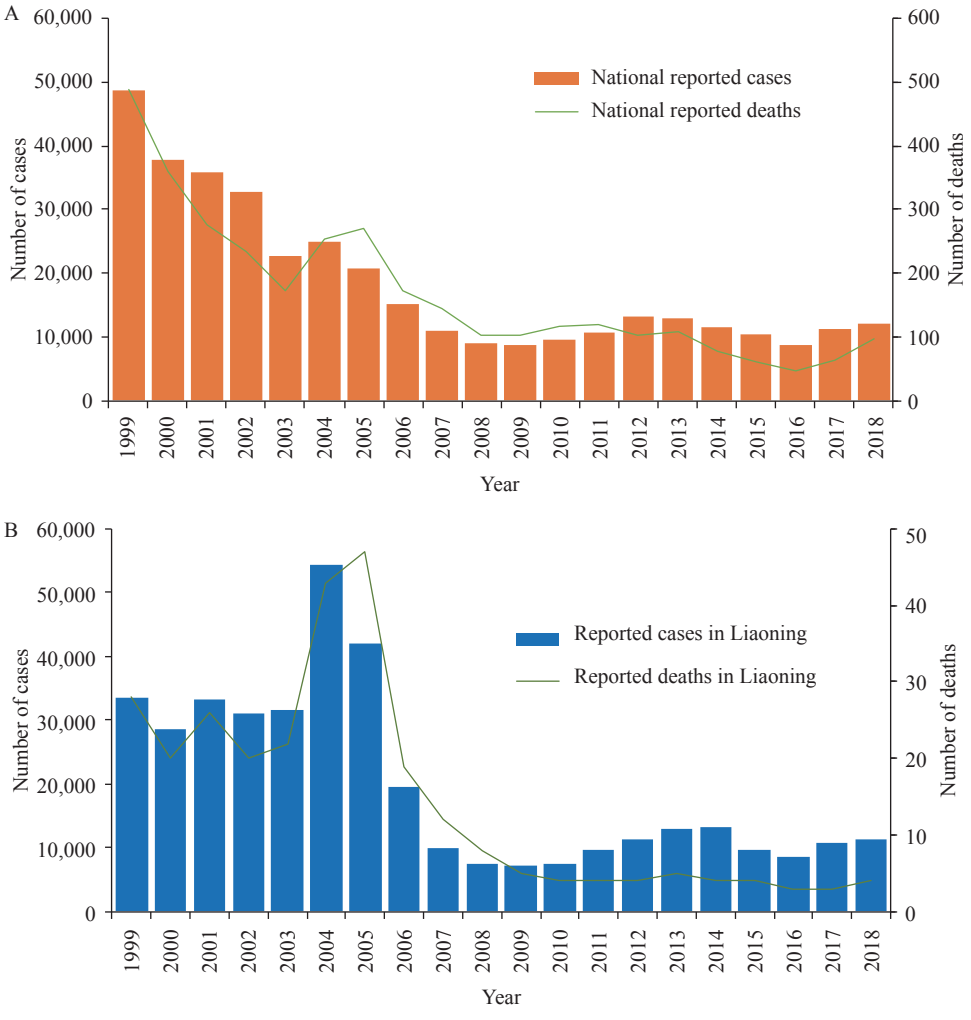

FIGURE 1. The reported cases and deaths of hemorrhagic fever with renal syndrome (HFRS) from 1999 to 2018 in China (A) and Liaoning Province (B).

