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Risk factors and molecular epidemiology of canine rabies in Beijing

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Abstract

Rabies is a serious public health problem in Asia and Africa. Approximately 99% of cases result from a bite by an infected dog. The high rabies risk countries are generally characterized by rapid urbanization; however, the prevalence and risk factors for rabies in urban dogs in developing countries remain unclear. Beijing, the capital of China, has been undergoing rapid urbanization, and implemented compulsory rabies immunization for dogs on October 1, 2014. We evaluated the effects of this immunization policy and investigated the dynamics and determinants of the spread of dog rabies virus (RABV) in Beijing. Between 2013 and 2018, the positive rate of rabies in biting dogs declined significantly; 79.6% of these were stray dogs. Reconstructed spatiotemporal diffusion for RABV presents a more central distribution within Beijing and a surrounding dissemination of virus lineage toward suburban areas, indicating that rabies is self-sustaining in Beijing. Continuous RABV phylogeographic reconstructions and logistic regression analysis of rabies cases revealed that rabies tended to remain in commercial areas, high-rise residences, demolition areas, and high-GDP, high-population and high-accessibility areas. Demolition areas, where many residents have abandoned their pets, are recognized as high-risk areas for rabies. Our findings reveal that the implementation of the immunization policy in Beijing has been effective in prevention and control of canine rabies, and because the prevalence of canine rabies results from multiple factors, emphasize the important role of multi-sectoral cooperation in rabies prevention and control.

Keywords Rabies, Urban areas, Prevalence, Risk factors

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Introduction

Rabies is estimated to cause 59,000 human deaths annually in over 150 countries and incur 8.6 billion USD cost annually worldwide [1–3]. Dogs, the principal vector for human rabies, are responsible for more than 99% of human cases. With effective vaccines against rabies for human beings and dogs, a global plan to eradicate rabies transmission from dogs to human beings by 2030 has been agreed by the Food and Agriculture Organization (FAO), the World Organization for Animal Health (WOAH, founded as OIE and the World Health Organization (WHO) [4].

Rabies has been recognized to be epidemic mostly in rural areas of Africa and Asia [5]. Lack of rabies awareness and the high costs and long distance from rural areas to health clinics in these areas are responsible for the rarity of patients seeking post-exposure prophylaxis (PEP) after a bite or scratch [4, 6]. The fewer cases of rabies in humans in urban than in rural areas has been partly attributed to PEP [4]. In recent years, repeated incidents of rabies cases transmitted from dogs have occurred in urban areas [7–11], and the circulation of rabies in the dog population of urban areas is a potential risk. Additionally, the higher number of dogs and greater population density in urban areas makes the control of rabies more complex. As the process of urbanization has accelerated in many developing countries, rabies control strategies in urban areas have become increasingly important. However, timely and accurate data about rabies cases and the risk factors for rabies infection, especially for dogs in urban areas in developing countries, are still lacking. These uncertainties inevitably hamper improvements in disease control strategies and the evaluation of control measures. Continuous phylogeographic analyses as well as related post hoc approaches have previously been used to study the dispersal history of RABV spread in both large and small geographical scales and to investigate the environmental factors impacting its dispersal dynamics [5]. More comprehensive data to define the distribution, reservoirs and dispersal dynamics of rabies are hence important in order to gain a better understanding of the role of environmental heterogeneity in the maintenance and spread of the disease as well as the development of control measures.

China used to be the second-largest reported number of global rabies deaths, after only India [12]. China has experienced the largest and fastest urbanization process in world history: the urbanization of the permanent population in China was 59.58% in 2018 compared to 10.64% in 1949. Beijing, the capital, covers an area of 16,800 square kilometers, and has a permanent population of 21.52 million, with 86.5% classified as urban. The Beijing municipal government has implemented several strategies for controlling rabies. In particular, compulsory rabies immunization for dogs has been performed since October 2014. However, rabies infections have persisted in humans in Beijing, suggesting that other factors are influencing the control effect.

