



Unique Epidemiological Patterns and Origin of the Outbreak of Human Infection with H7N9 AIV in China from 2013 to 2015

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Abstract

Background: Novel Human Avian Influenza (H7N9) (h-H7N9 AI) occurred in China in February, 2013 and continued today. Although there were many reports on epidemiology, the reservoir and origin have not been confirmed.

Methods: Until April 2015, 628 cases collected from WHO. Descriptive epidemiology was used to compare differences between h-H7N9 AI and other h-AI with statistical analysis.

Results: Compared with only 18 cases of h-H5N1 AI located just in Hong Kong during 7 months, 571 cases with 212 died (37%) occurred up to February, 2015 and only limited in the mainland. It is suggested h-H7N9 AI were fully different from other h-AI, and may belong to very new type of "Natural Focus Disease (Zoonosis)". H7N9 AIV was not detected in farms and wild birds in China before and during the early phase, and quite different from h-H5N1 AI occurred in geese of Guangdong in 1996 and in farms in 1997. So, h-H7N9 AI should have occurred in countries with it in birds and poultry for long time, rather than in China. The mean age was 62 years old in the beginning, then decreased to 59.0, 58.0 and 2 years later to 54.8, with correlation of the epidemic-lasting days ($r=-0.953P=0.047$). It indicated that the senior had no specific immunity and H7N9 AIV was absolutely new virus and never existed in China.

Interpretation: We have creatively identified that h-H7N9 AI is with unique pattern based on abnormalities in incidence and distributions and should have occurred in another country with it for long time.

Keywords: Outbreaks; Avian Influenza A (H7N9); Reservoir; Unique origin of Virus; Epidemiology

Introduction

An epidemic of human infection (h-H7N9 AI) with a novel Avian Influenza A (H7N9) virus (h-H7N9 AIV) broke out in China in 2013. The first case occurred in Shanghai on February 19, 2013, neighbouring provinces were involved in the next months. Until May 10, 2013, 131 confirmed cases (Figure 1) had been reported to the World Health Organization (WHO), of which 32 cases died (24.4%) [1]. It was considered that the incidence and mortality were both high (Table 1) [2]. Six hundred and twenty eight cases were confirmed up to April, 2015. Until June 2016, 767 cases had occurred only in the mainland with inputting cases in other regions and countries [3,4] (Figure 1). It was suggested that the human infection with H7N9 AIV is "Natural Focus Disease" and the "Natural Focus" is in mainland China, therefore, it cannot be spread outward. "Natural Focus Disease"

often is referred to as "Zoonosis" by most scholars in scientific papers. However, we believe that the "Natural Focus Disease" can better reflect the epidemiological characteristics, and now many of the "Natural Focus Diseases" have not yet adapted to the people and prevailed only among animals.

Meanwhile, so far, it is unable to make the appropriate explanation in epidemiology for its abnormal distribution of age and place, and also it is difficult to determine the natural reservoir and origin. Uyeki pointed out that the emergence of the new h-H7N9 AIV should be of concern to the global public health community and explains many of the issues that are eager to answer [5]. The objective of this study is to examine the possible sources and origin of h-H7N9 AI infection, through a comprehensive analysis of the outbreak, and a detailed comparison of the epidemiological patterns between the h-H7N9 AI epidemic with other previous ones, such as h-H5N1 avian influenza (first reported in 1997) (Table 1) and H1N1 swine influenza in 2009. This paper mainly focused on 628 confirmed cases (from February,

2013 to April, 2015) and guided with the “Chinese Theory of Infectious Disease Epidemiology” and combination of epidemiology practices.

