

RESEARCH ARTICLE

Mapping the Distribution of Anthrax in Mainland China, 2005–2013

Wan-Jun Chen¹, Sheng-Jie Lai^{2,3,4}, Yang Yang⁵, Kun Liu¹, Xin-Lou Li¹, Hong-Wu Yao¹, Yu Li², Hang Zhou², Li-Ping Wang², Di Mu², Wen-Wu Yin², Li-Qun Fang^{1*}, Hong-Jie Yu^{2*}, Wu-Chun Cao^{1*}

1 The State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, People's Republic of China, **2** Division of Infectious Disease, Key Laboratory of Surveillance and Early-warning on Infectious Disease, Chinese Centre for Disease Control and Prevention, Beijing, People's Republic of China, **3** Department of Geography and Environment, University of Southampton, Southampton, United Kingdom, **4** Flowminder Foundation, Stockholm, Sweden, **5** Department of Biostatistics, College of Public Health and Health Professions, and Emerging Pathogens Institute, University of Florida, Gainesville, Florida, United States of America

☞ These authors contributed equally to this work.

* caowc@bmi.ac.cn (WCC); yuhj@chinacdc.cn (HJY); fanglq@bmi.ac.cn (LQF)



OPEN ACCESS

Citation: Chen W-J, Lai S-J, Yang Y, Liu K, Li X-L, Yao H-W, et al. (2016) Mapping the Distribution of Anthrax in Mainland China, 2005–2013. *PLoS Negl Trop Dis* 10(4): e0004637. doi:10.1371/journal.pntd.0004637

Editor: Joseph M. Vinetz, University of California San Diego School of Medicine, UNITED STATES

Received: October 31, 2015

Accepted: March 28, 2016

Published: April 20, 2016

Copyright: © 2016 Chen et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The data of human anthrax are available from the Chinese Center for Disease Control and Prevention. For approved reasons, there may be access restrictions applied to this data. Anyone interested in it, please contact Xiang Ren, Email: renxiang@chinacdc.cn. All other relevant data is in the paper and its Supporting Information files.

Funding: The study was supported by the Special Program for Prevention and Control of Infectious Diseases in China (No. 2013ZX10004218), the Basic Work on Special Program for Science & Technology Research (2013FY114600), the Natural Science

Abstract

Background

Anthrax, a global re-emerging zoonotic disease in recent years is enzootic in mainland China. Despite its significance to the public health, spatiotemporal distributions of the disease in human and livestock and its potential driving factors remain poorly understood.

Methodology/Principal Findings

Using the national surveillance data of human and livestock anthrax from 2005 to 2013, we conducted a retrospective epidemiological study and risk assessment of anthrax in mainland China. The potential determinants for the temporal and spatial distributions of human anthrax were also explored. We found that the majority of human anthrax cases were located in six provinces in western and northeastern China, and five clustering areas with higher incidences were identified. The disease mostly peaked in July or August, and males aged 30–49 years had higher incidence than other subgroups. Monthly incidence of human anthrax was positively correlated with monthly average temperature, relative humidity and monthly accumulative rainfall with lags of 0–2 months. A boosted regression trees (BRT) model at the county level reveals that densities of cattle, sheep and human, coverage of meadow, coverage of typical grassland, elevation, coverage of topsoil with pH > 6.1, concentration of organic carbon in topsoil, and the meteorological factors have contributed substantially to the spatial distribution of the disease. The model-predicted probability of occurrence of human cases in mainland China was mapped at the county level.

Foundation of China (No. 81172728), YY was supported by the US National Institutes of Health grant for Modeling of Infectious Disease Agent Study Center for Excellence (grant U54-GM111274), HJY is funded by the Ministry of Science and Technology, China (grants 2012ZX10004-201 and 2014BA113B05), the China CDC's Key Laboratory of Surveillance and Early-warning on Infectious Disease, and grant from the US National Institutes of Health (Comprehensive International Program for Research on AIDS grant U19 AI51915), and SJL is funded by the Flowminder Foundation for his postgraduate research on population movement and infectious disease dynamics in the University of Southampton. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Conclusions/Significance

Anthrax in China was characterized by significant seasonality and spatial clustering. The spatial distribution of human anthrax was largely driven by livestock husbandry, human density, land cover, elevation, topsoil features and climate. Enhanced surveillance and intervention for livestock and human anthrax in the high-risk regions, particularly on the Qinghai-Tibetan Plateau, is the key to the prevention of human infections.

Author Summary

Anthrax is a worldwide zoonosis affecting mostly grazing herbivores, with occasional spillover to humans who have contact with infected animals or contaminated animal products. We characterized the distributional patterns of both human and livestock anthrax in China from 2005 to 2013, and identified agro-ecological, environmental and meteorological factors contributing to the temporal and spatial distributions of the disease. We found that the spatial distribution of human anthrax in China was mainly driven by densities of cattle, sheep and humans, coverage of meadow, coverage of typical grassland, elevation, pH level of topsoil, concentration of organic carbon in topsoil, and meteorological factors. We also identified the regions with higher probabilities for the occurrence of human cases. Our findings provided a clear qualitative and quantitative understanding of the epidemiological characteristics and risk recognition of anthrax in China, and can be helpful for prioritizing surveillance and control programs in the future.

Introduction

Anthrax is one of the ancient zoonoses caused by *Bacillus anthracis* [1]. It is primarily a disease in herbivores and sometimes sparks outbreaks in human with potentially serious consequences [2]. It is enzootic in most countries in Africa and Asia as well as some countries in Europe and America [3]. The disease occurs worldwide with an estimate of 20,000 to 100,000 new human cases each year [4]. According to the World Health Organization (WHO), developing countries in Africa and those in central and southern Asia have the highest human incidences of naturally occurred anthrax [5]. Because of its wide distribution and its potential use for bioterrorism, anthrax is considered as a global public health threat [6]. Concerns have been heightened by the persistent existence of human anthrax cases and outbreaks across continents in recent years, e.g., Zambia, Zimbabwe and Ethiopia in Africa [7–9], India, Bhutan, Bangladesh and Georgia in Asia [10–14], and Turkey, Greece and Serbia in Europe [15–17]. In addition, the emergence of “injectional anthrax” among heroin users in Europe highlights the possibility of new routes for the spread of human anthrax [18, 19].

Bacillus anthracis, the causative agent of anthrax, is a sporulating Gram-positive bacterium that manifests a particular bimodal lifestyle: the vegetative phase and the spore phase [2]. Bacteria in the vegetative phase are shed by infected animals and may die rapidly in most environmental conditions. Once sporulated from the vegetative cells, the bacteria can survive in soil for decades [20]. It has been speculated that levels of pH and calcium cation in the soil play an important role in the process of germination or in maintaining spore's viability. Besides, the organic matter in the soil affects spore adhesion [5, 21, 22]. As a result, topsoil conditions may geographically regulate the distribution of anthrax infections. Some other environmental

factors including climatic conditions could also be associated with anthrax infection in herbivores and humans [21, 23]. Herbivores, the primary hosts of this pathogen, are usually infected anthrax by ingestion of spores while grazing or browsing [5]. Human infection was usually a result of contacting ill animals during agricultural activities or processing contaminated animal products [24, 25]. Limited person-to-person transmission has been reported. Human anthrax cases are classified into three forms according to the transmission route: the cutaneous form accounts for about 95% of all human cases worldwide, the gastrointestinal form, and the inhalational form.

