

Infectious diseases emerging from Chinese wet-markets: zoonotic origins of severe respiratory viral infections

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Purpose of review

In China, close contacts between humans and food animals have resulted in the transmission of many microbes from animals to humans. The two most notable infectious diseases in recent years are severe acute respiratory syndrome and avian influenza. In this review, these two severe zoonotic viral infections transmitted by the respiratory route, with pandemic potential, are used as models to illustrate the role of Chinese wet-markets in their emergence, amplification and dissemination.

Recent findings

Two research groups independently discovered the presence of severe acute respiratory syndrome coronavirus-like viruses in horseshoe bats. An astonishing diversity of coronaviruses was also discovered in different species of bats. For the recent and still ongoing avian influenza H5N1 outbreak that originated in Southeast Asia, from 2003 to 21 April 2006, 204 humans have been infected, with 113 deaths. Most patients had recent direct contacts with poultry.

Summary

In Chinese wet-markets, unique epicenters for transmission of potential viral pathogens, new genes may be acquired or existing genes modified through various mechanisms such as genetic reassortment, recombination and mutation. The wet-markets, at closer proximity to humans, with high viral burden or strains of higher transmission efficiency, facilitate transmission of the viruses to humans.

Keywords

avian influenza, Chinese wet-market, respiratory viral infection, severe acute respiratory syndrome, zoonosis

Abbreviations

ACE angiotensin-converting enzyme
CoV coronavirus
SARS severe acute respiratory syndrome

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Introduction

'The people take food as their heaven (民以食為天)'. 'Beijing people talk about everything, Shanghai people buy everything and Guangdong people eat everything (北京人甚麼都講, 上海人甚麼都買, 廣東人甚麼都吃)'. These traditional Chinese sayings illustrate the importance of 'having good and delicious food' among the Chinese, especially those from southern China, where the severe acute respiratory syndrome (SARS) epidemic originated. In China, food is considered most nutritive and delicious if it is freshly prepared from live animals, whereas frozen meat is considered inferior. Therefore, wet-markets are located in the vicinity of residential areas in most parts of China, which allows frequent contacts between human and live food animals. In addition to ordinary food animals, many people in southern China, such as Guangdong Province, have the habits of eating a wide range of exotic wild animals, including civets and bats, as this is traditionally believed to improve health and sexual performance. This habit is especially popular in winter when most respiratory tract infections occur. In the past few years, such close contacts with live animals have resulted in the transmission of many zoonotic microbes to humans, with backyard farm dwellers and those working in wet-markets or handling food for cooking being most vulnerable. The types of microbes transmitted are in turn determined by the different modes of contacts. Ingestion of undercooked or contaminated freshwater fish has been associated with acquisition of gastrointestinal microbes such as *Clonorchis sinensis* and *Laribacter hongkongensis* [1,2], while direct contact or percutaneous injuries during food preparation have resulted in transmission of invasive tissue pathogens such as *Streptococcus suis* and *Streptococcus iniae* [3,4]. Although these infections carry significant mortalities and morbidities, their epidemic potential is limited by their relatively inefficient modes of transmission. In contrast, zoonotic microbes causing severe illnesses that are efficiently spread by the respiratory route, with the possibility of

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Table 1 Characteristics of influenza A virus H5N1 and SARS coronavirus (SARS-CoVs)