“The Chinese Theory of Infectious Disease Epidemiology” has special features: reasoning tight, well-structured and rigorous terminology. For decades, its guidance to the prevention and control of infectious diseases achieved a great deal in China. There are 3 key points in the theory. Firstly, the occurrence of infectious diseases is based on “3 links” called in China, that is, source of infection, route of transmission and susceptible population. Secondly, society factors and nature factors that are called “2 factors” in China put the “3 links” together and mutual relations, thus to cause the disease occurrence and outbreak in the population. Thirdly, the outbreak or epidemic can be measured and characterized by incidence and the distributions of time, place, crowd and etc. Meanwhile, “source of infection” refers to the infected person or animal individual carrying, reproducing and spreading the pathogens, and is different from “the reservoir” which refers to the special human or animal population. Among the reservoir-population, not only the individual can play to the source of infection, but also the more important is that the infected individuals have continually carried and spread the pathogens, in order to maintain the infectious disease in the population themselves from generation to generation. With reverse thinking, through the analysis of the characteristics and the 2 factors of the outbreak, the application of the philosophy principle about “Seeing the essence through the phenomena”, which can reveal the epidemiological features, especially the route of transmission and sources of infection. Therefore, this paper investigated and analysed the abnormalities in the distributions (the phenomena), then revealed the origin of h-H7N9 AI (the essence) guided by “the Chinese Theory of Infectious Disease Epidemiology”.

Materials and Methods

Subjects

Subjects were the 628 confirmed h-H7N9 AI cases reported to the WHO between February, 2013 and April, 2015 [3,4] which (except case 1 to 3) were collected from websites of WHO. Others were collected from National Health and Family Planning Commission (NHFPC) of the PRC [4], Chinese Center for Disease Control and Prevention (China CDC), Veterinary Bureau of the Ministry of Agriculture of China and the related literature. Chinese People infected with H7N9 avian influenza diagnostic and treatment program were applied as diagnostic criteria [6]. Therefore, we can carry out comparative analysis of data between this study and the literature published by the United States centers for disease control and prevention (US CDC) [7].

Data and statistical analysis

Descriptive epidemiological methods were used in the analysis. The 95% confidence intervals for the positive rates of swine influenza H1N1 in 2009 and human avian influenza H7N9 in different age groups were estimated. Differences across age groups were tested by Fisher’s exact test and/or chi-square test. Correlation between age and the positive rates was estimated by Spearman rank correlation. The correlation between epidemic-lasting days and mean age was examined by trend test. Statistical analysis was performed using SPSS 16.0.

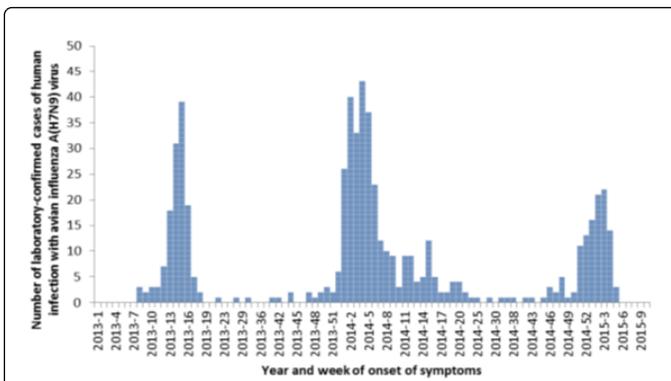


Figure 1. 571 Laboratory-confirmed cases of human infection with avian influenza A (H7N9) virus by week of onset [8]. **Note:** A total of 571 laboratory-confirmed cases of human infection with H7N9 virus, including 212 deaths (37%), have been reported to WHO: 568 cases from China including 552 cases by the China National Health and Family Planning Commission, four cases reported by the Taipei Centers for Disease Control (Taipei CDC), and 12 cases reported by the Centre for Health Protection, Hong Kong SAR. One Chinese traveller case was reported by Malaysia, and two were reported by Canada whom were travellers returning from China on 12 January 2015.