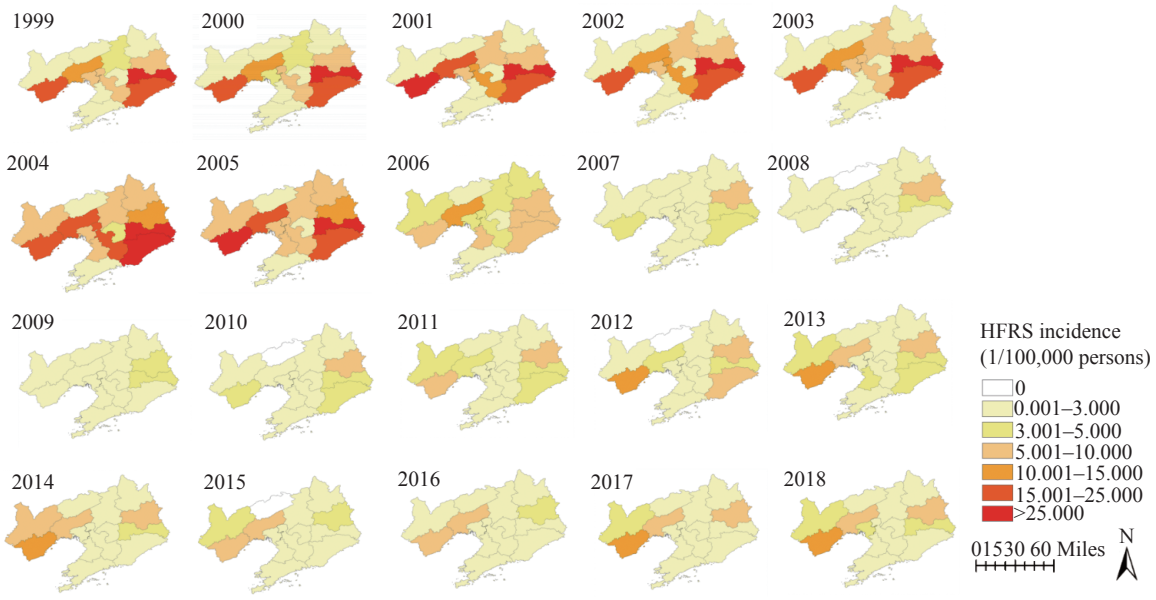

FIGURE 2. Maps showing prefecture incidence rate of hemorrhagic fever with renal syndrome (HFRS) from 1999 to 2018 in Liaoning Province.

the 55–65 years group has increased, while that of the 30–40 years group has decreased significantly (Supplementary Figure S1, available in http://weekly.chinacdc.cn/), which may be related to the fact that the old have replaced the young as the main agricultural production work force in most rural areas of China. The ratio of male to female patients was 2.88:1. Among female patients, the proportion of cases in the highest age group was higher than that in the male (Supplementary Figure S1). Most patients (62.74%) were farmers, followed by housewives and unemployed (12.94%), and students and non-agriculture workers were the other two occupations at relative high risk of acquiring HFRS (Supplementary Figure S2, available in http://weekly.chinacdc.cn/). There were 285 deaths in total from 1999 to 2018 with an average case fatality rate of 0.72%, which involved all 14 cities. Areas with high mortality were usually areas with low prevalence, such as Fuxin (4.84%, 3/62), Liaoyang (2.31%, 7/303), Dalian (2.01%, 17/847), and Tieling (1.99%, 11/553). To some extent, it showed that the experience and awareness of clinical doctors played an important role in the treatment of HFRS.

Previous laboratory surveillance data of hantavirus were collected and sorted. In 2018, 867 rodents were trapped with 25,336 snap-traps in residential areas and in fields in the cities and counties of Shenyang, Benxi, Dandong, Jinzhou, and Anshan. The predominant rodent species was *Rattus norvegicus* in residential areas and *Apodemus agrarius* in the fields, and the house mouse (*Mus musculus*) was next highest. The captured densities of rodents in residential areas was higher than in fields. In residential areas, the antigen detection rate was about 5.8% (29/500), ranging from 0% (0/50) in Benxi County to 14% (7/50) in Yuhong District and Haicheng City; and the serum antibody detection rate was about 17.2% (86/500), ranging from 2% (1/50) in Benxi county to 50% (25/50) in Fengcheng County and Dandong City. In the fields, the antigen detection rate was about 4.6% (23/500), ranging from 0% (0/50) in Yuhong District to 32% (16/50) in Haicheng City; and the serum antibody detection rate was about 9.2% (46/500), ranging from 0% (1/50) in Yuhong District to 46% (23/50) in Benxi County. Hantavirus-antigen positive rodents were further tested by RT-PCR assay, viral genome RNA were detected from 18 rodents using real-time RT-PCR (*3*), and the complete genome of L, M and S segments were obtained from 16 *Rattus norvegicus* lung samples directly, including 3 in Fushun, 4 in Shenyang, 2 in Jinzhou, and 7 in Tieling. Two viral isolates were obtained from those lung samples collected at Tieling, named LNTL001 and LNTL003. No significant antigenicity difference was observed between the two new isolates and the reference strain L99 in plaque reduction neutralization tests with blood from 30 recovered patients.