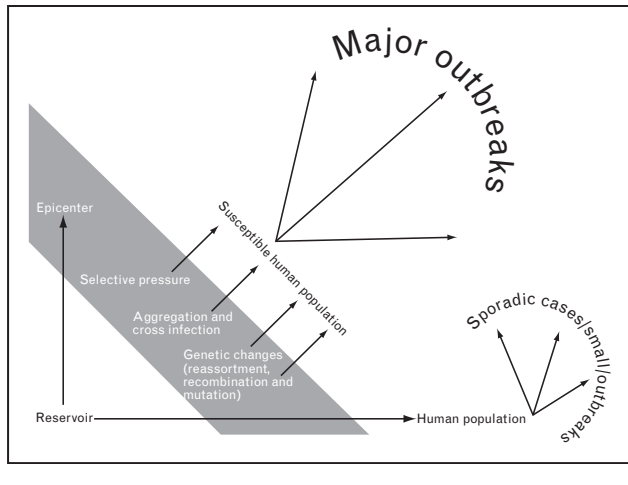
Characteristics	Influenza A virus H5N1	SARS-CoV
Geographical origin	south China	south China
Natural reservoir	wild water birds?	wild bats?
Amplification host	domestic poultry	civets and game food mammals
Epidemic center or premises of acquisition	wet-markets, backyard farms	wet-markets, restaurants, housing estate with faulty sewage system, hospital, laboratory, plane, taxi, hotel
Main type of transmission	poultry-to-human	human-to-human
Accepted modes of transmission	contact, droplet, airborne?	contact, droplet, airborne
Presenting clinical syndrome	community acquired pneumonia, influenza like illness	community or hospital acquired pneumonia
Common extrapulmonary manifestation	diarrhea	diarrhea
Radiological changes	extensive bilateral infiltration, lobar collapse, focal consolidation and air bronchograms; interstitial infiltrates are less common	focal or diffuse ground glass opacities or consolidations are common at presentation which rapidly progress to patchy or diffuse consolidations
Common changes in blood tests	leukopenia, lymphopenia, thrombocytopenia, increased alanine and aspartate aminotransferases	leukopenia, lymphopenia, thrombocytopenia, increased alanine and aspartate aminotransferases
Severe complications	Reye's syndrome (aspirin in children), encephalitis, rhabdomyolysis, adult respiratory distress syndrome	spontaneous pneumomediastinum or pneumothorax, disseminated intravascular coagulation, adult respiratory distress syndrome
Peak viral load in respiratory secretion and onset of neutralizing antibody	not clear	around day 10
Specimen for diagnosis with positive viral RNA (reverse transcription-polymerase chain reaction) or culture (cell culture)	respiratory secretions (nasopharyngeal swab, throat swab, nasopharyngeal aspirate, bronchoalveolar lavage), stool, rectal swab, serum, cerebrospinal fluid	lung tissue, liver biopsy, respiratory secretions (nasopharyngeal aspirate, nasopharyngeal swab, throat swab, bronchoalveolar lavage, tear), stool, urine, blood/serum, cerebrospinal fluid
Criteria for positive antibody testing	4-fold rise in serum (taken at least 14 days apart) neutralizing antibody against the circulating genotype (BSL3 facility)	4-fold rise in serum (taken at least 14 days apart) neutralizing antibody against SARS-CoV (often just 4-fold rise in immunofluorescence antibody against fixed whole SARS-CoV if BSL3 facility not available)
Antivirals (required randomized control trial for validation)	oseltamivir at increased dose with or without adamantanes	interferons (infacon1, interferon- β , leukocytic interferons)?; combinations of protease inhibitor with ribavirin?
Antiviral resistance	adamantine resistance prevalent in Indochina clade of genotype Z, emergence of oseltamivir resistance due to H274Y mutant during therapy	not applicable
Mainstay of therapy	antiviral and respiratory and intensive care support	respiratory and intensive care support
Crude mortality rate	50%	10–15%

human-to-human transmission, carry the greatest pandemic potential. Without doubt, the two infectious diseases that have drawn most public health concern in recent years are SARS and avian influenza [5,6,7^{**}]. The virology, clinical features, laboratory diagnosis and management of these two severe zoonotic viral infections have been extensively reviewed (Table 1) [7^{**},8[•],9[•],10^{**}]. In this review, they are used as models to illustrate the role of Chinese wet-markets in their emergence, amplification and dissemination.

Emergence, amplification and dissemination of emerging infectious diseases

Factors that determine the potential for the emergence of an infectious disease are complex. A model for the origin, amplification and dissemination of emerging infectious diseases is depicted in Fig. 1. The potential pathogen normally resides in a reservoir, which can be living (e.g.