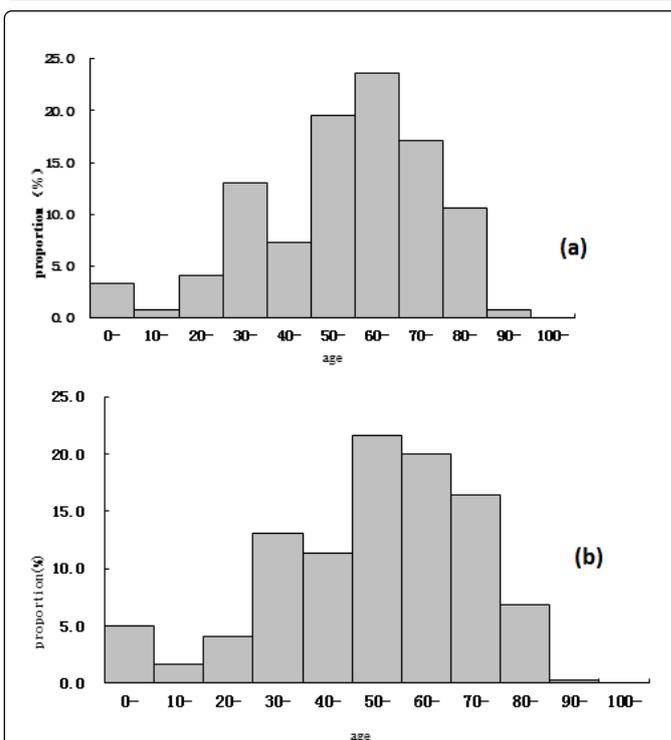


Figure 2. (a) The age distribution of h-H7N9 AI cases (N=135) in China, between February 19 and August 12, 2013 (age \geq 50: 71.6%). (b) The age distribution of h-H7N9 AI cases (N=627) in China, between February, 2013 and April, 2015 (age \geq 50: 65.1%).

Results

Abnormal incidence of human infection with a novel avian influenza A (H7N9) virus

Within three months of onset a total of 131 cases were reported in 11 provinces, as mentioned above (Figure 1) [1,7,9]. Since then, the epidemic continues in the mainland, happening all the year with the obvious winter and spring higher wave in time and involving more and more provinces in areas with absolutely focusing only in the mainland and inputting cases in Hong Kong, Macao, Taiwan and Southeast Asia and North America (Table 1). Up to February 13, 2015, a total of 571 cases occurred and 212 cases died (37%) (Figure 1) [8].

Therefore, two unusual results were found after 3 years observation. First, while natural focuses of h-H7N9 AI and the Middle East SARS (MERS) occurring [10] are very similar, this only limited in mainland China and Middle East, respectively. Cases in other regions or countries are imported. Because the occurrences of “natural focus disease” only limit to the “natural foci” in epidemiology, both h-H7N9 AI and MERS belong to the “natural focus disease”. However, it is surprising that although they are the same emerging “natural focus disease”, but the epidemiological features and/or the origin are quite different from each other, such as transmission mode, case fatality rate and the discovery of reservoir or not (published in another paper). It was suggested that the origin of h-H7N9 AI is quite distinctive and is a very new type of “Natural Focus Disease”. Second, the epidemical curve in time of h-H7N9 AI is different from that of the world’s first human infection with avian influenza virus (h-H5N1 AI) sixteen years ago (Table 1). The first case of h-H5N1 AI emerged in Hong Kong on May 9, 1997, followed by 17 more cases in the next seven months. Comparing with h-H7N9 AI, in first epidemic of h-H5N1 AI there were only fewer cases, yet during long period of time and limited to Hong Kong (Table 1) [7,11-14]. Not only researchers discussed the differences, but also WHO assistant director general took the same view: “there are more (h-H7N9 AI) cases of rapid increase in the past few weeks” [15].