More than 112 thousands human cases have been reported in China from 1956 to 1997 with three large-scale outbreaks in the years of 1957, 1963 and 1977, respectively [26]. In the recent decades, human and livestock anthrax outbreaks have been reported in many provinces across the nation, such as Liaoning, Inner Mongolia Autonomous Region, Jiangsu, Guizhou, and Xinjiang Autonomous Region [27–31]. Previous studies mainly focused on local case-report, outbreak investigations, or the spatial and temporal distribution of cutaneous anthrax of human cases in China [27–33]. Using the national surveillance data of human and livestock anthrax from 2005 to 2013, we conducted a comprehensive and in-depth retrospective epidemiological study on the spatiotemporal dynamics and risk determinants of anthrax in mainland China.

Materials and Methods

Ethical statement

The National Health and Family Planning Commission of China considers the collection of data from human and livestock anthrax cases as part of its routine surveillance, and such data collection is therefore exempt from approval by the institutional review board.

Data collection and management

In China, inhalational anthrax is managed as a class A infectious disease, while other forms of human anthrax are listed as one of the class B infectious diseases. Cases diagnosed at medical institutions were reported to the Chinese Centre for Disease Control and Prevention (CCDC) through the web-based national Notifiable Infectious Diseases Reporting Information System (NIDRIS) (<http://www.cdpc.chinacdc.cn/UVSSERVER2.0/login?fromSmp=true&fromCDC3=true&service=http%3A%2F%2Fwww.cdpc.chinacdc.cn%2Fportal%2FcasAuthUser%3Fvsite%3Dguojia>). All clinically-diagnosed and laboratory-confirmed cases during 2005–2013 were included in this study. Routine surveillance of livestock anthrax is conducted by the Ministry of Agriculture of the People's Republic of China. The surveillance data are published monthly on the Official Veterinary Bulletin (<http://www.moa.gov.cn/zwllm/tzgg/gb/sygb/>), from which we extracted the monthly numbers of livestock cases and outbreaks as well as affected species in each province during the study period. The case definitions for human and livestock anthrax are stated in the [S1 Text](#).

Data concerning agro-ecological, environmental and meteorological factors were collected for exploring potential determinants for the temporal and spatial distributions of human anthrax in mainland China ([S1 Table](#)). Raster-typed data with a 5 km² resolution regarding the density of cattle, sheep and goats were obtained from the Food and Agriculture Organization of the United Nations (http://www.fao.org/AG/againfo/resources/en/glw/GLW_dens.html). The human population size and the annual number of livestock including cattle, sheep, goats, pigs and horses were obtained from the National Bureau of Statistics of China (<http://www.stats.gov.cn/>). The land cover data with a 1 km² resolution in 2005 was collected from the Data Sharing Infrastructure of Earth System Science (<http://www.geodata.cn>). The elevation data

were obtained from Global digital elevation data products (<http://www.geodata.cn/data/datadetails.html?dataguid=201519481253546&docid=1301>). The soil-related variables including pH level, concentration of organic carbon, and concentration of calcium in topsoil with a 1 km² resolution were derived from the Harmonized World Soil Database (<http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/>). The climatic variables including monthly average temperature and relative humidity, as well as monthly accumulative rainfall and sunshine hours during the study period were obtained from the China Meteorological Data Sharing Service System (<http://data.cma.cn/>).

Using the spatial analytic methods in the ArcGIS 9.2 software (ESRI Inc., Redlands, CA, USA), we extracted the following 15 variables for each county: densities of cattle, sheep, goats and human, percentage coverages of meadow, typical grassland and alpine steppe, average elevation, percentage coverage of topsoil with pH > 6.1, average concentration levels of organic carbon and calcium in topsoil [21], monthly average temperature, relative humidity, and yearly accumulative rainfall, sunshine hours during the study period.

Analysis of distributional patterns of human and livestock anthrax

Monthly numbers of human cases were plotted to display the seasonal dynamic of the disease. Annual incidence rates in human and annual numbers of livestock cases were plotted to show the overall temporal trend. The bar charts of average age- and gender-specific incidences were created during the study period, and the proportions of human cases by occupation were calculated. Demographic data from the 2010 census were used to calculate the average annual incidences for each county, standardized by the national distribution of age (10-year age-group categories) and sex (male and female).

The Kulldorff retrospective spatial-temporal and spatial-only scan statistics were used to detect human anthrax clustering areas at the county level (SaTScan software, version 9.3, <http://www.satscan.org>) [34]. The spatial-temporal statistic was calculated by forming the cylindrical windows with a geographic circle up to 50% of the population at risk and a time span up to 90% of the study period. The spatial-only scan statistic was calculated for each year using the maximum spatial cluster size of 10% of the population at risk. Clustering areas were identified using the log likelihood ratio (LLR) test based on a Poisson model, and the significance levels were evaluated using 999 Monte Carlo replications. A *P* value ≤ 0.05 was considered statistically significant.

A thematic map of the standardized average annual incidences of human anthrax was created, and the cumulative numbers of livestock anthrax cases and the identified spatial-temporal clustering areas were overlapped on the thematic map. To illustrate the spatiotemporal dynamics of human anthrax cases from 2005 to 2013, a bar chart of the annual numbers of human anthrax cases over the study period were shown for each province on the map. In addition, a map series for the spatial distribution of annual clustering areas and annual incidence rates of human anthrax were created. Spearman correlation was used to examine the association between monthly human anthrax incidence and each climatic variable (temperature, relative humidity, rainfall and sunshine hours) within the most likely clustering area using the Stata software, version 11.0 (StataCorp LP, Texas, USA) [35].

Analysis of influencing factors of human anthrax occurrence

A boosted regression trees (BRT) model was applied to explore the potential determinants of spatial distribution of human anthrax at the county level. It is a method that combines the advantages of two algorithms, regression trees and boosting, and is able to accommodate non-linear relationships between outcomes and covariates and multiway interactions among

covariates [36]. The weight of each variable estimated from all identified trees represents the influence of that variable in predicting the outcome. The BRT approach has been applied to the risk mapping for infectious diseases such as avian influenza A (H7N9), highly pathogenic avian influenza (H5N1) and dengue fever [37–40]. We used the data from 2005 to 2011 to construct the BRT model and the data from 2012 to 2013 to assess the model's predictive power. We adopted a tree complexity of 5, a learning rate of 0.005 and a bag fraction of 75% to identify the optimal trees for each bootstrap data.

To address the issue of multicollinearity between climate variables, principal component analysis (PCA) was performed for these variables before the BRT modeling [41]. The principal component with the largest eigenvalue (>1.0) accounts for 84.61% of the total variability (S2 Table). We refer to this principal component as the meteorological index and included it as a covariate in the BRT model. Temperature, relative humidity, rainfall and sunshine hours contributed almost equally to the meteorological index, although only sunshine hours has a negative loading (S2 Table). The BRT modeling was carried out in sequential steps. First, 1,360 counties were randomly selected without replacement from all 2,650 counties without reported human anthrax cases throughout mainland China. These control counties were then combined with the 272 counties with human anthrax cases reported during 2005–2011 to form a bootstrap dataset with a 1-to-5 case-control ratio. Second, the bootstrap dataset was randomly portioned into a training dataset with 75% of the counties and a test dataset with 25%. Third, a BRT model was built using the training dataset and validated using the test dataset. Finally, the fitted model was used to predict the probability of human anthrax presence during 2012–2013 for all counties in mainland China. These steps were repeated for 50 times. Each time, a receiver-operating characteristic (ROC) curve was produced, and the area under the curve (AUC) was calculated to evaluate the predictive power of the model. A risk map of human anthrax infections in 2012–2013 was then created based on the average predicted probabilities of the 50 repetitions.