In the present study, we analyzed the prevalence and risk factors of rabies during 2013–2018. We found that although the rabies cases decreased after implementation of compulsory immunization measures, the rabies viruses continued to circulate in stray dogs. Rabies cases were closely related to human activity and urbanization construction.

Results

Rabies immune status of dogs and prevalence of human and canine rabies

Beijing officially implemented a compulsory rabies immunization for registered dogs on October 1, 2014. We assessed the effect of this rabies prevention and control policy change in Beijing. The serum samples of 24,878 registered dogs were collected (average of 4146 ± 906 samples/year). We found that the immunization coverage increased from 68.3% to 87.7% between 2013 and 2014, and has maintained in the 87.0%–89.5% range through 2015–2018 (Fig. 1A).

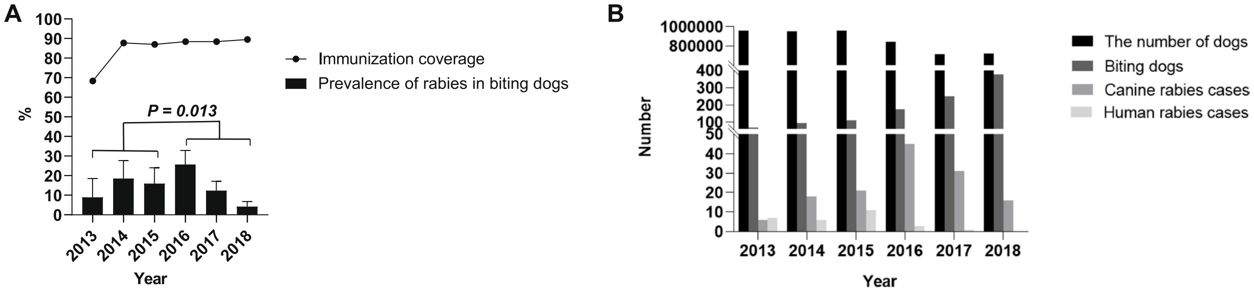

Fig. 1 Epidemiological data of rabies, 2013–2018, Beijing, China. **A** Immunization coverage in registered dogs and the prevalence of rabies in biting dogs, 2013–2018. **B** Number of dogs, biting dogs, canine rabies cases and human rabies cases, 2013–2018. Data compared by Fisher's exact test

Though the number of registered dogs decreased (Fig. 1B), the numbers of dogs biting humans increased continuously from 67 in 2013 to 377 in 2018 (Fig. 1B). A total of 137 (12.9%) brain tissue samples, including 28 from owned dogs and 109 from stray dogs, tested positive for rabies. The positive rate of rabies in biting dogs significantly reduced from 2013–2015 (45 cases of 258 (17.4%)) to 2016–2018 (92 (11.5%) of 802; $P=0.013$; Fig. 1A). Human rabies cases also declined, from 11 in 2015 to 0 in 2018 (Fig. 1B). Overall, although Beijing has an effective immunization policy for dogs, the RABV is nonetheless still circulating.

Phylogeny of RABV in Beijing

In order to understand the genetic relationship of canine RABVs in Beijing, we extracted viral RNA from brain tissue positive for rabies. Viral N genes and G genes were amplified and sequenced, of which 77 N sequences and 60 G sequences were successfully obtained. Combining these with the first 50 genetically close gene sequences based on BLASTn and reference sequences of the major lineages in China, a phylogenetic tree was constructed using the maximum likelihood method. All the N and G genes we sequenced belonged to the SEA I and China I lineages, respectively, which are currently the main lineages circulating in dogs in China (Fig. 2). All viruses were genetically close to the dog-, human- or livestock-associated strains in Beijing. Therefore, rabies in Beijing is currently a relatively independent public health issue originating from local rabid dogs. It is noteworthy that the sequences of RABVs isolated from stray and owned dogs were grouped closely, indicating that a two-way transmission between RABVs in stay and owned dogs occurred frequently in Beijing.