The abnormalities of area distribution and locations of the wild birds and poultry as the source of infection

Human avian influenza frequently occurs in human populations in the same location after similar outbreaks in wild birds and poultry. For example, avian influenza (H5N1) virus was found on farmed geese in Guangdong province in 1996, one year before the h-H5N1 AI epidemic in Hong Kong in 1997 (Table 1) [11,12]. Therefore, if the epidemical patterns of h-H7N9 AI and h-H5N1 AI are similar, the first case of h-H7N9 AI should have occurred in Korea, Mongolia and countries of Europe and North America where avian influenza (H7N9) virus had been detected, rather than in China [7,14-19].

Furthermore, the inhabitant locations for infectious source in Shanghai during the early phase of the epidemic were localized in three live poultry markets, while h-H7N9 AIV or H7N9 AIV was not detected in farmed fowls and swine, and wild birds in Shanghai, Anhui, Henan, Zhejiang, Fujian, and Jiangsu [15,18]. This was quite different from h-H5N1 AI, because widespread outbreaks of H5N1 AI occurred in many poultry farms (Table 1) [12,13,19]. Furthermore, H7 AIV was similar [20,21].

Abnormal age distribution of h-H7N9 AI cases

The peak incidence in elders of age group is abnormal obviously, its change also is very interesting and deserved to deeply thinking by researchers. The median age of confirmed h-H7N9 AI cases was 62 years old (age ≥ 50 : 71.6%) up to April 22, 2013 [18] (Figure 2a), while the mean age was soon down 60 up to August 12 [22], afterward it rapidly decreased to 54.8 (age ≥ 50 : 65.1%) until April 2015 (Figure 2b and Table 1). Linear Trend Test showed that there was significant difference ($P=0.047$) and correlation coefficient between epidemic-lasting days and mean age was -0.953 (Table 2). It showed that mean age was decreased with mounting up of epidemic-lasting days.

This is quite unusual. Most researchers have no reasonable explanation in such median or mean age [14,15,18]. Keiji, the assistant general secretary of WHO, pointed out in Shanghai: “We also see some unusual situation, for example, many patients are the seniors, so far we have no way to explain the reasons” [15].

But, in fact in this paper, with application of “the Chinese Theory of Infectious Disease Epidemiology” as a guide, we simultaneously combined with the epidemiological features and could prove that the reason was not hard to find and furthermore, could offer the convincing proof that the h-H7N9 AI should not happen in China! According to our principle, two factors are major impacts on age distribution of infectious diseases. One is immunity and the other is the chance of exposure to infectious agents. As well all know teenagers and young adults are more susceptible to previous human avian influenza viruses, such as H5N1 AIV. The WHO reported in 2008 that the mean age was 21.7 years and median age was 20 years with low incidence in elders in h-H5N1 AI outbreak [12]. Hence, it can be inferred in the epidemiology that h-H7N9 AIV is a complete new virus for Chinese population, namely, h-H7N9 AIV or new-H7N9 AIV or similar viruses with the H7N9 AIV has never happened in China, this also meet a lot of literature and history of avian flu epidemic in the world [15-19]. Although the host infected by pathogens of some infectious diseases, such as bacillary dysentery, common cold and influenza, can be also infected by the same pathogen again and again, because of temporary and unstable immunity. Accordingly, even in that situation, the seniors still tend to have the stronger specific immunity in the general population, because certain specific (humeral and cellular) immunities are still induced by multiple or cross immunity with growing age. So, we could answer why elder people also are with higher specific immunity to other h-AIV. Because these h-AI occurred in a few years or even more than ten years before the same type of AIV had already happened among the wild birds and poultry for long time, where both birds and poultry, and humans have more or less exposure. But this epidemic is different, H7N9 AIV has never detected among China’s birds and poultry. So the old man are with highest incidence, the most likely explanation is that h-H7N9 AIV is a very new virus in China, the Chinese old man has no specific immunity with lower nonspecific immunity, various functional degradation and some chronic diseases. Thereby, their susceptibility is certainly higher than young adults.

