Rabies surveillance and control in China over the last twenty years

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ABSTRACT

Globally, rabies remains a serious threat to public health. In particular, large number of cases continue to be detected in Africa and Asia. China has experienced three major outbreaks with the current epizootic starting in 1990s, peaking in 2007 with 3,300 cases, and consistently decreasing since this point. A key factor of the success in keeping the rabies outbreak under control is the National Public Health Reporting Program that was introduced to coordinate surveillance at provincial and regional levels. Through this program, it is possible to obtain an integrated overview of how rabies was spreading and how various factors were contributing to the outbreak. At the same time, samples have been collected from suspected rabies cases and a subset of positive samples selected for sequencing. In this way, it is possible to investigate the outbreak from a phylogenetic perspective, providing the comprehensive description of a rabies outbreak to date. In this work, we review rabies control efforts over the last 20 years, and show how this integrated approach has led to a deeper characterization of the outbreak and a better understanding of the factors driving the spread of the disease.

1. Background

Current estimates indicate that rabies kills about 59,000 people every year globally, and remains a major public health threat, especially in countries in Asia and Africa [1,2].

Until the 1950s, rabies virus (RABV) was considered to be the unique pathogen causing rabies in humans and animals [3]. Subsequently, Lagos bat virus (LBV) and Mokola virus (MOKV) were found in Nigeria in 1956 and 1968 respectively and were termed "rabies-related viruses" [4–6]. Duvenhage virus (DUVV) isolated in 1970 in South Africa also showed a serological relationship with RABV [7]. Later, bat viruses in Europe were confirmed to be closely related to but distinct from DUVV [3,8]. To accommodate the growing variety of "rabies-related" viruses, the genus Lyssavirus of the family Rhabdoviridae was established in the International Committee on Taxonomy of Viruses (ICTV) in 1975 [3]. Currently, there are 16 recognized species in the lyssavirus genus [9–11].

RABV, the most widely distributed species, mainly circulates in dogs and causes over 99% of all the human cases worldwide [2,12]. Although an Irkut Virus (IRKV) bat stain was isolated in Northeast China in 2012 [13], RABV remains the primary concern in the country [14–16]. A national reporting system was established in 1949 in mainland China, and rabies was on the initial list of notifiable diseases [17]. From 1950 to 2003, the number of cases and deaths in each province was reported monthly to the Ministry of Health (MOH). However, as a consequence of the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003, Chinese government introduced a more comprehensive public health information system and classified 37 pathogens as high threat. Thus, from 2004 onwards, each probable and confirmed case associated with these high threat pathogens, together with the relevant patient data was required to be reported from all health care facilities nationwide to the Chinese Center for Disease Control and Prevention (China CDC) [18]. Based on these surveillance data, it became possible to build a detailed picture of rabies cases in China over the course of time. Between 2002 and 2004, more than 1,000 human rabies cases were reported each year, and rabies became the leading cause of death in China among the 37 notifiable infectious diseases [17,19]. As a consequence, a national rabies surveillance system was established to investigate factors related to the epizootic, and to collect more human and animal samples for testing and sequencing at the national reference laboratory in 2005 [14,19,20]. The government has used these surveillance findings in the implementation of a number of preliminary control measures. Starting from 2008, the number of fatalities case number has begun to fall, and the number of fatalities had dropped by 91% by 2019, compared to 2007. Investigation of the diversity and evolution of viruses over the course of an epidemic has been demonstrated to play a role in the

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development of strategies to control viral diseases [21,22]. Here we review the evolution of RABV surveillance and the role of phylogenetic analysis in controlling the epizootic in China and examine how it can help to work towards the ultimate goal of eliminate rabies in China.

2. Three rabies epizootic waves

Rabies is continuously epizootic in China. Fig. 1 shows a summary of the annual number of human cases in China from 1950 to 2019. Regardless of the variation in the comprehensiveness of surveillance in earlier years, the figure shows three clear epizootic waves over this period [23]. Moreover, the gap between successive epizootic is brief, with a rapid recurrence of cases within a few years. The first wave occurred in the 1950s, reaching a peak in 1957 (1,933 cases). The second wave extended from the 1960s to the middle of 1990s and was the most serious epidemic, with 4,000–7,000 cases reported annually for most of the 1980s, with a peak of 7,037 cases occurring in 1981 [24]. China is currently in the midst of a third wave that peaked in 2007 (3,300 cases) [14]. Since then, the numbers have gradually decreased [17,19,23], with less than 2,000 cases reported in 2011, less than 1,000 cases in 2014, and 290 cases in 2019.