Epidemics concentrated in downtown of Beijing

We performed a phylogenetic relaxed random walk approach to measure the spatial spread of the outbreak. As shown in Fig. 3, the inferred RABV diffusion histories estimated from the N and G gene alignments are consistent with each other. When projected through space and time (Fig. 3), the phylogeny shows RABVs were more centrally distributed within Beijing, with a surrounding dissemination of virus lineage toward suburban areas. The diffusion coefficient of RABV in Beijing was 82.72 km^2/year (95% highest posterior distribution [HPD] = 58.44–110.54), which is substantially lower previously estimated in rural areas of Yunnan province in China (D = 1733 km^2/year; 95% HPD = 1082–2928).

Risk factors for rabies cases

Because we found rabies concentrated in downtown Beijing, we analyzed the implicit association of rabies cases with environmental and human-related factors. When

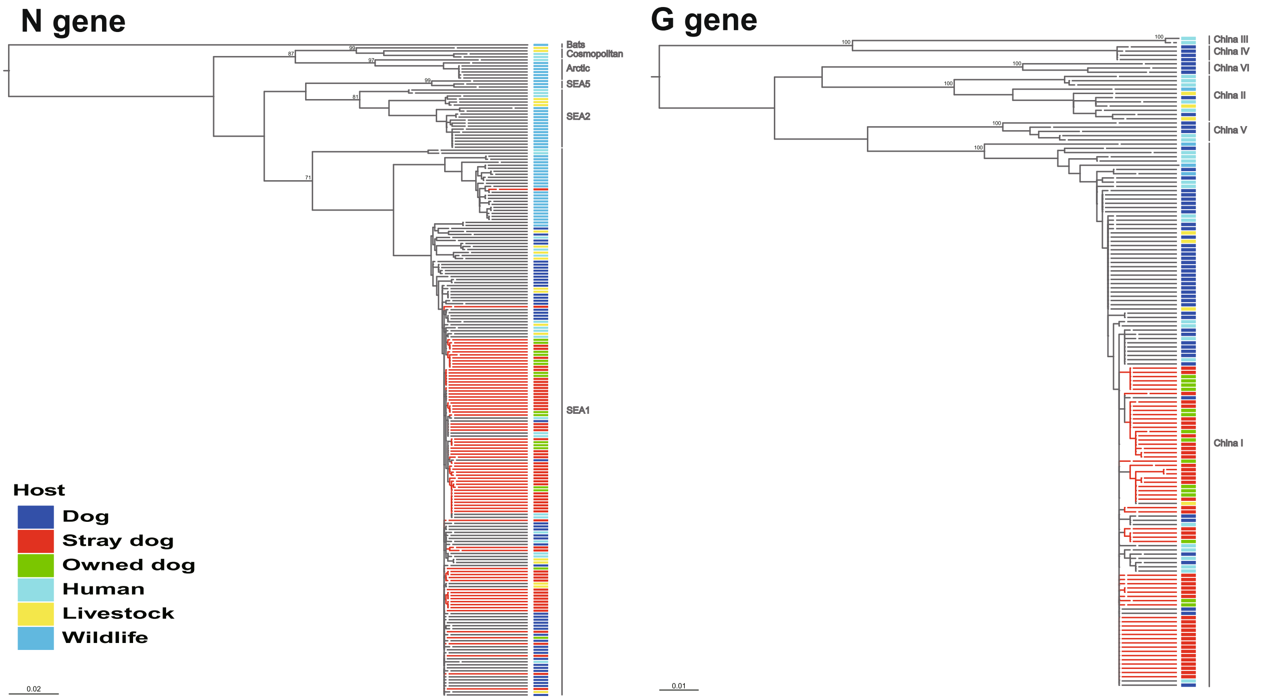

Fig. 2 Phylogenetic analysis of China N and G sequences. Viral hosts are identified by color. Named strains in red identify viruses isolated in this study