Of course, the old people have a greater chance to go to live poultry markets and get exposed to the virus. But, not only the exposure probability to h-H7N9 AIV should be very similar to that to other h-AIV, also the slightly higher exposure chance could not explain the vast difference in the mean age between h-H7N9 AI and other human avian influenza cases [23].

Meanwhile, median age was 62 years old [21] in first 63 days of the outbreak, only after 4 months, mean age decreased rapidly to 59.0 years old (age \geq 50: 71.6%). After more than a year, it was reduced to 58.0. The most surprised is that it decreased dramatically to 54.8 years old after 2 years and the epidemic-lasting day was correlated with mean age ($r=-0.953$, $P=0.047$) (Table 2). This phenomenon is unprecedented in the epidemical history of infectious diseases. The reason is obvious, many children are susceptible to most infectious diseases and with highest incidence, the young adults are susceptible to only a few ones. Hereafter, the mean or median age of cases rises in the late phase of the epidemic, or years, decades, even for a longer time after the epidemic, due to gradually increasing immunity among the age groups mentioned above. It is indicated that this condition above completely is enormous different from the h-H7N9 AI outbreak of China in which older cases are with high incidence and the mean age has obviously declined with continuing epidemic. From all appearances, this kind of difference has just demonstrated our view above, that is, h-H7N9 AIV is a new virus which never exists in population and nature in China. Therefore, elders are in the age group with highest incidence; hence, over a period of time, their specific immunity has relatively risen through subclinical infection and mean age has obviously decreased. Furthermore, should be pointed out that the age distribution of h-H7N9 AI just accords with the characteristics of "Natural Focus Disease", in other words, it can be used as the another evidence of the suggestion mentioned in the preface of this paper, that is, the human infection with H7N9 AIV is very new type of "natural focus disease".

Abnormal transmission pattern of h-H7N9 AI

There may be very limited person to person transmission during h-H7N9 AI epidemic. In the early epidemic period, familial cluster was showed in 3 cases [21]. There are 3 views to discuss the clusters [2,14,15,18,23]. Firstly, family cluster was caused by jointly or successively exposure of domestic poultry, pigeon, or the environment. Secondly, family cluster cannot be explained by limited person to person transmission which is unclear. Thirdly, limited person to person transmission cannot be excluded, but it needs further investigation and molecular virology evidence.

A couple who had not been analysed in other studies was investigated in our research. The wife was ill on March 27, 2013 and the husband followed 5 days later. The five day interval was less than the shortest incubation period. The wife died in seven days after the onset, so the virus seems highly virulent, and able to transmit it in a large amount and with a high frequency. Her husband was more likely to have been infected due to the long-time close contact with his wife

without any protective measures. The infection either caused by exposure to domestic poultry or by co-exposure had not been reported in the epidemic from February to May, so both can be excluded.

In contrast, even seven years after the H5N1 outbreak in 1997, limited human-to-human transmissions and a few clustered cases of families were just reported [11,12,13,24,25]. The evidence showed that transmission pattern of h-H7N9 AI is abnormal.

Abnormal distribution of the infectious source

So far the infectious source of human avian influenza viruses other than h-H7N9 AIV has been found to be from both wild birds and farm poultry, however, that of h-H7N9 AI is mainly confined to live poultry in markets. Closing these markets in Shanghai in the early phase of the outbreak was found to be very effective, which is a powerful evidence showing that the live poultry markets are the infectious source of h-H7N9 AIV.

Up to now only 1 exposure likely coming from a small-scale farm was reported in Jilin Province of China [26]. However, there are 3 questionable points. First, this farm fed more than 200 various types of poultry, mainly chickens, since 2013. In addition to larger scale the feeding way is similar to in the live poultry markets. Second, after the onset, H7N9 AIV of the poultry and the environment was detected positive by PCR; however, the poultry in the original large farms selling these poultry to the small farm and the nearby farms were negative. Third, except for the head of the household, 2 employees and members in the family were not infected. Obviously, it is difficult to judge whether this case due to exposure to the farm.