animals) or nonliving (e.g. soil), in equilibrium. This potential pathogen may occasionally be transmitted to humans, causing sporadic cases or small outbreaks which often go undetected. In addition to direct transmission to humans, the potential pathogen may be transmitted to other epicenters, such as other animals, that are not the natural reservoir of the microbe. Under the new environment conducive to the expansion of viral burden and the selective pressure within the epicenter, new genes may be acquired or existing genes modified through various mechanisms such as genetic reassortment in influenza viruses because of the segmented genome, recombination in coronaviruses (CoVs) because of the unique mechanism of replication and mutation (the rate is in the order of one per 10 000 nucleotides replicated, which makes RNA viruses especially plastic for rapid host switching), resulting in the emergence of variants with higher transmissibility or pathogenicity. Coupled with

Figure 1 Emergence, amplification and dissemination of emerging infectious diseases

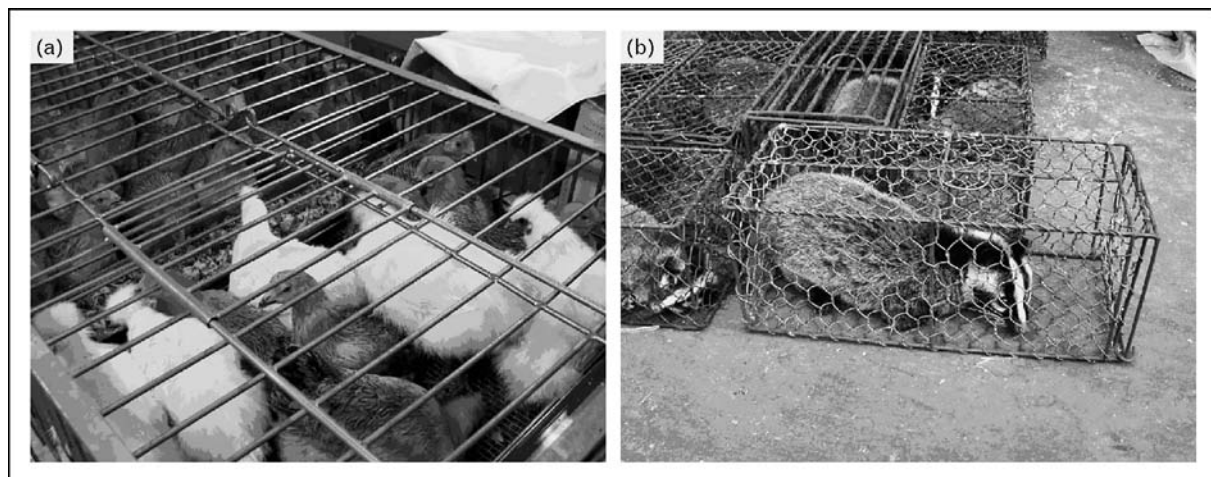
over-crowdedness, the microbial density at the epicenter can be much higher than that of the reservoir. With all these favorable conditions, the epicenter, at closer proximity to humans than the natural reservoir, facilitates transmission of the pathogen to humans. When a large number of humans are infected, especially if human-to-human transmission becomes effective with the basic reproductive number above unity [11], a major outbreak will occur.

Chinese wet-markets and zoonosis

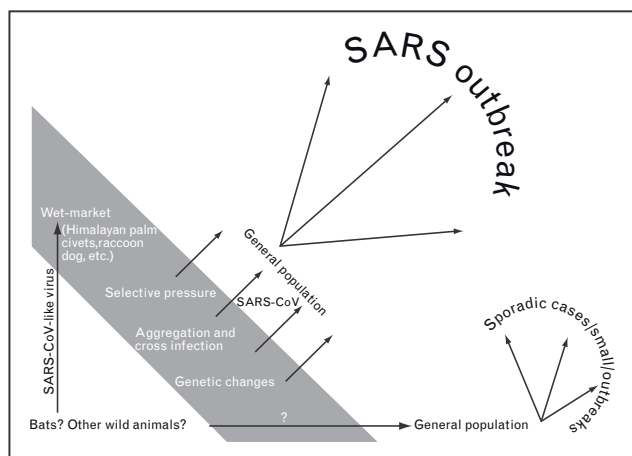
Zoonoses are diseases caused by pathogens with vertebrate intermediates or reservoirs in which the

pathogens may or may not cause disease. In developed countries, contacts between wild animals and humans are limited as a result of adoption of central slaughtering for food animals and immunization programs for most pets. Therefore, most zoonotic diseases in these countries are limited to the individuals who have personal close contact with the corresponding animals such as veterinarians, farmers, livestock and animal processing workers, and those engaged in outdoor occupation-related or recreational activities in the wild. In developing countries, however, most people live closely with animals for food supply, clothing, transportation and religious rituals. The whole population is thus continuously exposed and susceptible to zoonotic infections.