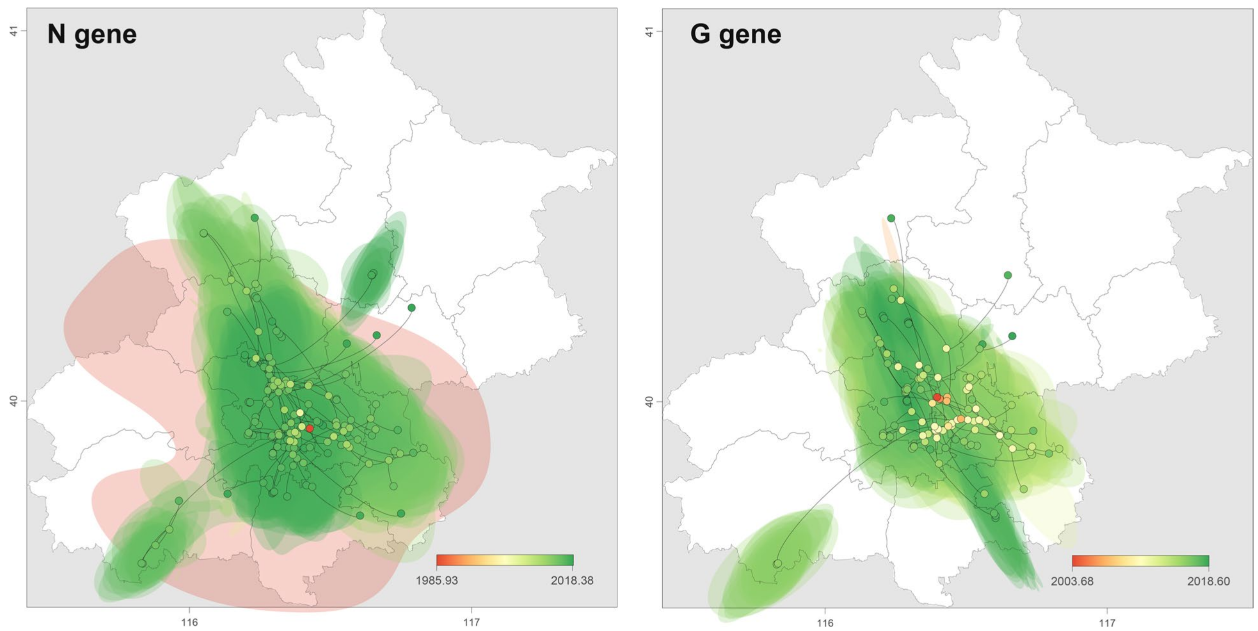

Fig. 3 Reconstructed spatio-temporal diffusion for RABV in Beijing. The plots show mapped trees and 95% highest posterior distribution (HPD) regions based on 1000 trees subsampled from the post burn-in posterior distribution of trees. Nodes of the trees are colored according to a color scale ranging from red (time to the most recent common ancestor, TMRCA) to green (most recent sampling time). Beijing borders are indicated by solid lines

analyzed by univariable analysis, 4 of 5 associations were shown to be significant ($P \leq 0.05$; Table 1). Hybrid dogs and small dogs were significant risk factors for rabies-positive cases (OR = 1.687, 95% confidence interval [CI]: 1.149–2.475; OR = 2.076, 95% CI: 1.421–3.033, respectively). Stray dogs accounted for 79.6% of the rabies-positive dogs and had significantly higher odds (OR = 3.004, 95% CI: 1.944–4.641) of rabies positivity. The odds of rabies positivity in winter (OR = 3.289, 95% CI: 1.934–5.593) was significantly higher than for other seasons. No observable difference was seen between males and females (OR = 1.037, 95% CI: 0.720–1.492). In the multivariable analysis (Table 2), small dogs had significantly higher odds (OR = 2.073, 95% CI: 1.171–3.670) of rabies positivity compared with large dogs. The single striking observation to emerge from the data comparison was that stray dogs were significantly associated with higher rabies prevalence (OR = 2.088, 95% CI: 1.318–3.308); winter also presented increased risk compared with summer (OR = 3.642, 95% CI: 2.102–6.309).