In order to clear main infectious sources further, surveillance data with animal H7N9 avian influenza viruses in China were collected in this study (Table 3) [27]. It was found that: (1) 97% of positive samples are from the live poultry markets, only 3% from farms. (2) The time distribution of positive samples is in the spring or winter, which is consistent with the peak of h-H7N9 AI epidemic. The reliability of this data is thereby supported by the epidemiological characteristics mentioned above. (3) All three farms with positive samples were detected in January and it was suggested that either live poultry in markets or cases were not infected from the farms.

Obviously, source of infection and route of transmission between h-H7N9 AI and h-H5N1, h-H9N2 AI and other h-AI are totally different. Cases of h-H7N9 AI only exposed to live poultry markets or family chicken, while other h-AI diseases could be infected from wild birds, farms and/or live poultry markets.

h- AI (Year)	Intensity		Distribution			Source of infection		H5N1 or H7N9 AIV in birds or farms of the region			Reservoir
	No. of cases/duration (Mon.)	Fatality (%)	time	areas	mean age (years)	Farm	Market	Before epidemic	Early phase	Duration	
h-H5N1 AI(1997)	18-Jul	33.3	7 months	Hong Kong	21.7#	Y*	Y	Y	Y	Y	Birds or poultry farms
h-H7N9 AI(2013)	131/3	24.4	To the present	Mainland of China	62.0 down to 54.8	N*	Y	N	N	##	**

#WHO: Weekly Epidemiological Record. ^Y: Yes; N: No, ##Only 3% of positive samples from farms, **Not sure.

Table 1. The differences of first epidemic between h-H5N1 AI and h-H7N9 AI.

Date**	Epidemic-lasting days#	Cases (No.)	Mean (years) age
20130422	63	104	62.0
20130812	174	135	59.0
20140627	493	450	58.0
20150430	800	627	54.8

*Trend test: $r=-0.953$; $P=0.047$; **Date: The date on which subject cases were collected; #Epidemic-lasting days: Days from February 19, 2013 to the "Date"

Table 2. Relationship between epidemic-lasting days and mean age.

Time (year/month)	H7N9 AIV detection			Source of positive H7N9 AIV samples	
	No. of samples	No. of positive samples	%	Live poultry markets (No.)	Farms (No.)*
2016/6	21,771	0	0	0	0
2016/5	17,511	1	0.01	1	0
2016/4	10,595	5	0.05	5	0
2016/3	10,733	1	0.01	1	0
2016/2	26,555	2	0.01	2#	0
2016/1	30,195	2	0.01	1	1
2015/12	53,765	12	0.02	12	0
2015/11	23,305	2	0.01	2	0
2015/10	19,106	1	0.01	1	0
2015/9	23,116	0	0	0	0
2015/8	8,762	0	0	0	0
2015/7	27,760	0	0	0	0
2015/6	29,557	0	0	0	0
2015/5	13,573	10	0.07	10	0
2015/4	18,385	26	0.14	26	0
2015/3	35,311	8	0.02	8	0
2015/2	27,307	18	0.07	18	0
2015/1	25,726	6	0.02	4	2
Total	423,033	92	0.02	89	3*

Table 3. Animal H7N9 AIV surveillance data in mainland China.

Discussion

In this study, we have creatively identified a unique epidemiological pattern of human infection with Avian Influenza A (H7N9) virus, based on an examination and analysis of the distributions in time, place, characteristics of case groups, locations of infectious source and etc. of China from February 2013 to April 2015.

From the epidemiological history of human avian influenza, it is well observed that most infections and outbreaks of avian influenza in wild birds and poultry usually appear first, then followed by an epidemic of the same type in human populations in the same region or country several years later [16,20,24,25]. For example, following the H5N1 avian influenza outbreaks in geese in Guangdong, an h-H5N1 AI outbreak occurred in Hong Kong in 1997 [11-13,20,24]. However, the epidemic of the h-H7N9 AI is very strange, even unprecedented. Mammals infected with type H7 AIV have been rare in Asia previously, while it has not yet found that AIV type N9 can infect people in the world [16,18].