Results

A total of 3,115 human anthrax cases were reported in mainland China during 2005–2013. Cutaneous anthrax accounted for 97.7% of all the cases. The majority of the cases (72.2%) occurred in summer and autumn, and the disease usually peaked in July or August (Fig 1A). Males had a higher average annual incidence than females in the age groups of 20 years and above (Chi-square test, $P < 0.01$). The highest age-specific incidences were found in the age groups of 30–39 and 40–49 years for both males and females (Fig 1B). Herdsmen and peasants accounted for 88.7% of all cases reported during 2010–2013, followed by children less than 6 years old (31 cases, 3.0%) (Table 1). During 2005–2013, a total of 2,261 livestock anthrax cases were reported, the majority of which were cattle, sheep, goats and pigs (S3 Table). The epizootic curve of livestock anthrax was more fluctuating than the human epidemic curve but still showed an overall decreasing trend (Cochran-Armitage trend test, $P < 0.01$). It was moderately correlated with the epidemic of human anthrax, with a Spearman correlation coefficient of 0.38 (95% CI: 0.20–0.54, $P < 0.01$).

Human anthrax cases were distributed in 299 counties of 19 provinces with an average annual incidence of 0.39 per 100,000 person years (range: 0.01–51.98). About 82% of all cases were located in six provinces/autonomous regions of western and northeastern China, including Sichuan, Xinjiang, Guizhou, Gansu, Qinghai, and Inner Mongolia (Table 1). The remaining cases were distributed sporadically in 13 provinces/autonomous regions (Fig 2). Four provinces/autonomous regions, Gansu, Qinghai, Yunnan and Inner Mongolia, showed a rebound in the number of human cases in recent years, despite the overall decreasing trend in the whole

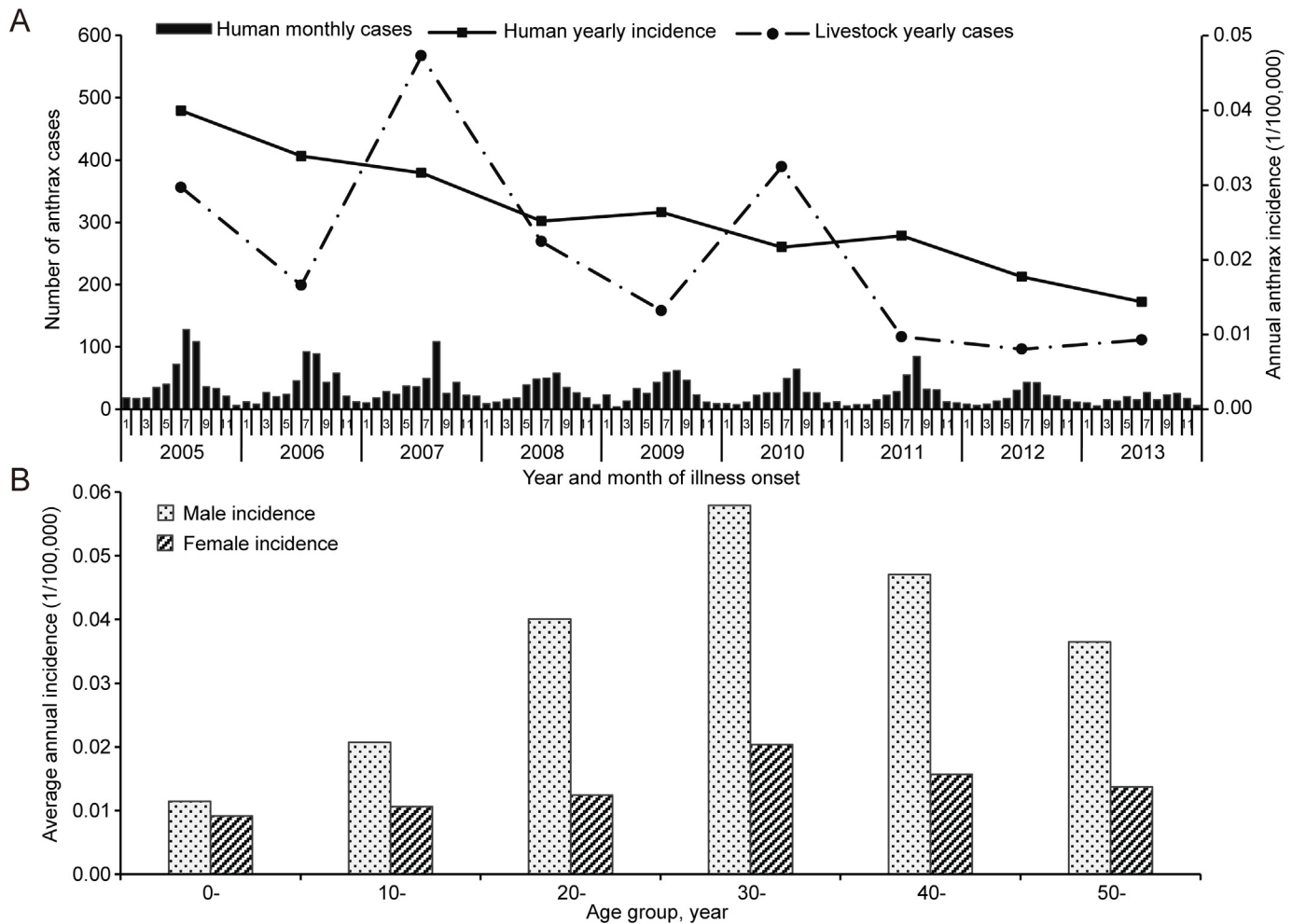


Fig 1. Temporal distribution and demographic profile of anthrax cases in mainland China during 2005–2013. (A) Temporal distribution of human and livestock cases of anthrax. (B) Average human anthrax incidences stratified by gender and age group.

doi:10.1371/journal.pntd.0004637.g001

China (S1 Fig). Qinghai is the province that suffered the most from livestock anthrax during the study period. The spatial distribution of human anthrax was mostly consistent with that of livestock anthrax, except for Sichuan Province (Fig 2, S1 Fig).

S2 Fig shows the dynamics of spatial clustering areas and standardized annual anthrax incidence of human anthrax. The most likely clusters were persistently located on the eastern Qinghai-Tibet Plateau, whereas the locations of secondary clusters varied over time. The spatiotemporal scan statistic identified one most likely cluster and four secondary clusters during the entire study period (2005–2013) (Fig 2). The most likely cluster consists of 34 counties on the junction of Sichuan, Gansu, Qinghai and Tibet provinces/autonomous regions, and spanned from January 2005 to January 2013, with a relative risk of 424.3 in comparison to counties outside the cluster. In the most likely cluster, monthly human anthrax incidence was positively correlated with monthly average temperature, relative humidity and monthly accumulative rainfall at lags of 0–2 months (S4 Table, S3 Fig). For the three climatic variables, the Spearman correlation coefficients ranged from 0.67 to 0.70 at lags of 0 or 1 month, and decreased with longer time lags. Sunshine hours at lags of 0–1 months were marginally correlated with monthly incidence of human anthrax ($P = 0.048$).