Impact of environmental and human-related factors on spatial dispersal dynamics

Our continuous RABV phylogeographic reconstructions and logistic regression analysis of rabies cases revealed potential correlations of the environmental and human-related factors and virus dispersal. We then used randomization tests to assess the level of significance of the

Table 1 Univariable analysis of rabies positivity in dogs that bit humans in Beijing, 2013–2018

Variable	RABV positive	RABV negative	OR(95% CI)	*P*-value
Genders				
Female	57(13.2%)	376(86.8%)	1.037 (0.720–1.492)	0.847
Male	80(12.8%)	547(87.2%)	1(ref)	
Species				
Hybrid	94(15.3%)	521(84.7%)	1.687(1.149–2.475)	0.008*
Other	43(9.7%)	402(90.3%)	1(ref)	
Type				
Small	92(16.7%)	458(83.3%)	2.076(1.421–3.033)	< 0.0001*
Large	45(8.8%)	465(91.2%)	1(ref)	
Owner				
Owned	28(6.5%)	402(93.5%)	1(ref)	
Stray	109(17.3%)	521(82.7%)	3.004(1.944–4.641)	< 0.0001*
Seasons				
Spring	33(14.0%)	203(86.0%)	1.470(0.910–2.375)	0.115
Summer	45(10.0%)	407(90.0%)	1(ref)	
Autumn	31(11.6%)	236(88.4%)	1.188(0.732–1.929)	0.486
Winter	28(26.7%)	77(73.3%)	3.289(1.934–5.593)	< 0.0001*

[*] Variables with $P < 0.05$ were entered into the multivariate model

Table 2 Multivariable logistic regression analysis of factors associated with rabies positivity in dogs that bit humans in Beijing, 2013–2018

Variable	OR(95% CI)	*P*-value
Species		
Hybrid	0.897(0.500–1.611)	0.716
Other	1(ref)	
Type		
Small	2.073(1.171–3.670)	0.012*
Large	1(ref)	
Owner		
Owned	1(ref)	
Stray	2.088(1.318–3.308)	0.002*
Seasons		
Spring	1.441(0.885–2.344)	0.142
Summer	1(ref)	
Autumn	1.226(0.750–2.003)	0.417
Winter	3.642(2.102–6.309)	< 0.0001*

* Variables with $P < 0.05$ were considered significantly associated with positivity

correlations between phylogeny branch durations and environmental distances. The analysis reveals a relatively low correlation between branch durations and geographical distances (Table S2). Although the woodland and accessibility variables were associated with a Bayes factor > 20, they do not appropriately explain the RABV dispersal velocity better than the geographical distance factor alone, due to the BF supports based on the randomization procedure being only reported when P (Q > 0) is at least 90%.

We investigated the impact of these factors on dispersal direction, that is, on the tendency of viral lineages to remain in or disperse toward specific environmental conditions. The analysis of the impact on dispersal tendency reveals several environmental factors associated with BF values > 20, which can be considered as strong support (Table 3). Viral lineages tended to remain in commercial, high-rise residences, demolition areas, and high-GDP, high-population and high-accessibility areas. In addition, this analysis also underlines that lineages were less likely to occur in woodland and agriculture areas. These results indicated that the prevalence of canine rabies in Beijing was closely related to human activities and urbanization.

Discussion

Dogs are responsible for more than 99% of human rabies cases. Therefore, understanding the dispersal dynamics of rabies in dogs is essential for preventing dog-to-human viral transmission. People living in urban areas have significant potential exposure to the rabies virus due to the larger number of dogs and higher population density. However, the low incidence of human rabies due to heavy PEP use has resulted in little attention being paid to the circulation of RABV in urban dogs. In fact, a study in Africa indicated that urbanization facilitated the spread and maintenance of dog RABV [13]. In the present study,

Table 3 Impact of environmental factors on the dispersal direction of RABV lineages in Beijing