Wet-markets are places where food animals are sold either alive or as fresh meat (Fig. 2a). Due to the predilection of the southern Chinese to special delicacies, their wet-markets are additionally filled with a large variety of wild animals (Fig. 2b). Here, the animals are closely packed in cages and hygienic conditions are inevitably poor, with the shedding of large amounts of animal excreta. These animal excreta may contain high concentrations of zoonotic microbes of potential hazard to human health. High-risk behaviors of customers, such as blowing the cloacae of chickens commonly practised to examine their healthiness, further increase the risk of transmission of these potential microbes. All these factors contribute to the role of these wet-markets as a unique place for transmission of zoonotic disease to humans.

Figure 2 Chinese wet-markets in Hong Kong and Guangdong

(a) A poultry wet-market in Hong Kong, with a large number of different species of chicken closely packed in cages. (b) A wild life wet-market in Guangdong, with Himalayan palm civets housed in cages. Other commonly seen wild animals in wet-markets include beavers, Chinese ferret badgers, Chinese hares, Chinese muntjacs, hog-badgers, raccoon dogs, red foxes, bats, lesser rice field rats, rabbits, red jungle fowls, spotbill ducks, greylag geese, Chinese francolins, common pheasants, pigeons, wild boars, minks, goats and green peafowls.

Figure 3 Chinese wet-markets and SARS

Chinese wet-markets and severe acute respiratory syndrome

SARS serves as an excellent example to illustrate the role of Chinese wet-markets in the emergence of zoonotic severe respiratory viral infections (Fig. 3). The first case of SARS occurred in Foshan, a city in the Guangdong Province, whereas the second case occurred in a chef from Heyuan who worked in a restaurant in Shenzhen and had regular contact with wild food animals. Shortly after the description of SARS and isolation of the causative agent, SARS-CoV [2], SARS-CoV-like viruses were isolated from caged animals, including Himalayan palm civets and a raccoon dog, from wild live markets in Guangdong [12], suggesting that wild animals could be the reservoir of SARS-CoV-like viruses.

These SARS-CoV-like viruses have overcome the host barrier to infect humans, likely as a result of mutations in the viral proteins responsible for host cell entry. Angiotensin-converting enzyme (ACE)2 is the receptor for SARS-CoV through interaction with the spike protein [13]. Previous studies have identified a 193-amino-acid residue receptor-binding domain within the S1 region [14]. Despite the high similarity between the receptor-binding domains from palm civet and human strains, which differ by only four residues, the affinity of the respective spike proteins for human ACE2 varies by more than 1000-fold [15]. This supports that the virus has acquired the ability to infect human cells by accumulation of only a few mutations, which represents the best example of the potential of zoonotic viruses in causing epidemics under a suitable environment.

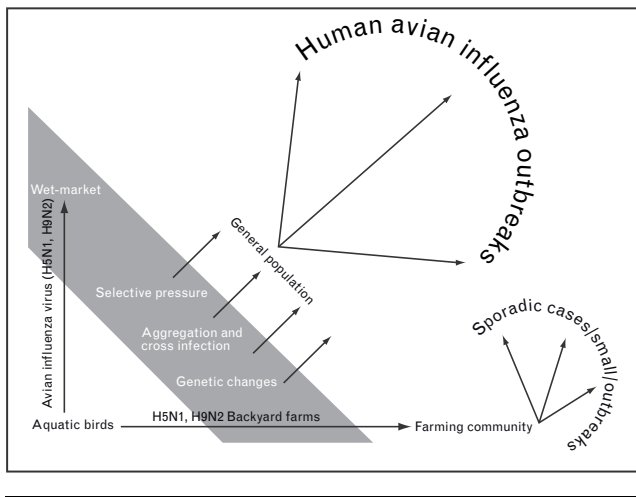
Although genome analysis supported that the direction of transmission and evolution is from civets to humans [16,17], several lines of evidence suggested that these wild game animals in Guangdong may have only served