Pair-wise alignment and comparison of complete genome sequences showed that both the nucleotide and amino acid sequences were closely related to each other, with similarity above 95%. The sequences

obtained in 2018 shared 95.6%–100% of amino acid homology and shared 94%–99.7% of nucleotide homology with sequences obtained previously. No significant recombination was observed among these sequences using recombination detection program version 4 (RDP4) software (*5*). Phylogenetic analyses showed that the hantavirus in Liaoning could be clustered into the clade of sub-genotype III (S3) and sub-genotype I (S1) of the Seoul virus, and sub-genotype VI of Hantaan virus (H6) (*2*) (Figure 3). Seoul virus was the predominant pathogen, and S3 was the major genotype currently circulating in the province. S4 and H7 of Hantaan virus were also found in the province (*6*–*7*).

DISCUSSION

HFRS is endemic in Liaoning, and the incidence peaked twice a year and varied in a cyclic fashion (*8*). The risk of occasional spillover of hantavirus from rodents to humans showed region-specific characteristics. Areas with high mortality were observed in areas with low prevalence, which suggests that lack of experience and awareness of diagnosis and treatment may cause patients to miss the best period of treatment and increase the patient's severity and mortality rate, which had been crucial to improving the prevention and treatment of HFRS. In China, the appropriate age for vaccination of current approved HFRS vaccines ranged from 15 to 60 years, while the population out the protection range of vaccination contributed about 15% of total reported cases, which is another challenge faced by current prevention and control work, especially with an increasing proportion of cases over 60 years old.

Both Seoul and Hantaan virus are circulating in Liaoning. Seoul virus is the main pathogen that cause HFRS, and the major sub-genotype is S3. The highly close relatedness of complete genome sequences