Environmental factor	Testing the tendency of lineages to remain in specific environmental conditions (E)		Testing the tendency of lineages to disperse towards specific environmental conditions (R)	
	BF for factors treated as negative drivers	BF for factors treated as positive drivers	BF for factors treated as negative drivers	BF for factors treated as positive drivers
Woodland	99.00	0.01	0.29	3.37
Water	1.65	0.25	0.70	0.61
Undeveloped	0.40	1.93	2.18	0.41
Transport	0.54	1.43	4.45	0.22
Green	0.08	9.47	3.48	0.23
Industrial	0.07	10.84	1.42	0.65
Institutional	0.12	6.38	12.24	0.06
Commercial	0.01	27.13	7.41	0.12
High-rise residences	0.00	449.00	13.06	0.07
Demolition areas	0.00	> 999	1.79	0.43
Agriculture	24.71	0.03	0.07	14.25
GDP	0.00	899.00	0.94	0.91
Population density	0.00	> 999	1.05	0.89
Inaccessibility	> 999	0.00	0.00	149.00

Each environmental variable was tested once as a positive and once as a negative driver of the viral dispersion. Bayes factors (BF) > 3 and > 20 can be considered as positive and strong evidence, respectively we found stray dogs to be an important factor responsible for the circulation of rabies in urban areas in China. RABV-positive dogs were concentrated in areas where intensive human activity and urbanization construction were related to concentrations of stray dogs. Thus, the effective vaccination of urban stray dogs is crucial for rabies prevention and control.

To control RABV in dogs, Beijing implemented compulsory rabies immunization for pet dogs on October 1, 2014. To assess the effect of this implementation in Beijing, we designed a survey to assess the rabies in biting dogs from 2013 to 2018. We found that despite immunization coverage in Beijing among registered dogs being maintained in the range 87%–89% since 2014, rabies infection persists in dogs. Further studies are urgently needed to assess the impact of implicit factors on this continued presence in the urban dog population. It is noteworthy that the risk of rabies positivity is higher in stray dogs than owned dogs. In our study, we found that the immunization coverage among registered dogs (owned dogs) was maintained in the range 87%–89% since 2014. However, 79.6% of rabies-positive dogs were stray dogs. Having an unknown immune background was significantly associated with higher rabies prevalence, and the stray dogs were usually not immunized. Using the data on biting dogs, we identified that stray dogs, hybrid dogs and small dogs were potentially associated with rabies positivity. Compared with purebred dogs and large dogs, hybrid and small dogs were more likely to live in the wild. According to our logistic regression analysis, it is found that rabies is high in winter which might due to the ambient condition in winter is facilitate the virus survive. However, the summer is the most dangerous season for rabies in humans as previously observed [14]. The reason for this observation is might be that more skin is exposed for human during this season which provide more opportunity for the transmission of rabies virus.

The potential importance of environmental factors was further determined by comparing environmental conditions at branch termination locations. Analysis of the environmental factors on spatial dispersal dynamics of RABVs showed that rabies tended to remain in commercial areas, near high-rise residences, in demolition areas, and in high-GDP, high-population and high-accessibility areas, which may result from stray dogs relying on human activities for survival. Previous studies have found that population density and roads were associated with rabies spread, while inaccessibility acted as a barrier [5, 15–18]. A higher risk of canine rabies was found in urban slums in El Salvador [19], which may be due to the lack of management of stray dogs within high poverty zones in cities. Our results indicated that if stray dogs are not effectively controlled, they will concentrate in downtown areas with more food sources. In addition, our study identified demolition areas as high-risk areas for rabies. This may be because many pet dogs are abandoned following changes of residence during demolition, and these areas were usually unsupervised. Compared with developed countries, many cities in developing countries are undergoing extensive renovation, with a resulting large number of demolition areas. To reduce risk of rabies, management of stray dogs in these areas should be considered.

Phylogenetic analysis in our study indicated that the sequences of RABVs isolated from stray and owned dogs were closely grouped, indicating frequent occurrence of two-way transmission between them. Therefore, we can conclude that stray dogs responsible for the continued circulation of rabies in Beijing.

An investigation of the spatiotemporal dynamics of rabies in domestic dogs in Bangui, Central African Republic, demonstrated that the rabies epidemic is not self-sustaining but caused by numerous reintroductions of virus from neighboring peri-urban and rural areas [20]. In contrast, our phylogeographic reconstructions showed that RABVs were concentrated in the center of Beijing and disseminated to the surrounding suburban areas. Therefore, control of canine rabies in city centers, especially those with many stray dogs and urbanized areas with significant human activity, is a crucial issue to be addressed.