Table 1. Epidemiological characteristics of human anthrax cases in China, 2005–2013.

Characteristic	Total cases (n = 3115)
Demographic	
Male, No. (%)	2292 (73.58%)
The major age group (%)	30–49: 1423 (45.68%)
Peasant/herdsman, No. (%) ^a	909 (88.68%)
Temporal distribution	
2005	532
2006	451
2007	421
2008	335
2009	351
2010	289
2011	309
2012	236
2013	191
Spatial distribution	
No. of affected provinces	19
No. of affected counties	299
Most affected provinces (No.)	
Sichuan	917
Xinjiang	447
Guizhou	392
Gansu	370
Qinghai	225
Inner Mongolia	196

^aThe proportion of human cases according to occupation was calculated using data from 2010 to 2013.

doi:10.1371/journal.pntd.0004637.t001

The BRT model found that the spatial distribution of human anthrax was significantly associated with the densities of cattle, sheep and human, coverage of meadow, coverage of typical grassland, elevation, coverage of topsoil with pH > 6.1, concentration of organic carbon in topsoil, and the meteorological index. All above potential predictors had weights (relative contribution) of more than 5.0 in the BRT models (Table 2). The maps of human anthrax incidence overlaid by these variables were also created to display the potential spatial association between them (S4 and S5 Figs). The probability of occurrence of human cases increased with higher values of densities of cattle and sheep, coverage of meadow, and concentration of organic carbon in topsoil. The risk rose quickly with higher elevations in the range of 500–1500 m, and plateaued or dropped for elevations above 1500m. In addition, the probability of occurrence of human anthrax cases was negatively associated with the density of human population, and the meteorological index (S6A Fig).

The estimated AUC value of 0.921 (95% CI: 0.899–0.943) indicates a decent predictive power for the probability of occurrence of human cases. The AUC estimates are 0.975 (95% CI: 0.966–0.984) and 0.892 (95% CI: 0.873–0.912) for the training and test dataset, respectively (S6B Fig). To avoid overfitting of the model, which is possible considering the enormous heterogeneity in all variables across the whole country, we performed a sensitivity analysis by restricting case counties and the sampling of control counties to seven provinces (Sichuan, Qinghai, Gansu, Guizhou, Inner Mongolia, Heilongjiang and Liaoning provinces /autonomous

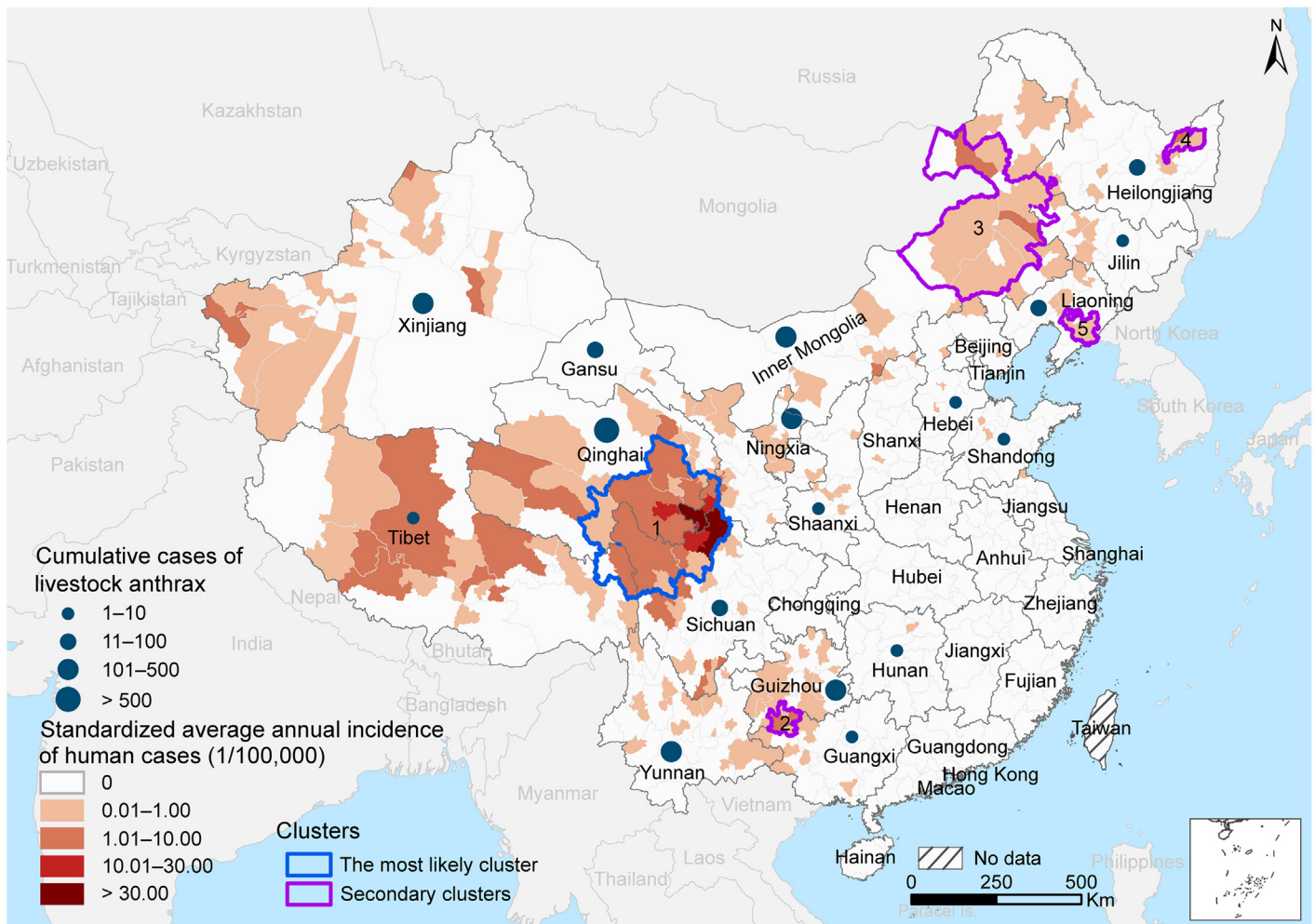


Fig 2. Standardized average annual incidences and spatial-temporal clusters of human anthrax at the county level. The cumulative numbers of livestock anthrax cases at the provincial level during 2005–2013 are overlapped.

doi:10.1371/journal.pntd.0004637.g002

regions) where spatiotemporal clusters of the disease were located. The resulted ROC and AUC's are similar to our final model based on the data of the whole country (S6C Fig). On the basis of the average predicted probability of occurrence of human cases for each county in 2012–2013, the high-risk areas of human anthrax were mainly distributed in four regions: (1) the central-west high-risk region that contains most of the Qinghai-Tibetan Plateau, and covers eastern Qinghai, northwestern Sichuan, southwestern Gansu, and central Tibet; (2) the south-west high-risk region that consists of Yunnan, Guizhou and western Guangxi provinces; (3) the northwest high-risk region that covers western and northwestern Xinjiang; and (4) the north high-risk region that covers central and eastern Inner Mongolia, western and eastern Heilongjiang, and Jilin provinces (Fig 3). By superimposing the locations of reported human anthrax cases on the predictive risk map during 2012–2013, we found that 93.1% of reported cases were located in the high risk counties each with a probability of occurrence of human cases more than 0.7. Coinciding with the most likely cluster, the eastern part of the central-west high-risk region has the highest risk of occurrence of human cases.