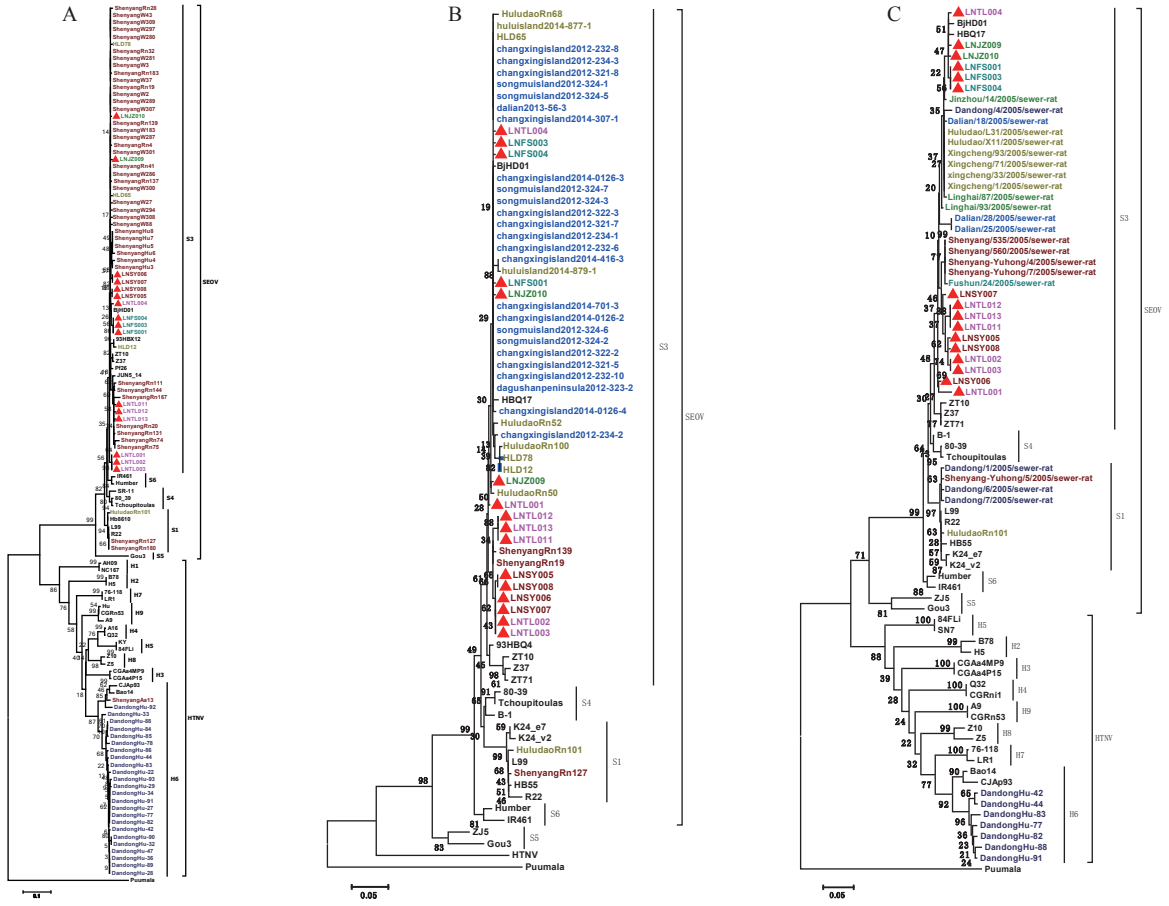

FIGURE 3. Phylogenetic analysis of hantavirus genomic sequences obtained in Liaoning province. (A) phylogenetic tree based on the Gn segment (1383–1630 nt). (B) phylogenetic tree based on the Gc segment (2000–2317 nt). (C) phylogenetic tree based on the S segment (633–991 nt). Sequences in different cities are marked by different colors, and isolates obtained in this study were marked by ▲.

obtained from different places in the province suggested that Liaoning belong to the same natural focus of infection. However, several sub-genotypes detected in rodents revealed the complexity of the natural focus of HFRS in Liaoning. Genetic and antigenic stabilities of the detected hantavirus over a long period of time may suggest the effectiveness of the approved inactivated vaccine currently used in China.

Hantaviruses are enzootic viruses that maintain persistent infections in their rodent hosts, and the spillover of these viruses to humans could occur at any time when contact happens between a susceptible person and an infective virion (*9*–*10*). After decades of implementation of comprehensive strategies for HFRS prevention and control, China has brought HFRS from highly common epidemic outbreaks to largely sporadic cases. Further improvement of HFRS prevention and control requires precise risk-based strategic practices that are integrated and regional to target highly-affected people in high-risk areas. These measures could improve public awareness of HFRS transmission, reduce human exposure to infected rodents and their excreta, and increase vaccination among at-risk populations.