If radical policy changes to efforts for the prevention and control of rabies in dogs are not made, dog-associated rabies will remain a continuous public health threat, and the economic and medical burden on individuals and society will increased in the long run. Our results indicate that the rabies immunization policy in Beijing has been effective in preventing dog-to-human rabies transmission, but rabies-positive dogs cannot be eliminated if the rabies host (unregistered or stray dogs) is disregarded. One Health approach has been recognized as the most promising way to manage and prevent rabies. It is important that we recognize the prevalence and risk factors of canine rabies in Beijing, and these results promote the cooperative rabies prevention and control work between the Center for Animal Disease Control and Prevention, the Public Security Bureau, and universities. The Department of Public Security strengthened the management of stray dogs, especially in high-risk areas such as densely populated and demolition areas. The numbers of canine rabies cases have consistently decreased since 2016, and great advances have been made in the control of human rabies cases, with the reported cases of rabies in humans in Beijing being 3, 1, 0, 1, 1 and 0 in 2016, 2017, 2018, 2019, 2020 and 2021, respectively, which further demonstrated that integrated rabies elimination is a model for One Health collaboration. Moreover, the awareness of responsibilities for pet owners should be raised to reduce the potential for abandonment; an ID chip program might help to prevent abandonment as well. Management of dogs and building immunity are measures capable of eliminating the rabies epidemic, as has been done in Europe, America, Japan, the Republic of Korea and other developed countries via largescale inoculation. In Mexico, media and community engagement, dog massive vaccination campaigns, national rabies notification and a decentralized surveillance system have resulted in a reduction of human rabies deaths to zero. In KwaZulu-Natal, South Africa, training and awareness materials, dog vaccine banks, and free access to PEP have led to the elimination of human rabies. In Sri Lanka, mass dog vaccination and sterilization campaigns, free PEP, and a system of national notification of human and animal rabies cases have reduced the number of human rabies cases by 85% from 1990–2014. These will promote a One Health approach, including community engagement to build rabies awareness, dog vaccination to interrupt rabies transmission and increased access to life-saving PEP for people. Therefore, collaboration between veterinarians, doctors and government officers to prevent and control rabies in the early stages of the transmission chain should be continually pursued in the future.

Materials and methods

Measurement of antibody titer against rabies

Serum samples were collected from dogs in animal hospitals, farms or houses. Sera were separated by centrifugation following incubation at ambient temperature for 3 h. Rabies virus neutralizing antibody (RVNA) was assayed using the standard FAVN method [21, 22]. Rabies virus neutralization antibody (VNA) titers were detected using the fluorescent antibody virus neutralization test. VNA titers > 0.5 IU/mL indicated positive immunization and VNA < 0.5 IU/mL indicated negative immunization. All animal experiments were performed in compliance with the Guidance for the Care and Use of Laboratory Animals produced by the China Agricultural University. The study was approved by the Animal Ethics Committee of China Agricultural University (No. AW01017102-2).

Data collection and statistical analysis

Data on biting human dog cases, including location, genders, species, size, immune background, with or without owner, and season of onset between 2013 and 2018, were obtained from local police stations in Beijing. The dogs who bit humans were kept in quarantine by the Municipal Public Security Bureau. The brain tissue samples from dogs suspected of rabies were collected and submitted to the reference laboratory at the Changchun Veterinary Research Institute of the Chinese Academy of Agriculture Sciences, where they were examined with a fluorescent antibody test (FAT) [23] using FITC-conjugated anti-rabies monoclonal antibody. Univariable analysis was conducted using logistic regression analysis in SPSS software (version 23.0). Variables with a $P \geq 0.05$ were kept for multivariable analysis. A multivariable logistic regression model adopted the backward stepwise process. Variables with a $P \leq 0.05$ were considered as a risk factor.