Table 2. Results of the boosted regression trees model applied to the human anthrax data in China during 2005–2011.

Variable	Boosted regression trees	
	Relative contribution (mean)	Relative contribution (sd)
Cattle density ^b	11.12	1.30
Sheep density ^b	8.18	1.21
Goats density	3.36	0.68
Human density ^b	8.04	1.88
Percentage coverage of meadow ^b	8.13	2.70
Percentage coverage of typical grassland ^b	5.55	1.21
Percentage coverage of alpine steppe	0.89	0.46
Elevation ^b	27.23	3.23
Percentage coverage of topsoil with pH > 6.1 ^b	5.03	1.20
Average concentration of organic carbon ^b	9.87	1.54
Average concentration of calcium	3.30	0.86
Meteorological index ^b	9.30	1.75

^bVariables reaching a weight (relative contribution) of more than 5 were considered significantly contributing to the occurrence of human anthrax infection.

doi:10.1371/journal.pntd.0004637.t002

Discussion

Our study provides a complete overview of spatiotemporal dynamics of human and livestock anthrax in mainland China from 2005 to 2013. It is the first national risk assessment of human anthrax occurrence in China. Our study identified five clustering areas of human anthrax cases and four potential high-risk regions for the occurrence of human cases. In addition, we quantified the relationship of climate factors to the temporal trend of human anthrax and the contribution of agro-ecological, environmental and meteorological factors to the spatial distribution of human anthrax.

We found that males had a higher incidence than females, in particular for adults, probably due to the occupational exposure. Among herders and farmers, men are usually more exposed to livestock than women by undertaking most agricultural activities such as pasturing and slaughtering [13,14]. Although incidences of human cases and livestock cases were significantly correlated, inconsistency was found in their spatial distributions in some provinces, e.g., Tibet and Sichuan, likely due to underreporting of anthrax case in livestock or the possibility that more than one person may contract the disease from a single animal [42]. A serological surveillance study carried out by the Chinese Institute of Epidemiology and Microbiology during 1990–1994 showed that 29.1% of human samples and 31.2% of livestock samples had detectable antibodies to the capsular of *Bacillus anthracis* at outbreak spots [43]. Since then, there have been very few studies on serological prevalence of anthrax both in human and livestock across the country. It is necessary to strengthen the surveillance of anthrax in livestock, especially in the four high-risk regions identified in this study.

Densities of cattle and sheep were identified as useful predictors for the risk of human anthrax. Suitable habitat conditions for these livestock were also important predictors, i.e., a higher coverage of meadow, a higher elevation or a lower human density. Unlike brucellosis (another zoonotic disease) [44], the presence of human anthrax was not found to be associated with the density of goats. This could be partially explained by the difference in the feeding habits between goats and other livestock. It was documented that cattle ingest lots of soil by pulling the plant out of the ground when grazing, whereas goats usually browse on grass only, which makes them less exposed to spores in soil [5, 21]. There were only two outbreaks of anthrax

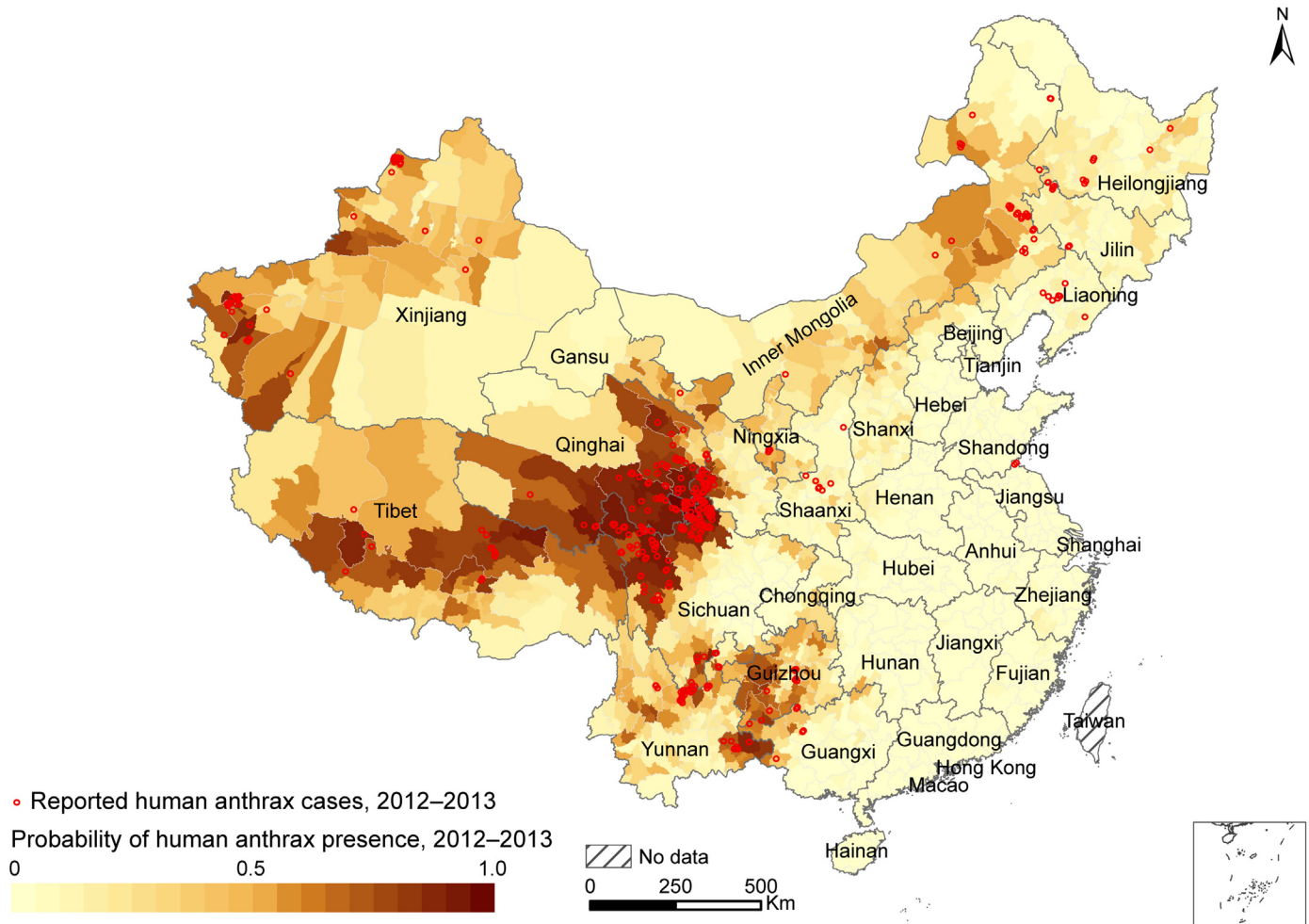


Fig 3. Predicted risks of human anthrax at the county level in China based on the BRT model and covariates for the period of 2012–2013. The BRT model was created and validated based on the data from 2005 to 2011, and the red circles represent the reported human anthrax cases from 2012 to 2013.

doi:10.1371/journal.pntd.0004637.g003

among goats during the study period, as compared to 107 outbreaks in cattle and 21 in sheep (S3 Table).