3. Pathogen surveillance from southern China

The national surveillance program was initially implemented in three southern provinces (Guizhou, Guangxi, and Hunan), where around half of the total number of cases in China were recorded [25–27]. 2,887 brain specimens were randomly collected from apparently healthy domestic dogs used as livestock meat in restaurants in 15 cities in the three provinces. A positive rate of 2.3% was confirmed by using a direct immunofluorescence assay (DFA), indicating rabies was present in dogs in these regions and represented a primary source of the high incidence of human rabies cases [26].

RABV has a 12-kb non-segmented negative sense RNA genome encoding five viral proteins (3′ to 5′): nucleoprotein (N), phosphoprotein (P), matrix protein (M), Glycoprotein (G) and RNA-dependent RNA polymerase (L) [28]. The N and G genes are most commonly used for genetic typing and evolutionary studies [29–31], although additional studies focus on other genes or whole genome analysis, they reveal consistent results [32–39]. An early phylogenetic analysis of positive samples based on the N gene sequences identified three primary lineages, originally designated as groups I, II, and III [26]. Within these lineages, human isolates were closely related to dog isolates from the same area, providing virologic evidence that domestic dogs were the major transmission source for the epizootic [26,40]. Within China, it was also observed

that the dissemination of RABVs from high-incidence regions was facilitated by long-distance migration and trans-provincial movement of dogs associated with human-related activities, [26].

4. The spatial and temporal dynamics of rabies in China

With the development of more rapid and cheaper nucleotide sequencing technologies, larger numbers of sequenced isolates have become available. At the same time, advances in spatial and temporal methods offers new analytical opportunities and insights into the epizootic [41]. In particular, the availability of these larger numbers of sequences revealed a larger number of distinct lineages with distinct properties and origins. Morever, migration analysis and estimates of the most recent common ancestor (TMRCA) [42–45], revealed migration events consistent with the dissemination of the virus based on human movement. Based on a more complete sequence set consisting of 210 N gene sequences from the entire 15 epidemic provinces in 2008, phylogenetic trees were constructed using the Maximum Likelihood (ML) method implemented in the PHYML and PHYLIP software packages, and isolates in China were divided into four major clades [46], with Clade IV identified as a new lineage. Consistent with previous reports [26], the majority of these samples were located in Clade I and II, but distinct differences were identified in the properties of these two clades. Firstly, spatial dynamics analysis using the MigraPhyla software package identified structure in the geographic diffusion of the rabies virus in China at the provincial level. In Clade I, Jiangsu Province was estimated to be a main source of translation events, with additional events originating in Guizhou and Henan. However, Clade II migratory centers were identified in southwestern and eastern provinces [46]. Secondly, using a Bayesian relaxed clock method, different population models were predicted with exponential population growth and constant population size determined to be the most appropriate population model for Clade I and Clade II, respectively. The evolutionary rates of each clade based on the selected population model were 1.274×10^{-3} (HPD95%: 8.3705^{-4}-1.2515×10^{-3}) substitutions per site per year for Clade I and 9.629×10^{-4} (HPD95%: 3.519^{-4}-1.628×10^{-3}) substitutions per site per year for Clade II. The TMRCA estimates for Clade I, II, and III were 15.5 years (about 1992), 48.0 years (about 1960) and 117.0 years (about 1891), respectively [46]. The corresponding Skyline plots indicated Clade I and Clade II possessed different demographic transition patterns. For Clade I, the genetic diversity decreased rapidly from 2004 to 2005, and continued a general downward trend until 2008, whereas Clade II experienced a rapid increase from 2000. Thus, the two major clades possessed distinct
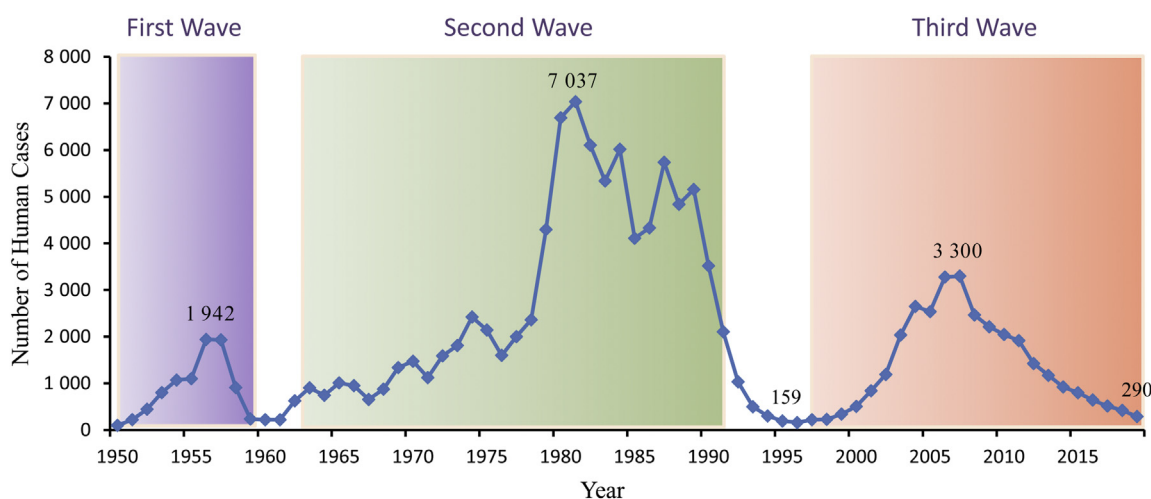