Sequencing and phylogenetic analysis

Viral RNA of the rabies-positive samples was extracted using the QIAamp Viral RNA Minikit (Qiagen). PCR was conducted using specific virus primers for nucleoprotein (N) and glycoprotein (G) genes. The primer sequences and amplification conditions are available from the authors upon request. PCR products were purified with a QIAquick PCR purification kit (Qiagen) and sequenced (Beijing BGI-GBI Biotech Co., Ltd.). Sequence data were analyzed with DNAMAN5.2 (Lynnon Biosoft, USA). The viral nucleotide sequences are available from the GenBank using the accession numbers OQ828466 to OQ828602.

All rabies virus sequences available from the GenBank database were downloaded. BLASTn was performed locally with default parameters against the downloaded sequences using each of the N and G genome sequences from this study as a query. The first 50 gene sequences in the BLASTn output were collected. Multiple sequence alignment was performed using Muscle for each gene [24]. The MEGA 7 program package was used to construct the phylogenetic trees using the maximum likelihood method with 1000 bootstrap replicates [25].

Continuous phylogeographic analyses

The history of virus dispersal in Beijing was inferred using the continuous phylogeographic method implemented in BEAST 1.10 [26]. A flexible Bayesian skygrid coalescent model and relaxed random walk diffusion model was used. Because the low numbers of genomes involved in these analyses did not allow adequately informing a molecular clock model, an informative prior on the substitution rate to obtain precise and realistic estimates of time-dependent estimates was used. We extracted the spatiotemporal information which contained in the inferred phylogenetic trees by using the SERAPHIM R package [27]. The spatiotemporal information from a subset of 1000 trees sampled at regular intervals from the posterior distribution of trees was extracted. Each phylogeny branch is represented as a distinct movement vector [28]. Based on these extracted movement vectors, we used SERAPHIM to estimate statistics of spatial dispersal. According to previous study, the mean branch velocity, the weighted dispersal velocity, the diffusion coefficient [28] and the weighted diffusion coefficient [29] was estimated.

Impact of environmental and human-related factors on spatial dispersal dynamics

The impact of environmental and human-related factors on lineage dispersal velocity was analyzed according to previous studies [15, 30, 31]. All scripts for these analyses are available in the SERAPHIM R package [27]. We investigated the impact of the following environmental and human-related variables: woodland, water, undeveloped, transport, green, industrial, institutional, commercial, high-rise residences, demolition areas, agriculture [32–36], GDP, population density, and inaccessibility. Table S1 shows the sources of the data in the original raster files. All factors were tested as potential conductance factors and as potential resistance factors. The correlations between phylogenetic branch durations and distances were quantified as a statistic Q. A factor was only considered as potentially explanatory if both its distribution of regression coefficients and its associated distribution of Q values were positive [37]. In a positive distribution of estimated Q values, statistical support was evaluated against a null distribution generated by a randomization procedure and formalized using a Bayes factor (BF) value [15].

In addition to the analyses based on lineage dispersal velocity, the impact of several environmental and human-related factors on dispersal tendency was analyzed. We compared the environmental and human-related conditions between the locations of the two nodes connected by a phylogeny branch. The difference in raster cell values between the start location and the end location was computed in each branch and environmental and human-related factor. We averaged these differences within each sampled tree and evaluated against a null distribution generated by the same randomization procedure.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-023-00018-8.

Additional file 1: Table S1. Source of data for each environmental or human-related variables raster. **Table S2.** Impact of environmental factors on the dispersal velocity of RABV lineages in Beijing.

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Authors' contributions

Z.W., Q.L., X.F., L.M., J.G., X.G. and B.Z. collected the data. Z.W., Q.L., C.C, X.F., Y.S. and Y.W analysed and interpreted the data. Z.W., Q.L., Y.S. and Y.W wrote the manuscript. All authors reviewed, revised, and approved the final report.

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Availability of data and materials

All the data supporting the conclusions of this article is included within the article.

Declarations

Ethics approval and consent to participate

Ethical approval was reviewed and given by China Agricultural University Animal Ethics Committee document (No. AW01017102-2).

Consent for publication

Not applicable.

Competing interests

No potential conflict of interest was reported by the authors.

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