The ability of *Bacillus anthracis* to form long-lasting, highly resistant spores is the key to the persistence of anthrax in any area [22]. Certain soil characteristics, such as high levels of organic matter, pH or calcium, were thought to facilitate the survival of spores [5, 21, 22, 45], although the role of the calcium level was not always clear [46]. We found the evidence for the ecological association of human anthrax with the pH value of topsoil and the concentration of organic carbon in topsoil (Table 2, S5C and S5D Fig), but the concentration of calcium in topsoil was not picked by our model (Table 2, S5E Fig). In our data, the presence of human anthrax was largely driven by the distribution of livestock and suitable habitats for them, and the calcium level in the topsoil of the habitats is generally lower than that of non-epidemic areas, as shown in S5E Fig. However, the possibility of spatial heterogeneity in the effect of the calcium level on the presence of the disease cannot be ruled out.

Temperature, relative humidity and rainfall were positively correlated over time with human anthrax in the most likely clustering area (S4 Table, S3 Fig). Increased rainfall and temperature in the summer could unearth the anthrax spores and facilitate the breeding of vectors,

such as tabanids and *Stomoxys* [5, 21, 47, 48]. Moreover, the transfer of *Bacillus anthracis* between the vegetative form and the spore was thought to be related to temperature and relative humidity [49]. However, the exact ecological mechanism of climatic influence on the seasonality of anthrax is not clear and may vary across geographic regions. For example, drought easily makes the herbivores more exposed to the spores in soil by inhibiting the growth of grass [5, 21]. Extreme temperatures may also depress innate immunity of the host, reducing the minimal dose of anthrax for infection [5, 47]. In contrast to the positive temporal association of the climate variables to the incidence of the disease, it is interesting that the spatial risk distribution of human anthrax was negatively associated with the meteorological index and thus negatively associated with average temperature, relative humidity and rainfall. The negative association in the spatial dimension and the positive association in the temporal dimension at any given location do not contradict each other. In addition, the spatial association was partially due to the relatively high elevations of livestock habitats where average annual temperature, relative humidity and rainfall are relatively low.

Our results should be interpreted with the following limitations in mind. First, human and livestock cases could have been under-reported as the surveillance was passive. The changes in the diagnostic criteria for human anthrax cases since 2008 might have affected the quantity of the reported data. Second, the BRT model is an ecological analysis of relative contributions of risk factors and offers no causal interpretation. The causality of the identified risk factors can be tested with appropriately designed studies in the future. Third, some relevant risk factors were not available to refine our exploration, including but not limited to seroprevalence in human and livestock, exposure level of people at risk, and the industrialization level of livestock production [21].

Although anthrax underwent an overall decreasing trend during the study period, incidences rebounded and outbreaks were reported in recent years in some provinces [27–31]. While imposing threats to both animal productivity and human health in affected communities, anthrax remains a largely neglected zoonosis [50]. Existing surveillance programs for anthrax should be improved and expanded to cover livestock, human, and environmental samples, a “One Health” approach [14, 51, 52]. In addition, vaccination of both livestock and human would be essential for disease prevention and can be prioritized for high-risk regions identified in our work [42, 53].

Supporting Information

S1 Text. The case definitions of human and livestock anthrax.

(DOCX)

S1 Table. Description of risk factors used in the spatial analyses.

(DOCX)

S2 Table. Details about the principal component analysis on climatic variables.

(DOCX)

S3 Table. Outbreaks of the livestock anthrax in mainland China, 2005–2013.

(DOCX)

S4 Table. Spearman correlation coefficients (95% confidence interval) between monthly incidence of human anthrax and climate variables within the most likely cluster.

(DOCX)

S1 Fig. The distribution of human and livestock anthrax cases at province level in mainland China, 2005–2013.

(TIF)

S2 Fig. The maps of human anthrax cases from 2005 to 2013 overlaid with yearly spatial clusters in mainland China, 2005–2013.

(TIF)

S3 Fig. The temporal relationship between monthly anthrax incidence and climate variables within the most likely cluster.

(TIF)

S4 Fig. The maps of human anthrax cases from 2005 to 2013 overlaid with the distributions of anthropogenic factors in mainland China. (A) Cattle density; (B) Sheep density; (C) Goats density; (D) Human density.

(TIF)

S5 Fig. The maps of human anthrax cases from 2005 to 2013 overlaid with environmental factors in mainland China. (A) Land cover; (B) Elevation; (C) pH in topsoil; (D) Concentration of organic carbon in topsoil; (E) Concentration of calcium in topsoil; (F) Monthly average temperature during the study period; (G) Monthly average relative humidity during the study period; (H) Yearly accumulative rainfall during the study period; (I) Yearly accumulative sunshine hours during the study period.

(TIF)

S6 Fig. The outcomes based on the BRT models. (A) Relationships between the risk of human anthrax occurrence and risk factors (contribution weights ≥ 5) based on the BRT model; (B) ROC curves for the BRT models built on national data; (C) ROC curves for the BRT models built on counties in the seven provinces that reported human anthrax cases. ROC curves for all 50 bootstrap datasets are colored in grey. The average ROCs based on the training set, the test set and the prediction set (2012–2013) are colored in blue, red and black, respectively.

(TIF)

Acknowledgments

The authors thank medical staff and health practitioners who have contributed to the reporting of human and livestock anthrax in mainland China.

Author Contributions

Conceived and designed the experiments: WCC HJY LQF WJC. Analyzed the data: WJC SJL LQF KL XLL. Contributed reagents/materials/analysis tools: WJC WCC HJY LQF SJL YY KL XLL HWY YL HZ LPW DM WWY. Wrote the paper: WJC LQF. Revised the manuscript: YY HJY WCC.

References

1. World Health Organization. The control of neglected zoonotic diseases—Community-based interventions for prevention and control. Geneva, 2010. Available:http://apps.who.int/iris/bitstream/10665/44746/1/9789241502528_eng.pdf.
2. Fasanella A, Galante D, Garofolo G, Jones MH. Anthrax undervalued zoonosis. *Vet Microbiol*. 2010; 140(3–4):318–331. doi: [10.1016/j.vetmic.2009.08.016](https://doi.org/10.1016/j.vetmic.2009.08.016) PMID: [19747785](https://pubmed.ncbi.nlm.nih.gov/19747785/)
3. The Food and Agriculture Organization, Anthrax is affecting animals and humans. *EMPRES Trans-bound Anim Dis Bull* 2011; No 39: . Available:<http://www.fao.org/docrep/015/i2530e/i2530e00.pdf>
4. Swartz MN. Recognition and management of anthrax—an update. *N Engl J Med*. 2001; 345(22):1621–1626. PMID: [11704686](https://pubmed.ncbi.nlm.nih.gov/11704686/)