as an epicenter for amplification of SARS-CoV and provided the environment for major genetic variations permitting efficient animal-to-human and human-to-human transmissions [18–21]. First, the K_a/K_s ratios of the *spike*, *orf3a* and *nsp3* genes of the civet strains in both the 2003 and the minor 2004 outbreaks were high, suggesting a rapidly evolving process of gene adaptation in the animals [18]. Second, while the spike proteins of SARS-CoV, isolated from humans during the 2003 epidemic, bound to and utilized both human and palm civet ACE2 efficiently, isolates from the 2004 minor outbreaks and those from palm civets utilized human ACE2 markedly less efficiently [19]. Furthermore, substantial functional changes have been detected in the spike proteins from a case in late 2003 from Guangdong Province and from two palm civets, which depended less on the human ACE2 receptor and were markedly resistant to antibody inhibition [20]. Third, while as many as 80% of the animals from markets in Guangzhou had significant levels of antibody to SARS-CoV, civets on farms and in the wild were largely free from SARS-CoV infection [21]. These facts represent strong evidence for the presence of yet unknown primary or introductory hosts of SARS-CoV. Recently, we and another group in mainland China independently discovered the presence of SARS-CoV-like viruses in horseshoe bats, which could be the reservoirs of SARS-CoV or related viruses [22^{••},23^{••}]. Comparison of the genomes of SARS-CoV from humans and civets, and the SARS-CoV-like virus in Chinese horseshoe bats showed that a 29-bp insertion in ORF8 is only found in civet SARS-CoV and bat SARS-CoV, but not in most human SARS-CoV genomes, which supports a common ancestor for the animal viruses. A 176-amino-acid fragment with 73% identities to the receptor-binding domain of SARS-CoV was found in bat SARS-CoV, within which the residues that are important for ACE2 association are also present [22^{••}]. These discoveries completed the model depicted in Fig. 1.

After the discovery of bat SARS-CoV, we conducted a surveillance study on the diversity of CoVs in bats. Results showed that an astonishing diversity of CoVs was observed in different species of bats, with the discovery of six novel CoVs [24^{••}]. These CoVs, with inherent high recombination and mutation rates, are a potential source of human infections in future.

Chinese wet-markets and avian influenza

The outbreak of avian influenza A H5N1 virus human infections in Hong Kong in 1997, with 18 cases and six deaths [6], serves as another excellent example to illustrate the role of Chinese wet-markets in the emergence of zoonotic severe respiratory viral infections (Fig. 4). Aquatic birds are the natural reservoirs of all known influenza A virus subtypes [25]. Before 1997, no purely avian influenza A viruses had been directly transmitted to

Figure 4 Chinese wet-markets and avian influenza

humans. In the 1997 H5N1 outbreak in Hong Kong, exposure to live poultry within a week before the onset of illness was shown to be a risk factor for acquiring the disease [26]. At that time, H5N1 virus was detected in about 20% of poultry in wet-markets in Hong Kong, which served as the epicenter and the source of the virus for human infections [27]. Fortunately, genetic changes that may lead to efficient animal-to-human or human-to-human transmission have not taken place. Therefore, the number of human cases was relatively small and the epidemic was terminated promptly by mass depopulation of all poultry in Hong Kong.

The recent and still ongoing avian influenza H5N1 outbreak that originated in Southeast Asia is of great concern. From 2003 to 21 April 2006, 204 humans had been infected, with 113 deaths (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_04_21/en/index.html). Epidemiological studies showed that most of the patients had recent direct contacts with poultry in the backyards [7••].

Although the molecular basis of the transmissibility of avian influenza viruses to mammals and humans is not completely understood, the increasing virulence of H5N1 influenza virus strains isolated in ducks in southern China is of great concern. Although the H5N1 influenza viruses isolated from China and Hong Kong between 1999 and 2004 showed a range of genotypes and considerable variability within the genotypes, a series of 21 H5N1 influenza virus isolates collected from apparently domestic ducks in coastal provinces of southern China between 1999 and 2002 demonstrated progressive acquisition of pathogenicity to mice [28]. The residues in PB2 and NS1 that have been shown by reverse genetics to be important for increased virulence were, however, not

present in the isolates [29–31]. Although a recent study showed that under laboratory conditions, a single amino acid substitution (Asp to Asn) at position 701 of PB2 enabled a strain of H5N1 avian influenza A virus to infect and become lethal for mice [32], a constellation of genetic changes is probably required for the virus to efficiently replicate in humans so that it can cross the species barrier and maintain itself in the human population with a basic reproduction number of above unity. Nevertheless, the wet-market could provide an excellent natural laboratory for such genetic changes to take place.