According to inferences from epidemiological history above, first epidemic of h-H7N9 AI should occur in countries in which H7N9 AIV detection were positive rather than in China [15-18,23]. It is certain an abnormal pattern in epidemiological distribution rather than an accident or coincidence.

Unique epidemiological distributions of human avian influenza (H7N9) cases

Although h-H7N9 AIV is a new virus, it still belongs to h-AIV with re-assortment of HA, N9 and H9N2 genes [15-18]. Therefore, it should entirely keep to the epidemiological principles of the human avian influenza or avian influenza infected by the h-AIV or AIV. However, the distributions of incidence, place, age, route of transmission and etc. of h-H7N9 AI were absolutely different from h-AI or AI above.

In the light of the epidemiological notion, the human avian influenza infected by a new h-AIV can present some differences from usual characteristics of ones caused by previous h-AIV. In other words, h-H7N9 AI could somehow be different from other h-AI in distributions of the time, place, characteristics of case groups and etc. But there are two very important fundamentals. Firstly, the differences cannot be abnormal and unusual. Secondly, all aspects of the new disease couldn't completely be different from old ones. Through this, it can reflect new disease being very special. The first case of h-H5N1 AI was in Hong Kong. Only total 18 cases occurred in nearly 7 months of the first epidemic, which limited to Hong Kong. Unlike h-H5N1 AI, 131 h-H7N9 AI cases occurred in first 3 months of the epidemic, involving 11 provinces [1,2,7,11-13]. Not only that, they own different disparity both in the family cluster and/or common exposure mentioned above.

A huge difference in age distribution is most surprising. Based on the results of the paper in detail, the elders were with highest incidence, and mean age of cases had gradually declined with epidemic progress. However, WHO reported that the mean age of h-H5N1 AI was 20 years old, 90% of cases < 40 years old [25]. In accordance with

epidemiological view, most of avian influenza viruses have not yet adapted to the people, some are in the process of adaptation; nevertheless, influenza A, such as H1N1, H2N2, H3N2, has better adapted to the people, can cause the global pandemic. Even have fully adapted to the people, however, the age distribution of influenza A was also completely different from h-H7N9 AI. For instance, the new influenza A H1N1 outbreak in 2009 showed that the incidence of 0-14 age group was 6.5 times as much as that of ≥ 65 age group in Denmark and the similar age distributions were reported in Britain and New Zealand [28-31]. In Hong Kong, the H1N1 incidence in 5-14 age years was 43.4%, while that in 30-59 age years was only 4%-4.6%, with a difference of up to about 10 times [30]. Some researchers suggested that the unapparent infection or mild cases in younger age groups may have contributed to the abnormal age distribution of h-H7N9 AI [7,15,18]. However, in our view, this cannot account for such an enormous difference in age distributions as observed above. A recent publication provides support to our view [23]. The Chinese National Influenza-Like Illness Surveillance Network (CNISN) collected 20,739 samples for detection from surveillance sites at 141 hospitals in ten provinces where h-H7N9 AI cases had occurred. The constituent ratios used in the original report were expressed as positive (unapparent infection and/or mild case) rates (per 10, 000) for comparison in this article. The positive rate (per 10, 000) of h-H7N9 AI was 2.9 (6/20,739) in all sampling population, 3.2 in 0-4 age group, 4.9 in 25-59 age group, and 7.2 in >60 age group, respectively. These rates were consistent with age distribution in epidemic [23]. Moreover, a report confirmed the above argument: the whole population including any age groups before the outbreak was no specific antibody to h-H7N9 AIV [32]. Therefore, the high incidence in elders of h-H7N9 AI was not mainly caused by the unapparent infection and/or mild disease.