5. Anthrax in humans and animals. 4th edition. Geneva: World Health Organization; 2008. PMID: [26269867](#)
6. Doganay M, Demiraslana H. Human anthrax as re-emerging disease. *Recent Pat Antiinfect Drug Discov.* 2015; 10(1):10–29. PMID: [25851429](#)
7. Hang'ombe MB, Mwansa JC, Muwowo S, Mulenga P, Kapina M, Musenga E, et al. Human-animal anthrax outbreak in the Luangwa valley of Zambia in 2011. *Trop Doct.* 2012; 42(3):136–139. doi: [10.1258/td.2012.110454](#) PMID: [22472314](#)
8. Gombe NT, Nkomo BM, Chadambuka A, Shambira G, Tshimanga M. Risk factors for contracting anthrax in Kuwirirana ward, Gokwe North, Zimbabwe. *Afr Health Sci.* 2010; 10(2):159–164. PMID: [21326969](#)
9. Gelaw Y, Asaminew T. Periocular cutaneous anthrax in Jimma Zone, Southwest Ethiopia: a case series. *BMC Res Notes.* 2013; 6:313–317. doi: [10.1186/1756-0500-6-313](#) PMID: [23924443](#)
10. Reddy R, Parasadini G, Rao P, Uthappa CK, Murhekar MV. Outbreak of cutaneous anthrax in Musalimadugu village, Chittoor district, Andhra Pradesh, India, July–August 2011. *J Infect Dev Ctries.* 2012; 6(10):695–699. doi: [10.3855/jidc.2635](#) PMID: [23103890](#)
11. Thapa NK, Tenzin, Wangdi K, Dorji T, Migma, Dorjee J, et al. Investigation and control of anthrax outbreak at the human-animal interface, Bhutan, 2010. *Emerg Infect Dis.* 2014; 20(9):1524–1526. doi: [10.3201/eid2009.140181](#) PMID: [25147965](#)
12. Chakraborty A, Khan SU, Hasnat MA, Parveen S, Islam MS, Mikolon A, et al. Anthrax outbreaks in Bangladesh, 2009–2010. *Am J Trop Med Hyg.* 2012; 86(4):703–710. doi: [10.4269/ajtmh.2012.11-0234](#) PMID: [22492157](#)
13. Navdarashvili A, Doker TJ, Geleishvili M, Haberling DL, Kharod GA, Rush TH, et al. Human anthrax outbreak associated with livestock exposure Georgia, 2012. *Epidemiol Infect.* 2015 Jun 19:1–12. "In press". PMID: [26088361](#)
14. Kracalik I, Malania L, Tsertsvadze N, Manvelyan J, Bakanidze L, Imnadze P, et al. Human cutaneous anthrax, Georgia 2010–2012. *Emerg Infect Dis.* 2014; 20(2):261–264. doi: [10.3201/eid2002.130522](#) PMID: [24447721](#)
15. Tekin R, Sula B, Deveci O, Tekin A, Bozkurt F, Ucmak D, et al. Cutaneous anthrax in Southeast Anatolia of Turkey. *Cutan Ocul Toxicol.* 2015; 34(1):7–11. doi: [10.3109/15569527.2014.880844](#) PMID: [24678748](#)
16. Stefanos A, Gatselis N, Goudelas A, Mpakarosi M, Papaparaskevas J, Dalekos GN, et al. Cutaneous infection caused by *Bacillus anthracis* in Larissa, Thessaly, central Greece, July 2012. *Euro Surveill.* 2012; 17(32):pii = 20245. PMID: [22913937](#)
17. Duric P, Cosic G, Rajcevic S, Petrovic V, Tomkovic M, Subic Z, et al. Three probable cases of cutaneous anthrax in autonomous province of Vojvodina, Serbia, June 2011. *Euro Surveill.* 2012; 17(1):pii = 20050. PMID: [22264812](#)
18. Booth M, Donaldson L, Cui XZ, Sun JF, Cole S, Dailsey S, et al. Confirmed *Bacillus anthracis* infection among persons who inject drugs, Scotland, 2009–2010. *Emerg Infect Dis.* 2014; 20(9):1452–1463. doi: [10.3201/eid2009.131481](#) PMID: [25148307](#)
19. Berger T, Kassirer M, Aran AA. Injectional anthrax—new presentation of an old disease. *Euro Surveill.* 2014; 19(32):pii = 20877. PMID: [25139073](#)
20. Driks A. The *Bacillus anthracis* spore. *Mol Aspects Med.* 2009; 30(6):368–373. doi: [10.1016/j.mam.2009.08.001](#)
21. Hugh-Jones M, Blackburn J. The ecology of *Bacillus anthracis*. *Mol Aspects Med.* 2009; 30(6):356–367. doi: [10.1016/j.mam.2009.08.003](#) PMID: [19720074](#)
22. Dragon DC, Rennie RP. The ecology of anthrax spores: tough but not invincible. *Can Vet J.* 1995; 36(5):295–301. PMID: [7773917](#)
23. Joyner TA, Lukhnova L, Pazilov Y, Temiralyeva G, Hugh-Jones ME, Aikimbayeva A, et al. Modeling the potential distribution of *Bacillus anthracis* under multiple climate change scenarios for Kazakhstan. *PLoS One.* 2010; 5(3):e9596. doi: [10.1371/journal.pone.0009596](#) PMID: [20231894](#)
24. Woods CW, Ospanov K, Myrzabekov A, Favorov M, Plikaytis B, Ashford DA. Risk factors for human anthrax among contacts of anthrax-infected livestock in Kazakhstan. *Am J Trop Med Hyg.* 2004; 71(1):48–52. PMID: [15238688](#)
25. Bales ME, Dannenberg AL, Brachman PS, Kaufmann AF, Klatsky PC, Ashford DA. Epidemiologic response to anthrax outbreaks: field investigations, 1950–2001. *Emerg Infect Dis.* 2002; 8(10):1163–1174. PMID: [12396934](#)
26. Liang XD. Anthrax surveillance situation in China [in Chinese]. *Disease Surveillance.* 1999; 14(2):69–71.