Prevention and control of Chinese wet-markets-related severe respiratory viral infections

Prevention of Chinese wet-markets-related severe respiratory viral infections is not only a scientific issue, but also involves political and cultural considerations. Theoretically, the most optimal strategy is to forbid all kinds of live animals at wet-markets, with enforcement of central slaughtering. Changing the culture of a huge ethnic group that has been established for thousands of years is, however, not an easy task. Therefore, less drastic transitional measures have to be looked for.

Hong Kong, as a more developed part of China, has always been facing the dilemma of prevention of another avian influenza outbreak and the desire of the general population to have fresh chicken. Furthermore, chicken farmers have always been pushing the government for more compensation for giving up their licenses. Although the ultimate goal is to do away with live chickens in retail markets, a series of transitional measures for prevention of avian influenza outbreaks have been implemented since 1997.

Since 1998, no live ducks and geese have been allowed in retail markets in Hong Kong, as they are natural carriers of avian influenza viruses. As of 2002, no live quails have been allowed since they can be the potential intermediate host for all subtypes of influenza viruses [33,34]. At the moment, chickens in Hong Kong come from two sources – raised in local farms and imported from mainland China. Since January 2004, both local and imported chickens have to be vaccinated against influenza virus H5. For local farms, biosecurity measures were tightened. All farms are situated at least 500 m apart, and use bird-proof net. Chicken farms must not keep any other bird species and pigs. Movement of chickens or feed between farms is tightly controlled. Footwear is disinfected before entering the farm, and all staff must shower and put on protective clothing before entering any production area. Chickens from local farms should only be transported in disinfected cages to a designated local poultry wholesale market. Vehicles that carry chickens from one farm to the wholesale market are not permitted to carry chickens or

any other poultry from another farm. No chickens that have been in a wholesale or retail market are allowed to enter local farms. As for chickens imported from mainland China, they must be from registered farms recognized by the authority in China. At the wholesale and retail market levels, market rest days (4 rest days for wholesale and 2 matching rest days for retail markets) are set up to break the virus replication cycle and reduce the viral load in the markets. During these rest days, all trading activities are stopped, all live poultry in the retail outlet slaughtered and the premises thoroughly cleansed and disinfected.

After the major outbreak in 1997, only one small outbreak of locally acquired human avian influenza involving two patients, due to H9N2 with no deaths, erupted in 1999, although two cases of human avian influenza H5N1 were imported from Guangdong Province in 2003, with one patient's death [35,36].

Concluding remarks

China, with one-quarter of the world's population, 16 of the 20 most polluted cities of the world and a huge diversity of animals closely associated with the human population, is one of the countries with the greatest potential for the emergence and spread of infectious diseases, such as SARS and avian influenza. Similar problems of overpopulation and wet-markets are also present in other countries in Southeast Asia, where the recent avian influenza outbreak apparently began. These emerging infectious disease problems are further magnified by the problem of poverty in these countries. When the avian influenza outbreak occurred in Hong Kong in 1997, we had the privilege of slaughtering all poultry in order to stop the outbreak. Poultry and eggs are, however, the principle source of proteins in these developing countries. If all poultry is slaughtered, many people will suffer from malnutrition rather than infectious diseases. This is probably one of the main reasons why the residents, or even the governments, of these countries may just report part of the real situations of the epidemics. Fortunately, unlike human immunodeficiency virus, the basic reproductive numbers of previous SARS (in the latter half of the epidemic) and avian influenza outbreaks were still below unity. After all, perhaps the most feasible way to prevent these wet-market-related severe respiratory tract viral infections is central slaughtering and cold chain for storing the meat for food.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 496).

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