Fig. 1. The three rabies epidemic waves in Mainland China since 1949. Y-axis shows annual number of human rabies cases in China from 1950 to 2019. Since the foundation of the People's Republic of China, the country has experienced three rabies epizootics. The country is currently at the tail end of a third epizootic that begun in 1996 (159 cases), peaked in 2007 (3,300 cases) and has consistently decreased since that point.

translocation patterns and evolutionary patterns, each contributing to the epidemic in ways [46]. For Clade I, the virus spread from Southwest to East China, constantly encountering new hosts consistent with the emergence of a new lineage, whereas Clade II appeared to be already present at low levels throughout the country and reemerged more gradually.

5. The global history of rabies

Previous phylogenetic analysis has identified six distinct rabies virus lineages: Indian subcontinent, Cosmopolitan, Arctic-related, Southeast Asia (SEA) SEA1, SEA2; and SEA3 [12]. Although there is limited data, various efforts have been made to establish an evolutionary history of lyssaviruses at the global level [47]. Previous investigations of the evolutionary history of the lyssaviruses have estimated the emergence of carnivore rabies from chiropteran lyssaviruses to have occurred 888 to 1,459 years ago [22], and RABV is estimated to have begun to diverge about 500 years ago [12,22,48]. With the emergence of new species and collection of greater numbers of isolates in more divergent regions, this question is periodically revisited. A more recent study generated a dataset of 533 G lyssavirus gene sequences which was investigated using the MODELTEST and BEAST tools [47]. The mean nucleotide substitution rate for lyssavirus G gene was estimated to be 3.0×10^{-4} substitutions per site per year (95% HPD values = $2.5 \times 10^{-4} - 3.5 \times 10^{-4}$ subs per site per year) [12,22]. The TMRCA was estimated to be approximately 5,030 years (95% HPD 3,988 – 6,069 years), consistent with evidence of the earliest human civilizations (ancient Mesopotamia, ancient Egypt, ancient India and ancient China) [47]. The Carnivora and Chiroptera host group of RABV species divided about 500 years ago, and the Cosmopolitan group, which is distributed throughout the world appears to have expanded within the last 200 years. In addition, evidence of host switching in lyssavirus history from the Chiroptera to the Carnivora orders was also identified [22,47]. Thus, several notable lyssavirus evolutionary events appear to coincide with important stages in the development of human civilization.

6. RABV lineages in China and phylogenetic characterization

As a consequence of the trial Chinese national surveillance program, as well as efforts by other rabies researchers [42,43,45], significant numbers of isolates have been collected and sequenced to expand the geographic distribution (23 provinces) and the host range of infected hosts (8 species) [14]. From 2004 to 2020, as a consequence of a national effort consisting of local and provincial CDCs and the military, 8,696 specimens were collected from dog brain, human brain or saliva, and ferret badger brain, and 516 were confirmed positive. The complete G gene coding regions (1,575 nt) of 112 specimens were sequenced [14]. Additionally, all complete G sequences of rabies street strains with full background information (isolation time/host/location) were combined with the newly sequenced samples to form a final set of 320 sequences.

This dataset is the most extensive collected for any rabies epizootic and has offered new opportunities to investigate the characteristics. In particular, phylogenetic based approaches have been used to identify how the virus has been dispersed and evolved over the course of the outbreak.