27. Li S, An X, Huang Y, Pei G, Cao D, Mi Z, et al. Source tracking of an anthrax outbreak in northeastern China using complete genome analysis and MLVA genotyping. *Eur J Clin Microbiol Infect Dis*. 2015; 34(1):89–100. doi: [10.1007/s10096-014-2195-7](https://doi.org/10.1007/s10096-014-2195-7) PMID: [25073769](https://pubmed.ncbi.nlm.nih.gov/25073769/)
28. Chen H, Bao W, Wang Y, Zhang K, Wang F. Clinical and epidemiological investigation of a fatal anthrax case in China. *J Infect Dev Ctries*. 2015; 9(2):214–217. doi: [10.3855/jidc.5242](https://doi.org/10.3855/jidc.5242) PMID: [25699498](https://pubmed.ncbi.nlm.nih.gov/25699498/)
29. Tan Z, Qi X, Gu L, Bao C, Tang F, Zhu YF. Molecular characterization of *Bacillus anthracis* directly from patients' eschar and beef in an anthrax outbreak in Jiangsu Province, China, 2012. *Am J Trop Med Hyg*. 2014; 91(3):574–576. doi: [10.4269/ajtmh.13-0633](https://doi.org/10.4269/ajtmh.13-0633) PMID: [25002304](https://pubmed.ncbi.nlm.nih.gov/25002304/)
30. Yao GH, Nie W, Wang D, Ma Q, Sun J, Zhu J, et al. Epidemiologic analysis on five cases involved in an outbreak of anthrax in a village of Wengan County, Guizhou, China [in Chinese]. *Chin J Zoonoses*. 2014; 30(8):871–874.
31. Zhao YP. A survey on human cutaneous anthrax outbreak caused by slaughtering infected cattle [in Chinese]. *Chin J derm venereal*. 2012; 26(10):917–918.
32. Yang YB, Yang XD, Yang FL, Zhang Q, Ma HB, Yu BB, et al. Survey of infection focus and the epidemic trend of anthrax in Yunnan province [in Chinese]. *Chin Trop Med*. 2013; 13(11):1332–1334.
33. Zhang WY, Wang LY, Zhang XS, Han ZH, Hu WB, Qian Q, et al. Spatiotemporal clustering analysis and risk assessments of human cutaneous anthrax in China, 2005–2012. *PLoS One*. 2015; 10(7):e0133736. doi: [10.1371/journal.pone.0133736](https://doi.org/10.1371/journal.pone.0133736) PMID: [26208355](https://pubmed.ncbi.nlm.nih.gov/26208355/)
34. Kracalik I, Abdullayev R, Asadov K, Ismayilova R, Baghirova M, Ustun N, et al. Changing patterns of human anthrax in Azerbaijan during the post-Soviet and preemptive livestock vaccination eras. *PLoS Negl Trop Dis*. 2014; 8(7):e2985. doi: [10.1371/journal.pntd.0002985](https://doi.org/10.1371/journal.pntd.0002985) PMID: [25032701](https://pubmed.ncbi.nlm.nih.gov/25032701/)
35. Wei JN, Hansen A, Liu QY, Sun YH, Weinstein P, Bi P. The effect of meteorological variables on the transmission of hand, foot and mouth disease in four major cities of Shanxi province, China: a time series data analysis (2009–2013). *PLoS Negl Trop Dis*. 2015; 9(3):e0003572. doi: [10.1371/journal.pntd.0003572](https://doi.org/10.1371/journal.pntd.0003572) PMID: [25742504](https://pubmed.ncbi.nlm.nih.gov/25742504/)
36. Elith J, Leathwick JR, Hastie T. A working guide to boosted regression trees. *J Anim Ecol*. 2008; 77(4):802–813. doi: [10.1111/j.1365-2656.2008.01390.x](https://doi.org/10.1111/j.1365-2656.2008.01390.x) PMID: [18397250](https://pubmed.ncbi.nlm.nih.gov/18397250/)
37. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan A, et al. Novel methods improve prediction of species' distributions from occurrence data. *Ecography*. 2006; 29(2):129–151. doi: [10.1111/j.2006.0906-7590.04596.x](https://doi.org/10.1111/j.2006.0906-7590.04596.x)
38. Fang LQ, Li XL, Liu K, Li YJ, Yao HW, Liang S, et al. Mapping spread and risk of avian influenza A (H7N9) in China. *Sci Rep*. 2013; 3:2722. doi: [10.1038/srep02722](https://doi.org/10.1038/srep02722) PMID: [24072008](https://pubmed.ncbi.nlm.nih.gov/24072008/)
39. Martin V, Pfeiffer DU, Zhou X, Xiao X, Prosser DJ, Guo FS, et al. Spatial distribution and risk factors of highly pathogenic avian influenza (HPAI) H5N1 in China. *PLoS Pathog*. 2011; 7(3):e1001308. doi: [10.1371/journal.ppat.1001308](https://doi.org/10.1371/journal.ppat.1001308) PMID: [21408202](https://pubmed.ncbi.nlm.nih.gov/21408202/)
40. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue [letter]. *Nature*. 2013; 496(7446):504–507. doi: [10.1038/nature12060](https://doi.org/10.1038/nature12060) PMID: [23563266](https://pubmed.ncbi.nlm.nih.gov/23563266/)
41. Sang SW, Yin WW, Bi P, Zhang HL, Wang CG, Liu XB, et al. Predicting local dengue transmission in Guangzhou, China, through the influence of imported cases, mosquito density and climate variability. *PLoS One*. 2014; 9(7):e102755. doi: [10.1371/journal.pone.0102755](https://doi.org/10.1371/journal.pone.0102755) PMID: [25019967](https://pubmed.ncbi.nlm.nih.gov/25019967/)
42. Ozkurt Z, Parlak M, Tastan R, Dinler U, Saglam YS, Ozyurek SF. Anthrax in eastern Turkey, 1992–2004. *Emerg Infect Dis*. 2005; 11(12):1939–1941. PMID: [16485484](https://pubmed.ncbi.nlm.nih.gov/16485484/)
43. Liang XD, Li AF, Ma FQ, Xiao DL, Yu DZ. The surveillance and control study of anthrax in high risk provinces, China [in Chinese]. *Med Res Commun*. 1997; 26(10):16–17.
44. Li YJ, Li XL, Liang S, Fang LQ, Cao WC. Epidemiological features and risk factors associated with the spatial and temporal distribution of human brucellosis in China. *BMC Infect Dis*. 2013; 13:547. PMID: [24238301](https://pubmed.ncbi.nlm.nih.gov/24238301/) doi: [10.1186/1471-2334-13-547](https://doi.org/10.1186/1471-2334-13-547)
45. Kracalik IT, Malania L, Tsertsvadze N, Manvelyan J, Bakanidze L, Imnadze P, et al. Evidence of local persistence of human anthrax in the country of Georgia associated with environmental and anthropogenic factors. *PLoS Negl Trop Dis*. 2013; 7(9):e2388. PMID: [24040426](https://pubmed.ncbi.nlm.nih.gov/24040426/) doi: [10.1371/journal.pntd.0002388](https://doi.org/10.1371/journal.pntd.0002388)
46. Mullins J, Lukhnova L, Aikimbayev A, Pazilov Y, Van Ert M, Blackburn JK. Ecological niche modelling of the *Bacillus anthracis* A1.a sub-lineage in Kazakhstan. *BMC Ecol*. 2011; 11:32. PMID: [22152056](https://pubmed.ncbi.nlm.nih.gov/22152056/) doi: [10.1186/1472-6785-11-32](https://doi.org/10.1186/1472-6785-11-32)
47. Hampson K, Lembo T, Bessell P, Auty H, Packer C, Halliday J, et al. Predictability of anthrax infection in the Serengeti, Tanzania. *J Appl Ecol*. 2011; 48(6):1333–1344. PMID: [22318563](https://pubmed.ncbi.nlm.nih.gov/22318563/)
48. Beyer W, Turnbull PC. Anthrax in animals. *Mol Aspects Med*. 2009; 30(6):481–489. doi: [10.1016/j.mam.2009.08.004](https://doi.org/10.1016/j.mam.2009.08.004) PMID: [19723532](https://pubmed.ncbi.nlm.nih.gov/19723532/)

49. Davies DG. The influence of temperature and humidity on spore formation and germination in *Bacillus anthracis*. *J Hyg (Lond)*. 1960; 58(2):177–186. PMID: [20475844](#)
50. Welburn SC, Beange I, Ducrotoy MJ, Okello AL. The neglected zoonosis—the case for integrated control and advocacy. *Clin Microbiol Infect*. 2015; 21(5):433–443. doi: [10.1016/j.cmi.2015.04.011](#) PMID: [25911990](#)
51. Coffin JL, Monje F, Asiimwe-Karimu G, Amuguni HJ, Odoch T. A One Health, participatory epidemiology assessment of anthrax (*Bacillus anthracis*) management in Western Uganda. *Soc Sci Med*. 2015; 129:44–50. doi: [10.1016/j.socscimed.2014.07.037](#) PMID: [25066946](#)
52. Cantas L, Suer K. Review: the important bacterial zoonoses in "one health" concept. *Front Public Health*. 2014; 2:144. doi: [10.3389/fpubh.2014.00144](#) PMID: [25353010](#)
53. Centers for Disease Control and Prevention. Use of Anthrax Vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep*. 2010; 59(RR-6):1–30. PMID: [20651644](#)