In conclusion, about 60 years old with the peak incidence in acute infectious diseases of human beings is exclusive. It strongly suggested that this should be unusual in natural epidemic history of human acute infectious disease, and H7N9 AIV was absolutely new virus and never existed in China, therefore, the first epidemic of h-H7N9 AI should not occur in China.

Origin of human avian influenza (H7N9) virus and its research strategy

Many studies on origin of h-H7N9AIV and its mechanisms have been carried on [33-39]; however, all of them were explored from microcosm and focused on two aspects: one is the application of molecular biology and molecular evolution [33-35,38,39]; second is the laboratory studies [36,37], attempting to explore the route of transmission and the possible source of infection with testing in birds and poultry infected by h-H7N9 AIV. Although they have obtained certain results, which provide the basis for further comprehensive study, the authors also believe that the origin or the reservoir of h-H7N9 AIV cannot be confirmed at present. In fact, these findings are very far from researcher's target, because the strategies of the studies had critical drawbacks.

First, the difference between h-H7N9 AIV and the previous h-AIV is tremendous, which has been discussed in the paper. Avian flu and human avian influenza in China have received much attention from international organizations and other countries for many years. Therefore, Chinese government at all levels and the relevant experts have tried to do their best with very close monitoring the epidemic situation and development trend of etiology. This has been visible in the world. The results showed that although H7N9 AIV were detected

in neighbouring countries and North American, Europe and Asia, h-H7N9 AIV or novel H7N9 AIV have never been detected in China [10,16,19-21]. Consequently, suddenly occurring in China was apparently against the natural history of h-AIV.

Second, although 8 genes of h-H7N9 AIV were clear, views on how did the recombinant among 7 of them have not reached an agreement [10,16,17,32,38]. So did the results of molecular evolution. The differences above not only reflected the complex of the origin and evolution process of h-H7N9 AIV or the new H7N9 AIV, but also reflected the problems of its research ideas.

According to the results of this study, researchers in the field must adjust their strategies. First, the authors have revealed that epidemiological distributions of h-H7N9 AI in China are against the natural history. That is, with applying the philosophy of "Seeing the Essence through the Phenomena", we can affirmatively determine that origin, source of infection, transmission and susceptible population and the reservoir of h-H7N9 AI are different totally from all kinds h-AI in the past. Therefore, it is very important and very urgent that the "Reverse Thinking" of the previous strategy used in the h-AIV and h-AI studies should be applied instead of general idea.

Second, macroscopic and microscopic should be combined in any modern scientific researches. However, many researchers in the life sciences especially pay attention to micro, which is likely to narrow their views. The more important concern is that there are macro researchers without widely investigation, deep thinking and painstaking analysis themselves, such as some epidemiological scholars have only listed many data of cases and population, but do not combine them with related epidemiological principles to explore the essence of the outbreaks. It seems to have found something new, but they have not revealed the nature of h-H7N9 AI epidemic and its origin till now. Therefore, in order to solve the mystery of h-H7N9 AIV origin, researchers should correctly apply infectious disease epidemiology and combine with molecular phylogenetic technique to reconstruct h-H7N9 AIV phylogenetic tree and to probe comprehensively into the relationship with h-H7N9 AI epidemic. In fact, this is the shortage of this article and the researches only limited to the epidemiological characteristics of h-H7N9 AI without molecular evolution investigation at the same time, because of the insufficiency in some conditions. Now, we have fortunately overcome difficulties to follow the strategy proposed by us for further study.

Otherwise, according to the results and analysis of this study, it can be speculated that the reservoir of h-H7N9 AIV or new-H7N9 AIV coming from some special wild birds or poultry inhabited only in the mainland of China should be firstly disclosed, but it should be a very long and tortuous process to identify the real direct ancestor. All is the unusual origin of h-H7N9 AIV.

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