In particular, phylogenetic analysis predicts six major clades (China I – China VI). This is confirmed by analysis of nucleotide sequences from both the G protein [14] and N sequences [49]. Fig. 2 shows the relationship of these clades with respect to global lineages [14]. China I, China II, China V and China VI are sub-lineages of the Asian clade; China III corresponds to Cosmopolitan, and China IV corresponds to Arctic-like. The limited number of sequences for China III to VI means it is difficult to resolve the exact relationship among these lineages, but it appears that China I represents the youngest strain in the China tree. Despite this, the lineage is distributed throughout 19 provinces and accounts for more than 80% of all strains, consistent with its emergence in the current epizootic. China II, the second most prevalent clade, appears to have originated from the previous epizootic and earlier analyses [14,26,46] included 49 strains from 7 provinces

and accounts for 15% of the samples. It suggests that China II was displaced by China I as the dominant lineage early in the current epizootic. However, more phylogenetic analysis of recent samples (unpublished data) indicates that China II continues be associated with a large fraction of sequenced isolates and has spread to additional provinces. The remaining four clades only account for 5% of the strains, are circulating in limited regions and have little association with the current epidemic. Investigation of the host of these isolates indicates they are primarily associated with spillover from wildlife [14].

Additionally, investigation of the relationship between China lineages and those from neighboring countries indicated that though sharing common lineages, they were distinct in terms of the time scale of the current epizootic. Thus, although they share a common ancestor, these lineages diverged long before the current outbreak. This suggests that current control efforts at national borders were effective at halting the transmissions of rabies into other countries [14,49]. However, in 2017, a new lineage (China VII) appeared in Tibet in 2017, and phylogenetic analysis indicated a common ancestor with isolates from Nepal [16], indicating that increased trade between bordering nations challenged efforts to halt the spread of rabies between neighboring countries [20,49,50].

7. The gradual displacement of China II by China I

Analysis of the geographical composition of these lineages shows it is consistent with human case data, indicating the surveillance and sample collection process is effectively capturing key features of the outbreak. It also reveals the gradual emergence of China I in the third epizootic [14]. In the early stages, both China I and China II remained confined to Southern China, but there were indications that newer cases in neighboring provinces were predominantly China I. By the latter part of the surveillance period, almost all isolates were China I, and the contributions from the remaining lineages were minimal [15,39]. The prevalence of China II in the early stages of the third epidemic and the presence in wildlife suggests it originated from a previous epizootic, which is supported by estimates of TMRCA for the different lineages. Thus, control efforts in previous outbreaks, which mainly involved dog culling, had the effect of reducing dominant strains to levels comparable with other localized background strains without completely eradicating the lineage, allowing it treemerge when the right conditions presented. This highlights the importance of continuing surveillance in high risk regions as well as establishing similar programs in low incidence regions. In this way, localized outbreaks can be rapidly controlled before it spreads to other regions. With the implementation of the notifiable infectious diseases program [17,19], this vision has become a reality.

Finally, in low incidence regions, China III and China IV have also been found in Western China in additional to China I [15,51,52]. In particular, all of the identified rabies lineages have been isolated in Inner Mongolia, suggesting this is a potential portal for the future spread of rabies to neighboring provinces [20]. However, as there has been limited sample collection in Western China, it is difficult to establish the dynamics of the situation in these regions. Therefore, further and more comprehensive surveillance should be conducted to improve our understanding of the current situation.