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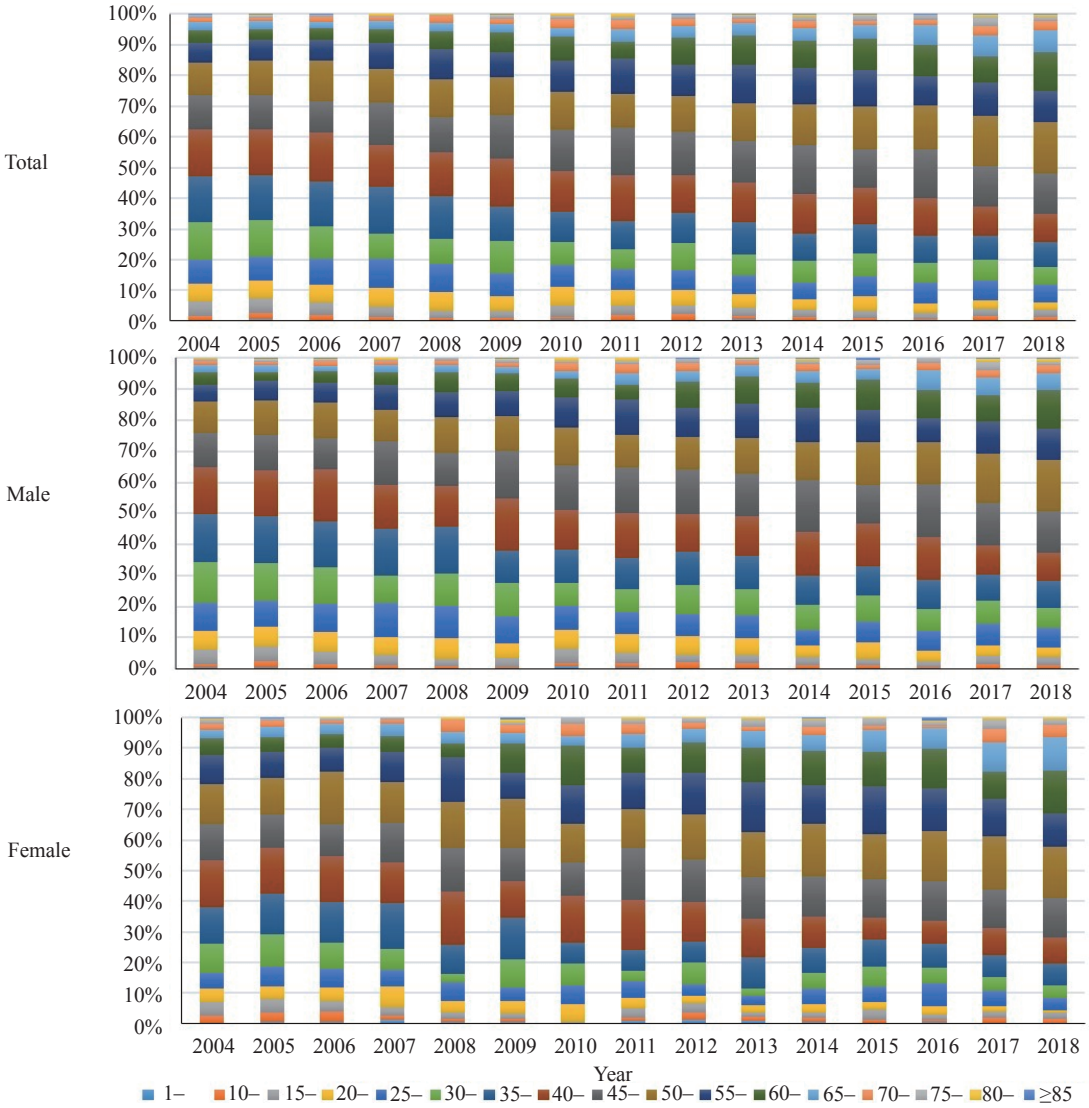
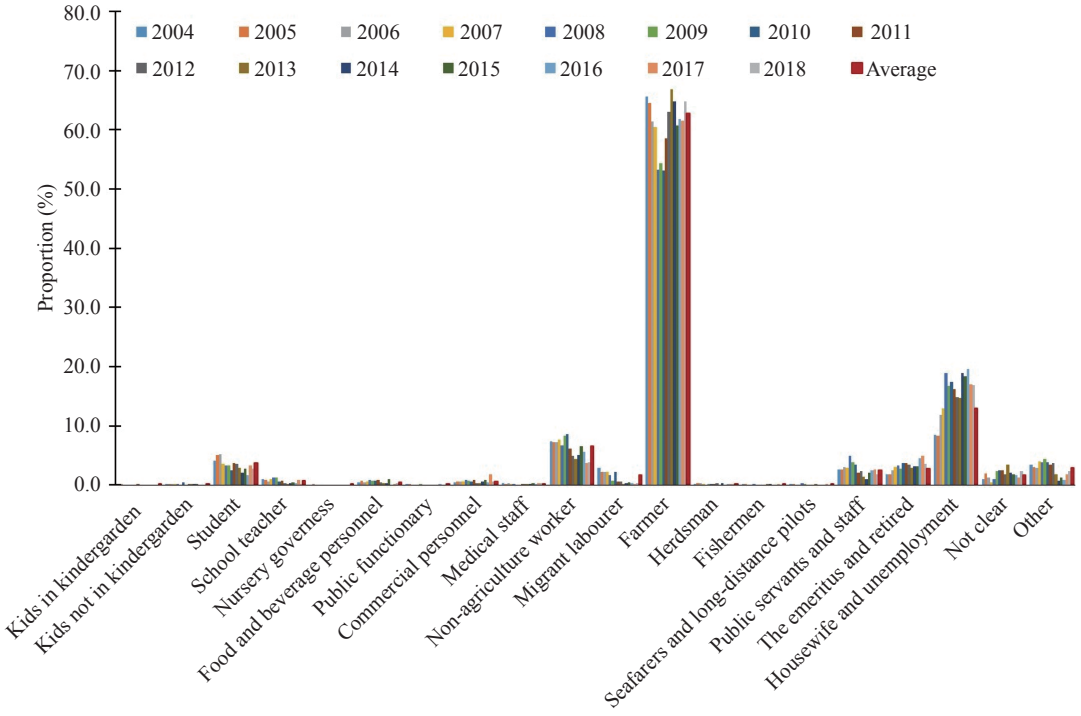


SUPPLEMENTARY FIGURE S1. Age distribution of reported hemorrhagic fever with renal syndrome (HFRS) cases in Liaoning Province.



SUPPLEMENTARY FIGURE S2. Occupational distribution of the reported cases of hemorrhagic fever with renal syndrome (HFRS) in Liaoning Province.