8. Rabies in wildlife in China

The situation is further complicated by lineages circulating in wildlife [53]. Rabies virus has been isolated in many different wild species, including bat, Chinese ferret badger, raccoon dog, rat, fox, and wolf. In particular, Chinese ferret badger [54], and raccoon dog [55] appear to play an important role in maintaining rabies in wildlife and are often responsible for spillover events into dog populations, and occasional reported human cases due to bites from these animals. However, due to ongoing extensive surveillance efforts, it is possible to characterize the strains associated with wildlife and it appears they can be attributed to the China III, IV, V & VI lineages [49]. This knowledge has been used in low incidence provinces in Western China. Samples from sporadic rabies cases have been collected,
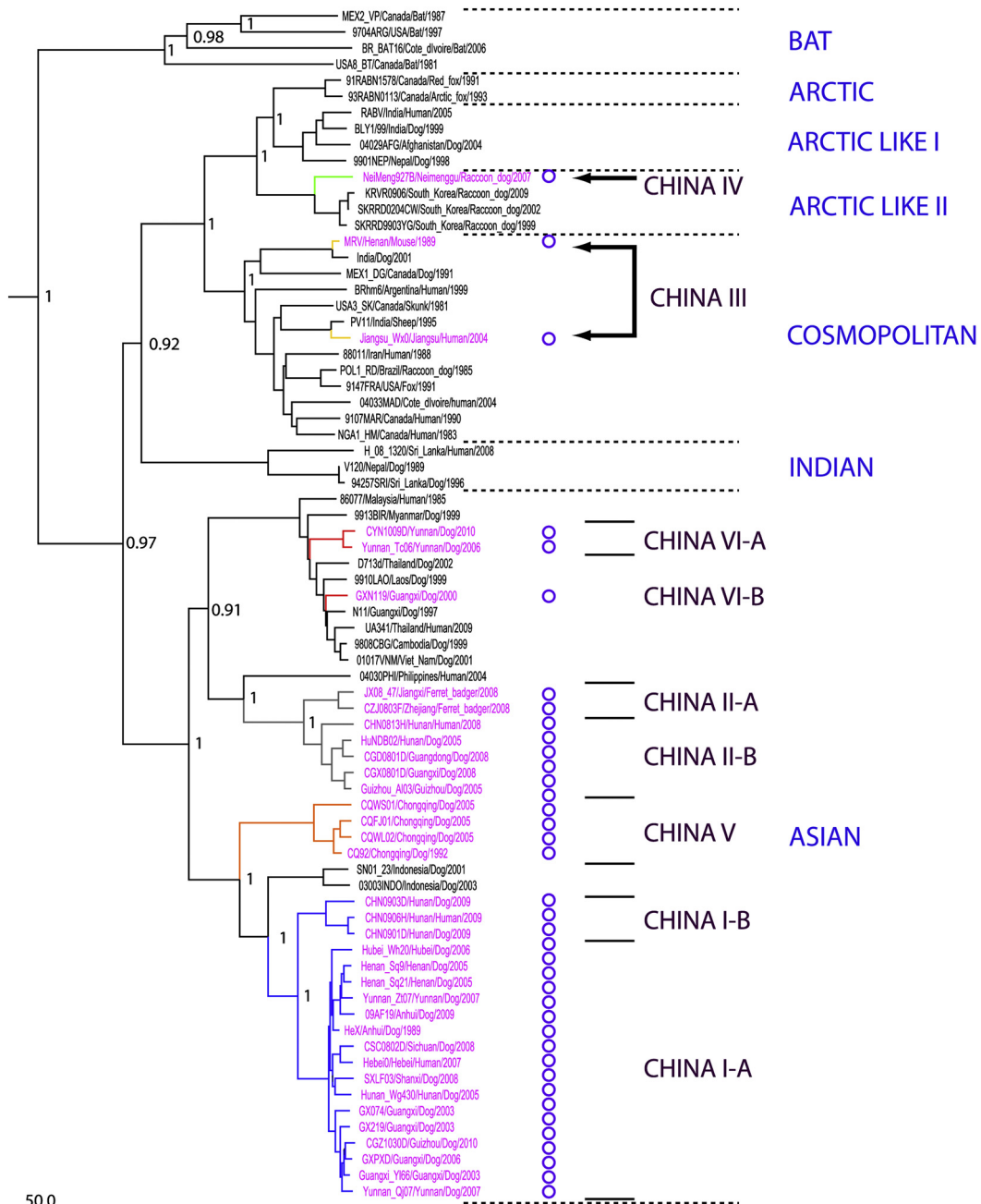
Fig. 2. Phylogenetic analysis of world sequence dataset [14]. Estimated phylogenetic tree for a representative dataset of 74 global sequences consisting of 37 sequences from the China dataset (marked with a circle after the sequence name). The tree indicates that China I, II, V&VI are sublineages of the Asian clade, China III corresponds to Cosmopolitan and China IV corresponds to the Arctic-like clade.

sequenced and analyzed to identify lineages to determine whether their likely origin is from wildlife or the current epizootic. This can provide important insight into whether current control and surveillance efforts are effective.

9. Polices and outlook of rabies surveillance and control

The rabies surveillance network is now well established in China, providing a detailed overview of the situation at the national, provincial and county level [19]. Moreover, the various control policies, including education, implemented by the government has played a key role in reducing rabies incidence in China. Lastly, regulation of postexposure prophylaxis (PEP) quality, together with improved management and vaccination of domesticated animals have also helped to improve prevention and control of

rabies [19]. However, level of PEP treatment varies according to sex, age, degree and site of exposure, as well as the source of infection. Thus, combined efforts on these front needs to be continued to ensure the situation remains under control [14,16].

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Xiaoyan Tao: Writing – Original Draft. **Siqing Liu:** Writing – Review & Editing. **Wuyang Zhu:** Conceptualization. **Simon Rayner:** Conceptualization, Writing – Original Draft.

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